BRIEF COMMUNICATIONS

Evolution, 58(3), 2004, pp. 651–655

SPERM-LIMITED FECUNDITY IN NEMATODES: HOW MANY SPERM ARE ENOUGH?

ASHER D. CUTTER
Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85719
E-mail: acutter@email.arizona.edu

Abstract.—The Bateman principle, which holds that oocytes are the limiting gamete in reproduction, is violated in a variety of species. Self-fertilizing hermaphrodites of the nematode *Caenorhabditis elegans* provide an example of a system in which sperm number limits lifetime reproductive output, in this species due to the protandrous nature of sperm production that in turn delays the onset of fertilization. This reproductive delay forms the basis of a trade-off between generation time and total fecundity, in which sperm number plays a pivotal role. I use an age-structured population model to describe the number of sperm that maximize fitness, given larval development time and rates of gamete production. The model predicts the evolution of sperm numbers that are consistent with empirical data for *C. elegans* provided that precocious larval sperm production is taken into account. Several testable hypotheses follow from the model regarding how natural selection and environmental variation may influence patterns of sperm production among populations or species with a similar mode of reproduction.

Key words.—*Caenorhabditis elegans*, life-history evolution, self-fertilization, sperm-limitation.

Received July 16, 2003. Accepted October 21, 2003.

Bateman's principle (Bateman 1948) holds that female gametes tend to limit reproduction, an assumption that underlies a broad and successful literature on sexual selection and sperm competition (Birkhead and Møller 1998). However, breeding systems in a variety of taxa do not conform to this generalization that oocytes limit fecundity. Examples of sperm- or pollen-limited systems extend from liverworts (McLetchie 1996) to spiny lobsters (MacDiarmid and Butler 1999) and a number of species of *Drosophila* (Pitnick 1993; Pitnick and Markow 1994), a variety of flowering plants (Bierzychudek 1981), multiple marine broadcast-spawning species (Levitan and Petersen 1995) such as gorgonian corals (Lasker et al. 1996), and many nematode taxa (Poinar and Hansen 1983), including the nematodes *Caenorhabditis elegans* (Ward and Carrel 1979) and *C. briggsae* (Fodor et al. 1983). The processes leading to sperm-limited reproduction in these taxa are varied, and here I focus on the simple mechanism underlying sperm limitation in *C. elegans* and its protandrous, self-fertilizing hermaphrodite relatives. This system provides a novel model for a quantitative investigation of the physiological forces involved in the evolution of sperm production.

Reproduction in the nematode *C. elegans* occurs primarily by self-fertilization, whereby a hermaphrodite's gonad first produces about 280 sperm prior to an irreversible switch to oogenesis, potentially leading to the production of more than 1000 oocytes (Kimble and Ward 1988). Because fertilization may commence only upon the completion of spermatogenesis and onset of oogenesis, the time required to produce sperm delays the onset of reproduction and results in sperm-limited fertilization among self-fertilizing hermaphrodites (Ward and Carrel 1979; Kimble and Ward 1988; Hodgkin and Barnes 1991; Barker 1992). Thus, there is a trade-off between total fecundity, which depends directly on the number of sperm produced, and growth rate, which depends on the time delay in the onset of fertilization caused by sperm production. There is experimental support for this trade-off (Hodgkin and Barnes 1991), and a theoretical justification examined the influence of different survival probability functions and the ratio of temporal costs of sperm and egg production on this trade-off (Barker 1992). However, it remains an open question whether this simple trade-off mechanism can provide an adequate quantitative explanation for the numbers of sperm produced by hermaphrodites.

The small number of sperm produced by the nematode *C. elegans* and the simple mechanism underlying sperm limitation in this species provides a novel set of circumstances to dissect sperm production from a physiological and evolutionary perspective. In this study, I extend the modeling framework employed by Barker (1992) to infer the expected number of sperm that a hermaphrodite will produce to maximize fitness (growth rate). I focus on how such factors as sperm production rates, oviposition rates, larval development time, and precocious larval production of sperm may quantitatively influence the number of sperm produced by a hermaphrodite. I then apply empirical data to the model from experiments and from the literature and argue that the trade-off mechanism may reasonably describe sperm numbers in *C. elegans* once precocious larval sperm production is taken into account.

MODEL

Caenorhabditis elegans populations reproduce continuously, consequently I follow the standard Euler equation approach to age-structured population growth to describe the birth and death process in an exponentially growing population (Charlesworth 1994; Barker 1992). Using this approach, the relation

$$1 = \int_a^d \exp(-rx) l(x) b(x)\, dx \qquad (1)$$

must be conserved (Charlesworth 1994), where a is the age at which hermaphrodites begin laying fertilized eggs as adults, d is the age at which hermaphrodites cease reproducing, r is the intrinsic rate of growth, and $l(x)$ and $b(x)$ are the mortality and birth schedules, respectively. The bounds of the duration

of reproduction (a and d) depend on: (1) the duration of larval development, t_j; (2) the duration of the sperm production period, $f(n)$, where n = number of sperm; and (3) the duration of the oviposition period, $g(n)$. The duration of the oviposition period is a function of the total number of sperm because zygote production is limited by the number of sperm. Thus, $a = t_j + f(n)$ and $d = a + g(n)$: progeny production initiates after all sperm are produced following the larval stage and terminates when all sperm have been used to fertilize oocytes. I numerically solve for the equilibrium number of sperm (n) for a simplified set of assumptions regarding the form of $f(n)$, $g(n)$, $b(x)$, and $l(x)$ and compare the effects of constant gamete production rates with gamete production when all or only some fraction of sperm production delays reproduction. For simplicity, I use the exponential survival function $l(x) = \exp^{-ux}$, with mortality parameter u, which may approximate mortality in nature (Barker 1992).

When both sperm production and egg production occur at constant rates s and e, respectively, then $f(n) = n/s$, $g(n) = n/e$, and $b(x) = e$. However, because sperm production by hermaphrodites initiates during the last larval stage so only some fraction of sperm production delays the onset of reproduction (Hirsh et al. 1976; L'Hernault 1997), it is more appropriate to define $f(n) = (1 - p)n/s$, where p represents the fraction of sperm that are produced precociously during the last larval stage. This leaves us with the equation

$$1 = e \int_a^d \exp[-(r + u)x]\, dx. \tag{2}$$

Differentiating this with respect to n (where n lies within the limits of integration a and d) and solving for r gives us an equation for the maximum growth rate,

$$r_{\max} = \frac{e \times \ln\left(1 + \frac{s}{e}\right)}{(1 - p)n^*} - u, \tag{3}$$

where n^* is the value of n that maximizes the growth rate, given the other parameters. We can solve for n^* (with numerical methods) by substituting $r_{\max}$ for r in equation (2). By virtue of the exponential survival probability function, the mortality parameter u falls out of the solution for n^* and thus mortality does not influence the estimates for the number of sperm that maximizes fitness. As Barker (1992) noted, other types of survival probability functions do not drop out of the equations, but an exponential survival probability function may be similar to the mortality schedule in nature. Furthermore, he showed that the influence of mortality, when it occurs, is relatively weak compared to variation in the duration of sperm and egg production.

Empirical Model Parameter Values

The extensive laboratory study of *C. elegans* life history in the published literature provides empirical estimates of the parameters incorporated into this model. Because sperm limit fecundity and are used in hermaphrodite self-fertilization with nearly 100% efficiency (Ward and Carrel 1979), hermaphrodite self-fecundity can be used as a proxy for sperm count. Among 23 strains of *C. elegans*, mean hermaphrodite fecundity was 286.3 progeny (± 8.2 SE, range of strain means = 187–353) at 20°C (Hodgkin and Doniach 1997), implying that an equivalent number of sperm were produced. In the canonical N2 strain at 20°C, the temperature at which population growth rate is maximal (Venette and Ferris 1997), mean oviposition rate has been reported as 3 eggs/h (Ward and Carrel 1979), 4.4 eggs/h (Byerly et al. 1976), and 5.3 eggs/h (Hodgkin and Barnes 1991), with a maximum of 9.1 eggs/h during the egg laying period (Byerly et al. 1976). Rough calculations suggest that sperm production occurs at a rate of 0.5–1.5 sperm/min (Hirsh et al. 1976; Hodgkin and Barnes 1991; L'Hernault 1997; C. Lamunyon, pers. comm.). Although I know of no exact published measures, the schedule of development provided by Hirsh et al. (1976) suggests that hermaphrodites may produce slightly more than half of their sperm prior to the adult molt. At 20°C, embryonic and larval development requires about 64 h and oviposition begins about 14 h later (Lewis and Fleming 1995). Lower temperatures lengthen development time (~6 days at 15°C vs. ~3 days at 20°C) and higher temperatures shorten development time (~2 days at 25°C; Byerly et al. 1976).

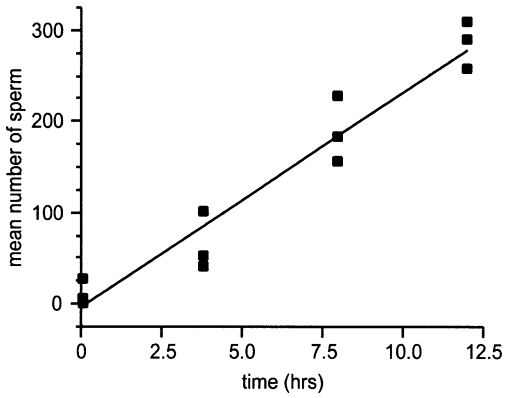

FIG. 1. Observed increase in sperm numbers over time. Each point is the average number of sperm counted from 10 hermaphrodite individuals. The first three time points correspond to the last larval stage; the final time point corresponds to young adults. The slope of the linear regression line (23.6 sperm/h = 0.39 sperm/min) corresponds to the rate of sperm production.

To obtain more precise estimates of sperm production rates and the fraction of sperm produced during the larval period, I performed a simple set of experiments. I sampled individuals from three replicate, synchronized populations of the *C. elegans* strain N2 (reared at 20°C) at intervals of 4 h during late larval and early adult development to assay sperm complements. Using methanol–acetic acid fixation and DAPI staining, nematode samples were squashed on microscope slides (Sulston and Hodgkin 1988). I then counted the number of spermatid nuclei located within the gonad of at least 10 hermaphrodite individuals from each sample. Average sperm numbers among individuals for each sample-replicate were used in linear regression (Fig. 1) to estimate the rate of sperm production as the slope of the regression line. Using this approach, I estimate that sperm were produced at a rate of 23.6 ± 1.86 (SE) sperm/h (0.39 ± 0.031 SE sperm/min; $r^2 = 0.94$, $F_{1,10} = 160.8$, $P < 0.0001$), which contrasts with the faster rate of sperm production reported for males (Lamunyon and Ward 1998). The lack of significance of a qua-

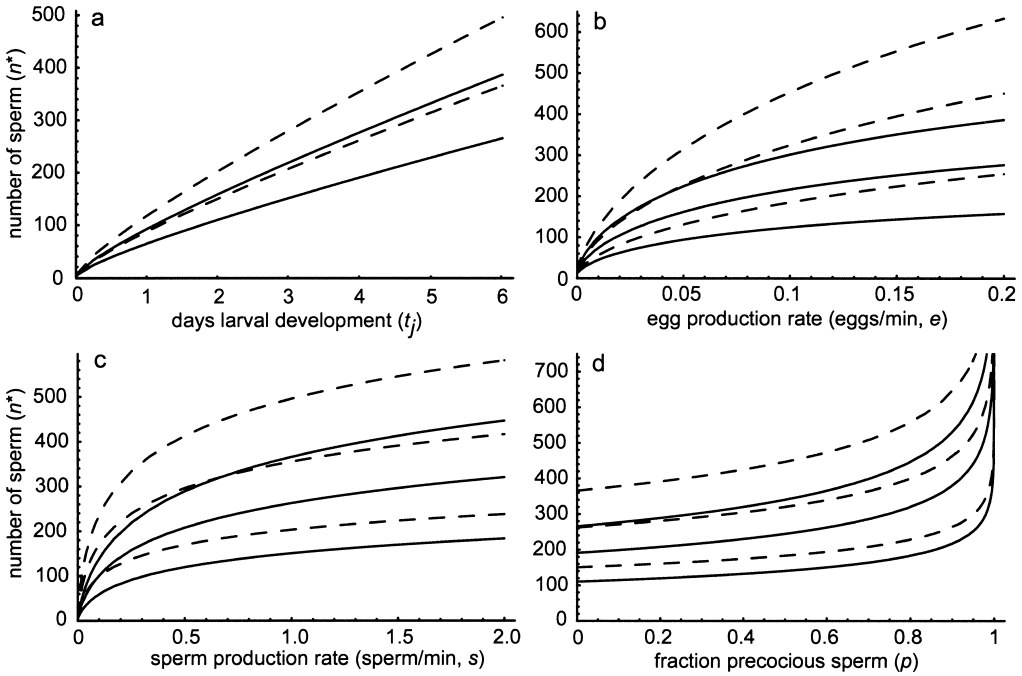

FIG. 2. Equilibrium number of sperm as a function of (a) larval development, (b) egg production rate, (c) sperm production rate, and (d) fraction precocious sperm. In (a, b, d), solid curves indicate $s = 0.4$ sperm/min, dashed $s = 1$ sperm/min. The bottom curve for each set with identical dashing in (a) corresponds to $p = 0$, top $p = 0.67$. In (c), solid curves indicate $p = 0$, dashed $p = 0.67$. The triplets of similarly dashed curves in (b, c, d) correspond to top $t_j = 6$ days, middle $t_j = 4$ days, bottom $t_j = 2$ days. In (a, c, d), $e = 4.4$ eggs/h (0.073 eggs/min), whereas in (b) $s = 0.4$ sperm/min.

dratic term ($P = 0.3$) indicates constancy of sperm production over time, consistent with the model assumption of a constant rate of sperm production, *s*. An average of 188.5 of 284.9 sperm were produced in the three sample points preceding the final, young adult time point. This suggests that 66.2% of sperm are produced during the pre-reproductive developmental stages (i.e., $p \approx 2/3$), so only about one-third of sperm production delays the onset of fertilization.

RESULTS

From the general trends implied by the model, it is apparent that the number of sperm that maximizes fitness (growth rate) increases with the duration of the larval phase, for given rates of gamete production (Fig. 2a). This is likely a consequence of sperm count contributing less of a delay, in relative terms, to the onset of fertilization with a longer prereproductive life span:

$$\lim_{t_j \to \infty} \frac{f(n)}{t_j} = 0. \tag{4}$$

As the rate of egg production increases for a given rate of sperm production, the expected number of sperm produced increases (Fig. 2b). In other words, more rapid production of eggs counteracts the temporal costs of producing more sperm. Third, increasing the rate of sperm production increases the expected number of sperm (Fig. 2c). Figure 2d shows that precocious larval sperm production exerts a positive influence on the expected total number of sperm produced by hermaphrodites. Point estimates of n^* are summarized in Tables 1 and 2 for several biologically relevant values of *s*, *p*, *e*, and t_j. Using parameter estimates that correspond to standard laboratory conditions at 20°C, the model predicts an equilibrium of about 220 sperm versus the observed 286 sperm.

TABLE 1. Equilibrium number of sperm (n^*); $t_j = 3$ days; $e = 4.4$ eggs/h.

Sperm production rate per min (*s*)	Fraction precocious sperm (*p*)			
	0	0.50	0.67	0.75
0.2	114	152	176	194
0.4	152	194	220	239
1.0	208	254	281	301
1.5	235	281	309	330

TABLE 2. Equilibrium number of sperm (n^*); $s = 0.4$ sperm/min, $p = 0.67$.

Egg production rate per h (*e*)	Days larval development (t_j)			
	2	3	4	6
3.0	131	181	227	315
4.4	159	220	278	387
5.3	174	242	305	426

DISCUSSION

Sex allocation theory applied to hermaphrodites predicts that the allocation of resources to male function should be low in highly selfing individuals because selfing hermaphrodites should produce the fewest number of sperm required to fertilize all ova (Charnov 1982). Because growth rate is the appropriate measure of net fitness in *C. elegans* (rather

than total fecundity), the specious violation of Bateman's principle in that *C. elegans* hermaphrodites produce fewer sperm than oocytes is actually fully consistent with sex allocation theory. Here I have shown that a model of a tradeoff may be used to estimate hermaphrodite sperm counts, the predicted numbers of sperm are quantitatively consistent with empirical observations, and failure to account for late-larval sperm production leads to gross underestimation of sperm counts. The expected numbers of sperm produced by hermaphrodites are increased by longer larval development, faster rates of gamete production, and a greater fraction of sperm produced during the larval phase (Fig. 2).

The simple mechanisms captured in this model seem sufficient to provide a reasonable quantitative description of sperm production in *C. elegans* when precocious production of sperm by larvae is taken into account (about two-thirds of hermaphrodite sperm produced during the last larval stage). The model indicates that for a life history marked by long larval development an individual's fitness will be maximal when many sperm are produced relative to when larval development progresses rapidly (Fig. 2a). This inference assumes that gamete production rates and the rate of ontogenetic growth are sufficiently decoupled (i.e., independence of t_j relative to e and s) and might be influenced by both heritable and nonheritable factors. The equilibrium number of sperm indicated by the model for long larval development may be revised downward if rates of gametogenesis and development covary (e.g., slower production of oocytes and sperm with longer larval development), although the degree of covariation between gametogenesis and overall development has not been well characterized empirically. Heritable factors may contribute to variation in sperm number among populations or species that have adapted to different environmental conditions that also affect larval development time (e.g., temperature differences across latitudes or habitats). Genetic differences between nematode populations (or species) native to warm regions may be expected to underlie the production of fewer sperm than those from cooler regions because development is more rapid at higher temperatures. Consistent with this hypothesis, hermaphrodites of the congener *C. briggsae* generally develop faster and produce fewer progeny than *C. elegans* hermaphrodites, and *C. briggsae* individuals are also able to tolerate and proliferate at higher temperatures than *C. elegans* (Fodor et al. 1983).

Nonheritable developmental and physiological lability may allow variation in sperm number for a given genetic background when sperm and oocyte production rates are impacted to a lesser extent than the pace of larval development. Consistent with this idea, *C. elegans* self-fecundity is greatest at low-to-moderate temperatures (~16°C) and drops off as temperature increases to 25°C (Hirsh et al. 1976; Grewal 1991), provided that the fecundity measures reasonably represent sperm count and not some factor that reduces zygote viability at higher temperatures. However, rates of chromosomal nondisjunction are known to increase at elevated temperatures (Rose and Baillie 1979), which may lead to reduced fecundity independently of total sperm production (Hodgkin et al. 1979; Cutter et al. 2003). In contrast to the pattern in *C. elegans* and inconsistent with the hypothesis of labile sperm production with respect to temperature, hermaphrodites of the congener *C. briggsae* do not exhibit greater fecundity and sperm production when reared at lower temperatures (Fodor et al. 1983). Validation of relationships between genetics, development, temperature, and sperm production warrants rigorous biogeographic, comparative, and experimental analyses.

Although sperm counts are generally consistent between the model and empirical data, laboratory strains do produce somewhat higher numbers of sperm than predicted by the model. This may point to some interesting features of the biology of *C. elegans* or may simply reflect some of the simplifying assumptions of the model. First, because the rate of egg production by hermaphrodites varies over time (Byerly et al. 1976) and the presence of sperm influences oocyte maturation (McCarter et al. 1999), the accommodation of the additional biological realism of temporally varying oogenesis must be weighed against the inclusion of extra parameters. The incorporation of a more complicated, linearly increasing gametogenesis function tends to yield higher equilibrium numbers of sperm relative to the case with constant rates of gamete production (not shown), more in line with observed sperm counts.

Second, the underprediction of sperm numbers might also be due to the existence of the facultative dauer larval stage in nature, in which larval animals follow an alternate developmental trajectory under conditions of low food and high population density to become a stress-resistant, quiescent ''enduring'' larva that can persist for months (Riddle 1988). If the incidence of dauer formation could be taken into account in terms of average larval development time, it would effectively extend the expected duration of the pre-reproductive phase. Consequently, the failure to account for dauer development suggests that larval duration may be underestimated when based solely on the standard developmental pathway, leading to an underestimation of sperm production because of the positive association between t_j and n^*. Such a phenomenon would predict that selfing hermaphrodites of species with obligate dauer stages, as in many parasitic nematodes, will make more sperm than relatives with facultative dauer development.

Third, alternative mortality schedules can influence gamete production, although the exponential survivorship function that I employed is likely to be biologically appropriate in nature (Barker 1992), and mortality is essentially absent over the reproductive life of hermaphrodites (i.e., $l[x] = 1$ from age a to d) under laboratory conditions (Gems and Riddle 2000), both of which eliminate any influence of the death process on sperm count.

Fourth, the model considers reproduction only in hermaphrodites, but *C. elegans* populations are in fact androdioecious; thus, the propensity for the presence of males to influence hermaphrodite sperm production may be modeled by letting $g(n) = (n + m)/e$, where m is the number of male sperm present in the hermaphrodite reproductive tract (neglecting sexual selection on sperm count). This adjustment indicates that the presence of male sperm would tend to cause hermaphrodites to produce fewer self-sperm. If males occur in the low frequencies predicted (Hodgkin and Doniach 1997; Chasnov and Chow 2002; Stewart and Phillips 2002; Cutter and Payseur 2003; Cutter et al. 2003), then their influence on hermaphrodite sperm count will be minimal (e.g., ~1%

outcrossing would result in only about three male sperm per hermaphrodite, on average), although sperm competition between males and hermaphrodites would be expected to act to increase hermaphrodite sperm counts.

Finally, genetic or developmental constraints may limit the ability of nematode populations to evolve optimal levels of sperm production, particularly under laboratory conditions, or favor a strategy of elevated levels of sperm production.

Among the varied taxa that do not conform to the expectation of sperm excess under Bateman's principle (Bateman 1948), the nematode *C. elegans* and its hermaphrodite relatives afford us a tractable system in which to dissect the forces that affect sperm limitation. In particular, the trade-off mechanism of sperm limitation in *C. elegans* allows for quantitative investigation of sperm production. I have shown that extension of a common population model of fitness maximization in terms of the intrinsic rate of growth may be used effectively to describe the evolution of hermaphrodite sperm number. Furthermore, this modeling framework provides testable hypotheses relating life history and gamete production; for example, all else being equal, hermaphrodite nematodes may be expected to produce more sperm in populations or species that experience long larval development periods.

Acknowledgments

I am grateful to the members of the Avilés and Ward laboratories for their support and suggestions. I particularly thank L. Avilés, T. Bukowski, C. LaMunyon, K. Powers, S. Ward, and an anonymous reviewer for constructive comments on earlier drafts of the manuscript and N. Washington for help with microscopy. This work was facilitated by a National Defense Science and Engineering Graduate Fellowship.

Literature Cited

Barker, D. M. 1992. Evolution of sperm shortage in a selfing hermaphrodite. Evolution 46:1951–1955.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.

Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. Am. Nat. 117:838–840.

Birkhead, T. R., and A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press, New York.

Byerly, L., R. C. Cassada, and R. L. Russell. 1976. Life-cycle of nematode *Caenorhabditis elegans*. 1. Wild-type growth and reproduction. Dev. Biol. 51:23–33.

Charlesworth, B. 1994. Evolution in age-structured populations. Cambridge Univ. Press, New York.

Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. Biol. J. Linn. Soc. 22:333–348.

Charnov, E. L. 1982. The theory of sex allocation. Princeton Univ. Press, Princeton, NJ.

Chasnov, J. R., and K. L. Chow. 2002. Why are there males in the hermaphroditic species *Caenorhabditis elegans*? Genetics 160: 983–994.

Cutter, A. D., and B. A. Payseur. 2003. Selection at linked sites in the partial selfer *Caenorhabditis elegans*. Mol. Biol. Evol. 20: 665–673.

Cutter, A. D., L. Avilés, and S. Ward. 2003. The proximate determinants of sex ratio in *C. elegans* populations. Genet. Res. 81: 91–102.

Fodor, A., D. L. Riddle, F. K. Nelson, and J. W. Golden. 1983. Comparison of a new wild-type *Caenorhabditis briggsae* with laboratory strains of *Caenorhabditis briggsae* and *C. elegans*. Nematologica 29:203–217.

Gems, D., and D. L. Riddle. 2000. Defining wild-type life span in *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. 55: B215–B219.

Grewal, P. S. 1991. Influence of bacteria and temperature on the reproduction of *Caenorhabditis elegans* (Nematoda, Rhabditidae) infesting mushrooms (*Agaricus bisporus*). Nematologica 37:72–82.

Hirsh, D., D. Oppenheim, and M. Klass. 1976. Development of reproductive-system of *Caenorhabditis elegans*. Dev. Biol. 49: 200–219.

Hodgkin, J., and T. M. Barnes. 1991. More is not better: brood size and population growth in a self-fertilizing nematode. Proc. R. Soc. Lond. B 246:19–24.

Hodgkin, J., and T. Doniach. 1997. Natural variation and copulatory plug formation in *Caenorhabditis elegans*. Genetics 146: 149–164.

Hodgkin, J., H. R. Horvitz, and S. Brenner. 1979. Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics 91: 67–94.

Kimble, J., and S. Ward. 1988. Germ-line development and fertilization. Pp. 191–213 *in* W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, New York.

Lamunyon, C. W., and S. Ward. 1998. Larger sperm outcompete smaller sperm in the nematode *Caenorhabditis elegans*. Proc. R. Soc. Lond. B 265:1997–2002.

Lasker, H. R., D. A. Brazeau, J. Calderon, M. A. Coffroth, R. Coma, and K. Kim. 1996. In situ rates of fertilization among broadcast spawning gorgonian corals. Biol. Bull. 190:45–55.

Levitan, D. R., and C. Petersen. 1995. Sperm limitation in the sea. Trends Ecol. Evol. 10:228–231.

Lewis, J. A., and J. T. Fleming. 1995. Basic culture methods. Pp. 3–29 *in* H. F. Epstein and D. C. Shakes, ed. *Caenorhabditis elegans*: modern biological analysis of an organism. Academic Press, New York.

L'Hernault, S. W. 1997. Spermatogenesis. Pp. 271–294 *in* D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, ed. *Caenorhabditis elegans* Vol. II. Cold Spring Harbor Laboratory Press, New York.

MacDiarmid, A. B., and M. J. Butler. 1999. Sperm economy and limitation in spiny lobsters. Behav. Ecol. Sociobiol. 46:14–24.

McCarter, J., B. Bartlett, T. Dang, and T. Schedl. 1999. On the control of oocyte meiotic maturation and ovulation in *Caenorhabditis elegans*. Dev. Biol. 205:111–128.

McLetchie, D. N. 1996. Sperm limitation and genetic effects on fecundity in the dioecious liverwort *Sphaerocarpos texanus*. Sexual Plant Reprod. 9:87–92.

Pitnick, S., 1993. Operational sex-ratios and sperm limitation in populations of *Drosophila pachea*. Behav. Ecol. Sociobiol. 33: 383–391.

Pitnick, S., and T. A. Markow. 1994. Male gametic strategies: sperm size, testes size, and the allocation of ejaculate among successive mates by the spermlimited fly *Drosophila pachea* and its relatives. Am. Nat. 143:785–819.

Poinar, G. O., and E. L. Hansen. 1983. Sex and reproductive modifications in nematodes. Helminthol. Abstr. B 52:145–163.

Riddle, D. L. 1988. The dauer larva. Pp. 393–412 *in* W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, New York.

Rose, A. M., and D. L. Baillie. 1979. Effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics 92:409–418.

Stewart, A. D., and P. C. Phillips. 2002. Selection and maintenance of androdioecy in *Caenorhabditis elegans*. Genetics 160: 975–982.

Sulston, J. E., and J. Hodgkin. 1988. Methods. Pp. 587–606 *in* W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, New York.

Venette, R. C., and H. Ferris. 1997. Thermal constraints to population growth of bacterial-feeding nematodes. Soil Biol. Biochem. 29:63–74.

Ward, S., and J. S. Carrel. 1979. Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. Dev. Biol. 73:304–321.

Corresponding Editor: S. Pitnick