Environmental heterogeneity generates opposite gene-by-environment interactions for two fitness-related traits within a population

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Theory predicts that environmental heterogeneity offers a potential solution to the maintenance of genetic variation within populations, but empirical evidence remains sparse. The live-bearing fish Xiphophorus variatus exhibits polymorphism at a single locus, with different alleles resulting in up to five distinct melanistic “tailspot” patterns within populations. We investigated the effects of heterogeneity in two ubiquitous environmental variables (temperature and food availability) on two fitness-related traits (upper thermal limits and body condition) in two different tailspot types (wild-type and upper cut crescent). We found gene-by-environment (G × E) interactions between tailspot type and food level affecting upper thermal limits (UTL), as well as between tailspot type and thermal environment affecting body condition. Exploring mechanistic bases underlying these G × E patterns, we found no differences between tailspot types in hsp70 gene expression despite significant overall increases in expression under both thermal and food stress. Similarly, there was no difference in routine metabolic rates between the tailspot types. The reversal of relative performance of the two tailspot types under different environmental conditions revealed a mechanism by which environmental heterogeneity can balance polymorphism within populations through selection on different fitness-related traits.

KEY WORDS: Balancing selection, body condition, CT_{max}, poeciliidae, polymorphism, upper thermal limit.

Understanding the functional mechanisms by which genetic variation is maintained in natural populations despite the depleting effects of natural and sexual selection remains a central problem in evolutionary biology (Mitchell-Olds et al. 2007; Ingleby et al. 2011). A rich history of theoretical work dating back several decades has generally found that the conditions for the maintenance of genetic polymorphisms are restrictive (reviewed in Felsenstein 1976). However, cyclical selection in which the fitness of discrete genotypes or phenotypes varies with some predictability can maintain polymorphism under certain conditions (Felsenstein 1976). Environmental heterogeneity, which is often cyclical in nature, may offer an opportunity to explain the maintenance of genetic variation by generating temporally variable selection on fitness-related traits (Via and Lande 1987; Zhang and Hill 2005). Specifically, when the relative performance of different genotypes varies with changing environmental conditions (a gene-by-environment or G × E interaction), balancing selection exerted by a heterogeneous environment can promote the
maintenance of multiple genotypes (Byers 2005). There is considerable evidence corroborating the role of spatial environmental heterogeneity in generating local adaptation, thus maintaining genetic variation among species and populations (for reviews see Hedrick et al. 1976; Linhart and Grant 1996; Mitchell-Olds et al. 2007). For example, pea aphids exhibit genetic variation for performance on different host plants, generating locally adapted populations due to fitness differences among clones on alfalfa and red clover (Via 1991). There are noticeably fewer empirical examples of the role of environmental heterogeneity in maintaining genetic variation within populations (Hedrick 2006; Yeaman et al. 2010), despite recurrent predictions from theoretical models (Via and Lande 1987; Gillespie and Turelli 1989; Bürger and Gimelfarb 2002; Spichtig and Kawecki 2004; Turelli and Barton 2004; Zhang and Hill 2005). However, Shaw et al. (1995) demonstrated within-population genetic variance for fitness across different environments for the annual plant, *Nemophila menziesii*. Similarly, populations of the lesser wax moth exhibit genetic variation for sexual signals affecting reproductive fitness that vary with environmental context, suggesting that changing environmental conditions could promote the maintenance of multiple genotypes by modulating attractiveness (Jia et al. 2000).

Temperature and food availability are pervasive environmental variables. These two variables have a critical influence on organismal survival and reproduction. Most organisms experience heterogeneity in temperature and/or food availability periodically throughout their lives (Clarke 2003; Wang et al. 2006). The functional importance of heterogeneity in these two environmental variables is amplified by global climate change as evidenced by an increased frequency and intensity of extreme temperature events (Meehl and Tebaldi 2004; Smith et al. 2009; Hansen et al. 2012), mass mortality events associated with them (Donner et al. 2007; Harley 2008; Coma et al. 2009), and a decoupling of trophic interactions (Walther 2010). The disruption of these interactions has led to asynchronies in predator–prey life cycles and resultant decreases in resource availability in terrestrial, marine, and freshwater ecosystems (Winder and Schindler 2004; Both et al. 2009; Hansen et al. 2012). Studying the genetic basis of fitness differences across environments and its functional basis is important to better understand the evolution and maintenance of polymorphisms and to predicting the impact of global change on levels of standing genetic variation in the wild (Pauls et al. 2012).

We investigated two pertinent fitness-relevant traits associated with temperature and food availability: upper thermal limits (UTLs) and energetic state (i.e., body condition). UTLs provide direct limits to survival in the face of thermal stress (Harley 2008; Garrabou et al. 2009). Plasticity in UTLs is critical to mitigating the effects of thermal stress and plasticity plays a key role in adaptation to novel environments (Ghalambor et al. 2007). An individual’s energetic state (Froese 2006) has important influence on survival via starvation resistance (Reim et al. 2006; Plath et al. 2007), immune function (Lochmiller and Deerenberg 2000; Alonso-Alvarez and Tella 2001; Seppala and Jokela 2010), and risk taking behavior (Krause et al. 1998). It also shapes reproductive success through effects on reproductive investment (Kodric-Brown 1989; Elkin and Reid 2005) and expression of sexual ornaments (Johnstone 1995; but see Cotton et al. 2004). An individual’s energetic state therefore affects fitness through survival and reproduction.

To investigate potential G × E interactions related to the aforementioned traits, we focused on a polymorphic species of platyfish. The platyfishes of the genus *Xiphophorus* are small-bodied, live-bearing fishes distributed primarily throughout the coastal plain of eastern Mexico. Several species of platyfish exhibit polymorphism at a single autosomal locus that controls melanistic tailspot patterns expressed by both sexes (Culumber 2014). *Xiphophorus variatus* has been documented to have six tailspot alleles (Borowsky 1984), with up to five tailspot alleles occurring in single populations at any given time (Culumber et al. 2014). Wild-type homozygotes lack any melanistic tailspot pattern, whereas alternative alleles induce a variety of spotting patterns on the caudal peduncle. Pattern alleles are codominant to one another and dominant over the wild-type allele (Borowsky 1981). Although color patterns are frequent targets of female mate choice in *Xiphophorus* (Morris 1998; Kingston et al. 2003; Fernandez and Morris 2008), female mate choice appears to have limited influence on the maintenance of tailspots in *X. variatus* (Culumber and Rosenthal 2013), consistent with random mating patterns inferred from offspring tailspot phenotypes (Borowsky and Khouri 1976). The maintenance of the tailspot polymorphism in *X. variatus* appears to be dependent on environmental factors. Fish of different tailspot types exhibit differences in physiological tolerances to environmental stressors, such as low-dissolved oxygen concentrations (Borowsky 1990) and elevated temperatures (Culumber et al. 2014). Culumber et al. (2014) found that wild-type fish had significantly lower UTLs than fish of three pattern types collected from the same stream pool of one population. Findings for UTLs were consistent with female fecundity and wild-type allele frequencies, both of which exhibit negative correlations with temperature (Borowsky 1984). Overall, these data suggest that heterogeneity in environmental variables likely contributes to balancing polymorphism at the tailspot locus within populations, yet mechanistic tests of this hypothesis are lacking.

Here, we examined whether the relative physiological performance of individuals of different tailspot types changes between environmental conditions, indicating G × E interactions that could contribute to the maintenance of polymorphism within
populations. To address this question, we manipulated temperature and food availability in the laboratory and measured performance of two tailspot types for UTLs and energetic state. Based on the lower UTLs of wild-type individuals (Culumber et al. 2014) and aforementioned correlations between wild-type individuals and temperature (Borowsky 1984), we predicted that relative performance of the two tailspot types would change between temperature conditions. There were no a priori predictions of relative performance of tailspots under food restriction. Evidence of potential energetic constraints on response to extreme temperature events (Coma et al. 2009) led to a general prediction of reduced UTLs under food restriction. Furthermore, we explored potential mechanisms underlying physiological performance by measuring gene expression under each environmental condition and metabolic rate (MR) variation between tailspot types.

**Methods**

Adult male, *X. variatus*, of two tailspot types (wild-type and upper cut crescent) were collected from the Arroyo Grande in the Tuxpan drainage at Guaguaco, Veracruz (latitude/longitude: 20.7835°—98.0701°). Wild-type and upper cut crescent were the only tailspot types present, and both types were caught in the same stream pools and within the same minnow traps. Water temperature at the time of collection was 27°C. Fish were transported to the Centro de Investigaciones Científicas de las Huastecas Aguazarca (CICHAZ), slowly acclimated to the ambient air temperature of 25°C by bubbling the water in which they were transported, and then maintained in 200 L aquaria at 25°C for five days. During this time, fish were fed ad libitum twice daily with a combination of decapsulated brine shrimp eggs and Tetramin flake food. Only males were used for this study due to the fact that variation in female reproductive state precludes meaningful measurement of body condition (Reznick and Braun 1987). Female poeciliids can also reabsorb nutrients from their eggs (Trexler 1997), making it difficult to accurately determine the level of resource stress that any given female experienced during an experiment. Prior to the start of the experiment, temperature records were evaluated to rule out recent thermal stress in their natural habitat (see Supporting Information).

**EXPERIMENTAL CONDITIONS**

Following the five-day acclimation period, all individuals were photographed, weighed to the nearest 0.001 g using a digital scale, and randomly assigned to an experimental treatment representing a fully factorial design of two temperature treatments and two food treatments. Fish were exposed to experimental conditions for five days. The time course of our experiment was chosen to mimic the incidence of extreme temperature events. Significant effects of thermal stress and potential G × E interactions even over this short period of time would demonstrate the power of environmental stress in generating changes in relative performance among individuals within populations. Thermal stress—and limitations in resource availability—can last even longer in the wild. In lowland streams inhabited by *X. variatus*, water temperatures closely track air temperatures (Borowsky 1984). For six months out of the year, the monthly maximum air temperatures in nearby Atlapexco, Hidalgo have ranged from 35 to 41°C since 1981 (April to September; Servicio Meteorologico Nacional 2010), indicating that high temperatures can occur for extended periods of time.

Our experiment included two levels of resource availability (fed or food deprived). Fish in the treatment with food were given 0.01 g of Tetramin per fish at 900, 1100, 1300, and 1500 h based on Culumber and Monks (2014b). Low resource treatments were food deprived for the duration of the five-day experiment. Food deprivation for this length of time has previously been shown to generate biologically relevant changes in poeciliid body condition without damage to fish (Plath et al. 2005; Fisher and Rosenthal 2006), and all fish from our experiment recovered. Although food deprivation represents an extreme form of food limitation, fishes do experience extended periods of resource limitation and even periods of complete starvation (Wang et al. 2006; McCue 2010). Some streams inhabited by *X. variatus* dry down to isolated pools with elevated temperatures, reduced dissolved oxygen concentrations, and increased population densities during the dry season (Borowsky 1984). Thus, *X. variatus* likely experiences considerable resource limitation at times during the year.

For the manipulation of temperature, fish were either exposed to 25 or 34°C. Temperature treatments were based on water temperatures in natural populations of *X. variatus*. For the focal population of this study, the mean water temperature from two collections made in winter (January/February) and two in summer (August) was 25°C. Mean water temperature in 16 collections of 12 populations in various months from 2012 to 2014 was 26.5°C, comparable to our 25°C treatment. Maximum water temperatures in *X. variatus* habitats reach at least 32.5°C (Z. W. Culumber, pers. obs.), and the mean maximum air temperature between April and September at a nearby meteorological station in Atlapexco, Hidalgo, has averaged 34.2°C since 1981 (Servicio Meteorologico Nacional 2010). Monthly absolute maximum temperatures over that same period ranged from 35.0 to 41.1°C. Water temperatures in some populations therefore can easily approach 34°C. Consequently, depending on the treatment group, water temperature was either maintained at 25°C or increased to 34°C over 18 h using aquarium heaters with adjustable thermostats. This rate of temperature change represents an ecologically relevant rate of change in that similar rates are observed in the wild (Culumber and Monks 2014b). Once temperatures were at the desired levels, the experiment began and ran for five days.
ASSAYS OF UTLs AND ENERGETIC STATE

Thermal tolerance assays followed standard protocols for estimating UTLs of freshwater fishes, including the focal species (Culumber et al. 2012, 2014). An air stone and 1.5 W air pump were used to aerate the testing chamber during all trials to alleviate any effects of oxygen limitation (Pörtner 2001). Four liters of aged tap water were preheated to the treatment temperature of the focal fish and then placed in a ceramic chamber that was suspended over a gas burner calibrated to heat the water at a rate of 0.3°C/min (Lutterschmidt and Hutchison 1997; Beitinger et al. 2000). Temperature was monitored with digital thermometers to the nearest 0.1°C to assure proper temperature increase. The point at which a fish lost all ability to right itself was considered its UTL (loss of equilibrium assay; Culumber et al. 2012). At this point the temperature was recorded, and the fish was removed and placed in a separate container to recover. When the water in the recovery container had reached ambient temperature, a final body mass measurement was taken to assess the change in energetic state over the course of the experiment.

To test for differences in performance between tailspot types, we conducted two separate analyses of covariance (ANCOVA) with UTLs and change in body mass as the dependent variables in the respective analyses. Because calculating relative change in body mass (i.e., dividing change by the initial mass) can introduce unwanted bias in the data (Kelly and Price 2005), we directly analyzed change in body mass as the final mass subtracted from initial mass. We included temperature, food level, tailspot type, and their interaction terms as fixed effects. Fishes including poeciliids commonly exhibit MRs (i.e., energy use) that are dependent upon body size (C. Passow et al., unpubl. ms.), and body condition is typically measured as mass controlled for body length (Froese 2006). We therefore used standard length (SL) as a covariate in our analysis of body mass. In the analysis of body mass, the only interaction involving the covariate that was included was that with food level. All other interactions were non-significant and their inclusion did not change the results, but their removal reduced model complexity. Photographs were used to measure SL for each fish as the distance from the tip of the snout to the caudal peduncle using ImageJ software (Rasband 1997). Because SL and body mass were significantly correlated ($R^2 = 0.908, N = 117, P < 0.001$), only SL was included as a covariate. There were no differences in SL among treatment groups (see Supporting Information).

Finally, we tested potential mechanisms underlying variation in UTLs and energetic state. We quantified the gene expression of a heat shock protein ($hsp70$) for individuals from each of the four experimental treatments. Lastly, we tested whether the two tailspot types differed in routine MRs, which could directly affect body condition. Methods and results of these components are in the online Supporting Information.

Results

UTLs

There was a significant interaction of resource treatment and tailspot type on UTLs (Table 1A; Fig. 1). Upper cut crescent fish (Cu) had higher UTLs than wild-type fish at the normal food level but had lower UTLs than wild-type fish when food deprived. The UTLs of Cu fish were reduced by a factor of $1.7 \times$ greater than wild-type fish during food deprivation. There was a significant overall effect of acclimation temperature on UTLs (Table 1A), but no difference in response to temperature between tailspot types. Both tailspot types exhibited upward shifts in UTLs in response to thermal stress and downward shifts in response to resource limitation (Fig. S1). The mean ($\pm$SE) upward acclimation capacity of UTLs in response to thermal stress was $1.77^\circ$C ($\pm$0.076) and was nearly identical for wild-type ($1.76 \pm 0.079^\circ$C) and Cu fish ($1.78 \pm 0.075^\circ$C; Fig. S1). No other terms involving temperature were significant (Table 1).

ENERGETIC STATE

There were significant main effects of temperature and resource availability on change in body mass (Table 1B). However, there was also a significant three-way interaction (temperature $\times$ resource level $\times$ tailspot type; Table 1B and Fig. 2), indicating that the relative performance of the tailspot types depended upon the combination of temperature and resource conditions. Within the warm temperature treatment, the wild-type tailspot performed more poorly at both food levels. This contrasted with the pattern observed at the moderate temperature in which wild-type fish performed better than Cu under normal food, but there was no difference in performance under food limitation (Fig. 2). The difference in performance between tailspot types was driven largely by the interaction with temperature (Table 1B; Fig. S2),
suggesting a somewhat smaller but still significant role of resources. There was also a significant interaction between SL and resource level on change in body mass; larger individuals lost less body mass than smaller individuals during food deprivation, but gained less mass when fed (Fig. S3).

**Discussion**

Environmental heterogeneity can balance polymorphisms within populations, if the relative fitness of genotypes changes depending on environmental conditions. Populations of *X. variatus* exhibit high allelic variation in tailspot patterns. Our study indicated that two different fitness-related traits are characterized by G × E interactions within populations. The relative UTLs of the two tailspot types changed between well-fed and food-deprived conditions, whereas the relative energetic state changed depending on thermal and resource conditions. Overall, our findings have implications for the maintenance of genetic variation, demonstrating how environmental heterogeneity can cause changes in relative performance among genotypes within populations and generate opposing G × E interactions on different traits.

**UTLs**

There was a G × E interaction between tailspot pattern and food treatment on UTLs. Under normal food conditions, wild-type fish had marginally lower UTLs than upper cut crescents, but upper cut crescent fish exhibited a substantially stronger, negative effect of food deprivation on UTLs, resulting in reversal of relative performance. Food limitation also increased expression of a heat shock protein (*hsp70*), an inducible molecular defense associated primarily with thermal stress (see Supporting Information). However, there was no evidence for a G × E interaction on gene expression, suggesting that *hsp* expression is not the primary mechanism underlying differences in UTLs between the tailspot types. Food stress has previously been shown to reduce adult heat resistance in the butterfly, *Bicyclus anaynana* (Fischer et al. 2010). Nonetheless, it remains to be tested how food stress is mechanistically tied to heat resistance, but it appears that there may be energetic constraints to mounting a heat shock response. Indeed, a study in the Mediterranean sea recently suggested a link between energetic constraints due to resource limitation and increased susceptibility to extreme temperature events, though it is not clear whether this constraint acted on UTLs or some other trait.
that led to increased mortality (Coma et al. 2009). Food limitation has also been shown to precipitate G × E interactions shaping immune function, lifespan, and other life-history traits (Seppälä and Jokela 2010; Jacquin et al. 2012, Savory et al. 2014). Collectively, these results suggest that G × E interactions induced by environmental heterogeneity in food availability may play an important role in evolution via the maintenance of polymorphism in fitness-related traits.

An increasing focus has been placed on evolution in light of climate change and the ability of species not only to adapt to gradual changes in mean temperatures (Walther et al. 2002; Parmesan 2006), but withstand abrupt fluctuations, such as extreme temperature events (Jentsch et al. 2007; Karl et al. 2011; Adamo et al. 2012). Under both temperature treatments in our experiment, UTLs were reduced by food deprivation. Given the role that energetic constraints play in susceptibility to extreme temperatures (Coma et al. 2009), climate-driven resource limitation (Walther 2010) could increase susceptibility to extreme events by reducing heat tolerance. Large-scale die-offs of plants and animals associated with extreme temperatures not only directly increase mortality rates (Harley 2008; Coma et al. 2009; Royer et al. 2011), but could indirectly reduce adaptive potential of populations through bottleneck effects (Pauls et al. 2012). Food constraints on UTLs have been observed in a marine cnidarian (Coma et al. 2009), terrestrial invertebrate (Fischer et al. 2010), and freshwater fish (this study), suggesting that the negative effects of resource limitation on resilience to environmental stress may be of wider importance than is currently considered. It has recently been shown that strong selection by the more gradual change in climate means can result in the loss of adaptive genetic variation during rapid range expansion (Buckley and Bridle 2014). Collectively, these examples demonstrate that both short- and long-term responses to climate change can limit adaptive potential.

### ENERGETIC STATE

There was also a G × E interaction effect between temperature, resource availability, and tailspot pattern on change in body mass. Upper cut crescent fish outperformed wild-type at both food levels under thermal stress. However, at the more moderate temperature, relative performance was modulated by food availability with wild-type outperforming upper cut crescent fish when food was available, but no difference in performance at this temperature when food was absent. Overall, the G × E interaction for body mass was most pronounced when food was abundant but temperatures differed (25 vs. 34°C). Our findings were consistent with previous work in X. variatus, documenting that wild-type female fecundity and allele frequencies were negatively correlated with temperature (Borowsky 1984). Temperature induces known G × E interactions in other systems, including host–parasite dynamics in Daphnia (Mitchell et al. 2005; Vale et al. 2008) and immune function and fecundity in Drosophila (Lazzaro et al. 2008). Our results add to those studies in support of the evolutionary importance of temperature heterogeneity in maintaining genetic variation within populations.

The hypothesis that environmental heterogeneity in variables, such as resource availability or temperature, maintains variation in tailspots assumes strong genetic or pleiotropic associations between the tailspot locus and other traits, such as behavior or metabolic physiology. For example, differential changes in body mass could be affected by variation in activity rates (e.g., in social interactions) between tailspot types under different temperature treatments (Wang et al. 2006). Social interactions also could affect energy budgets of individual fish. Although we can rule out variation in courtship or mating activity (females were absent from experimental tanks), variation in male–male aggression between tailspot types could induce differential changes in body mass. However, such interactions are size-dependent in X. variatus,
with small males showing little or no aggression (Culumber and Monks 2014a), suggesting that agonistic interactions would have favored greater activity and mass loss among large males, which was not consistent with our data. Finally, our data also rule out variation in MR as a driving mechanism in energetic state differences, because tailspot types did not differ in routine MR (see Supporting Information). MR is a key trait affecting energy budgets and exhibits temperature sensitivity (McNab 2002), making it an attractive candidate for variation in body mass changes associated with thermal stress. To clarify the mechanistic links between temperature-induced G × E interactions and energetic state, future studies will need to test for potential G × E interactions in behavioral and physiological traits that affect organismal energy budgets and consequently energetic state.

Irrespective of the proximate mechanisms affecting changes in body mass in our experiment, temperature-induced variation in energetic state should have pronounced fitness consequences. Variation in energetic state could directly affect survival through increasing starvation risk and adversely influencing immunity (Seppala and Jokela 2010). In addition, variation in body condition could have important influence on reproduction and sexual selection, because female poeciliids prefer males in better condition (Plath et al. 2005; Fisher and Rosenthal 2006), and better condition leads to higher quality and number of gametes (Franssen et al. 2008; Devigili et al. 2013; Gasparini et al. 2013). The effects of temperature on male attractiveness through effects on body condition may contribute to seasonal variation in relative reproductive success among males of different tailspot types. Indeed, wild-type male success appears to decline at the end of the breeding season, which coincides with higher temperatures in the summer months (Borowsky 1981).

THE MAINTENANCE OF GENETIC VARIATION: ENVIRONMENTAL HETEROGENEITY, ITS LIMITATIONS, AND ALTERNATIVES

The conditions to maintain genetic polymorphisms within populations are fairly restrictive, relying on some degree or combination of frequency-dependent selection, overdominance, and overlapping generations (Felsenstein 1976). Besides G × E interactions, frequency-dependent selection represents a powerful, alternative mechanism to maintain polymorphisms in natural populations (Ayala and Campbell 1974; Clarke et al. 1988). For example, color polymorphisms in male guppies are maintained by negative frequency-dependent selection mediated by female mate choice (Hughes et al. 2013) and a survival advantage of rare males (Olendorf et al. 2006). Frequency-dependent selection has been reported and implicated in the maintenance of genetic polymorphisms in a variety of other systems from fish to plants and insects (Hori 1993; Gigord et al. 2001; Fitzpatrick et al. 2007; Takahashi et al. 2010). Cyclical selection that varies in a predictable manner, such as frequency-dependent predation or mate choice, is therefore sufficient to maintain polymorphism. Frequency-dependence in mate choice and predation have not yet been tested in X. variatus. However, it is important to note that—just like negative frequency-dependent selection—seasonal variation in environmental heterogeneity is often cyclical, relatively stable over short periods, and predictable over evolutionary time scales. The existence of G × E interactions in X. variatus suggests that cyclical environmental variation may contribute to maintaining polymorphism. Other sources of frequency-dependent selection must also be considered in future studies, because G × E interactions may be only one source of selection that contributes to the maintenance of polymorphism. Alternatively, overdominance could also fulfill the requirements needed to maintain polymorphism purely through selection in the heterogeneous environment (reviewed in Felsenstein 1976). Dominance of pattern alleles over the wild-type precluded distinguishing between homozygous and heterozygous individuals in this study. Some of our phenotypically upper cut crescent fish were likely heterozygotes. Nonetheless, the two tailspot types exhibited robust phenotypic differences as in other studies where heterozygotes were included (Borowsky 1984; Borowsky 1990; Culumber et al. 2014), and although pattern tailspot alleles exhibit dominance over wild-type, the pleiotropic effects of the tailspot locus may not. Future studies conducting controlled crosses will be necessary to explicitly test for differences between heterozygotes and homozygotes and the potential for overdominance.

Finally, a further requirement for the maintenance of polymorphism in a heterogeneous environment is the presence of overlapping generations (Ellner and Hairston 1994). There are no published data on lifespan and age of maturity in the wild for X. variatus. However, in the laboratory and outdoor mesocosms, laboratory born X. variatus and wild-caught adults of unknown age have been observed to live at least 20 months (Borowsky 1973; Z. W. Culumber, pers. obs.). Closely related female X. birchmanni in sympatry with X. variatus can be mature and reproductively active as early as three to four months of age (Kindsvater et al. 2013), and several other species of Xiphophorus, including a platyfish, exhibit continuous breeding throughout the year implying an overlap of generations. Even though X. variatus in some parts of their range may be most reproductively active only during six months of the year, brood intervals average just over 40 days (Borowsky and Diffley 1981), such that populations can produce multiple generations within a breeding season.

Conclusions

Environmental variables are important drivers of broad scale evolutionary patterns, setting limits to survival and reproduction, but there are surprisingly few examples of how environmental
heterogeneity maintains polymorphism within populations. Our findings indicate that multiple \( G \times E \) interactions stemming from different environmental variables and acting on different fitness-related traits could be a major contributor to the maintenance of intrapopulation polymorphisms. Our study illustrates the power of the platyfish model for investigating the maintenance of polymorphism in complex environments and highlights areas for further empirical work in this as well as other systems including (1) detailed data on temporal variation in multiple environmental variables together with allele frequencies, (2) the extent to which environmental and sexual selection result in selection for and against certain genotypes, and (3) determining the ultimate mechanisms and underlying genes responsible for \( G \times E \) interactions. Although considerable theoretical work has focused on polymorphisms and the conditions necessary to balance them, those models have not been widely applied to empirical systems. In natural environments it remains unclear the extent to which predictable, cyclical selection is sufficient to maintain polymorphism or how cyclical selection may reinforce weak frequency-dependent selection.

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**DATA ARCHIVING**

The doi for our data is 10.5061/dryad.hd3st.

**LITERATURE CITED**


Supporting Information
Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Table S1. Results from an analysis of variance demonstrating that there was no difference in the mean standard length of individuals among treatment groups in our experiment.

Table S2. Results from an analysis of variance from the gene expression experiment.

Figure S1. Mean (± SE) UTLs for the two tailspot types at each temperature and resource level.

Figure S2. Change in body mass (mean ± SE) controlled for body length exhibited a G × E interaction with temperature treatment in which upper cut crescent fish outperformed under thermal stress.

Figure S3. There was a significant interaction between body size and resource level on change in body mass.

Figure S4. Mean (± SE) gene expression of hsp70 from males in each experimental group.

Figure S5. Routine metabolic rates (mean ± SE) did not differ between the wild-type and upper cut crescent tailspot types.

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