Vol. 95: 245–250, 1993 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published May 27

Genetic variation within and between two Tyrrhenian populations of the Mediterranean alcyonarian *Corallium rubrum*

Marco Abbiati, Giovanni Santangelo, Silvia Novelli

Dipartimento di Scienze dell'Ambiente e del Territorio, Università di Pisa, Via Volta 6, I-56126 Pisa, Italy

ABSTRACT: Allozymic variation of 13 gene loci was investigated in 2 samples of *Corallium rubrum* (L.) collected in the Tyrrhenian Sea (Tuscany, Italy), at the coastal Calafuria cliff (south of Leghorn) and at Elba Island (Tuscan Archipelago) respectively. Among 5 polymorphic loci, *Pgm* and *Pgi* showed the highest level of heterozygosity. In the Elba sample all polymorphic loci were in Hardy-Weinberg equilibrium, whereas in the Calafuria sample, *Pgm* and *Pgi* were not. Mixing of individuals from genetically different subpopulations (Wahlund effect) may cause this disequilibrium. Nei's indices together with *F*-statistics show that the samples investigated can be considered as 2 distinct local populations. The results suggest that limited larval dispersal may be more important in promoting *C. rubrum* population differentiation than differential selection on the migrating larvae.

INTRODUCTION

Corallium rubrum (L.) is a gorgonian endemic species of the Mediterranean and contiguous Atlantic coasts, from southern Portugal to the Cabo Verde Islands. This species settles mainly on rocky cliffs and in submarine caves, from about 20 to 200 m in depth, representing a specific facies in the biocenosis of the 'hemidark caves' (Pérès & Picard 1964). It is a dioecious species brooding pelagic larvae (planulae). Commercial exploitation, over thousands of years, of this long-living but slow-growing gorgonian, mainly for use in jewellery, has resulted in severe depletion of its Mediterranean beds.

Because of its over-harvesting, *Corallium rubrum* has attracted more attention in recent years from a number of researchers (FAO 1984, 1989a). Their studies have concerned some biological and ecological aspects of coral life as well as population dynamics.

Corallium rubrum larval dispersal capability is not yet known, although Vighi (1972) and Weinberg (1979) made a rough evaluation of planulae survival and swimming ability under laboratory conditions. Estimation of larval dispersal capability by *in situ* observations or by tracking, common techniques for reef corals (Willis & Oliver 1990), are not easy to apply on a species lacking massive spawning like red coral. In such cases biochemical genetics represents a useful approach to study species dispersal capability (Ward 1989). In addition, population genetic studies provide useful information on population structure of commercially significant species, which, together with data on population structure and dynamics (Abbiati et al. 1991, 1992a), might be relevant for their commercial exploitation.

In this research, electrophoretic techniques have been applied to *Corallium rubrum* in order to characterize its population genetic structure.

MATERIALS AND METHODS

Corallium rubrum samples were collected by SCUBA diving at 2 sites along the Tuscan coast (Italy), respectively Calafuria (south of Leghorn, 43° 30' N, 10° 20' E) and Elba (Tuscan Archipelago, 42° 44' N, 10° 17' E), (Fig. 1). The distance between the 2 investigated sites is about 80 km. At Calafuria, *C. rubrum* is

distributed along a 10 km cliff, which extends about 200 m off the coast, from 25 to 45 m in depth. This cliff is characterized by small caves, where coral colonies are thickly settled on the vaults. Colonies for electrophoretic analysis were collected from adjacent caves on 23 October and 6 November 1990 and 21 January 1991. At Elba, a small, dense coral settlement was located on a rocky wall, which extends for no more than 200 m, at a depth of 40 to 50 m. Colonies were collected on 27 February and 20 March 1991.

Samples were kept alive for ca 10 d in aerated darkened aquaria provided with circulating filtered seawater at 17 °C. Each coral colony was considered as an individual, because of its origin from a single planula larva (Vighi 1972). Colony size ranged between 2 and 5 cm in length. The polyps and a few coenosarcs were gently removed from the calcareous skeleton with tweezers. This material was then homogenised with Potter pestles in Eppendorf tubes containing 75 µl of extracting buffer (0.2 M Tris buffer, 1 mM Na_4-EDTA and 25 mM 2-mercaptoethanol, pH 7.0), enough to obtain at least 50 µl of supernatant. The homogenate was centrifuged for 10 min at 4000 × *g* and the supernatant used for electrophoresis.

Electrophoretic separation on cellulose acetate was done for 12 enzymes (Table 1) corresponding to 13 loci. Four buffer systems were used, referred to in Table 1 as:

A: Tris-EDTA-maleate pH 7.8 (Schneppenheim & MacDonald 1984);
B: Tris-EDTA-maleate pH 7.4 (Grunbaum 1981);
C: Tris-citrate pH 7.2 (Grunbaum 1981);
D: Tris-phosphate pH 7.8 (Grunbaum 1981).

Because of the small quantity of organic matter available per colony, increased concentrations of the enzyme staining mixtures were used (Harris & Hopkinson 1976, Grunbaum 1981).

The allelic and genotypic frequencies were calculated from the enzyme banding patterns. Statistical analysis of the electrophoretic data was done using the computer program BIOSYS-1 (Swofford & Selander 1981). For each population, genetic variability was expressed in terms of polymorphism (0.99 and 0.95 criteria) and mean heterozygosity per locus. The level of genetic differentiation among populations was analysed by Nei's identity (*I*) and distance (*D*) indices (Nei 1987) and by *F*-statistics (Wright 1965, Nei 1987). Significance of F_{IS} (fixation index of subpopulations) was tested according to Li (1955) and significance of F_{IT} (fixation index of compound population) was tested according to Brown (1970). To test the significance of F_{ST}-values (standardized variance in allele frequencies among subpopulations), contingency chi-square values were calculated using the method of Workman & Niswander (1970). To apply *F*-statistics, the 2 coral samples were considered as subpopulations of a hypothetical compound population resulting from the sum of all the individuals of the 2 samples.

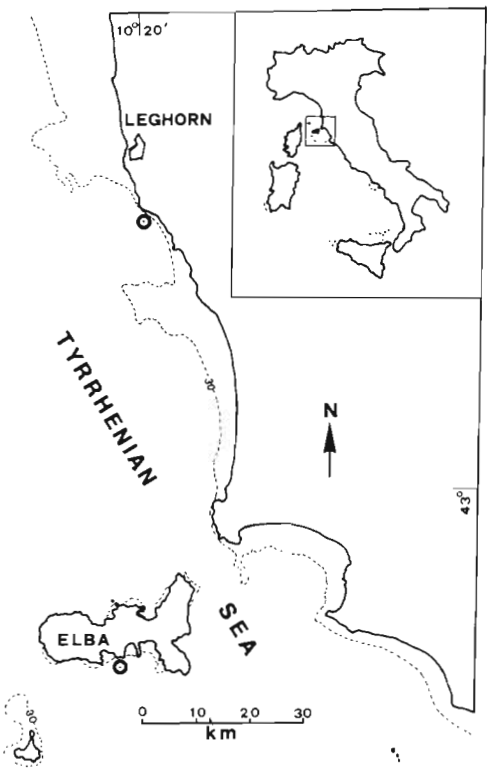

Fig. 1 Location of sampling sites in the Tyrrhenian Sea: Calafuria cliff south of Leghorn; and Elba Island

Table 1. Enzymes assayed and buffer systems used in the study of *Corallium rubrum* from Tyrrhenian Sea. See text for explanation of buffer systems

Enzyme	Abbrev.	E.C. no.	Buffer system
Lactate dehydrogenase	LDH	1.1.1.27	A
Malate dehydrogenase	MDH	1.1.1.37	A
Malic enzyme	ME	1.1.1.40	A
Phosphogluconate dehydrogenase	PGD	1.1.1.43	C
Glucose-6-phosphate dehydrogenase	GD	1.1.1.49	B
Purinenucleoside phosphorilase	NP	2.4.2.11	B
Hexokinase	HK	2.7.1.1	A
Adenylate kinase	AK	2.7.4.3	B
Phosphoglucomutase	PGM	2.7.5.1	A
Aldolase	ALD	4.1.2.13	B
Mannosephosphate isomerase	MPI	5.3.1.8	D
Phosphoglucose isomerase	PGI	5.3.1.9	A

RESULTS

Thirteen loci were successfully resolved in both *Corallium rubrum* samples (Table 2); the mean number of scored colonies per locus was 91.1 for the Calafuria sample and 76.1 for the Elba sample. Eight loci in both samples were monomorphic: *Ldh, Mdh1, Mdh2, Me, Gd, Np, Ak* and *Mpi*; 3 loci were polymorphic at 0.95 criterion: *Pgm, Pgi* and *Ald*. Loci *Hk* and *Pgd* in the Elba sample were polymorphic at 0.95 and 0.99 criteria respectively, while in Calafuria only *Hk* was polymorphic at 0.99 criterion. The percentage of polymorphic loci was greater in the Elba sample (38.46 % with 0.99 criterion) than in the Calafuria sample (30.77 % with 0.99 criterion) (Abbiati et al. 1992b).

The mean heterozygosity was $\overline{H}_L$ = 0.104 (± 0.054) and $\overline{H}_L$ = 0.073 (± 0.035) in the Calafuria and Elba samples respectively; these values agree with the average values reported for marine invertebrates (Ayala & Kiger 1984, Nevo et al. 1984). A slight difference, not significant by *t*-test, was observed between the mean heterozygosity values of the 2 samples. *Pgm* and *Pgi* showed the highest mean heterozygosity values. *Pgm* was the most variable locus with 3 alleles (Fig. 2). Neither locus was in Hardy-Weinberg equilibrium in the Calafuria sample due to heterozygote deficiency (Table 3). In the Elba sample only *Ald* was not in Hardy-Weinberg equilibrium (Table 3). Considering the low polymorphism values of this locus, however, this fact may be explained by the presence of 2 individuals with rare homozygous phenotypes (102-102).

Table 2. *Corallium rubrum*. Allelic frequencies (F) at 13 examined loci in Calafuria and Elba populations. N: no. of colonies scored

		Calafuria		Elba	
Locus	Allele	F	N	F	N
Ldh	100	1.000	83	1.000	40
Mdh1	104	1.000	124	1.000	69
Mdh2	100	1.000	134	1.000	98
Me	100	1.000	31	1.000	35
Pgd	102	0.009	113	0.038	78
	100	0.991		0.962	
Gd	100	1.000	37	1.000	60
Np	100	1.000	56	1.000	83
Hk	100	0.955	132	0.929	77
	98	0.045		0.071	
Ak	100	1.000	58	1.000	87
Pgm	102	0.086	128	0.053	76
	100	0.563		0.717	
	96	0.352		0.230	
Ald	102	0.122	41	0.069	94
	100	0.878		0.931	
Pgi	100	0.600	95	0.104	96
	98	0.400		0.896	
Mpi	100	1.000	152	1.000	99

Table 3. *Corallium rubrum*. Chi-square test for deviations from Hardy-Weinberg equilibrium. Only cases where significant deviations ($p < 0.05$) were found are included. H_{obs}: observed frequency of heterozygotes. H_{exp}: expected frequency of heterozygotes

Sample	Locus	χ^2	df	p	H_{obs}	H_{exp}
Calafuria	*Pgm*	50.640	3	< 0.001	52	70.7
	Pgi	6.148	1	< 0.05	34	45.6
Elba	*Ald*	6.173	1	< 0.05	9	12.1

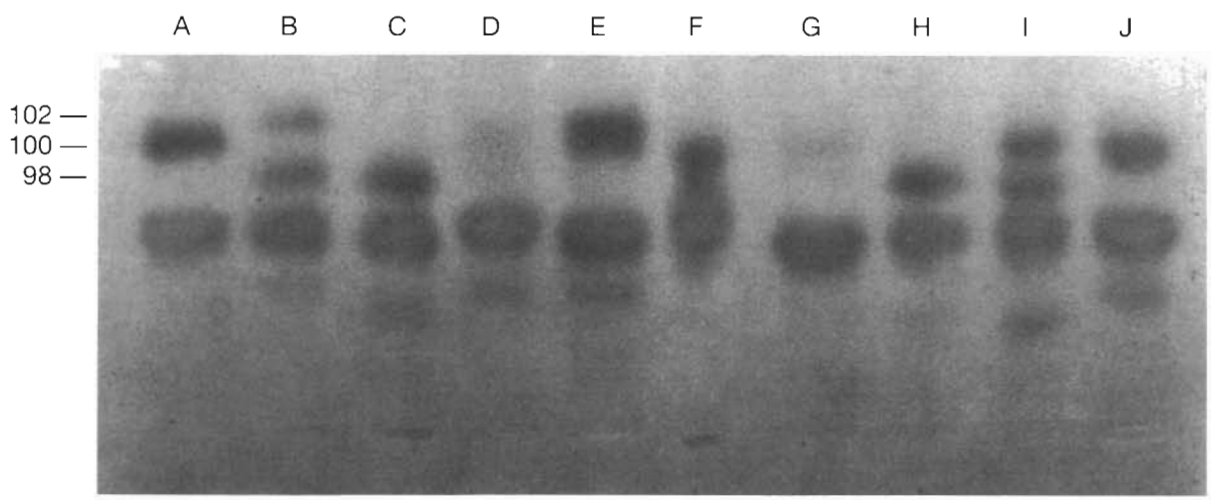

Fig. 2. Zymogram of *Pgm* locus, showing the 3 scored alleles, respectively 98, 100 and 102, represented by the isozyme bands. Genotypes are: Lanes A & J, 100/100; Lane B, 98/102; Lanes C & H, 98/98; Lanes D, F, G & I, 98/100; Lane E, 100/102

The weighted averages of the F_{IS} over the 2 samples revealed their overall conformity to Hardy-Weinberg expectations (Table 4). On the contrary, significant F_{IT} values (Table 4) show deviation from the random mating conditions of the compound population. *Pgi* and *Pgm* showed highest values of F_{IT}. In *Pgm* this is due to the higher frequency of allele 100 in the Calafuria sample and of allele 98 in the Elba one, while for *Pgm* we observed slight differences between the samples in the frequencies of the 3 alleles. F_{IT} and F_{ST} show that the variation at *Pgi* and *Pgm* is such that the compound population deviates significantly from Hardy-Weinberg equilibrium, and that the differentiation of these loci is to a significant extent caused by variation among the samples (27 and 2 % for *Pgi* and *Pgm* respectively), still leaving substantial variation allocated within the samples (73 and 98 % for *Pgi* and *Pgm* respectively).

The genetic differences between the 2 investigated samples of *Corallium rubrum* are summarised by the F_{ST} mean value (Table 4) and by Nei's (1987) genetic identity and distance indices ($I = 0.978$, $D = 0.022$).

Table 4. *Corallium rubrum*. Summary of *F*-statistics at the 5 polymorphic loci over the 2 samples. See text for definitions of F_{IS}, F_{IT} and F_{ST}

Locus	F_{IS}	F_{IT}	F_{ST}
Pgd	−0.034	−0.024	0.009*
Hk	0.004	0.007	0.003
Pgm	0.225	0.240	0.020**
Ald	0.009	0.017	0.008
Pgi	0.213	0.425	0.269***
Weighted mean	0.158	0.246*	0.105***

*p < 0.05, **p < 0.01, ***p < 0.001

DISCUSSION

The above results indicate that Calafuria and Elba red coral populations are genetically heterogeneous and provide evidence of some breeding isolation and population substructuring in the Tyrrhenian sea.

The mean F_{ST} value found for Calafuria and Elba red coral populations is clearly higher than those reported in populations of marine invertebrates with planktonic larvae (Johnson & Black 1984, Hunt & Ayre 1989, Watts et al. 1990) and agrees with those reported by Stoddart (1984a) for the brooding reef coral *Pocillopora damicornis*, even though the 2 sites investigated cover only a small area in relation to the broad geographic distribution of red coral in the Mediterranean (Marchetti 1965).

In sessile marine organisms with planktonic larvae, restricted gene flow may be due to limited larval dispersal capability or to a reduced fitness of the migrating larvae (Hedgecock 1986). Population genetic studies help us to estimate the relative importance of these 2 events (Johnson & Black 1984, Stoddart 1984a, Ward 1989). These studies show that, even in some species with long-living pelagic larvae, genetic heterogeneity among adult groups is more probably due to the genetic history of the recruits rather than to post-recruitment selection (Watts et al. 1990). There is evidence that larvae of reef coral brooding species, even if able to survive long periods in the plankton, generally settle within a relatively short distance from the parent colony (Babcock 1988).

Little research has been carried out to characterize red coral larval survival and dispersal capability. Most alcyonarian larvae are lecithotrophic, passive swimmers, remain close to the bottom during their larval life and generally settle within a few days to a week (Thorson 1946). Red coral is a brooding species releasing planulae that, under laboratory conditions, survive 4 to 12 d (Vighi 1972, Weinberg 1979, Grillo & Chessa 1992). Further research on red coral reproductive biology is needed to reveal effective larval dispersal capability, nevertheless these data suggest that a reduced larval dispersal may promote population differentiation.

If the level of analysis is shifted to the single population in Calafuria, *Pgm* and *Pgi* loci show the highest level of heterozygosity and significant deviations from Hardy-Weinberg expectations. Available data on red coral biology and ecology are not adequate to explain the heterozygous deficit observed in the Calafuria population. Such a phenomenon frequently occurs in marine invertebrates (Johnson & Black 1984, Stoddart 1984b, Hunt & Ayre 1989, Watts et al. 1990) and is commonly explained by the Wahlund effect: mixing of individuals from genetically different units (Wahlund 1928, Nei 1987). The occurrence of the Wahlund effect in the Calafuria population is suggested by the peculiarity of this site, where colonies of a large red coral population are mainly settled on the vaults of small isolated caves. Non-conformity to Hardy-Weinberg expectations may be due to a reduced gene flow among different caves. The occurrence of the Wahlund effect in this red coral population is also suggested by Weinberg's (1979) observations on red coral planulae behaviour. Weinberg showed, in fact, that planulae are indifferent to light and negatively geotactic. This implies that planulae, released by cave-dwelling colonies, may be trapped at the ceiling of the cave till settlement (Weinberg 1979). Nevertheless, the degree of differentiation between Calafuria and Elba populations shows that some dispersion occurs, in spite of the geonegative behaviour of planulae tending to keep different units apart. Present data do not allow us to quantify the heterozygosity deficit due to the Wahlund

effect. For this we need a specific analysis of a significant number of colonies, collected in different caves, located along a linear transect.

These data suggest that larval dispersal by *Corallium rubrum* does not ensure sufficient gene flow to preserve the genetic homogeneity of the species. Reduced genetic exchange could result in species genetic structuring in the Mediterranean. The presence of discrete distinct populations of *C. rubrum* does not support the proposal of a rotating harvesting for the management of Mediterranean red coral resources (FAO 1989b). In a rotating harvesting regime, stock areas designated for inclusion in the scheme are opened in sequence on a defined temporal basis. Intensive harvesting in one geographical area comprising several different coral populations could lead to the local extinction of one or more of them. One approach to fostering conservation of the species would involve independent harvesting strategies, based on local population peculiarities, designed to preserve the genetic units of the species. In order to reveal the effective population structuring of this species, it would be of great interest to investigate red coral populations from other areas of the Mediterranean.

Acknowledgements. We thank Professor S. Bonotto (University of Turin) for his stimulating discussions and valuable suggestions, and the anonymous referees for their criticism of the manuscript.

LITERATURE CITED

Abbiati, M., Buffoni, G., Caforio, G., Di Cola, G., Santangelo, G. (1992a). Harvesting, predation and competition effects on a red coral population. Neth. J. Sea Res. 30(1): 219–218

Abbiati, M., Buffoni, G., Di Cola, G., Santangelo, G. (1991). Red coral population dynamics: stability analysis and numerical simulation of time evolution of perturbed states. In: Ravera, O. (ed.) Terrestrial and aquatic ecosystems: perturbation and recovery. Ellis Horwood Limited, London, p. 219–228

Abbiati, M., Santangelo, G., Novelli, S. (1992b). Genetic structure of *Corallium rubrum* L. 1758 populations from Tyrrhenian Sea. Rapp. P.-v. Commn int. Explor. scient. Mer Méditerr. 33: 25

Ayala, F. J., Kiger, J. A. Jr (1984). Modern genetics, 2nd edn. Benjamin Cummings, Davis

Babcock, R. C. (1988). Fine-scale spatial and temporal patterns in coral settlement. Proc. 6th int. coral Reef Symp. 2: 635–639

Brown, A. H. D. (1970). The estimation of Wright's fixation index from genotypic frequencies. Genetica 41: 399–406

FAO (1984). Technical consultation on red coral resources of the Western Mediterranean and their rational exploitation. FAO Fish. Rep. 306: 1–142

FAO (1989a). GFCM Technical consultation on red coral of the Mediterranean. FAO Fish. Rep. 413: 1–162

FAO (1989b). Report on the ad hoc GFCM WORKING GROUP on the development of a scheme for rotating harvest of Mediterranean red coral resources. GFCM/RM/VII/89 Inf. 6 suppl.: 1–15

Grillo, M. C., Chessa, L. A. (1992). Développement larvaire de *Corallium rubrum*: étude. Rapp. P.-v. Commn int. Explor. scient. Mer Méditerr. 33: 40

Grunbaum, B. W. (1981). Handbook for forensic individualization of human blood and bloodstain. Library of Congress Catalog Card no. 80. Sartorius, Hayward

Harris, H., Hopkinson, D. A. (1976). Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co., Amsterdam

Hedgecock, D. (1986). Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull. mar. Sci. 39(2): 550–564

Hunt, A., Ayre, D. J. (1989). Population structure in the sexually reproducing sea anemone *Oulactis muscosa*. Mar. Biol. 102: 537–544

Johnson, M. S., Black, R. (1984). The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. Mar. Biol. 79: 295–302

Li, C. C. (1955). Population genetics. University of Chicago Press, Chicago

Marchetti, R. (1965). Ricerche sul corallo rosso della costa ligure e toscana. Distribuzione geografica. Rend. Ist. Lomb. Sci. Lett. B 99: 255–278

Nei, M. (1987). Molecular evolutionary genetics. Columbia University Press, New York

Nevo, E., Beiles, A., Ben-Shlomo, R. (1984). The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In: Mani, G. F. (ed.) Evolutionary dynamics of genetic diversity. Lecture notes in biomathematics, Vol. 53. Springer-Verlag, Berlin, p. 13–213

Pérès, J. M., Picard, J. (1964). Nouveau manuel de bionomie benthique de la mer Méditerranée. Recl. Trav. Stn mar. Endoume 47(5): 1–137

Schneppenheim, R., MacDonald, C. M. (1984). Genetic variation and population structure of krill (*Euphausia superba*) in the Atlantic sector of Antarctic waters and off the Antarctic Peninsula. Polar Biol. 3: 19–28

Stoddart, J. A. (1984a). Genetic differentiation amongst populations of the coral *Pocillopora damicornis* off southwestern Australia. Coral Reefs 3: 149–156

Stoddart, J. A. (1984b). Genetical structure within populations of the coral *Pocillopora damicornis*. Mar. Biol. 81: 19–30

Swofford, D. L., Selander, R. B. (1981). Biosys-1 a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 282–283

Thorson, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates. Medd. Dan. Fisk. Havunders. Ser. Plankton 4(1): 1–523

Vighi, M. (1972). Etude sur la reproduction du *Corallium rubrum* (L.). Vie Milieu 23(1,A): 21–32

Wahlund, S. (1928). Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11: 65–106

Ward, R. D. (1989). Molecular population genetics of marine organisms. In: Ryland, J. S., Taylor, P. A. (eds.) Reproduction, genetics and distribution of marine organisms. Olsen & Olsen, Fredensborg, p. 395–420

Watts, R. J., Johnson, M. S., Black, R. (1990). Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Mar. Biol. 105: 145–151

Weinberg, S. (1979). The light-dependent behaviour of planulae larvae of *Eunicella singularis* and *Corallium rubrum* and its implication for octocorallian ecology. Bijd. Dierkd. 49(1): 16–30

Willis, B. L., Oliver, J. K. (1990). Direct tracking of coral larvae: implications for dispersal studies of planktonic larvae in topographically complex environments. Ophelia 32 (1-2): 145–162

Workman, P. L., Niswander, J D. (1970). Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Ann. J. Human Genet. 22: 24–49

Wright, S. (1965). The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19: 395–420

This article was submitted to the editor

Manuscript first received: July 23, 1992
Revised version accepted: February 11, 1993