BULLETIN OF MARINE SCIENCE, 63(2): 257–264, 1998

GENETIC EVIDENCE FOR THE ASEXUAL ORIGIN OF SMALL INDIVIDUALS FOUND IN THE COELENTERON OF THE SEA ANEMONE *ACTINIA BERMUDENSIS* MCMURRICH

Fernando A. Monteiro, Claudia A. M. Russo and Antonio M. Solé-Cava

ABSTRACT

Some sea anemone species brood small individuals in their coelenteron. This paper studies the genetic relationship between brooding and brooded individuals in the tropical sea anemone, *Actinia bermudensis*, to verify whether the offspring was produced asexually or not. Horizontal starch gels were stained for 14 enzymes, two of which showed high gel resolution and were polymorphic for some brooding anemones. Those anemones were analyzed again along with their offspring to see whether their genotype was identical to that of the brooding adults. As observed in other species of the genus, a total genotypic agreement was found between brooding and brooded *A. bermudensis*. The probability of this result occurring by chance if the young were produced sexually was at most 1.1×10^{-35}. It was concluded therefore, that the young anemones found in the coelenteron of *A. bermudensis* are produced asexually. This suggests that asexual brooding may be more common in sea anemone species than it was previously thought.

Actinia bermudensis (McMurrich, 1889) is a tropical sea anemone that occurs in the intertidal zone of marine and estuarine rocky shores from Florida (USA) to South Brazil (Schlenz, 1983). Adults of this species often brood small anemones in their coelenteron. There is some controversy over the sexual or asexual origin of the juveniles found inside the coelenteron of many sea anemone species (Chia, 1976; Rostron and Rostron, 1978; Gashout and Ormond, 1979). Subsequently, the complete genotype identity found between brooding and brooded anemones at some allozyme loci showed that the temperate species *Actinia equina, A. tenebrosa, Sagartia ornata,* and *Epiactis prolifera* brood asexually reproduced offspring (Black and Johnson, 1979; Orr et al., 1982; Shaw et al., 1987; Edmands, 1995, respectively). How common this phenomenon is in actiniarians still remains to be demonstrated (Shick, 1991).

The aim of this paper was to determine, by means of allozyme analysis, the sexual or asexual origin of the juveniles found inside the coelenteron of *Actinia bermudensis*.

MATERIALS AND METHODS

This paper examined the origin of the juveniles in the red and the brown colortype of *Actinia bermudensis*. Samples of the red colortype were collected at three sites along the Brazilian coast: Barra de Itabapoana (21°16′S, 41°03′W), Itaipú (22°57′S, 43°00′W), and Florianópolis (27°35′S, 48°30′W), whereas the brown colortype was collected only in Florianópolis. Adult anemones and their young were transported on ice to the laboratory, where they were kept at –20°C and analyzed within one month of collection.

Tissue samples were ground with an equal volume of distilled water and analyzed by horizontal 13% starch gel electrophoresis as previously described (Solé-Cava et al., 1985; Russo et al., 1994). The buffer system used was the Tris-Citrate pH 8.0 (Ward and Beardmore, 1977). Enzyme nomenclature and staining recipes followed Harris and Hopkinson (1978) and Richardson et al. (1986).

Table 1. Gene frequencies for *Mpi* and *Gdh* loci in *Actinia bermudensis*. n = number of individuals analyzed. RD: red color type; BR: brown color type. I: Itaipú; F: Florianó polis; B: Barra de Itabapoana.

Mpi	RDI	RDF	BRF	RDB
a	0.229	0.143	0.607	0.100
b	0.479	0.857	0.393	0.840
c	0.292	0.000	0.000	0.060
n	72	49	42	25
Gdh	RDI			
a	0.594			
b	0.406			
n	32			

Gene frequencies were estimated for 15 gene loci of 188 adult anemones from the three localities studied. Eight loci were found to be polymorphic. On four of these, polymorphism was restricted to a single geographical site and the most common allele was nearly fixed; two others showed only average gel resolution. The two polymorphic loci with best gel resolution *Mpi* (mannose phosphate isomerase) and *Gdh* (glutamate dehydrogenase) were, therefore, chosen for the comparison between the adults and their brood (Table 1). For this comparison, proteins from each adult were electrophoresed alongside those from its brood. Twenty two adults and 88 brooded young were used in this phase.

Voucher specimens of *Actinia bermudensis* were deposited in the Cnidarian Collection of the Department of Zoology of the Federal University of Rio de Janeiro, under the number DZIBUFRJ 2-896. This was done to allow the correct specific assignment of the populations studied if the systematics of the genus is changed in the future.

Results

A complete color and genotype identity (Table 2, Fig. 1) was found between the adults and their brood. In the case of sexually produced juveniles and considering the brooding anemone as one of the parents, it would be expected that for a brooding anemone with the genotype AA, the frequency of offspring with the same genotype would be equal to the frequency of the allele A on the overall population (f_A). In locally structured populations, one should add to that the probability that the sperm came from an adult with an allele A identical by descent to that of the egg. However, although some *Actinia equina* populations can be highly structured locally (Solé-Cava and Thorpe, 1992), this does not seem to be the case in the phylogenetically close (Solé-Cava et al., 1994) *A. bermudensis* studied in this paper ($F_{IS} = 0.092$; Russo et al., 1994).

Furthermore, the populations studied here were found on exposed parts of rocky shores, and the maximum distance between any two individuals collected in each site was 20 m. Therefore, it is reasonable to assume that the sperm came from a locally panmitic pool and, thus, the effect of local inbreeding can be ignored. Consequently, the probability of observing *n* sexually produced young with the same diploid genotype as the parent (genotype ij) by chance can be calculated as:

$$P = (^1/_2 f_i + {}^1/_2 f_j)^n$$

Table 2. Individual and total probabilities of observing the complete identity of the genotypes between each adult anemone and its offspring, considering the young sexually produced and the adult anemone as one of the parents. P = probability. RD: red color type. BR: brown color type. B: Barra de Itabapoana. I: Itaipú F: Florianó polis.

			Mpi		*Gdh*		
	Adult	No. of juveniles	Genotype	P	Genotype	P	Total
RDI	1	2	AB	0.1253000	BB	0.1648000	0.0206494
	2	5	BC	0.0085130	AA	0.0739000	0.0006291
	3	11	AC	0.0000003	BB	0.0000490	1.5×10^{-11}
	4	6	BC	0.0033000	AA	0.0439000	0.0001449
	5	5	AC	0.0012000	BB	0.0110000	0.0000132
	6	2	AC	0.0679000	BB	0.1648000	0.0111899
	7	1	BC	0.3855000	AA	0.5940000	0.2289870
	8	2	BB	0.2294000	AB	0.2500000	0.0573500
	9	2	BC	0.1486000	AA	0.3528000	0.0524261
	10	6	BB	0.0120800	AB	0.0156000	0.0001884
	11	2	BB	0.2294000	AA	0.3528000	0.0809323
	12	4	AC	0.0046000	BB	0.0272000	0.0001251
	13	3	BC	0.0573000	AA	0.2096000	0.0120101
	14	5	AC	0.0012000	BB	0.0110000	0.0000132
	15	5	BB	0.0252000	AB	0.0313000	0.0007888
	16	4	BC	0.0221000	AB	0.0625000	0.0013813
	Total	65		5.5×10^{-31}		1.7×10^{-21}	9.3×10^{-52}
RDF	17	4	AB	0.0625000	—	—	0.0625000
	18	5	BB	0.4623000	—	—	0.4623000
	Total	9		0.0288938	—	—	0.0288938
BRF	19	2	AB	0.2500000	—	—	0.2500000
	20	5	BB	0.0094000	—	—	0.0094000
	Total	7		0.0023500	—	—	0.0023500
RDB	21	5	BB	0.4182000	—	—	0.4182000
	22	2	BB	0.7056000	—	—	0.7056000
	Total	7		0.2950819	—	—	0.2950819
Total	22	88		1.1×10^{-35}	—	1.7×10^{-21}	1.9×10^{-56}

where f_i and f_j are the frequencies of the i and j alleles in the population, respectively. This reduces to $P = f_i^n$, in the case of homozygotes (where $i = j$, and therefore $f_i = f_j$) and to $P = (^1/_2)^n$ for the heterozygotes on two-allele systems (where $f_i + f_j = 1$). To illustrate, let us consider an example of one sea anemone (adult #15, on Table 2) and its five juveniles that were all homozygotes for allele B for *Mpi*. The probability of such event, if the young were produced sexually, can be calculated as $f_i = f_j =$ *frequency of B in the population* $= 0.479$; $P = (^1/_2 0.479 + {}^1/_2 0.479)^5$; $P = 0.479^5 = 0.0252$.

In the case of adult # 14, (heterozygote AC for *Mpi*) the probability is $f_i =$ *frequency of A in the population* $= 0.229$; $f_j =$ *frequency of C in the population* $= 0.292$; $P = (^1/_2 0.229 + {}^1/_2 0.292)^4$ and then $P = (0.2605)^4 = 0.0012$, and thus, the cumulative probability, considering all the anemones studied for the *Mpi* locus, is $P = 1.1 \times 10^{-35}$ and for the *Gdh* locus $P = 1.7 \times 10^{-21}$ (Table 2).

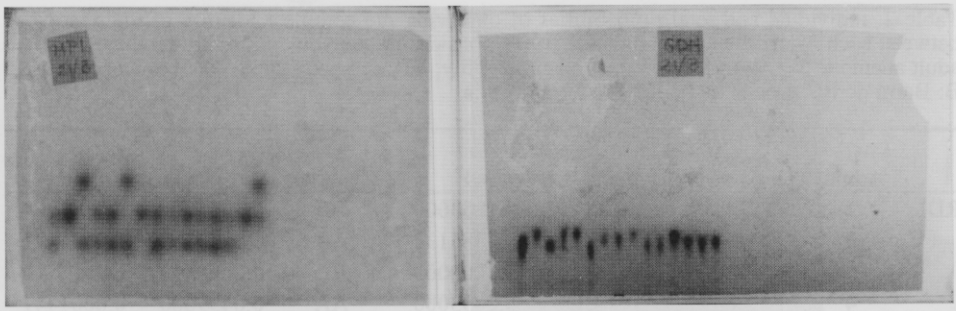

Figure 1. Gel patterns for the *Actinia bermudensis* population for the *Mpi* (left) and *Gdh* (right) enzymes, showing the polymorphism of the population.

Even though each one of these probabilities is by itself sufficient to ascertain the asexual origin of the juveniles, it is not obvious whether they are independent or not. Table 3 shows the relationship between the genotypes in *Mpi* and *Gdh* loci for each individual of the red colortype of Itaipú. For example, all five individuals that were AC for *Mpi* were also BB for *Gdh*. Examining the rest of the table, there seems to be far more association between the two genotypes than what would be expected by chance or even by linkage disequilibrium (see Hartl and Clark, 1991). This indicates that although the minimum distance between any two individuals collected was 2 m, this was not sufficient to avoid sampling within clones.

However, as it will be shown next, this would not invalidate the composite probabilities for each locus shown in Table 2. For instance, let us consider that the 5 *Mpi*-AC/*Gdh*-BB are from the same clone, i.e., they are a single genetic individual (genet, sensu Harper 1977). The composite probabilities would still be correct because they depend only on the number of juveniles with the same genotype as the parent and that would have remained the same whether we had one or five genets. This holds because for any X, a, and b,

$$X^{a+b} = X^a \times X^b.$$

This would mean that the overall probability of observing a complete genotype identity between those 5 *Mpi*-AC/*Gdh*-BB adults and their offspring (11, 5, 2, 4, and 5 juveniles, respectively) will be the same as if there were just one adult with 27 juveniles.

Table 3. *Mpi* and *Gdh* genotypes association in the red color type of Itaipú (RDI).

No. Ind.	*Mpi*	No. Ind.	*Gdh*	%
1	AB	1	AB	100
6	BC	5	AA	83
		1	AB	17
5	AC	5	BB	100
4	BB	3	AB	75
		1	AA	25

Discussion

The results clearly show that the young *Actinia bermudensis* are identical to the adults harboring them and are therefore produced asexually. In addition, all brooded juveniles were of the same color as the adults, which could be a further indication that the juveniles were asexually produced (see Gashout and Ormond, 1979). However, the column color is not a reliable characteristic for the identification of clones, since transplant experiments showed that the color of the brooded juveniles of *A. equina* can be altered by the adults containing them (Lubbock and Allbut, 1981). The instability of the column color of this species is a phenomenon that deserves attention, since column and pedal disc colors are important characteristics in the taxonomy of this genus (Carter and Thorpe, 1981; Haylor et al., 1984; Solé-Cava and Thorpe, 1987, 1992; Perrin et al., 1996).

The genetic data derived from allozyme electrophoresis are usually more reliable than morphological characteristics because allozymes are less likely to be influenced by external factors. In the case of the two polymorphic enzymes studied here it is extremely unlikely that the young were produced sexually and were identical to the adult by chance (for *Mpi* $P < 1.1 \times 10^{-35}$ and for *Gdh* $P < 1.7 \times 10^{-21}$, Table 2). These results agree well with those described for other sea anemone species (Black and Johnson, 1979; Orr et al., 1982) and strongly indicate that some form of asexual reproduction is involved.

Some authors in the past have argued that the high similarity found between adults and their brooded young should not be used alone as hard evidence for asexual reproduction. They stated that this similarity could be the result of selection in which the adult anemone would let enter its coelenteron only genetically similar juveniles (Ottaway and Kirby, 1975; Carter and Thorp, 1979). Although the idea of selection of genetically similar young by the adults might have made sense at the time it was issued, it is now difficult to support. This is because most sea anemones studied to date have presented very high levels of gene variation (e.g., Shaw et al. 1987; Solé-Cava and Thorpe, 1991; Russo et al., 1994; Perrin et al., 1996), and it would be practically impossible for a sexually produced larva (or adult) to encounter an adult anemone (or larva) genetically identical to it.

It could still be argued that the selection by the adult/larva is occurring based only on a few "compatibility" or "recognition" loci, but that would require that these were genetically linked to the enzyme loci identical between adult and young anemones. Finally, the continuous appearance of juveniles inside isolated anemones kept in filtered sea water for many months (Chia and Rostron, 1970; Gashout and Ormond, 1979) shows that they could not have come from the water and further rejects the "selection of the young hypothesis."

A possible source of bias is the putative expression of the maternal mRNA in the early stages of larval development, leading to an apparent identity in enzyme phenotype with the mother (Stoddart et al., 1988). Not surprisingly, the intensity of that expression decreases with larval age (Stoddart et al., 1988). However, this could not be the case for the young anemones studied here, since they had the same levels of activity for their allozymes, regardless of body size.

In a recent paper, Edmands (1995) examined the asexual or sexual origin of juveniles of *Epiactis* spp. using both allozymes and DNA fingerprinting. Although, in *Epiactis prolifera* both methods indicated asexual reproduction, in *E. lisbethae* mother and offspring were all identical for allozymes, but some of the young had different fingerprints from their parents. The fingerprinting analysis clearly indicates that about half of the

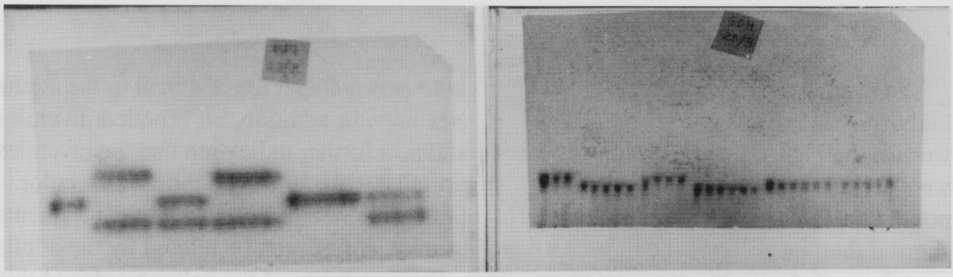

Figure 2. Gel patterns of the enzymes *Mpi* (left) and *Gdh* (right) for some *Actinia bermudensis* adults (lanes # 1, 4, 9, 13, 19 and 25, which correspond to adults #11–16 on Table 2, respectively) followed by their juveniles.

juveniles were not asexually produced by the adults from which they were taken. The apparent discrepancy between isozymes and fingerprinting data, however, is easily explained by the fact that no heterozygotes were used in that work. This is true because, a homozygous parent can produce a progeny identical to it at a few loci by chance or by a number of sexual processes, such as selfing or inbreeding in an extremely locally structured population. In fact, the *E. lisbethae* population presented an extremely high F_{IS} = 0.98! Heterozygotes, on the other hand, will only produce n offspring identical to it sexually with a probability of $(1/2)^n$, no matter the type of sexual reproduction involved. For *E. lisbethae*, isozyme analysis certainly is not the method to be used, which draws attention to the importance of not using that method indiscriminately (see Avise 1994).

The case for *A. bermudensis* is quite different, because the observed heterozygosity is high (Fig. 1) and agrees well with the Hardy-Weinberg expected values (Russo et al., 1994). Furthermore, even heterozygote adults (such as #14 for *Mpi* in Fig. 2) always had identical progeny and local structuring was low (F_{IS} = 0.092; Russo et al., 1994).

Although this paper shows the existence of asexual reproduction in *A. bermudensis*, the mechanism involved is still not clear (Ayre, 1988). For other sea anemone species, some authors have suggested parthenogenesis (Gashout and Ormond, 1979; Shaw et al., 1987), while others proposed some form of internal budding (= somatic embryogenesis) as the main type of asexual reproduction (Orr et al., 1982). The high proportion of females in the population and the non development of small fragments of adults into juveniles led Gashout and Ormond (1979) to conclude that parthenogenesis was the main type of asexual reproduction in *A. equina*. Apomytic parthenogenesis was also chosen as the most likely type of asexual reproduction in *Sagartia ornata*, since it would explain the high proportion of females, the possible tetraploidy found in that species, and the lack of evidence for any other mechanism of reproduction (Shaw et al., 1987).

On the other hand, Ayre (1988) found that even male and immature adults can incubate juveniles in *Actinia tenebrosa*. Since these juveniles are asexually produced (Black and Johnson, 1979) and in this species sex is genetically determined (Ayre 1988), and therefore stable, this is strong evidence for internal budding in *A. tenebrosa*.

In a population of *Actinia bermudensis* from Florida a female:male ratio of 10:1 has been reported (Jennison, 1983), which might be used to support the apomytic parthenogenesis hypothesis. However, the identification of the species in that paper was dubious (Jennison, 1983) and that result, therefore, must be taken with extreme caution. We still do not know the sex ratio or the number of chromosomes of the Brazilian population of *A. bermudensis*. The conclusion of this work is that the young found within *A. bermudensis*

are being produced asexually, possibly by apomytic parthenogenesis or some type of somatic embryogenesis. The confirmation of the type of reproduction awaits further cytological and cytogenetic work.

Acknowledgments

The authors thank M. R. Monteiro, and J. P. Thorpe for comments and revision of an earlier version of the manuscript. Some of the data analyses were conducted while Claudia Russo was at the Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, and A.M. Solé-Cava was at the Plymouth Marine Laboratory, Plymouth, England. This work was supported by CNPq (National Research Council, Brazil) and FAPERJ (Rio de Janeiro Research Foundation, Brazil).

Literature Cited

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York. 511 p.

Ayre, D. J. 1988. Evidence for genetic determination of sex in *Actinia tenebrosa*. J. Exp. Mar. Biol. Ecol. 116: 23–34.

Black, R. and M. S. Johnson. 1979. Asexual viviparity and population genetics of *Actinia tenebrosa*. Mar. Biol. 53: 27–31.

Carter, M. A. and C. H. Thorp. 1979. The reproduction of *Actinia equina* var. *mesembryanthemum*. J. Mar. Biol. Ass. U.K. 59: 989–1001.

_____ and _____. 1981. *Actinia* reproduction and the species problem. Porcupine Soc. News. 2: 44–45.

Chia, F. S. 1976. Sea anemone reproduction: patterns and adaptive radiation. Pages 261–270 *in* G. O. Mackie, ed. Coelenterate ecology and behaviour. Plenum Press, New York.

_____ and M. A. Rostron. 1970. Some aspects of the reproductive biology of *Actinia equina* (Cnidaria: Anthozoa). J. Mar. Biol. Ass. UK 50: 253–264.

Edmands, S. 1995. Mating systems in the sea anemone genus *Epiactis*. Mar. Biol. 123: 723–733.

Gashout, S. E. and G. F. Ormond. 1979. Evidence for parthenogenetic reproduction in the sea anemone *Actinia equina* L. J. Mar. Biol. Ass., U.K. 59: 975–987.

Harper, J. L. 1977. Population biology of plants. Academic Press, London. 892 p.

Harris, H. and D. A. Hopkinson. 1978. Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam. 259 p.

Hartl, D. L. and A. G. Clark. 1991. Principles of population genetics. Sinauer Press, Sunderland, Massachusetts. 682 p.

Haylor, G. S., J. P. Thorpe and M. A. Carter. 1984. Genetical and ecological differentiation between sympatric colour morphs of the common intertidal sea anemone *Actinia equina*. Mar. Ecol. Prog. Ser. 16: 281–290.

Jennison, B. L. 1983. Reproductive biology of three species of sea anemones from the central Atlantic Coast of Florida. Fla. Sci. 46: 168–178.

Lubbock, R. and C. Allbut. 1981. The sea anemone tolerates allogeneic juveniles but alters their phenotype. Nature 293: 474–475.

McMurrich, J. P. 1889. A contribution to the actinology of the Bermudas. Proc. Acad. Sci. Philadelphia, 1889: 102–126.

Orr, J., J. P. Thorpe and M. A. Carter. 1982. Biochemical genetic confirmation of the asexual production of brooded offspring in the sea anemone *Actinia equina*. Mar. Ecol. Prog. Ser. 7: 227–229.

Ottaway, J. R. and G. C. Kirby. 1975. Genetic relationships between brooding and brooded *Actinia tenebrosa*. Nature 255: 221–223.

Perrin, M. C., J. P. Thorpe, and A. M. Solé-Cava. 1996. *Actinia equina*: a genetic role model and reproductive enigma. *In* R. G. Hartnoll and S. J. Hawkins, eds. Marine biology: A Port Erin perspective (Centenary Volume of the Port Erin Marine Laboratory). Immel Publishing, London (in press).

Richardson, B. J., P. R. Baverstock and M. Adams. 1986. Allozyme electrophoresis. Academic Press, Sydney. 410 p.

Rostron, M. A. and J. Rostron. 1978. Fecundity and reproductive ecology of a natural population of *Actinia equina* L. (Cnidaria: Anthozoa). J. Exp. Mar. Biol. Ecol. 33: 251–259.

Russo, C.A.M., A.M. Solé-Cava and J. P. Thorpe. 1994. Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actiniidae) with different reproductive strategies. Mar. Biol. 119: 267–276.

Schlenz, E. 1983. Anêmonas (Cnidaria: Actiniaria) do Brasil. An. Acad. Bras. Cienc. 55: 330–331.

Shaw, P. W., J. A. Beardmore and J. S. Ryland. 1987. *Sagartia troglodytes* (Anthozoa: Actiniaria) consists of two species. Mar. Ecol. Prog. Ser. 41: 21–28.

Shick, J. M. 1991. A functional biology of sea anemones. Chapman and Hall, New York. 395 p.

Solé-Cava, A. M., C. A. M. Russo, M. E. Araújo and J. P. Thorpe. 1994. Cladistic and phenetic analysis of allozyme data for nine species of anemones of the family Actiniidae (Cnidaria: Anthozoa). Biol. J. Linn. Soc. 52/53: 225–239.

_____, J. P. Thorpe and J. G. Kaye. 1985. Reproductive isolation with little genetic divergence between *Urticina* (=*Tealia*) *felina* and *U. eques* (Anthozoa: Actiniaria). Mar. Biol. 85: 279–284.

_____ and _____. 1987. Further genetic evidence for the reproductive isolation of the green colour morph of the intertidal sea anemone *Actinia equina*. Mar. Ecol. Prog. Ser. 38: 225–229.

_____ and _____. 1991. High levels of gene variation in marine invertebrates. Biol. J. Linn. Soc. 44: 65–80.

_____ and _____. 1992. Genetic divergence between sympatric and allopatric populations of the various morphs of the common intertidal sea anemone *Actinia* (Anthozoa: Actiniaria). Mar. Biol. 112: 243–252.

Stoddart, J. A., R. C. Babcock and A. J. Heyward. 1988. Self-fertilization and maternal enzymes in the planulae of the coral *Goniastrea favulus*. Mar. Biol. 99: 489–494.

Ward, R. D. and J. A. Beardmore. 1977. Protein variation in the plaice (*Pleuronectes platessa*). Genet. Res. 30: 45–62.

DATE SUBMITTED: September 14, 1994. DATE ACCEPTED: September 19, 1996.

ADDRESSES: (C.R., F.M., A.M.S.-C.) *Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro Bl. A, CCS, Av. Brigadeiro Trompowsky s/n CEP 21941-590, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ, Brazil;* (A.M.S.-C.) *Departamento de Biologia Geral, Instituto de Biologia, Universidade Federal Fluminense, Morro do Valonguinho, Centro CEP 24000, Niterói, RJ, Brazil.*