

High Genetic Variability in a Population of *Tridacna maxima* from the Great Barrier Reef

C. A. Campbell¹, J. W. Valentine² and F. J. Ayala¹

¹ Department of Genetics, University of California; Davis, California, USA, and

² Department of Geology, University of California; Davis, California, USA

Abstract

A population of the bivalve mollusk *Tridacna maxima* (Röding) from Heron Island, Great Barrier Reef, Australia, was studied by gel electrophoresis, and proved to be highly variable genetically, with an average heterozygosity of about 22%. This compares closely with a population of *T. maxima* from Enewetak (Eniwetok) Atoll, with an average heterozygosity of about 20%, very high for marine organisms. Enewetak Atoll was the site of a series of nuclear tests. The Heron Island study verifies that the high variability is natural, and supports the hypothesis that species from trophically stable environments tend to be highly variable genetically.

Introduction

The genetic variabilities of populations are affected by their breeding system, by their population size and by their recent history. Genetic variability may also be regulated by environmental regimes through natural selection. During an investigation of genetic variability in marine invertebrate species from a variety of environments, a population of *Tridacna maxima* (Röding) was collected from Enewetak Atoll, Marshall Islands. This population was studied by gel electrophoresis and proved to have high genetic variability (Ayala *et al.*, 1973; Valentine *et al.*, 1973). Of 30 loci studied, 25 have detectable allelic variation (average sample size, 176 genes/locus); using a conservative definition of polymorphism (that the most common allele has a frequency no greater than 0.95), 63.3% of the loci are polymorphic. The average individual is heterozygous at 20.2% of the loci. This was the most genetically variable marine population then studied.

Enewetak Atoll has been the site of a series of nuclear tests. The pattern of the allelic frequencies of this *Tridacna maxima* population does not conform to what is expected if the variability were caused by radioactive mutagens (Ayala *et al.*, 1973). Yet, it seems desirable to verify this unusual variability through studies of a *T. maxima* population remote

from nuclear test sites. Here we report on genetic variability in a population of *T. maxima* from the Great Barrier Reef, approximately 2,400 miles from Enewetak Atoll.

Materials and Methods

Population Sample

A collection of 50 specimens of *Tridacna maxima* (Röding) was taken during September, 1973 (by CAC) from reef flats within the Capricorn Group, Great Barrier Reef, Queensland, Australia (23°30'S; 151°30'E). Approximately equal numbers of mollusks were taken intertidally from the south side of Heron Island and from the north side of the neighboring Wistari Reef, one mile to the west. All the specimens were removed from the shell, frozen, and stored on dry ice, and thus returned to our laboratories at Davis (California, USA), where they were maintained at -70°C until studied electrophoretically. Some specimens thawed while in the freezer at Heron Island; gels from these individuals could not be read reliably. Forty-two specimens were scored for some or all of the loci studied.

Sample Preparation

Each specimen was dissected for 5 tissues: adductor muscles, mantle ventral

to zooxanthellae, stomach, kidney, and gill. The samples were homogenized in an equal volume of distilled water and centrifuged at 17,000 revs/min for 20 min; the supernatant was stored at -70°C until used for electrophoresis.

We have followed the methods previously employed (Ayala et al., 1972, 1973) with the following modifications and additions: Adenylate kinase: buffer system A; 5 h at 20 v/cm; stain as described. Glyceraldehyde-3-phosphate dehydrogenase: buffer system B; 6 h at 20 v/cm; stain as described. Hexokinase: buffer system B; 6 h at 25 v/cm; stain: 20 mg nitro blue tetrazolium, 25 mg nicotinamide adenine dinucleotide phosphate (TPN), 25 mg adenosine triphosphate, 20 mg MgCl_2 , 900 mg glucose, 80 units glucose-6-phosphate dehydrogenase in 100 ml 0.1 M Tris-HCl, pH 7.1; incubate at 37°C for 1 h; add 5 mg phenazine methosulfate. Isocitrate dehydrogenase: buffer system D; 6 h at 25 v/cm; Phosphoglucumutase: buffer system B; 6 h at 25 v/cm. Ten milligrams of TPN was added to starch when preparing gels for the following enzymes: glyceraldehyde-3-phosphate-dehydrogenase, hexokinase, lactate dehydrogenase, malate dehydrogenase, malic enzyme, phosphoglucumutase, tetrazolium oxidase, triosephosphate isomerase. Samples of the fruit fly *Drosophila willistoni* were prepared in the same manner as the *Tridacna maxima* specimens and used as controls on the gels.

Observations

Genetic Variability

We have studied the Heron Island population at 28 loci, with an average sample of 64 ± 3 genes/locus. Table 1 lists the allelic frequencies at the 23 polymorphic loci. Five loci - *Adk-1*, *Adk-2*, *Hk-2*, *Hk-4*, and malic enzyme - exhibit no detectable variation. A sixth locus, *Ldh*, appears to be represented by two alleles whose products migrate nearly identically in our gels. We had some difficulty scoring the genotypes at this locus, and thus in Table 1 we have summarized the data in two ways - one uses the allelic frequencies that we consider to be the case, the other considers this locus as monomorphic. If a locus is assumed to be polymorphic when the second most common allele has a frequency equal to or greater than 1%, then this population has 82% polymorphic loci if *Ldh* is considered to be polymorphic, and 79% if it is not. If the criterion of polymorphism is that the most common allele be present at a frequency no greater than

95%, then this population has 57 or 54% polymorphic loci depending on whether *Ldh* is considered to be polymorphic or not. The average individual is heterozygous at $22.4 \pm 1.4\%$ of the loci studied if *Ldh* is considered to be polymorphic, and at $21.3 \pm 1.3\%$ if it is not. These observed heterozygosities do not differ significantly from the expected heterozygosities calculated assuming Hardy-Weinberg equilibrium. We are apparently dealing with a single interbreeding population, and it is highly variable genetically. Indeed, the Heron Island population is ostensibly equally polymorphic to the Enewetak Atoll population, whose observed heterozygosity was $20.2 \pm 2.9\%$, although somewhat different sets of loci were studied in the two cases.

The data from the two populations can be combined to give an estimate of average heterozygosity for *Tridacna maxima*. A total of 37 gene loci have been sampled (30 loci in the Enewetak population plus 7 new ones in the present study); the average proportions of heterozygous individuals per locus are $20.9 \pm 2.7\%$ and $21.6 \pm 3.2\%$, for the observed and expected heterozygosities, respectively.

Genetic Similarity

Of the 28 loci studied in our sample, 21 were also studied in the Enewetak Atoll population. It is worth asking how similar the allelic frequencies are in the two populations. Unfortunately, the homogenates from the Enewetak Atoll population were not preserved, and thus direct comparisons in the same gel are not possible. Comparisons can, nevertheless, be made using as references the *Drosophila willistoni* controls placed in every gel. This is not completely satisfactory, since small differences in the amount of migration of the enzymes cannot be unambiguously ascertained. We have followed a conservative criterion - whenever a given enzyme appears to migrate equally in the two populations we have assumed that such is indeed the case. For estimation of genetic similarity between the two populations we have used only 18 of the 21 loci studied in both of them; the equivalences between alleles at the other three loci could not be established.

Using the statistics developed by Nei (1972), the genetic similarity between the Enewetak Atoll and Heron Island populations is 0.968, and the genetic distance is 0.032. These values fall well within the norm of the amount of genetic differentiation usually found between

Table 1. *Tridacna maxima*. Allelic frequencies at 23 polymorphic loci in a population from Heron Island, Australia

Locus ^a	N ^b	Allelic frequencies ^c					Frequency (number) of heterozygous individuals	
		1	2	3	4	5	Observed	Expected
<i>Adk-3</i>	54	0.98	0.02				0.037(1)	0.036(1.0)
<i>Adk-4</i>	84	0.96	0.04				0.071(3)	0.069(2.9)
<i>Adk-5</i>	82	0.01	0.94	0.01	0.01	0.02	0.122(5)	0.117(4.8)
<i>Adk-6</i>	84	0.92	0.08				0.119(5)	0.153(6.4)
<i>Est-2</i>	52	0.15	0.02	0.79	0.04		0.423(11)	0.353(9.2)
<i>Est-4</i>	84	0.02	0.81	0.17			0.286(12)	0.316(13.3)
<i>Est-5</i>	66	0.02	0.97	0.01			0.061(2)	0.059(2.0)
<i>Est-6</i>	68	0.15	0.13	0.68	0.02	0.03	0.500(17)	0.502(17.1)
<i>G3pdh</i>	54	0.07	0.76	0.17			0.481(13)	0.390(10.5)
<i>Hk-3</i>	54	0.02	0.98				0.037(1)	0.036(1.0)
<i>Idh</i>	74	0.01	0.68	0.22	0.09		0.405(15)	0.488(18.0)
<i>Ldh</i>	54	0.44	0.56				0.444(12)	0.494(13.3)
<i>Lap-3</i>	82	0.04	0.74	0.18	0.04		0.341(14)	0.410(16.8)
<i>Lap-4</i>	78	0.04	0.62	0.35			0.410(16)	0.500(19.5)
<i>Mdh-2</i>	52	0.08	0.04	0.86	0.02		0.269(7)	0.243(6.3)
<i>Mdh-3</i>	54	0.98	0.02				0.037(1)	0.036(1.0)
<i>Mdh-4</i>	48	0.92	0.08				0.167(4)	0.153(3.7)
<i>Mdh-5</i>	54	0.06	0.83	0.11			0.333(9)	0.290(7.8)
<i>Odh</i>	66	0.17	0.80	0.03			0.273(9)	0.326(10.8)
<i>Pgm-1</i>	54	0.04	0.46	0.48	0.02		0.704(19)	0.552(14.9)
<i>To-1</i>	80	0.02	0.97				0.050(2)	0.049(1.9)
<i>To-3</i>	74	0.57	0.41	0.03			0.838(31)	0.513(19.0)
<i>Tpi</i>	24	0.04	0.96				0.083(1)	0.080(1.0)
Average (includes invariant loci) ^d								
(1)	63.8±3.0						0.232±0.043 (210)	0.222±0.038 (202.1)
(2)	63.8±3.0						0.216±0.043 (198)	0.203±0.037 (188.8)

^aThe enzymes coded by the gene loci are as follows: *Adk*, adenylate kinase; *Est*, esterase; *G3pdh*, glyceraldehyde-3-phosphate dehydrogenase; *Hk*, hexokinase; *Idh*, isocitrate dehydrogenase; *Ldh*, lactate dehydrogenase; *Lap*, leucine amino peptidase; *Mdh*, malate dehydrogenase; *Odh*, octanol dehydrogenase; *Pgm*, phosphoglucumutase; *To*, tetrazolium oxidase; *Tpi*, triosephosphate isomerase.

^bNumber of genomes studied at each locus.

^cAlleles are listed in order of increasing migration towards anode.

^d(1) Assumes, and (2) does not, that the *Ldh* locus is polymorphic.

geographic populations of the same species (Ayala et al., 1974; Avise et al., 1975). We must, however, emphasize that our assumptions may lead to underestimation of genetic differentiation, and thus that the values given represent only the lower limit of the genetic differentiation between the two populations.

Discussion

The "stability" hypothesis (see Levins, 1968) is a widely accepted explanation for patterns of genetic variability. It has been suggested for marine systems by

Bretsky and Lorenz (1969), Grassle (1972), and Grassle and Sanders (1973) among others. The hypothesis exists in several variations. A general expectation is that species in more stable (constant, predictable, certain) environments will have low levels of genetic variability, while those in more unstable (variable, unpredictable, uncertain) environments will have much genetic polymorphism. Although "stable", "constant" and "predictable" do not necessarily refer to the same properties of environments, they are not distinguished here because previous authors have either not distinguished them or expressed similar expectations for all as

regards genetic variability. The rationale is that in unstable environments populations require high levels of genetic variability in order to cope with the variety of environmental states to which they are subjected, while in stable environments, selection would favor those few alleles optimal for the narrow range of prevailing conditions and thus maintain little genetic variability. Such an argument has led to the specific prediction that coral reef animals should have low genetic variabilities (Grassle, 1973). It is not always clear just which environmental parameters should be stable or unstable in order to elicit the purported effects. Some authors specify climatic factors. Another possibility is that biological factors such as primary productivity, that are sensitive in turn to seasonality, could be involved.

Since many environmental factors display characteristic patterns of variability, both globally and locally, it is possible to contrast the pattern of each factor with the observed pattern of genetic variability, to determine whether or not a relationship exists. The pattern of genetic variability thus far observed in marine species is easily summarized (reviewed in Ayala et al., 1975; Valentine, in press). For benthic invertebrates, data are available for 13 species. Those living in low latitudes or in the deep sea have much genetic variability (17 to 21% average heterozygosity), while those living in intermediate to high latitudes are less polymorphic (1 or 2% to 12% average heterozygosity). Only three planktonic species have been studied. They are closely related species of krill (*Euphausia*), and they display a pattern similar to that of benthic species. One species from circumpolar waters off Antarctica has relatively low variability (about 6% average heterozygosity), one from temperate waters off northern Chile has higher variability (about 15% average heterozygosity), and one from equatorial waters off Central America is the most polymorphic (about 21% average heterozygosity) (Valentine and Ayala, in press). The limited data available for marine fishes display a pattern similar to that of the invertebrates (Somero and Soulé, 1974).

The observed patterns of genetic variability are inconsistent with predictions of the stability hypothesis. Physical factors such as temperature fluctuate most highly in intermediate latitudes, where intermediate rather than high genetic variabilities are found. Animals from the more stable tropics

have higher levels, and animals from the more stable Antarctic waters lower levels of genetic variability. With respect to primary productivity, the observations are just the opposite of what is expected by the stability hypothesis: species living in regimes of stable productivity (tropics, deep sea) are characterized by high levels of genetic variability, while those from the more seasonal regimes (Antarctic waters, northeast Atlantic shelf) have low genetic variability.

Tridacna maxima fits the observed pattern well. It is essentially tropical in distribution, ranging from East Africa to Pitcairn Island, but extending northward to Japan and southward along the Australian coasts beyond the margins of the tropics (Rosewater, 1965).

Although there certainly are errors inherent in the estimation of genetic variability by electrophoresis, we have seen that clear differences in variability levels occur between marine species from distinctive climatic zones, as from the tropics and from temperate latitudes. The difference in genetic variabilities between central and marginal tropical species expected on the basis of our limited experience with tropical and temperate forms, may be about 2 to 4% at most. This difference is so small relative to the accuracy of the estimates that we can draw no conclusions from the similarity of the estimates for the Heron Island population, which lives at the southern border of the tropics, and the more centrally located Enewetak population.

A hypothesis has been proposed to explain the high genetic variability found in tropically stable environments (Ayala et al., 1975; Valentine, in press). In such environments, species tend to pursue a relatively "coarse-grained" spatial strategy, owing to the stable resource base. Animals are narrowly specialized trophically and/or in habitat requirements. Alleles, the products of which perform efficiently over narrow functional ranges, may accumulate in the gene pool. Different environmental parameters have different local patterns, resulting in a mosaic of conditions even within a narrow habitat range. The "specialized" alleles are selected because they exhibit higher fitnesses in slightly different habitat conditions. Forms of balancing selection may hold large numbers of alleles in the gene pool to create a rich variety of genotypes specialized for many combinations of environmental parameters.

Conversely, in tropically unstable environments, species tend to pursue

"fine-grained" spatial strategies, and thus to be flexibly adapted to a wide range of food and/or habitat conditions. A single allele (or a few) may be selected at each locus that is functionally most flexible. Thus, the species would come to possess relatively little genetic variation but would be ecologically a generalist.

Conclusions

The amount of genetic variation in the Heron Island population of *Tridacna maxima* is very similar to that found earlier in a population from Enewetak Atoll. This result effectively lays to rest any doubts about the variability of the Enewetak Atoll population being due to natural processes, rather than to man-generated radiation. One might conceivably argue that mutations caused by radioactive fall-out at nuclear test sites might have migrated swiftly and widely, and that their deployment has greatly enriched such distant gene pools as that at Heron Island. This is too unlikely to merit further consideration.

The cause of the high genetic variability of *Tridacna maxima* has been inferred to have a selective basis related to the trophic stability of the tropical environment (see Ayala et al., 1975). In trophically stable regions, genetic variability accumulates and enhances the adaptation of highly specialized populations by producing large numbers of genotypes, many of which may be especially fit in particular microhabitats (Ayala et al., 1975). The Heron Island *T. maxima* population is another instance of high genetic variability in a tropical population, and thus supports the trend observed earlier.

Acknowledgements. The extensive help of H. Campbell in collecting and preparing the specimens is gratefully acknowledged. Thanks are also due to Dr. J.H. Campbell for his interest in the project. Research was supported in part by contract AT(O4-3)34 with the Atomic Energy Commission of the United States, and by a Faculty Research Grant, University of California, Davis. The facilities at Heron Island Research Station were used for preparing specimens.

Literature Cited

Avise, J.C., J.J. Smith and F.J. Ayala: Adaptive differentiation with little genic change between two native California minnows. *Evolution*, Lancaster, Pa. (1975). (In press)

- Ayala, F.J., D. Hedgecock, G.S. Zumwalt and J.W. Valentine: Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution*, Lancaster, Pa. 27, 177-191 (1973)
- , J.R. Powell, M.L. Tracey, C.A. Mourão and S. Pérez-Salas: Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics*, Austin, Tex. 70, 113-139 (1972)
- , M.L. Tracey, D. Hedgecock and R.C. Richmond: Genetic differentiation during the speciation process. *Evolution*, Lancaster, Pa. 28, 576-592 (1974)
- , J.W. Valentine, T.E. DeLaca and G.S. Zumwalt: Genetic variability of the Antarctic brachiopod *Liothyrella notorcadensis* and its bearing on mass extinction hypotheses. *J. Paleont.* 49, 1-9 (1975)
- Bretsky, P.W. and D.M. Lorenz: Adaptive response to environmental stability: a unifying concept in paleoecology. *Proc. N. Am. paleont. Conv., Part E*, 522-550 (1969)
- Grassle, J.F.: Species diversity, genetic variability and environmental uncertainty. In: Fifth European Marine Biology Symposium, pp 28-37. Ed. by B. Battaglia, Padova, Italy: Piccin Editore 1972
- , Variety in coral reef communities. In: *Biology and geology of coral reefs*, Vol. 2. Biology, Part 1. pp 247-270. Ed. by O.A. Jones and R. Enean. N.Y.: Academic Press 1973
- and H.L. Sanders: Life histories and the role of disturbance. *Deep-Sea Res.* 20, 643-659 (1973)
- Levins, R.: *Evolution in changing environments*, 120 pp. Princeton, N.J.: Princeton University Press 1968
- Nei, M.: Genetic distance between populations. *Am. Nat.* 106, 283-292 (1972)
- Rosewater, J.: The family Tridacnidae in the Indo-Pacific. *Indo-Pacif. Mollusca* 1, 347-396 (1965)
- Somero, G.N. and M. Soulé: Genetic variation in marine fishes as a test of the niche-variation hypothesis. *Nature*, Lond. 249, 670-672 (1974)
- Valentine, J.W.: Genetic strategies of adaptation. In: *Molecular evolution*, Ed. by F.J. Ayala. Sunderland, Mass.: Sinauer Ass. In press
- , and F.J. Ayala: Genetic variability in krill. *Proc. natn. Acad. Sci. U.S.A.* (In press)
- , G.S. Zumwalt, D. Hedgecock and F.J. Ayala: Mass extinctions and genetic polymorphism in the killer clam, *Tridacna maxima*. *Bull. geol. Soc. Am.* 84, 3411-3414 (1973)

Cathryn A. Campbell
Department of Genetics
University of California
Davis, California 95616
USA