

Auxin-Mediated Sterility Induction System for Longevity and Mating Studies in *Caenorhabditis elegans*

Katja R. Kasimatis,¹ Megan J. Moerdyk-Schauwecker,¹ and Patrick C. Phillips²

Institute of Ecology and Evolution, 5289 University of Oregon, University of Oregon, Eugene, OR 97403

ORCID ID: 0000-0001-7271-342X (P.C.P.)

ABSTRACT The ability to control both the means and timing of sexual reproduction provides a powerful tool to understand not only fertilization but also life history trade-offs resulting from sexual reproduction. However, precisely controlling fertilization has proved a major challenge across model systems. An ideal sterility induction system should be external, non-toxic, and reversible. Using the auxin-inducible degradation system targeting the *spe-44* gene within the nematode *Caenorhabditis elegans*, we designed a means of externally inducing spermatogenesis arrest. We show that exposure to auxin during larval development induces both hermaphrodite self-sterility and male sterility. Moreover, male sterility can be reversed upon cessation of auxin exposure. The sterility induction system developed here has multiple applications in the fields of spermatogenesis and mating systems evolution. Importantly, this system is also a highly applicable tool for aging studies. In particular, we show that auxin-induced self-sterility is comparable to the commonly used chemically-induced FUDR sterility, while offering multiple benefits, including being less labor intensive, being non-toxic, and avoiding compound interactions with other experimental treatments.

KEYWORDS

inducible sterility
auxin inducible
degradation
longevity
spermatogenesis
C. elegans
genetics of sex

Sexual reproduction is among the most fundamental of biological processes, and the ability to control the means and mode of sexual reproduction provides a powerful tool for studying a wide variety of important questions. First and foremost, interactions within and between the sexes are mediated by fertilization. Thus, precise control of fertilization allows for the nature of reproductive interactions to be directly manipulated, addressing questions regarding potentially antagonistic interactions between members of the same sex (e.g., sperm competition) (Karr and Pitnick 1999; Edward *et al.* 2015), between members of the opposite sex (e.g., sexual conflict) (Arnqvist and Rowe 2005), and

between parents and offspring (e.g., parent-offspring conflict) (Trivers 1972). In addition, reproduction itself is a critical field of study. For example, investment in offspring is often thought to represent a trade-off with other aspects of an individual's life history, including overall lifespan (Stearns 1989; Schluter *et al.* 1991). Directly manipulating the dynamics of reproduction allows these trade-offs to be specifically assessed. More prosaically, some experiments, such as longevity studies, require the separation of parents and offspring and in some systems this separation is best accomplished by simply not allowing the adults to reproduce in the first place (Park *et al.* 2017).

Currently there are few techniques available to control reproduction short of direct physical manipulation and/or separation of the sexes. In these cases, mostly in model organisms, chemical interventions or genetic mutations can be used to induce sterility in one of the sexes. For example, sterility induction mechanisms—both genetic and chemical—are common in the agricultural industry as a method of preventing cross-pollination (Kempe and Gils 2011). In *Drosophila*, several genetic mutations can be used to generate either female (Schüpbach and Wieschaus 1991; Volpe *et al.* 2001) or male sterility (Castrillon *et al.* 1993). However, since these mutations tend to be recessive, they must be maintained over a balancer chromosome or in a heterozygous population, making them manually intensive to use. Vertebrate models

Copyright © 2018 Kasimatis *et al.*

doi: <https://doi.org/10.1534/g3.118.200278>

Manuscript received March 28, 2018; accepted for publication June 6, 2018; published Early Online June 7, 2018.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Supplemental material available at Figshare: <https://doi.org/10.6084/m9.figshare.6446228>.

¹These authors contributed equally to this work.

²Corresponding Author: Institute of Ecology of Evolution, 5289 University of Oregon, Onyx Bridge 272, Eugene, OR 97403-5289, E-mail: pphil@uoregon.edu

offer many more challenges to reproductive control, and therefore few sterility induction approaches exist in these systems (see Hsu *et al.* 2009). *Caenorhabditis elegans* is a major model system for genetics, development, neurobiology, and aging. Within *C. elegans*, a limited number of sterility mutants are available (L'Hernault 2006; Nishimura and L'Hernault 2010; Ellis and Stanfield 2014) and can be maintained by mating hermaphrodites to males. In some cases, temperature sensitive sterility mutants (Hirsh and Vanderslice 1976; Ward and Miwa 1978) exist. While these mutants can be an effective tool, by necessity they require a temperature shift, which can affect lifespan (Park *et al.* 2017). Lifespan can also be affected due to the pleiotropic effects of reproductive genes (Murakami and Johnson 1996). An alternative scheme is to prevent progeny production in hermaphrodites using chemical treatments (Mitchell *et al.* 1979), though these techniques are manually intensive and not conducive to high-throughput assays. Further, chemical intervention can potentially generate unaccounted for fitness effects, which not only confound the biological interpretation of results but also make reproducibility a challenge.

An ideal sterility system would be inducible, driven by an external treatment, and, when possible, reversible. To the best of our knowledge such an approach does not exist, even within model organisms. To address this need, we used the non-toxic, non-native auxin inducible degradation (AID) system (Nishimura *et al.* 2009) coupled with knowledge of a critical spermatogenesis gene to create an external sterility induction system in *C. elegans*. We show that this system induces hermaphrodite self-sterility and complete, but reversible sterility of males. This method has broad applications in nematode biology, including studies of aging, gametogenesis, and mating systems evolution.

Constructing an inducible spermatogenesis arrest

The AID system (Nishimura *et al.* 2009; Zhang *et al.* 2015) was chosen as the optimal method for an external sterility induction system in *C. elegans*, as auxin is non-native, non-toxic, and cost-effective. The auxin hormone regulates gene expression in *Arabidopsis thaliana* by activating the F-Box transport inhibitor response 1 (TIR1) protein – the substrate recognition component of a Skp1-Cullin-F-box E3 ubiquitin ligase complex which ubiquitinates degron-tagged proteins for degradation by the proteasome (Tan *et al.* 2007; Nishimura *et al.* 2009). This system has been co-opted as an inducible genetic mechanism in a variety of organisms by degron-tagging a protein of interest and choosing a promoter to drive TIR1 expression in the necessary cell type (Kanke *et al.* 2011; Zhang *et al.* 2015; Trost *et al.* 2016; Natsume *et al.* 2016). We targeted a necessary spermatogenesis gene *spe-44*, causing a spermatogenesis arrest and therefore sterility. Specifically, *spe-44* is one of eleven sperm-specific transcription factors (Reinke 2003) and is predicted to have hundreds of downstream targets, including the critical Major Sperm Protein (Kulkarni *et al.* 2012). Constitutive TIR1 expression was driven using the germline promoter of *pie-1*, which is one of few genes known to have strong sperm expression in hermaphrodites and males (Merritt *et al.* 2008). These three components—auxin, *P_{pie-1}::TIR1*, *spe-44::degron*—generate a fully controllable sterility induction system in *C. elegans*.

MATERIALS AND METHODS

Molecular biology

Guide sequences were chosen using the tools CRISPRdirect (Naito *et al.* 2015), MIT CRISPR Design (<http://crispr.mit.edu>) and Sequence Scan for CRISPR (Xu *et al.* 2015). For the TIR1 insertion, a guide targeting the sequence GAAATCGCCGACTTGCGAGGAGG near the ttT14348 MosSCI site was inserted into pDD162 (Dickinson *et al.* 2013) using the

Q5 site-directed mutagenesis kit (NEB) to create pMS18. This insertion region was previously shown to be permissive for germline expression (Frøkjær-Jensen *et al.* 2012). The plasmid pMS30 was created by Gibson assembly using the NEBuilder HiFi Kit (NEB) and included: homology arms amplified from N2 genomic DNA, the *pie-1* promoter amplified from pCM1.127 (Addgene #21384) (Merritt *et al.* 2008), the *C. elegans* optimized *AtTIR1::mRuby* fusion and *unc-54* terminator amplified from pLZ31 (Addgene #71720) (Zhang *et al.* 2015), and the self-excising drug selection cassette (SEC) amplified from pDD282 (Addgene #66823) (Dickinson *et al.* 2013). The plasmid backbone was also derived from pDD282. An 11 bp segment of the genomic DNA sequence was omitted from the homology arms to prevent re-cutting. All plasmid assembly junctions were confirmed by Sanger sequencing. Sequencing showed that pMS30 contained a single nucleotide substitution in one of the LoxP sites of the SEC. However, this substitution did not notably impact SEC removal.

The degron::3X-FLAG tag utilized asymmetric homology arms (Richardson *et al.* 2016) for *spe-44* insertion and contained appropriate silent sites to prevent re-cutting. The insert was synthesized as a GeneArt String (ThermoFisher) and amplified by PCR prior to injecting.

Strain generation by CRISPR/Cas9

The *P_{pie-1}::TIR1::mRuby* construct was injecting into the gonad of young adult hermaphrodites (standard laboratory strain N2) using a mixture of 50 ng/μl pMS18, 10 ng/μl pMS30 and 2.5 ng/μl pCFJ421 (Addgene #34876) (Frøkjær-Jensen *et al.* 2012). Screening and removal of the SEC was done following Dickinson *et al.* (2013). Presence of the insertion and removal of the SEC was confirmed by PCR and Sanger sequencing.

To degron tag *spe-44*, a cr:tracrRNA (Synthego) targeting the sequence ATTGAATATGACTAGGTCTGG near the C-terminus of *spe-44* was annealed and pre-incubated with Cas9 (PNA Bio) in accordance with manufacturer protocol. A mix of 1.7 μM cr:tracrRNA, 1.65 μg/μl Cas9 (PNA Bio), and 80 ng/μl of the PCR repair template, was then injected into the gonad of young adult N2 hermaphrodites containing the *P_{pie-1}::TIR1::mRuby* construct. Included in the injection mix was an additional cr:tracrRNA and oligonucleotide repair template, allowing for screening through *dpy-10* co-conversion (Paix *et al.* 2015). Progeny from broods containing individuals with a Dumpy or Roller phenotype were then screened for the *spe-44::degron* insertion by PCR and confirmed by Sanger sequencing.

Confirmed double mutants were backcrossed 5 times to N2 to create the final strain PX627 (*fxIs1[P_{pie-1}::TIR1::mRuby*, I:2851009]; *spe-44(fx110[*spe-44::degron*])*). This strain was crossed to strain CB4088, to create the male-rich strain PX629 (*fxIs1[P_{pie-1}::TIR1::mRuby*, I:2851009]; *spe-44(fx110[*spe-44::degron*])* IV; *him-5* (e1490) V).

Worm culture and strains

The *C. elegans* strains PX627, PX629, N2, and JK574 (*fog-2(q71)* V) were maintained on NGM-agar plates seeded with OP50 *Escherichia coli* at 20° (Brenner 1974). The *fog-2* mutation blocks self-sperm production in hermaphrodites, making them functionally female. Synchronized cultures of larval stage 1 (L1) animals were obtained through hypochloride treatment of gravid adults (Kenyon 1988). To induce sterility, worms were transferred to NGM-agar plates containing 1 mM indole-3-acetic acid (Auxin, Alfa Aesar) following Zhang *et al.* (2015). Zhang *et al.* (2015) showed this auxin concentration to be non-toxic to adults with no larval development defects or fecundity effects. Auxin plates were stored in the dark at 4° to prevent compound degradation.

Auxin exposure assays were carried out on small plates (35 mm) seeded with 100 μ L *E. coli* and a sample size of 130 hermaphrodites per developmental stage and 100 males per stage. Developmental stages were scored using the known growth rate of animals at 20° (Byerly *et al.* 1976). Animals were considered fertile if at least one viable progeny was produced. Adult male developmental exposure assays were done by plating synchronized L1 PX629 worms on NGM-agar plates until day 1 of adulthood. Males were then transferred to small auxin plates seeded with 10 μ L *E. coli* along with two virgin females (strain JK547). Males were transferred to new virgin females twice a day until no fertilized eggs were seen on plates. Sterility induction was analyzed using a general linear model (GLM) with a binomial distribution in the R statistical language (R Core Team 2015). Male sterility recovery experiments were done by plating synchronized L1 animals on auxin plates and leaving worms on auxin until day 1 or day 2 of adulthood. Males were then transferred to small NGM plates seeded with 10 μ L *E. coli* and given three virgin females (strain JK574) with which to mate. Plates were monitored until fertilized eggs appeared. Experiments within a given replicate set were conducted contemporaneously.

The baseline fertility for PX627 hermaphrodites was determined by counting the total number of progeny produced and compared to wild-type hermaphrodites (strain N2). Twenty hermaphrodites of each strain were maintained on small NGM-agar plates seeded with 10 μ L *E. coli* until they used all their self-sperm. Self-progeny data were analyzed using a *t*-test in R. For the hermaphrodite self-sterility mating experiments, synchronized L1 PX627 worms were plated onto auxin plates and removed three hours into adulthood. Virgin females (strain JK574) were used as a control. Individual pseudo-females were mated with two males (strain JK574) overnight on small NGM-agar plates seeded with 10 μ L *E. coli*, after which males were removed. All the progeny laid over the subsequent 24 hr were counted. Two independent biological replicates were done with 23 to 35 pseudo-females in each treatment. Mating data were analyzed using a GLM framework with random effects and a Poisson distribution using the *lme4* v.1.1.3 package (Bates *et al.* 2015) in R.

Lifespan assays

Lifespan data were collected using automated lifespan machines following Stroustrup *et al.* (2013). Briefly, worms were synchronized by letting day 2 adults (strains PX627 and N2) lay eggs over a two hour time period. Auxin self-sterility was achieved by allowing PX627 hermaphrodites to lay directly on auxin plates or by transferring larval stage 4 (L4) progeny to auxin plates. Both self-sterility treatments were transferred to NGM-agar plates on day 1 of adulthood. As a control, egg lays for both PX627 and N2 hermaphrodites were done on NGM-agar plates. At day 1 of adulthood, these animals were transferred to small plates containing 51 μ M 5-fluoro-2'-deoxyuridine (FUdR, VCI America) to inhibit reproduction (Mitchell *et al.* 1979). Control worms were transferred to fresh FUdR plates 24 hr later.

On day 5 of adulthood, all worms were transferred onto medium scanner plates (60 mm) with sealable lids to minimize dehydration. NGM-agar scanner plates contained 40 mM potassium phosphate buffer (pH 6.0), 1mM magnesium sulfate, and 5 mg/mL cholesterol, along with 100 mg/mL nystatin to prevent fungal growth while on the automated lifespan system. Control plates also included 51 μ M FUdR. All scanner plates were seeded with 200 μ L *E. coli*. A total of 35 to 60 adult hermaphrodites were transferred to each plate with four technical replicates of each treatment. The 16 plates were randomly arranged on a modified Epson v700 scanner in a temperature controlled 20° room and held in place by rubber mat. Plates were imaged

approximately every hour for twenty days across two independent biological replicates.

Images were analyzed using the Worm Browser software developed with the automated lifespan system (Stroustrup *et al.* 2013). This process includes specifying the location of individual plates on the scanner, detecting individual worms, and analyzing worm movement. The resulting data are time of death calls for each individual worm based on the cessation of movement. All plates were hand annotated to ensure that non-worm objects were excluded. Additionally, the time of death calls for the first and last 10% of worms on each plate were checked as these time points are more error prone. The final lifespans were calculated using the egg lay as day zero.

To analyze the influence of our sterilization approach on longevity, we used a mix-model survival analysis as outlined in Lucanic *et al.* (2017). Longevity effects were evaluated using both a mixed-model Cox Proportional Hazard (CPH) model (Therneau *et al.* 2012) using the *coxme* v.2.2-5 package (Therneau 2017), as well as via GLM using the *lme4* package in R. In each case, the *coxme* and GLM approaches yield equivalent results and so only the *coxme* results are presented as they represent the more comprehensive analytical framework for these data. Using the automated lifespan machine, a small subset of individuals initially placed on a plate are missing and presumed lost over the course of an assay. Such individuals would normally be classified as “censored” in normal survivorship analysis. However, because mortality is determined retrospectively when an individual ceased to move, the moment of loss of such individuals cannot be determined and so they must simply be classified as missing rather than censored at a given time point. For these analyses, the environmental treatment within which each individual was raised (FUdR, auxin) and the genotype of the individual (wild-type or *P_{pie-1::TIR1::mRuby}; spe-44::degron*) were treated as fixed effects, while replicate and plate (nested within replicate) were treated as random effects. Specific *a priori* hypotheses about effects of FUdR and genetic background were tested via contrast coefficients using the *mcp* procedure of the multcomp procedure in R (Hothorn *et al.* 2008).

Data availability

The oligonucleotides and synthetic constructs used in this study, as well as the sterility induction, fertility, and longevity data and its associated R script, are available via the Genetics Figshare.com archive (<https://doi.org/10.6084/m9.figshare.6446228>). Worm strains N2, JK574, PX627, and PX629 are available from the *Caenorhabditis* Genetics Center. Supplemental material available at Figshare: <https://doi.org/10.6084/m9.figshare.6446228>.

RESULTS

Self-sterility induction in hermaphrodites

Caenorhabditis elegans hermaphrodites are protandrous, such that they produce several hundred sperm cells during their final larval stage and then switch to oocyte production for the remainder of their lifespan (Hirsh *et al.* 1976). Tagging the *spe-44* gene resulted in a slight reduction in progeny production (~7%) relative to wild-type hermaphrodites, likely due to problems during spermatogenesis resulting from the degron tag. This trend was most notable on day 2 of adulthood with wild-type hermaphrodites laying significantly more progeny ($t = 3.55$, d.f. = 37, $P < 0.01$; Figure 1). However, while overall life time reproductive success was marginally different between wild-type and *spe-44::degron* hermaphrodites, it was not significantly so (mean \pm SD: N2 = 311.7 \pm 32, PX627 = 289.3 \pm 39, $t = 1.99$, d.f. = 37, $P = 0.054$).

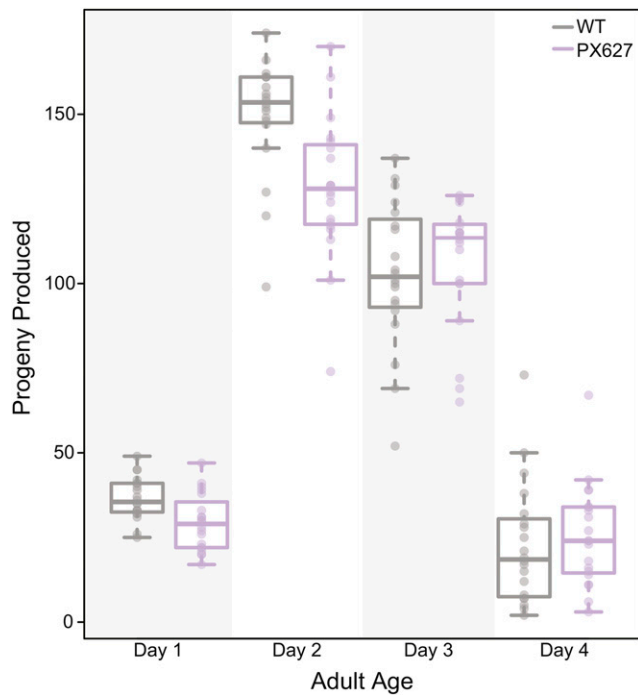


Figure 1 Baseline fecundity for *spe-44::degron* hermaphrodites. Progeny production per day is shown for wild-type hermaphrodite (gray) and PX627 hermaphrodites (purple). While overall reproductive success is not significantly different ($t = 1.99$, d.f. = 37, $P = 0.054$), wild-type hermaphrodites laid significantly more progeny on day 2 of adulthood ($t = 3.55$, d.f. = 37, $P < 0.01$) than PX627 hermaphrodites.

We examined the necessary and sufficient windows of auxin exposure during hermaphrodite development to induce self-sterility. To prevent sperm production, hermaphrodites must be exposed to auxin during their larval development (Figure 2A). When systematically analyzing exposure starting at the L4 stage—the developmental stage during which sperm are produced—through the first 30, 60, or 90 min of adulthood, all were hermaphrodites self-sterile ($n = 50$ per exposure time). In fact, the L4 window alone was both necessary and sufficient to drive self-sterility ($n = 50$). Self-sterile hermaphrodites continued to lay unfertilized oocytes throughout their adult life, as is characteristic of certain classes of spermatogenesis mutants (L’Hernault 2006). Adult exposure to auxin had no effect on progeny production (Figure 2A).

Despite being self-sterile, hermaphrodite oogenesis was unaffected. In particular, when mated to a male, auxin-treated hermaphrodites had comparable progeny counts to hermaphrodites made functionally female through the *fog-2* mutation (Figure 3). Interestingly, these mated self-sterile hermaphrodites were highly consistent in the number of progeny they produced. However, control *fog-2* females laid significantly more progeny than self-sterile hermaphrodites ($z = -2.78$, $P < 0.01$), potentially due to adaptation to obligate outcrossing within the laboratory strain (see e.g., Teotonio *et al.* 2012; Palopoli *et al.* 2015), although it is equally possible that there are subtle partial spermiogenesis effects at play in the knock-down lines.

Inducible sterility of males is reversible within a single generation

We tested the sterility induction of males using a male-enriched *C. elegans* strain. Like hermaphrodites, males begin spermatogenesis

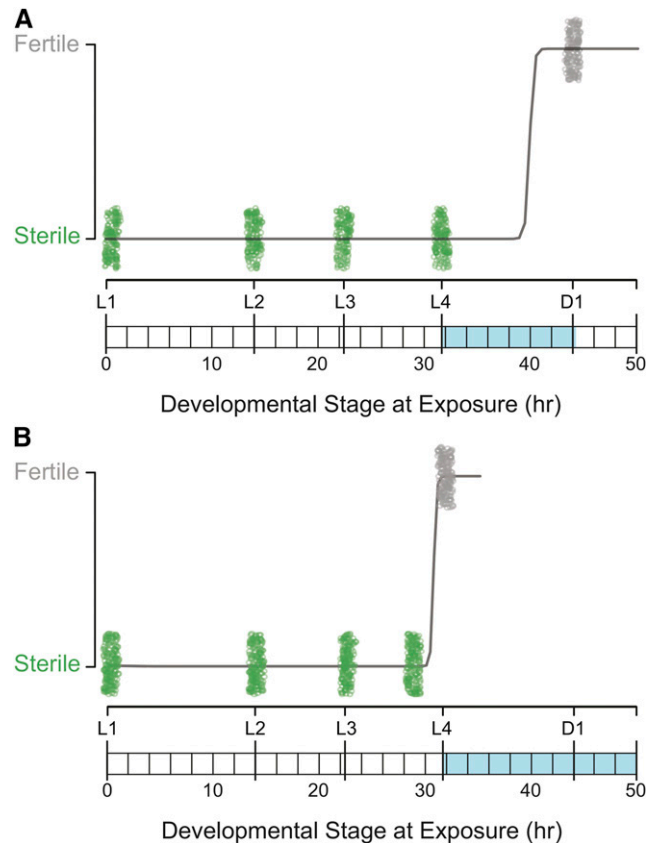


Figure 2 Auxin exposure induces hermaphrodite self-sterility and male sterility. A) Hermaphrodites were exposed to auxin starting at each of the four larval stages (L1 – L4) and first day of adulthood (D1). Spermatogenesis (highlighted in blue) occurs during L4 and continues approximately 30 min into adulthood (Hirsh *et al.* 1976). Each point represents an individual screened ($n = 130$ per stage). When exposed to auxin during larval development, all hermaphrodites were self-sterile. However, adults exposed to auxin were fully fertile. The logistic regression is shown in black (residual deviance < 0.0001). B) Males were exposed to auxin starting at each of the four larval stages (L1 – L4). Spermatogenesis (highlighted in blue) begins during L4 and continues throughout adulthood (L’Hernault 2006). Each point represents an individual screened ($n = 100$ per stage). Males exposed to auxin prior to L4 were sterile, however, males exposed to auxin at L4 produced a low number of progeny. The logistic regression is shown in black (residual deviance < 0.0001).

during L4, however they continue producing sperm throughout adulthood, whereas hermaphrodites do not (L’Hernault 2006). We examined the window of auxin exposure during larval male development sufficient to induce sterility. Interestingly, L4 exposure alone was not sufficient to induce complete sterility, as these males still produced a low number of progeny. Rather males had to be exposed to auxin at least 2 hr prior to the L3/L4 molt (Figure 2B). To measure the sterility induction onset at adulthood, males were raised on standard NGM plates and exposed to auxin starting at day 1 of adulthood. Within 24 hr of auxin exposure, no progeny were observed from male-virgin female matings, indicating that males were fully sterile ($n = 44$).

To determine if sterility in males could be reversed following consistent exposure to auxin during larval development, males were transferred from auxin to standard NGM plates at day 1 and day 2 of adulthood. Day 1 adult males began to recover their fertility within

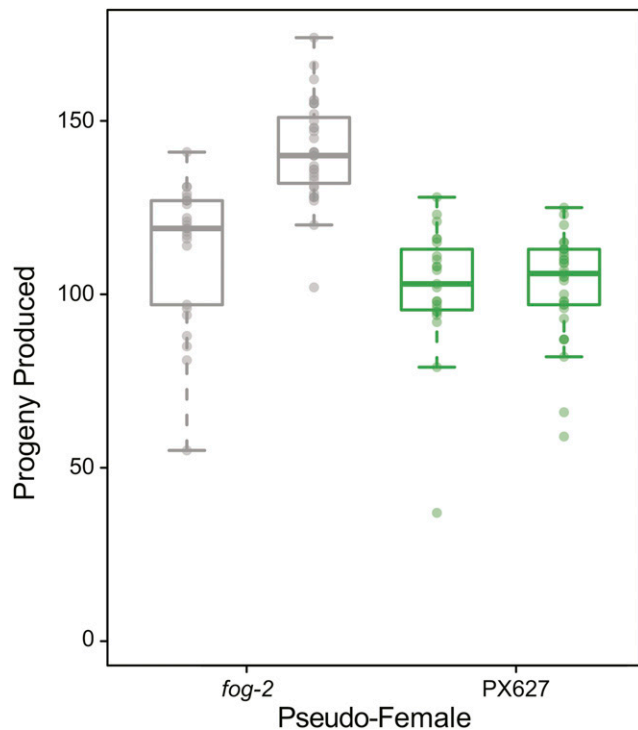


Figure 3 Self-sterile PX627 hermaphrodites (green) can recover their fertility when mated with a wild-type male, as compared to *fog-2* functional females (gray). Each bar within a genotypic set represents an independent replicate. While self-sterile hermaphrodites produced fewer progeny than *fog-2* females ($z = -2.78$, $P < 0.01$), their progeny production was invariable across replicates ($t = -0.097$, $df = 40.73$, $P = 0.92$).

approximately 12 hr and all males were fully fertile within 24 hr ($n = 30$ of 30). Day 2 adult males, however, had a much slower recovery period and not all males became fertile ($n = 16$ of 30).

Hermaphrodite self-sterility induction as a tool for aging research

Lifespan assays in *C. elegans* are complicated by the difficult and relatively labor intensive process of separating individuals of an aging cohort from their offspring. A variety of approaches to address this problem are used in the literature, with treatment of adults by the pyrimidine analog FUdR (a chemotherapy agent) being the most widely used. FUdR interferes with DNA synthesis, thereby preventing the production of viable offspring. The sterility induction system developed here allows hermaphrodites to be treated during larval development in order to induce self-sterilization and to then be transferred to whatever media type is necessitated by a given experiment, such as plates treated with bioactive compounds (see Lucanic *et al.* 2017). We examined this potential use by contrasting longevity in wild-type (N2) and *P_{pie-1}::TIR1::mRuby; spe-44::degron* (PX627) adults living on plates containing FUdR with those of PX627 individuals reared on auxin plates either throughout the entire larval development period or during the L4 stage alone before being transferred to standard NGM plates for the remainder of their lives.

The survivorship curves of individuals sterilized via either FUdR or auxin were similar to one another, although they differed slightly in quantitative details (Figure 4). A comparison of adult wild-type and PX627 individuals raised on FUdR in the absence of auxin yielded highly similar

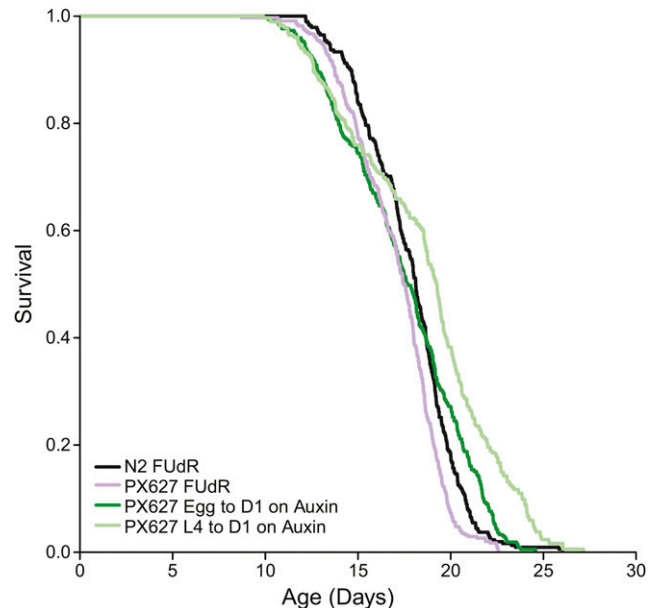


Figure 4 Lifespan curves comparing FUdR sterility to auxin-induced self-sterility. Wild-type (N2, black) and (PX627, purple) adults were FUdR treated. PX627 individuals were exposed to auxin from egg to day 1 of adulthood (dark green) or during the L4 stage alone (light green). Each survivorship curve represents six to eight pooled replicates each with over 100 individuals. The survivorship profiles were very similar across treatments and genetic backgrounds, though the PX627 L4 auxin treatment showed a quantitatively distinct profile.

survivorship profiles and median lifespans (N2_FUdR = 18.1 days, PX627_FUdR = 17.5 days; CPH contrast: $z = 2.17$, $P = 0.0996$). PX627 individuals sterilized with FUdR also displayed quite similar overall longevity profiles, with individuals raised on auxin for their entire larval periods displaying nearly identical median lifespans to those treated with FUdR (whole larval period PX627_auxin = 17.7 days). However, auxin-exposed worms tended to display a lower rate of mortality late in life, yielding an overall significant difference between these treatments (CPH contrast: $z = 3.04$, $P = 0.0089$). The largest difference in lifespan was observed in PX627 individuals exposed to auxin during only the L4 stage of development, which had both longer median and maximum lifespans than matched FUdR treated individuals (L4 PX627_auxin = 19.2; CPH contrast: $z = 5.76$, $P < 0.0001$). Additionally, replicate trials from individuals treated only during the L4 stage tended to display more error variance (total variance attributable to replicate + plate effects) than the other experimental treatments (12% vs. 2–4%, respectively).

DISCUSSION

Sexual reproduction integrates multiple processes across an organism's life, including the generation of gametes, the act of finding and securing mates, and the production of offspring. Each of these steps has an associated cost (Lehtonen *et al.* 2012). On top of these direct consequences, antagonistic interactions between the sexes during the process of mating as well as conflicts between parents and offspring can further exacerbate reproductive costs. Additionally, the interplay between reproduction and other major life history processes, such as aging and stress response, can add additional fitness trade-offs (Adler and Bonduriansky 2014). However, precise manipulation and quantification of reproductive trade-offs in an experimental setting has proved challenging.

Sterility induction system

Using the AID system, we designed an external, non-toxic spermatogenesis arrest in *C. elegans*, resulting in hermaphrodite self-sterility and reversible male sterility. Hermaphrodite self-sterility could be induced through auxin exposure during the spermatogenesis developmental window alone. However, auxin exposure throughout larval development also induced complete self-sterility and had no noticeable effects on development (also see Zhang *et al.* 2015). Since this continued larval exposure required very little manual intervention, it is the preferred method for sterility induction, rather than multiple transfers of individuals on and off auxin during late larval development. While tagging the critical spermatogenesis gene *spe-44* gave complete self-sterility, the degron tag itself seems to create some inherent sperm loss. This observation is perhaps unsurprising given the wide-ranging role of this transcription factor. Specifically, while wild-type progeny production peaks on day 2, tagged hermaphrodites had more consistent progeny production over days 2 and 3 again likely due an overall decrease in the number of sperm produced. Despite the degron tag effect, overall progeny production was not significantly different from wild-type hermaphrodites in the absence of auxin and self-sterile hermaphrodites recovered their full fertility when mated with a wild-type male.

In males, auxin exposure during larval spermatogenesis initiation alone was not sufficient to induce sterility, but rather had to occur within the L3 stage. This earlier exposure window corresponds to the earlier expression profile of *spe-44* relative to other spermatogenesis genes observed by Kulkarni *et al.* (2012). However, males could be fully sterilized using auxin exposure throughout larval development or early adult development. Moreover, males could recover their fertility, though in an age-dependent manner. The inability of all day 2 males to recover their fertility could be due to a decrease in the transcription level of *spe-44* over time or reduced mating behavior.

Our sterility induction system has a broad range of applications within the fields of spermatogenesis, sperm competition, and mating systems evolution. The temporal control over male sperm production allows for an increased understanding of sperm dynamics, including the rate at which sperm are produced and the amount of sperm stored. Additionally, this temporal control could be co-opted for precise studies of sperm competitive behavior under multiple mating scenarios. A particularly interesting application of this system is the study of mating systems evolution. For example, a genetically identical population could be simultaneously evolved under hermaphroditic and obligate male-female mating regimes. Alternatively, populations could be evolved to switch between mating regimes to better understand the genomic implications of these transitions.

A new approach for aging studies

C. elegans is one of the premiere model systems for studying the biology of aging. The first life-extending mutations were discovered in *C. elegans* (Friedman and Johnson 1988; Kenyon *et al.* 1993) and since then this system has been used in hundreds of studies to investigate a wide variety of questions in aging research (reviewed in Park *et al.* 2017). In particular, a number of studies have shown that the reproductive state of an individual, especially those controlled by germline-soma signaling systems, can have important consequences for longevity (Shi and Murphy 2014; Angeles-Albores *et al.* 2017). From a practical standpoint, reproduction can greatly complicate longevity assays in nematodes. Since the age at first reproduction is much shorter than median lifespan, there is the potential for several generations to be living on a plate at the same time, even if one starts with an initial age synchronized cohort. In most longevity studies this problem is solved either by manually removing (“picking”)

adults to fresh media every day, which is very labor intensive and prone to error, or using a chemical means to sterilize reproductive adults. The most common sterilization technique involves the use of FUDR, which disrupts DNA replication in proliferating tissues such as the germline. Actively poisoning a subject while trying to accurately track their health and lifespan is obviously less than ideal.

The sterility induction system developed here provides an ideal alternative to existing chemical sterilization approaches in *C. elegans* and other nematodes. First, worms only need to be exposed to auxin during their larval development and can then be transferred to regular media as adults. This exposure window cuts down on expense as well as the need to constantly replenish an environmental toxin throughout adult life. Additionally, unlike FUDR treatment, auxin sterilization has no impact on oogenesis—a major cellular process throughout hermaphrodite adulthood. Further, there is no concern about potential interactions between the sterilization agent and other external treatments such as food quality or chemical interventions (Lucanic *et al.* 2017). Overall, we find that longevity trajectories of self-sterilized individuals are very similar to individuals raised on FUDR (Figure 4). The only substantive difference that we observed was in individuals that had only been exposed to auxin during the L4 stage that immediately precedes sexual maturity. These individuals lived longer and displayed more variable outcomes than those that were exposed to auxin throughout their entire larval period. Potentially, even those these individuals are sterile, there may be some progression through spermatogenesis that has lifespan ramifications. This observation will require further study and provides an opportunity for deeper investigation of the relationship between reproduction and lifespan.

Overall, inducible sterility implemented during larval development followed by a transfer to standard media appears to be a viable, non-toxic, and more natural means of conducting long-term longevity studies with *C. elegans*. The only major disadvantage to this approach is the dependence upon the genetic background that we have constructed here or reconstructing the required degron system components in other genetic backgrounds. While these components may limit the applicability in some genetic studies, there is a very large advantage for direct environmental and/or chemical manipulation studies. Further, new aging related mutant screens might be initiated using the presented *spe-44::degron* hermaphrodites as the parental strain.

Conclusion

Our targeted approach of a critical spermatogenesis gene and the potential applications should in principle be transferable to other systems where auxin-induction is viable, such as *Drosophila* and zebrafish. Additionally, many other types of cell-specific arrests should be targetable using the auxin-inducible system. Now firmly within the era of CRISPR/Cas9 transgenics, targeted, external induction systems, such as the method presented here, are possible. When coupled with the power of automated assays and next-generation sequencing techniques, the field is poised to gain a wealth of information previously unattainable.

ACKNOWLEDGMENTS

We would like to thank C. Sedore for assistance with the automated lifespan set-up and data processing. We would like to thank S. Banse for advice and the Phillips Lab and two anonymous reviewers for constructive comments. This work was supported by the National Institutes of Health (training grant T32 GM007413 to KRK and R01GM102511 and R01AG049396 to PCP) and the ARCS Foundation Oregon Chapter (KRK).

LITERATURE CITED

- Adler, M. I., and R. Bonduriansky, 2014 Sexual Conflict, Life Span, and Aging. *Cold Spring Harb. Perspect. Biol.* 6: a017566. <https://doi.org/10.1101/cshperspect.a017566>
- Angeles-Albores, D., D. H. W. Leighton, T. Tsou, T. H. Khaw, I. Antoshechkin *et al.*, 2017 The *Caenorhabditis elegans* female state: decoupling the transcriptomic effects of aging and sperm-status. *G3 (Bethesda)* 7: 2969–2977. <https://doi.org/10.1534/g3.117.300080>
- Arnqvist, G., and L. Rowe, 2005 *Sexual conflict*, Princeton University Press, Princeton, NJ. <https://doi.org/10.1515/9781400850600>
- Bates, D., M. Mächler, B. Bolker, and S. Walker, 2015 Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67: 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71–94.
- Byerly, L., R. C. Cassada, and R. L. Russell, 1976 The life cycle of the nematode *Caenorhabditis elegans*. I. Wild-type growth and reproduction. *Dev. Biol.* 51: 23–33. [https://doi.org/10.1016/0012-1606\(76\)90119-6](https://doi.org/10.1016/0012-1606(76)90119-6)
- Castrillon, D. H., P. Gönczy, S. Alexander, R. Rawson, C. G. Eberhart *et al.*, 1993 Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile mutants generated by single P element mutagenesis. *Genetics* 135: 489–505.
- Dickinson, D. J., J. D. Ward, D. J. Reiner, and B. Goldstein, 2013 Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. *Nat. Methods* 10: 1028–1034. <https://doi.org/10.1038/nmeth.2641>
- Edward, D. A., P. Stockley, and D. J. Hosken, 2015 Sexual Conflict and Sperm Competition. *Cold Spring Harb. Perspect. Biol.* 7: a017707. <https://doi.org/10.1101/cshperspect.a017707>
- Ellis, R. E., and G. M. Stanfield, 2014 The regulation of spermatogenesis and sperm function in nematodes. *Semin. Cell Dev. Biol.* 29: 17–30. <https://doi.org/10.1016/j.semcdb.2014.04.005>
- Friedman, D. B., and T. E. Johnson, 1988 A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118: 75–86.
- Frøkjær-Jensen, C., M. W. Davis, M. Ailion, and E. M. Jørgensen, 2012 Improved Mos1-mediated transgenesis in *C. elegans*. *Nat. Methods* 9: 117–118. <https://doi.org/10.1038/nmeth.1865>
- Hirsh, D., and R. Vanderslice, 1976 Temperature-sensitive developmental mutants of *Caenorhabditis elegans*. *Dev. Biol.* 49: 220–235. [https://doi.org/10.1016/0012-1606\(76\)90268-2](https://doi.org/10.1016/0012-1606(76)90268-2)
- Hirsh, D., D. Oppenheim, and M. Klass, 1976 Development of the reproductive system of *Caenorhabditis elegans*. *Dev. Biol.* 49: 200–219. [https://doi.org/10.1016/0012-1606\(76\)90267-0](https://doi.org/10.1016/0012-1606(76)90267-0)
- Hothorn, T., F. Bretz, and P. Westfall, 2008 Simultaneous inference in general parametric models. *Biom. J.* 50: 346–363. <https://doi.org/10.1002/bimj.200810425>
- Hsu, C.-C., M.-F. Hou, J.-R. Hong, J.-L. Wu, and G. M. Her, 2009 Inducible Male Infertility by Targeted Cell Ablation in Zebrafish Testis. *Mar. Biotechnol. (NY)* 12: 466–478. <https://doi.org/10.1007/s10126-009-9248-4>
- Kanke, M., K. Nishimura, M. Kanemaki, T. Kakimoto, T. S. Takahashi *et al.*, 2011 Auxin-inducible protein depletion system in fission yeast. *BMC Cell Biol.* 12: 8. <https://doi.org/10.1186/1471-2121-12-8>
- Karr, T. L., and S. Pitnick, 1999 Sperm competition: defining the rules of engagement. *Curr. Biol.* 9: R787–R790. [https://doi.org/10.1016/S0960-9822\(00\)80014-7](https://doi.org/10.1016/S0960-9822(00)80014-7)
- Kempe, K., and M. Gils, 2011 Pollination control technologies for hybrid breeding. *Mol. Breed.* 27: 417–437. <https://doi.org/10.1007/s11032-011-9555-0>
- Kenyon, C., 1988 The nematode *Caenorhabditis elegans*. *Science* 240: 1448–1453. <https://doi.org/10.1126/science.3287621>
- Kenyon, C., J. Chang, E. Gensch, A. Rudner, and R. Tabtiang, 1993 A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366: 461–464. <https://doi.org/10.1038/366461a0>
- Kulkarni, M., D. C. Shakes, K. Guevel, and H. E. Smith, 2012 SPE-44 implements sperm cell fate. *PLoS Genet.* 8: e1002678. <https://doi.org/10.1371/journal.pgen.1002678>
- L'Hernault, S. W., 2006 Spermatogenesis (February 20, 2006), *WormBook*, ed. The *C. elegans* Research Community WormBook, doi/10.1895/wormbook.1.85.1, <http://www.wormbook.org>.
- Lehtonen, J., M. D. Jennions, and H. Kokko, 2012 The many costs of sex. *Trends Ecol. Evol.* 27: 172–178. <https://doi.org/10.1016/j.tree.2011.09.016>
- Lucanic, M., W. T. Plummer, E. Chen, J. Harke, A. C. Foulger *et al.*, 2017 Impact of genetic background and experimental reproducibility on identifying chemical compounds with robust longevity effects. *Nat. Commun.* 8: 14256. <https://doi.org/10.1038/ncomms14256>
- Merritt, C., D. Rasoloson, D. Ko, and G. Seydoux, 2008 3' UTRs Are the Primary Regulators of Gene Expression in the *C. elegans* Germline. *Curr. Biol.* 18: 1476–1482. <https://doi.org/10.1016/j.cub.2008.08.013>
- Mitchell, D. H., J. W. Stiles, J. Santelli, and D. R. Sanadi, 1979 Synchronous growth and aging of *Caenorhabditis elegans* in the presence of fluoro-deoxyuridine. *J. Gerontol.* 34: 28–36. <https://doi.org/10.1093/geronj/34.1.28>
- Murakami, S., and T. E. Johnson, 1996 A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*. *Genetics* 143: 1207–1218.
- Naito, Y., K. Hino, H. Bono, and K. Ui-Tei, 2015 CRISPRdirect: software for designing CRISPR/Cas guide RNA with reduced off-target sites. *Bioinformatics* 31: 1120–1123. <https://doi.org/10.1093/bioinformatics/btu743>
- Natsume, T., T. Kiyomitsu, Y. Saga, and M. T. Kanemaki, 2016 Rapid Protein Depletion in Human Cells by Auxin-Inducible Degron Tagging with Short Homology Donors. *Cell Reports* 15: 210–218. <https://doi.org/10.1016/j.celrep.2016.03.001>
- Nishimura, H., and S. W. L'Hernault, 2010 Spermatogenesis-defective (*spe*) mutants of the nematode *Caenorhabditis elegans* provide clues to solve the puzzle of male germline functions during reproduction. *Dev. Dyn.* 110: 1502–1514. <https://doi.org/10.1002/dvdy.22271>
- Nishimura, K., T. Fukagawa, H. Takisawa, T. Kakimoto, and M. Kanemaki, 2009 An auxin-based degron system for the rapid depletion of proteins in nonplasmid cells. *Nat. Methods* 6: 917–922. <https://doi.org/10.1038/nmeth.1401>
- Paix, A., A. Folkmann, D. Rasoloson, and G. Seydoux, 2015 High Efficiency, Homology-Directed Genome Editing in *Caenorhabditis elegans* Using CRISPR-Cas9 Ribonucleoprotein Complexes. *Genetics* 201: 47–54. <https://doi.org/10.1534/genetics.115.179382>
- Palopoli, M. F., C. Peden, C. Woo, K. Akiha, M. Ary *et al.*, 2015 Natural and experimental evolution of sexual conflict within *Caenorhabditis* nematodes. *BMC Evol. Biol.* 15: 93. <https://doi.org/10.1186/s12862-015-0377-2>
- Park, H.-E. H., Y. Jung, and S.-J. V. Lee, 2017 Survival assays using *Caenorhabditis elegans*. *Mol. Cells* 40: 90–99. <https://doi.org/10.14348/molcells.2017.0017>
- R Core Team, 2015 *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reinke, V., 2003 Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. *Development* 131: 311–323. <https://doi.org/10.1242/dev.00914>
- Richardson, C. D., G. J. Ray, M. A. DeWitt, G. L. Curie, and J. E. Corn, 2016 Enhancing homology-directed genome editing by catalytically active and inactive CRISPR-Cas9 using asymmetric donor DNA. *Nat. Biotech.* 2016 34: 339–344. <https://doi.org/10.1038/nbt.3481>
- Schluter, D., T. D. Price, and L. Rowe, 1991 Conflicting selection pressures and life history trade-offs. *Proc. Biol. Sci.* 246: 11–17. <https://doi.org/10.1098/rspb.1991.0118>
- Schüpbach, T., and E. Wieschaus, 1991 Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations

- blocking oogenesis or altering egg morphology. *Genetics* 129: 1119–1136.
- Shi, C., and C. T. Murphy, 2014 Mating Induces Shrinking and Death in *Caenorhabditis* Mothers. *Science* 343: 536–540. <https://doi.org/10.1126/science.1242958>
- Stearns, S. C., 1989 Trade-Offs in Life-History Evolution. *Funct. Ecol.* 3: 259. <https://doi.org/10.2307/2389364>
- Stroustrup, N., B. E. Ulmschneider, Z. M. Nash, I. F. López-Moyado, J. Apfeld *et al.*, 2013 The *Caenorhabditis elegans* Lifespan Machine. *Nat. Methods* 10: 665–670. <https://doi.org/10.1038/nmeth.2475>
- Tan, X., L. I. A. Calderon-Villalobos, M. Sharon, C. Zheng, C. V. Robinson *et al.*, 2007 Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* 446: 640–645. <https://doi.org/10.1038/nature05731>
- Teotonio, H., S. Carvalho, D. Manoel, M. Roque, and I. M. Chelo, 2012 Evolution of Outcrossing in Experimental Populations of *Caenorhabditis elegans*. *PLoS One* 7: e35811–e35813. <https://doi.org/10.1371/journal.pone.0035811>
- Therneau, T. M., P. M. Grambsch, and V. S. Pankratz, 2012 Penalized Survival Models and Frailty. *J. Comput. Graph. Stat.* 12: 156–175. <https://doi.org/10.1198/1061860031365>
- Therneau, T. M., 2017 *coxme: Mixed Effects Cox Models*. R package version 2.2–5. <https://CRAN.R-project.org/package=coxme>
- Trivers, R. L., 1972 *Sexual selection and parental investment*, Aldine Publishing Company, Piscataway, NJ.
- Trost, M., A. C. Blattner, and C. F. Lehner, 2016 Regulated protein depletion by the auxin-inducible degradation system in *Drosophila melanogaster*. *Fly (Austin)* 10: 35–46. <https://doi.org/10.1080/19336934.2016.1168552>
- Volpe, A. M., H. Horowitz, C. M. Grafer, S. M. Jackson, and C. A. Berg, 2001 *Drosophila rhino* Encodes a Female-Specific Chromo-domain Protein That Affects Chromosome Structure and Egg Polarity. *Genetics* 159: 1117–1134.
- Ward, S., and J. Miwa, 1978 Characterization of Temperature-Sensitive, Fertilization-Defective Mutants of the Nematode *Caenorhabditis Elegans*. *Genetics* 88: 285–303.
- Xu, H., T. Xiao, C.-H. Chen, W. Li, C. A. Meyer *et al.*, 2015 Sequence determinants of improved CRISPR sgRNA design. *Genome Res.* 25: 1147–1157. <https://doi.org/10.1101/gr.191452.115>
- Zhang, L., J. D. Ward, Z. Cheng, and A. F. Dernburg, 2015 The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*. *Development* 142: 4374–4384. <https://doi.org/10.1242/dev.129635>

Communicating editor: M. Félix