The Inheritance of Albinism in a Freshwater Snail, *Physa heterostropha*

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Complementation tests revealed that albinism in four laboratory strains of *Physa* (*Physella*) *heterostropha pomilia* resulted from two recessive, nonallelic genes. F_2 dihybrid progeny displayed the 9:7 ratio classically associated with reciprocal recessive epistasis between unlinked loci. This offers a contrast to the situation in the better known planorbid snails and provides a valuable tool for the study of reproductive biology in these facultatively self-fertilizing hermaphrodites.

Pulmonate snails (Subclass Pulmonata, Order Basommatophora or Lymnophilia) are among the most diverse and conspicuous inhabitants of the world's rivers, lakes, and ponds. The attention of geneticists has quite naturally focused on the members of the family Planorbidae that serve as intermediate hosts for human schistosomiasis in Africa and in the Americas—*Biomphalaria* and *Bulinus.* Albinism in *Biomphalaria* was first described and characterized as a single-locus recessive trait by Newton (1954). Subsequent work has demonstrated two or three additional alleles at this locus (depending on species), coding for varying amounts of pigmentation (Richards 1970, 1973, 1975, 1978). A second, unlinked locus controls the distribution of pigment on the mantle, with three alleles thus far recognized (Richards 1985). Of 15 enzyme loci examined to date, linkage has been detected only between the mantle pigmentation locus and the locus that encodes 6-phosphogluconate dehydrogenase (Mulvey and Vrijenhoek 1984; Mulvey and Woodruff 1985; Mulvey et al. 1988).

Helisoma duryi, a large planorbid native to Florida, has received some attention as a possible biological control agent against *Biomphalaria* and *Bulinus.* Madsen and colleagues (1983) demonstrated that albinism in a strain of *Helisoma* introduced to Kenya was due to a recessive allele at a single locus. Jelnes (1982) detected no linkage between the albino locus of *Helisoma* and four enzyme loci. Albinism has not been described in *Bulinus,* but Rudolph and Bailey (1983) reported that two codominant alleles control mantle pigmentation pattern.

Much of the interest in the pigment mutants of planorbids has been due to their utility as markers in breeding studies. Pulmonate snails are hermaphroditic, and although they prefer to outcross, most retain the ability to self-fertilize (Paraense 1956; Paraense and Correa 1988; Richards 1970). They are capable of multiple mating and long-term storage of exogenous sperm (Rollinson et al. 1989; Rudolph and Bailey 1985; Vianey-Liaud et al. 1987). Monteiro and colleagues (1984) described the phenomenon of "sperm sharing" in *Biomphalaria,* where an individual snail passes a second snail's sperm along to a third. This has been controversial (Paraense 1987; Rollinson et al. 1989). To date, most published studies on these subjects have involved pigmentation as a genetic marker.

Much less attention has been paid to the three other major families of pulmonates. There is older literature describing a single locus for albinism in the family Lymnaeidae (Boycott and Diver 1927; Cain 1956). But to our knowledge, the inheritance of body coloration has not been investigated in the Ancylidae or the Physidae. This is unfortunate, for physids in particular are easier to obtain and culture, and thus they could serve as lab animals for studies of general importance, such as those on sex allocation. In 1989 we initiated genetic studies of *Physa* (*Physella*) *heterostropha pomilia* (Conrad), an extremely common and widespread inhabitant of North American rivers and ponds. We isolated 35 individuals collected at a local pond (described in Dillon and Dutra-Clarke, in press) and separately reared all the egg masses laid by each over a period

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of 60 days. By comparing the leucine-aminopeptidase genotype of each parent to a sample of its progeny, we demonstrated that wild-caught *Physa* generally carry large stores of exogenous sperm, just as has been demonstrated in lab studies of planorbids (Wethington and Dillon, 1991). We also found evidence of multiple insemination.

All 35 of the original parents collected from the wild showed typical body coloration. But in the course of rearing hundreds of F_1 offspring from these parents, we noticed individual albinos that had no pigment whatsoever in the body or eye. We isolated albinos from four different parents, numbered 7, 15, 27, and 29. Here we report the results of our experiments on the inheritance of albinism in *Physa*.

Methods

We reared snails for these experiments in 10-ounce (230-ml) clear plastic cups of pond water, with plastic petri dish lids. Snails were fed ground commercial fish food weekly, and their water was changed monthly. Mature, outcrossed adults commonly produce an egg mass of 20–40 embryos every 24–48 h, which is then attached by a gelatinous capsule to the cup wall. We transferred actively reproducing adults into new cups every few days to avoid overcrowding in the next generation.

We established pure albino cultures of the four lines, 7, 15, 27, and 29, by rearing individuals in isolation until they self-fertilized. (Reproduction is both delayed and reduced in isolated snails.) Then we made complementation tests between individuals from all pairs of cultures. Two to four matings were set up for each complementation test; each mating consisted of snails paired at age 2 weeks, which is long before sexual maturity. At maturity, each pair of snails was allowed to lay several hundred offspring, in multiple cups. We characterized the F_1 juveniles according to their phenotype: "wild type" if eyes were black or "albino" if eyes were unpigmented.

In cases where the F_1 progeny were wild type (i.e., the genes "complemented" one another), three pairs of F_1 were isolated at age 2 weeks and allowed to mate to produce an F_2 generation. We counted and scored the F_2 offspring to a sample size of about 300, approximately the number at which a 1:1 ratio will be rejected by a goodness-of-fit test to a 9:7 ratio. This was done by scoring whole cups of offspring, evenly distributed among the three pairs of F_1 mates. The 9:7 ratio was then tested using chi-square.

In cases where only albino F_1 progeny were obtained, self-fertilization, although highly unlikely, could not initially be ruled out. Thus we compared the isozyme phenotypes in a sample of the F_1 progeny to those of their parents using horizontal starch gel electrophoresis. Tissues were prepared and samples were analyzed using techniques described previously (Dillon 1982, 1985, 1986). Because the wild population from which these cultures were founded is polymorphic at three presumptive esterase loci, esterases were especially helpful in verifying outcrosses. In an analysis that used electrophoretic techniques similar to ours (TEB8 buffer and α-naphthyl acetate as substrate), Mulvey and Vrijenhoek (1984) demonstrated Mendelian inheritance at three esterase loci in *Biomphalaria*.

Results

The results of our complementation tests are shown in Table 1. Even though several separate matings were performed for each complementation test, and hundreds of F_1 offspring were examined for each mating, all results were consistent. Each pair of strains produced either 100% albino offspring or 100% wild type. Table 1 suggests that the albino mutations fixed in lines 15, 27, and 29 are allelic, but that the line 7 mutation seems to be in a different gene.

Examination of the isozyme phenotypes in P and F_1 snails for each of the 15 × 27, 15 × 29, and 27 × 29 crosses revealed no results inconsistent with outcrossing. Indeed, isozyme frequencies provided strong evidence for outcrossing in several cases. For example, one pair of parents in the 15 × 29 test included a heterozygote and a homozygote at the *Est2* locus (numbering from anode to cathode, as Mulvey and Vrijenhoek 1984). The eight offspring examined included six snails homozygous for the parental allele and two heterozygotes,

Table 1. Results of complementation tests between four albino strains of *Physa heterostropha*; a plus sign (+) indicates wild-type coloration in the F_1, while a minus sign (−) indicates that F_1 offspring were albinos

Strain no.	Strain no. 7	15	27	29
7	−	+	+	+
15		−	−	−
27			−	−
29				−

Table 2. Phenotypes of the F_2 offspring from crosses of complementing *Physa* albino strains; values of χ^2 are goodness-of-fit to a 9:7 ratio, all nonsignificant with 1 df

Strain	Cross 7 × 15	7 × 27	7 × 29
Wild type	205	160	178
Albino	149	128	117
χ^2	1.99	0.06	0.41

a result difficult to reconcile with selfing by either parent. One of the 27 × 29 crosses included a pair of parents heterozygous at the *Est1* locus, one a fast/medium and the other a medium/slow. Offspring displayed all three alleles in Mendelian proportions, clearly demonstrating an outcross.

We isolated and reared pairs of F_1 snails from the complementary 7 × 15, 7 × 27, and 7 × 29 tests, examining the phenotypes of their F_2 offspring. Table 2 shows a good match to the nine wild type : seven albino ratio expected for reciprocal recessive epistasis. If two loci showing this type of interaction were linked so tightly as to preclude recombination, one would expect a 1:1 F_2 phenotypic ratio from a cross such as we have performed. The 1:1 hypothesis can be rejected in two of the three tests ($\chi^2 = 12.6$ in 7 × 15 and 8.86 in 7 × 29). Were recombination frequency to increase, the expected frequency of wild-type F_2 offspring would increase slowly, to 51% at 20% recombination, 52.2% at 30% recombination, and 56% for independent assortment. Note, however, that the net deviation shown in Table 2 is toward higher frequencies of wild-type offspring than expected, not lower. Thus although the test is weak, these data show no evidence of linkage between the two loci responsible for albinism.

Discussion

The results we report here indicate that albinism in *Physa* may be inherited as a recessive trait at two apparently unlinked genes. The trait would seem to be more commonly due to the locus for which our lines 15, 27, and 29 are now fixed. This locus we will call "*alb1*." The albinism locus for which our line 7 is fixed we will call "*alb2*." In contrast, only a single albino locus has been recognized over the scores of *Biomphalaria* strains isolated from 1954 to date. Complementation tests have yet to be performed among all pairs of *Biomphalaria* albino strains, held as they are on four continents, so the existence of mul-

tiple albinism loci in planorbids cannot be ruled out. But if this distinction between physids and planorbids is genuine, substantial biochemical divergence between the two taxa is suggested.

Although albinism seems to occur commonly enough in laboratory populations of freshwater snails to have attracted the attention of geneticists on numerous occasions, the trait generally seems to be rare in natural populations. For example, we are aware of only a very few documented cases of albinism among the (over 10) families of prosobranch (gilled) snails that inhabit fresh water. The senior author has never encountered an albino *Goniobasis* in 10 years of observations involving scores of populations (e.g., Dillon 1984). Since the investigations described above, we have taken six monthly quantitative samples of both *Physa* and *Biomphalaria* at the pond from which our original 35 snails were collected. Among thousands of individuals, we have to date found only one albino *Biomphalaria* and two albino *Physa* in the wild.

None of the 35 parental snails we isolated in 1989 showed abnormal body color, yet four of them produced albino offspring, in some cases on several occasions. We suggest that these original parents, numbers 7, 15, 27, and 29, were heterozygous for the trait and, hence, that the actual frequency of albinism alleles in the wild might be greater than one would infer from the observed frequency of albinos. We think it likely that albinism is deleterious in the wild and is present at frequencies determined by mutation/selection balance. Then the small number of homozygous albino F_1 offspring we observed might have resulted from low background levels of self-fertilization, and their subsequent survival is probably a consequence of our relatively benign laboratory conditions. In our earlier work using leucine-aminopeptidase isozyme markers, we failed to detect any such shift to selfing after 60 days of isolation (Wethington and Dillon, in press). But our F_1 sample sizes were probably insufficient to detect selfing rates as low as those implied by the small numbers of albinos recovered. Among labmated snails, we have recently obtained some experimental data suggesting that outcrossed *Physa* may revert to partial selfing as little as 1 week after insemination.

Less likely explanations for the apparent commonness of albinos among our F_1 progeny include new mutation and multiple insemination. Regarding the latter, it is certainly possible that snails 7, 15, 27, and 29 may have stored small quantities of exogenous albino sperm in addition to their apparently large stores of wild-type sperm. But an outcross explanation seems especially unlikely for the rarer *alb2* and implies that the frequencies of the albino alleles are even higher in the wild than they would be in a selfing explanation.

We are continuing a study of sex allocation in *Physa* using both *alb1* and *alb2* markers in which we periodically introduce a growing *alb1* homozygote to a fully mature *alb2* homozygote, ascribing female function to the *alb1* snail when it produces pigmented embryos and male function when its partner produces pigmented embryos. Other experiments on sperm competition and "sperm sharing" are projected.

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