Behav Ecol Sociobiol (1994) 35:413–421

Bruno Baur

Multiple paternity and individual variation in sperm precedence in the simultaneously hermaphroditic land snail *Arianta arbustorum*

Received: 1 February 1994/Accepted after revision: 2 September 1994

Abstract Intraspecific variation in the proportion of offspring sired by the second mate with a female (P_2) is an aspect of sperm competition that has received little attention. I examined the effects of delay between copulations (range 9–380 days) and size of sperm donor on sperm precedence in double-mated individuals of the simultaneously hermaphroditic land snail *Arianta arbustorum*. Using shell colour as a genetic marker, paternity was analysed in 132 broods produced by 35 snails that had mated with two partners of different genotype. Sperm precedence (P_2) was influenced by the time between the two matings when the mating delay exceeded 70 days (one reproductive season). P_2 averaged 0.34 in the first brood of snails that mated twice within 70 days indicating first mate sperm precedence. In contrast, P_2 averaged 0.76 in broods of snails that remated in the following season, indicating a decreased viability of sperm from the first mate. The size of sperm-donating individuals had no effect on the fertilization success of their sperm in the first brood produced after the second copulation. Analysis of long-term sperm utilization in 23 snails that laid three to nine egg batches over 2 years revealed striking differences among individuals. Five snails (21.7%) exhibited first-mate sperm precedence throughout, eight snails (34.8%) showed second-mate sperm precedence throughout, whereas ten snails (43.5%) exhibited sperm mixing in successive batches. It is suggested that the individual variation in sperm precedence in *A. arbustorum* may partly be due to differences in the amount of sperm transferred. Paternity analysis in 34 batches laid by 19 wild-caught individuals of *A. arbustorum* indicated that at least 12 snails (63.2%) used sperm from two or more mates for the fertilization of their eggs. This suggests a high incidence of multiple paternity in broods of *A. arbustorum* under field conditions.

Key words Sperm precedence · Multiple paternity Remating interval · Mate size · Gastropods

Introduction

Since Parker (1970) first pointed out the importance of competition between sperm from different males for the fertilization of eggs of a single female, differential sperm usage from consecutive matings has been documented across a wide diversity of animal taxa, including insects, spiders, fishes, reptiles, birds, bats, and primates (for reviews see Walker 1980; Smith 1984; Ginsberg and Huck 1989; Birkhead and Hunter 1990; Birkhead and Møller 1992). These studies typically have measured species-specific sperm precedence as mean proportion of eggs fertilized by the second of the two mates (mean P_2 value; Boorman and Parker 1976). However, many of the species examined show a wide, and as yet unexplained, range of variation around these P_2 values. This intraspecific variability may be due to random variation, to differences among males in the competitive ability of their sperm, to female sperm preference or to unknown details of sperm transfer and storage that were not controlled for in the experiments. Furthermore, most studies and examples of sperm precedence have focused on the relative number of fertilizations achieved by two males in eggs laid immediately after a double copulation (see Gwynne 1984). Studies that extend their observation period to cover several egg-laying episodes have demonstrated that sperm precedence may change and thus may reduce the advantage of a particular male enjoyed immediately after the last

---

B. Baur[1]
Institute of Zoology, University of Basel, Rheinsprung 9, CH - 4051 Basel, Switzerland
Present address:
[1] Conservation Biology Research Group (NLU), University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland

copulation (Siva-Jothy and Tsubaki 1989, and references therein).

Gastropods show diverse mating behaviour and sperm transfer methods (Duncan 1975; Tompa 1984), yet little is known about sperm competition among these animals. In natural populations of the simultaneously hermaphroditic land snails *Arianta arbustorum* and *Helix pomatia* mating was found to be random with respect to shell size (Baur 1992a). In *A. arbustorum*, repeated mating has been observed both in a field cage experiment and in the laboratory (Baur and Raboud 1988; Baur 1992a). Mate-choice tests revealed that individuals of *A. arbustorum* discriminate neither between potential mates of different size nor between snails of different reproductive status (virgin/mated) and different degrees of relatedness (Baur 1992a; B. Baur and A. Baur, unpublished data). Time constraints on locomotor activity and high costs of searching for a mate might explain the prevalence of random mating patterns in land snails (Baur 1992a). However, a random mating pattern does not necessarily imply random fertilization of eggs, for these snails mate repeatedly and are able to store viable sperm for long periods. This may promote sperm competition and differential "male" fertilization success in this hermaphroditic gastropod.

The purpose of this study was to examine two variables of potential importance to "male" fertilization success in *A. arbustorum*. A laboratory experiment was designed to investigate the effects of the delay between two matings and the size of the sperm-donating snails on sperm precedence in double mated individuals of *A. arbustorum*. Paternity in broods was analysed using shell colour as a genetic marker. The observation period following the second mating was extended to 1–2 years to cover several egg-laying episodes and to examine any possible changes in sperm precedence. I also present data on hatchlings deriving from wild-caught snails that demonstrate a high incidence of multiple paternity and sperm competition in a natural population of *A. arbustorum*.

Methods

Study organism

Arianta arbustorum is a simultaneously hermaphroditic land snail, which is common in moist habitats of north-western and central Europe (Kerney and Cameron 1979). Individuals become sexually mature at an age of 2–4 years. On average, adult snails live 3–4 years (maximum 14 years; Baur and Raboud 1988). In the field, snails deposit 1–3 clutches, each containing 20–50 eggs, per reproductive season (Baur and Raboud 1988; Baur 1990).

Mating of *A. arbustorum* includes elaborate courtship behaviour and lasts 2–18 h (Hofmann 1923; Baur 1992a). Copulation is reciprocal; spermatophores are transferred after simultaneous intromission. *A. arbustorum* mates repeatedly in the course of a reproductive season and viable sperm can be stored for more than 1 year (Baur 1988a). The sperm storage organ (receptaculum seminis) of *A. arbustorum* consists of several blind sacs that have a common entrance into the duct of the fertilization pouch (M. Haase, unpublished data). *A. arbustorum* has been reported to be self-incompatible (Lang 1904). More recently, extensive laboratory breeding revealed that 12 of 44 virgin individuals (27.3%) prevented from mating produced a few hatchlings by self-fertilization in the 2nd or 3rd year (Chen 1993). However, their reproductive success was less than 2% of that of mated snails (Chen 1993).

Using genetic markers

The genetic background of shell polymorphism including ground colour and banding pattern is well studied in *A. arbustorum* (Oldham 1934; Cook and King 1966). One locus controls the ground colour of the shell, which may be yellow or brown (brown being dominant). I used shell colour as genetic marker to analyse paternity in broods of double-mated individuals of known genotype because shell colour is already distinctly expressed in hatchlings.

General methods

Virgin snails with yellow shells (subadult individuals that had not yet completed shell growth) were collected in an alpine pasture near Amden, Switzerland (47°10′ N, 9°11′ E; altitude 1430 m above sea level), where the brown phenotype is not known to occur. The snails were kept isolated in transparent plastic containers ($14 \times 10 \times 5$ cm in size) until they reached sexual maturity as indicated by the formation of a reflected lip at the shell aperture. The bottoms of the containers were lined with moist soil mixed with powdered limestone. The containers were cleaned one or two times per week and fresh lettuce was provided ad libitum as food. Sexually mature snails were marked individually with numbers written on their shells with a waterproof felt-tipped pen on a spot of correction fluid (Tipp-Ex). The animals showed no visible reaction to the marking procedure.

As mating partner (sperm donor) either adult snails from the same population (homozygous recessive for shell colour) or individuals that were homozygous dominant for shell colour were used. The latter animals were obtained from the embankment along a track in a subalpine forest near Gurnigel, Switzerland (46°44′ N, 7°27′ E; altitude 1430 m). All snails from this population had brown shells, and no yellow phenotype was observed during 4 years' field work in this population. Furthermore, preliminary breeding experiments with yellow snails indicated that all individuals from the Gurnigel population were homozygous for the shell colour "brown". The snails that served as sperm donors were kept isolated in containers as described above. Each snail was used only once as sperm donor.

Mating trials were initiated in the evening and run during the night between May and July (period of maximum mating activity in natural snail populations) in 1989–1991. All mating trials were carried out outdoors to expose snails to natural temperature and light conditions. The experimental snail and a sperm donor of known phenotype were placed in a transparent plastic container ($14 \times 10 \times 7$ cm in size) whose bottom was covered with moistened paper towelling to maintain snail activity. The snails' behaviour was observed at intervals of 30 min (at night using a torch), and copulating pairs were carefully checked to see if reciprocal intromission had taken place. Most of the snails were mated first with a yellow-shelled partner and then with a brown-shelled partner. However, to test for equal competitive ability of sperm from the two populations, I changed the mating sequence in seven individuals. These animals showed no difference in sperm utilization pattern. This suggests that the sequence of mating does not change the competitive ability of sperm from the two populations.

After mating, snails were kept isolated in plastic containers lined with moist soil (as described above) in an environmental chamber at 19° ± 1°C and with a light/dark cycle of 18:6 h. The containers were checked weekly for eggs. Eggs were counted and incubated in plastic dishes lined with moist paper towelling at 19°C. Newly-hatched snails were separated daily from remaining unhatched eggs to prevent egg cannibalism (Baur and Baur 1986; Baur 1992b). Eggs laid by single mated snails in the period between the two matings were also checked for successful fertilizations by sperm from the first mate by counting the hatchlings.

Newly-hatched snails were assigned to the first or second sperm-donating snail (mate) depending on their shell colour. Sperm precedence (P_2) was calculated as the proportion of total offspring sired by the second mate after a virgin snail had mated twice (Boorman and Parker 1976). P_2 values range from 0 to 1: a P_2 of 0 indicates first-mate sperm precedence, a P_2 of 0.5 indicates equal fertilization success for both mates, and a P_2 of 1 indicates second-mate sperm precedence. I only calculated P_2 values when a minimum of two hatchlings emerged from an egg batch.

To assess the influence of the interval between two consecutive matings on sperm precedence, the snails were allowed to remate either within the same reproductive season (interval between matings 9–70 days; $n = 27$ snails) or in the following year (interval between matings 300–380 days; $n = 8$). To assess any possible effect of the size of the sperm donor and receptor on sperm precedence, I measured the size (shell breadth and height) of each mating snail (except for four snails acting as sperm donors) to the nearest 0.1 mm using vernier callipers and calculated the shell volume using the following formula: Shell volume $= 0.312 \times [(\text{breadth})^2 \times \text{height}] - 0.038$ (measurements in mm; B. Baur, unpublished data). Shell volume is a more reliable measurement of snail size than weight, because weight depends on the state of hydration and thus is highly variable in terrestrial gastropods.

Eggs of double-mated snails were collected over 2 years (until the snails died). To simulate seasonality of the reproductive period as it may occur in the field, the snails were allowed to hibernate from October to April at 4°C in darkness. During this period the snails were kept singly in plastic beakers (6 cm diameter, 8 cm high) provided with a 3- to 4-cm layer of moist soil, but no food.

Double-mated snails that laid three and more clutches were assigned to one of the following groups: individuals with a mean P_2 value significantly smaller than 0.5 (paired *t* -test) showed a first mate sperm precedence pattern, those with a mean P_2 value significantly larger than 0.5 showed a second-mate sperm precedence pattern. Snails whose mean P_2 value did not differ from 0.5 were considered to show a sperm mixing pattern over successive clutches.

Means are given ± SEs unless otherwise stated. Statistical analyses were performed using the SAS program package (SAS Institute 1991). Non-parametric correlation analysis was used to evaluate any relationship between P_2 and shell size of the mother snail, shell size of the two sperm donors and several reproductive variables (clutch size, time between the two matings, number of batches and fertilized eggs produced between the two matings).

Multiple paternity in a natural population

Twenty-two adult *A. arbustorum* with yellow shells were collected in a pasture on the north-facing slope of Mont Raimeux (Jura mountains, Switzerland; 47°18′ N, 7°26′ E) at an altitude of 1290 m on 20 May 1993. This population is dimorphic with respect to shell colour. The percentage of adults with yellow shells was recorded twice in 1993. Both estimates revealed very similar results: 27.7% ($n = 94$) of the adult *A. arbustorum* had yellow shells on 20 May, and 27.4% ($n = 124$) on 24 July ($\chi^2 = 0.002$, $df = 1$, $P > 0.9$). Assuming Hardy-Weinberg equilibrium, these data allowed an estimate of the frequencies of the allele "brown" ($p = 0.475$) and "yellow" ($q = 0.525$) for this population.

Adult snails were kept isolated in containers lined with moist soil for a period of 65 days. Maintenance of snails and treatment of eggs were as described above. A total of 892 hatchlings that emerged from 34 clutches were scored for their shell colour. The percentage of hatchlings with yellow shells (25.7%) was similar to that of adult snails recorded in the field (mean of both field records: 27.5%; $\chi^2 = 0.31$, $df = 1$, $P > 0.5$). This suggests that there is no selection on shell colour during the juvenile stage in *A. arbustorum* of the Mont Raimeux population.

Because the wild-caught snails were homozygous recessive for shell colour, the shell colour of the offspring gives some indication of their paternity. According to the null hypothesis of single paternity, each clutch should consist either of only yellow or only brown hatchlings or a 1:1 segregation of each. Significant deviations from Mendelian ratios indicate double or multiple paternity within a brood. However, ratios in accordance with the null hypothesis do not necessarily represent single paternity. Obviously, repeated matings with snails of the same genotype will produce results that are indistinguishable from the broods of single matings. In the present population, the probability of a particular snail mating successively with two homozygous recessive individuals is 0.076 [= prob (homozygous "yellow") × prob(homozygous "yellow") $= 0.525^2 \times 0.525^2$], with two homozygous dominant individuals is 0.051 [= prob(homozygous "brown") × prob(homozygous "brown") $= 0.475^2 \times 0.475^2$], and with two heterozygous individuals is 0.249 [= prob(heterozygous) × prob(heterozygous) $= (2 \times 0.525 \times 0.475) \times (2 \times 0.525 \times 0.475)$]. Thus, this technique allows an accurate estimate of multiple paternity in 62.4% of the cases when the snails have mated twice. Consequently, the actual frequency of multiple paternity in broods of wild-caught *A. arbustorum* may be underestimated.

Results

Immediate sperm precedence

Following the second mating, the snails produced their first egg batch in 8 days (median; range = 1 – 338 days; $n = 35$). Figure 1 shows the distribution of P_2 values of the first egg batches laid subsequent to the second mating. Sperm precedence, as expressed by the P_2 value, averaged 0.43 ± 0.06 (range 0 – 1.00, $n = 35$). Of 35 snails 24 (68.6%) used sperm from both mating partners for the fertilization of the first egg batch,

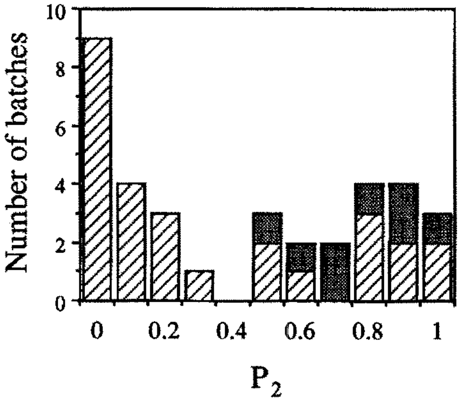

Fig. 1 Frequency distribution of P_2 values of the first egg batches laid subsequent to the second mating by *Arianta arbustorum* ($n = 35$). *Lightly shaded bars* refer to snails that mated within 70 days, *dark bars* to snails that remated after 300 and more days

Table 1 Effect of time between two matings on sperm precedence of the second mate in *Arianta arbustorum*. P_2, clutch size and hatching success of the first batch laid subsequent to the second mating are given. Data are means ± SE [range]

Interval between matings (days)	*n*	P_2	Clutch size (no. of eggs)	Hatching success (%)
9–21	9	0.32 ± 0.14 [0–1.00]	44.4 ± 5.1	45.4 ± 10.7
23–34	13	0.35 ± 0.10 [0–0.93]	42.6 ± 6.0	49.2 ± 8.6
47–70	5	0.33 ± 0.16 [0–0.94]	41.1 ± 4.8	35.9 ± 7.5
300–380	8	0.76 ± 0.06 [0.50–1.00]	33.0 ± 4.5	41.1 ± 6.8

resulting in multiple paternity in their offspring, whereas 8 snails (22.9%) exclusively used sperm from the first sperm donor ($P_2 = 0$) and 3 snails (8.6%) sperm from the second sperm donor ($P_2 = 1$). Snails using sperm from only one individual did not differ in clutch size and hatching success of eggs from snails using sperm from two mates (Mann-Whitney *U*-test; clutch size: $P = 0.59$; hatching success: $P = 0.18$).

Considering snails that remated in the same reproductive period (time between matings 9–70 days), P_2 averaged 0.34 ± 0.07 ($n = 27$; Table 1). In these snails the time between the two matings had no influence on sperm precedence ($r_s = 0.04$, $n = 27$, $P = 0.83$). However, snails that remated in the following reproductive season (after 300–380 days) showed a significant second mate sperm precedence with a P_2 averaging 0.76 ± 0.06 ($n = 8$) (Mann-Whitney *U*-test, $P = 0.013$). This indicates that the time between two matings affects sperm precedence if the delay exceeds one reproductive season ($r_s = 0.41$, $n = 35$, $P = 0.013$). The interval between second mating and oviposition of the first clutch did not influence sperm precedence ($r_s = -0.20$, $n = 35$, $P = 0.25$).

In the period between the two matings the snails produced 3.3 ± 0.4 (range 1–10) clutches with a total of 117 ± 10 (41–312) eggs. Both the number of clutches and total number of eggs produced were positively correlated with the time between the two matings (clutches: $r_s = 0.78$, $n = 35$, $P < 0.001$; eggs: $r_s = 0.62$, $n = 35$, $P < 0.001$). However, P_2 was not correlated with the number of hatchlings sired by the first sperm donor in the period between the two matings ($r_s = 0.03$, $n = 35$, $P = 0.85$). This suggests that the increase in P_2 after remating in the successive season is unlikely to be caused by depletion of first mate's sperm due to fertilization of eggs in the period between the two matings. Furthermore, P_2 was not correlated with clutch size ($r_s = -0.11$, $n = 35$, $P = 0.53$).

"Female" fecundity (i.e. the number of clutches produced per season, clutch size and egg size) is positively correlated with shell size in *A. arbustorum* (Baur 1984, 1988b; Baur and Raboud 1988). Assuming that the number of sperm received by the mother snail is related to the size of the sperm donor, one could expect first-mate sperm precedence if the first sperm donor is larger than the second sperm donor, whereas second-mate sperm precedence should occur if the first sperm donor is smaller than the second one. Thus, P_2 should negatively correlate with the shell size ratio first/second sperm donor. However, no correlation between immediate P_2 and the shell size ratio of the two sperm donors was found (all snails: $r_s = -0.17$, $n = 31$, $P = 0.35$; only snails that remated within 70 days: $r_s = -0.19$, $n = 26$, $P = 0.35$).

Long-term sperm precedence

In 23 double-mated snails, I obtained P_2 values for three and more successive egg batches (mean = 5.0, maximum = 9 batches). The snails showed different patterns of sperm utilization. Of the 23 snails 5 (21.7%) exhibited first-mate sperm precedence ($P_2 = 0.087 \pm 0.014$) in successive batches. Eight snails (34.8%) showed

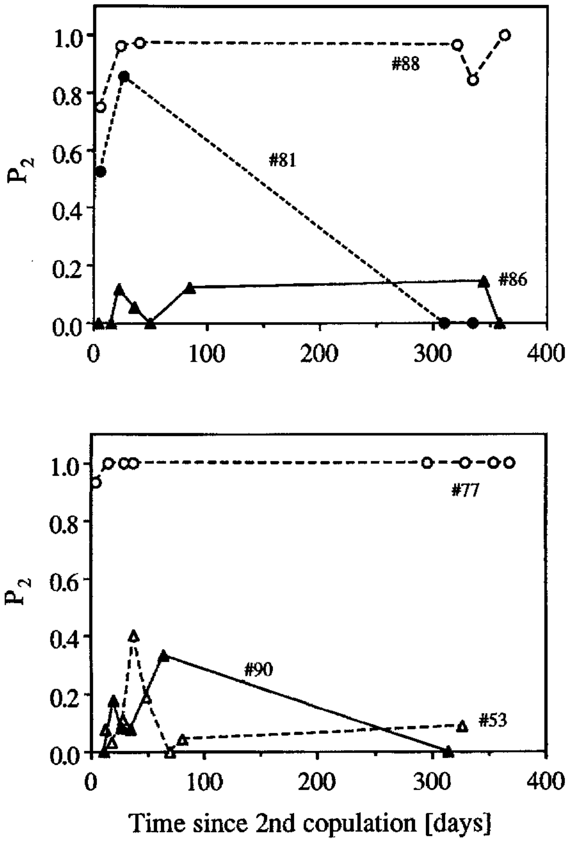

Fig. 2 Variation in P_2 of successive egg batches for individual snails. *Triangles* refer to snails showing first-mate sperm precedence, *open dots* to snails with second-mate sperm precedence and *full dots* to snails with sperm mixing. The snails hibernated approximately from day 100 to day 280

second-mate sperm precedence throughout ($P_2 = 0.842 \pm 0.046$), whereas ten snails (43.5%) exhibited sperm mixing in successive batches ($P_2 = 0.447 \pm 0.049$). Figure 2 shows the variation in P_2 with time in successive batches for individual snails.

Snails that showed different sperm utilization patterns did not differ in shell size (Table 2). The size ratio of successive mating partners indicates that the second sperm donor tended to be larger than the first sperm donor in individuals with second-mate sperm precedence, whereas the reverse occurred in snails with first-mate sperm precedence and sperm mixing (Table 2). However, the differences in size ratio of successive mates did not quite reach the 5% significance level ($P = 0.079$). Individuals with second-mate sperm precedence had a larger mating delay than snails with first-mate precedence or sperm mixing (Table 2). As a result of the longer period between the two matings, snails of the group "second-mate sperm precedence" produced more eggs and hatchlings than snails from the other two groups (Table 2). Considering reproduction after the second mating, snails of the three groups did not differ in number of clutches produced, clutch size or hatching success of their eggs (Kruskal-Wallis test, in each case $P > 0.2$).

Multiple paternity in a natural population

Of the 22 adult *A. arbustorum* collected on Mont Raimeux 19 produced 34 clutches with fertilized eggs in the laboratory. Of the 34 clutches 9 (26.5%) deviated significantly from Mendelian ratios of single paternity, providing evidence for multiple paternity (Table 3; two-tailed binomial test, in 9 clutches, $P < 0.05$). As explained in Methods, this figure may underestimate the actual frequency of multiple paternity in broods of *A. arbustorum*.

Several snails deposited two or three clutches. Considering the total number of offspring produced by single snails during 65 days, the progeny of 12 of 19 individuals (63.2%) deviated significantly from Mendelian ratios of a single copulation. Again, this figure may underestimate the actual frequency of multiple mating in *A. arbustorum*.

For a two-allele, autosomal locus, the proportion of offspring of different genotypes from homozygous recessive females can be calculated for random mating with a single mate, with two, or with three mates (Johnson 1977). The distribution of genotypes found in hatchlings of the 19 snails of the Mont Raimeux population fitted the expected distribution of random mating with two mates per snail best ($\chi^2 = 1.54$, $df = 2$, $P > 0.4$), and that with three mates rather well ($\chi^2 = 3.00$, $df = 2$, $P > 0.2$), but it deviated significantly from the expected distribution of random mating with a single mate ($\chi^2 = 15.42$, $df = 2$, $P < 0.001$). This indicates that adult snails had copulated with 2 or 3 mates before they were collected for this study.

Of the 19 snails 3 produced three batches within 65 days. Figure 3 shows the percentage of brown hatchlings for each snail and batch. Considering single batches, the offspring of the first and third batch from snail 2 might have multiple paternity, while the offspring from the snails 11 and 13 did not deviate significantly from Mendelian ratios of single paternity (Table 3). Considering successive batches, snail 11 must have mated with two snails of different genotype.

Discussion

The present study demonstrates that individual variation in sperm precedence in *A. arbustorum* is related to the time between matings. However, there may be additional factors, such as the amount of sperm transferred and the way in which sperm are stored in the spermatheca, that influence the individual variation in P_2 (see below). To my knowledge, this is the first study which experimentally examines sperm competition in a simultaneously hermaphroditic land snail.

Table 2 Reproductive characteristics of 23 *A. arbustorum* that exhibited different sperm utilization patterns over 2 years (see Methods). Data are means ± SE

Variable	Sperm utilization pattern			Kruskal-Wallis test *P*
	First-mate precedence	Sperm mixing	Second-mate precedence	
Number of snails	5	10	8	
Shell size mother (cm^3)	1.54 ±0.06	1.57 ±0.04	1.63 ±0.07	0.573
Size ratio of two successive mates	1.24 ±0.06	1.19 ±0.07	0.98 ±0.09	0.079
Time between two matings (days)	23.6 ±2.0	52.5 ±35.2	192.4 ±61.6	0.004
Number of eggs produced between two matings	104.0 ±10.4	84.9 ±14.0	147.5 ±28.4	0.047
Number of hatchlings between two matings	27.4 ±10.0	34.9 ±8.4	74.4 ±18.3	0.070

Table 3 Paternity in offspring of homozygous recessive *A. arbustorum* which were collected as adults in a natural population on Mont Raimeux. Deviations from the Mendelian ratios of the null hypothesis of single paternity were evaluated using two-tailed binomial tests (see Methods)

Snail no	Batch no	Number of hatchlings	Yellow hatchlings	Brown hatchlings	P to reject H_0 single paternity
1	1	26	26	0	–
	2	11	2	9	0.066
2	1	19	3	16	0.004
	2	30	11	19	0.201
	3	35	4	31	< 0.001
4	1	37	1	36	< 0.001
5	1	48	8	40	< 0.001
6	1	51	0	51	–
7	1	22	6	16	0.052
	2	29	8	21	0.026
9	1	24	0	24	–
	2	30	9	21	0.044
10	1	31	5	26	< 0.001
11	1	36	0	36	–
	2	28	0	28	–
	3	19	6	13	0.168
12	1	9	6	3	0.508
13	1	27	13	14	1.000
	2	39	16	23	0.337
	3	32	13	19	0.379
14	1	43	6	37	< 0.001
15	1	13	3	10	0.092
	2	8	2	6	0.290
16	1	29	13	16	0.711
	2	45	20	25	0.549
18	1	11	5	6	1.000
	2	10	5	5	1.000
19	1	16	0	16	–
	2	11	5	6	1.000
20	1	42	15	27	0.089
	2	6	1	5	0.218
21	1	35	5	30	< 0.001
	2	12	0	12	–
22	1	28	12	16	0.569

An understanding of variation in sperm competition success is important for understanding the mechanisms and evolutionary significance of sperm competition. Lewis and Austad (1990) examined sources of variation in sperm precedence in the flour beetle *Tribolium castaneum* and found consistent differences among males which accounted for 17.8% of the total variation in sperm precedence. The degree of second-male sperm precedence in *T. castaneum* was positively correlated with body size of mating beetles. However, sperm precedence was not correlated with any aspect of mating behaviour (number of mounts by the mates or copulation duration). Simmons and Parker (1992) demonstrated that individual variation in the fertilization success of male dung flies, *Scatophaga stercoraria*, is related to body size. P_2 values were dependent on the relative sizes of first and second males; large males transferred more sperm per unit time than small ones. The diet of dung flies and probably the level of prey availability during the maturation period between emergence and onset of sexual activity also contributed to the variation in P_2 (Simmons and Parker 1992).

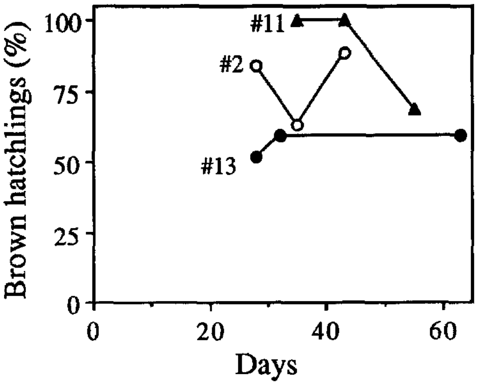

Fig. 3 Variation in percentage of hatchlings with brown shells in three successive batches laid by three wild-caught *A. arbustorum* from the Mont Raimeux population

In the present study, the size of the sperm donor had no effect on immediate sperm precedence. In *A. arbustorum*, the spermatophore is formed and filled with sperm during copulation (Hofmann 1923). The spermatophore has a distinctive form consisting of head, body (sperm container) and a 2–3 cm long tail. Spermatophores of *A. arbustorum* vary considerably in sperm content: in a sample of 55 spermatophores from snails of the Gurnigel population sperm number ranged from 115,000 to 2,850,000 (median: 700,000) (Bernasconi 1993). Sperm number was positively correlated both with spermatophore size and with the shell size of the sperm donor (G. Bernasconi and B. Baur, unpublished data). However, the number of sperm transferred in a spermatophore was not correlated with copulation duration (Bernasconi 1993). Thus, like female fecundity, male fecundity might be size-related in this simultaneously hermaphroditic land snail. The number of sperm transferred, however, must not necessarily correlate with fertilization success (female sperm preference; see below). This could explain the lack of a significant effect of the size of the sperm donor on sperm precedence in this study (although a tendency for an increased fertilization success over a longer period was found in relatively large sperm-donating individuals).

Sperm precedence in *A. arbustorum* could also be influenced by anatomical features of the reproductive tract and, especially, of the spermatheca that consists of several blind sacs with a common entrance. The way in which sperm from different mates are stored in the spermatheca is not known. However, a snail could manipulate the fertilization success of sperm from different mates by storing sperm in different sacs (female control of paternity). The adaptive significance of the spermatophore in helicid land snails with well-developed copulation organs and internal fertilization is unclear. In *Helix pomatia*, sperm leave the spermatophore body through the spermatophore tail in the bursa stalk and migrate into the spermatheca (Lind 1973). The spermatophore and any remaining sperm are dissolved later in the bursa copulatrix. Lind (1973) suggested that the function of the spermatophore is to ensure that a number of sperm can migrate into the oviduct (and reach the spermatheca) without coming into contact with the digesting bursa copulatrix. Thus, the significance of the peculiar way of transferring sperm in *H. pomatia* (and most probably in *A. arbustorum*) may be to allow only the most active sperm to pass to the spermatheca. This kind of sperm selection would also allow "female" manipulation resulting in particular sperm preferences. Anyhow, the way in which the sperm have to escape from the bursa stalk implies that large numbers of sperm are lost. Lind (1973) stated (without presenting any actual data) that only about 0.1% of the sperm of a spermatophore reach the spermatheca.

Repeated mating has been observed in *A. arbustorum* (Baur and Raboud 1988; Baur 1992a). It is, however, not exactly known how frequently *A. arbustorum* mates in the course of a reproductive season under natural conditions. A controlled laboratory experiment demonstrated that one copulation per reproductive season is sufficient to fertilize all the eggs produced by an individual (Chen and Baur 1993). However, there is a possibility that a particular copulation will not lead to successful fertilization of eggs. Furthermore, egg production in *A. arbustorum* tends to increase with the number of matings. As in several other stylommatophoran land snails, mating behaviour and/or the transfer of a spermatophore stimulates egg production via hormones (Griffond and Gomot 1989; Saleuddin et al. 1991; Baur and Baur 1992). Furthermore, multiple mating with different partners may lead to multiple parentage and thus increase genetic variability within a brood. Hence, repeated mating within a season might be adaptive for the female function of *A. arbustorum* by stimulating egg production and serving as a hedge against sterile mates (Chen and Baur 1993).

Recently, several models have been developed to explain sperm competition mechanisms, and particularly to explain the P_2 characteristics for various types of reproductive systems (Lessells and Birkhead 1990; Parker 1990a, b, 1992, 1993; Parker et al. 1990; Parker and Simmons 1991). Sperm precedence may be influenced by the number of sperm received from either of two mates (Parker 1984). The more sperm a particular male has in the female tract or in her sperm storage organ, the higher his chances for fertilization success. Parker (1990a) described this as the raffle principle, which is based on the assumption of random sperm mixing. The number of sperm transferred in a spermatophore of *A. arbustorum* varies enormously (see above). Sperm precedence could therefore result from different amounts of sperm received in two successive matings. Thus, sperm competition could be described by the fair raffle principle. However, complete sperm mixing is unlikely considering the anatomical structure of the spermatheca of *A. arbustorum* (several tubules). In contrast, second-mate sperm precedence becomes predictable with an increasing delay between two matings. This suggests that the first male's sperm became exhausted or that some spermatozoa died.

Little information concerning the viability of allosperm in the storage organs of pulmonates is available. Viable sperm have been found 520 days after the last copulation in the tropical land snail *Limicolaria martensiana* (Owiny 1974), 341 days in *Achatina fulica* and 476 days in *Macrochlamys indica* (Raut and Ghose 1979). However, sperm viability in terrestrial gastropods may not be a simple function of time. In *A. fulica* and *M. indica*, the viability of sperm stored is influenced by the length of the aestivation period. Sperm viability decreased to 105 days in aestivating *A. fulica* and to 150 days in *M. indica* (Raut and Ghose 1982). Most probably sperm viability will also decrease in hibernating *A. arbustorum*, although detailed information is not available. Thus, considering a period longer than a year, a loaded raffle with time-related sperm viability may best describe the outcome of sperm competition in *A. arbustorum*. Much remains to be learnt about the physiological and behavioural mechanisms of sperm uptake, maintenance and release from the spermatheca in hermaphroditic gastropods.

The results in *Tribolium castaneum* (Lewis and Austad 1990) and dung flies (Simmons and Parker 1992) and a reexamination of published data by Lewis and Austad (1990) demonstrated a high degree of intraspecific variation in sperm precedence, suggesting that mean values are insufficient to adequately characterize sperm precedence patterns. The present study supports this conclusion.

The present study also provides evidence for a high incidence of multiple paternity in a natural population of *Arianta arbustorum*. Until recently, evidence for multiple paternity was scarce in gastropods, an exception being *Cepaea nemoralis* (Murray 1964). Using enzyme electrophoresis, multiple paternity has been demonstrated in a few species of freshwater snails (*Biomphalaria obstructa*, Mulvey and Vrijenhoek 1981; *Bulinus cernicus*, Rollinson et al. 1989; *Physa heterostropha pomilia*, Wethington and Dillon 1991) and in a marine gastropod (*Crepidula fornicata*, a protandric hermaphrodite, Gaffney and McGee 1992). Sperm competition may occur more frequently among gastropods than commonly assumed. Several snail species may be well-suited for studies on sperm competition, but their potential as experimental organisms has not yet begun to be exploited by evolutionary biologists.

Acknowledgements I thank A. Baur, T. Birchler and C. Seiberth for technical assistance, and A. Baur, G. Bernasconi, M. Haase, K. Lessells and S.C. Stearns for constructive comments on the manuscript. Financial support was received from the Swiss National Science Foundation (grants 31–26258.89 and 31–33511.92), and the Treubel Foundation of the Freiwillige Akademische Gesellschaft, University of Basel.

References

Baur B (1984) Shell size and growth rate differences for alpine populations of *Arianta arbustorum* (L.) (Pulmonata: Helicidae). Rev Suisse Zool 91:37–46

Baur B (1988a) Repeated mating and female fecundity in the simultaneously hermaphroditic land snail *Arianta arbustorum*. Invert Reprod Develop 14:197–204

Baur B (1988b) Population regulation in the land snail *Arianta arbustorum*: density effects on adult size, clutch size and incidence of egg cannibalism. Oecologia 77:390–394

Baur B (1990) Seasonal changes in clutch size, egg size and mode of oviposition in *Arianta arbustorum* (L.) (Gastropoda) from alpine populations. Zool Anz 225:253–264

Baur B (1992a) Random mating by size in the simultaneously hermaphroditic land snail *Arianta arbustorum*: experiments and an explanation. Anim Behav 43:511–518

Baur B (1992b) Cannibalism in gastropods. In: Elgar MA, Crespi BJ (eds) Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, Oxford, pp 102–127

Baur B, Baur A (1986) Proximate factors influencing egg cannibalism in the land snail *Arianta arbustorum* (Pulmonata, Helicidae). Oecologia 70:283–287

Baur B, Baur A (1992) Effect of courtship and repeated copulation on egg production in the simultaneously hermaphroditic land snail *Arianta arbustorum*. Invert Reprod Develop 21:201–206

Baur B, Raboud C (1988) Life history of the land snail *Arianta arbustorum* along an altitudinal gradient. J Anim Ecol 57:71–87

Bernasconi G (1993) Outnumbering the rivals' sperm: intraspecific variation in sperm expenditure in a hermaphroditic land snail. Master thesis, University of Basel

Birkhead TR, Hunter FM (1990) Mechanisms of sperm competition. Trends Ecol Evol 5:48–52

Birkhead TR, Møller AP (1992) Sperm competition in birds. Evolutionary causes and consequences. Academic Press, London

Boorman E, Parker GA (1976) Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to females age and mating status. Ecol Entomol 1:145–155

Chen X (1993) On reproductive strategies in hermaphroditic land snails. PhD thesis, University of Basel

Chen X, Baur B (1993) The effect of multiple mating on female reproductive success in the simultaneously hermaphroditic land snail *Arianta arbustorum*. Can J Zool 71:2431–2436

Cook LM, King JMB (1966) Some data on the genetics of shell-character polymorphism in the snail *Arianta arbustorum*. Genetics 53:415–425

Duncan CJ (1975) Reproduction. In: Fretter V, Peake J (eds) Pulmonates, vol. 1. Academic Press, London, pp 309–365

Gaffney PM, McGee B (1992) Multiple paternity in *Crepidula fornicata* (Linnaeus). Veliger 35:12–15

Ginsberg JR, Huck UW (1989) Sperm competition in mammals. Trends Ecol Evol 4:74–79

Griffond B, Gomot L (1989) Endocrinology of reproduction in stylommatophoran pulmonate gastropods with special reference to *Helix*. Comp Endocrinol 8:23–32

Gwynne DT (1984) Male mating effort, confidence of paternity, and insect sperm competition. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic Press, New York, pp 117–149

Hofmann E (1923) Über den Begattungsvorgang von *Arianta arbustorum* L. Jen Zeitsch Naturw 59 (NF 52):363–400

Johnson C (1977) The use of gene frequencies in estimating the mean number of mates in a multiple-mate and stored-sperm system of mating. Theor Appl Genet 49:181–185

Kerney MP, Cameron RAD (1979) A field guide to the land snails of Britain and North-West Europe. Collins, London

Lang A (1904) Über Vorversuche zu Untersuchungen über die Varietätenbildung von *Helix hortensis* Müller und *Helix nemoralis* L. Denkschr Med Naturw Ges Jena 11:437–506

Lessells CM, Birkhead TR (1990) Mechanisms of sperm competition in birds: mathematical models. Behav Ecol Sociobiol 27: 325–337

Lewis SM, Austad SN (1990) Sources of intraspecific variation in sperm precedence in red flour beetles. Am Nat 135:351–359

Lind H (1973) The functional significance of the spermatophore and the fate of spermatozoa in the genital tract of *Helix pomatia* (Gastropoda: Stylommatophora). J Zool Lond 169:39–64

Mulvey M, Vrijenhoek RC (1981) Multiple paternity in the hermaphroditic snail, *Biomphalaria obstructa*. J Hered 72:308–312

Murray J (1964) Multiple mating and effective population size in *Cepaea nemoralis*. Evolution 18:283–291

Oldham C (1934) Some albinistic varieties of *Arianta arbustorum* (L.). Proc Malacol Soc Lond 21:103–108

Owiny AM (1974) Some aspects of the breeding biology of the equatorial land snail *Limicolaria martensiana* (Achatinidae: Pulmonata). J Zool Lond 172:191–206

Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. Biol Rev 45:525–568

Parker GA (1984) Sperm competition and the evolution of animal mating strategies. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic Press, New York, pp 1–60

Parker GA (1990a) Sperm competition games: raffles and roles. Proc R Soc Lond B 242: 120–126

Parker GA (1990b) Sperm competition games: sneaks and extra-pair copulations. Proc R Soc Lond B 242: 127–133

Parker GA (1992) Marginal value theorem with exploitation time costs: diet, sperm reserves, and optimal copulation duration in dung flies. Am Nat 139: 1237–1256

Parker GA (1993) Sperm competition games: sperm size and sperm number. Proc R Soc Lond B 253: 245–254

Parker GA, Simmons LW (1991) A model of constant random sperm displacement during mating: evidence from *Scatophaga*. Proc R Soc Lond B 246: 107–115

Parker GA, Simmons LW, Kirk H (1990) Analysing sperm competition data: simple models for predicting mechanisms. Behav Ecol Sociobiol 27: 55–65

Raut SK, Ghose KC (1979) Viability of sperm in two land snails, *Achatina fulica* Bodwich and *Macrochlamys indica* Godwin-Austen. Veliger 21: 486–487

Raut SK, Ghose KC (1982) Viability of sperms in aestivating *Achatina fulica* Bodwich and *Macrochlamys indica* Godwin-Austen. J Mollusc Stud 48: 84–86

Rollinson D, Kane RA, Linnes JRL (1989) An analysis of fertilization in *Bulinus cernicus* (Gastropoda: Planorbidae). J Zool Lond 217: 295–310

Saleuddin ASM, Griffond B, Ashton ML (1991) An ultrastructural study of the activation of the endocrine dorsal bodies in the snail *Helix aspersa* by mating. Can J Zool 69: 1203–1215

SAS Institute (1991) SAS user's guide: statistics (1991 edn). SAS Institute, Cary

Simmons LW, Parker GA (1992) Individual variation in sperm competition success of yellow dung flies, *Scatophaga stercoraria*. Evolution 46: 366–375

Siva-Jothy MT, Tsubaki Y (1989) Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae). 1. Alternative mate-securing tactics and sperm precedence. Behav Ecol Sociobiol 24: 39–45

Smith RL (ed) (1984) Sperm competition and the evolution of animal mating systems. Academic Press, New York

Tompa AS (1984) Land snails (Stylommatophora). In: Tompa AS, Verdonk NH, Biggelaar JAM van den (eds) The Mollusca, vol. 7. Academic Press, London, pp 47–140

Walker WF (1980) Sperm utilization strategies in nonsocial insects. Am Nat 115: 780–799

Wethington AR, Dillon RT (1991) Sperm storage and evidence for multiple insemination in a natural population of the freshwater snail, *Physa*. Am Malacol Bull 9: 99–102

Communicated by M.A. Elgar