Sperm Status Regulates Sexual Attraction in *Caenorhabditis elegans*

Natalia S. Morsci,*,†,1 **Leonard A. Haas,*** **and Maureen M. Barr***

*Department of Genetics, Rutgers University, Piscataway, New Jersey 08854, and †Cell and Molecular Biology Program, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT Mating behavior of animals is regulated by the sensory stimuli provided by the other sex. Sexually receptive females emit mating signals that can be inhibited by male ejaculate. The genetic mechanisms controlling the release of mating signals and encoding behavioral responses remain enigmatic. Here we present evidence of a *Caenorhabditis elegans* hermaphrodite-derived cue that stimulates male mating-response behavior and is dynamically regulated by her reproductive status. Wild-type males preferentially mated with older hermaphrodites. Increased sex appeal of older hermaphrodites was potent enough to stimulate robust response from mating-deficient *pkd-2* and *lov-1* polycystin mutant males. This enhanced response of *pkd-2* males toward older hermaphrodites was independent of short-chain ascaroside pheromones, but was contingent on the absence of active sperm in the hermaphrodites. The improved *pkd-2* male response toward spermless hermaphrodites was blocked by prior insemination or by genetic ablation of the *ceh-18*-dependent sperm-sensing pathway of the hermaphrodite somatic gonad. Our work suggests an interaction between sperm and the soma that has a negative but reversible effect on a hermaphrodite-derived mating cue that regulates male mating response, a phenomenon to date attributed to gonochoristic species only.

THE immense variety of reproductive strategies of metazoans underlie the diversity of their reproductive behaviors. To maximize its lifetime reproductive fitness, females of dioecious species release mating signals to attract males, and female mating receptiveness can be inhibited by male ejaculate (Gillott. 2003; Chasnov *et al.* 2007; Avila *et al.* 2011). Animals continuously adjust their sexual behavior according to internal reproductive needs and stimuli provided by the opposite sex. The repercussions of the evolution of self-fertility on mating cues and behavior output of the progeny-bearing parent have not been intensively investigated. The genetic mechanisms controlling the release of mating signals and regulating behavioral responses of the opposite sex remain enigmatic.

We used the nematode *Caenorhabditis elegans* as a model to investigate the genetic regulation of mating cues by the changing reproductive status of an animal. *C. elegans* is an androdioecious (male/hermaphrodite) species that evolved from gonochoristic (male/female) ancestors (Kiontke *et al.* 2004; Cutter *et al.* 2008). During larval development, *C. elegans* hermaphrodites produce limited amount of sperm that can self-fertilize oocytes. *C. elegans* hermaphrodites can also be cross-fertilized by males, although self-fertile hermaphrodites exhibit physical resistance to male mating (Garcia *et al.* 2001; Kleemann and Basolo. 2007). *C. elegans* males chemotax toward hermaphrodite-secreted cues and are retained by a signal in the hermaphrodite cuticle (Simon and Sternberg. 2002; White *et al.* 2007; Barrios *et al.* 2008). Once in physical proximity of a potential mate, males display response behavior, which is defined as the purposeful contact by male tail copulatory structures (Barr and Garcia. 2006). The hermaphrodite cue that regulates male contact-based mating response is not known. In this report, we present evidence of a hermaphrodite-derived cue that dynamically regulates male mating-response behavior in *C. elegans*.

Sperm has a profound effect on the reproductive physiology of the *C. elegans* hermaphrodite: it maintains gonadal morphological integrity and organization and positively regulates oocyte maturation and ovulation (McCarter *et al.* 1999; Miller *et al.* 2003; Mendenhall *et al.* 2011). The hermaphrodite gonad remains responsive to sperm and capable

of producing cross-progeny for several days after self-sperm depletion (Mendenhall *et al.* 2011). We reasoned that this non-self-reproductive, but functional, state of the adult hermaphrodite gonad might influence her mating signals and affect male mating behavioral responses. To explore this idea, we compared male mating response toward wild-type sperm-containing, wild-type sperm-depleted, and reproductive mutant *C. elegans* hermaphrodites. We found that sperm-depleted hermaphrodites elicited preferential response from wild-type males and robust male response from mating-defective polycystin males. This hermaphrodite-derived cue for male response is negatively regulated by activated sperm and positively regulated by her somatic gonad tissues. The inhibitory effect of sperm-carrying females on male mating response was also observed in the dioecious *Caenorhabditis remanei* species, suggesting conservation of function. We suggest that the reproductive system of the *C. elegans* hermaphrodite regulates mating cue output to suppress or elicit mating and cross-fertilization by males.

Materials and Methods

Stocks

Nematodes were maintained using standard conditions (Brenner 1974). Males were isolated at L4 stage ≥24 hr prior to experiments and kept at 20–22° overnight. Hermaphrodites were isolated at L4 stage 1 or 3 days prior to experiments, labeled 1DA (days of adulthood) and 3DA, respectively. 3DA animals were replated on day 2 to avoid overcrowding from progeny. To achieve cross-insemination, *C. elegans* N2, *fog-2*, and *C. remanei* females were exposed to isogenic young males throughout their aging period. Nematodes were considered fertilized on the basis of the presence of embryos in the uterus.

Sperm content in spermatheca of 1DA and 3DA *C. elegans* N2, *daf-22*, *fog-2*, *vab-1*, *ceh-18* prior to the response assays was inferred on the basis of the presence or absence of embryos in the uterus and confirmed by DAPI staining of age-matched hermaphrodites. Sperm content in spermatheca of 1DA and 3DA *C. elegans* *spe-19*, *spe-8*, and *spe-38* was inferred on the basis of the DAPI staining of age-matched hermaphrodites.

Mating assay

Response efficiency (RE) reflects the percentage of a population of males successfully responding to hermaphrodite contact within 4 min. The reported *n* is the total number of males tested over the course of at least three tests performed on separate days. Response assay plates were prepared by dropping 13 µl of OP50 *Escherichia coli* culture onto a fresh NGM plate the night before assay, making a lawn of ~5 mm diameter. Twenty hermaphrodites were placed onto the mating dot and allowed to acclimate for 10 min prior to the assay. Response was assayed as follows: five males of the one genotype were placed in the center of the mating lawn and observed for a 4-min time period, during which response (1/yes or 0/no) was recorded for each male. Response was scored as positive when a male made flush contact with the ventral side of his tail against the hermaphrodite and initiated scanning along her body. After 4 min, all males were removed from the assay plate and five males of the next genotype were assayed against the same hermaphrodites.

Statistical analysis

All the statistical analyses were performed using GraphPad Prism 5 software. To control for type I error rate, multiple group comparisons were done by Kruskal –Wallis test for nonparametric data with Dunn's multiple comparison post-hoc test.

Results

Aged hermaphrodites have more sex appeal

The first step of *C. elegans* male mating repertoire is response, the act of physical contact between a male tail copulatory structures and hermaphrodite body (Barr and Garcia 2006). Male response is mediated by nine bilateral pairs of tail rays, each composed of two sensory neurons (A and B types) and a structural cell (Sulston *et al.* 1980; Liu and Sternberg 1995). Male-specific polycystin genes *lov-1* and *pkd-2* are expressed in the B-type ray neurons, act non-redundantly in the same genetic pathway, and are required for efficient male response to hermaphrodite contact (Barr and Sternberg. 1999; Barr *et al.* 2001). The low RE of *pkd*-pathway mutant males is attributed to defects in sensory transduction, rather than structure, of the male cilia (Barr and Sternberg 1999; Barr *et al.* 2001).

When tested with young immobilized hermaphrodites, *pkd-2(sy606)* male RE is 20–22% (Barr *et al.* 2001 and Figure 1). Strikingly, we observed that when exposed to aged hermaphrodites of the same genotype, *pkd-2* male RE increases dramatically to 75% (Figure 1). Male RE of other polycystin pathway mutants, *lov-1(sy582)* and *klp-6(my8)*, also significantly increased toward aged hermaphrodites (Figure 1). Because hermaphrodites in these assays were genetically immobilized *unc-31(e169)* mutants, we conclude that the difference in male response was not due to changes in hermaphrodites locomotory behavior, as described by Kleeman and Basolo (2007).

Wild-type male RE was high with either young or aged hermaphrodites, suggesting that, in the parameters of our response assay (see *Materials and Methods*), *pkd-2* males are more sensitive to variation in hermaphrodite-derived mating cues than wild-type males (Figure 1). To determine whether wild-type males can distinguish between young and aged hermaphrodites, we developed a "choice assay," a modification of response assay in which males are exposed to an even mixture of young and aged wild-type (N2) hermaphrodites. Of wild-type males, 66% responded to an aged hermaphrodite during the choice assay, thus showing preference for
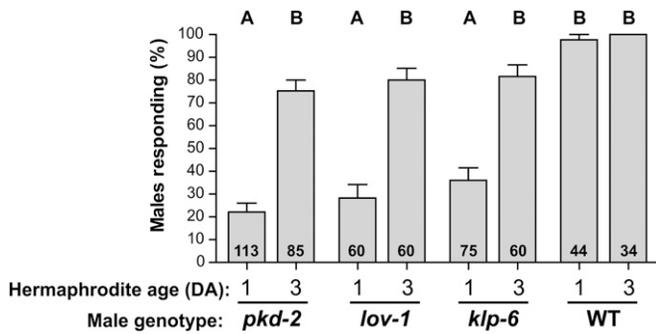
Figure 1 Male response to aged *unc-31(e169)* hermaphrodites. *x*-axis: hermaphrodite age groups (DA, days of adulthood) and male genotypes, *pkd-2(sy606)*, *lov-1(sy582)*, *klp-6(my8)*, *him-5(e1490)*. *y*-axis: mean ± SEM percentage of responding males out of total males tested (number within column). Letters above columns indicate results of the Kruskal–Wallis test with Dunn's Multiple Comparison post-hoc test. Columns marked with the same letter were not statistically different at *P* < 0.01 cutoff.

Table 1 *pkd-2(sy606)* males' response to mutant hermaphrodites

H. Genotype[a]	H. Age[b]	Male RE[c]	n[d]	1DA vs. 3DA[e]
N2	1DA	16 ± 4	95	*P* < 0.001
N2	3DA	65 ± 5	85	*P* < 0.001
daf-22(m130)	1DA	7 ± 3	56	*P* < 0.001
daf-22(m130)	3DA	66 ± 6	56	*P* < 0.001
fog-2(q71)	1DA	63 ± 4*	133	NS
fog-2(q71)	3DA	63 ± 6	72	NS
spe-19(hc41)	1DA	56 ± 5*	100	NS
spe-19(hc41)	3DA	53 ± 6	60	NS
spe-8(hc40)	1DA	58 ± 6*	60	NS
spe-8(hc40)	3DA	60 ± 6	60	NS
spe-38(eb44)	1DA	8 ± 3	95	NS
spe-38(eb44)	3DA[f]	32 ± 5*	95	NS
vab-1(dx31)	1DA	23 ± 6	60	*P* < 0.001
vab-1(dx31)	3DA	78 ± 5	60	*P* < 0.001
ceh-18(mg57)	1DA	23 ± 4	88	NS
ceh-18(mg57)	3DA	28 ± 4*	108	NS
fog-2; *ceh-18*	1DA	18 ± 4**	106	

* *P* < 0.001 vs. same-age N2, Kruskal–Wallis test with Dunn's posthoc, **P < 0.001 vs. same-age *fog-2*, Kruskal–Wallis test with Dunn's posthoc.
[a] Hermaphrodite genotype.
[b] Hermaphrodite age; DA, days of adulthood, number of days since L4 stage.
[c] RE, response efficiency (mean ± SE), percentage of *pkd-2* males responding.
[d] Number of observations.
[e] Analysis of statistical significance between age groups of same genotype.
[f] Some *spe-38* hermaphrodites retained self-sperm at 3DA age.

aged over young hermaphrodites (Fisher's exact *P* < 0.0001, *n* = 125). This observation suggests that when placed in a more complex environment of choice assay, wild-type males distinguish age-dependent differences in hermaphrodite sex appeal. In conclusion, the qualitative change in aged hermaphrodites improves *pkd-2* male mating behavior and may also have relevance for wild-type male behavior.

Hermaphrodite sex appeal is not dependent on short-chain ascarosides

Hermaphrodite short-chain ascarosides, glycosides of the dideoxysugar ascarylose, act as male chemoattractants at a distance (Srinivasan *et al.* 2008). To test whether the increased male response was dependent on ascarosides, we assayed the *pkd-2* male response with *daf-22(m130)* hermaphrodites, which are defective in ascaroside synthesis and whose extracts have no male-attraction activity (Srinivasan *et al.* 2008). *pkd-2* RE to *daf-22* hermaphrodites was comparable to control N2 hermaphrodites in either age group (Table 1). Therefore, short-chain ascarosides are not responsible for the increased *pkd-2* RE toward aged hermaphrodites.

Hermaphrodite sex appeal is inhibited by active spermatozoa

The quantity of self-sperm in a *C. elegans* hermaphrodite is finite. The self-reproductive period spans roughly 3–5 days following last larval molt, depending on the temperature, during which about 300 oocytes are fertilized and deposited (Maupas 1900; Argon and Ward 1980). We observed that the onset of increased *pkd-2* male RE toward aging hermaphrodites temporally coincided with their self-sperm depletion (supporting information, Figure S1), which led us to the hypothesis that self-sperm was a negative regulator of hermaphrodite sex appeal during early adulthood. To test this hypothesis, we used *fog-2(q71)* mutants, which do not produce self-sperm and are essentially female (Schedl and Kimble 1988). Young *fog-2* females elicited high RE from *pkd-2* males, comparable to aged N2 (Table 1). When

spermless hermaphrodites (either aged N2 or young *fog-2*) were inseminated prior to the response assay, *pkd-2* male RE was reduced (Figure 2). We conclude that the presence of active sperm reduces hermaphrodite mating receptiveness, and the absence of sperm due to age-dependent depletion or genetic mutation triggers a physiological change in the hermaphrodite body that stimulates male response. The robust sex appeal of the spermless hermaphrodite is dynamic and can be reversed by sperm transferred from the male.

To test whether the sperm activity or the mere presence of sperm inhibits hermaphrodite sex appeal, we assayed *pkd-2* male response to *spe-19(hc41)* mutant hermaphrodites whose spermatids fail to activate and do not form motile spermatozoa (Geldziler *et al.* 2005). Young *spe-19* hermaphrodites enhanced *pkd-2* male RE to levels similar to *fog-2* females and spermless aged N2 hermaphrodites (Table 1). These results were confirmed with *spe-8(hc40)* mutant hermaphrodites of the same spermatid activation pathway (Table 1) (L'Hernault *et al.* 1988; Geldziler *et al.* 2005). Because young *spe-19* and *spe-8* hermaphrodites bearing inactive spermatids elicited significantly higher response than young N2 hermaphrodites, we conclude that spermatid activation is necessary for inhibition of mating receptiveness in young hermaphrodites.

We reasoned that reduced mating receptiveness of sperm-containing hermaphrodites might be not due to sperm, but rather due to the presence of embryos in the reproductive tract. To determine whether internal fertilization inhibits hermaphrodite sex appeal, we assayed *pkd-2* RE toward *spe-38 (eb44)* hermaphrodites whose spermatozoa fail to fertilize oocytes despite being morphologically indistinguishable from wild type (Chatterjee *et al.* 2005). Despite the lack of embryos
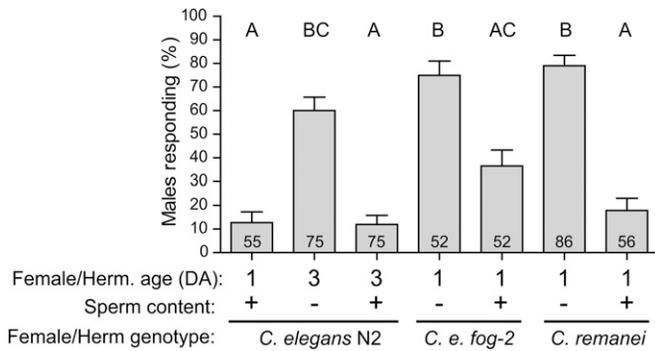
Figure 2 Response of *pkd-2* males to virgin and fertilized partners. *x*-axis: hermaphrodite/female age (DA, days of adulthood), sperm status, and genotype. *y*-axis: mean ± SEM percentage of responding males out of total males tested (number within column). Letters above columns indicate results of the Kruskal–Wallis test with Dunn's Multiple Comparison post-hoc test. Genotypes marked with the same letter were not statistically different at *P* < 0.01 cutoff.

in the uterus, young adult *spe-38* hermaphrodites elicited low RE from *pkd-2* males, comparable to RE toward wild-type N2 hermaphrodites (Table 1). Many aged *spe-38* hermaphrodites retained self-sperm in the spermatheca and elicited low *pkd-2* RE at an age when wild-type N2 hermaphrodites become highly attractive to *pkd-2* males (Table 1). These results suggest that active sperm, but not embryos, in the reproductive system of the hermaphrodite negatively regulate her mating receptiveness to male response.

Hermaphrodite sex appeal is dependent on the CEH-18 sperm-sensing pathway

In the *C. elegans* hermaphrodite, sperm positively regulate oocyte maturation and ovulation by antagonizing the inhibitory signaling of parallel and partially redundant sperm-sensing pathways in the gonad represented by Eph receptor protein–tyrosine kinase VAB-1 and POU-class transcription factor CEH-18 (Greenstein *et al.* 1994; McCarter *et al.* 1999; Miller *et al.* 2003). To test whether sperm regulation of the hermaphrodite mating cue occurs via VAB-1 or CEH-18 sperm sensing pathways, we assayed *pkd-2* RE toward *vab-1(dx31)* and *ceh-18(mg57)* hermaphrodites. Male response to *vab-1* was comparable to N2 hermaphrodites of either age group, but *ceh-18* failed to elicit elevated male RE at a sperm-depleted age, indicating that *ceh-18* is a positive genetic regulator of the hermaphrodite attractiveness after self-sperm depletion. The dependence of the hermaphrodite mating cue on *ceh-18* pathway was confirmed by the complete suppression of *fog-2* attraction phenotype in *fog-2; ceh-18* double mutants (Table 1). Cumulatively, the data indicate that sperm regulate the hermaphrodite mating cue at least in part via the *ceh-18* sperm-sensing pathway.

Sperm regulation of the response cue is evolutionarily conserved

Caenorhabditis species may be gonochoristic (such as male–female *C. remanei*) as well as androdioecious (male–hermaphrodite *C. elegans*). To determine whether sperm-based

inhibition of the response cue occurs in other *Caenorhabditis* species, we tested the ability of *C. remanei* females to elicit response from the sensitized *C. elegans pkd-2* males. Similar to sperm-depleted N2 and feminized *fog-2 C. elegans*, young spermless *C. remanei* females elicited high RE from *pkd-2* males; the RE reduced dramatically when *C. remanei* females were inseminated prior to assay (Figure 2). Hence, the mechanism of sperm-dependent negative regulation of female–hermaphrodite response cue is likely to be conserved in the *Caenorhabditis* genus.

Discussion

Here we show that the self-fertilizing *C. elegans* hermaphrodites play an active regulating role in their sexual attractiveness and male mating-response behavior. In the *C. elegans* literature, the term "mating cue" has been applied to other hermaphrodite-derived chemicals that act as distant chemoattractants or modulators of male locomotion (Simon and Sternberg. 2002; Srinivasan *et al.* 2008). It is important to distinguish cues that regulate social behaviors and development (*e.g.*, short-chain ascarosides) from the cues that regulate physical aspects of male mating. In this report, we present evidence of a hermaphrodite cue that regulates male mating response, is correlated to hermaphrodite reproductive status, and does not depend on known sex pheromones or behavioral changes in locomotion. At this point, we cannot distinguish whether this cue is secreted by the hermaphrodite or represents a cuticle-bound modification.

The polycystin family of proteins play important and evolutionarily conserved roles in mating and fertilization. Polycystins have been shown to be required for mouse sperm competition (Sutton *et al.* 2008), *Drosophila* sperm guidance (Watnick *et al.* 2003; Kottgen *et al.* 2011), self-recognition of ascidian gametes (Harada *et al.* 2008), human sperm motility (Torra *et al.* 2008), flagella-dependent mating in *Chlamydomonas* (Huang *et al.* 2007), and *C. elegans* male mating behavior (Barr and Sternberg. 1999; Barr *et al.* 2001). Our data illustrate that in *C. elegans*, male-specific polycystin pathway is particularly required for male response toward sperm-containing unreceptive hermaphrodites. We speculate that, in *Caenorhabditis* species, polycystins may have evolved to give selective reproductive advantage to males by enabling mating with partners with low mating signals. The observed improvement of response efficiency in polycystin mutant males toward spermless hermaphrodites is an indication that hermaphrodite mating cues are perceived by males using both polycystin-dependent and polycystin-independent pathways. Activation of a polycystin-independent pathway by a cue from sperm-depleted hermaphrodite compensates for the sensory defects of polycystin pathway and increases the efficiency of male response. The identification of this pathway (s) will be a topic of future study.

We utilized *pkd-2* mutant males as a sensitized background to detect and quantify hermaphrodite sex cues.

The experimental need for the sensitized background was based on high response efficiency of wild-type males to all hermaphrodites tested. It is important to be aware, however, of the artificially optimal conditions of male growth and chance of hermaphrodite encounter during the response assay on a small two-dimensional bacterial lawn (see *Materials and Methods*). In the wild, the task of male mating is more challenging. Under native conditions, *C. elegans* male encounters a complex three-dimensional environment, suboptimal nutrition, exposure to infectious or toxic pathogens, and other obstacles. Hence, hermaphrodite-derived cues would be a significant variable in determining wild-type male response efficiency and, ultimately, reproductive success.

We observed that activated sperm inhibits mating cue(s) of *C. elegans* hermaphrodites. This negative effect is reversible and does not depend on fertilization competence of sperm, but depends on a functional sperm-sensing pathway of the ceh-18 transcription factor. In *Drosophila*, female mating receptiveness is inhibited by a sex peptide transferred in the male seminal fluid. Male sex peptide signals by binding to the sex peptide receptor on sensory neurons that innervate female uterus and control female reproductive behaviors (Yapici *et al.* 2008). In *C. elegans* hermaphrodites, spermatids promote oocyte ovulation and uterine contractions via secreted major sperm protein (MSP) signaling (McCarter *et al.* 1999; Miller *et al.* 2003). Importantly, MSP signaling does not depend on spermatid activation or even proper spermatocyte formation (McCarter *et al.* 1999), which makes MSP an unlikely candidate for the spermatid activation-dependent inhibitory cue of hermaphrodite mating attractiveness. *C. elegans* spermatid activation involves dramatic changes in cellular morphology and includes fusion of the membranous organelles to the plasma membrane, extending a pseudopod, and generating plasma membrane flow (Ward *et al.* 1981; Roberts and Ward. 1982; Arduengo *et al.* 1998). Determination of the molecular mechanisms by which activated sperm inhibits *C. elegans* hermaphrodite mating cue(s) awaits further studies.

The goal of mating behavior is to maximize the lifetime reproductive success of an individual. What may be the advantage of producing positive mating cues by a self-fertile hermaphrodite? The ancestral *Caenorhabditis* species was gonochoristic, like most of the known *Caenorhabditis* species that rely on sex pheromones to attract opposite-sex partners (Chasnov *et al.* 2007). *C. elegans* evolved the ability to make self-sperm, which could relax the selective pressures on genes for mating behaviors. For example, Garcia *et al.* (2007) demonstrated that *C. remanei* females, but not *C. elegans* hermaphrodites, are sensitive to the soporific-inducing factor of males. However, sperm remains the limiting factor of lifetime reproductive fitness of the *C. elegans* hermaphrodite, as illustrated by the fact that a mated hermaphrodite can produce up to 1400 progeny as compared to ~300 of self-progeny (Kimble and Ward. 1988). We propose that the *C. elegans* hermaphrodite retained male attraction ability of the ancestral gon-

ochoristic *Caenorhabditis* and that genes regulating *C. elegans* mating cues may be under positive selection due to the longer lifetime reproductive fitness of a fertilized hermaphrodite over an unfertilized one. In addition, the ability of sperm-depleted hermaphrodites (effectively females) to elicit preferential male mating response in a mixed population may give them selective reproductive advantage over younger, sperm-containing hermaphrodites.

Why don't *C. elegans* hermaphrodites release the mating cue in early adulthood? Male sperm outcompete hermaphrodite self-sperm in fertilization of oocytes (Ward and Carrel. 1979). This fertilization superiority of male sperm makes it disadvantageous for the hermaphrodite to mate while in a self-fertile stage. The inhibitory effect of the self-sperm on hermaphrodite attractiveness may act to minimize her likelihood of cross-fertilization by males. For the male, however, it is advantageous to mate with a hermaphrodite as soon as she is sexually mature. Consequently, this may create an overt sexual conflict over the timing of mating, which may drive evolutionary adaptations in *C. elegans* to manipulate hermaphrodite mating cues in order to increase self-reproduction. In summary, our results illustrate how males and hermaphrodites adopt different strategies to maximize their reproductive success under the condition of conflicting sexual interests.

Acknowledgments

Literature Cited

Arduengo, P. M., O. K. Appleberry, P. Chuang, and S. W. L'Hernault, 1998 The presenilin protein family member SPE-4 localizes to an ER/Golgi derived organelle and is required for proper cytoplasmic partitioning during *Caenorhabditis elegans* spermatogenesis. J. Cell Sci. 111(24): 3645–3654.

Argon, Y., and S. Ward, 1980 *Caenorhabditis elegans* fertilization-defective mutants with abnormal sperm. Genetics 96: 413–433.

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner, 2011 Insect seminal fluid proteins: identification and function. Annu. Rev. Entomol. 56: 21–40.

Barr, M. M., and L. R. Garcia, 2006 Male mating behavior. *WormBook,* ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.78.1, http://www.wormbook.org

Barr, M. M., and P. W. Sternberg, 1999 A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. Nature 401: 386–389.

Barr, M. M., J. DeModena, D. Braun, C. Q. Nguyen, D. H. Hall *et al.*, 2001 The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs lov-1 and pkd-2 act in the same pathway. Curr. Biol. 11: 1341–1346.

Barrios, A., S. Nurrish, and S. W. Emmons, 2008 Sensory regulation of *C. elegans* male mate-searching behavior. Curr. Biol. 18: 1865–1871.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics 77: 71–94.

Chasnov, J. R., W. K. So, C. M. Chan, and K. L. Chow, 2007 The species, sex, and stage specificity of a Caenorhabditis sex pheromone. Proc. Natl. Acad. Sci. USA 104: 6730–6735.

Chatterjee, I., A. Richmond, E. Putiri, D. C. Shakes, and A. Singson, 2005 The *Caenorhabditis elegans* spe-38 gene encodes a novel four-pass integral membrane protein required for sperm function at fertilization. Development 132: 2795–2808.

Cutter, A. D., J. D. Wasmuth, and N. L. Washington, 2008 Patterns of molecular evolution in Caenorhabditis preclude ancient origins of selfing. Genetics 178: 2093–2104.

Garcia, L. R., P. Mehta, and P. W. Sternberg, 2001 Regulation of distinct muscle behaviors controls the *C. elegans* male's copulatory spicules during mating. Cell 107: 777–788.

Garcia, L. R., B. LeBoeuf, and P. Koo, 2007 Diversity in mating behavior of hermaphroditic and male-female Caenorhabditis nematodes. Genetics 175: 1761–1771.

Geldziler, B., I. Chatterjee, and A. Singson, 2005 The genetic and molecular analysis of spe-19, a gene required for sperm activation in *Caenorhabditis elegans*. Dev. Biol. 283: 424–436.

Gillott, C., 2003 Male accessory gland secretions: modulators of female reproductive physiology and behavior. Annu. Rev. Entomol. 48: 163–184.

Greenstein, D., S. Hird, R. H. Plasterk, Y. Andachi, Y. Kohara *et al.*, 1994 Targeted mutations in the *Caenorhabditis elegans* POU homeo box gene ceh-18 cause defects in oocyte cell cycle arrest, gonad migration, and epidermal differentiation. Genes Dev. 8: 1935–1948.

Harada, Y., Y. Takagaki, M. Sunagawa, T. Saito, L. Yamada *et al.*, 2008 Mechanism of self-sterility in a hermaphroditic chordate. Science 320: 548–550.

Huang, K., D. R. Diener, A. Mitchell, G. J. Pazour, G. B. Witman *et al.*, 2007 Function and dynamics of PKD2 in *Chlamydomonas reinhardtii* flagella. J. Cell Biol. 179: 501–514.

Kimble, J., and S. Ward, 1988 Germ-line development and fertilization, pp. 191–213 in: *The Nematode Caenorhabditis elegans*, edited by W. B. Wood. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kiontke, K., N. P. Gavin, Y. Raynes, C. Roehrig, F. Piano *et al.*, 2004 Caenorhabditis phylogeny predicts convergence of hermaphroditism and extensive intron loss. Proc. Natl. Acad. Sci. USA 101: 9003–9008.

Kleemann, G. A., and A. L. Basolo, 2007 Facultative decrease in mating resistance in hermaphroditic *Caenorhabditis elegans* with self-sperm depletion. Anim. Behav. 74: 1339–1347.

Kottgen, M., A. Hofherr, W. Li, K. Chu, S. Cook *et al.*, 2011 Drosophila sperm swim backwards in the female reproductive tract and are activated via TRPP2 ion channels. PLoS ONE 6: e20031.

L'Hernault, S. W., D. C. Shakes, and S. Ward, 1988 Developmental genetics of chromosome I spermatogenesis-defective mutants in the nematode *Caenorhabditis elegans*. Genetics 120: 435–452.

Liu, K. S., and P. W. Sternberg, 1995 Sensory regulation of male mating behavior in *Caenorhabditis elegans*. Neuron 14: 79–89.

Maupas, E., 1900 Modes et formes de reproduction des nématodes. Arch. Zool. Exp. Gen. 8: 463–624.

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.

Mendenhall, A. R., D. Wu, S. K. Park, J. R. Cypser, P. M. Tedesco *et al*, 2011 Genetic dissection of late-life fertility in *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. 66: 842–854.

Miller, M. A., P. J. Ruest, M. Kosinski, S. K. Hanks, and D. Greenstein, 2003 An eph receptor sperm-sensing control mechanism for oocyte meiotic maturation in *Caenorhabditis elegans*. Genes Dev. 17: 187–200.

Roberts, T. M., and S. Ward, 1982 Membrane flow during nematode spermiogenesis. J. Cell Biol. 92: 113–120.

Schedl, T., and J. Kimble, 1988 *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics 119: 43–61.

Simon, J. M., and P. W. Sternberg, 2002 Evidence of a mate-finding cue in the hermaphrodite nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 99: 1598–1603.

Srinivasan, J., F. Kaplan, R. Ajredini, C. Zachariah, H. T. Alborn *et al.*, 2008 A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature 454: 1115–1118.

Sulston, J. E., D. G. Albertson, and J. N. Thomson, 1980 The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. 78: 542–576.

Sutton, K. A., M. K. Jungnickel, and H. M. Florman, 2008 A polycystin-1 controls postcopulatory reproductive selection in mice. Proc. Natl. Acad. Sci. USA 105: 8661–8666.

Torra, R., J. Sarquella, J. Calabia, J. Marti, E. Ars *et al.*, 2008 Prevalence of cysts in seminal tract and abnormal semen parameters in patients with autosomal dominant polycystic kidney disease. Clin. J. Am. Soc. Nephrol. 3: 790–793.

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

Ward, S., Y. Argon, and G. A. Nelson, 1981 Sperm morphogenesis in wild-type and fertilization-defective mutants of *Caenorhabditis elegans*. J. Cell Biol. 91: 26–44.

Watnick, T. J., Y. Jin, E. Matunis, M. J. Kernan, and C. Montell, 2003 A flagellar polycystin-2 homolog required for male fertility in Drosophila. Curr. Biol. 13: 2179–2184.

White, J. Q., T. J. Nicholas, J. Gritton, L. Truong, E. R. Davidson *et al.*, 2007 The sensory circuitry for sexual attraction in C. elegans males. Curr. Biol. 17: 1847–1857.

Yapici, N., Y. J. Kim, C. Ribeiro, and B. J. Dickson, 2008 A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. Nature 451: 33–37.

Communicating editor: R. Anholt

GENETICS

Sperm Status Regulates Sexual Attraction in *Caenorhabditis elegans*

Natalia S. Morsci, Leonard A. Haas, and Maureen M. Barr

Figure S1 Timeline of *pkd-2* male response to aged unc-31 hermaphrodites