1   Title: Auxin-Mediated Sterility Induction System for Longevity and Mating Studies in

2   *Caenorhabditis elegans*

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ABSTRACT

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24   The ability to control both the means and timing of sexual reproduction provides a powerful tool

25   to understand not only fertilization, but also life history trade-offs resulting from sexual

26   reproduction. However, precisely controlling fertilization has proved a major challenge across

27   model systems. An ideal sterility induction system should be external, non-toxic, and reversible.

28   Using the auxin-inducible degradation system targeting the *spe-44* gene within the nematode

29   *Caenorhabditis elegans*, we designed a means of externally inducing spermatogenesis arrest.

30   We show that exposure to auxin during larval development induces both hermaphrodite self-

31   sterility and male sterility. Moreover, male sterility can be reversed upon cessation of auxin

32   exposure. The sterility induction system developed here has multiple applications in the fields of

33   spermatogenesis and mating systems evolution. Importantly, this system is also a highly

34   applicable tool for aging studies. We show that auxin-induced self-sterility is comparable to the

35   commonly used chemically-induced FUdR sterility, while offering multiple benefits, including

36   being less labor intensive, being non-toxic, and avoiding compound interactions with other

37   experimental treatments.

38

INTRODUCTION

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 the process of fertilization. Thus, precise control of fertilization allows for the nature of those 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 of 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 offer many more challenges to reproductive control, and therefore few sterility induction approaches

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65   exist in these systems (see Hsu *et al.* 2009). *Caenorhabditis elegans* is a major model system

66   for genetics, development, neurobiology, and aging. Within *C. elegans*, a limited number of

67   sterility mutants are available (L'Hernault 2006; Ellis and Stanfield 2014) and can be maintained

68   by mating hermaphrodites to males. In some cases, temperature sensitive sterility mutants

69   (Hirsh and Vanderslice 1976; Ward and Miwa 1978) exist. However, these tend to be general

70   germline mutations with pleiotropic effects rather than fine-scale control of gametogenesis *per*

71   *se*. Additionally, these mutants by necessity require a temperature shift, which can affect

72   lifespan (Park *et al.* 2017). An alternative scheme is to prevent progeny production in

73   hermaphrodites using chemical treatments (Mitchell *et al.* 1979), though these techniques are

74   manually intensive and not conducive to high-throughput assays. Further, chemical intervention

75   can potentially generate unaccounted for fitness effects, which not only confound the biological

76   interpretation of results but also make reproducibility a challenge.

77   An ideal sterility system would be inducible, driven by an external treatment, and, when

78   possible, reversible. To the best of our knowledge such an approach does not exist, even within

79   model organisms. To address this need, we used the non-toxic, non-native auxin inducible

80   degradation (AID) system (Nishimura *et al.* 2009) coupled with knowledge of a critical

81   spermatogenesis gene to create an external sterility induction system in *C. elegans*. We show

82   that this system induces self-sterility of hermaphrodites and complete, but reversible sterility of

83   males. This method has broad applications in nematode biology, including studies of aging,

84   gametogenesis, and mating systems evolution.

Constructing an inducible spermatogenesis arrest

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86   The AID system (Nishimura *et al.* 2009; Zhang *et al.* 2015) was chosen as the optimal method

87   for an external sterility induction system in *C. elegans*, as auxin is non-native, non-toxic, and

88   cost-effective. The auxin hormone regulates gene expression in *Arabidopsis thaliana* by

89   activating the F-Box transport inhibitor response 1 (TIR1) protein – the substrate recognition

90   component of a Skp1-Cullin-F-box E3 ubiquitin ligase complex which ubiquitinates degron-

91   tagged proteins for degradation by the proteasome (Tan *et al.* 2007; Nishimura *et al.* 2009). This

92   system has been co-opted as an inducible genetic mechanism in a variety of organisms by

93   degron-tagging a protein of interest and choosing a promoter to drive TIR1 expression in the

94   necessary cell type (Kanke *et al.* 2011; Zhang *et al.* 2015; Trost *et al.* 2016; Natsume *et al.*

95   2016). We targeted a necessary spermatogenesis gene *spe-44*, causing a spermatogenesis

96   arrest and therefore sterility. Specifically, *spe-44* is one of eleven sperm-specific transcription

97   factors (Reinke 2003) and is predicted to have hundreds of downstream targets, including the

98   critical Major Sperm Protein (Kulkarni *et al.* 2012). Constitutive TIR1 expression was driven

99   using the germline promoter of *pie-1*, which is one of few genes known to have strong sperm

100   expression in hermaphrodites and males (Merritt *et al.* 2008). These three components—auxin,

101   $P_{pie-1}::TIR1$, *spe-44::degron*—generate a fully controllable sterility induction system in *C.*

102   *elegans.*

103

104   **MATERIALS AND METHODS**

105   **Molecular biology**

106   Guide sequences were chosen using the tools CRISPRdirect (Naito *et al.* 2015), MIT CRISPR

107   Design (http://crispr.mit.edu) and Sequence Scan for CRISPR (Xu *et al.* 2015). For the TIR1

108   insertion, a guide targeting the sequence GAAATCGCCGACTTGCGAGG<u>AGG</u> near the

109   ttTi4348 MosSCI site was inserted into pDD162 (Dickinson *et al.* 2013) using the Q5 site-

110   directed mutagenesis kit (NEB) to create pMS18. This insertion region was previously shown to

111   be permissive for germline expression (Frøkjær-Jensen *et al.* 2012). The plasmid pMS30 was

112   created by Gibson assembly using the NEBuilder HiFI Kit (NEB) and included: homology arms

113   amplified from N2 genomic DNA, the *pie-1* promoter amplified from pCM1.127 (Addgene

114   #21384) (Merritt *et al.* 2008), the *C. elegans* optimized *AtTIR1::mRuby* fusion and *unc-54*

115   terminator amplified from pLZ31 (Addgene #71720) (Zhang *et al.* 2015), and the self-excising

116   drug selection cassette (SEC) amplified from pDD282 (Addgene #66823) (Dickinson *et al.*

117   2013). The plasmid backbone was also derived from pDD282. An 11 bp segment of the

118   genomic DNA sequence was omitted from the homology arms to prevent re-cutting. All plasmid

119   assembly junctions were confirmed by Sanger sequencing. Sequencing showed that pMS30

120   contained a single nucleotide substitution in one of the LoxP sites of the SEC. However, this

121   substitution did not significantly impact SEC removal.

122   The degron:3X-FLAG tag utilized asymmetric homology arms (Richardson *et al.* 2016)

123   for *spe-44* insertion and contained appropriate silent sites to prevent re-cutting. The insert was

124   synthesized as a GeneArt String (ThermoFisher) and amplified by PCR prior to injection.

125   **Strain generation by CRISPR/Cas9**

126   The $P_{pie-1}::TIR1::mRuby$ construct was injecting into the gonad of young adult hermaphrodites

127   (standard laboratory strain N2) using a mixture of 50 ng/µl pMS18, 10 ng/µl pMS30 and 2.5

128   ng/µl pCFJ421 (Addgene #34876) (Frøkjær-Jensen *et al.* 2012). Screening and removal of the

129   SEC was done following Dickinson *et al.* (2013). Presence of the insertion and removal of the

130   SEC was confirmed by PCR and Sanger sequencing.

131   To degron tag *spe-44*, a cr:tracrRNA (Synthego) targeting the sequence

132   ATTGAATATGACTAGGTCC<u>TGG</u> near the C-terminus of *spe-44* was annealed and pre-

133   incubated with Cas9 (PNA Bio) in accordance with manufacturer protocol. A mix of 1.7 µM

134   cr:tracrRNA, 1.65 µg/µl Cas9, and 80 ng/µl of the PCR repair template, was then injected into

135   the gonad of young adult N2 hermaphrodites containing the $P_{pie-1}::TIR1::mRuby$ construct.

136   Included in the injection mix was an additional cr:tracrRNA and oligonucleotide repair template,

137   allowing for screening through *dpy-10* co-conversion (Paix *et al.* 2015). Progeny from broods

138   containing individuals with a dumpy or roller phenotype were then screened for the *spe-*

139   *44::degron* insertion by PCR and confirmed by Sanger sequencing.

140   Confirmed double mutants were backcrossed 5 times to N2 to create the final strain

141   PX627 (fx*Is*1[*P~pie-1~::TIR1::mRuby*, I:2851009]; *spe-44*(fx110[*spe-44*::*degron*]). This strain was

142   crossed to strain CB4088, to create the male-rich strain PX629 (fx*Is*1[*P~pie-1~::TIR1::mRuby*,

143   I:2851009]; *spe-44*(fx110[*spe-44*::*degron*]) IV; *him-5* e(1490) V).

144   **Worm culture and strains**

145   The *C. elegans* strains PX627, PX629, N2, and JK574 (*fog-2*(q71) V) were maintained on NGM-

146   agar plates seeded with OP50 *Escherichia coli* at 20ºC (Brenner 1974). The *fog-2* mutation

147   blocks self-sperm production in hermaphrodites, making them a functionally female.

148   Synchronized cultures of larval stage 1 (L1) animals were obtained through hypochloride

149   treatment of gravid adults (Kenyon 1988). To induce sterility, worms were transferred to NGM-

150   agar plates containing 1 mM indole-3-acetic acid (Auxin, Alfa Aesar) following Zhang *et al.*

151   (2015). Zhang *et al.* (2015) showed this auxin concentration to be non-toxic to adults with no

152   larval development defects or fecundity effects. Auxin plates were stored in the dark at 4ºC to

153   prevent compound degradation.

154   All larval exposure assays were carried out on small plates (35 mm) seeded with 100 µL

155   *E. coli* and a sample size of 130 hermaphrodites per developmental stage and 100 males per

156   stage. Adult male developmental exposure assays were done by plating synchronized L1

157   PX629 worms on NGM-agar plates until day 1 of adulthood. Males were then transferred to

158   small auxin plates seeded with 10 µL *E. coli* along with two virgin females (strain JK547). Males

159   were transferred to new virgin females twice a day until no fertilized eggs were seen on plates.

160   Male sterility recovery experiments were done by plating synchronized L1 animals on auxin

161   plates and leaving worms on auxin until day 1 or day 2 of adulthood. Males were then

162   transferred to small NGM plates seeded with 10 µL *E. coli* and given three virgin females (strain

163   JK574) with which to mate. Plates were monitored until fertile eggs appeared.

164   For the hermaphrodite self-sterility mating experiments, synchronized L1 PX627 worms

165   were plated onto auxin plates and removed three hours into adulthood. Virgin females (strain

166   JK574) were used as a control. Individual pseudo-females were mated with two males (strain

167   JK574) overnight on small NGM-agar plates seeded with 10 µL *E. coli*, after which males were

168   removed. All the progeny laid over the subsequent 24 hours were counted. Two independt

169   biological replicates were done with 23 to 35 pseudo-females in each treatment. Fertility data

170   were analyzed using a generalized linear model (GLM) framework with random effects and a

171   Poisson distribution using the *lme4* v.1.13 package (Bates *et al.* 2015) in the R statistical

172   language (R Core Team 2015).

173   **Lifespan assays**

174   Lifespan data were collected using automated lifespan machines following Stroustrup *et al.*

175   (2013). Briefly, worms were synchronized by letting day 2 adults (strains PX627 and N2) lay

176   eggs over a two hour time period. Auxin self-sterility was achieved by allowing PX627

177   hermaphrodites to lay directly on auxin plates or by transferring larval stage 4 (L4) progeny to

178   auxin plates. Both self-sterility treatments were transferred to NGM-agar plates on day 1 of

179   adulthood. As a control, egg lays for both PX627 and N2 hermaphrodites were done on NGM-

180   agar plates. At day 1 of adulthood, these animals were transferred to small plates containing 51

181   µM 5-fluoro-2'-deoxyuridine (FUdR, VCI America) to inhibit reproduction (Mitchell *et al.* 1979).

182   Control worms were transferred to fresh FUdR plates 24 hours later.

183   On day 5 of adulthood, all worms were transferred onto medium scanner plates (60 mm)

184   with sealable lids to minimize dehydration. NGM-agar scanner plates contained 40 mM

185   potassium phosphate buffer (pH 6.0), 1mM magnesium sulfate, and 5 mg/mL cholesterol, along

186   with 100 mg/mL nystatin to prevent fungal growth while on the automated lifespan system.

187   Control plates also included 51 µM FUdR. All scanner plates were seeded with 200 µL *E. coli*. A

188   total of 35 to 60 adult hermaphrodites were transferred to each plate with four technical

189   replicates of each treatment. The 16 plates were randomly arranged on a modified Epson v700

190   scanner in a temperature controlled 20°C room and held in place by rubber mat. Plates were

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

192   Images were analyzed using the Worm Browser software developed with the automated

193   lifespan system (Stroustrup *et al.* 2013). This process includes specifying the location of

194   individual plates on the scanner, detecting individual worms, and analyzing worm movement.

195   The resulting data are time of death calls for each individual worm based on the cessation of

196   movement. All plates were hand annotated to ensure that non-worm objects were excluded.

197   Additionally, the time of death calls for the first and last 10% of worms on each plate were

198   checked as these time points are more error prone. The final lifespans were calculated using

199   the egg lay as day zero.

200   To analyze the influence of our sterilization approach on longevity, we used a mix-model

201   survival analysis as outlined in Lucanic *et al.* (2017). Longevity effects were evaluated using

202   both a mixed-model Cox Proportional Hazard (CPH) model (Therneau *et al.* 2012) using the

203   *coxme* v.2.2-5 package {coxme:DjUq3nzA}, as well as via GLM using the *lme4* package in R. In

204   each case, the coxme and GLM approaches yield equivalent results and so only the *coxme*

205   results are presented as they represent the more comprehensive analytical framework for these

206   data. Using the automated lifespan machine, a small subset of individuals initially placed on a

207   plate are missing and presumed lost over the course of an assay. Such individuals would

208   normally be classified as "censored" in normal survivorship analysis. However, because

209   mortality is determined retrospectively when an individual ceases to move, the moment of loss

210   of such individuals cannot be determined and so they must simply be classified as missing

211   rather than censored at a given time point. For these analyses, the environmental treatment

212   within which each individual was raised (FUdR, auxin) and the genotype of the individual

213   (wildtype or $P_{pie-1}::TIR1::mRuby; spe-44::degron$) were treated as fixed effects, while replicate

214   and plate (nested within replicate) were treated as random effects. Specific *a priori* hypotheses

215   about effects of FUdR and genetic background were tested via contrast coefficients using the

216   *mcp* procedure of the multcomp procedure in R (Hothorn *et al.* 2008).

217   **Data accessibility**

218   The oligonucleotides and synthetic constructs used in this study are listed in Table S1. The

219   fertility data are given in File S1 and the lifespan data in File S2. Worm strains N2, JK574,

220   PX627, and PX629 are available from the *Caenorhabditis* Genetics Center.

221

222   **RESULTS**

223   **Self-sterility induction in hermaphrodites**

224   *Caenorhabditis elegans* hermaphrodites are protandrous, such that they produce several

225   hundred sperm cells during their final larval stage and then switch to oocyte production for the

226   remainder of their lifespan (Hirsh *et al.* 1976). We examined the necessary and sufficient

227   windows of auxin exposure during hermaphrodite development to induce self-sterility. To

228   prevent sperm production, hermaphrodites must be exposed to auxin during their larval

229   development (Fig. 1A). Specifically, the L4 window alone—the developmental stage during

230   which sperm are produced—was both necessary and sufficient to drive self-sterility (Fig. 1B). As

231   observed in other spermatogenesis mutants, self-sterile hermaphrodites continue to lay

232   unfertilized oocytes throughout their adult life. Adult exposure to auxin had no effect on progeny

233   production.

234   Despite being self-sterile, hermaphrodites could recover their fertility when mated to a

235   male, comparable to hermaphrodites made functionally female through the *fog-2* mutation (Fig.

236   2). Interestingly, these mated self-sterile hermaphrodites were highly consistent in the number

237   of progeny they produced. However, control *fog-2* females laid significantly more progeny than

238   self-sterile hermaphrodites ($z = -2.78$, $p < 0.01$), likely due to adaptation to obligate outcrossing.

239   **Inducible sterility of males is reversible within a single generation**

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240   We tested the sterility induction of males using a male-enriched *C. elegans* strain. Like

241   hermaphrodites, males begin spermatogenesis during L4, however they continue producing

242   sperm throughout adulthood, whereas hermaphrodites do not (L'Hernault 2006). We examined

243   the window of auxin exposure during larval male development sufficient to induce sterility.

244   Interestingly, L4 exposure alone was not sufficient to induce complete sterility, as these males

245   still produced a low number of progeny. Rather males had to be exposed to auxin at least 2

246   hours prior to the L3/L4 molt (Fig. 3A).

247   To determine if sterility in males could be reversed following consistent exposure to

248   auxin during larval development, males were transferred from auxin to standard NGM plates at

249   day 1 and day 2 of adulthood. Day 1 adult males began to recover their fertility within

250   approximately 12 hours and all males were fully fertile within 24 hours (Fig. 3B). Day 2 adult

251   males, however, had a much slower recovery period and not all males became fertile (n = 16

252   out of 30). Alternatively, we measured the sterility induction onset for adult male auxin exposure.

253   Here males were raised on standard NGM plates and exposed to auxin starting at day 1 of

254   adulthood. Within 24 hours of auxin exposure, no progeny were observed from male-virgin

255   female matings, indicating that males were fully sterile (Fig. 3C).

Hermaphrodite self-sterility induction as a tool for aging research

257   Lifespan assays in *C. elegans* are complicated by the difficult and relatively labor

258   intensive process of separating individuals of an aging cohort from their offspring. A variety of

259   approaches to address this problem are used in the literature, with treatment of adults by the

260   pyrimidine analog FUdR (a chemotherapy agent) being the most widely used. FUdR interferes

261   with DNA synthesis, thereby preventing the production of viable offspring. The sterility induction

262   system developed here allows hermaphrodites to be treated during larval development in order

263   to induce self-sterilization and to then be transferred to whatever media type is necessitated by

264   a given experiment, such as plates treated with bioactive compounds (see Lucanic *et al.* 2017).

265 We examined this potential use by contrasting longevities in wildtype (N2) and P_{pie-}

266 $_1$::TIR1::mRuby; spe-44::degron (PX627) adults living on plates containing FUdR with those of

267 PX627 individuals reared on auxin plates either throughout the entire larval development period

268 or during the L4 stage alone before being transferred to standard NGM plates for the remainder

269 of their lives.

270 Median lifespan of individuals sterilized via either FUdR or auxin were similar to one

271 another, although they differ slightly in quantitative details (Fig. 4). A comparison of adult

272 wildtype and PX627 individuals raised on FUdR in the absence of auxin yielded highly similar

273 survivorship profiles and median lifespans (N2_FUdR = 18.1 days, PX627_FUdR = 17.5 days;

274 CPH contrast: z = 2.17, p = 0.0996). PX627 individuals sterilized with FUdR also displayed quite

275 similar overall longevity profiles, with individuals raised on auxin for their entire larval periods

276 displaying nearly identical median lifespans to those treated with FUdR (whole larval period

277 PX627_auxin = 17.7 days). However, auxin-exposed worms tended to display a lower rate of

278 mortality late in life, yielding an overall significant difference between these treatments (CPH

279 contrast: z = 3.04, p = 0.0089). The largest difference in lifespan was observed in PX627

280 individuals exposed to auxin during only the L4 stage of development, which had both longer

281 median and maximum lifespans than matched FUdR treated individuals (L4 PX627_auxin =

282 19.2; CPH contrast: z = 5.76, p < 0.0001). Additionally, replicate trials from individuals treated

283 only during the L4 stage tended to display more error variance (total variance attributable to

284 replicate + plate effects) than the other experimental treatments (12% versus 2-4%,

285 respectively).

286

287 **DISCUSSION**

288 Sexual reproduction integrates multiple processes across an organism's life, including the

289 generation of gametes, the act of finding and securing mates, and the production of offspring.

290 Each of these steps has an associated cost (Lehtonen et al. 2012). On top of these direct

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291   consequences, antagonistic interactions between the sexes during the process of mating as

292   well as conflicts between parents and offspring can further exacerbate reproductive costs.

293   Additionally, the interplay between reproduction and other major life history processes, such as

294   aging and stress response, can add additional fitness trade-offs (Adler and Bonduriansky 2014).

295   However, precise manipulation and quantification of reproductive trade-offs in an experimental

296   setting has proved challenging.

297   **Sterility induction system**

298   Using the AID system, we designed an external, non-toxic spermatogenesis arrest in *C.*

299   *elegans*, resulting in hermaphrodite self-sterility and reversible male sterility. Hermaphrodite

300   self-sterility could be induced through auxin exposure during the spermatogenesis

301   developmental window alone. However, auxin exposure throughout larval development also

302   induced complete self-sterility and had no noticeable effects on development (also see Zhang *et*

303   *al.* 2015). Since this continued larval exposure required very little manual intervention, it is the

304   preferred method for sterility induction, rather than multiple transfers of individuals on and off

305   auxin during late larval development. Additionally, self-sterile hermaphrodites recovered their full

306   fertility when mated with a wildtype male. In males, auxin exposure during larval

307   spermatogenesis initiation alone was not sufficient to induce sterility, likely because transcription

308   of *spe-44* is increasing going into L4 (Kulkarni *et al.* 2012). However, males could be fully

309   sterilized using auxin exposure throughout larval development or early adult development.

310   Moreover, males could recovery their fertility in an age-dependent manner, presumably again

311   due to the transcription level of *spe-44*.

312   Our sterility induction system has a broad range of applications within the fields of

313   spermatogenesis, sperm competition, and mating systems evolution. The temporal control over

314   male sperm production allows for developments in our understanding of spermatogenesis,

315   including the rate at which sperm are produced and the amount of sperm stored. Additionally,

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316   this temporal control could be co-opted for precise studies of sperm competitive behavior under

317   multiple mating scenarios. A particularly interesting application of this system is the study

318   mating systems evolution. For example, a genetically identical population could be

319   simultaneously evolved under hermaphroditic and obligate male-female mating regimes.

320   Alternatively, populations could be evolved to switch between mating regimes to better

321   understand the genomic implications of these transitions.

322   **A new approach for aging studies**

323   *C. elegans* is one of the premiere model systems for studying the biology of aging. The first life-

324   extending mutations were discovered in *C. elegans* (Friedman and Johnson 1988; Kenyon *et al.*

325   1993) and since then this system has been used in hundreds of studies to investigate a wide

326   variety of questions in aging research (reviewed in Park *et al.* 2017). In particular, a number of

327   studies have shown that the reproductive state of an individual, especially those controlled by

328   germline-soma signaling systems, can have important consequences for longevity (Shi and

329   Murphy 2014; Angeles-Albores *et al.* 2017). From a practical standpoint, reproduction can

330   greatly complicate longevity assays in nematodes. Since the age at first reproduction is much

331   shorter than median lifespan, there is the potential for several generations to be living on a plate

332   at the same time, even if one starts with an initial age synchronized cohort. In most longevity

333   studies this problem is solved either by manually removing ("picking") adults to fresh media

334   every day, which is very labor intensive and prone to error, or using a chemical means to

335   sterilize reproductive adults. The most common sterilization technique involves the use of

336   FUdR, which disrupts DNA replication in proliferating tissues such as the germline. Actively

337   poisoning a subject while trying to accurately track their health and lifespan is obviously less

338   than ideal.

339   The sterility induction system developed here provides an ideal alternative to existing

340   chemical sterilization approaches in *C. elegans* and other nematodes. First, worms only need to

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341   be exposed to auxin during their larval development and can then be transferred to regular

342   media as adults. This exposure window cuts down on expense as well as the need to constantly

343   replenish an environmental toxin throughout adult life. Further, there is no concern about

344   potential interactions between the sterilization agent and other external treatments such as food

345   quality or chemical interventions (Lucanic *et al.* 2017). Overall, we find that longevity trajectories

346   of self-sterilized individuals are very similar to individuals raised on FUdR (Fig. 4). The only

347   substantive difference that we observed was in individuals that had only been exposed to auxin

348   during the L4 stage that immediately precedes sexual maturity. These individuals lived longer

349   and displayed more variable outcomes than those that were exposed to auxin throughout their

350   entire larval period. Potentially, even those these individuals are sterile, there may be some

351   progression through spermatogenesis that has lifespan ramifications. This observation will

352   require further study and provides an opportunity for deeper investigation of the relationship

353   between reproduction and lifespan.

354   Overall, inducible sterility implemented during larval development followed by a transfer

355   to standard media appears to be a viable, non-toxic, and more natural means of conducting

356   long-term longevity studies with *C. elegans.* The only major disadvantage to this approach is the

357   dependence upon the genetic background that we have constructed here or reconstructing the

358   required degron system components in other genetic backgrounds. While these components

359   may limit the applicability in some genetic studies, there is a very large advantage for direct

360   environmental and/or chemical manipulation studies. Further, new aging related mutant screens

361   might be initiated using PX627 as the parental strain.

Conclusion

363   Our targeted approach of a critical spermatogenesis gene and the potential applications

364   should in principle be transferable to other systems where auxin-induction is viable, such as

365   *Drosophila* and zebrafish. Additionally, many other types of cell-specific arrests should be

366   targetable using the auxin-inducible system. Now firmly within the era of CRISPR/Cas9

367   transgenics, targeted, external induction systems, such as the method presented here, are

368   possible. When coupled with the power of automated assays and next-generation sequencing

369   techniques, the field is poised to gain a wealth of information previously unattainable.

370

376

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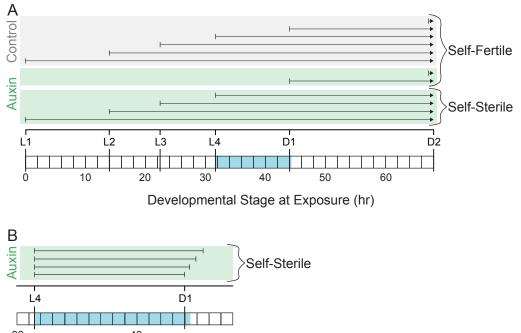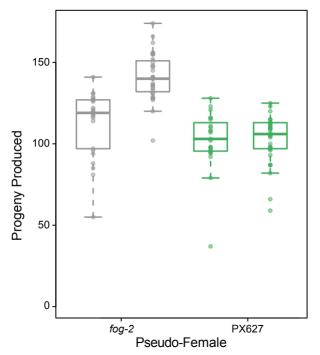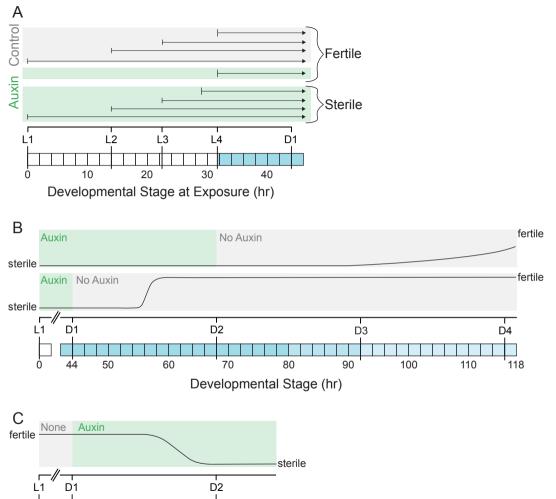
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478 **Figure Legends**

479 **Figure 1.** Auxin exposure induces hermaphrodite self-sterility. A) Hermaphrodites were exposed

480 to auxin (shown in green; n = 130 hermaphrodites per stage) starting at each of the four larval

481 stages (L1 – L4) and first two days of adulthood (D1 – D2). Spermatogenesis (highlighted in

482 blue) occurs during L4 and continues approximately 30 minutes into adulthood (Hirsh *et al.*

483 1976). When exposed to auxin during larval development, all hermaphrodites were self-sterile.

484 However, adults exposed to auxin were fully fertile. B) Hermaphrodites were exposed to auxin

485 at L4 and transferred off auxin in 30 minute increments starting at adulthood (n = 50

486 hermaphrodites per stage). All worms were self-sterile, indicating that L4 alone is sufficient to

487 induce self-sterility.

488 **Figure 2.** Self-sterile PX627 hermaphrodites (green) can recover their fertility when mated with

489 a wildtype male, as compared to *fog-2* functional females (gray). Each bar within a genotypic set

490 represents an independent replicate. While self-sterile hermaphrodites produced fewer progeny

491 than *fog-2* females (z = -2.78, p < 0.01), their progeny production was invariable across

492 replicates (t = -0.097, df = 40.73, p = 0.92).

493 **Figure 3.** Auxin exposure induces male sterility. A) Males were exposed to auxin (shown in

494 green; n = 100 males per stage) during larval development. Spermatogenesis (highlighted in

495 blue) begins during L4 and continues throughout adulthood (L'Hernault 2006). Males exposed to

496 auxin prior to L4 were sterile, however, males exposed to auxin at L4 produced a low number of

497 progeny. B) Males exposed to auxin throughout larval development up until day 1 (D1; n = 30)

498 or day 2 (D2; n = 30) of adulthood recovered their fertility when removed from auxin in a time-

499 dependent manner. However, not all males removed from auxin at day 2 of adulthood became

500 fertile (n = 16). C) Adult male sterility could be induced by exposing males to auxin at day 1 of

501 adulthood. All males were sterile within 24 hours (n = 44).

21

502   **Figure 4.** Lifespan curves comparing FUdR sterility to auxin-induced self-sterility. Wildtype (N2,

503   black) and (PX627, purple) adults were FUdR treated. PX627 individuals were exposed to auxin

504   from egg to day 1 of adulthood (dark green) or during the L4 stage alone (light green). Each

505   survivorship curve represents six to eight pooled replicates each with over 100 individuals. The

506   survivorship profiles were very similar across treatments and genetic backgrounds, though the

507   PX627 L4 auxin treatment showed a quantitatively distinct profile.

A

Control / Auxin

Fertile

Sterile

L1   L2   L3   L4   D1

0   10   20   30   40

Developmental Stage at Exposure (hr)

B

Auxin   No Auxin   fertile

sterile

Auxin No Auxin   fertile

sterile

L1   D1   D2   D3   D4

0   44   50   60   70   80   90   100   110   118

Developmental Stage (hr)

C

None   Auxin

fertile

sterile

L1   D1   D2

0   44   50   60   70

Developmental Stage (hr)