Selection and Maintenance of Androdioecy in *Caenorhabditis elegans*

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ABSTRACT

Caenorhabditis elegans is an androdioecious nematode composed of selfing hermaphrodites and rare males. A model of male maintenance demonstrates that selfing rates in hermaphrodites cannot be too high or else the frequency of males will be driven down to the rate of spontaneous nondisjunction of the X chromosome. After their outcrossing ability is assessed, males are found to skirt the frequency range in which they would be maintained. When male maintenance is directly assessed by elevating male frequency and observing the frequency change through time, males are gradually eliminated from the population. Males, therefore, appear to reproduce at a rate just below that necessary for them to be maintained. Populations polymorphic for a mutation (*fog-2*) that effectively changes hermaphrodites into females demonstrate that there is strong selection against dioecy. Factors such as variation in male mating ability and inbreeding depression could potentially lead to the long-term maintenance of males.

ANDRODIOECY is a sexual system composed of males and hermaphrodites. Androdioecy has been well studied in terms of theories predicting conditions under which it could evolve and be maintained (Lloyd 1975; Charlesworth and Charlesworth 1978; Charlesworth 1984; Otto *et al.* 1993; Pannell 1997a). Unfortunately, finding functional androdioecy in natural populations has proved difficult. Many species have been suggested to be androdioecious, but with few exceptions, they have proved to be functionally dioecious (having separate sexes; Charlesworth 1984; Pannell 1997b; Vassiliadis *et al.* 2000). To date only a few plants, including *Datisca glomerata* (Liston *et al.* 1990), *Mercurialis annua* (Pannell 1997b), *Schizopepon bryoniaefolius* (Akimoto *et al.* 1999), and *Fraxinus ornus* (Bertand *et al.* 1999), and two shrimp genera, Eulimnadia (Sassaman 1989; Weeks *et al.* 1999, 2000) and Triops (Sassaman 1991), have been clearly demonstrated as androdioecious. Here, we suggest that many nematode species, including *Caenorhabditis elegans*, should also be considered to be androdioecious, and we evaluate the conditions under which males can be maintained in these populations.

Why is androdioecy so rare? For a male to compete in a population of self-incompatible hermaphrodites, it must be able to sire twice as many offspring as the hermaphrodites (Lloyd 1975; Charlesworth and Charlesworth 1978; Otto *et al.* 1993). Furthermore, if hermaphrodites are capable of even a little selfing, males must account for an even greater proportion of the offspring to compensate (Lloyd 1975; Charlesworth and Charlesworth 1978; Charlesworth 1984; Otto *et al.* 1993). Finally, androdioecious populations will tend to maintain males at such a low frequency that they may not be easily recognized as such (Charlesworth 1984). Thus, it is perhaps not surprising that few examples of androdioecy are known.

Androdioecy in *C. elegans*: *C. elegans* is a protandrous nematode comprised of both selfing hermaphrodites and males. All individuals go through four distinct larval (L1–L4) stages and an adult stage. Hermaphrodites (XX) undergo spermatogenesis at the L4 stage (Wood 1988) and produce between 270 and 373 sperm (Hodgkin and Barnes 1991) in the standard laboratory strain N2. After the L4 molt, spermatogenesis is irreversibly stopped and oogenesis starts. Hermaphrodites are completely self-compatible, utilizing their sperm with almost 100% efficiency (Ward and Carrel 1979), but are incapable of outcrossing with other hermaphrodites. Males arise spontaneously through nondisjunction of the X chromosome during gametogenesis and the subsequent fusion with a normal gamete, yielding an XO individual. Males begin spermatogenesis at the L4 stage and continue throughout adulthood. Males are capable of outcrossing with hermaphrodites by injecting their sperm through the vulva via a specialized mating structure (fan) on the tail. In standard laboratory populations, males are maintained through nondisjunction at a frequency between 0.001 (Ward and Carrel 1979) and 0.002 (Hodgkin and Doniach 1997).

The extremely low frequency of males observed in laboratory populations is seemingly at odds with many favorable features of male behavior and function. Simple observation of males reveals that they attempt to mate with every individual they contact. Hermaphrodites also display no obvious avoidance of mating with

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males even though it significantly shortens their life span (Gems and Riddle 1996). Males, however, do not suffer decreased life span due to mating or sperm production (Gems and Riddle 1996; but see Van Voorhies 1992). Furthermore, male sperm physically displaces sperm produced and stored by hermaphrodites (Ward and Carrel 1979; Singson *et al.* 1999). In fact, virtually all offspring are outcrossed after mating (Hodgkin and Barnes 1991). Moreover, sperm production in hermaphrodites is limited to ~300 sperm (Ward and Carrel 1979; Hodgkin and Barnes 1991; A. D. Stewart, unpublished results), while males have no such limitation, so that outcrossing can yield two to four times the offspring normally obtained through selfing (Hodgkin and Barnes 1991; A. D. Stewart, unpublished results). Still, despite all these advantages, males have not been reported to persist in laboratory populations at a frequency >0.001, the rate of spontaneous male production.

The presence of males in this and another similar nematode species is therefore something of a paradox. They are not maintained in appreciable frequencies, yet a large portion of the genome is devoted to male function (Jiang *et al.* 2001). This study presents the first step along the path to addressing this paradox. First, a model analyzing the factors affecting the maintenance of males is developed. Second, frequency-dependent male mating success is measured. Third, the rate of loss of males in a laboratory population is assessed. And finally, the influence of introducing a mutation leading to female-only function on the maintenance of males is examined. These studies combine to produce a picture of male function in these laboratory-adapted populations and provide the basis for future experimental studies examining the factors influencing the evolution of androdioecy.

Androdioecy to dioecy: *C. elegans* is an especially appropriate organism for exploring the evolution of androdioecy, because there are a large number of mutations that change the functional sex of individuals (see Schedl and Kimble 1988; Clifford *et al.* 1994). *fog-2* (*f*eminization *o*f the *g*erm line) is a recessive mutant in a gene believed to be responsible for male somatic sex determination in hermaphrodites (Schedl and Kimble 1988). In hermaphrodites homozygous for the *fog-2* allele, sperm production is totally inhibited, making them functionally females. However, sperm production is unaffected in males (Schedl and Kimble 1988). When the *fog-2* allele has become fixed, the populations are functionally dioecious and males are maintained at ~50% through outcrossing and XO males producing nearly equal frequencies of X-bearing and O-bearing gametes (A. D. Stewart, unpublished results). The prediction for mixed populations of hermaphrodites and *fog-2* females is that males should perform better because of this obligate outcrossing and potentially be maintained in contrast to populations lacking the mutation.

MATERIALS AND METHODS

The model: General models for the evolution of androdioecy must include the selfing rate, fecundity through hermaphroditic and male fertilization, and the frequencies of the two classes of individuals (*e.g.*, Ross and Weir 1976; Charlesworth 1984). Hedgecock (1976) provides a model of male frequency change in *C. elegans*, but his analysis requires a number of equilibrium assumptions (*e.g.*, equality of the reproductive potentials of males and hermaphrodites) that limit its applicability in the current case. Otto *et al.* (1993) developed a comprehensive model for the slightly more complex case of autosomal sex determination. Differences in *C. elegans* include hemizygous sex determination and production of males directly from hermaphrodites via chromosomal nondisjunction. Modifying the Otto *et al.* (1993) model using the mating table shown in Table 1, the change in male frequency (m) over time is given by

$$m' = (1-\sigma)(1+2u)\alpha m/2T + \beta(1-\delta)(1-\alpha m)u/T, \tag{1}$$

where $T = \alpha m[1-\sigma(1/2+u)] + \beta(1-\delta)(1-\sigma u)(1-\alpha m)$, α is equal to the fertilization success of males (αm is the proportion of female eggs that are outcrossed, $0 \leq \alpha m \leq 1$), β is equal to the proportion of eggs not fertilized by males that are self-fertilized, σ is equal to the relative viability difference between males and hermaphrodites, δ is equal to the degree of inbreeding depression in selfed offspring, and u is the rate of nondisjunction at the X chromosome (*cf.* Otto *et al.* 1993, Equations 1 and 2). The existence of nondisjunction ensures that the males will always be maintained in the population at least on the order of the rate of nondisjunction (u; Chasnov and Chow 2002; A. Cutter, personal communication). Since u is known to be very low, it is easier to examine potential equilibria at higher frequencies by assuming that the contribution by nondisjunction to these equilibria is negligible. Under this parameterization, the equilibrium condition for the maintenance of androdioecy,

$$\alpha(1-\sigma) > 2\beta(1-\delta), \tag{2}$$

is the same in both the autosomal and hemizygous models (Otto *et al.* 1993, Equation 4). Thus, depending on the degree of inbreeding depression, for males to be maintained other than at a nondisjunction-selection equilibrium they have to be significantly better at fertilizing eggs than hermaphrodites. Since inbreeding depression is thought to be fairly minor in *C. elegans* (Johnson and Wood 1982; Chasnov and Chow 2002), most of the influence on the equilibrium is likely to be through the relative fertilization success of males *vs.* hermaphrodites.

Equation 2 is very useful for illustrating the underlying factors that are important in maintaining males. If the parameters are allowed to become frequency dependent, however, potential solutions become more complex (Otto *et al.* 1993). It is clear that the selfing rate in hermaphrodites must in general be frequency dependent because when the frequency of males is low it is unlikely that the males will be able to mate with all of the available hermaphrodites. This is especially the case in a species like *C. elegans*, where hermaphroditic sperm production precedes egg production (Wood 1988). Hermaphrodites that remain unmated shortly after reaching sexual maturity are therefore very likely to produce selfed offspring. As the frequency of males increases, the encounter rate between males and hermaphrodites should also increase, driving down the effective selfing rate. In *C. elegans*, however, the timing of sperm production makes it likely that there will always be some residual selfing regardless of male frequency, if only because some hermaphrodites go temporarily unmated just by chance. Some of the frequency dependence of this

TABLE 1

Mating table

	Frequency	Inbreeding	Offspring XX ⚥	Offspring XO♂
Mating type				
XX ⚥ with XO ♂	$\alpha m(1 - m)$	1	$1/2 - u$	$1/2 + u$
XX ⚥ selfed	$\beta(1 - m)(1 - \alpha m)$	$1 - \delta$	$1 - u$	u
Viability			1	$1 - \sigma$

situation is encompassed in the above model since the selfing rate is itself frequency dependent [$\beta(1 - m)(1 - \alpha m)$; Table 1]. The underlying parameters themselves may also be frequency dependent, however. In particular, male fertilization success (α) may strongly depend on male frequency. For instance, mating success may be higher when males are rare than when they are common, especially because of male-male interference.

In the hemizygous model, we can solve for the frequency-dependent dynamics for male frequencies greater than the nondisjunction rate directly by noting that any male offspring in the population must be the result of a male-hermaphrodite cross. In the absence of meiotic drive (see LaMunyon and Ward 1997) and barring rare nondisjunction events, one-half of the offspring of a male-hermaphrodite cross will be males, and the frequency change of males across generations is given by

$$m' = (1 - s[m])/2, \tag{3}$$

where $s[m]$ is the frequency-dependent hermaphroditic "selfing rate" (the fraction of all fertilized eggs that are self-fertilized). Here $s[m]$ is a composite of the mating and viability parameters given above, generalized to be frequency dependent. Rather than attempt to fully parameterize this model in terms of its component parts, we use a graphical approach that is well suited for empirical estimation.

The selfing function $s[m]$ can in principle take any form, although it is constrained by the condition that $s[0] = 1$. For example, one trivial equilibrium can occur when the selfing rate approaches 0 as the male frequency becomes high ($s[m] \rightarrow 0$ as $m \rightarrow \frac{1}{2}$), which results in a stable male frequency of $m = \frac{1}{2}$, or what would effectively be a dioecious population. In practice, however, a great number of stable and unstable equilibria are possible depending on the form of $s[m]$. Rather than working directly with $s[m]$, we have found it easier to use the entire left-hand side of Equation 3, which describes male frequency in the next generation as a function of the male frequency in the current generation. We call this the male maintenance function (MMF), because the male/hermaphrodite dynamics and equilibria can be described by the relationship between this function and the line of equilibrium, $m' = m$. This general approach allows all of the potential influences on selection for or against males, including nondisjunction, to be determined empirically.

The MMF behaves in a very similar manner to a Ricker recruitment curve or return function used to describe population size dynamics (May and Oster 1976; Murray 1993). As shown in Figure 1A (using a complex curve chosen for illustrative purposes only), any time m' goes above the equilibrium line, the population will evolve back toward it in the direction of increasing frequency, whereas when m' goes below the line, the population will evolve back toward it in the direction of decreasing frequency. Thus, points where the MMF crosses the line starting above and ending below will be stable equilibria, while crossing points starting below and ending above will be unstable equilibria (Figure 1). Equation 1 yields MMFs with a single inflection point, which will either generate the Equation 2 equilibrium or not, depending on whether the curve starts above or below the equilibrium line. Equation 3 and the graphical analysis allows for potentially more complex equilibrium relationships.

MMF measurement: Estimation of the MMF requires measuring the change in male frequency from one generation to the next. Four male-enriched populations of the N2 (Bristol) strain of *C. elegans* were each started from three hermaphrodites and 20 males and grown on 6-cm nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50 (Brenner 1974). From these plates, large numbers of eggs (>200) were selected at random and "picked" with a platinum wire onto five or six seeded 6-cm plates. Maintained at 20°, the worms were then sexed at the L4 stage and allowed to lay eggs. The sex ratio in this population served as an estimate of male frequency in the "parental" population (m). To avoid food depletion and to maintain constant density, the worms from each plate were suspended in ~1 ml of M9 solution, and a 300-μl fraction of this solution was transferred to a fresh plate when the progeny reached the L2 stage. The offspring developed to the L4 stage and were sexed, providing an estimate of male frequency in the "offspring" population (m'). This procedure was repeated 40 times using the same male-enriched population over a period of 4 weeks, allowing a wide variety of initial male frequencies to be sampled. Approximately 400 individuals per replicate were assayed for male frequency in two independent estimates, in both the parental and offspring generations, yielding a total of 70,753 individuals assayed.

Because we had no *a priori* expectation as to the shape of the response curve, we used the best-fitting polynomial derived from a stepwise regression to fit the data (Sokal and Rohlf 1994).

Male and *fog-2* frequency assessment: Two male-enriched populations of the N2 strain of *C. elegans* were prepared as above. From these matings 150 eggs were selected at random, picked onto each of five 10-cm seeded NGM agar plates, and maintained at 20°. After maturing to the L4 stage, all individuals were sexed and allowed to lay eggs. Their progeny, likewise, matured to the L4 stage and were sexed. Because of the large number of worms (~1500/plate), all subsequent sexing was done using a cross-secting grid representing ~20% of the total plate area. To avoid food depletion and to keep the population size constant at ~1500 worms/plate, the worms from each plate were suspended in ~1 ml of M9 solution and a fraction of this solution (between 200 and 350 μl) was transferred to a fresh plate. The worms were sexed and transferred 6 of every 7 days. During transfers, all five lines were kept independent. The lines were maintained for 54 days, or ~15 generations.

For the *fog-2* competition assays, five independent lines were established and maintained for ~15 generations as above except that eggs from hermaphroditic N2 and dioecious *fog-2*

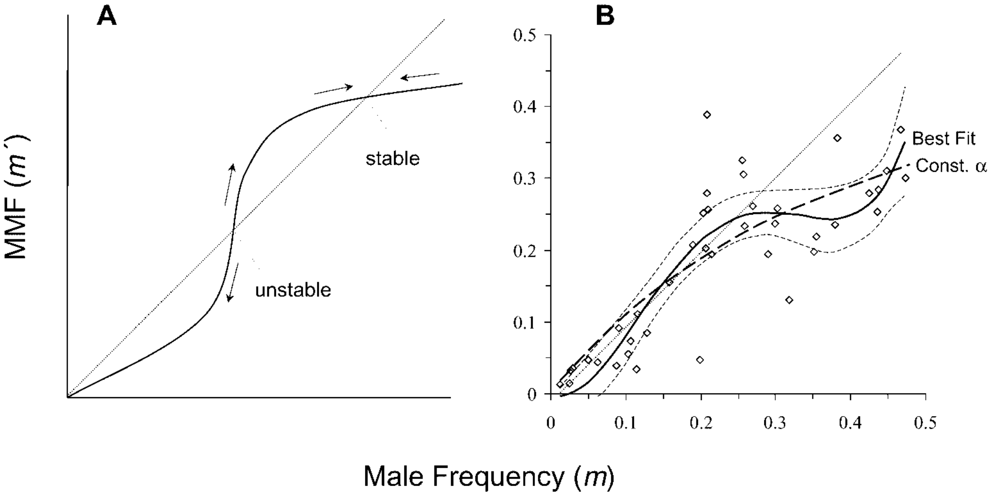

Figure 1.—Male maintenance function for an androdioecious population. (A) A schematic representation of male frequency dynamics as theoretically predicted by the MMF. The dotted line gives the line of equilibrium at which there is no change in male frequency from one generation to the next ($m' = m$). If the MMF falls above this line, then the population will evolve toward points of higher male frequency (*i.e.*, male frequency is predicted to increase each generation). When the MMF falls below the equilibrium line, then the population evolves toward points of lower frequency. Stable and unstable equilibrium points are both possible, depending on the orientation of the MMF as it crosses the equilibrium line. This curve was chosen for illustrative purposes only and is not based on any theoretical prediction. (B) Experimental assessment of the MMF in *C. elegans*. The ability of males to outcross is observed (open diamonds) to vary with male frequency. Given an initial male frequency, m, the quartic function fit to the observed data predicts the frequency of males in the offspring, m' (solid curve). Also depicted is the 95% confidence interval for the quartic fit curve (dotted curves). The equation for the quartic curve is given as $m_1 = \beta_1 m + \beta_2 m^2\ \beta_3 m^3 + \beta_4 m^4 + \varepsilon$ with the y-intercept set to 0 (Table 2). For male frequency to remain constant, the quartic curve must cross the maintenance line (dashed line). Any point where such a crossing exists (0, 0.14, 0.24) is predicted to be an equilibrium point, although the confidence region includes many points on either side of the maintenance line. The least-squares best fit of the model with frequency-independent parameters (Equation 1) is shown as the boldface dashed line ($\alpha = 0.52$, $\beta = 0.2$, $\sigma = 0$, $\delta = 0$, $u = 0.002$).

populations were combined at the outset (50 N2 and 100 *fog-2* eggs, *i.e.*, 33% of each sex), creating what may be called a "trioecious" population. Female *fog-2* individuals lay only one unfertilized egg every 8 hr if they are not mated (T. Schedl, personal communication, 1997), with the great excess of unlaid maturing oocytes accumulating in the ovaries. As more oocytes mature, they visibly compact against each other until they resemble the keys of a piano. This "piano effect" allows the *fog-2* gene frequency to be assessed using offspring production and self-fertility criteria as described in Figure 2. The *fog-2* populations used below were derived from an N2 (wild-type) background and backcrossed into N2 for eight generations (Schedl and Kimble 1988). These stocks were kindly provided by Tim Schedl (Washington University, St. Louis).

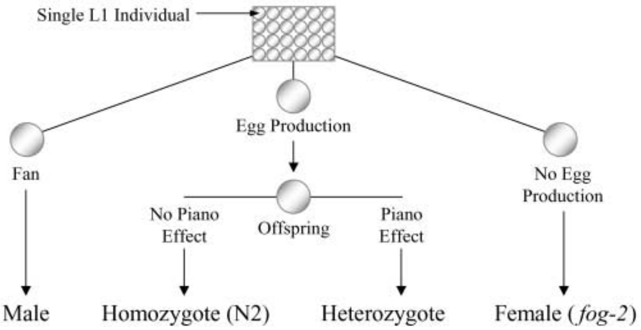

Figure 2.—Schematic overview of the *fog-2* gene assay. Young larval individuals were placed into wells. If they developed male morphology (fans) or had hermaphroditic morphology but laid no eggs (piano effect), they were classified, respectively, as males or females. True hermaphrodites laid eggs and were classified as homozygous wild type or heterozygous by whether their offspring all laid eggs (homozygotes) or some did not (heterozygotes).

The count data were analyzed using a logistic regression approach with the CATMOD procedure of SAS (SAS Institute 1988). The model used is analogous to an analysis of covariance using categorical data (Hagenaars 1990). A completely factorial model was used with line as a main effect and day as a logistic covariate. Residuals displayed no significant serial autocorrelation, so no autocovariance function was fitted to the data (Chatfield 1984). Roughly 3000 individuals were counted per day across the five lines for a total of 112,977 and 193,512 individuals sexed in the androdioecious and *fog-2* assays, respectively. Parameter solutions and significance tests were obtained using an iterative maximum-likelihood approach (SAS Institute 1988).

RESULTS

MMF measurement: Measurement of the single generational change in male frequencies shows that males often maintain or even occasionally increase in frequency depending on their frequency in the initial generation (Figure 1B). The maintenance line (slope = 1) shows the points at which males would replace themselves in the population every generation and, thus, maintain their current frequency. From the model developed above, any point where the MMF crosses the maintenance line is predicted to be an equilibrium point (Figure 1A). A quartic polynomial was found to be the best-fit curve using a stepwise approach and accounted for the majority of variation among the observations ($r^2 = 0.93$; Table 2). Adding more terms did not significantly increase the fit of the model. This curve suggests possible stable equilibria at male frequencies of 0 and 0.24 and an unstable equilibrium at 0.14. How-

TABLE 2

Regression summary for the observed MMF data

Variable	Estimate	Standard error	*t* value	*P* value
m	−0.417	0.652	−0.64	0.5266
m^2	18.609	7.385	2.52	0.0163
m^3	−72.167	26.327	−2.74	0.0095
m^4	80.283	29.359	2.74	0.0096

m, frequency of males.

ever, the 95% confidence interval around this predicted line includes area on either side of the maintenance line, so the existence of an equilibrium is not strongly predicted by this data. Instead, the striking feature of the MMF is the way in which male reproductive success causes the population to skirt the maintenance line when male frequency is $< \sim 25\%$ and then fall well below this line when the male frequency is $>25\%$.

The frequency-independent model given by Equation 1 can also be fit to the data. Using a nondisjunction rate of $u = 0.002$ (Hodgkin and Doniach 1997) and assuming negligible inbreeding depression ($\delta = 0$; Johnson and Wood 1982; Chasnov and Chow 2002), the least-squares estimators of the other parameters are $\alpha = 0.52$, $\beta = 0.20$, and $\sigma = 0$ ($r^2 = 0.69$; Figure 1B). In practice, u has virtually no effect on the results as long as it is small. This model predicts that males should be maintained in the population at an equlibrium frequency of $\sim$0.16 (see also Equation 2). If the empirically fit quartic curve above is taken as the "true" maintenance function and it is assumed that only male fertilization success (α) varies with male frequency, then the frequency-dependent form of Equation 1 can also be fit. This approach suggests that male fertilization success is low when male frequency is low, reaches a maximum around $m = 0.2$, and then declines again as male frequency increases (Figure 3).

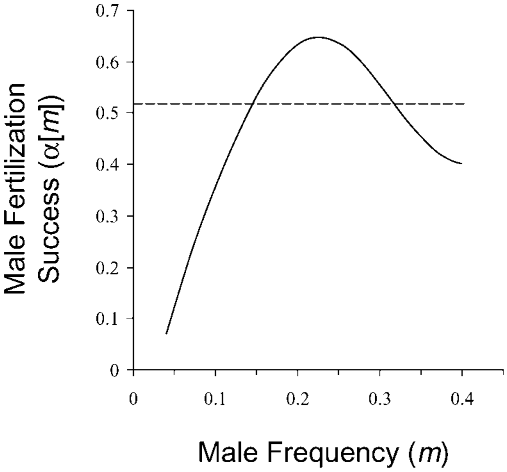

Figure 3.—Frequency-dependent male fertilization success. The fit of Equation 1 to the male-maintenance function data when α is assumed to be fixed (dashed line) or allowed to be frequency dependent (solid curve). The qualitative shape of the curve is fairly independent of the values of the other parameters in the model.

Maintenance of males in laboratory populations: In the androdioecious populations, male frequency rapidly declines in the five lines over time and shows no evidence of an equilibrium (Figure 4A). The starting male frequencies were all near the desired 0.50, with the average across the five lines being 0.45 (SD = 0.02).

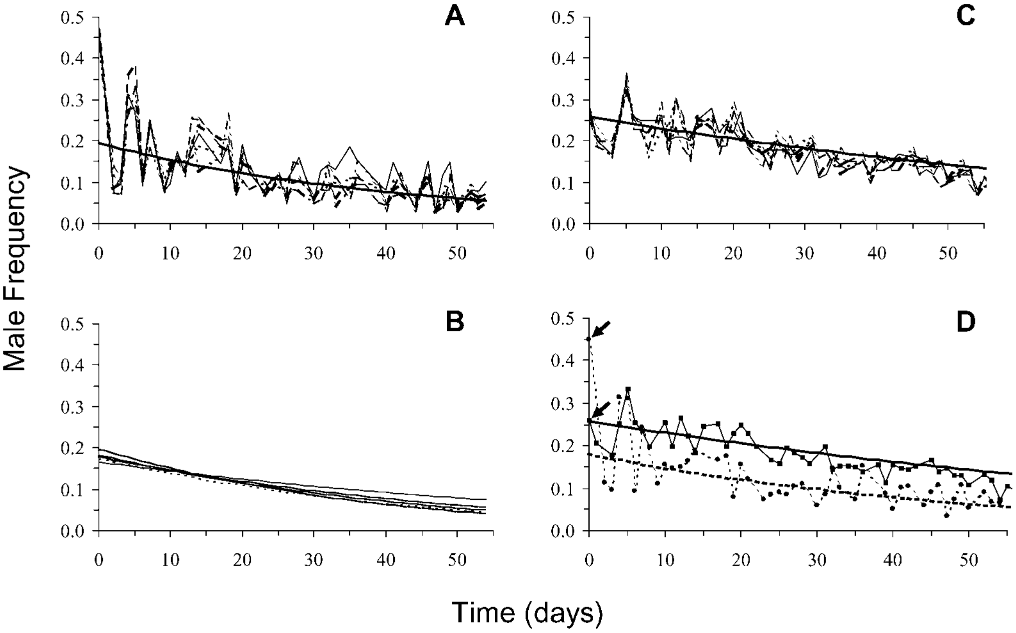

Figure 4.—Decline in male frequency over time. (A) Androdioecious population. Male frequency of the five populations (dashed lines) is seen to fluctuate but generally decline in an approximate exponential decay over the 54 days of measurement. The solid line is the model fit using the logistic regression (Table 3). (B) The line-by-day interaction results largely from the crossing of the individual logistic fit curves for each of the five androdioecious replicates. However, the amount of crossing and gross differences across these lines is seen to be slight. (C) Trioecious population including the *fog-2* mutation. (D) Average responses shown in A and C. Dashed lines are the average and fitted frequencies for the androdioecious populations; solid lines are average and fitted frequencies for the *fog-2* populations. Note the similarity in the rate of loss but the difference in the initial degree of loss (arrows).

TABLE 3

Logistic regression summary for the male frequency assessment of androdioecious and trioecious populations

		Androdioecious		Trioecious (*fog-2*)	
Source of variation	d.f.	χ^2	*P*	χ^2	*P*
Intercept	1	7570.6	<0.0001	6863.0	<0.0001
Day	1	1754.3	<0.0001	3102.7	<0.0001
Line	4	32.8	<0.0001	16.3	0.0027
Day × line	4	82.5	<0.0001	3.6	0.4677

Over the course of the experiment, the male frequency dropped to an average of 0.07 (SD = 0.02). In general, the males are lost with an approximately exponential decay (Figure 4A). All five lines showed very similar declines throughout the experiment, although they did significantly differ from one another (Table 3). This effect is most likely caused by initial sampling variance in the frequency of males. The lines behaved qualitatively similar to each other (Figure 4B), although there is some slight crossing of the decay trajectories, which leads to a significant day-by-line interaction (Table 3). There are large synchronized fluctuations in frequency, particularly at the outset of the experiment, which may be caused by age structure effects. All individuals began as eggs at the start of the experiment but after a few weeks the entire gamut of developmental stages was observed interacting simultaneously.

The male frequencies in the five lines also declined in the trioecious populations including *fog-2* and showed no evidence that males would be maintained (Figure 4C; Table 3). The starting male frequencies were all somewhat lower than the theoretical 0.33, with the average across the five lines being 0.26 (SD = 0.01). Over the course of the experiment, the male frequency dropped to an average of 0.10 (SD = 0.02). In general, the males were again lost in an approximate exponential decay (Figure 4C). Despite beginning at initially lower frequencies, males in the trioecious population were on average maintained at higher frequencies than in the dioecious population (Figure 4D).

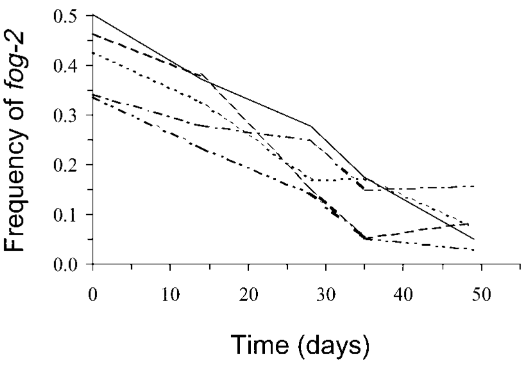

FIGURE 5.—Gene frequency change of an allele causing trioecy. *fog-2* gene frequencies of the five replicate populations are all seen to sharply decline over the course of the experiment. While there is variation visible between lines, all five lines show nearly the same magnitude of *fog-2* decrease.

***fog-2* gene assay:** The frequency of the *fog-2* allele showed an extremely steep decline over the course of the experiment (Figure 5). The initial *fog-2* frequency in the population was calculated by doubling the initial frequency of males, since all males were from the *fog-2* population, where they exist in a 50:50 ratio. After 56 days in the presence of hermaphrodites, the *fog-2* allele frequency in hermaphrodites and females dropped to an average of 0.08 (SD = 0.05), a sixfold decline. Thus, the *fog-2* allele was lost at the rate of ~0.7% per day or ~2.5% per generation.

DISCUSSION

Male maintenance: Under normal laboratory conditions, *C. elegans* males are selected against and reduced to low frequencies (Figure 4A). This result agrees with anecdotal evidence from many researchers and laboratories. The regression model predicts that males will be reduced to their nondisjunction equilibrium after ~200 days or ~57 generations. The best-fit line for the MMF predicts a possible equilibrium point ~25%, although the confidence region around this curve includes large areas in which there would be no equilibrium. Combined with potential stochastic variation generated by intergenerational effects, it is perhaps not surprising that the populations might move in and out of an equilibrium condition, if one were to actually exist. Simulation studies of stochastic variation around the estimated MMF confirm that males are frequently lost in fluctuating populations even when there is a deterministic equilibrium (results not shown). Results from the selection experiment clearly demonstrate the absence of an equilibrium. Thus, males are not maintained in these populations but are lost at a rate just beyond that required for their persistence.

Some of the complex shape of the MMF can be explained by frequency dependence in male fertilization success (Figure 3). For example, it is possible that when males are in low frequency, more hermaphrodites would be able to self and their offspring would be hermaphrodites only. Therefore, males at low frequencies would be effectively diluted from the population despite their ability to sire two to four times the offspring through outcrossing than a selfing hermaphrodite (Hodgkin and Barnes 1991; A. D. Stewart, unpublished data). This is as likely to be a density-dependent as frequency-dependent effect. Additionally, males are also quite indiscriminate in their attempts to mate. As male frequency rises, males could be interfering with each other's ability to mate, driving down male fertilization success at higher male frequencies. Weak male fertilization success at low frequencies makes it especially unlikely that they can move beyond the nondisjunction-selection equilibrium to become more prevalent in the population (see also Chasnov and Chow 2002).

It is possible that males from other *C. elegans* strains will have different mating abilities that could allow them to be maintained at higher frequencies. Males from different natural isolates do have differences in fertility and mating characteristics (Hodgkin and Doniach 1997), and a number of different genes are known to affect mating ability (Hodgkin 1983). Examining Equation 2 shows that differences in male mating ability will affect the left-hand side of the equilibrium equation, with more capable males making stable androdioecy more likely.

Selection against dioecy: Despite its introduction at a high initial frequency, a mutation leading to dioecy (*fog-2*) was rapidly eliminated from each of the five replicate lines (Figure 5). It therefore seems unlikely that such a mutation would be able to invade this androdioecious population. Indeed, neither females nor males are maintained in the populations, which would be predicted to become almost exclusively hermaphroditic after ~400 days or 115 generations. The overall rate of loss of males with and without *fog-2* is approximately the same (Figure 4D), but the initial loss is much less in the presence of *fog-2*, leading to an ~1.4-fold increase in male frequencies over time and a doubling in the predicted time to eventual elimination. This shift is most likely caused by the presence of the *fog-2* females, which can only reproduce by outcrossing with males and which produce ~50% males. Similar results have been obtained by Chasnov and Chow (2002), who demonstrated strong selection against a mutation in the *him-5* gene that leads to a higher rate of nondisjunction (and therefore males).

Inbreeding depression and the evolution of androdioecy: The major feature of the Equation 2 equilibrium that is likely to be lacking in this laboratory population is inbreeding depression. Inbreeding depression increases the fitness impact of male mating and can be a major force in the maintenance of outcrossing and males in androdioecious species (Charlesworth 1984; Otto *et al.* 1993; Rieseberg *et al.* 1993; Weeks *et al.* 1999, 2000). The N2 strain used in this study has been maintained in the laboratory for many generations under high levels of selfing and does not show much evidence of inbreeding depression (Johnson and Wood 1982; Chasnov and Chow 2002). Natural isolates may have higher frequencies of deleterious alleles that would increase the influence of inbreeding depression on the maintenance of males. There does appear to be variation among isolates in the number of males produced and maintained within populations (P. Phillips, personal observation). The fact that the MMF curve comes fairly close to maintenance levels suggests that alternative equilibria are at least possible in natural populations and could influence the evolution of the nondisjunction rate itself (Chasnov and Chow 2002).

Estimation of the among-population variation in the parameters that influence the evolution of androdioecy still needs to be addressed. More importantly, the question of at which frequencies males actually exist in natural populations remains completely unknown at this point. The environmental context in which selection on males occurs is also likely to be important. The laboratory environment used here is obviously artificial, although one might expect the male-hermaphrodite encounter rate to be much lower in the soil. Nevertheless, we can make the strong prediction on the basis of this study that increasing the level of inbreeding depression in this system should readily lead to equilibrium conditions in which males can be maintained, perhaps even leading to conditions that would favor dioecy via the *fog-2* mutation.

The vast majority of nematodes are, in fact, dioecious, with Rhabditids being the major exception (Lee 1965; Jenkins and Taylor 1967; Nicholas 1984). Although well-rooted phylogenies are currently lacking for Rhabditiae, it is very likely that they evolved from a dioecious ancestor (Fitch *et al.* 1995; Blaxter *et al.* 1998). Furthermore, there are both dioecious and androdioecious species within Caenorhabditis itself (Fitch and Thomas 1997). *C. elegans* thus provides an ideal system both for understanding the proximal basis of the genetics of sex determination (Haag and Kimble 2000; Kuwabara and Perry 2001) and for testing theories relating to major transitions in mating system evolution.

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