POPULATION STRUCTURING, GENE DISPERSAL AND REPRODUCTION IN THE *ACTINIA EQUINA* SPECIES GROUP

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Abstract Sea anemones of the genus *Actinia* are common on many shores worldwide and hence have received much attention from ecologists and many other workers. The most extensively studied species, *A. equina*, is abundant on many rocky shores around Britain and elsewhere in Europe. It has long been known to be phenotypically highly variable with many colour morphs and great variation, for example, in size and mode of reproduction. More recently molecular and other studies have revealed unexpected levels of population structuring with genetic divergence and, in some cases, evidence for reproductive isolation between morphs. This has resulted in taxonomic problems, exacerbated by our lack of detailed understanding of processes of reproduction and recruitment in *Actinia* species in Europe. Work on population genetics, systematics and speciation in *A. equina* and other *Actinia* species is discussed and attention is drawn to areas in which further work is needed.

Introduction

Sea anemones (Phylum Cnidaria, Class Anthozoa, Subclass Hexacorallia, Order Actiniaria) are common organisms in many benthic marine communities (Sebens 1986, Longhurst & Pauly 1987). Because of their ubiquity, ecological importance and a variety of reproductive modes, various sea anemones have, in the past few years, attracted much attention from research workers in several fields. It has become clear that in several species of sea anemone the genetic structuring of populations is more complex than previously suspected, leading to problems in understanding species boundaries and modes of speciation and, of course, consequent taxonomic and systematic difficulties. The problems are illustrated particularly well by species of the genus *Actinia* Linnaeus 1767, especially the abundant and widely distributed European *A. equina* (*sensu* Gosse 1860). The aims of this paper are to review the literature on the taxonomy, population genetics and breeding systems of the genus, particularly *A. equina*, and to use the available information as a starting point to indicate possible future studies.

Sea anemones are opportunistic predators (Sebens 1981, Zamer 1986, Kruger & Griffiths 1996) or filter feeders (Rubenstein & Koehl 1977) and are themselves eaten by a few invertebrate and fish species (Ottaway 1977, Hall et al. 1982, 1984, Ates 1989). Sea anemones also interact both interspecifically and intraspecifically via aggression (Bonnin 1964, Bigger 1980, Ayre 1982, Sebens 1984) and commensal or mutualistic symbiosis (Mariscal 1970,

Ross 1983). In general, sea anemones show variety in form (Stephenson 1935, Hand 1955a,b, Schmidt 1971, 1972, Manuel 1988), reproduction (Chia 1976, Fautin 1991, Shick 1991) and behaviour (Herndl 1984, Brace & Reynolds 1989), making them useful model species for the investigation of theories of speciation (Quicke & Brace 1984, Shaw et al. 1987, Solé-Cava & Thorpe 1992), larval dispersal and the genetic structuring of populations (Hunt & Ayre 1989, Ayre et al. 1991, Russo & Solé-Cava 1991, Solé-Cava et al. 1994a, Monteiro et al. 1997) and evolutionary strategies (Ayre 1982, Shaw 1989).

The genus *Actinia* has been the focus of a significant number of wide ranging biological studies. Physiological investigations have included those of reproduction (e.g. Polteva 1963, Schaefer 1981), growth (e.g. Abeloos 1955, Ottaway 1980), nutrition (Kruger & Griffiths 1996) and environmental tolerances (e.g. Ottaway 1973, Griffiths 1977a,b), together with fields as diverse as allorecognition (e.g. Bigger 1980, Lubbock 1980) and strength of attachment to substrata (Young et al. 1988). Behavioural investigations of species in this group have included examinations of feeding (e.g. Herndl 1984), movement and spacing (e.g. Parker 1917, Ottaway & Thomas 1971, Ottaway 1978, 1979a, Brace & Quicke 1985, 1986a,b, Ayre 1987), and aggression (e.g. Bonnin 1964, Brace et al. 1979, Ayre 1982, Brace & Reynolds 1989). Ecological studies have been concerned essentially with reproductive ecology (e.g. Chia & Rostron 1970, Carter & Thorp 1979, Gashout & Ormond 1979, Carter & Thorpe 1981, Lubbock & Allbut 1981, Orr et al. 1982, Ayre 1984a,b, Carter & Miles 1989) and population dynamics (e.g. Ottaway 1979b, Rees 1984, Ayre 1985, Brace & Quicke 1986a,b). The taxonomy of the group has received continuing interest through both traditional morphological investigations (e.g. Carlgren 1949, Schmidt 1971, 1972, Chintiroglou & Simsiridou 1997, Allcock et al. 1998) and more recently through the application of biochemical genetic techniques (Carter & Thorpe 1981, Quicke et al. 1983, Quicke & Brace 1983, Quicke et al. 1985, Haylor et al. 1984, Solé-Cava et al. 1994b, Solé-Cava & Thorpe 1992, Russo et al. 1994, Monteiro et al. 1997, 1998). The genetic structuring of *Actinia* populations on small and large scales has also received some attention (Ayre 1983a, 1984a, Quicke & Brace 1983, Brace & Quicke 1985, Ayre et al. 1991, Russo et al. 1994, Monteiro et al. 1997).

In addition to their ecological importance, there are other obvious reasons for the apparent scientific popularity of *Actinia*. Individuals of this genus are small, they are exclusively occupants of hard substrata in the intertidal and shallow subtidal and commonly occur at high densities (Ottaway 1979a, 1980, Brace & Quicke 1986b). They are easily collected owing to their often conspicuous and almost sedentary nature (Rees 1984, Brace & Quicke 1986b) and in the laboratory they are simple to maintain, requiring far less husbandry than most marine invertebrates (Brenowitz 1972).

Systematics of *Actinia*

The genus *Actinia* is represented globally by about nine nominate species. Although some of these are poorly described and may be synonyms, other species are considered to be very polymorphic and may encompass groups of distinct species. Traditionally, discrimination between species in this genus is based on external morphological characteristics, such as the number of tentacles, the size and the colour of the column and foot, nematocyst type and distribution, and the type, colour, size and number of acrorhagi (special organs of defence that have abundant nematocysts and that are found on the top of the column, close to the tentacles). The species currently recognized are described below. Only the last three are

known to occur in the British Isles, although three others are described from the shores of continental Europe.

Actinia bermudensis *McMurrich 1889*

A. bermudensis is a tropical sea anemone of the west Atlantic and Caribbean, recorded from the shores of Florida southwards to southern Brazil. It may sexually reproduce a planktonic larva (Jennison 1983), but it is also documented as reproducing asexually through the production of brooded young (Russo et al. 1994, Monteiro et al. 1998). As a species it shows phenotypic variability with red, brown and yellow column morphs having been described (Russo et al. 1994). Using enzyme electrophoresis, Russo et al. (1994) report that at one location, Florianopolis (Brazil), populations of red and brown morphs were genetically different enough to suggest that the two groups were not the same species. Electrophoretic comparison of enzymes has shown *A. bermudensis* from Brazil to be genetically distinct (i.e. a separate species) from *Actinia* from Britain (*A. equina*, *A. prasina* and *A. fragacea*) (Solé-Cava et al. 1994b).

Actinia cari *Delle Chiaje 1825*

This is a Mediterranean species, which, like other *Actinia*, has a history of taxonomic revision, being seen as a separate species by most workers, often under a different species name (Risso 1826, Gravenhorst 1831, Andres 1884, Carlgren 1949). Other biologists have viewed the form merely as a variety of *A. equina* (Pax 1907). *A. cari* typically has a greenish brown column, on which there are 28 unevenly distributed brownish blue to brown black rings which are often open or can blend. The pedal disc of this species is said to be 5.5 cm in diameter and brown to greenish brown in colour. *A. cari* shows variability in the colour and form of the rings, leading Andres (1884) to detail several varieties. The sexes are separate in this species, the female is oviparous with no record of individuals brooding young internally (Schmidt 1972).

Actinia sali *Monteiro et al. 1997*

A. sali was described by Monteiro et al. (1997) from specimens collected on the Cape Verde Island of Sal (off the west coast of Africa). It is apparently morphologically indistinguishable from red footed red column morphs of *A. equina* from elsewhere, but it is genetically very divergent and may show small nematocyst differences (see Monteiro et al. 1997). It is known to brood small anemones in the enteron and these are presumably produced asexually. Distribution is unknown other than that it occurs on Sal, but presumably it may occur also on other islands in the Cape Verde group and possibly further afield.

Actinia schmidti *Monteiro et al. 1997*

This species was recently described by Monteiro et al. (1997) following molecular work on samples collected from the coast of the Mediterranean Sea near Marseille. It is a large

species (mean pedal disc diameter 3.7 cm), with red column, tentacles and pedal disc, blue acrorhagi and a blue rim at the edge of the pedal disc (limbus). In this species there is a clear nematocyst difference from most other *Actinia* in that the microbasic B-mastigophores of the mesenteric filaments are smaller than the basitrichs of the actinopharynx (see Monteiro et al. 1997; also Chintiroglou & Simsiridou 1997). The species is unusual because as far as is known it never broods young in the enteron and hence may not reproduce asexually (cf. *A. fragacea*). *A. schmidti* appears to be indistinguishable from, and is probably conspecific with, the *A. equina mediterranea* I of Schmidt (1971, 1972). If so, its distribution probably includes most of the Mediterranean and the Atlantic coast as far north as northern Portugal.

Actinia striata *Rizzi 1907*

This is another Mediterranean species and has a red-brown column on which there are superimposed numerous continuous and broken dark brown-red vertical stripes (Schmidt 1972). The pedal disc of this species is approximately 6 cm and opaque red-brown with visible mesenterial insertions. The sexes are separate and reproduction is by viviparity. Schmidt (1972) notes that most of these forms and species of Mediterranean *Actinia* show diagnostic differences in their cnidae (nematocyst complements) and have differing littoral and large-scale distributions.

Actinia tenebrosa *Farquhar 1898*

A. tenebrosa is a very common anemone on New Zealand and Australian shores (Ottaway 1979b, Ayre 1983b). This species is dark red-brown in column colour (Dakin 1952) with no apparent documented evidence for phenotypic variation. Enzyme electrophoretic studies of *A. tenebrosa* report high levels of genetic variation within and between some populations (Ayre 1984a, Ayre et al. 1991), but have offered no evidence for cryptic speciation. A genetic comparison of Australian *A. tenebrosa* populations with *A. equina* populations from South African shores, has confirmed that they are distinct species (Ayre 1984a).

Actinia fragacea *Tugwell 1856*

The "strawberry" anemone, so called because of its regular yellow or green spotting on a red column was, until recently, regarded as being a morph of *A. equina* (e.g. Gosse 1860, Schmidt 1971). Stephenson (1935) agreed with Gosse in grouping all *Actinia* occurring in Britain under the name *A. equina*. He mentioned that he considered the "strawberry" morph to be a possible separate species, but he cited it merely as *A. equina* var. *fragacea*.

Although, because of its larger size and the lack of viviparity, var. *fragacea* was recognized to be conspicuously different from other varieties of *A. equina*, its systematic position remained unchallenged until 1981, when Carter & Thorpe demonstrated, through the use of allozyme electrophoresis, that sympatric samples of the strawberry and the beadlet anemone (from Wembury, south Devon, UK), were consistently genetically different and were, therefore, reproductively isolated. Genetic identity (Nei 1972) between *A. fragacea* and *A. equina* was estimated as 0.726, a value usually associated to interspecific comparisons (see Thorpe 1979, 1982) and therefore further supporting the hypothesis of the distinctness of the two species. This was the first use of molecular systematics in the genus *Actinia*, and it started a

series of discoveries on the fine genetic differentiation between many of the so-called varieties of *A. equina*. Perrin (1993) re-affirmed the specific status of *A. fragacea* through genetic comparisons with a number of *Actinia* morphs and produced identity values of 0.50, 0.85 and 0.87 between *A. fragacea* and the *A. equina* morphs, a range comparable with the value of 0.726 calculated by Carter & Thorpe (1981), although obtained over a slightly different mix of loci.

A. fragacea is probably nowhere abundant and its range is apparently restricted to certain rocky shores in southwest England (Devon and Cornwall) and northern France (Brittany Peninsula) (Manuel 1988).

Actinia prasina *Gosse 1860*

Gosse (1860) considered the "green colour morph" of the beadlet anemone to be conspecific with *A. equina*, a trend that was followed later by other sea anemone systematists (Stephenson 1935, Manuel 1981). However, Haylor et al. (1984) found significant differences between the gene frequencies of the green morph and sympatric individuals of the red morph of *A. equina*, at four out of 17 loci examined (*Est**, *Sod–2**, *Pgm**, *Odh**). This, together with other studies showing differences in internal morphology (Schmidt 1971), behaviour (Brace et al. 1979) and predation (Hall et al. 1982, 1984) led the authors to suggest that, for the Isle of Man populations at least, the green morph should be afforded specific status, suggesting the name *A. prasina* Gosse (1860). Subsequent work by Solé-Cava & Thorpe (1987) confirmed the differentiation between *A. prasina* and the red morph of *A. equina*.

Actinia equina *(Linnaeus 1758)*

This is the most studied species, the beadlet anemone. It is thought to be distributed from the coasts of north Russia (Kola Peninsula) to those of South Africa (Stephenson 1935, Branch & Branch 1981, Manuel 1988). It is very common on shores around the British Isles and western Europe and extends into the Mediterranean. *A. equina* is typically intertidal, occurring as high on the shore as mean high water neaps, although it may be found subtidally down to 20 m below sea level (Manuel 1988). In common with many sea anemone species, *A. equina* is highly variable in both colour and form throughout its geographical range from Europe (Schmidt 1972, Manuel 1988) to South Africa (C. Russo, pers. comm. 1998). This variability has led to much taxonomic debate. On shores in the British Isles the existence of various green, orange, brown, red and striped or spotted column forms has long been recognized, with early natural historians considering certain of these morphs to be separate species (Templeton 1836, Johnston 1847, Dalyell 1848, Cocks 1850, Tugwell 1856, Milne-Edwards 1857). Gosse (1860) considered all colour forms to be *A. equina*, but did detail and name 11 colour varieties. Stephenson (1935) considered only the "strawberry" morph (his var. *fragacea*) to be a possible separate species. All other forms, were considered by Stephenson to be *A. equina* var. *mesembryanthemum*. Generally the various forms of *A. "equina"* examined from Britain have not shown reliable nematocyst differences, but recent work indicates that there may be some useful nematocyst differences (Allcock et al. 1998).

A. equina also shows a large degree of polymorphism on the mainland shores of Europe and shores of North Africa. Taxonomic studies of these forms (Schmidt 1971, 1972) have involved only the more conventional morphological comparisons of cnidae, internal

structure, reproductive physiology and general body form. Schmidt described several different subspecies and varieties of *A. equina*.

1. *Actinia equina equina*, is considered by Schmidt to be identical to the type of the species *A. equina* of Linnaeus (1758). Within *A. equina equina* he recognizes a "brown-red" column form I, a "green" column form II and a "crimson to purple red" column form III, all of which have differing mesenterial arrangements. These forms are all viviparous and are described from the coastline of Britain and the northwest mainland of Europe.

2. *Actinia equina atlantica*, which can be found from Arcachon, France, to northwest Spain and in the Azores, exhibits two distinct forms: *A. equina atlantica* I has a red column, is the larger of the two forms (Schmidt does not detail size) and is oviparous. *A. equina atlantica* II has a red or green column and is viviparous. Schmidt (1971) notes that *A. equina atlantica* form I is found lower on the shore than form II.

3. *Actinia equina mediterranea* also has two forms: form I is relatively large (pedal disc diameter 7.5 cm), dioecious and oviparous, with a red column and a red pedal disc. Its distribution is limited essentially to the Mediterranean, but Schmidt reports finding this form also in northern Portugal. *A. equina mediterranea* form II is smaller than form I (pedal disc size 3 cm), is dioecious and oviparous, has a brown-red column with a greenish "shimmer" and a light red pedal disc. Outside of the Mediterranean Schmidt found this form on the northern coastline of Spain and in the Canary Islands. As noted above, it is likely that Schmidt's *A. equina mediterranea* form I is conspecific with the species *A. schmidti*, recently described by Monteiro et al. (1997).
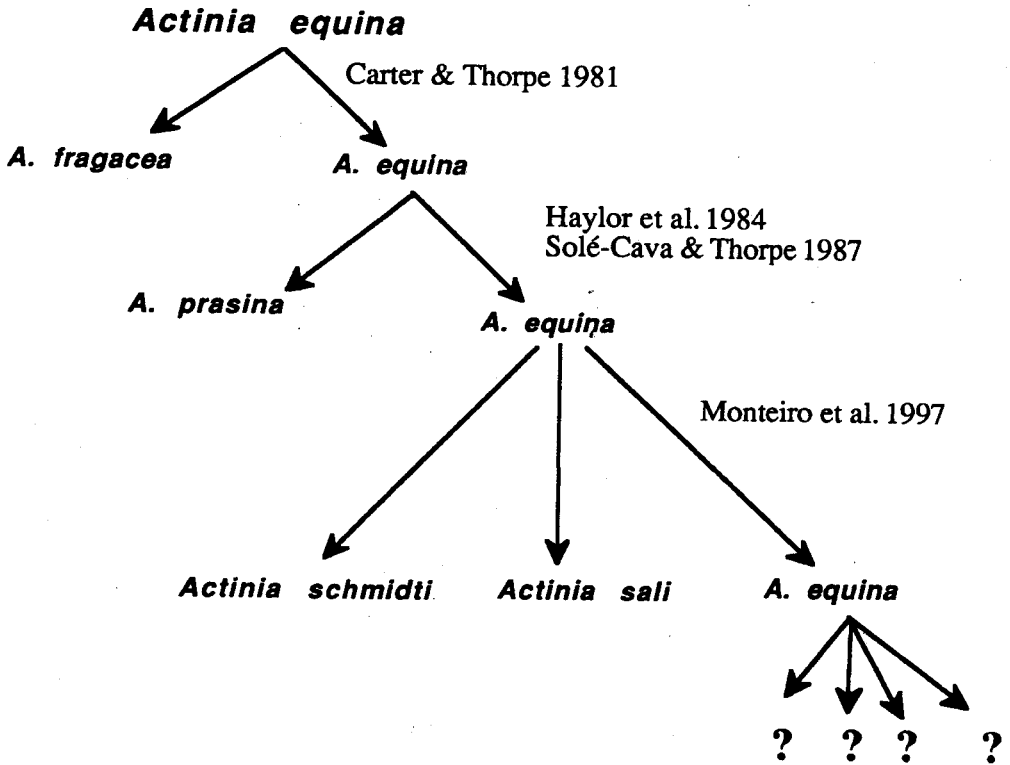

Figure 1 Diagram to illustrate the main taxonomic "splits" following genetic studies of population structure in *Actinia "equina"*.

To date there is only one published study examining the genetic relatedness of populations of *A. equina* across its broader range. This is the work of Monteiro et al. (1997), who compared genetic divergence between samples of red *Actinia* from the Isle of Man, the Mediterranean and the Cape Verde Islands. These all showed large-scale genetic differences and consequently the last two populations were considered to be separate species (*A. schmidti* and *A. sali*; see above). This work combined with reports of morphological variability and the discovery of genetically different colour forms or cryptic speciation at some localities in Britain (e.g. Carter & Thorpe 1981, Quicke & Brace 1983, Quicke et al. 1983, 1985, Haylor et al. 1984, Solé-Cava & Thorpe 1987, 1992, Solé-Cava et al. 1994b) lead to doubts as to whether *A. equina* is likely to constitute a single species across its entire range (Fig. 1). The extensive cryptic speciation within *A. equina* (and also possibly in *A. bermudensis*, Russo et al. 1994) suggests that the many "colour morphs" may conceal further cryptic species.

Population genetics of *Actinia* in the British Isles

The application of enzyme electrophoresis to the study of sea anemones has had a significant impact on the field of taxonomy and systematics (McCommas & Lester 1980, Carter & Thorpe 1981, Bucklin & Hedgecock 1982, Haylor et al. 1984, Quicke & Brace 1983, Solé-Cava et al. 1985, Smith & Potts 1987, Solé-Cava & Thorpe 1992, McFadden et al. 1997, Monteiro et al. 1997). It has also been important in studies of factors influencing levels of genetic polymorphism (Hoffman 1983, Solé-Cava & Thorpe 1989, 1991, Zamer & Hoffman 1989), in investigations of population structure (Black & Johnson 1979, Ayre 1983a, 1984a, Hoffman 1987, Hunt & Ayre 1989, Ayre et al. 1991, Russo et al. 1994) and in the study of breeding systems (Ottaway & Kirby 1975, Black & Johnson 1979, Carter & Thorp 1979, Orr et al. 1982, Bucklin et al. 1984, Shaw et al. 1987, Monteiro et al., 1998).

Column and foot colour

Evidence for a relationship between the phenotypic diversity of *A. equina* and genotype was first produced by Quicke & Brace (1983). In the course of examining the phenotypic and genotypic spacing of a group of individuals at one site (Burniston, North Yorkshire) they reported associations between pedal disc (foot) colour and allele frequency at two loci. This association was used to describe two distinct morphs within the red-brown column coloured individuals of the species (Quicke et al. 1983). One of the morphs described had a pink or red pedal disc and was considered to differ in enzyme genotype from the other morph group which contained individuals with green (H), grey (G) or radially striped (L) pedal discs. Linkage between alleles at two enzyme loci was also suggested.

Quicke et al. (1983) further reported some potentially important ecological differences between morphs. Transect data showed that at two British shores (Burniston, North Yorkshire and Trevone, Cornwall), anemones with red or pink pedal discs were more common on upper mid-shore areas while anemones with green or grey pedal discs were more abundant on the low shore. Furthermore, data collected from one site showed that anemones with red or pink pedal discs were more common on vertical rock surfaces and were attached more strongly to the substratum than the other morph which was found more commonly on horizontal surfaces and was generally weakly attached. The authors put forward the theory of

two co-adapted gene complexes maintained by selection and involving the two enzyme loci and pedal disc colour.

Further transect surveys on two shores (Trevone, Cornwall and Burniston, North Yorkshire), combined with electrophoretic investigations of genotypes, suggested distributional differences for three groups of *Actinia* morphs (Quicke et al. 1985). Individuals with red or pink pedal discs were most common on the upper to midshore. These individuals were termed upper (U) shore morphs and differed genetically from the mid-shore (M) morph which had red or pink pedal discs and was relatively more abundant at the lower mid-shore level. The low (L) shore morph was also described as being genetically distinct and had a green, grey or lined pedal disc. It was suggested that the genetic differences between the upper and lower shore morphs and the mid-shore "hybrid" are maintained by selective forces varying with shore level and acting on linked loci.

Donoghue et al. (1985), studying anemones from Burniston, North Yorkshire, found significant differences in genotype frequencies between morphs which they termed red (Rd) and pink (P) pedal disk morphs. They also reported differences in the form of the acrorhagi between *Actinia* possessing different coloured pedal discs. Their examinations of acrorhagial form considered four parameters (type, size, colour, and number) in six pedal disc colour groups termed: dark red (DRd); light red (LRd); pink (P); grey (G); green (H); and grey or green with red or orange radiating lines (L). Comparisons of acrorhagial type between groups revealed differences between DRd pedal disc individuals and all other morphs, with the first possessing a higher proportion of "compound" acrorhagi than the other groups. Significant differences in size of acrorhagi were found between some groups. DRd pedal disc anemones had significantly larger acrorhagi than the other morphs. The colour of the acrorhagi was considered to separate the anemones into three groups: DRd, LRd and P, and G, H and L. Anemones with DRd pedal discs had on average significantly more acrorhagi than individuals of any of the other pedal disc colour groups. Thus Quicke, Brace and their colleagues (e.g. Quicke et al. 1983, 1985, Quicke & Brace 1983, 1984, Donoghue et al. 1985) working with *Actinia* they described as having red or brown columns, distinguished various groups of column and pedal disc colour morphs.

More recently our own and other work (Lynch 1996) at Port Erin has also provided evidence of three apparent gene pools in *A. equina*. Results to date suggest that both the morphology and genotypes at key loci of the anemones constituting these gene pools are similar from shore to shore. Thus, there is some evidence of morphs, characterized by particular combinations of genotype and phenotype, being consistent over a geographical range. Although interpretations of the genetic differences do not entirely concur, the morphs concerned appear partially similar to those differentiated originally by Quicke, Brace and co-workers (see e.g. Quicke et al. 1983, Quicke & Brace 1984). However, we doubt whether the three "morphs" are maintained solely by selection. This work is currently continuing.

Origins of the brooded offspring in Actinia

Several early studies of *A. equina* document viviparity and brooding in this species (Dalyell 1848, Gosse 1860, Gravier 1916). The brooded young of *A. equina* were considered of sexual origin primarily because of the presence of separate adult male and female individuals in the population (Stephenson 1929). Young anemones at several developmental stages are found in the enteron (gastric cavity) of the parent individual. The youngest form is the pre-planula larva which is merely a bundle of ciliated cells and is often seen in the tentacles of adults

(Carter & Funnell 1980). It is assumed that these larvae then become planulae which are approximately 300×200 μm (Chia & Rostron 1970). In metamorphosis of the planulae the development of tentacle buds is seen first followed by the development of a pedal disc. Tentaculate young may be brooded up to 10 mm in pedal disc diameter (Rees 1984), a size well above that of the smallest free living brooding adults (M. R. Brown & J. P. Thorpe, in prep.). The colour of brooded young has generally been observed to be the same as that of the parent (e.g. Cain 1974, Carter & Thorp 1979, Gashout & Ormond 1979).

Given the early hypothesis of sexual reproduction, it seemed peculiar that *A. equina*, which shows such a large degree of colour polymorphism (Gosse 1860, Stephenson 1935), should show such consistency in overall brood phenotype. Cain (1974) recognized this discrepancy and offered four possible explanations for individuals brooding coloured offspring of the same colour as the parent.

1. Each colour variety may in fact be a separate species.
2. Planula larvae may return from the plankton to enter only the adults of their own colour.
3. The colour of juveniles is in some way controlled by the fostering adult.
4. Juveniles may be produced by asexual or parthenogenetic means.

The second of these suggestions formed the basis of a theory that was initially proposed by Gravier (1916). Chia & Rostron (1970) were also in support of this "re-entry" theory. Their support for this idea followed the failure to find pre-planula larval forms in the enterons of adult *Actinia*. They explained this absence as being due to larval release into the plankton after only short periods of incubation. In order to test the "re-entry" theory attempts have been made by several groups of workers to induce adult *Actinia* to accept juveniles into the enteron; these have all failed (Chia & Rostron 1970, Carter & Thorp 1979, Gashout & Ormond 1979).

Using enzyme electrophoresis Carter & Thorp (1979) discovered that brooded young were invariably of the same esterase genotype as their parent individual. This was in line with discoveries that the Australasian congener, *A. tenebrosa* also brooded offspring genetically identical to the parent (Ottaway & Kirby 1975, Black & Johnson 1979). Carter & Thorp (1979) suggested a modification of the larval re-entry theory. They proposed that given the ability of *Actinia* to recognize self and non-self, like genotype larvae could be selected from the plankton and subsequently be accepted into the enteron. Following this, they proposed that vegetative proliferation (cloning) of such juveniles leads to increased brood size.

At about the same time, Gashout & Ormond (1979) were developing the idea that brooded young might be of parthenogenetic origin (hypothesis 4 of Cain 1974). One obvious failing of this hypothesis was the inability to explain why all *A. equina* populations studied were dioic, with both sexes brooding young in their enteron (Chia & Rostron 1970, Rostron & Rostron 1978, Carter & Thorp 1979, Carter & Miles 1989). The same studies also reveal that *Actinia* populations contain a large proportion of non-sexual individuals (i.e. individuals that possess no gonads) which also commonly brood. (These observations would clearly preclude self-fertilization in the species). By way of explaining these patterns of brooding Gashout & Ormond, (1979) suggested phasic hermaphroditism. This in itself was not a new suggestion for the species (Carter & Thorp 1979) and its existence has been implied for another actinian, *Epiactis prolifera*, by Dunn (1975). Interestingly, Gashout & Ormond (1979) rejected the possibility of somatic embryogenesis for the production of brooded young because attempts to induce tissue fragments to metamorphose into juveniles had been unsuccessful. Somatic embryogenesis as the mode of juvenile production was, however, supported by Orr et al. (1982). Using enzyme electrophoresis to analyze samples from the Isle of Man, those authors showed that broods shared the same genotype as their parents across all of the

five polymorphic loci investigated. Parents heterozygous for any of these loci brooded similarly heterozygous young. If the young had been produced by self-fertilization or inbreeding a degree of segregation would be expected in their genotypes. To date there is only the indirect evidence of apparently functional gonads (Larkman 1980, Larkman & Carter 1980, 1982, 1984, Carter & Miles 1989) and the detection of substantial amounts of genetic variation in populations (Solé-Cava & Thorpe 1992, Perrin 1993) to support the existence of any sexual reproduction in *Actinia equina*. A mixed mode reproductive strategy cannot be ruled out in this species (see below for further discussion of possible sexual reproduction).

Under the preconception that the brooded young of *Actinia* were sexually derived, studies concerning the reproductive ecology of *A. equina* have documented levels of brooding and sexual condition in an attempt to relate these two reproductive parameters (see e.g. Rostron & Rostron 1978, Gashout & Ormond 1979). Available data indicate that the brooding of juveniles in the enteron is a year round phenomenon, although peaks in brooding and gametogenic cycles have been identified at differing times of the year for differing populations. The first study of its kind by Chia & Rostron (1970) reported higher levels of brooding (as percentage of individuals brooding) during winter months in two populations on the English east coast. Gonads in individuals from these populations were seen to regress during November, December and January. Additional data from one of these locations (Broadstairs, Kent) provided by Rostron & Rostron (1978) suggests that gonads may also regress towards the end of the summer months, re-developing again through September and October. A study of a population in the south of England revealed brooding peaks (as mean number of brooding adults) in two successive August samples, but otherwise there was no detectable seasonality in brooding (Carter & Thorp 1979). In a North Yorkshire *Actinia* population, Gashout & Ormond (1979) found main brooding peaks (as percentage of individuals brooding and mean brood size) in May, June and July over the period 1977–78. The percentage of individuals bearing gonads appeared to peak around two periods, March to April and September to October. Rees (1984) reported the highest levels of brooding in early summer months for populations at Burniston, North Yorkshire and Rhossili, southwest Wales.

A critical evaluation of gametogenesis is provided by Carter & Miles (1989). Results from this study suggest that oogenesis and spermatogenesis are synchronized, occurring over the months of February to May before subsequent shedding of gametes during the summer. A pattern of spawning in the warmer months of the year appears to be a common feature in littoral species of sea anemone (Dunn 1975, Jennison 1978, Ottaway 1979b, Bucklin 1980, Schaefer 1981, Shaw et al. 1987).

Very few studies of *A. equina* have attempted to relate fecundity or gametogenic cycles to changes in environmental conditions, although the study of the Broadstairs population (Chia & Rostron 1970, Rostron & Rostron 1978) does highlight an inverse relationship between sea temperature and levels of brooding. It may also be of note that the higher proportions of individuals bearing gonads in spring and autumn is coincident with plankton blooms in the area (Colebrook & Robinson 1965), suggesting that food availability may influence breeding. Other environmental conditions that may effect levels of brooding in *A. equina*, include tidal height and exposure which have been shown to be negatively correlated with brood size and with frequency of brooding for populations studied in the Isle of Man (M. R. Brown & J. P. Thorpe, in prep.). This study also found marked differences in both frequency of brooding and mean brood size between *A. prasina* and several morphs of *A. equina* (with both being highest in *A. prasina*), but there was no apparent relationship between either of these parameters and estimates of population density or nearest neighbour distance for the brooding adult.

Biologists studying the reproductive ecology of *A. equina* have been almost entirely preoccupied by the investigations of natural populations, with little attention having been directed towards laboratory-based mating experiments. One inherent problem with such experiments is that sex in *A. equina* can only be assessed by histological examination of the gonads. In the course of laboratory-based investigations of gametogenic cycles, anemone isolation, and pairing experiments Carter & Miles (1989) successfully used a biopsy technique to remove gonad material for histological inspection and were able to identify the sex of experimental animals. Sex determination mechanisms are unknown in *A. equina*, but are thought to be genetic in *A. tenebrosa* (Ayre 1988). Carter & Miles (1989) found that males kept in isolation or paired with females released sperm during spring and summer months. In addition, isolated males, females and non-sexual individuals were observed to produce and brood young. Results from this study suggest that sex is stable in the species. Observations of females producing broods shortly after contact with males that had released sperm, in conjunction with the finding that larger broods tended to be carried by females, led the authors to suggest that females may brood sexually derived young over the summer months. They do acknowledge, however, that, as a result of observation of non-sexual individuals producing young, some brooded offspring must be produced asexually. Subsequently, Perrin (1993) examined brooded offspring from several morphs at different times of the year and concluded that all of the large number of offspring he examined were reproduced asexually.

Cloned offspring are also known to be similarly reared in the enteron in the Australian *A. tenebrosa* (Ottaway & Kirby 1975, Black & Johnson 1979) and in Brazilian populations of *A. bermudensis* (Russo et al. 1994, Monteiro et al., in press), but apparently do not occur in *A. fragacea* (Carter & Thorpe 1981, Larkman 1983, 1984a,b) or in *A. schmidti* (Monteiro et al. 1997). They also apparently occur (Perrin 1993) in the related species *Bunodactis verrucosa*. In some European populations of *Actinia equina* it appears that some females may reproduce asexually also through the parthenogenetic development of unfertilized eggs (Schaefer 1981).

In *Actinia* the relative advantages of asexual reproduction are difficult to assess because, for such a well studied species, remarkably little is known of its sexual reproduction. As yet no species of *Actinia* has been found to brood sexually reproduced offspring. It is clear that sexual reproduction must occur in *A. equina*, because at any time of the year (but particularly in summer, Carter & Miles 1989) and in any population some individuals can be seen (in histological sections) to have male or female gonads in the mesenteries, although in many individuals (often most) gonads are apparently absent (see Carter & Thorpe 1981, Carter & Miles 1989). Possible advantages of internal brooding of asexually reproduced juveniles are the maintenance and amplification of co-adapted genotypes (see, e.g. Hughes 1989) and for the brooded young better conditions with the elimination of competition from other invertebrates for space or other resources. The asexually reproduced young are released at an advanced stage of development and growth, ready to settle onto the substratum. Ottaway (1979b) has shown that in *A. tenebrosa* the size of the brooded offspring at release significantly affects the chance of survival. When compared with the microscopic anemones which could be produced following the settlement of a putative larva the far greater initial size must give the brooded cloned offspring a huge survival advantage in the very stressful intertidal environments that the species occupies. Hence, asexual reproduction may be particularly advantageous in *Actinia*, thus possibly explaining the widespread incidence of internal brooding within the genus.

Further evidence that sexual reproduction does occur is that populations generally show high levels of genetic variability and allele frequencies approximate to Hardy-Weinberg

expectations (e.g. Solé-Cava & Thorpe 1992). The few studies of the timing of asexual reproduction have led to contradictory conclusions (see above), but the more recent data from the major study of Carter & Miles (1989) show evidence of seasonality in both brooding and in the production of gonads. The monitoring of recruitment on the shore appears unlikely to shed much light on the problems of understanding reproductive activity because currently available data (authors' unpublished results) suggest that longevity is very high (possibly centuries) and both recruitment and natural mortality are minimal (in some populations both recruitment and loss of "adult" sized anemones are possibly under 1% a year).

Dispersal and gene flow

Adult *Actinia* are essentially non-dispersive and, as it is difficult to envisage cross-fertilization occurring over other than very short distances, there is likely to be little gene flow between shores unless larval dispersal occurs. The generally high levels of genetic divergence between allopatric populations of most morphs of *Actinia*, even when sampled over only short geographical distances, indicate that genetic differentiation in *Actinia* takes place on a very small geographical scale (Solé-Cava & Thorpe 1992) and, thus, the likelihood of larval dispersal is, at best, very low.

The reason that dispersal is low could be simply that *Actinia* may not produce a dispersive larva. The brooded offspring in the enteron usually can be seen to be present in various stages of development from small balls of cells, to tiny "embryo" anemones with tentacle buds, to various sizes of juvenile anemones commonly up to about 6 mm high. Various ciliated balls of cells found in the enteron approximate planulae; some look convincingly like the planula larvae of other anemone species and were assumed by Chia & Rostron (1970) to be sexually produced. Whether any of these are sexually reproduced larvae, which may be subsequently released and disperse, or whether they are all merely developmental stages in the asexually produced brooded offspring is not clear. In any case, the "planula larvae" found in the enteron do not seem to be capable of much dispersal, since rearing experiments (Chia & Rostron 1970, Perrin 1993) indicate that outside the enteron they are incapable of metamorphosis or of long-term survival.

Problems of the definition of species in *Actinia*

Before discussing the process of speciation or the existence of cryptic species in sea anemones, it is pertinent to outline what is meant by the term species. By definition some current concepts may not be applicable to *Actinia* and other similar species of sea anemone. Population geneticists and ecologists have generally embraced what is known as the biological concept of species (e.g. Lewontin 1974, Endler 1977, Nei 1987). There are currently several widely accepted forms of biological concept, the first of which is commonly termed simply "The Biological Species Concept", but is more accurately known as the isolation species concept (Paterson 1985). Under the isolation species concept, Mayr (1963) described species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". This is the most widely used concept in evolutionary

literature. The recognition species concept (Paterson 1981, 1982, 1985), however, views the isolating mechanisms of the isolation species concept as mechanisms facilitating reproduction among members of a given population. This concept defines the species as "the most inclusive population of individual biparental organisms which share a common fertilization system" (Paterson 1985). Neither the recognition nor the isolation species concept is considered applicable to asexually reproducing organisms or syngameons (units of interbreeding in a hybridizing group of species).

According to the evolutionary species concept, a species is a population or group of populations that shares a common evolutionary fate through time. This concept is applicable to both living and extinct groups and to sexual and non-sexual organisms and is, for practical purposes, the species definition used by most practising taxonomists and palaeontologists. Independent species status is usually attributed on the basis of patterns of phenotypic cohesion within, and discontinuity between, groups of organisms. The failures of this concept are that it does not set out clearly which phenotypic traits are important in defining species, and that it is vague in its judgment of just how common a "common evolutionary fate" should be. In addition, this concept works with the end product of cohesion but does not address the evolutionary mechanisms that result in cohesion.

Templeton (1989), in an attempt to define a species concept that can be applied to all organisms, has defined the cohesion species concept. Through this concept he defines a species as "the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms". The cohesive mechanisms to which he alludes can be classified as genetic exchangeability: "the factors that define the limits of spread of new genetic variants through gene flow", and demographic exchangeability: "the factors that define the fundamental niche and the limits of new genetic variants through genetic drift and natural selection". The inclusion of considerations of demographic exchangeability makes this concept applicable to asexual organisms. The factors Templeton identifies under his two cohesion mechanisms are too numerous to discuss here, but the summary table in his paper (Templeton 1989) presents an overview.

Ascribing specific status to colour morphs of *A. equina* using current species concepts may be a difficult task for a number of reasons. Any investigation of the genetics of *A. equina* colour morphs will rely to an extent on the pooling of similar colour morphs. Where concerted efforts are made to collect and use near identical or identical morphs it is likely that rarer morphs will be neglected. Thus, while an understanding of the genetic relationship between more common forms may be arrived at, this may be at the expense of understanding the genetics of the group as a whole. Even in instances where near identical morphs are used in investigations the possibility remains that cryptic species may exist within such well defined groups.

Species concepts that are based to any degree on phenotypic cohesion may falter when used to describe species of *Actinia* because of, first, the high phenotypic variability of *Actinia* within shores and, secondly, the similarity of colour morphs from different shores which can show high levels of genetic divergence (Solé-Cava & Thorpe 1992) that in instances would imply separate specific status.

A. equina may be considered reproductively "awkward". This species can reproduce clonally (Carter & Thorp 1979, Orr et al. 1982, Perrin 1993), is dioecious, and is presumed to be able to reproduce sexually (Carter & Miles 1989). *A. equina* may be considered (see below) to challenge the isolation and recognition species concepts through engaging in both "too little" and "too much" sex (see Templeton 1989).

As detailed above there is, as yet, no direct evidence for successful sexual reproduction in *A. equina*. If non-sexual *Actinia* never develop gonads and produce brooded young which are also non-sexual for the whole of their lives, then it is possible to envisage clonal races of *Actinia* that take their own evolutionary path with specialization occurring via mutation and selection. Some populations of the sea anemone *Haliplanella luciae* are thought to be obligately clonal (Shick & Lamb 1977), although this species appears to possess functional gonads. The application of isolation or recognition species concepts to obligately clonal races of anemones is not possible, although these races do constitute separate species under definitions of the evolutionary and Templeton's cohesion species concepts (Templeton 1989).

The possible existence of hybrids (the middle shore morph of Quicke et al. 1985) within *Actinia* on British shores could also present problems for the future definition of species in the group. If the putative middle shore morph is indeed a hybrid of the upper and lower shore morphs (Quicke et al. 1985), it is pertinent to ask whether hybridization between the two ancestral forms is current (as proposed by Quicke et al. 1985) or whether this morph is the result of past hybridization events.

With the increased understanding of the genetics and reproduction of *A. equina*, the use of the conventional biological species concepts to define species in the group may not be viable. As highlighted above, any concept relying on phenotypic cohesion as a means of defining species in *Actinia* will face the problems of cryptic speciation or allopatric speciation of like colour morphs. Of the concepts defined above, the cohesion species concept outlined by Templeton (1989) may present the most useful approach to describing species in the group. The strength of this concept in defining species in *Actinia*, given the concerns over 'too much' and 'too little' sex in the group, lies with the relative importance that this concept places on demographic rather than genetic exchangeability for asexual taxa and syngameons.

Our understanding of the genetics of *A. equina*, at both the individual and population level, remains in its infancy. Added to this are the gaps in our knowledge surrounding reproduction in the group. Despite the obvious and potential flaws exposed for the reproductive and isolation species concepts, at present these serve to crudely describe species in what is taxonomically a generally poorly understood genus.

Possible mechanisms of speciation in *Actinia*

The increasing number of studies reporting genetic differentiation within sea anemone species suggest that cryptic species may be common within the group (McCommas & Lester 1980, Carter & Thorpe 1981, Bucklin & Hedgecock 1982, Haylor et al. 1984, Solé-Cava et al. 1985, Shaw et al. 1987, Solé-Cava & Thorpe 1987, 1992, Shaw 1988, Perrin 1993, McFadden et al. 1997, Monteiro et al. 1997). Considerations of the life history and life styles of *A. equina* suggest a number of possibilities for mechanisms of speciation.

Allopatric species may have arisen in *A. equina* through "founder effect speciation". Here, geographically isolated populations are thought to be established by a very small number of founder individuals, and subsequently grow through the localized recruitment of clonally produced young. This "genotype amplification effect" (Ayre 1984a, Bucklin 1985), combined with limited dispersal between populations, may consequently lead to divergence and speciation. Stochastic events, e.g. storm disruption of boulder fields (Ottaway 1979a)

or climatic extremes (Crisp 1964), may cause bottlenecks in small populations of *Actinia*, enhancing rates of divergence.

The high genetic variability of *A. equina*, and of sea anemones in general, may facilitate speciation in these groups (Solé-Cava & Thorpe 1992). There is evidence to suggest a positive relationship between the heterozygosity of loci and their rates of divergence (Skibinski & Ward 1981, Ward & Skibinski 1985) and consequently species with high genetic variability, such as sea anemones (Solé-Cava & Thorpe 1991, Perrin 1993), may evolve more rapidly than less polymorphic species.

Hybridization in nature is a widely recognized and investigated phenomenon among plants (Stebbins 1950) and occurs in some animals such as marine molluscs (e.g. Skibinski et al. 1978), *Drosophila* (e.g. Bock 1980), fish (e.g. Brassington & Ferguson 1976, Child & Solomon 1977), and mammals (e.g. Hutchison et al. 1974, Serov et al. 1978). The application of enzyme electrophoresis to the investigation of animal and plant populations has made identifying hybrid individuals easier than reliance on purely morphological criteria (e.g. Littlejohn et al. 1971, Moran et al. 1980). Where different alleles are present at a locus in each parent the hybrid will exhibit a "heterozygote" type banding pattern. Results from the use of electrophoresis in the study of sea anemones suggest that past hybridization may have been an important speciating mechanism in this group (Quicke et al. 1985, Shaw et al. 1987, Perrin 1993). The existence of a hybrid form of *Actinia* on British shores seems likely with evidence to suggest that the ancestral and hybrid forms may currently coexist (Quicke et al. 1985, Perrin 1993), but in our opinion the hybridization, if it has occurred, has done so at some time during the past evolutionary history of the species. Quicke et al. (1985) considered that the differences between upper and lower shore morphs and the putative mid-shore hybrids resulted from, and were maintained by, selection acting on linked gene loci. The possibility that limited amounts of gene flow may be mediated through backcrossing with ancestral morphs (introgression) should also be considered, although the suggestion that differences in morphology and in alleles at various enzyme loci ("co-adapted gene complexes") between lower and upper shore morphs and in the mid-shore "hybrids" are maintained by selection seems intuitively improbable. More geographically wide-ranging studies should determine whether there is a distinct hybrid zone in this group or whether the hybrid form exists out of the range of one or both of its ancestors.

Shaw et al. (1987) suggest that the actinian *Sagartia ornata* is of hybrid origin. They identify *S. troglodytes* (formerly *S. troglodytes* var. *decorata*) as being one ancestral "parent" to *S. ornata*, and report finding both species in sympatry at certain locations. Shaw et al. (1987), however, failed to identify the other ancestor in the populations they examined, leading them to suggest that it may be a geographically distant or a now extinct species. Furthermore, the authors report that *S. ornata* is polyploid. An alternative explanation to hybridization leading to fixed and near fixed heterozygosity (in the absence of two parental forms) is that autoploidy in *S. troglodytes* gave rise to the tetraploid *S. ornata*. The fixed heterozygotes seen in this species would therefore be the result of divergence of alleles in the two genomes.

Evidence for historical duplication of genetic material in the Actiniidae is seen in the existence of duplicate loci in most species of actiniid sea anemones investigated by enzyme electrophoresis (e.g. McCommas & Lester 1980, Carter & Thorpe 1981, Bucklin & Hedgecock 1982, Bucklin et al. 1984, Ayre et al. 1991, Solé-Cava & Thorpe 1992, Russo et al. 1994). These studies in particular report the existence of two loci for both PGI and MDH in the species studied. Whether the existence of these multiple loci has resulted from duplication of restricted portions of the genome or from polyploidy remains unclear.

Actinia equina as a model species

The "strawberry-coral" model of Williams (1975) predicts that in organisms which possess the ability to reproduce both asexually and sexually, asexual reproduction acts to preserve locally adapted multi-locus genotypes, whereas the maintenance of sex is used to produce widely dispersed, genotypically diverse colonists. Furthermore, the model predicts intense competition among genotypically distinct colonists and that the area occupied by a clone will show an inverse relationship with habitat heterogeneity. Intertidal sea anemone species have become increasingly important as test organisms for this theory (e.g. Ayre 1982, 1983a, 1984a,b, 1985, Fujii 1987, Shaw 1988, Ayre et al. 1991). In particular, studies involving *Actinia tenebrosa* have shown this species to be highly conformative to the predictions of the model (Ottaway 1979b, Ayre 1982, 1983a, 1984b, Ayre et al. 1991). In common with this congener, *A. equina* is believed to employ both asexual (Carter & Thorp 1979, Orr et al. 1982, Perrin 1993) and sexual reproductive modes (Carter & Miles 1989). Competition for space via aggression is also demonstrated by *A. equina* (Brace & Pavey 1978, Brace et al. 1979, Rees 1984). Like *A. tenebrosa*, *A. equina* is found distributed through a range of microhabitats (Ayre 1983a, Haylor et al. 1984, Manuel 1988) some of which, e.g. boulder fields, are markedly heterogeneous. Despite these attributes, explicit attempts to relate the life history and population genetics of *A. equina* to the strawberry-coral model have not been made. Significant genetic differences between neighbouring populations of like morphs (Solé-Cava & Thorpe 1992) may indicate that larval dispersal and consequently panmixis are limited in this species. However, in the absence of any detailed genetic investigation of disparate populations of *A. equina*, the extent of gene flow and the role that sex plays in this process remain poorly understood. Localized clonal grouping, predicted by the strawberry-coral model, has been found for *A. tenebrosa* (Ayre 1983a, 1984a), but as yet has not been identified in *A. equina* (e.g. Quicke & Brace 1983).

Aspects of the behaviour as well as the various gene pools of *A. equina* on shores in the British Isles may create problems in investigations designed to apply this model to the species. The existence of cryptic species within *A. "equina"* (Haylor et al. 1984, Quicke et al. 1985, Solé-Cava & Thorpe 1992, Perrin 1993) presents the most obvious difficulty. Investigative and manipulative studies of *A. tenebrosa* have been facilitated by the absence of taxonomic problems within the species and the subsequent fact that this species forms monospecific stands (e.g. Ayre 1985). In comparison, a large number of *A. equina* aggregations are likely to be composed of more than one genetically distinct morph or species.

Ayre (1985) used reciprocal transplant experiments to confirm that clones of *A. tenebrosa* were locally adapted. In these experiments asexual fecundity and survival were used as indicators of relative fitness in transplanted and native colonies. Any similar investigations using *A. equina* must recognize the existence of genetic differentiation in the group in order to avoid highlighting possible species specific differences. This is particularly important given that life history characteristics (e.g. asexual fecundity) may vary between putative species irrespective of their location in the environment (see M. R. Brown & J. P. Thorpe in prep.).

Perrin (1993) provided estimates for genetic variability in *Actinia* colour morphs indicating markedly differing levels of heterozygosity between various morph groups. The genus *Actinia* on British shores would seem to present itself as an ideal group with which to examine the "niche-width" hypothesis (Van Valen 1965). This theory holds that there is a positive correlation between niche-breadth and the level of genetic diversity within an organism. This phenomenon has been reported to occur in a wide variety of organisms (e.g.

Beardmore 1961, Mackay 1980, Lavie & Nevo 1981, 1986, Lacy 1982, Noy et al. 1987), although its existence in some species is disputed (Schopf & Gooch 1971, Somero & Soule 1974, Solé-Cava & Thorpe 1991). If the niche-width hypothesis holds true the most genetically variable *Actinia* groups may be expected to have broader ecological niches. This may manifest itself as a wider vertical distribution or occupation of a greater variety of microhabitats. Alternatively, as found by Noy et al. (1987) for littorinid species, it may be that more heterozygous groups are found at higher shore levels where environmental fluctuations are greatest. A vertical distribution study conducted at Perwick Bay, Isle of Man (Perrin 1993) provided no clear evidence for a broader niche in relation to tidal height for any morph. In a study of three vertical transects (Quicke et al. 1985) only a red or pink pedal disc morph was found over the entire range of each transect, but the pooling of red and pink individuals in this and other studies is likely to be an oversimplification in sampling strategy, given that individuals of these groups show genetic differentiation at some locations (Quicke et al. 1985, Perrin 1993).

Suggestions for future work

At present there is limited information available as to the long-term stability of colour in individuals of *Actinia* (Gosse 1860, Elmhirst & Sharpe 1920). Such information, although elementary, would enable particular morphs or colour morph groups to be identified with greater confidence. Investigations should include both the field monitoring of different colour morphs and laboratory-based examination of colour stability under different environmental regimes.

The scope for further biochemical genetic investigation of *A. equina* populations in the British Isles remains great. To date populations from relatively few locations have been investigated and in general studies have used only small sample sizes. Within further studies of this nature the putative *Actinia* morphs of Quicke et al. (1985) and of Donoghue et al. (1985) need to be re-examined. The use of molecular genetic methodology could be helpful to approach some basic problems. The techniques of DNA fingerprinting and the random amplified polymorphic DNA (RAPD) analysis, for example could be used for the investigation of genetic identity, which would provide additional data about the origin of brooded individuals and an accurate recognition of clonal genotypes (Turner et al. 1990, Carvalho et al. 1991, Coffroth et al. 1992), and hybrid speciation (Hadrys et al. 1992). Single locus and multi loci polymerase chain reaction analysis would be an important tool to estimate levels of gene flow between populations (Bruford et al. 1992, Karl & Avise 1993), and DNA sequencing of nuclear or mitochondrial genes would help to formulate hypotheses about the phylogenetic relationships between the different species of the genus.

Of equal priority is the need for cytological studies of colour morphs of *A. equina*. Estimates of chromosome number, banding patterns and nucleolar organizing regions may shed light on whether the "fixed heterozygote" patterns observed in the group result from polyploidy and, thus, whether these should be interpreted as products of one, or more than one, locus.

If further investigation confirms the existence of "upper" and "lower" shore morphs in *A. equina* and these forms are shown to have differing ecologies (e.g distributional and behavioural), then additional attempts to find morphologically distinguishing characteristics should be made. Characteristics that allow the separation of these forms in the field would

be of greatest value to ecologists and may be found in further, more objective and critical attention to differences in column and pedal disc pigmentation and acrorhagial form (Donoghue et al. 1985).

The question of how many species exist within *A. equina* on British shores remains to be answered. The existing body of evidence points to *A. equina* being a species complex. While continued efforts are being made to fully understand the genetics of the "species", it would seem sensible that researchers whose studies involve *A. equina* should document to as fine a level as is practicable, the colour variation in individuals they encounter.

Acknowledgements

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