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The proximate determinants of sex ratio in *C. elegans* populations

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Summary

The soil nematode *Caenorhabditis elegans* is an example of a species in which self-fertilizing hermaphrodites predominate, but functional males continue to persist – allowing outcrossing to persevere at low levels. Hermaphrodites can produce male progeny as a consequence of sex chromosome non-disjunction or via outcrossing with males. Consequently, the genetics of sex determination coupled with the efficiency by which males find, inseminate and obtain fertilizations with hermaphrodites will influence the frequency at which males and outcrossing occurs in such populations. Behavioural and physiological traits with a heritable basis, as well as ecological characters, may influence male reproductive success and therefore sex ratio. Because sex ratio is tied to male reproductive success, sex ratio greatly affects outcrossing rates, patterns of genetic variation, and the ability of natural selection to act within populations. In this paper we explore the determinants of male frequency in *C. elegans* with a mathematical model and experimental data. We address the role of the genetic machinery of sex determination via sex chromosome non-disjunction on sex ratio and the influence of physiological components of *C. elegans*' life history that contribute to variation in sex ratio by way of male reproductive success. Finally, we discuss the short-term and long-term factors that are likely to affect sex ratio and breeding system evolution in species like *C. elegans*.

1. Introduction

The bacteriophagous soil nematode *Caenorhabditis elegans* exhibits an androdioecious mode of reproduction: *C. elegans* hermaphrodites are self-fertile, but can outcross only with males. It is conceivable that a simple genetic basis underlay the evolution of androdioecy from gonochorism (male and female sexes) in *C. elegans*' ancestors, because, for example, gonochorism can be restored by loss-of-function mutation of the *fog-2* gene (Schedl & Kimble, 1988; Hodgkin, 2002). Self-fertilizing hermaphrodites and parthenogenesis have arisen independently multiple times from gonochoric ancestors within the Rhabditidae (Fitch & Thomas, 1997), although the forces favouring different reproductive modes in these taxa remain unknown. In species with modes of sex determination like *C. elegans*, the relative contribution of self-fertilization and outcrossing to progeny production in natural populations is directly related to sex ratio. Thus, regardless of the origins of androdioecy, the relative frequency of the two sexes influences the degree of outcrossing, the amount of standing genetic variation and population subdivision, and the strength with which natural and sexual selection may operate.

The relatively rare phenomenon of androdioecy among animals occurs in *C. elegans*, in part, as a consequence of its sex-determination mechanism. Like *Drosophila* but unlike mammalian species (Marin & Baker, 1998), the well-characterized sex-determination pathway in *C. elegans* depends primarily upon the ratio of sex chromosomes to autosomes (X : A) (Brenner, 1974; Hodgkin, 1987; Cline & Meyer, 1996; Meyer, 2000). Hermaphrodite individuals exhibit $X:A \geqslant 1$ by harbouring two copies of each of the five autosomes and two (or rarely three) X chromosomes. Males, on the other hand, contain only a single X chromosome in addition to their diploid complement of autosomes, resulting in $X:A = \frac{1}{2}$.

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C. elegans hermaphrodites differ from females only in that the germ line transiently produces sperm prior to an irreversible switch to oogenesis (Ward & Carrel, 1979; Hodgkin, 1988; Kimble & Ward, 1988). This leads to self-fertilization in hermaphrodites, unless they mate with a male; morphology precludes mating between hermaphrodite pairs. The relative frequency of the different sexes is governed primarily by two forces: the ability of males to obtain fertilizations relative to a hermaphrodite's own sperm and the rate of random sex chromosome non-disjunction during hermaphrodite meiosis. Normally, each hermaphrodite gamete contains one X chromosome. However, occasional chromosomal non-disjunction events lead to the production of viable gametes that lack a sex chromosome (nullo-X), which, when fused with a normal mono-X-bearing self-gamete, results in the formation of a male zygote (XO) (Hodgkin *et al*., 1979). Such males are fertile and fully functional. Thus, rare chromosomal allocation errors during meiosis result in the spontaneous production of males by unmated, selfing hermaphrodites. Male worms produce nullo-X and X-bearing sperm with equal frequency, so male × hermaphrodite cross-progeny observe a 1 : 1 sex ratio (Hodgkin *et al*., 1979). Likewise, diplo-X gametes can result from sex chromosome non-disjunction which can lead to the formation of viable, fertile triplo-X hermaphrodites.

Among other factors, the competitive ability of male sperm inside the hermaphrodite reproductive tract will influence male reproductive success. Male sperm outcompete hermaphrodite self-sperm for access to oocytes within the spermathecae, as a consequence of their larger size and greater motility (Ward & Carrel, 1979; Lamunyon & Ward, 1995, 1998; Singson *et al*., 1999). This precedence of male sperm promotes male zygote formation via cross-fertilization relative to the rate at which male production would occur as a consequence of X non-disjunction events. However, hermaphrodite self-sperm will be used for fertilization prior to an insemination by a male or, if only a small number of male sperm are transferred, following depletion of male sperm. Such utilization of self-sperm for fertilization results in hermaphrodite broods that contain much fewer than 50% males on average.

Here, we explore these counteracting forces in a mathematical model to describe the expected equilibrium frequency of males in *C. elegans* populations. Although we focus on the biology of *C. elegans*, the general form of the model we derive applies to any species that exhibits a similar mechanism of sex determination and contains hermaphrodites that cannot mate with each other. We then apply a set of experiments to the model framework. In particular, we use the model to describe quantitatively the phenotypic effects of a mutant strain of *C. elegans* with elevated rates of X-chromosome-specific non-disjunction in hermaphrodites and reduced sperm count in males relative to these attributes in the canonical N2 strain.

Androdioecy enjoys extensive theoretical treatments relating specifically to plant mating systems (Lloyd, 1975; Charlesworth & Charlesworth, 1981; Charlesworth, 1984; Seger & Eckhart, 1996; Vassiliadis *et al*., 2000). However, models of plant androdioecy presume that hermaphrodite–hermaphrodite matings can occur – an assumption which *C. elegans* and other androdioecious animals violate. Sex ratio in *C. elegans* was first treated in a model that utilized Fisher's concept of reproductive value (Fisher, 1930; Hedgecock, 1976). Stewart & Phillips (2002) extended to *C. elegans* the population genetic modelling approach taken to explore androdioecy in the self-fertilizing clam shrimp *Eulimnadia texana* (Otto *et al*., 1993; Weeks & Zucker, 1999; Medland *et al*., 2000; Weeks *et al*., 2000). Another recent theoretical study developed a simple model of the influence of male reproductive ability and sex chromosome non-disjunction on sex ratio in *C. elegans* (Chasnov & Chow, 2002). However, all these approaches de-emphasize (or exclude) the biological mechanisms that underlie sex chromosome non-disjunction and its role in making males (and the alternative triplo-X genotype of hermaphrodites). Our treatment of male frequency in *C. elegans* provides a more complete mechanistic framework for sex ratio in this species that characterizes the dynamics of males and the two hermaphrodite genotypes (diplo- and triplo-X). This model applies both to populations of wild-type worms and to genetic mutants with extreme phenotypes. Males will be absent deterministically only when no non-disjunction occurs or when every gamete is the product of non-disjunction (which are both biologically implausible). Because sex chromosome non-disjunction is unlikely to be zero due to biochemical constraints, we focus on determining the positive equilibrium frequency of males.

2. A mechanistic model of sex ratio in *C. elegans*

(i) *Model formulation*

The following mathematical model characterizes the dynamics of the different genders in *C. elegans*. We model the formation of gametes by diplo-X hermaphrodites as described above such that X chromosome non-disjunction leads to the formation of diplo-X and nullo-X gametes with equal frequency ($\frac{1}{2}c$, where c is the rate of non-disjunction) in both sperm and oocytes (mono-X-bearing gametes thus occur with frequency $1-c$). This assumption of equivalent rates of X non-disjunction in hermaphrodite sperm and oocytes appears to be valid (Hedgecock, 1976). However, diplo-X gametes exhibit reduced viability relative to nullo-X and mono-X gametes (Hodgkin *et al*., 1979). To account for this difference in gametic

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Table 1. *The normalized sum of the frequencies of zygotes formed by selfing and crossing yield the composition of each zygote type in progeny* (*see equation 1*)

Zygote type	Gametes produced	Self-zygotes	Cross-zygotes
OO	0	$y_{OO}=(1-s)f_{XX}(h_O^2)+(1-s)f_{3X}(t_O^2)$	$z_{OO}=sf_{XX}(m_Oh_O)+sf_{3X}(m_Ot_O)$
XO	$m_O=\frac{v_O}{\hat{m}}$, $m_X=\frac{v_X}{\hat{m}}$, $\hat{m}=v_O+v_X$	$y_{XO}=(1-s)f_{XX}(2h_Oh_X)$ $+(1-s)f_{3X}(2t_Ot_X)$	$z_{XO}=sf_{XX}(m_Xh_O+m_Oh_X)$ $+sf_{3X}(m_Xt_O+m_Ot_X)$
XX	$h_O=\frac{v_Oc}{2\hat{h}}$, $h_X=\frac{v_X(1-c)}{\hat{h}}$, $h_{XX}=\frac{v_{XX}c}{2\hat{h}}$, $\hat{h}=\frac{1}{2}v_Oc+v_X(1-c)+\frac{1}{2}v_{XX}c$	$y_{XX}=(1-s)f_{XX}(2h_Oh_{XX}+h_X^2)$ $+(1-s)f_{3X}(2t_Ot_{XX}+t_X^2)$	$z_{XX}=sf_{XX}(m_Xh_X+m_Oh_{XX})$ $+sf_{3X}(m_Xt_X+m_Ot_{XX})$
XXX	$t_O=\frac{v_Oc}{4\hat{t}}$, $t_X=\frac{v_X\frac{1}{2}(1-c)+\frac{1}{4}c}{\hat{t}}$, $t_{XX}=\frac{v_{XX}\frac{1}{2}(1-c)+\frac{1}{4}c}{\hat{t}}$, $t_{3X}=\frac{v_Oc}{4\hat{t}}$, $\hat{t}=\frac{1}{4}v_Oc+v_X\frac{1}{2}(1-c)+v_{XX}\frac{1}{2}(1-c)+\frac{1}{4}v_{3X}c$	$y_{3X}=(1-s)f_{XX}(2h_Xh_{XX})$ $+(1-s)f_{3X}(2t_Xt_{XX}+2t_Ot_{3X})$	$z_{3X}=sf_{XX}(m_Xh_{XX})$ $+sf_{3X}(m_Xt_{XX}+m_Ot_{3X})$
4X	0	$y_{4X}=(1-s)f_{XX}(h_{XX}^2)$ $+(1-s)f_{3X}(2t_Xt_{3X}+t_{XX}^2)$	$z_{4X}=sf_{3X}(m_Xt_{3X})$
5X	0	$y_{5X}=(1-s)f_{3X}(2t_{XX}t_{3X})$	$z_{5X}=0$
6X	0	$y_{6X}=(1-s)f_{3X}(t_{3X}^2)$	$z_{6X}=0$

The fraction of gametes in which non-disjunction occurs is described by c and v_i represents the relative viability of the ith gamete type in hermaphrodites. The fitness of self-progeny relative to cross-progeny is $1-d$. Diplo-X hermaphrodite zygotes may be formed from the union of two X-bearing gametes or from the union of a nullo-X gamete with a diplo-X gamete, and triplo-X hermaphrodite zygotes may derive from the union of nullo-X with triplo-X gametes, or of mono-X with diplo-X gametes. The fraction of oocytes fertilized by male sperm (s) is the product of ρ and q_{XO}. The frequency of diplo-X hermaphrodites among all hermaphrodites is $f_{XX}=q_{XX}/1-q_{XO}$ and of triplo-X hermaphrodites is $f_{3X}=1-f_{XX}$.

viability, let the fractions of surviving gametes with a given number of sex chromosomes be v_O, v_X and v_{XX}. In contrast, males produce X-bearing and nullo-X gametes with equal frequency (provided $v_O=v_X$), regardless of the rate of X chromosome non-disjunction in hermaphrodites (Hodgkin *et al*., 1979). Sex chromosome non-disjunction during meiosis in triplo-X hermaphrodite individuals results in the formation of O, X, XX and triplo-X gametes with probabilities $\frac{1}{4}c$, $\frac{1}{2}(1-c)+\frac{1}{4}c$, $\frac{1}{2}(1-c)+\frac{1}{4}c$ and $\frac{1}{4}c$, respectively. Assuming random assortment of gametes during hermaphrodite self-fertilization, then seven zygotic karyotypes are possible (Table 1). Four of these zygote types are inviable (nullo-X 'OO' and $\geqslant$4X; Hodgkin *et al*., 1979), but the remaining three are equally viable (XO male, XX and 3X hermaphrodite; Hodgkin *et al*., 1979). By using these simple probabilistic rules of meiosis in conjunction with parameters representing the reproductive efficiency of males (ρ) and the influence of inbreeding depression due to selfing (d), we may derive predictions of XO male frequency among progeny.

Some fraction of the sperm that hermaphrodites use to fertilize oocytes, s, is produced by males and transferred to hermaphrodites by insemination (and therefore the fraction of self-sperm used in fertilization is $1-s$). This variable s is analogous to the quantity αu in the androdioecy model of Otto *et al*. (1993) and b in Chasnov & Chow (2002). Like the Otto *et al*. (1993) model, the proportion of cross-sperm depends on the frequency of males in the population (q_{XO}) and their reproductive efficiency, leading to $s=\rho q_{XO}$. The coefficient describing the reproductive efficiency of males (ρ) is constrained only such that it results in $0\leqslant s\leqslant 1$. In other words, ρ is an index of the ability of males to obtain fertilizations. The function ρ could be partitioned to account for the individual effects of all factors that influence male reproductive success, such as worm motility, mate-finding ability, hermaphrodite receptivity to mating, male copulatory ability, and the number and competitive ability of transferred male sperm. Note that male mating ability comprises only part of male reproductive efficiency, because of the other factors that contribute to male reproductive success. Ecological factors such as population density or temperature could also influence ρ by altering encounter rates among individuals, if encounters limit mating. Any genetic or environmental variation among populations in such factors would tend to result in different realized values of ρ for those populations. Although ρ itself may vary as a function of male frequency, we will use the simpler definition of constant male reproductive efficiency $\rho=2r$, such that it must satisfy $0\leqslant 2rq_{XO}\leqslant 1$ (so for $q_{XO}\leqslant\frac{1}{2}$, $0\leqslant r\leqslant 1$). This representation of male reproductive success as a constant assumes that frequency-dependent male reproductive efficiency (interference competition among

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males) is absent. Consistent with this assumption, we find no evidence that male reproductive success is frequency-dependent at the population densities used in this study (see Section 4).

Inbreeding depression is likely to be an important factor in maintaining outcrossing in some taxa, such as the clam shrimp *E. texana* (Otto *et al*., 1993; Weeks & Zucker, 1999; Weeks *et al*., 2000). Despite its long history of selfing, inbreeding depression would still play a role in determining fitness in *C. elegans* following the occurrence of new deleterious recessive mutations. Also, inbreeding depression should have been important in the origin of selfing hermaphrodites from outcrossed ancestors. We thus allow for the possibility of inbreeding depression by introducing the parameter d such that the fitness of self-progeny is only a fraction $1-d$ of that of cross-progeny (Table 1). Although no evidence for inbreeding depression in *C. elegans* currently exists for populations reared under benign laboratory conditions (Johnson & Hutchinson, 1993; Chasnov & Chow, 2002), studies with harsher conditions that approximate nature could reveal a significant influence of inbreeding on fitness (Kondrashov & Houle, 1994; Jimenez *et al*., 1994).

The frequency of individuals of zygote type i among viable progeny may be described by:

$$q_i' = \frac{k_i[(1-d)\cdot y_i + z_i]}{\sum_j k_j[(1-d)\cdot y_j + z_j]}, \tag{1}$$

where y and z represent self- and cross-progeny, respectively, the relative viability of the ith zygote genotype is k_i (e.g. $k_{OO}=0$, $k_{XO}=1$, etc.), and a prime denotes the subsequent generation (Table 1). The non-linear return map of q_{XO}' on q_{XO} will have a positive y-intercept – i.e. some male progeny will always be produced even when no male parents exist – as long as the rate of X non-disjunction is non-zero. In the special cases where males are absent ($q_{XO}=0$) or males are incapable of obtaining fertilizations ($\rho=0$), then all reproduction is via selfing of hermaphrodites. When reproduction does occur strictly via selfing ($s=0$), we can predict the rate of spontaneous male production ($q_{XO}'|_{s=0} = q_{XO}^{spont}$) based on (1) from a population of diplo-X hermaphrodites:

$$q_{XO}^{spont} = \frac{2v_O v_X c(1-c)}{2v_X^2 + 2cv_X(v_O - 2v_X + v_{XX}) - c^2(2v_O v_X - v_O v_{XX} + 2v_X v_{XX} - 2v_X^2)}. \tag{2}$$

The formulation of q_{XO}^{spont} is necessary to compare the model with empirical studies because they typically report the frequency of spontaneously produced male progeny, rather than an estimate of the rate of X non-disjunction (Hodgkin *et al*., 1979; Hodgkin & Doniach, 1997). An expression similar to (2) may also be derived to describe the rate of spontaneous male production from triplo-X hermaphrodites. However, for the assumptions of v_i appropriate for *C. elegans* (see below), X chromosome non-disjunction by diplo-X hermaphrodites gives rise to more spontaneous male progeny than triplo-X hermaphrodites over most of the range of c (not shown). Consequently, equilibrium male frequency is relatively unaffected by triplo-X reproduction due to the rarity of triplo-X individuals coupled with their lower rate of spontaneous production of males.

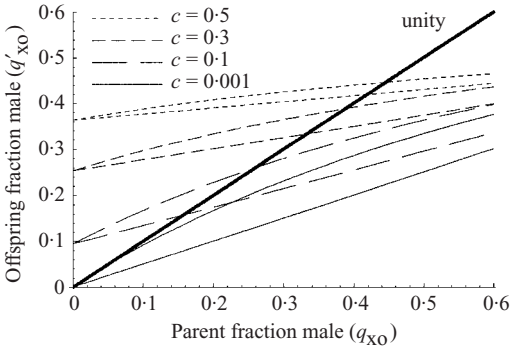

Fig. 1. The relation between fraction of male offspring and parents as a function of the rate of non-disjunction. The upper line in each pair of curves with identical dashing corresponds to $d=\frac{1}{2}$, the lower curve to $d=0$. In all cases, $r=\frac{1}{2}$, $v_O=v_X=1$, and $v_{XX}=\frac{1}{2}$. The points at which the unity line intersects the curves indicate equilibrium male frequencies (q_{XO}^*).

The equilibrium frequency of males (q_{XO}^*) occurs when male frequency among parents (q_{XO}) and progeny (q_{XO}') equilibrates at the point $q_{XO}=q_{XO}'=q_{XO}^*$. This can be seen graphically as the intersection of the curve describing q_{XO}' with the unity line (Fig. 1). Thus, q_{XO}^* provides an expectation for equilibrium male frequency as a function of X non-disjunction (c), male reproductive efficiency (ρ), inbreeding depression (d) and gametic viability (v_O, v_X, v_{XX}, v_{3X}) (Figs 1, 4). For the general case, we could solve for q_{XO}^* only numerically. However, for the special case of populations lacking triplo-X hermaphrodite parents, we derived an analytical solution (not shown), which was used to generate the figures. Also, when c is small, we can neglect higher-order terms of c. Doing so allows an approximation of q_{XO}^* as

$$q_{XO}^*|_{d+r<1} = \frac{c(1-d)}{1-(d+r)} + 0[c^2] \tag{3a}$$

$$q_{XO}^*|_{d+r\geq 1} = \frac{1-(d+r)}{-2dr} + 0[c]. \tag{3b}$$

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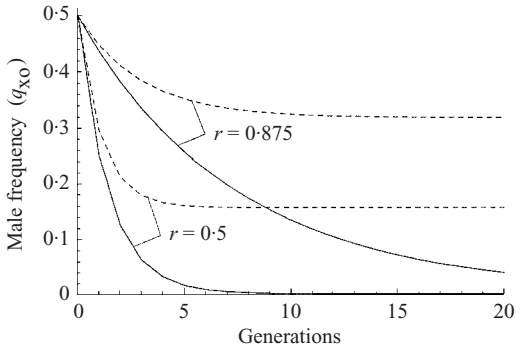

Fig. 2. The approach to equilibrium male frequency as a function of male reproductive efficiency and the rate of non-disjunction. Dashed curves correspond to $c=0{\cdot}1$, continuous curves to $c=0{\cdot}001$.

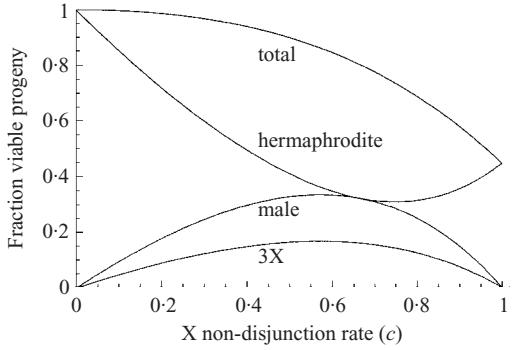

Fig. 3. Self-progeny composition as a function of non-disjunction rate. Curves correspond to (4) with $v_O=v_X=1$, $v_{XX}=\frac{1}{2}$ and $s=2rq_{XO}=0$.

When $r=1$ or $d=1$, equilibrium male frequency is constant at $\frac{1}{2}$. Equation (3a) is appropriate for many *C. elegans* strains because no inbreeding depression is observed ($d=0$), male reproductive efficiency is imperfect ($r<1$) and rates of X non-disjunction in wild isolates are likely to be low (Hodgkin & Doniach, 1997). However, these approximations will not hold for mutant strains with elevated rates of X non-disjunction.

(ii) *Model results*

As the rate of X non-disjunction increases and inbreeding depression increases, the return map of male frequency shifts upward, increasing equilibrium male frequency (Fig. 1). Although the curves in Fig. 1 were derived from a non-linear relationship between q_{XO} and q'_{XO}, they appear linear due to a dominant linear term (see equation 6). The overall fecundity of hermaphrodites declines with increasing rates of X non-disjunction because more inviable zygotes with zero, four or more X chromosomes form at higher rates of X non-disjunction (Fig. 3). Because the prevalence of spontaneous males (q_{XO}^{spont}) increases with the X non-disjunction rate (c), but the maximum possible male frequency remains constant ($\frac{1}{2}$), the steepness of the return map decreases with c (Fig. 1). Likewise, reduced reproductive efficiency decreases the return map steepness, but without altering the y-intercept (not shown). Fig. 2 shows the decline toward the expected equilibrium sex ratio over time for populations initiated with 50% males (based on parameter values used in (4), below). Equilibrium is reached faster when male reproductive efficiency is low and X non-disjunction is high. When X non-disjunction is very high in a diplo-X hermaphrodite, most of the hermaphrodite's gametes are either nullo-X or diplo-X (rather than the typical mono-X). Consequently, most self-zygotes that form are either OO, XX or 4X (because very few mono-X gametes are available), which results in a rise in relative hermaphrodite frequency and a fall in relative male and 3X frequency at extremely high non-disjunction rates (Fig. 3; although total progeny production is diminished due to the inviability of the zygotes with zero, four or more X chromosomes).

High male reproductive efficiency, high (but not extreme) rates of spontaneous male production due to frequent X non-disjunction and strong inbreeding depression all result in greater equilibrium male frequencies (Fig. 4). When male equilibrium frequency is evaluated as a function of c, a value of c exists that maximizes the equilibrium male frequency, and its magnitude depends on male reproductive efficiency and the strength of inbreeding depression (Fig. 4*A*). Although male frequency is greater when X non-disjunction occurs in a large fraction of hermaphrodite gametes (Fig. 4*A*), the overall fecundity of such individuals will also be greatly reduced relative to hermaphrodites with lower rates of X non-disjunction (Fig. 3). The model considers non-disjunction ranging from 0 to 1, but, based on the phenotypic effects of a variety of genes (Hodgkin *et al.*, 1979), it is unlikely that non-disjunction rates will exceed $\sim 0{\cdot}5$ in *C. elegans*. Equilibrium male frequency drops rapidly as the strength of inbreeding depression weakens for low to moderate rates of X non-disjunction, but falls less dramatically when X non-disjunction is high (Fig. 4*B*). Strong inbreeding depression can result in high equilibrium frequencies of males, even when non-disjunction rates are low (Fig. 4*A*, *B*). This effect occurs because the relative frequency of cross-zygotes (which are $\sim\frac{1}{2}$ male) among progeny increases as the viability of self-zygotes diminishes with the strength of inbreeding depression. In the extreme, where self-zygotes are all inviable ($d=1$), dioecy results (Fig. 4*B*).

Based on estimates of equilibrium male frequency, we may also infer the proportion of individuals in the population that result from mating rather than selfing – the rate of outcrossing (C). The outcrossing rate is simply $C=s^*=\rho q^*_{XO}=2rq^*_{XO}$.

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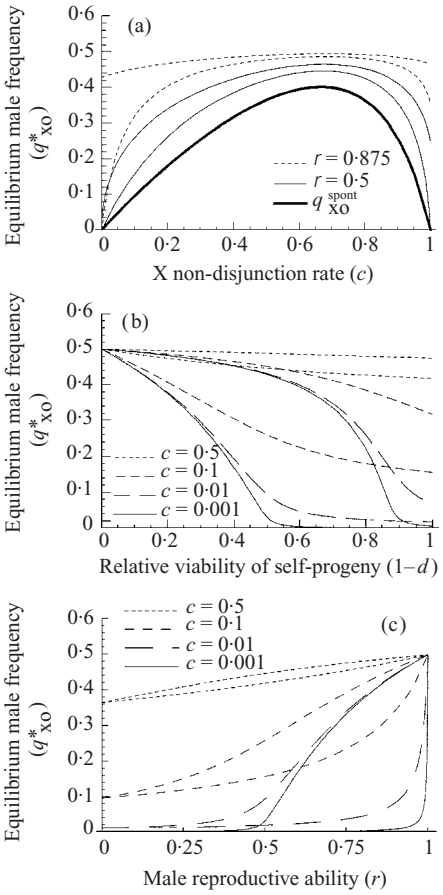

Fig. 4. Equilibrium male frequency as a function of (*A*) rate of non-disjunction, (*B*) inbreeding depression, and (*C*) male reproductive efficiency. Outcrossing rate is simply a multiple of equilibrium male frequency ($C = 2rq^*_{XO}$). In (*A*) and (*C*), the upper line in each pair of identically-dashed curves corresponds to $d = \frac{1}{2}$ and the lower curve to $d = 0$ (as in Fig. 1). In (*B*) the upper and lower lines in each pair correspond to $r = 0{\cdot}875$ and $r = \frac{1}{2}$, respectively.

3. Experimental application of the sex ratio model

We conducted a set of experiments to which the above model can be easily applied. The experiments yield sex ratio data that approximate the form of the curves in Fig. 1, where progeny sex ratios are derived from parental populations that vary in sex ratio. We included two worm strains: the standard 'wild-type' and a mutant strain with an elevated rate of X chromosome non-disjunction. We used a non-linear regression model fitting procedure of (4), below, to the resulting data to estimate the parameters that correspond to male reproductive efficiency (r) and hermaphrodite X non-disjunction rate (c). On the basis of these parameter estimates, we were able to calculate expectations of equilibrium male frequency (q^*_{XO}) and outcrossing rate (C), and rates of spontaneous male production (q^{spont}_{XO}). We then compared our empirical and model predictions with data from the literature.

(i) *Methods*

We seeded 35 mm Petri dishes, each of which contained a single spot ($\sim$10 mm diameter) of *E. coli* OP50 food source, with populations of 10 virgin worms in their last larval stage. These parental populations contained from 0 to 5 males and 5–10 diplo-X hermaphrodites, with each of these six treatments replicated 6- to 12-fold. We transferred the parental worms to the bacterial spots of two new sets of plates daily for 6 days. We sampled eggs from hermaphrodite worms during a period of 1–5 h on each of the 6 days for the first set of fresh plates before we transferred them to a second set of plates in which they resided (eating, mating and ovipositing unsampled eggs) until the following day. Hermaphrodite worms were in the presence of male worms (except in the zero male treatment) on all plates before, during and after the egg-sampling period. The daily egg-sampling period duration differed (from 1 to 5 h) between treatments with different absolute numbers of hermaphrodites, so that the total sampled egg yield per day would be similar among treatments. The first plate set provided progeny samples with synchronized development and yielded a reasonable sample size of offspring ($\sim$100 maximum per plate) to ease scoring. Populations were incubated at 15 °C. We counted and identified the sex of offspring that were deposited during the oviposition periods after they developed into adults. We used total progeny counts from the 6-day sampling period in further analyses. Males and hermaphrodites of late larval and adult age are easily distinguished with a dissecting microscope by the presence of a conspicuous tail reproductive structure (cloaca) in males. Some mortality of parental worms of both sexes occurred over the experimental period, so we used a weighted average frequency of male parents in analyses. Analyses based on initial, rather than the weighted average, parent sex ratio did not significantly alter resulting parameter estimates (not shown).

Populations for the four experiments were comprised of parental worms containing (1) all 'wild-type' N2 worms, (2) all worms homozygous for the *him-5* (*e1490*) allele (hereafter *e1490* individuals), (3) *e1490* males and N2 hermaphrodites, and (4) N2 males and *e1490* hermaphrodites. The autosomal *him-5* (*e1490*) allele confers a high rate of X-chromosome-specific non-disjunction during meiosis in hermaphrodite worms (with no apparent effect on autosome segregation), resulting in high rates of spontaneous male production (Hodgkin *et al.*, 1979). Males from the *e1490* mutant strain have reduced sperm counts, although the two male gamete karyotypes are produced with equal frequency (Hodgkin *et al.*, 1979). The genetic background of the *e1490* strain is otherwise identical to the N2 strain.

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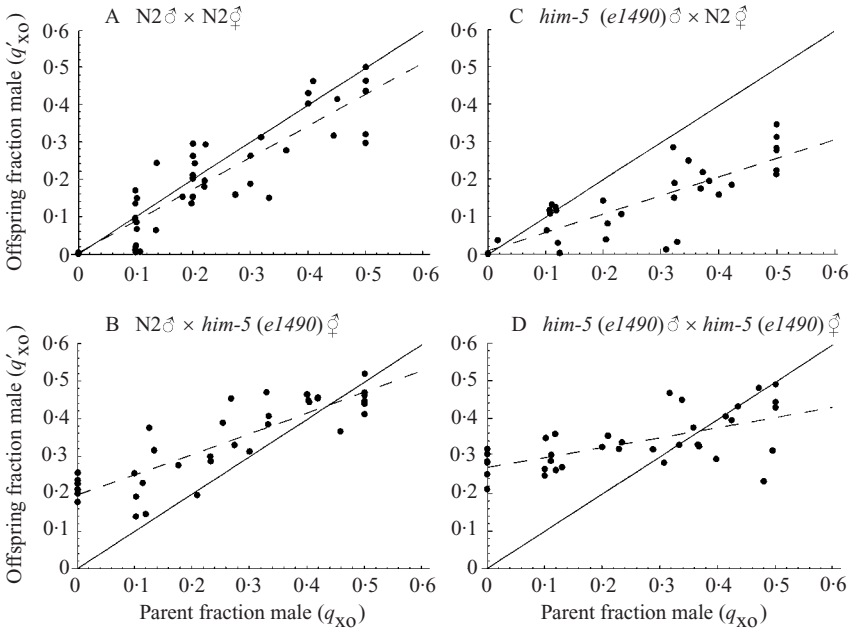

Fig. 5. Non-linear regressions of (4) to parent and offspring sex ratio data from the four mating experiments. See Table 2 for variable values and parameter estimates. Points of intersection between the continuous unity line and the dashed non-linear regression curves in (*A*) and (*D*) indicate the expected equilibrium male frequency (q^*_{XO}) for N2 and *e1490* populations, respectively.

We analysed the parent and progeny sex ratio data with plots of offspring male frequency on parent male frequency – concordant with the outline of the model presented above (Fig. 1). Non-linear regression analysis of the data with (4), below, a simplification of (1) in which reproduction by triplo-X hermaphrodites is neglected (because triplo-X hermaphrodites were excluded from the experimental parent populations), allowed us to evaluate the significance of the parent–offspring sex ratio relationship and to estimate the parameters r and c simultaneously (Systat v. 9). We used exact formulae to calculate equilibrium male frequencies rather than the approximate (3a). We used estimates for v_O, v_X, v_{XX} and v_{3X} (see below) based on the findings of Hodgkin *et al.* (1979), which we included in the formulation of the non-linear regressions. Based on our estimates of r, c, v_O, v_X, v_{XX} and v_{3X}, we make predictions for the equilibrium frequency of males in these populations based on (1). In all cases, significance was conserved whether untransformed or arcsin-square-root transformed data were analysed; here we report statistics and parameter estimates from untransformed data only.

(ii) *Empirical results and model parameterization*

The plots of progeny male frequency on parent male frequency show a positive, nearly linear relationship between the sex ratio of parents and offspring (Fig. 5). Each point in Fig. 5 summarizes the fraction of males in approximately 10 parental individuals and a mean of (*A*) 506, (*B*) 190, (*C*) 460 and (*D*) 238 progeny for a total of (*A*) 24 302, (*B*) 6843, (*C*) 17 042 and (*D*) 8552 progeny in all. We used non-linear regression to fit (4) to the data from each mating experiment. All four model fits were significant (5*A*: $F_{2,46}=310{\cdot}5$, $P<0{\cdot}001$, mean corrected $R^2=R^2_{mc}=0{\cdot}82$; 5*B*: $F_{2,34}=662{\cdot}3$, $P<0{\cdot}001$, $R^2_{mc}=0{\cdot}74$; 5*C*: $F_{2,34}=134{\cdot}2$, $P<{\cdot}001$, $R^2_{mc}=0{\cdot}71$; 5*D*: $F_{2,34}=631{\cdot}6$, $P<0{\cdot}001$, $R^2_{mc}=0{\cdot}41$). Wald 95% confidence intervals for estimates of r did not overlap with $\frac{1}{2}$ in N2 male treatments (5*A*, 5*B*), 95% CI of c did not overlap zero in *e1490* hermaphrodite treatments (5*B*, 5*D*). Fig. 6 shows the sex ratio of progeny across the duration of the experiment for N2 populations with differing initial parental sex ratios. Overall, progeny sex ratio is fairly even over time. The trend toward lower male progeny production at later sampling dates could reflect reduced male mating efficiency or reduced sperm production among aged male parents.

Hodgkin *et al.* (1979) demonstrated that nullo-X and diplo-X gametes derived from diplo-X hermaphrodites occur in a 2 : 1 ratio, probably due to loss of diplo-X gametes. This suggests that it is appropriate to let $v_O=v_X=1$ and $v_{XX}=\frac{1}{2}$. We assume $v_{3X}=0$. Studies investigating inbreeding depression in laboratory populations of *C. elegans* have found no supporting evidence (Johnson & Hutchinson, 1993; Chasnov & Chow, 2002), so we let $d=0$ (self- and cross-progeny equally viable). We also use the definition $\rho=2r$ for the male reproductive efficiency function, as described above. Using these values and assuming all parent hermaphrodites have two X chromosomes ($f_{XX}=1$), (1) and (2) can be simplified

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Table 2. *Summary of non-linear regression parameter estimates for male reproductive efficiency* ($\rho = 2r$) *and X non-disjunction rate* (c) *and corresponding model predictions from* (4) *and* (6) *for the equilibrium frequency of males* (q^*_{XO}), *spontaneous rate of male production* (q^{spont}_{XO}), *and fraction of outcrossed individuals* (C)

Parents (male × hermaphrodite)	Fig. 5	n	r (SE)	c (SE)	q^*_{XO}	q^{spont}_{XO}	C
N2 × N2	A	48	0·858 (0·040)	0·0036 (0·017)	0·024	0·0036	0·041
N2 × *him-5* (*e1490*)	B	36	0·913 (0·055)	0·223 (0·022)	0·437	0·198	0·798
him-5 (*e1490*) × N2	C	36	0·508 (0·042)	0·0092 (0·016)	0·018	0·0092	0·019
him-5 (*e1490*) × *him-5* (*e1490*)	D	36	0·599 (0·089)	0·324 (0·026)	0·369	0·270	0·441

For the two cases (B, C) in which males mated hermaphrodites from a different strain, q^*_{XO} indicates the equilibrium male frequency of a hypothetical population with corresponding parameter values. In all cases, $v_O = v_X = 1$, $v_{XX} = \frac{1}{2}$, $v_{3X} = 0$ and $d = 0$ (see text).

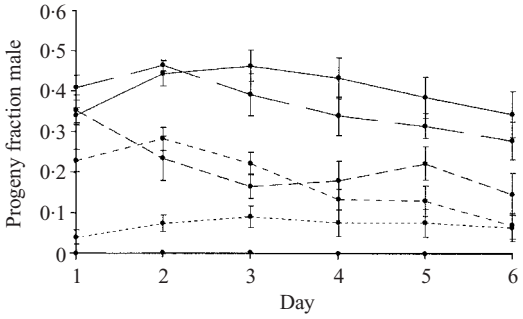

Fig. 6. Progeny sex ratio over days of oviposition. Points connected with curves with smaller dashing correspond to initial parent sex ratios descending from 50% to 0 males in 10% increments. Vertical error bars are standard errors.

to, respectively:

$$q'_{XO} = \frac{c^2(17rq_{XO} - 8) + c(8 - 22rq_{XO}) + 8rq_{XO}}{c^2(6rq_{XO} - 2) - 4c(rq_{XO} + 1) + 8}, \qquad (4)$$

and

$$q^{spont}_{XO} = \frac{4c(c-1)}{c^2 + 2c - 4} \qquad (5)$$

When the rate of X non-disjunction is low ($c \ll 1$), and terms of second order in c may be neglected, the above equations may be approximated as

$$q'_{XO} = rq_{XO} + c\left[\left(1 - \tfrac{9}{4}rq_{XO}\right) + \left(\tfrac{1}{2}r^2q^2_{XO}\right)\right] + 0[c^2], \qquad (6)$$

and

$$q^{spont}_{XO} = c + 0[c^2]. \qquad (7)$$

Although (6) and (7) are appropriate for strains such as N2, they are likely to be poor estimators for strains with high X non-disjunction rates such as *e1490*. Note that for these simplified models, the equilibrium frequency of males may be derived from (4) and (6) as the positive solution to $q_{XO} = q'_{XO}$, where $q_{XO} = q'_{XO} = q^*_{XO}$.

The non-linear regression model of (4) provided a strong fit to the data (see legend to Fig. 5). Although a simple linear regression model would also fit these data, the parameters from such an analysis have no straightforward biological interpretation. Therefore, we present only the results from the model represented by (4), the parameters of which have predefined biological meaning. Table 2 summarizes the non-linear regression estimates for X non-disjunction rate (c) and male reproductive efficiency (r) for each experiment. These parameter estimates proved to be consistent for sex and strain: males of the same strain showed similar reproductive efficiencies regardless of which hermaphrodite strain they mated, and hermaphrodites of the same strain showed similar rates of X non-disjunction. In particular, N2 male reproductive efficiency greatly exceeds the reproductive efficiency of *e1490* males, and *e1490* hermaphrodites demonstrate much higher rates of X non-disjunction than N2 hermaphrodites.

4. Discussion

A clear understanding of the factors and mechanisms that influence male frequency in androdioecious populations is necessary to interpret the extent to which outcrossing might influence evolution in such species. We have developed a mechanistic model of sex frequency for androdioecious species that provides a framework for experimental quantification of the effects of variation in ecological conditions and genetically controlled behaviour and physiology. In a set of experiments applied to the model, we describe the quantitative differences between the canonical N2 strain and a mutant strain with elevated X non-disjunction in hermaphrodites and reduced reproductive efficiency of males. Nematodes comprise a taxonomically rich clade (Dusenbery, 1980; Blaxter, 1998; Adoutte *et al.*, 1999) and soil nematodes in particular are both diverse and extremely abundant (Yeates & Bongers, 1999). XO sex determination is widespread in nematodes and androdioecy is common in taxa with

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a hermaphrodite sex (Triantaphyllou, 1983). Thus, although we developed this model specifically to address sex ratio and outcrossing in the nematode *C. elegans*, it also is appropriate for the array of taxa that exhibit a similar sex determination system and in which hermaphrodites cannot mate with each other.

(i) *Experimental verification of the sex ratio model*

The fitted parameter estimates indicate that X non-disjunction rate and male reproductive efficiency are consistent within strains for the two strains examined, in that, relative to N2, *e1490* hermaphrodites exhibited high rates of X non-disjunction and *e1490* males experienced low reproductive efficiency. Individuals from these two strains differ in allelic state at only one locus (*him-5*), the only phenotypic effect on males of which is a difference in the number of sperm produced (both male types produce X-bearing and nullo-X sperm with equal frequency; Hodgkin *et al.* 1979). Hodgkin *et al.* (1979) showed that *e1490* males produce only approximately 55% as many sperm as N2 males and suggested that their reproductive ability was proportionately reduced. This value approximates the ratios of the reproductive efficiency parameters, r, for the two male strains (mean r *e1490*/N2:0·552/0·885 = 0·62; Table 2), implicating differences in male sperm production between the two strains as the primary agent responsible for the observed differences in male reproductive efficiency. However, differences in hermaphrodite sperm production between the two strains may also contribute to realized male reproductive efficiency because *e1490* hermaphrodites produce fewer sperm to compete with male sperm than do N2 hermaphrodites (Hodgkin *et al.*, 1979). Consistent with this hypothesis, males of both strains show a trend towards reduced reproductive efficiency when mated to N2 hermaphrodites relative to *e1490* hermaphrodites (0·51 : 0·60 for *e1490* males; 0·86 : 0·91 for N2 males). These differences in r suggest that the N2 allele of *him-5* could invade a population fixed for the *e1490* allele based solely on superior N2 male reproductive efficiency, before accounting for differences in hermaphrodite fecundity. Reports that males exhibit variation in sperm production among strains that were collected at different geographic locations (Hodgkin & Doniach, 1997) suggest that male sperm production could potentially influence male reproductive efficiency in natural populations, and therefore affect male frequency and outcrossing rates. Likewise, differences in sperm competitive ability between males may be important in comparisons of strains that vary significantly in sperm size, as seen among several natural isolates of *C. elegans* (Lamunyon & Ward, 2002).

It is conceivable that male reproductive efficiency is frequency-dependent such that males experience disproportionately lower reproductive success when common. Such interference competition seems to occur in the androdioecious clam shrimp *E. texana* (Medland *et al.*, 2000). A simple model using a power function of male frequency could describe such a scenario in *C. elegans*: $m' = pm^a + b$ (male frequency m, strength of frequency dependence a where $a < 1$ is expected for frequency-dependent interference among males, spontaneous male production rate b, and scaling parameter p). When we fit this model to our experimental data, Wald 95% confidence intervals for a overlap 1 in all four strain mating pairs, suggesting that frequency dependence does not accurately describe the dynamics of mating under the conditions of our experiments. This also suggests that our formulation of the male reproductive efficiency function as $\rho = 2r$ in the mechanistic model is appropriate for these data. The primary advantage of our mechanistic model over such an alternative model is that the parameters of the mechanistic model have more straightforward biological analogues (e.g. p is not independent of b because male frequency has an effective maximum at 50%).

The rates of spontaneous male production given in Table 2 that are based on (5) closely approximate previously published reports and our raw empirical data that estimated spontaneous male production as the actual observed frequency of males produced by unmated hermaphrodites. In our study, unmated, diplo-X N2 hermaphrodites produced 3 males out of 5915 total progeny (0·05%). Other reports of spontaneous male progeny production in N2 provide values of 0·3% (Hodgkin *et al.*, 1979) and 0·14% (Hodgkin & Doniach, 1997) when worms were reared at the higher incubation temperature of 20 °C. Equilibrium frequencies of males in laboratory populations of N2 at 20 °C have been estimated as 0·2% (Hodgkin, 1983) and 0·077% (Chasnov & Chow, 2002). Out of 1963 total progeny, unmated, diplo-X *e1490* hermaphrodites yielded 500 males (25·5%). For this same strain reared at 20 °C, spontaneous male progeny have been reported to occur at frequencies of 32·9% (Hodgkin *et al.*, 1979) and 32·6% (Chasnov & Chow, 2002). Higher temperatures promote X non-disjunction in *C. elegans* (Hodgkin, 1983), which may explain the trend of lower values for our estimates of the fraction male progeny produced by unmated hermaphrodites relative to those in previous studies.

The model prediction for the equilibrium frequency of males (q^*_{XO}) in N2 is approximately 7 times higher than the rate of spontaneous male production (Table 2). This is due to the high reproductive efficiency of N2 males under the experimental conditions examined in this study. While a value of $r = 0{\cdot}858$ seems high for a species expected to reproduce largely via self-fertilization (compared with the $r = 1$ required for

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dioecy), the non-linear effect of r on equilibrium male frequency (Fig. 4*C*) coupled with low rates of X non-disjunction should be kept in mind when evaluating the nominal parameter values. The value we estimate for N2 male reproductive efficiency accords with the time series of sex ratio described by Stewart & Phillips (2002): after 15 generations, our model predicts male frequency to be 7·4% (given $r = 0{\cdot}858$, $c = 0{\cdot}0036$, and initial male frequency 0·45) compared with their observed 7% (±2% SD). Also, the outcrossing rate has been estimated to occur on the order of a few per cent, based on the fit of the background selection model to the genomic distribution of *C. elegans* single-nucleotide polymorphisms between two strains (Cutter & Payseur, 2003). However, until the potential effects of population density, overlapping generations, and genetic and environmental factors become clear, extrapolation of the numerical value of q^*_{XO} from laboratory studies to natural populations may be premature. For example, high temperatures promote X non-disjunction (Hodgkin, 1983) and high population densities may promote male mating. Likewise, male reproductive efficiency may be influenced by such genetically controlled traits as copulatory plug formation (Hodgkin & Doniach, 1997), aggregation behaviour (de Bono & Bargmann, 1998), ability of males to obtain copulations (Liu & Sternberg, 1995; Emmons & Sternberg, 1997) and rate of production and size of sperm and oocytes (Hodgkin & Barnes, 1991). Furthermore, the influence of stochastic events on male frequency and outcrossing rates may be particularly important when the predicted sex ratio is so low.

(ii) *Natural selection on sex ratio in* C. elegans

In populations of *C. elegans*, two forces will select against very high rates of X chromosome non-disjunction. First, the higher frequency of inviable nullo-X zygotes and zygotes with four or more X chromosomes formed by individuals with higher X non-disjunction rates will place them at a reproductive disadvantage due to overall reduced fecundity (Fig. 3). Related to this, diplo-X and triplo-X gametes (which are more common at higher X non-disjunction rates) experience reduced survival, which reduces the pool of available gametes – an important limited resource. Second, the higher rate of male progeny production will further reduce the net reproductive rate of lineages with higher rates of X non-disjunction ('cost of males'; Maynard Smith, 1971) if the males have a low reproductive efficiency (Williams, 1975; Bell, 1982). However, the probability of X non-disjunction is unlikely to be zero due to biochemical constraints in meiosis, so male individuals will continue to be produced spontaneously by selfing hermaphrodites – as long as the genetic and developmental machinery required to form the male phenotype remains functional.

Because males are ostensibly unnecessary for reproduction in this species, their persistence requires explanation. If male *C. elegans* are evolutionarily unimportant (i.e. neutral), then the many loci present in the genomes of both sexes but expressed only in males (Jiang *et al.*, 2001) would be expected to experience relaxed selection and a concomitant accumulation of deleterious mutations. Mutation accumulation would lead to the eventual loss of function of the products of male-specific genes and, thus, cause failure of male development. Available evidence for estimates of mutation rate (Drake *et al.*, 1998; Shabalina & Kondrashov, 1999; Keightley & Bataillon, 2000; A. D. Cutter & B. A. Payseur, unpublished data 2002) and lineage age (Kennedy *et al.*, 1993; Thacker *et al.*, 1999; Coghlan & Wolfe, 2002) suggests that sufficient time has elapsed for such a process to occur.

Chasnov & Chow (2002) argued, on the basis of a mutational model, that mating is likely to occur with sufficient frequency to overcompensate for deleterious mutations in male specific genes – implying that an evolutionary advantage to outcrossing is not responsible for the persistence of males. Their model is appropriate, but we believe that variation in our ability to estimate key elements of their model's parameters brings into question the definitiveness of their conclusion. First, they assume *e1490* and N2 males have equivalent reproductive efficiencies. Our data (Table 2), and those of Hodgkin *et al.* (1979), suggest that N2 male reproductive success is nearly twice that of *e1490* males (due to greater sperm production), and some natural isolates have even more virile males (Hodgkin & Doniach, 1997; Lamunyon & Ward, 2002). Second, the precise genomic deleterious mutation rate in *C. elegans* is unknown, but probably lies in the range 0·005–0·12 (Davies *et al.*, 1999; Vassilieva *et al.*, 2000; Keightley & Bataillon, 2000). Third, their estimate of 50–60 male-specific loci may be an underestimate by a factor of approximately 2. An analysis of multiple microarray gene expression experiments suggests that 83–95 of 17 661 loci assayed are likely male-specific, implying 91–104 male-specific genes in a genome of 19 282 loci (Kim *et al.*, 2001). As a consequence of these issues, the threshold mutation rate ($v_c = 1{\cdot}6 \times 10^{-5}$ to $1{\cdot}7 \times 10^{-5}$) may not be sufficiently greater than the per gene mutation rate ($u_g = 5 \times 10^{-6}$ to $1{\cdot}1 \times 10^{-7}$) that would be necessary to support the number of male-specific genes in *C. elegans*' genome (Chasnov & Chow, 2002). If true, this implies that male maintenance may be subject to selection, or, that loci involved in the production of males effectively act as selfish genetic elements.

Evidence from several sources indicates that *C. elegans*' history has been affected by outcrossing (Egilmez *et al.*, 1995; Koch *et al.*, 2000; Cutter &

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Payseur, 2003), despite low genetic variation among known wild isolate strains (Thomas & Wilson, 1991; Fitch & Thomas, 1997; Koch *et al*., 2000; Wicks *et al*., 2001; Graustein *et al*., 2002). The theoretical advantages to outcrossing in facilitating adaptive evolution (Fisher, 1930; Muller, 1932; Hamilton *et al*., 1990) and in eliminating deleterious mutations (Muller, 1964; Felsenstein, 1974; Kondrashov, 1988; Charlesworth, 1990; Gabriel *et al*., 1993) form enticing hypotheses for male persistence in this species, but other possibilities – namely the notion that male-specific genes and loci that influence X non-disjunction might act as selfish genetic elements – must be excluded before this may be considered definitive. Clearly, dissection of the evolutionary forces responsible for the persistence of males in populations of *C. elegans* and other androdioecious nematodes remains a topic requiring further inquiry.

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