ORIGINAL RESEARCH

Physiological stress and the maintenance of adaptive genetic variation in a livebearing fish

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Abstract The importance of genetic variation in evolution is well established. Yet, the mechanisms by which genetic variation—particularly variation in traits under selection—is maintained in natural populations has long been an evolutionary puzzle. Understanding individual variables driving selection and their functional mechanisms is increasingly important in the context of global change and its potential consequences for biodiversity. Here we examined intra-population performance among allelic variants of a pleiotropic locus in response to thermal stress in the variable platyfish, *Xiphophorus variatus*. The wild-type tailspot allele exhibited significantly lower heat tolerance than all three pattern alleles found in the population, conforming to predictions based on previously observed correlations between temperature and pattern frequencies in the wild. Furthermore, differences between tailspot pattern frequencies in adults and juveniles were broadly consistent with this trend. Thus, it appears that physiological stress and reduced performance of the wild-type allele at higher relative temperatures is a mechanism balancing its frequency in natural populations. Temperature variation and not dissolved oxygen alone, as previously reported, is likely a important abiotic variable contributing to the maintenance of adaptive polymorphism. Furthermore, our findings underscore the potential implications of rising temperatures and physiological stress for levels of genetic variation in natural populations.

Keywords Pleiotropy · *Xiphophorus* · Poeciliidae · Abiotic environment

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Introduction

The significance of adaptive genetic variation for natural populations is well recognized in terms of providing evolutionary potential in the face of novel environmental stressors (Reed and Frankham 2003; Spielman et al. 2004; Frankham 2005). The importance of such variation might be particularly crucial in the context of global climate change and associated environmental selection from increasing temperatures and consequent alterations of ecological interactions, increased physiological stress, and parasite and pathogen risks (Helmuth et al. 2002; Parmesan 2006; Marcogliese 2008). Understanding the mechanisms that promote and maintain adaptive genetic variation is a longstanding focus in evolutionary biology, particularly for traits of ecological importance (Hedrick et al. 1976; Piertney and Oliver 2006; Hedrick 2006; Manel 2012). Variable selection, such as that owing to spatial or temporal heterogeneity in the environment, has long been proposed as a mechanism maintaining genetic polymorphism (Felsenstein 1976; Hedrick et al. 1976). Over the last several decades, this theory has gained increasing support (reviewed in Hedrick 2006).

The effects of environmental heterogeneity on the maintenance of adaptive genetic variation have been documented in a variety of taxa (see Schmidt and Rand 2001; Schmidt and Conde 2006; Bockelmann et al. 2003; Huestis et al. 2009 for examples). Two classic examples involve ecological selection favoring the maintenance of variation in melanism in the peppered moth (*Biston betularia*; Cook 2003) and two species of mice (*Chaetodipus intermedius*, Nachman et al. 2003; *Peromyscus polionotus*; Belk and Smith 1996). In those examples, selection due to increased predation on uncamouflaged individuals has given rise to melanism that matches the color of the background. Populations of the rock pocket mouse, *C. intermedius*, that live on dark volcanic rock are nearly monomorphic for dark coat color while populations with lighter backgrounds have much lower frequencies of dark colored individuals (Hoekstra et al. 2004). Moreover, as the studies above highlight, the growing body of literature on the maintenance of adaptive variation primarily centers on effects of heterogeneous environments that occur between or among populations or across broad geographic areas (e.g. altitudinal or latitudinal clines) rather than temporal or spatial variation that can maintain polymorphism within a single population (Mitchell-Olds et al. 2007; Manel 2012).

A specific mechanism thought to promote the maintenance of polymorphism in heterogenous environments is pleiotropy, in which a single locus has affects on more than one trait. Specifically, when alleles at a pleiotropic locus have different relative fitness under different environmental conditions, spatial or temporal variation in the environment could promote a stable polymorphism (Hedrick et al. 1976). However, the role that ecological selection—particularly heterogeneity of abiotic environmental variables—plays in maintaining polymorphism by pleiotropy remains largely unanswered. Some studies have demonstrated the potential for large fitness effects of pleiotropy due to environment-specific fitness (Lukens and Doebley 1999; Scarcelli et al. 2007; Mensch et al. 2008; Kover et al. 2009). However, this work has been dominated by studies in model organisms including *Arabidopsis*, *Drosophila* and maize and remains comparatively understudied in non-model systems (but see Barrett et al. 2009).

Xiphophorus variatus is a small, livebearing fish that is polymorphic—within and among populations—for a melanic pigmentation pattern expressed at the junction of the caudal peduncle and caudal fin (Fig. 1). These tailspot patterns are coded by different alleles at a single, autosomal locus exhibiting pleiotropic effects on physiology (Borowsky and Khouri 1984). Pattern alleles exhibit dominance over the wild-type but are codominant

to one another (Borowsky and Khouri 1978, 1984). Up to five patterns can occur within the same populations (i.e. sympatry), however, there is geographic variation in tailspot occurrences. In other words, while some populations may have 4 or five pattern types occurring together, others have only 1 or 2 tailspot types. Across the species broad range from the eastern gulf coast of Mexico to the foothills of the Sierra Madre Oriental there is considerable heterogeneity in habitat type and quality, as well as temporal and spatial variation in abiotic environmental variables (e.g. temperature and dissolved oxygen). Collections across multiple localities and years have revealed correlations between relative condition of males and dissolved oxygen, and further laboratory experiments demonstrated differences in physiology and metabolism of 'wild-type' (+) and 'cut crescent' (Ct) patterns under varying levels of dissolved oxygen (Borowsky and Khouri 1984). Field data demonstrated that those two tailspot patterns are distributed non-randomly among pools that vary in levels of dissolved oxygen; males of the Ct pattern are found at higher relative frequency in conditions of low dissolved oxygen while wild-types preferred higher dissolved oxygen (Borowsky and Khouri 1990) Temperature was not controlled nor accounted for in that study, thus it may have contributed to those patterns, as temperature and dissolved oxygen are inversely related. Thus, field and experimental data both confirmed that the Ct pattern is favored by specific environmental conditions (i.e. low dissolved oxygen) under which this pattern is physiologically superior to other tailspot patterns.

Temperature, like dissolved oxygen, is an abiotic variable exhibiting considerable temporal variation within localities. A recent study combining correlative and mechanistic approaches in hybrid zones between swordtails *X. malinche* and *X. birchmanni* demonstrated the role of temperature in delimiting niche space of parental species and influencing
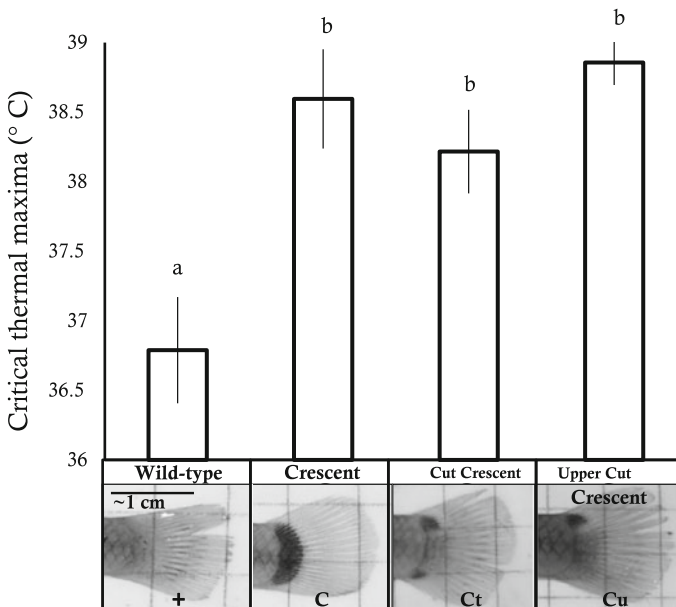
Fig. 1 Individuals with wild-type tailspot pattern exhibited significantly lower heat tolerance ($\pm$SE) than those with all other patterns from the San Pedro population. *Different letters* above the *bars* indicate significant differences ($p < 0.003$). The C, Ct, and Cu patterns did not differ ($p > 0.05$)

allele frequencies in hybrid populations (Culumber et al. 2012). Preliminary field observations of *X. variatus* suggested that temperature may be an important variable in the physiological niche of the wild-type pattern. In particular, Borowsky and Khouri (1984) reported significant negative relationships between temperature and wild-type female fecundity and wild-type allele frequency. These relationships between the wild-type pattern and temperature were consistent across collections in numerous populations, but no other tailspot patterns exhibited such relationships. However, correlative analyses can be misleading due to correlation among potential selective variables and do not address the functional mechanisms that generate selection to maintain genetic variation in these populations.

Here we address whether physiological tolerance arising from pleiotropic effects of the tailspot locus differ among allelic variants within a population. We test the performance of male and female *X. variatus* of four tailspot patterns—collected from the same pool—in response to stress from increasing temperature. We hypothesized that if stress associated with elevated temperatures underlies previous correlations with wild-type female fecundity and wild-type allele frequency, then wild-type fish should exhibit reduced physiological performance (disproportionately greater stress) under increasing temperatures. Specifically, we predicted that the wild-type pattern would have lower thermal tolerance than the Ct pattern. As previous studies have not closely investigated the 'crescent' (C) nor 'upper cut crescent' (Cu) patterns, this is the first study to evaluate the physiological response of these two allelic variants. Finally, we tested for evidence of potential selection associated with temperature in the population by comparing phenotype distributions of adults and juveniles. Temperatures are highest on average in the months preceding our sample. Thus, if wild-type female fecundity is negatively affected by stress resulting from increased temperatures, then we should detect deviations in the frequencies of the tailspot patterns of juveniles compared to the adult generation.

Methods

Study site and specimen collection

The locality where fish were collected for this study was San Pedro Huazalingo (384 m; 20° 57′ 3″N 98° 31′ 26″W) on the Rio Huazalingo in NE Hidalgo, Mexico. Adult and juvenile *X. variatus* were collected with baited minnow traps and seine net in August 2012. All fish were collected from the same pool and individuals of the different tailspot types were collected even within the same minnow traps. All specimens were transported to the Centro de Investigaciones de las Huastecas Aguazarca (CICHAZ) inside thermally insulated coolers in water from the collection locality to maintain the same temperature as when the fish were collected (25.5 °C). Only adults were used for tests of thermal tolerance, but adults and juveniles were used to calculate phenotype frequencies. A second collection using identical methods was made within one week in order to increase the sample sizes only for calculating phenotype frequencies of adults and juveniles, not for thermal tolerance. Adults not used in thermal tolerance tests and all juveniles were released back into the population at the conclusion of the second collection. The second collection, therefore, did not recount any of the individuals from the first collection.

Thermal tolerance

All fish were tested on the same day as they were collected and were maintained in water from their source population at the collection temperature with air bubblers to keep oxygen levels at or near saturated levels, until time of testing. Methods for heat tolerance trials closely followed Culumber et al. (2012). Adult fish were placed inside an enamel container with 4-L of river water from the collection locality on an inverted ceramic plate inside a larger enamel container. The container was suspended over a gas burner and the water was heated at a rate of 0.3 °C min^{-1}. The container was an 'open' system in that it was not covered and allowed for oxygen exchange with the outside air throughout the trial. Temperature was measured using two digital thermometers (Coralife, Inc.) to the nearest 0.1 °C. All temperatures used in data analyses were obtained by averaging the readings of the two thermometers for greater precision. Four to six fish were introduced to the container at the start of a trial. Fish were monitored and the critical thermal maxima, the point at which the fish lose and are unable to regain equilibrium, was recorded for each individual. At final loss of equilibrium fish were removed and temporarily placed in individual containers to recover. At the end of the experiment all fish were anesthetized in tricaine methanesulfonate (1 mg L^{-1}), digitally photographed, fin clipped (tissues stored in 95 % ethanol) and preserved in 10 % formalin. After 2 days in 10 % formalin, specimens were rinsed and soaked in water for 24 h and stored in 70 % ethanol. During trials no fish were observed performing aquatic surface respiration (ASR). ASR is a typical response of poeciliids and other fishes to stress induced by declining oxygen concentrations (Kramer and McClure 1982). ASR simply consists of performing aquatic respiration at the surface of the water because oxygen is typically at its highest concentration near the air–water interface in oxygen limited environments (Kramer and Mehegan 1981). Additionally, *X. variatus* of similar size (mean body mass = 0.655 g) do not reach their critical oxygen tension until 15–19 h in closed chamber of 250 mL volume (ZWC unpublished data). Thus, on a per fish basis (mean number of fish per trial = 5), our open, 4-L container had 3.2 times greater volume and oxygen. Based on these factors and the absence of ASR behavior, it is improbable that oxygen limitation occurred in our thermal tolerance trials that lasted only 30 min.

Tailspot phenotype frequencies

Adults (N = 87) and juveniles (N = 79) were phenotyped for tailspot pattern in order to compare phenotype distributions. If fitness is equal among all tailspot patterns, then genotype frequencies (i.e. phenotypes) should be equal in adults and juveniles. The tailspot pattern of all individuals was classified according to nomenclature in Borowsky and Khouri (1984). A single adult individual of the 'peduncular spot' (Ps) pattern was observed, and four pattern heterozygotes were observed in the juveniles. These low frequency individuals were considered outliers and excluded from the comparison of adults and juveniles. To compare adult and juvenile phenotype distributions, we used the tailspot pattern counts of juveniles as the observed category and adults as the expected. The observed category was derived from the adults by multiplying the tailspot frequencies of adults by the total sample size of juveniles (N = 79) in order to have an equal count number in the expected and observed categories.

Statistical analyses

We conducted an analysis of variance (ANOVA) to assess differences in thermal tolerance among tailspot patterns. The ANOVA was conducted on temperature at final loss of equilibrium (FLOE) with tailspot pattern and sex as factors. Since previous work has suggested that males and females of a given tailspot pattern may differ physiologically (Borowsky and Khouri 1984, 1990), we also tested the interaction of tailspot × sex. Post-hoc Fisher's LSD was used to evaluate pairwise differences in thermal tolerance among the tailspot patterns within the population. Since body size could affect individual physiology, we conducted an analysis of covariance using the same variables as above but with standard length as a covariate. There was no effect of standard length, thus we report the results of the original ANOVA. Furthermore, there was no difference in mean size ($\pm$SE) of males 37.46 $\pm$ 0.96) and females (37.14 $\pm$ 1.51) in our data set ($t = 0.184$, $df = 54$, $p = 0.856$). We used a Pearson χ^2 test to evaluate deviations in the phenotype distributions of adults and juveniles.

Results

Thermal tolerance

Tailspot pattern had a significant effect on thermal tolerance ($F_{3,57} = 5.165$, $p = 0.003$; Fig. 1). Post-hoc tests on tailspot pattern revealed that the wild-type pattern had lower heat tolerance than all other tailspot patterns ($p \leq 0.003$ for all comparisons). No other patterns differed significantly from one another in post hoc tests ($p > 0.05$). There also was a significant difference in the heat tolerance of males and females, with males exhibiting a slightly lower heat tolerance than females ($F_{1,57} = 6.584$, $p = 0.013$), but no interaction between tailspot pattern and sex ($F_{3,57} = 1.004$, $p = 0.399$).

Tailspot phenotype frequencies

Five total tailspot patterns were observed in adults from San Pedro: +, C, Ct, Cu and Ps (Table 1). Only one individual of 87 adults exhibited the Ps pattern. Pattern heterozygotes were altogether absent in the adult sample. Juveniles also exhibited five tailspot patterns, including the four most common in adults (+, C, Ct and Cu) as well as four C/Ct pattern heterozygotes. There was a highly significant deviation between adult and juvenile phenotype frequencies ($\chi^2 = 21.59$, $df = 3$, $p < 0.0001$; Fig. 2). Both the + and Ct patterns were observed at lower frequencies in juveniles than adults, while the C pattern was at a much higher frequency in juveniles.

Discussion

The role that pleiotropy plays in the maintenance of genetic variation—and particularly the underlying mechanisms—are not well understood outside of a small number of model systems. Characterization of selective agents and the mechanisms by which abiotic environmental variables figure into the maintenance of variation in adaptive traits are areas where further study is needed (MacColl 2011). Here we demonstrated that alleles at a single locus for the tailspot pattern have significant effects on physiological performance

Table 1 Sample sizes and counts of tailspot patterns in adults and juveniles collected within a pool of the San Pedro population in August 2012

Tailspot pattern	Adults N	Juveniles N
Wildtype (+)	26	16
Crescent (C)	11	22
Cut crescent (Ct)	25	13
Upper cut crescent (Cu)	24	24
Peduncular spot (Ps)	1	–
C/Ct (heterozygote)	–	4
Total	87	79

Fig. 2 Phenotype frequencies of adults and juveniles for the four tailspot patterns observed at San Pedro. *Filled bars* indicate adult pattern frequencies and *open bars* are juvenile frequencies. The phenotype distribution of juveniles was significantly different from that of adults ($p < 0.0001$)
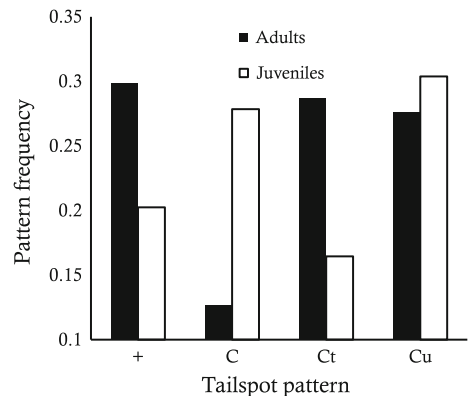
under thermal stress. Though other sources of selection cannot be ruled out, reduced frequency of the wild-type pattern in juveniles was consistent with expectations based on thermal tolerance. Temperature varies substantially with the seasons and our results together with previous observations of female fecundity provide insight into how heterogeneity in the abiotic environment can maintain adaptive genetic variation at a single, pleiotropic locus within natural populations.

Temperature and dissolved oxygen

Oxygen limitation is known to influence critical thermal limits (Pörtner 2001; Pörtner et al. 2004) and dissolved oxygen levels are negatively correlated with water temperature (APHA 1992). Thus, the potential exists for increasing temperature and decreasing oxygen to have worked in concert to induce stress in our experimental fish. However, several observations suggest that oxygen was not limiting including some that were mentioned in the methods on thermal tolerance. No fish were observed performing aquatic surface respiration, a typical behavior of oxygen limited poeciliids and other fishes (Kramer and McClure 1982). In fact, individuals that are allowed to perform ASR during thermal tolerance trials—by providing access to the water surface—have higher critical thermal maxima because oxygen limitation effects on thermal tolerance can be mitigated by the ability to obtain more oxygen by performing ASR (Rutledge and Beitinger 1989). Fish in

our trials had free access to the water surface for the duration of the trial, yet no individuals were observed performing ASR. This suggests that they were not oxygen limited.

Nonetheless, we cannot entirely rule out that oxygen limitation may have contributed to stress under increasing temperatures. This does not change our results. Our study was intended to measure differences in physiological response to thermal stress. In natural freshwater environments where fish occur, temperature and dissolved oxygen are inexorably correlated. Fish experience stress from the cumulative environment. As such, oxygen limitation is an unavoidable aspect of thermal stress in natural environments. By using an open system our study likely mimicked the manner in which fish experience thermal stress in their natural stream environment. In any case, since reduction in oxygen would have been due to increasing temperature, the ultimate underlying cause of variation in physiological performance in our trials was stress induced by increasing temperatures, even if oxygen limitation contributed to performance. Our data demonstrate that regardless of the specific mechanism (heat or oxygen stress), tailspot patterns exhibit different physiological responses to increasing temperatures. Future experiments can focus on the specific cellular and molecular pathways that give rise to the observed differences in physiology to determine the relative contributions of stress from oxygen and temperature.

Thermal tolerance

Lower heat tolerance indicates disproportionately greater stress for wild-type individuals at relatively lower temperatures compared to patterned individuals from the same population. These results are consistent with observations previously made in *X. variatus*. Correlations derived over multiple collections and localities revealed that temperature exhibited a negative relationship with wild-type female relative fecundity (Borowsky and Khouri 1984). Furthermore, as would be expected if increased physiological stress at higher temperatures lowers female fecundity, the wild-type allele frequency is typically lower following periods of warmer temperatures (Borowsky and Khouri 1984). Our results on performance of tailspot patterns in thermal tolerance trials provide empirical evidence of the mechanistic relationship between temperature and physiological stress, which may stem either from temperature directly or the interaction of temperature and dissolved oxygen.

Temperature is an important source of selection in other species of *Xiphophorus*, affecting the distribution of two parental species and their hybrids, as well as temporal variation in allele frequencies within populations (Culumber et al. 2012). As demonstrated in a common-garden rearing experiment in those closely related swordtails, plasticity is unlikely to explain variation in thermal tolerances (Culumber et al. 2012). However, even if plasticity plays a role in *X. variatus*—for example, if the wild-type pattern exhibits less plasticity in thermal tolerance—temperature would nonetheless be the selective agent affecting the frequency and maintenance of the wild-type pattern. It is unclear why males exhibited lower overall heat tolerance than females across all tailspot patterns. It was not entirely unexpected to observe differences between the sexes of *X. variatus* (Borowsky and Khouri 1984, 1990). However, in contrast to those studies which observed differences between the sexes within tailspot patterns, our differences were between the sexes as a whole. There were no differences between the two sexes within any tailspot patterns. Previously observed differences were between sexes within tailspot patterns. Female reproductive state could play a role, and females have previously been shown to differ from males in physiology related to fat distribution and cycling (Borowsky and Kallman 1993). Additionally, males of another livebearer, *Gambusia affinis*, have a relatively

complex pattern of fat cycling, having their lowest energy reserves at the end of the summer, and with the oldest males not exhibiting energy storage (Reznick and Braun 1987). Low fat reserves at the end of the summer are due to a range of factors including reproductive schedule and social cues (Reznick and Braun 1987), but could also be related to the substantial amount of time that male livebearers invest in courting, harassing, and attempting copulations with females during the mating season (Magurran and Seghers 1990; Bisazza and Marin 1995; Plath et al. 2007). Given these observations and that lower energy reserves may be a sign of poorer condition and reduced ability to resist environmental stress (Borowsky and Khouri 1990; Hoffman and Harshman 1999; Neff and Cargnelli 2004), differences in energy reserves between the sexes may explain the patterns observed in thermal tolerance. Further work will be needed to investigate whether differences in thermal tolerance between the sexes are consistent among multiple populations or may be seasonal in nature and, if so, what might be the basis.

The critical thermal limits observed in these fish, as with many studies of critical thermal limits, are slightly out of the range of temperatures observed in the wild. However, it is important to consider that the critical thermal limit was defined as the point at which fish lost all motion control. If these temperatures were reached in the wild, it would almost certainly be fatal to fish. The important consideration is that fish experience physiological stress prior to losing all motion control and death. The significantly lower critical thermal maximum of wild-type compared to patterned fish indicates that wild-type fish experience physiological stress at a comparatively lower threshold. The previously reported, negative correlations between wild-type allele frequency and wild-type female fecundity with temperatures recorded in natural populations suggest that wild-type individuals experience reduced fitness at lower temperatures compared to patterned fish (Borowsky and Khouri 1984). Similarly, temperature is a significant predictor of allele frequency in both space and time in closely related swordtails that exhibit different critical thermal limits along an elevational gradient, even though critical thermal limits are not reached in natural populations (Culumber et al. 2012). Thus, temperatures need not reach levels sufficient to kill fish in natural populations for physiological stress to be biologically relevant.

Tailspot phenotype frequencies

Temperatures in the area of the collecting locality, and in the broader region, reach their mean monthly maximums and daily maximums between April and August, the time period directly preceding fish collection for this study (Servicio Meteorologico Nacional Mexico).

The frequency of the wild-type pattern was substantially lower in juveniles than adults, consistent with the expectation of reduced wild-type allele frequency following warm temperatures, possibly resulting from lower fecundity of wild-type females (Borowsky and Khouri 1984). Environmental stress is known to negatively affect reproduction in fishes and such stressors often result in more acute effects for juveniles, affecting their growth and survival (Pankhurst and Munday 2011 and references therein). The fact that wild-type was the most frequent phenotype in adults but only the third most frequent in juveniles, suggests that increased environmental stress from higher temperatures may impose selection on female fecundity and/or juvenile survival at early life stages. Similar effects of environmental stress have been observed for the Ct pattern under varying concentrations of dissolved oxygen. At low dissolved oxygen where Ct is favored due to lower metabolic oxygen demand, juvenile growth rate of the Ct pattern exceeded that of wild-type juveniles (Borowsky and Khouri 1984). While the discrepancy between adult and juvenile wild-type frequencies coincided with the warmer summer period, consistent with results of thermal

tolerance, ultimately, longer-term data from within this population is necessary to confirm this relationship.

The Ct and C patterns also exhibited considerable changes in frequency between adults and juveniles. The frequency of Ct and C were 12 % lower and 15 % higher, respectively, in juveniles compared to adults. Whether these deviations stem from selection relating to temperature is not clear, but thermal tolerance data suggest that this is unlikely since these patterns had similar critical thermal maxima. Thus, it seems probable that other environmental factors (e.g. dissolved oxygen) act simultaneously and have asymmetric effects among the tailspot patterns within a population. We cannot entirely rule out the contribution other processes such as migration to changes in pattern frequencies. While there is no evidence to suggest that any pattern types are inherently more likely or capable to migrate, migration could itself be affected by environmental variables. For example, Borowsky and Khouri (1990) study on dissolved oxygen revealed that individuals of the + and Ct pattern types distributed non-randomly among pools according to the microhabitat conditions most favourable for each pattern. Additionally, as physiology of these allelic variants are clearly affected by abiotic variables, migration could be biased against individuals who are under greater physiological stress due to unfavourable environmental conditions. These processes would nonetheless indicate a role of environmental selection and physiological stress underlying deviations in phenotype frequencies. Historical frequencies due to founder effects or bottlenecks are unlikely to account for differences in present day frequencies unless contemporary selection maintains frequencies at historical levels. Random mating and equal fitness should return populations to Hardy–Weinberg equilibrium and, through evolutionary time, random genetic drift would result in the fixation of different tailspot alleles among different populations.

The maintenance of adaptive genetic variation and associated phenotypic variation is complex. Even when a morphological trait exhibits adaptive variation in a heterogeneous environment, heterogeneity in the environment may not be involved in the maintenance of trait variation, as demonstrated by a study of wing shape in *Drosophila melanogaster* (Yeaman et al. 2010). Alternatively, environmental heterogeneity may be only one among a variety of factors contributing to maintenance of a polymorphism. In the case of *X. variatus*, environmental factors have strong effects on physiology of tailspot allelic variants. However, other sources of selection may contribute to maintaining the polymorphism. In particular, sexual selection related to the pigmented tailspots and ecological selection stemming from variation in predation or habitat complexity are plausible mechanisms for maintaining variation.

Sexual selection due to female mate choice on male morphology is common in live-bearing fishes (Rosenthal and García de León 2011). Female guppies, *Poecilia reticulata*, prefer males with novel color patterns, promoting the maintenance of the extreme levels of color polymorphism observed in the species (Hughes et al. 1999). Pigmentation patterns factor prominently in mate choice for several species of *Xiphophorus*, as well. *Xiphophorus* females exhibit variation in preference for pigmented vertical bars (Morris et al. 2003), body coloration (Kingston et al. 2003) and indirect selection for a macromelanophore pattern, 'spotted caudal', controlled by an oncogene conferring larger male body size favored by females (Fernandez and Bowser 2010). Thus, female preferences for particular pigmented tailspot patterns in *X. variatus* or preferences for rare patterns (e.g. negative frequency dependence) could contribute to the maintenance of the polymorphism within populations. Previous work on mating patterns in the southern platyfish, *X. maculatus*, showed strong disassortative mating at the tailspot locus (Borowsky and Kallman 1976). However, when investigated in *X. variatus*, no evidence of non-random mating was

observed (Borowsky and Khouri 1976). Therefore, it appears that any effects of sexual selection on maintenance of the polymorphism are likely minimal in *X. variatus*.

Alternatively, pigmentation patterns and melanism commonly arise from or are maintained by environmental factors or ecological selection via predation or competition. Two classic examples involve adaptive melanism that evolved in response to variation in the background color of the environment of the pepper moth (*Biston betularia*; Cook 2003) and the mice *C. intermedius* (Nachman et al. 2003) and *Peromyscus polionotus* (Belk and Smith 1996). In both the moth and mice examples, matching the color of the environment provides camouflage from predators and thus a fitness advantage. In the guppy, multiple characteristics of polymorphic color patterns expressed by males have evolved and are maintained by differences in predation regimes (Endler 1995). Both predation and substrate size influence the number, size and color of spots on males (Endler 1978, 1980). In *Xiphophorus pygmaeus*, agonistic behavior between males plays an important role in maintaining color polymorphism within populations (Kingston et al. 2003). The extent to which aggressive behaviors may exist among males of different tailspot patterns is not known. If such encounters occur, and are sufficiently strong to maintain polymorphism, they should likely be apparent in mating patterns. Previous work demonstrated no deviations from non-random mating in *X. variatus* (Borowsky and Khouri 1976). Differential mortality from predators such as the Mexican tetra (*Astyanax mexicanus*), sleeper goby (*Gobiomorus dormitor*) or the amazon kingfisher (Chloroceryle amazona) among tailspot variants cannot be ruled out. Although, it seems unlikely that variation in tailspots is maintained by variation in the substrate of the habitat. High tailspot polymorphism is maintained within populations in pools that have uniform substrate type and size (ZWC unpublished data). Nonetheless, ecological factors such as aggressive behavior and predation cannot be ruled out entirely.

Conclusion

Physiological effects of increasing temperature differed between wild-type and patterned fish, which is consistent with the hypothesis that individuals with different tailspot patterns have different physiological niches. However, changes in the frequency of multiple patterns suggest the possibility of simultaneous selection on tailspot frequencies stemming from other biotic or abiotic factors. Both spatial and temporal variation in environmental conditions likely contribute to maintaining adaptive polymorphism at this pleiotropic locus within individual populations. These results highlight the potential for climate change to impact levels of adaptive polymorphism. Based on the current data for *X. variatus*, increasing temperatures—whether acting alone or through interactions with oxygen levels—could provoke a reduction of genetic variation in some geographic areas as physiological tolerances of some tailspot allelic variants are exceeded. This could also potentially lead to expansion of the species' distribution by enabling upward migration of *X. variatus* into higher-elevation areas that previously were unsuitable. This work provides new insight into the relationship between environmental heterogeneity and the maintenance of polymorphism at a pleiotropic locus and a mechanism by which rising global temperatures may affect levels of genetic variation in this and other species.

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