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Expandable spermatheca influences sperm storage in the simultaneously hermaphroditic snail *Arianta arbustorum*

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Summary

In many simultaneously hermaphroditic land snail species, the sperm storage organ (spermatheca) is highly structured, suggesting that the female function might be able to influence offspring paternity. Physical properties of the sperm storage organ, including its initial size and sperm storage capacity, may also affect fertilization patterns in multiply mated snails. We examined the structure, volume and tubule length of empty spermathecae in the land snail, *Arianta arbustorum*, and assessed differences in spermatheca size following a single copulation. The number of spermathecal tubules ranged from 2–7, but was not correlated with the volume of empty spermathecae. The volume of sperm stored in the spermatheca after a copulation was correlated with neither the number of spermathecal tubules nor copulation duration. Mean spermathecal volume more than doubled between two and thirty-six hours after sperm uptake, but the length of the spermathecal tubules did not change. Interestingly, the volume of sperm stored in the spermatheca seems not to be related to the size of the spermatophore and thus not to the number of sperm received (= allosperm). The amount of allosperm digested in the bursa copulatrix was highly variable and no significant relationship with the size of the spermatophore received was found. These findings suggest that numerical aspects of sperm transfer are less important in influencing fertilization success of sperm in *A. arbustorum* than properties of the female reproductive tract of the sperm receiver.

Key words: *Arianta arbustorum*, sexual selection, simultaneous hermaphrodite, sperm storage, spermathecal morphology

Introduction

In internally fertilizing species, mating with multiple partners and long-term storage of sperm can lead to intersexual conflicts over the post-copulatory processes involved in sperm storage and sperm use (Arnqvist and Rowe, 2005). The sperm donor may develop strategies that increase the ability to outcompete the ejaculates of rival mates inside the sperm receiver and thus to enhance its own fertilization success. For its part, the sperm receiver, which may directly and indirectly benefit from multiple matings (e.g., Jennions and Petrie, 2000), should respond to the restrictions of polyandry with adaptations to allow for post-copulatory mate choice to be retained. Adaptations of the sperm receiver

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to exert control over paternity may include the selective storage of sperm in complex storage organs (Hellriegel and Ward, 1998; Pitnick et al., 1999) and the selective extrusion or digestion of sperm, thereby reducing the amount of sperm reaching the storage organ (Eberhard, 1985; Birkhead et al., 1993).

Highly structured sperm storage organs occur in species of several animal groups, e.g., in millipedes (Barnett et al., 1995), spiders and flies (Eberhard, 1985, 1996), lizards (Fox, 1963) and birds (Briskie, 1996). The spatial separation of sperm from multiple males in different spermathecal compartments has been documented in the yellow dung fly (e.g., Hellriegel and Bernasconi, 2000). The shape of the sperm storage organ (spherical or tubular) and the variation in physical properties (e.g., elasticity) may also influence sperm utilization (Walker, 1980; Simmons, 2001). For example, sperm stratification in the tubular spermatheca of red flour beetles influences fertilization success (Lewis et al., 2005), whereas the expandable spermatheca in some gryllids can accommodate successive ejaculates, resulting in random utilization of sperm (Simmons, 1986).

Recently, it has been recognized that sexual selection (including sexual conflict) is also an important factor in the evolution of reproductive traits in simultaneous hermaphrodites, where both sexes are combined within one individual (Charnov, 1979; Michiels, 1998). As in species with separate sexes, multiple matings and long-term sperm storage are common in hermaphrodites. Consequently, strategies of the sperm receiver to influence fertilization may also exist (Baur, 1998; Michiels, 1998). In terrestrial pulmonate snails, which are all simultaneous hermaphrodites, the spermatheca can be highly structured and there is considerable among- and within-species variation in the number of sperm storing tubules (Baur, 1998). Furthermore, the female reproductive tract of land snails is extremely hostile to sperm received (= allosperm). Compared with the number of sperm received, the proportion of allosperm stored is very low (Lind, 1973; Rogers and Chase, 2001). The remaining sperm are digested in a specialized organ called the bursa copulatrix.

In the helicid land snail, *Arianta arbustorum* (Linnaeus, 1758), a controlled experiment with double-mated individuals revealed a considerable variation in sperm utilization patterns (Baur, 1994). This variation may be due to differences among sperm donors, e.g., in the amount of sperm transferred and/or sperm quality, or be influenced by the morphology and hostility of the sperm receiver's reproductive tract.

The aim of the present study was to examine the potential importance of complexity, shape and physical properties of the sperm receiver's spermatheca for paternity in *A. arbustorum*. This gastropod is common in moist habitats of northern, western and central Europe (Kerney and Cameron, 1979). The snail has determinate growth, with individuals becoming sexually mature at an age of 2–4 years and adults living another 3–4 years (maximum 14 years; Baur and Raboud, 1988). The snails mate repeatedly during a reproductive season and store viable allosperm for more than one year (Baur, 1988). Individuals usually reproduce through outcrossing, but self-fertilization may occur after isolation for 2–3 years (Chen, 1994). Mating includes elaborate courtship with optional dart shooting and lasts 2–18 h (Hofmann, 1923). Copulation is reciprocal and after simultaneous intromission each snail transfers one spermatophore, consisting of a hardened secretion that encapsulates the sperm, into the partner's bursa tract diverticulum (Hofmann, 1923). Previous studies showed that mating partners do not adjust the number of sperm transferred (Baur et al., 1998). Furthermore, the number of sperm transferred is unaffected by copulation duration, degree of sperm competition risk or nutritional stress (Locher and Baur, 1999, 2000, 2002). Three to four weeks after successful copulations, *A. arbustorum* has entirely replenished its autosperm reserves (Hänggi et al., 2002). In addition, no direct effects of dart-shooting on sperm transfer and sperm storage have been found (Baminger et al., 2000; Bojat and Haase, 2002).

Allosperm received reach the spermoviduct via the spermatophore's tail and travel up to the spermatheca, where they are stored (Lind, 1973). However, the vast majority of sperm received is transported to the sperm-digesting bursa copulatrix. The spermatheca of *A. arbustorum* consists of 2–9 blind-ended tubules uniting to a common duct, which opens into the fertilization pouch (e.g., Baminger et al., 2000). Morphological studies have shown that the stored allosperm are not equally distributed among all spermathecal tubules, suggesting that sperm from different mates might be both stored and used separately (Haase and Baur, 1995; Baminger and Haase, 1999; Baminger et al., 2000; Bojat and Haase, 2002). Furthermore, ultrastructural analyses indicate that ciliation of the spermathecal epithelium and musculature surrounding the spermathecal tubules may allow manipulations of sperm storage and release (Bojat et al., 2001a, 2001b). So far, however, the physical properties of the spermatheca, such as its initial size and sperm storage capacity have not been investigated. We therefore examined the volume and tubule length of empty spermathecae in relation to the variable spermatheca structure. We also assessed changes in spermatheca size (volume, tubule length) after the uptake of sperm following a single copulation and

examined whether copulation duration influenced the amount of sperm stored. The relationship between sperm transfer and sperm use was estimated by assessing the assumed initial size of the received spermatophore and number of sperm still confined in the spermatophore and comparing these with the volume of allosperm stored in the spermatheca and the volume of the sperm digesting bursa copulatrix.

Materials and Methods

A sample of 120 sub-adult specimens of *A. arbustorum* was collected in a subalpine forest near Gurnigelbad, 30 km south of Bern, Switzerland (46°45′N, 7°27′E) on 10 May 2003. Snails were raised individually to adulthood in transparent plastic beakers (6.5 cm in diameter, 8 cm deep) on moist soil mixed with powdered limestone. The snails were kept under a light:dark regime of 16:8 h and a constant temperature of 19 ± 1°C. The beakers were cleaned 1–2 times per week, and fresh lettuce was provided *ad libitum* as food. Sexual maturity, as indicated by the formation of a reflected shell lip, was attained within 6 weeks.

Mating trials were set up at night under natural ambient temperature and light conditions. Two groups each of 60 snails were placed together in transparent plastic boxes (34 × 22 × 9.5 cm) in which snails were allowed to court. The snail's mating behaviour was checked at intervals of 15–30 min (at night using a torch). When two snails began to court they were separated from the others by placing them in a smaller transparent plastic container. This prevents interference by other snails (cf. Adamo and Chase, 1990). Copulating snails were observed at intervals of 15 min. We recorded the courtship duration for each mating pair. Snails that did not mate were used in succeeding trials.

We assumed that, after reception, the spermatophore has been moved into the final position 2 h after copulation (cf. Baumgartner, 1997) and that sperm migration from the spermatophore to the spermatheca had ceased after 36 h (cf. Lind, 1973; Bojat and Haase, 2002). Consequently, one randomly chosen mating partner was killed by decapitation 2 h after copulation, the other mating partner 36 h after copulation. Prior to dissection, shell breadth and height of each mated snail were measured to the nearest 0.1 mm using a vernier calliper. Shell volume was calculated using the formula: $V = 0.312 \times [(\text{breadth})^2 \times \text{height}] - 0.038$ (B. Baur, unpubl. 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.

Together with the fertilization pouch, parts of the spermoviduct and hermaphroditic duct, the spermatheca forms the so-called fertilization pouch-spermatheca complex (FPSC; Tompa 1984; see insert in Fig. 3). FPSC's of all mated snails were embedded in paraplast, serially cross-sectioned at 8 µm and stained with haematoxylin-eosin. For each mated snail, the structure of the spermatheca was examined by counting the number of spermathecal tubules. The length of each tubule was approximated by counting the number of cross-sections (cf. Cuellar, 1966), starting with that section in which a tubule was clearly separated from the tubule from which it branched off. The main tubule of the spermatheca was always the longest tubule. For each tubule a semi-quantitative assessment of the amount of allosperm stored was made using the following classes: no sperm found, sporadic sperm occurrence, sperm loosely packed, sperm densely packed. We estimated the spermatheca volume by measuring the area of the lumen on digitized microscopic images and multiplying it with the corresponding thickness of the slices. The volume of allosperm stored in the spermatheca was measured in the same way. Spermatheca volume and the volume of allosperm stored are expressed as the sum of single tubule measurements.

The spermatophores were dissected from the diverticulum and digital images were taken to assess spermatophore volume [expressed as the volume of the sperm-containing part of the spermatophore; see Beese et al. (2006) for an illustration of a spermatophore]. We calculated the volume by using the formula for a truncated cone: $V = \pi L/12 \times (d_1^2 + d_2^2 + d_1 d_2)$ with L = length along the midline, d_1 = diameter at the narrow end, and d_2 = diameter at the broad end. To assess the assumed initial spermatophore volume of snails 36 h after copulation, we multiplied the obtained volume with a factor of 1.33. This factor was obtained by dividing the mean spermatophore volume 2 h after copulation by the mean spermatophore volume 36 h after copulation.

The number of sperm transferred to the mating partner was assessed by counting the number of sperm heads in a spermatophore (Locher and Baur, 1997). The spermatophore was mechanically disrupted in 200 µl phosphate-buffered saline (PBS) for 10–15 min using a pair of microscissors. Subsequently, we added an equal volume of a gallocyanin-chromium complex to the homogenate to stain the sperm heads (Einarson, 1951). Each sample was treated three times with a sonicator (35 kHz) for 12 h to separate sperm clusters. Subsamples of known volume were diluted 1:1 or 1:2 with PBS, depending on the sperm density, and transferred to a Bürker–Türk counting chamber. We counted all sperm heads in randomly chosen cells until the total number of

sperm heads exceeded 400 and used the average of two sub-samples to calculate the total number of sperm in a spermatophore. This technique is accurate and repeatable (Locher and Baur, 1997).

The bursa copulatrix volume was assessed on digital images and calculated by using the formula for an ellipsoid body: $V = 4/3\pi \times L/2 \times (W/2)^2$ with L = length axis diameter and W = width axis diameter.

Images were obtained using a Sony CCD-Iris camera mounted on a Leica DML compound microscope for spermatheca cross-sections or on a Leica MZ 8 binocular microscope for spermatophore volume and bursa copulatrix volume. Length and area measurements of digital images were determined using NIH Image public domain software Version 1.63 (http://rsb.info.nih.gov/nih-image). For spermatophore volume and bursa copulatrix volume, each measurement was repeated three times and means were used for statistics. The repeatability of measurements was assessed following Lessells and Boag (1987) and ranged from 0.93–0.97, indicating a high accuracy.

Means ± SD are given unless otherwise stated. Statistical analyses were performed using SPSS 11 for Mac OS X. Possible differences in reproductive traits were determined using analyses of covariance (ANCOVA, type III model) with time after mating (2 h or 36 h) as factor and shell volume (an indicator of snail size) as covariate. In no analysis did shell volume have any significant influence, and we therefore removed it from the analyses and presented the results of one-way ANOVAs with time after mating as factor. Prior to analysis, length data were transformed by raising them to the power of three to have volume measures in all characters. The power of r (alpha = 0.05) for non-significant correlations was calculated following Cohen (1988). Spearman rank correlations were used to examine possible relationships between the number of spermathecal tubules and reproductive traits. In two individuals, no allosperm were stored after 36 h. These animals were not considered in the data analyses.

Results

Spermatheca size in relation to sperm storage

The volume of empty spermathecae (2 h after mating) ranged from 0.68×10^{-3} to 3.04×10^{-3} mm^3 (Table 1). Cumulative tubule length varied between 2.38 and 5.23 mm and main tubule length between 1.11 and 2.14 mm. The number of spermathecal tubules ranged from 2 to 7 (median 4.5). The volume of empty spermathecae, cumulative tubule length, main tubule length and tubule number were not found to be correlated with shell size (in all cases $P > 0.15$). Furthermore, we found no correlation between the volume of empty spermathecae and the number of tubules ($r_s = 0.20$, $N = 18$, $P = 0.42$).

The storage of allosperm resulted in an increase in spermatheca volume (Fig. 1; Table 1). The volume of sperm stored in the spermatheca 36 h after copulation averaged $1.78\times10^{-3} \pm 1.63\times10^{-3}$ mm^3 ($N = 18$; range: 0.02×10^{-3}–4.47×10^{-3} mm^3). The volume of sperm stored and the volume of the spermatheca were positively correlated ($r = 0.95$, $N = 18$, $P < 0.001$; Fig. 2). However, cumulative length of spermathecal tubules and the length of the main tubule did not differ before and after sperm storage (Table 1). The volume of the spermatheca 36 h after copulation ($r_s = -0.31$, $N = 18$, $P = 0.21$) and the volume of sperm stored ($r_s = -0.42$, $N = 18$, $P = 0.09$) were not correlated with the number of spermathecal tubules. These results indicate that the volume of sperm stored from a single spermatophore is not influenced by the number of spermathecal tubules. Spermatheca volume and volume of sperm stored in the spermathecae were correlated with neither the shell size of the sperm receiver nor with that of the sperm donor (in all cases $P > 0.35$).

As in previous studies (Baminger and Haase, 1999, 2000; Bojat and Haase, 2002), most of the allosperm were stored in the main spermathecal tubule (Fig. 3). The amount of sperm stored in lateral tubules was variable. In five out of 18 individuals all lateral tubules were empty. In the remaining snails one or more lateral tubules and in four individuals all lateral tubules contained at least a few sperm. In most snails, the first lateral tubules contained more allosperm than the remaining lateral tubules.

The volume of sperm stored in the spermatheca was not influenced by copulation duration (median: 97.5 min, range: 65–135 min; $r_s = -0.05$, $N = 18$, $P = 0.83$).

Spermatophore volume and sperm number

The spermatophore volume and the number of sperm it contained 2 h after copulation were positively correlated ($r = 0.80$, $N = 19$, $P < 0.001$). Both spermatophore volume and the number of sperm it contained differed between snails examined 2 h and 36 h after mating (Table 1). The volume of full spermatophores (2 h after mating) and the number of sperm transferred were correlated with neither the shell size of the sperm donor, nor that of the sperm receiver (in all cases $P > 0.10$).

The transfer of larger spermatophores did not result in more sperm reaching the storage organ 36 h after mating ($r = 0.35$, $N = 14$, $P = 0.22$), assuming that the spermatophore volume decreased to the same degree in all individuals. Furthermore, if a larger number of sperm delivered also results in a larger number of sperm stored,

Table 1. Reproductive traits in individuals of *A. arbustorum* at 2 h and 36 h after copulation. Mean values ± SD are presented with sample sizes in parentheses. *F*- and *P*-values and degrees of freedom result from one-way ANOVA to test for differences in reproductive traits at different times after copulation

	Time after copulation		*df*	*F*	*P*
	2 h	36 h			
Spermatheca volume ($\times 10^{-3}$ mm^3)	1.62 ± 0.61 (18)	3.80 ± 1.96 (18)	1, 34	20.16	<0.001
Cumulative tubule length (mm)	3.20 ± 0.78 (19)	3.34 ± 0.84 (18)	1, 35	0.28	0.598
Main tubule length (mm)	1.54 ± 0.32 (19)	1.63 ± 0.29 (18)	1, 35	0.66	0.421
Volume of spermatophore transferred (mm^3)	1.79 ± 0.50 (19)	1.35 ± 0.60 (17)	1, 34	5.29	0.028
Sperm content of spermatophore (number of sperm $\times 10^6$)	1.34 ± 0.68 (19)	0.25 ± 0.34 (17)	1, 34	36.99	<0.001

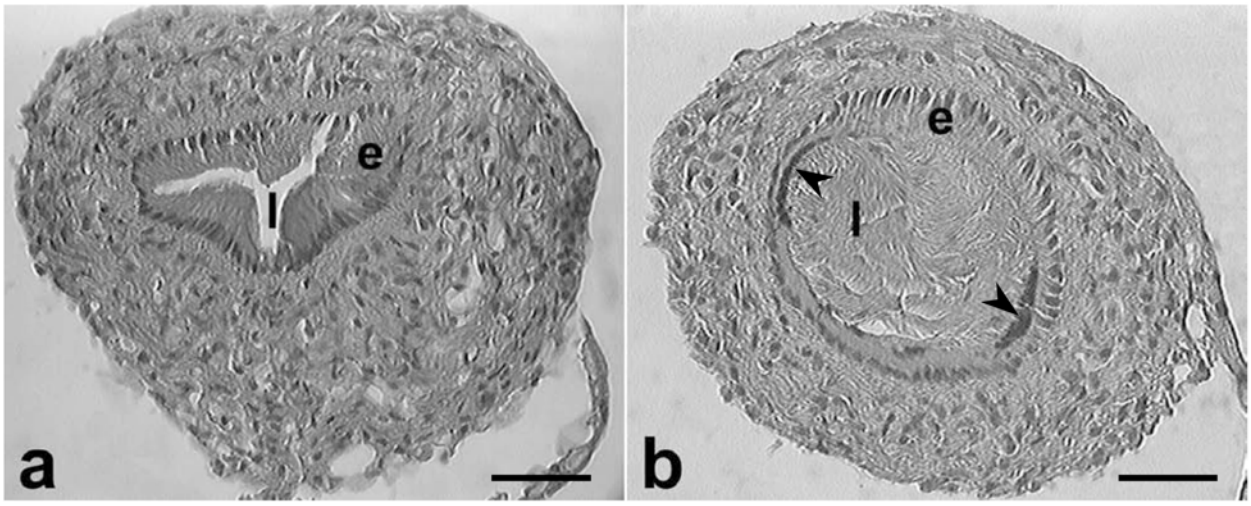

Fig. 1. Cross-section through the proximal end of the main spermathecal tubule of (a) an empty spermatheca (2 h after copulation), and (b) a spermatheca filled with sperm (36 h after copulation; sperm heads indicated by arrowheads). e, epithelium; l, lumen of main tubule. Scale bar = 50 µm.

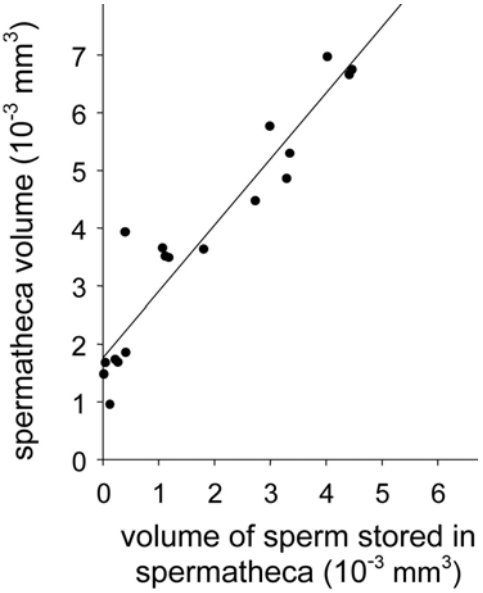

Fig. 2. Correlation between the volume of sperm stored in the spermatheca 36 h after copulation and spermatheca volume in 18 individuals of *A. arbustorum*.

we would expect a negative correlation between the amount of sperm remaining in the spermatophore 36 h after mating and the volume of sperm stored in the spermatheca. There was no correlation between these two traits ($r = 0.03$, $N = 14$, $P = 0.92$). However, in both correlations the statistical power was too low to provide sufficient evidence that there is no effect (power of 0.25 and 0.02, respectively).

Assuming that a fixed proportion of sperm is digested, then the transfer of large spermatophores with larger numbers of sperm should result in more sperm being transferred into the digesting bursa copulatrix. Furthermore, the less sperm that remain in the spermatophore the more should be found in the bursa. Indeed, we found a positive correlation between spermatophore volume and bursa copulatrix volume 36 h after mating ($r = 0.67$, $N = 9$, $P = 0.049$), but also between number of sperm remaining in the spermatophore and bursa volume ($r = 0.67$, $N = 9$, $P = 0.050$). However, after removing a single outlier, both correlations were no longer significant ($P > 0.58$).

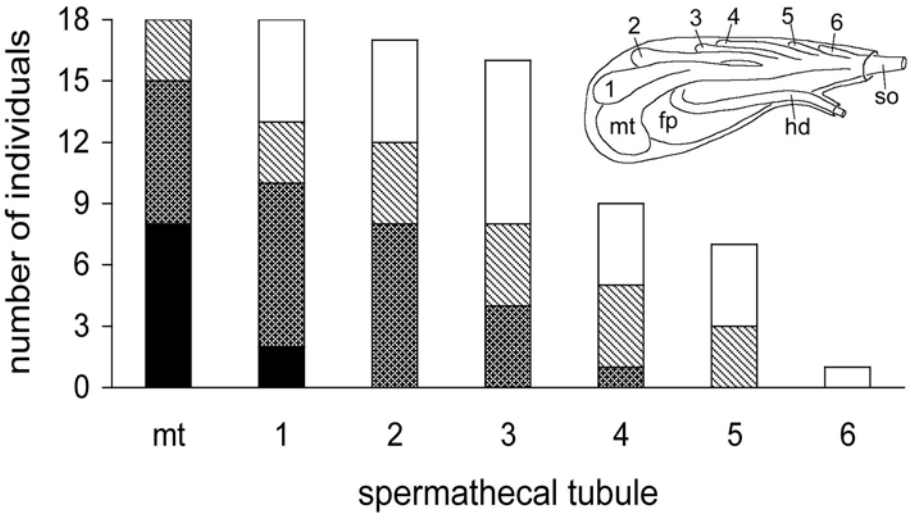

Fig. 3. Frequency distribution of the amount of sperm stored in the spermathecal tubules of *A. arbustorum* (mt = main tubule, 1–6 = lateral tubules; see insert). The amount of sperm is indicated as sperm densily packed (black), sperm loosely packed (chequered), sporadic sperm occurrence (diagonally hatched) and no sperm found (white). The insert shows a schematic representation of the fertilization pouch-spermatheca complex (fp, fertilization pouch; hd, hermaphroditic duct; so, spermoviduct).

The amount of sperm reaching the spermatheca was very low, corresponding to only 0.09% of the amount of sperm received (based on mean spermatophore volume 2 h after copulation, $N = 16$). However, this is a very rough estimate because the spermatheca shrinks during processing for histology.

Discussion

Our study shows that the spermatheca of the helicid land snail, *A. arbustorum*, is expandable and can accommodate more sperm than would be expected from measuring its initial volume. After a single copulation, 45% of the spermatheca volume was filled with sperm. The volume of sperm stored in the spermatheca 36 h after copulation corresponded to 98% of the volume of empty spermathecae. The increase in spermatheca volume must solely be due to the expansion of tubule diameter, because sperm uptake did not influence the length of the spermathecal tubules. Histological investigations revealed that the increase in spermathecal volume may be associated with stretching of the spermathecal epithelium (Beese, pers. obs.), which possesses extensive interdigitations between lateral and basal cell membranes in the unfilled state (Bojat et al., 2001b).

Our results confirmed the morphological structure of the spermatheca previously described (Haase and Baur, 1995; Baminger and Haase, 1999; Bojat and Haase, 2002). However, in contrast to Bojat and Haase (2002), we found no correlation between tubule number and amount of sperm stored. Furthermore, tubule number did not influence the volume of empty spermathecae, which contradicts the assumption of Bojat and Haase (2002).

Consistent with previous studies, there are indications that the spermathecal tubules are filled with sperm in a certain sequence, starting with the main tubule and continuing with the first lateral tubule, followed by the second lateral tubule and so on (Baminger and Haase, 1999). This indicates a potential for spatially separating sperm from multiple mates in different tubules and thus may represent a mechanism for cryptic mate choice. However, the tubular structure of the spermatheca could also be a naturally selected adaptation for efficient sperm storage and use. The nutrition of spermatozoa may be an important function of the spermathecal epithelium in *A. arbustorum* and the observed sperm storage pattern may be the result of sperm being nourished during storage (Bojat et al., 2003). Sperm are stored with their heads in tight contact with the spermathecal epithelium, usually at the bulbous blind tubule ends (Bojat et al., 2001b). The tails of the spermatozoa beat in synchronous waves that are directed towards the entrance of the tubules (Rogers and Chase, 2002). The activity of sperm during storage and the long periods during which sperm remain viable suggest that sperm may require energy in the spermatheca. Long tubules provide a large surface for sperm attachment and, potentially, for nourishment. However, an uptake of nutrients by stored sperm has not yet been demonstrated in *A. arbustorum* (Bojat et al., 2001b) and tubular spermathecae may have evolved simply to reduce sperm loss, as suggested for the red flour beetle

(Fedina and Lewis, 2004), or to match storage capacity demands arising through divergence in female longevity or egg productivity (Pitnick et al., 1999).

Spermatheca shape and capacity potentially influence sperm utilization patterns (Walker, 1980; Simmons, 2001). A tubular structure, as found in *A. arbustorum* and other snail species, may lead to sperm stratification and thereby result in last-mate sperm precedence. However, there is no evidence for sperm stratification nor for consistent last-mate sperm precedence in helicid snails (Baur, 1994; Rogers and Chase, 2002; Evanno et al., 2005). On the contrary, first-mate sperm precedence has been found in the first brood of individuals of *A. arbustorum* that mated twice within 70 days (Baur, 1994) and also in *Helix aspersa* (Evanno et al., 2005). The observed pattern could be due to the spermatheca being filled to capacity during the first mating (Retnakaran, 1974), but it might also be explained by the activity of allosperm during storage. Rogers and Chase (2002) suggested that the beating of the flagella of sperm from the first mate may generate resistance to incoming sperm from subsequent mates; the higher the number of resident sperm, the stronger the resistive force. Thus, the probability of sperm gaining access to the spermathecal tubules would decrease with each successive mating and this may provide paternity assurance for the first mate. A spermatheca that is elastic and expands to accommodate successive ejaculates potentially enables the female function to store and to use sperm of more than one mating partner, which could enhance the chance of cryptic mate choice (Simmons, 2001). However, this hypothesis is difficult to test, because the amount of sperm stored from each mating partner has to be assessed.

Sperm utilization in *A. arbustorum* changed to second-mate precedence when the interval between matings exceeded 300 days (Baur, 1994), suggesting a reduced viability or a reduced resistance of "old" allosperm (Rogers and Chase, 2002). However, analyses of long-term sperm utilization revealed clear differences among individuals, ranging from first-mate sperm precedence, through sperm mixing, to last-mate sperm precedence (Baur, 1994). A variable sperm precedence pattern might be explained by the differential use of sperm from different mates, but might also be due to differences in the amount of sperm received from each mate as well as sperm quality (Baur, 1994). Thus, different factors may influence sperm utilization patterns in this snail species.

The transfer of large numbers of sperm should lead to a numerical superiority of these sperm in the storage organ. In *A. arbustorum*, however, the amount of sperm stored seems to be unaffected by the amount of sperm received (see also Bojat and Haase, 2002). This suggests either an active role of the sperm receiver in transferring sperm into the storage organ, as has been found in insects (Bloch Qazi et al., 1998; Hellriegel and Bernasconi, 2000), or different quality (swimming speed, longevity) of sperm from different sperm donors (e.g., Garcia-Gonzalez and Simmons, 2005; Minoretti and Baur, 2006). We found no indication of a fixed proportion of sperm being digested and this also might be related to flexible adjustments of sperm use by the sperm receiver. The low amount of sperm reaching the storage organ of *A. arbustorum* is comparable to that in other snail species [0.1% in *H. pomatia*, Lind (1973) and 0.025% in *H. aspersa*, Rogers and Chase (2001)].

In many animal taxa, the spermatheca coevolves with sperm or ejaculate traits, possibly due to a conflict between the sex functions over the control of paternity. This indicates that the storage organ allows the sperm receiver at least some control over sperm use and fertilization (Birkhead and Pizzari, 2002). Consistent with this idea, there are indications for a coevolution between spermatheca volume and the number of sperm transferred in *A. arbustorum* (Beese et al., 2006). Thus, post-copulatory sexual selection seems to drive evolutionary changes in reproductive trait morphology and may therefore also be the reason for the complexity and diversity of sperm storage organs in helicid snails.

The type of spermatheca found in *A. arbustorum* allows a spatial separation of sperm from different mating partners. However, sperm uptake, storage and use might be influenced by both the sperm donor and the sperm receiver in different ways. This makes it difficult to determine single effects on paternity success. Nevertheless, the identification of different mechanisms underlying sperm transfer and sperm storage is an important step to explain the observed patterns of sperm precedence.

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