chores, as seen under the electron microscope. Marks[11] in *Nigella damascena* has also compared the paired centromeric Giemsa stained dots with the kinetochores. Our results also indicate kinetochorelike structures in *H. lupulus* L.

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2 M.L. Pardue and J.G. Gall, Science *168*, 1356 (1970).
3 C.G. Vosa and P. Marchi, Nature, New Biol. *237*, 191 (1972).
4 A.T. Natarajan and S. Natarajan, Hereditas *72*, 323 (1972).
5 D. Schweizer, Chromosoma *40*, 307 (1973).
6 C.G. Vosa, Heredity *36*, 383 (1976).
7 H. Eiberg, Nature *248*, 55 (1974).
8 H.J. Evans and A. Ross, Nature *249*, 861 (1974).
9 P. Luykx, Int. Rev. Cytol. *27*, Suppl. 3, 1 (1970).
10 S.M. Stack, Chromosoma *47*, 361 (1974).
11 G.E. Marks, J. cell Sci. *18*, 19 (1975).

Biochemical divergence between cavernicolous and marine Sphaeromidae and the Mediterranean salinity crisis

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Summary. Allozymic variation in proteins encoded by 14 loci was analyzed in 3 cavernicolous *Monolistra* and in 2 marine *Sphaeroma* species. Genetic distance data, high levels of heterozygosity and the divergence time calculations support the hypothesis that *Monolistra* diverged from its Sphaeroma-like marine ancestor during the Messinian, in connection with the Mediterranean salinity crisis.

The occurrence of Monolistrini (Crustacea, Isopoda), of the typically marine family Sphaeromidae in freshwater cave environments in continental Europe is a remarkable example of relict distribution. The present range of the cavernicolous species, all included in the genera *Caecosphaeroma* and *Monolistra,* extends from France to Herzegovina, including karst areas of the foothills of the Alps, Slovenia and Istria[2]. This distribution has been related to Miocene palaeogeography[3] in connection with the occurrence of deep, long-lasting inlets over these areas (figure 1). It has been speculated that the subterranean evolution of Monolistrini originated during the Miocene when their brackish water ancestors began to adapt gradually to freshwater environments[4]. This hypothesis and recent progress in the palaeogeography of the Mediterranean during the Tertiary suggested to us a study of the genetic structure and degree of divergence between 3 cavernicolous *Monolistra* and 2 marine *Sphaeroma* species. An attempt was made to identify *Sphaeroma* as a possible prototype for the marine ancestor of the cave-dwelling *Monolistra* by evaluating the divergence time between the 2 genera. The biochemical data presented here support this hypothesis.

The 2 *Sphaeroma* species selected for this study are both abundant and widespread in the Mediterranean[5]. *S. serratum* (Fabricius) is an intertidal species, whereas *S. hookeri* Leach lives in brackish waters[6] and may represent an adaptively intermediate stage in the evolution from marine to cave-dwelling forms. Population samples for electrophoresis of these species were collected in 2 localities along the Tyrrhenian coast. 3 *Monolistra* population samples were taken from underground streams in limestone caves situated along the foothills of the Alps in northern Italy, respectively in prealpine Lombardy near Bergamo, *M. boldorii bergomas* Arcangeli, in the Colli Berici near Vicenza, *M. berica* (Fabiani) and in prealpine Friuli near Tarcento (Udine), *M. caeca* Gerstaecker.

Samples of about 50 individuals per population were assayed using current electrophoretic techniques[7]. Genetic variation was analyzed in enzymes and other proteins encoded by 22 gene loci. A minimum of 18 loci were assayed for each species. 14 loci were common to all the species studied and the indices of genetic distance were calculated on this basis. Other details of collecting sites and electrophoretic methods and results are available.

Table 1 shows the genetic distance between the various species, calculated by the Nei method[8]. Figure 2 shows the phylogenetic relations among the Sphaeromidae studied as revealed by our data. It is immediately clear that the distances between *Sphaeroma* and *Monolistra* are higher than those recorded for the congeneric species, either *Sphaeroma* or *Monolistra.* Also, among the *Monolistra* the 2 easternmost species, *M. caeca* and *M. berica,* show the highest degree of similarity. These findings are consistent with the greater part of available data on comparisons between true species and between different genera[9–11]. Estimates of absolute divergence time between pairs of Sphaeromidae species were made using Nei's formula[11]. We used 2 alternative values for α (figure 2). 2 reservations must be made, however, concerning this methodology. a) Nei's formula is correct only under certain assumptions about protein structure and evolution[12], and b), the standard errors in the genetic distance estimates are fairly large

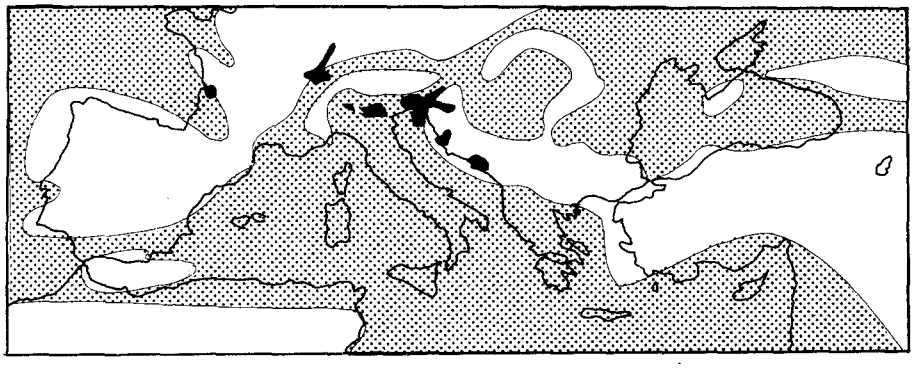

Fig. 1. Present distribution of the Monolistrini (black areas) superimposed on the Middle Miocene Palaeogeography of the Mediterranean (reconstruction after Hsü et al., 1977). White areas: land; dotted areas: seas.

due to the small set of loci assayed. However, even the raw estimates of divergence time obtained with this method provide valuable information when associated with other available genetic and geological data. The average divergence time calculated between *Sphaeroma serratum* and the *Monolistra* species is 6.8–7.7 million years; that calculated between *S. hookeri* and *Monolistra* is 5.6–6.4 myr. These estimates fall around the Messinian, and the Mediterranean salinity crisis, a dramatic ecological event which has also been dated as far back as 6 myr[13,14]. There is disagreement among geologists as to the conditions which resulted from this phenomenon. Some claim that the basin dried up completely[13-15] while others hypothesize only a dramatic increase in salinity[16]. In either case, the salinity crisis must have created a drastic situation of selection for the majority of marine species including those which were saved by opportune ecological refuges such as brackish waters from which they might eventually have found their way into subterranean freshwater habitats via surface streams and pools. An evolutionary model of this type seems a probable explanation for the adaptation of Sphaeromidae to the cave environment. Interestingly, *S. hookeri,* which may represent an intermediate stage in the marine to freshwater cavernicolous Sphaeromidae, is more closely related to the *Monolistra* species than is *S. serratum.* The time of divergence and the beginning of the process of *Monolistra* speciation falls, in our estimation, in the middle and upper Pliocene. This dating, already hypothesized by Racovitza[4], is in close agreement with palaeogeographic and palaeoclimatic evidence relative to the prealpine Po plain during this period, when tectonic and erosive phenomena would have produced a definitive isolation of the various caves and karst areas[17-19].

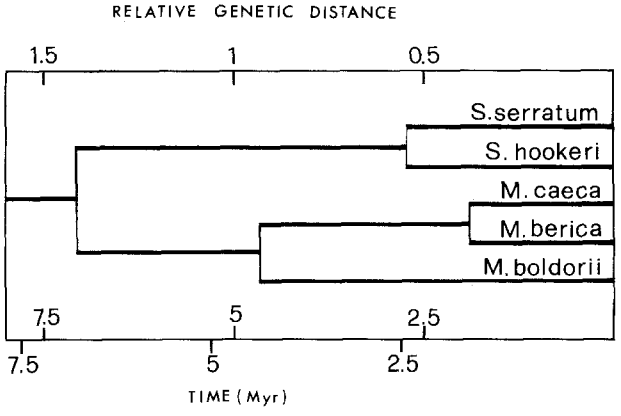

Fig. 2. Biochemical similarity dendrogram and absolute divergence times based on genetic distance data for *Sphaeroma* and *Monolistra* species. The evolutionary time scale is calculated according to 2 different values for a ($a = 10^{-7}$ above[11]; $a = 1.136 \times 10^{-7}$ below) from the formula $t = D/2a = D/2cn_t\lambda_a$ where t is the time since isolation of 2 populations, D is the genetic distance, c is the proportion of amino acid substitutions which are electrophoretically detectable, n_t is the average number of amino acid per protein, and λ_a is the rate of amino acid substitutions per polypeptide per year.

In addition to the evidence of genetic divergence, an early isolation of the cavernicolous populations is also indicated by the polymorphism which they display. Table 2 shows some estimates of genetic variability for the populations studied. Surprisingly the populations of *Monolistra,* notwithstanding their rather small size, estimated at most at a few thousand individuals per population, show levels of heterozygosity even higher than those found in *Sphaeroma.* A finding of this kind is not compatible with recent colonization of the caves by surface water forms. In fact, during colonization the founder effect plays a considerable role in the drastic reduction of variability[21,22]. Variability would be expected to reemerge little by little in the cave populations if further bottle necks did not come into play. However, because of the notable stability of these environments, it is unlikely that bottle necks would occur. Available genetic data for cavernicolous populations of various organisms are all in agreement with this model[7,23-25]. On the other hand, because of the very low vagility of the *Monolistra* and the notable degree of isolation of prealpine subterranean water systems, it is highly unlikely that genic flow could explain the high level of heterozygosity observed. Therefore, the high variability of the populations of *Monolistra,* which appears to be maintained by balancing selection[26], is in our view further evidence in establishing the early isolation of these species.

Table 1. Coefficients of genic identity (above) and distance (below)[8] between species of *Monolistra* and *Sphaeroma*

	M. boldorii	*M. berica*	*M. caeca*	*S. hookeri*
M. berica	0.4150			
	0.8795			
M. caeca	0.3742	0.6849		
	0.9829	0.3786		
S. hookeri	0.3326	0.2954	0.2232	
	1.1007	1.2193	1.4997	
S. serratum	0.1895	0.2187	0.2331	0.5702
	1.6633	1.5199	1.4564	0.5618

Table 2. Estimates of genetic variation in *Monolistra* and *Sphaeroma* species

	P	A	H_e	H′
M. boldorii	0.714	2.000	0.303	0.206
M. berica	0.714	2.000	0.321	0.216
M. caeca	0.786	2.143	0.318	0.213
S. serratum	0.714	1.929	0.277	0.185
S. hookeri	0.714	1.929	0.216	0.155

P, frequency of polymorphic loci (those with a frequency of the rarest allele > 0.01); A, mean number of alleles per locus; H_e, average heterozygosity (expected under Hardy-Weinberg equilibrium); H′, genic diversity (Shannon information index)[20].

1 This research was supported by the National Research Council, Italy, grant No. 77.01490.04, and by funds of the Faculty of Sciences, University of Rome.
2 B. Sket, Int. J. Speleol. *1,* 249 (1965).
3 A. Vandel (ed.), Biospéologie. La Biologie des Animaux cavernicoles. Gauthier-Villars, Paris 1964.
4 E. G. Racovitza, Arch. Zool. exp. Gén. *4,* 625 (1910).
5 R. Lejuez, Arch. Zool. exp. Gén. *107,* 469 (1966).
6 P. Kerambrun, Mar. Biol. *6,* 128 (1970).
7 V. Sbordoni, M. Cobolli Sbordoni and E. De Matthaeis, Lavori Soc. it. Biogeogr. *6,* 329 (1979).
8 M. Nei, Am. Nat. *106,* 283 (1972).
9 F. J. Ayala, Evol. Biol. *8,* 1 (1975).
10 J. C. Avise, in: Molecular Evolution, p. 106. Ed. F. J. Ayala. Sinauer, Sunderland, MS, 1976.
11 M. Nei (ed.), Molecular population genetics and evolution. North-Holland, Amsterdam 1975.
12 J. C. Avise, J. J. Smith and F. J. Ayala, Evolution *29,* 411 (1975).
13 K. J. Hsü, Sci. Am. *227,* 26 (1972).
14 K. J. Hsü, L. Montadert, D. Bernoulli, M. B. Cita, A. Erickson, R. E. Garrison, R. B. Kidd, F. Mèlierés, C. Müller and R. Wright, Nature *267,* 399 (1977).
15 K. J. Hsü, Sci. Am. *238,* 52 (1978).
16 R. Selli, Scienze *56,* 20 (1973).
17 F. Carraro, R. Malaroda, G. Piccoli, C. Sturani and S. Venzo, Note illustrative della Carta Geologica d'Italia. Foglio 48. Servizio Geologico d'Italia, Roma 1969.

18 M. Chardon, Les Prealpes Lombardes et leurs Bordures, vol 1. Librairie Honoré Champion, Paris 1975.
19 G. Nangeroni, Atti Soc. it.Sc. nat. *109*, 97 (1969).
20 R.C. Lewontin, Evol. Biol. *6*, 381 (1972).
21 T.C. Barr, Evol. Biol. *2*, 35 (1968).
22 M. Soulé, in: Molecular Evolution, p.60. Ed. F.J. Ayala. Sinauer, Sunderland, MS, 1976.
23 J.C. Avise and R.K. Selander, Evolution *26*, 1 (1972).
24 C. Laing, G.R. Carmody and S.B. Peck, Evolution *30*, 484 (1976).
25 D.E. Cockley, J.L. Gooch and D.P. Weston, Evolution *31*, 313 (1977).
26 V. Sbordoni, A. Caccone, E. De Matthaeis and M. Cobolli Sbordoni, in preparation.

Genetic variability in natural populations, evidence in support of the selectionist view

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Summary. Genetic variability in 6 neotropical anopheline species, analyzed, by zymogram technique, has been described. The results support the 'selectionist' theory of the evolutionary significance of high levels of molecular diversity in natural populations.

Thanks to the monumental work of Lewontin and his group, showing a high level of genetic diversity in *Drosophila pseudoobscura*, by zymogram technique[2,3], the long controversy between the 'classical' school and 'balanced' view finally started to turn around. Until then, according to the 'classical' school championed by H.J. Muller, later led by J.F. Crow and M. Kimura, natural populations possess very low genetic variability. Most mutations are deleterious and are removed by natural selection which thus acts primarily as a cleansing agent of 'wild type' gene pool. A rare favourable mutation may replace the old less-fit gene, leading to evolution of the gene pool[4-6]. On the other extreme, the 'balanced' view proposed by T. Dobzhansky and later led by B. Wallace holds quite an opposite view of the evolutionary process. According to this school, natural populations possess a high level of genetic variation maintained by various forms of balancing selection. An occasional favourable mutation contributes just a simple fraction to the high level of genetic diversity always present. This variation enables populations to adapt to diverse temporal and spatial environmental conditions.

Since then a great amount of genetic diversity has been reported in populations of diverse types of organisms[3,7-9]. These findings thus tended to support the 'balanced' school. With the controversy among the 2 schools on the amount of genetic diversity in populations thus resolved, the next 3 central questions of the population or evolutionary genetics persist; 1. what is the evolutionary significance of this high level of molecular polymorphism or in other words its adaptive significance?; 2. what is the important 'unit of selection'?; 3. what evolutionary mechanisms maintain the high level of genetic variability[3,10,11]. The 1st question again drew diverse answers from 2 schools of thought. Kimura[12], the proponent of the 'classical' school put forward the 'neutral' theory of genic polymorphism suggesting that most of the observed biochemical variation is random and physiologically irrelevant, whereas according to the 'selection' school, this variation is the direct result of balancing forms of natural selection, and is thus not selectively neutral. If so, it would then be necessary to support this with experimental data. Thus the 'balanced vs. classical' controversy changed into 'selectionist vs. neutralist' controversy.

2 types of approaches commonly used can be phrased into the following questions. Does each isoenzyme of a given locus possess a specific physiological or metabolic function and if the enzyme diversity indeed provides the adaptive raw material necessary for a population to explore and adapt to the changing environment, then there should be an overall correlation between the degree and nature of molecular diversity in a population or species and the proportion of the diverse ecological niches it occupies in its geographical distribution range. In other words the more extensive the distribution of a population or species over wide environmental conditions, more genetically heterozygous or polymorphic it should be and conversely if a population or species has its distribution limited or restricted to specialized ecological niches it should be less polymorphic. Far more studies have been addressed to the 1st question[13], for references, than to the second. It is the latter question that our studies have attempted. We initiated studies on genetic structure of 6 neotropical anophelines, of which *A. aquasalis* has a very rigid requirement of salt water during the larval stages and thus the distribution of this species is restricted to the coastal region only. On the other hand, other species (table) are widely distributed, both in coastal and interior regions, and consequently these species have been able to explore regions of high and low

Measures of genetic variability in neotropical anopheline species

Species	No. of enzyme loci analyzed	Proportion of genome heterozygous per individual	Proportion of polymorphic loci crit. 1	crit. 2	Proportion of heterozygous individuals
A. aquasalis	26	0.081	0.23	0.34	0.084
A. darlingi	19	0.21	0.579	0.632	0.125
A. nuneztovari	26	0.171	0.46	0.54	0.111
A. argyritarsis	27	0.188	0.46	0.682	0.113
A. albitarsis	18	0.27	0.42	0.65	0.17
A. evansae	19	0.23	0.56	0.63	0.149

Enzymes used for this table included esterases, ODH, XDH, MDH, ME, HK, GOT, AO of larval stages; LAP of pupal and ACPH and a-GPDH of adults. Crit. 1 = Most common allele with a frequency of 0.95 or less. Crit. 2 = Second most common allele has a frequency not smaller than 0.01.