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## RESEARCH NOTE

### The genetic basis of albinism in the hermaphroditic freshwater snail *Planorbella trivolvis*

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**Abstract:** In many species, the difference between pigmented and non-pigmented individuals is due to a single recessive Mendelian gene. We have demonstrated that this is also the case in the freshwater hermaphroditic snail, *Planorbella trivolvis* (Say, 1817), and established an albino population with comparable genetic background to a wild population so that pigmentation can be used as a visible genetic marker to better understand the reproductive biology of these mollusks. We carried out Mendelian crosses between albino (A) individuals from an inbred laboratory strain and pigmented (P) individuals one generation removed from a natural population and assessed pigmentation of progeny from eggs collected immediately after mating and 11 weeks later. Results of parental, F<sub>1</sub>, and F<sub>2</sub> generations and backcrosses to pure-breeding albinos were consistent with a Mendelian single gene inheritance pattern. Because we never observed albino progeny from any albino snails mated to pigmented sperm donors, we also confirmed that selfing is rare in *P. trivolvis*. A simple non-invasive paternity marker in this unusual hermaphrodite snail that displays almost no self-fertilization will facilitate experiments to understand its reproductive biology and provide a more complete picture of hermaphrodite mating strategies.

**Key words:** genetic marker, self-fertilization, *Helisoma trivolvis*

Evolutionary ecologists strive to understand how natural selection, sexual selection and sexual conflict influence reproductive strategies (Andersson 1994, Arnqvist and Rowe 2005). Most of this work (both theoretical and empirical) has concerned gonochoristic species – those with two separate sexes, male and female. In hermaphrodites, organisms which have both male and female structures, reproductive decisions also involve sex allocation (how much energy an organism devotes to male vs female function, Charnov 1982) and the extent of self-fertilization vs outcrossing (Escobar *et al.* 2011). Studying hermaphrodites adds to the general understanding of evolutionary principles and expands our view of the diversity of sexual strategies present in natural systems. Although increasing attention has been devoted to these species, recent reviews call for empirical studies to complement theoretical work and to determine whether results are generalizable among hermaphrodite species (Anthes *et al.* 2010, Schärer *et al.* 2014). Freshwater snails are emerging as model systems for addressing key questions about reproduction and mating strategies in hermaphrodites (Nakadera and Koene 2013). Yet these efforts are often hampered by the fact that mating often occurs in the dark and positioning of mating partners makes it difficult to determine behaviorally not only which individual is acting in the male or female role (or both), but also when actual mating and sperm transfer begin and end (Jarne *et al.* 1993). Methods to track individuals and identify parentage are therefore essential to data collection. Although DNA fingerprinting and microsatellite markers are now commonly used and

becoming less expensive, we focused on a simple phenotypic marker, pigmentation, as this marker can easily and non-invasively be used to track paternity.

In freshwater hermaphroditic snails, albinism has often been used as a genetic marker, since in most species the difference between pigmented and non-pigmented individuals is due to segregation of a single recessive Mendelian gene. As early as 1927, the simple genetic basis of albinism was implicated in *Lymnaea peregra* (now *Radix peregra* (Müller 1774), Boycott and Diver 1927), and this pattern of inheritance has been confirmed in Pulmonate snails including: *Lymnaea stagnalis* (Linnaeus 1758, Cain 1956), *Australorbis glabratus* (now *Biomphalaria glabrata* (Say 1818), Newton 1954), *Biomphalaria* (Preston 1910) spp. (Richards 1967, 1975, 1978), *Bulinus africanus* (Kraus 1848, Rudolph and Bailey 1983), *Physa heterostropha* (now *Physella heterostropha* (Say 1817) synonymous with *Physella acuta* (Draparnaud 1805), Dillon and Wethington 1992) and *Helisoma duryi* (now *Planorbella duryi* (Wetherby 1879), Madsen *et al.* 1983). These albino alleles are generally rare in natural populations where outcrossing is common, but may reach high frequencies in self-fertilizing species such as *Biomphalaria Pfeifferi* (Kraus 1848, Mouahid *et al.* 2010 and references therein). Our aim was to determine whether albinism is similarly inherited as a Mendelian recessive in the hermaphroditic freshwater snail, *Planorbella trivolvis* (Say 1817), and to establish an albino population with a more diverse genetic background than available laboratory populations so this simple visible marker can be used to investigate factors contributing to reproductive strategies.

*Planorbella trivolvis* (Say, 1817 Hygrophila: Planorbidae, formerly known as *Helisoma trivolvis*) is a hermaphroditic freshwater Pulmonate snail widely distributed across the US and Canada (Johnson *et al.* 2013). Individuals may mate as male, delivering sperm to a partner, or as female, producing eggs, and often mate reciprocally, acting simultaneously as male and female (Abdel-Malek 1952). Following mating, eggs are laid approximately daily in jelly-enclosed masses containing on average 20 individual eggs (Norton and Newman 2015). A single mating opportunity can provide enough sperm to fertilize several thousand eggs, as sperm can be stored for at least 16 weeks. Although most hermaphroditic freshwater snails will produce self-fertilized eggs when isolated, *P. trivolvis* have rarely produced selfed offspring in the laboratory (Crabb 1927, Paraense and Correa 1988, Escobar *et al.* 2011, Norton and Newman 2015), which makes them a unique system for studying hermaphrodite reproductive strategies since matings can be easily controlled and progeny assumed to result from cross-fertilization. Individuals in the wild are typically pigmented, but several labs maintain albino populations.

Our first goal was to determine the genetic basis of albinism in *P. trivolvis* using Mendelian crosses between snails from a laboratory albino population with non-pigmented tegument and eyes and pigmented individuals derived from a wild population where albinism has never been seen (Dillon, pers. comm.). Based on the mode of inheritance in other Pulmonate snails, we hypothesized that the pigmentation difference between wild-type and albino individuals is caused by a single locus autosomal recessive Mendelian gene. Secondly, we were able to use these experiments to investigate the extent of self-fertilization in mated individuals. Based on earlier studies of isolated snails (Norton and Newman 2015), we hypothesized that selfing would be rare.

## MATERIALS AND METHODS

### Study populations

Albino (A) *Planorbella trivolvis* were the progeny of snails with non-pigmented tegument and eyes sampled from a laboratory population originally derived from two non-pigmented individuals found in a laboratory stock in 2002. Pigmented (P) snails were the progeny of individuals collected from a pond at the entrance to Charles Towne Landing State Park (Charleston, NC 32.8068 °N; 79.9902 °W) where albinism has never been observed in years of collecting (Dillon pers. comm.). Small percentages of albino snails in wild *Helisoma trivolvis* (Say 1817, now considered *Planorbella*) populations were reported by Page (pers. comm. as reported in Studier *et al.* 1975), but these are the only published estimates. Although it is possible that the pigmented snails could have mated with albinos in the field, it is unlikely. The test snails came from

eggs laid by nine individuals from each population within a one week period.

### Crosses

We isolated fifty-four snails of each type (6 from each adult) at 10 weeks of age in 296 ml plastic cups filled with dechlorinated tap water. We fed them approximately equal amounts of boiled romaine lettuce (approx. 2x2 cm, an amount typically eaten in 3–4 days by adult snails housed alone in the lab) and changed water twice per week. Trays of snails were kept in the laboratory at room temperature (~22 °C) and with ambient light on a rolling cart with multiple shelves. Parental and F<sub>1</sub> matings were set up at 18 weeks, when snails were approximately 126 days old, well after sexual maturity ( $104.3 \pm 9.7$  d = 14.9 weeks; Escobar *et al.* 2011). Snails were paired randomly with a partner in a single cup for one week, and then separated in individual cups one week later. Since both members of a pair typically lay eggs, each pair generates two sets of offspring, one from each partner; the individual laying eggs is considered the sperm recipient and the partner the sperm donor. We set up 18 crosses of each type: Albino x Albino, Pigmented x Pigmented, and Albino x Pigmented. The first two crosses (comparable to Mendelian parental crosses), designated AA and PP (N = 36 snails in each cross), allowed us to characterize each original population. Since both parents produce eggs as well as contribute sperm to their partner, the Albino x Pigmented crosses served as reciprocal F<sub>1</sub> crosses with 36 focal individuals designated AP (N = 18) or PA (N = 18); the first letter indicates the maternal parent/sperm recipient and second the paternal parent/sperm donor. AP snails were thus albino snails with pigmented sperm donors, and PA snails were their partners, pigmented snails with albino sperm donors. Progeny of these matings (collected from the second week of egg production post-mating) were then used to establish the F<sub>2</sub> generation (AP x AP, N = 6; PA x PA, N = 10; and AP x PA, N = 15) and backcrosses of F<sub>1</sub> to the albino stock (PA x A, N = 13 and AP x A, N = 15).

### Phenotypes

To determine the pigmentation phenotypes of progeny, we collected up to three egg masses from individuals in each cross type during the first week after mating. We collected eggs from ¼ of the parental snails (9 PP and 9 AA individuals), all of the F<sub>1</sub> snails (18 PA and 18AP individuals), and all of the F<sub>2</sub> and backcross snails. Egg masses were cut out of the plastic cups and maintained in separate wells of 12-well tissue culture plates filled with dechlorinated tap water. We identified whether the developing embryos were albino or pigmented (the eyes of pigmented individuals contain dark pigment and those of albinos lack pigment) by observing each egg mass under an inverted tissue culture microscope at 40x. For the parental and F<sub>1</sub> crosses, we collected egg masses from these same individuals 10 weeks later to determine

whether any individuals may have been producing selfed eggs once allosperm (contributed by the mate) was becoming depleted. We also assessed the pigmentation phenotypes of juveniles hatched from egg masses not observed earlier to verify pigmentation of the body as well as eyes.

If the original populations are true breeding (homozygous for either the albino or pigmented alleles) and the pigmentation differences between them are caused by a single locus Mendelian recessive gene, A x A crosses are expected to produce all albino offspring, P x P crosses are expected to produce all pigmented, and in the absence of self-fertilization all F<sub>1</sub> progeny should be pigmented. F<sub>2</sub> progeny are similarly expected to be ¾ pigmented and ¼ albino, and backcross progeny ½ pigmented and ½ albino. To test whether the outcomes of these crosses conformed to expected Mendelian ratios, we carried out chi-square goodness of fit tests with one degree of freedom and an  $\alpha$  level of 0.05. Any individuals that produced fewer than 18 embryos in collected masses (8 from the F<sub>2</sub> and 4 from backcrosses) were excluded from the analysis to avoid small biased samples.

## RESULTS

### Phenotypes

Progeny from the parental A x A crosses were all albino (as indicated by non-pigmented eyes), and those from P x P crosses all had pigmented eyes (Table 1). This was the case for embryos collected both one week and 11 weeks after mating. Juveniles from eggs laid 1–3 weeks post mating and observed 12 weeks later exhibited the same pattern – those from A x A crosses had non-pigmented eyes and tegument and those from the P x P crosses had pigmented eyes and tegument. All progeny from the F<sub>1</sub> crosses (embryos week 1, embryos week 11 and juveniles) whether produced by albino (AP) or pigmented (PA) individuals were also pigmented. In no case did an albino snail mated to a pigmented snail produce albino offspring.

Phenotypic ratios in the F<sub>2</sub> progeny conformed to the expected 3:1 pigmented to albino Mendelian ratios (Table 2). Progeny from each of the cross combinations as well as the combined totals show no significant differences from those expected. Similarly, for the backcrosses, there were no significant differences from the expected 1 pigmented: 1 albino phenotypic ratio (Table 2).

## DISCUSSION

### Genetics of albinism in *Planorbella trivolvis*

Our data demonstrate a single gene basis for albinism in *Planorbella trivolvis*, with phenotypic ratios conforming to Mendelian expectations. Phenotypic ratios of parental, F<sub>1</sub>, F<sub>2</sub>

**Table 1.** Phenotypes of offspring from parental (AA and PP) and F<sub>1</sub> (AP and PA) crosses. Sample size (N) indicates the number of individuals from which eggs were collected at each time period.

Cross	Source of Individuals (time post-mating)	N	Albino	Pigmented
AA	embryos (1 week)	9	345	0
	embryos (11 weeks)	6	151	0
	juveniles (1 week)	17	124	0
	Total		620	0
AP	embryos (1 week)	17	0	714
	embryos (11 weeks)	15	0	583
	juveniles (1 week)	12	0	61
	Total		0	1358
PA	embryos (1 week)	14	0	536
	embryos (11 weeks)	6	0	114
	juveniles (1 week)	12	0	65
	Total		0	715
PP	embryos (1 week)	7	0	309
	embryos (11 weeks)	8	0	366
	juveniles (1 week)	27	0	89
	Total		0	764

generations and backcrosses are all consistent with those expected from a single locus autosomal recessive mode of inheritance. These results confirm our hypothesis and are consistent with the mode of inheritance of albinism in other freshwater hermaphroditic snails (Boycott and Diver 1927, Cain 1956, Newton 1954, Richards 1967, 1975, 1978, Rudolph and Bailey 1983, Dillon and Wethington 1992, Madsen *et al.* 1983). The ability to use pigmentation differences as a genetic marker in *P. trivolvis* will help to further our goal of developing this species as a model system for understanding hermaphrodite reproductive strategies. Albinism has been used in many other snail species to study frequencies of self- vs cross-fertilization (Paraense 1955, Guimarães *et al.* 2016), the age at which female and male function mature (Wethington and Dillon 1993), duration of sperm storage (Cain 1956), the influence of mating history on gender roles and gender conflict (Wethington and Dillon 1996), and flexibility of gender roles (Vianey-Liaud 1989). We have now established a population of albino snails derived from homozygous albino offspring of F<sub>2</sub> crosses which has a genetic background more like that of wild populations than current existing laboratory populations (they share approximately half their genome with the wild caught populations) which will facilitate similar studies.

### Self-fertilization

In the current study we were also able to assess the frequency of self- and cross-fertilization in *P. trivolvis*. In no instance did albino snails mated to a pigmented partner (in the F<sub>1</sub> A x P crosses) produce albino offspring. The absence of

**Table 2.** Phenotypes of embryos from F<sub>2</sub> generation and backcrosses. We counted an average of 42 (range = 18-76) progeny from the 3 egg masses collected from each F<sub>2</sub> individual and 31 (range = 18-47) from each of the backcross parents. All were collected from eggs laid during the first week post-mating. Values are observed numbers of progeny in each phenotypic class with expected numbers in parentheses. Expected ratios for the F<sub>2</sub> crosses are 3:1 pigmented:albino progeny and for backcrosses 1:1 pigmented:albino.  $\chi^2$  is the Chi-square value from a goodness of fit test with 1 degree of freedom; P is the probability of getting the observed deviation from expectation by chance alone.

F <sub>2</sub> Crosses	Albino	Pigmented	$\chi^2$	P
AP x AP N = 5	62 (63)	190 (189)	0.021	0.88
AP x PA N = 15	141 (140.25)	420 (420.75)	0.005	0.94
PA x PA N = 11	128 (116.25)	337 (348.75)	1.584	0.21
all F <sub>2</sub> N = 30	331 (319.5)	947 (958.5)	0.552	0.46
Backcrosses	Albino	Pigmented	$\chi^2$	P
A x AP N = 11	144 (141.5)	139 (141.5)	0.088	0.77
A x PA N = 14	246 (226)	206 (226)	3.540	0.06
all B N = 25	390 (367.5)	345 (367.5)	2.76	0.10

albino progeny from these crosses not only confirms the rarity of albinism in this wild population, as albino progeny could also have resulted if a pigmented sperm donor carried a recessive albino allele, but it confirms that these snails exclusively use allosperm to fertilize their eggs, even when they have mated with another individual. This was the case in the first week post-mating as well as at 10 weeks later, when snails were laying fewer egg masses and fewer eggs per mass, presumably as a result of sperm depletion. These results from the albino laboratory stock are consistent with the extremely low level of self-fertilization we have observed in pigmented snails from the wild-caught South Carolina population isolated before sexual maturity and monitored their entire lives (Norton and Newman 2015). This absence of self-fertilization makes *P. trivolvis* particularly interesting as a model system for investigating reproductive strategies in hermaphrodites without the complication of autosperm production or selfed offspring.

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#### LITERATURE CITED

- Abdel-Malek, E. T. 1952. The preputial organ of snails in the genus *Helisoma* (Gastropoda: Pulmonata). *American Midland Naturalist* **48**: 94–102.
- Andersson, M. 1994. *Sexual Selection*. Princeton University Press: Princeton, NJ.
- Anthes, N., P. David, J. Auld, J. Hoffer, P. Jarne, J. Koene, H. Kokko, M. C. Lorenzi, B. Péliissié, D. Sprenger, A. Staikou, L. Schärer. 2010. Bateman gradients in hermaphrodites: An extended approach to quantify sexual selection. *American Naturalist* **176**: 249–263.
- Arnqvist, G. and L. Rowe. 2005. *Sexual Conflict*. Princeton University Press: Princeton, NJ.
- Boycott, A. and C. Diver. 1927. The origin of an albino mutation in *Limnaea peregra*. *Nature* **119**: 9.
- Cain, G. L. 1956. Studies on cross-fertilization and self-fertilization in *Limnaea stagnalis appressa* Say. *Biological Bulletin* **111**: 45–52.
- Charnov, E. L. 1982. *The Theory of Sex Allocation*. Princeton University Press: Princeton, NJ.
- Crabb, E. D. 1927. The fertilization process in the snail, *Limnaea stagnalis appressa* Say. *Biological Bulletin* **53**: 67–108.
- Dillon, R. T. and A. R. Wethington. 1992. The inheritance of albinism in a freshwater snail, *Physa heterostropha*. *Journal of Heredity* **83**: 203–210.
- Escobar, J. S., J. R. Auld, A. C. Correa, J. M. Alonso, Y. K. Bony, M. Coutellec, J. M. Koene, J. Pointier, P. Jarne, P. David. 2011. Patterns of mating-system evolution in hermaphroditic animals: correlations among selfing rate, inbreeding depressions, and the timing of reproduction. *Evolution* **65**: 1233–1253.
- Guimarães, M. C. D. A., R. M. T. D. Menezes, R. Tuan. 2016. Experimental study on reproduction of the freshwater snail *Biomphalaria tenagophila* (d'Orbigny, 1835). *Invertebrate Reproduction and Development* **60**: 145–151.
- Jarne, P., M. Vianey-Liaud, B. Delay. 1993. Selfing and outcrossing in hermaphrodite freshwater gastropods (Basommatophora): where, when and why. *Biological Journal of the Linnean Society* **49**: 99–125.
- Johnson, P. D., A. E. Bogan, K. M. Brown, N. M. Burkhead, J. R. Cordeiro, J. T. Garner, P. D. Hartfield, D. A. W. Lepitzki, G. L. Mackie, E. Pip, T. A. Tarpley, J. S. Tiemann, N. V. Whelan, E. Strong. 2013. Conservation status of freshwater gastropods of Canada and the United States. *Fisheries* **38**: 247–282.
- Madsen, H., F. W. Thiongo, J. H. Ouma. 1983. Egg laying and growth in *Helisoma duryi* (Wetherby) (Pulmonata: Planorbidae): Effect of population density and mode of fertilization. *Hydrobiologia* **106**: 185–191.
- Mouahid, G., R. Mintsá Nguéma, M. A. Idris, M. A. Shaban, S. A. Yafee, J. Languard, M. Verdoit-Jarraya, R. Galinier, H. Moné. 2010. High phenotypic frequencies of complete albinism in wild populations of *Biomphalaria pfeifferi* (Gastropoda: pulmonata). *Malacologia* **53**: 161–166.

- Nakadera, Y., J. M. Koene. 2013. Reproductive strategies in hermaphroditic gastropods: conceptual and empirical approaches. *Canadian Journal of Zoology* **91**: 367–381.
- Newton, W. L. 1954. Albinism in *Australorbis glabratus*. *Proceedings of the Helminthological Society of Washington* **21**: 72–74.
- Norton, C. G. and B. R. Newman. 2015. Growth, reproduction and longevity in the hermaphroditic freshwater snail *Helisoma trivolvis*. *Journal of Molluscan Studies* **82**: 178–186.
- Paraense, W. L. 1955. Self- and cross-fertilization in *Australorbis glabratus*. *Memorias do Instituto Oswaldo Cruz* **53**: 285–291.
- Paraense, W. L. and L. R. Correa. 1988. Self-fertilization in the freshwater snails *Helisoma duryi* and *Helisoma trivolvis*. *Memorias do Instituto Oswaldo Cruz* **83**: 405–409.
- Richards, C. S. 1967. Genetic studies on *Biomphalaria glabrata* (Basommatophora: Planorbidae), a third pigmentation allele. *Malacologia* **5**: 335–340.
- Richards, C. S. 1975. Genetics of pigmentation in *Biomphalaria straminea*. *American Journal of Tropical Medicine and Hygiene* **24**: 154–156.
- Richards, C. S. 1978. Genetic studies on *Biomphalaria straminea*: occurrence of a 4<sup>th</sup> allele of a gene determining pigmentation variations. *Malacologia* **17**: 111–115.
- Rudolph, P. H. and J. B. Bailey. 1983. Inheritance of mantle pigmentation patterns in *Bulinus (Physopsis) africanus* (Basommatophora: Planorbidae). *Freshwater Invertebrate Biology* **2**: 56–59.
- Schärer, L., T. Janicke, and S. A. Ramm. 2014. Sexual conflict in hermaphrodites. *Cold Spring Harbor Perspectives in Biology* **7**: a017673.
- Studier, E. H., K. E. Edwards, M. D. Thompson. 1975. Bioenergetics in two pulmonate snails, *Helisoma* and *Physa*. *Comparative Biochemistry and Physiology Part A: Physiology* **51**: 859–861.
- Vianey-Liaud, M. 1989. Growth and fecundity in a black-pigmented and an albino strain of *Biomphalaria glabrata* (Gastropoda: Pulmonata). *Malacological Review* **22**: 25–32.
- Wethington, A. R. and R. T. Dillon. 1993. Reproductive development in the hermaphroditic freshwater snail *Physa* monitored with complementing albino lines. *Proceedings of the Royal Society of London B: Biological Sciences* **252**: 109–114.
- Wethington, A. R. and R. T. Dillon. 1996. Gender choice and gender conflict in a non-reciprocally mating simultaneous hermaphrodite, the freshwater snail, *Physa*. *Animal Behaviour* **51**: 1107–1118.

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