

SHORT NOTE

Biochemical Genetic Confirmation of the Asexual Reproduction of Brooded Offspring in the Sea Anemone *Actinia equina*

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ABSTRACT: The reproductive biology of the beadlet anemone *Actinia equina* L. has been a topic of considerable research interest in recent years. Several theories have been put forward to explain the origin of young anemones found brooded in the enteron of female, male and apparently asexual individuals, but to date, conclusive evidence has not been produced to substantiate any of these hypotheses. In this paper, evidence is presented – based on the results of an investigation of isozymes from adult *A. equina* and their broods – which demonstrates that the young are asexually produced.

The sea anemone *Actinia equina* L. is extremely common on rocky shores throughout Europe (e. g. Stephenson, 1935; Manuel, 1981). As a result of its abundance and ecological importance the species has been extensively studied (e. g. Fischer-Piette, 1932; Crisp and Southward, 1958; Chia and Rostron, 1970; Schmidt, 1971; Francis, 1973; Ottaway and Kirby, 1975; Edmunds et al., 1976; Jones et al., 1977; Brace and Pavey, 1978; Rostron and Rostron, 1978; Brace et al., 1979; Carter and Thorp, 1979; Gashout and Ormond, 1979; Carter and Thorpe, 1981) and remains a subject of considerable research interest. Because of its ecological importance, it is essential that the reproductive strategy of the species is fully understood but, to date, there is still great controversy concerning its mode of reproduction (c.f. Chia and Rostron, 1970; Cain, 1974; Rostron and Rostron, 1978; Carter and Thorp, 1979; Gashout and Ormond, 1979; Carter and Funnel, 1981).

It has long been known (Dalyell, 1848) that young anemones are brooded inside the enteron (gastric cavity) of an adult. More recent work (Chia and Rostron, 1970; Rostron and Rostron, 1978; Carter and Thorp, 1979; Gashout and Ormond, 1979) has shown that irrespective of sex, time of year or gonad condition, most adult *Actinia equina* will, under natural conditions, contain young anemones.

It has been proposed by Chia and Rostron (1970) that

these young are derived from a sexually reproduced free swimming planula larva which metamorphosed to a young anemone only after seeking out and entering the enteron of an adult. However, in a species which shows extensive colour polymorphism, it is difficult to reconcile this suggestion with the data of several authors (Cain, 1974; Carter and Thorp, 1979; Gashout and Ormond, 1979) showing that the brooded young are, at least generally, if not invariably, of the same colour as the parent.

Cain (1974) proposed 4 possible mechanisms to explain these various observations.

- (1) Each colour variety (i.e. red, brown, green) may represent a different species.
- (2) Planula larvae may re-enter and be brooded by adults of their own colouration only.
- (3) A foster parent effect could control the colouration of the brooded offspring.
- (4) The juveniles may be produced asexually or by parthenogenesis within the adult in which they are found.

In the light of subsequent work 3 of Cain's proposals can now be rejected.

- (1) Recent studies (Orr, 1980; Haylor, 1981; Haylor et al., in preparation) have demonstrated that isoenzyme allele frequencies are randomly distributed between red and brown specimens, hence showing at least the red and brown colour morphs to be conspecific. (Green individuals, which were comparatively rare in the population, were not used in this study).
- (2) Pre and post metamorphic larval forms will not re-enter the enteron of an adult (Carter and Funnel, 1980).
- (3) Larval colour is stable in the laboratory both outside and within the enteron of a contrasting-coloured foster parent (Carter, in preparation).

Cain's fourth hypothesis, that asexual or parthenogenic reproduction could account for the brooded juveniles has not, to date been adequately tested.

Another suggestion (Carter and Thorp, 1979) is that sexual reproduction is followed by post-fertilisational selection for genotypes similar or identical to that of the brooding parent. However, observations (Gashout and Ormond, 1979) that individuals maintained in total isolation in filtered or artificial seawater will continue to produce broods of young over periods of time indicate the probability of asexual reproduction. Self fertilisation cannot account for the majority of these broods since many authors (Chia and Rostron, 1970; Carter and Thorp, 1979; Gashout and Ormond, 1979) have concluded that *Actinia equina* is very infrequently if ever hermaphrodite.

Here we present data from isoenzyme studies which we consider demonstrate conclusively that the brooded young of *Actinia equina* are asexually produced. Specimens were obtained from a population at Fleshwick Bay, Isle of Man. Horizontal starch gel electrophoresis was used to screen these for 32 enzymes, of which 6 enzymes (7 loci) were found to be usefully polymorphic.

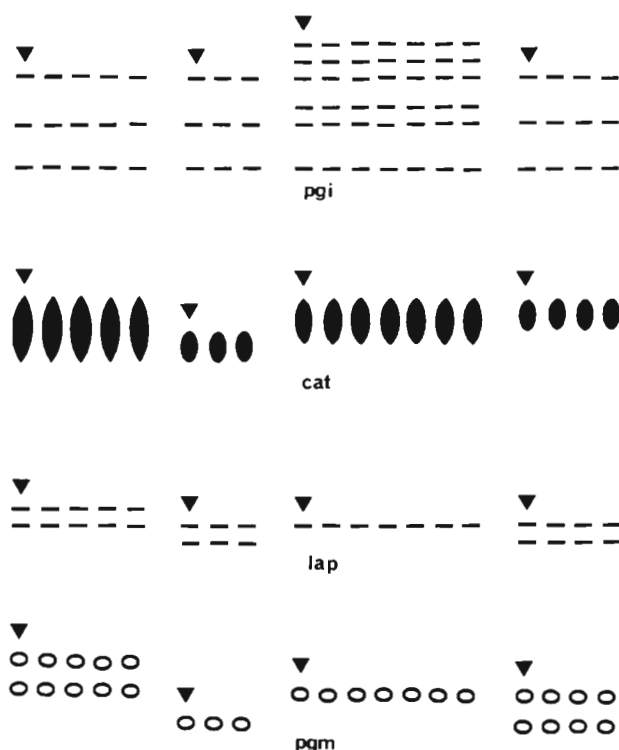


Fig. 1 *Actinia equina*. Electrophoretic banding patterns from parent and brooded young for 4 enzymes. In each parent/brood group triangles denote parental tissue samples. The two-banded phenotypes for Lap and Pgm are interpreted as being heterozygotes at a single locus. For Pgi the banding patterns indicate the presence of 2 loci with a central hybrid zone (this is to be expected from the dimeric molecular structure of Pgi). The phenotype with 3 bands at the upper locus is considered to show heterozygosity at this locus with the expected 2 bands in the hybrid zone

Juveniles released from adult specimens were reared until they reached a size at which they could be used for electrophoresis. The broods of young anemones and adults were then compared by electrophoresis over 5 of the previously identified polymorphic enzyme loci (Fig. 1). It is clear that in every case the offspring are of identical genotype to the parent, although there is considerable genetic variation between broods.

The results are essentially similar to those briefly described from investigations of the Australian *Actinia tenebrosa* (Ottaway and Kirby, 1975; Black and Johnson, 1979). It is likely therefore that in both species asexual reproduction is used to produce the brooded offspring. Self fertilization or inbreeding cannot account for the results since genetic segregation would be expected at least in the broods from heterozygous parents. The most likely mechanism for this is some form of budding in the enteron of the parent, although to date this has not been observed in histological studies (Chia and Rostron, 1970; Carter and Thorp, 1979; Gashout and Ormond, 1979). Gashout and Ormond (1979) suggested tentatively that somatic embryogenesis could account for the observations made on the reproduction of *A. equina*. They describe small pieces of tissue, sometimes resembling misshapen embryos, many of which were clearly derived from parts of the adult anemone. Gashout and Ormond (1979) also found that small pieces of adult tissue would, if cultivated in a Petri dish containing sea water, produce a membrane about themselves and swim by ciliary action. However, these authors failed to observe metamorphosis *in vitro*.

In the light of this work, and the demonstration by Polteva (1963) of somatic embryogenesis resulting from excision experiments with *Actinia equina* juveniles, it would appear possible that this mechanism could account for the production of the brooded young.

Such a mode of reproduction has not been previously described within the Anthozoa but budding and fragmentation are known to occur (reviewed by Chia, 1976). Somatic embryogenesis is therefore not a radical departure from previously described methods of asexual reproduction within the group.

It should be noted that, although all the brooded young which we have examined were asexually reproduced, the presence of gonad in adult *Actinia equina* and of genetic variation at isozyme loci within the species (Orr, 1980; Carter and Thorpe, 1981; Haylor, 1981) provide clear evidence that sexual reproduction also occurs. Whether sexually reproduced offspring are ever brooded is not known; it is possible that they are released early in ontogeny as a dispersive phase of the life cycle.

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