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Reproductive isolation with little genetic divergence between *Urticina* (= *Tealia*) *felina* and *U. eques* (Anthozoa: Actiniaria)

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Abstract

There has long been doubt as to whether there are one or two British species of the sea anemone genus *Urticina*. In the present study, populations of both putative species, which occur sympatrically in the Isle of Man, have been compared by electrophoresis of isozymes and nematocyst analysis. The analysis of fourteen isozyme loci exhibited a high genetic identity between the two taxa ($I = 0.907$), but four loci showed significant differences in gene frequencies; thus indicating reproductive isolation. The populations displayed highly significant differences in the sizes of the various nematocyst types. The individual analysis of data for each type of nematocyst revealed that the basitrics from the actinopharynx and from the tentacles contribute most to the observed difference. Consequently, *U. felina* (L.) and *U. eques* (Gosse) are assumed to be valid species, as suggested by Manuel (1981). The mean heterozygosities for each species (0.410 and 0.436, respectively) are the highest reported for cnidarians.

Introduction

Sea anemones of the genus *Urticina* Ehrenberg, 1834 (formerly *Tealia* Gosse, 1858) are very abundant in the North Atlantic Ocean, from the lower intertidal zone down to 400 m. They are considered to be boreal-artic and circumpolar in distribution (Carlgren, 1949; Manuel, 1981). It has been assumed by some authors (Elmhirst and Sharpe, 1923; Stephenson, 1935; Carlgren, 1949) that this genus has only one highly polymorphic species. Hand (1955), however, considered that several of the "varieties" of the genus were not conspecific. In the most recent major work on British anthozoans, Manuel (1981) considered there to be two valid species, *U. felina* (L.) and *U. eques* (Gosse). The differentiation between *U. felina* and *U. eques* is based principally on colouration (dark in *U. felina*, paler in *U. eques*) and presence of gravel encrustations on the column (abundant in *felina* but rare or absent in *eques*) (Hand, 1955; Manuel, 1981). However, Elmhirst and Sharpe (1923) argue that these characters are extremely variable, and depend on the type of microhabitat in which the anemone is found. The nematocysts used by Hand (1955) to differentiate several "species" of *Tealia*, were considered by Carlgren (1921) to be variable and of little or no use in distinguishing what he considered to be varieties of a single species. The taxonomic status of the various morphotypes of *Urticina* is of considerable significance, since the proper identification of species in such a common genus is clearly important for meaningful ecology.

The considerable potential of biochemical taxonomy based upon enzyme electrophoresis has been clear for some time (see Gottlieb, 1971; Avise, 1974) and has been used to solve taxonomic problems in a wide variety of groups (for reviews see Ferguson, 1980; Thorpe, 1982, 1983; Ayala, 1983). Previous biochemical genetic studies on sea anemones include those on species of *Actinia* (e.g. Ottaway and Kirby, 1975; Black and Johnson, 1979; Carter and Thorpe, 1981; Orr *et al.*, 1982; Ayre, 1983; Quicke and Brace, 1983; Haylor *et al.*, 1984), *Metridium* (e.g. Hoffman, 1976; Bucklin and Hedgecock, 1982), *Haliplanella* (e.g. Shick and Lamb, 1977; Shick *et al.*, 1979), and *Bunodosoma* (e.g. McCommas and Lester, 1980). From some of the above and other biochemical genetic studies it is clear that many "varieties" of sea anemone species have proved to be reproductively isolated and therefore cannot be conspecific. Whether this problem is common in anthozoans or perhaps largely restricted to only a few species is, at present, unclear.

The objective of the present work was to assess the population structure of the taxa *Urticina felina* and *U. eques* that occur sympatrically in the Isle of Man, and to try to establish their taxonomic status. The methods used were enzyme electrophoresis in conjunction with a detailed study of nematocyst types in the two putative species.

Materials and methods

Over the period November 1983–March 1984, 49 specimens of *Urticina felina* (L.) and 29 specimens of *U. eques* (Gosse) were collected by diving in shallow (< 10 m) subtidal areas of rocky substratum in the inner part of Port Erin Bay (Isle of Man). All samples could be easily and unambiguously assigned to *U. felina* or to *U. eques* using the characters of colour and of abundance and size of verrucae (see Hand, 1955; Manuel, 1980, 1981). In both species the size (diameter of expanded oral disc) ranged approximately from 3 to 7 cm. After collection, the sea anemones were kept in the laboratory in running sea water and fed regularly with scallop adductor muscle.

For electrophoresis, sections were cut from the sea anemones to include both oral disc tissue (including tentacles) and column. Samples were homogenised in not more than an equal volume of distilled water. The rest of the sea anemone was divided into two parts, one being frozen at −20 °C (for no longer than one week) to be used for further electrophoresis, and the other stored in 4% Formalin for nematocyst analysis.

Electrophoresis was by standard horizontal starch-gel methods (reviews by Brewer, 1970; Harris and Hopkinson, 1978; Ferguson, 1980). Gels used 12.5% starch (Sigma Chemical Company, Poole, Dorset, England). Extensive trials were conducted with several buffer systems, principal among which were a discontinuous histidine/citrate system, pH 8.0 (Brewer, 1970); a discontinuous tris-citrate/Borate pH 8.3 (Poulik, 1957) and a discontinuous tris-maleic, pH 7.4 (Carter and Thorpe, 1981). However, a range of buffers was found to be unnecessary and most of the results were obtained using a continuous tris-citrate, pH 8.0 buffer from Ward and Beardmore (1977). (Composition, electrode buffer: 30.3 g Tris + 12.0 g citric acid + 1 000 ml distilled water; gel buffer: 38.5 ml of electrode buffer diluted to 1 000 ml with distilled water). An exception was Aconitase, which was run on a continuous tris-citrate, pH 7.0 buffer (Brewer, 1970).

The staining of the gels followed standard procedures, using recipes from Shaw and Prasad (1970) and Harris and Hopkinson (1978). Enzyme nomenclature is in accordance with Harris and Hopkinson (1978).

The nematocyst types were identified as basitrics, spirocysts and microbasics following the terminology of Carlgren (1949) and Hand (1955). Ten to fifty nematocysts of each type were measured in macerated samples of each of three tissues (mesenteric filaments, actinopharynx and tentacles) from ten individuals of each species.

Results

Genetic analysis

Allele frequencies for the loci studied in *Urticina felina* and *U. eques* are presented in Table 1. Apart from the locus *Pgi-1*, none of the genotypic frequencies differed significantly from those expected for populations in Hardy-Weinberg equilibrium. *Pgi-1* showed a significant ($P < 0.01$) deviation from equilibrium in *U. felina* samples. This deviation was due to a heterozygote deficiency.

The mean observed (H_o) and expected (H_e) heterozygosities were not significantly different either in *Urticina felina* ($H_o = 0.348$; $H_e = 0.410$; $\chi^2 = 8.35$; DF = 12; $P = 0.68$) or *U. eques* ($H_o = 0.424$; $H_e = 0.436$; $\chi^2 = 8.41$; DF = 11;

Table 1. *Urticina felina* and *U. eques*. Allele frequencies at 14 enzyme loci. Enzymes are ACON (aconitase EC 4.2.1.3), EST (esterase EC 3.1.1.1.), GOT (glutamate-oxaloacetate transaminase EC 2.6.1.1.), HK (hexokinase EC 2.7.1.1.), ME (malic enzyme EC 1.1.1.40), ODH (octanol dehydrogenase EC 1.1.1.1), PGD (6-phosphogluconate dehydrogenase EC 1.1.1.44), PGM (phosphoglucomutase EC 2.7.5.1), PGI (phosphoglucose isomerase EC 5.3.1.9), SOD (superoxide dismutase EC 1.15.1.1)

Locus	Allele	*U. felina*	*n*	*U. eques*	*n*
Acon	1	0.667	18	0.417	12
	2	0.333		0.583	
Est-1	1	0.210	30	0.214	14
	2	0.710		0.286	
	3	0.080		0.500	
Est-2	1	0.385	28	0.000	14
	2	0.385		0.070	
	3	0.115		0.000	
	4	0.115		0.571	
	5	0.000		0.357	
Got-1	1	0.404	94	0.464	56
	2	0.521		0.500	
	3	0.075		0.036	
Got-2	1	0.063	96	0.000	56
	2	0.937		1.000	
Hk	1	0.183	94	0.121	58
	2	0.312		0.276	
	3	0.237		0.207	
	4	0.269		0.397	
Me	1	0.012	86	0.040	58
	2	0.788		0.900	
	3	0.165		0.040	
	4	0.035		0.020	
Odh	1	0.233	86	0.086	58
	2	0.709		0.810	
	3	0.058		0.103	
Pgd	1	0.087	92	0.121	58
	2	0.837		0.603	
	3	0.076		0.241	
	4	0.000		0.035	
Pgm	1	0.269	78	0.273	44
	2	0.205		0.364	
	3	0.513		0.341	
	4	0.013		0.022	
Pgi-1	1	0.088	68	0.350	40
	2	0.912		0.650	
Pgi-2	1	0.806	72	0.750	40
	2	0.194		0.250	
Sod-1	1	1.000	80	1.000	38
Sod-2	1	0.088	80	0.081	38
	2	0.300		0.297	
	3	0.550		0.514	
	4	0.062		0.081	
	5	0.000		0.027	

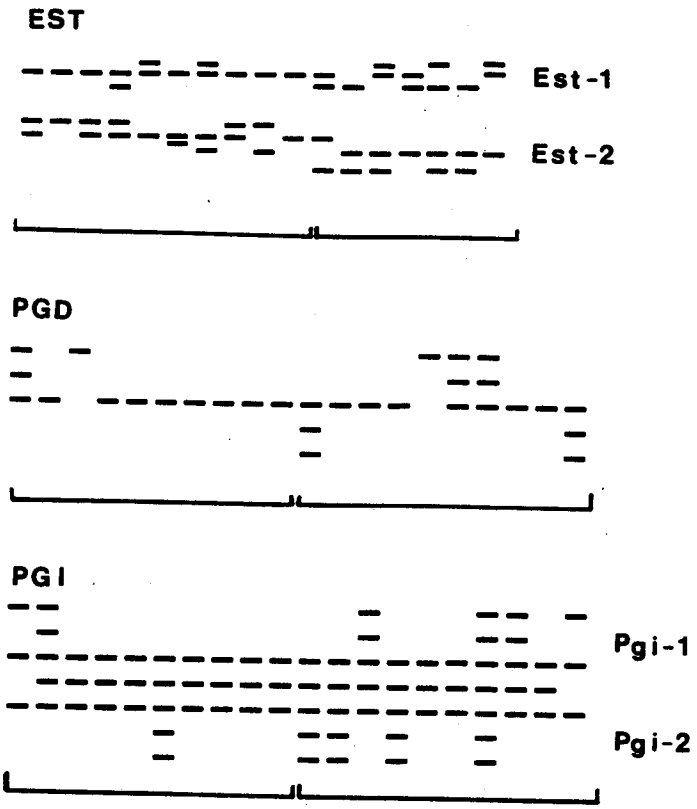

Fig. 1. *Urticina felina* and *U. eques*. Typical gels for EST, PGD and PGI. The first ten individuals are *U. felina*, the others *U. eques*. All the enzymes migrate anodally

Table 2. *Urticina felina* and *U. eques*. Results of contingency table comparisons between allele frequencies at four isozyme loci

Locus	χ^2	DF	*P*
Est-1	9.53	2	0.009
Est-2	22.51	4	0.0002
Pgd	12.66	2	0.003
Pgi-2	11.44	1	0.0007

$P=0.63$). Allele frequencies in *U. felina* and *U. eques* were significantly different at four of the fourteen isozyme loci (Table 2, Fig. 1). Whilst none of these loci is individually diagnostic [i.e., loci that allow the identification with 99% of confidence of one individual to its species (Ayala, 1983)], the probability of error in the identification of one individual, when the four loci are used in conjunction is extremely small ($P=4.6\times10^{-4}$). An incorrect conclusion will be reached less than once each two thousand times when identifying one unknown individual as *U. felina* or *eques* based exclusively on its genotypes at the loci *Est-1*, *Est-2*, *Pgd* and *Pgi-1*.

It is also of note that a blue pigment which migrated cathodally was present in all 49 samples of *Urticina felina*, but absent in the 29 *U. eques* analysed. Unlike the blue water-soluble pigment of the anemone *Corynactis californica* (see West, 1979), the colour of the pigment disappeared if the gel was immersed in solutions of HCl (0.01 *N*), NaOH (0.01 *N*), trichloroacetic acid (30% w/v) or Formalin (4% v/v). Extractions of homogenates of *Urticina* anemones with organic solvents, such as hexane, ethyl ether or acetone produced only yellow and red pigments, whereas the blue pigment was soluble in water or methanol. From its chemical behaviour, it is likely that this pigment is of proteinaceous nature (see Cheesman *et al.*, 1967), as previously suggested by Elmhirst and Sharpe (1923).

Nematocyst analysis

No difference was found between the qualitative distribution of nematocysts in the sea anemones studied. Both *Urticina felina* and *U. eques* have basitrics in the mesenteric filaments, actinopharynx and tentacles, three size classes of microbasic p-mastigophors in the mesenteric filaments, and spirocysts in the tentacles. This distribution has been previously described by Hand (1955). However, there is a

Table 3. *Urticina felina* and *U. eques*. Mean size (μm), standard deviation (σ) and number of nematocysts (n) analysed for each nematocyst type (total in 10 individuals). Student's t test is calculated on the individual means of the 10 individuals of each population (DF = 18). Fisher's F test is calculated from a nested analysis of variance (Sokal and Rohlf, 1981)

Organ and nematocyst type	*U. felina*			*U. eques*			t	*P*
	Mean	σ	n	Mean	σ	n		
Mesenteric filaments								
Basitric	18.67	1.00	130	20.68	2.32	114	2.39	0.03
Microbasic 1	12.72	0.81	153	13.57	0.73	180	2.35	0.03
Microbasic 2	22.67	1.46	148	25.04	1.38	140	3.54	0.03
Microbasic 3	39.27	3.44	161	43.07	3.95	145	2.18	0.04
Actinopharynx								
Basitric	65.37	4.81	230	73.68	3.72	263	4.10	0.0009
Tentacles								
Basitric	23.61	1.70	156	27.16	2.39	146	3.63	0.002

F. (U. felina/U. eques) = 40.77 DF = 1 and 113 $P=2\times10^{-6}$

significant difference in the size of the nematocysts between *U. felina* and *U. eques* (Table 3). Individual means for each nematocyst type in each tissue were compared between the two taxa by a nested analysis of variance with replicates (Sokal and Rohlf, 1981). Spirocysts were not included in this analysis because they are considered as being of little taxonomic significance (Manuel, 1981). The individual analysis of data for each nematocyst class showed that basitrics from the actinopharynx and from the tentacles contribute most to the overall difference between the taxa (Table 3).

Discussion

Two important and related, but very different uses of isozyme analysis are as diagnostic taxonomic characters (Ayala, 1983) or as indicators of reproductive isolation. Naturally, the existence of a genetic diagnostic character between two populations also indicates reproductive isolation between them. However, populations can be reproductively isolated and genetically divergent without showing any diagnostic locus. Isozymes as diagnostic characters are specially important to distinguish sibling species (e.g. Thorpe *et al.*, 1978; McCommas and Lester, 1980) or early stages of development of different species (e.g. Bryce and Hobart, 1972). However, more commonly the question is to decide whether two or more populations, well characterized morphologically (and ideally sympatric) are conspecific. It is common for loci to show significant differences in their allele frequencies, without being necessarily diagnostic. This is the case for the loci *Est-1, Est-2, Pgd* and *Pgi-2* in *Urticina felina* and *U. eques*. None of these loci was diagnostic, but the accumulated probability that the analysed samples were from the same population and that the differences found were produced by chance was extremely low ($P < 10^{-16}$). The calculation of accumulated probability for several loci assumes that they are independent. The possible existence of linkage between some isozyme loci in *Actinia equina* (Quicke and Brace, 1983) could be a warning against the assumption of independence of our isozyme loci. However, in the present work, since all samples were from known individuals, it was possible to test for linkage between the four loci. Our results show no significant correlation between them and provide no indication of any possible linkage.

It is interesting to note that we did not find any two anemones with identical genotypes over the fourteen loci analysed. This indicates that sexual reproduction is, if not the only, at least by far the most important method of reproduction in both species of *Urticina*. This is contrary to the situation in *Haliplanella luciae* (Shick *et al.*, 1979), *Metridium senile* (Hoffman, 1976), *Actinia equina* (Orr *et al.*, 1982; Quicke and Brace, 1983) or *A. tenebrosa* (Black and Johnson, 1979; Ayre, 1983). In these anemones, asexual reproduction may be significant and presumably can play an important role when colonizing new environments. This also can lead to electrophoretically detectable clones in some populations. It is not known whether *Urticina* species reproduce asexually (Stephenson, 1935; Chia, 1976), but the gametes and planktonic larvae of *U. felina* (as *Tealia crassicornis*) have been described (Chia and Spaulding, 1972).

The significant ($P < 0.01$) heterozygote deficiency at the *Pgi-1* locus in *Urticina felina* is puzzling and may merely result from sampling error. Alternative explanations based upon disruptive selection (e.g. Thoday and Gibson, 1962, but see Lewontin, 1974, pp 161–162) or population mixing (Wahlund, 1928) are not supported by data for the other loci, and also necessitate an exceptionally strong selection against the heterozygotes or some improbably high differences in allele frequency between the populations concerned (see Manwell and Baker, 1970, pp 20–21). A homozygote excess could also result from asexual reproduction, but this is clearly not the explanation since no two individuals were identical over all the enzyme loci (see above).

The blue pigment protein provides a clear difference between *Urticina felina* and *U. eques* although, of course, the genetic basis of this difference is not known. The protein may well be coded by a single genetic locus which is absent (or at least not functional) in *U. eques*. It is also of note that in *U. felina* the mesoglea is blue compared to the pink or white colour of this tissue in *U. eques*. This difference may be due to the presence of the blue pigmented protein in *U. felina*.

The nematocyst differences (Table 1) between *Urticina felina* and *U. eques* are also very significant and provide a further clear diagnostic character. Differences in allele frequencies between the sympatric populations of *U. eques* and *U. felina*, in conjunction with the diagnostic blue protein and the highly significant differences in the nematocyst sizes, indicate that the populations are reproductively isolated and must be given specific status. This is in full agreement with the conclusion of Manuel (1981). Morphologically, the two species can be readily distinguished using the diagnostic characters described by Hand (1955) and Manuel (1980, 1981).

Despite the reproductive isolation between *Urticina felina* and *U. eques*, the genetic identity (Nei, 1972) and similarity (Thorpe, 1979) indices between the two species are high ($I = 0.847$; $S = 0.745$). If the putative locus (*Pt-1*), coding for the blue protein, is not considered, the indices are even higher ($I = 0.907$; $S = 0.798$) [the unusually large differences between I and S values are due to the very high genetic variability within the populations (see Thorpe, 1979, 1982; Hillis, 1984)]. The values are similar to those encountered between *Actinia equina* and *A. prasina* (Gosse, 1860) (see Haylor *et al.*, 1984), but are well above average for general comparisons of congeneric species of plants and animals (Thorpe, 1979, 1982, 1983; Ayala, 1983). High levels of genetic identity between different species could be related to recent (Avise and Ayala, 1976) or saltational (Ayala, 1975, 1983; Gottlieb, 1976) speciation. The contrast between the small genetic distances and the clear morphological differences between *U. felina* and *U. eques* could be

due to differential evolutionary forces acting on the isozymes and morphological genes (Turner, 1974). However, any kind of speculation on the comparison of morphological and genetical data must be made with caution, because of the common polygenic nature of many morphological characteristics as opposed to the per locus analysis of isozymes (see Lewontin, 1984). A better knowledge of the ecology and the natural history of the genus *Urticina* is needed before conclusions can be drawn about the evolution of its species.

The very high levels of heterozygosity found in both populations may indicate that they have not suffered any major reduction in effective population size in the recent past. The predominance of sexual reproduction is important in maintaining panmixia among local populations, but the lecithotrophic short-lived planktonic larvae that these anemones produce (Chia and Spaulding, 1972) may not enable the species to sustain a reasonable gene flow between geographically distant populations. An analysis of samples from other localities inside and outside the Irish Sea could be helpful in understanding further the population structure of these species.

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Literature cited

Avise, J. C.: Systematic value of electrophoretic data. Syst. Zool. *23*, 465–481 (1974)

Avise, J. C. and F. J. Ayala: Genetic differentiation in speciose versus depauperate phylads: evidence from the California minnows. Evolution, Lawrence, Kansas *30*, 46–58 (1976)

Ayala, F. J.: Genetic differentiation during the speciation process. Evolutionary Biol. *8*, 1–78 (1975)

Ayala, F. J.: Enzymes as taxonomic characters. *In:* Protein polymorphism: adaptative and taxonomic significance, pp 3–26. Ed. by G. S. Oxford and D. Rollinson. London: Academic Press 1983

Ayre, D. J.: The effects of asexual reproduction and inter-genotypic aggression on the genotypic structure of populations of the sea anemone *Actinia tenebrosa*. Oecologia *57*, 158–165 (1983)

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

Brewer, G. J.: An introduction to isozyme techniques, 186 pp. New York: Academic Press 1970

Bryce, D. and A. Hobart: The biology and identification of the larvae of the Chironomidae (Diptera). Entomologist's Gaz. *23*, 175–217 (1972)

Bucklin, A. and D. Hedgecock: Biochemical genetic evidence for a third species of *Metridium* (Coelenterata: Actiniaria). Mar. Biol. *66*, 1–7 (1982)

Carlgren, O.: Actiniaria, Part 1. Dan. Ingolf Exped. *5*, 1–241 (1921)

Carlgren, O.: A survey of the Phytodactiaria, Corallimorpharia and Actiniaria. K. svenska. VetenskAkad. Handl. *1*, 1–121 (1949)

Carter, M. A. and J. P. Thorpe: Reproductive, genetic and ecological evidence that *Actinia equina* var. *mesembryanthemum* and var. *fragacea* are not conspecific. J. mar. biol. Ass. U.K. *61*, 79–83 (1981)

Cheesman, D. F., W. L. Lee and P. F. Zagalsky: Carotenoproteins in invertebrates. Biol. Rev. *42*, 132–160 (1967)

Chia, F.-S.: Sea anemone reproduction: patterns and adaptative radiation. *In:* Coelenterate ecology and behaviour, pp 261–270. Ed. by G. O. Mackie. New York: Plenum Press 1976

Chia, F.-S. and J. G. Spaulding: Development and juvenile growth of the sea anemone *Tealia crassicornis*. Biol. Bull. mar. biol. Lab., Woods Hole *142*, 206–218 (1972)

Elmhirst, R. and J. S. Sharpe: On the colours of the sea anemone *Tealia crassicornis*. Ann. Mag. nat. Hist. *11*, 615–621 (1923)

Ferguson, A.: Biochemical systematics and evolution, 194 pp. Glasgow: Blackie 1980

Gottlieb, L. D.: Gel electrophoresis: a new approach to the study of evolution. BioSci *21*, 939–944 (1971)

Gottlieb, L. D.: Biochemical consequences of speciation in plants. *In:* Molecular evolution, pp 123–140. Ed. by F. J. Ayala. Sunderland, Massachusetts: Sinauer Associates 1976

Hand, C.: The sea anemones of central California, Part 2. The endomyarian and mesomyarian anemones. Wasmann J. Biol. *13*, 37–99 (1955)

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

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

Hillis, D. M.: Misuse and modification of Nei's genetic distance. Syst. Zool. *33*, 238–240 (1984)

Hoffman, R. J.: Genetics and asexual reproduction of the sea anemone *Metridium senile*. Biol. Bull. mar. biol. Lab., Woods Hole *151*, 478–488 (1976)

Lewontin, R. C.: The genetic basis of evolutionary change, 346 pp. New York: Columbia University Press 1974

Lewontin, R. C.: Detecting population differences in quantitative characters as opposed to gene frequencies. Am. Nat. *123*, 115–124 (1984)

Manuel, R. L.: The Anthozoa of the British Isles – a colour guide, 60 pp. Manchester: Underwater Conservation Society 1980

Manuel, R. L.: British Anthozoa, 241 pp. London: Academic Press 1981

Manwell, C. and C. M. A. Baker: Molecular biology and the origin of species, 394 pp. Seattle: University of Washington Press 1970

McCommas, S. A. and L. J. Lester: Electrophoretic evaluation of the taxonomic status of two species of sea anemone. Biochem. Syst. Ecol. *8*, 289–292 (1980)

Nei, M.: Genetic distance between population. Am. Nat. *106*, 283–292 (1972)

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

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

Poulik, M. D.: Starch gel electrophoresis in a discontinuous system of buffers. Nature, Lond. *180*, 1477–1479 (1957)

Quicke, D. L. J. and R. C. Brace: Phenotypic and genotypic spacing within an aggregation of the sea anemone, *Actinia equina*. J. mar. biol. Ass. U.K. *63*, 493–515 (1983)

Shaw, P. R. and R. Prasad: Starch gel electrophoresis of enzymes – a compilation of recipes. Biochem. Genet. *4*, 297–320 (1970)

Shick, J. M., R. J. Hoffmann and A. N. Lamb: Asexual reproduction, population structure, and genotype-environment interactions in sea anemones. Am. Zool. *19*, 699–713 (1979)

Shick, J. M. and A. N. Lamb: Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. Biol. Bull. mar. biol. Lab., Woods Hole *153*, 604–617 (1977)

Sokal, R. R. and F. J. Rohlf: Biometry. The principles and practice of statistics in biological research. 2nd ed. 859 pp. San Francisco: Freeman & Co. 1981

Stephenson, T. A.: The British sea anemones. Vol 2. 426 pp. London: Ray Society 1935

Thoday, J. M. and J. B. Gibson: Isolation by disruptive selection. Nature, Lond. *193*, 1164–1166 (1962)

Thorpe, J. P.: Enzyme variation and taxonomy: the estimation of sampling errors in measurements of interspecific genetic similarity. Biol. J. Linn. Soc. *11*, 369–386 (1979)

Thorpe, J. P.: The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. A. Rev. Ecol. Syst. *13*, 139–168 (1982)

Thorpe, J. P.: Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. *In:* Protein polymorphism: adaptative and taxonomic significance. pp 131–152. Ed. by G. S. Oxford and D. Rollinson. London: Academic Press 1983

Thorpe, J. P., J. A. Beardmore and J. S. Ryland: Genetic evidence for cryptic speciation in the marine bryozoan *Alcyonidium gelatinosum*. Mar. Biol. *49*, 27–32 (1978)

Turner, B. R.: Genetic divergence of Death Valley pupfish species: biochemical versus morphological evidence. Evolution, Lawrence, Kansas *28*, 281–294 (1974)

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

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

West, H. H.: Pigmentation in the sea anemone *Corynactis californica*. Comp. Biochem. Physiol. *64 B*, 195–200 (1979)

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