Resting oxygen consumption varies among lactate dehydrogenase genotypes in the sow bug, *Porcellio scaber*

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SUMMARY

Laboratory studies of respiration in the sow bug, *Porcellio scaber*, reveal that respiration rates are related to genetic variation at the lactate dehydrogenase (Ldh) locus. In population samples taken from Burlington, North Carolina and Pacific Grove, California, respiration rates differed among Ldh genotypes, but not among genotypes at the other enzyme polymorphisms. In both population samples, the respiration rate of the common Ldh homozygote exceeded the respiration rate of the heterozygote by more than 50%. The differences in respiration rates are consistent with previously reported viability differentials at the Ldh polymorphism.

1. INTRODUCTION

The fields of physiological ecology and population biology have been linked together by a series of studies that relate allozyme variation to physiological variation associated with components of fitness (Watt 1991; Clark & Koehn 1992; Feder & Watt 1992; Garland & Carter 1994; Mitton 1993a,b, 1997). Several of these studies have reported a single enzyme polymorphism to have a surprisingly strong influence on oxygen consumption. For example, the lactate dehydrogenase (Ldh) genotypes in the killifish, *Fundulus heteroclitus* (Powers *et al.* 1994), differ in a variety of kinetic parameters, most notably k_{cat}/K_M , and the kinetic variation is related to the rate of development of eggs (DiMichele & Powers 1982a), the amount of oxygen consumed (DiMichele & Powers 1984; Paynter *et al.* 1991) and delivered to muscles (DiMichele & Powers 1982b), and the sustained swimming speed of the fish (DiMichele & Powers 1982b). The Adh genotypes of the tiger salamander, *Ambystoma tigrinum*, have significant kinetic variation for the k_{cat}/K_M ratio, and this variation is associated with variation in oxygen consumption, growth rate, and success at metamorphosis (Mitton *et al.* 1986; Carter 1997; Carter *et al.* 1997; Mitton 1997). Red blood cell levels of adenosine triphosphate (ATP), which modulate the affinity of haemoglobin for oxygen, differ among Adh genotypes. The frequency of Adh-SS homozygotes increases with levels of dissolved oxygen in ponds, as a consequence of differential growth and

success at metamorphosis. In the honey bee, *Apis mellifera*, malate dehydrogenase genotypes respire at different rates during hovering (Coelho & Mitton 1988), and exhibit different levels of developmental stability (Messier & Mitton 1996).

Here we test for variation in rates of respiration among allozyme genotypes in the sow bug, *Porcellio scaber*. Sassaman (1978) conducted a demographic analysis of *P. scaber*, and reported average viabilities at the Ldh locus to be 0.86, 1.00, and 0.64 for the common homozygote, heterozygote, and rare homozygote, respectively. This consistent pattern of viability selection detected at the Ldh locus suggested the hypothesis that rates of respiration vary among Ldh genotypes.

2. MATERIALS AND METHODS

Population samples of sow bugs were obtained from Carolina Biological Supply in Burlington, North Carolina, and Hopkins Marine Station, Pacific Grove, California. At both localities, animals were taken from natural populations. The populations were maintained in the laboratory in containers with damp soil, potatoes, and carrots.

Two studies of oxygen consumption differed in details, and are described separately below. In both experiments, we placed sow bugs in sealed syringes, and left them undisturbed, in the dark, to estimate resting rates of respiration. We assumed that differences among individuals were attributable to metabolic rate, and not differences in activity. We also assumed that the respiratory exchange rate (CO_2/O_2) was 1.0 to estimate

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the respiration rate from CO₂ production and O₂ consumption. Mass-specific CO₂ production rates were standardized to 273 K and 760 mmHg pressure. Finally, each individual was weighed on a sartorius 1207 MP2 top loading balance.

(a) *Experiment one*

Forty individuals sampled from Burlington, NC, were maintained in the laboratory for several weeks. To obtain estimates of the rate of respiration, individuals were placed into 10 ml sealed polyethylene syringes. Sow bugs usually attempt to escape from bright light, but are quiescent for extended periods of time in the dark. The syringes were placed in a darkened area and the sow bugs were left undisturbed for 4 h. The air in each syringe was injected as a single bolus through a drierite chamber to remove water and then into an Anarad flow-through CO₂ analyser to measure CO₂ content. The air was passed through a chamber of drierite to remove water. For the calculation of CO₂ production, the concentration of CO₂ in ambient air was assumed to be 0.03%.

(b) *Experiment two*

Individuals were randomly chosen from the Hopkins Marine Station population sample and placed in open 50 ml plastic syringes. A damp piece of paper towel was added to the syringe to minimize water stress and its effects on respiration. The sow bugs were allowed 2 h to adjust to their new surroundings by placing the syringes in the dark. After 2 h, the plunger of the syringe was placed into the bore, sealing the sow bug in an air-tight cylinder. Animals were left in the cylinder for 12 h. The air in each syringe was injected as a single bolus through a drierite chamber to remove water and then into an Anarad AR400 respirometer, and CO₂ and O₂ levels were measured. Assumptions of 20.96% O₂ and 0.03% CO₂ in ambient air were used to calculate changes of both gases during the 12 h period.

(c) *Electrophoresis*

Protein homogenates were prepared by homogenizing each sow bug by hand in an equal volume of grinding solution (0.5 g dithiothreitol in 10 ml of distilled water), in a 1.5 ml eppendorf microcentrifuge tube. Homogenates were prepared just prior to electrophoresis, and kept on ice until they were loaded into starch gels. Phosphoglucosmutase (Pgm), malate dehydrogenase (Mdh), lactate dehydrogenase (Ldh) and phosphoglucose isomerase (Pgi) were resolved on the tris-citric acid (pH 8.0) buffer system described by Tanaka *et al.* (1980).

(d) *Statistical analyses*

Heterogeneity of allelic frequencies was tested with the χ^2 test of Workman & Niswander (1970), which is equivalent to a row by columns test of independence

on the numbers of alleles in two or more population samples.

The sow bugs in experiment two were measured over two days, and although the weights of animals did not differ between days, both CO₂ production and O₂ consumption were heterogeneous between days. This heterogeneity was treated with a dummy variable in either multiple regressions or ANCOVA.

The allometric relationship between metabolic rate and organismic mass was tested by regression on a log-log plot. Although this relationship was not statistically significant in either set of data, the trends for mass-specific measures of respiration to decline with mass were apparent. To remove this influence of mass, measures of CO₂ production and O₂ consumption were regressed against mass. Residuals from this regression were then used in analyses of variance to test for the influence of genotypes, at a particular locus, on measures of respiration. These analyses are equivalent to ANCOVA, using mass and day as covariates. Although residuals were used in the ANCOVA and regressions, the values presented in tables and figures are the estimates of O₂ consumption and CO₂ production, with the units ml g⁻¹ h⁻¹. Presentation of data with these units makes it easier to examine differences among groups, and facilitates comparisons with other studies.

Heterogeneity of allelic frequencies was tested with the χ^2 test of Workman & Niswander (1970). The fit of observed genotypic frequencies to Hardy-Weinberg expectations was tested with a χ^2 test (Spiess 1977).

3. RESULTS

In all population samples, observed genotypic frequencies had acceptable fits to Hardy-Weinberg equilibrium expectations. Two alleles were segregating at the Ldh locus, and allelic frequencies were similar in the two population samples (table 1). Three alleles were segregating at the Pgm and Pgi loci, and once again, allelic frequencies were similar between localities. Allelic frequencies were significantly differentiated between localities at the Mdh locus ($p < 0.001$, table 1). Three Mdh alleles reached frequencies of 0.10 or greater in Burlington, NC, but only two alleles segregated at this locus in Pacific Grove, CA, and the common allele had a frequency of 0.96. For each of these loci, the same allele was the most common allele in both populations.

In both experiments one and two, CO₂ production was significantly lower in Ldh heterozygotes than in Ldh homozygotes (table 2). In experiment one, the mean values of VCO₂ for Ldh homozygotes and heterozygotes were 0.0962 and 0.0568 ml g⁻¹ h⁻¹, respectively ($p < 0.001$). In experiment two, the mean values of VCO₂ for Ldh homozygotes and heterozygotes were 0.1058 and 0.684 ml g⁻¹ h⁻¹, respectively ($p < 0.05$). In both experiments, there were no differences in CO₂ production among the genotypes at the loci coding for Pgm, Pgi, and Mdh. Thus, in both experiments, CO₂ production was related only to variation at the Ldh locus, and in both experiments, mean

Table 1. Genotype counts and allele frequencies at allozymes in two populations of the sow bug, *Porcellio scaber*

(χ^2 test of the heterogeneity of allelic frequencies between localities. p is the probability associated with χ^2 . n.s. means not significant.)

enzyme	locality	genotype counts						allele frequencies				
		11	12	22	13	23	33	F(1)	F(2)	F(3)	χ^2	p
Ldh	Burlington	40	8	0	—	—	—	0.92	0.08	—	0.3	n.s.
	Pacific Grove	41	6	0	—	—	—	0.94	0.06	—		
Mdh	Burlington	3	15	14	4	4	0	0.31	0.59	0.10	39.6	<0.001
	Pacific Grove	0	0	44	0	4	0	0.00	0.96	0.04		
Pgm	Burlington	0	4	18	1	13	4	0.06	0.066	0.028	1.4	n.s.
	Pacific Grove	0	3	28	0	14	3	0.03	0.76	0.21		
Pgi	Burlington	8	15	4	8	3	2	0.49	0.33	0.19	4.0	n.s.
	Pacific Grove	7	10	3	19	7	2	0.45	0.24	0.31		

VCO₂ was more than 50% higher in homozygotes than in heterozygotes.

Oxygen consumption, measured only in experiment two, differed between heterozygotes and homozygotes at the Ldh locus (table 2). The mean values of oxygen consumption for homozygotes and heterozygotes were 0.1179 and 0.0758 ml g⁻¹ h⁻¹, respectively ($p < 0.05$). Once again, this measure of respiration was more than 50% higher in Ldh homozygotes than in heterozygotes. Oxygen consumption did not vary among the genotypes at the loci coding for Pgm, Pgi, or Mdh.

4. DISCUSSION

The population samples for experiments one and two were taken from geographic localities 3900 km apart, yet they revealed the same differential; the respiration rate of the common Ldh homozygote was 50% higher than the respiration rate of the heterozygote (table 2). The lower respiration rate of heterozygotes is consistent with their superior viability (Sassaman 1978). In population biology, it is always difficult to reject the hypothesis that the important genetic variation is one or more loci that are in linkage disequilibrium with the marker (Ldh) locus. However, the consistency among the geographic locations (table 2), and between Sassaman's study (1978) and the present study, suggest that the major effects on respiration and viability are due to the Ldh locus, and that a functional study of Ldh allozymes in *P. scaber* would reveal the presence of

causal relationships between Ldh variation and variation in estimates of Darwinian fitness.

An active debate focuses on the underlying cause of relationships between heterozygosity and rates of respiration and growth (Mitton & Grant 1984; Zouros & Foltz 1987; Mitton 1993a,b, 1994, 1995, 1997; Gaffney 1994; Zouros & Pogson 1994). The decreases in respiration rates with heterozygosity have been hypothesized to be caused by a range of inbreeding coefficients in the population, by associative overdominance, by null alleles, and by kinetic differences among allozyme genotypes. Zouros & Pogson (1994) recently reported that growth rate of the deep-sea scallop, *Placopecten magellanicus*, increases with allozyme heterozygosity, but not with the heterozygosity of anonymous nuclear DNA polymorphisms. That result, and the results of Koehn *et al.* (1988) and the present study, suggest that relationships between allozyme heterozygosity and components of fitness are locus-specific, and therefore probably not caused by variation in the degree of inbreeding in the population.

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Table 2. Mass-specific measures of CO₂ production and O₂ consumption for Ldh homozygotes and heterozygotes

(Both VCO₂ and VO₂ have the units ml g⁻¹ h⁻¹. N is the sample size. Asterisks indicate significant differences between genotypes revealed by analyses of variance: *** indicates $p < 0.001$, * indicates $p < 0.05$.)

locality	genotype	N	VCO ₂	VO ₂
Burlington, NC	11	32	0.0962***	
	12	8	0.0568	
Pacific Grove, CA	11	41	0.1058*	0.1179*
	12	5	0.0684	0.0758

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