analyzed with relatively little effort. The results reported in this article constitute an attempt to characterize such a system in preparation for further studies. On this basis, we are presently investigating the question of where selective forces are operating within a modifier system, in the hope of finding out whether selection is principally acting on modifiers of major genes, or whether selective changes are occurring primarily on the major genes themselves.

1 We are grateful to Dr M. Green for valuable comments on the experimental design used in this paper.
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Mercury selection of allozyme genotypes in shrimps

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Summary. The effects of mercury pollution on the allozymic variation of 15 phosphoglucomutase (PGM) genotypes was tested in the Mediterranean shrimp *Palaemon elegans* in 79 laboratory tests involving 2765 shrimps, with 1560 survivors (767 test and 793 control). Our results indicate differential tolerance of genotypes in variable mercury concentrations, suggesting that they are adaptive. The genetic structure can possibly be explored and potentially be used as a monitoring system for the quality and quantity of marine pollutants.

The proportion of adaptive[2] or neutral[3] allozyme polymorphisms, commonly found in natural populations, is still a major unresolved problem of evolutionary genetics. Usually, tests utilize differences in gene frequencies in natural populations observed after the operation of unknown natural forces. In contrast with this routine, we have tested in controlled experiments the cause-effect influence of mercury on allozymic variation in the shrimp *Palaemon elegans*. Our results indicate differential tolerance of some phosphoglucomutase (PGM) genotypes in variable mercury concentrations, suggesting that they are adaptive.

The shrimp *Palaemon elegans* is a widespread species in the Eastern Atlantic and Mediterranean coasts in rocky pools and lagoons[4,5]. This species was chosen because of its abundance and small size, which permitted the testing of relatively large samples. We have decided to concentrate on the PGM system owing to its high variability in comparison with 24 other tested systems. Animals were collected from rocky pools near Haifa and introduced into 10 aquaria in the laboratory. Fresh water was pumped for the experiments from 30 m depth at the Shikmona National Institute of Oceanography. Conditions in all aquaria were identical (22 °C; pH = 8.3, and constant aeration). No food was provided during any of the experiments which involved 0.02–0.40 ppm $HgCl_2$ and lasted 1–11 days according to concentration (table). All tests conducted simultaneously were matched with only 1 control. The test and the control differed solely in the addition of the pollutant to the former. Each experiment included at least 25 shrimps (for experimental details, see Nevo et al.[6]). We conducted 79 experiments involving 2765 shrimps. The survivors were deep-frozen (−80 °C) and 1560 shrimps (767 test and 793 control) were homogenized and studied by horizontal starch gel electrophoresis[7]. To avoid temporal variation in gene frequencies, we employed the method of paired comparison design, where control and tests were randomly taken from the same batch of shrimps and run simultaneously. Differential survivorship of genotypes was analyzed by the Wilcoxon matched-pairs signed-ranks test[8].

Results and discussion. A total of 5 alleles were found and designated: S^-, S, M, F, F^+ for *slow*, *medium*, and *fast*

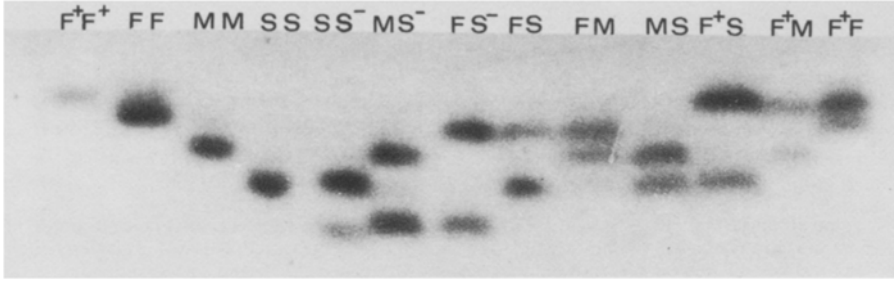

Figure 1. Zymogram of phosphoglucomutase (PGM) of *Palaemon elegans*, involving all possible genotypes except S^-S^- and F^+S^-.

migrating allozymes. We recognized all possible genotypes, 5 homozygotes and 10 heterozygotes (fig. 1). Our major significant results relate to the following 2 genotypes: *MS* and *MM*. a) The *MS* heterozygote displayed a concave survivorship curve. It increased in frequency from 23 to 39% within the range 0.00–0.18 ppm $HgCl_2$. In contrast, its frequency decreased in high $HgCl_2$ concentrations at 0.26–0.40 ppm (table and fig. 2). The level of the *MS* frequency in the range 0.02–0.24 ppm was significantly higher than its frequency in the matched controls ($N=34$, Wilcoxon's $T=63$, $p<0.0001$). In the higher $HgCl_2$ concentration, 6 out of 7 paired comparisons showed a consistent decrease in *MS* frequency. b) The *MM* homozygote displayed, in contrast to the *MS* pattern, a conspicuous depression at 0.12–0.24 ppm $HgCl_2$, and exhibited a distinct high frequency at the high $HgCl_2$ concentration at 0.26–0.40 ppm. Both its low and high frequencies were significantly different from their matched controls (at 0.12–0.24 ppm, $N=10$, $T=3.5$, $p<0.02$; at 0.26–0.40 ppm, $N=8$, $T=2.5$, $p<0.05$).

Thus we found differential survival of the 2 genotypes *MS* and *MM* in different mercury-polluted environments. The *MS* heterozygote was superior in the low and intermediate, whereas the *MM* was superior in the high $HgCl_2$ concentration. It is noteworthy that the range of $HgCl_2$ concentrations in our experiments is within the range of mercury concentrations in the sediments in the immediate vicinity of chemical plant outfalls, and that accumulated in marine organisms such as fish and invertebrate organisms[9]. For the total mercury content accumulated in our test animals see reference 10.

If the changes in allozyme structure of organisms are indeed sensitive to, and vary with, the level and type of pollutant, then such changes can be explored and can potentially be used as a promising biological monitoring system for detecting the level of specific pollutants[6]. The present study is a pre-hoc rather than a post-hoc demonstration of the effects of a specific pollutant on a specific enzymatic marker and/or its linked sequence of genes[11]. Our results seem to be inconsistent in this case with the neutral theory of protein variation, and suggest the adaptive nature of the genotypes tested.

Differential frequencies of *MS* and *MM* genotypes in control and $HgCl_2$ tests

Date	No. of test days	Concentration of $HgCl_2$ (ppm)	Percent survivors		No. of analyzed survivors		Frequency of *MS* genotype		Increase in frequency of *MS*	Frequency of *MS* genotype		Increase in frequency of *MM*
			Control	Test	Control	Test	Control	Test		Control	Test	
11.7.79	10	0.023	100	66	24	23	0.12	0.22	+0.10	0.37	0.30	−0.07
11.7.79	11	0.023	(100)	52	(24)	15	(0.12)	0.13	+0.01	(0.37)	0.13	−0.24
28.9.78	10	0.030	90	78	20	24	0.20	0.42	+0.22	0.25	0.33	+0.08
22.7.79	5	0.030	80	60	24	24	0.33	0.29	−0.04	0.21	0.13	−0.08
1.8.79	5	0.030	96	64	41	24	0.12	0.17	+0.05	0.34	0.25	−0.09
9.10.78	6	0.035	87	77	23	23	0.30	0.35	+0.05	0.17	0.26	+0.09
26.10.78	7	0.035	64	76	24	23	0.25	0.26	+0.01	0.25	0.30	+0.05
26.7.79	5	0.035	94	84	24	24	0.29	0.50	+0.21	0.29	0.21	−0.08
7.11.78	9	0.040	100	54	19	23	0.05	0.00	−0.05	0.16	0.43	+0.27
4.1.79	8	0.040	70	77	19	20	0.11	0.30	+0.19	0.16	0.25	+0.09
14.8.79	5	0.040	100	64	43	32	0.26	0.25	−0.01	0.19	0.41	+0.22
22.7.79	5	0.040	(80)	56	(24)	28	(0.33)	0.25	−0.08	(0.21)	0.14	−0.07
24.8.79	5	0.040	96	82	47	39	0.17	0.36	+0.19	0.19	0.28	+0.09
29.11.79	5	0.040	100	88	25	22	0.36	0.32	−0.04	0.20	0.23	+0.03
11.12.79	5	0.040	100	84	25	21	0.20	0.33	+0.13	0.44	0.14	−0.30
16.12.79	5	0.040	96	64	23	16	0.26	0.25	−0.01	0.26	0.31	+0.05
26.7.79	5	0.050	(94)	80	(24)	24	(0.29)	0.46	+0.17	(0.29)	0.13	−0.16
1.8.79	5	0.050	(96)	64	(41)	23	(0.12)	0.26	+0.14	(0.34)	0.26	−0.08
14.8.79	5	0.050	(100)	74	(43)	15	(0.26)	0.33	+0.07	(0.19)	0.27	+0.08
14.8.79	8	0.060	(100)	20	(43)	10	(0.26)	0.40	+0.14	(0.19)	0.20	+0.01
24.8.79	5	0.060	(96)	78	(47)	24	(0.17)	0.21	+0.04	(0.19)	0.17	−0.02
15.9.79	5	0.070	84	20	21	5	0.24	0.40	+0.16	0.19	0.40	+0.21
23.1.80	5	0.080	95	95	19	18	0.21	0.33	+0.12	0.37	0.22	−0.15
24.2.80	5	0.090	100	80	24	20	0.42	0.60	+0.18	0.21	0.20	−0.01
15.9.79	5	0.120	(84)	24	(21)	6	(0.24)	0.83	+0.59	(0.19)	0.00	−0.19
5.10.79	5	0.120	96	72	22	18	0.18	0.33	+0.15	0.27	0.17	−0.10
16.10.79	5	0.120	92	66	43	33	0.23	0.39	+0.16	0.30	0.15	−0.15
24.10.79	5	0.180	92	36	22	9	0.27	0.33	+0.06	0.18	0.22	+0.04
30.10.79	5	0.180	84	32	32	16	0.22	0.44	+0.22	0.38	0.13	−0.25
27.4.80	5	0.180	96	72	22	18	0.32	0.28	−0.04	0.32	0.28	−0.04
11.6.79	1	0.240	96	8	19	2	0.16	1.00	+0.84	0.26	0.00	−0.26
12.6.79	6	0.240	96	80	17	18	0.18	0.11	−0.07	0.41	0.17	−0.24
24.6.79	7	0.240	80	52	11	13	0.18	0.31	+0.13	0.36	0.15	−0.21
15.5.80	5	0.240	96	60	24	15	0.21	0.47	+0.26	0.17	0.20	+0.03
19.6.79	5	0.260	100	40	21	9	0.38	0.22	−0.16	0.10	0.22	+0.12
28.5.79	1	0.300	92	40	10	10	0.00	0.00	–	0.10	0.10	–
29.5.79	1	0.300	100	76	12	12	0.42	0.17	−0.25	0.25	0.42	+0.17
30.5.79	6	0.300	92	80	9	21	0.33	0.19	−0.14	0.22	0.38	+0.16
7.5.79	1	0.400	100	52	12	12	0.42	0.00	−0.42	0.17	0.25	+0.08
23.5.79	1	0.400	100	4	16	1	0.25	0.00	−0.25	0.38	1.00	+0.62
5.6.79	1	0.400	100	8	20	2	0.00	1.00	+1.00	0.10	0.00	−0.10
6.6.79	5	0.400	100	48	16	12	0.31	0.17	−0.14	0.19	0.58	+0.39
3.6.80	2	0.400	100	48	20	11	0.25	0.18	−0.07	0.35	0.45	+0.10

Parentheses indicate repetitive reference to a previous control conducted at the same day.

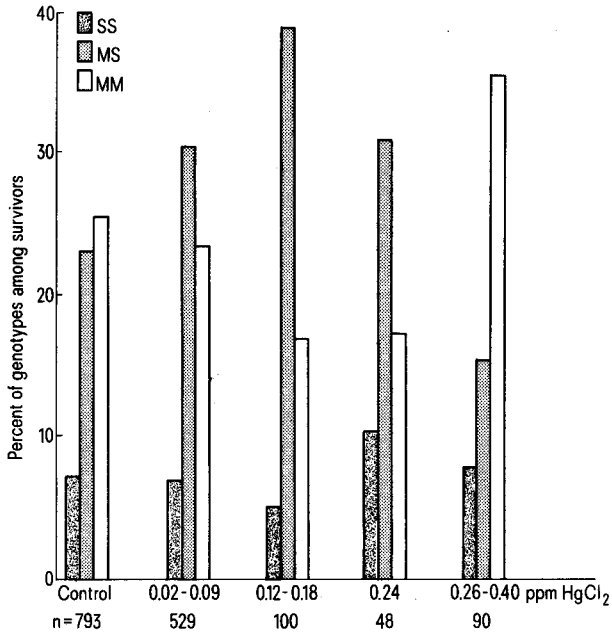

Figure 2. Differential survivorship of three PGM genotypes of *Palaemon elegans* as a function of increasing concentration of $HgCl_2$.

1 Acknowledgments. This research was supported by grants from FAO/UNEP, The United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel, the Motzkin family and the Israel Electric Corporation. We thank Uri Zoller for technical advice and P. Hertz for reading and commenting on the manuscript. - D. Wool is working at Tel Aviv University.

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Rhodamine isothiocyanate coupled peanut lectin for quantitative studies of D-galactosyl receptors of neuroblastoma cells[1]

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Summary. Rhodamine-peanut agglutinin conjugate has been obtained without alteration of the lectin activity. This conjugate interacts specifically with terminal D-galactosyl glycoconjugates. Such receptors are found on neuroblastoma differentiated or undifferentiated cell membranes. The density and distribution of these galactosyl sites are different after neuraminidase treatment.

The study of specific interactions involving glycoconjugates is of practical and theoretical importance. The acquisition of glycosylated structures appears to be linked to biological evolution, during ontogenesis and differentiation[2,3]. Their variation may cause the disappearance or the acquisition of certain biological properties. A quantitative study of these variations can be performed using fluorescent labelled lectins. We report the results of the conjugation of peanut agglutinin (PNA), a lectin specific for terminal D-galactosyl residues[4,5], with tetramethylrhodamine isothiocyanate (TRITC), as well as the characterization of the conjugate. Furthermore, a new approach was used to quantify the fluorescence of neuroblastoma cells. These cells provide a well-studied model of in vitro morphological and biochemical differentiation[6,7].

Materials and methods. The seeds of *Arachis hypogaea* were obtained from Lesieur Cotelle S.A. The lectin, specific for D-galactose, was purified by affinity chromatography on desialylated polymerized human red cell ghosts[8]. Hemagglutination assays were performed in microtiter plates with human red blood cells treated with 60 µl/ml of neuraminidase (*Vibrio cholerae* Behring) for 90 min at 37 °C. Polyacrylamide gel electrophoresis was performed on 7.25% slab gels in Tris-glycine buffer, pH 8.9 or in acetate buffer, pH 4.3[9] and on gradient gels, 4-30% in Tris-buffer, pH 4.8 and Tris SDS, pH 7.4[10]. The gels were stained for protein detection with Coomassie brilliant blue. Ouchterlony double diffusion[11] and immunoelectrophoresis[12] were performed in 1.5% agarose in Na-barbiturate buffer, pH 8.6, against anti-peanut lectin obtained against our preparation. To conjugate the lectin with TRITC (isomer R-BBL) we used a method derived from that reported for immunoglobulin[13]. 15 µl of 1 M $NaHCO_3$ was added to 10 ml of the lectin solution in phosphate buffered saline before addition of TRITC solution in dimethylsulfoxide (1 mg/ml). Conjugation was attempted with different quantities of TRITC. The pH was adjusted to 9 with Na_2CO_3M. The mixture was incubated for 3 h, in the dark and at room temperature.