2020-12

Evidence of fostering in an internally brooding sea anemone

Lane, SM

10.1111/eth.13088
Ethology
Wiley

EVIDENCE OF FOSTERING IN AN INTERNALLY BROODING SEA ANEMONE

Running title: Alloparental care in a sea anemone

Sarah M. Lane[1]*, Mark Briffa[1], Alastair J. Wilson[2], Manuela Truebano[1], & Nicola L. Foster[1]

[1] School of Biological and Marine Sciences, Animal Behaviour Research Group, University of Plymouth, Plymouth, Devon, PL4 8AA, UK.

[2] Centre for Ecology and Conservation, University of Exeter (Penryn Campus), Cornwall, TR10 9FE, UK.

*Corresponding email: sarah.lane@plymouth.ac.uk

This is a manuscript version of the accepted article. The definitive version of this article is available at https://doi.org/10.1111/eth.13088.

ACKNOWLEDGEMENTS

We thank Ann Torr for help with collecting anemones and two anonymous reviewers for their constructive comments on the manuscript. This work was funded by Biotechnology and Biological Sciences Research Council grants awarded to MB (grant no: BB/M019772/1) and AJW (grant no: BB/M019748/1).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Evidence of alloparental care during the incubation stage has largely been demonstrated for species that incubate their offspring externally in a nest. Alloparental care in these species generally consists of the rearing of mixed broods which contain a low proportion of 'foreign' young alongside the host's own offspring. However many animals, including sea anemones, incubate offspring either on or within their bodies. The beadlet anemone *Actinia equina* incubate their young internally, and as many sea anemones are capable of reproducing both sexually and asexually, the origin of these internally brooded young has been the subject of much debate. While genetically identical young are brooded internally under the juvenile stage, it is thought that those produced sexually are released as larvae into the water and must return to the gastric cavity of an adult in order for metamorphosis to occur. As the likelihood of a planula larva finding its way back to its parent is slim, this suggests that alloparental care may play a role in the survival of juveniles in this species, a hypothesis first suggested a century ago but rarely tested. Here, using highly polymorphic microsatellite markers we find evidence of alloparental care in *A. equina*. Our results indicate that while a high proportion of juveniles were genetically identical to their brooding adult, the remaining juveniles showed stark genetic differences to their brooding adult. These juveniles shared far fewer alleles with their 'parent' than expected under sexual reproduction, indicating that they were not the adult's offspring. Furthermore, we found variation in the genetic composition of broods, which consisted either of (a) entirely genetically identical individuals, (b) a mix of unique individuals and clonemates or (c) entirely unique individuals i.e. no shared genotype. Our results thus indicate that adult *A. equina* tolerate the presence of non-offspring within their gastric cavity and furthermore that they may incubate entirely 'foreign' broods.

Keywords: Alloparental care; Asexual reproduction; Brooding; Sea anemones

1. INTRODUCTION

Alloparental care – parental care directed towards non-offspring – seems counterintuitive, but an understanding of the costs and benefits may explain its adaptive value. The costs associated with alloparental care derive from the allocation of resources to non-offspring which could otherwise be invested in an individual's own reproduction. However, the relative costs and benefits are expected to depend on several factors that determine the extent to which taking in additional offspring impacts the host's own survival and reproduction (Lopez-Sepulchre & Kokko, 2002; Sefc et al., 2012). Specifically, when the host is genetically related to the fostered young, the indirect fitness benefits gained by the host may outweigh any costs to its direct fitness. Furthermore, it may be of net benefit for an individual to take in unrelated offspring if it is unable to discriminate or selectively abandon 'foreign' young from amongst its own offspring (Eadie, Kehoe & Nudds, 1988). Alloparental care often occurs during the incubation phase of offspring development, resulting in adults rearing mixed broods in which 'foreign' offspring make up a small percentage of the total clutch. Evidence for this phenomenon is perhaps most readily observed in animals that incubate their offspring externally in a nest (e.g. fish - Wisenden, 1999; and birds – Riedman, 1982). However, not all animals brood their young externally and examples of alloparental care in species that brood young either on or within their bodies has begun to emerge. For instance, multiple species of mouth brooding cichlids have been found to recall mixed broods into their mouths for protection (Sefc et al., 2012; Kellogg et al., 1998; Schaedelin, van Dongen & Wagner, 2012) and mixed maternity has been identified in the clutches of embryos carried on the underside of female six-rayed sea stars *Leptasterias* spp. (Bareto & Bauer, 2019).

Sea anemones exhibit an incredibly diverse array of reproductive strategies, possessing the capacity to reproduce both sexually and asexually via a multitude of mechanisms. The use of sexual and asexual reproduction varies greatly both between and within species, with some anemone species capable of utilising both modes (Chia 1976). Asexual methods of reproduction include somatic embryogenesis, whereby juveniles are derived from a single cell and all organs are developed anew (Bocharova & Kozevich, 2011). Somatic embryogenesis that involves internal brooding of genetically identical offspring within the coelenteron (gastrovascular cavity) of the adult (see Larson, 2017 for a review). This internal incubation is a critical step in anemone development whether offspring are reproduced asexually via somatic embryogenesis, or sexually, as larvae are unable to metamorphose through the juvenile stage outside of the coelenteron (Gravier, 1916; Chia & Rostron, 1970). While asexually produced offspring are brooded internally until the juvenile stage, it has been hypothesised that sexually produced young are released into the water column as planula larvae, and that these larvae then return to an adult's coelenteron wherein they can metamorphose (Gravier, 1916; Chia & Rostron, 1970). Intuitively, the likelihood of a planula larva finding its way back to its parent after being in the water column for an unknown length of time is very slim. One possibility suggested is that larvae enter the coelenteron of other, potentially unrelated, adults in order to complete their development. However, this hypothesis has rarely been investigated and thus remains highly disputed. To date evidence has been demonstrated by a single study of the actiniid *Aulactinia stella*, in which almost a third of the adults sampled were shown to contain 'foreign' (genetically distinct) offspring (Bocharova & Mugue, 2012; Bocharova, 2015). However, the molecular markers utilised in this study (rRNA sequences) did not enable the extent to which the brooding adults differed genetically to these 'foreign' offspring to be determined.

The beadlet sea anemone *Actinia equina* is found in the intertidal zone across the UK and much of Europe. In recent years it has become a model species for the study of agonistic contest behaviour as adults (Rudin & Briffa, 2011; 2012; Lane & Briffa 2018a, b) and juveniles (Lane, Wilson & Briffa, 2020) compete aggressively for space on the shore. *A. equina* are dioecious and both females and males are known to brood offspring (Carter & Miles, 1989), with a range of developmental stages (from planula larvae to juveniles) being found simultaneously within the gastric cavity of a single adult (Chia & Rostron, 1970). The origin (sexual or asexual) of internally brooded juveniles in this species has been the subject of many studies over the last 40 years (Chia & Rostron, 1970; Carter & Miles, 1989; Carter & Throp, 1979; Gashout & Ormond, 1989; Lubbock & Allbut, 1981; Orr, Thorpe & Carter, 1982; Perrin, Thorpe, Solé-Cava, 1999; Douek et al., 2002; Chomsky et al., 2009; Pereira, Cadeireiro & Robalo, 2016) yet still remains unclear.

Microsatellites are highly polymorphic, co-dominant markers, which offer greater resolution for examining individual-level genetic differences. Here, we develop eight highly polymorphic microsatellite loci for *A. equina*. Then, using these microsatellites we investigate the origin (asexual, sexual, non-offspring) of internally brooded juveniles by analysing the genetic relationship between internally brooded juveniles and, moreover, between juveniles and their brooding adult.

2. METHODS

2.1 Anemone collection and tissue sampling

Adult *Actinia equina* of the red/brown colour morph (>2 cm in diameter, n=24) were collected from Portwrinkle (Cornwall, UK; grid reference: SX 357539) between December 2015 and October 2017, and taken back to the laboratory within 1-2 hours of collection. Anemones were collected a minimum of 1 m apart from one another to minimise the chances of collecting genetically identical adults (clones) and immediately isolated in screw-top pots in order to prevent any accidental cross-contamination of broods across adults (i.e. in case any juveniles were released during transit). Once in the laboratory, anemones were placed individually in plastic tanks (23 x 16 cm and 17.5 cm high) containing 700 mL of filtered sea water (with an air stone to provide constant aeration), maintained in at 15 ± 0.5°C on a 12L:12D lighting cycle and monitored for the release of juveniles. Anemones were fed *ad libitum* on aquaria marine fish flakes (Vitalis Aquatic Nutrition, Thorne, UK) every 2-3 days and sea water was changed fully every 7 days, taking care not to inadvertently transfer any released juveniles between tanks. Juveniles released by adults were maintained at 15°C in the same tanks as their brood-mates and parent until being removed for genetic analysis in October 2017. *A. equina* can produce multiple 'batches' of juveniles over time and as it is not possible to identify when each juvenile was released without immediate isolation, we use the word 'brood' to refer to all juveniles released by a single isolated adult during our experiment.

Brood size varied greatly between individuals (range = 1 – 25 offspring per breeding adult) and, in order to maximise the number of broods sampled, an average of 2.7 juveniles were sampled per brood. In order to ascertain enough tissue to extract a sufficient amount of DNA juveniles had to be sampled whole and any individuals with a pedal disc of <3 mm in diameter could not be used. Juveniles were placed individually in 1.5 mL microcentrifuge tubes containing 100% molecular grade ethanol and stored at -20°C until further use. For

adults, a small piece of pedal disc (~1 cm x 1 cm) was removed using a scalpel and preserved as above until use. A total of 18 adults and 69 juveniles were sampled (*N* = 87).

2.2 Microsatellite genotyping

DNA was extracted from tissue using a GeneJet genomic DNA purification kit (Thermo Fisher Scientific, UK) following the manufacturer's instructions. Purity and concentration of DNA samples were determined using a NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific, UK). DNA concentrations ranged from 12 to 175 ng μL^{-1}. The quality of extracted DNA samples was monitored on 2% agarose gels.

Thirteen polymorphic microsatellite DNA markers were developed for *A. equina* by Ecogenics GmbH (Balgach, Switzerland) (see supplementary material for details of development). Due to logistical constraints, however, we used nine out of the 13 microsatellite markers in the following protocol. The nine chosen were the most polymorphic of the 13 markers.

PCR amplifications were carried out in house following the protocol described in Lane et al. (2020). PCR products were then analysed by Ecogenics GmbH (Balgach, Switzerland) using an ABI3730 (Applied Biosystems) DNA analyser with an internal size standard (GeneScanTM-500 LIZ, Applied Biosystems) for accurate sizing. Electropherograms were visualised using Peak Scanner Software v1.0 (Applied Biosystems) and alleles scored based on amplicon size. Due to the presence of null alleles, only eight out of nine microsatellites were used in the following analysis. The microsatellite sequences developed and used in this paper have been deposited in GenBank (see table S1 in Lane et al., 2020 for accession numbers).

GenAIEx v6.5 (Peakall & Smouse, 2006; 2012) was used to calculate the number of multilocus genotypes present and to match individuals by genotype. Individuals that had

identical alleles at all eight loci were classified as clonemates possessing the same genotype. MLGSim was used to calculate significance values for the likelihood that a multilocus genotype observed more than once in the population results from sexual reproduction (Stenberg et al. 2003). On inspection of the genetic composition of the 24 broods, five were found to contain two genotypes which differed by just one out of 16 alleles. In four out of five of these broods, the scored alleles differed by a single repeat unit and thus this difference was assumed to be a scoring error and corrected.

Ethical note

The research described in this study adheres to the ASAB Guidelines for the Use of Animals in Research. After use in this study adult anemones were returned to the collection site. No permits or licenses were required for this work.

3. Results

3.1 Genotypic diversity

A total of 18 adults and 64 juveniles (*N* = 82) comprising 24 broods (18 sampled with adult, six without - due to adult death prior to sampling) were successfully genotyped and a total of 25 unique genotypes identified. Of these genotypes, six were singleton genotypes, fourteen were found only in one brood and the remaining five were found across multiple broods (table 1). The results of MLGSim analysis were statistically significant for all 19 multilocus genotypes that occurred more than once in the population ($P < 0.001$ in all cases – table S1), confirming the asexual origin of these shared genotypes.

3.2 Genetic composition of broods sampled with parents

Of 36 juveniles for which the parent was genotyped, 31 juveniles (86.1%) had identical genotypes to their parent. The genotypes of the remaining five juveniles (13.8%) differed from their parental genotype by an average of 8.7 alleles (range = 1 to 13 alleles) out of a possible 16.

3.3 Genetic composition of broodmates

Of the 24 broods sampled in total, 17 (71%) appeared to be fully clonal (i.e. all individuals sampled within that brood were genetically identical), while the remaining seven broods (29%) contained up to four unique genotypes (see table 1). Two broods (8.3%) consisted entirely of unique individuals, while five (20.8%) consisted of a mix of clonemates and non-clonemates (Table 1). Of these five broods, two contained multiple clonemates from multiple genotypes (e.g. two individuals of genotype A and two individuals of genotype B - see figure 1 for examples of the different brood compositions).

4. DISCUSSION

Our analysis based on microsatellite data demonstrates that internally brooded juveniles of *Actinia equina* originate from at least two sources. A large proportion of the juveniles sampled in this study (86.1%) were identical to their brooding parent at all microsatellite loci examined,

indicating a very high likelihood that they are the product of asexual somatic embryogenesis. The remaining juveniles (13.8%) however, exhibited stark genetic differences to their brooding adult, differing by as many as 13 out of the 16 alleles sampled. If juveniles were the sexual progeny of their brooding adult, we would expect them to share at least one allele per locus with the brooding adult. However, in the majority of instances, this was not the case and thus our results indicate that this latter set of juveniles are non-offspring, 'fostered' within the coelenteron of adults that are not their parents.

The high proportion of juveniles that were genetically identical to the brooding adult (and thus definitely offspring) might indicate low levels of tolerance to non-offspring. However, seven of the broods sampled consisted either entirely of unique individuals or of a mix of clonemates and unique individuals. For the three broods in which the adult genotype was known, we found that juveniles were genetically distinct from their brooding adult. While in one of the broods this difference was minimal (one allele), for the other two broods the difference was substantial (from 7-14 alleles different out of a possible 16). Furthermore, even in broods for which the parent could not be sampled, the difference between non-identical brooded juveniles was greater than expected under sexual reproduction i.e. they shared less than 50% of alleles. Thus, it appears that a least a subset of adults are very tolerant of non-offspring. Variation in tolerance to non-offspring has been observed in allonursing species such as southern right whales *Eubalaena australis* (Best et al., 2015) and African lions *Panthera leo*. (Pusey & Packer, 1994). In *P. leo*, tolerance appears to relate to the size of the female's own litter, those with smaller litters demonstrating a higher proportion of nursing to non-offspring, presumably because they can afford to spare resources (in this instance milk) (Pusey & Packer, 1994). The factors that drive tolerance of non-offspring in *A. equina* are currently unclear. Previous studies in which juveniles have been experimentally

introduced into the coelenteron of unrelated adults suggest that the tolerance of non-offspring relies on phenotype matching (e.g. red adults tolerate red juveniles – Lubbock & Allbut, 1981), however this is not a pattern we have observed in this study, with juvenile phenotype varying greatly within broods (SML *personal observation*).

Five out of the 19 multilocus genotypes identified in our study were shared across broods, including between broods which contained multiple genotypes. This result has several possible implications. First of all, it indicates that there may a limited number of genotypes within the sample population, most likely due to a lack of sexual reproduction. Indeed, recent evidence suggests that *A. equina* may actually lack some of the key genes necessary for sexual reproduction (Wilding et al. 2020). Taken together the results of Wilding et al. (2020) and those presented here suggest that adults may not be taking in sexually produced larvae but fostering asexually produced clones. It has been previously stated that asexual young are brooded internally by their parent until the juvenile stage (Gravier, 1916; Chia & Rostron, 1970), thus why clonal juveniles would enter the coelenteron of another adult at this life stage is unclear. Two of the broods sampled in this experiment (for which parental genotype was unknown) contained multiple clonemates of multiple genotypes, which could be indicative either of adults taking in multiple 'foreign' juveniles of the same genotypes (which would imply alloparental care of asexual clones rather than sexual larvae) or, if sexual reproduction is occurring, of some juveniles within those broods being sexually reproduced genetically identical siblings (i.e. twins). Polyembrony, which results from a single zygote dividing into two genetically identical embryos (similar to the production of monozygotic twins in humans), has recently been described for colonies of the Indo-Pacific coral *Pocillopora damicornis* (Yeoh & Dai, 2010, Combosch & Vollmer, 2013). However, further

research is required to disentangle these possibilities, in particular data in which the genotype of the brooding adult is known for all broods sampled.

The finding that juveniles within a single brood can possess different genotypes and, moreover, that genetically identical individuals can experience different brooding environments (i.e. non-parental genetically distinct adults) has interesting implications for behavioural studies of *Actinia equina*. As mentioned above, *A. equina* have become a model system for studying fighting behaviour and there is evidence to suggest that relatedness has significant effects on the likelihood and intensity of aggression expressed between individuals. Specifically, *A. equina* are capable of discriminating between self and non-self (i.e. clonemates and non-clonemates) and appear to only exhibit aggression towards non-clonemates (Turner et al., 2003). Furthermore, the levels of aggression expressed towards non-clonemates has been shown to increase with relatedness (Foster & Briffa, 2014; Lane, Wilson & Briffa, 2020). Together with the findings of the current study, this suggests that levels of aggression exhibited within a brood should vary with the level of genetic diversity expressed. As *A. equina* fight over territory on the shore, intra-brood aggression between juveniles of different genotypes could also provide a mechanism by which to ensure dispersal, albeit on a smaller scale. Finally, *A. equina* could be an ideal system in which to separate and study the relative effects of genotype and early life environment (i.e. brooding adult) on a vast range of traits from behaviour, to physiology and development.

The data presented in this study suggest that *A. equina* may provide a rare example of adults raising entire 'foreign' broods and moreover, raising them internally. There are multiple possibilities as to why adults of this species would brood foreign offspring. The first and perhaps most obvious reason is that adults are unable to distinguish their own young

from others and so are forced to tolerate 'foreign' young rather than risk ejecting their own. However, as previous evidence suggests that *A. equina* are capable of discriminating self (genetically identical) and non-self (Turner et al., 2003), this explanation seems unlikely. A second possibility then is that adults have the capacity to distinguish between young but are unable to eject 'foreign' young once they have entered the coelenteron. This scenario could result in aggression between adults and unrelated juveniles once the brood is released from the coelenteron. Indeed, acrorhagial peels have been observed on the columns of juvenile *A. equina* in the field (SML *personal observation*), and as only adult anemones possess acrorhagi, this damage indicates the occurrence of direct aggression by adults to juveniles. A third and perhaps least likely explanation is that adults are able to distinguish between young, have the capacity to selectively eject 'foreign' offspring, but willingly take in non-offspring. Why an adult would tolerate the presence of 'foreign' young in this last scenario is unclear, especially as any resources utilised by these non-offspring would be unavailable for the adult's own young. Further studies are required to gain a greater understanding of the causes, costs and benefits of this behaviour.

Data availability

Upon acceptance for publication, data from this study will be accessible via PEARL, the open access research repository for the University of Plymouth.

REFERENCES

Bareto, F. S., Bauer, K. K. (2019). Genetic evidence for alloparental care and frequent multiple paternity in the brooding sea star (*Leptasterias* sp.). *Marine Biology,* 166, 38. https://doi.org/10.1007/s00227-019-3487-3

Benson, G. (1999). Tandem repeats finder: a program to analyse DNA sequences. *Nucleic Acids Research,* 27, 573-580.

Best, P. B., Elwen, S. H., Palsbøll, P. J., Thornton, M., Austin, E., Vinding, K. (2015) Possible non-offspring nursing in the southern right whale, *Eubalaena australis*. *Journal of Mammalogy*, 96, 405-416. https://doi.org/10.1093/jmammal/gyv042

Bocharova, E. S. (2015). Reproductive biology and genetic diversity of the sea anemones *Aulactinia stella* (verrill, 1864). *Hydrobiologia,* 759, 27-38. https://doi.org/10.1007/s10750-015-2250-0

Bocharova, E. S., Kozevich, I. A. (2011). Modes of reproduction in sea anemones (Cnidaria, Anthozoa). *Biology Bulletin,* 38, 849-860. https://doi.org/10.1134/S1062359011090020

Bocharova, E. S., Mugue, N. S. (2012). Sea anemones *Aulactinia stella* (Verrill, 1864) (Hexacorallia, Actiniidae) can brood offspring from other individuals of the same species. *Doklady Biological Sciences,* 444, 227-229. https://doi.org/10.1134/S0012496612030040

Carter, M. A., Miles, J. (1989). Gametogenic cycles and reproduction in the beadlet sea anemone *Actinia equina* (Cnidaria: Anthozoa). *Biologoical Journal of the Linnean Society,* 36, 129-155.

Carter, M. A., Throp, C. H. (1979). The reproduction of *Actinia equina* L. var. *Mesembryanthemum*. *Journal of the Marine Biological Association of the United Kingdom,* 59, 989-1001.

Chia, F-S. (1976). Sea anemone reproduction: patterns and adaptive radiation. In: G. O. Mackie (Ed), *Coelenterate Ecology and Behaviour* (pp 261-271). Springer International Publishing, Switzerland.

Chia, F-S., Rostron, M. A. (1970). Some aspects of the reproductive biology of *Actinia equina* [Cnidaria: Anthozoa]. *Journal of the Marine Biological Association of the United Kingdom,* 50, 253-264.

Chomsky, O., Douek, J., Chadwick, N. E., Dubinsky, Z., Rinkevich, B. (2009). Biological and population-genetic aspects of the sea anemone *Actinia equina* (Cndiara: Anthozoa) along the Mediterranean coast of Israel. *Journal of Experimental Marine Biology and Ecology,* 375, 16-20. https://doi.org/10.1016/j.jembe.2009.04.017

Combosch, D. J., Vollmer, S. V. (2013). Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. *Ecology and Evolution,* 3, 3379-3387. https://doi.org/ 10.1002/ece3.721

Douek, J., Barki, Y., Gateño, D., Rinkevich, B. (2002). Possible cryptic speciation within the sea anemone *Actinia equina* complex detected by AFLP markers. *Zoological Journal of the Linnean Society,* 136, 315-320.

Eadie, J. McA., Kehoe, F. P., Nudds, T. D. (1988). Pre-hatch and post-hatch brood amalgamation in North American Anatidae: a review of hypotheses. *Canadian Journal of Zoology,* 66, 1709-1721.

336 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
337 *Bioinformatics,* 26, 2460–1. https://doi.org/10.1093/bioinformatics/btq461

338 Foster, N. L., Briffa, M. (2014). Familial strife on the seashore: Aggression increases with
339 relatedness in the sea anemone *Actinia equina*. *Behavioural Processes*, 103, 243-245.
340 http://dx.doi.org/10.1016/j.beproc.2014.01.009

341 Gashout, S. E., Ormond, R. F. G. (1979). Evidence for parthenogenetic reproduction in the sea
342 anemone *Actinia equina* L. *Journal of the Marine Biological Association of the United Kingdom,*
343 59, 975-987.

344 Gravier, C. J. (1916). Sur l'incubation chez l'Actinia equina L. à l'ile de San Thome (golfe de
345 Guinée). *Comptes rendus de l'Académie des Sciences,* 162, 986–988.

346 Kellogg, K. A., Markert, J. A., Stauffer Jr, J. R., Kocher, T. D. (1998). Intraspecific brood mixing
347 and reduced polyandry in a maternal mouth-brooding cichlid. *Behavioural Ecology*, 9, 309-
348 312.

349 Lane, S. M., Briffa, M. (2018a). Immune function and the decision to deploy weapons during
350 fights in the beadlet anemone, *Actinia equina*. *Journal of Experimental Biology*,
351 221:jeb169201. https:// doi:10.1242/jeb.169201

352 Lane, S.M., Briffa, M. (2018b). How does the environment affect fighting? The interaction
353 between extrinsic fighting ability and resource value during contests. *Journal of Experimental*
354 *Biology*, 221:jeb187740. https://doi:10.1242/jeb.187740

355 Lane, S. M., Wilson, A. J., Briffa, M. (2020). Analysis of direct and indirect genetic effects in
356 fighting sea anemones. *Behavioural Ecology*, 31, 540-547.
357 https://doi:10.1093/beheco/arz217

358 Larson, P. (2017). Brooding sea anemones (Cnidaria: Anthozoa: Actiniaria): paragons of
359 diversity in mode, morphology, and maternity. *Invertebrate Biology*, 136, 92-112.
360 https://doi:10.1111/ivb.12159

361 Lopez-Sepulchre, A., Kokko, H. (2002). The role of kin recognition in the evolution of
362 conspecific brood parasitism. *Animal Behaviour*, 64, 215-222.
363 https://doi:10.1006/anbe.2002.3043

364 Lubbock, R., Allbut, C. (1981). The sea anemone *Actinia equina* tolerates allogenic juveniles
365 but alters their phenotype. *Nature*, 293, 474-475.

366 Orr, J., Thorpe, J. P., Carter, M. A. (1982). Biochemical genetic confirmation of the asexual
367 reproduction of brooded offspring in the sea anemone *Actinia equina*. *Marine Ecology*
368 *Progress Series*, 7, 227-229.

369 Peakall, R., Smouse, P. E. (2006). GenAIEx 6: genetic analysis in Excel. Population genetic
370 software for teaching and research. *Molecular Ecology Notes*, 6, 288-295.
371 https://doi.org/10.1111/j.1471-8286.2005.01155.x

372 Peakall, R., Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic
373 software for teaching and research-an update. *Bioinformatics*, 28, 2537-2539.
374 https://doi:10.1093/bioinformatics/bts460

375 Pereira, A. M., Cadeirero, E., Robalo, J. I. (2016). Asexual origin of brooding in the sea
376 anemones *Actinia equina* and *A. schmidti*: molecular evidence from the Portuguese shore.
377 *New Zealand Journal of Marine and Freshwater Research*, 51, 316-320.
378 https://doi.org/10.1080/00288330.2016.1236737

379

Perrin, M. C., Thorpe, J. P., Solé-Cava, A. M. (1999). Population structuring, gene dispersal and reproduction in *Actinia equina* species group. *Oceanography and Marine Biology: an Annual Review*, 37, 129-152.

Pusey, A. E., Packer, C. (1994) Non-offspring nursing in social carnivores: minimising the costs. *Behavioural Ecology*, 5, 362-374.

Raymond, M., Russet, F. (1995). GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248-249.

Riedman, M. L. (1982). The evolution of alloparental care and adoption in mammals and birds. *The Quarterly Review of Biology*, 57, 405-435.

RStudio Team (2016). RStudio: Integrated development for R. RStudio, Inc., Boston, MA, http://www.rstudio.com/.

Rudin, F. S., Briffa, M. (2011). The logical polyp: Assessments and decisions during contests in the beadlet anemone *Actinia equina*. *Behavioural Ecology*, 22, 1278-1285. https://doi.org/10.1093/beheco/arr125

Rudin, F. S., Briffa, M. (2012). Is boldness a resource-holding potential trait? Fighting prowess and changes in startle response in the sea anemone *Actinia equina*. *Proceedings of the Royal Society B,* 279, 1904-1910. https://doi:10.1098/rspb.2011.2418

Schaedelin, F. C., van Dongen, W. E. D., Wagner, R. H. (2012). Nonrandom brood mixing suggests adoption in a colonial cichlid. *Behavioural Ecology*, 24, 540-546. https://doi.org/10.1093/beheco/ars195

401 Sefc, K. A. M., Hermann, C. M., Taborsky, B., Kobmüller, S. (2012). Brood mixing and reduced
402 polyandry in a maternally mouthbrooding cichlid with elevated among-breeder relatedness.
403 *Molecular Ecology*, 21, 2805-2815. https://doi:10.1111/j.1365-294X.2012.05573.x

404 Stenberg, P., Lundmark, M., Saura, A. (2003). MLGsim: a program for detecting clones using a
405 simulation approach. *Molecular Ecology*, 3, 329-331. https://doi.org/10.1046/j.1471-
406 8286.2003.00408.x

407 Turner, V. L. G., Lynch, S. M., Paterson, L., León-Cortés, J. L., Thorpe, J. P. (2003). Aggression
408 as a function of genetic relatedness in the sea anemone *Actinia equina* (Anthozoa: Actiniaria).
409 *Marine Ecology Progress Series*, 247, 85-92. https://doi:10.3354/meps247085

410 Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., Rozen, S. G.
411 (2012). Primer3--new capabilities and interfaces. Nucleic Acids Research. 40(15), e115.
412 https://doi: 10.1093/nar/gks596

413 Valiere, N. (2002). GIMLET: a computer program for analysing genetic individual identification
414 data. *Molecular Ecology Notes*, 2, 377-379. https://doi.org/10.1046/j.1471-
415 8286.2002.00228.x-i2

416 Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., Shipley, P. (2007). MICRO-CHECKER:
417 software for identifying and correcting genotyping errors in microsatellite data. *Molecular*
418 *Ecology Notes*, 4, 535-538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

419 Waits, L. P., Luikart, G., Taberlet, P. (2001). Estimating the probability of identity among
420 genotypes in natural populations: causations and guidelines. *Molecular Ecology*, 10, 249-256.
421 https://doi:10.1046/j.1365-294x.2001.01185.x

422

Wilding, C. S., Fletcher, N., Smith, E. K., Prentis, P., Weedall, G. D., Stewart, Z. (2020). The genome of the sea anemone *Actinia equina* (L.): Meiotic toolkit genes and the question of sexual reproduction. *Marine Genomics,* 100753. https://doi.org/10.1016/j.margen.2020.100753

Wisenden, B. D. (1999). Alloparental care in fishes. *Reviews in Fish Biology and Fisheries*, 9, 45-70.

Yeoh, S-R., Dai, C-F. (2010). The production of sexual and asexual larvae within single broods of the scleractinian coral, *Pocillopora damicornis*. *Marine Biology*, 157, 351-359. https://doi:10.1007/s00227-009-1322-y

Table 1 Genotypic composition of 24 broods sampled. Genotypes shared across broods are colour coded. Unique genotypes are signified by the prefix 'Gen_U'. Individuals could differ between a maximum of 16 alleles sampled.

Brood ID	Parent genotype	Juvenile genotype(s)	Difference between genotypes (no. alleles)	
ALL UNIQUE INDIVIDUALS				
A	Gen_1	Gen_U2 (n=1) Gen_U5 (n=1)	Gen_1 – Gen_U2 Gen_1 – Gen_U5 Gen_U2 – Gen_U5	7 10 10
B	Unknown	Gen_U1 (n=1) Gen_U3 (n=1) Gen_U6 (n=1)	Gen_U1 – Gen_U3 Gen_U1 – Gen_U6 Gen_U3 – Gen_U6	7 9 2
MIX OF CLONEMATES AND UNIQUE INDIVIDUALS				
C	Gen_6	Gen_4 (n=2)	Gen_6 – Gen_4	14
D	Gen_2	Gen_2 (n=4) Gen_U4 (n=1)	Gen_2 – Gen_U4	1
E	Unknown	Gen_4 (n=1) Gen_14 (n=3)	Gen_4 – Gen_14	9
F	Unknown	Gen_4 (n=2) Gen_6 (n=1) Gen_14 (n=2)	Gen_4 – Gen_6 Gen_4 – Gen_14 Gen_6 – Gen_14	14 9 10
G	Unknown	Gen_1 (n=1) Gen_6 (n=1) Gen_14 (n=4) Gen_15 (n=2)	Gen_1 – Gen_6 Gen_1 – Gen_14 Gen_1 – Gen_15 Gen_6 – Gen_14 Gen_6 – Gen_15 Gen_14 – Gen_15	10 7 11 10 13 8
ALL CLONEMATES				
H	Gen_10	Gen_10 (n=2)		
I	Unknown	Gen_15 (n=3)		
J	Gen_8	Gen_8 (n=1)		
K	Gen_9	Gen_9 (n=3)		
L	Gen_5	Gen_5 (n=1)		
M	Gen_19	Gen_19 (n=2)		
N	Gen_18	Gen_18 (n=3)		
O	Gen_13	Gen_13 (n=1)		
P	Gen_12	Gen_12 (n=3)		
Q	Gen_3	Gen_3 (n=1)		
R	Gen_15	Gen_15 (n=5)		
S	Gen_17	Gen_17 (n=4)		
T	Gen_16	Gen_16 (n=1)		
U	Gen_4	Gen_4 (n=1)		
V	Unknown	Gen_15 (n=2)		
W	Gen_7	Gen_7 (n=1)		
X	Gen_11	Gen_11 (n=1)		

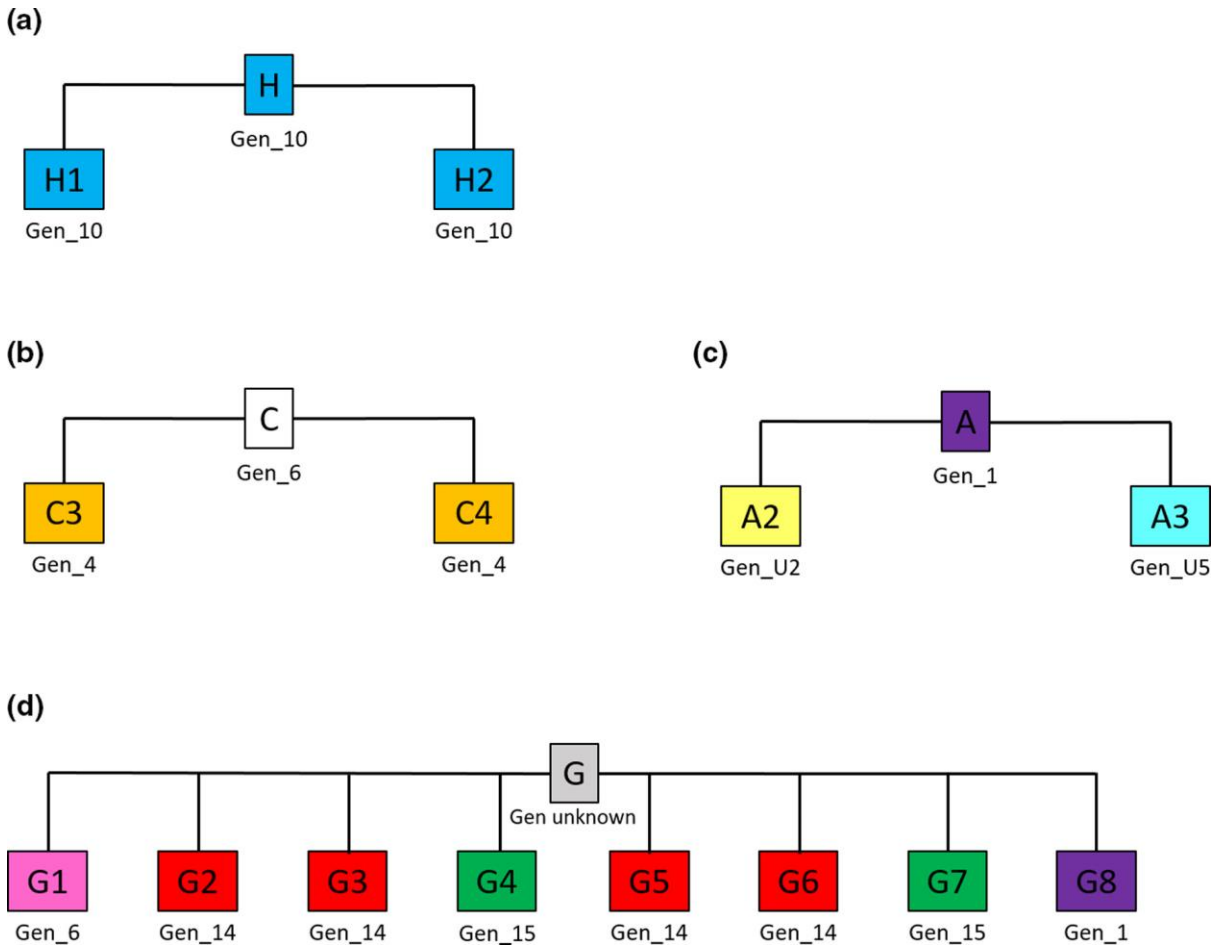

Figure 1 Examples of the different genetic brood compositions seen in *Actinia equina.* (a) A fully clonal brood - all juveniles are genetically identical to parent, (b) juveniles are genetically identical to each other but not to parent, (c) All individuals within brood possess unique genotypes, (d) Multiple unique genotypes are expressed by juveniles with multiple clonemates for each genotype. Matching genotypes are signified by matching colours. Grey boxes signify that the genotype of that individual is unknown (i.e. not sampled).