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Mother–offspring data in a study of the mating system in a natural population of *Bulinus globosus* (Gastropoda: Planorbidae) in Zimbabwe

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Summary

The mating system of a natural population of *Bulinus globosus* from the Chiweshe area, Zimbabwe, was studied with mother–offspring data using isozyme genetic markers. The study was done in response to work on the genetic structure of this population which suggested a limited extent of cross-fertilization. Of the 24 adults whose progenies were analysed, at least 15 showed evidence of outcrossing and 9 had results consistent with selfing. These results show that the two modes of reproduction are important under natural conditions and the mating system of this population is considered to be 'partially-selfing'.

1. Introduction

Most basommatophorans are simultaneous hermaphrodites (Geraerts & Joose, 1984). The usual mode of reproduction seems to be outcrossing, but in certain conditions selfing does occur (Jarne *et al.* 1993). The generally low electrophoretic variability in natural populations of *Bulinus* (Mimpfoundi & Greer, 1990; Njiokou *et al.* 1993) has hindered studies of their mating system and hence no clear conclusion has been reached in this regard.

Analysis of the mating system or reproductive mode in *Bulinus globosus* requires Mendelian inherited genetic markers. Foltz *et al.* (1982), Brown & Richardson (1988) and Njiokou *et al.* (1994) have inferred the mating system of molluscs from the genetic structure of populations, while parent–offspring analysis has been used as an alternative way of studying the mating system in *Biomphalaria obstructa* (Mulvey & Vrijenhoek, 1981), *B. cernicus* (Rollinson & Wright, 1984), *Ancylus fluviatilis* (Städler *et al.* 1993) and *B. alexandrina* (Vrijenhoek & Graven, 1992). This analysis allows one to estimate multiple paternity and to test for the occurrence of selfing in hermaphroditic species, using polymorphic markers (Jarne & Delay, 1991).

Stochastic factors which influence snail populations in natural conditions could lead to variability in the selfing rate between populations of the same species, and Jarne *et al.* (1993) emphasize the importance of comparative analyses of mating systems among populations occupying different environments. Attempts to correlate the frequency of selfing with factors such as habitat complexity and stability, population structure, history and demography are necessary.

This study had the aim of elucidating the mating system of a natural population of *B. globosus* using parent–offspring combinations analysed with isozyme genetic markers. It is an extension to the population genetic studies of *B. globosus* from Kakwidibire (Chiweshe), Zimbabwe (Mukaratirwa *et al.* 1996*a*), where inference of the mating system of this population from the population genetic structure revealed deviation from Hardy–Weinberg proportions with a deficiency of heterozygotes, and partial selfing was suggested as the prevalent mating system.

2. Materials and methods

Wild-caught *B. globosus* snails used in this study were collected from Kakwidibire (Chiweshe) in November 1993. The snails were collected within a single square

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Table 1. *Parent–offspring combinations for the polymorphic loci*

Parent	Locus	Genotype	No. of offspring of type: F/F	F/M	M/M	F/S	M/S	S/S	50% heterozygotes	Self-fertilization	Single mating	Multilocus selfing rate	Mating behaviour
A	*Est-1*	M/M	—	4	1	—	—	—	—	−	0·188	—	O
	Est-2	F/F	5	—	—	—	—	—	—	+	—	0·10	
	Idh	F/F	5	—	—	—	—	—	—	+	—	—	
B	*Est-1*	F/F	8	5	—	—	—	—	—	−	0·290	—	O
	Est-2	F/M	—	6	7	—	—	—	0·500	−	—	—	
	Idh	M/M	—	4	9	—	—	—	—	−	0·133	0·00	
	Hbdh	M/M	—	—	9	—	4	—	—	−	0·133	—	
C	*Est-1*	F/M	3	11	—	—	—	—	0·028	+	—	—	O
	Idh	M/M	—	—	14	—	—	—	—	+	—	—	
	Hbdh	M/M	—	4	10	—	—	—	—	−	0·089	0·01	
D	*Est-1*	F/F	1	19	—	—	—	—	—	−	0·999*	—	O
	Idh	M/S	—	—	10	—	8	2	0·252	−	—	0·00	
	Hbdh	M/M	—	—	20	—	—	—	—	+	—	—	
E	*Est-1*	F/M	5	4	—	—	—	—	0·500	−	—	—	O
	Hbdh	M/M	2	7	—	—	—	—	—	−	0·089	—	
	Idh	M/S	—	—	1	—	2	4	0·226	−	—	0·00	
F	*Est-1*	F/M	6	7	—	—	—	—	0·935	−	—	—	O
	Est-2	F/F	7	6	—	—	—	—	—	−	0·395	—	
	Idh	M/M	—	—	8	—	4	—	—	−	0·194	—	
	Hbdh	M/M	—	—	13	—	—	—	—	+	—	0·05	
G	*Est-1*	F/F	10	—	—	—	—	—	—	+	—	—	S
	Idh	M/M	—	—	10	—	—	—	—	+	—	—	
	Hbdh	M/M	—	—	10	—	—	—	—	+	—	1·00	
H	*Est-1*	F/F	9	1	—	—	—	—	—	−	0·01*	—	S
	Est-2	M/M	—	—	10	—	—	—	—	+	—	0·89	
	Idh	M/M	—	—	10	—	—	—	—	+	—	—	
I	*Est-1*	M/M	—	—	8	—	—	—	—	+	—	—	S
	Est-2	F/M	2	5	1	—	—	—	0·363	+	—	1·00	
	Idh	M/M	—	—	8	—	—	—	—	+	—	—	
J	*Est-1*	F/M	—	2	3	—	—	—	0·500	−	—	—	O
	Est-2	F/F	5	—	—	—	—	—	—	+	—	0·00	
	Idh	M/S	—	—	4	—	1	1	0·109	+	—	—	
K	*Est-1*	F/F	8	—	—	—	—	—	—	+	—	—	S
	Est-2	F/F	8	—	—	—	—	—	—	+	—	1·00	
	Idh	M/M	—	—	8	—	—	—	—	+	—	—	
L	*Est-1*	F/M	3	2	2	—	—	—	0·226	+	—	—	S
	Est-2	F/F	7	—	—	—	—	—	—	+	—	0·92	
	Idh	M/S	—	—	4	—	2	1	0·226	+	—	—	
M	*Est-1*	F/F	4	—	—	—	—	—	—	+	—	—	S
	Est-2	F/F	4	—	—	—	—	—	—	+	—	1·00	
	Idh	M/S	—	—	3	—	1	—	0·312	+	—	—	
N	*Est-1*	F/F	6	—	—	—	—	—	—	+	—	—	S
	Est-2	F/F	6	—	—	—	—	—	—	+	—	1·00	
	Idh	M/M	6	—	—	—	—	—	—	+	—	—	
O	*Est-1*	M/M	—	7	1	—	—	—	—	−	0·996*	—	O
	Est-2	F/F	3	5	—	—	—	—	—	−	0·363	0·00	
	Idh	M/M	—	6	2	—	—	—	—	−	0·144	—	
P	*Est-1*	—	1	2	1	—	—	—	—	.	—	0·01	O
	Est-2	—	1	2	1	—	—	—	—	.	—	—	
Q	*Est-1*	M/M	—	1	9	—	—	—	—	−	0·01*	—	S
	Est-2	F/F	9	1	—	—	—	—	—	−	0·01*	0·89	
	Idh	M/M	—	1	9	—	—	—	—	−	0·01*	—	
R	*Est-1*	—	2	5	—	—	—	—	—	.	—	0·00	O
	Idh	—	—	—	2	—	4	—	—	.	—	—	
S	*Est-1*	—	1	9	8	—	—	—	—	.	—	0·01	O
	Idh	—	—	—	2	—	10	6	—	.	—	—	
T	*Idh*	—	—	—	1	—	8	4	—	.	—	0·01	O
U	*Est-1*	F/M	1	3	—	—	—	—	0·312	+	—	—	O

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Table 1. (*cont.*)

Parent	Locus	Genotype	No. of offspring of type: F/F	F/M	M/M	F/S	M/S	S/S	50% heterozygotes	Self-fertilization	Single mating	Multilocus selfing rate	Mating behaviour
	Idh	M/S	—	—	—	—	2	2	—	−	—	0·00	
V	*Est-1*	F/M	—	4	10	—	—	—	0·089	+	—	0·00	O
	Idh	F/S	4	—	—	9	—	1	0·211	+	—	—	
W	*Est-1*	F/M	4	6	—	—	—	—	0·376	−	—	—	O
	Idh	F/M	—	4	5	—	1	—	—	−	—	0·01	
X	*Est-1*	F/F	10	—	—	—	—	—	—	+	—	—	S
	Est-2	M/M	—	—	10	—	—	—	—	+	—	1·00	
	Idh	M/M	—	—	10	—	—	—	—	+	—	—	

+, consistent with selfing; −, not consistent with selfing; O, outcrossing; S, selfing.
Probability values are calculated for the single mating hypothesis and for the hypothesis of 50% heterozygotes in the progeny of heterozygous parents.
* $P < 0{\cdot}05$.

metre, which excluded the possibility of accidentally pooling genetically heterogeneous subpopulations.

Dense populations of *B. globosus* are found in these sites in the middle of the dry season (July and August) and densities decrease towards the start of the rainy season. The snails were mature (length of shell 10–13 mm). Immediately after collection snails were screened for trematode infection and uninfected snails separated individually into plastic containers (with approximately 400 ml of dechlorinated water). Each snail was given an identification number and left to lay eggs. After deposition of several egg capsules, parental snails were removed and stored at −70 °C prior to electrophoresis.

The snails were allowed to lay eggs for a maximum of 5 days. It is important to analyse the first individuals born in the laboratory as sperm storage is a highly variable parameter among individuals and animals might switch to selfing after some time under laboratory conditions (Jarne & Delay, 1991). After hatching, the F_1 generation was raised: feeding was with dried lettuce and trout pellets alternately twice a week until 3 weeks of age. The shell length at that age was approximately 5 mm or more before the snails were processed for electrophoresis.

From previous studies of *B. globosus* populations in Zimbabwe by Mukaratirwa *et al.* (1996*a*), esterases (*Est*), isocitrate dehydrogenase (*Idh*) and hydroxybutyrate dehydrogenase (*Hbdh*) were found to be reliable genetic markers. Mothers and offspring were examined at these polymorphic loci and each parental snail was analysed simultaneously with its offspring to ensure easy comparison of the genotype of each mother with that of her offspring.

Electrophoretic techniques and sample preparations were as described previously by Jelnes (1979) and Mukaratirwa *et al.* (1996*b*). The alleles of the genetic markers were as follows; *Est-1* (150 and 167), *Est-2* (100 and 133), *Hbdh* (112 and 132) and *Idh* (76, 91 and 100). All the loci produced clear-cut bands which segregated in a Mendelian manner.

The approach of Mulvey & Vrijenhoek (1981) was used to assess the degree of selfing and outcrossing. With Mendelian inheritance, homozygous mothers cannot have progeny homozygous for the alternative autosomal allele. At a diallelic locus, heterozygous mothers will produce a 1:1 ratio of homozygous to heterozygous progeny regardless of paternal genotypes or the number of inseminations. The same test can be carried out for the triallelic *Idh* locus in the cases where the same two alleles that a mother carries appear in the offspring. An analysis was also carried out to determine whether the snails which were consistent with outcrossing had undergone single or multiple matings. This was done by testing for a 1:1 ratio of heterozygotes to homozygotes among segregating offspring of homozygote mothers. A rejection of this hypothesis can either be caused by non-Mendelian segregation in the male partner or by multiple matings. A binomial distribution probability test was used in both cases whereas a chi-squared test was used on the pooled data of offspring from heterozygous parents at each locus. The binomial test statistic was considered significant at the 5% level if the cumulative probability value was outside the range 0·025 to 0·975. Assuming that offspring of a single snail are produced either by selfing or by outcrossing (with either one or several partners), the multilocus outcrossing rate (1-selfing rate) was calculated using a multilocus mating system estimation program written by Ritland (1990). The Expectation-Maximization (EM) method was used.

The probability test of the Genepop (version 1.2) package of Raymond & Rousset (1995) was used for an exact test of Hardy–Weinberg proportions. The program quantifies excesses or deficiencies of heterozygotes by F_{IS} values according to Weir & Cockerham (1984).

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Table 2. *Parent–offspring combinations for the polymorphic loci*

Locus	Parental genotypes	No. of parents	Offspring phenotypes F/F	F/M	M/M	F/S	M/S	S/S	*n*	χ^2
Est-1	F/F	8	56	25	—	—	—	—	81	—
	F/M	8	22	40	15	—	—	—	77	0·117
	M/M	4	—	12	19	—	—	—	31	—
Est-2	F/F	9	55	12	—	—	—	—	67	—
	F/M	2	2	11	8	—	—	—	21	0·048
	M/M	2	—	—	20	—	—	—	20	—
Idh	F/F	1	5	—	—	—	—	—	5	—
	F/M	1	—	4	5	—	1	—	10	—
	M/M	11	6	7	88	—	8	—	101	—
	F/S	1	4	—	—	9	—	1	14	1·143
	M/S	6	—	—	22	—	18	8	48	3·000
Hbdh	M/M	6	2	15	63	—	—	—	80	—

χ^2 values are presented for the hypothesis of a 1:1 ratio of homozygous to heterozygous progeny of heterozygous parents.

Table 3. *Allele frequencies and genotype frequencies of* B. globosus *from Kakwidibire*

Locus	Allele frequency			F_{IS}	*P* value	Observed genotype frequency					
Est-1	150	167				150/150	150/167	167/167	—	—	—
	0·24	0·76		0·268	0·0008	19	48	112	—	—	—
Est-2	100	133				100/100	100/133	133/133	—	—	—
	0·69	0·31		0·215	0·0050	94	60	25	—	—	—
Hbdh	112	132				112/112	112/132	132/132	—	—	—
	0·01	0·99		−0·003	1·0000	0	2	177	—	—	—
Idh	76	91	100			76/76	76/91	76/100	91/91	91/100	100/100
	0·07	0·27	0·66	0·164	0·0237	3	6	14	18	53	85
Gdh	178	189				178/178	178/189	189/189	—	—	—
	0·02	0·98		0·067	0·3621	1	16	162	—	—	—

F_{IS} measures the deviation from Hardy–Weinberg proportions according to Weir & Cockerham (1984). *P* value is the probability value for the exact Hardy–Weinberg test (Raymond & Rousset, 1995).

3. Results

Of the 24 adults collected from the field, all produced at least one egg capsule within the first 3 days. The progenies of the adult snails were examined for patterns of segregation of parental alleles at the polymorphic loci (Table 1). Comparison of mother–offspring genotypes for the loci failed to uncover combinations inconsistent with hypotheses of single loci with co-dominant alleles undergoing simple Mendelian segregation.

Four snails (D, H, O and Q) were homozygous at loci that segregated progenies in non-Mendelian (i.e. non-1:1) proportions, but it is impossible from these data alone to distinguish whether these distortions were due to multiple matings or partial selfing.

At the *Est-1*, *Est-2*, *Idh* and *Hbdh* loci tested for segregation in heterozygotes there were no significant deviations from the expected 1:1 ratio of heterozygotes to homozygotes (Table 1), and likewise the pooled progenies from heterozygous parents at each locus showed no significant deviation from the expected ratio (Table 2). One adult (snail W) was heterozygous at the *Idh* locus and produced progeny with three different phenotypes. A single cross or selfing cannot produce these results, but either a combination of outcrossing (e.g. F/M × M/S) or a combination of outcrossing with two mates (e.g. F/M × M/M + M/S) could produce similar results.

The computed estimates of the selfing rates are also shown in Table 1. The overall selfing rate estimate of the population was 0·553. The multilocus selfing rate estimates of individual maternal snails (Table 1) showed that at least 15 adults outcross and 9 adults were consistent with selfing.

4. Discussion

From the results, a mixed mating system is proposed with a high selfing rate. The findings from this study resemble those from many plant populations in maintaining a stable mixed mating system. Plants

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express a diversity of breeding systems and theoretical and empirical studies have predominantly been focused on their mating systems (Charlesworth & Charlesworth, 1987; Brown, 1990). The evolutionary mechanism(s) acting to maintain this diversity, particularly within species, are unclear (Knight & Waller, 1987).

In natural populations of *B. globosus*, self-fertilization may occur when a virgin snail is isolated or when a snail which has copulated at least once is isolated long enough to switch to self-fertilization (Jarne *et al.* 1991). Snails collected for our study can be assumed to have had ample opportunity to receive allosperm, considering their size (adult) and the proximity to each other in the locality where they were collected. Our results, however, show that this population self-fertilizes at a high frequency.

Further support for this view is provided by the data on the genetic structure of this population (Table 3), which shows pronounced heterozygote deficiency at three of the five loci tested according to the method of Weir & Cockerham (1984). Other possible causes of heterozygote deficiency have been discussed (Mukaratirwa *et al.* 1996*a*, *b*) and the possibility of null or silent alleles was rejected. Null alleles exhibit no activity or bands in the *in vitro* staining systems, and heterozygotes with normally active alleles may be mis-scored as homozygotes (Crouau-Roy, 1988). It is very unlikely that the heterozygote deficiency observed at the three loci in our study was due to null alleles. Estimation of the frequency of the null allele for each locus from the genotype data in Table 3 using the EM algorithm (Hartl & Clarke, 1989) revealed high estimates (*Est-1* = 0·083; *Est-2* = 0·069; *Idh* = 0·054). With such high frequencies of null alleles, one would expect that a relatively large fraction of individuals classified as homozygotes carry silent alleles. This could result in inconsistencies in mother–offspring combinations, but none were observed among the 24 mothers and their 179 offspring, suggesting that null alleles probably cannot be responsible for the excess homozygotes.

The study of population structure, and parent–offspring analysis, in molluscs have often failed to reveal significant selfing rates (Jarne *et al.* 1993; but see Städler *et al.* 1993, in *A. fluviatilis*). A possible reason is that most of the experiments were laboratory based and might not reflect the role of selfing in natural populations. Our studies are consistent with those of Jarne *et al.* (1991) and Njiokou *et al.* (1994) who reported selfing in populations of *B. globosus*.

To gain a better understanding of the mating systems of populations of *B. globosus*, it is necessary to consider that a population's history of colonization and extinctions and subsequent mating structure combine to determine extant patterns of variation. Data on the mating systems of populations in unstable environments subjected to severe seasonal desiccation and floods are scarce. In these populations, as in our study, self-fertilization is expected to increase in seasonally drying environments due to repeated isolation and reduction in population size.

Multiple paternity has been reported in *Biomphalaria obstructa* by Mulvey & Vrijenhoek (1981) and in *Bulinus cernicus* by Rollinson *et al.* (1989). The lack of comparative mother–offspring data for *B. globosus* makes the estimation of multiple paternity in our study difficult. Also, cases of repeated matings with males of the same genotype will go unnoticed. The inclusion in the study of many polymorphic loci with more than three alleles would have enhanced the chances of detecting multiple matings, if they occur.

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