The consequences of environmental heterogeneity for fitness, selection, and inheritance

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ABSTRACT

The environment influences two key drivers of adaptive evolution: the relationship between phenotype and fitness (selection) and the translation of genotype to phenotype (inheritance). The same environmental conditions may simultaneously affect both of these components in natural populations, thereby shaping evolutionary trajectories in heterogeneous landscapes, although such concurrent environmental effects remain largely neglected by evolutionary ecologists. I employed empirical, meta-analytical, and conceptual approaches to address the evolutionary consequences of separate and simultaneous environmental effects on selection and inheritance. I explored the direct and indirect fitness consequences of multiple resource use in *Bolitotherus cornutus*, a mycophagous beetle that exploits three sympatric species of host fungus. My results demonstrate that the focal *B. cornutus* metapopulation does not exhibit resource specialization, in spite of the extreme and complex fitness consequences of the host fungi at multiple life stages. Cannibalism, which accelerates larval growth and is most common in a high-quality fungus, exacerbates phenotypic and fitness differences among larvae developing in the three host fungi. The heterogeneous host community therefore generates substantial fitness variation in *B. cornutus* through a combination of direct and indirect effects throughout the life cycle. I reached beyond the *B. cornutus* system to investigate environmental effects on inheritance. In a meta-analysis of published studies, I found that the environment is a potentially pervasive source of change in multivariate genetic architecture, suggesting that labile genetic constraints shape evolutionary trajectories in changing environments. Finally, I developed a conceptual framework to examine the evolutionary implications of an environmentally driven correlation between

selection and inheritance. I found that a correlation between the strength of selection and the availability of genetic variation considerably impacts the rate of the evolutionary response. I demonstrate that concurrent environmental effects on the central components of adaptive evolution have the potential to alter evolutionary dynamics in heterogeneous landscapes.

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INTRODUCTION

Adaptive evolution depends on the translation of genotype to phenotype (inheritance) and phenotype to fitness (selection) (Falconer and Mackay 1996). The environment—both the abiotic and biotic conditions a population experiences—shapes both processes (Hoffman and Merilä 1999, MacColl 2011). Therefore, the separate and simultaneous effects of the environment on inheritance and selection govern evolution in heterogeneous landscapes (Pemberton 2010). In this dissertation, I explore the causes and consequences of environmental effects on inheritance and selection using a combination of empirical, conceptual, and meta-analytical approaches. I focus specifically on how the manifold effects of the environment on multiple aspects of the adaptive process—fitness consequences of the environment throughout the life cycle, or the covariance between selection and heritability that may emerge in variable environments—contribute to evolution in natural populations.

Environmental effects on selection

The environment is a major source of variation in fitness (Wade and Kalisz 1990). Differences in the strength and direction of selection among environments are common across taxa (Grant and Grant 1995, Brodie et al. 2002, Gosden and Svensson 2008). The underlying ecological causes of this variation have been identified in many cases (Boughman 2001, Calsbeek and Cox 2010, Reznick and Ghalambour 2005), pinpointing the sources of selection on suites of traits, disentangling the functional basis of individual traits from correlated ones, and elucidating how changes in the ecological agents of selection shape adaptive evolution (Wade and Kalisz 1990, MacColl 2011). However, our

understanding of environmental effects on evolution is limited by the fact that most such studies are phenotype-centric, focusing on the ecological sources of phenotypic selection in a single episode. Environment-centric approaches that explore the cumulative fitness consequences of a single ecological agent throughout the life cycle are underrepresented in evolutionary ecology.

The prevailing focus on single episodes of selection may underestimate the complexity underlying fitness variation in heterogeneous environments. Even when the ecological sources of selection in any given selective episode are well understood, the cumulative fitness consequences of the environment throughout the life cycle can be substantially more complicated (Grant and Grant 2011). In spite of this, it remains rare for studies of selection in the wild to incorporate multiple episodes of selection (Kingsolver et al. 2001, Kingsolver and Pfennig 2007).

This is a crucial deficiency in the literature because the same environmental conditions may have opposite fitness effects at different life stages, or result in cumulatively greater consequences than are observed in any single episode of selection as a result of both direct and indirect environmental effects on fitness (Arnold and Wade 1984). Some environments are associated with lower survival or reproductive success than others, resulting in direct fitness consequences for individuals that inhabit them (Kindvall 1996, Awmack and Leather 2002). In addition to these direct effects, the developmental environment can indirectly affect fitness later in life by modifying the expression of a phenotype with fitness consequences in adulthood. This effect is common in condition-dependent traits such as sexually selected ornaments and weapons, which are highly sensitive to the developmental environment and closely associated with fitness in

adulthood (Emlen 1994, Emlen and Nijhout 2000, Miller and Emlen 2008). Simultaneous environmental effects on the expression of multiple traits (e.g., morphology and behavior) can ameliorate or exacerbate fitness differences among environments (Kasumovic 2013). Finally, because the environment can concurrently influence phenotypic expression and the relationship between phenotype and fitness, the indirect fitness consequences of the developmental environment may depend on the selective landscape experienced later in life, a complication that is rarely considered (but see Cockburn et al. 2008). The complex nature of environmental effects on phenotype, fitness, and selection suggests that it is crucial to integrate direct and indirect fitness consequences of the environment in natural populations.

Ultimately, the fitness consequences of the environment depend on patterns of habitat use on the landscape scale. Specialization can ameliorate the fitness consequences of environmental variation because individuals primarily experience the conditions to which they are best adapted (Kawecki and Ebert 2004, Nosil and Crespi 2006). In the absence of specialization, however, the environment may be the primary source of fitness variation in natural metapopulations, and the abundance of different habitats dictates which environments influence adaptive processes. High-fitness environments are often assumed to dominate evolutionary dynamics in heterogeneous landscapes, but this may not be the case if low-fitness environments are disproportionately abundant (Pulliam 1988, Pulliam 2000, Kawecki 2008). It is therefore necessary to couple the fitness effects of different environments with patterns of habitat use to understand the evolutionary consequences of environmental heterogeneity in metapopulations that exploit multiple habitats (Kawecki et al. 2008, Hanski et al. 2011).

Environmental effects on inheritance

The evolutionary response depends jointly on selection and heritable genetic variation (Falconer and MacKay 1996). Inferring the evolutionary consequences of environmental effects on fitness and selection is complicated by the fact that the environment shapes genetic variances and covariances as well as selection (McGuigan and Sgrò 2009, Walsh and Lynch 2015).

In the decades since Waddington (1956) demonstrated that novel conditions uncover previously unobserved genetic variation in the laboratory, evolutionary ecologists have documented environmentally induced changes in genetic variances and covariances in ecologically significant phenotypes in natural populations (Weinig et al. 2003, Barrett et al. 2009, McGuigan et al. 2011). For example, additive genetic variation for body size in marine stickleback is expressed only in novel freshwater conditions, suggesting that this "cryptic genetic variation" (sensu McGuigan and Sgrò 2009) may have contributed to repeated adaptation to freshwater habitats in the stickleback system (McGuigan et al. 2011). Environment-specific gene expression is thought to underlie such changes in genetic architecture (Barrett et al. 2009), and molecular geneticists have identified molecular capacitors that are associated with the release of genetic variation in new environments (e.g., heat shock chaperone proteins, Queitsch et al. 2002). At least one of these capacitors has been implicated in the evolution of complex morphological phenotypes in natural systems (Rohner et al. 2013). Cryptic genetic variation may therefore be an important source of standing genetic variation that contributes to

adaptation to rapidly changing environments (Gibson and Dworkin 2004, Barrett and Schluter 2008, McGuigan and Sgrò 2009).

Although the existence of environmental effects on genetic architecture is well substantiated, our understanding of its implications for adaptive evolution remains limited (Ledón-Rettig 2014, Paaby and Rockman 2014). The scope of environmental effects on genetic architecture is largely uncharacterized, especially for the multivariate phenotype (McGuigan and Sgrò 2009). Synthetic meta-analyses of environmental effects on genetic architecture to date have focused on univariate changes—differences in heritabilities and genetic variances between environments—and their implications for evolutionary rates (Hoffman and Merilä 1999, Charmantier and Garant 2005). Analogous effects on genetic covariances have the potential to alter both the rate and direction of evolutionary change, shaping patterns of multivariate constraint in heterogeneous environments (Sgrò and Hoffman 2004, Blows and Hoffman 2005). If multivariate genetic architecture is environmentally sensitive, environmental change may alter evolutionary trajectories even in the absence of variation in selection (McGuigan and Sgrò 2009).

Simultaneous environmental effects on selection and inheritance

The evolutionary consequences of environmentally induced change in genetic architecture are inextricably linked to environmental effects on selection. Because the environment can alter both selection and the expression of genetic variation, change in selection may frequently accompany the release of genetic variance in heterogeneous environments (Wilson et al. 2006, Pemberton 2010). If the same environmental

conditions affect selection and genetic variation, the two may covary in heterogeneous landscapes, accelerating evolution in some environments and slowing it in others. A number of environmental conditions—most notably, environmental stressors, novelty, and quality—have been independently shown to affect selection and genetic variation (Stanton et al. 2000, Charmantier and Garant 2005). As a result, correlations between selection and genetic variation may be pervasive in heterogeneous and rapidly changing environments, such as those affected by invasive species, anthropogenic habitat modification, or climate change.

In spite of the potentially broad evolutionary consequences of non-independence between selection and inheritance, this phenomenon remains virtually unexplored theoretically and empirically. Joint estimation of selection and genetic variance in wild populations is surprisingly rare (Ritland 2011), perhaps because measuring the two parameters across multiple environments is a data-hungry enterprise. The few studies that have done so have found evidence for environmentally driven correlations between selection and genetic variance in natural populations (Wilson et al 2006, Husby et al. 2011). However, we lack a theoretical or conceptual framework for the evolutionary consequences of this correlation, which is necessary to evaluate the degree to which it may shape the evolutionary response in heterogeneous landscapes.

Dissertation objectives, approaches, and contributions

My dissertation addresses the evolutionary consequences of separate and simultaneous environmental effects on selection and inheritance using a combination of empirical, conceptual, and meta-analytical approaches. The empirical component of this

dissertation includes investigations of the direct and indirect fitness consequences of the resource environment in *Bolitotherus cornutus*, a beetle that exploits three host fungi (Chapters 1-3). Insects that rely on multiple hosts are excellent systems in which to study the effects of environmental variation because they use discrete resources that differ in quality and abundance (Jaenike 1990, Matsubayashi et al. 2010). Because the consequences of environmental heterogeneity for inheritance turned out to be empirically intractable in the *B. cornutus* system, I performed a meta-analysis of environmental effects on genetic correlations to assess the scope of environmentally induced change in multivariate genetic architecture (Chapter 4). Finally, I conducted a conceptual exploration of the response to selection when selection and genetic variance covary to characterize the evolutionary implications of simultaneous changes in selection and inheritance in heterogeneous environments (Chapter 5).

The forked fungus beetle (*Bolitotherus cornutus*; Coleoptera: Tenebrionidae) relies on fruiting bodies ("brackets") of three wood-rotting fungi: *Fomes fomentarius*, *Ganoderma applanatum*, and *G. tsugae* (Holliday et al. 2009). *Bolitotherus cornutus* populations exhibit characteristic metapopulation structure: they are comprised of subpopulations (all the beetles on a log) that exchange migrants (Conner and Hartl 2004). Approximately 25-30% of adults migrate between logs (Whitlock 1992, Ludwig 2008). Females lay eggs singly on the brackets and larvae burrow into and feed on the brackets until they emerge as adults (Liles 1956). Effects of the developmental environment on adult phenotype are likely to have fitness consequences in both sexes. Female fecundity is associated with body size in most insects (Bonduriansky 2001), and male *B. cornutus* have elaborate thoracic horns, the length of which is under strong sexual selection

(Conner 1988, Conner 1989, Formica et al. 2011). The empirical components of this dissertation were conducted at Mountain Lake Biological Station in Giles County, Virginia. The three fungi grow intermixed in the woods around the station (Graff 1947), and the local *B. cornutus* metapopulation has been the subject of ecological and evolutionary research for three decades (Brown and Bartalon 1986, Whitlock 1992, Formica et al. 2011, Ludwig 2008).

I demonstrate that the three host fungi have profound and complex fitness consequences across multiple life stages in *B. cornutus*, generating substantial fitness variation in the focal metapopulation. In Chapter 1, I demonstrate that *B. cornutus* does not exhibit resource specialization that might otherwise mitigate the negative fitness consequences of multiple host use. *Bolitotherus cornutus* frequently migrate among fungal hosts, as evidenced by the complete absence of fungus-associated population genetic structure in the focal metapopulation.

In Chapter 2, I demonstrate that the fungi have complex fitness consequences for *B. cornutus* throughout its life cycle. Larval survival is so low in *F. fomentarius* that few adults emerge from it. Beyond these direct effects, the developmental environment has indirect fitness consequences in adulthood in both sexes through its effects on adult body size. Selection favors larger adults, although its strength differs among fungi. As a result, lifetime fitness depends jointly on the fungus environment experienced during development and in adulthood. In the field, *B. cornutus* uses all three fungi even though ovipositing females prefer a high-quality fungus (*G. tsugae*) in laboratory trials. Landscape ecology may underlie the absence of specialization in *B. cornutus*: the landscape is dominated by the poorest-quality fungus, while the preferred host is rare and

temporally variable, conditions that facilitate multiple resource use in natural populations (Dias 1996, Holt et al. 2003).

Chapter 3 examines the role of conspecific interactions in the larval stage in generating fitness differences among beetles on the three fungi. I found that early larval growth depends strongly on the fungus environment under controlled laboratory conditions. These effects are exacerbated by context-dependent competitive interactions (cannibalism) among larvae. Cannibalism is most common in a high-quality fungus (*G. applanatum*) and confers growth benefits, which likely aggravates phenotypic and fitness differences among larvae from different developmental fungus environments.

Chapter 4 demonstrates that the environment is an underappreciated source of change in the structure of multivariate inheritance. Differences in the structure of the genetic variance-covariance (**G**) matrix—which describes multivariate genetic architecture—were as large between environments as the differences that accumulate between diverging conspecific populations. This comparison reveals that the environment can produce changes in genetic architecture in a single generation that are on a par with those driven by the cumulative effects of selection, mutation, migration, and drift, and challenges the prevailing assumption that **G** is stable over the short term. Environmentally sensitive genetic constraints may play an important role in shaping the trajectory of evolution in changing environments.

Finally, in Chapter 5, I found that simultaneous effects of the environment on selection and inheritance are predicted to substantially impact the rate of evolution. While a correlation between selection and genetic variance has only a modest effect on the mean evolutionary response, it dramatically alters the variation in the response among

populations. A positive correlation inflates among-population differences in the evolutionary response, while a negative correlation nearly eliminates the response in all populations. This association between selection and inheritance may be common in novel conditions, but remains virtually unmeasured in natural populations. Its magnitude, direction, and evolutionary consequences merit empirical and theoretical attention from evolutionary biologists.

My dissertation establishes that environmental effects on each of the two central components of adaptive evolution—selection and inheritance—are pervasive and complex. The empirical components of this work demonstrate that the fitness consequences of a single environmental variable can propagate throughout the life cycle, emphasizing the importance of considering simultaneous environmental effects on multiple episodes of selection. Concurrent environmentally induced changes in the genetic architecture of the phenotypes under selection may be an underappreciated component of the evolutionary response in heterogeneous landscapes. As a result of environmental effects on both selection and inheritance, non-independence between these parameters may be an overlooked determinant of evolutionary trajectories. This work underscores the primacy of ecology in the study of adaptive evolution.

Literature cited

Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: applications. *Evolution* 38:720–734.

Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity. *Annual Review of Entomology* 47:817–844.

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. *Trends in Ecology and Evolution* 23:38–44.

Barrett, R. D. H., S. M. Rogers, and D. Schluter. 2009. Environment-specific pleiotropy facilitates divergence at the Ectodysplasin locus in threespine stickleback. *Evolution* 63:2831–7.

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86:1371–1384.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews of the Cambridge Philosophical Society* 76:305–39.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–8.

Brodie, E. D., Jr., B. J. Ridenhour, and E. D. Brodie III. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* 56:2067–82.

Brown, L., and J. Bartalon. 1986. Behavioral correlates of male morphology in a horned beetle. *The American Naturalist* 127:565–570.

Calsbeek, R., and R. M. Cox. 2010. Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* 465:613–6.

Charmantier, A., and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society—B* 272:1415–25.

Cockburn, A., H. L. Osmond, and M. C. Double. 2008. Swingin' in the rain: condition dependence and sexual selection in a capricious world. *Proceedings of the Royal Society—B* 275:605–12.

Conner, J. K. 1989. Density-dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 43:1378–1386.

Conner, J. K. 1988. Field measurements of natural and sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 42:736–749.

Conner, J. K. and D. L. Hartl. 2004. A Primer of Ecological Genetics. Sinauer Associates, Inc., Sunderland, MA.

Dias, P. C. 1996. Sources and sinks in population biology. *Trends in Ecology and Evolution* 11:326–30.

Emlen, D. J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society—B* 256:131–136.

Emlen, D. J., and H. F. Nijhout. 2000. The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology* 45:661–708.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics, Fourth Edition. Essex, UK: Longman Group Ltd.

Formica, V. A., J. W. McGlothlin, C. W. Wood, M. E. Augat, R. E. Butterfield, M. E. Barnard, and E. D. Brodie III. 2011. Phenotypic assortment mediates the effect of social selection in a wild beetle population. *Evolution* 65:2771–81.

Gibson, G., and I. Dworkin. 2004. Uncovering cryptic genetic variation. *Nature Reviews Genetics* 5:681–90.

Gosden, T. P., and E. I. Svensson. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution* 62:845–856.

Graff, P. W. 1947. Fungi from the Mountain Lake region of Virginia. *Castanea* 12:9–24.

Grant, P. R., and B. R. Grant. 1995. Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* 49:241–251.

Grant, P. R., and B. R. Grant. 2011. Causes of lifetime fitness of Darwin's finches in a fluctuating environment. *Proceedings of the National Academy of Sciences* 108:674–9.

Hanski, I., T. Mononen, and O. Ovaskainen. 2011. Eco-evolutionary metapopulation dynamics and the spatial scale of adaptation. *The American Naturalist* 177:29–43.

Hoffmann, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution* 14:96–101.

Holliday, A. E., F. M. Walker, E. D. Brodie III, and V. A. Formica. 2009. Differences in defensive volatiles of the forked fungus beetle, *Bolitotherus cornutus*, living on two species of fungus. *Journal of Chemical Ecology* 35:1302–8.

Holt, R. D., R. Gomulkiewicz, and M. Barfield. 2003. The phenomenology of niche evolution via quantitative traits in a "black-hole" sink. *Proceedings of the Royal Society—B* 270:215–224.

Husby, A., M. E. Visser, and L. E. B. Kruuk. 2011. Speeding up microevolution : the effects of increasing temperature on selection and genetic variance in a wild bird population. *PLoS Biology* 9: e1000585.

Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics* 21:243–273.

Kasumovic, M. M. 2013. The multidimensional consequences of the juvenile environment: Towards an integrative view of the adult phenotype. *Animal Behaviour* 85:1049–1059.

Kawecki, T. J. 2008. Adaptation to marginal habitats. *Annual Review of Ecology and Systematics* 39:321–342.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7:1225–1241.

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

Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and power of phenotypic selection in nature. *BioScience* 57:561–572.

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

Ledón-Rettig, C. C., D. W. Pfennig, A. J. Chunco, and I. Dworkin. 2014. Cryptic genetic variation in natural populations: a predictive framework. *Integrative and Comparative Biology* 54:783–93.

Liles, M. P. 1956. A study of the life history of the forked fungus beetle, *Bolitotherus cornutus* (Panzer) (Coleoptera: Tenebrionidae). *Ohio Journal of Science* 56:329–337.

Ludwig P. 2008. Causes and consequences of dispersal in the forked fungus beetle, *Bolitotherus cornutus*. Ph.D Dissertation: Department of Biology. University of Virginia, Charlottesville, VA.

MacColl, A. D. C. 2011. The ecological causes of evolution. *Trends in Ecology and Evolution* 26:514–22.

Matsubayashi, K. W., I. Ohshima, and P. Nosil. 2010. Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata* 134:1–27.

McGuigan, K., and C. M. Sgrò. 2009. Evolutionary consequences of cryptic genetic variation. *Trends in Ecology and Evolution* 24:305–11.

McGuigan, K., N. Nishimura, M. Currey, D. Hurwit, and W. A. Cresko. 2011. Cryptic genetic variation and body size evolution in threespine stickleback. *Evolution* 65:1203–11.

Miller, C. W., and D. J. Emlen. 2009. Dynamic effects of oviposition site on offspring sexually-selected traits and scaling relationships. *Evolutionary Ecology* 24:375–390.

Nosil, P., and B. J. Crespi. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences* 103:9090–5.

Paaby, A. B., and M. V Rockman. 2014. Cryptic genetic variation: evolution's hidden substrate. *Nature Reviews Genetics* 15:247–58.

Pemberton, J. M. 2010. Evolution of quantitative traits in the wild: mind the ecology. *Philosophical Transactions of the Royal Society—B* 365:2431–8.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. *The American Naturalist* 132:652–661.

Pulliam, H. R. 2000. On the relationship between niche and distribution. *Ecology Letters* 3:349–361.

Queitsch, C., T. A. Sangster, and S. Lindquist. 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417:618–624.

Reznick, D. N., and C. K. Ghalambor. 2005. Selection in nature: Experimental manipulations of natural populations. *Integrative and Comparative Biology* 45:456–462.

Ritland, K. 2011. Evolutionary potential in the wild: more than meets the eye. *Molecular Ecology* 20:3494–5.

Rohner, N., D. F. Jarosz, J. E. Kowalko, M. Yoshizawa, W. R. Jeffery, R. L. Borowsky, S. Lindquist, and C. J. Tabin. 2013. Cryptic variation in morphological evolution: Hsp90 as a capacitor for loss of eyes in cavefish. *Science* 342:1372–1375.

Sgrò, C. M., and A. A. Hoffmann. 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* 93:241–8.

Stanton, M. L., B. A. Roy, and D. A. Thiede. 2000. Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution* 54:93–111.

Waddington, C. H. 1956. Genetic assimilation of the bithorax phenotype. *Evolution* 10:1–13.

Wade, M. J., and S. Kalisz. 1990. The causes of natural selection. *Evolution* 44:1947–1955.

Walsh, B. and M. Lynch. Evolution and Selection of Quantitative Traits. Chapter 36: Comparisons of **G** and its Stability. Last modified 26 June 2014; accessed 25 February 2015. http://nitro.biosci.arizona.edu/zbook/NewVolume_2/pdf/Chapter36.pdf.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments. *Molecular Ecology* 12:1153–63.

Whitlock, M. C. 1992. Nonequilibrium population structure in forked fungus beetles: extinction, colonization, and the genetic variance among populations. *The American Naturalist* 139:952–970.

Wilson, A. J., J. M. Pemberton, J. G. Pilkington, D. W. Coltman, D. V Mifsud, T. H. Clutton-Brock, and L. E. B. Kruuk. 2006. Environmental coupling of selection and heritability limits evolution. *PLoS Biology* 4:e216.

CHAPTER ONE:

Surprisingly little population genetic structure in a fungus-associated beetle despite its exploitation of multiple hosts[1]

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Abstract

In heterogeneous environments, landscape features directly affect the structure of genetic variation among populations by functioning as barriers to gene flow. Resource-associated population genetic structure, in which populations that use different resources (e.g., host plants) are genetically distinct, is a well-studied example of how environmental heterogeneity structures populations. However, the pattern that emerges in a given landscape should depend on its particular combination of resources. If resources constitute barriers to gene flow, population differentiation should be lowest in homogeneous landscapes, and highest where resources exist in equal proportions. In this study we tested whether host community diversity affects population genetic structure in a beetle (*Bolitotherus cornutus*) that exploits three sympatric host fungi. We collected *B. cornutus* from plots containing the three host fungi in different proportions and quantified population genetic structure in each plot using a panel of microsatellite loci. We found no relationship between host community diversity and population differentiation in this species; however, we also found no evidence of resource-associated differentiation, suggesting that host fungi are not substantial barriers to gene flow. Moreover, we detected no genetic differentiation among *B. cornutus* populations separated by several kilometers, even though a previous study demonstrated moderate genetic structure on the scale of a few hundred meters. Although we found no effect of community diversity on population genetic structure in this study, the role of host communities in the structuring of genetic variation in heterogeneous landscapes should be further explored in a species that exhibits resource-associated population genetic structure.

Introduction

Most natural populations exist in spatially heterogeneous environments. Because environmental heterogeneity impacts two forces of evolution—selection and gene flow—it often shapes patterns of genetic variation at the landscape level (Manel et al. 2003, Hanski et al. 2011). Environmental features as diverse as physical impediments (e.g., mountain ranges, Rueness et al. 2003; waterfalls, Castric et al. 2001), habitat fragmentation (Haag et al. 2010), and microhabitat variation (Stireman et al. 2005, Cano et al. 2008, Ferrari et al. 2008) all function as barriers to gene flow, producing patterns of population genetic structure (hereafter, "population structure") that are coincident with the landscape. Understanding when and how the landscape influences population structure is essential to delineate the circumstances that constrain and foster phenotypic evolution and local adaptation.

One such pattern typically found in heterogeneous environments is resource-associated population structure, in which populations exploiting distinct resources are genetically differentiated (Stireman et al. 2005, Ferrari et al. 2012). It generally results from divergent selection in different environments, which selects against migrants, and habitat choice, which limits migration between environments (Kawecki and Ebert 2004, Ficetola and Bonin 2011). Resource-associated population structure is well documented in natural populations and demonstrates how the environment can impact gene flow, even in the absence of physical barriers (Feder et al. 1994, Mopper 1996).

Most studies, however, stop short of investigating how resource community diversity on the landscape level affects patterns of population structure. The pattern of resource-associated population structure that emerges in a given landscape should depend

on its particular combination of resources: a population in an environment composed of a single resource should be less structured than one found in a landscape in which multiple resources are equally abundant. Furthermore, the cost of dispersal may be higher—and therefore dispersal less frequent—in heterogeneous environments, because only a subset of encountered resources will be suitable. These effects of resource community composition on population genetic structure can be detected by measuring population genetic differentiation in communities that differ in the degree of resource heterogeneity. In a highly heterogeneous resource community, differentiation should be high because most pairwise comparisons will involve populations inhabiting different resources, between which gene flow is infrequent. By contrast, in a homogenous environment all pairwise comparisons should be between populations on the same resource, between which gene flow is uninhibited, and population differentiation will be low.

Insects that exploit multiple sympatric host species are good systems in which to explore this question, because the relative abundance of the hosts is spatially heterogeneous, creating a landscape that varies in terms of resource community structure. This heterogeneity of host availability may impact patterns of genetic structure in associated insects due to differential host preferences, as well as host-specific selection regimes (Nosil and Crespi 2004, Resetarits 1996, Refsnider and Janzen 2010).

Here we investigate the relationship between host community diversity and population structure in forked fungus beetles (*Bolitotherus cornutus*; Tenebrionidae; Figure 1). These beetles rely on three species of polypore fungi (*Fomes fomentarius, Ganoderma applanatum*, and *Ganoderma tsugae*) that are sympatric over much of their

range, and are often found growing within a few meters of each other (Gilbertson and Ryvarden 1986; Figure 2). Host community diversity varies on a small spatial scale, allowing us to address the impact of the host community on population structure in a single metapopulation. A previous study conducted at approximately the same scale and location as the present study, but only examining a single host (G. *applanatum*), found a moderate level of genetic differentiation (F_{ST} = 0.017; Whitlock 1992). This suggests that population structure exists on a micro-geographic scale in *B. cornutus*.

Several lines of evidence suggest that the host fungi may contribute to population differentiation in *B. cornutus*. First, throughout their life cycle these beetles rely on live bracket fungi, which are probably well defended by secondary chemical compounds (Liles 1956, Jonsell and Nordlander 2004). Coevolution between insects and the chemical defenses of their hosts is common, and tends to drive specialization on a single host (Cornell and Hawkins 2003). Second, defensive volatiles differ among beetles collected from different host fungi, suggesting that individual beetles tend to associate with a single fungus type and that the fungi are characterized by distinct chemical compositions (Holliday et al. 2009). Moreover, endophagous insects like *B. cornutus*, whose larvae develop and pupate inside the brackets (Liles 1956), disproportionately exhibit local adaptation to a single host (Mopper 1996, Stireman et al. 2005). Third, the fungi themselves grow on different trees and have contrasting life histories, so their nutrient profiles may differ (Gilbertson and Ryvarden 1986). *F. fomentarius* and *G. applanatum* produce perennial brackets and grow mostly on hardwoods, while *G. tsugae* produces brackets annually and specializes on hemlock (*Tsuga* spp.; Brown and Rockwood 1986). Finally, experimental evidence suggests that *B. cornutus* discriminate among the three

host fungi. In lab-based choice experiments, *B. cornutus* preferred to eat *G. tsugae*, and the same experiment suggested that females might prefer to oviposit on *G. applanatum* (Heatwole and Heatwole 1968). Discrimination among fungi is evident in the field as well. *B. cornutus* that originate on *G. tsugae* tended to disperse to other *G. tsugae*-infected logs (Schwarz 2006).

Here we assess the effect of a heterogeneous host community on *B. cornutus* population structure on a micro-geographic scale: an Appalachian forest in which all three host fungi are intermixed. We address two questions. First, does host community diversity affect the degree of genetic differentiation among *B. cornutus* subpopulations? We predict that in a community with low host species diversity, gene flow will be relatively unrestricted and genetic differentiation low. By contrast, gene flow will be more restricted and genetic differentiation high in a community in which all three hosts are equally abundant. The expected effect of host community diversity on population structure is based on the assumption that fungi contribute to population structure through resource-associated differentiation. To directly test for resource-associated differentiation, we ask whether host fungus contributes to population structure in *B. cornutus*, which would suggest that divergent selection and/or habitat choice may be acting as a barrier to gene flow between beetle populations on different host fungi.

Methods

Study Species

Adult *B. cornutus* feed, mate, and lay eggs on the fruiting bodies ("brackets"; Liles 1956) of host fungus, and larvae burrow into the brackets and consume fungal

tissue throughout development (Figure 1). A single subpopulation consists of all individuals inhabiting a single log and the associated fungal fruiting bodies. Approximately 25%-30% of individuals migrate to other logs during their lifespan (Whitlock 1992; Ludwig 2008), though these estimates were obtained in studies of subpopulations inhabiting only one of the main host fungi (*G. applanatum*). The average adult lifespan of *B. cornutus* is 69 days (Conner 1988), though some individuals live for several years (Pace 1967).

Field Sampling and Genotyping

The study site was located on Salt Pond Mountain near Mountain Lake Biological Station in Giles County, Virginia. In May-June 2011 we surveyed an area of approximately 0.25 km^2 (250 m x 1000 m) along two drainages, Hunters Branch and Pond Drain (37.3738 N, 80.5351 W), for the three host fungi (Figure 2). We geo-referenced all logs infected with *F. fomentarius*, *G. applanatum*, and *G. tsugae* with a handheld global positioning system (Garmin, Kansas City, KS).

Within the surveyed area we defined five circular study plots (Figure 2) using geographic information systems software (ArcGIS, ESRI, Redlands, CA). Each of the plots had a radius of 90 m, a distance chosen because 75% of beetles disperse fewer than 90 m (Ludwig 2008). Plots were separated by 180 m. In June-July 2011, we visited each plot once per week for three weeks, and during each visit we searched all infected logs for *B. cornutus* adults. All *B. cornutus* were collected and brought back to the lab. Each individual received a unique ID, which was painted on its elytra using Testors Gloss Enamel in earth tone colors. We collected 0.2-5 µL of hemolymph from the defensive

glands of all beetles using the method described in Donald et al. (2012), which did not affect either survival or reproduction in laboratory trials. Hemolymph was stored in prepared lysis buffer (Promega DNA IQ system). All beetles were returned to their location of capture within 72 hours.

DNA extractions were performed with Promega's DNA IQ System (Promega 2010), and PCR was performed using Qiagen's Multiplex PCR kit and microsatellite protocol (Qiagen 2010). Fragment analysis was completed by GeneWiz Inc. (South Plainfield, NJ, USA) using Applied Biosystems 3730xl DNA Analyzers. All individuals' genotypes at nine microsatellite loci (Donald et al. 2012) were scored using GeneMarker (SoftGenetics, State College, PA).

Statistical Analysis: Population Genetic Structure

We used hierarchical analysis of molecular variance (AMOVA) to test whether host fungi contribute to population structure in this system. Population differentiation was assessed using *F*-statistics, which rely on allele identity information (Weir and Cockerham 1984), rather than with *R*-statistics, analogs to *F*-statistics that are based on a stepwise mutation model and are often used for microsatellites (Slatkin 1995). To determine whether *F*- or *R*-statistics were appropriate for our dataset, we performed a test of mutation effect on genetic structure in SPAGeDi 1.3 (Hardy and Vekemans 2002) based on 10,000 permutations (Hardy et al. 2003, Galligan et al. 2012). Because there was no significant effect of mutation on genetic structure in our dataset (P=0.483, n=508 individuals), we performed all tests using *F*-statistics.

AMOVA partitions total genetic variance into covariance components that describe the correlation between random haplotypes within the same subgroup, relative to a larger group. For our analysis, individuals were nested within logs (subpopulations), and logs were nested within fungus species. Based on these hierarchical groupings we calculated the following *F*-statistics: F_{IS}, the correlation between haplotypes within individuals relative to their subpopulation; F_{SC}, the correlation between haplotypes within subpopulations relative to all individuals from the same host fungus; F_{CT}, the correlation between haplotypes within host fungi relative to the entire metapopulation; and F_{ST}, the correlation between haplotypes within subpopulations relative to the entire metapopulation. A significant value for any of these *F*-statistics indicates significant population genetic structure with respect to the relevant group (i.e., a significant F_{ST} indicates genetic structure among subpopulations; a significant F_{CT} indicates significant genetic structure among host fungi).

All genetic analyses were performed in Arlequin (Excoffier et al. 2005), and significance testing was conducted using 10,000 permutations. A subpopulation was only included in these analyses if it had at least five genotyped individuals. We performed locus-by-locus AMOVA because some loci had missing data, and we are presenting the results for each of the nine microsatellite loci separately, as well as the weighted average over all loci. 95% confidence intervals for the multilocus *F*-statistics were obtained by bootstrapping over loci.

Because some logs were co-infected by more than one species of fungus (N = 9; 20.9% of all logs), we included a co-infected category as a fourth level of the fungus factor (*G. applanatum*, *G. tsugae*, *F. fomentarius*, and co-infected logs) in the AMOVA

analysis. All but one of the co-infected logs were infected by *G. applanatum* and *F. fomentarius*; the remaining co-infected log hosted *G. applanatum* and *G. tsugae*. Excluding co-infected logs did not qualitatively alter our results.

Before performing the combined analysis, we ran a separate AMOVA to test for a significant effect of plot. Plot did not contribute significantly to total genetic variance (P = 0.91), allowing us to pool individuals across plots to test forest-wide structure. Finally, because sex-biased dispersal may result in sex-specific patterns of population structure, we also ran the above AMOVA separately for females and males. Only populations with at least five females or five males, respectively, were included in this analysis.

Statistical Analysis: Isolation-by-Distance

Because we did not detect population structure in the above analyses (see Results), we tested for isolation-by-distance on a larger geographic scale by augmenting our dataset with 88 individuals collected from two sites 6 km (37.4220 N, -80.5031 W) and 9 km (37.4594 N, -80.5334 W) away from our primary study site on Salt Pond Mountain. At each of these sites, beetles were collected from several logs that were infected by either *F. fomentarius* or *G. applanatum* (6-km site: 9 logs; 9-km site: 11 logs). Because the host fungi did not affect population structure in the Salt Pond Mountain dataset, these beetles were pooled by site for the isolation-by-distance analysis. Pairwise genetic distances (Slatkin's linearized F_{ST}, $D=F_{ST}/(1-F_{ST})$) were calculated in Arlequin, and pairwise geographic distances were calculated using the Geographic Distance Matrix Generator v 1.2.3. (Ersts 2012) and log-transformed for analysis (Rousset 1997). The significance of this relationship was tested using a Mantel test with

10,000 randomizations in the Isolation by Distance Web Service v. 3.23 (Jensen et al. 2005).

Statistical Analysis: Host Fungus Community Diversity

Host fungus community diversity in each of the five plots was quantified with the Shannon-Wiener equitability index (also known as Pielou's *J*; Pielou 1969), which measures the relative abundance of species that comprise a community. The index (J') is given by

$$J' = -\Sigma[p_i * \ln(p_i)] / \ln(S)$$

where p_i is the frequency of the i^{th} species, and S is the total number of species in the community. The numerator is the Shannon diversity index, H'; the denominator is equivalent to H'_{max}, the maximum value that H' can take, given S species. As a result, J' ranges from zero to one, with zero corresponding to a community with a single species, and one to a community where all species are equally abundant. We chose a measure of evenness instead of richness (the number of species in the community) in order to distinguish between host communities in which all host species are present, but in very different proportions.

To calculate J' for each plot, we counted all logs in the plot that were infected by the three host fungi. Because we intended this index to reflect the community of available host fungi, we excluded logs that did not exhibit any evidence of *B. cornutus* (i.e., neither adults or eggs were present) or on which all brackets were in an advanced state of decay.

Logs that were co-infected by two of the host fungi (20.9% of all logs) were counted twice, once for each fungus species. All three fungi were present in all five plots.

We performed an AMOVA separately for each plot in order to test the hypothesis that community diversity affects population differentiation. This analysis calculated F_{ST} in each plot. We examined the relationship between the Shannon-Wiener equitability index and these F_{ST} values using Spearman's rank correlation. Each observation in this dataset was one of the five study plots. This analysis was performed in R 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

We genotyped a total of 596 individuals from 63 logs, including 508 individuals from 43 logs at the main study site at Mountain Lake Biological Station. In this sample, 173 beetles were collected from *F. fomentarius*, 56 from *G. applanatum*, 161 from *G. tsugae*, and 118 from co-infected logs.

We found no significant relationship between host community diversity and F_{ST} across the five study plots (Spearman's $\rho = 0.0$, N = 5, P = 1.0; Figure 3). We also found that the three host fungi did not significantly contribute to population structure ($F_{CT} = 0.001$, P = 0.36; Table 1). This pattern held across all nine microsatellite loci. Overall, the level of population differentiation was extremely low ($F_{ST} = 0.0021$ [95% confidence interval: -0.0003-0.0049], P = 0.31; Table 1), a result that is inconsistent with a previously published study that estimated a moderate level of population structure on the same mountain along one of the same drainages ($F_{ST} = 0.017$; Whitlock 1992). When F_{ST} is calculated separately for each locus, only one locus approaches the level of

population structure measured by Whitlock (Boco_128: F_{ST} = 0.012), but this value was not significant. However, we did detect a significant inbreeding coefficient (F_{IS} = 0.02; Table 1).

When the data were analyzed separately for the two sexes, the host fungi still did not affect population structure within each sex (Table 2). Moreover, there was no evidence of sex-biased dispersal irrespective of fungus species, as F_{ST} was not significantly different from zero in either sex. We detected a significant inbreeding coefficient in males but not in females.

The isolation-by-distance analysis did not reveal a significant relationship between genetic distance and geographic distance (Mantel test; r = -0.016, P = 0.51; Figure 4). Populations separated by nine kilometers were not more likely to be genetically distinct than those separated by a few meters.

Discussion

We found no evidence that the host community contributes to population structure in this *B. cornutus* metapopulation. However, we also found that population structure appears to be unrelated to the host fungi in this species, indicating that host fungi do not constitute a substantial barrier to gene flow among *B. cornutus* subpopulations. In contrast to an earlier study, we also found no evidence for population structure, even between populations separated by nearly ten kilometers and located on another mountain.

The lack of a relationship between host community diversity and population differentiation is consistent with the fact that the host fungi do not seem to pose a barrier to gene flow in *B. cornutus*. There are a number of reasons that may account for the

absence of host-associated differentiation in this system. First, the three hosts may not be characterized by divergent selection, allowing migrants to move freely among habitats. Two of the three hosts are congeners, so adaptation to the chemical profile of one host may have positive effects on performance on the other. Moreover, in a survey of bracket-feeding insects, Jonsell and Nordlander (2004) found that *F. fomentarius* and *G. applanatum* was the most commonly used pair of hosts; insects that fed on one were likely to use the other as well. As a result, tradeoffs in performance across the three host fungi may be relatively minor, unlike in species that exhibit pronounced local adaptation to a host (e.g., aphids, Ferrari et al. 2008).

An alternative possibility is that although *B. cornutus* populations on different hosts experience divergent selection, frequent migration on the micro-geographic scale of this study prevents the emergence of host-associated population structure. One aspect of the ecology of the system that may promote migration among fungi is intra- or interannual variation in relative abundance of the hosts. One of the host fungi (*G. tsugae*) produces brackets on an annual basis, so it tends to be less abundant in the spring and becomes more common as the summer progresses. As it becomes a more abundant resource, it likely absorbs migrants from the other two host fungi, erasing genetic differentiation between populations on different fungi. Interannual variation in the relative abundance of the three host fungi would have a similar effect. Such temporal heterogeneity in the host fungus community could generate selection for ecological generalists that are able to exploit whichever fungus is most abundant during a given month or year (Kassen 2002).

In contrast to a previous mark-recapture study of dispersal in *B. cornutus* (Schwarz 2006), there is no genetic evidence from our study that the fungus on which they originate affects *B. cornutus* dispersal. Furthermore, because the contribution of host fungus to genetic structure does not differ between males and females, there is no evidence for habitat choice in either sex. This result is somewhat surprising because ovipositing females often exhibit habitat choice for a particular host, especially when their offspring rely on the host throughout development (Resetarits 1996), as is the case in *B. cornutus*. Females of some species are more sensitive to chemical volatiles diagnostic of a particular host species, probably for this reason (Faldt et al. 1999). We did find a significant positive inbreeding coefficient in the complete dataset ($F_{IS} = 0.02$; Table 1), but only in males when the two sexes were analyzed separately (Table 2). The biological interpretation and significance of this result are unclear. It may be indicative of kin structuring within subpopulations, though it is not immediately evident why such an effect should be stronger in males.

It may be that the costs of habitat choice in this system outweigh the benefits. This is more likely to be true if habitat choice is energetically expensive, either because choosy individuals migrate longer distances before locating a log infected with their preferred fungus, or because the sensory apparatus required to discriminate among habitats is costly to maintain. In the latter case, constraint may play a role in preventing the development of host-associated population structure in *B. cornutus*. Bracket fungi emit cocktails of volatile compounds that dispersing insects exploit to locate infected logs (Faldt et al. 1999). Closely related bracket fungi differ in the chemical composition of these volatiles, and some, but not all, insects are able to discriminate between hosts on

this basis (Faldt et al. 1999). A previous lab-based experiment demonstrated that *B. cornutus* prefer *G. applanatum*, suggesting that *B. cornutus* can discriminate amongst the hosts (Heatwole and Heatwole 1968). It is possible that even though they are capable of distinguishing hosts, the cost of habitat choice in the field, imposed by dispersal, outweighs the host preference exhibited in the lab.

Many canonical examples of microhabitat specialization and concomitant patterns of population genetic structure are found in host-associated insects (Feder et al. 1994, Mopper 1996,Via et al. 2000, Stireman et al. 2005). However, host-associated differentiation in insects is certainly not ubiquitous; there are a number of species in which the host resource has no measureable impact on patterns of population differentiation (Van Zandt and Mopper 1998, Stireman et al. 2005, Jourdie et al. 2010, Kohnen et al. 2011). Certain aspects of insect life history seem to predispose some species to host specialization, which is disproportionately common in endophagous and parthenogenetic species (Van Zandt and Mopper 1998, Stireman et al. 2005). Endophagous species like gall-makers come into especially close contact with the host defenses, generating stronger selection for specialization; parthenogenesis results in a tighter association between insect and host genotypes, accelerating divergence among hosts.

Yet exceptions to this pattern exist even among gall-makers and parthenogens. There is no signature of host-associated differentiation in a rose gall wasp (*Diplolepis rosae*), a species in which *Wolbachia* infection results in parthenogenesis (Kohnen et al. 2011). Population genetic structure in bruchid beetles, the larvae of which consume the developing seeds of legumes, is not affected by the host bean species (Restoux et al.

2010). In a study of two insects associated with the hosts in the genus *Pinus* but differing in life histories traits such as endophagy, Kerdelhué et al. (2006) found no effect of host plant on genetic structure in either species. Finally, the results of the present study demonstrate that host fungi are not associated with population differentiation in *B. cornutus*, an endophagous beetle. This body of results underscores the fact that host-associated population differentiation is a complex phenomenon that can be difficult to predict (Kohnen et al. 2011). A diversity of ecological factors—including but not limited to the grain of environmental heterogeneity, the magnitude of differences among hosts, insect life histories, and the frequency of migration among hosts—govern the extent to which gene flow between populations on different hosts is constrained.

A comparison between this study and a previously published study in the same geographic area (Whitlock 1992) suggests that population structure in *B. cornutus* may be temporally labile. We found little or no population structure in our study population: beetles on logs separated by nearly ten kilometers were not more genetically distinct than those on neighboring logs (Figure 4). However, Whitlock (1992) reported moderate differentiation (F_{ST} = 0.017) on the same mountain. It is worth noting that Whitlock (1992) analyzed allozyme variation, while our present study examined microsatellites. However, these two different marker types typically produce similar estimates of population differentiation, especially after outlier allozymes are excluded (Estoup et al. 1998, Dhuyvetter et al. 2004, Roberts and Weeks 2011). Although it is difficult to speculate about the mechanism responsible for driving the change in population structure in the past two decades, one possibility is that the age structure of populations in the study area has changed substantially. Whitlock's (1992) estimate of moderate population

differentiation was primarily driven by the younger (more recently colonized) logs in his sample. He concluded that younger populations were more genetically differentiated than older ones due to founder effects, so a decline in the proportion of young populations could result in a corresponding decrease in F_{ST}. The magnitude of the reduction in F_{ST} between younger and older populations differed between the two sites in Whitlock's study; although there was no detectable genetic differentiation among older populations at one site, at the site corresponding to the location of the present study F_{ST} among older populations was 0.015. This suggests that even a substantial change in age structure may not be sufficient to account for the difference between Whitlock (1992) and the present study.

An alternative hypothesis for disappearance of micro-geographic population structure in *B. cornutus* involves the dynamic nature of the host fungus community on a longer time scale. Temporal variation in host community structure could have erased resource-associated population differentiation, because a decline in the frequency of one host would force beetles previously associated with it to colonize the other two. It is possible that host fungus community diversity has changed substantially on Salt Pond Mountain in the two decades separating this study from Whitlock's, due to the invasion of the hemlock woolly adelgid (*Adelges tsugae*). Widespread eastern hemlock (*Tsuga canadensis*) decline, driven by the invasion of hemlock woolly adelgid, may have led to an increase in the frequency of *G. tsugae*—a hemlock specialist. Hemlock woolly adelgid is a sap-sucking insect that feeds at the base of hemlock needles, killing the tree (Young 1995, Stadler et al. 2006, Evans and Gregoire 2007). Its impact on tree communities—and corresponding effect on their associated fungi—is likely most pronounced in riparian

forests in the southern Appalachians, such as our study site, where hemlock is the dominant tree species (Webster et al. 2012). Both standing dead hemlock snags and adelgid-infected live hemlocks are common at our study site.

An invasive from Asia, hemlock woolly adelgid was first recorded in North America in 1951 near Richmond, VA, and since has spread into the Appalachian Mountains south to Georgia and north to Maine (Fitzpatrick et al. 2012). The first hemlock woolly adelgid infestation in Giles County, VA (the location of this study) was recorded in the early 1990s (Fitzpatrick et al. 2012; USFS data available online), shortly after Whitlock's data were collected in 1988. As a result, dead hemlocks are probably much more abundant in the study site than they were in the late 1980s, and may have driven a corresponding increase in the frequency of *G. tsugae* brackets. Whether exploitation of this newly abundant resource may have erased population genetic structure in *B. cornutus* that Whitlock (1992) observed remains to be tested.

This study accords with a growing body of evidence suggesting that population genetic structure can be surprisingly dynamic (Charbonnel et al. 2002, Heath et al. 2002, Østergaard et al. 2003, Apodaca et al. 2013). In some cases, temporal changes in population structure are associated with catastrophic events such as hurricanes (e.g., sailfin mollies; Apodaca et al. 2013), while in others, population structure varies among years in the absence of obvious large-scale ecological drivers (e.g., steelhead trout; Heath et al. 2002). Many more temporally replicated estimates of population structure are necessary to assess its lability in the short and long term, and to identify the environmental factors associated with the restructuring of genetic variance in natural populations.

Finally, the degree to which genetic structure reflects the environment may differ across different regions of the genome, particularly in the early stages of local adaptation (Via 2009). Under divergent selection in different habitats, loci that are associated with resource utilization exhibit pronounced divergence relative to neutral markers like microsatellites. This is the principle underlying methods such as Q_{ST}-F_{ST} comparisons: regions associated with ecologically important quantitative traits may show high levels of divergence not reflected in neutral genetic variation (Scheffer and Hawthorne 2007). Thus, the geographic distribution of genetic variance at non-neutral loci may be very different from that at neutral loci. For example, in a study of moor frogs, a wetland-breeding amphibian, Richter-Boix et al. (2011) found that although population structure at most microsatellite loci was unaffected by environmental differences among wetlands, one locus exhibited habitat-associated structure. The authors inferred that this single locus was likely experiencing selection for local adaptation not reflected in the neutral loci. The *B. cornutus* genome could be a mosaic in which loci that affect habitat use are characterized by host-associated genetic structure, while neutral loci are not. However, all nine microsatellite loci included in the present study yielded similar estimates of population structure, providing little evidence for this interpretation.

Although this study did not find a relationship between host community diversity and population differentiation, such a relationship may exist in populations that do exhibit habitat-associated population structure, unlike *B. cornutus*. Because habitat-associated population structure is common in other systems, the most robust test of our hypothesis would be conducted in a species in which gene flow among environments is known to be restricted (e.g., Hoeskstra et al. 2004, Nosil and Crespi 2004, Stireman et al.

2005). Future studies in species that are characterized by pronounced habitat-associated genetic differentiation are necessary to elucidate the effect of landscape-level habitat diversity on patterns of population genetic structure.

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Literature Cited

Apodaca J. J., J. C. Trexler, N. K. Jue, M. Schrader, and J. Travis. 2013. Large-scale natural disturbance alters genetic population structure of the Sailfin Molly, *Poecilia latipinna*. *The American Naturalist* 181:254-263.

Brown L. and L. L. Rockwood. 1986. On the dilemma of horns—if long horns give fungus beetles an edge in mating, why do so many males have short horns? *Natural History* 95:54-61.

Cano J. M., H. S. Makinen, T. Leinonen, J. Freyhof, and J. Merila. 2008. Extreme neutral genetic and morphological divergence supports classification of Adriatic three-spined stickleback (*Gasterosteus aculeatus*) populations as distinct conservation units. *Biological Conservation.* 141:1055-1066.

Castric V., F. Bonney, and L. Bernatchez. 2001. Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution* 55:1016-1028.

Charbonnel N., M. Quesnoit, R. Razatavonjizay, P. Brémond, and P. Jarne. 2002. A spatial and temporal approach to microevolutionary forces affecting population biology in the freshwater snail *Biomphalaria pfeifferi*. *The American Naturalist* 160:741-755.

Conner J. 1988. Field measurements of natural and sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 42:736-749.

Cornell H. V. and B. A. Hawkins. 2003. Herbivore responses to plant secondary compounds: A test of phytochemical coevolution theory. *The American Naturalist* 161:507-522.

Dhuyvetter H., E. Gaublomme, and K. Desender. 2004. Genetic differentiation and local adaptation in the salt-marsh beetle *Pogonus chalceus*: A comparison between allozyme and microsatellite loci. *Molecular Ecology* 13:1065-1074.

Donald H. M., C. W. Wood, K. M. Benowitz, R. A. Johnson, E. D. Brodie III, and V. A. Formica. 2012. Nondestructive sampling of insect DNA from defensive secretion. *Molecular Ecology Resources* 12:856-860.

Ersts, P.J. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available at http://biodiversityinformatics.amnh.org/open_source/gdmg. Accessed on 18 Oct. 2012.

Estoup A., F. Rousset, Y. Michalakis, J. M. Cornuet, M. Adriamanga, and R. Guyomard. 1998. Comparative analysis of microsatellite and allozyme markers: A case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology* 7:339-353.

Evans A. M. and T. G. Gregoire. 2007. A geographically variable model of hemlock woolly adelgid spread. *Biological Invasions* 9:369-382.

Excoffier L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1:47-50.

Faldt J., M. Jonsell, G. Nordlander, and A. K. Borg-Karlson. 1999. Volatiles of bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* and their functions as insect attractants. *Journal of Chemical Ecology.* 25:567-590.

Feder J. L., S. B. Opp, B. Wlazlo, K. Reynolds, W. Go, and S. Spisak. 1994. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *Proceedings of the National Academy of Sciences of the United States of America* 91:7990-7994.

Ferrari J., S. Via, and H. C. J. Godfray. 2008. Population differentiation and genetic variation in performance on eight hosts in the pea aphid complex. *Evolution* 62:2508-2524.

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.

Ficetola G. F. and A. Bonin. 2011. Conserving adaptive genetic diversity in dynamic landscapes. *Molecular Ecology* 20:1569-1571.

Fitzpatrick M. C., E. L. Preisser, A. Porter, J. Elkinton, and A. M. Ellison. 2012. Modeling range dynamics in heterogeneous landscapes: invasion of the hemlock woolly adelgid in eastern North America. *Ecological Applications* 22:472-486.

Galligan T. H., S. C. Donnellan, F. J. Sulloway, A. J. Fitch, T. Bertozzi, and S. Kleindorfer. 2012. Panmixia supports divergence with gene flow in Darwin's small ground finch, *Geospiza fuliginosa*, on Santa Cruz, Galapagos Islands. *Molecular Ecology* 21:2106-2115.

Gilbertson R.L. and L. Ryvarden. 1986. North American Polypores: Volume 1. Olso, Norway: Fungiflora. 433 pages.

Haag T., A. S. Santos, D. A. Sana, R. G. Morato, L. Cullen Jr., P. G. Crawshaw Jr., C. de Angelo, M. S. di Bitetti, F. M. Salzano, and E. Eizirik. 2010. The effect of habitat

fragmentation on the genetic structure of a top predator: loss of diversity and high differentiation among remnant populations of Atlantic Forest jaguars (*Panthera onca*). *Molecular Ecology* 19:4906-4921.

Hanski I., T. Mononen, and O. Ovaskainen. 2011. Eco-evolutionary metapopulation dynamics and the spatial scale of adaptation. *The American Naturalist* 177:29-43.

Hardy O. J. and X. Vekemans. 2002. SPAGEDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2:618-620.

Hardy O. J., N. Charbonnel, H. Freville, and M. Heuertz. 2003. Microsatellite allele sizes: A simple test to assess their significance on genetic differentiation. *Genetics* 163:1467-1482.

Heath D. D., C. Busch, J. Kelly, and D. Y. Atagi. 2002. Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Molecular Ecology* 11:197-214.

Heatwole H., and A. Heatwole. 1968. Movements, host fungus preferences, and longevity of *Bolitotherus cornutus* (Coleoptera: Tenebrionidae). *Annals of the Entomological Society of America* 61:18-23.

Hoekstra H. E., K. E. Drumm, and M. W. Nachman. 2004. Ecological genetics of adaptive color polymorphism in pocket mice: Geographic variation in selected and neutral genes. *Evolution* 58:1329-1341.

Holliday A. E., F. M. Walker, E. D. Brodie III, and V. A. Formica. 2009. Differences in defensive volatiles of the forked fungus beetle, *Bolitotherus cornutus*, living on two species of fungus. *Journal of Chemical Ecology* 35:1302-1308.

Jensen, J. L., A. J. Bohonak, and S. T. Kelley. 2005. Isolation by distance, web service (v.3.23). *BMC Genetics* 6:13. Available at http://ibdws.sdsu.edu/. Accessed on 18 Oct. 2012.

Jonsell M. and G. Nordlander. 2004. Host selection patterns in insects breeding in bracket fungi. *Ecological Entomology* 29:697-705.

Jourdie V., N. Alvarez, J. Molina-Ochoa, T. Williams, D. Bergvinson, B. Benrey, T. C. J. Turlings, and P. Franck. 2010. Population genetic structure of two primary parasitoids of *Spodoptera frugiperda* (Lepidoptera), *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera): to what extent is the host plant important? *Molecular Ecology* 19:2168-2179.

Kassen R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology* 15:173-190.

Kawecki T. J. and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7:1225-1241.

Kerdelhué C., E. Magnoux, F. Lieutier, A. Roques, and J. Rousselet. 2006. Comparative population genetic study of two oligophagous insects associated with the same hosts. *Heredity* 97:38-45.

Kohnen A., V. Wissemann, and R. Brandl. 2011. No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species. *Biological Journal of the Linnean Society* 102:369-377.

Liles M. P. 1956. A study of the life history of the forked fungus beetle, *Bolitotherus cornutus* (Panzer) (Coleoptera: Tenebrionidae). *The Ohio Journal of Science* 56: 329-337.

Ludwig P. 2008. Causes and consequences of dispersal in the forked fungus beetle, *Bolitotherus cornutus*. Doctoral dissertation: University of Virginia, Charlottesville, VA.

Manel S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology and Evolution* 18:189-197.

Mopper S. 1996. Adaptive genetic structure in phytophagous insect populations. *Trends in Ecology and Evolution* 11:235-238.

Nosil P. and B. J. Crespi. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in *Timema cristinae* walking-sticks. *Evolution* 58:102-112.

Østergaard S., M. M. Hansen, V. Loeschcke, and E. E. Nielsen. 2003. Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. *Molecular Ecology* 12:3123-3135.

Pace A. E. 1967. Life history and behavior of a fungus beetle, *Bolitotherus cornutus* (Tenebrionidae). *Occasional Papers of the Museum of Zoology, University of Michigan* 653:1114-1128.

Pielou E. C. 1969. An Introduction to Mathematical Ecology. New York, New York: John Wiley & Sons. 286 pp.

Promega. 2010. DNA IQ System—Small Sample Casework Protocol. Section 4C. DNA isolation from liquid samples. Promega Corporation, Madison, WI.

Qiagen. 2010. QIAGEN Multiplex PCR Handbook: Amplification of microsatellite loci using multiplex PCR. Hilden, Germany.

Refsnider J. M. and F. J. Janzen. 2010. Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics* 41:39-57.

Resetarits W. J. 1996. Oviposition site choice and life history evolution. *American Zoologist* 36:205-215.

Restoux G., M. Hossaert-McKey, B. Benrey, and N. Alvarez. 2010. The effect of host plant and isolation on the genetic structure of phytophagous insects: A preliminary study on a bruchid beetle. *European Journal of Entomology* 107:299-304.

Richter-Boix A., M. Quintela, G. Segelbacher, and A. Laurila. 2011. Genetic analysis of differentiation among breeding ponds reveals a candidate gene for local adaptation in *Rana arvalis*. *Molecular Ecology* 20:1582-1600.

Roberts J. M. K. and A. R. Weeks. 2011. Genetic structure and long-distance dispersal in populations of the wingless pest springtail, *Sminthurus viridis* (Collembola: Sminthuridae). *Genetics Research* 93:1-12.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145:1219-1228.

Rueness E. K., N. C. Stenseth, M. O'Donoghue, S. Boutin, H. Ellegren, and K. S. Jakobsen. 2003. Ecological and genetic spatial structuring in the Canadian lynx. *Nature* 425:69-72.

Scheffer S. J. and D. J. Hawthorne. 2007. Molecular evidence of host-associated genetic divergence in the holly leafminer *Phytomyza glabricola* (Diptera: Agromyzidae): Apparent discordance among marker systems. *Molecular Ecology* 16:2627-2637.

Schwarz C. 2006. The role of habitat heterogeneity in population dynamics: from individual behavior to metapopulation structure. Doctoral dissertation: The Pennsylvania State University, State College, PA.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457-462.

Stadler B., T. Müller, and D. Orwig. 2006. The ecology of energy and nutrient fluxes in hemlock forests invaded by hemlock woolly adelgid. *Ecology* 87:1792-1804.

Stireman J. O., J. D. Nason, and S. B. Heard. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *Evolution* 59:2573-2587.

Van Zandt P. A. and S. Mopper. 1998. A meta-analysis of adaptive deme formation in phytophagous insect populations. *The American Naturalist* 152:595-604.

Via S. 2009. Natural selection in action during speciation. *Proceedings of the National Academy of Sciences of the United States of America* 106:9939-9946.

Via S., A. C. Bouck, and S. Skillman. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution* 54:1626-1637.

Webster J. R., K. Morkeski, C. A. Wojculewski, B. R. Niederlehner, E. F. Benfield, and K. J. Elliot. 2012. Effects of hemlock mortality on streams in the southern Appalachian Mountains. *The American Midland Naturalist* 168:112-131.

Weir B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population-structure. *Evolution* 38:1358-1370.

Whitlock M. C. 1992. Nonequilibrium population-structure in forked fungus beetles - extinction, colonization, and the genetic variance among populations. *The American Naturalist* 139:952-970.

Young R. F., K. S. Shields, and G. P. Berlyn. 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America* 88:827-835.

Figures and Tables

Figure 1. A female forked fungus beetle (*B. cornutus*) on a fruiting body of *Fomes fomentarius*. Photo by V. A. Formica.

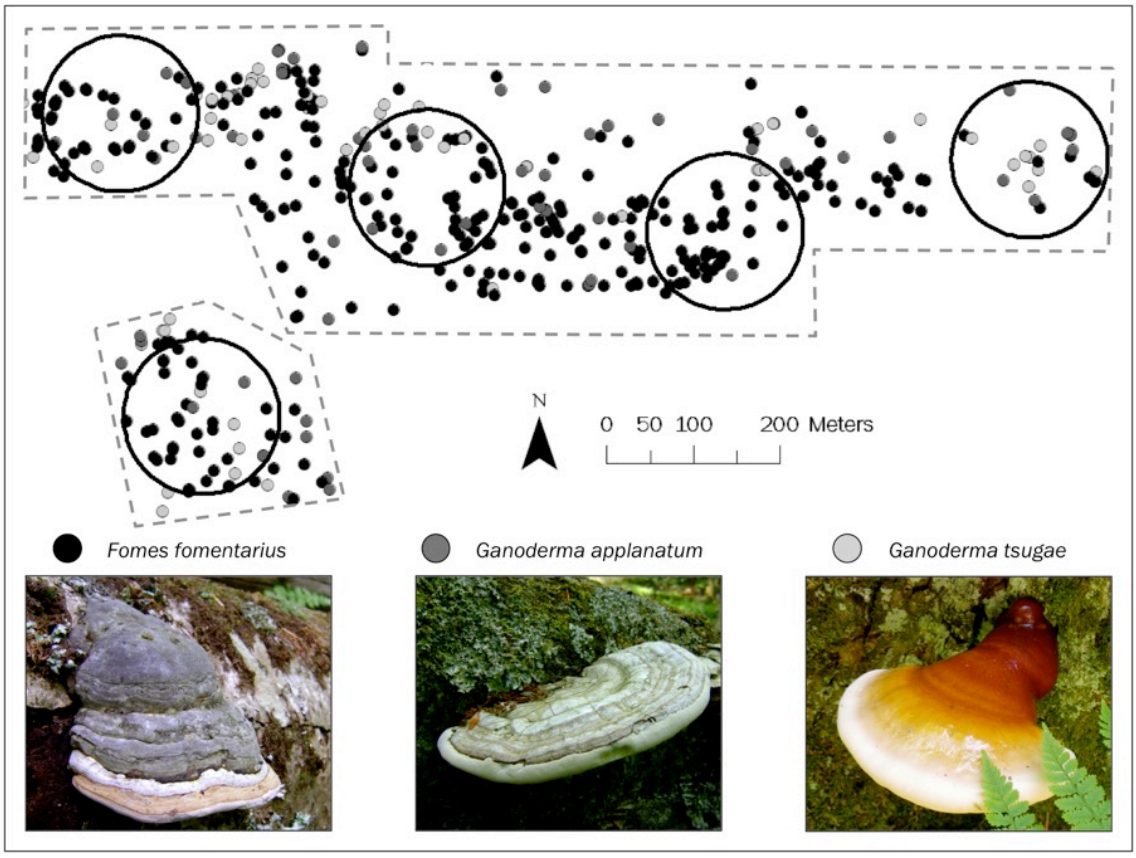

Figure 2. The distribution of the three host fungi in the landscape near Mountain Lake Biological Station in Giles County, VA. Top: Map of the study area. The circles delineate the five study plots, and the dotted lines, the area surveyed. Bottom: a fruiting body of each of the three fungi. Photos by C. W. Wood.

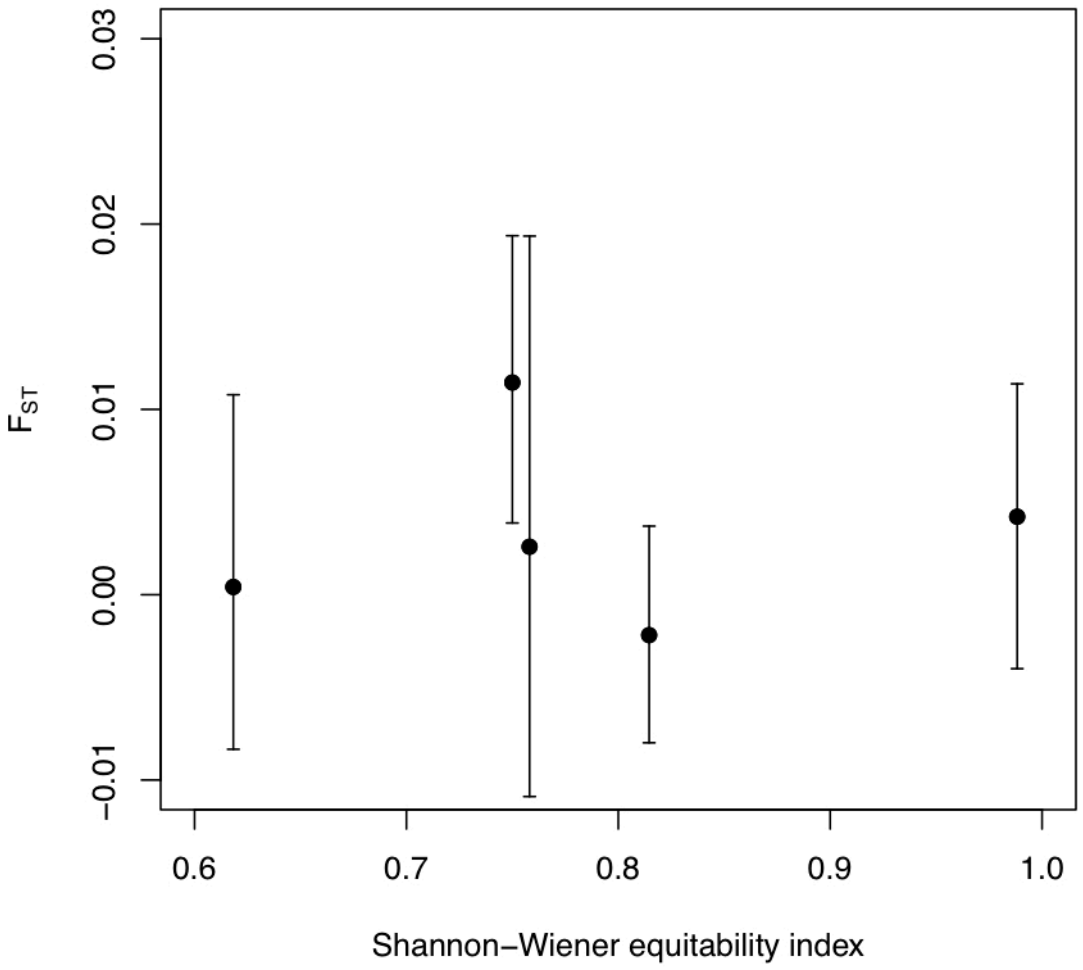

Figure 3. The relationship between community diversity (Shannon-Wiener equitability index) and population differentiation (F_{ST}). Each point corresponds to one of the five study plots. Error bars represent 95% confidence intervals obtained by bootstrapping over loci. This relationship was not significant (Spearman's $\rho = 0.0$, N = 5, P = 1.0).

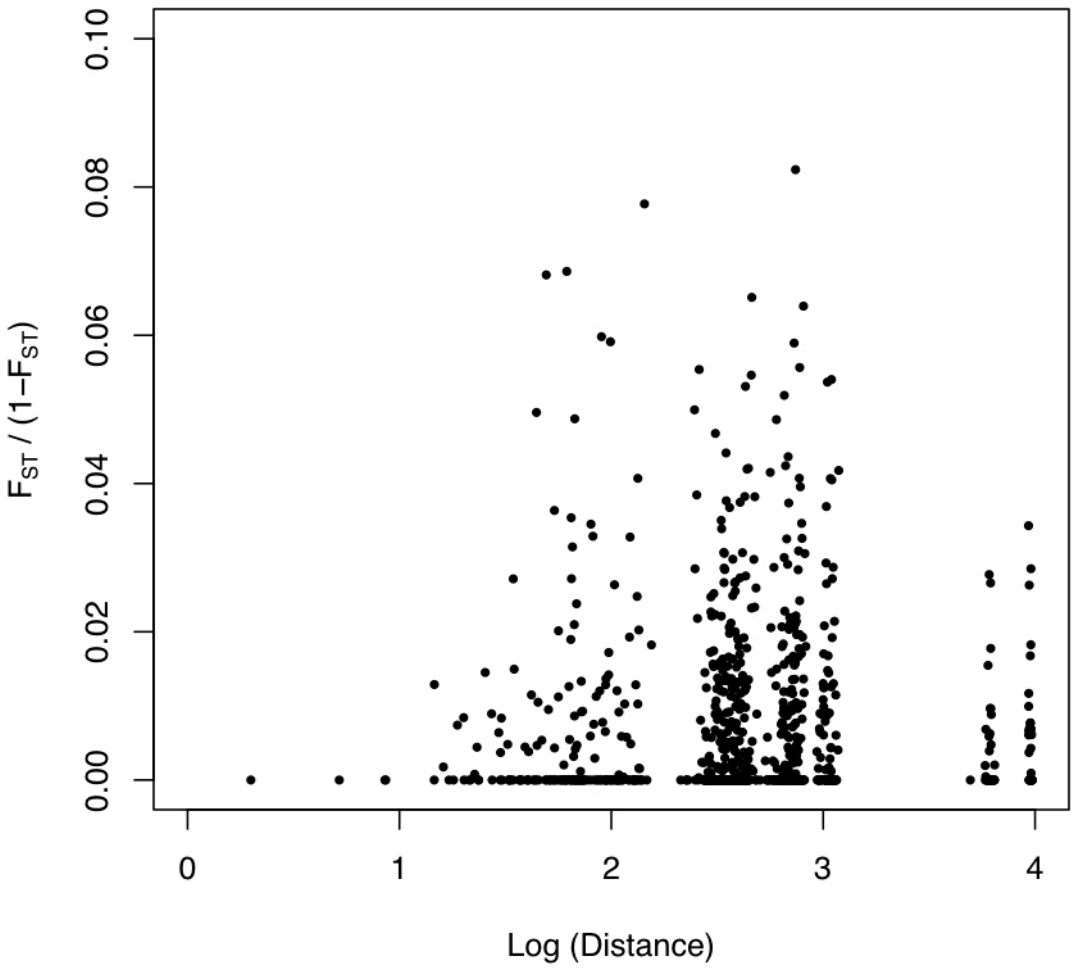

Figure 4. The relationship between Slatkin's linearized F_{ST} (D = F_{ST}/(1-F_{ST})) and the logarithm of geographic distance. This relationship was not significant (Mantel test; r = -0.016, P = 0.51).

Table 1. Hierarchical analysis of molecular variance (AMOVA), partitioning total genetic variance into the following components: within logs (F_{IS}), within fungi (F_{SC}), among fungi (F_{CT}), and among all logs (F_{ST}).

Locus	F_{IS} *within logs*	P	F_{SC} *within fungi*	P	F_{CT} *among fungi*	P	F_{ST} *among logs*	P
Boco_049	0.0077	0.41	0.0002	0.47	0.0020	0.19	0.0022	0.40
Boco_030	0.0189	0.28	-0.0008	0.60	0.0025	0.11	0.0018	0.46
Boco_007	0.0343	0.14	-0.0055	0.85	0.0043	0.04	-0.0012	0.71
Boco_006	0.0399	0.18	-0.0005	0.58	-0.0020	0.71	-0.0025	0.63
Boco_061	0.0358	0.19	0.0009	0.53	-0.0023	0.80	-0.0014	0.60
Boco_065	0.0372	0.06	0.0010	0.50	-0.0016	0.83	-0.0006	0.63
Boco_034	-0.0458	0.93	0.0019	0.28	0.0007	0.28	0.0026	0.23
Boco_128	0.0421	0.16	0.0151	0.05	-0.0031	0.65	0.0120	0.10
Boco_084	0.0253	0.15	0.0093	0.10	-0.0016	0.70	0.0077	0.10
All loci	**0.0209** (0.0013-0.0345)	**0.02***	0.0020 (-0.0024-0.0047)	0.35	0.0001 (-0.0014-0.0018)	0.36	0.0021 (-0.0003-0.0049)	0.31

See Methods for a detailed explanation of the four *F*-statistics. Results are presented for each of the nine microsatellite loci, as well as the weighted average over all loci. 95% confidence intervals for the multilocus *F*-statistics are reported in parentheses and were obtained by bootstrapping over loci. Significant values ($P < 0.05$) are indicated in bold, with an asterisk.

Table 2. Hierarchical analysis of molecular variance (AMOVA) performed separately for the two sexes, partitioning total genetic variance into the following components: within logs (F_{IS}), within fungi (F_{SC}), among fungi (F_{CT}), and among all logs (F_{ST}).

	F_{IS} (within logs)	P	F_{SC} (within fungi)	P	F_{CT} (among fungi)	P	F_{ST} (among logs)	P
Females	-0.0016 (-0.0392-0.0262)	0.52	-0.0005 (-0.0086-0.0078)	0.54	-0.0027 (-0.0051--0.0004)	0.96	-0.0033 (-0.0108-0.0045)	0.72
Males	**0.0396** (0.0101-0.0709)	**0.02***	-0.0021 (-0.0137-0.0110)	0.63	0.0028 (-0.0043-0.0100)	0.16	0.0033 (-0.0054-0.0152)	0.49

See Methods for a detailed explanation of the four *F*-statistics. 95% confidence intervals are reported in parentheses and were obtained by bootstrapping over loci. Significant values ($P < 0.05$) are indicated in bold, with an asterisk.

CHAPTER TWO:

The use of multiple host resources generates fitness variation in a mycophagous beetle[2]

[2] Formatted as a co-authored manuscript: Wood, C. W., J. del Sol, S. Paul, E. W. Wice, and E. D. Brodie III.

Abstract

Heterogeneous environments can generate substantial variation in fitness in populations that exploit multiple habitats. The cumulative fitness consequences of the environment in these landscapes depend jointly on the fitness effects of the available habitats and realized patterns of habitat use in the field. However, these two components are rarely coupled in studies of multiple habitat use, limiting our understanding of sources of fitness variation in heterogeneous environments. In this study, we paired estimates of the fitness consequences of the resource environment across multiple life stages with patterns of resource use in a natural metapopulation of *Bolitotherus cornutus*, a beetle that occurs on multiple species of fungus. We found that the three fungi have strong fitness consequences that are most pronounced in the larval stage, and indirect fitness consequences in adulthood via fungus effects on adult phenotypes that experience selection. Ovipositing females exhibit a clear preference for a high-quality fungus in the lab, but do not oviposit most frequently on it in the field, perhaps because the lowest-quality fungus is most abundant in the local landscape. Our results suggest that the focal *B. cornutus* metapopulation depends disproportionately on the two rare, higher-quality fungi, and that the most common fungus may function as a population sink. Use of multiple host resources may generate substantial fitness variation in many natural populations, even in those that appear to specialize on a single resource under artificial laboratory conditions.

Introduction

The environment is a major source of variation in fitness (Kruuk et al. 2002, Zikovitz and Agrawal 2013). The conditions that individuals experience account for a substantial proportion of variation in survival and reproductive success in heterogeneous landscapes (Price and Schluter 1991, Rowe and Houle 1996, Awmack and Leather 2002). However, because the same environmental conditions often affect fitness throughout the life cycle—with opposing or concordant effects at different life stages—the cumulative contribution of the environment to variation in fitness can be more complex than is apparent in any single episode of selection.

Environmental effects on fitness occur in multiple ways. The environment can affect fitness directly by driving differences in mortality or fecundity among individuals in different habitats (Awmack and Leather 2002, Merrill et al. 2013). Alternatively, the environment can indirectly affect fitness by modifying the expression of a phenotype with fitness consequences (Emlen 1994, Kasumovic 2013). For example, if the developmental environment influences adult body size and selection favors larger adults, variation in fitness among adults will be due to conditions experienced during development. These indirect environmental effects on fitness via trait expression are common in sexually selected traits, which tend to exhibit heightened condition dependence and experience strong selection (Bonduriansky 2007, Emlen et al. 2012). Finally, the environment can modify the relationship between phenotype and fitness (i.e., selection; Grant and Grant 1995, Kingsolver and Gomulkiewicz 2003, Gosden and Svensson 2008, Kasumovic et al. 2008). Differences in the strength, direction, and form of selection among environments are widespread (Kingsolver et al. 2012, Siepielski et al.

2013), and as a result, can substantially impact fitness even in the absence of environmental effects on phenotypic expression or on mean survival or fecundity.

The cumulative fitness consequences of the environment are complex as a result of its manifold effects on fitness. If environmental effects on phenotypic expression co-occur with environmental effects on selection, fitness will depend jointly on the environment that determines phenotypic expression and on the environment in which that phenotype experiences selection. Environmental effects on fitness early in life are especially impactful because they reduce effective population size (Zikovitz and Agrawal 2013), and interfere with the estimation of selection on traits expressed at later life stages if juvenile mortality is non-random with respect to traits expressed only in adulthood (the "invisible fraction" problem; Hadfield 2008, Mojica and Kelly 2010).

Patterns of habitat use in the field have the potential to ameliorate or exacerbate the effects of the environment on fitness (Doak et al. 2006, Forister and Wilson 2013). Even if differences in survival or reproductive success among environments are extremely large, they will only manifest as fitness variation in natural populations if multiple environments are used frequently. Environments with high mean fitness (high-quality habitats) are typically thought to exert a larger influence on evolutionary dynamics than low-quality ones (Kawecki 2008), but this may not be the case when the majority of the population occupies low-quality environments (Pulliam 1988, Holt 1997, Forister and Wilson 2013). As a result, the fitness consequences of environmental variation depend jointly on the direct and indirect fitness effects of the same environmental variable throughout the life cycle, and the frequency with which different environments are used in the field (With 2004).

However, most studies stop short of coupling the cumulative fitness effects of the environment with realized patterns of habitat use in natural landscapes. This omission is critical because inferring habitat use from laboratory studies is insufficient (Thompson 1988). Habitat use is shaped by many factors (Forister and Wilson 2013), leading many populations to fail to specialize on a single high-quality habitat due to genetic constraints, costs associated with specialization, or ecological characteristics of the environment that favor niche breadth (Jaenike 1990, Kawecki and Ebert 2004). Consequently, even in species that exhibit strong habitat preferences in the lab, the use of multiple habitats is common in the field (Thompson 1988, Doak et al. 2006, Refsnider and Janzen 2010). Therefore, comprehensive datasets that combine the fitness effects of the environment throughout the life cycle with patterns of habitat use in the wild are necessary to understand the evolutionary consequences of environmental heterogeneity in metapopulations that exploit multiple habitats (With 2004, Kawecki 2004).

In the present study we addressed the following questions. First, is the environment a major source of variation in fitness, and is fitness variation predominately the result of differences in mean fitness or in selection among environments? Second, do patterns of habitat use in the field ameliorate these effects? Insects that exploit multiple hosts are excellent systems in which to answer these questions. These species rely on multiple discrete environments—host species—that often differ substantially in quality (Jaenike 1990, Matsubayashi et al. 2010, Forister et al. 2015), and host use is easily quantifiable. Many species lack parental care, so host resources often strongly influence juvenile phenotype and fitness, and these effects may persist into adulthood (Refsnider and Janzen 2010). Finally, host-associated insects often rely on the same host as juveniles

and adults, so the fitness effects of a single environmental variable can be measured throughout the life cycle.

We measured the fitness consequences of three co-occurring host fungi for a mycophagous beetle (*Bolitotherus cornutus*), and coupled these data with patterns of resource use in a natural metapopulation. The three host fungi strongly affect early larval growth in *B. cornutus* in the lab (Chapter 3), but there is no evidence for resource specialization in the focal metapopulation, suggesting that *B. cornutus* uses all three fungi in the field (Chapter 1). To address the fitness consequences of the three fungus hosts in *B. cornutus*, we measured juvenile mortality and development time, as well as the expression of, and selection on, adult body size in each fungus. We complemented these data with a lab-based oviposition choice experiment and field-based survey of oviposition patterns to assess whether patterns of host use are likely to ameliorate fungus effects on fitness.

Methods

These data were collected in three field studies and one lab experiment that took place at or near Mountain Lake Biological Station in Giles County, Virginia. All statistical analyses were performed in R v3.1.0 (R Core Team 2014).

Study species and study population

The forked fungus beetle (*Bolitotherus cornutus*, Coleoptera: Tenebrionidae) is a mycophagous beetle found in eastern North America (Liles 1956). *Bolitotherus cornutus* relies on the fruiting bodies ("brackets") of three wood-rotting fungi: *Fomes fomentarius*,

Ganoderma applanatum, and *G. tsugae*. Adult beetles feed on the fungal tissue and the spores. Female beetles lay single eggs on the surface of the brackets, and larvae burrow into the bracket tissue, on which they feed throughout development (Liles 1956). The three fungi affect early larval growth differentially in the lab, suggesting that they vary in quality (Chapter 3). In spite of the strong fitness consequences of the early larval environment, there is no evidence for local adaptation to one of the three fungi in the lab or in the field. There is no family × fungus interaction for larval growth in the lab (Chapter 3), and there is a complete absence of resource-associated population genetic structure in the field (Chapter 1).

In a previously published study (Chapter 1), we surveyed a 0.25 km^2 (250m x 1000m) area along Hunters Branch and Pond Drain on Salt Pond Mountain, near Mountain Lake Biological Station (37.3738 N, 80.5351 W). We geo-referenced all logs infected with any of the three host fungi, recorded the fungus species with which they were infected, and estimated the number of brackets on each log. Because all data reported herein were collected within this site (Parts 2-4 below) or nearby in the surrounding woods (Part 1 below), we used these data to estimate the relative abundance of the three host fungi in the local landscape (Table 1). The landscape is dominated by *F. fomentarius*; together, the two *Ganoderma* species account for only 30.5% of the fungus brackets in the study site.

Part 1: Fungus effects on larval fitness and adult body size

In the summer of 2013, we collected 65 fungus brackets from 25 logs on and around Salt Pond Mountain (N = 28 *F. fomentarius* brackets, 14 *G. applanatum*, and 23

G. tsugae). We chose brackets that exhibited signs of beetle presence (e.g., egg scars or exit holes), and sampled brackets across all stages of senescence. Before dissection, we estimated bracket volume by submerging the bracket in a graduated cylinder filled with water. Brackets were dissected in the lab within two weeks and any larvae found within were weighed to the nearest 0.01 mg using a Mettler Toledo AX205 DeltaRange balance and imaged on an Epson Perfection v300 flatbed scanner. After dissection, each bracket was dried and ground in an industrial blender (Blendtec, Orem, UT), and all larvae were maintained on the bracket from which they were collected. Large larvae (> 0.5 cm) were housed in plastic soufflé cups and small larvae (< 0.5 cm) in 1.5mL tubes in an incubator on a 16:8 light:dark cycle at 23°C. Larvae were checked every two-three weeks through the winter of 2013, and were imaged when they eclosed as adults. Adult elytra length was measured from these images in ImageJ (Abramoff et al. 2004).

To test whether the mass of collected larvae differed among fungi, we ran a linear mixed model using the lmer function in the lme4 package (Bates et al. 2014) with log-transformed larval mass as the dependent variable, fungus as the independent variable, and bracket as a random effect. To test whether fungus effects on larval phenotype persist into adulthood, we used the subset of larvae from the field collection described above that metamorphosed into adults in the fall of 2013 (N = 45). We regressed adult elytra length on fungus species, late-instar larval mass, and their interaction. We tested the significance in both models using the Anova function in the car package (Fox and Weisberg 2011) and type III sums of squares.

To test whether the number of collected larvae differed among fungi, we ran a general linear model with log-transformed larval density (larvae per cm^3) as the

dependent variable and fungus as the independent variable. We used a similar model to test for differences among fungi in the number of larvae that metamorphosed into adults within three months of collection, with log-transformed adult density (adults per cm^3) as the dependent variable. We assessed the significance of pairwise differences between fungi using Tukey post-hoc tests executed with the glht function in the multcomp package (Hothorn et al. 2008).

Part 2: Fungus effects on adult fitness and selection on adult phenotype

In the summer of 2012 we estimated selection on adult body size in two of the five plots that were established in a previous study (Chapter 1). These plots were 180 meters in diameter and were 0.5 kilometers apart. We visited these plots multiple times during daylight hours in May-July 2012 and on each visit we searched all logs infected with the three host fungi (N = 47 logs). We captured all adult *B. cornutus*, imaged them on a flatbed scanner, and sampled 0.2-5μL of hemolymph following the methods of Donald et al. (2012). Hemolymph was stored in lysis buffer (Promega DNA IQ system) until DNA extraction. Each individual was painted with a unique ID using Testors Gloss Enamel (Chapter 1). All beetles were released at their log of capture within 72 hours. We marked 190 adult females and 107 adult males in the summer of 2012.

We used two fitness components to estimate selection: survival and reproductive success. To measure survival, we visited the plots in the middle of July 2012 and the beginning of September 2012 to recapture marked beetles. We surveyed all logs in the two study plots during these visits, and included all recaptured beetles in the analysis, regardless of whether they were recaptured on the log on which they originated. Any

individuals that were recaptured during either of these visits were assigned a survivorship of 1, and all other individuals, 0. Because we did not incorporate detection probabilities into our analysis (Nichols 1992), we almost certainly underestimated mean survival in our study site. However, as long as detection probabilities do not differ among fungus species, this should not generate spurious differences in the strength of selection or mean fitness among fungi.

To estimate reproductive success, we measured number of offspring. We collected and assigned parentage to all eggs laid on a subset of the logs at the center of the plots used in the mark-recapture study (three *F. fomentarius* logs, with 105 brackets total; two *G. applanatum* logs, with 78 brackets; and four *G tsugae* logs, with 128 brackets). *Bolitotherus cornutus* females lay eggs singly on the brackets and cover them with a casing that remains on the bracket after the egg hatches (Liles 1956). To be certain that we only collected eggs laid in the summer of 2012, we removed all eggs and egg casings from all brackets in mid June using dissecting probes. Two weeks later we collected all new eggs on these logs. We repeated this process two more times, in late July and mid August, leaving two weeks between each collection. *Bolitotherus cornutus* eggs hatch in two to three weeks (Liles 1956), so this sampling scheme ensured that we collected nearly all eggs laid between late June and mid August. Eggs were maintained in an incubator on a 16:8 light:dark cycle at 23°C until they hatched. Upon hatching, larvae were transferred to lysis buffer (Promega DNA IQ system). When an egg was punctured inadvertently in the field during collection, it was transferred directly to lysis buffer with a microcapillary tube.

DNA was extracted from all offspring and adult samples using Promega's DNA IQ system (Promega 2010). All samples were genotyped at 28 microsatellite loci, including the nine loci described in Donald et al. (2012) (Supplementary Table 1). PCR was performed with Qiagen's Multiplex PCR kit and microsatellite protocol (Qiagen 2010), and fragment analysis was performed at Yale's DNA Analysis Facility. Microsatellite genotypes were scored in GeneMarker (SoftGenetics, State College, PA). Maternity and paternity assignment included all individuals with genotypes at 14 or more loci (1407 offspring, 146 males, and 238 females), and were obtained using full likelihood scores from a single medium-precision run in COLONY (Jones and Wang 2009, Wang 2013). We specified that inbreeding was present and that polygamy could occur in both sexes, and included the estimated frequencies of null alleles calculated in MICRO-CHECKER (Van Oosterhout et al. 2004). This analysis assigned paternity to 689 offspring (49.0%) and maternity to 887 (63.0%) with greater than 95% confidence.

We estimated variance-standardized selection gradients on adult body size using linear regression of relative fitness on elytra length (Lande and Arnold 1983). For the purposes of the selection analysis, adults were assigned to the fungus species on which they were first captured, regardless of whether they later migrated. Adult elytra length was standardized to a mean of zero and variance of one before analysis. Fitness was relativized by dividing each individual's absolute fitness (survival: 0 or 1; reproductive success: number of offspring) by the population mean fitness. Elytra length was standardized and fitness relativized within each sex separately, and we ran separate selection analyses for each sex. Our selection analyses included relative fitness (survival or reproductive success) as the dependent variable, and standardized elytra length,

fungus, and their interactions as independent variables. We tested significance of the estimated gradients using a generalized linear mixed model with binomial (survival) or Poisson (reproductive success) error distributions. Population (log of collection) was included in both models as a random effect, and these models were run using the glmer function in the lme4 package. Fitness functions were visualized with cubic splines in the ggplot2 package (Wickham 2009).

Part 3: Fungus effects on development time

To assess fungus effects on development time, we enclosed the brackets on all nine logs from analysis of selection via reproductive success in aluminum screen bags in March 2013 to capture emerging adults over the next two years. Brackets were bagged in the spring to allow eggs to be laid until the end of the breeding season in the late fall, following the end of egg collection for the selection analysis in August. Few adults are likely to have emerged earlier in the spring before the brackets were bagged because *Bolitotherus cornutus* do not appear to be active before May near Mountain Lake Biological Station (C. Wood, personal observation). Bags were constructed by shaping aluminum screening into a tube, folding the edges, and sealing them with a stapler. Because *G. tsugae* brackets are produced annually and senesce at the end of the summer, they were removed from the log and sealed individually into screen bags, which were nailed onto the log. *Ganoderma applanatum* and *F. fomentarius* produce brackets perennially, so they were bagged while attached to the log to avoid killing the brackets. Bark at the base of each bracket was removed with a chisel, and a screen bag was stapled and sealed to the log with fast-drying waterproof silicone caulk (GE, Huntersville, NC).

All bagged *G. tsugae* brackets (N = 159) were collected and dissected in September and October 2013. A small, biased sample of *G. applanatum* (N = 9) and *F. fomentarius* (N = 27) brackets that exhibited signs of beetle presence (e.g., egg cases) were dissected at the same time. Because we collected equal numbers of larvae and adults from the *G. applanatum* and *F. fomentarius* brackets (see Results), the remaining bagged brackets of these two species were left in the field for another year to allow the larvae to complete development under field conditions. In September-October 2014, a random sample of the remaining bagged *G. applanatum* (N = 10) and *F. fomentarius* (N = 18) brackets were dissected. We calculated beetle density (individuals per cm^2) by dividing the number of adults or larvae collected from each bracket by its surface area, which we estimated by overlaying the bracket with a 3 × 3cm grid. We were only able to measure the surface area of 87 *G. tsugae* brackets because many were too decayed to assess volume.

To test whether development time of larvae differs among fungi, we compared the number of adults and larvae collected from each fungus species in each year in two ways. First, we ran a general linear model with the number of individuals per cm^2 as the dependent variable, and fungus, life stage (larvae or adults), and their interaction as independent variables. We ran separate models for the two collection years, and log-transformed the dependent variable to meet normality assumptions. Second, because the three fungi were sampled differently in 2013 (we collected all *G.* tsugae brackets, but only a few high-density *G. applanatum* and *F. fomentarius* brackets), we ran separate models for each fungus species in each year. These models were run using the lm

function, and we tested significance using the Anova function in the car package and type III sums of squares.

Part 4: Patterns of oviposition in the lab and field

To test whether ovipositing females prefer one of the three fungi, we performed an oviposition choice experiment using females from known developmental environments collected in Parts 1 and 3 (see above). Because so few adults emerged from *F. fomentarius* (see Results), this experiment only included females raised in *G. applanatum* (N = 17) and *G. tsugae* (N = 32). Each female was paired with a *G. tsugae*-reared male and maintained in an incubator on an 18:6 light:dark cycle at 23° C and 80% humidity for three to four weeks. After courtship and mate guarding were observed in the majority of pairs, females were transferred to choice arenas. These arenas were round clear-lidded plastic containers 18.42 cm in diameter, the bottom of which was covered in plaster of Paris and topped with hardwood mulch. A five cm^2 piece of each fungus was imbedded in the plaster equidistant from the other fungi and the center of the arena. At the beginning of the trials, females were placed in the centers of their arenas. Arenas were checked daily for 15 days, and the locations of new eggs were recorded. Each egg was marked with Testors Gloss Enamel to distinguish new eggs from old ones. The experiment was conducted in two rounds (round 1: N = 12 *G. applanatum* and 11 *G. tsugae* females; round 2: N = 5 *G. applanatum* and 21 *G. tsugae* females) in December 2013 and February-March 2014.

We tested whether females preferred one of the three fungi in two ways. First, we used a chi-square goodness-of-fit test to test whether the first egg was laid on one of the

fungi more often than expected by chance. Second, we used a generalized linear mixed model with a Poisson error distribution to test whether the number of eggs laid differed among fungi. This model included the number of eggs laid on each fungus as the dependent variable, and fungus, female origin (developmental environment), and their interaction as independent variables. Female ID was included in this model as a random effect. This model was run using the lmer function in the lme4 package. Finally, to test whether a female's developmental environment affected her oviposition preference, we used a MANOVA with the number of eggs laid on each of the fungi as the three dependent variables and female origin as the independent variable. Females that did not lay eggs were excluded from the analyses.

To test whether the oviposition preference expressed in the lab manifested in the field, we compared egg densities among the three fungi in the field. For this analysis, we used the first two egg collections from analysis of selection via reproductive success (Part 3 above). We measured egg density (eggs per cm^2) by divided the number of eggs collected per bracket by its surface area, which we estimated by overlaying a 3 × 3cm grid onto each bracket. Because egg density was severely zero inflated, we were not able to transform it to meet parametric statistical assumptions, so we ran two separate models. First, we used a generalized linear model with a binomial error distribution to test whether egg presence (the probability that at least one egg was laid on a bracket) differed among fungi, using egg presence (0 or 1) as the dependent variable and fungus, collection (June-July or July-August), and their interaction as independent variables. Second, using only the brackets with at least one egg (omitting the zero class) we ran a generalized linear model with a Poisson error distribution to test whether egg densities differed

among fungi, with the same independent variables as the egg presence analysis. We tested significance using the Anova function in the car package and type III sums of squares.

Methods summary

We synthesized the results from Parts 1-3 to assess the direct and indirect fitness consequences of the three host fungi in *B. cornutus*, and used the results of Part 4 to assess patterns of host resource use in the focal metapopulation. We tested for direct fungus effects on survival using the cross-sectional sample of larvae collected in Part 1, and direct fungus effects on development time by comparing the relative proportions of adults and larvae collected from bagged brackets in Part 3. We used the results from Parts 1 and 2 to test whether the fungi indirectly affect adult fitness through their effects on body size. We compared the adult body size of larvae collected from different fungi in Part 1 to test for fungus effects on adult phenotype, and measured selection on adult body size in populations on each of the three fungi in Part 2 to assess the fitness consequences of adult body size. Finally, in Part 4 we tested whether *B. cornutus* exhibit a host preference in the lab, and whether this preference is evident in patterns of oviposition on the three fungi under field conditions in the focal metapopulation.

Results

Part 1: Fungus effects on larval fitness and adult body size

We collected 83 larvae from *F. fomentarius*, 141 from *G. applanatum*, and 91 from *G. tsugae*. Larval densities differed significantly among the three fungi ($F_{2,62} = 8.787$, $P <$

0.001). Significantly fewer larvae per cm^3 were collected from *G. tsugae* than from the other two species (Figure 1A). Larvae collected from *F. fomentarius* were smaller than those from the two *Ganoderma* species (LR $\chi^2 = 20.571$, df = 2, $P < 0.001$; Figure 1B), which suggests that very few larvae developing in *F. fomentarius* reach later larval stages. However, because these larvae are field collected, these size differences among fungi may reflect a combination of fungus effects on growth, mortality, or genetic differences between larvae from different fungi.

Significantly fewer larvae collected from *F. fomentarius* metamorphosed into adults within three months of collection ($F_{2,62} = 4.442$, P = 0.012; Figure 1C). This result suggests that larval survival is low in *F. fomentarius* and especially high in *G. tsugae*. Size differences among beetles collected from the three fungi persist into adulthood. Late-instar larval mass significantly predicted adult elytra length ($F_{1, 25} = 5.108$, P = 0.033; Figure 1D), and adult elytra length differed significantly between fungi ($F_{1, 25} = 8.960$, P = 0.006; Figure 1D). Adults that developed in *G. applanatum* were significantly larger than those collected from *G. tsugae*. The relationship between larval mass and adult body size did not differ significantly between the two *Ganoderma* species ($F_{1, 25} = 1.925$, P = 0.178). Because only one adult eclosed from *F. fomentarius* from these field-collected brackets, we were unable to compare the size of *F. fomentarius* adults to the other two species.

Part 2: Fungus effects on adult fitness and selection on adult body size

Fungal hosts do not differentially affect adult survival in either sex (fungus main effect, Table 2), indicating that the fitness consequences of resource environment due to

survival are most severe in the larval stage (Figure 2A and 2C). Selection gradients using survival as the fitness component were weakly positive on all fungi, though we did not detect significant viability selection on body size (elytra main effect, Table 2). These selection gradients are comparable in magnitude to those reported in previous studies of *B. cornutus* (Conner 1988, Formica et al. 2011).

Using reproductive success as the fitness component, we detected significant positive selection on body size in both sexes: larger individuals produced more offspring (elytra main effect, Table 2). Selection was primarily directional; there were no strong intermediate peaks in the fitness functions (Figure 2B and 2D). The strength of selection differed among fungi, significantly so in females and marginally significantly in males (elytra × fungus interactions, Table 2). Selection on body size was weaker on *G. tsugae* than on *F. fomentarius* and *G. applanatum* (Table 2, Figure 2B and 2D). Reproductive success differed significantly among fungi in females but not in males (fungus main effect, Table 2), and was lowest on *G. tsugae* (Figure 2).

Part 3: Fungus effects on development time

Larval development time was shorter in *G. tsugae* than in *G. applanatum* and *F. fomentarius* (Figure 3). Significantly more adults than larvae were collected from *G. tsugae* in the fall of 2013, suggesting that most larvae developing in *G. tsugae* emerge as adults within a year (Table 3). During the same collection in the fall of 2013, there was no significant difference between the numbers of larvae and adults collected from *G. applanatum* and *F. fomentarius*. Equal numbers of adults and larvae were collected from *G. applanatum* and *F. fomentarius* in the fall of 2014, two years after brackets were

bagged, demonstrating that larval development lasts at least two years in these fungi (Table 3).

Significantly more individuals (both adults and larvae) were collected from *G. applanatum* than from the other two fungi in the fall of 2013 (Figure 3). However, we caution against drawing strong inferences from comparisons among fungi in the fall of 2013 because the sampling regime differed among fungi (see Methods). While we dissected all bagged *G. tsugae* brackets, we only sampled a small fraction of *G. applanatum* and *F. fomentarius* brackets that we expected to have high beetle densities.

Part 4: Patterns of oviposition in the lab and field

Ovipositing females exhibited a significant preference for *G. tsugae* in the lab choice experiment (Figure 4). Twenty-seven of the 49 females laid at least one egg in their choice arenas. Most females laid their first egg on *G. tsugae* ($\chi^2 = 14.889$, df = 2, $P < 0.001$), and significantly more eggs were laid on *G. tsugae* than on the other two fungi (Wald $\chi^2 = 133.228$, df = 2, $P < 0.001$). Females that developed in *G. applanatum* and *G tsugae* did not lay different numbers of eggs (Wald $\chi^2 = 1.003$, df = 1, $P = 0.317$). Oviposition choice did not depend on a female's developmental environment ($F_{3,46} = 0.665$, Pillai's trace = 0.042, $P = 0.578$). Both *G. applanatum* and *G. tsugae* females laid most of their eggs on *G. tsugae* (Figure 4).

Egg presence and egg density differed significantly among fungi in the field. Across both collection periods, eggs were more likely to be present on *G. tsugae* than on *G. applanatum* (post-hoc Tukey test, $P = 0.005$; Table 4, Figure 5), although there was no significant difference in egg presence between *F. fomentarius* and *G. applanatum*, or

between *F. fomentarius* and *G. tsugae*. The opposite was true for egg densities on brackets with at least one egg, which were significantly lower on *G. tsugae* than the other two fungi (post-hoc Tukey test, GT-FF: $P < 0.001$, GT-GA: $P < 0.001$; Table 4, Figure 5). Differences among fungi in egg presence and density remained significant when the number of females collected from each log was included in the analysis as a covariate to control for differences in population size (presence: fungus main effect: $F_{2,615} = 4.816$, $P = 0.008$; density: fungus main effect: $F_{2,374} = 28.997$, $P < 0.001$).

Discussion

The three species of host fungus generate substantial variation in fitness in the focal *B. cornutus* metapopulation. The fitness effects of the host resource manifest predominately in the larval stage, but extend to adulthood through fungus effects on body size, which is under strong selection in adults of both sexes. Differences in host quality are so pronounced that very few larvae survive to adulthood on *F. fomentarius*, even after accounting for striking plasticity in development time among fungal hosts. Patterns of host use in the field are unlikely to mitigate these fitness consequences. Although *B. cornutus* exhibits strong preference for a high-quality host in the lab, the preference is not apparent in the field, where the poorest-quality host is the most abundant. Our results suggest that our focal population exhibits many of the characteristics of a source-sink metapopulation, which is primarily dependent on high-quality resources that make up a small fraction of the host community.

Fitness consequences of the resource environment in multiple life stages

Host differences in larval survival and performance indicate that the three fungi differ substantially in quality as developmental environments, and that resource heterogeneity generates variation in fitness in the focal metapopulation. Field-collected larvae from *F. fomentarius* were significantly smaller in mass than those collected from the two *Ganoderma* species (Figure 1A), the same qualitative pattern that has been observed in the laboratory (Chapter 3). Our data strongly suggest that larval mortality is elevated in *F. fomentarius*. Larvae collected from *F. fomentarius* were smaller than those from the other two fungi, and they were also less likely to metamorphose into adults, indicating that late-stage larvae are largely absent from *F. fomentarius* (Figure 1C). This effect cannot be attributed solely to differences in larval growth among fungi; even if growth were much slower in *F. fomentarius*, our cross-sectional sample should have identified some late-stage larvae if they survived to reach the end of development. Similarly, it is unlikely that host effects on larval mass reflect genetic differences among larvae, as there was no genetic subdivision in the adult population (Chapter 1).

In addition to their pronounced consequences for larval mortality, the three fungi induce striking plasticity in development time. The majority of larvae developing in *G. tsugae* emerged as adults within a year, while we found equal numbers of adults and larvae in *F. fomentarius* and *G. applanatum* two years after brackets were bagged (Figure 3). This developmental plasticity matches the life histories of the three fungi. *G. tsugae* brackets senesce within a year, while the other two fungi produce brackets that live for several years (Gilbertson and Ryvarden 1986). Because of these life history differences, our data may underestimate disparities in development time between the three fungi. The

G. tsugae brackets that we dissected in 2013 grew in the summer of 2012, so the individuals collected from these brackets cannot be older than one year of age. By contrast, *B. cornutus* collected from *G. applanatum* and *F. fomentarius* brackets could have been older than two years of age because many of those brackets were produced before the summer of 2012. Overall, our data indicate that larval development in the two perennial fungi lasts for several years, contrary to published estimates (Liles 1956).

Plasticity in phenology is common in heterogeneous environments (Galloway 2005, van Asch et al. 2010, Burghardt et al. 2015). Life history plasticity in response to the environment is divisible into adaptive plasticity, which occurs in response to reliable cues of future conditions (Simons 2014), and bet hedging, in which plasticity is apparently random in response to unpredictable environments (Slatkin 1974, Simons 2014). Because the developmental plasticity that we observed in *B. cornutus* matches the life history of the brackets produced by the three fungi, adaptive plasticity in response to fungal cues or indicators of resource depletion, rather than bet hedging, may underlie differences among fungi in the timing of metamorphosis.

The most dramatic fitness consequences of the three fungi are largely confined to the larval stage. The fungi did not strongly affect survival in adults of either sex (Table 2, Figure 2). However, the developmental fungus environment affected adult fitness indirectly through its effects on adult phenotype and selection on adult phenotype, because fungus effects on body size persisted into adulthood (Figure 1D). Adults that developed in *G. applanatum* were significantly larger than those from *G. tsugae*, and selection tended to favor larger body size in adults. As a result, beetles that developed in *G. applanatum* likely have higher mean fitness in adulthood than those from *G. tsugae*.

Indirect effects of the developmental fungus environment on adult fitness are primarily due to strong selection via reproductive success on body size in adults of both sexes. Selection via survival on body size was positive but weak in both sexes (Figures 2A and 2C), in accordance with previous studies (Conner 1988, Formica et al. 2011). Selection via reproductive success, on the other hand, strongly favored larger body size in both sexes (Figures 2B and 2D). Variation in fitness resulting from selection on adult body size is at least partially due to the host fungi because of the effect of the developmental fungus environment on body size in adults. Therefore, the fitness consequences of the fungi extend to multiple life stages as a result of fungus effects on adult phenotype. Selection on male body size has been previously reported in *B. cornutus* and is driven by male-male competition (Conner 1988, Conner 1989, Formica et al. 2011). Our estimate of selection on male body size using number of offspring as the fitness component qualitatively agrees with previous studies that used mate guarding, indicating that the sneaker male tactic—an alternative way to gain copulations often used by small males—is absent in *B. cornutus*. Although not previously documented in *B. cornutus*, selection via reproductive success on female body size is common in holometabolous insects. Fecundity is tightly associated with body size in these species, and male mate choice based on female body size is widespread (Bonduriansky 2001).

The strength of selection on adult body size differed among fungi in both sexes (elytra × fungus interactions in Table 2; Figure 2). Selection was weakest in populations on *G. tsugae* in both males and females. It is possible, but unlikely, that these differences in selection are an artifact of differences among fungi in patterns of adult dispersal. If, for example, large adults from *G. tsugae* were more likely to disperse out of the study site,

we would have under-sampled their offspring and underestimated selection in *G. tsugae* populations. Alternatively, the differences in the strength of selection may reflect differences among fungi as competitive environments. The strength of sexual selection on male combat traits depends on whether resources are easily defensible, which is governed by population density (Kokko and Rankin 2006), sex ratio (Kasumovic et al. 2008), and the spatial geometry of the resource environment (Emlen and Oring 1977, Reichard et al. 2009). Population density is known to affect the strength of sexual selection (Conner 1989) and social selection (Formica et al. 2011) in male *B. cornutus*, so consistent differences among fungi in beetle demographics may contribute to the pattern reported herein.

Similar demographic characteristics of the three fungi may be responsible for the differences in selection on female body size among fungi. Although understudied relative to male-male competition, female-female competition over mates, food resources and oviposition sites has been observed in many taxa, and is affected by similar ecological and demographic factors (Rosvall 2011). Regardless of the environmental characteristics underlying differences in selection among fungi, these results indicate that the indirect fitness consequences of the developmental environment in adult *B. cornutus* of both sexes are determined jointly by fungus effects on adult body size and on the relationship between body size and fitness.

Patterns of resource use in the lab and in the field

Bolitotherus cornutus are capable of discriminating among their fungal hosts. Ovipositing females exhibit a strong preference for *G. tsugae* in laboratory trials

regardless of their own developmental environment (Figure 4). This preference may be adaptive, as larval development is rapid and mortality is low in *G. tsugae* (Figures 1 and 3). It is unclear why females discriminate between the two *Ganoderma* species, both of which are associated with rapid larval growth and low mortality. One possibility is that *G. applanatum* is a highly competitive environment, characterized by high egg and larval densities in the field (Figures 1 and 5) and elevated larval cannibalism in the lab (Chapter 3). The risk of juvenile competition deters oviposition in many species (Ellis 2008, Refsnider and Janzen 2010), and may be an important factor in oviposition site choice in *B. cornutus* as well. Alternatively, sensory constraint may shape host preferences in *B. cornutus*, as it does in many other species (Fox and Lalonde 1993, Cunningham 2012). *F. fomentarius* and *G. applanatum* are a commonly used pair of hosts for many polyphagous insects (Jonsell and Nordlander 2004), suggesting that they produce similar chemical cues and are difficult to distinguish. Because larval survival is so low in *F. fomentarius*, females may avoid it and any hosts that appear similar, manifesting as a preference for *G. tsugae* in our experiment.

Notwithstanding clear differences in resource quality, and in spite of strong host preferences in the lab, *B. cornutus* uses all three fungi in the field (Figure 5). Egg densities were lowest on *G.* tsugae, the fungal host preferred in the lab. It is not uncommon for oviposition site choice in the field to deviate from preferences expressed in the lab because it depends on multiple factors, only one of which is offspring performance (Thompson 1988, Refsnider and Janzen 2010). Oviposition on a poor habitat can persist if some individuals are competitively excluded from high-quality habitats (the “ideal despotic distribution”; Fretwell 1972), or if costs or constraints

prevent the avoidance of undesirable oviposition sites (Holt 1997, Doak et al. 2006), although our data do not directly address these hypotheses.

The landscape ecology and life history of the three host fungi may play an important role in shaping host use in our focal metapopulation. The use of a low-quality resource can persist in heterogeneous environments when high-quality resources are temporally unpredictable or extremely rare (Jaenike 1990). The poorest-quality fungus is the most abundant in our study site. *F. fomentarius* accounts for 60% of infected logs on Salt Pond Mountain (Table 1), and is available year-round because it produces brackets perennially. By contrast, the preferred fungus in oviposition choice trials—*G. tsugae*—is functionally absent from the host community during the early breeding season. Its annual brackets do not mature until several weeks after *B. cornutus* begin to lay eggs (C. Wood, personal observation). Moreover, *G. tsugae* is rare, comprising only 13% of infected logs, and anecdotal evidence indicates that it was even rarer in the recent past. The invasion of the hemlock woolly adelgid, a sap-sucking insect that has caused widespread hemlock death in eastern North America (Fitzpatrick et al. 2012), likely altered the host community on Salt Pond Mountain (Chapter 1). *Ganoderma tsugae*, a hemlock specialist as its name implies, has probably increased substantially in abundance since the adelgid invasion reached our study site in the 1990s and began killing hemlock trees (Chapter 1, Fitzpatrick et al. 2012). Therefore, the historical scarcity and temporally variable availability of the preferred host may contribute to the frequent oviposition on *F. fomentarius* despite severe negative fitness consequences for developing larvae.

Demographic and evolutionary implications

Fomes fomentarius appears to exhibit many of the hallmark characteristics of a sink habitat. Sinks are marginal habitats in which reproduction is not sufficient to counterbalance mortality, and which are therefore dependent on migration from high-fitness source habitats (Pulliam 1998, Holt 1997, Forister and Wilson 2013). Sink habitats facilitate population persistence when high-quality resources are temporally variable or extremely rare (Holt 1997, Hanski 1999), as is likely the case in our focal metapopulation.

Source-sink metapopulation dynamics may contribute to the lack of resource specialization in species like *B. cornutus* that might otherwise be expected to specialize on a single high quality host. Adaptation to sink habitats is hampered by immigration each generation, which dilutes locally adapted alleles (Holt 1996, Kawecki 2004). Source-sink dynamics may account for the lack of fungus-associated population structure in *B. cornutus* (Chapter 1). If source environments like the two *Ganoderma* species contribute the majority of individuals to the adult population, most individuals in sink environments like *F. fomentarius* must be migrants, impeding genetic differentiation among populations on different fungi.

Although they are often considered inconsequential, marginal habitats can have profound demographic and evolutionary consequences (Kawecki 2008). Our results demonstrate that the resource environment that our focal metapopulation inhabits generates substantial fitness variation. These fitness effects are complex, reflecting a combination of environmental effects on juvenile mortality, adult phenotype, and the fitness consequences of adult phenotype. We underscore the importance of synthesizing

these fitness consequences with ecological attributes of the landscape in studies of evolution in heterogeneous environments.

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Literature cited

Abramoff, M. D., P. J. Magalhaes, S. J. Ram. 2004. Image processing with ImageJ. *Biophotonics International* 11:36–42.

Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity. *Annual Review of Entomology* 47:817–844.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-6. URL http://CRAN.R-project.org/package=lme4

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews of the Cambridge Philosophical Society* 76:305–39.

Bonduriansky, R. 2007. The evolution of condition-dependent sexual dimorphism. *The American Naturalist* 169:9–19.

Burghardt, L. T., C. J. E. Metcalf, A. M. Wilczek, J. Schmitt, and K. Donohue. 2015. Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. *The American Naturalist* 185:212–227.

Conner, J. K. 1988. Field measurements of natural and sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 42:736–749.

Conner, J. K. 1989. Density-dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 43:1378–1386.

Cunningham, J. P. 2012. Can mechanism help explain insect host choice? *Journal of Evolutionary Biology* 25:244–51.

Doak, P., P. Kareiva, and J. Kingsolver. 2006. Fitness consequences of choosy oviposition for a time-limited butterfly. *Ecology* 87:395–408.

Donald, H. M., C. W. Wood, K. M. Benowitz, R. A. Johnson, E. D. Brodie III, and V. A. Formica. 2012. Nondestructive sampling of insect DNA from defensive secretion. *Molecular Ecology Resources* 12:856–60.

Ellis, A. M. 2008. Incorporating density dependence into the oviposition preference-offspring performance hypothesis. *Journal of Animal Ecology* 77:247–56.

Emlen, D. J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society—B* 256:131–136.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215–223.

Emlen, D. J., I. A. Warren, A. Johns, I. Dworkin, and L. C. Lavine. 2012. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* 337:860–865.

Fitzpatrick, M. C., E. L. Preisser, A. Porter, J. Elkinton, and A. M. Ellison. 2012. Modeling range dynamics in heterogeneous landscapes: invasion of the hemlock woolly adelgid in eastern North America. *Ecological Applications* 22:472–486.

Forister, M. L., V. Novotny, A. K. Panorska, L. Baje, Y. Basset, and P. T. Butterill. 2015. The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences* 112:442–447.

Forister, M. L., and J. S. Wilson. 2013. The population ecology of novel plant-herbivore interactions. *Oikos* 122:657–666.

Formica, V. A., J. W. McGlothlin, C. W. Wood, M. E. Augat, R. E. Butterfield, M. E. Barnard, and E. D. Brodie III. 2011. Phenotypic assortment mediates the effect of social selection in a wild beetle population. *Evolution* 65:2771–81.

Fox, C. W. and R. G. Lalonde. 1993. Host confusion and the evolution of insect diet breadths. *Oikos* 67:577–581.

Fox, J. and S. Weisberg. 2011. An R Companion to Applied Regression, Second Edition. Thousand Oaks, CA: Sage. URL http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Fretwell, S. D. 1972. Populations in a Seasonal Environment. Princeton University Press, Princeton, NJ.

Galloway, L. F. 2005. Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist* 166:93–9.

Gilbertson, R. L. and L. Ryvarden. 1986. North American Polypores: Volume 1. Olso, Norway.

Gosden, T. P., and E. I. Svensson. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution* 62:845–56.

Grant, P. R., and B. R. Grant. 1995. Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* 49:241–251.

Hadfield, J. D. 2008. Estimating evolutionary parameters when viability selection is operating. *Proceedings of the Royal Society—B* 275:723–734.

Hanski, I. 1999. Metapopulation Ecology. Oxford University Press, New York, NY.

Holt, R. D. 1996. Adaptive evolution in source-sink environments: direct and indirect effects of density-dependence on niche evolution. *Oikos* 75:182-192.

Holt, R. D. 1997. On the evolutionary stability of sink populations. *Evolutionary Ecology* 11:723–731.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346--363.

Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics* 21:243–273.

Jones, O. and J. Wang. 2009. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10:551–555.

Jonsell, M., and G. Nordlander. 2004. Host selection patterns in insects breeding in bracket fungi. *Ecological Entomology* 29:697–705.

Kasumovic, M. M. 2013. The multidimensional consequences of the juvenile environment: Towards an integrative view of the adult phenotype. *Animal Behaviour* 85:1049–1059.

Kasumovic, M. M., M. J. Bruce, M. C. B. Andrade, and M. E. Herberstein. 2008. Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. *Evolution* 62:2316–25.

Kawecki, T. J. 2004. Ecological and evolutionary consequences of source-sink population dynamics. In Ecology, Genetics, and Evolution of Metapopulations, eds. I. Hanski and O. Gaggiotti. Elsevier Academic Press, Burlington, MA.

Kawecki, T. J. 2008. Adaptation to marginal habitats. *Annual Review of Ecology and Systematics* 39:321–342.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7:1225–1241.

Kingsolver, J. G., and R. Gomulkiewicz. 2003. Environmental variation and selection on performance curves. *Integrative and Comparative Biology* 43:470–7.

Kingsolver, J. G., S. E. Diamond, A. M. Siepielski, and S. M. Carlson. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evolutionary Ecology* 26:1101–1118.

Kokko, H., and D. J. Rankin. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society—B* 361:319–34.

Kruuk, L. E. B., J. Slate, J. M. Pemberton, S. Brotherstone, F. Guinness, and T. Clutton-Brock. 2002. Antler size in red deer: heritability and selection but no evolution. *Evolution* 56:1683–1695.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.

Liles, M. P. 1956. A study of the life history of the forked fungus beetle, *Bolitotherus cornutus* (Panzer) (Coleoptera: Tenebrionidae). *Ohio Journal of Science* 56:329–337.

Matsubayashi, K. W., I. Ohshima, and P. Nosil. 2010. Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata* 134:1–27.

Merrill, R. M., R. E. Naisbit, J. Mallet, and C. D. Jiggins. 2013. Ecological and genetic factors influencing the transition between host-use strategies in sympatric *Heliconius* butterflies. *Journal of Evolutionary Biology* 26:1959–67.

Mojica, J. P., and J. K. Kelly. 2010. Viability selection prior to trait expression is an essential component of natural selection. *Proceedings of the Royal Society—B* 277:2945–2950.

Nichols, J. D. 1992. Capture-recapture models. *BioScience* 42:94-102.

Price, T. and D. Schluter. 1991. On the low heritability of life-history traits. *Evolution* 45:853–861.

Promega. 2010. DNA IQ System—small sample casework protocol. Section 4C. DNA isolation from liquid samples. Promega Corporation, Madison, WI. Qiagen.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. *The American Naturalist* 132:652–661.

Qiagen. 2010. QIAGEN Multiplex PCR Handbook: amplification of microsatellite loci using multiplex PCR. Hilden, Germany.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Refsnider, J. M., and F. J. Janzen. 2010. Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics* 41:39–57.

Reichard, M., M. Ondracková, A. Bryjová, C. Smith, and J. Bryja. 2009. Breeding resource distribution affects selection gradients on male phenotypic traits: experimental study on lifetime reproductive success in the bitterling fish (*Rhodeus amarus*). *Evolution* 63:377–90.

Rosvall, K. A. 2011. Intrasexual competition in females: evidence for sexual selection? *Behavioral Ecology* 22:1131–1140.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variation by condition dependent traits. *Proceedings of the Royal Society—B* 263:1415–1421.

Siepielski, A. M., K. M. Gotanda, M. B. Morrissey, S. E. Diamond, J. D. DiBattista, and S. M. Carlson. 2013. The spatial patterns of directional phenotypic selection. *Ecology Letters*16:1382–92.

Simons, A. M. 2014. Playing smart vs. playing safe: the joint expression of phenotypic plasticity and potential bet hedging across and within thermal environments. *Journal of Evolutionary Biology* 27:1047–1056.

Slatkin, M. 1974. Hedging one's evolutionary bets. *Nature* 250:704–705.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47:3–14.

Van Asch, M., R. Julkunen-Tiito, and M. E. Visser. 2010. Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology. *Functional Ecology* 24:1103–1109.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535-538.

Wang, J. 2013. An improvement on the maximum likelihood reconstruction of pedigrees from marker data. *Heredity* 111:165-174.

Wickham, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, New York, NY. URL http://had.co.nz/ggplot2/book

With, K.A. 2004. Metapopulation dynamics: perspectives from landscape ecology. In Ecology, Genetics, and Evolution of Metapopulations, eds. I. Hanski and O. Gaggiotti. Elsevier Academic Press, Burlington, MA.

Wood, C. W., H. M. Donald, V. A. Formica, and E. D. Brodie III. 2013. Surprisingly little population genetic structure in a fungus-associated beetle despite its exploitation of multiple hosts. *Ecology and Evolution* 3:1484–94.

Wood, C. W., H. E. Marti, and E. D. Brodie III. 2014. The effect of ecological context and relatedness on larval cannibalism in a fungus-associated beetle. *Behavioral Ecology* 25:951–959.

Zikovitz, A. E., and A. F. Agrawal. 2013. The condition dependency of fitness in males and females: the fitness consequences of juvenile diet assessed in environments differing in key adult resources. *Evolution* 67:2849–2860.

Figures and Tables

Table 1. Abundance of the three fungi on Salt Pond Mountain near Mountain Lake Biological Station. The percentages of total logs or brackets are in parentheses.

	Fungus	N. logs		N. brackets	
Single infections	*F. fomentarius* (FF)	368	(59.6%)	8,787	(69.5%)
	G. applanatum (GA)	107	(17.3%)	2,105	(16.7%)
	G. tsugae (GT)	83	(13.5%)	1,745	(13.8%)
Co-infections	FF and GA	47	(7.6%)	-	
	FF and GT	8	(1.3%)	-	
	GA and GT	4	(0.6%)	-	

Table 2. Selection gradients on elytra length estimated on each of the three fungi from general linear models (left) and significance tests from generalized linear models (right). FF = *F. fomentarius*, GA = *G. applanatum*, and GT = *G. tsugae*. Bold values are significant ($\alpha = 0.05$).

		β						
		FF	GA	GT		Wald χ^2	df	P
Survival	*Males*	0.04	0.25	0.17	Elytra	0.004	1	0.948
					Fungus	3.371	2	0.185
					Elytra × Fungus	0.565	2	0.754
	Females	0.24	0.13	0.10	Elytra	2.567	1	0.109
					Fungus	1.769	2	0.413
					Elytra × Fungus	0.668	2	0.716
Reproductive success	*Males*	0.50	0.29	0.04	**Elytra**	**14.000**	**1**	**<0.001**
					Fungus	3.428	2	0.180
					Elytra × Fungus	5.700	2	0.058
	Females	0.29	0.57	0.13	**Elytra**	**16.704**	**1**	**<0.001**
					Fungus	**21.008**	**2**	**<0.001**
					Elytra × Fungus	**6.726**	**2**	**0.035**

Table 3. Results from general linear models testing for differences in the number of individuals of each life stage (larvae and adults) collected from bagged brackets of the three fungi. The top of the table ("All fungi") contains the results from models with all three fungi; the bottom of the table contains the results from models run separately for each fungus. Bold values are significant ($\alpha = 0.05$).

		Fall 2013			*Fall 2014*		
		F	df	P	F	df	P
All fungi	**Fungus**	**8.496**	**2, 218**	**<0.001**	2.382	1, 52	0.129
	Life stage	0.041	1, 218	0.839	0.778	1, 52	0.382
	Fungus × Life stage	1.769	2, 218	0.173	0.035	1, 52	0.852
F. fomentarius	Life stage	0.142	1, 50	0.708	3.079	1, 34	0.089
G. applanatum	Life stage	0.026	1, 16	0.873	0.330	1, 18	0.573
G. tsugae	**Life stage**	**13.286**	**1, 152**	**<0.001**	-	-	-

Table 4. Results from generalized linear models testing for effects of fungus species and collection time (June-July or July-August) on egg presence and egg density. Bold values are significant ($\alpha = 0.05$).

		F	df	P
Egg presence	**Fungus**	**7.915**	**2, 616**	**<0.001**
	Collection	7.720	1, 616	0.006
	Fungus × collection	0.658	2, 616	0.518
Egg density	**Fungus**	**33.589**	**2, 375**	**<0.001**
	Collection	**15.038**	**1, 375**	**<0.001**
	Fungus × collection	0.157	2, 375	0.855

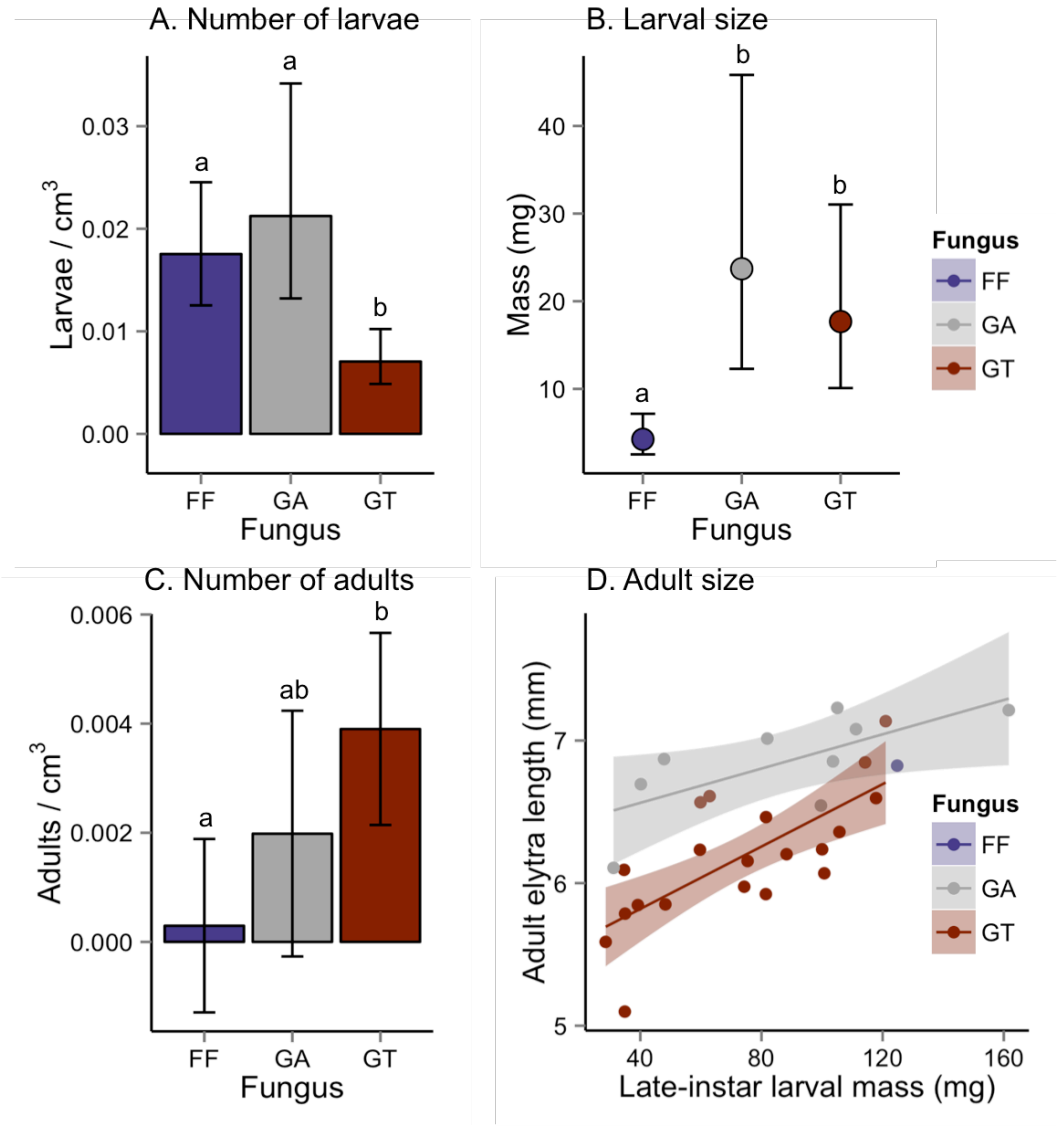

Figure 1. The number and size of larvae and adults from field-collected fungus brackets FF = *F. fomentarius*, GA = *G. applanatum*, and GT = *G. tsugae*. (A) Number of larvae per cm^3 of bracket sampled. (B) Larval mass. (C) Number of larvae that eclosed as adults within three months of collection per cm^3 of bracket sampled. (D) Body size of eclosing adults. Only one adult emerged from *F. fomentarius*, so the regression was only estimable for *G. applanatum* and *G. tsugae*. Comparisons with different letters are significantly different according to Tukey post-hoc tests. Error bars are 95% confidence intervals.

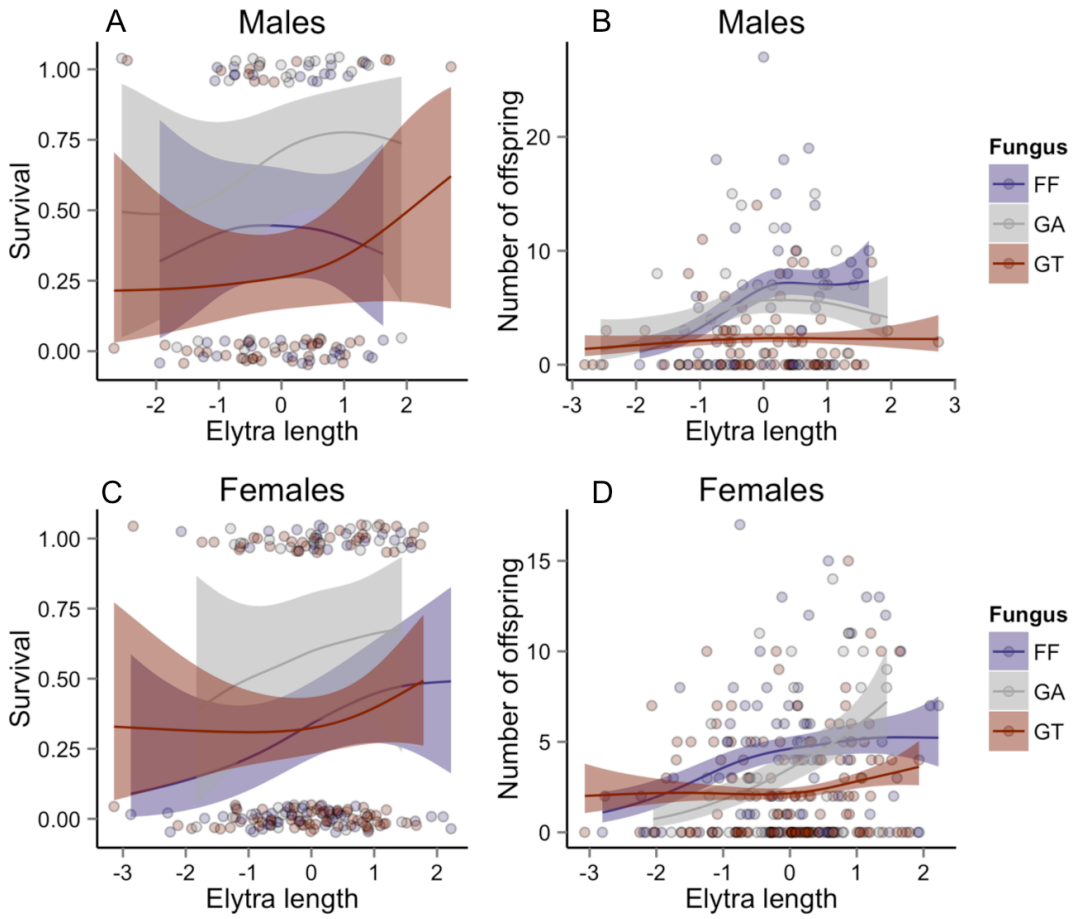

Figure 2. Selection via survival (A and C) and reproductive success (B and D) on adult body size in populations on the three fungi. FF = *F. fomentarius*, GA = *G. applanatum*, and GT = *G. tsugae*. Elytra length was standardized to zero mean and unit variance within each sex separately. Fitness functions for males are show in the top row and females, in the bottom row. Fitness functions were visualized with cubic splines. Bands are 95% confidence intervals.

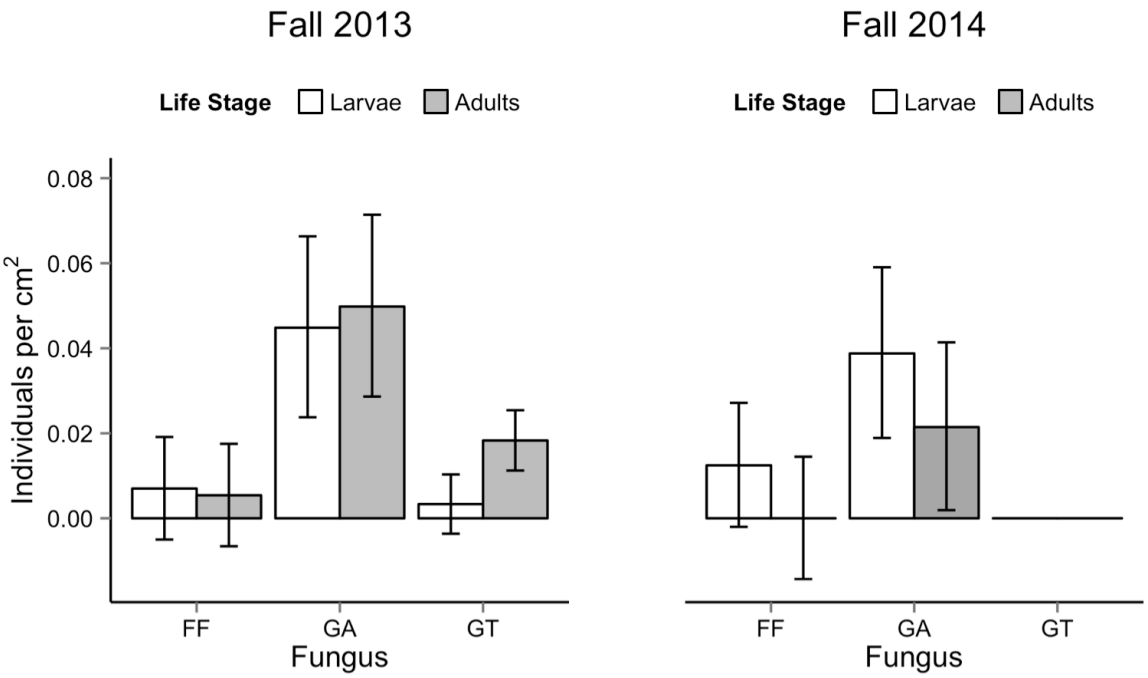

Figure 3. Number of larvae and adults collected from bagged brackets of the three fungi in two consecutive years. FF = *F. fomentarius*, GA = *G. applanatum*, and GT = *G. tsugae*. See Table 3 for statistics. Note that GT was not sampled in fall 2014 because all bagged brackets were collected in fall 2013. Error bars are 95% confidence intervals.

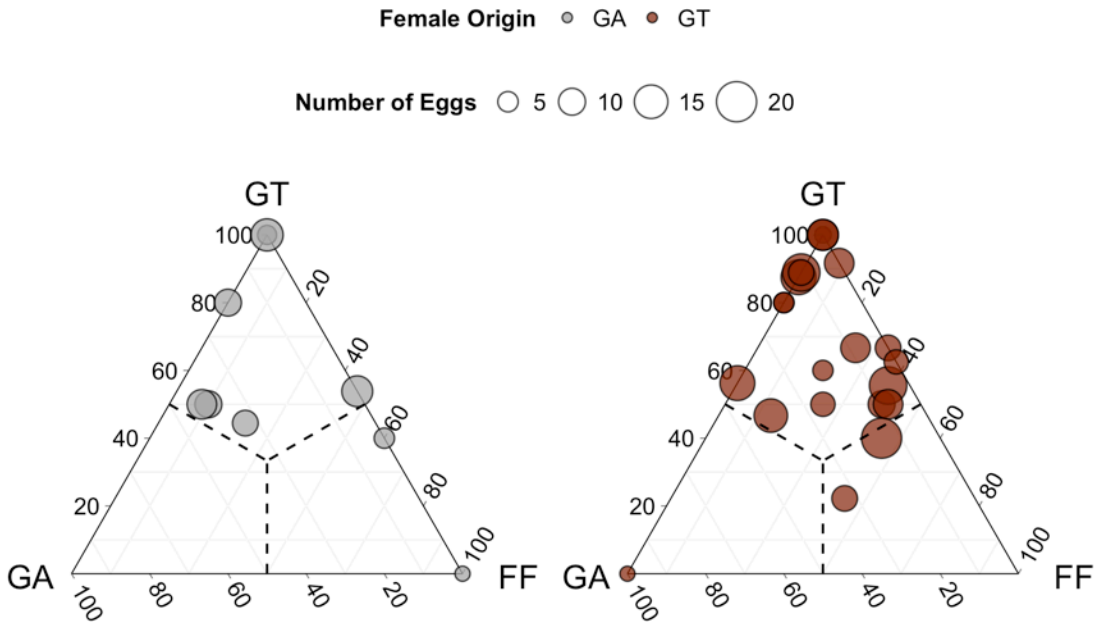

Figure 4. Ovipositing females prefer *G. tsugae* in lab choice trials (FF = *F. fomentarius*, GA = *G. applanatum*; GT = *G. tsugae*). Each dot is a female, and its location in the ternary plot (triangle) represents the proportion of eggs she laid on each of the three species. Females that developed in *G. applanatum* are shown in the left plot in gray, and females that developed in *G. tsugae* are shown in the right plot in red-orange.

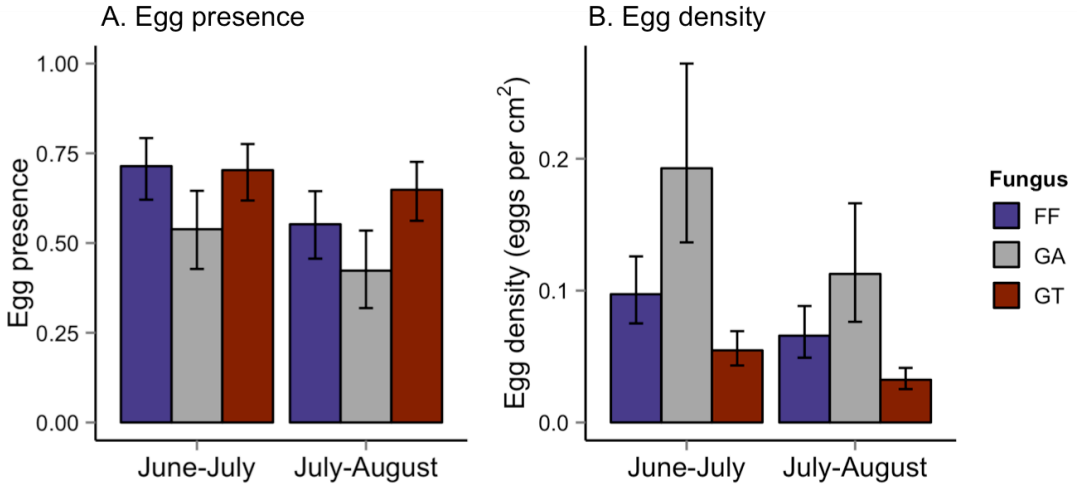

Figure 5. (A) Egg presence (the proportion of brackets with at least one egg) and (B) egg density (only brackets with at least one egg) on the three fungi in the field. FF = *F. fomentarius*, GA = *G. applanatum*, and GT = *G. tsugae*. Error bars are 95% confidence intervals.

Supplementary Tables

Supplementary Table 1. Microsatellite loci used for parentage assignment in this study. Effective number of alleles (eff. n. alleles), observed (H_O) and expected (H_E) heterozygosities, and deviations from Hardy-Weinberg were estimated in GenoDive (Miermans and Van Tienderen 2004). Null allele presence and frequency was estimated in MicroChecker using the "Brookfield 1" estimator (Van Oosterhout et al. 2004). F_{IS} is measure of inbreeding used to test whether genotype frequencies at a locus deviate from Hardy-Weinberg expectations, and P is the probability that the locus is significantly out of Hardy-Weinberg equilibrium.

Literature cited

Meirmans, P.G. and P.H. Van Tienderen. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4:792-794.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535-538.

Locus	N. alleles	Eff. n. alleles	H_O	H_E	F_{IS}	P	Null allele?	Null allele freq.
Boco049	4	2.080	0.481	0.520	0.076	**0.043**	no	0.007
Boco030	8	2.772	0.615	0.641	0.040	0.188	no	0.004
Boco068	4	1.858	0.448	0.463	0.033	0.316	no	-0.007
Boco065	13	4.332	0.759	0.771	0.016	0.302	no	0.004
Boco006	4	2.118	0.520	0.529	0.016	0.396	no	-0.013
Boco128	3	1.666	0.332	0.401	0.172	**0.001**	yes	0.037
Boco084	10	4.905	0.765	0.798	0.041	0.096	no	0.032
Boco004	6	2.496	0.387	0.601	0.357	**0.001**	yes	0.124
Boco113	3	1.301	0.164	0.232	0.291	**0.001**	yes	0.038
Boco286	8	2.371	0.320	0.580	0.448	**0.001**	yes	0.178
Boco034	6	2.387	0.567	0.582	0.026	0.299	no	-0.016
Boco167	3	1.882	0.490	0.469	-0.045	0.217	no	-0.022
Boco082	3	1.317	0.238	0.241	0.013	0.441	no	-0.003
Boco213	5	1.872	0.427	0.467	0.085	0.056	no	0.020
Boco172	3	1.331	0.264	0.249	-0.061	0.202	no	-0.022
Boco061	6	1.742	0.417	0.427	0.022	0.345	no	-0.001
Boco185	4	2.016	0.453	0.505	0.103	0.060	no	-0.003
Boco150	6	2.426	0.581	0.589	0.013	0.387	no	0.017
Boco263	3	2.055	0.243	0.515	0.528	**0.001**	yes	0.17
Boco270	8	3.612	0.708	0.725	0.023	0.307	no	-0.005
Boco223	8	4.948	0.498	0.800	0.378	**0.001**	yes	0.177
Boco045	5	2.442	0.294	0.592	0.504	**0.001**	yes	0.172
Boco148	3	1.309	0.233	0.236	0.015	0.409	no	0.008
Boco080	4	1.345	0.216	0.257	0.161	**0.009**	yes	0.034
Boco278	5	2.262	0.279	0.560	0.502	**0.001**	yes	0.150
Boco117	9	4.144	0.792	0.760	-0.041	0.138	no	-0.001
Boco017	7	2.497	0.494	0.602	0.178	**0.001**	yes	0.100
Boco092	4	1.320	0.236	0.243	0.028	0.337	no	0.004

CHAPTER THREE:

The effect of ecological context and relatedness on larval cannibalism in a fungus-associated beetle[3]

[3] As published: Wood, C. W., H. E. Marti, and E. D. Brodie III. 2014. The effect of ecological context and relatedness on larval cannibalism in a fungus-associated beetle. *Behavioral Ecology* 25:951–959.

Abstract

The fitness consequences of extreme competitive interactions such as cannibalism are often governed by the environment, because the costs and benefits of such behaviors depend on the ecological and social context of the participants. However, most studies of context-dependent cannibalism are conducted under extreme circumstances or examine only a single environmental context, conditions that are unlikely to exist in natural populations. In this study we tested the effect of multiple environmental contexts on the frequency of cannibalism in forked fungus beetle larvae (*Bolitotherus cornutus*), which develop in three different fungus species. We paired larvae in laboratory trials and measured the effect of (1) ecological context (the three fungi) and (2) the relatedness of the paired larvae on the frequency of cannibalism. We found a strong effect of ecological context on cannibalism: larvae in one fungus cannibalized nearly twice as often as larvae in the other two. We did not detect an effect of relatedness on cannibalism in the one species of fungus in which trials were conducted. Cannibalism conferred benefits in the form of accelerated growth rates in all measured traits relative to non-cannibals. However, contrary to most studies, cannibalism was most common in the highest-quality fungus, contradicting the hypothesis that cannibalism occurs in poor environments to compensate for resource deficiencies. We discuss alternative mechanisms that may drive the ecological context-dependence of cannibalism in *B. cornutus*, and emphasize the importance of studying context-dependent behavior in naturally occurring environments.

Introduction

The environment in which a behavior is expressed affects its associated costs and benefits. Consequently, behaviors as diverse as foraging activity (MacArthur and Pianka 1966), reproductive investment (Badyaev and Duckworth 2003), altruism (Hamilton 1964), mate choice (Gasparini et al. 2013), and aggression (Tanner and Adler 2009) depend on the ecological or social context in which they occur. Identifying the axes of environmental variation that drive differences in behavior, as well as the phenotypic and fitness consequences of such variable behaviors, is necessary to understand the forces that govern behavioral evolution in heterogeneous environments.

Cannibalism provides an excellent opportunity to explore the context-dependence of behavior. It is taxonomically widespread, pervasive in natural populations even in the absence of stress, and has profound fitness consequences that often depend on the environment in which it occurs (Fox 1975, Rudolf et al. 2010). The propensity for cannibalism is heritable (reviewed in Polis 1981) and has been shown to respond to selection in laboratory populations (Stevens 1989), suggesting that differences in the frequency of cannibalism among environments are potentially adaptive. The conflicting benefits and costs of cannibalism are well documented. Cannibals often develop faster (Chapman et al. 1999, Via 1999), attain larger body size and increased fecundity at maturity, and experience lower mortality than their non-cannibalistic counterparts (Church and Sherratt 1996, Vijendravarma et al. 2013), either as a direct result of the nutritional benefits of cannibalism or as an indirect consequence of reduced competition for otherwise shared resources (Fox 1975). These benefits are countered by the costs of injury due to reciprocal violence or disease transmission, and the loss of inclusive fitness

if cannibals consume relatives such as offspring or siblings (Polis 1981, Collie et al 2013).

The ecological context in which cannibalism occurs and the relatedness of interacting individuals both influence the relative magnitude of these costs and benefits. Most research on the effect of ecological context on cannibalism focuses on food-restricted circumstances. Cannibalism tends to be more common under resource-deficient conditions (Fox 1975, Polis 1981), in which the caloric benefits of cannibalism outweigh the risks to the cannibal. For example, cannibalistic larvae of the fall armyworm developed faster than non-cannibals only when raised in a limited food environment (Chapman et al. 1999). Similarly, neotropical mosquitos that cannibalized other larvae survived longer than those that did not, but only in low-food conditions (Church and Sherratt 1996). These results accord with experimental demonstrations that the propensity for risky foraging behavior increases when organisms are operating at or below the break-even point of their energy budgets (Caraco et al. 1990, Cartar and Dill 1990). Very little data exist on the response of cannibalism to more complex ecological contexts (e.g., alternate food resources); in the few studies that have examined a realistic range of ecological contexts, it is often difficult to disentangle direct effects on cannibalism from other plastic responses to the same environmental differences. For example, because the diet differences that affect cannibalism propensity in spadefoot toad tadpoles also trigger sweeping morphological changes (Pfennig and Murphy 2000), it difficult to isolate the effects of food resource on cannibalism alone.

The costs of cannibalistic behavior are also influenced by the relatedness of cannibals to their victims. Inclusive fitness costs can constrain the evolution of

cannibalism (Rudolf et al. 2010) because in kin-structured populations individuals engaging in cannibalism are likely to eliminate a relative (Pfennig 1997). Kin recognition and avoidance mitigate this cost in many species (Parsons et al. 2013). Some exhibit a reduced propensity for cannibalism when paired with relatives (Joseph et al. 1999), whereas others preferentially associate with non-relatives (Pfennig 1999). A canonical example of the latter is the tadpoles of spadefoot toads (*Spea*), which exhibit diet-induced behavioral and morphological polymorphism. The non-cannibalistic omnivores school with siblings, while the carnivores, which are more cannibalistic, preferentially associate with non-relatives, ameliorating the inclusive fitness cost that cannibalism would otherwise incur (Pfennig 1992).

Despite the wide-ranging phenotypic and fitness consequences of context-dependent cannibalism, however, the effects of ecological context and relatedness on cannibalism are rarely explored in the same system under a realistic range of ecological environments. The paucity of data on the effects of multiple contexts on cannibalism constrains our ability to understand its dynamics in natural populations that inhabit heterogeneous environments. If both social and ecological factors independently affect cannibalism, then in combination they may oppose or reinforce each other, resulting in the consistent underestimation of cannibalism risk in some contexts, and overestimation in others. This limitation is compounded by the fact that studies of the ecological context-dependence of cannibalism have been confined primarily to oversimplified or extreme circumstances (e.g., food limitation), leaving the natural range of developmental environments largely unexplored (but see Laycock et al. 2006). If ecological environments that natural populations experience influence the frequency and

consequences of cannibalism, then the ecological and evolutionary consequences of cannibalistic behavior may be more widespread than we currently appreciate.

In this study we examined the effect of ecological context (food resource) and relatedness on the frequency of larval cannibalism in *Bolitotherus cornutus*, a beetle that spends its entire life cycle on one of three fungus species. Because larvae are confined to a discrete ecological environment—a fungus fruiting body—throughout development and are unable to disperse, larval competition is likely intense. Moreover, the risk of cannibalizing siblings in the same fruiting body may alter costs incurred by cannibalistic larvae.

We tested whether the fungal host affected the propensity of *B. cornutus* larvae to cannibalize, and whether larvae avoided cannibalizing siblings. To assess differences in environmental quality among the fungi, we first reared larvae individually in each of the three fungi and measured larval growth. We then paired these lab-reared larvae and scored the frequency of cannibalism in (1) the three fungus species and (2) between sibling and non-sibling larval pairs. Finally, to assess the benefits of cannibalism in the three fungi, we compared the growth rate of cannibals to non-cannibals and measured the risk of non-cannibalistic mortality in the three fungi. We hypothesized that cannibalism would be most common in the lowest-quality fungus and in non-sibling pairs, and that growth benefits of cannibalism would be most pronounced in the lowest-quality fungus.

Methods

Study system

Forked fungus beetles (*Bolitotherus cornutus*; Coleoptera: Tenebrionidae) develop in the fruiting bodies ("brackets") of three wood-decaying fungi: *Fomes fomentarius*, *Ganoderma applanatum*, and *Ganoderma tsugae*. Females lay eggs singly on the surface of the brackets, and the larvae burrow into the brackets and remain inside with other conspecific larvae and pupae until they eclose as adults several months to a year later (Liles 1956). Preliminary trials under laboratory conditions demonstrated that *B. cornutus* larvae engage in cannibalism (Wood and Marti, unpublished data). Cannibalism likely occurs only between larvae; because the eggs are laid on the bracket surface they are protected from foraging larvae, and their hard frass-like covering protects them from foraging adults. Growth effects associated with cannibalism in the larval stage may have broad fitness consequences later in life: in insects, larger females tend to be more fecund (Bonduriansky 2001), and both body size and thoracic horn length positively affect fitness in *B. cornutus* males (Conner 1988, Formica et al. 2011).

The three host fungi provide different social and ecological environments for developing larvae in natural populations. Dissection of field-collected brackets indicates that the potential for larval competition differs among the three fungi: in brackets that contained at least one larva, larval density was higher in *G. applanatum* (10.07 larvae per bracket, which translates to 0.034 ± 0.031 larvae/cm^3) than in either *G. tsugae* (3.95 larvae per bracket, or 0.010 ± 0.010 larvae/cm^3) or *F. fomentarius* (2.96 larvae per bracket, or 0.023 ± 0.023 larvae/cm^3; del Sol and Wood, unpublished data). The fungi appear to be characterized by distinct chemical compositions, as adult beetles collected

from *G. applanatum* and *F. fomentarius* can be distinguished by the chemistry of their defensive secretions (Holliday et al. 2009). Moreover, adults discriminate between the fungi in lab-based feeding choice trials (Heatwole and Heatwole 1968).

Ecological environment and larval growth

One hundred and twenty-seven mating pairs of *B. cornutus* were established from collections on and around Salt Pond Mountain in Giles County, Virginia during the summers of 2012 and 2013. Adult pairs were maintained on *G. tsugae* in an incubator on 16:8 light:dark cycle at 23°C. Water and fungus were provided *ad libitum*. Pairs were checked for eggs 1-2 times per week and all eggs were collected. Collected eggs were randomly assigned to one of the three fungi, and each egg was transferred to a 1.5 mL tube filled with pulverized fungus of its assigned species. Several brackets of each species were dried, frozen, and ground in an industrial blender (Blendtec, Orem, UT) to ensure resource homogeneity within each fungus species. These eggs were housed in the same conditions as adults, and water was added and mold removed when necessary. Eggs were checked approximately every other day and hatch date was recorded when an empty egg case was found. Approximately 10 days after a larva hatched it was weighed to the nearest 0.01 mg using an Ax205 DeltaRange balance (Mettler-Toledo, Columbus, OH).

All statistical analyses were performed in R 3.0.1 (R Core Team 2013). We used deviation coding ("contr.sum" in R) for unordered categorical variables. To test for an effect of the fungus environment on larval growth, we compared the mass of 10-day-old larvae reared individually in the three fungi. Our model (executed using the function *lmer* in the *lme4* package) included log-transformed larval mass as the dependent variable,

fungus as a fixed effect, and family and the family × fungus interaction as random effects. We log-transformed larval mass because the residuals of untransformed data were non-normally distributed and violated the assumption of homogeneity of variance. We tested the significance of the fungus effect with a likelihood-ratio test using the *drop1* function. To test for the presence of a significant genotype-by-environment interaction for growth, we used a likelihood-ratio test to compare the full model to a reduced model in which the family × fungus interaction was omitted.

Ecological environment and cannibalism

To test the effect of the fungus environment on the frequency of cannibalism, larvae from the above experiment were then assigned to one of six treatment groups. Three of these groups were cannibalism trials, in which each larva was paired with one other unrelated larva (from a different mating pair of adults) that had been reared on the same fungus (N=35 larval pairs for each fungus). The remaining three groups were controls to allow us to measure growth benefits of cannibalism and the rate of non-cannibalistic mortality. These larvae were housed unpaired on the fungus on which they had been reared (N=35 for *F. fomentarius* and *G. applanatum*; N=38 for *G. tsugae*). The paired larvae in the cannibalism trials were size-matched within 1 mg whenever possible because size asymmetries between competitors can determine the outcome of cannibalistic interactions (Petersen et al. 2010). We chose to size-match larvae to avoid confounding the effects of size differences and fungus environment on cannibalism frequency. Because the fungi had a strong effect on the mean and variance in larval size (see Results), if we had not size-matched the larvae the average size difference would

have been much larger in *G. tsugae* (in which variance in larval size was large) than in *F. fomentarius* (in which variance in larval size was small), rendering it difficult to isolate the influence of the fungus environment on cannibalism.

All trials were housed in clear 1.5 mL tubes with a hole in each lid for ventilation, and water was added to each tube as needed throughout the experiment. Tubes with paired larvae were filled with 0.050-0.070 g of dried ground fungus, lightly packed into 0.5 mL volume. Control tubes were filled with 0.025-0.035 g of dried ground fungus, lightly packed into 0.25 mL volume. Larval densities in the experiment were equal across fungus types, and higher than those generally observed in the field to increase the potential for interactions among larvae.

Paired trials were checked daily for 14 days, or until cannibalism occurred, or until both larvae died from other causes. Although the larval stage can last a year in *B. cornutus* (Liles 1956), we chose this experimental duration because most cannibalism occurred in the first two to three days in all fungi (see Results), suggesting that the initial encounter was disproportionately responsible for determining whether a pair would cannibalize. We scored a trial as cannibalism whenever we found one larva that was either partially or completely consumed. The remains of cannibalized larvae could be distinguished from molted exoskeletons by the presence of soft tissue inside the exoskeleton or head capsule of the cannibalized larva. The severity of cannibalism ranged from a single wound to consumption of nearly the entire larva. Larvae that were found dead but not wounded were scored as non-cannibalistic deaths. Control groups were checked daily for the first 3 days to replicate the disturbance to the paired larvae, and

after the third day were measured on the same schedule as the larvae that cannibalized their partners (see Cannibalism and Growth Rate section below).

We used a generalized linear model (the *lmer* function in R) with a binomial error distribution and logit link to test for differences in the probability of cannibalism among fungi. In this model, trial outcome was the dependent variable and fungus species was an independent variable. To control for any effect of initial larval size or the size difference between paired larvae on the probability of cannibalism, we included the mass difference between larvae and average initial mass of each pair as covariates. We performed a likelihood ratio chi-square test using the *drop1* function to test for significance of the fungus effect. We used a Kruskal-Wallis rank sum test to test for an effect of the fungus environment on the latency to cannibalism (measured in days) because the number of days until cannibalism occurred was non-normally distributed. We used Fisher's exact tests to test for differences in the frequency of non-cannibalistic death among fungi, after excluding the trials in which cannibalism had occurred. We performed these tests separately for paired and control larvae, which were unpaired and raised individually. We tested for differences in the latency to non-cannibalistic death among fungi using Kruskal-Wallis rank sum tests.

Relatedness and cannibalism

To test the effect of relatedness on cannibalism, larvae were paired with a sibling (a larva from the same mating pair of adults; N=35 larval pairs). These trials were conducted in *G. tsugae*. The frequency of cannibalism in this group was compared to the frequency of cannibalism in the pairs of unrelated larvae raised in *G. tsugae* from the

ecological environment experiment (N=35 pairs; see Ecological Environment and Cannibalism section above). Sibling trials were conducted only in *G. tsugae* because logistical constraints prevented us from raising sufficient numbers of larvae in each of the three fungi. The sibling pairs may have included some half-siblings because wild-caught females could have previously mated. These trials were conducted under the same experimental protocol as the ecological environment trials described above.

We used a generalized linear model with a binomial error distribution and logit link to test for differences in the probability of cannibalism between sibling and non-sibling pairs; this model was identical to the model used to test for differences among fungi, except that a relatedness fixed effect replaced the fungus fixed effect. We used Kruskal-Wallis rank sum tests to test for differences in the latency to cannibalism between sibling and non-sibling pairs. We used Fisher's exact tests to test for differences in the frequency of non-cannibalistic death between sibling and non-sibling pairs (after excluding the trials in which cannibalism had occurred), and tested for differences in the latency to non-cannibalistic death using Kruskal-Wallis rank sum tests.

Cannibalism and growth rate

To test for growth benefits of cannibalism, we examined whether cannibals (larvae that consumed one other larva) grew significantly faster than control larvae, which never had the opportunity to cannibalize. This analysis included all paired larvae that cannibalized, including the sibling pairs raised on *G. tsugae.* Control larvae were weighed and an image was taken with a microscope-mounted camera at the start of the experiment and again 7-12 days after they were placed in their control tubes; cannibals

were weighed and an image was taken on the day that they cannibalized, and again 7-12 days later. The variance in the number of days between initial and final measurements was due to the fact that imaging the larvae was time-consuming, so we were unable to process all larvae exactly 10 days after they cannibalized. However, this is unlikely to have affected our results because we controlled for the number of days between the initial and final measurements in our statistical analysis (see below), and because the number of days between initial and final measurements was random with respect to fungus treatment.

We measured three traits from the microscope images using ImageJ (Abramoff et al. 2004): larval length, head capsule width, and mandible length. Length was measured from the base of the head capsule to the end of the larva, using a segmented line to incorporate curvature. Head capsule width was measured as the straight-line distance between the antennal insertions, and mandible width was measured as the straight-line distance from the base of the mandible to the tip. Because sclerotized structures like the head capsule and mandibles grow most rapidly following progression from one developmental stage ("instar") to the next (Daly 1985), accelerated growth in these traits may indicate an effect of cannibalism on the rate of larval development.

We calculated growth rates for the four traits (mass, length, head capsule width, and mandible length) for both controls and cannibals by subtracting each larva's initial measurement from its final measurement and dividing by the number of days separating the two measurements. We used ANOVA to test for an effect of the fungus environment, cannibalism, and their interaction on larval growth, and ran a separate model for each of the four traits. In these models growth rate was the dependent variable and fungus,

cannibalism, the fungus × cannibalism interaction, and initial size were included as fixed effects. We conducted significance tests for all fixed effects using the *Anova* function in the *car* package.

Results

Ecological environment and larval growth

The fungus environment had a significant effect on the mass of 10-day-old larvae (Figure 1; df = 2, likelihood-ratio $\chi^2 = 164.430$, $P < 0.001$). This effect was driven by larvae raised on *F. fomentarius*, which were smaller than those reared on either *G. applanatum* or *G. tsugae*. We found no evidence for a genotype-by-environment interaction for larval growth (family × fungus interaction: df = 1, likelihood-ratio $\chi^2 = 0.321$, $P = 0.571$).

Ecological environment and cannibalism

The probability of cannibalism differed significantly among fungus environments, and was most common in *G. applanatum* (Figure 2; Table 1), but was not affected by the average initial size of the paired larvae or by their size difference (Table 1). There was no significant effect of the fungus environment on the number of days until cannibalism occurred, although there was a trend towards a longer latency to cannibalism in *G. applanatum* (df = 2, Kruskal-Wallis $\chi^2 = 5.010$, $P = 0.082$). This pattern was driven by larvae in *G. applanatum*, which continued to cannibalize for several days after the start of the experiment, while *F. fomentarius* and *G. tsugae* rarely cannibalized after the second

day. In all three fungi the highest rates of cannibalism occurred in the first two days of the experiment. The fact that most cannibalism took place at the start of the experiment suggests that larvae assess whether to cannibalize the first time they encounter another larva (and that in the small test arenas the time until first encounter was short). The rapid decline in cannibalism after the first few days of the experiment further suggests that a larva that does not cannibalize after the first encounter is unlikely to cannibalize in the future.

The fungus environment affected the frequency of non-cannibalistic mortality in the paired trials in which cannibalism did not occur: mortality was high in *G. tsugae* and low in *G. applanatum* (Figure 2; Fisher's exact test, P = 0.029). Mortality rates in the control larvae were not significantly different among the three fungi (Figure 2; Fisher's exact test, P = 0.103), although more control larvae died in *G. tsugae*, similar to the pattern observed among paired larvae. There was no effect of the fungus environment on latency to death (df = 2, Kruskal-Wallis $\chi^2 = 1.425$, P = 0.491).

Relatedness and cannibalism

Relatedness did not affect the probability of cannibalism (Figure 2; Table 1). The size difference of the paired larvae also had no effect on the probability of cannibalism, although the probability of cannibalism increased with the pair's average initial size (Table 1). There was no difference in the latency to cannibalism between sibling and non-sibling pairs (df = 1, Kruskal-Wallis $\chi^2 = 0.488$, P = 0.485), as would be expected if siblings refrained from cannibalism longer than non-siblings.

Neither non-cannibalistic mortality (Fisher's exact test, P = 0.243) nor the latency to death (df = 1, Kruskal-Wallis $\chi^2 = 0.440$, P = 0.501) differed between the sibling and non-sibling pairs.

Cannibalism and growth rate

Cannibals grew at a faster rate than control larvae for all four measured traits (Figure 3; Table 2). Growth rates in all traits were significantly different among fungi, but we found no strong evidence for a cannibalism × fungus interaction for growth rate (Table 2). The cannibalism × fungus interaction was not significant for mass, head capsule width, or mandible length, and was only marginally significant for larval length.

Discussion

Cannibalism was strongly influenced by ecological context in *B. cornutus*, and conferred measurable benefits in the form of accelerated growth rate in cannibals. Cannibalism was nearly twice as common in *G. applanatum* as in the other two fungus species. Contrary to most studies on cannibalism across resource environments (King and Dawson 1972, Polis 1981, Wolcott and Wolcott 1984), the highest rate of cannibalism occurred in the highest-quality resource. We did not detect an effect of relatedness on cannibalism in this study, but these trials were conducted only in the fungus in which cannibalism was rare. If the propensity to cannibalize siblings depends on the ecological context, results obtained in the other two fungi (where cannibalism was more common) may be very different from those obtained in *G. tsugae*, with complex evolutionary ramifications. Finally, because cannibalism was not universal under any of these

experimental conditions—and occurred only in the minority of trials in *F. fomentarius* and *G. tsugae*—there may be unmeasured costs of cannibalism in this system that limit its prevalence.

Our results underscore the importance of incorporating the ecological complexity characteristic of natural environments into experimental studies of context dependence. Implicit in the use of realistic ecological treatments is the recognition that many variables that may simultaneously contribute to context dependence of behavior. Because natural environments differ in many dimensions, focusing the effect on a single isolated variable (e.g., calorie content) has the potential to lead to inaccurate predictions when extrapolated to natural populations. Therefore, comparing complex ecological environments is crucial to an understanding of the dynamics of context-dependent behaviors like cannibalism in heterogeneous environments.

Ecological environment and larval growth

The three fungi appear to be substantially different in quality (Figure 1). The effect of the fungus environment on mass is strong—already pronounced in 10-day-old larvae—and complex. *Fomes fomentarius* is clearly the lowest quality environment. The average mass of 10-day-old larvae reared in the two *Ganoderma* species was similar, but variance in both mass and growth rate was higher in *G. tsugae* than *G. applanatum* (Figures 1 and 3). It is unlikely that this amplified variance reflects higher resource heterogeneity in *G. tsugae* because the fungus was blended and homogenized prior to the experiment to minimize within-fungus species variation. Instead, *B. cornutus* larvae may vary in their ability to metabolize *G. tsugae* to a larger degree than the other two fungal

hosts. We found no evidence for a genotype-by-environment interaction for larval size; all families performed poorly on *F. fomentarius*.

These observed differences in environmental "quality" likely encompass three distinct types of food stress: food limitation (i.e., caloric restriction), nutrient limitation (i.e., shortage of an essential nutrient), and food toxicity (i.e., the abundance of defensive compounds). Variance in the abundance of defensive compounds may in fact drive the strong effect of the fungus environment on larval growth in *B. cornutus*, rather than caloric or nutrient content differences among the three fungi. Wood-decaying bracket fungi are well defended by chemical volatiles (Jonsell and Nordlander 2004), and negative effects of these compounds may inhibit larval growth in *F. fomentarius* or contribute to the high variance observed in *G. tsugae*. Furthermore, differences in nutritional quality may reflect differences in the invertebrate prey communities within each fungus species, which would have been homogenized along with the brackets in our experiment. The abundance of other invertebrate species appeared to be especially low in *F. fomentarius* relative to the other species in a sample of field-collected brackets (del Sol and Wood, unpublished data).

Ecological environment and cannibalism

Although we documented strong differences in resource quality among the ecological environments, and a large effect of the ecological environment on the frequency of cannibalism, no clear relationship existed between the quality of the ecological environment and the frequency of cannibalism. Cannibalism was twice as common in *G applanatum* as the other two species, contradicting our original hypothesis

that the lowest-quality environment—*F. fomentarius*—would induce the highest rate of cannibalism. Cannibalism conferred large benefits in the form of accelerated growth rates in all traits, but this effect was not significantly different among environments, contrary to our hypothesis that cannibalism would confer the greatest benefit in the lowest-quality environment. This pattern of context-dependence may function to compound, rather than minimize, phenotypic differences between individuals developing in low- and high-quality environments.

Three alternative hypotheses may explain why the pattern we observed was opposite the pattern seen in most other studies (e.g., King and Dawson 1972, Polis 1981, Wolcott and Wolcott 1984). First, the high rate of cannibalism in the "best" environment may reflect the ability of individuals in high-quality environments to maintain high activity or aggression levels. If individuals across all environments are equally likely to cannibalize when they encounter another larva, but food- or nutrient-limited individuals are less active, encounter rates—and, as a result, cannibalism—will be elevated in high-quality environments. This pattern has been documented in other cannibalistic systems (e.g., Mayntz and Toft 2006). However, this hypothesis does not account for differences in the rate of cannibalism in the two *Ganoderma* species, in which larval growth rates were similar.

Second, the deficiencies in the low-quality environment (*F. fomentarius*) in our study may be indicative of a type of food stress that cannot be mitigated through cannibalism. Naturally occurring food resources such as fungi likely differ in multiple dimensions, including calorie content, the availability of essential nutrients, and the abundance of defensive compounds. As a result, it is unsurprising that the pattern of

context-dependent cannibalism documented in this study is more complex than that in studies that confined to simplified or extreme circumstances that only incorporate a single dimension of food stress (e.g., starvation). If cannibalism does not directly compensate for the deficiencies of a low-quality ecological environment, then in these circumstances the costs of cannibalism may outweigh its benefits and it should remain infrequent. For example, if the observed differences in larval growth in the three fungal environments are due to defensive compounds and not nutrient limitation, then the nutrients gained from cannibalism may not confer any benefit in poor environments like *F. fomentarius*. That the growth benefits of cannibalism were not larger in *F. fomentarius* than in the two *Ganoderma* species suggests that whatever benefits are obtained from cannibalism are not directly ameliorating the main source of stress in that environment. However, this hypothesis fails to address the difference in cannibalism frequencies in the two *Ganoderma* environments.

Third, the rate of cannibalism may be an indirect consequence of the resource environment, responding to an environmental variable that covaries with it in wild populations. One such variable that may underlie the pattern reported here is conspecific density, which triggers cannibalism in other taxa (Fox 1975), and is one aspect of the social environment that distinguishes *G. applanatum* from the other two fungi. Field observations show that the density of *B. cornutus* eggs (E. Wice, unpublished data) and larvae (J. del Sol, unpublished data) is highest on *G. applanatum* brackets. If these observations reflect consistent differences in density among the three environments, *G. applanatum* may trigger cannibalism in developing larvae as a strategy to eliminate abundant competitors. However, because density did not differ among the three fungal

environments in our experiment, this hypothesis requires that larvae respond to a cue in the fungus itself by facultatively increasing their propensity for cannibalism in *G. applanatum*, rather than responding to direct indicators of larval density (e.g., vibrational cues).

Finally, it is important to note that the effect of fungus environment on the frequency of cannibalism is not independent of other sources of mortality that vary among fungi (Figure 2). Non-cannibalistic mortality was not attributable to physical injury because we scored all wounded larvae as cannibalism, nor is variation in non-cannibalistic mortality among environments an artifact of size-matching the larvae, which would have affected all environments equally. Instead, differences among fungus environments in the relative frequency of cannibalistic and non-cannibalistic mortality may reflect context-dependent responses to stress imposed by conspecifics. That is to say, in some environments (e.g., *G. applanatum*), larvae respond to elevated stress by attacking and cannibalizing conspecifics, while in others (e.g., *G. tsugae*), larvae are equally stressed—and often die as a result—but do not respond by cannibalizing their competitor.

Relatedness and cannibalism

Cannibalistic *B. cornutus* larvae displayed no kin-avoidance behavior, a surprising result given that kin competition tends to be most common when juveniles are confined to discrete, ephemeral resources (Resetarits 1996). Several explanations may account for this result. First, kin-avoidance behavior may only manifest when the risk of cannibalism is high. Our experiment assessed the effect of relatedness on cannibalism in

an environment in which cannibalism turned out to be rare (*G. tsugae*). If the effect of relatedness on cannibalism depends on frequency of cannibalism in a given ecological environment—for example, if larvae avoid relatives only when cannibalism risk is very high—then the dynamics of cannibalism in natural populations, in which larvae may encounter siblings in all fungus species, may be significantly more complex.

Second, larval *B. cornutus* may not be capable of kin recognition, in which case cannibals would be unable to discriminate between related and unrelated larvae. Instead, kin avoidance may occur at the maternal level during oviposition site selection. Because *B. cornutus* females lay eggs singly rather than in clutches, females may spatially separate offspring to minimize kin competition. This pattern could reduce selection for kin recognition in the larval stage because larvae would not likely encounter relatives during development.

Third, the benefits of cannibalism may outweigh the costs of consuming a sibling. We documented strong benefits of cannibalism in *B. cornutus* larvae. The rate of non-cannibalistic mortality was significantly higher in the paired trials than in the controls (Figure 2), suggesting that high larval densities are stressful. Cannibalism may be a mechanism to alleviate that stress, counteracting the inclusive fitness cost of consuming a relative (Collie et al. 2013). Cannibals experienced accelerated growth (Figure 3), which is likely doubly beneficial due to direct advantages of larger body size and decreased vulnerability to conspecific predation. It is unlikely that growth rate differences reflect differences in food availability because the control larvae, which were raised in less food than the pairs, did not exhaust available food by the end of the experiment. Accelerated head capsule and mandible (traits that grow primarily between instars) growth in

cannibals indicates that cannibalism also shortens larval development time. Such elevated growth rates allow larvae to quickly escape the smallest size classes, when they are likely most vulnerable to cannibalism from larger larvae. This may be especially beneficial in *B. cornutus* because larvae of many age and size classes are often found in the same bracket (C. Wood, unpublished data). Taken together, these data suggest that strong benefits of cannibalism could offset the inclusive fitness cost of eliminating a sibling.

Consequences of context-dependent cannibalism in heterogeneous environments

The benefits of cannibalism that we detected in the form of accelerated growth rates, in combination with differences in the frequency of cannibalism among fungi, may have broad ramifications in heterogeneous environments. In *B. cornutus* and other species in which fecundity and mating success depend on body size (Conner 1988, Bonduriansky 2001, Formica et al. 2011), juvenile cannibalism will increase adult fitness if the morphological effects of cannibalism persist into adulthood. When, as in the present study, high-quality environments induce cannibalism that increases growth, this combination should exaggerate the morphological differences between individuals developing in low- and high-quality environments.

As a result, the evolutionary implications of context-dependent juvenile behavior may extend to traits expressed at other life stages. *Bolitotherus cornutus* adults from different environments likely compete directly with each other for mates due to frequent migration among fungi (Chapter 1). Because males possess elaborate sexually selected horns, a class of traits that are used in competition with conspecifics and exhibit

heightened nutrient sensitivity (Emlen 1994, Bonduriansky 2007, Emlen et al. 2012), cannibalism expressed in juveniles could exaggerate asymmetries in competitive interactions between adults. Similarly, variation in the risk of cannibalistic mortality, along with differences in fungus quality, may generate selection on female oviposition site choice, because females that choose environments that maximize offspring performance and minimize risk will have higher fitness than those that do not (Wolf et al. 1998, Wolf et al. 1999, Refsnider and Janzen 2010, Buser et al. 2013).

In this study we have shown that naturally occurring environmental contexts can influence the expression of extreme competitive behaviors like cannibalism. Furthermore, our data contradict the results of studies conducted in simplistic ecological conditions, which have found that cannibalism is most common in nutrient-poor environments (Fox 1975; Polis 1981). The incorporation of natural environmental variation into studies of the context-dependent behaviors may be necessary in order to extrapolate experimental inferences to the dynamics of context-dependent behaviors in wild populations.

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Literature cited

Abramoff MD, Magalhaes PJ, Ram SJ. 2004. Image processing with ImageJ. Biophotonics International 11: 36-42.

Badyaev AV, Duckworth RA. 2003. Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. J Evolution Biol. 16: 1066-1076.

Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev. 76: 305-339.

Bonduriansky R. 2007. The evolution of condition-dependent sexual dimorphism. Am Nat. 169: 9-19.

Buser CC, Ward PI, Bussière LF. 2013. Adaptive maternal plasticity in response to perceptions of larval competition. Funct Ecol. doi: 10.1111/1365-2435.12188

Caraco T, Blanckenhorn WU, Gregory GM, Newman JA, Recer GM, Zwicker SM. 1990. Risk-sensitivity: ambient temperature affects foraging choice. Anim Behav. 39: 388-345.

Cartar RV, Dill LM. 1990. Why are bumble bees risk-sensitive foragers? Behav Ecol Sociobiol. 26: 121-127.

Chapman JW, Williams T, Esscribano A, Caballero P, Cave RD, Goulson D. 1999. Fitness consequences of cannibalism in the fall armyworm, *Spodoptera frugiperda*. Behav Ecol. 10: 298-303.

Church SC, Sherratt TN. 1996. The selective advantages of cannibalism in a Neotropical mosquito. Behav Ecol Sociobiol. 39: 117-123.

Collie K, Kim SJ, Baker MB. 2013. Fitness consequences of sibling egg cannibalism by neonates of the Colorado potato beetle, *Leptinotarsa decemlineata.* Anim Behav. 85: 329-338.

Conner JK. 1988. Field measurements of natural and sexual selection in the fungus beetle, *Bolitotherus cornutus*. Evolution 42: 736-749.

Daly HV. 1985. Insect morphometrics. Annu Rev Entomol. 30: 415-438.

Emlen DJ. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera, Scarabaeidae). P Roy Soc B-Biol Sci. 256: 131-136.

Emlen DJ, Warren IA, Johns AJ, Dworkin I, Corley Lavine L. 2012. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. Science 337 860-864.

Formica VA, McGlothlin JW, Wood CW, Augat ME, Butterfield RE, Barnard ME, Brodie III ED. 2011. Phenotypic assortment mediates the effect of social selection in a wild beetle population. Evolution 65: 2771-2781.

Fox RF. 1975. Cannibalism in natural populations. Annu Rev Ecol Syst. 6: 87-106.

Gasparini C, Serena G, Pilastro A. 2013. Do unattractive friends make you look better? Context-dependent male mating preferences in the guppy. P Roy Soc B-Biol Sci. 280: 20123072.

Hamilton WD. 1964. The genetical evolution of social behavior. I. J Theor Biol. 7: 1-16.

Heatwole H, Heatwole A. 1968. Movements, host-fungus preferences, and longevity of *Bolitotherus cornutus* (Coleoptera: Tenebrionidae). Ann Entomol Soc Am. 61: 18-23.

Holliday AE, Walker FM, Brodie III ED, Formica VA. 2009. Differences in defensive volatiles of the forked fungus beetle, *Bolitotherus cornutus*, living on two species of fungus. J Chem Ecol. 35: 1302-1308.

Jonsell M, Nordlander G. 2004. Host selection patterns in insects breeding in bracket fungi. Ecol Entomol. 29: 697-705.

Joseph SB, Snyder WE, Moore AJ. 1999. Cannibalizing *Harmonia axyridis* (Coleoptera: Coccinellidae) larvae use endogenous cues to avoid eating relatives. J Evolution Biol. 12: 792-797.

King CE, Dawson PS. 1972. Population biology and the *Tribolium* model. Evol Biol. 5:133-227.

Laycock A, Camm E, van Laerhoven S, Gillespie D. 2006. Cannibalism in a zoophytophagous omnivore is mediated by prey availability and plant substrate. J Insect Behav. 19: 219-229.

Liles MP. 1956. A study of the life history of the forked fungus beetle, *Bolitotherus cornutus*. Ohio J Sci. 56: 329-337.

MacArthur RH, Pianka ER. 1966. On optimal use of a patchy environment. Am Nat. 100: 603-609.

Mayntz D, Toft S. 2006. Nutritional value of cannibalism and the role of starvation and nutrient imbalance for cannibalism tendencies in a generalist predator. J Anim Ecol. 75: 288-297.

Parsons W, Zhong W, Rudolf VHW. 2013. Mating status and kin recognition influence the strength of cannibalism. Anim Behav. 85: 365-369.

Petersen A, Nielsen KT, Christensen CB, Toft S. 2010. The advantage of starving: success in cannibalistic encounters among wolf spiders. Behav Ecol. 21: 1112-1117.

Pfennig DW. 1992. Polyphenism in spadefoot toad tadpoles as a logically adjusted evolutionarily stable strategy. Evolution 46: 1408-1420.

Pfennig DW. 1997. Kinship and cannibalism. BioScience 47: 667-675.

Pfennig DW. 1999. Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin. P Roy Soc B-Biol Sci. 266: 57-61.

Pfennig DW, Murphy PJ. 2000. Character displacement in polyphenic tadpoles. Evolution 54: 1738-1749.

Polis GA. 1981. The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst. 12: 225-251.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Refsnider JM, Janzen FJ. 2010. Ecological and evolutionary hypotheses for variation in oviposition-site choice. Annu Rev Ecol Evol Syst. 41: 39-57.

Resetarits Jr. WJ. 1996. Oviposition site choice and life history evolution. Am Zool. 36: 205-215.

Rudolf VHW, Kamo M, Boots M. 2010. Cannibals in space: The coevolution of cannibalism and dispersal in spatially structured populations. Am Nat. 175: 513-524.

Stevens L. 1989. The genetics and evolution of cannibalism in flour beetles (genus *Tribolium*). Evolution 43: 169-179.

Tanner CJ, Adler FR. 2009. To fight or not to fight: context-dependent interspecific aggression in competing ants. Anim Behav. 77: 297-305.

Via S. 1999. Cannibalism facilitates the use of a novel environment in the flour beetle *Tribolium castaneum*. Heredity 82: 267-275.

Vijendravarma RK, Narasimha S, Kawecki TJ. 2013. Predatory cannibalism in *Drosophila melanogaster* larvae. Nature 4: 1789.

Wolcott DL, Wolcott TG. 1984. Food quality and cannibalism in the red land crab, *Gecarcinus lateralis*. Physiol Zool. 57: 318-324.

Wolf JB, Brodie III ED, Cheverud JM, Moore AJ, Wade MJ. 1998. Evolutionary consequences of indirect genetic effects. Trends Ecol Evol. 13:64-69.

Wolf JB, Brodie III ED, Moore AJ. 1999. Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. Am Nat. 153: 254-266.

Wood CW, Donald HM, Formica VA, Brodie III ED. 2013. Surprisingly little population genetic structure in a fungus-associated beetle despite its exploitation of multiple hosts. Ecol Evol. 3: 1484-1494.

Figures and Tables

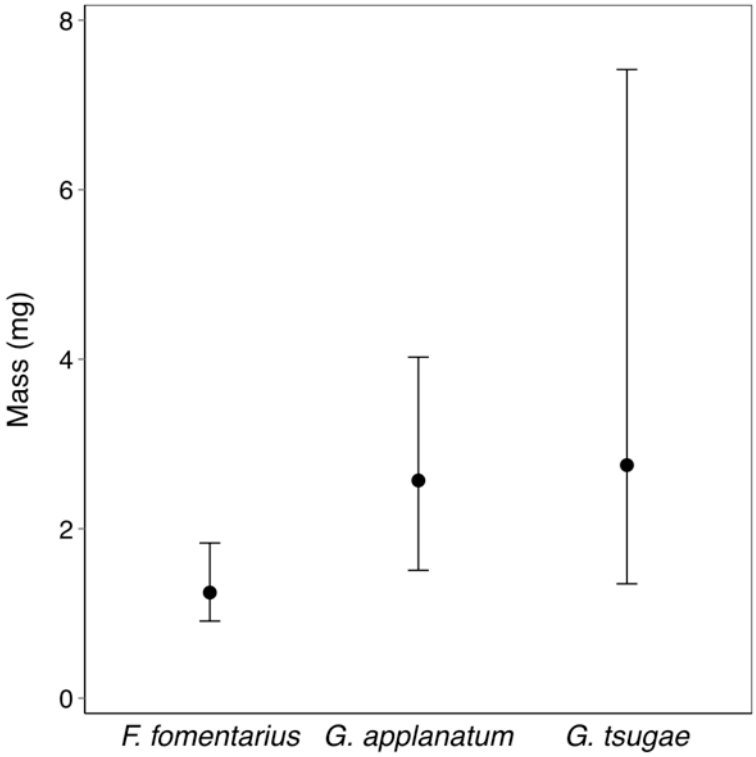

Figure 1. The fungus environment affects the mass of 10-day-old larvae. Error bars are 95% confidence intervals.

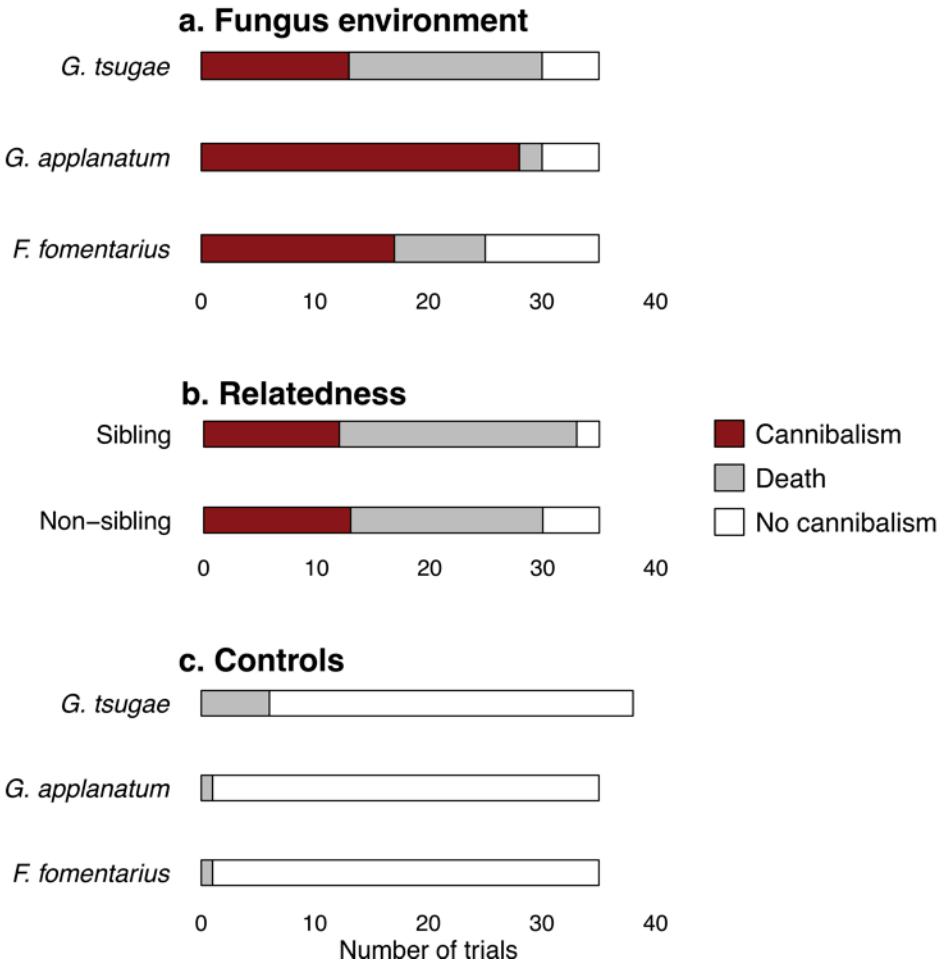

Figure 2. The frequency of cannibalism and non-cannibalistic death in different (a) fungus environments, (b) between siblings and non-siblings, and (c) in the controls. The probability of cannibalism differed significantly among fungi, but not between siblings and non-siblings. There was no significant effect of the fungus environment on mortality in the control larvae.

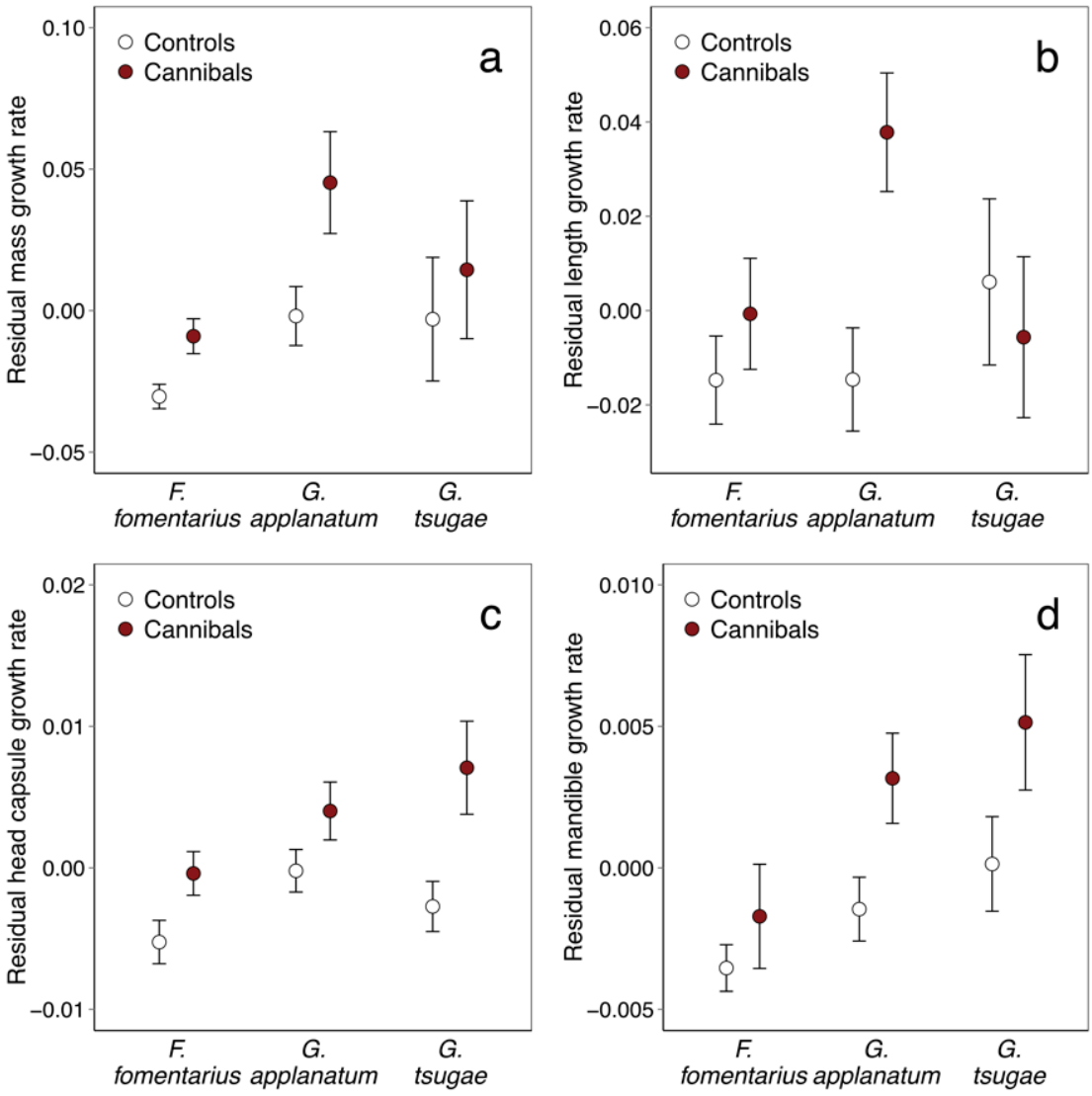

Figure 3. Growth rates in (a) mass, (b) length, (c) head capsule width, and (d) mandible length of control larvae and larvae that cannibalized. The growth rate of cannibals was significantly greater than that of controls for all traits (Table 2). Error bars are standard errors, and values on the y-axis are the residuals from the regression of larval growth rate on initial larval size for each trait.

Table 1. Likelihood-ratio tests for the effect of the fungus environment and relatedness on the probability of cannibalism.

Model	Effect	df	Likelihood-ratio χ^2	P
Fungus environment	**Fungus**	**2**	**15.780**	**0.001**
	Initial size	1	0.349	0.555
	Size difference	1	0.247	0.627
Relatedness	Relatedness	1	0.277	0.782
	Initial size	**1**	**2.092**	**0.036**
	Size difference	1	-0.309	0.757

Table 2. ANOVAs of the effect of the fungus environment, cannibalism, and their interaction on larval growth rate in four traits.

Trait	Effect	df	F	P
Mass	**Cannibalism**	**1**	**6.538**	**0.012**
	Fungus	**2**	**4.925**	**0.008**
	Fungus × cannibalism	2	0.555	0.575
	Initial mass	1	0.805	0.371
Length	**Cannibalism**	**1**	**5.002**	**0.027**
	Fungus	**2**	**3.109**	**0.048**
	Fungus × cannibalism	**2**	**3.062**	**0.050**
	Initial length	1	2.316	0.130
Head capsule width	**Cannibalism**	**1**	**25.116**	**<0.001**
	Fungus	**2**	**15.603**	**<0.001**
	Fungus × cannibalism	2	2.450	0.090
	Initial head capsule width	**1**	**20.409**	**<0.001**
Mandible length	**Cannibalism**	**1**	**17.030**	**<0.001**
	Fungus	**2**	**18.637**	**<0.001**
	Fungus × cannibalism	2	1.698	0.187
	Initial mandible length	**1**	**37.342**	**<0.001**

CHAPTER FOUR:

Environmentally induced changes in genetic correlation structure are comparable to the divergence between conspecific populations[4]

[4] Formatted as a co-authored manuscript: Wood, C. W. and E. D. Brodie III.

Abstract

Genetic correlations between traits determine the multivariate response to selection in the short term, and thereby play a causal role in evolutionary change. The environment is known to shape the expression of genetic variation in single traits, suggesting that it has the potential to influence genetic correlations between traits as well. While individual studies have documented environmentally induced changes in genetic correlations, the nature and extent of environmental effects on multivariate genetic architecture across species and environments remains largely uncharacterized. We reviewed the literature for studies that estimated the genetic variance-covariance (**G**) matrix in multiple environments, and compared differences in **G** between environments to the divergence in **G** between conspecific populations (measured in a common garden), which reflects the change in genetic correlations that occurs due to the combined action of selection, mutation, migration, and drift. We found that between-environment differences in total genetic variance and the relative magnitude of genetic correlations were as large as between-population differences, and that the orientation of genetic correlations differed more between environments than between populations. The predicted evolutionary trajectory differed as strongly between environments as it did between conspecific populations. The effect of the environment on phenotypic correlations—but not phenotype means—predicted these differences in **G**. Our results suggest that environmental effects on genetic architecture in a single generation may be comparable to the divergence that accumulates over dozens or hundreds of generations. Lability in multivariate genetic architecture may therefore play an important role in determining evolutionary trajectories in changing environments.

Introduction

The genetic variance-covariance matrix (**G**) describes the magnitude and orientation of the axes of quantitative genetic variation in a population (Lande 1979). Because **G** affects the rate and direction of the response to selection (Schluter 1996, Chenoweth et al. 2010), the processes that shape **G** have cascading effects on patterns of genetic constraint and, ultimately, on multivariate evolutionary trajectories (McGuigan 2006, Arnold et al. 2008).

G is not static. Changes in genetic covariances have been documented over both short and long evolutionary timescales (reviewed in Roff and Mousseau 1999, Arnold et al. 2008). Given that **G** evolves, the crucial question is now how quickly and under what circumstances it does so (Steppan et al. 2002, Doroszuk et al. 2008). If **G** is conserved over long timescales, existing genetic covariances will accurately reflect historical and future genetic constraints on the multivariate evolutionary response in most populations. However, if rapid change in **G** is common, the rate and direction of the evolutionary response may be unpredictable on timescales as short as a few generations (Phillips and McGuigan 2006).

The rate of change in **G** depends on the building blocks of genetic variances and covariances—allele frequencies and allelic effects (Falconer and Mackay 1996)—and the processes that shape them. Most research on the lability of **G** focuses on change in allele frequencies driven by selection, migration, mutation, and genetic drift (Roff 2000, Phillips and McGuigan 2006, Arnold et al. 2008). Allelic effects, the realized effects of alleles on phenotype (Falconer and Mackay 1996), are an underappreciated but potentially pervasive source of change in **G**. The average effect of an allele depends on

the environment in which it is expressed (de Jong 1990), typically as a result of among-family differences in environment-specific gene expression. For example, the effect of the *Eda* locus on growth rate in stickleback depends on the salinity of the developmental environment (Barrett et al. 2009), and herbivory resistance is governed by different QTL in spring- and fall-germinating *Arabidopsis* (Weinig et al. 2003). Environment-dependent allelic effects have the potential to change genetic covariances in a single generation—much more rapidly than allele frequencies are likely to shift through selection or drift.

Environmental effects on **G** challenge the prevailing assumption that genetic architecture is stable over short periods of time, the evolutionary consequences of which may be profound in heterogeneous or rapidly changing environments (Sgrò and Hoffman 2004). If the environment strongly impacts genetic architecture, environmental change may release prior genetic constraints or introduce new ones, shaping the rate and direction of adaptive evolution even in the absence of variation in selection among environments (McGuigan and Sgrò 2009). Even under identical selection regimes, environmental effects on genetic architecture may contribute to the maintenance of genetic variation by altering the genetic basis of phenotypic variation, and therefore, the genomic regions that are subject to selection (McGuigan and Sgrò 2009, Paaby and Rockman 2014). Finally, adaptation to changing climates, anthropogenic disturbance, and novel ecological niches may be considerably less predictable than is currently appreciated if existing genetic architecture does not persist in new environments.

A growing body of literature demonstrates that the environment can change genetic variances and covariances (Sgrò and Hoffman 2004, Pigliucci 2005, Doroszuk et

al. 2008, Eroukhmanoff and Svensson 2011, Johansson et al. 2012, Sikkink et al. *in press*). However, it remains unclear whether environmental effects on **G** are common enough to challenge the assumption that **G** is predominately stable in the short term (Scheiner 1993). The evolutionary consequences of environmental effects on **G** depend on two unresolved questions. First, how strong are environmental effects relative to selection, mutation, migration and drift? If differences in genetic architecture between environments are negligible relative to the differences that accumulate between diverging populations, short-term changes in genetic architecture may not exert strong influence on the evolutionary response. On the other hand, if the environment induces change in **G** that is comparable to the divergence observed over dozens or hundreds of generations, **G** may be vary substantially in heterogeneous environments over short timescales.

Second, what environments precipitate the largest changes in **G**? The evolutionary consequences of change in **G** to natural populations depend on the environmental conditions that trigger change (Charmantier and Garant 2005, Paaby and Rockman 2014). Answering this question requires a metric of differences between environments that can be applied regardless of the manipulation employed in any given study. One potential approach is to identify the types of environmental conditions that restructure **G**. A promising candidate is environmental novelty. Novel environments are thought to perturb the genotype-to-phenotype map and reshape genetic architecture, although empirical evidence linking novelty to the release of genetic variation remains equivocal (McGuigan and Sgrò 2009, Ledón-Rettig et al. 2014). An alternative to this environment-centric approach is a phenotype-centric one: using the phenotype as a

barometer of environmental difference to determine whether the environments that change phenotype means and (co)variances drive corresponding change in **G**.

To assess the evolutionary consequences of environmentally induced change in **G**, we surveyed the literature for studies that measured **G** in different environments. We compared differences in **G** between environments to differences in **G** between conspecific populations to determine how strong environmental effects on **G** are relative to the combined action of selection, mutation, migration, and drift. We asked three main questions: How large are between-environment differences in the structure of **G** relative to differences between conspecific populations (when the latter are compared in a common environment)? How large are between-environment differences in the predicted evolutionary trajectory relative to differences between populations? Finally, what patterns of environmental difference precipitate the largest changes in **G**?

Methods

Dataset

We searched the literature for studies that reported broad- or narrow-sense genetic covariances or correlations (variance-scaled covariances) between at least two traits. We performed our search in Web of Science and Dryad using the search terms "G matrix," "genetic correlation," "genetic covariance," "comparison," and "environment," and supplemented the results of this search with studies cited in the papers identified. Several of the studies in our dataset came from two previous reviews (Sgrò and Hoffman 2004 and Pitchers et al. 2014). Two studies estimated **G** separately for males and females; in these cases we arbitrarily selected only one sex for the analysis. When **G** was estimated

in more than two environments, we compared the two most extreme environments (Charmantier and Garant 2005).

For our between-environment G-matrix comparisons, we only included experimental studies that reared the same source population under different environmental conditions. This dataset thus reflects, as closely as possible, differences in **G** due only to environment-specific allelic effects. For our between-population G-matrix comparisons, we only included studies that reared different conspecific source populations under common garden conditions. This dataset reflects differences in **G** due to allele frequencies or evolved differences in allelic effects in the source populations.

The studies in our dataset used a variety of methods to compare G-matrices, so we could not directly incorporate the results of their analyses into our meta-analysis. Instead, we ran our own set of matrix comparison tests on the G-matrices that they reported, treating each matrix as a point estimate. To account for the fact that this approach ignores error in estimating **G** (which is substantial; Cheverud 1988), we included the sample size used to build each original G-matrix as a covariate in our analyses to statistically control for systematic differences in estimation error between studies. The sample size for narrow-sense G-matrices is the number of half-sib families; for broad-sense G-matrices, it is the number of full-sib families or genotypes (when clones or recombinant inbred lines were used).

Genetic covariance matrices were only available for half of the studies in our dataset (47 of 99, the remainder reported correlation matrices), so we converted covariance matrices to correlation matrices for all comparisons except total genetic variance (see below). We omitted any traits with an estimated genetic variance of zero. A

third of the G-matrices in our dataset (65 of 198 matrices) contained negative eigenvalues, which is not uncommon when matrices are built using variance component estimation (Hill and Thompson 1978). We "bent" these matrices by replacing all negative eigenvalues with small positive numbers (1.0×10^{-6}) because many matrix comparison tests require positive definite matrices (Hayes and Hill 1981, Chapuis et al. 2008, Phillips and Arnold 1999). Matrix bending is a potential source of bias (Phillips and Arnold 1989), but the bias that it introduced in our dataset was likely small, because the negative eigenvalues accounted for a small percentage of the total variance in the 65 non-positive definite matrices (mean: 6.8%, median: 3.0%, range: 0.00002%-42.8%). All analyses were performed in R v3.1.0 (R Core Team 2014).

Comparisons of G-matrix structure

We used three metrics of G-matrix structure in our comparisons: total genetic variance, and the relative magnitude and orientation of genetic correlations (Phillips and Arnold 1999) (Figure 1). Total genetic variance reflects the genetic variation available to selection across all traits; changes in total genetic variance affect the rate of the evolutionary response (Figure 1A). The relative magnitude and orientation of genetic correlations affect both the rate and direction of the evolutionary response (Roff et al. 2012). The relative magnitude of genetic correlations describes the proportion of genetic variation that falls along the major axis (Figure 1B). A change in relative magnitude results in differences in the degree of genetic constraint in the most accessible evolutionary direction. The orientation of genetic correlations describes which dimensions of the multivariate phenotype are most strongly correlated (Figure 1C). A

change in orientation implies that traits that are correlated in one environment or population are uncorrelated in another, or that the sign of the correlation changes. Differences in the orientation of **G** reflect fundamental differences in genetic constraint and in the most accessible direction of evolution.

For each pair of G-matrices sampled from two environments or two populations, we calculated the difference in the three metrics of matrix structure described above. To measure the difference in total genetic variance for a pair of G-matrices, we calculated the total genetic variance of each matrix by summing the eigenvalues of **G** (its "volume"; Kirkpatrick 2009). For each comparison, we calculated the absolute value of the difference in total genetic variance and standardized each difference by dividing by the average of the two. This analysis was only performed for the 47 comparisons for which covariance matrices were available because the total genetic variance of a correlation matrix is constrained to equal the number of traits.

To measure the difference in the magnitude of genetic correlations for a pair of G-matrices, we calculated the difference in the proportion of variance along the major axis of variation (hereafter, "$\mathbf{g}_{max}$") for each comparison. The proportion of variance along $\mathbf{g}_{max}$ measures the fraction of total genetic variation that is found along the first eigenvector (Kirkpatrick 2009), and is given by

$$\lambda_1 \Big/ \sum_{i=1}^{n} \lambda_i$$

where λ_i are the eigenvalues ordered from largest to smallest. For each matrix pair, we calculated the absolute value of the difference between these fractions, and standardized each difference by dividing by the average proportion of variance along $\mathbf{g}_{max}$.

To measure the difference in the orientation of genetic correlations for a pair of G-matrices, we calculated the angle between their major axes of variation (i.e., $\mathbf{g}_{max}$). The angle between the $\mathbf{g}_{max}$ vectors was calculated as

$$\frac{180}{\pi} * \cos^{-1}\left(\frac{\boldsymbol{g}_{max_{E1}} \cdot \boldsymbol{g}_{max_{E2}}}{\sqrt{\boldsymbol{g}_{max_{E1}} \cdot \boldsymbol{g}_{max_{E1}}}\sqrt{\boldsymbol{g}_{max_{E2}} \cdot \boldsymbol{g}_{max_{E2}}}}\right)$$

(Ingleby et al. 2014, Teplitsky et al. 2014). This calculation results in values ranging from 0° to 180°, so we subtracted from 180° any angles in the range $90° \leq x \leq 180°$ so all angles were between 0° and 90°. An angle of 0° indicates no difference in orientation, and an angle of 90° indicates that the major axes of genetic variation in the two environments are orthogonal.

To test whether the difference in the structure of **G** between populations was larger than the difference in the structure of **G** between environments, we ran a separate general linear model for each matrix comparison metric as the dependent variable using the lm function in R. The independent variables in these regressions were the comparison type (between environments or between populations), the number of traits in **G**, and their interaction, as well as the sample size used to build **G**. We tested significance using the Anova function and type III sums of squares in the *car* package (Fox and Weisberg 2011).

Comparisons of the predicted evolutionary trajectory

We compared the predicted evolutionary trajectories caused by each pair of G-matrices to evaluate the evolutionary consequences of change in the structure of **G**. We used random skewers to measure the difference in the predicted evolutionary trajectory

for each pair of G-matrices (Cheverud 1996). Random skewers applies random selection gradients to two matrices and measures the difference in their responses to selection. We applied 1000 random selection gradients drawn from a uniform distribution on the interval (-1,1) to each G-matrix, and calculated the response to selection for each gradient, resulting in 1000 response vectors per matrix. We used a modified version of the R function RAND.SKEWER provided in Roff et al. (2012) for this analysis.

We compared the predicted evolutionary trajectory for each pair of G-matrices using two metrics: the direction of the response to selection and the overall response difference (Figure 1D and 1E). To measure the difference in the direction of the response between two matrices (Figure 1D), we calculated the angle between their response vectors, using the same approach that we used to find the angle between their major axes (see above). This metric captures the difference in the direction but not rate of the evolutionary response. To measure the overall difference in the response between each pair of G-matrices (Figure 1E), we calculated the distance between the endpoints of the two response vectors (eq. 7 in Hansen and Houle 2008). This metric incorporates differences in both the rate and direction of the evolutionary response because it depends on the length of the two response vectors and the angle between them.

To test whether the difference in the evolutionary response between populations was larger than the difference between environments, we ran two separate general linear models using the lm function in R. The first used the difference in the direct of the response as the dependent variable, and the second, the overall response difference. These models included the same independent variables as the models used to compare the structure of **G,** and we used the same approach to test significance (see above).

*What environmental conditions change **G**?*

A crucial step in evaluating the evolutionary consequences of environmental effects on **G** is identifying the environmental conditions that change **G**. We employed two metrics of environmental difference that were available for most studies in our dataset: environmental novelty and the environmental effect on phenotype.

Novel environments are thought to alter genetic architecture because they perturb evolved phenotypic buffering mechanisms (Paaby and Rockman 2014). We tested whether there was a larger change in **G** in comparisons between novel and non-novel environments than in comparisons between two non-novel environments. We only included comparisons for which the original study categorized both environments as novel or non-novel (N = 44) based on whether the environmental treatment was outside the typical range of conditions the organism experiences. We ran a separate model for each matrix comparison metric using the lm function, with each metric as the dependent variable and environmental novelty as the independent variable, and tested significance using the Anova function and type III sums of squares in the *car* package (Fox and Weisberg 2011). We included number of traits in **G** as a covariate in this analysis.

For our second metric of environmental difference we used the environmental effect on phenotype means and phenotypic correlations, assuming that very different environmental conditions are more likely to produce large differences in phenotypic expression. To measure the effect of the environment on phenotype means, we calculated the difference between the phenotype mean in the two environments, standardized by dividing by their pooled standard deviation, and took the absolute value (eq. 1 in

Nakagawa and Cuthill 2007). We then took the median of this standardized difference across all phenotypes reported in each study. We calculated this index for all studies in which the phenotype means were available in text, in supplementary materials, estimable from figures, or obtainable from Dryad (N = 34).

We tested whether the difference in phenotype means between environments predicted the environmental effect on **G** by regressing each matrix comparison metric on this index. We included the number of traits in each G-matrix in our analysis as a covariate, and tested significance using the Anova function and type III sums of squares in the *car* package (Fox and Weisberg 2011). We repeated this analysis with the maximum of the standardized phenotype difference across all traits in each study, in case the most environmentally responsive traits were the best predictors of change in **G**. This analysis produced qualitatively similar results to the median-based analysis, so we do not report it here.

To measure the effect of the environment on phenotypic correlations, we obtained phenotypic correlation matrices (**P**) from the original text, supplementary materials, or Dryad (N = 20). We quantified the effect of the environment on the relative magnitude of phenotypic correlations, the orientation of phenotypic correlations, and the predicted evolutionary trajectory using the same comparison metrics as for **G**. We tested for a relationship between the environmental effect on **P** and the environmental effect on **G** using Pearson's correlations. We were unable to compare the effect of the environment on total phenotypic and genetic variance because only 6 studies reported both genetic and phenotypic covariance matrices. Matrix bending was unnecessary for the **P**-matrices because all were positive definite.

Results

We identified 99 G-matrix comparisons (pairs of G-matrices) from 85 studies published between 1981 and 2014 that reported genetic covariance or correlation matrices for 2-12 traits (Supplementary Table 1). Sixty-two of these were comparisons between environments and 37 were comparisons between populations. The data are heavily taxonomically biased: 77 comparisons are from plant or insect systems, with only three genera (*Drosophila*, *Arabidopsis*, and *Gryllus*) accounting for nearly a quarter of all comparisons. This distribution almost certainly reflects the state of the field, rather than systematic bias in our literature search, because the measurement of **G** is a time-consuming and data-hungry endeavor that is most feasible in species with short generation times that can be raised in the lab. Among the studies that compared **G** in different environments, the environmental manipulation varied broadly. Most involved lab-based manipulations of diet, temperature, water, or light availability and photoperiod (43 of 62; Supplementary Table 1).

Our dataset included narrow- and broad-sense estimates of **G** in approximately equal proportions (N = 43 and 56 comparisons, respectively). There was no significant difference between narrow- and broad-sense estimates of **G** in the change observed for any matrix comparison metric (Supplementary Table 2), indicating that this methodological difference among studies does not contribute to our results.

Comparisons of G-matrix structure

Changes in different aspects of G-matrix structure were only weakly correlated with one another (Table 1). The only significant correlation was between the relative

magnitude and the orientation of genetic correlations, indicating that change in one aspect of **G** does not necessarily translate to strong effects on another. The change in the orientation of **G** was the only aspect of G-matrix structure that was significantly correlated with the change in the predicted evolutionary response, an unsurprising result given that changes in the orientation of genetic correlations constitute major change in fundamental genetic constraints.

Between-environments differences in total genetic variance were highly variable, ranging from zero to nearly 200% (Figure 2A). Differences between environments in the relative magnitude of genetic correlations were small (Figure 2B), suggesting that the environment does not strongly affect the degree to which **G** is dominated by a major axis of variation. However, between-environment differences in the orientation of genetic correlations were moderate to large, in some cases changing the direction of the major axis of variation nearly 90 degrees (Figure 2C). The largest differences occurred between matrices that included 2-4 traits, a pattern that was especially pronounced for between-environment comparisons (Figure 2). This result may reflect investigation bias: studies that examined only a few traits may have selected those traits because they were hypothesized to be environmentally responsive.

Differences in **G** between environments were equal to or greater than differences in **G** between conspecific populations. For two of the three metrics of G-matrix structure—total genetic variation and the relative magnitude of genetic correlations—divergence in **G** between environments was not significantly different from divergence in **G** between conspecific populations (Figure 2, Table 2). The orientation of genetic

correlations differed significantly more between environments than between populations (Figure 2, Table 2).

Comparisons of the predicted evolutionary trajectory

There was no significant difference between the change in the predicted evolutionary response in between-environment comparisons and between-population comparisons (Figure 2, Table 2). However, the comparison × number of traits interaction was marginally significant for both the angle between the responses and the response difference, suggesting that the effect of the number of traits in **G** may differ between comparison types (Table 2). While the change in the predicted evolutionary trajectory for G-matrices with few traits was similar in between-environment and between-population comparisons, the predicted evolutionary trajectory for G-matrices with many traits differed more between populations than between environment comparisons (Figure 2).

In most cases, the changes in G-matrix structure documented above did not result in major differences in the predicted evolutionary response of most matrix pairs (median angle between the response vectors: 24.8°). The results were qualitatively similar for the direction of the response and the overall response difference, which indicates that the majority of the overall difference in the evolutionary response is due to a change in the angle of the response vector rather than its magnitude.

*What environmental conditions change **G**?*

Comparisons involving novel environments were not significantly more likely to affect the structure of **G** (total genetic variance, the relative magnitude of genetic

correlations, and the orientation of genetic correlations; Table 3) or the predicted evolutionary response (the direction of the response and the response difference; Table 3).

Similarly, the environmental effect on mean phenotype was a poor predictor of the effect of the environment on **G** (Table 3). There was no relationship between the environmental effect on mean phenotype and the change in total genetic variance, relative magnitude of genetic correlations, or the orientation of genetic correlations. The same was true for both metrics of the predicted evolutionary trajectory (Table 3).

By contrast, the effect of the environment on phenotypic correlations (**P**) was a strong predictor of environmental effects on the structure of **G** and the predicted evolutionary trajectory. Large differences in **P** between environments coincided with large differences in **G** in the orientation of genetic correlations ($r = 0.736$, $t_{18} = 4.616$, $P < 0.001$), although there was no significant correlation between environmental effects on the relative magnitude of genetic correlations ($r = 0.160$, $t_{18} = 0.688$, $P = 0.500$; Figure 3). The evolutionary trajectory based on **P** was significantly correlated with the evolutionary trajectory based on **G** for both response metrics: the direction of the response ($r = 0.496$, $t_{18} = 2.426$, $P = 0.026$) and the response difference ($r = 0.720$, $t_{18} = 4.408$, $P < 0.001$; Figure 3).

The difference in **G** between environments tended to be larger than the difference in **P** (i.e., most of the points in Figures 4 and 5 lie above the dashed 1:1 line). Although this finding may indicate that environmental effects on genetic correlations are stronger than on phenotypic ones, it is possible that this result is an artifact of the error inherent in estimating **G**, which is substantially larger than that associated with **P** (Cheverud 1988).

Discussion

Our results suggest that the environment is capable of driving change in **G** as large as differences between conspecific populations. This pattern held for both the structure of **G** and the evolutionary trajectory that it would generate. These data indicate that the environment is capable of inducing changes in genetic architecture within a single generation comparable to those that accumulate in many generations as populations diverge due to the combined action of selection, mutation, migration, and drift. Neither environmental novelty nor differences in phenotype means predicted environmental effects on the structure of **G** and on the predicted evolutionary trajectory. Instead, change in **G** was associated with change in phenotypic correlations, indicating that a multivariate view of phenotypic plasticity is necessary to identify the circumstances under which the environment affects genetic architecture. Our results suggest that the environment can shape genetic architecture directly, and deserves attention as an important factor that governs the structure of genetic constraint (Jones et al. 2003, Eroukhmanoff and Svensson 2011, Björklund et al. 2013, Sikkink et al. *in press*).

Environmental effects on G-matrix structure

The environment tended to have the strongest effect on total genetic variance (the volumne of **G**) and the orientation of genetic correlations (the angle between g_{max}; Figure 2A and 2C). Change in different metrics of G-matrix structure were only weakly correlated with one another (Table 2), implying that change in one aspect of G-matrix structure is not accompanied by sweeping changes in all aspects of genetic architecture.

Our analysis corroborates previous research that provides substantial evidence for environmental effects on genetic variances (Ledón-Rettig et al. 2010, Berger et al. 2011, McGuigan et al. 2011, Clark et al. 2013). Heritable variation released by the environment ("cryptic genetic (co)variation"; Ledón-Rettig et al. 2012, Sikkink et al. *in press*) is thought to provide a reservoir of standing genetic variation that is exposed under conditions of environmental perturbation, and can contribute to the evolutionary response under new selection regimes (Waddington 1956, Paaby and Rockman 2014).

We detected surprisingly strong effects of the environment on the orientation of genetic correlations, indicative of common among-environment differences in trait integration and multivariate genetic constraint. Between-environment differences in the orientation of **G** were significantly greater than between-population differences (Figure 2), suggesting that environmental effects on genetic tradeoffs between traits can exceed evolved changes due to selection or drift. Reorientation of genetic correlations constitutes a major change in **G**—arguably the largest—because it alters the most accessible direction of evolution and affects sources of indirect selection on each trait (Phillips and Arnold 1999, Arnold et al. 2008). The impact of the orientation of **G** on the response to selection is evident in the fact that change in the orientation was significantly correlated with change in the predicted evolutionary trajectory in our analysis (Table 1).

It is important to note that our dataset likely overestimates differences in **G** between environments and between populations due to observation and publication bias. Many studies of environmental effects on **G** in our dataset chose conditions that they expected to weaken or reverse tradeoffs between traits, or selected traits based on *a priori* expectations of environmental responsiveness (e.g., Czesak and Fox 2003, Haselhorst et

al. 2011, King et al. 2011). The latter may be especially likely to affect matrices with few traits (Figure 2). Similarly, studies that performed between-population G-matrix comparisons often chose populations on the basis of large differences between them (e.g., Doroszuk et al. 2008, Bacigalupe et al. 2013). Finally, negative results are less likely to be published (the "file-drawer problem"; Kingsolver et al. 2012), so literature reviews and meta-analyses perennially overestimate effect of interest (Kingsolver et al. 2001, Charmantier and Garant 2005). There is no reason to expect observation and publication bias to affect between-environment and between-population comparisons unequally, so systematic bias is unlikely to influence our comparison of between-environment and -population differences in **G**.

The fact that between-environment differences in the structure of **G** are comparable to between-population differences imply that allelic effects—and, by extension, the environment—may be a crucial component of change in genetic architecture over short evolutionary timescales. Differences in allelic effects appear to most strongly affect the orientation of **G** and the structure of genetic tradeoffs between traits. These results contrast with the current literature on the stability of **G**, which focuses predominately on changing allele frequencies, often minimizing or entirely omitting the role of allelic effects (Phillips and McGuigan 2006, Arnold 2008). The between-environment G-matrix comparisons in our dataset isolate the role of allelic effects in changing **G** because they minimized allele frequency differences by splitting families or populations across environments. Therefore, our results suggest that differences in allelic effects alone can change **G** as much as the differences in allele frequencies and allelic effects that accumulate between populations.

*What are the evolutionary consequences of environmental effects on **G**?*

The predicted evolutionary trajectory differed as strongly between environments as it did between conspecific populations (Figure 2, Table 2), implying that changes in **G** in a single generation can affect the evolutionary response as much as changes that accumulate over hundreds of generations. However, while the difference in the predicted evolutionary trajectory in between-environment and between-population comparisons was similar for small matrices, there was a trend towards larger differences in between-population comparisons of matrices with many traits (comparison × number of traits interaction; Table 2, Figure 2). This statistical interaction may indicate that differences in the evolutionary trajectory between environments are largely due to a small number of traits that are strongly environmentally sensitive, while differences between populations result from accumulated changes in the genetic architecture of the entire multivariate phenotype.

The absolute change in the evolutionary response (between environments and between populations) was fairly small in most cases (Figure 2D). Ultimately, the long-term evolutionary consequences of the differences in **G** between environments and populations may be minor if they are relatively ephemeral, average out over longer timescales, or decay in the face of strong selection (Delph 2011, Walsh and Lynch 2015). The impact of environmentally induced change in **G** on evolutionary trajectories therefore depends on whether it accumulates over time or is amplified by parallel effects of the environment on selection.

Differences in selection among environments complicate inferences about the implications of environmental effects on **G** for evolutionary constraint. Because genetic

correlations function as constraints only if they oppose selection (Agrawal and Stinchcombe 2009, Conner 2012), two similar G-matrices can impose very different constraints under different selection regimes. The assumption that selection is constant across environments is inherent in our analysis of change in the predicted evolutionary trajectory because we applied identical random vectors of selection gradients to the two G-matrices. In heterogeneous environments, however, variation in selection is common (MacColl 2011), and may frequently accompany change in **G**. If differences in selection among environments amplify small differences in **G**, our approach may underestimate the effect of environmentally induced change in **G** on the evolutionary response, or overestimate it if differences in selection among environments counterbalance differences in **G**.

*What environmental conditions change **G**?*

A striking result from our literature survey is the variance in the effect of the environment on **G** and on the predicted evolutionary response (Figure 2). Given the considerable variation in environmental effects on **G**, characterizing the conditions that change **G** is necessary to assess the ramifications of environmentally induced change in **G** in natural populations. Novel environments are often implicated in the release of cryptic genetic variation because unfamiliar conditions disrupt phenotypic buffering mechanisms (Hansen 2006, Paaby and Rockman 2014). Our results do not support this hypothesis (Table 3), although our power to detect an effect was fairly low due to the sample size for this analysis (N = 44). Support for this hypothesis in the literature remains equivocal, perhaps because our mechanistic understanding of environmental

effects on genetic variance comes primarily from molecular genetics (McGuigan and Sgrò 2009, Ledón-Rettig et al. 2014). It is unclear how the agents that release genetic variation in the lab (e.g., Hsp90; Queitsch et al. 2002) affect quantitative traits in genetically variable populations under realistic ecological conditions (Mittler 2006).

Although environmental effects on mean phenotype are often more apparent to empirical biologists than effects on phenotypic (co)variances, the latter is a better indicator of environmentally induced change in genetic architecture. Our data underscore the importance of distinguishing between the environmental responsiveness in means and (co)variances, as plasticity of phenotypic means was a poor predictor of changes in **G**. Instead, plasticity of phenotypic correlations was our strongest predictor of environmental effects on genetic correlations. Changes in phenotypic correlations were significantly correlated with changes in genetic correlations for all metrics (Figure 3). Previous studies have suggested that **P** is a reasonable proxy for **G**, largely due to fact that estimates of **G** are imprecise (the unit of observation in **G** is the family or genotype, not the individual**;** Cheverud 1988, Roff 1995). Though our sample size is small, our data suggest that just as **P** is a good proxy for **G**, change in **P** is a good proxy for change in **G**. Using changes in phenotypic correlations as a surrogate for genetic correlations may simplify the identification of circumstances in which environmental effects on genetic (co)variances have the potential to alter evolutionary trajectories.

Conclusion

The short-term stability of genetic constraints remains an unsolved problem in evolutionary biology. Theoretical approaches have produced equivocal results (Walsh

and Lynch 2015), and consequently, the stability of **G** has been considered an empirical question for several decades (Turelli 1988, Arnold 2008). In the present study, we demonstrate that environmental effects on genetic architecture equal or exceed evolved differences in genetic architecture between diverging conspecific populations. Our results corroborate recent empirical studies that document extremely rapid changes in **G** over only a few generations (Delph et al. 2011, Sikkink et al. *in press*). The evolutionary consequences of this short-term change in **G** depend on whether it represents ephemeral "wobbling" around a mean, or persistent change in the structure of genetic architecture that may shape the evolutionary response in the long term (Walsh and Lynch 2015).

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Literature cited

Agrawal, A. F. and J. R. Stinchcombe. 2009. How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society—B* 276:1183-1191.

Arnold, S. J., R. Bürger, P. A. Hohenlohe, B. C. Ajie, and A. G. Jones. 2008. Understanding the evolution and stability of the G-matrix. *Evolution* 62:2451-2461.

Bacigalupe, L. D., K. Barrientos, A. P. Beckerman, M. J. Carter, C. C. Figueroa, S. P. Foster, A. J. Moore, A. X. Silva, and R. F. Nespolo. 2013. Can invasions occur without change? A comparison of G-matrices and selection in the peach-potato aphid, *Myzus persicae*. *Ecology and Evolution* 3:5109–5118.

Barrett, R. D. H., S. M. Rogers, and D. Schluter. 2009. Environment specific pleiotropy facilitates divergence and the *Ectodysplasin* locus in threespine stickleback. *Evolution* 63:2831-2837.

Berger, D., S. S. Bauerfeind, W. U. Blanckenhorn, and M. A. Schäfer. 2011. High temperatures reveal cryptic genetic variation in a polymorphic female sperm storage organ. *Evolution* 65:2830-2842.

Björklund, M., A. Husby, and L. Gustafsson. 2013. Rapid and unpredictable changes of the G-matrix in a natural bird population over 25 years. *Journal of Evolutionary Biology* 26:1-13.

Blows, M. W., S. F. Chenoweth, and E. Hine. 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. *The American Naturalist* 163:329-340.

Chapuis, E., G. Martin, and J. Goudet. 2008. Effects of selection and drift on G matrix evolution in a heterogeneous environment: a multivariate Q_{ST}-F_{ST} test with the freshwater snail *Galba truncatula*. *Genetics* 180:2151-2161.

Charmantier, A. and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society—B* 272:1415-1425.

Chenoweth, S. F., H. D. Rundle, and M. W. Blows. 2010. The contribution of selection and genetic constraints to phenotypic divergence. *The American Naturalist* 175:186-196.

Cheverud, J. M. 1988. A comparison of genetic and phenotypic correlations. *Evolution* 42:958-968.

Cheverud, J. M. 1996. Quantitative genetic analysis of cranial morphology in the cotton-top (*Saguinus oedipus*) and saddle-back (*S. fuscicollis*) tamarins. *Journal of Evolutionary Biology* 9:5-42.

Clark, E. S., L. G. E. Wilkins, and C. Wedekind. 2013. MHC class I expression dependent on bacterial infection and parental factors in whitefish embryos (Salmonidae). *Molecular Ecology* 22:5256-5269.

Conner, J. K. 2012. Quantitative genetic approaches to evolutionary constraint: how useful? *Evolution* 66:3313-3320.

Czesak, M. E. and C. W. Fox. 2003. Evolutionary ecology of egg size and number in a seed beetle: genetic trade-off differs between environments. *Evolution* 57:1121-1132.

Delph, L. F., J. C. Steven, I. A. Anderson, C. R. Herlihy, and E. D. Brodie III. 2011. Elimination of a genetic correlation between the sexes via artificial correlational selection. *Evolution* 65:2872–2880.

Doroszuk, A., M. W. Wojewodzic, G. Gort, and J. E. Kammenga. 2008. Rapid divergence of genetic variance-covariance matrix within a natural population. *The American Naturalist* 3:291-304.

Eroukhmanoff, F. and E. I. Svensson. 2011. Evolution and stability of the G-matrix during the colonization of a novel environment. *Journal of Evolutionary Biology* 24:1363-1373.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics, Fourth Edition. Essex, UK: Longman Group Ltd.

Fox, J. and S. Weisberg. 2011. An R Companion to Applied Regression, Second Edition. Thousand Oaks, CA: Sage. URL http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Hansen, T. F. 2006. The evolution of genetic architecture. *Annual Review of Ecology, Evolution, and Systematics* 37:123-157.

Hansen, T. F. and D. Houle. 2008. Measuring and comparing evolvability and constraint in multivariate characters. *Journal of Evolutionary Biology* 21:1201-1219.

Haselhorst, M. S., C. E. Edwards, M. J. Rubin, and C. Weinig. 2011. Genetic architecture of life history traits and environment-specific trade-offs. *Molecular Ecology* 20:4042-4058.

Hayes, J. F., and W. G. Hill. 1981. Modifications of estimates of parameters in the construction of genetic selection indices (‘bending’). *Biometrics* 37:483–493.

Hill, W. G. and R. Thompson. 1978. Probabilities of non-positive definite between-group or genetic covariance matrices. *Biometrics* 34: 429-439.

Ingleby, F. C., D. J. Hosken, K. Flowers, M. F. Hawkes, S. M. Lane, J. Rapkin, C. M. House, M. D. Sharma, and J. Hunt. 2014. Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in *Drosophila simulans*. *Journal of Evolutionary Biology* 27: 700-713.

Johansson, F., M. I. Lind, P. K. Ingvarsson, and F. Bokma. 2012. Evolution of the G-matrix in life history traits in the common frog during a recent colonization of an island system. *Evolutionary Ecology* 26:863-878.

Jones, A. G., S. J. Arnold, and R. Bürger. 2003. Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution* 57:1747-1760.

de Jong, G. 1990. Genetics of reaction norms. *Journal of Evolutionary Biology* 3:447-468.

King, E. G., D. A. Roff, and D. J. Fairbairn. 2011. The evolutionary genetics of acquisition and allocation in the wing dimorphic cricket, *Gryllus firmus*. *Evolution* 65:2273-2285.

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

Kingsolver, J. G., S. E. Diamond, A. M. Siepielski, and S. M. Carlson. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations, and future directions. *Evolutionary Ecology* 26:1101-1118.

Kirkpatrick, M. 2009. Patterns of quantitative genetic variation in multiple dimensions. *Genetica* 136:271-284.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution* 33:402–416.

Ledón-Rettig, C. C., D. W. Pfennig, and E. J. Crespi. 2010. Diet and hormonal manipulation reveal cryptic genetic variation: implications for the evolution of novel feeding strategies. *Proceedings of the Royal Society—B* 277: 3569-3578.

Ledón-Rettig, C. C., D. W. Pfennig, A. J. Chunco, and I. Dworkin. 2014. Cryptic genetic variation in natural populations: a predictive framework. *Integrative and Comparative Biology*. 54:783–93.

MacColl, A. D. C. 2011. The ecological causes of evolution. *Trends in Ecology and Evolution* 26:514–22.

McGuigan, K. 2006. Studying phenotypic evolution using multivariate quantitative genetics. *Molecular Ecology* 15:883-896.

McGuigan, K. and C. Sgrò. 2009. Evolutionary consequences of cryptic genetic variation. *Trends in Ecology and Evolution* 6:305-311.

McGuigan, K., N. Nishimura, M. Currey, D. Hurwit, and W. A. Cresko. 2011. Cryptic genetic variation and body size evolution in threespine stickleback. *Evolution* 65:1203-1211.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* 11:15–9.

Nakagawa, S. and I. C. Cuthill. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews* 82:591-605.

Paaby, A. B. and M. V. Rockman. 2014. Cryptic genetic variation: evolution's hidden substrate. *Nature Reviews Genetics* 15:247-258.

Phillips, P. C. and S. J. Arnold. 1989. Visualizing multivariate selection. *Evolution* 43:1209-1222.

Phillips, P. C. and S. J. Arnold. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution* 53:1506-1515.

Phillips, P. C., and K. L. McGuigan. 2006. Evolution of genetic variance-covariance structure. In C. W. Fox and J. B. Wolf, eds. Evolutionary genetics, concepts and case studies. pp 310-325. Oxford University Press, Oxford.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology and Evolution* 20:481-486.

Pitchers, W., J.B. Wolf, T. Tregenza, J. Hunt, and I. Dworkin. 2014. Evolutionary rates for multivariate traits: the role of selection and genetic variation. *Philosophical Transactions of the Royal Society B* 369:20130252.

Queitsch, C., T. A. Sangster, and S. Lindquist. 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417:618–624.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Roff, D. A. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture.

Roff, D. A. 2000. The evolution of the G matrix: selection or drift? *Heredity* 84:135-142.

Roff, D. A. and T. Mousseau. 1999. Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. *Journal of Evolutionary Biology* 12:361-369.

Roff, D. A., J. M. Prokkola, I. Krams, and M. J. Rantala. 2012. There is more than one way to skin a G matrix. *Journal of Evolutionary Biology* 25:1113-1126.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* 24:35-68.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* 50:1766-1774.

Sgrò, C. M. and A. A. Hoffman. 2004. Genetic correlations, tradeoffs, and environmental variation. *Heredity* 93:241-248.

Sikkink, K. L., R. M. Reynolds, W. A. Cresko, and P. C. Phillips. In press. Environmentally induced changes in correlated responses to selection reveal variable pleiotropy across a complex genetic network. *Evolution*.

Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics. *Trends in Ecology and Evolution* 7:320-327.

Teplitsky, C., M. R. Robinson, and J. Merilä. 2014. Evolutionary potential and constraints in wild populations. in Quantitative Genetics in the Wild, ed. A. Charmantier, D. Garant, and L. E. B. Kruuk. Oxford University Press, Oxford, UK.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive variation. *Evolution* 42:1342–1347.

Waddington, C. H. 1956. Genetic assimilation of the bithorax phenotype. *Evolution* 10:1-13.

Walsh, B. and M. Lynch. Evolution and Selection of Quantitative Traits. Chapter 36: Comparisons of **G** and its Stability. Last modified 26 June 2014; accessed 25 February 2015. http://nitro.biosci.arizona.edu/zbook/NewVolume_2/pdf/Chapter36.pdf.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments. *Molecular Ecology* 5:1153-1163.

Studies included in the meta-analysis

Andersson, S. 1997. Genetic constraints on phenotypic evolution in Nigella (Ranunculaceae). *Biological Journal of the Linnean Society* 62:519–532.

Arnold, S. J. 1981. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. *Evolution* 35:489–509.

Arnold, S. J. 1988. Quantitative genetics and selection in natural populations: microevolution of vertebral numbers in the garter snake *Thamnophis elegans*. pp. 619–636 *in* B. S. Weir, M. M. Goodman, E. J. Eisen, and G. Namkoong, eds. Proceedings of the Second International Conference on Quantitative Genetics. Sinauer Associates, Inc., Sunderland, Massachusetts.

Arnold, S. J., and P. C. Phillips. 1999. Hierarchical comparison of genetic variance-covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. *Evolution* 53:1516–1527.

Ashman, T.-L. 2003. Constraints on the evolution of males and sexual dimorphism: field estimates of genetic architecture of reproductive traits in three populations of gynodioecious *Fragaria virginiana*. *Evolution* 57:2012–2025.

Auld, J. R. 2010. The effects of predation risk on mating system expression in a freshwater snail. *Evolution* 64:3476-3494.

Auld, J. R. 2010. Data from: The effects of predation risk on mating system expression in a freshwater snail. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.1690

Bacigalupe, L. D., K. Barrientos, A. P. Beckerman, M. J. Carter, C. C. Figueroa, S. P. Foster, A. J. Moore, A. X. Silva, and R. F. Nespolo. 2013. Can invasions occur without change? A comparison of G-matrices and selection in the peach-potato aphid, *Myzus persicae*. *Ecology and Evolution* 3:5109–5118.

Bégin, M. and D. A. Roff. 2001. An analysis of G matrix variation in two closely related cricket species, *Gryllus firmus* and *G. pennsylvanicus*. *Journal of Evolutionary Biology* 14:1-13.

Bégin, M., D. A. Roff, and V. Debat. 2004. The effect of temperature and wing morphology on quantitative genetic variation in the cricket *Gryllus firmus*, with an appendix examining the statistical properties of the Jackknife-MANOVA method of matrix comparison. *Journal of Evolutionary Biology* 17:1255-1267.

Bennington, C. C. and J. B. McGraw. 1996. Environment-dependence of quantitative genetic parameters in *Impatiens pallida*. *Evolution* 50:1083-1097.

Berger, D., E. Postma, W. U. Blanckenhorn, and R. J. Walters. 2013. Quantitative genetic divergence and standing genetic (co)variance in thermal reaction norms along latitude. *Evolution* 67:2385–2399.

Brock, M. T. and C. Weinig. 2007. Plasticity and environment-specific covariances: an investigation of floral-vegetative and within flower correlations. *Evolution* 61:2913-2924.

Brock, M. T., J. M. Dechaine, F. L. Iniguez-Luy, J. N. Maloof, J. R. Stinchcombe, and C. Weinig. 2010. Floral genetic architecture: an examination of QTL architecture underlying floral (co)variation across environments. *Genetics* 186:1451-1465.

Brodie III, E. D. 1993. Homogeneity of the genetic variance-covariance matrix for antipredator traits in two natural populations of the garter snake *Thamnophis ordinoides*. *Evolution* 47:844–854.

Calsbeek, B., S. Lavergne, M. Patel, and J. Molofsky. 2011. Comparing the genetic architecture and potential response to selection of invasive and native populations of reed canary grass. *Evolutionary Applications* 4:726–735.

Cano, J. M., A. Laurila, J. Palo, and J. Merilä. 2004. Population differentiation in **G** matrix structure due to natural selection in *Rana temporaria*. *Evolution* 58:2013-2020.

Carr, D. E., and C. B. Fenster. 1994. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. *Heredity* 72:606–618.

Caruso, C. M. 2004. The quantitative genetics of floral trait variation in *Lobelia*: potential constraints on adaptive evolution. *Evolution* 58:732–740.

Collins, R. D., Y. Jang, K. Reinhold, and M. D. Greenfield. 1999. Quantitative genetics of ultrasonic advertisement signalling in the lesser waxmoth *Achroia grisella* (Lepidoptera: Pyralidae). *Heredity* 83:644–651.

Czesak, M. E. and C. W. Fox. 2003. Evolutionary ecology of egg size and number in a seed beetle: genetic trade-off differs between environments. *Evolution* 57:1121-1132.

Dai, X., S. Gao, and D. Liu. 2014. Genetic basis and selection for life-history trait plasticity on alternative host plants for the cereal aphid *Sitobion avenae*. *PLoS One* 9:e106179.

Delcourt, M., and H. D. Rundle. 2011. Condition dependence of a multicomponent sexual display trait in *Drosophila serrata*. *The American Naturalist* 177:812–23.

Delcourt, M., M. W. Blows, and H. D. Rundle. 2009. Sexually antagonistic genetic variance for fitness in an ancestral and a novel environment. *Proceedings of the Royal Society—B* 276:2009-2014.

Delcourt M., M. W. Blows, H.D. Rundle. 2009. Data from: Sexually antagonistic genetic variance for fitness in an ancestral and a novel environment. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.0pn48s2t

Delesalle, V. A, and S. J. Maze. 1995. The structure of phenotypic variation in gender and floral traits within and among populations of *Spergularia marina* (Caryophyllaceae). *American Journal of Botany* 82:798–810.

Donohue, K., E. H. Pyle, D. Messiqua, M. S. Heschel, and J. Schmitt. 2000. Density dependence and population differentiation of genetic architecture in *Impatiens capensis* in natural environments. *Evolution* 54:1969-1981.

Doroszuk, A., M. W. Wojewodzic, G. Gort, and J. E. Kammenga. 2008. Rapid divergence of genetic variance-covariance matrix within a natural population. *The American Naturalist* 3:291-304.

Elle, E. 1998. The quantitative genetics of sex allocation in the andromonoecious perennial, *Solanum carolinense* (L.). *Heredity* 80:481–488.

Engqvist, L. 2007. Environment-dependent genetic correlations between development time and body mass in a scorpionfly. *Zoology* 110:344-353.

Eroukhmanoff, F. and E. I. Svensson. 2011. Evolution and stability of the G-matrix during the colonization of a novel environment. *Journal of Evolutionary Biology* 24:1363-1373.

Etterson, J. R. and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. *Science* 294:151-154.

Fong, D.W. 1989. Morphological evolution of the amphipod *Gammarus minus* in caves: Quantitative genetic analysis. *The American Midland Naturalist* 121:361–378.

Fournier-Level, A., A. M. Wilczek, M. D. Cooper, J. L. Roe, J. Anderson, D. Eaton, B. T. Moyers, R. H. Petipas, R. N. Schaeffer, B. Pieper, M. Reymond, M. Koornneef, S. M. Welch, D. L. Remington, and J. Schmitt. 2013. Paths to selection on life history loci in different natural environments across the native range of *Arabidopsis thaliana*. *Molecular Ecology* 22:3552-3566.

Fournier-Level, A., A. M. Wilczek, M. D. Cooper, J. L. Roe, J. Anderson, D. Eaton, B. T. Moyers, R. H. Petipas, R. N. Schaeffer, B. Pieper, M. Reymond, M. Koornneef, S. M. Welch, D. L. Remington, and J. Schmitt. 2013. Data from: Paths to selection on life history loci in different natural environments across the native range of Arabidopsis thaliana. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.1pg3n

Franks, S. J., G. S. Wheeler, and C. Goodnight. 2012. Genetic variation and evolution of secondary compounds in native and introduced populations of the invasive plant *Melaleuca quinquenervia*. *Evolution* 66:1398–1412.

Garant, D., J. D. Hadfield, L. E. B. Kruuk, and B. C. Sheldon. 2008. Stability of genetic variance and covariance for reproductive characters in the face of climate change in a wild bird population. *Molecular Ecology* 17:179-188.

Gebhardt, M. D. and S. C. Stearns. 1988. Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*. *Journal of Evolutionary Biology* 1:335-354.

Gemeno, C., A. J. Moore, R. F. Preziosi, and K. F. Haynes. 2001. Quantitative genetics of signal evolution: A comparison of the pheromonal signal in two populations of the cabbage looper, *Trichoplusia ni*. *Behavior Genetics* 31:157–165.

Giesel, J. T. 1986. Genetic correlation structure of life history variables in outbred, wild *Drosophila melanogaster*: effects of photoperiod regimen. *The American Naturalist* 128:593-603.

Grill, C. P., A. J. Moore, and E. D. Brodie III. 1997. The genetics of phenotypic plasticity in a colonizing population of the ladybird beetle, *Harmonia axyridis*. *Heredity* 78:261–269.

Guntrip, J., R. M. Sibly, and G. J. Holloway. 1997. The effect of novel environment and sex on the additive genetic variation and covariation in and between emergence body weight and development period in the cowpea weevil, *Callosobruchus maculatus* (Coleoptera, Bruchidae). *Heredity* 78:158-165.

Gutteling, E. W., A. Doroszuk, J. A. G. Riksen, Z. Prokop, J. Reszka, and J. E. Kammenga. 2007. Environmental influence on the genetic correlations between life-history traits in *Caenorhabditis elegans*. *Heredity* 98:206-213.

Haselhorst, M. S., C. E. Edwards, M. J. Rubin, and C. Weinig. 2011. Genetic architecture of life history traits and environment-specific trade-offs. *Molecular Ecology* 20:4042-4058.

Heath, K. D., and K. E. McGhee. 2012. Coevolutionary constraints? The environment alters tripartite interaction traits in a legume. *PLoS One* 7:e41567.

Higgie, M., and M. W. Blows. 2007. Are traits that experience reinforcement also under sexual selection? *The American Naturalist* 170:409–420.

Holloway, G. J., S. R. Povey, and R. M. Sibly. 1990. The effect of new environment on adapted genetic architecture. *Heredity* 64:323-330.

Hughes, K. A., F. H. Rodd, and D. N. Reznick. 2005. Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *Journal of Evolutionary Biology* 18:35–45.

Ingleby, F. C., D. J. Hosken, K. Flowers, M. F. Hawkes, S. M. Lane, J. Rapkin, C. M. House, M. D. Sharma, and J. Hunt. 2014. Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in *Drosophila simulans*. *Journal of Evolutionary Biology* 27: 700-713.

Ingleby, F. C., D. J. Hosken, K. Flowers, M. F. Hawkes, S. M. Lane, J. Rapkin, C. M. House, M. D. Sharma, and J. Hunt. 2014. Data from: Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in Drosophila simulans. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.r06rn

Johansson, F., M. I. Lind, P. K. Ingvarsson, and F. Bokma. 2012. Evolution of the G-matrix in life history traits in the common frog during a recent colonization of an island system. *Evolutionary Ecology* 26:863-878.

Kause, A. and J. P. Morin. 2001. Seasonality and genetic architecture of development time and body size of the birch feeding sawfly *Priophorus pallipes*. *Genetics Research* 78:31-40.

King, E. G., D. A. Roff, and D. J. Fairbairn. 2011. The evolutionary genetics of acquisition and allocation in the wing dimorphic cricket, *Gryllus firmus*. *Evolution* 65:2273-2285.

King, E. G., D. A. Roff, and D. J. Fairbairn. 2011. Data from: The evolutionary genetics of acquisition and allocation in the wing dimorphic cricket, Gryllus firmus. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.8727

Kingsolver, J. G., J. G. Shlichta, G. J. Ragland, and K. R. Massie. 2006. Thermal reaction norms for caterpillar growth depend on diet. *Evolutionary Ecology Research* 8:703-715.

Kraft, P. G., R. S. Wilson, C. E. Franklin, and M. W. Blows. 2006. Substantial changes in the genetic basis of tadpole morphology of *Rana lessonae* in the presence of predators. *Journal of Evolutionary Biology* 19:1813–1818.

Larsson, K. 1993. Inheritance of body size in the Barnacle Goose under different environmental conditions. *Journal of Evolutionary Biology* 6:195-208.

Lau, J. A., R. G. Shaw, P. B. Reich, P. Tiffin. 2014. Indirect effects drive evolutionary responses to global change. *New Phytologist* 201:335-343.

Lau, J. A., R. G. Shaw, P. B. Reich, P. Tiffin. 2013. Data from: Indirect effects drive evolutionary responses to global change. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.6sf28

Mallitt, K. L., S. P. Bonser, and J. Hunt. 2010. The plasticity of phenotypic integration in response to light and water availability in the pepper grass, *Lepidium bonariense*. *Evolutionary Ecology* 24:1321-1337.

McDaniel, S. F. 2005. Genetic correlations do not constrain the evolution of sexual dimorphism in the moss *Ceratodon purpureus*. *Evolution* 59:2353–2361.

Messina, F. J. and J. D. Fry. 2003. Environment-dependent reversal of a life-history trade-off in the seed beetle *Callosobruchus maculatus*. *Journal of Evolutionary Biology* 16:501-509.

Paccard, A., M. Vance, and Y. Willi. 2013. Weak impact of fine-scale landscape heterogeneity on evolutionary potential in *Arabidopsis lyrata*. *Journal of Evolutionary Biology* 26:2331-2340.

Podolsky, R. H., R. G. Shaw, and F. H. Shaw. 1997. Population structure of morphological traits in *Clarkia dudleyana*. II. Constancy of within-population genetic variance. *Evolution* 51:1785–1796.

Punzalan, D., M. Delcourt, and H. D. Rundle. 2014. Comparing the intersex genetic correlation for fitness across novel environments in the fruit fly, *Drosophila serrata*. *Heredity* 112:143-148.

Punzalan, D., M. Delcourt, and H. D. Rundle. 2014. Data from: Comparing the intersex genetic correlation for fitness across novel environments in the fruit fly,

Drosophila serrata. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.5892n

Rauter, C. M. and A. J. Moore. 2002. Quantitative genetics of growth and development time in the burying beetle *Nicrophorus pustulatus* in the presence and absence of post-hatching parental care. *Evolution* 56:96-110.

Relyea, R. A. 2005. The heritability of inducible defenses in tadpoles. *Journal of Evolutionary Biology* 18:856–66.

Rodríguez, R. L., and M. D. Greenfield. 2003. Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution* 57:1304–1313.

Roff, D. A. and T. Mousseau. 1999. Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. *Journal of Evolutionary Biology* 12:361-369.

Roff, D. A., T. Mousseau, A. P. Møller, F. de Lope, and N. Saino. 2004. Geographic variation in the G matrices of wild populations of the barn swallow. *Heredity* 93:8–14.

Ronsheim, M. L., and J. D. Bever. 2000. Genetic variation and evolutionary trade-offs for sexual and asexual reproductive modes in *Allium vineale* (Liliaceae). *American Journal of Botany* 87:1769–1777.

Sandquist, D. R., and J. R. Ehleringer. 2003. Population- and family-level variation of brittlebush (*Encelia farinosa*, Asteraceae) pubescence: Its relation to drought and implications for selection in variable environments. *American Journal of Botany* 90:1481–1486.

Scheiner, S. M., R. L. Caplan, and R. F. Lyman. 1991. The genetics of phenotypic plasticity. III. Genetic correlations and fluctuating asymmetries. *Journal of Evolutionary Biology* 4:51-68.

Seko, T., T. Miyatake, S. Fujioka, and F. Nakasuji. 2006. Genetic and environmental sources of egg size, fecundity and body size in the migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperiidae). *Population Ecology* 48:225–232.

Service, P. M. 2000. The genetic structure of female life history in *D. melanogaster*: comparisons among populations. *Genetical Research* 75:153–166.

Service, P. M. and M. R. Rose. 1985. Genetic covariation among life-history components: the effect of novel environments. *Evolution* 39:943-945.

Shakhatreh, Y., O. Kafawin, S. Ceccarelli, and H. Saoub. 2001. Selection of barley lines for drought tolerance in low-rainfall areas. *Journal of Agronomy and Crop Science* 186:119-127.

Sherrard, M. E., H. Maherali, and R. G. Latta. 2009. Water stress alters the genetic architecture of functional traits associated with drought adaptation in *Avena barbata*. *Evolution* 63:702-715.

Simmons, L. W. 2004. Genotypic variation in calling song and female preferences of the field cricket *Teleogryllus oceanicus*. *Animal Behaviour* 68:313–322.

Simons, A. M. and D. A. Roff. 1996. The effect of a variable environment on the genetic correlation structure in a field cricket. *Evolution* 50: 267-275.

Stanton, M. L., D. A. Thiede, and B. A. Roy. 2004. Consequences of intraspecific competition and environmental variation for selection in the mustard *Sinapsis*

arvensis: Contrasting ecological and evolutionary perspectives. *The American Naturalist* 164:736–752.

Stinchcombe, J. R. 2002. Environmental dependency in the expression of costs of tolerance to deer herbivory. *Evolution* 56:1063-1067.

Stinchcombe, J. R. and J. Schmitt. 2006. Ecosystem engineers as selective agents: the effects of leaf litter on emergence time and early growth in *Impatiens capensis*. *Ecology Letters* 9:258-270.

Strobbe, F., and R. Stoks. 2004. Life history reaction norms to time constraints in a damselfly: Differential effects on size and mass. *Biological Journal of the Linnean Society* 83:187–196.

Teplitsky, C., M. Tarka, A. P. Møller, S. Nakagawa, J. Balbontin, T. A. Burke, C. Doutrelant, A. Gregoire, B. Hansson, D. Hasselquist, L. Gustafsson, F. De Lope, A. Marzal, J. A. Mills, N. T. Wheelwright, J. W. Yarrall, and A. Charmantier. 2014. Assessing multivariate constraints to evolution across ten long-term avian studies. *PLoS ONE* 9:e90444.

Tonsor S. J., S. M. Scheiner, and T. W. Elnaccash. 2013. Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness. *Evolution* 67: 2923–2935.

Tonsor S. J., S. M. Scheiner, and T. W. Elnaccash. 2013. Data from: Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.j00tn

Via, S. 1984. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. *Evolution* 38:896-905.

Via, S. and J. Conner. 1995. Evolution in heterogeneous environments: genetic variability within and across different grains in *Tribolium castaneum*. *Heredity* 74:80-90.

Waldmann, and Andersson. 2000. Comparison of genetic (co)variance matrices within and between *Scabiosa canescens* and *S. columbaria*. *Journal of Evolutionary Biology* 13:826–835.

Widén, B., S. Andersson, G. Y. Rao, and M. Widén. 2002. Population divergence of genetic (co)variance matrices in a subdivided plant species, *Brassica cretica*. *Journal of Evolutionary Biology* 15:961–970.

Windig, J. J. 1994. Genetic correlations and reaction norms in wing pattern of the tropical butterfly *Bicyclus anynana*. *Heredity* 73:459-470.

Figures and Tables

Table 1. Pearson's correlations between changes in different aspects of **G**. P-values are in parentheses, and significant correlations after Bonferroni correction ($\alpha = 0.005$) are indicated in bold. Sample sizes for each correlation are along the diagonal.

	Structure of **G**			Evolutionary trajectory	
	Total genetic variance	Relative magnitude	Orientation	Direction of the response	Response difference
Total genetic variance	*47*	0.18 (0.225)	0.21 (0.162)	0.05 (0.739)	0.22 (0.139)
Relative magnitude		*99*	0.19 (0.054)	**0.30 (0.002)**	0.15 (0.146)
Orientation			*99*	**0.57 (<0.001)**	**0.32 (0.001)**
Direction of the response				*99*	**0.73 (<0.001)**
Response difference					*99*

Table 2. Results from general linear models testing for differences in **G** in between environments and between populations ("Comparison" in the table). A separate model was run with each of the five matrix comparison metrics as the dependent variable. "N. traits": number of traits in **G**; "sample size": number of half-sib families (narrow-sense G-matrices) or full-sib families or genotypes (broad-sense G-matrices) used to estimate **G**. Bold entries are significant ($\alpha = 0.05$).

	Structure of **G**						Evolutionary trajectory			
	Total genetic variance		Relative magnitude		Orientation		Direction of the response		Response difference	
	$F_{1,39}$	P	$F_{1,91}$	P	$F_{1,91}$	P	$F_{1,91}$	P	$F_{1,91}$	P
Comparison	0.053	0.819	3.374	0.070	**4.812**	**0.031**	1.692	0.197	1.146	0.287
N. traits	0.057	0.813	2.827	0.096	**6.189**	**0.015**	0.350	0.555	2.667	0.106
Comparison × n. traits	0.300	0.587	**4.552**	**0.036**	**4.957**	**0.028**	3.686	0.058	2.818	0.097
Sample size	3.184	0.082	1.007	0.318	0.573	0.451	1.307	0.256	0.219	0.641

Table 3. Results from general linear models testing the effect of environmental novelty and the change in mean phenotype on between-environment differences in **G**. A separate model was run with each of the five matrix comparison metrics as the dependent variable. Bold entries are significant ($\alpha = 0.05$).

	Structure of **G**						Evolutionary trajectory			
	Total genetic variance		Relative magnitude		Orientation		Direction of the response		Response difference	
	$F_{1,15}$	P	$F_{1,40}$	P	$F_{1,40}$	P	$F_{1,40}$	P	$F_{1,40}$	P
Novelty	0.240	0.631	0.020	0.888	0.127	0.723	0.000	0.999	1.649	0.207
N. traits	0.007	0.933	2.249	0.142	3.857	0.057	0.086	0.771	2.065	0.159
Sample size	1.566	0.230	0.029	0.865	0.119	0.732	1.576	0.217	0.182	0.672
	$F_{1,17}$	P	$F_{1,31}$	P	$F_{1,31}$	P	$F_{1,31}$	P	$F_{1,31}$	P
Change in mean phen.	2.046	0.171	1.531	0.225	2.196	0.148	2.002	0.167	0.496	0.486
N. traits	0.237	0.632	0.730	0.399	1.700	0.202	0.659	0.423	**14.226**	**<0.001**

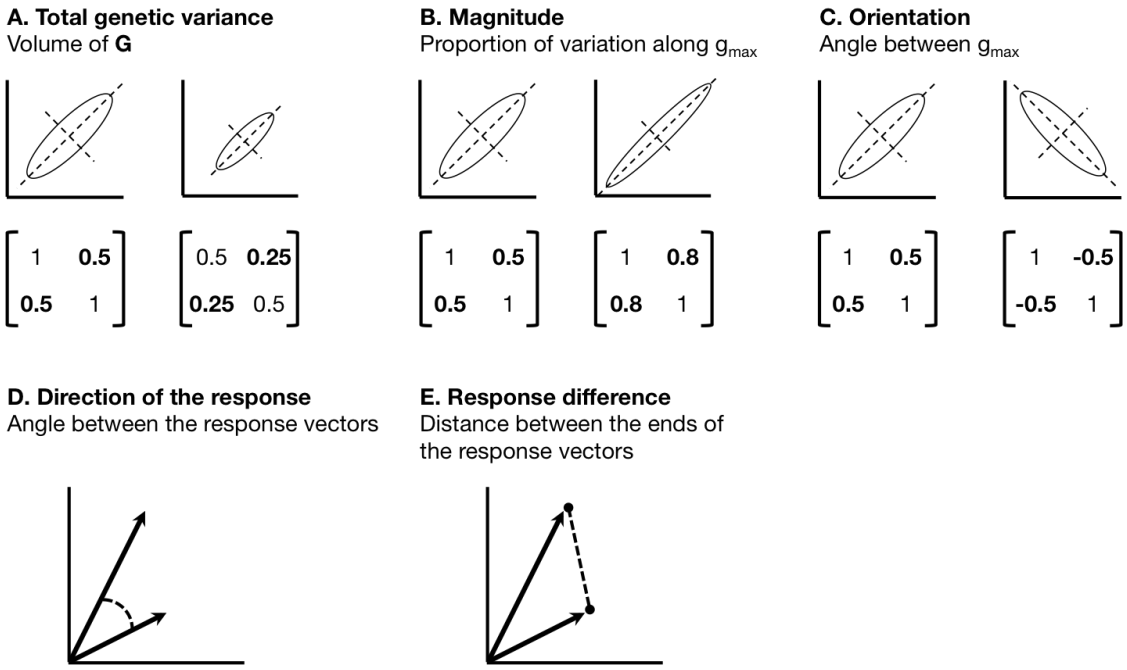

Figure 1. The metrics used to compare **G**. (A-C) Matrix comparison metrics that measure differences in the structure of two matrices. The two graphs in each panel show the correlation between two hypothetical traits (one hypothetical trait is on the x-axis and one is on the y-axis), with the corresponding G-matrix depicted in the bottom row. (D-E) Metrics of change in the predicted evolutionary trajectory. The graph in each panel shows the multivariate response to selection for two hypothetical traits (one on the x-axis and one on the y-axis). The two vectors in each panel indicate the direction of the response to selection in two G-matrices.

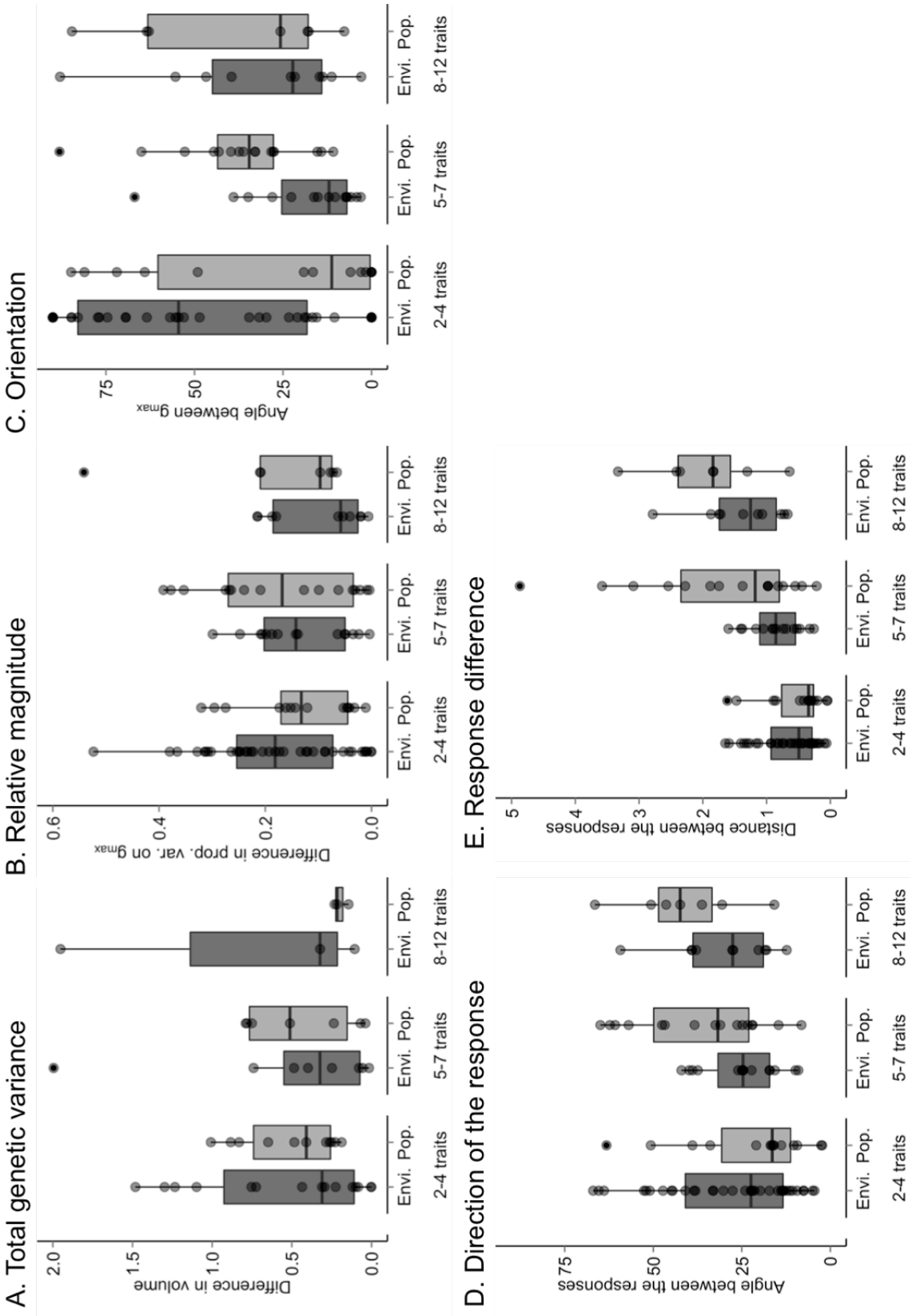
A. Total genetic variance
B. Relative magnitude
C. Orientation
D. Direction of the response
E. Response difference
Difference in volume
Difference in prop. var. on gmax
Angle between gmax
Angle between the responses
Distance between the responses
Envi. Pop.
2-4 traits
5-7 traits
8-12 traits

Figure 2. Box-and whisker plots of differences in the structure of **G** (A-C) and the predicted evolutionary trajectory (D-E) in between-environment (dark gray) and between-population (light gray) comparisons. Each data point is a single comparison of two G-matrices. See Table 2 for statistics.

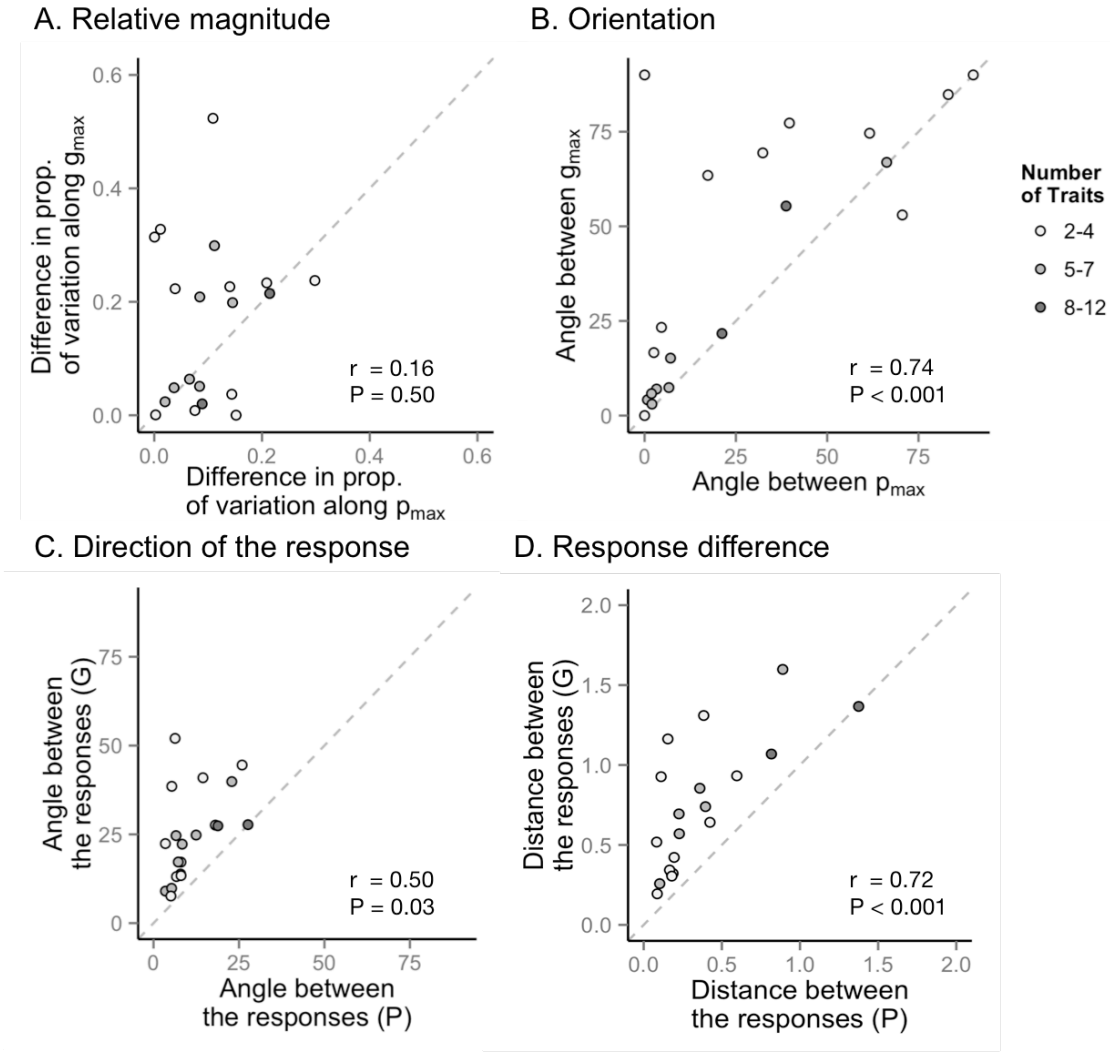

Figure 3. Plots of the correlation between (A-B) between-environment differences in the structure of phenotypic correlations (**P**) and genetic correlations (**G**) and (C-D) between-environment differences in the predicted evolutionary trajectory based on **P** and based on **G**. Total genetic variance was omitted because there were not enough phenotypic covariance matrices available. The dashed gray line is the 1:1 line.

Supplementary Tables

Supplementary Table 1. Studies included in the meta-analysis. Abbreviations: “Y” = yes, “N” = no, “NA” = not applicable, “UK” = unknown.

(*Table begins on the next page*)

Supplementary Table 2. Results of five separate general linear models testing for an effect of G-matrix type (narrow- or broad-sense) on the change in **G**. In each of these models, the type of estimate (narrow- or broad-sense) was the independent variable, and each of the five matrix comparison metrics was the dependent variable.

Metric	df	F	P
Total genetic variance	1, 45	0.177	0.676
Relative magnitude	1, 97	0.053	0.818
Orientation	1, 97	0.686	0.410
Direction of the response	1, 97	3.059	0.083
Response difference	1, 97	2.515	0.116

Reference	Comparison	Gen. cov.	Phen. cor.	Phen. means	Taxon	Species	Sample unit	Sample size	N. traits	Environment	Novel
Andersson 1997	Populations	N	NA	NA	Plant	Nigella degenii	Full_Sib	80	5	NA	NA
Arnold 1981	Populations	N	NA	NA	Reptile	Thamnophis elegans	Full_Sib	14.5	12	NA	NA
Arnold 1988	Populations	Y	NA	NA	Reptile	Thamnophis elegans	Full_Sib	100	2	NA	NA
Arnold and Phillips 1999	Populations	Y	NA	NA	Reptile	Thamnophis elegans	Full_Sib	129	6	NA	NA
Ashman 2003	Populations	N	NA	NA	Plant	Fragaria virginiana	Half_Sib	68	5	NA	NA
Auld 2010	Environments	N	N	Y	Snail	Physa acuta	Full_Sib	30	4	Predator, Mating	N
Bacigalupe et al. 2013	Populations	Y	NA	NA	Insect	Myzus persicae	Genotype	22	3	NA	NA
Begin and Roff 2001	Environments	Y	Y	Y	Insect	Gryllus firmus	Full_Sib	46	5	Temperature, Photoperiod	N
Begin and Roff 2001	Environments	Y	Y	Y	Insect	Gryllus pennsylvanicus	Full_Sib	39	5	LabField	N
Begin et al. 2004	Environments	Y	Y	Y	Insect	Gryllus firmus	Full_Sib	58	5	Temperature	N
Begin et al. 2004	Environments	Y	Y	Y	Insect	Gryllus firmus	Full_Sib	38.5	5	Temperature	N
Bennington and McGraw 1996	Environments	Y	N	N	Plant	Impatiens pallida	Half_Sib	60	4	Field	Y
Berger et al. 2013	Environments	Y	N	N	Insect	Sepsis punctum	Isofemale Line	71	5	Diet	N
Berger et al. 2013	Populations	Y	NA	NA	Insect	Sepsis punctum	Isofemale Line	27	5	NA	NA
Brock and Weinig 2007	Environments	N	N	N	Plant	Arabidopsis thaliana	Genotype	15	7	Light	N
Brock et al. 2010	Environments	N	Y	Y	Plant	Brassica rapa	RIL	150	11	LabField	N

Reference	Comparison	Gen. cov.	Phen. cor.	Phen. means	Taxon	Species	Sample unit	Sample size	N. traits	Environment	Novel
Brodie 1993	Populations	N	NA	NA	Reptile	Thamnophis ordinoides	Full_Sib	101.5	4	NA	NA
Calsbeek et al. 2011	Populations	Y	NA	NA	Plant	Phalaris arundinacea	Genotype	24.5	2	NA	NA
Cano et al. 2004	Environments	Y	N	N	Amphibian	Rana temporaria	Half_Sib	5	4	Water	N
Cano et al. 2004	Environments	Y	N	N	Amphibian	Rana temporaria	Half_Sib	5	4	Water	N
Cano et al. 2004	Populations	Y	NA	NA	Amphibian	Rana temporaria	Half_Sib	5	4	NA	NA
Carr and Fenster 1994	Populations	N	NA	NA	Plant	Mimulus guttatus	Full_Sib	24	7	NA	NA
Carr and Fenster 1994	Populations	N	NA	NA	Plant	Mimulus micranthus	Full_Sib	8.5	7	NA	NA
Caruso 2004	Populations	N	NA	NA	Plant	Lobelia siphilitica	Half_Sib	41	7	NA	NA
Collins et al. 1999	Environments	N	Y	Y	Insect	Achroia grisella	Half_Sib	47	3	Social	N
Czesak and Fox 2003	Environments	N	Y	Y	Insect	Stator limbatus	Half_Sib	126	3	Diet	N
Dai et al. 2014	Environments	N	N	N	Insect	Sitobion avenae	Genotype	8	6	Diet	N
Dai et al. 2014	Populations	N	NA	NA	Insect	Sitobion avenae	Genotype	16	6	NA	NA
Delcourt and Rundle 2011	Environments	Y	N	Y	Insect	Drosophila serrata	Half_Sib	92	8	Diet	N
Delcourt et al. 2009	Environments	Y	N	Y	Insect	Drosophila serrata	Half_Sib	81	2	Diet	Y
Delesalle and Maze 1995	Populations	N	NA	NA	Plant	Spergularia marina	Full_Sib	15	8	NA	NA
Donohue et al. 2000	Environments	N	N	N	Plant	Impatiens capensis	Genotype	18	9	Light, Competition	N
Doroszuk et al. 2008	Populations	Y	NA	NA	Nematode	Acrobeloides nanus	Genotype	51.5	3	NA	NA

Reference	Comparison	Gen. cov.	Phen. cor.	Phen. means	Taxon	Species	Sample unit	Sample size	N. traits	Environment	Novel
Elle 1998	Populations	N	NA	NA	Plant	Solanum carolinense	Half_Sib	28	6	NA	NA
Enqvist 2007	Environments	N	N	Y	Insect	Panorpa cognata	Full_Sib	27	2	Diet	N
Eroukhmanoff and Svensson 2011	Populations	Y	NA	NA	Crustacean	Asellus aquaticus	Half_Sib	24	7	NA	NA
Eroukhmanoff and Svensson 2011	Populations	Y	NA	NA	Crustacean	Asellus aquaticus	Half_Sib	26	7	NA	NA
Etterson and Shaw 2001	Environments	N	N	N	Plant	Chamaecrista fasciculata	Half_Sib	45	3	Field	Y
Etterson and Shaw 2001	Environments	N	N	N	Plant	Chamaecrista fasciculata	Half_Sib	48	3	Field	Y
Etterson and Shaw 2001	Environments	N	N	N	Plant	Chamaecrista fasciculata	Half_Sib	50	3	Field	Y
Fong 1989	Populations	N	NA	NA	Crustacean	Gammarus minus	Full_Sib	53	8	NA	NA
Fournier-Level et al. 2013	Environments	Y	Y	Y	Plant	Arabidopsis thaliana	RIL	116	5	Field	UK
Franks et al. 2012	Populations	Y	NA	NA	Plant	Melaleuca quinquenervia	Full_Sib	60	3	NA	NA
Garant et al. 2008	Environments	Y	N	Y	Bird	Parus major	Animal Model	NA	3	Temperature	UK
Gebhardt and Stearns 1988	Environments	Y	N	Y	Insect	Drosophila mercatorum	Half_Sib	11	2	Diet	N
Gemeno et al. 2001	Populations	N	NA	NA	Insect	Trichoplusia ni	Half_Sib	17.5	6	NA	NA
Giesel 1986	Environments	N	N	N	Insect	Drosophila melanogaster	Half_Sib	12	3	Photoperiod	N
Grill et al. 1997	Environments	N	Y	Y	Insect	Harmonia axyridis	Full_Sib	24	4	Diet	Y
Guntrip et al. 1997	Environments	Y	N	Y	Insect	Callosobruchus maculatus	Half_Sib	40	2	Humidity	Y
Gutteling et al. 2007	Environments	N	N	Y	Nematode	Caenorhabditis elegans	RIL	80	3	Temperature	UK

Reference	Comparison	Gen. cov.	Phen. cor.	Phen. means	Taxon	Species	Sample unit	Sample size	N. traits	Environment	Novel
Haselhorst et al. 2011	Environments	N	N	Y	Plant	Brassica rapa	RIL	152	7	Temperature, Photoperiod	UK
Heath and McGhee 2012	Environments	Y	Y	Y	Plant	Medicago truncatula	Genotype	55	2	Light	N
Higgie and Blows 2007	Populations	Y	NA	NA	Insect	Drosophila serrata	Isofemale Line	128.5	8	NA	NA
Holloway et al. 1990	Environments	N	N	Y	Insect	Sitophilus oryzae	Half_Sib	67	4	Diet	Y
Hughes et al. 2005	Populations	N	NA	NA	Fish	Poecilia reticulata	Full_Sib	8	3	NA	NA
Ingleby et al. 2014	Environments	Y	Y	Y	Insect	Drosophila simulans	RIL	60	3	Temperature, Diet	Y
Johansson et al. 2011	Populations	Y	NA	NA	Amphibian	Rana temporaria	Full_Sib	10	2	NA	NA
Kause and Morin 2001	Environments	N	Y	N	Insect	Priophorus pallipes	Full_Sib	60	2	Diet	N
King et al. 2011	Environments	N	Y	Y	Insect	Gryllus firmus	Half_Sib	63	3	Diet	UK
Kingsolver et al. 2006	Environments	Y	N	N	Insect	Pieris rapae	Full_Sib	30	4	Diet	UK
Kraft et al. 2006	Environments	Y	N	N	Amphibian	Rana lessonae	Half_Sib	13	3	Predator	N
Larsson 1993	Environments	Y	N	Y	Bird	Branta leucopis	Full_Sib	24	3	Quality	N
Lau et al. 2014	Environments	Y	Y	Y	Plant	Arabidopsis thaliana	RIL	60	4	CO_2, Competition	UK
Mallitt et al. 2010	Environments	N	N	N	Plant	Lepidium bonariense	Genotype	13	12	Light, Water	UK
McDaniel 2005	Populations	N	NA	NA	Plant	Ceratodon purpureus	Haploid_Sib	33	6	NA	NA
Messina and Fry 2003	Environments	N	Y	Y	Insect	Callosobruchus maculatus	Half_Sib	94	3	Diet	N
Paccard et al. 2013	Environments	Y	N	Y	Plant	Arabidopsis lyrata	Full_Sib	22	7	Water	UK
Paccard et al. 2013	Environments	Y	N	Y	Plant	Arabidopsis lyrata	Full_Sib	22	7	Water	UK

Reference	Comparison	Gen. cov.	Phen. cor.	Phen. means	Taxon	Species	Sample unit	Sample size	N. traits	Environment	Novel
Podolsky et al. 1997	Populations	Y	NA	NA	Plant	Clarkia dudleyana	Half_Sib	82.5	5	NA	NA
Punzalan et al. 2014	Environments	Y	N	Y	Insect	Drosophila serrata	RIL	42	2	Diet	Y
Rauter and Moore 2002	Environments	N	Y	N	Insect	Nicrophorus pustulatus	Half_Sib	69	8	Parental care	N
Relyea 2005	Environments	N	N	N	Amphibian	Rana sylvatica	Half_Sib	21	10	Predator	N
Rodriguez and Greenfield 2003	Environments	N	N	N	Insect	Achroia grisella	Full_Sib	20	4	Temperature	UK
Roff and Mousseau 1999	Populations	Y	NA	NA	Insect	Allonemobius socius	Full_Sib	30	2	NA	NA
Roff et al. 2004	Populations	Y	NA	NA	Bird	Hirundo rustica	Full_Sib	60	6	NA	NA
Ronsheim and Bever 2000	Environments	N	N	N	Plant	Allium vineale	Genotype	16	7	Diet	UK
Sandquist and Ehleringer 2003	Populations	N	NA	NA	Plant	Encelia farinosa	Full_Sib	10	3	NA	NA
Scheiner et al. 1991	Environments	N	Y	Y	Insect	Drosophila melanogaster	Half_Sib	587	6	Temperature	UK
Seko et al. 2006	Environments	N	Y	Y	Insect	Parnara guttata	Full_Sib	17	3	Temperature, Photoperiod	UK
Service 2000	Populations	Y	NA	NA	Insect	Drosophila melanogaster	Half_Sib	127.5	4	NA	NA
Service and Rose 1985	Environments	Y	N	Y	Insect	Drosophila melanogaster	Half_Sib	46.5	2	Diet	Y
Shakhatreh et al. 2001	Environments	N	N	N	Plant	Hordeum vulgare	Genotype	84	2	Water	UK
Sherrard et al. 2009	Environments	N	N	Y	Plant	Avena barbata	RIL	26	11	Water	UK
Simmons 2004	Populations	N	NA	NA	Insect	Teleogryllus oceanicus	Full_Sib	12	9	NA	NA
Simons and Roff 1996	Environments	N	Y	N	Insect	Gryllus pennsylvanicus	Full_Sib	69	7	LabField	UK

Reference	Comparison	Gen. cov.	Phen. cor.	Phen. means	Taxon	Species	Sample unit	Sample size	N. traits	Environment	Novel
Stanton et al. 2004	Environments	N	N	N	Plant	Sinapsis arvensis	Half_Sib	94	9	Light, Competition	N
Stinchcombe 2002	Environments	N	N	N	Plant	Ipomoea hederacea	Genotype	18	2	Herbivore	N
Stinchcombe and Schmitt 2006	Environments	Y	N	Y	Plant	Impatiens capensis	RIL	45	8	Soil	UK
Strobbe and Stoks 2004	Environments	N	N	N	Insect	Enallagma cyathigerum	Full_Sib	13	5	Photoperiod	N
Teplitsky et al. 2014	Populations	Y	NA	NA	Bird	Hirundo rustica	Animal Model	NA	4	NA	NA
Teplitsky et al. 2014	Populations	Y	NA	NA	Bird	Cyanistes caeruleus	Animal Model	NA	4	NA	NA
Tonsor et al. 2013	Environments	Y	N	Y	Plant	Arabidopsis thaliana	RIL	160	8	Nitrogen	N
Via 1984	Environments	N	Y	N	Insect	Liriomyza sativae	Full_Sib	45	2	Diet	Y
Via and Conner 1995	Environments	N	N	N	Insect	Tribolium castaneum	Half_Sib	20	2	Diet	Y
Via and Conner 1995	Environments	N	N	N	Insect	Tribolium castaneum	Half_Sib	20	2	Diet	Y
Waldmann and Andersson 2000	Populations	Y	NA	NA	Plant	Scabiosa columbaria	Full_Sib	18	8	NA	NA
Waldmann and Andersson 2000	Populations	Y	NA	NA	Plant	Scabiosa canescens	Full_Sib	20	8	NA	NA
Widen et al. 2002	Populations	Y	NA	NA	Plant	Brassica cretica	Half_Sib	33	7	NA	NA
Windig 1994	Environments	N	N	N	Insect	Bicyclus anynana	Full_Sib	17	4	Temperature	N

CHAPTER FIVE:

Evolutionary response when selection and heritability covary across environments[5]

[5] Formatted as a co-authored manuscript: Wood, C. W. and E. D. Brodie III.

Abstract

The environment affects the two primary determinants of adaptive evolution: selection and heritability. When environmental heterogeneity simultaneously impacts both, a correlation can arise between selection and heritability that could magnify the evolutionary response under some circumstances and diminish it under others. We used a simulation to explore the evolutionary consequences of a correlation between selection and heritability. We found that the correlation has only a modest effect on the mean, but dramatically alters the variance, of the predicted evolutionary response to selection. Depending on its magnitude and sign in the wild, this correlation may impact the adaptive potential of populations in rapidly changing environments, complicate predictions of trait evolution, and partially account for stasis in the face of strong selection. Ultimately, the impact of the correlation between selection and heritability depends on its distribution in natural populations, which remains unknown.

Introduction

Adaptive evolution is governed by two main factors: the strength of selection on a trait, and the proportion of variation in the trait that is heritable (Falconer and Mackay 1996). Together, selection and heritable variation determine the evolutionary response; a trait under strong selection will evolve rapidly only when heritable variation is abundant. Models of trait evolution usually assume that variation in selection and heritability are independent, but because the environment affects both, non-random associations between the two might arise and alter the adaptive potential of natural populations. Here we explore the evolutionary consequences of an environmentally driven correlation between these two parameters.

In natural populations, selection is highly variable among environments and among months or years (Grant and Grant 1995, Kingsolver et al. 2001, Brodie et al. 2002, Gosden and Svensson 2008, Kasumovic et al. 2008, Siepielski et al. 2009). The ecological agents of selection have been identified in a number of systems (e.g., Cain and Sheppard 1954, Grant and Grant 1995, Boughman 2001, Vignieri et al. 2010), directly linking the environment to variation in selection and allowing us to predict the circumstances that consistently produce strong or weak selection (MacColl 2011). In conjunction with estimates of additive genetic variance (V_A), differences in selection (β) among environments can be translated into the evolutionary response ($\Delta\bar{z}$) with the breeder's equation:

$$\Delta\bar{z} = V_A\,\beta \qquad \text{(eq. 1)}$$

The evolutionary response is greatest in environments that generate strong selection as long as heritable variation is equally abundant in all environments.

However, heritable variation is unlikely to be equally abundant in all environments because it, like selection, is sensitive to the environment (Figure 1, Hoffman and Merilä 1999, Sgrò and Hoffman 2004, Garant et al. 2005, Le Rouzic and Carlborg 2008, Schlichting 2008). Although environmental effects on heritable variation remain less well characterized than on selection, particularly under ecologically relevant conditions, environmental quality, stressors, and novelty are all known to affect heritability (Bennington and McGraw 1996, Conner et al. 2003, Charmantier and Garant 2005, Ledon-Rettig et al. 2010, Tibbetts 2010, McGuigan et al. 2011). The environment affects heritability (the proportion of phenotypic variance attributable to additive genetic effects) in two ways: by affecting total phenotypic variance—included in β in eq. 1—or by altering additive genetic variance (V_A) through effects on gene expression (Figure 1). Environmental effects on V_A, rather than on non-genetic sources of phenotypic variance, are our main focus because V_A governs the response to selection (Houle 1992, Hansen et al. 2011).

Because the environment influences both selection and V_A, it has the potential to generate a correlation between these factors among populations in heterogeneous environments (Figure 2; Wilson et al. 2006, McGuigan and Sgrò 2009, Pemberton 2010). When V_A is abundant in the same environments that generate strong selection, the correlation is positive; when there is little V_A in the environments that generate strong selection (or vice versa), the correlation is negative. The correlation will be strongest when the scale of environmental heterogeneity is large relative to the scale of gene flow, because movement among environments homogenizes the environment experienced by individuals in different populations. When the environment varies temporally, the

strongest correlations between selection and V_A may exist in species with short lifespans relative to the scale of environmental fluctuation, maximizing among-generation differences in the environment. The only two studies that have measured the correlation between selection and V_A both detected strong environmentally driven correlations, one negative (birth weight in Soay sheep: Wilson et al. 2006) and one positive (timing of breeding in great tits: Husby et al. 2011).

A correlation between selection and V_A will impact both the mean and variance in the evolutionary response of a set of populations. This is true whether the correlation is generated by environmental variation that occurs spatially (e.g., among logs in a forest, ponds in a watershed, or islands in the sea) or temporally (e.g., among generations on the scale of months, years or decades). The impact on the evolutionary response depends on the magnitude and sign of the correlation. The mean response to selection $E(\Delta\bar{z})$ is given by analogy to the expectation of the product of two random variables,

$$E(\Delta\bar{z}) = E(V_A)\,E(\beta) + cov(V_A, \beta) \qquad \text{(eq. 2)}$$

where $E(V_A)$ and $E(\beta)$ are the mean additive genetic variance and mean selection gradient, and $cov(V_A, \beta)$ is the covariance between the two. $cov(V_A, \beta)$ is equivalent to the correlation between V_A and β when both are standardized to a variance of one. Equation 2 demonstrates that positive covariance increases the mean response and negative covariance decreases it.

Covarying selection and V_A will also impact the degree to which the evolutionary outcomes differ among populations, described by the variance in the response. By analogy to variance of the product of two random variables, the variance in response is

$$var(\Delta\bar{z}) = [E(\beta)]^2 var(V_A) + [E(V_A)]^2 var(\beta) + E\left[\left(V_A - E(V_A)\right)^2\left(\beta - E(\beta)\right)^2\right] + 2\,E(V_A)\,E\left[\left(V_A - E(V_A)\right)\left(\beta - E(\beta)\right)^2\right] + 2\,E(\beta)\,E\left[\left(V_A - E(V_A)\right)^2\left(\beta - E(\beta)\right)\right] + 2\,E(V_A)E(\beta)\,cov(V_A, \beta) - [cov(V_A, \beta)]^2 \quad \text{(eq. 3)}$$

(Bohrnstedt and Goldberger 1969). Covariance between V_A and β changes the variance in the response to selection (note the last two terms in eq. 3), but the effect on the variance is more complex than the effect on the mean. Positive covariance inflates variance in the response, because the populations with strong selection and high V_A exhibit an extremely large response while the response in others is near zero. Negative covariance reduces variance in the response because strong selection is tempered by low V_A wherever it occurs.

The evolutionary implications of a correlation between selection and V_A are heightened by the possibility that it is pervasive in heterogeneous environments. Many aspects of the environment—environmental stressors, novelty, density, and quality—affect both selection and V_A (e.g., Bennington and McGraw 1995, Hoffman and Merila 1999, Stanton et al. 2000, Charmantier and Garant 2005, Plough 2012). Variation among populations in these environmental parameters is common when populations invade a novel environment (Huang et al. 2010), when their native habitat is invaded (Kandori et al. 2009) or when patterns of temperature or precipitation are altered (Etterson 2004). This suggests that the correlation may be prevalent in environments affected by invasive species, anthropogenic habitat modification, or climate change, and therefore may be a

key component of evolutionary change or stasis across taxa. The ability of natural populations to keep pace with rapid environmental change depends in part upon their ability to respond to selection. If positive correlations predominate, adaptation to environmental stressors may be accelerated because at least some populations will harbor the genetic variation to respond to strong selection. Negative correlations, on the other hand, may result in evolutionary stasis because genetic variation will be scarce wherever selection is strong.

However, these general predictions do not evaluate how strong the correlation need be in order to drive substantial departures from the expected evolutionary response. Ultimately, the consequences will depend on its magnitude and sign in natural populations, and its realized effect under known distributions of selection and V_A. Here we explore the evolutionary consequences of the correlation between selection and V_A using a simulation approach to examine its effect on the evolutionary response under biologically reasonable distributions.

Methods

We simulated nine datasets of 10,000 populations each. Each population was assigned a selection gradient and additive genetic variance randomly drawn from distributions with specified correlation. We varied the magnitude and sign of the correlation among the nine datasets, and calculated the mean and variance in the response to selection for each dataset, using R version 3.0.1 (R Core Team 2013). This approach assumes no impact of migration between populations on the correlation; although

migration certainly may alter the correlation, its effect will depend on whether it is random with respect to the environment.

Selection gradients were drawn from a folded normal distribution, with parameter $\theta = 4.75$. This corresponds to a mean of 0.21, a median of 0.18, and a variance of 0.025, which approximates the distribution of directional selection gradients reported in Kingsolver et al. (2001, 2012). Because the absolute value of a standard normal random variable follows a folded normal distribution, this is appropriate if the magnitude (but not the sign) of a variable is of interest, as is the case in the present study. Additive genetic variances were drawn from a beta distribution with parameters $\alpha = 0.95$ and $\beta = 1.90$, which corresponds to a mean of 0.33, a median of 0.29, and a variance of 0.058 (Geber and Griffen 2003, Hansen et al. 2011). To estimate α and β, we used the function *fitdist* in package fitdistrplus in R to fit a beta distribution to the heritabilities in Hansen et al. (2011 Supplementary Table 1). Because a beta distribution is defined on the interval [0,1], we excluded cases where $h^2 < 0$ and $h^2 > 1$ (n=20 out of 1,460).

We used the inverse transform method to generate two random variables with the desired distributions with correlation r (Law and Kelton 2000, p. 440). Each set of 10,000 populations was generated by creating a matrix with 10,000 observations of two multivariate normal random variables with zero mean and unit variance using the function *mvrnorm* in the MASS package in R. We applied the normal cumulative distribution function (CDF) to these variables, which resulted in uniformly distributed variables on the interval [0,1]. We then applied the inverse CDFs of the desired marginal distributions for V_A and β (see above) to the two variables. Applying the inverse CDF of any distribution to a uniform [0,1] random variable produces a variable with the desired

distribution, and preserves the correlation (r) that was originally specified between the two normally distributed variables. This resulted in two variables, V_A and β, with correlation r, that were beta and half-normally distributed, respectively.

We repeated this process for the following correlations: $r = 0.0$, ± 0.25, ± 0.5, ± 0.75, and ± 0.95. This produced nine datasets each comprising 10,000 simulated populations. Finally, we calculated the response to selection in each population in all nine datasets using $\Delta\bar{z} = V_A\,\beta$. Because the response to selection was non-normally distributed, we used a Box-Cox transformation to select the best transformation in the family of power transformations (Sokal and Rohlf 1995, pp. 417-419), using the *boxcox* function in the MASS package. The estimate of λ that maximized the log-likelihood function was 0.22, so we fourth-root transformed the response to selection for analysis. We tested whether the correlation affected the variance in the response using a Brown-Forsythe test. We used an ANOVA to test for differences in the mean response to selection among the nine values of the correlation. Because the correlation affected the variance in the response (see Results) and transformation did not eliminate heteroscedasticity in the residuals, we used White's heteroscedasticity-corrected covariance matrix in this test (White 1980). Figure 3 was created using the ggplot2 package (Wickham 2009).

Results

We found that a correlation between selection and V_A significantly alters the mean ($F_{8,\,89{,}991} = 147.36$, $P < 0.001$) and variance (Brown-Forsyth test, $F_{8,\,89{,}991} = 1883.5$, $P < 0.001$) of the response to selection (Figure 3). Although the effect on the mean was

moderate, the effect on the variance was extremely large. As we predicted, the positive correlation increased both the mean and variance, while a negative correlation decreased both. In fact, a negative correlation almost eliminated variance in the response to selection for high values of r (Figure 3).

Discussion

We found that correlation between selection and V_A affects the evolutionary response. We expected the correlation to have the strongest effect on the mean response, which was the focus of the two studies that have measured the correlation in the wild (Wilson et al. 2006, Husby et al. 2011). However, the effect on the mean was dwarfed by the impact on the variance in the evolutionary response. The degree to which evolutionary outcomes differed among populations was dramatically inflated when the correlation was positive and nearly eliminated when it was negative. The evolutionary consequences of this pattern are similar when the correlation between selection and V_A is driven by temporal, rather than spatial, environmental variation. When the correlation is positive, the population will exhibit a large evolutionary response in at least some years, while a negative correlation will preclude a response in all years

The impact of the correlation on the variance in the evolutionary response has broad implications (Vuilleumier et al. 2008). The inflation of the variance when the correlation is positive results in an extremely rapid response in a small number of populations (or years, if the environmental variation is temporal rather than spatial), and a negligible response in most others. In a heterogeneous landscape, positive correlations have the potential to create a mosaic of evolutionary hotspots, in which evolution

proceeds quickly, and coldspots in which evolution is slow (sensu Brodie et al. 2002). However, if the few populations with the capacity to respond to selection export migrants to other populations, they can drive a change in the trait mean even in populations that do not experience strong selection or harbor the genetic variance to respond to it. Therefore, a small number of rapidly evolving populations can disproportionately impact the evolutionary dynamics in a metapopulation by facilitating phenotypic change in other populations.

Conversely, when the correlation between selection and V_A is strongly negative, differences in the evolutionary response among populations are eliminated. The response is virtually eradicated in each population because V_A is rarely exposed to strong selection (Figure 3). Therefore, negative correlations may constitute a mechanism for the maintenance of genetic variance in heterogeneous environments. Although correlations strong enough to completely halt evolution may be extremely rare, even moderate negative correlations may hamper adaptation to changing environments if novel environments tend to generate strong selection but mask genetic variance. Under these conditions, the elimination of the variance in the evolutionary response is critical: because no population exhibits an appreciable response, populations that lack the genetic variance to respond to selection cannot be rescued by migrants from other populations (or, when environment varies temporally, by a strong response in some years).

Whether species predominantly respond evolutionarily or plastically to environmental change may depend on the relative frequency of positive and negative correlations between selection and genetic variance. While positive correlation promotes genetic responses, negative correlation favors plastic responses because the genetic

response is constrained by the paucity of genetic variance in the face of strong selection. A recent meta-analysis found that plasticity plays an important role in the large rates of phenotypic evolution in anthropogenically altered environments, a result that is consistent with pervasive negative correlations between selection and V_A in changing environments (Hendry et al. 2008). Because plasticity alone can facilitate response to environmental change (at least in the short term; Charmantier et al. 2008), ubiquitous negative correlations do not necessarily translate to increased vulnerability, but instead may affect the relative importance of genetic and plastic responses in the adaptive process.

Although the scope of our analysis was restricted to the evolutionary response in a single trait, the consequences of environmentally driven correlation between selection and genetic variance extend to multivariate evolution if the environment affects genetic covariances among traits as well (Travis et al. 1999, Sgrò and Hoffman 2004). Environmental effects on genetic covariances have received less empirical attention than environmental effects on genetic variances, but are known to exist (Conner et al. 2003, Pigliucci 2003, Robinson et al. 2009). The evolutionary response of each trait will be less constrained by selection on correlated traits if the environments that generate strong selection also weaken genetic covariances. The opposite association will increase the role of genetic constraint in heterogeneous environments. The implications of correlation between selection and genetic covariances merits further attention.

On a pragmatic note, correlation between selection and V_A affects our ability to predict trait evolution in natural populations, potentially confounding assessment of adaptive potential in rapidly changing environments (Geinapp et al. 2008). When

selection and genetic variance positively covary, the ability to generalize predictions of the response to selection in one population to others is extremely limited, because evolutionary outcomes differ dramatically among populations. However, if negative correlations are common, the evolutionary response observed in one population is a reasonably accurate estimate of the response in other populations, because among-population variance in the response is minimal even in the face of large differences in selection or in the abundance of genetic variation.

In spite of the potentially broad evolutionary consequences of covarying selection and V_A, few empirical estimates of the correlation exist, so it remains difficult to predict the net effect of the correlation in the wild. Although novel, stressful, and dynamic environments are known to affect V_A while simultaneously generating strong selection (Figure 1; Ledon-Rettig et al. 2010, Kvist et al. 2013), there remains a lack of consensus as to whether these environments mask or release genetic variation (Gebhardt-Henrich and van Noordwijk 1991, Charmantier and Garant 2005, Tibbetts 2010). While the pervasive effects of environmental heterogeneity on both selection and V_A imply that the correlation may be common, it remains difficult to predict whether a positive or negative correlation—and their respective evolutionary consequences—is more likely. Our results underscore the complex nature of the consequences of environmental heterogeneity for adaptive evolution in the wild, and suggest avenues for future empirical work.

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Literature cited

Barrett, R. D. H., S. M. Rogers, and D. Schluter. 2009. Environment specific pleiotropy facilitates divergence and the *Ectodysplasin* locus in threespine stickleback. *Evolution* 63:2831-2837.

Bennington, C. C. and J. B. McGraw. 1995. Natural selection and ecotypic differentiation in *Impatiens pallida*. *Ecological Monographs* 65:303-324.

Bennington, C. C. and J. B. McGraw. 1996. Environment-dependence of quantitative genetic parameters in *Impatiens pallida*. *Evolution* 50:1083-1097.

Bohrnstedt, G. B. and A. S. Goldberger. 1969. On the exact covariance of the product of random variables. *Journal of the American Statistical Association* 64:1439–1442.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411: 944-947.

Brodie, E. D. Jr., B. J. Ridenhour, and E. D. Brodie III. 2002. The evolutionary response of predators to dangerous prey: Hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* 56:2067-2082.

Cain, A. J. and P. M. Sheppard. 1954. Natural selection in *Cepaea*. Genetics 39:89-116.

Charmantier, A. and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society—B* 272:1415-1425.

Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk, and B. C. Sheldon. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* 320:800-803.

Conner, J. K., R. Franks, and C. Stewart. 2003. Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution* 57:487-495.

Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculate* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution* 58:1446-1458.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. Pearson Education Limited, Essex, England.

Garant, D., L. E. B. Kruuk, T. A. Wilkin, R. H. McCleery, and B. C. Sheldon. 2005. Evolution driven by differential dispersal within a wild bird population. *Nature* 433:60-65.

Geber, M. A. and L. R. Griffen. 2003. Inheritance and natural selection on functional traits. *International Journal of Plant Science* 164:S21-S42.

Gebhardt-Henrich, S. G. and A. J. van Noordwijk. 1991. Nesting growth in the Great Tit I. Heritability estimates under different environmental conditions. *Journal of Evolutionary Biology* 3:341-362.

Geinapp, P., C. Teplitsky, J. S. Alho, J. A. Mills, and J. Merilä. 2008. Climate change and evolution: disentangling evolutionary and genetic responses. *Molecular Ecology* 17:167-178.

Gosden, T. P. and E. I. Svensson. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution* 62:845-856.

Grant, P. R. and B. R. Grant. 1995. Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* 49:241-251.

Hansen, T. F., C. Pélabon, and D. Houle. 2011. Heritability is not evolvability. *Evolutionary Biology* 38:258-277.

Hendry, A. P., T. J. Farrugia, and M. T. Kinnison. 2008. Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology 17:20-29.

Hoffman, A. A. and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution* 14:96-101.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics 130:195-204.

Huang, X., J. Schmitt, L. Dorn, C. Griffith, S. Effgen, S. Takao, M. Koornneef, and K. Donohue. 2010. The earliest stages of adaptation in an experimental plant population: strong selection on QTLs for seed dormancy. *Molecular Ecology* 19:1335-1351.

Husby, A., M. E. Visser, L. E. B. Kruuk. 2011. Speeding up microevolution: the effects of increasing temperature on selection and genetic variance in a wild bird population. *PLoS Biology* 9:1-9.

Kandori, I., T. Hirao, S. Matsunaga, and T. Kurosaki. 2009. An invasive dandelion unilaterally reduces the reproduction of a native congener through competition for pollination. *Oecologia* 159:559-569.

Kasumovic, M. M., M. J. Bruce, M. C. B. Andrade, and M. E. Herberstein. 2008. Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. *Evolution* 62:2316-2325.

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

Kingsolver, J. G., S. E. Diamond, A. M. Siepielski, and S. M. Carlson. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations, and future directions. *Evolutionary Ecology* 26:1101-1118.

Kvist, J., C. W. Wheat, E. Kallioniemi, M. Saastamoinen, I. Hanski, and M. J. Frilander. 2013. Temperature treatments during larval development reveal extensive heritable and plastic variation in gene expression and life history traits. *Molecular Ecology* 22:602-619.

Law, A. M. and W. D. Kelton. 2000. Simulation Modeling and Analysis. Third edition. McGraw-Hill, New York, NY.

Le Rouzic, A. and Ö. Carlborg. 2008. Evolutionary potential of hidden genetic variation. *Trends in Ecology and Evolution* 23:33-37.

Ledon-Rettig, C. C., D. W. Pfennig, and E. J. Crespi. 2010. Diet and hormonal manipulation reveal cryptic genetic variation: implications for the evolution of novel feeding strategies. *Proceedings of the Royal Society—B* 277:3569-3578.

MacColl, A. D. C. 2011. The ecological causes of evolution. *Trends in Ecology and Evolution* 26:514-522.

McGuigan, K. and C. M. Sgrò. 2009. Evolutionary consequences of cryptic genetic variation. *Trends in Ecology and Evolution* 24:305-311.

McGuigan, K., N. Nishimura, M. Currey, D. Hurwit, and W. A. Cresko. 2011. Cryptic genetic variation and body size evolution in threespine stickleback. *Evolution* 65:1203-1211.

Pemberton, J. M. 2010. Evolution of quantitative traits in the wild: mind the ecology. *Philosophical Transactions of the Royal Society—B* 365:2431-2438.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecology Letters* 6:265-272.

Plough, L. V. 2012. Environmental stress increases selection against and dominance of deleterious mutations in inbred families of the Pacific oyster *Crassostrea gigas*. *Molecular Ecology* 21:3974-3987.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Robinson, M. R., A. J. Wilson, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and L. E. B. Kruuk. 2009. The impact of environmental heterogeneity on genetic architecture in a wild population of Soay sheep. *Genetics* 181:1639-1648.

Schlichting, C. D. 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. Annals of the New York Academic of Sciences 1133:187-203.

Sgrò, C. M. and A. A. Hoffman. 2004. Genetic correlations, tradeoffs, and environmental variation. *Heredity* 93:241-248.

Siepielski, A. M., J. D. DiBattista, and S. M. Carlson. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters* 12:1261-1276.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry. Third edition. W.H. Freeman and Company, New York.

Stanton, M. L., B. A. Roy, and D. A. Thiede. 2000. Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution* 54:93-111.

Tibbetts, E. A. 2010. The condition dependence and heritability of signaling and nonsignaling color traits in paper wasps. *The American Naturalist* 175:495-503.

Travis, J., M. G. McManus, and C. F. Baer. 1999. Sources of variation in physiological phenotypes and their evolutionary significance. *American Zoologist* 39:422-433.

Vignieri, S. N., J. G. Larson, and H. E. Hoekstra. 2010. The selective advantage of crypsis in mice. *Evolution* 64:2153-2158.

Vuilleumier, S., J. M. Yearsley, and N. Perrin. 2008. The fixation of locally beneficial alleles in a metapopulation. *Genetics* 178:467-475.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments. *Molecular Ecology* 12:1153-1163.

White, H. 1980. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica* 48:817-838.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer, New York.

Wilson, A. J., J. M. Pemberton, J. G. Pilkington, D. W. Coltman, D. V. Mifsud, T. H. Clutton-Brock, L. E. B. Kruuk. 2006. Environmental coupling of selection and heritability limits evolution. *PLoS Biology* 4:1270-1275.

Figures and Tables

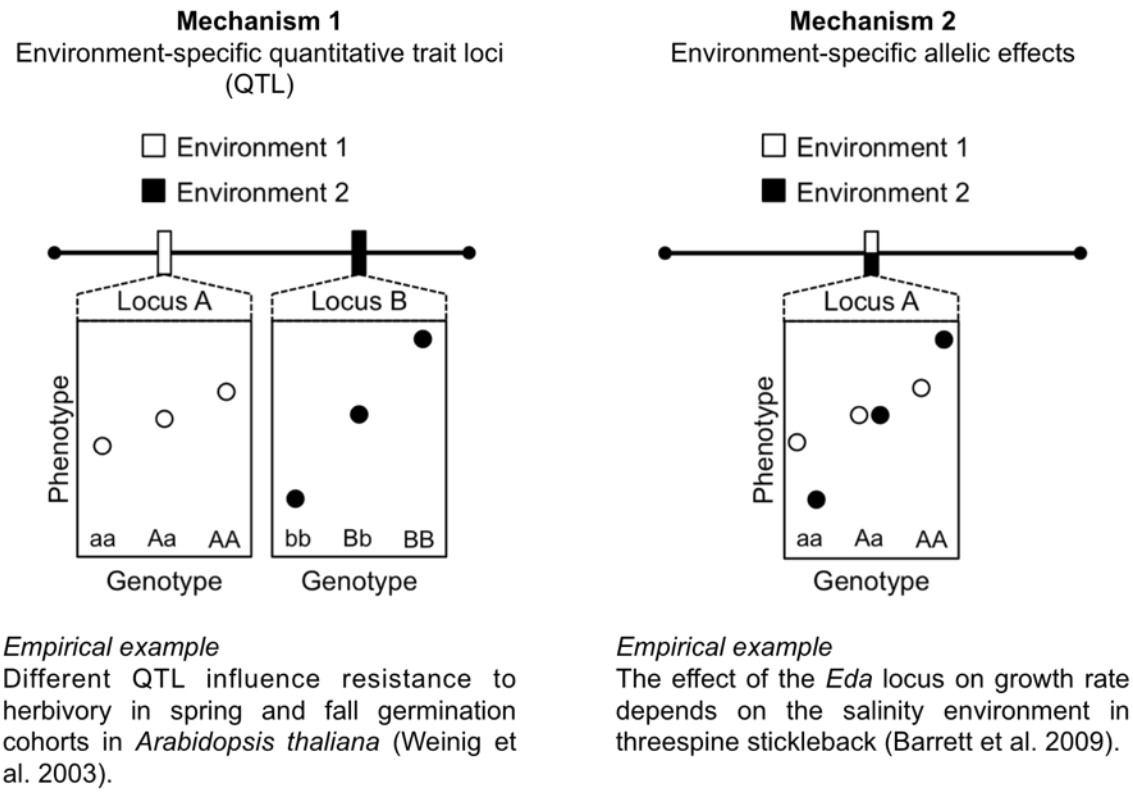

Figure 1. Environmental effects on heritable variation in a phenotype are driven by two mechanisms. Mechanism 1: Different loci contribute to the phenotype in different environments (Locus A in Environment 1 and Locus B in Environment 2). The expression of Locus B results in greater variation in Environment 2. Mechanism 2: The contribution of alleles at a single locus to the phenotype differs among environments. Above, the substitution of **A** for **a** has a larger effect in Environment 2. The evolutionary implications are similar regardless of mechanism: trait evolution is more rapid in environments with abundant additive genetic variance.

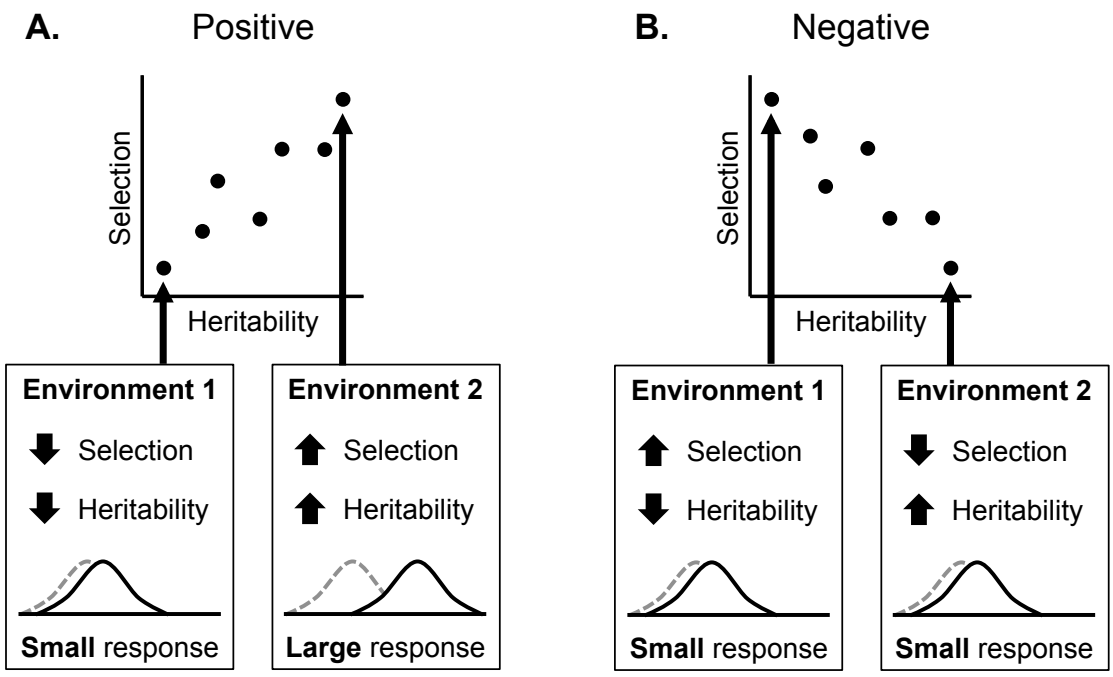

Figure 2. The hypothesized effect of an environmentally-driven correlation between selection and heritability on the evolutionary response to selection. Each point in the scatterplots represents a single population. A. Positive correlation: the same environments that generate strong selection also produce high heritability and exhibit a large evolutionary response, and the environments that generate weak selection also produce low heritability and exhibit a small response. B. Negative correlation: the environments that generate strong selection produce low heritability, and the environments that generate weak selection generate high heritability. When the correlation is negative, the evolutionary response is always small.

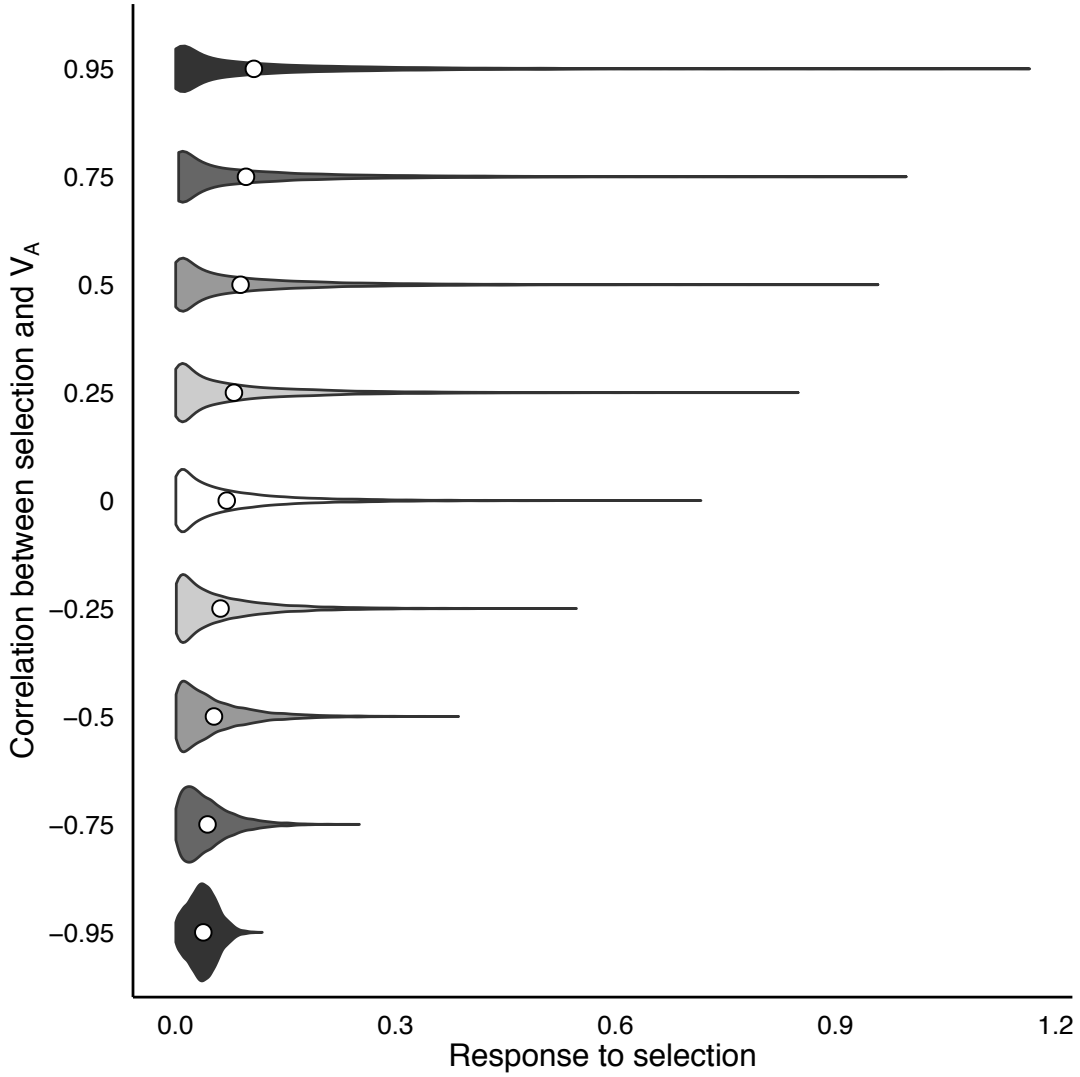

Figure 3. Violin plots of the effect of a correlation between selection and V_A on the evolutionary response. The distribution of the response when selection and V_A are uncorrelated is shown at $r = 0$ on the vertical axis; above, the correlation becomes increasingly positive, and below, increasingly negative. The mean response is indicated by the white circle on each distribution.