HERITABILITY OF SPERM LENGTH AND ADULT SHELL SIZE IN THE LAND SNAIL *ARIANTA ARBUSTORUM* (LINNAEUS, 1758)

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ABSTRACT

Sperm length varies considerably, both between and within species, but the evolutionary implications of this variation are poorly understood. Sexual selection on sperm length requires a significant additive genetic variance, but few studies have actually measured this. Stylommatophoran gastropods have extraordinarily long sperm. However, the extent of intraspecific variation has rarely been examined. Here we present the first estimates of heritability of sperm length in the land snail *Arianta arbustorum* using two complementary approaches (one-parent–offspring regression and full-sibling split design). We also examined whether sperm length is influenced by the shell size of the snail and estimated heritability of shell size. Sperm delivered by the same individuals in 2–4 matings over two reproductive seasons did not differ in length, indicating a high repeatability of this trait. Offspring of 10 families were kept at three temperatures (11, 15 and 20°C) to examine the influence of different environmental conditions on sperm length and adult shell breadth. Independent of shell breadth, sperm length was affected by temperature but not by family of origin (the variance component associated with family was not significantly different from zero), while adult shell breadth was influenced by temperature and family of origin. Higher temperatures resulted in shorter sperm, but larger shells. The heritability of sperm length derived from the two different approaches (one-parent–offspring regression: $h^2 \pm \mathrm{SE} = 0.52 \pm 0.55$; full-sibling split design: $H^2 \pm \mathrm{SE} = -0.19 \pm 0.28$) suggests relatively little genetic variation in this trait in the studied population. In contrast, the heritability of adult shell breadth indicates a strong genetic effect (mother–offspring regression, $h^2 \pm \mathrm{SE} = 0.90 \pm 0.33$). The heritability ($h^2 \pm \mathrm{SE}$) of adult shell breadth obtained from the father–offspring regression was 0.18 ± 0.42, i.e. five times smaller than that of the mother–offspring regression, suggesting a maternal effect on shell size.

INTRODUCTION

Sperm length shows an extraordinary variation both within and among species (Snook, 2005; Pitnick, Hosken & Birkhead, 2009a). This variation may reflect population- and species-specific differences in fertilization mode, allometry and strength of postcopulatory sexual selection. Within species, sperm–female interactions have been shown to be a major factor influencing sperm length evolution (e.g. Miller & Pitnick, 2002; Pattarini *et al.*, 2006; Pitnick, Wolfner & Suarez, 2009b). The length of sperm may influence their power and swimming speed as well as longevity because of differences in the energetic demands between longer and shorter flagella (e.g. Mossman *et al.*, 2009; Helfenstein, Podevin & Richner, 2010). In taxa with sperm storage organs, sperm length may determine the ability to reach the storage organs first and to move to the ovum from the storage organ once ovulation takes place (e.g. in the land snail *Helix aspersa*, Roger & Chase, 2002; in the domestic fowl, Froman, 2003).

The influence of sperm competition on sperm length is less well understood (Pizzari & Parker, 2009). In some species, sperm length appears to be relevant for male reproductive success under conditions of intensive sperm competition. For example, short sperm were favoured during sperm competition in the cricket *Gryllus bimaculatus* (Gage & Morrow, 2003) and sperm morphometry was adjusted in males of the polymorphic Gouldian finch (*Erythrura gouldiae*) across social environments (Immler, Calhim & Birkhead, 2010). Similarly, studies on simultaneous hermaphrodites indicate that the intensity of sperm competition can affect sperm length (Crean & Marshall, 2008; but see Janicke and Schärer 2010). In contrast, no correlation between sperm length and male reproductive success could be found in a variety of species including insects (Tomkins & Simmons, 2000; Simmons *et al.*, 2003), fish (Gage *et al.*, 2004) and mammals (Gage & Freckleton, 2003).

Sperm length usually exhibits little variation across ejaculates of single males (Morrow & Gage, 2001a; Birkhead *et al.*,

2005; Immler *et al.*, 2008; Fitzpatrick & Baer, 2011), indicating strong genetic determination (Morrow & Gage, 2001b; Simmons & Kotiaho, 2002). Simmons & Moore (2009) summarized available information on additive genetic and phenotypic variation and heritabilities of sperm and sperm-related traits in various taxa. Heritabilities of sperm morphology including sperm length varied around 0.5. In contrast, heritabilities of sperm performance traits such as sperm motility, viability and fertilization success were lower. Indeed, fitness traits are considered to show a substantial genetic variation, but low heritabilities due to a large fraction of residual variation (Houle, 1992). However, there is some evidence that environmental factors may influence sperm length. Sperm size increased with temperature in dung flies (Blanckenhorn & Hellriegel, 2002; but see Gage & Cook, 1994), and with the males' age in the rove beetle *Alleocharo bilineata* (Green, 2003), but decreased with larval density in *Drosophila melanogaster* (Morrow, Leijon & Meerupati, 2008; but see Gay *et al.*, 2009). The type of nutrition showed only a weak effect on sperm size (Gage & Cook, 1994; Amitin & Pitnick, 2007). Furthermore, maternal effects may partly determine sperm length. In the seed beetle *Callosobruchus maculates*, older mothers produced sons with longer sperm than did younger mothers (Dowling, Nowostawski & Arnqvist, 2007; Gay *et al.*, 2009). However, most aspects of the control of sperm length, including physiological processes and temperature, have so far not been investigated (Engel, Ludington & Marshall, 2009).

Gastropods exhibit a large interspecific variation in sperm morphology (Thompson, 1973; Healy, 1988, 1996; Luchtel *et al.*, 1997). Spermatozoa of stylommatophorans are among the largest of the gastropods (e.g. 800 μm in *Arianta arbustorum*, Bojat, Sauder & Haase, 2001; 850 μm in *Helix pomatia* and 1140–1400 μm in *Hedleyella falconeri*, Thompson, 1973). Information on intraspecific variation in sperm length is restricted to a single species, the land snail *Arianta arbustorum* (Linnaeus, 1758) (Minoretti & Baur, 2006). However, the significance of the variation in sperm length in terrestrial gastropods is largely unknown.

In the present study, we assessed the repeatability of sperm length in spermatophores delivered in successive matings by individuals of *A. arbustorum*. We also conducted a breeding experiment to estimate the heritability of sperm length and adult shell breadth in this species. We considered the relationship between shell breadth and sperm length because most reproductive traits (e.g. egg size, clutch size) are size-related in *A. arbustorum* (Baur, 1988b, 1990; Baur & Raboud, 1988; Baur, Locher & Baur, 1998). We used two different methods to estimate the heritability of sperm length and shell breadth. Firstly, we calculated the one-parent–offspring regression to obtain an estimate of narrow-sense heritability h^2. Secondly, we used a full-sibling split design to raise the offspring of several snails under different environmental conditions (three temperatures) until their first mating. Spermatogenesis of pulmonate gastropods is sensitive to both temperature and photoperiod (Tompa, 1984). This second approach allowed us to partition the genetic variance from the total phenotypic variance and thus to estimate the broad-sense heritability H^2 of sperm length and shell breadth (Lynch & Walsh, 1998).

MATERIAL AND METHODS

Study organism

Arianta arbustorum is common in moist habitats of northwestern and central Europe (Kerney & Cameron, 1979). The snail has determinate growth (shell breadth of adults 16–24 mm; Baur, 1984). Individuals become sexually mature at 2–4 years and adults live another 3–4 years (Baur & Raboud, 1988). Outcrossing is the dominant mode of reproduction in *A.*

arbustorum (Chen & Baur, 1993). Breeding experiments showed that 27% of virgin snails prevented from mating produced a few hatchlings by self-fertilization in the second and third year of isolation (Chen & Baur, 1993). However, the reproductive success of selfing individuals was less than 2% of that of outcrossing snails. Mating in *A. arbustorum* includes elaborate courtship behaviour, which lasts 2–18 h (Baur & Baur, 1992). Copulation is reciprocal; after intromission each snail simultaneously transfers one spermatophore, which is formed and filled with sperm during copulation (Hofmann, 1923; Baminger & Haase, 2001). Sperm are monomorphic in this species. Sperm length differed among populations (mean values of four populations: 878, 898, 913 and 939 μm), and—to a minor extent—even among individuals (Minoretti & Baur, 2006). Fertile sperm can be stored for more than 1 year (Baur, 1988a). In the field, *A. arbustorum* mates repeatedly in the course of a reproductive season. Snails deposit 1–3 egg batches, each consisting of 20–50 eggs (Baur, 1990). Multiple mating and sperm storage might promote postcopulatory processes in terms of competition among sperm from different partners, and/or selective storage and use of allosperm from the receiver (Baur, 1994; Kupfernagel, Rusterholz & Baur, 2010).

Sampling site and snail maintenance

Virgin individuals (subadult snails that had not yet completed shell growth) of *A. arbustorum* were collected from an embankment along a track in the subalpine forest near Gurnigelbad in Switzerland (46°45′N, 7°28′E, elevation 1230 m a.s.l.) in spring 2001 and 2002. The snails occurred at densities of 4–8 adults per m² on the embankment (Kupfernagel *et al.*, 2010). The snails in the sampling site were connected via streams with other populations. The snails collected were kept individually in transparent beakers (6.5 cm diameter, 8 cm deep) lined with moist soil (*c.* 4 cm) at 20°C with a light:dark cycle of 18:6 h. Within 4 weeks the snails reached sexual maturity as indicated by the formation of a reflected lip at the shell aperture. The beakers were cleaned twice per week and fresh lettuce was provided *ad libitum* as food. During winter (November 2001–March 2002) the snails were allowed to hibernate in darkness at 4°C and no food was provided.

Three criteria had to be fulfilled for a snail to be considered as virgin: (1) it had to be collected before the mating season; (2) its shell growth at the time of sampling was not yet completed (indicated by absence of reflected lip); and (3) no eggs should be laid when the animal was kept isolated for 3 weeks. A previous study showed that this procedure is highly accurate for assessing virginity in snails collected from natural populations (Kupfernagel & Baur, 2011).

The snails were marked individually with letters and 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. After shell growth was completed, we measured the shell breadth of each snail to the nearest 0.1 mm using vernier callipers.

Repeatability of sperm length

To assess the repeatability of sperm length in spermatophores delivered by the same individual in successive matings, we allowed snails to mate 2–4 times over two reproductive seasons. Mating trials were performed outdoors to expose snails to natural temperature and light conditions. Two randomly chosen active snails (individuals with an extended soft body and everted tentacles) were allowed to copulate in a transparent plastic container, measuring 14 × 10 × 7 cm, whose bottom had been covered with moistened paper towelling to maintain activity. Mating trials were initiated in the evening

and ran during several nights in June and July. Snails that did not mate within 8 h were tested again 3 days later with a new partner. Between trials, unmated snails were kept isolated as described above.

After copulation, one mating partner (hereafter referred to as sperm donor) was kept isolated. The other mating partner (hereafter referred to as sperm recipient) was frozen immediately after copulation in a freezer at $-18°C$. Sperm donors were allowed to remate with a second partner in the same reproductive season and with a third and fourth partner in the following season (all sperm recipients were virgin individuals). The recipients were dissected and their diverticulum removed to obtain the spermatophore received from the sperm donor. We measured the length (L) of the sperm-containing part of each spermatophore and its diameter at both ends (D_1 and D_2) to the nearest 0.1 mm using a dissecting microscope. Spermatophore volume was approximated, by the formula $V = 1/12\pi L(D_1^2 + D_1 D_2 + D_2^2)$, assuming a truncated-cone volume. Spermatophores were kept singly in Eppendorf tubes at $-30°C$ until required.

The beakers of the sperm donors were checked twice per week for eggs. The eggs deposited after the first mating were collected and kept in plastic dishes (6.5 cm in diameter) lined with moist paper towelling at $19°C$. The families of hatchlings were used for the breeding experiment (see below).

We assessed sperm length in all spermatophores obtained. We digitized randomly chosen sperm using a camera (SONY CCD-Iris) mounted on a 190 light microscope (Leica DMLD, magnification $\times$ 200) connected to a Macintosh computer. We measured the total length (head and tail) of 25–30 sperm per spermatophore using an image-analysis system (Minoretti & Baur, 2006), and calculated mean sperm length. Using this technique, measurements of sperm length are highly repeatable (calculated as intraclass correlation: 0.92; Minoretti & Baur, 2006).

Sperm length might be influenced by the number of sperm produced (e.g. Snook, 2005). To examine the potential trade-off between sperm length and sperm number, we counted the number of sperm in the spermatophores, following Locher & Baur (1997), in a subsample of 15 snails, which mated twice ($n = 30$ spermatophores). Briefly, the sperm suspension obtained from the mechanically disrupted spermatophore was stained with a DNA marker (gallocyanin–chromium complex). Two subsamples of known volume of the sperm suspension were 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 the two subsamples to calculate the total number of sperm transferred in a spermatophore. We adjusted our estimate of sperm number by multiplying the value with a correction factor of 1.00068, which considers the proportion of sperm removed for sperm length measurements (following Minoretti & Baur, 2006).

Breeding experiment to estimate heritability

To estimate the heritability of sperm length and shell breadth in *A. arbustorum*, hatchlings of single-mated mother snails were raised at three different temperatures using a full-sibling split design, and sperm length and shell breadth were measured in individuals, which completed shell growth and attained sexual maturity. Newly hatched snails from 29 singly mated mothers ($n = 1095$) were randomly assigned to one of three temperature treatments: $11°C$ (mean temperature $\pm$ SD measured by data loggers: $10.7 \pm 0.2°C$), $15°C$ ($14.7 \pm 0.2°C$) and $20°C$ ($20.0 \pm 0.2°C$) in such a way that similar numbers of offspring from each family were raised at each temperature. Three climate chambers, with a light:dark cycle of 16:8 h, were used for the three temperature environments. To minimize effects of

intraspecific competition, a maximum of 38 hatchlings (siblings) were kept in 750-ml plastic containers lined with moist soil. After 4 weeks, the number was reduced to a maximum of 20 juvenile snails per 750-ml container, and subsequently to 3 individuals per 225-ml container after 12 weeks. The containers were cleaned twice per week and fresh lettuce was provided *ad libitum* as food. At an age of 6 months, the snails were allowed to hibernate in darkness at $4°C$ for 3 months. The breeding experiment was started with 1095 hatchlings from 29 families. In spring, the 166 offspring belonging to 20 families that survived the overwintering period were kept isolated in transparent plastic beakers lined with moist soil (as described above). However, only 78 of the 166 snails reached sexual maturity in the three temperature treatments within 1 year in summer 2002 (the others attained sexual maturity later). Sixty of 78 offspring from 13 mothers (3–10 full-sibs per family) mated in the experiment. However, not all temperature treatments contained full-siblings from the same family, reducing the number of families to 10 (with a total of 48 offspring). The offspring were allowed to mate with an adult *A. arbustorum* collected in the wild in spring 2002. The spermatophores of the mating offspring were obtained by dissecting the recipients and sperm length was measured as described above. In a subsample of 19 offspring from 5 families we also assessed the volume of the spermatophore and the number of sperm delivered in each spermatophore (as described above). Adult shell breadth was measured in all offspring ($n = 48$).

Statistical analyses

All statistical analyses were carried out with SPSS® version 20 (IBM® SPSS®, 2011).

Mean values $\pm$ 1 standard error are presented. Possible relationships between shell breadth, sperm length, number of sperm delivered and spermatophore volume were analysed using Pearson correlations. To examine the effects of repeated mating and individual snail on sperm length in the parent generation, we used a two-way ANOVA with the fixed factor mating and the random factor individual. Variance components for sperm length were partitioned among sources (among 27 snails, between 2–4 matings for each snail) by using a nested analysis of variance. Differences in the coefficient of variation between snails (CV, adjusted to sample size: $CV_{adj.} = (1 + \frac{1}{4} n)$ CV) were examined using Levene's test.

We estimated repeatability of sperm length in successive matings following Lessells & Boag (1987). We calculated the intraclass correlation in sperm length derived from a one-way ANOVA, and adjusted it for unequal samples size (n_o).

To assess the influence of different environmental conditions on sperm length, offspring of 10 families were kept at three temperatures (full-sibling split design, see Methods). As a result of mortality, unbalanced sample sizes were obtained for the families and the three temperatures ($11°C$, $n = 13$; $15°C$, $n = 16$; $20°C$, $n = 19$). In the analyses we only considered families with at least one offspring at each temperature. In the case that a family had more than one offspring at a given temperature, we calculated the mean value of the offspring per family at this particular temperature. This procedure balanced the design and allowed us to use an ANOVA with temperature as a fixed factor and family of origin as a random factor. The degrees of freedom did not allow any calculation of the interaction between the factors. However, an interaction between family and temperature exists when the reaction norms, i.e. the lines connecting the sperm length of offspring of single mothers kept at different temperatures cross each other (see Fig. 1). We did not use an analysis of covariance correcting for shell breadth, because the correlations of sperm length with shell
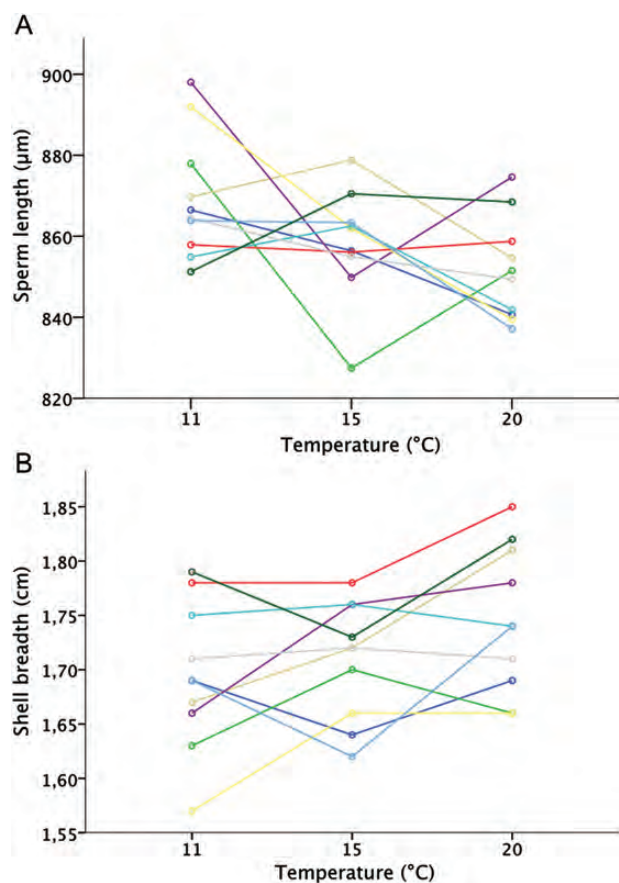
Figure 1. Sperm length and shell breadth of offspring of the land snail *Arianta arbustorum* raised at three temperatures. Sperm length (**A**) and shell breadth (**B**) for full-siblings of 10 families. In the case that a family had more than one offspring at a given temperature, we calculated and plotted the mean value of the offspring per family at this particular temperature.

breadth depended on the temperatures, which rendered an ANCOVA invalid (Sokal & Rohlf, 1995).

Narrow-sense heritability h^2 was estimated using the traditional one-parent−offspring regression, for which the level of genetic determination is given by multiplying the slope (b) of the regression by 2, i.e. $h^2 = 2b$, and the associated standard error equals twice the SE of the slope of the regression (Roff, 1997). This model uses information from the mother and the offspring generation, but does not consider temperature-induced variability in offspring traits. In a second approach, i.e. the full-sibling split design, we used the variance components of sperm length and shell breadth of the ANOVA to calculate H^2, their broad-sense heritability (H^2 = intraclass correlation coefficient multiplied by 2) and the associated SE following Roff (1997).

RESULTS

Repeatability estimates

Repeatability of sperm length was assessed in 27 snails that copulated 2−4 times. Mean sperm length differed among snails but not between successive matings of the same individual (inter-individual range: 844–922 μm; grand mean: 879 μm, $n = 27$; Table 1). The inter-individual variation in mean sperm length did not differ in successive matings (CV range: 1.9–2.6%; Levene's test: df$_1$ = 3, df$_2$ = 63, $P = 0.59$). Approximately half of the variation (49.1%) in sperm length can be attributed to

differences among snails, 5.5% to differences between matings of the same snail and 45.4% to differences within individuals. The repeatability of mean sperm length in successive matings of a single snail was 85.9% (ANOVA: $F_{26,40} = 16.10$, $P < 0.0001$).

Mean sperm length of a snail was not correlated with its shell breadth ($r = 0.03$, $n = 27$, $P = 0.89$). The number of sperm delivered ranged from 2.0×10^6 to 3.4×10^6 (grand mean: 2.6×10^6, $n = 15$; Table 1). Mean sperm length was neither correlated with the number of sperm delivered ($r = 0.16$, $n = 15$, $P = 0.58$), nor with the spermatophore volume ($r = 0.25$, $n = 26$, $P = 0.23$).

Sperm length and shell breadth in offspring

The ANOVA with a balanced design revealed that sperm length significantly decreased with increasing temperature ($F_{2,18} = 3.82$, $P = 0.042$; Fig. 1A). The variance component associated with the random factor family was not significantly different from zero. The interindividual variation measured as CV in sperm length of the offspring generation was 2.0% ($n = 48$), and did not differ from the variation found in the parent generation (1.3%, $n = 10$; Levene's test: df$_1$ = 1, df$_2$ = 56, $P = 0.13$).

Shell breadth increased significantly with temperature ($F_{2,18} = 4.61$, $P = 0.024$; Fig. 1B). The variance component of family (0.0024 or 61% of total) was significantly different from zero (likelihood ratio test: $\chi^2 = 9.6$, df = 1, $P = 0.002$) for shell breadth. The interindividual variation in shell breadth of the offspring generation was 4.3% ($n = 48$), which was similar to the value calculated for the parent generation (CV = 4.0%, $n = 10$; Levene's test: df$_1$ = 1, df$_2$ = 56, $P = 0.97$).

Heritability of sperm length and shell breadth

We used two approaches to estimate the heritability of sperm length. First, the one-parent−offspring regression (following Roff, 1997) revealed an h^2 of 0.52 (SE = 0.55, $n = 10$; $t = 1.44$, $P = 0.16$) for sperm length. Second, we used the ratio of the genetic variance to the total phenotypic variance extracted from the ANOVA of the offspring kept at different temperatures as an estimate of H^2 (i.e. data from the full-sibling split design). Heritability of sperm length assessed in offspring was not significantly different from zero (in fact the estimated variance component was negative, -0.19 ± 0.28). A minor part of the variance can be attributed to the family of origin (9.3%). The variance due to different temperatures was 24.4%. Unfortunately, many individuals died in the course of the experiment resulting in a low sample size. Thus, due to the low sample size, the experiment has little statistical power to test for low or moderate heritabilities.

Based on the mother−offspring regression, shell breadth showed a significant high heritability ($h^2 = 0.90$, SE = 0.33, $n = 10$; $t = 2.68$, $P = 0.012$). The father−offspring regression revealed a lower heritability than that obtained from the mother−offspring regression ($h^2 = 0.18$, SE = 0.42, $n = 10$; $t = 0.49$, $P = 0.63$). Heritability of shell breadth (H^2) assessed from the ANOVA of the offspring kept at different temperatures was 1.08 (SE = 0.27, $n = 3$ snails for each of the 10 families). The variance explained by the family of origin was 53.9% and that attributable to the different temperatures 21.7%.

DISCUSSION

The present study showed that individuals of *Arianta arbustorum* delivered sperm of constant length in four successive matings. The high repeatability of sperm length suggests a genetic determination of this trait, while the results of our breeding experiment, in which full-siblings were raised at different temperatures,

Table 1. Summary of two-way ANOVAs examining the effects of individual snails and repeated matings on sperm traits in *Arianta arbustorum*.

Sperm trait	Grand mean $\pm$ SE	Mean for each mating $\pm$ SE				Individual snail			Mating		
		1st	2nd	3rd	4th	df	F	P	df	F	P
Sperm length (μm)	879 ± 4 (27)	878 ± 4 (27)	881 ± 4 (27)	865 ± 6 (7)	859 ± 6 (6)	26, 37	12.22	<0.0001	3, 37	1.12	0.36
Number of sperm delivered ($\times 10^6$)	2.6 ± 0.1 (15)	1.9 ± 0.2 (15)	3.3 ± 0.2 (15)	–	–	14, 14	0.38	0.96	1, 14	17.36	0.001
Spermatophore volume (mm^3)	2.7 ± 0.1 (26)	2.9 ± 0.2 (26)	2.5 ± 0.2 (26)	2.5 ± 0.4 (5)	2.6 ± 0.4 (5)	25, 34	2.61	0.005	3, 34	1.62	0.20

Sample sizes are given in parentheses.

revealed both environmental and—to a minor extent—genetic effects on sperm length. Our results indicated that sperm length may be affected by the temperature, and this fact should be considered when studying genetic components of sperm length.

In the case of systematic maternal or environmental effects, repeatability may overestimate the corresponding narrow-sense heritability (Falconer, 1981). The high repeatability of sperm length between matings could result from the same environmental conditions to which snails were exposed in the laboratory culture. Interindividual differences in sperm length may derive from both genetic and environmental influences. We used a full-sibling split design to estimate the heritability corrected for environmental effects (Roff, 1997). A higher temperature (20°C) resulted in shorter sperm (Fig. 1A). Approximately a quarter of the variance (24.4%) in sperm length could be explained by temperature and only 9.3% by the family of origin. The sample size with the resulting degrees of freedom did not allow us to calculate the interaction between the two factors. However, an interaction between family of origin and temperature exists if the reaction norms, i.e. the lines connecting the sperm length of full-siblings raised at the three temperatures from different mothers, cross each other. Figure 1A indicates that this was the case in our breeding experiment. In pulmonate gastropods, spermatogenesis is sensitive to both temperature and photoperiod (Tompa, 1984). For example, the rate of spermatogenesis in *Helix aspersa* decreased at temperatures below 15°C and stopped at 5°C. At temperatures of 20–25°C, the multiplication of sperm cells and differentiation of spermatozoa proceeded within 3–4 weeks (Gomot de Vaufleury, 2001). Similar information is not available for *A. arbustorum*.

We used two different approaches to determine the level of genetic determination of sperm length. The one-parent–offspring regression revealed a relatively high estimate of heritability ($h^2 = 0.52$) but this value was not significantly different from zero. The parent–offspring regression reflects the influence of the genes transmitted from parents to their offspring combined with environmental effects (Roff, 1997). In the second approach, we used the ratio of the genetic variance to the total phenotypic variance extracted from the ANOVA of the offspring kept at different temperatures as an estimate of H^2, the broad-sense heritability. Based on families of full-siblings, this approach revealed a heritability estimate, which was not significantly different from zero. Thus, the data suggest relatively little genetic variation in sperm length in the studied population, because both approaches indicate almost no statistically significant resemblance between related individuals. However, due to the small sample size, the absence of a family effect or of a significant slope from the parent–offspring regression is only suggestive of an absence of a very strong genetic effect.

There are at least three explanations for the different heritability values of sperm length obtained by the two approaches. First, the sample size of offspring was relatively small. We started the breeding experiment with 29 families and 1095 hatchlings. However, only 10 families with 48 offspring could be considered in the analyses (see Methods). In our experiment, juvenile survival (15.2% after first hibernation) was approximately twice as high as recorded in natural populations of *A. arbustorum* (7.6%; Andreassen, 1981; Akçakaya & Baur, 1996). This demonstrates that huge breeding stocks are required to receive adequate sample sizes for experimental heritability estimates in traits with low genetic determination. The negative heritability indicates that offspring deviated consistently and in the opposite direction from the population mean of their parents (Palmer, 2000). As sample size decreases, the likelihood of obtaining a negative heritability (<0) or an extremely positive (>1) heritability increases substantially (Palmer, 2000).

Second, the heritability estimate obtained in the full-siblings split design is based on the ratio of the genetic variance to the total phenotypic variance. The total phenotypic variance includes the variance among individuals resulting from environmental differences. Therefore, an increase in the environmental variation (temperature) decreases heritability (Hartl, 2000).

Finally, we did not consider offspring that reached sexual maturity after one year. Thus, our heritability estimate is based on a subsample of fast growing snails. Individual growth rate is known to influence adult size in *Cepaea nemoralis* (Oosterhoff, 1977). However, it is not known whether growth rate affects sperm length in terrestrial gastropods.

The adaptive significance of sperm length variation is still unknown in *A. arbustorum*. Post-copulatory mechanisms of sexual selection could be a selective force for sperm length evolution (Pitnick *et al.*, 2009a). In the wild, sperm length differs among populations of *A. arbustorum* and even among individuals (Minoretti & Baur, 2006). In snails from two populations, no correlations between sperm length, velocity, percentage motility and longevity of sperm were found (Minoretti & Baur, 2006). Spermatozoa received from the mating partner are stored in the blind-ending tubules of the spermatheca, attached by the heads to the spermathecal epithelium (Bojat *et al.*, 2001). Rogers & Chase (2002) suggested that the unified beating of the flagella of sperm from the first mating provide resistance to incoming sperm from subsequent matings entering the tubules and in this way function as a paternity assurance. It is possible that longer sperm provide an increased resistance to incoming sperm, which would increase their chances for fertilization success. This hypothesis needs to be tested.

Shell breadth of offspring was significantly affected by both the family of origin and the temperature treatment. A higher temperature resulted in larger adult shells (Fig. 1B). The parallel-running reaction norms in Figure 1B suggest that no genotype–environment interaction occurs. Both methods revealed high heritabilities for shell breadth in *A. arbustorum* (one-parent–offspring regression: $h^2 = 0.90$; offspring estimate: $H^2 = 1.08$). The latter, as discussed above, may be a result of

the relatively small sample size, which can lead to an extreme positive (>1) heritability. Thus, offspring exhibit consistently more extreme phenotypes than their parents (Palmer, 2000). On the other site, heritability estimates derived from full-sibling split design that are larger than 1 more likely indicate that some of the genetic variance is due to dominance, epistasis or common environment in early life (Simmons & Moore, 2009). However, the effect of the family of origin was so strong that it was significant even with a small sample size. Our results confirmed the relatively high heritability of shell breadth in pulmonate land snails (*A. arbustorum*: 0.70 (Cook, 1965) and 0.54 (Baur, 1984); *Partula taeniata*: 0.40 and *P. suturalis*: 0.53 (Murray & Clarke, 1968)).

We also assessed the heritability of shell breadth using separate one-parent–offspring regressions for both mother and father snails. Interestingly, h^2 of shell breadth estimated with the father-offspring regression was 0.18, i.e. 5 times smaller than that of the mother–offspring regression ($h^2 = 0.90$), suggesting a maternal effect on shell size. This result is of importance because female reproductive traits including egg size and clutch size are positively correlated with shell size in *A. arbustorum* (Baur 1988b, 1990; Baur & Raboud, 1988; Baur *et al.*, 1998) as well as in other helicid snails (Dupont-Nivet *et al.*, 2000).

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