Physiological stress and the fitness effects of *Mpi* genotypes in the acorn barnacle *Semibalanus balanoides*

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Supplement

Experimental design for the laboratory treatments, further information on Hardy-Weinberg equilibrium and linkage disequilibrium, and mtDNA genotyping data

Experimental design

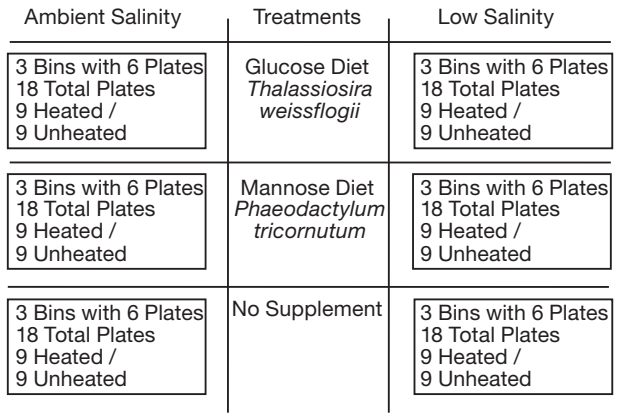

Fig. S1. Experimental design detailing each treatment and level of replication. Plates within treatments were randomized among the bins daily. Bins were also rinsed during the imposed low tide periods throughout treatment to further eliminate bin effects

Hardy Weinberg equilibrium and linkage disequilibrium

Hardy Weinberg equilibrium (HWE) was assessed for each locus in each of the treatments using the 'genhw' package in Stata (StataCorp 2007). No treatments showed significant deviations from HWE at either locus. Values in Table S1 are p-values from Fisher exact tests of HWE. There was also no significant linkage disequilibrium (LD) observed between the *Mpi* and *Gpi* genotypes in any of the treatments. P-values below refer to Fisher exact tests of genotypic LD between *Mpi* and *Gpi*.

Table S1. Hardy-Weinberg equilibrium and linkage disequilibrium (LD) p-values for the *Mpi* and *Gpi* genotypes. Values correspond to significance in Fisher exact tests

Treatment	Hardy-Weinberg p-value *Mpi*	*Gpi*	LD p-value
Field collected	0.310	0.886	0.904
Laboratory – no heat			
No supplement	0.881	0.282	0.439
Mannose diet	0.227	0.156	0.797
Glucose diet	1.000	0.052	0.103
Laboratory – heat			
No supplement	0.664	0.362	0.670
Mannose diet	0.393	0.056	0.478
Glucose diet	0.588	0.782	0.659
Total	0.956	0.212	0.207

mtDNA analysis

Genotyping of mtDNA was done as an internal genomic control following procedures described in Schmidt & Rand (1999). Previous work shows that it does not respond to heat stress as its frequency does not change in hot and cold microhabitats (Schmidt et al. 2000, Schmidt & Rand 2001). One alternative hypothesis for this experiment is that selection could act on cohorts of individuals that have different allele frequencies at the allozyme loci or the mtDNA. A shift in allele frequency could then be observed if selection was on a cohort of individuals, and it would have little to do with the allozyme loci in question. If mtDNA frequencies had changed, this would have provided compelling evidence for this cohort-level selection as opposed to direct selection on metabolic pathways.

Cirri were removed from *Semibalanus balanoides* individuals and DNA was extracted following incuba-

Table S2. Frequency and fitness data for mtDNA genotypes. The number of individuals and plates surveyed are shown. Fitness values correspond to frequencies in each treatment divided by the initial frequency of the genotype in the field population. n/a: not applicable

Treatment	No. plates	No. individuals	mtDNA type 1		mtDNA type 2	
			Frequency	Fitness	Frequency	Fitness
Field collected	4	96	0.6250	n/a	0.3750	n/a
No heat, no supplement	4	125	0.6320	1.0112	0.3680	0.9813
Heat, no supplement	4	95	0.6000	0.9600	0.4000	1.0667
No heat, mannose supplement	4	110	0.5909	0.9454	0.4091	1.0909
Heat, mannose supplement	2	66	0.6667	1.0667	0.3333	0.8888
No heat, glucose supplement	4	115	0.6261	1.0017	0.3739	0.9971
Heat, glucose supplement	4	136	0.6471	1.0353	0.3529	0.9411
No heat, pooled	12	350	0.6171	0.9873	0.3829	1.0211
Heat, pooled	10	297	0.6364	1.0182	0.3636	0.9696
Total	26	743	0.6258	n/a	0.3742	n/a

tion in TE buffer with proteinase K added. Following heat inactivation of the proteinase K, the control region of the mtDNA was PCR amplified using primers from Brown et al. (2001) and digested with *Dde*I to resolve mtDNA haplotypes on a 2% agarose gel. The polymorphic site leading to enzymatic digestion is present in about 40% of *S. balanoides* mtDNA haplotypes. Overall, 743 individuals were genotyped and no significant or consistent shifts were observed in the frequencies of the 2 mtDNA haplotypes across all treatments (Table S2). Logistic regression with diet and heat terms was not significant ($\chi^2_5 = 1.6$, $p = 0.9017$), and neither was separation by the 3 treatment groups ($\chi^2_2 = 0.25$, $p = 0.8807$). These results confirm previous findings that mtDNA can be treated as a neutral marker in this system (Brown et al. 2001).

LITERATURE CITED

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