



# Sperm allocation in the simultaneously hermaphroditic land snail *Arianta arbustorum*

BRUNO BAUR, ROLF LOCHER & ANETTE BAUR

Department of Integrative Biology, Section of Conservation Biology (NLU), University of Basel

(Received 3 November 1997; accepted 13 January 1998;  
final acceptance 6 March 1998; MS. number: 5697)

## ABSTRACT

To test the idea that individuals of the simultaneously hermaphroditic land snail *Arianta arbustorum* can control the number of spermatozoa in their spermatophores, we investigated whether they differentially release sperm to virgin or nonvirgin partners with respect to the potential risk of sperm competition in a given mating. The number of sperm transferred ranged from 802 620 to 3 968 800 ( $\bar{X}$  = 2 185 100;  $N$  = 91), but was related neither to the mating history of the partner nor to copulation duration. This indicates that individuals of *A. arbustorum* are not able to adjust sperm expenditure to the mating history of the partner. Furthermore, the number of sperm transferred was correlated neither with the size of the donor nor with the size of the recipient. It has been proposed that the sexual conflict between the two genders in simultaneous hermaphrodites could be resolved by gamete trading. Theory predicts that sperm trading should occur in hermaphrodites in which the female role controls fertilization, for example in gastropods with a gametolytic gland and/or sperm storage such as *A. arbustorum*. To see whether sperm trading occurs, we also examined whether individuals of *A. arbustorum* adjust the number of sperm they release to the number they receive from their mating partner. There was a high degree of reciprocity in spermatophore transfer: in 45 of the 46 mating pairs investigated both partners delivered a spermatophore that contained spermatozoa. The numbers of sperm transferred by the two mating partners were not correlated, however. This indicates that sperm trading does not occur in this simultaneously hermaphroditic land snail.

© 1998 The Association for the Study of Animal Behaviour

Sperm number is, in some cases, an important determinant for achieving successful fertilization in sperm competition (Parker 1970; Smith 1984; Birkhead & Møller 1998). Theoretical models and empirical evidence from various studies suggest that, fundamentally, numerical superiority is an adaptive strategy for sperm competition (Parker 1990a, b; Birkhead & Møller 1998). Males may incur a substantial cost, however, in ejaculate and spermatophore production (Dewsbury 1982; Nakatsuru & Kramer 1982). Parker's model (1990a, b) predicts that males faced with an increased risk of sperm competition should maximize fertilization prospects by inseminating more sperm per ejaculate. For example, owing to sperm storage from previous copulations, mating with a nonvirgin partner may result in a higher risk of sperm competition than mating with a virgin partner. Experimental evidence for adjustment of ejaculate size with respect to mating history has been provided for insects (meal

worm beetles, *Tenebrio molitor*: Gage & Baker 1991; Mediterranean fruit flies, *Ceratitis capitata*: Gage 1991; bush crickets, *Requena verticalis*: Simmons et al. 1993), salamanders, *Ambystoma texanum* (McWilliams 1992), rats, *Rattus norvegicus* (Bellis et al. 1990) and humans (Baker & Bellis 1989), but not for zebra finches, *Taeniopygia guttata* (Birkhead & Fletcher 1995). Gastropods show diverse sexual systems and reproductive strategies with very complex mating behaviours (Duncan 1975; Tompa 1984; Baur 1998). The variety of complex genitalia suggests that sexual selection is likely to be important in this group (cf. Eberhard 1996). No information on adaptive variation in the number of sperm delivered at mating is available for terrestrial gastropods, however.

One of our aims in the present study was to examine whether individuals of the simultaneously hermaphroditic land snail *Arianta arbustorum* adjust sperm release according to the potential risk of sperm competition incurred with a virgin or nonvirgin mating partner. An adjustment of the number of sperm released is also a prerequisite for sperm trading. In simultaneous hermaphrodites, a sexual conflict may arise when there is a

Correspondence: B. Baur, Department of Integrative Biology, Section of Conservation Biology (NLU), University of Basel, St Johannis-Vorstadt 10, CH-4056 Basel, Switzerland (email: baur@ubaclu.unibas.ch).

difference in potential fitness gain between the role of the sperm donor and that of the sperm receiver (Charnov 1979; Fischer 1980; Leonard 1991). Hermaphroditic individuals in a population would benefit from mating primarily in the more fitness-enhancing sexual role leading to a conflict of interest between two prospective mating partners (Charnov 1979). It has been suggested that gamete trading might have evolved to resolve the sexual conflict in simultaneous hermaphrodites (Leonard & Lukowiak 1984, 1985; Leonard 1991). The gamete trading model is based on the premise that the preferred role for a simultaneous hermaphrodite will be the one that controls fertilization (Leonard 1991). In particular, this model predicts that where the female function controls fertilization, the mating system will be based on sperm trading. Evidence for sperm trading is so far only available for two species, however: the sea slug *Navanax inermis* (Leonard & Lukowiak 1984, 1985) and the freshwater flatworm *Dugesia gonocephala* (Vreys & Michiels 1998). Leonard (1991) suggested that sperm trading should occur in all simultaneously hermaphroditic gastropods, except those that lack a gametolytic gland and/or sperm storage. Sperm trading has not yet been examined in any pulmonate land snail species, however. A further aim of this study was to examine whether sperm trading occurs in mating pairs of *A. arbustorum*.

In *A. arbustorum*, repeated mating has been observed both in a field cage experiment and in the laboratory (Baur & Raboud 1988; Baur 1992). In natural populations, mating was found to be random with respect to shell size (Baur 1992). In the laboratory, mate choice tests revealed that individuals of *A. arbustorum* do not discriminate between potential mates of different size nor between partners of different degrees of relatedness (Baur 1992; Baur & Baur 1997). 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 1992). Sperm stored from previous copulations may promote sperm competition in *A. arbustorum*. Paternity analysis in broods of wild-caught *A. arbustorum* showed a high frequency of multiple insemination (Baur 1994a). Furthermore, differential 'male' fertilization success has been observed in double-mated individuals of *A. arbustorum* (Baur 1994a).

Here we present the results of an experiment designed to test whether the male function of the simultaneously hermaphroditic land snail *A. arbustorum* can control the number of sperm released. In particular, we asked (1) whether individuals of *A. arbustorum* adjust sperm release according to the potential risk of sperm competition incurred with a virgin or nonvirgin mating partner, and (2) whether individuals adjust the number of sperm they release to the number they receive from their mating partner.

## METHODS

### Study Animals

*Arianta arbustorum* is a simultaneously hermaphroditic land snail, which is common in moist habitats of north-

western and central Europe. The snail has determinate growth (shell breadth of adults 17–22 mm). Individuals become sexually mature at 2–4 years, and adults live another 3–4 years (maximum 14 years; Baur & Raboud 1988). In the field, snails deposit one to three egg batches consisting of 20–50 eggs, per reproductive season (Baur & Raboud 1988; Baur 1990). Breeding experiments showed that 12 of 44 virgin individuals (27%) prevented from mating produced a few hatchlings by self-fertilization in the second and third reproductive year (Chen 1993). The reproductive success of selfing individuals, however, was less than 2% of that of mated snails, suggesting high costs for selfing (Chen 1994).

Mating in *A. arbustorum* includes elaborate courtship behaviour with optional dart shooting (i.e. the pushing of a calcareous dart into the mating partner's body), and lasts 2–18 h (Hofmann 1923; Baur 1992). Copulation is reciprocal; after intromission each snail transfers simultaneously one spermatophore (Haase & Baur 1995). The spermatophore is formed and filled with sperm during copulation (Hofmann 1923). It has a distinctive form consisting of a head, a body (sperm container) and a tail 2–3 cm long. The snails mate repeatedly in the course of a reproductive season, and viable sperm can be stored for more than 1 year (Baur 1988a).

### General Methods

We collected subadult individuals that had not yet completed shell growth and adult snails (fully grown individuals with a reflected lip at the shell aperture) from an embankment along a track in a subalpine forest near Gurnigelbad, 30 km south of Bern, Switzerland, on 27 April 1996 (46°45'N, 7°28'E; at an altitude of 1250 m above sea level). Fully grown snails (hereafter referred to as nonvirgins) were sexually mature and stored viable sperm from previous matings (most likely from the preceding year) as indicated by the production of fertilized eggs in the laboratory.

The snails were kept isolated in transparent beakers (8 cm deep, 6.5 cm in diameter) lined with moist soil (approximately 4 cm) at 19°C and on a light:dark cycle of 18:6 h for 4 weeks. During this period, subadult individuals reached sexual maturity as indicated by the formation of a reflected lip at the shell aperture (snails from this group are hereafter referred to as virgins). We kept the snails singly to prevent them mating repeatedly, which may deplete their autosperm supply. In the field, *A. arbustorum* may copulate two to five times each year, although one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by one individual (Chen & Baur 1993). An isolation period of 4 weeks may represent an average interval between two copulations under natural conditions and does not lead to allosperm depletion. We cleaned and checked the beakers for eggs twice per week, and provided fresh lettuce ad libitum as food. Over the 4 weeks, each nonvirgin snail laid one to three batches of fertilized eggs, whereas virgin individuals produced no fertilized eggs. Snails of both groups 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.

Snails were allowed to mate in transparent plastic containers, measuring  $14 \times 10 \times 7$  cm, whose bottoms were covered with moistened paper towelling to encourage activity. We placed two randomly chosen snails of known mating history (either virgin or nonvirgin) in a mating arena. There were three types of pairs: virgin–virgin, virgin–nonvirgin and nonvirgin–nonvirgin. Mating trials were initiated in the evening and ran during 3 nights between 24 May and 5 June 1996. This period is the time of maximum mating activity in subalpine populations of *A. arbustorum*. We performed all mating trials outdoors to expose snails to natural temperature and light conditions.

We observed the snails' behaviour at intervals of 30 min (at night using a torch). Records included time until initiation of courtship, courtship duration (time interval from courtship initiation to copulation) and copulation duration. The initiation of courtship was defined as the first simultaneous oral contact (which was usually accompanied by a slight eversion of the penial lobe in one of the snails). The beginning of copulation was defined as the first simultaneous intromission. We observed copulating snails at intervals of 10–15 min. Observation sessions were terminated either when a pair finished copulation or after 8 h if no snail initiated courtship behaviour in a test arena. Snails that did not mate were tested again 5 or 13 days later with a new partner. Between two trials, unmated snails were kept in isolation as described above.

In all, 94 trials lasting 722 h were run. Snails that copulated were frozen immediately after copulation. To obtain the spermatophore we dissected out the female reproductive duct of the recipient. Spermatophores were kept singly in Eppendorf tubes at  $-40^{\circ}\text{C}$  until required.

To assess any effect of the size of the sperm donor and recipient on the number of sperm transferred, we measured the size (shell breadth and height) of each mating snail to the nearest 0.1 mm using vernier callipers and calculated the shell volume using the 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. We measured the length ( $L$ ) and width ( $W$ ) of the sperm-containing part of each spermatophore to the nearest 0.1 mm using a dissecting microscope at  $16 \times$  magnification. Spermatophore size (in  $\text{mm}^3$ ) was approximated, by the formula ( $\pi LW^2/4$ ), assuming a cylindrical volume.

### Procedure for Sperm Counting

The procedure for sperm counting is described in detail in Locher & Baur (1997). We evaluated the number of sperm that an individual delivered to a mating partner by counting the number of sperm in the spermatophore transferred. The spermatophore of *A. arbustorum* consists of a hardened secretion which encapsulates the

spermatozoa (Hofmann 1923). We mechanically disrupted the spermatophore in 200 ml PBS-buffer ( $138.6 \text{ mM NaCl}$ ,  $2.7 \text{ mM KCl}$ ,  $8.1 \text{ mM Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$  and  $1.5 \text{ mM KH}_2\text{PO}_4$ ) using a pair of microscissors. The sperm suspension was homogenized with a set of Gilson pipettes (in decreasing order  $1000 \mu\text{l}$ ,  $200 \mu\text{l}$  and  $20 \mu\text{l}$  pipettes) for 5–15 min. To obtain openings of different widths, we cut pieces of different lengths from the tips of the pipettes. To count the sperm, we stained the homogenate for 1–3 h with an equal volume of a galloxyanin–chromium complex which stains the DNA in the head of the spermatozoa. To check for sperm clusters we used a light microscope (magnification  $\times 400$ ). If spermatozoa still occurred in clusters, we treated the sample with a sonicator (35 kHz) for 16 h (in cases of extremely dense clusters up to 80 h). If the spermatozoa were well separated in the sperm suspension, two subsamples of known volume were diluted 1:3 with PBS-buffer and transferred to a Bürker–Türk counting chamber. This counting chamber consists of 16 cells each with a volume of 25 nl. We counted all sperm heads in randomly chosen cells until the total number of sperm heads exceeded 400 and used the average of two subsamples to calculate the total number of sperm in a spermatophore. We assessed the reliability of multiple sperm counts ( $N=8$ ) on the same spermatophore ( $N=6$ ) by calculating the repeatability, which was 0.997 indicating the technique was very accurate (Locher & Baur 1997).

### Statistical Analyses

Means  $\pm$  SE are given unless otherwise stated. For statistical analyses we used the SAS program package (SAS Institute 1991) and for post hoc power tests, the GPower program (Erdfelder et al. 1996).

In simultaneous hermaphrodites, the correlation coefficient between the amount of sperm transferred by the two mating partners can be distorted by the way the animals are assigned to the axes. To overcome this problem, we used a randomizing method to assign the number of sperm transferred by one individual of the pair to the  $X$  axis and that transferred by the other individual to the  $Y$  axis (see Baur 1992). Having calculated the correlation coefficient  $r$ , we again randomly assigned the individuals of each mating pair to the axes. This procedure was done 100 times and  $r$  is presented as the mean value of 100 runs. Deviations from  $r=0$  were tested using a two-tailed  $t$  test (Sokal & Rohlf 1981).

## RESULTS

### Adjustment of Sperm Expenditure

Copulations were observed in 46 (48.6%) of the 94 trials. Different types of pairs did not differ in frequency of copulation (virgin–virgin 56.3%, virgin–nonvirgin 41.7% and nonvirgin–nonvirgin 57.1%;  $\chi^2_2=2.08$ ,  $P>0.3$ ). Virgin and nonvirgin individuals did not differ in the number of sperm transferred ( $\bar{X} \pm \text{SE} = 2\,143\,157 \pm 88\,705$ ,  $N=55$ , versus  $2\,249\,285 \pm 153\,238$ ,  $N=36$ ; unpaired  $t$  test:

**Table 1.** Number of sperm delivered by virgin and nonvirgin *A. arbustorum* in relation to the mating history of the partner

Sperm donor	Sperm recipient	Number of snails	Number of spermatozoa		Size (volume) of spermatophore (mm <sup>3</sup> ) $\bar{X} \pm \text{SE}$	Shell size of donor (cm <sup>3</sup> ) $\bar{X} \pm \text{SE}$	Shell size of recipient (cm <sup>3</sup> ) $\bar{X} \pm \text{SE}$
			$\bar{X} \pm \text{SE}$	Range			
Virgin	Virgin	35	2 093 143 ± 110 943	853 650–3 763 100	2.61 ± 0.16	1.40 ± 0.03	1.39 ± 0.03
Virgin	Nonvirgin	20	2 230 683 ± 149 402	1 157 450–3 541 800	2.61 ± 0.13	1.37 ± 0.04	1.42 ± 0.03
Nonvirgin	Virgin	20	2 282 074 ± 220 489	802 620–3 719 050	2.77 ± 0.15	1.42 ± 0.03	1.37 ± 0.04
Nonvirgin	Nonvirgin	16	2 208 300 ± 214 719	1 160 400–3 968 800	2.63 ± 0.16	1.37 ± 0.03	1.37 ± 0.03
			$P=0.91$		$P=0.72$	$P=0.73$	$P=0.75$

*P* values result from Kruskal–Wallis tests.

**Table 2.** Courtship and copulation duration in pairs consisting of virgin and nonvirgin individuals of *A. arbustorum*

Type of pair	Courtship duration (min)			Copulation duration (min)		
	Median	Range	<i>N</i>	Median	Range	<i>N</i>
Virgin–virgin	150	60–540	18	130	60–180	17
Virgin–nonvirgin	165	90–450	20	120	65–185	19
Nonvirgin–nonvirgin	240	90–900	8	107.5	70–165	6
			$P=0.49$	$P=0.73$		

*N* indicates the number of pairs (copulation duration could not be recorded in four pairs). *P* values result from Kruskal–Wallis tests.

$t_{89}=0.64$ ,  $P=0.52$ ). To reach a power of 80% with the variance of the present data a true between-group difference (*d*) in number of sperm delivered of 0.608 would be required (observed  $d=0.133$ ; cf. Cohen 1988). Furthermore, virgin and nonvirgin snails did not differ in spermatophore size ( $2.61 \pm 0.11 \text{ mm}^3$ ,  $N=55$ , versus  $2.71 \pm 0.11 \text{ mm}^3$ ,  $N=36$ ; unpaired *t* test:  $t_{89}=0.59$ ,  $P=0.56$ ) or in shell size ( $1.39 \pm 0.02 \text{ cm}^3$ ,  $N=55$ , versus  $1.39 \pm 0.02 \text{ cm}^3$ ,  $N=36$ ; unpaired *t* test:  $t_{89}=0.16$ ,  $P=0.88$ ). Of the 56 virgin snails, one individual (1.8%) transferred a spermatophore that contained no spermatozoa. Data from this individual were omitted in the analyses on sperm number. All spermatophores from the 36 nonvirgin snails contained spermatozoa.

Table 1 gives the mean number of sperm transferred and the size of the spermatophores for each type of pair. Irrespective of the mating history of the partner (virgin or nonvirgin), snails received similar quantities of sperm. Combining data from virgin and nonvirgin sperm donors, virgin and nonvirgin snails did not differ in number of sperm received ( $2\ 161\ 845 \pm 106\ 324$ ,  $N=55$ , versus  $2\ 220\ 735 \pm 124\ 567$ ,  $N=36$ ; unpaired *t* test:  $t_{89}=0.36$ ,  $P=0.72$ ). To reach a power of 80% with the variance of the present data a true between-group difference in number of sperm received of 0.608 would be required (observed  $d=0.077$ ). Furthermore, the size of the spermatophore did not differ with respect to the mating history of the partner (Table 1). This indicates that individuals of *A. arbustorum* do not adjust sperm expenditure to the mating history of the partner and thus to the potential risk of sperm competition in a given mating.

Sperm number was positively correlated with the size of the spermatophore ( $r=0.65$ ,  $N=91$ ,  $P<0.0001$ ); however, neither sperm number nor spermatophore size was correlated with the size of the sperm donor (sperm number:  $r=0.05$ ,  $N=91$ ,  $P=0.65$ ; spermatophore size:  $r=0.03$ ,  $N=91$ ,  $P=0.82$ ). This suggests that, in contrast to clutch size (female fecundity), sperm expenditure (male fecundity) is not size related in this simultaneously hermaphroditic snail. Furthermore, neither sperm number nor spermatophore size was correlated with the size of the recipient (sperm number:  $r=-0.03$ ,  $N=91$ ,  $P=0.79$ ; spermatophore size:  $r=-0.10$ ,  $N=91$ ,  $P=0.36$ ).

The median courtship time was 180 min (range 60–900 min,  $N=46$  pairs) in snails that copulated successfully. Courtship duration did not differ between different types of pairs (Table 2). Similarly, no difference in copulation duration was recorded between different types of pairs (median 120 min, range 60–185 min,  $N=42$  pairs; Table 2). The number of sperm transferred in a spermatophore was not correlated with copulation duration (Spearman rank correlation:  $r_s=-0.02$ ,  $N=83$ ,  $P=0.85$ ).

### Sperm Trading

There was a high degree of reciprocity in spermatophore transfer: in 45 of the 46 mating pairs both partners delivered a spermatophore that contained spermatozoa. Only one virgin individual transferred an empty spermatophore. In copulating pairs, the numbers of sperm transferred by both partners were not correlated (mean  $r_{43}=0.026$ ,  $P=0.86$ ; range of *r* in the 100 runs 0.013–0.113). None of the 100 randomized runs revealed any

significant correlation coefficient (for  $df=43$  the critical value of  $r$  is 0.288 at the 5% level). Thus, individuals of *A. arbustorum* do not adjust the number of sperm they deliver to the number of sperm they receive from their mating partner, indicating that sperm trading does not occur in this species.

## DISCUSSION

Sperm competition theory predicts that males will be selected to deliver larger quantities of sperm when the risks of sperm competition are high (Parker 1990a, b). However, this presupposes mechanisms that allow males to adjust the quantity of sperm delivered. Our results provide no support for the idea that individuals of *A. arbustorum* can control the number of sperm they transfer during copulation with respect to the potential risk of sperm competition and to the quantity of sperm they receive from the partner.

The present study is to our knowledge the first to examine quantitatively sperm transfer in a simultaneously hermaphroditic land snail. We found a pronounced interindividual variation in number of sperm delivered. For several reasons it is unlikely that this variation was due to autosperm depletion. Nonvirgin snails were prevented from remating for at least 4 weeks. The results of an ongoing study show that individuals of *A. arbustorum* replenish their autosperm supply within 8 days of a copulation (R. Locher & B. Baur, unpublished data). Furthermore, the interindividual variation in number of sperm released was similar in nonvirgin and virgin individuals (4.9- and 4.4-fold variation, respectively).

In terrestrial gastropods, the intensity of sperm competition may vary with population density, which in turn varies to a large extent both between sites and in time (e.g. Andreassen 1981; Baur 1986; Baur et al. 1993). The risk of sperm competition incurred with a potential mating partner may also vary because of individual variation in long-term sperm storage. It has been suggested that the elaborate courtship may allow simultaneous hermaphrodites to assess potential mating partners (Chung 1987). Courtship behaviour in *A. arbustorum* includes introductory behaviour (foreplay) with reciprocal tactile and oral contacts and curving turns to reach the optimal position with respect to the genital opening of the partner (Hofmann 1923). This can be followed by dart shooting, the pushing of a calcareous dart into the body of the mating partner (although this is not obligatory). Courtship and copulation last several hours in *A. arbustorum* and may provide ample opportunities to assess the mating history, size and perhaps even the quality of the partner, probably on an olfactory basis (cf. Croll 1983; Chase 1986). However, individuals of *A. arbustorum* did not deliver larger quantities of sperm to larger mating partners, which have a higher female fecundity than smaller individuals (Baur 1984, 1988b, 1994b). This finding supports the observation of random mating with respect to shell size in this species (Baur 1992). By contrast, sperm delivery increases with the size of the recipient in the opisthobranch sea hare, *Aplysia parvula* (Yusa

1994); in this hermaphroditic gastropod copulations are unilateral and sperm donors prefer to mate with large individuals which produce more eggs.

The adaptive significance of increasing sperm number comes from an increased confidence of paternity for males that deliver larger quantities of sperm (Parker 1990a, b). This is true when sperm mix randomly within the female (e.g. in the field cricket *Gryllus bimaculatus*; Simmons 1987) or in cases where clear patterns of sperm precedence occur (e.g. in the dragonfly *Mnais pruinosa pruinosa*; Siva-Jothy & Tsubaki 1989). Paternity confidence might be relatively low in *A. arbustorum*, as indicated by the variable pattern of sperm precedence found in a laboratory experiment (Baur 1994a). Analysis of long-term sperm use in 23 double-mated snails that laid three to nine batches over 2 years revealed striking differences between individuals. Five snails (22%) showed first-mate sperm precedence throughout, eight snails (35%) showed second-mate sperm precedence throughout, whereas 10 snails (43%) showed sperm mixing in successive batches. The individual variation in sperm precedence in *A. arbustorum* could have arisen for several reasons, including differences in the amount of sperm transferred, differential fertilizing capacity of sperm from different 'males', or selective sperm storage by the female function. There is some evidence that sperm selection in the female reproductive tract (cryptic female choice; cf. Thornhill 1983; Eberhard 1996) is crucial for the fertilization success in *A. arbustorum*. In this species the sperm storage organ (spermatheca) consists of two to eight tubules with a common entrance into the duct of the fertilization pouch (Haase & Baur 1995). Haase & Baur (1995) examined the sites of sperm storage in individuals that remated successfully. In several snails some spermathecal tubules were filled with spermatozoa, while in others no sperm were found in the spermatheca, only in the sperm-digesting bursa copulatrix. This suggests that the female reproductive system of *A. arbustorum* may be able to control fertilization by selectively storing or digesting sperm from different mating partners.

## Sperm Trading

The outcome of the sexual conflict between the gender roles in simultaneous hermaphrodites is controversial (for a review see Michiels 1998). Simultaneous hermaphrodites with internal fertilization are expected to mate in order to donate rather than to receive sperm (Charnov 1979). In *A. arbustorum* one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by an individual (Chen & Baur 1993). Yet, the snails may copulate two to five times each year as observed in the helicid snail *Helix pomatia* (Lind 1988).

Theoretical models of gamete trading have been developed to predict the outcome of sexual conflict in simultaneous hermaphrodites (Leonard & Lukowiak 1984, 1985; Leonard 1991). There is substantial empirical evidence for the occurrence of egg trading in some coral fish, *Hypoplectrus nigricans*, *Serranus tortugarum* (Fischer & Petersen 1987) and *S. tabacarius* (Petersen 1991), and polychaete worms, *Ophryotrocha diadema* (Sella 1985) and

*O. gracilis* (Sella et al. 1997). Sperm trading is expected in species in which the female function controls fertilization or when both partners want to donate but not to receive sperm (Michiels 1998). However, there is relatively little evidence for its occurrence. In the sea slug *Navanax inermis*, sperm donation is unilateral, but sexual roles are alternated repeatedly during a single mating bout (Leonard & Lukowiak 1984, 1985). In simultaneous hermaphrodites with mutual penis intromission, sperm trading has been found only in the freshwater flatworm *Dugesia gonocephala* (Vreys & Michiels 1998). Yet, Leonard (1991) suggested that sperm trading should occur in all simultaneously hermaphroditic gastropods, except those that lack a gametolytic gland and/or sperm storage. The present study is the first to examine this hypothesis in a pulmonate land snail, but we could not find any evidence for sperm trading. Copulating individuals of *A. arbustorum* exchange sperm encapsulated in a spermatophore (Hofmann 1923), which they make during copulation (Lind 1973). The autospem, which are stored in the sperm duct, might be introduced into the spermatophore in the final phase of the copulation immediately before spermatophore exchange. Copulating snails that were mechanically separated released spermatophores that were free of sperm in all cases (H. Baminger, R. Locher & B. Baur, unpublished data). It may be difficult to assess the number of sperm that the mating partner transfers when sperm are encapsulated in a spermatophore, even when the size of the sperm container is related to the amount of sperm. We propose that the theory of sperm trading should be refined taking into account possible mechanisms of sperm transfer.

#### Acknowledgments

We thank A. Blarer, A. Erhardt, S. Zschokke and an anonymous referee for constructive comments on the manuscript. Financial support was received from the Swiss National Science Foundation (grant No. 31-43092.95).

#### References

- Andreassen, E. M. 1981. Population dynamics of *Arianta arbustorum* and *Cepaea hortensis* in western Norway. *Fauna norvegica, Series A*, 2, 1–13.
- Baker, R. R. & Bellis, M. A. 1989. Number of sperm in human ejaculates varies in accordance with sperm competition theory. *Animal Behaviour*, 37, 867–869.
- Baur, B. 1984. Shell size and growth rate differences for alpine populations of *Arianta arbustorum* (L.) (Pulmonata: Helicidae). *Revue suisse de Zoologie*, 91, 37–46.
- Baur, B. 1986. Patterns of dispersion, density and dispersal in alpine populations of the land snail *Arianta arbustorum* (L.) (Helicidae). *Holarctic Ecology*, 9, 117–125.
- Baur, B. 1988a. Repeated mating and female fecundity in the simultaneously hermaphroditic land snail *Arianta arbustorum*. *Invertebrate Reproduction and Development*, 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. *Zoologischer Anzeiger*, 225, 253–264.
- Baur, B. 1992. Random mating by size in the simultaneously hermaphroditic land snail *Arianta arbustorum*: experiments and an explanation. *Animal Behaviour*, 43, 511–518.
- Baur, B. 1994a. Multiple paternity and individual variation in sperm precedence in the simultaneously hermaphroditic land snail *Arianta arbustorum*. *Behavioral Ecology and Sociobiology*, 35, 413–421.
- Baur, B. 1994b. Parental care in terrestrial gastropods. *Experientia*, 50, 5–14.
- Baur, B. 1998. Sperm competition in molluscs. In: *Sperm Competition and Sexual Selection* (Ed. by T. R. Birkhead & A. P. Møller), pp. 255–305. London: Academic Press.
- Baur, B. & Baur, A. 1997. Random mating with respect to relatedness in the simultaneously hermaphroditic land snail *Arianta arbustorum*. *Invertebrate Biology*, 116, 294–298.
- Baur, B. & Raboud, C. 1988. Life history of the land snail *Arianta arbustorum* along an altitudinal gradient. *Journal of Animal Ecology*, 57, 71–87.
- Baur, B., Chen, X. & Baur, A. 1993. Genital dimorphism in natural populations of the land snail *Chondrina clienta* and the influence of the environment on its expression. *Journal of Zoology*, 231, 275–284.
- Bellis, M. A., Baker, R. R. & Gage, M. J. G. 1990. Variation in rat ejaculates is consistent with the kamikaze-sperm hypothesis. *Journal of Mammalogy*, 71, 479–480.
- Birkhead, T. R. & Fletcher, F. 1995. Depletion determines sperm numbers in male zebra finches. *Animal Behaviour*, 49, 451–456.
- Birkhead, T. R. & Møller, A. P. (Eds) 1998. *Sperm Competition and Sexual Selection*. London: Academic Press.
- Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences U.S.A.*, 76, 2480–2484.
- Chase, R. 1986. Lessons from snail tentacles. *Chemical Senses*, 11, 411–426.
- Chen, X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic *Arianta arbustorum* (L.) (land snail). *Heredity*, 71, 456–461.
- Chen, X. 1994. Self-fertilization and cross-fertilization in the land snail *Arianta arbustorum* (Mollusca, Pulmonata: Helicidae). *Journal of Zoology*, 232, 465–471.
- Chen, X. & Baur, B. 1993. The effect of multiple mating on female reproductive success in the simultaneously hermaphroditic land snail *Arianta arbustorum*. *Canadian Journal of Zoology*, 71, 2431–2436.
- Chung, D. J. D. 1987. Courtship and dart shooting behavior of the land snail *Helix aspersa*. *Veliger*, 30, 24–39.
- Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. 2nd edn. Hillsdale, New Jersey: L. Erlbaum.
- Croll, R. P. 1983. Gastropod chemoreception. *Biological Reviews*, 58, 293–319.
- Dewsbury, D. A. 1982. Ejaculate cost and male choice. *American Naturalist*, 119, 601–610.
- Duncan, C. J. 1975. Reproduction. In: *Pulmonates*, Vol. 1 (Ed. by V. Fretter & J. Peake), pp. 309–365. London: Academic Press.
- Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton, New Jersey: Princeton University Press.
- Erdfelder, E., Faul, F. & Buchner, A. 1996. GPOWER: a general power analysis program. *Behavior Research Methods, Instruments, and Computers*, 28, 1–11.
- Fischer, E. A. 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish *Hypoplectrus nigricans*. *Animal Behaviour*, 28, 620–633.
- Fischer, E. A. & Petersen, C. W. 1987. The evolution of social patterns in the seabasses. *Bioscience*, 37, 482–489.
- Gage, M. J. G. 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Animal Behaviour*, 42, 1036–1037.

- Gage, M. J. G. & Baker, R. R. 1991. Ejaculate size varies with socio-sexual situation in an insect. *Ecological Entomology*, **16**, 331–337.
- Haase, M. & Baur, B. 1995. Variation in spermathecal morphology and storage of spermatozoa in the simultaneously hermaphroditic land snail *Arianta arbustorum* (Gastropoda: Pulmonata: Stylommatophora). *Invertebrate Reproduction and Development*, **28**, 33–41.
- Hofmann, E. 1923. Über den Begattungsvorgang von *Arianta arbustorum* (L.). *Jena Zeitschrift für Naturwissenschaften*, **59**, 363–400.
- Leonard, J. L. 1991. Sexual conflict and the mating systems of simultaneously hermaphroditic gastropods. *American Malacological Bulletin*, **9**, 45–58.
- Leonard, J. L. & Lukowiak, K. 1984. Male–female conflict in a simultaneous hermaphrodite resolved by sperm trading. *American Naturalist*, **124**, 282–286.
- Leonard, J. L. & Lukowiak, K. 1985. Courtship, copulation and sperm trading in the sea slug, *Navanax inermis* (Opisthobranchia: Cephalaspidea). *Canadian Journal of Zoology*, **63**, 2719–2729.
- Lind, H. 1973. The functional significance of the spermatophore and the fate of spermatozoa in the genital tract of *Helix pomatia* (Gastropoda: Stylommatophora). *Journal of Zoology*, **169**, 39–64.
- Lind, H. 1988. The behaviour of *Helix pomatia* L. (Gastropoda, Pulmonata) in a natural habitat. *Videnskabelige Meddelelser fra Dansk naturhistorisk Forening i København*, **147**, 67–92.
- Locher, R. & Baur, B. 1997. A new technique to assess the number of spermatozoa in spermatophores of stylommatophoran gastropods. *Journal of Molluscan Studies*, **63**, 555–556.
- McWilliams, S. R. 1992. Courtship behaviour of the small-mouthed salamander (*Ambystoma texanum*): the effects of conspecific males on male mating tactics. *Behaviour*, **121**, 1–19.
- Michiels, N. K. 1998. Mating conflicts and sperm competition in simultaneous hermaphrodites. In: *Sperm Competition and Sexual Selection* (Ed. by T. R. Birkhead & A. P. Møller), pp. 219–254. London: Academic Press.
- Nakatsuru, K. & Kramer, D. L. 1982. Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science*, **216**, 753–755.
- Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biological Review*, **45**, 535–567.
- Parker, G. A. 1990a. Sperm competition games: raffles and roles. *Proceedings of the Royal Society of London, Series B*, **242**, 120–126.
- Parker, G. A. 1990b. Sperm competition games: sneaks and extra-pair copulations. *Proceedings of the Royal Society of London, Series B*, **242**, 127–133.
- Petersen, C. W. 1991. Sex allocation in hermaphroditic seabasses. *American Naturalist*, **138**, 650–667.
- SAS Institute. 1991. *SAS User's Guide: Statistics* (1991 edition). Cary, North Carolina: SAS Institute.
- Sella, G. 1985. Reciprocal egg trading and brood care in a hermaphroditic polychaete worm. *Animal Behaviour*, **33**, 938–944.
- Sella, G., Premoli, M. C. & Turri, F. 1997. Egg trading in the simultaneously hermaphroditic polychaete worm *Ophryotrocha gracilis* (Huth). *Behavioral Ecology*, **8**, 83–86.
- Simmons, L. W. 1987. Sperm competition as a mechanism of female choice in the cricket, *Gryllus bimaculatus*. *Behavioral Ecology and Sociobiology*, **21**, 197–202.
- Simmons, L. W., Craig, M., Llorens, T., Schinzig, M. & Hosken, D. 1993. Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. *Proceedings of the Royal Society of London, Series B*, **251**, 183–186.
- Siva-Jothy, M. T. & Tsubaki, Y. 1989. Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae). 1. Alternative mate-securing tactics and sperm precedence. *Behavioral Ecology and Sociobiology*, **24**, 39–45.
- Smith, R. L. (Ed.) 1984. *Sperm Competition and the Evolution of Animal Mating Systems*. New York: Academic Press.
- Sokal, R. R. & Rohlf, F. J. 1981. *Biometry*. 2nd edn. San Francisco: W. H. Freeman.
- Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *American Naturalist*, **122**, 765–788.
- Tompa, A. S. 1984. Land snails (Stylommatophora). In: *The Mollusca*, Vol. 7 (Ed. by A. S. Tompa, N. H. Verdonk & J. A. M. van den Biggelaar), pp. 47–140. London: Academic Press.
- Vreys, C. & Michiels, N. K. 1998. Sperm trading by volume in a hermaphroditic flatworm with mutual penis intromission. *Animal Behaviour*, **56**, 777–785.
- Yusa, Y. 1994. Factors regulating sperm transfer in an hermaphroditic sea hare, *Aplysia parvula* Mörch, 1863 (Gastropoda: Opisthobranchia). *Journal of Experimental Marine Biology and Ecology*, **181**, 213–221.