SHORT COMMUNICATION

Artificial thermal gradients alter the distribution of Daphnia mendotae genotypes

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Previous work on Daphnia found that phosphoglucose isomerase (Pgi) homozygotes produced a more thermally stable form of the enzyme compared with heterozygotes. We found a positive relationship between temperature and homozygote frequency in a cooling reservoir with strong thermal gradients. Laboratory experiments revealed that heterozygotes had lower thermal tolerance. Integrating existing information about biochemical properties of Pgi, and data from thermal tolerance assays helped explain the population genetic consequences of temperature shifts in this lake.

KEYWORDS: allozymes; genotypic distribution; phosphoglucose isomerase; thermal tolerance; zooplankton

INTRODUCTION

Freshwater ecosystems are particularly sensitive to environmental change (Kernan et al., 2010). Utilizing information at one level of organization to make predictions at another level have been particularly useful in advancing our understanding of the consequences of environmental change on freshwater ecosystems (Woodward et al., 2010). Shifts in water temperature could displace thermally sensitive species, and allow for invasion by thermally resistant species (Krenkel and Parker, 1969). At the intraspecific
level, variation in thermal biology can affect genotypic distribution (Rice and Emery, 2003). For example, changing ambient temperatures might favor some genotypes compared with others if there is genetic variation in the thermal stability of key enzymes within a population (Watt and Dean, 2000).

It is well known that there is considerable genetic variation in several genes involved in glycolysis, the fundamental metabolic process that utilizes glucose as a substrate to generate ATP (e.g. Bulfield et al., 1978). Moreover, extensive information on specific glycolytic genes (e.g. phosphoglucose isomerase, \( Pgi \)) in response to temperature exists (reviewed in Riddoch, 1993; Wheat, 2010). Variation in key enzyme characteristics (e.g. activation energy, thermal stability) among genotypes explains, with marked consistency, the fitness and distribution of genotypes in environments naturally differing in temperature (e.g. Watt, 1977; Hoffmann, 1983; Carvalho and Crisp, 1987; Zera, 1987), and also due to progressive global warming (Dahlhoff and Rank, 2000; Rank and Dahlhoff, 2002).

\( Pgi \) variants of aquatic arthropods such as \( Daphnia \) differ considerably in their response to natural (seasonal) variation in temperature (Carvalho, 1988). Nevertheless, little is known about the response of \( Pgi \) variants to changes in ambient temperature within a lake in any given time (i.e. spatial). Studying populations of \( Daphnia \) inhabiting artificial lakes constructed as water-cooling units for power plants allows us to test whether spatial variations in water temperature have noticeable effects on population genetic structure. As in all other organisms, \( Pgi \) catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate in the preparative phase of glycolysis in \( Daphnia \) (Boriss, 2001). Previous work found that \( Pgi \) homozygotes produce a more thermally stable form of the enzyme than heterozygotes, while heterozygotes have lower activation energy than homozygotes, which enable it to carry out glycolysis at a higher rate in colder temperatures (Boriss, 2001). Whether changing ambient temperature alters the distribution of \( Pgi \) genotypes in a field setting remains to be ascertained.

The aim of this study was to test whether spatial variation in water temperature has noticeable effects on the frequency and distribution of \( Pgi \) genotypes in the natural population of \( Daphnia \) inhabiting a cooling reservoir with steep temperature clines. Specifically, we tested whether the proportion of \( Pgi \) heterozygotes varied spatially among sites differing in water temperature, and temporally, as water temperature at all sites increased over the sampling period (i.e. from April to June). Furthermore, we tested whether the \( Pgi \) genotype is associated with differential thermal tolerance that could potentially explain such distributional shifts due to temperature.

TEMPERATURE GRADIENT IN SOONER LAKE

Sooner Lake is an impoundment of Greasy Creek (a tributary of the Arkansas River) built in 1976 by the Oklahoma Gas and Electric Company (OG&E) as a cooling reservoir. The lake has a capacity of 0.2 km\(^2\) and an average depth of 8.5 m, and maximum depth of 27 m (Boeckman, 2011). OG&E discharges warm water into the lake causing a gradient in water temperature downstream from the point of disposal. Sooner Lake was initially explored to establish the water temperature gradient. Eight sites were selected spanning the gradient of temperature from warmest (near the plant disposal) to the coldest (by the plant intake). Four cold, and four hot sites were determined depending on their respective distance from the plant intake, and average surface temperatures recorded from 2009 to 2011 (Boeckman, 2011). The sites were sampled once in each of April, May and June 2011. In addition, water temperature at the surface was recorded at each sample site with a Hydrolab Quanta multi-parameter probe (Hach Hydromet Corporation, Loveland, CO). ANOVA performed in SPSS (IBM Corp, 2011) revealed that sampling date had a major effect on water temperature (\( F_{2,22} = 27.21, P < 0.0001 \)). The warm sites had significantly higher water temperature compared with cold sites (\( F_{2,22} = 4.13, P = 0.04 \)), while no significant differences in temperature were found within the cold and hot sites (\( F_{2,22} = 0.81, P = 0.59 \)). These trends are consistent with long-term studies in Sooner Lake that have observed striking temperature variation both spatially, as well as seasonally (Boeckman, 2011).

PGI FREQUENCY

\( Daphnia mendotae \), the most abundant daphniid species in the lake, were collected by vertical towing with a 64-\( \mu \)m plankton net at depths ranging from 3 to 4 m. Five tows were done per site, and placed in 800 mL of lake water and transported in coolers to the laboratory. Individuals were placed in 96-well plates and stored at \(-80^\circ\)C for subsequent allozyme screening. Allozyme electrophoresis was done on cellulose acetate gels, and stained based on the methods of Hebert and Beaton (Hebert and Beaton, 1986). We examined two loci: phosphoglucoisomerase (\( Pgi \)) and phosphoglucomutase (\( Pgm \)). \( Pgm \) was screened as control loci to verify whether changes in \( Pgi \) frequency are driven by thermal ecology. \( Pgi \) alleles were scored on a 1–5 scale: 5 being the fastest travelling allele and 1 being the slowest. Frequency of \( Pgi \) heterozygotes and homozygotes per site was recorded. The majority of heterozygotes (495 total individuals genotyped) found was
scored as 14, but 12 individuals with 45, and 9 individuals with 15 genotypes were observed. The majority of homozygotes (422 total individuals genotyped) found was scored as 44, but 48 individuals with 11 genotypes were found. Alleles two and three were not observed in this population. We discarded data from the extremely rare genotypes, and only used 14 and 44 genotypes in this study. These genotypes are comparable to the SM and MM genotypes reported in Boriss (Boriss, 2001), respectively.

We used a binomial generalized linear mixed model to test whether water temperature predicted the probability of finding a Pgi heterozygote. This test revealed that temperature predicted heterozygote occurrence with a high degree of significance (Fig. 1a; \(F_{1,384} = 134.69, P < 0.00001\)). We ran another model with sampling date as the fixed effect and found that occurrence of Pgi heterozygotes varied significantly among the three sampling dates (\(F_{2,384} = 101.94, P < 0.00001\)). On the other hand, neither temperature (Fig. 1b; \(F_{1,384} = 0.168, P = 0.68\)), nor sampling date (\(F_{2,384} = 0.134, P = 0.87\)) significantly predicted the occurrence of Pgm heterozygotes. These observations indicate that water temperature is correlated with the distribution of Pgi, but not Pgm, genotypes in Sooner Lake. Nevertheless, the precise mechanisms underlying such distributional patterns remain unclear. Based on Boriss (Boriss, 2001), we would expect Pgi heterozygotes to have lower thermal tolerance because they possess an enzyme with lower thermal stability.

**TEMPERATURE TOLERANCE OF PGI GENOTYPES**

During sampling, 10 additional individuals per site were placed in individual 100-mL jars with filtered (80-μm mesh size) water from Sooner Lake and cultured for further temperature tolerance experiments. These were used to test whether changes in Pgi frequency was indeed driven by differences in thermal stability of the Pgi enzyme between heterozygotes and homozygotes. Individual Daphnia isolated from Sooner Lake were maintained in culture in the laboratory after genotyping as described above. From these laboratory cultures 30 heterozygote and 30 homozygote gravid females were selected for generation of the 540 individuals required for experiments. A majority of the heterozygotes (i.e. 25) were isolated from colder sites, while the majority of the homozygotes (i.e. 21) were isolated from warmer sites of Sooner Lake. Following Kiivivouri and Ladhes (Kiivivouri and Ladhes, 1996), three 3-day-old neonates were shocked for 30 min at 31, 32, 33, 34, 35 and 36°C in a hot water bath. Fifteen replicates per temperature, per Pgi genotype were performed (i.e. \(15 	imes 6 	imes 2 = 180\) jars of 3 individuals each). Mortality was determined by lack of movement and heartbeat using a dissection microscope at 4× magnification (Leica S8APO, Leica Microsystems, IL) after 24 h. Individuals living after 24 h were considered to have recovered from the heat shock treatment and thereby possess superior thermal tolerance. A significant binomial generalized linear mixed model revealed that stress temperature had a major impact on mortality (Fig. 2; \(F_{1,536} = 136.51, P < 0.0001\)), with mortality increasing with temperature. There was a significant temperature treatment by Pgi genotype interaction (\(F_{1,536} = 1.222; P = 0.039\)). This interaction arises because homozygotes exhibited significantly higher thermal tolerance compared with heterozygotes, specifically at 34 and 35°C (Fig. 2).

Our data clearly show that Pgi heterozygotes had lower thermal tolerance compared with homozygotes (Fig. 2). Enzyme kinetic work on Pgi from heterozygotes and homozygotes in not only Daphnia (Boriss, 2001), but also other arthropods (Watt, 1977) found that homozygotes produce an enzyme that has lower thermal stability, and requires higher activation energy compared with heterozygotes. It is possible that such biochemical differences between genotypes may underlie the distributional dynamics of genotypes in the D. mendotae population inhabiting Sooner Lake. No doubt, other loci linked to Pgi may confer a fitness advantage that could explain the distribution of Pgi genotypes. For example, Dalhoff and Rank (Dalhoff and Rank, 2000) found that Pgi homozygotes of the montane beetle Chrysomela aeneicollis exhibited higher Hsp70 levels compared with homozygotes that, in addition to variation in enzyme kinetic parameters at Pgi elaborated above, explained the frequency distribution of Pgi genotypes in this beetle population at a regional scale. It is likely that similar covariation between the Pgi genotype and other genomic regions underlies the spatio-temporal distribution of Pgi genotypes in Sooner Lake and warrants further study. In addition, it is possible that other environmental parameters associated with temperature could interact to determine Pgi frequencies at various locations in Sooner Lake. For example, Ross et al. (Ross et al., 1996) found that the distribution of Daphnia pulicaria Pgi genotypes was correlated with dissolved oxygen. Further studies isolating the covarying components at both the genomic, as well as the ecological levels, are needed to fully understand the precise mechanisms that underlie the clines in the Pgi genotype reported here.

**CONCLUSION**

Daphnia inhabiting Sooner Lake are subjected to strong temperature gradients because of the lake’s function as a cooling reservoir for a power plant. Sooner Lake offers a
A "semi-natural" system to tease apart the consequences of temperature shifts on population genetics, and ultimately microevolutionary trajectories. We predicted that homozygotes at \( P_{gi} \) will be more frequent in the warmer zones of the lake (downstream from the effluent source) and heterozygotes at \( P_{gi} \) would be more frequent in colder zones of the lake. Systematic spatial sampling and genotyping using allozyme electrophoresis supported our predictions. Furthermore, laboratory thermal tolerance assays revealed that \( P_{gi} \) heterozygotes exhibited lower tolerance and recovery compared with homozygotes. Further studies on the enzymatic properties of \( P_{gi} \) from Sooner Lake \textit{Daphnia}, and its associations with other key loci such as heat shock proteins is warranted to identify the mechanisms underlying the observed distribution patterns. Our study demonstrates that information on sub-cellular biochemical properties can be used to generate and test predictions about the distribution of genotypes in space and time. Populations inhabiting ecosystems that are under substantial anthropogenic influence are ideal models to test and refine such predictions. Such predictive power, generated by mechanistically linking processes at multiple levels of organization will be useful to better understand and manage the impacts of global environmental change on natural populations.

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