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Estimates of natural allosperm storage capacity and self-fertilization rate in the hermaphroditic freshwater pulmonate snail, *Physa acuta*

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Summary

Previous estimates of allosperm storage capacities and self-fertilization rates in pulmonate snails have been derived almost exclusively from laboratory mating experiments. Here we report observations on a single adult albino *Physa acuta* collected from a natural pond in Charleston, South Carolina. Cultured in isolation from August 2002 until its death in February, this individual laid an average of 78.3 eggs per week (s.e.m. = 6.2), with a hatching success of 35.1% and an overall frequency of albino (putatively selfed) offspring of 6.4%. We found no evidence of reduced fecundity, reduced offspring viability, or increased self-fertilization over 20 weeks of observation, suggesting that stores of allosperm at no time approached exhaustion. We suggest that self-fertilization in primarily outcrossing populations of pulmonate snails may not be an adaptation, but rather may be a consequence of inefficiency in the mechanism sorting autosperm from allosperm.

Key words: Self fertilization, sperm storage, pulmonate snail, albinism

Introduction

The reproductive diversity of pulmonate snails, which may include selfing, outcrossing, or a mixture of the two, is generally viewed as a key adaptation to the challenges of colonization (Dillon, 2000). Gonads (“ovotestes”) are regionally differentiated to produce both ova and spermatozoa (“autosperm”) which are delivered down a common hermaphrodite duct. The hermaphrodite duct terminates in a region called the carrefour in freshwater (basommatophoran) pulmonates or the fertilization pouch in terrestrial (stylommatophoran) pulmonates. Although the function of the latter organ is uncontroversial (Bojat et al., 2001), the site of fertilization in the Basommatophora is less clear (Tomé and Ribeiro, 1998; Monteiro and Kawano, 2000). Further details regarding the anatomy and physiology of pulmonate reproductive systems are available in Duncan (1975), Tompa (1984), Geraerts and Joose (1984) and Runham (1988).

Although there are exceptions (Stadler et al., 1995; Doums et al., 1996; Wirth et al., 1997; Jordaens et al., 2000), outcrossing is generally favored over self-fertilization in pulmonate snails (Jarne et al., 1993; Jarne, 1995). In the cosmopolitan freshwater pulmonate *Physa acuta*, for example, snails paired in the laboratory mature at an average of 6.8 weeks and have a weighted net reproductive rate of 823 offspring/parent (Wethington and Dillon, 1997). Isolated individuals delay reproduction to an average age of 12.2 weeks, with a reproductive rate reduced to 286 viable offspring/parent, for an estimated inbreeding depression of 0.353 and an overall self-fertilization depression of 0.646. But a substantial fraction of paired *P. acuta*, while primarily outcrossing, nevertheless produce greater than 1% self-fertilized progeny. Such "mixed mating" does not appear to affect lifetime fitness.

Given the adverse fitness consequences typically expected from reproduction by complete self-fertilization, allosperm storage capacity is a critical variable in the success of an individual pulmonate colonist. The middle region of the hermaphrodite duct (the "seminal vesicle") has been attributed some function in sperm storage (Lind, 1973; Hodgson and Shachak, 1991; Clelland et al., 2001) as well as spermathecal tubules within the fertilization pouch (Haase and Baur, 1995; Rogers and Chase, 2001; Bojat and Haase, 2002). The literature contains numerous estimates of the maximum duration of allosperm storage in basommatophorans: 116 days for *Lymnaea* (Cain, 1956), 70 or 120 days for *Bulinus* (Rollinson and Wright, 1984; Rudolph and Bailey, 1985), 68, 100 or 147 days for *Biomphalaria* (Paraense, 1955; Monteiro et al., 1984; Vianey-Liaud et al., 1991) and 150 days for *Helisoma* (Madsen et al., 1983). Albinism or similar somatic pigmentation genes have been used as markers in almost all of these studies to monitor the switch from allosperm to autosperm. The duration of allosperm storage is less well documented in stylommatophorans, as most estimates rest on the assumption that self-fertilization is precluded in the study population (Owiny, 1974; Duncan, 1975; Raut and Ghose, 1979; Baur, 1988).

All of the estimates of allosperm storage capacity in the Basommatophora cited above have resulted from controlled mating experiments in the laboratory. It is not clear how well such experiments may generalize to the natural situation where a single random colonist might be transported to a new environment to found a new population. Wethington and Dillon (1991) used a naturally-occurring allozyme polymorphism to estimate the allosperm storage capacity of wild-collected *P. acuta.* They collected 35 adult snails from a pond in Charleston, SC, isolated them in individual containers, and reared all offspring produced over 60 days. None of the five largest sibships displaying suitable allozyme polymorphism showed any evidence of maternal allosperm depletion during the course of the study, suggesting that the natural capacity to store allosperm in *P. acuta* may exceed 60 days and 300–600 viable progeny.

The allozyme technique of Wethington and Dillon yielded sample sizes too small to detect self-fertilization at the level it typically occurs in *P. acuta*. Pigmentation markers, such as albinism, are a more powerful tool in this regard. It was during the 1991 study that Wethington and Dillon first discovered albino *P. acuta*, occurring in very low frequency among the progeny of four normally pigmented parents. Dillon and Wethington (1992, 1994) showed this trait to be encoded by simple recessive alleles at two complementing loci, and subsequently used albinism as a genetic marker in several studies of the reproductive biology of *P. acuta* (Wethington and Dillon, 1993, 1996, 1997).

On September 8, 2002, we collected a single albino *P. acuta* during routine sampling of the main pond at Charles Towne Landing State Park, Charleston, SC. The population inhabiting this pond (CTL, of Dillon and Wethington, 1995) was the source of the 35 wild snails initially isolated by Wethington and Dillon (1991), and the albino lines of Dillon and Wethington (1992, 1994). Note that the specific designation of the CTL population has recently changed from *Physa heterostropha* to *Physa acuta* in light of our results on reproductive isolation in the worldwide Physidae (Dillon et al., 2002).

In many years of collection at CTL involving hundreds of samples, this was the first homozygous albino individual we have encountered. The albino was a mature adult (shell length 8.9 mm), almost certainly born in the spring 4–5 months prior to collection. Given the rarity of the albinism gene in this population, it would seem reasonable to assume that such an animal would have been previously inseminated by a male (or males) homozygous for normal, pigmented genes. Hence this discovery represented an opportunity to estimate sperm storage and the natural rate of self-fertilization in the wild, using the more powerful pigmentation marker-based approach previously restricted to the laboratory.

Materials and Methods

The albino animal was isolated immediately, transferred to laboratory culture in a standard (10 oz, 210 ml) clear plastic drinking cup of filtered pond water (with a 95×15 mm Petri dish cover), and fed a commercially available flake fish food with a *Spirulina* base. At weekly intervals it was transferred to a new cup, with complete water change and feeding. The eggs (adhering in gelatinous masses to the wall of the old cup) were counted and the water changed. Old cups were monitored until hatch (approximately 2–3 weeks after adult removal), and all viable hatchlings counted and scored as to phenotype.

We used runs tests for trend data (Sokal and Rohlf, 1995) to test three hypotheses. The first hypothesis was that fecundity, measured as weekly egg production, would decrease with weeks in isolation. The second hypothesis was that offspring viability, measured as percent hatching success, would decrease as the parent switched to self-fertilization. The third was that the rate of self-fertilization, measured as percent albino hatchlings, would increase. Significant trends in any of these three variables would constitute evidence that allosperm stores in our subject snail approached depletion during the period of the experiment.

Results

The albino parent died on February 14, 2003, after 20 weeks in isolation. It bore a total of 1,566 eggs over its lifetime in culture, yielding 550 viable hatchlings for a net survivorship of 35.1%. A total of 35 hatchlings were albinos, suggesting that the overall rate of self-fertilization was effectively 6.4% during this period.

The general impression presented by the weekly results (Fig. 1) is one of volatility. Fecundity ranged from 130 eggs in week 8 to 40 eggs in week 14, and hatchling survivorship from 70.6% in week 4 to 4.8% in week 13. The highest effective rate of self-fertilization was recorded on the first week of the experiment when six albinos were counted among 36 hatchlings, and zero self-fertilization was recorded in weeks 10, 13, and 19. Weekly means were 78.3 eggs (s.e.m = 6.2), 27.5 total viable hatchlings (s.e.m. = 3.7), and 1.75 albino hatchlings (s.e.m. = 0.38).

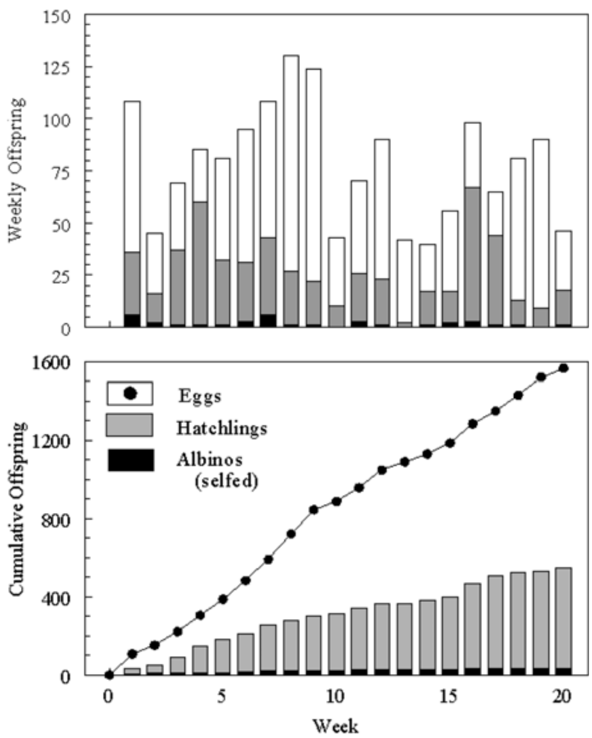

Fig. 1. Reproductive output of a single albino snail collected from a pond in Charleston, SC. Eggs (points or open bars), viable wild type hatchlings (shaded bars) and viable albino hatchlings (black bars) are shown on both a weekly basis (above) and cumulative (below).

All three tests for trend (in fecundity, in offspring viability, and in self-fertilization rate) returned insignificant results. Thus, the data in Fig. 1 contain no evidence that stores of allosperm approached depletion during the 20-week study period.

Discussion

As commonly observed in natural populations of freshwater pulmonates (Dillon, 2000), the maximum longevity of *P. acuta* in the main pond at the CTL population is approximately 1 year (Wethington and Dillon, 1997). Thus, our data suggest that the allosperm storage capacity of a typical adult pulmonate sampled during the growing season is effectively inexhaustible. At the time of its death 20 weeks after isolation, our study animal showed no evidence of reduced fecundity, reduced hatchling viability, or increased incidence of self-fertilization.

Our experiment was designed to model the natural situation where a single adult colonist might be dispersed to found a new population of snails. Given the capacity for multiple insemination displayed by pulmonates (Mulvey and Vrijenhoek, 1981; Rollinson et al., 1989; Baur, 1994; Rogers and Chase, 2002), the effective colonizing size in such an event is potentially much greater than the head count. The actual rate of self-fertilization in single colonists may be greater than the 6.4% we report here, given the reduced hatchability of selfed eggs (Wethington and Dillon, 1997), but an estimate on the basis of viable hatchlings is consistent with the model.

Using progeny arrays, Jarne et al. (2000) estimated a self-fertilization rate of 10% for a population of *P. acuta* from France. The 26 paired *P. acuta* monitored for fitness by Wethington and Dillon (1997) displayed a wide range of self-fertilization in culture, from 34.9% to 0.0% (12 instances) over their lifetimes. Wethington and Dillon divided these animals into two groups for analysis, using 1% self-fertilization as a (rather arbitrary) criterion: 16 outcrossers and 10 mixed-maters. But combined over all 26 laboratory-mated animals, the lifetime average rate of self-fertilization was 5.7%, a figure notably close to the 6.4% recorded by the single wild-collected snail analyzed here.

A 6.4% self-fertilization rate generalized over the population would imply a coefficient of inbreeding ($F = 0.061$). Dillon and Wethington (1995) surveyed allele frequencies at polymorphic allozyme loci over ten populations of *P. acuta* from the Charleston area, including the CTL population from which the present study animal was collected. Fits to Hardy-Weinberg expectations were poor in many cases; seven populations (including CTL) showing significant deficits of heterozygotes at one or more loci. Over six loci, the average coefficient of inbreeding at CTL was $F = 0.054$. The average coefficient of inbreeding within all ten populations was $F_{is} = 0.198$, with ten loci and samples of 30–70 individuals per population.

Coefficients of inbreeding ranging from 5% to 10% are commonly observed in natural populations of pulmonate snails generally (Jarne, 1995). Although not necessarily maladaptive (Jarne and Charlesworth, 1993), the mixed mating this implies would seem to have adverse fitness consequences in populations (such as CTL) where inbreeding depression is substantial. We suggest that any loss of progeny due to the low levels of self-fertilization typically observed in otherwise outcrossing populations of pulmonate snails may constitute "soft selection," substituted for nonselective background mortality within the (hundreds of) offspring sired by each parent. Hence self-fertilization in pulmonates with ready stores of allosperm may best be interpreted not as an adaptation, but rather as the product of inefficiency in internal sperm sorting processes, with little evolutionary consequence.

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