
Evolution of sperm size in nematodes: sperm competition favours larger sperm

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In the free-living rhabditid nematode *Caenorhabditis elegans*, sperm size is a determinant of sperm competitiveness. Larger sperm crawl faster and physically displace smaller sperm to take fertilization priority, but not without a cost: larger sperm are produced at a slower rate. Here, we investigate the evolution of sperm size in the family Rhabditidae by comparing sperm among 19 species, seven of which are hermaphroditic (self-fertile hermaphrodites and males), the rest being gonochoristic (females and males). We found that sperm size differed significantly with reproductive mode: males of gonochoristic species had significantly larger sperm than did males of the hermaphroditic species. Because males compose 50% of the populations of gonochoristic species but are rare in hermaphroditic species, the risk of male–male sperm competition is greater in gonochoristic species. Larger sperm have thus evolved in species with a greater risk of sperm competition. Our results support recent studies contending that sperm size may increase in response to sperm competition.

Keywords: evolution; comparative; nematodes; sperm competition; sperm size

1. INTRODUCTION

Female polyandry and, as a result, sperm competition are now recognized as almost universal features of reproductive ecology across animal phyla (Birkhead & Møller 1992, 1998; Smith 1984). Selective pressure on males to succeed at fertilization has resulted in the evolution of myriad traits including behaviours (e.g. frequent copulations, mate-guarding), morphologies (e.g. penile accessories for sperm removal), and secretions (e.g. mating plugs and anti-aphrodisiacs). Less clear is the role of sperm competition in the evolution of sperm themselves, including morphology, motility, and number. When considered simply as a lottery, sperm competition favours evolution of high sperm counts. If germ-line resources are limited, theory predicts that increased sperm numbers will lead to reduced sperm size (Parker 1982, 1984). Recently, however, theoretical work has also suggested that sperm size may instead increase in response to sperm competition when larger sperm experience greater survivorship and/or when larger sperm are better competitors (Parker 1993). Comparative studies have shown that sperm size increases with greater risk of sperm competition in butterflies (Gage 1994), birds (Briskie *et al.* 1997) and mammals (Gomendio & Roldan 1991; but see Harcourt 1991; Hosken 1997). Finally, empirical studies have shown that the larger, amoeboid sperm of mites take precedence over smaller sperm (Radwan 1996), and that larger sperm in dung flies are preferentially stored in the spermathecae (Otronen *et al.* 1997).

Nematode sperm compete to fertilize eggs by vying to gain access to the spermatheca and then to maintain residence in this organ where the eggs are fertilized. In the hermaphroditic nematode *C. elegans*, sperm from hermaphrodites are always outcompeted by sperm from males. Hermaphrodites resemble females but undergo a brief period of spermatogenesis before producing eggs, so they are self-fertile. Although hermaphrodites cannot pass sperm to others, they can receive sperm from males and produce outcrossed progeny. Male sperm outcompete hermaphrodite sperm by excluding them from the fertilization process (LaMunyon & Ward 1995; Ward & Carrel 1979) and we have found that male sperm are much larger than hermaphrodite sperm (LaMunyon & Ward 1998). Larger sperm crawl faster than smaller sperm and are able to physically displace them from the spermatheca, taking precedence in fertilization (LaMunyon & Ward 1998). However, there is a cost to larger sperm: they are produced at a slower rate. Male sperm compete equally with other male sperm (Barker 1994; Ward & Carrel 1979), which are similar in size (LaMunyon & Ward 1998).

Based on patterns found in *C. elegans*, we hypothesized that a greater risk of sperm competition would favour evolution of larger sperm. Here, we test this hypothesis by comparing sperm size among 19 hermaphroditic and gonochoristic (male or female) species of rhabditid nematode, whose risk of sperm competition varies with reproductive mode. In hermaphroditic nematodes, males generally make up less than 1% of populations. Thus, self-fertilization predominates, and sperm competition is rare. However, in gonochoristic species, males compose 50% of populations, and mating is more common. Therefore, there exists a greater risk of sperm competition in gonochoristic species than in hermaphroditic species.

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2. METHODS

The nematode species investigated here are all free-living feeders on bacteria. They were obtained either from the *Caenorhabditis* Genetics Center (CGC) or from David Fitch (New York University) and maintained in the laboratory in Petri plates on agar seeded with the *E. coli* strain OP50 (Brenner 1974). The 19 species studied belong to five genera, which group into three higher taxa based on recent molecular data (Fitch & Thomas 1997; D. Fitch, personal communication) as follows. Group I: *Caenorhabditis elegans* (Maupas, 1899) Dougherty, 1953; *C. briggsae* (Dougherty & Nigon, 1949) Dougherty, 1953; *C. remanei* (Sudhaus, 1974) Andrassy, 1983; and *C. sp.* (CGC strain CB5161). Group II: *Rhabditis brassicae* Southern, 1909; *R. blumi* Sudhaus, 1974; *R. dolichuroides* Anderson & Sudhaus, 1985; *R. guentheri* Sudhaus & Hooper, 1994; *R. sp.* (CGC strain CEW1); *R. dolichura* (Schneider, 1866) Sudhaus & Hooper, 1994; *R. tipulae* (Lam & Webster, 1971) Sudhaus, 1993; *R. myriophila* Poinar, 1986; *R. dudichi* (Andrassy, 1970) Sudhaus, 1976; *R. sp.* (CGC strain AF5); *Rhabditella axei* (Cobbold, 1884) Chitwood, 1933; *Pellioiditis typica* (Stefanski, 1922) Andrassy, 1983; and *P. sp.* (CGC strain EM434). Group III: *Pelodera strongyloides* (Schneider, 1860) Schneider, 1866; and *P. tenes* Schneider, 1866. Of these, all are gonochoristic except *R. myriophila*, *R. sp.* (AF5), *R. sp.* (CEW1), *R. tipulae*, *R. guentheri*, *C. elegans*, and *C. briggsae*, which are hermaphroditic.

Our measurements of sperm size were taken from spermatids, which are haploid gametes that sprout a pseudopod and become the mature amoeboid spermatozoa. Owing to their irregular shape, spermatozoa are difficult to measure, but in *C. elegans* they retain the same volume they had as spermatids (Roberts *et al.* 1986), which are generally spherical and easy to measure. Male worms were standardized for age by isolating them as last-stage juveniles and leaving them for 15–20 h, during which time they became adult but remained virgin. These males, which store sperm as spermatids, were dissected in sperm medium (Nelson & Ward 1980) and observed under Nomarski optics. The cross-sectional area of each spermatid was measured from video-captured images by using the analysis software NIH Image. Hermaphrodites, whose sperm remain as spermatids only transiently, were dissected as freshly moulted adults in sperm medium, and the spermatids were measured. For all species except *R. guentheri*, an average of 175 sperm (range 94–378) from 7–10 males were measured, and in hermaphroditic species, an average of 92 sperm (range 38–220) from 3–19 hermaphrodites were measured. While working with *R. guentheri*, it was extremely difficult to obtain males. After screening many thousands of worms, only three males were found. We measured body size and sperm size ($n=138$) from these males.

To determine if sperm size scales with body size, we measured male body size. Males, isolated as last-stage juveniles 15–20 h earlier, were anaesthetized with sodium azide (LaMunyon & Ward 1994) to quell their undulatory movements. Once motionless, the males were placed in a depression slide to prevent compression by the coverslip and observed under a compound microscope; the area occupied by their silhouettes (=longitudinal section) was measured by means of NIH Image. Eight to sixteen young adult males from each species were measured. Mean sperm sizes were calculated for male sperm from all species and for hermaphrodite sperm from hermaphroditic species.

To remove the effects of phylogenetic relationships on sperm size, the data were analysed by nested ANOVA, nesting genus within the three higher-level groupings, and the residuals, representing variation among species, were saved (Gage 1994;

Stearns 1983). More sophisticated methods were not used because they require a phylogeny, which is not available for many of the species used in this study. Indeed, several of the species examined here are, as yet, undescribed.

3. RESULTS

Sperm size varied greatly in our sample of worms (figure 1). Although we measured cross-sectional area, as shown in figure 1, the actual volumetric variation would be much greater, scaling exponentially. Within species, sperm size varied significantly among worms (analyses not shown), but the range of sperm from each worm was generally greater than 50% of the species range. Thus, even from individual worms, the sperm showed large variation in size. Sperm from hermaphrodites were the smallest observed, ranging from $2\ \mu\text{m}^2$ to $30\ \mu\text{m}^2$ in cross-section. Male sperm ranged from $20\ \mu\text{m}^2$ to $100\ \mu\text{m}^2$. Generally, those species with the largest sperm also had the greatest range of sperm size (figure 1). One species, *Rhabditis dudichi*, had a preponderance of small sperm (figure 1), which is apparent in micrographs of the spermatids themselves (figure 2). We wondered whether male age played a role in the production of the odd distribution of sperm size in this species. Therefore, we measured sperm from virgin males which were two days old, in addition to the one-day-old virgins already measured. The distribution did indeed change in the older males: larger sperm increased in proportion to the smaller sperm (figure 3). Thus, male *R. dudichi* make smaller sperm as young adults and larger sperm later.

The observed variation in male sperm size is explained, in part, by reproductive mode (hermaphroditic or gonochoristic), which reflects the risk of sperm competition. Hermaphroditic species had smaller male sperm than did the gonochoristic species ($F_{1,18}=13.179$, $p=0.002$; figure 4a). This difference in sperm size was not the result of phylogeny: sperm size did not vary significantly with affiliation in either genus ($F_{4,13}=0.701$, $p=0.605$) or higher-level phylogeny ($F_{2,13}=1.284$, $p=0.310$). Even though sperm size did not differ with phylogeny, we saved residuals from this analysis and examined their relation with reproductive mode. Residual male sperm size was still significantly reduced in hermaphroditic species compared with gonochoristic ones ($F_{1,17}=7.685$, $p=0.013$; figure 4b).

The difference in male sperm size between reproductive modes was also not due to allometry with body size. Sperm size did, in fact, correlate positively with body size ($r=0.520$, $p=0.022$). However, when body size was included as a covariate, sperm size still varied significantly with reproductive mode ($F_{1,16}=5.918$, $p=0.027$), even when considering residual sperm size after removing phylogenetic effects ($F_{1,16}=6.333$, $p=0.023$). Thus, although sperm size increases with body size, reproductive mode has an independent effect on sperm size.

4. DISCUSSION

Our results indicate that male sperm size in nematodes varies with reproductive mode: sperm are larger in gonochoristic species than in hermaphroditic ones. Because males are more common in gonochoristic species,

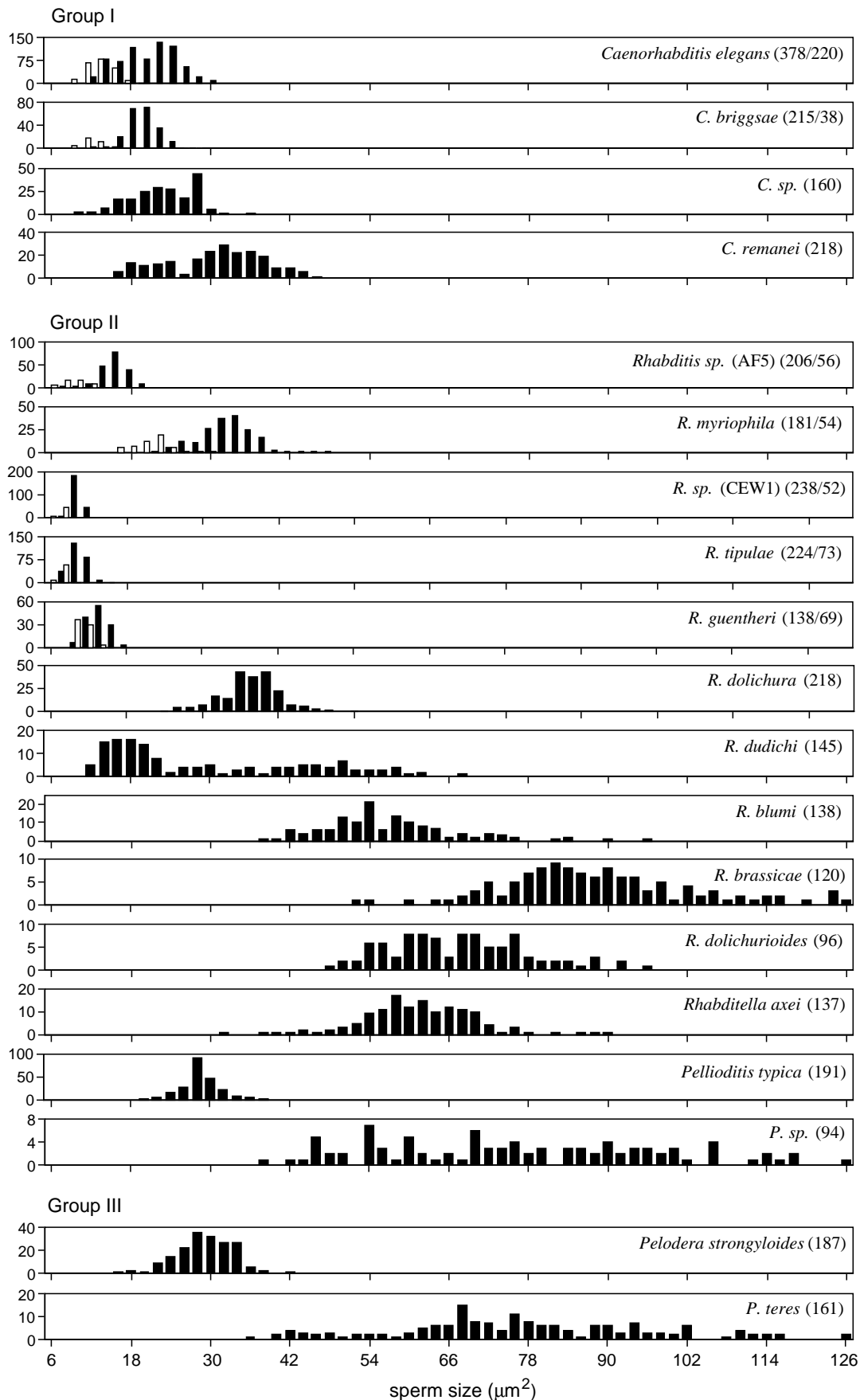


Figure 1. Distribution of spermatid sizes, measured as cross-sectional area, for 19 species of rhabditid nematode. Closed bars represent male sperm; open bars represent hermaphrodite sperm. Sample sizes are listed parenthetically (male sperm/hermaphrodite sperm).



Figure 2. Micrograph of spermatids from male *Rhabditis dudichi*, one day old. Bar, 10 μm .

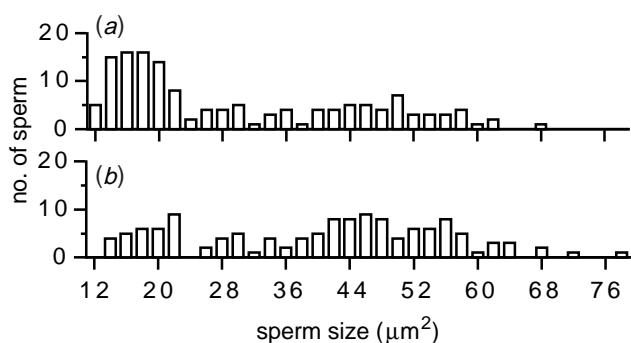


Figure 3. Size distributions of spermatids from (a) one- ($n=145$) and (b) two-day-old ($n=130$) *Rhabditis dudichi* males. Sperm from males two days old were the larger (ANOVA with observations nested within the worm of origin: $F_{1,259}=36.851$, $p<0.001$).

polyandry and the risk of sperm competition probably occur to a greater degree in gonochoristic than in hermaphroditic species. Larger sperm have thus evolved in species with a greater risk of sperm competition. Phylogeny had no effect on sperm size. This lack of a phylogenetic effect is not surprising, given the fact that molecular divergence within this one family of morphologically similar nematodes is fivefold greater than that between different classes of tetrapod (Fitch & Thomas 1997). Thus, these species are apparently sufficiently diverged to effectively eliminate phylogenetic constraint. Allometry also did not affect the outcome of our analyses, even though sperm size correlated with body size. Our comparative results support other studies that have found a relationship between sperm size and risk of sperm competition across species (Briskie *et al.* 1997; Gage 1994; Gomendio & Roldan 1991) and add credence to theoretical studies that suggest that larger sperm may be favoured when larger sperm are more competitive (Parker 1993).

Sperm from hermaphrodites were generally the smallest we observed. In hermaphroditic species, sperm competition is primarily male–hermaphrodite, because male sperm always encounter hermaphrodite sperm after mating (unless a male copulates with an old hermaphrodite depleted of self-sperm). However, all hermaphrodites in our study made smaller sperm than did the males of

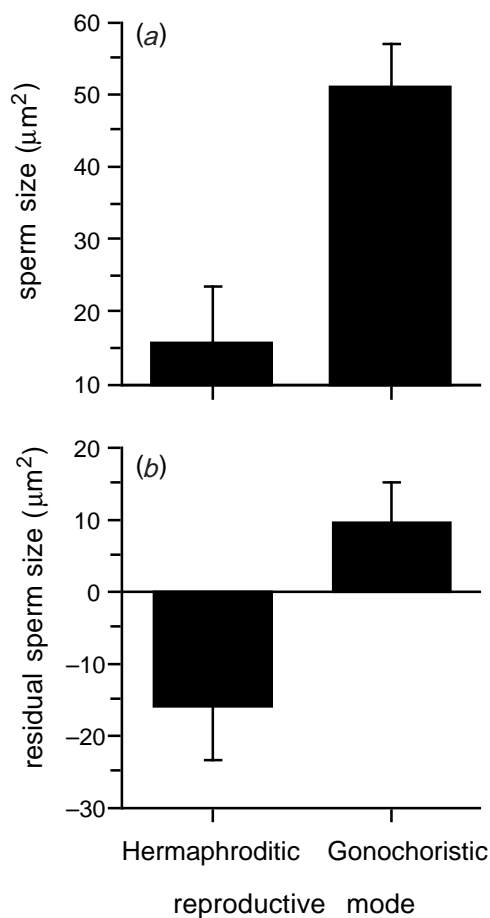


Figure 4. Spermatid size as a function of the species' reproductive mode, hermaphroditic ($n=7$) or gonochoristic ($n=12$). Data analysed before (a), and after (b), removing effects of phylogeny (see text). Error bars equal 1 s.e.m.

their species. This curious strategy of making small, less competitive sperm may have several advantages for hermaphrodites. First, outcrossing may provide a number of benefits (e.g. complementation of deleterious alleles, increased genetic variability, etc.) that are maximized by allowing male sperm to take precedence. Second, smaller sperm in *C. elegans* are produced faster (LaMunyon & Ward 1998) reducing the time to egg-laying (because spermatogenesis precedes oogenesis) and thereby shortening generation time; in *C. elegans*, this shorter generation time allows for more rapid colonization of new habitats (Hodgkin & Barnes 1991). Hermaphroditism is probably a derived character in the Rhabditidae (Poinar 1983) and has arisen at least once in both *Caenorhabditis* and *Rhabditis*, although this is probably an underestimation (Sudhaus & Kiontke 1996). Thus, the benefits of making small sperm are common to both lineages of hermaphrodites. Males in hermaphroditic species need only outcompete the hermaphrodite sperm, and therefore are free to track the decreasing size of hermaphrodite sperm and thus reduce the costs of making large sperm, as long as the male sperm remain larger than hermaphrodite sperm.

The cost of making larger sperm in *C. elegans* is a reduction in the rate at which they are produced (LaMunyon & Ward 1998). Males in gonochoristic species are likely to suffer this cost to a much greater degree, because they construct very large sperm. Perhaps the wide distribution of sperm sizes

produced by males is a response to this cost: they make some large, highly competitive sperm and some smaller, cheaper sperm that increase fertilizations when passed to a virgin female. Male *R. dudichi* show this strategy temporally, producing small sperm initially (to rapidly boost sperm count) and large sperm later (to increase ejaculate competitiveness).

Sperm competition has apparently had a profound influence on the size of sperm in nematodes. Sperm size has been tailored by both the benefits of increased competitiveness and the costs of producing highly competitive sperm. Larger sperm have evolved where competitiveness is more important; smaller sperm have evolved where competitiveness is less important. This may be the case in other animals, such as mites, where larger sperm are more competitive (Radwan 1996), or mammals, where a preliminary study suggested larger sperm swim faster and may reach the egg sooner than smaller sperm (Gomendio & Roldan 1991), or birds, where larger sperm appear to compete more effectively for sole possession of the female's sperm-storage tubules (Birkhead & Møller 1998; Briskie *et al.* 1997). Thus, where sperm competition is a selective force and where the sperm themselves are active competitors and not passive 'lottery tickets', larger sperm, owing to their increased competitiveness, will probably evolve (Parker 1993).

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