

## Polymorphisms of Octopine dehydrogenase (Odh) in mollusks and implications for the neutralism-selectionism hypothesis

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**Abstract:** Octopine dehydrogenase (Odh) was examined in several species of bivalves and gastropods and complemented with bibliographic data, to assess the controversy between neutralism and selectionism in explaining the maintenance of genetic variation in natural populations. This debate was the center of the molecular evolution and population genetic research in the 1970s and 1980s, but waned thereafter, without resolution. Although DNA data have been produced, implications are not understood. We examined the polymorphisms of Odh in several species of bivalves and gastropods, and the kinetic properties (apparent  $K_m$ ) of the different isozymes in the scallop *Euvola ziczac* that indicates an apparent case of overdominance of the heterozygous individuals. The question "which of the two hypothesis is correct" has shifted with time to "how much influence did each factor have in the maintenance of genetic variation".

**Key words:** Polymorphism, octopine dehydrogenase, neutralism, selectionism.

The possible adaptive significance and mechanisms that maintain genetic variation in natural populations, has been a central controversy in evolution (Beumont and Zouros 1991). In contrast to the Darwinian theory of evolution through natural selection, the neutralist theory (Kimura 1968) stated that evolutionary changes at the molecular level are caused by random fixation of neutral mutants; therefore many polymorphisms are not adaptive. The neutralist-selectionist debate was a very important issue in population genetic and molecular evolution in the 1970s and 1980s. There was hope that far more powerful tests based in DNA sequence data would help. However, as DNA data become avail-

able, the debate waned without resolution because it what DNA data can reveal about the subject is not clear (Hey 1999).

Both hypotheses seem to be applicable (Pérez *et al.* 1995) in some fishes that possess polymorphic hemoglobins with identical properties (isohemoglobins) whose presence can be explained by the neutralist hypothesis and polymorphic hemoglobins with different properties (Pérez 1986) that gives advantages to the organisms in variable environments.

The polymorphism levels of different enzymes could be interrelated with the structure and its regulatory function. Monomeric enzymes and those that perform a regulatory function, showed increased levels of poly-

morphisms. This was found in 23 kinds of coelenterates and sponges with an average of 14.3 enzymes by species (Sole -Cava and Thorpe 1989), which apparently contradicts neutralism but favors selectionism. However, knowledge of biochemistry and ecology of coelenterates and sponges is scarce and any suggestion of relationship among function and genetic variation, in these groups, must be made with caution. Sole-Cava and Thorpe (1989) urged employing a greater number and variety of enzymes to understand the possible interactions among structure and function of the enzymes.

We believe that there is another way to study a highly polymorphic enzyme. We have selected the monomeric and no regulatory enzyme octopine dehydrogenase (Odh), that is frequently polymorphic in mollusks. This enzyme has a relevant function in energy production, presents a high activity in the adductor muscle of some mollusks and its principal function is to maintain the balance redox NADH/NAD to allow anaerobic glycolytic ATP production.

## MATERIAL AND METHODS

The organisms *Euvola ziczac* (L.1758), *Nodipecten nodosus* (L. 1758), *Amussium papyraceum* (Gabb 1873), *Spondylus americanus* (Hermann 1781), *Murex pomum* Gmelin 1791, *Murex brevifrons* Lamarck 1822, *Fasciolaria tulipa* (L. 1758), *Strombus pugilis* (L. 1758), *Donax denticulatus* (L. 1758), *Perna perna* (L. 1758) and *Perna viridis* (L. 1758) were collected in the eastern coast of Venezuela. To determine possible polymorphism for octopine dehydrogenase (Odh: 1.5.1.11), a fraction of the adductor muscle of each organism was homogenized in proportion 1: 1 W/V with distilled water at 0-4°C, and centrifuged to 3 000 r.p.m. for 20 min. The supernatants were analyzed by horizontal 12% starch electrophoresis. Gels were stained in buffer 0.2 mol l<sup>-1</sup> Tris/0.025 mol l<sup>-1</sup> citric acid (Morizot and Schmidt 1990).

## RESULTS

Our results indicate the presence of polymorphism in the bivalves *Euvola ziczac*, *Amussium papyraceum* and *Spondylus americanus* (Table 1). To these species we can add, as product of a bibliographical review (Beaumont *et al.* 1980, Beaumont and Beveridge 1984, Menairdus and Gäde 1986, Moraga and Laubier 1986, Durand and Blanc 1989, Galleguillos *et al.* 1989, Volckaert and Zouros 1989, Mc Donald *et al.* 1991, Hoare and Beaumont 1995), the bivalves: *Cerastoderma (Cardium) edule* (L. 1758), *Mytilus edulis* (L. 1758), *M. galloprovincialis* Lamarck 1819, *M. trossulus* Gould 1850, *Modiolus modiolus* (L. 1758), *Chlamys distorta* (da Costa), *C. varia* (L. 1767), *C. opercularis* (L. 1767), *Pecten maximus* (L. 1758), *Placopecten magellanicus* (Gmelin 1791), *Argopecten purpuratus* (Lamarck 1819), *Tapes philippinarum* (Adams & Reeve 1850), and *Pinctada margaritifera* (L. 1758).

TABLE 1

Species, number of organism analyzed (N); and number (N<sub>a</sub>) and frequencies of alleles.

Species	N	N <sub>a</sub>	Alleles	Frequencies
<i>Euvola ziczac</i>	103	5	0.005 0.505 0.312 0.153 0.025	
<i>Amussium papyraceum</i>	30	3	0.300 0.550 0.150	
<i>Spondylus americanu</i>	4	4	0.625 0.125 0.125 0.125	
<i>Nodipecten nodosus</i>	80	2	0.990 0.010	
<i>Murex pomum</i>	40	3	0.275 0.325 0.400	
<i>Murex brevifrons</i>	36	1	1.000	
<i>Strombus pugilis</i>	62	1	1.000	

The only species of bivalves found to be monomorphic (although there was a second allele, but in a frequency lower than 0.05, see Table 1) was *Nodipecten nodosus* (L.). Only four specimens of *S. americanus* were analyzed and the polymorphism was evident.

For gastropods, we observed polymorphisms in *Murex pomum* (Table 1), and Baldwin & England (1982) found it in *Strombus luhuanus*. *Murex brevifrons* and *Strombus pugilis* were monomorphic. No activity for the enzyme (by histochemical

staining) was found in *Donax denticulatus*, *Perna perna*, *Perna viridis*, *Fasciolaria tulipa* and *Thais haemastoma*.

The species analyzed in our laboratory and described in the literature include temperate and tropical, sedentary and motile organisms. A sympatric species of *Murex* was polymorphic (*M. pomum*) and the other monomorphic (*M. brevifrons*). What is the meaning of these results? And why is Odh so frequently polymorphic in bivalves?

### DISCUSSION

Allozyme heterozygosity and the rate of growth have been positively correlated in many species of bivalves (Beaumont and Zouros 1991, Zouros *et al.* 1992, Hedgecock *et al.* 1996). High levels of heterozygosity have been associated with a decrease rate of the basal metabolism (Koehn and Shumway 1982). Presumably this allows heterozygous individuals to use their aerobic potential to increase adaptability after complying with basic requirements (Garton *et al.* 1984, Rodhouse *et al.* 1986, Volckaert and Zouros 1989). Recently, Hedgecock *et al.* (1996) found that the oxygen consumption rate decreases in the hybrid larvae of the Pacific oyster *Crassostrea gigas* (Tumberg 1793), in comparison with consanguineous larvae, and that net efficiency of protein synthesis is higher in the hybrids.

In sedentary mollusks, metabolic energy can be invested in growth during the juvenile stages, and in reproduction in the adults. In scallops that can escape predators by swimming, this energy may be invested to increase locomotive capacity (Pérez *et al.*, unpublished).

In the scallop *Euvola ziczac*, the apparent  $K_m$  of Odh during the formation of octopine for several enzyme genotypes was calculated to determine if the correlation among enzyme heterozygosity and affinity for substrates is present. Our results (Pérez *et al.* 2000) indicated a greater affinity (lower apparent  $K_m$ ) for pyruvate in the heterozygotes; this would explain the maintenance of the polymorphism

by overdominance.

Heterozygotes presented a lower  $K_m$  value (higher affinity) than homozygotes in the sea anemone *Metridium senile* (Walsh 1981), but Baldwin and England (1982) and Meinardus and Gäde (1986) did not find differences in the apparent  $K_m$  for different isozymes of Odh in *Strombus luhuanus* (gastropod) and *Cardium tuberculatum* (bivalve), respectively.

Alfonsi *et al.* 1995 found a correlation between the degree of enzyme heterozygosity and the maximal activity of pyruvate kinase, octopine dehydrogenase, and NADP-dependent dehydrogenase in the adductor muscle in *E. ziczac*.

Identifying the mechanism(s) that maintain genetic variation in natural populations whether by selection (selectionism) or by chance (neutralism) is a very complex task that may not be solved by any simple theory. The differences between the two hypotheses are sometimes so subtle that some evidences may be used to support one or another according to personal bias (Sole-Cava and Thorpe 1991).

It is possible that genetic drift (underestimated as evolutionary factor until recently) and selection will have different relative importance at different times for a given group (Gillespie 1981); neutral processes can predominate in some more stable periods, whilst selection can act more intensely in other. Recently DNA data and analysis of many genes in *Drosophila melanogaster* have revealed that many loci have at least one region suggestive of the action of natural selection, and balancing selection in particular. Other loci show evidence that natural selection removed variation, probably by the recent increase in frequency of a favored sequence (Hey 1999).

In the beginning, the controversy between neutralism and selectionism was "which one of the two hypothesis was right". With time it has shifted to "how much influence did each factor had in the maintenance of genetic variation".

## RESUMEN

Se estudió un polimorfismo frecuente en moluscos, el de la enzima octopina deshidrogenasa (Odh), en varias especies de bivalvos y gastrópodos. El estudio se complementa con datos bibliográficos para entrar en la controversia entre el neutralismo y el seleccionismo como explicaciones para el mantenimiento de la variación genética en las poblaciones naturales. Este debate fue el centro de las investigaciones en evolución molecular y genética de poblaciones en las décadas de 1970s y 1980s y aún permanece sin resolverse. A pesar de que se ha generado mucha información sobre el ADN, ha tomado tiempo entender sus aportes. Se examinó el polimorfismo de Odh en algunas especies de bivalvos y gastrópodos, y las propiedades cinéticas ( $K_m$  aparente) de las diferentes isozimas de *E. ziczac*, que indican un caso aparente de sobredominancia de los individuos heterocigotos. La evidencia sugiere que la pregunta no es "¿Cuál de las dos hipótesis es correcta?", sino "¿Cuánta influencia tiene cada factor en el mantenimiento de la variación genética?"

## REFERENCES

- Alfonsi, C., O. Nussetti & J.E. Pérez. 1995. Heterozygosity and metabolic efficiency in the scallop *Euvola ziczac* (L.1758). *J. Shellfish. Res.* 14: 389-393.
- Baldwin, J. & W. R. England. 1982. Multiple forms of octopine dehydrogenase in *Strombus luhuanus* (Mollusca, Gastropoda, Strombidae): genetic basis of polymorphism, properties of the enzymes, and relationship between the octopine dehydrogenase phenotype and the accumulation of anaerobic end products during exercise. *Biochem. Genet.* 20: 1015-1024.
- Beaumont, A. R., T. R. Daug & G. Gade. 1980. Genetic variation at the octopine dehydrogenase locus in the adductor muscle of the *Cerastoderma edule* (L.) and six other bivalve species. *Mar. Biol. Lett.* 1: 137-148.
- Beaumont, A. R. & C. M. Beveridge. 1984. Electrophoretic survey of genetic variation in *Pecten maximus*, *Chlamys opercularis*, *C. varia*, *C. distorta* from the Irish Sea. *Mar. Biol.* 81: 299-306.
- Beaumont, A. R. & E. Zouros. 1991. Genetics of scallops: 585-623. *In*: Shumway, S. E. (ed), *Scallops: Biology, Ecology and Aquaculture*. Elsevier, Amsterdam.
- Durand, P. & F. Blanc. 1989. Diversité génétique chez un bivalve Marin Tropical: *Pinctada margaritifera* (Linné, 1758). *Bull. Soc. Zool. France.* 113: 293-304.
- Galleguillos, R., L. Troncoso & E. Tarifeño. 1989. Variación proteínica en poblaciones del ostión del norte *Argopecten purpuratus*. *Bol. Red Acuic.* 3: 15-17.
- Garton D. W., R. K. Koehn & T. M. Scott. 1984. Multiple-locus heterozygosity and the physiological energetics of growth in the coot clam, *Mulinia lateralis*, from a natural population. *Genetics* 108: 445-455.
- Gillespie, J. H. 1986. Natural selection and the molecular clock. *Mol. Biol. Evol.* 3: 138-155.
- Hedgecock, D. D. J. McGoldrick, D. T. Manahan, J. Vavra, N. Appelmans & B. L. Bayne. 1996. Quantitative and molecular genetic analyses of heterosis in bivalve mollusks. *J. Exp. Mar. Biol. Ecol.* 203: 49-59.
- Hey, J. 1999. The neutralist, the fly and the selectionist. *Trends Ecol. Evol.* 14: 35-38.
- Hoare, K. & A. R. Beaumont. 1995. Effects of an Odh null allele and a GPI low-activity allozyme on shell length in laboratory-reared *Mytilus edulis*. *Mar. Biol.* 123: 775-780.
- Kimura, M. 1968. Evolutionary rate at the molecular level. *Nature* 217: 624-626.
- Koehn R. K. & S. E. Shumway. 1982. A genetic physiological explanation for differential growth rate among individuals of the American oyster *Crassostrea virginica*. *Mar. Biol. Lett.* 3: 35-42.
- McDonald, J. H., R. Seed & R. K. Koehn. 1991. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern hemispheres. *Mar. Biol.* 111: 323-333.
- Menairdus, G. & Gäde, G. 1986. The pyruvate branch point in the anaerobic metabolism of the jumping cockle, *Cardium tuberculatum*. *Exp. Biol.* 45: 91-110.
- Moraga, D. & L. Laubier. 1986. Polymorphisme génétique de populationns cultivées de la palourde du pacifique *Tapes philippinarum*. *C.R. Acad. Sci. Paris*, t. 302, serie III, n° 17: 621-624.
- Morizot, D. C. & M. E. Schmidt. 1990. Starch gel electrophoresis and histochemical visualization of proteins. Chapter 2: 23-80. *In*: Whitmore, D. (ed.), *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management*.
- Pérez, J. E. 1986. Hemoglobin polymorphism in the toadfish *Thalassophrine maculosa*. *Gunther. J. Exp. Biol. Ecol.* 100: 287-294.
- Pérez, J. E., O. Nussetti, N. Ramírez & C. Alfonsi. 2000. Allozyme and biochemical variation at the octopine dehydrogenase locus in the scallop *Euvola ziczac*. *J. Shellfish Res.* 19(1): In Press.
- Pérez, J. E., K. Rylander & M. Nirchio. 1995. The evolution of multiple hemoglobins in fishes. *Rev. Fish Biol. Fisher.* 5: 304-319.
- Rodhause, P. G., J. M. McDonald, R. J. Newell & R. K. Koehn. 1986. Gametic production, somatic growth and multiples locus enzyme heterozygosity in

- Mytilus edulis*. Mar. Biol. 90: 209-214.
- Sole-Cava, A. M. & J. P. Thorpe. 1989. Biochemical correlates of genetic variation in natural populations of marine lower invertebrates. Biochem. Genetics 27: 303-312
- Sole-Cava, A. M. & J. P. Thorpe. 1991. High levels of genetic variation in natural populations of marine lower invertebrates. Biol. J. Linneaus Soc. 44: 65-80.
- Volckaert, F. & E. Zouros. 1989. Allozyme and physiological variation in the scallop *Placopecten magellanicus*, and general model for the effects of heterozygosity on fitness in marine mollusks. Mar. Biol. 101: 1-10.
- Walsh, P. J. 1981. Purification and characterization of two allozymic forms of octopine dehydrogenase from California population of *Metridium senile*. J. Comp. Physiol. 143B: 213-222.
- Zouros, E., G. H. Pogson, D. I. Cook & M. J. Dadswell. 1992. Apparent selective neutrality of mitochondrial DNA size variation: a test in the deep-sea scallop *Placopecten magellanicus*. Evolution 46: 1466-1476.