THE ROYAL SOCIETY

Received 13 May 2003
Accepted 17 July 2003
Published online 7 October 2003

Plastic reproductive strategies in a clonal marine invertebrate

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Plastic reproductive allocation may allow individuals to maximize their fitness when conditions vary. Mate availability is one condition that may determine the fitness of an individual's allocation strategy. Using a variety of methods, I detected evidence of plastic allocation to asexual (clonal) reproduction in response to mate availability in the brittle star *Ophiactis savignyi*. There were more mature individuals in populations in which both sexes were present, and clones from these populations had fewer clone-mates than clones from single-sex populations. Animals placed with mates in a field experiment divided less frequently than animals without a mate. These findings demonstrate that animals reduce their allocation to asexual reproduction when mates are present and when a loss of fecundity associated with cloning would decrease offspring production. This plasticity is probably adaptive because it maximizes sexual-reproductive potential when fertilization is more likely, but maximizes survival of the clone when mates are absent and gametes are unlikely to be converted to offspring.

Keywords: asexual (clonal) reproduction; mate limitation; phenotypic plasticity; reproductive success; risk spreading; trade-offs

1. INTRODUCTION

Clonal or asexual reproduction is an important aspect of the life histories of many plant (Holsinger 2000) and animal (Hughes 1989) species. The contribution of clonal reproduction to population dynamics may often rival or exceed that of sexual reproduction (for a few recent examples, see McFadden (1997), Procaccini & Mazzella (1998) and Dorken & Eckert (2001)). Clonal reproduction may increase the survival of the clone through risk spreading (Cook 1979; Highsmith 1982; Schmid 1990; Hughes *et al.* 1992), allowing the clone to survive for many reproductive events. The production of clone-mates could also increase the number of bodies producing gametes (Highsmith 1982; Mladenov & Emson 1990; Mladenov 1996), and may increase the likelihood that at least some clone-mates are near potential mates (Handel 1985; Lasker *et al.* 1996; McLetchie & Puterbaugh 2000). However, clonal reproduction may divert resources away from the production of gametes by individual clone-mates (Emson & Wilkie 1980; Gasser *et al.* 2000) and put individuals below their sexual-reproductive size thresholds (Karlson 1986; Smith & Hughes 1999; McGovern 2002*a*,*b*).

Most discussions regarding the evolution of asexual strategies have considered their average costs and benefits (Caswell 1985; Stearns 1992), but few individuals may experience average conditions. How the theoretical costs and benefits of allocation decisions are realized for individuals at specific times will be affected by numerous factors, including the availability of mates (Muenchow 1978) because mate availability will determine how successfully individuals convert gamete expenditure into offspring production. For example, many clonal marine organisms reproduce sexually by broadcasting their gametes into the water, and fertilization success in broadcast spawners is dependent on the proximity of mates (reviewed in Levitan 1998). If mates are not available, reduced gamete production associated with the decision to clone may not incur a cost above that already experienced owing to fertilization limitation. In the presence of mates, however, when fertilization is relatively more likely, the costs of asexual reproduction could be severe because reduced gamete production would result in the production of fewer offspring.

Variation in the costs and benefits of cloning depending on an individual's immediate environment, including an individual's sexual neighbourhood, suggests that plastic allocation strategies could increase fitness (Stearns 1989; Winn 1996). Phenotypic plasticity, or the expression of different traits in different environments by the same genotype (Bradshaw 1965), offers organisms the opportunity to produce phenotypes that confer high fitness regardless of their surroundings (Bradshaw 1965; Schlichting 1986; West-Eberhard 1989). This ability is thought to be particularly important for sessile organisms, which cannot move to better habitats (Schlichting 1986), and long-lived organisms that are likely to experience a range of environmental conditions during their lifetimes (Bradshaw 1965), traits that describe many clonal organisms.

In this study, I question whether the allocation to asexual reproduction is a plastic response to the availability of mates in the brittle star *Ophiactis savignyi*. I first examined the number of mature animals in naturally occurring mixed-sex (MS) populations relative to those with only a single sex present (SS). I supplemented this inferential assessment of reproductive strategies with observations of the clonal structures of SS and MS populations, predicting that clones from SS populations would have more clone-mates if the absence of a mate induced a switch to asexual

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reproduction. I also conducted a field experiment to determine whether animals with mates were less likely to undergo clonal reproduction. Finally, I present evidence of both the costs and the benefits of clonal reproduction to the sexual capabilities of clone-mates in order to address the potential adaptive consequences of plastic clonal strategies in this species.

2. MATERIAL AND METHODS

(a) *Study organism*

Ophiactis savignyi is a small brittle star (0.5–11.5 mm disc diameter; McGovern 2002*a*) with a circumtropical distribution (Mladenov & Emson 1990; Chao & Tsai 1995). Typically, it lives in marine sponges (the animals contained within a single sponge are hereafter referred to as a 'population') where it can occur at high densities (up to 370 individuals per sponge; McGovern 2002*a*). Various details regarding the biology of *O. savignyi* and the methods are located in electronic Appendix A (available on The Royal Society's Publications Web site).

Ophiactis savignyi is dioecious. Mature animals bear a pair of gonads associated with each arm, so the typical six-armed animal carries 12 gonads. There is little information regarding the spawning behaviour of individuals, but I have observed a male broadcasting sperm and have never observed a brooded embryo, indicating that both sexes broadcast their gametes. In addition to reproducing sexually, this species also reproduces clonally by dividing across the disc and regenerating the lost portions of the body (Emson & Wilkie 1980).

Limitation of mating opportunities may occur in *O. savignyi* despite large population sizes. Typically, only 2–16% of animals within a sponge population are mature (McGovern 2002*a*) and most populations (74%) with mature animals harbour individuals of only one sex. Whereas the distance between individuals within a population is millimetres to centimetres, the distance between populations may be metres (mean of 0.81 m). Because this species is a broadcast spawner, the distance between mates should be a strong factor determining fertilization success (reviewed in Levitan 1998). Individuals in populations without mates (the majority of animals) should have much lower fertilization successes than those in populations with mates.

(b) *Field observations*

I examined the relationship between mate availability and the number of mature animals by collecting 283 populations of *O. savignyi* between March 1996 and October 1998. Populations were collected from Long Key Bight, Long Key, FL, USA, from an area of several hundred square metres. Out of the 283 populations examined, 117 (41%) contained at least one mature animal. Out of the populations with mature animals, 31 (26%) contained mature animals of both sexes, 63 (54%) contained only mature males and 23 (20%) contained only mature females. The numbers of sexually mature individuals in the 86 SS and 31 MS populations were regressed against population size (both variables were log-transformed to meet assumptions of normality) and an analysis of covariance (ANCOVA) was performed. For this and all following analyses (unless otherwise stated), all assumptions were met.

(c) *Observations of clonal structure*

I examined clonal structure in eight naturally occurring MS populations and 16 naturally occurring SS populations (eight female-only and eight male-only) using patterns of randomly amplified polymorphic DNA (RAPDs). Although the population types do not occur with equal frequencies in nature, I chose equal numbers for statistical testing. RAPDs are relatively easy and inexpensive to generate, and have been used to examine clonal structure in a variety of other organisms (e.g. Eppley *et al.* 1998; Hatton-Ellis *et al.* 1998). Because I was interested only in clonal structure, I was less concerned about the problems of dominance and non-homology that may be associated with RAPDs (Grosberg *et al.* 1996). I selected the eight populations of each type randomly, except for the proviso that each had, at least, approximately 15 individuals (the median population size of female-only populations, which was the smallest group). Population size still varied by over an order of magnitude (McGovern 2002*a*) so I chose to sample a fixed number, rather than a fixed proportion, of individuals from each group. I sampled all mature individuals from each population to collect information from as many clones of known sex as possible. I also chose up to 20 non-mature animals semi-randomly to reflect population size structure: I randomly chose animals from within 1 mm size classes in proportion to the relative abundance of each class.

Following the protocol of Levitan & Grosberg (1993), I extracted and amplified DNA from the selected animals using two 10-mer primers (AK16 and AN14, Operon Technologies, Alameda, CA, USA). The amplified product was run on 0.6% agarose gels with 1.0% Synergel (Diversified Biotech). Gels were stained with ethidium bromide, photographed and scored from the photographic negatives. The repeatability of banding patterns was not quantified for every animal because of the large number of individuals, but early trials, in addition to periodic re-amplification of individuals, indicated that banding patterns were repeatable.

Because clone-mates should produce identical banding patterns, I used pattern identity to group individuals into clones. I determined band sharing between individuals within populations from a matrix describing the presence or absence of bands at each locus. Individuals with identical banding patterns (band-sharing = 1) were considered to be clone-mates. If correlations were less than 1.0 but greater than 0.85 (which occurred in only a few cases), the individuals in question were re-scored side-by-side. I calculated band sharing between representatives of each clone from all populations to determine whether clones were distributed across multiple populations. I then assigned a sex to each clone for which the sex of at least one member was known.

I used the Box–Cox procedure in JMP (SAS Institute, Cary, NC, USA) to determine that $(N^{0.2} - 1)/0.05996$ provided the best approximation to normality (N is the clone size or the number of individuals with the same multilocus genotype). I examined the effects of a potential mate on clone size using a two-way analysis of variance (ANOVA) with sex as the second factor, since females and males have different division probabilities (McGovern 2002*b*) and clones of different sexes could therefore differ in size.

(d) *Rates of clonal division*

To determine whether individuals altered their clonal strategies in the presence of a mate, I monitored the division of mature animals both with and without mates in a field experiment. After measuring and sexing each animal, I marked each with an identifying sequence of spots using vital dyes (McGovern 2002*b*) before placing them into treatment groups. I made every attempt to equalize the sizes of animals in the

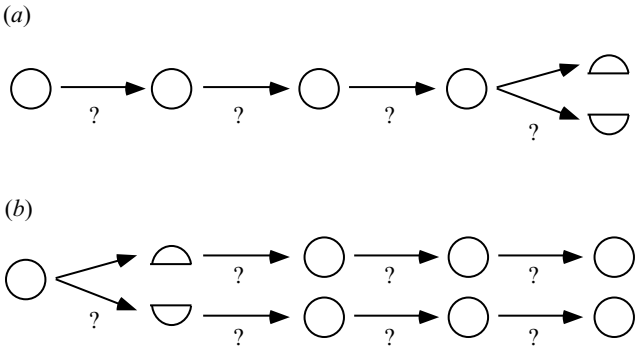

Figure 1. The calculation of actual versus possible divisions. In (*a*), there are four divisions possible (indicated by question marks), but only one actual division, so the division probability (*D*) is 0.25. In (*b*), halves produced during the first period are free to divide again independently of one another. The number of possible divisions is therefore seven, with one actual division, giving $D = 0.14$.

various treatment groups and to prevent the over-representation of potential clone-mates (individuals of the same sex from the same population) in the experiment.

SS treatments included single animals ($n = 8$ for each sex) and same-sex pairs ($n = 8$ for each sex). In prior analyses (McGovern 2002*b*), it was found that single animals and those from same-sex pairs had identical division probabilities so these treatments were pooled within sex. The MS treatment ($n = 8$) was initiated with a single animal of each sex. Animals were placed into enclosures consisting of small pieces of plastic tubing encased in large-mesh fabric. The enclosures were suspended from PVC and monofilament frames in the field *ca.* 35 m offshore from the Keys Marine Laboratory on Long Key, FL, USA at a depth of 1.5 m.

I began the experiment in October 1998 and censused animals once a month for eight months. At each census, using both arm length and disc asymmetry, I determined whether animals had divided. I repainted each individual and gave new clone-mates different marks. Division probability (*D*) was calculated as the number of divisions that occurred divided by the number of possible divisions (McGovern 2002*b*). The number of possible divisions was calculated by counting the number of instances in which it was possible to determine whether division had occurred (figure 1). This required that an animal (or its descendant clone-mates) was present in consecutive censuses. Following their separation, clone-mates produced during the experiment were treated as separate individuals with independent chances of division. This metric accounted for multiple divisions and/or the disappearance of animals during the experiment.

Division probabilities were analysed using a two-way ANOVA with the presence of a potential mate and sex as factors. The Box–Cox procedure demonstrated that $[(D + 0.01)^{-0.4} - 1]/-40.48876$ provided the best approximation to normality.

(e) *Costs and benefits of clonal reproduction to gamete production*

Both females (75%) and males (36%) may lose all gonads in one or both halves following division, representing a 50–100% loss of sexual potential (McGovern 2002*b*). I determined whether animals that retained gonads produced fewer gametes by regressing the number of gametes per gonad on a metric of the recency of division, the average length of the three shortest adjacent arms divided by the average length of the three longest arms (Mladenov & Emson 1988; McGovern 2002*a*). Arm-length ratios near zero indicate a greater disparity and, hence, more recent division.

In autumn 1997, I measured 29 female and 49 male brittle stars. I removed a single gonad from each individual and placed it in 1 ml of sea water. For females, I counted eggs in three 0.1 ml aliquots, and used the average to estimate the total number of eggs in the gonad. Estimates of sperm numbers were made from eight replicate counts of formalin-fixed sperm on a haemocytometer. I performed an ANCOVA to estimate the effects of both sex and the arm-length ratio on gamete number. The number of gametes per gonad was log-transformed, and the best approximation to a normal distribution for the arm-length ratio (*S*/*L*) was calculated as $[(S/L + 0.01)^{0.8} - 1]/0.39358$ using the Box–Cox procedure.

Examination of clonal structure allowed determination of whether cloning increased the number of clone-mates contributing to gamete production by the clone. I calculated the mean and 95% confidence intervals for the number of mature clone-mates in all clones with two or more clone-mates. I compared the proportion of mature clone-mates (in clones with more than a single clone-mate) using a two-way ANOVA with potential mate and sex as factors. The proportion of mature clone-mates was transformed (log (proportion mature)) $\times$ 0.314) using the Box–Cox procedure.

3. RESULTS

(a) *Field observations*

In the 86 SS ($r^2 = 0.33$) and 31 MS ($r^2 = 0.40$) populations, the number of mature animals increased with population size ($F = 41.76$ and 19.65, respectively, $p < 0.001$ for both), but MS populations had more mature animals than SS populations at all population sizes ($F = 6.06$, d.f. = 1, $p = 0.015$). There was no interaction between population size and the presence of a mate ($F = 0.13$, d.f. = 1, $p = 0.716$).

(b) *Observations of clonal structure*

Data on population sizes and sampling intensity are located in electronic Appendix B (available on The Royal Society's Publications Web site). PCR amplification with the two oligonucleotide primers generated 50 bands present in some, but not all, individuals. On average, I could distinguish over 90% of the clones in each population with only the first 12 bands from a single primer. In early trials, I was able to discriminate clearly between non-clone-mates using either primer. Known females and males were never assigned to the same clone, and no clones were distributed in multiple populations. The analysis identified 21 and 24 clones of known sex from SS and MS populations, respectively. Clones from SS populations had significantly more clone-mates (mean untransformed size = 9.14, s.d. = 6.57, median = 8) than clones from MS populations (untransformed mean = 4.79, s.d. = 4.13, median = 3, $F = 7.60$, d.f. = 1, $p = 0.01$; see figure 2). Neither clone sex ($F = 0.24$, d.f. = 1, $p = 0.63$) nor the interaction of sex and a potential mate ($F = 0.92$, d.f. = 1, $p = 0.34$) significantly affected clone size.

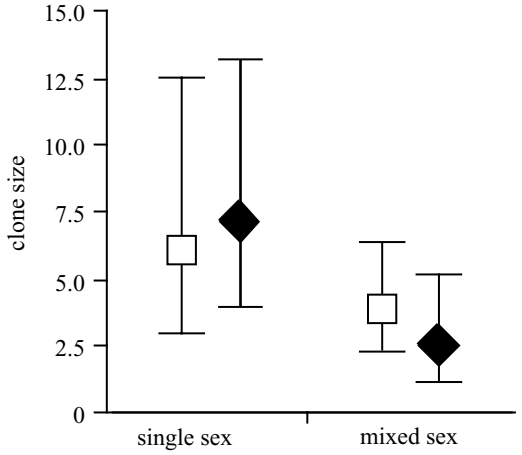

Figure 2. Sizes of female (filled diamonds) and male (open squares) clones in SS and MS populations. Shown are back-transformed means and 95% confidence intervals.

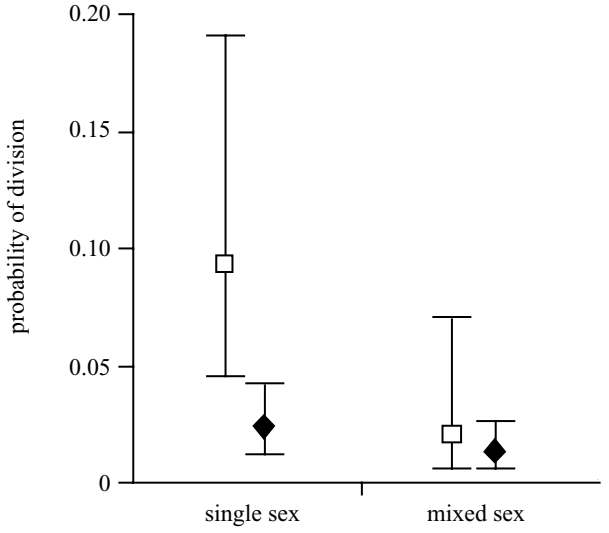

Figure 3. Probabilities of division for females (filled diamonds) and males (open squares) in SS and MS treatments. Shown are back-transformed means and 95% confidence intervals.

(c) *Rates of clonal division*

Animals with mates were more than four times less likely to divide than those without a mate ($F = 5.86$, d.f. = 1, $p = 0.019$; figure 3). Females were approximately four times less likely to divide than males (reported in McGovern 2002*b*) and there was no interaction between sex and the presence of a potential mate ($F = 1.150$, d.f. = 1, $p = 0.288$).

(d) *Costs and benefits of clonal reproduction to gamete production*

In the ANCOVA to determine the effects of sex and the recency of division on the number of gametes per gonad, the number of gametes decreased significantly with decreasing arm-length ratios, or more recent clonal division ($F = 7.43$, d.f. = 1, $p = 0.008$). There were more sperm per testes than eggs per ovary at all arm-length ratios ($F = 280.26$, d.f. = 1, $p < 0.001$), but the sexes did not differ in the relationship between gamete number and arm-length ratio ($F = 0.73$, d.f. = 1, $p = 0.395$). The slope of the relationship between arm-length ratio and gamete number was significantly positive for males ($r^2 = 0.17$, $p = 0.004$, $n = 49$) but not significant for females when considered alone ($r^2 = 0.05$, $p = 0.222$, $n = 29$).

Clones with more than one clone-mate averaged 2.1 sexually mature clone members (s.d. = 2.6). The 95% confidence interval ranged from 1.2 to 2.9, indicating that clones with multiple clone-mates contained significantly more than one mature animal. On average, 20.2% of the clone-mates from clones in SS populations were sexually mature (17.2% and 23.2% in female and male clones, respectively), whereas 40.4% of clone-mates from clones in MS populations were mature (48.2% and 36.4% in female and male clones, respectively). Although clones from MS populations were smaller, a greater proportion of their clone-mates were sexually mature ($F = 9.28$, d.f. = 1, $p = 0.005$). Sex ($F < 0.01$, d.f. = 1, $p = 0.953$) and the interaction of sex and a potential mate ($F = 2.07$, d.f. = 1, $p = 0.160$) were not significant factors.

4. DISCUSSION

(a) *Plastic reproductive strategies*

Several lines of evidence indicate that a brittle star's reproductive strategy is plastic with respect to its sexual neighbourhood. Across a range of population sizes, MS populations had more mature animals and, despite being smaller, clones from MS populations had a greater proportion of mature clone-mates than clones from SS populations. Clones of known sex from SS populations had almost twice as many clone-mates as clones from MS populations, suggesting that asexual reproduction was more prevalent in the absence of mates. However, a comparison of clone sizes in naturally occurring SS and MS populations of *O. savignyi* may be complicated. For example, the distribution of clones across multiple sponge populations may make estimation of clone sizes difficult. Disappearance of animals, particularly males, from the field experiment following the breeding season (McGovern 2002*b*) indicates that males may have greater emigration rates, which would disproportionately affect the estimation of male clone sizes. This could explain the similar sizes of female and male clones despite the large differences in division rates (McGovern 2002*b*). While no clones were detected in multiple sponges in this study, clone-mates in other sponge hosts might have remained undetected owing to incomplete sampling of populations and failure to collect neighbouring sponges, which are more likely to harbour clone-mates. More information on the migration behaviour of individuals as well as analyses of clonal structure within an explicit spatial framework could indicate whether migration might affect the estimation of clone size.

Results from the field experiment support the suggestion that clonal reproduction decreased in the presence of a potential mate. Individuals with mates, regardless of sex, were much less likely to divide than animals without mates. Though the mechanism by which mates are detected by *O. savignyi* is not known, many animals can communicate their sexual status through the release and reception of pheromones and other chemicals (Kaplan 1983; reviewed in Hardege 1999; Mitchell & Carvalho 2002). *Ophiactis savignyi* probably uses some sort of

waterborne cue to sense the presence of mates and adjusts its reproductive allocation strategies accordingly.

(b) *Costs and benefits of plastic clonal strategies*

Animals that divide may lose gonads in one or both new clone-mates (McGovern 2002*b*). This study indicates that there is a loss of fecundity even in animals that retain gonads. Though the relationship was not significant for females when they were considered alone, the power to detect the relationship in females (0.22) was lower than in males (0.86). Nevertheless, both the complete loss of gonads in one or both halves (particularly in females) and the decreased number of gametes per gonad (particularly in males) suggest that division results in reduced gamete production in animals of both sexes. This study also demonstrates the potential for long-term benefits of cloning in *O. savignyi*. The presence of multiple mature clone-mates means that several of them can produce gametes during any single mating season, in addition to the benefits of enhanced longevity resulting from risk spreading (Cook 1979; Highsmith 1982; Hughes *et al.* 1992).

The net fitness effect of these costs and benefits will depend on an individual's sexual neighbourhood. For animals near mates, the conversion of gametes into offspring is likely to be high, and loss of gametes following division would result in reduced offspring production. For mature animals in SS populations, however, reduced gamete-producing capabilities following division may result in little fitness loss beyond that already associated with mate limitation. In other words, because there is little likelihood of fertilization success, cloning in SS populations may not involve the same trade-off between asexual and sexual reproduction. Because individual brittle stars appear to be adopting strategies that are likely to maximize fitness in their particular sexual neighbourhood, the observed plasticity in reproductive strategy may be adaptive in *O. savignyi*. Other studies of altered asexual strategies in response to pollination failure in plants (Paige & Whitham 1987; Westley 1993) seem to represent passive plasticity (i.e. making the best of a bad situation) rather than active plasticity that is more likely to be adaptive (Schmid & Weiner 1993; Cheplick 1995; Dorn *et al.* 2000).

Limitation of mating opportunities is ubiquitous in nature and may be one of the major engines driving adaptive evolution (Darwin 1874; Andersson 1994; Burd 1994; Levitan 1998). A variety of traits may help circumvent limited mating opportunities, including self-fertilization in the absence of outcrossing (Jain 1976; Lloyd 1992; but see Herlihy & Eckert 2002) and aggregative spawning in species with external fertilization (Levitan 1998). This study of *O. savignyi* demonstrates active plasticity in clonal reproductive strategies in response to the mate limitation. Our understanding of the evolution of life-history traits such as reproductive allocation has previously relied on discussions of the average costs and benefits of the behaviour to individuals (Caswell 1985; Stearns 1992). However, this study and the growing number of demonstrations of plasticity in reproductive traits (Clauss & Venable 2000; Hughes *et al*. 2002; Patricelli *et al*. 2002, among others) emphasize the need to consider the costs and benefits of reproductive traits across the range of potential 'environments' experienced by individuals.

The author thanks C. Eckert, R. Ellington, J. Grubich, M. Hellberg, D. Houle, D. Levitan, M. Mesterton-Gibbons, K. McGhee, P. Munguia, D. Simberloff, C. Swanson, M. Taylor, J. Travis and several anonymous reviewers for comments on the manuscript. She also thanks D. Padilla and A. Winn for useful discussions on phenotypic plasticity. Financial support was provided by Sigma Xi, the Houston Underwater Club, the Florida Institute of Oceanography and National Science Foundation grant OCE-9702178 to D. Levitan.

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