Exploring the Envelope: Systematic Alteration in the Sex-Determination System of the Nematode *Caenorhabditis elegans*

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Manuscript received April 7, 2002
Accepted for publication July 1, 2002

ABSTRACT

The natural sexes of the nematode *Caenorhabditis elegans* are the self-fertilizing hermaphrodite (XX) and the male (XO). The underlying genetic pathway controlling sexual phenotype has been extensively investigated. Mutations in key regulatory genes have been used to create a series of stable populations in which sex is determined not by X chromosome dosage, but in a variety of other ways, many of which mimic the diverse sex-determination systems found in different animal species. Most of these artificial strains have male and female sexes. Each of seven autosomal genes can be made to adopt a role as the primary determinant of sex, and each of the five autosomes can carry the primary determinant, thereby becoming a sex chromosome. Strains with sex determination by fragment chromosomes, episomes, compound chromosomes, or environmental factors have also been constructed. The creation of these strains demonstrates the ease with which one sex-determination system can be transformed into another.

The diversity of sex-determination systems in the animal kingdom is a remarkable feature of the natural world. Here, it is demonstrated that much of this diversity can be artificially created within a single species, by utilizing control gene mutations and chromosomal rearrangements to modify the sex-determination system of *Caenorhabditis elegans*.

Sexuality is almost universal in the animal kingdom, despite the existence of rare exceptions such as the bdelloid rotifers (Welch and Meselson 2000). Many different sex-determination mechanisms occur, and the particular nature of the sex-determination system can have important evolutionary consequences. One striking example is provided by the order Hymenoptera, containing over half a million species of bees, wasps, and ants, all of which exhibit a common pattern of sex determination by ploidy with haploid males and diploid females (Beukeboom 1995). Social behavior has arisen on multiple independent occasions within the Hymenoptera, almost certainly because the sexual system leads to greater genetic similarity between related individuals, and therefore facilitates evolution of the kinds of altruistic behavior that are essential for sociality. Another, more specific, example is found in the case of brood-parasitizing cuckoos. Within a single cuckoo species, multiple races can exist, each of which is specialized for parasitizing a different host and produces eggs that specifically mimic the eggs of that host. The specialization probably depends on female birds being heterogametic (ZW), allowing the female-specific W chromosome to evolve to carry race-specific traits (Gibbs et al. 2000).

Chromosome and gene evolution can also be strongly affected by the nature of sex-determination systems. There is abundant evidence for the repeated evolution of sex chromosomes and sex chromosome heteromorphy, followed by phenomena such as dosage compensation and imprinting. The majority of genes on sex chromosomes are usually not involved in controlling sex per se, but they nevertheless experience its consequences.

The diversity of animal sex-determination systems is conspicuous (for general reviews, see Bell 1982; Bull 1983). Many groups have the familiar XX female, XY male system encountered in most mammals, but others, such as birds and butterflies, have heterogametic ZW females and homogametic ZZ males. Chromosomal sex determination can also be more complex, with multiple X and Y chromosomes, or maternal, as in the carrion fly *Chrysomya rufifacies*. Sex can also be determined by environment rather than by genome, as in the many groups exhibiting temperature-dependent sex determination or sex determination in response to nutritional conditions, crowding, and other social factors. The existence of all these different systems raises many questions about what selective forces may have caused their evolution and how one system can evolve into another.

These problems have been extensively studied at a theoretical level with some success, but it seems likely that adequate understanding will depend also on knowledge of the basic molecular machinery involved in sex determination. Here, too, a surprising diversity is encountered. The molecular genetics of sex has been studied most extensively in three types of animals: mammals, the fruitfly *Drosophila melanogaster*, and the nematode...
The primary determinants of sex on the X chromosome are a small number of genes, most notably sex-1 and fox-1, which act as counting elements to distinguish high from low X dose (Akerib and Meyer 1994; Hodgkin et al. 1994; Carmi and Meyer 1999). In XX individuals, the higher dose of these genes and their products results in downregulation of xol-1. Low activity of xol-1 permits high activity of sdc-2, which appears to be the most important of the three sdc genes; these genes coordinate to regulate both X chromosome dosage compensation and sex determination (Dawes et al. 1999). The sdc gene products have two functions. First, they activate recruitment of dosage compensation proteins to the X chromosome, thereby leading to a general depression of gene activity on both X chromosomes in the XX sex.
The phenotypes of relevant mutations affecting sex determination are shown in Table 1. Relevant phenotypes are more fully described at appropriate points in the text. The abbreviation (m/H11001z/H11002) refers to animals homozygous for a given mutation but derived from a heterozygous mother carrying a wild-type allele for the relevant gene; (m/H11002z/H11002) refers to homozygous mutants derived from homozygous mutant mothers.

### TABLE 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>XX phenotype</th>
<th>XO phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>—</td>
<td>Viable hermaphrodite</td>
<td>Viable mating male</td>
</tr>
<tr>
<td>tra-1 III</td>
<td>e1099</td>
<td>Poor mating male</td>
<td>Poor mating male</td>
</tr>
<tr>
<td></td>
<td>e1834</td>
<td>Poor mating male</td>
<td>Poor mating male</td>
</tr>
<tr>
<td></td>
<td>e1575</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>tra-2 II</td>
<td>e1095</td>
<td>Nonmating male</td>
<td>Mating male</td>
</tr>
<tr>
<td></td>
<td>ar221</td>
<td>Nonmating male (25°) or hermaphrodite (15°)</td>
<td>Mating male</td>
</tr>
<tr>
<td></td>
<td>q276</td>
<td>Mating male</td>
<td>Mating male</td>
</tr>
<tr>
<td></td>
<td>e2020</td>
<td>Female</td>
<td>Mating male</td>
</tr>
<tr>
<td></td>
<td>e2046/2531</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>tra-3 IV</td>
<td>e1767</td>
<td>Nonmating male (m−z−) or hermaphrodite (m+z−)</td>
<td>Mating male</td>
</tr>
<tr>
<td>her-1 V</td>
<td>e1518</td>
<td>Hermaphrodite</td>
<td>Hermaphrodite</td>
</tr>
<tr>
<td></td>
<td>e1561</td>
<td>Hermaphrodite</td>
<td>Hermaphrodite (25°) or male (15°)</td>
</tr>
<tr>
<td>fem-1 IV</td>
<td>e1927</td>
<td>Female</td>
<td>Female (m−z−)</td>
</tr>
<tr>
<td>fem-2 III</td>
<td>e2105</td>
<td>Female (m−z−)</td>
<td>Female (m−z−, 25°)</td>
</tr>
<tr>
<td>fem-3 IV</td>
<td>e1996</td>
<td>Female</td>
<td>Female (m−z−)</td>
</tr>
<tr>
<td>xol-1 X</td>
<td>y9</td>
<td>Viable hermaphrodite (Tra enhanced)</td>
<td>Dead, feminized</td>
</tr>
<tr>
<td>sdc-2 X</td>
<td>y15</td>
<td>Dead, masculinized</td>
<td>Viable mating male</td>
</tr>
<tr>
<td>sdc-3 V</td>
<td>y52</td>
<td>Viable, masculinized</td>
<td>Viable mating male</td>
</tr>
<tr>
<td>dpy-26 IV</td>
<td>n199</td>
<td>Dead, hermaphrodite</td>
<td>Viable mating male</td>
</tr>
<tr>
<td>dpy-27 III</td>
<td>rh18</td>
<td>Dead, hermaphrodite</td>
<td>Viable mating male</td>
</tr>
<tr>
<td>fog-2 V</td>
<td>q71</td>
<td>Female</td>
<td>Mating male</td>
</tr>
</tbody>
</table>

Relevant phenotypes are more fully described at appropriate points in the text. The abbreviation (m+z−) refers to animals homozygous for a given mutation but derived from a heterozygous mother carrying a wild-type allele for the relevant gene; (m−z−) refers to homozygous mutants derived from homozygous mutant mothers.

In the absence of dosage compensation, excess X chromosome gene expression has lethal effects on XX animals. Dosage compensation proteins are encoded by a set of genes specific to this process, such as *dpy-26* and *dpy-27* (Hodgkin 1983b, 1987b; Plenefisch et al. 1989). These genes do not affect sexual phenotype, only the relative viability of XX and XO individuals.

Second, *sdc-2* gene activity leads to transcriptional repression of the sex-determination gene *her-1*. This gene is able to encode a diffusible protein, believed to act as a repressive ligand for a membrane receptor encoded by *tra-2* (Kuwabara et al. 1992; Perry et al. 1993). In the absence of *her-1* activity, however, the TRA-2A receptor is active and able to inhibit one or more of the three FEM proteins encoded by *fem-1*, *fem-2*, and *fem-3* (Spence et al. 1990; Ahringer et al. 1992; Pilgrim et al. 1995). Activity of TRA-2A, and therefore inhibition of FEM proteins, is largely dependent on TRA-3, a regulatory protease encoded by *tra-3* (Barnes and Hodgkin 1996). At least part of the FEM inhibition involves direct protein-protein interaction between TRA-2A and FEM-3, perhaps leading to the sequestration of a FEM protein complex at the cell membrane (Mehra et al. 1999). The inhibition or sequestration of FEM proteins in turn prevents them from inhibiting the activity of the transcription factor *tra-1*, encoded by *tra-1* (Zarkower and Hodgkin 1992). TRA-1A is therefore fully active in the soma of XX animals, resulting in female somatic development.

Regulation in the germline of XX animals is modified to permit both spermatogenesis (early) and oogenesis (late). This modification involves multiple factors and translational regulation, acting at the level of the TRA-2 and FEM proteins (for a more complete review, see Kuwabara and Perry 2001). One important and specific gene here is *fog-2*, which is necessary for the modulation of TRA-2 activity that permits spermatogenesis in XX hermaphrodites (Schedl and Kimble 1988).

In XO animals, a reciprocal set of regulatory events...
occurs. The lower dose of counting elements on the X chromosome results in higher activity of xol-1, which in turn represses sdc-2. In the absence of SDC-2, her-1 is transcriptionally active, resulting in synthesis of the secreted protein HER-1A. This probably acts as a direct inhibitory ligand for the TRA-2A transmembrane receptor, which is therefore downregulated in activity. Consequently, the FEM proteins are no longer inhibited and can act to reduce TRA-1A activity. This lower activity results in male development of both soma and germline.

This outline of major regulatory events omits a number of significant additional interactions and effects, some of which need to be considered in the context of this article. First, regulation of her-1 is not the only event transducing X chromosome dosage to the downstream sex-determination genes, although it is the most important factor. There is good genetic evidence for an additional bypass effect that affects activity of the fem genes and/or tra-1 (Hodgkin 1980). This bypass involves xol-1 activity, but in this context xol-1 has a paradoxical feminizing function, in contrast to its masculinizing function at the initial steps of X chromosome dosage assessment (Miller et al. 1988). Second, there is evidence for a number of feedback effects within the pathway, which probably reinforce adoption of either the female or the male state (Okkema and Kimber 1991; Delong et al. 1993). Third, feed-forward effects, such as a direct interaction between a C-terminal portion of TRA-2 and TRA-1A, apparently bypass the FEM proteins (Lum et al. 2000). Fourth, the action of TRA-1A is more complex than the simple binary ON/OFF choice that describes regulation for many of the other genes in the pathway. Completely normal male development and full male fertility require low activity of tra-1, as opposed to complete inactivity (Hodgkin 1987a). Consequently, tra-1(null) XX animals are fully male in almost all features, but are variable in a few somatic features and have poor male germline development.

These effects and others (Goodwin and Ellis 2002) can legitimately be regarded as minor modulations or reinforcements of the major interactions that direct sexual development along either the male or the female path. Their existence is characteristic of the kind of fine tuning that becomes apparent when any piece of biological regulation is examined in detail. However, their significance can obviously change over evolutionary time. On the one hand, some minor interactions may be relics of regulatory events that were much more important in the past. On the other hand, some may acquire a major role in the future. Recent discoveries in the evolution of development suggest that developmental regulation is in a state of constant flux. This may be especially true of sex determination, as indicated by the rapid evolution of sex-determination genes. The manipulations described here demonstrate that the pathway in C. elegans is malleable to a remarkable degree.

MATERIALS AND METHODS

Nematode handling: Standard methods for culture of C. elegans were used (Brenner 1974). Strain construction and maintenance were carried out at 20°C unless otherwise noted. Irrelevant markers were often used in the construction of these strains, but were removed by segregation from the final stable strains. Genotypes were verified by appropriate test-crosses.

Nomenclature: The nomenclature used is as in Horvitz et al. (1979). Allele abbreviations are mostly used in the text, for simplicity: gf (gain of function), lf (loss of function), eg (enhanced gain of function), am (amber nonsense), ts (temperature sensitive).

Strains and alleles used: The names and full genotypes of strains described are as follows:

Strain 1 (CB3470): females, tra-1(e1575)/tra-1(e1575e1816e1828) males, tra-1(e1575e1816e1828)
Strain 2 (BS508): females, tra-2(q276); tra-1(az6)/tra-1(+), males, tra-2(q276)
Strain 3 (CB5190): females, tra-2(e2020)/tra-1(e1099)/tra-1(+); xol-1(y9) males, tra-2(e2020); tra-1(e1099); xol-1(y9)
Strain 4 (CB5081): females, tra-2(e2046e2531); tra-1(e1099)/tra-1(+) males, tra-2(e2046e2531); tra-1(e1099)
Strain 5: see Miller et al. (1988)
Strain 6 (CB4734): females, tra-2(e1095)/tra-2(+) ; fog-2(q71); xol-1(y9) males, tra-2(e1095) ; fog-2(q71); xol-1(y9)
Strain 7 (CB5023): females, tra-2(e2531 e2046)/+; dpy-26(n199) males, dpy-26(n199)
Strain 8 (CB4733): T females, tra-3(e1767)/tra-3(+) ; fog-2(q71); xol-1(y9) males, tra-3(e1767) ; fog-2(q71); xol-1(y9)
Strain 9 (CB5590): hermaphrodites, her-1(e11518) sdc-3(y52); xol-1(y9) males, her-1(e11518) sdc-3(y52)/her-1(+) sdc-3(y52); xol-1(y9)
Strain 10 (CB5666): females, her-1(e1518) sdc-2(y15); xol-1(y9) males, her-1(e1518) her-1(+) ; sdc-2(y15)
Strain 11 (CB4628): females, tra-2(e1095); fem-1(e1927)/ xol-1(y9) males, tra-2(e1095); fem-1(e1927)/ fem-1(+) ; xol-1(y9)
Strain 12 (CB4722): females, tra-2(e1095); fem-2(e2105)/ xol-1(y9) males, tra-2(e1095); fem-2(e2105)/ fem-2(+) ; xol-1(y9)
Strain 13 (CB4692): females, tra-2(e1095); fem-3(e1965)/ xol-1(y9) males, tra-2(e1095); fem-3(e1965)/ fem-3(+) ; xol-1(y9)
Strain 14 (CB5638): hermaphrodites, sup-34(e2227)/ sup-34(+) ; tra-3(e1107); xol-1(y9) males, tra-3(e1107); xol-1(y9)
Strain 15 (CB5035): females, tra-1(e1834)
Sex determination by the autosomal gene tra-1: The gene tra-1 encodes a zinc-finger transcription factor, TRA-1A, which is related to the CI protein in Drosophila and GLI proteins in mammals (Zarkower and Hodgkin 1992). TRA-1A plays an irreplaceable role in regulating all aspects of sexual differentiation in C. elegans. For at least some genes, TRA-1A acts as a transcriptional repressor, and it is possible that its major function is to prevent expression of male-specific genes, either globally or in specific cells. Consequently, null mutations of tra-1 result in the transformation of XX animals into males, and gain-of-function mutations result in feminization of XO animals. The first artificial sex-determination system to be reported for C. elegans (Hodgkin 1983a) was based on control by tra-1, exploiting the isolation of point mutations that cause either complete masculinization of XX animals or complete feminization of XO animals. Null mutations of tra-1 are recessive and cause both XX and XO animals to develop as low-fertility males, whereas gf mutations are dominant and cause both XX and XO to develop as females (Hodgkin 1987a). Consequently, a stable male/female strain can be constructed:

Strain 1: Females, tra-1(gf)/tra-1(null)
Males, tra-1(null)/tra-1(null)

Both females and males can be either XX or XO. However, in the absence of other factors, strains of this type tend to stabilize as pure XX. This occurs for several reasons: the XO females are less fertile than the XX females, the XO males are not more fertile than the XX males, and XO × XO crosses are intrinsically less productive because of the production of inviable nullo-X zygotes.

Two variants of this basic strain have been constructed to demonstrate the complete epistasis of tra-1 over mutations in the upstream gene tra-2. Most gain-of-function mutations in tra-2, including null mutations, result in the transformation of XX animals into abnormal males with fully masculinized gonads but incompletely transformed genitalia. Consequently, these tra-2 XX males are unable to mate and are often described as pseudomales.

One unusual non-null tra-2 allele has been isolated, q276, which results when homozygous in the development of more completely transformed XX males (T. B. Schedl, personal communication). These are frequently able to mate and sire progeny, although less frequently than tra-2(q276) XO males. Construction of double mutants of q276 with gf (feminizing) mutations of tra-1 resulted in fertile female development of both XX and XO, showing that the masculinization caused by q276 can be completely overridden by tra-1 activity. A stable strain of the following type was constructed and kindly made available by T. B. Schedl:

Strain 2: Females, tra-2(q276); tra-1(gf)/+  
Males, tra-2(q276)

This strain grows with greater fertility than strain 1 because tra-2(q276) XX males are more fertile than tra-1(null) XX males. tra-1(q276) XO males are still more fertile, and therefore strain 2 tends to grow as a mixture of XX and XO animals, because this greater XO male fertility outweighs the lower female or occasional hermaphrodite fertility of the tra-1(gf)/+ XO animals.

Gain-of-function mutations in tra-2, which cause either partial or complete feminization of XO animals, have also been isolated. The gf mutations first isolated behave as hypermorphs, probably by reducing translational repression of tra-2. The strongest of these, e2020 (Doniach 1986; Okkema and Kimble 1991), results in a deletion within the 3′ untranslated region of TRA-2A mRNA; this mutation causes XX animals to develop as females, while XO animals usually develop as fertile but slightly feminized males. Under starvation conditions, the feminization of the XO animals is much stronger, so that they develop as sterile intersexes with abnormal gonads and essentially female tails. Homozygous double mutants of e2020 with tra-1(null) alleles were constructed and found to develop as males, irrespective of nutritional state. To test this further, a strain of tra-2(e2020); tra-1(null)/+ XX females and tra-2(e2020); tra-1(null) XX males was constructed and several lines were propagated for at least five generations. Testcrosses confirmed the genotype of both types of males and females. This strain (CB5172) is not stable, however, because spontaneous tra-2(e2020); tra-1(null)/+ XO animals eventually arise. These develop into high-fertility males, and in successive generations the population rapidly converts to a pure tra-2(e2020) culture of XX females and XO males. To make a stable strain, a xol-1 mutation was introduced, which results in the death of all XO animals but does not affect XX animals:

Strain 3: Females, tra-2(e2020); tra-1(null)/+; xol-1 XX
Males, tra-2(e2020); tra-1(null); xol-1 XX
This strain was propagated for >10 generations, 50% female and 50% male, without change in properties. The males in the population have the striking property that when outcrossed to any normal hermaphrodite, they invariably sire 100% female progeny, despite their own male phenotype. This is because the e2020 mutation is dominant and all progeny are necessarily tra-2(e2020)/+; tra-1(null)/+ XX.

A related stable strain was also constructed using the eg mutation e2531 (Hodgkin and Albertson 1995; Kuwabara 1996a). The e2531 mutation results in a misense change in an extracellular domain of the receptor TRA-2A, which probably prevents binding to the inhibitory ligand HER-1A, and as a result XO animals carrying one or two doses of e2531 are completely transformed into fertile hermaphrodites. The e2531 mutation was originally isolated in cis with the gf mutation e2046, and e2046/e2531 mutants therefore develop as females, irrespective of X chromosome dosage. A null mutation of tra-1 is fully epistatic to tra-2(e2046/e2531), despite the presence of two different dominant feminizing mutations in the tra-2 gene (Hodgkin and Albertson 1995): Strain 4: Females, tra-2(e2046/e2531); tra-1(null)/tra-1(+)
Males, tra-2(e2046/e2531); tra-1(null)/tra-1(null)

In the four strains above, an active tra-1 gene [either tra-1(+) or tra-1(gf)] acts as a dominant feminizing factor, so the sex-determination system resembles that found in birds, with heterogametic ZW females and homogametic ZZ males. However, the Z and W chromosomes (derived from LGIII, the location of tra-1) are isomorphous.

**Sex determination by the autosomal gene tra-2:** The most important product of the tra-2 gene is a transcript encoding the membrane protein, TRA-2A, which is believed to act as a receptor for the masculinizing HER-1A ligand. Null mutations of tra-2 result in male development of XX animals, but the XX males are abnormal and mating defective, as a result of the bypass effect described in the Introduction. If the bypass is eliminated by also mutating xol-1, then the double-mutant tra-2[+]; xol-1 XX males are able to mate. This allowed Miller et al. (1988) to construct a stable strain in which tra-2 acts as a dominant feminizing factor: Strain 5: Females, tra-2(gf)/tra-2(null); xol-1
Males, tra-2(null); xol-1

In this strain, xol-1 acts as an enhancer of the masculinization caused by tra-2, such that tra-2(null); xol-1 XX animals are fertile mating males. The presence of the xol-1 mutation also means that all XO animals are inviable, so this is an obligate XX strain. A variant on this strain was constructed using if mutations only: Strain 6: Females, tra-2(null)/tra-2(+/); xol-1
Males, tra-2(null)/tra-2(+/); xol-1

This strain, like strain 5, grows as a stable male/female population. The fog-2 mutation results in transformation of hermaphrodites into females because the gametes normally destined to develop as sperm develop as oocytes instead, but fog-2 does not affect differentiation of sperm within the testis of a male (Schedl and Kimble 1988).

These various strains, which are useful for strain construction, complementation of sex-linked genes, and other purposes, provide convenient sources of XX mating males. XX males of different genotypes differ markedly in fertility, and their fertility has a major effect on how well the different artificial strains grow. A systematic comparison of XX male fertility was carried out by testing males under standard conditions (Table 2). The data show that wild-type XO males are more fertile than any of the XX males, as might be expected. Both tra-2(q276) XX and tra-2(e1095); xol-1(y9) XX males are much more fertile than any tra-1 XX male, with maximal male fertility seen with the latter. However, combining tra-2(q276) and xol-1(y9) does not lead to any further increase in male fertility, probably because the q276 mutation is a complex allele with both masculinizing and feminizing effects.

A different tra-2-determined strain, in which all viable animals are XO, was constructed using the dosage compensation mutant dpy-26(n199), which results in the death of almost all XX animals but does not affect XO animals (Hodgkin 1983b; Plenefisch et al. 1989): Strain 7: Females, tra-2(gf,eg)/+; dpy-26 XO
Males, tra-2(+)/tra-2(+); dpy-26 XO

Like other obligate XO strains, this strain does not grow with great fecundity, but is stable. In all tra-2-dependent strains, a dominant feminizing factor is provided by an active tra-2 gene on LGII, hence, this behaves as a W chromosome.

**Maternal sex determination by the autosomal gene tra-3:** The tra-3 gene encodes a calpain protease, which can act to cleave TRA-2A and thereby to enhance its activity (Sokol and Kuwabara 2000). It may also increase tra-2 activity by modulating translational repression of TRA-2A (Goodwin et al. 1997). Null mutations of tra-3 resemble weak mutations of tra-2 in their effect, causing XX animals to develop into infertile males or intersexes (Hodgkin and Brenner 1977). XO animals are unaffected and develop as fertile males. In addition, the gene exhibits a strong maternal rescue effect: tra-3 homozygous XX progeny generated from tra-3/+ heterozygous mothers develop as normal fertile hermaphrodites because they have inherited sufficient tra-3 product (either mRNA or protein) to sustain normal development. In the next generation, however, these rescued hermaphrodites produce self-progeny broods consisting entirely of XX pseudomales.

The maternal rescue effect made it possible to use a
**TABLE 2**

Male fertility measurements

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fraction fertile</th>
<th>No. assayed</th>
<th>Mean progeny sired</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type XO</td>
<td>11/11</td>
<td>11</td>
<td>1881</td>
<td>213–2871</td>
</tr>
<tr>
<td>tra-1(e1099) XX</td>
<td>10/46</td>
<td>10</td>
<td>43</td>
<td>2–262</td>
</tr>
<tr>
<td>tra-1(e1781) XX</td>
<td>3/36</td>
<td>3</td>
<td>27</td>
<td>15–63</td>
</tr>
<tr>
<td>tra-2(q276) XX</td>
<td>12/30</td>
<td>4</td>
<td>408</td>
<td>260–728</td>
</tr>
<tr>
<td>tra-2(e1095); xol-1(y9) XX</td>
<td>9/9</td>
<td>3</td>
<td>1202</td>
<td>956–1412</td>
</tr>
<tr>
<td>tra-2(q276); xol-1(y9) XX</td>
<td>13/20</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>tra-2(ar221); xol-1(y9) XX</td>
<td>10/20</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Tests and lifetime mating assays were carried out as in Hodgkin and Doniach (1997). The No. assayed column gives the number of fertile males for which complete lifetime fertilities were measured. For comparison, the table also includes data previously published in Hodgkin (1987a; tra-1 alleles, lines 2 and 3) and Hodgkin and Doniach (1997; wild type, line 1). NA, not applicable.

**tra-3 mutation to construct a stable male/female strain** in which sex is determined by maternal genotype. Populations of this strain consist of males and two kinds of female, thelygenic (producing entirely female progeny, abbreviated T) and arrhenogenic (producing entirely male progeny, abbreviated A):

**Strain 8**: T females, tra-3(null)/tra-3(+) ; fog-2; xol-1

A females, tra-3(null)/tra-3(null); fog-2; xol-1

Males, tra-3(null)/tra-3(null); fog-2; xol-1

Crosses between T females and males produce all-female broods consisting of equal numbers of T females and A females. Crosses between A females and males produce only males. Consequently, the strain is stable and grows as a mixture of 25% T females, 25% A females, and 50% males. Only a few of the males are capable of siring progeny because of the relatively weak effect of tra-3 mutations even when enhanced by xol-1, so this strain grows very poorly.

**Strain 8** provides an example of maternal sex determination comparable to that naturally observed in the carrion fly C. rufifacies (Schutt and Nothiger 2000).

**Sex determination by the autosomal gene her-1**: The her-1 gene encodes a small secreted protein, HER-1A, which is believed to be an extracellular ligand for the transmembrane receptor protein TRA-2A (Kuwabara et al. 1992; Perry et al. 1993). HER-1A mRNA is produced only in XO animals, as a result of transcriptional regulation of her-1 by SDC-2 (Dawes et al. 1999). Loss-of-function mutations of her-1 are recessive and result in transformation of XO animals into fertile hermaphrodites (Hodgkin 1980). Gain-of-function mutations of her-1 are dominant and cause partial masculinization of XX animals (Trent et al. 1988). More extreme masculinization is seen in homozygous her-1(gf); xol-1 XX animals, some of which develop into mating males. However, heterozygous her-1(gf)/+; xol-1 XX males are unable to mate successfully, which precludes use of her-1(gf) as a simple dominant masculinizing factor.

Instead, to construct a strain in which her-1(+) could act as a dominant masculinizing factor, an unusual sdc-3 mutation, sdc-3(y52), was used, which results in masculinization of XX animals but does not affect dosage compensation, unlike most sdc-3 alleles. XX animals of genotype sdc-3(y52); xol-1 develop into mating males (Delong et al. 1993) As expected from the standard model for sex determination, a her-1(null) mutation was fully epistatic to sdc-3(y52). This allowed construction of a male/hermaphrodite strain:

**Strain 9**: Hermaphrodites, her-1(null) sdc-3(y52)/her-1(null) sdc-3(y52); xol-1

Males, her-1(null) sdc-3(y52)/her-1(+) sdc-3(y52); xol-1

This strain was cultured successfully as a mixture of hermaphrodites and males for six generations and also preserved as a male/hermaphrodite frozen stock. Further propagation, without deliberate picking of males, resulted in reversion to a pure hermaphrodite population, with loss of her-1(+), as expected. A stable male/female XX strain could presumably be constructed by introducing fog-2, as in strain 10 (below).

A male/female XO strain in which sex is controlled by her-1 was assembled by making use of a different sdc mutation, a strong allele of sdc-2. This results in the death and masculinization of all XX progeny. XO animals are not affected by sdc-2, and her-1(null); sdc-2 XO animals develop into fertile hermaphrodites. Incorporation of fog-2 permitted the establishment of a male/female strain:

**Strain 10**: Females, her-1(iff) fog-2; sdc-2 XO

Males, her-1(iff) fog-2/her-1(+) fog-2; sdc-2 XO

In strains 9 and 10, her-1(+) behaves as a dominant masculinizing factor, and the presence of a single active
her-1 gene is sufficient to cause completely male development. This therefore corresponds to a single-locus, dominant masculinizing system such as a dominant Y, prevalent throughout the animal kingdom.

**Sex determination by fem-1:** The three fem genes, fem-1, fem-2, and fem-3, encode cytoplasmic proteins believed to act as second messengers connecting modulation of TRA-2A activity at the cell membrane to regulation of TRA-1A activity in the nucleus.

Various protein-protein interactions between these five proteins have been discovered, but the full details of their roles and regulation in soma and germline are far from clear. Moreover, in addition to their roles as regulators of tra-1 activity, the FEM proteins are also required for spermatogenesis. FEM-1 contains ankyrin repeats and has mammalian homologs; FEM-2 is a protein phosphatase, while FEM-3 lacks obvious homologs in taxa other than nematodes (Spence et al. 1990; Ahrringer et al. 1992; Pilgrim et al. 1995).

All three fem genes exhibit similar genetic properties: loss-of-function mutations result in fertile female development of both XX and XO individuals, but only if fem activity is absent from both maternal parent and zygote (Doniaich and Hodgkin 1984; Hodgkin 1986). If fem(+) activity is present in the mother, incomplete feminization occurs even if the zygote is homozygous fem(null). The residual male development occurs because fem(+) products can be inherited from the mother via ooplasm.

Strong mutations of either fem-1 or fem-3 are epistatic to masculinizing mutations of tra-2 at all temperatures, allowing construction of:

**Strain 11:** Females, tra-2(null); fem-1(null); xol-1

**Males,** tra-2(null); fem-1(null)/fem-1(+); xol-1

A fem-1(+) allele is never present in the females of this strain, so the maternal rescue effect does not interfere. Strain 11 is stable and grows well. In this strain, LGIV, containing the dominant masculinizing activity of fem-1(+), behaves like a mammalian Y chromosome, so the strain mimics XX female/XY male patterns of sex determination.

**Sex determination by fem-2:** An analogous strain was constructed for fem-2. All mutations of fem-2 are temperature sensitive, probably as a result of intrinsic temperature sensitivity in the whole pathway such that fem-2(+) activity is essential only at higher growth temperatures (Hodgkin 1986). At a temperature of 25°, fem-2(null) mutations result in complete feminization of XX and XO, as in mutations in fem-1 and fem-2. As a result, it was possible to construct a male/female strain dependent on fem-2:

**Strain 12:** Females, tra-2(null); fem-2(null); xol-1

**Males,** tra-2(null); fem-2(null)/fem-2(+); xol-1

This strain was propagated successfully at 25°, but on shifting to a lower temperature, all animals become partly or wholly masculinized, and the strain dies out. This effect should allow selection for modifiers that increase feminization by fem-2(null) at lower temperatures, but no success occurred in several shift-down experiments of this type.

In contrast to strains 1–4, in which LGII behaved as a sex chromosome carrying a dominant feminizing element and therefore as a W or Z chromosome, in strain 12 LGII carries (at a different location) a dominant masculinizing element and therefore behaves as a Y or an X chromosome.

**Sex determination by fem-3:** A strain comparable to strains 11 and 12 was constructed using fem-3 instead of fem-1:

**Strain 13:** Females, tra-2(null); fem-3(null); xol-1

**Males,** tra-2(null); fem-3(null)/fem-3(+); xol-1

In this strain, LGIV carries either a dominant masculinizing fem-3(+) allele or a recessive feminizing fem-3(null) allele and therefore behaves as a Y or an X chromosome.

**Sex determination by a suppressor tRNA:** The strains described in preceding sections all utilize one or more of the seven major sex-determining genes to control sexual phenotype. Additional genes can be artificially recruited into the network of interacting genes, and thereby given the status of sex-determining factors, by making use of suppression effects. An example is provided:

**Strain 14:** Hermaphrodites, sup-34(+); tra-3(am); xol-1

**Males,** +/+; tra-3(am); xol-1

The amber nonsense mutation used in this strain, e1107, is efficiently suppressed by a single dose of sup-34, which encodes an amber-suppressing Trp tRNA (Kondo et al. 1990). In the absence of sup-34, the tra-3; xol-1 animals develop as low-fertility males. The strain grows as a sustained mixture of hermaphrodites and males because sup-34/sup-34 homozygotes are much less fertile than sup-34(+)/+ heterozygotes. An obligate female/male strain could be constructed by introducing a fog-2 mutation, as in strain 8.

In this strain, the presence or absence of a tRNA mutation determines the difference between hermaphrodite and male development, and the mutation behaves as a dominant feminizing element. The tRNA gene in question is located on LGI, which therefore behaves as a sex chromosome although it does not carry any of the major sex-determination genes discussed above.

**Sex determination by a fragment chromosome:** Experiments investigating the properties of a small chromosomal inversion mutation, eC2, which includes a tra-1(gf) mutation, led to the generation of a derived free duplication, Dp25 (Zarkower et al. 1994). This consists of two fused right ends of LGIII (from dpy-18 to the right telomere) and also carries a tra-1(gf) mutation, which feminizes both XX and XO animals. A male/female strain based on this duplication was constructed:
Strain 15: Females, trai-1(ly); eDp25(trai-1(gf))

Males, trai-1(ly)

This strain is comparable to strain 1, being trai-1 based, but in this case the functional copy of trai-1 is located on a small chromosomal fragment, so the determining factor is epigenetic rather than chromosomal. Fragment chromosomes are frequently lost at meiosis in C. elegans, especially ones such as eDp25, which tend to pair with themselves and therefore lack a partner to disjoin from. Consequently, the sex ratio in strain 15 is extremely male biased, in contrast to most of the preceding strains. Six fertilized females from strain 15 produced a total of 143 females and 639 males (82% male). This potentially creates a situation selecting for modifiers that would shift the ratio toward equality, but in fact the mating efficiency of trai-1(ly) males is so low that such selection is unlikely to operate. However, possible modification of this type has been observed with a different strain, as described below (strain 16).

Sex determination by an extrachromosomal element:
Further variations on the sex-determination system can be achieved by making use of artificial transgene arrays that carry normal or mutant versions of cloned sex-determination genes. This was demonstrated by making use of arrays carrying a wild-type copy of fem-1; these arrays are able to rescue both the somatic and germline phenotypes of fem-1(null) mutants (Spence et al. 1990). Strain 11 (above) was modified by introducing such an array to replace the chromosomal copy of fem-1(+) with an extrachromosomal array:

Strain 16: Females, trai-2(null); fem-1(null); xol-1

Males, trai-2(null); fem-1(null); xol-1; eEx[fem-1(+)]

This strain was propagated successfully for >50 generations as a male/female culture. Two features of construction and propagation of strain 16 are noteworthy. First, during early generations of propagation one unusual self-fertile animal was observed; this was picked and used to establish a line of mixed hermaphrodites and females. In this derived line (strain CB4707), the properties of eEx14 appear to have been modified so that it is active in the germline but not in the soma. Consequently, the trai-2; fem-1; xol-1; eEx14 animals develop as somatic females, but are able to produce enough sperm for substantial self-fertility. The CB4707 line is no longer dependent on mating for propagation, so there is strong selection for this kind of modification of array expression. However, the effect is surprising in that most transgenic arrays are expressed much better in somatic tissues than in the germline, rather than vice versa, as in this case. In later generations of propagation of strain 16, no self-fertile animals appeared, suggesting that eEx14 had stabilized in its properties.

Second, the sex ratio in populations of strain 16 is determined by the efficiency with which the masculinizing array is transmitted and expressed, rather than by the meiotic segregation of chromosomes (as in most of the other strains discussed so far). In strain 11, for example, the observed sex ratio in total progeny from 11 single pair matings was 1708 females:1766 males. This ratio is not significantly different from the 1:1 ratio expected for normal meiotic segregation of chromosome IV, which bears the sex-determining locus in strain 11. Extrachromosomal arrays, however, are not reliably handled by the meiotic machinery, and therefore ratios very different from 1:1 can be generated in principle. Populations that start at a sex ratio different from 50:50 are under selection to move to a more equal sex ratio, according to the argument ascribed to Fisher (1930).

In strain 16, a small shift in the sex ratio produced from single pair crosses, from 56% female (133/237) at an early generation to 49% (508/1042) at a later generation, was indeed observed during long-term propagation. However, neither of these ratios is significantly different from 50%. It is nevertheless likely that a strongly biased starting sex ratio could be created by using an extrachromosomal array with different transmission properties, which could be examined over time, thereby allowing a direct test of the Fisher principle.

Sex determination by temperature: Temperature-sensitive mutations have been obtained for most, although not all, of the seven major sex-determination genes. This was demonstrated by making use of arrays carrying a wild-type copy of fem-1; these arrays are able to rescue both the somatic and germline phenotypes of fem-1(null) mutants (Spence et al. 1990). Strain 11 (above) was modified by introducing such an array to replace the chromosomal copy of fem-1(+) with an extrachromosomal array:

Strain 17: Hermaphrodites, trai-2(ar221); xol-1 XX (15°)

Males, trai-2(ar221); xol-1 XX (25°)

Several different temperature-sensitive mutations of trai-2 have been isolated during work on this gene (Klass et al. 1976; Hodgkin and Albertson 1995). The ar221 mutation (isolated and generously made available by J. Hubbard) is more extreme in its range of variation than those previously reported, such that trai-2 is almost inactive at the high temperature and substantially active at the low temperature. Animals of strain 17 grown at 25° (after shifting the parent hermaphrodites to this temperature at late L4 stage) are invariably male in phenotype and exhibit mating behavior. Of 25 individuals examined in detail by Nomarski microscopy, all had well-formed male gonads with abundant spermatogenesis and no signs of oogenesis. Most had fully developed male tails, but 5/25 exhibited some defects, such as stunted copulatory spicules. In mating tests, half (10/20) were capable of siring progeny (Table 2). Animals of strain 17 grown at 15° were usually self-fertile hermaphrodites, although most exhibited signs of mascu-
linization such as an Egl (egg-laying defective) phenotype and a truncated tail spike. Increased masculinization was seen when the strain underwent partial starvation. Strain 17 (CB5362) is potentially useful for genomic and biochemical studies because populations can be grown in bulk at low temperature, synchronized, and then shifted to high temperature. The resulting population will consist of pure phenotypic XX males, at any desired stage of development, which can be used as starting material for extraction of protein or RNA. These males will not be completely identical to wild-type XO males (which cannot easily be prepared in pure populations), but differences may be minimal and irrelevant for many purposes.

The contrasting strain, which is male at low temperature and hermaphrodite at high temperature, was made possible by a temperature-sensitive mutation in the gene her-1:

Strain 18: Hermaphrodites, her-1(e1561); dpy-26(n199) XO (25°)
Males, her-1(e1561); dpy-26(n199) XO (15°)

The dpy-26 mutation results in XX inviability, so the only surviving animals are XO in karyotype, as in some previous strains (strains 7 and 10). At low temperature, the her-1 gene has sufficient activity to direct male development, while at high temperature, the her-1 gene is inactive and consequently animals develop as hermaphrodites. Approximately 90% of animals grown at low temperature are male, with 10% intersexes; 100% of animals grown at high temperature are hermaphrodite in phenotype, although of low fertility like XO hermaphrodites of other genotypes.

Sex determination by a 3X/2X mechanism: As a result of X chromosome overexpression, mutations in the dosage compensation gene dpy-27 result in lethality to diploid XX animals (Plenefisch et al. 1989). Similarly, tetraploid 4A; 4X animals mutant for dpy-27 are also inviable. However, dpy-27 4A; 3X hermaphrodites are viable and non-Dpy, presumably because of the lesser overexpression from the X chromosome (Hodgkin 1987b). As a result, a tetraploid strain homozygous for dpy-27(rh18) can be maintained. Because the X chromosome is present in three copies, self-progeny consist of a mixture of 4X (inviable), 3X (hermaphrodite), and 2X (male) individuals:

Strain 19: Hermaphrodites, dpy-27(rh18) 4A; 3X
Males, dpy-27(rh18) 4A; 2X

This strain grows well as an obligate tetraploid population. Complete broods for eight hermaphrodites were counted: average self-progeny consisted of 92 zygotes, of which 23% were inviable, 47% were hermaphrodite, and 30% were male. Occasional intersexes (<1%) were also seen. In this strain, the X chromosome dose is still sex determining, but the distinction is now between 3X (hermaphrodite) and 2X (male), rather than between 2X (hermaphrodite) and XO (male).

Sex determination by neo-X and neo-Y chromosomes: Most of the previous strains have a karyotype related to that of wild-type strains of C. elegans. Chromosomal rearrangements can be used to create distinctly different sex karyotypes, however. This was first done by Stetson et al. (1986), who used mnt12 (a fusion between the right end of LGX and the left end of LGIV) to generate a neo-X neo-X hermaphrodite, neo-X neo-Y male strain:

Strain 20: Hermaphrodites, mnt12(X;IV)/mnt12(X;IV) Males, mnt12(X;IV)/+ IV

In this strain, the normal XX hermaphrodite / XO male system is converted into an XX hermaphrodite / XY male system, with a normal LGIV behaving as a neo-Y. However, the neo-Y does not itself have male-determining properties.

Isolation of mutants defective in telomere maintenance has allowed the generation of many other X autosomal fusions in C. elegans (Ahmed and Hodgkin 2000 and our unpublished results). All of these are like mnt12 in being homozygous viable and could be used to generate corresponding neo-X/neo-Y systems. One of them, eT5 (a fusion between the left end of LGX and the right end of LGIII), was combined with fog-2 and an autosomal translocation, eT1, to create a male/female strain of a more complicated kind, with multiple X and Y chromosomes, such as occur in many insect species. The resulting strain has an arrangement resembling that found in the beetle Cynium volkameriae (Virkki 1968), with X1X2Y1Y2 females and X1X2Y1Y2 males:

Strain 21: Females, eT5(X;III)/eT5(X;III); fog-2 V/fog-2 V Males, eT5(X;III)/eT1(III;V); fog-2 V/eT1 (fog-2 V;III)

The translocation eT1 is a reciprocal translocation of the right arm of LGIII and the right arm of LGV (Rosenbluth and Baillie 1981). In strain 21, eT5 and a normal LGV behave as neo-X, and neo-X cha- somes, and the reciprocal parts of eT1 behave as neo-Y, and neo-Y chromosomes. The strain propagates as a male/female strain because of the presence of the fog-2 mutation. In the males of this strain, the eT1 reciprocal translocation acts as an efficient recombination suppressor for the right arm of LGIII and the left arm of LGV. Therefore, all genes in these regions are effectively insulated from recombination with their homologs, and they are also transmitted solely in and by males. They will never be exposed to selection in a female individual. This sets up a situation for the long-term accumulation of male-biased mutations in these regions, and perhaps for the development of further chromosomal dimorphism. In principle, it may be feasible to propagate this or comparable strains for long enough to see if such
effects occur because populations of *C. elegans* will go through at least 100 generations a year, if maintained with adequate nutrition.

The promiscuous chromosome fusions made possible by *mrt-2* and related mutants, together with existing translocations and rearrangements, should also allow creation of yet further variations in sex karyotype. Construction of variant strains has been far from exhausted by the present study.

**DISCUSSION**

The strains described here demonstrate the considerable variety of sex-determination systems that can be artificially created for *C. elegans*. In most of these strains, a single factor assumes the major role for determining sex, but this factor can be a transcription factor (strains 1, 2, 3, 4, and 15), a transmembrane receptor (strains 5, 6, 7, and 17), a calpain-related protease (strain 8), an extracellular protein (strains 9, 10, and 18), a conserved cytoplasmic protein (strains 11, and 16), a phosphatase (strain 12), a novel cytoplasmic protein (strain 13), or a tRNA (strain 14). It follows that in animals with unknown sex-determination mechanisms, more or less any kind of molecule might be the primary determinant.

Moreover, the genes encoding these critical factors are scattered across the genome, so any one of autosomes II, III, IV, or V can easily be turned into a sex chromosome. The only one of the five autosomes that does not naturally carry a major sex-determination gene is LG1, and even this chromosome can be recruited into playing the sex-determining role by making use of the amber suppressor *sup-34* (strain 14). An alternative route to recruiting LG1 would be to fuse X and I or to integrate the *fem-1(+) extrachromosomal transgene used in strain 16 into LG1*. Gross chromosomal rearrangements or chromosome fusions can also be used to create karyotypically diverse stable strains, as in strains 19–21. All the strains described in this article have different sex chromosome constitutions and different life history parameters from the natural *C. elegans*, and this will impose different selective pressures on these strains as compared to the ancestral race. Since the strains are stable, it should be possible to grow them indefinitely, creating multiple possible scenarios for *in vitro* chromosome evolution. The 3-day generation time of *C. elegans* means that cultivation for hundreds of generations is feasible. On the other hand, the amount of variation available for selection *in vitro* is limited, so it is hard to predict whether interesting changes would arise if these were mainly dependent on *de novo* mutation.

In some of these strains, the sex-determination system has been altered so that the dosage compensation apparatus is no longer necessary. The compensation machinery might therefore be expected to undergo change or loss during prolonged cultivation of such strains. Dosage compensation mechanisms have probably arisen and decayed multiple times during animal evolution as a result of comparable changes in the sex-determination system.

The construction of the assorted strains described in this article involved the creation of many double- and triple-mutant combinations that have not been created previously. None of them exhibited a phenotype unpredicted from the standard genetic model for sex determination, and our analyses provide strong support for the correctness of the model and for an essentially hierarchical organization. The pathway, however, does remain far from fully described or understood, particularly in molecular details.

The natural *C. elegans* system has several properties that may have favored these artificial manipulations of sex determination. First, the existence of a self-fertile XX hermaphrodite sex means that both XX and XO germ cells must be able to naturally undergo spermatogenesis. In other animals, sex-transformed XX males are usually defective in spermatogenesis. Oogenesis seems to be less critically dependent on correct sex karyotype, so that, for example, XO female mice are fertile (*Ashworth et al. 1991*). Second, the seven major sex-specific regulatory genes affect both somatic and germline phenotypes in parallel ways, so any one of these seven major genes can be made to act as a switch. Third, these genes are largely or completely specific to sex determination, so mutants do not exhibit pleiotropic phenotypes. Fourth, the sex-determination genes are organized in a relatively simple hierarchy, so that epistasis in double-mutant combinations is usually complete. Fifth, nematode chromosomes are holocentric, so that fragment chromosomes and extrachromosomal arrays are transmitted fairly stably through meioses. Similarly, chromosomal fusions are stable.

The malleability of *C. elegans* with respect to sex determination is not a unique property of this species. Some animal species appear to be comparably flexible and have the potential to adopt several different sex-determination systems. Different races of housefly (*Musca domestica*) exhibit a variety of sex-determination schemes (*Schutt and Nothiger 2000*). Similar variability is encountered in certain mammalian species such as wood lemmings (*Liu et al. 2001*). These systems are poorly understood at present, so it is difficult to speculate about the factors underlying their malleability or about whether they share any of these contributory factors with *C. elegans*.

The strains reported here provide mimics of many but by no means all of the different sex-determination systems encountered in the biological world. Four important categories that have not been imitated in the present study are control by nutrition, control by social factors, control by endosymbionts, and control by ploidy (as in Hymenoptera). The first two may well become possible by means of further manipulation. In the case
of nutritional control, some of the existing C. elegans sex-determination mutants exhibit striking changes in phenotype under starvation conditions, suggesting that they might be used in this way. For example, the hyper-morphic tra-2 mutation e2020 has little effect on XO animals when they are well fed, so they develop into fertile males; but under starvation conditions, the XO animals are extensively feminized in the nongonadal soma (Doniaich 1986). Combination with other starvation-sensitive mutants might allow complete transformation from fertile male to fertile female, depending on food supply.

Environmental sex determination by social factors is a related and potentially overlapping effect, because population crowding can affect both nutrition and social signals. In the case of various parasitic nematodes, some of which tend to develop into males at high population density and females at low population density, it seems more likely that the relevant factor is social rather than nutritional (Triantaphyllou 1973). Peckol et al. (2001) recently demonstrated that the regulation of some genes in C. elegans is dependent on social factors. They found that expression of an srd-1 reporter transgene is very sensitive to population density, probably detected by means of dauer pheromone, and modulated even at population densities well below those that trigger dauer larva formation. If the pheromone-sensitive regulatory region in srd-1 were identified and cloned into an appropriate sex-determination gene construct, it would be possible to create crowding-based sex determination in C. elegans.

Endosymbiotic bacteria, which are known to affect sex determination in many arthropods (Bandi et al. 2001), are absent from the Caenorhabditis species. However, they exist and are important in related nematodes such as the filarial genera Brugia and Wuchereria (Bandi et al. 1998). If it becomes possible to introduce such bacteria (Wolbachia species) into C. elegans, conceivably by ooplasm injection, then further modification might be feasible, leading to an endosymbiont-based sex-determination system.

Haplo-diploid sex determination may be impossible to re-create in C. elegans, because haploids of this species are unable to complete embryogenesis (Schierenberg and WOod 1985). Their inviability is somewhat puzzling in that chromosomal balance should be identical between a normal diploid (AA;XX) and a haploid (A;X). Furthermore, no recessive lethals are segregating, and no imprinting appears to occur in C. elegans (Haack and Hodgkin 1991). One possible explanation is that excess dosage compensation occurs, resulting in insufficient X chromosome expression and therefore in lethality. Another possibility is that the nuclear volume of a haploid C. elegans cell is simply too small to sustain normal gene regulation and expression. Nuclear volume seems to be determined largely by DNA content, and the 50-Mb DNA content of a haploid C. elegans nucleus would be smaller than any known animal genome. If this is the critical factor, perhaps it might be possible to rescue haploid development by adding extraneous transgenic DNA, but unless the problem of haploid inviability can be solved, this realm of the sex-determination universe will remain inaccessible to C. elegans.

Other limitations are also apparent from the work so far. For example, it appears to be much easier to create usable temperature-sensitive mutations in some genes, such as tra-2 or fem-1, than in others, such as tra-1. One can imagine a variety of possible explanations for this difference, but whatever the explanation, it has potential evolutionary consequences for transitions between chromosomal and environmental sex determination. It appears likely that it is much easier to put some genes under environmental control than others, so those genes would be more likely to evolve into the critical factor or factors.

Part of the enduring importance and power of classical mutational analysis is that it can be regarded as accelerated evolution and that it explores the envelope of possible past and future change in any biological system, thereby revealing potential courses for natural evolutionary events. Overall, this survey demonstrates that a few mutational changes can radically change the nature of sex determination in a single species in many different ways. By extension, any change that can be achieved by simple genetic manipulations in the laboratory must surely be even easier to achieve in nature, often in ways that we may not have imagined yet. The principle known as Orgel’s Second Rule is apposite here: “Natural selection is cleverer than Leslie Orgel” (F. H. C. Crick, personal communication). Transitions between different sexual systems are evidently not so difficult. The more challenging questions are to determine the past course of evolution for any of these sex-determination mechanisms and to identify what selective forces drive the transitions in the real world.

I am grateful to Tim Schedl and Jane Hubbard for providing unpublished strains, to Debbie Whittington for technical assistance, and to the Medical Research Council for support. Some of the strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health, National Center for Research Resources.

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