Selfing, Outcrossing, and Mixed Mating in the Freshwater Snail Physa heterostropha: Lifetime Fitness and Inbreeding Depression

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Selfing, outcrossing, and mixed mating in the freshwater snail *Physa heterostropha:* lifetime fitness and inbreeding depression

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Abstract. The purpose of this investigation was to estimate inbreeding depression and self-fertilization depression from a comparison of self-fertilizing and outcrossing pulmonate snails, *Physa heterostropha.* We monitored lifetime survival and fecundity in 26 paired snails from three isofemale lines, using recessive alleles at two complementing albino loci to verify outcrossing. While demonstrably holding allosperm reserves, 10 of these individuals bore self-fertilized progeny at frequencies ranging from 1% to 35%. We designated these 10 individuals "mixed maters," leaving 16 individuals in our sample as more strictly outcrossers. Mixed maters and outcrossers produced comparable numbers of viable embryos per parent, and their embryos displayed negligible differences in survival to 7–14 days post-hatch. Outcrossing and mixed groups displayed nearly identical values for net reproductive rate (808 and 848 offspring/parent, respectively) and intrinsic rate of natural increase (2.86 and 2.82 per four weeks). Thus in the benign environment of artificial culture, the low levels of self-fertilization occasionally displayed by the previously inseminated snails seem adaptively neutral. The fitness of both the outcrossers and mixed maters greatly exceeded that of 15 individuals isolated as juveniles and hence forced to self-fertilize entirely ($R=286$, $r=1.57$). There were no significant differences among selfers, outcrossers, and mixed-maters with respect to parental survival. But selfing parents produced fewer eggs over their lifetimes, higher proportions of apparently infertile eggs, and embryos of much-reduced survival. The estimated inbreeding depression of 0.353, together with an overall self-fertilization depression of 0.646, suggests strong selective pressure against self-fertilization, and evolution toward at least partial outcrossing.

Additional key words: hermaphroditism, self-fertilization, mating system, albinism, Pulmonata

Inbreeding depression is a key factor in the origin of mating systems (Charnov et al. 1976; Lande & Schemske 1985; Holsinger 1991). But though levels of inbreeding depression have been fairly well documented in hermaphroditic plants (Jain 1976; Schemske & Lande 1985; Charlesworth & Charlesworth 1987), the phenomenon remains comparatively unexplored in animal hermaphrodites (Jarne 1995). Few animals seem as well suited for such studies as freshwater pulmonate snails.

Pulmonate snails are simultaneous hermaphrodites generally retaining the ability to self-fertilize (reviews by Duncan 1975; Geraerts & Joosse 1984; Jarne & Delay 1991; Jarne et al. 1993). Judging from the fits to Hardy-Weinberg equilibrium obtained in allozyme studies, most wild populations of freshwater pulmonates appear to be primarily or entirely outcrossing (Jarne & Städler 1995). Individuals forced to self-fertilize in isolation usually delay reproduction and/or lay eggs at a rate less than that of outcrossing controls. Provided a partner, previously isolated snails generally copulate immediately and switch from self-fertilization (with autosperm) to outcrossing (with allosperm) within 24 hours (Richards 1973; Rollinson et al. 1989). Allosperm are then generally stored and used exclusively for hundreds of fertilizations, over periods that may range to several months, until depleted. The persistent production of both selfed and outcrossed progeny by individual snails ("mixed mating") has been noted on rare occasions, especially in polyploids (Städler et al. 1995; Doums et al. 1996).

Under some circumstances, self-fertilization has been shown to have positive effects on fitness in pulmonate snails (Noland & Carriker 1946; Smith 1981; Van Duivenboden et al. 1985). Copulation interferes with egg-laying, is energetically expensive, and may shorten total life expectancy. Evidence suggests that some allotetraploid populations of the freshwater lim-

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pet, *Ancylus*, are highly self-fertilizing (Städler et al. 1993). Aphally, the complete absence of a male reproductive system, seems to be associated with high levels of self-fertilization in some populations of *Bulinus* (Jarne et al. 1992; Schrag et al. 1994).

The tadpole snail, *Physa* (*Physella*) *heterostropha pomilia* (CONRAD 1834), is a small, rapidly growing basommatophoran pulmonate common in lakes, ponds, and ditches throughout North America. The genetic variability of *P. heterostropha*, together with its adaptability to laboratory culture, have made it a model organism for the study of hermaphroditic reproduction (Wethington & Dillon 1991, 1993, 1996). The animals used in the present experiments were from three albino lines of different complementation groups described by Dillon & Wethington (1992, 1994). These lines were isolated in the summer of 1989 from individual snails wild-collected from a single population in the Charleston area, but were mass-cultured subsequently (perhaps 10 generations total) to minimize further inbreeding effects. Although estimates of inbreeding depression derived from laboratory lines are not as interesting as those derived from the wild, we judged that the benefits of fixed genetic markers in verifying outcrossing outweighed the disadvantages.

Most comparisons of selfing and outcrossing fitness in pulmonate snails published to date have not involved genetic markers, and hence the extent to which paired snails produce offspring by self-fertilization, rather than by outcrossing as expected, is generally unknown. Most published studies also have not extended over the lifetimes of the parental snails, focusing instead on fecundities during brief periods of maturity. Previous researchers have often combined pre-fertilization components of fitness with post-fertilization components, rendering dubious the calculation of inbreeding depression.

Here we define the fitness of an individual snail (n_a) as its lifetime production of offspring alive (crawling) 7–14 days post-hatch. There may be two components to any reduction in individual fitness suffered by a self-fertilizing snail relative to an outcrossing one. A pre-fertilization component may result from reproductive delays or from any other factor that might decrease the total eggs ovulated by a selfing parent. A post-fertilization (or "progeny") component would derive from any reduction in the fitness of its offspring. "Inbreeding depression" is properly calculated only from the latter component of reduction in fitness, as it is the progeny that are inbred, not the self-fertilizing parent itself. We estimated the progeny fitness of the j^th^ population (w_j) by summing the apparently viable, fertile eggs (n_e) laid by all snails in population j and comparing this figure to the summed offspring (n_a) alive 7–14 days post-hatch ($w_j = \Sigma n_a / \Sigma n_e$). Then inbreeding depression was calculated as 1 minus the ratio of selfing progeny fitness to outcrossing progeny fitness ($1 - w_s/w_o$, Lande & Schemske 1985).

There is currently no terminology uniquely describing the pre-fertilization component of the reduction in fitness suffered by self-fertilizing snails. But the overall reduction, including both pre- and post-fertilization components, has been labeled "self-fertilization depression" (Jarne et al. 1991). For the j^{th} population, the overall fitness has been defined as either net reproductive rate R_j ($=\Sigma l_x m_x$) or intrinsic rate of natural increase r_j (Kozlowski 1993). In this paper, relative fitness was defined as $W_j = R_j/R_{max}$, where R_{max} was the largest R measured. "Self-fertilization depression" was calculated as $1 - W_s/W_o$, by analogy to the definition for inbreeding depression of Lande & Schemske (1985).

The purpose of this investigation is to compare the lifetime fitness of outcrossing laboratory lines of the freshwater pulmonate snail *P. heterostropha* to the fitness of those reared in isolation and forced to self-fertilize. In so doing, we distinguish mixed-mating individuals from those more purely outcrossing, and inbreeding depression (in its strict, post-fertilization sense) from the larger phenomenon of self-fertilization depression.

Methods

The three lines involved in these experiments have been described by Dillon & Wethington (1992). Line-27 and line-29 are fixed for a recessive albino allele at a locus we have designated *alb1*, and line-7 is fixed for a recessive albino allele at a complementing locus *alb2*. The lines differ in their mean size at reproduction, but not their mean age at reproduction, under our culture conditions. The mean age at onset of male maturity is 5.7 weeks, female maturity arrives at 7.3 weeks, and (in isolation) self-fertility commences at a mean age of 22 weeks (Wethington & Dillon 1993). Complete inability to self-fertilize seems to vary greatly, from 44% of individuals in line-27 to a negligible fraction of line-29. We have also noted 11% outcross sterility, primarily in male function alone, across all three lines.

For the self-fertilization treatment, 15 parent snails (7 from line-7, 4 from line-27, and 4 from line-29) were isolated at age two weeks, well before the onset of reproductive activity (Wethington & Dillon 1993). They were held singly in 10 oz. (~220 ml) clear plastic drinking cups of filtered pond water and fed weekly (Tetra Conditioning Food with vegetable enriched flakes), at water change. We counted the number of

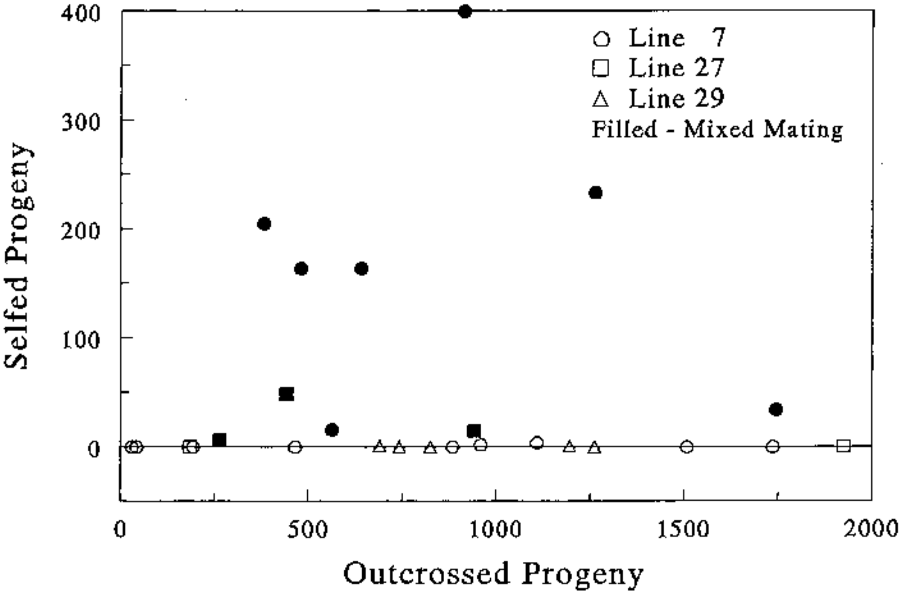

Fig. 1. The proportion of albino (selfed) offspring and pigmented (outcrossed) offspring produced by the 26 parents in the outcrossing treatment. Individuals displaying greater than 1% self-fertilization were designated "mixed maters."

eggs in any egg masses present at each water change, noting any (entirely clear, misshapen) eggs that appeared infertile. We then transferred each parent to a new cup. We returned to count total viable offspring in all cups 7–14 days post-hatch, as well as any empty shells that accumulated on the vessel bottom. This procedure was followed throughout the life of each parent snail. Cups containing all snails (both parents and offspring) were held randomly on large tables at room temperature (23°C). Logistical constraints precluded the culture of offspring beyond 14 days.

We simultaneously estimated the lifetime fecundity of 26 outcrossing parents for comparison to our self-fertilizing sample. We paired 16 snails from line-7, 5 from line-27, and 5 from line-29 with marked, complementing partners for 24 h weekly from age two weeks until onset of egg laying. (That is, homozygous *alb2* individuals of line-7 were paired with homozygous *alb1* partners of either line-27 or line-29.) We then removed each paired snail to a separate cup, to avoid any crowding effects on fecundity (Doums et al. 1994). Partner snails were randomized. As the sperm storage capacity of individuals of *P. heterostropha* exceeds 60 days (Wethington & Dillon 1991), we felt safe pairing adult outcrossers for 24-h periods at intervals as great as 30 days after their maturity. Egg masses were evaluated, counted, and isolated weekly as in the selfing experiment, empty shells counted, and viable offspring counted and scored for pigmentation. Egg masses laid during 24-h periods of pairing could not be attributed to a parent, and were discounted.

Albino offspring were in fact observed among the progeny of some of the parents in our "outcrossing" treatment. This confirmed that facultative self-fertilization may occur in *P. heterostropha* holding allosperm reserves, as has been previously described in planorbid pulmonates (Vianey-Liaud et al. 1991). We thus subdivided our "outcrossing" parents into the 10 individuals showing rates of self-fertilization greater than 1% (the "mixed maters") and the 16 individuals more truly outcrossing.

We tested for differences in median individual fitness (n_a) within mating treatment between isofemale lines using Kruskal-Wallis and Mann-Whitney tests, and finding none, combined data across lines. We then estimated the progeny fitness (w) and inbreeding depression for the groups of 15 selfers, 10 mixed maters, and 16 outcrossers from the fractions of their total, apparently fertile, eggs yielding young snails alive 7–14 days post-hatch. We also calculated survival (l_x) and fecundity (m_x) schedules for each of the three groups by dividing our study period into standard "months" of four weeks' duration. We calculated net reproductive rate R ($=\Sigma l_x m_x$) and estimated intrinsic rate of natural increase r from population growth rates projected to stable age distribution using spreadsheet software. Then taking overall fitness as R, we calculated relative fitnesses (W) and self-fertilization depression.

All 41 experimental animals survived into month 4, by which time reproduction was well under way. Reproduction peaked in months 2 through 6, and had become negligible by month 16, when the last parent died.

Results

Albinos were detected among the progeny of 14 of the 26 parents in our outcrossing treatment, represent-

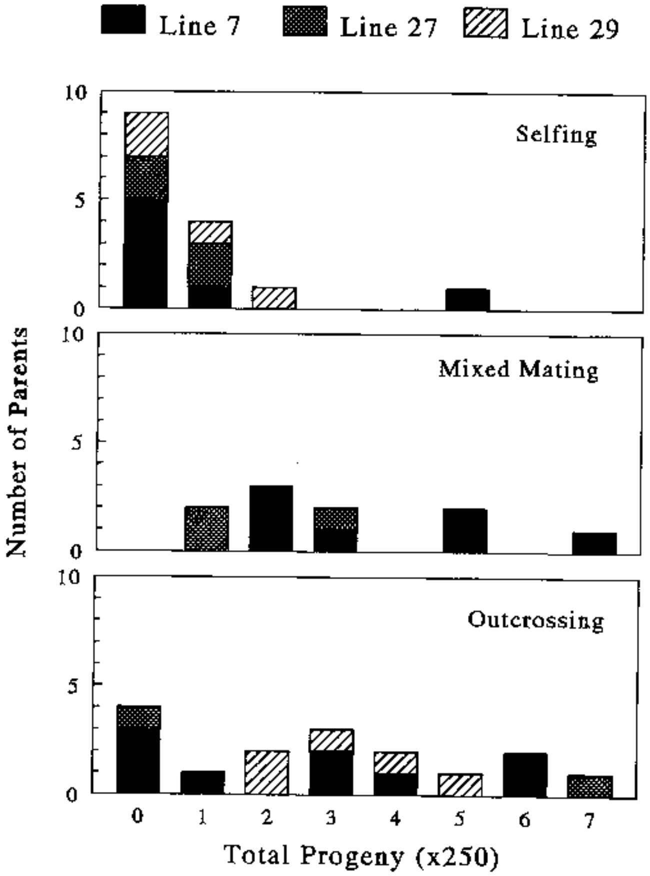

Fig. 2. Comparison of individual fitness (n_a) in three reproductive groups of *P. heterostropha*. The X-axis, divided for convenience, shows lower bounds; i.e., "category 1" includes parents producing 250–499 offspring.

ing all three lines, and were in evidence throughout the 13 months of "outcrossing" reproduction. Individual fractions of selfed progeny ranged up to 35% (Fig. 1), with no detectable relationship to overall fecundity (product-moment correlation = 0.02). Single albino offspring were found among the progeny of 2 line-29 parents, and 2 additional line-7 parents bore 2 and 4 albinos each. Since these 4 cases represented such low self-fertilization frequencies ($\ll$1%), we elected to retain them in the "outcrossing" group. The data seemed to suggest a natural division between 10 mixed maters, all of which had selfing fractions greater than 1%, and 16 outcrossers.

Figure 2 shows the individual fitnesses of the 41 parents in the (now three) reproductive groups. Although sample sizes rendered statistical tests rather weak, we found no difference between the three lines. Kruskal-Wallis tests uncovered no among-line difference in the selfing group (exact $p = 0.23$) or the outcrossing group ($p = 0.84$), and a Mann-Whitney test found no difference between lines 7 and 27 in the mixed mating group ($p = 0.38$).

Combining across lines, the survival of the parents in the three reproductive groups (Table 1) was not significantly different (Kruskal-Wallis test, $p = 0.46$). The last outcrosser died in month 15, while the last selfer died in month 16 (Fig. 3). All groups began reproduction in month 2, at 11.2 viable offspring per selfing parent, 154 offspring per mixed mater, and 157 per outcrosser in that month (Fig. 4). Production of viable offspring peaked for mixed maters and outcrossers at month 3 (196 and 192 offspring per parent, respectively) and at month 4 for the selfers (57.7 per parent). Net reproductive rates (R) were 286, 848, and 808 offspring for selfers, mixed maters, and outcrossers, respectively, corresponding to intrinsic rates of natural increase (r) of 1.57, 2.82, and 2.86 per standard (four week) month. Then the relative fitness (W) of the mixed maters was 1.0 with fitnesses of 0.953 and 0.337 for outcrossers and selfers, respectively. The cal-

Table 1. Comparison of fitness traits in the three reproductive categories of *P. heterostropha*, summed over all individuals and months.

	Selfing	Mixed mating	Outcrossing
Number of parents	15	10	16
Median parent survival (weeks)	46	54	45
Total eggs laid	18,340	20,076	29,012
Eggs apparently fertile (Σn_e)	13,125	17,044	26,637
Live offspring (Σn_a)	4,388	8,925	13,753
Dead offspring recovered	1,048	1,997	2,553
Overall progeny fitness ($w = \Sigma n_a/\Sigma n_e$)	0.334	0.524	0.516
Inbreeding depression ($1 - w_s/w_o$)	0.353	—	—
R ($\Sigma l_x m_x$)	286	848	808
r (per 4 weeks)	1.57	2.82	2.86
Relative population fitness (W)	0.337	1.00	0.953
Self-fertilization depression ($1 - W_s/W_o$)	0.646	—	—

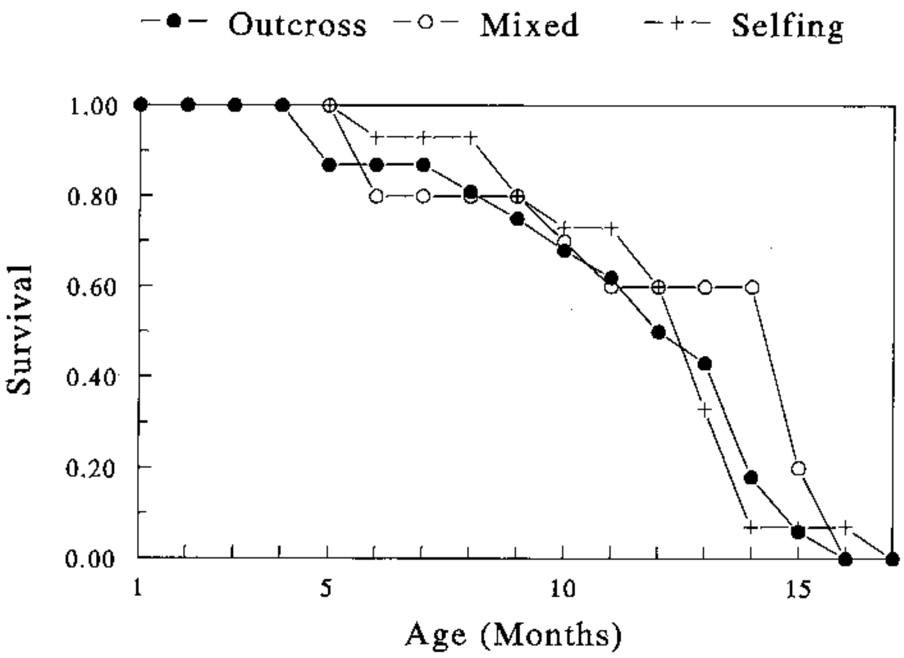

Fig. 3. Proportions of N = 15 self-fertilizing snails, N = 10 mixed maters, and N = 16 outcrossers alive at the beginning of month *x*.

culated self-fertilization depression, comparing selfers to pure outcrossers, was 0.646.

Summed over the entire experiment, selfers laid fewer fertile eggs and their offspring were less viable (Table 1). Per parent, selfers laid an average of 1,223 eggs over their lifetimes, of which about 28% appeared infertile on gross inspection, as compared to 2,008 per mixed mater (15% infertile) and 1,813 per outcrosser (8% infertile). The 13,125 apparently fertile eggs laid by selfers yielded only 4,388 offspring assessed at age 7–14 days, for a progeny fitness of 0.334. The outcrossed offspring had a much greater progeny fitness (w = 0.516), for a calculated inbreeding depression of 0.353. The offspring of mixed maters showed no better progeny fitness (w = 0.524) than those of pure outcrossers; the slight difference was not statistically significant (Yates-corrected $\chi^2 = 2.21$, 1 d.f.).

The recovery rate for empty shells of dead offspring was low, as might be expected from their small size ($\ll 1$ mm). The empty shells recovered represented 12% of the mortality among selfed offspring, very significantly less than the 20% of the outcrossed mortality and the 25% of the mixed mating mortality represented ($p < 0.0001$ in both cases, Yates-corrected χ^2). One might therefore infer that self-fertilized embryos tend to suffer problems earlier in their development than do outcrossed, and hence their dead are less likely to leave a recoverable shell record.

Discussion

Prior comparisons of fitness in selfing and outcrossing freshwater pulmonates have yielded quite diverse results, reflecting the diversity of the experimental approaches taken. Early workers (Noland & Carriker 1946; DeWitt 1954a,b) recorded higher lifetime fecundities in isolated snails than in outcrossers, perhaps because the fitness of their outcrossers was assessed in mass culture, where snails are subject to crowding and resource competition. Working with *Pseudosuccinea columella,* DeWitt & Sloan (1958) estimated lifetime fecundity as 756 viable offspring per isolated parent, and only 360 per paired parent. But because reproduction began earlier in paired snails, their intrinsic rate of increase was slightly better than that of the isolates. DeWitt & Sloan (1959) reported 501 viable offspring per paired parent, and only 157 per isolated parent, in a Florida population of *Physa heterostropha.* Although carried through the lifetimes of the parental snails, both studies involved only three pairs and three isolates (DeWitt & Sloan 1958, 1959). In neither study did the researchers distinguish inbreeding depression (as reduced egg hatchability and survival of offspring) from overall self-fertilization depression (including reduced fecundity of parents).

Several more recent studies have distinguished inbreeding depression from self-fertilization depression, but have not extended over the lifetimes of the parents. Jarne & Delay (1990) compared the reproductive output of selfed and outcrossed individuals of *Lymnaea peregra* over 40 days, the hatchability of their eggs, and survival of offspring to 30 days. Selfers were only 6% as fit as outcrossers, with inbreeding depression estimated at 0.88. Jarne et al. (1991) gathered data on two generations, comparing selfing and outcrossing fit-

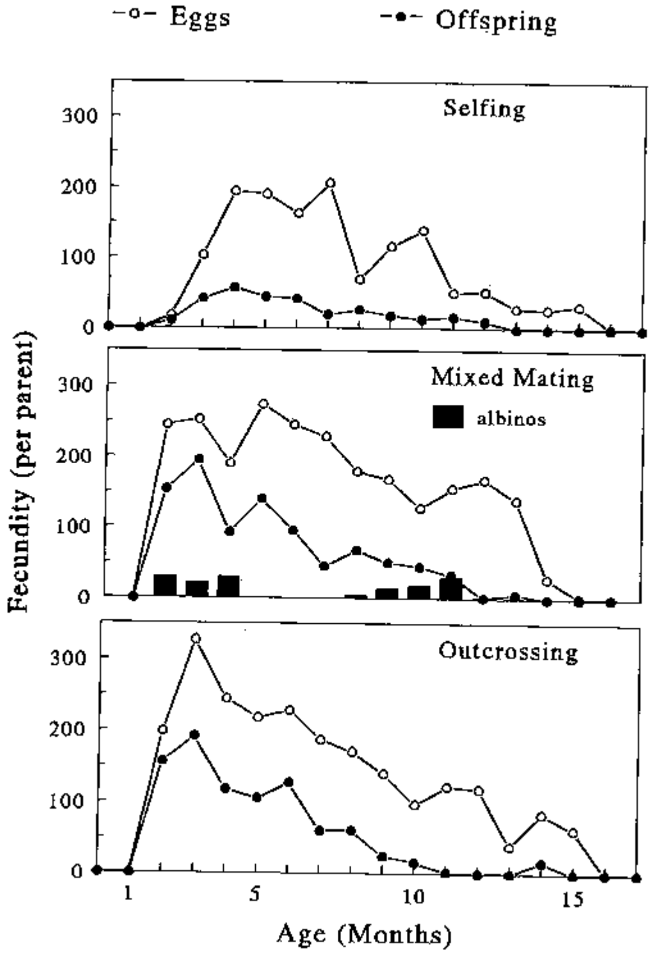

Fig. 4. Total eggs laid (apparently viable and non-viable together) and living offspring counted (7–14 days post-hatch) per parent surviving into month *x*. The mixed-mating graph also shows the fraction of albinos among the living offspring.

ness of *Bulinus globosus* from Niger. The 30-day fecundities were depressed about 50% in isolated parents, the hatchability of selfed eggs was only about 80% that of the outcrossed, and the fertility of inbred snails at age 90 days was reduced to about 60% of the outcrossed. The overall estimate of self-fertilization depression derived by Jarne et al. was 0.92. Njiokou et al. (1992) compared selfing and outcrossing abilities in populations of *Bulinus globosus* originating from two sites in Africa: Niger (normally outcrossing, to judge from allozyme frequencies) and the Ivory Coast (apparently naturally selfing to some extent). Isolated Niger snails showed a 20% reduction in 11-day fecundity, and their offspring showed an inbreeding depression of about 0.77 (egg to 30 d). Ivory Coast snails showed improved fertility and offspring survival when reared in isolation. Doums et al. (1996) confirmed a reduction in fecundity for isolated *B. globosus* of Niger, but showed improved fertility for isolates from (apparently normally self-fertilizing) populations of *Bulinus truncatus* and *Biomphalaria straminea.*

Our value of 0.353 for inbreeding depression may in fact underestimate its true value in the wild. We have no observations on the long-term viability or fertility of progeny, but such data often reflect additional adverse consequences of inbreeding. Extension of the period over which we monitored offspring viability from 14 to 30 days (as in some previous studies) might have yielded a higher inbreeding depression estimate. Our value of 0.646 for self-fertilization depression may also be artificially low, since the isofemale lines upon which these estimates are based were initially derived from demonstrably self-fertile parents. Furthermore, our estimates of outcross fecundity were reduced by about 3%, roughly the proportion of egg masses laid while snails shared culture facilities with complementing partners. In any case, such levels of inbreeding depression and self-fertilization depression, displayed even by laboratory lines, strongly suggest selective pressure against selfing and evolution toward outcrossing.

However, as might be expected from our favorable culture conditions, rates of population increase for all groups were much greater than could be supported in the wild. Values of λ (the factor by which each population increases per four weeks) were 4.8 for the selfing group, 16.8 for the mixed mating group, and 17.4 for the outcrossing group, corresponding to population doubling times of 12.3 days, 6.9 days, and 6.8 days, respectively.

There may be both within-line and between-line components to the fitness advantage enjoyed by our outcrossers. Part of the benefit may have been attributable to the provision of a partner from a second line carrying beneficial traits previously unavailable, and part of the benefit may have been due to outcrossing *per se,* even within line. Evidence of substantial fitness variation within lines was indeed found (Figs. 1, 2). A partition of the benefits of outcrossing is beyond the scope of the present work, but would provide some opportunity for further analysis in the future.

Given the disadvantage that apparently attends self-fertilization, one might expect mixed maters to display lower fitness than outcrossers. But the fitness of our mixed mating group was comparable to that of our outcrossing group in all respects. Although mixed maters laid more eggs over their lifetimes, their higher proportion of apparently infertile eggs brought their average output of viable eggs to 1,704, almost identical to the 1,665 posted by outcrossers. Progeny fitnesses to 7–14 days (*w*) were not different. The mixed maters achieved a slightly higher net reproductive rate over this study period, but comparison of intrinsic rates of increase indicates that the outcrossers would have an advantage at equilibrium. Thus, self-fertilization in individuals of *P. heterostropha* known to hold reservoirs of allosperm seems neither adaptive nor detrimental in our culture conditions.

Mixed mating is commonly observed in hermaphroditic plant populations, and some plants even show special floral adaptations for it. Schemske & Lande (1985) suggested that partial selfing in species which primarily outcross may be an "incidental by-product" of incomplete autosterility, and that mixed mating is not evolutionarily stable. Holsinger (1991) has observed, however, that mixed mating strategies can evolve in situations where a selfer's contribution to outcrossed progeny is reduced in proportion to its selfing rate ("pollen discounting"). Mixed mating may also arise should asexual reproduction yield more offspring, but parasites select for outcrossed progeny (Lively & Howard 1994). The circumstances under which selfing rates may stabilize between zero and one have been reviewed by Jarne & Charlesworth (1993) and Waller (1993).

Judging from gene frequencies at 5 polymorphic isozyme loci, the natural population from which our isofemale lines were founded appears to be primarily or entirely outcrossing (Dillon & Wethington 1995). But a survey of 9 other populations of *P. heterostropha* in the vicinity of Charleston uncovered several with quite striking deficiencies of heterozygotes at multiple loci. Thus, there is some evidence that inbreeding, perhaps including partial self-fertilization, may be as widespread a phenomenon in natural pulmonate populations as it seems in populations of hermaphroditic plants.

The occasional self-fertilized brood that some individuals of *P. heterostropha* mix with their outcrossed progeny may simply represent inefficiency in the fertilization process, so close to selective neutrality as to persist in the population. Or mixed mating may have evolved as an adaptation to "sperm discounting" or similar phenomena. Data regarding the heritability of mixed mating in this situation would clearly be of interest. This issue will remain an area of active research in the future.

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