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The evolution of chromosomal sex determination

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Abstract. There is a great diversity of sex determination mechanisms, with evidence for numerous evolutionary transitions between different systems. For example, environmental sex determination is widespread in lower vertebrates, and genetic sex determination has probably evolved from it several times. This requires the establishment of genes that override environmental cues. Close linkage between male and female determining loci is favoured by selection, and represents the first step towards the evolution of highly differentiated sex chromosomes. Once crossing over between primitive sex chromosomes has been suppressed, the primitive Y (W) chromosome is vulnerable to the operation of forces that lead to a reduction in its effective population size. This reduces the ability of natural selection to maintain the functionality of genes on the proto-Y, so that it gradually degenerates. Primitive sex chromosome systems, and systems of neo-X and neo-Y chromosomes formed by translocations involving autosomes and sex chromosomes, provide an opportunity to test evolutionary models of the degeneration of Y chromosomes and to determine the time-scales involved. Recent data confirm that newly-evolving Y or neo-Y chromosomes experience a sharp reduction in effective population size, and indicate that degeneration can occur over a few million generations.

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Sexual reproduction is prevalent throughout eukaryotes, and probably represents their ancestral state. Gamete dimorphism (numerous, small, motile gametes versus few, large and immotile gametes) is the basis of the male–female distinction and is not required for sexuality: many sexual lower eukaryotes produce gametes of equal size (Hoekstra 1987). Even if there is gamete dimorphism, cosexuality (in which an individual produces both male and female gametes), is widely distributed in animals and plants (Jarne & Charlesworth 1993). As Darwin (1859) pointed out, cosexuality may well have been the ancestral state in chordates; this is certainly the case in flowering plants (Bull 1983, Charlesworth & Guttman 1999). In these groups, the distinct developmental programmes required for the production of male and female reproductive structures and gametes must have evolved before

the establishment of separate males and females. Sex determination is then simply a decision to restrict an individual's development to one of two potential, pre-existing, pathways.

It is, therefore, not surprising that there is an enormous diversity of sex determining mechanisms (Table 1), as well as many evolutionary transitions between different systems (Bull 1983, 1987). For example, environmental sex determination (ESD), usually involving effects of temperature at critical stages during embryonic development, is widely distributed among cold-blooded vertebrates (Sinclair et al 2002, this volume). Phylogenetic analysis indicates that ESD may have been ancestral in the vertebrate lineage, and that several transitions from ESD to genetic sex determination (GSD) have occurred (Janzen & Paukstis 1991, Kraak & Pen 2001).

We can only speculate about the evolutionary causes of most of these systems (Bull 1983, 1987), except when within-species variation allows experimental analysis of the fitness effects of different sexual phenotypes or genotypes. Theoretical analysis shows that ESD is favoured when there is spatial heterogeneity in the environment, such that the relative fitnesses of males and females vary between different environments. For ESD in turtles, there is evidence for higher survival at a given temperature of the sex which is produced most frequently at that temperature (Janzen 1995). In contrast, GSD is commonly thought to be favoured over ESD if the environment fluctuates over a suitable time-scale, since it is disadvantageous to produce a highly skewed sex ratio over a long run of generations (Bull 1983, 1987). An alternative model has recently been proposed (Kraak & Pen 2001), but experimental evidence is currently lacking.

In other cases, the mode of transmission of the sex determinant itself generates a selective advantage. For example, a maternally transmitted cytoplasmic factor can prevent the production of male offspring, which cannot pass it on to future generations (Rigaud 1997). Similarly, systems such as haplodiploidy and female XY lemmings are associated with intrinsic transmission advantages to the genetic factors involved (Bull 1983, Fredga 1994). In other cases, such as the *M* factor of houseflies (Table 1), the change in sex determination system is in itself selectively neutral, and may have been established by genetic drift or by pleiotropic effects on fitness (Bull 1983, 1987).

The evolution of sex chromosomes

The existence of structurally and genetically highly divergent sex-determining chromosomes presents a challenge to evolutionists. In advanced systems of this kind, there is lack of crossing over between the X and Y chromosomes over all or most of their length (from now on, Z and W will be treated as equivalent to X

TABLE 1 Modes of sex determination

	Environmental sex determination
	Sex is determined by temperature during embryonic development in chelonians, crocodilians, and some lizard and fish species; by nutritional status in mermithid nematodes; by the presence or absence of female individuals in the marine echiurid worm *Bonnellia*.
	Genetic sex determination
Two factor systems	Sex is determined by a pair of Mendelian alternatives, either with male heterogamety (XX females and XY males, as in mammals, *Drosophila*, and most dioecious plants) or female heterogamety (WZ females and ZZ males, as in birds, Lepidoptera, many lower vertebrates, and strawberries). The sex-determining chromosomes may be highly distinct structurally and genetically, as in mammals, *Drosophila* and the white campion *Silene latifolia*. Alternatively, there may be a single genetic factor or small genetic region distinguishing the two, as in many dioecious plants, fishes such as the guppy (*Poecilia*), and many Dipterans such as blackflies. Intermediates with a limited amount of structural differences between the sex chromosomes are also found, as in the newt *Triturus* and the lizard *Cnemidophorus*.
Multiple factor systems	Additional factors interact with the basic sex determination system e.g. a dominant gene (M) that causes both XX and XY individuals to develop as males, so that females are always XX mm and males are either XX Mm or XY Mm. Such a gene is polymorphic in natural populations of the house fly, *Musca domestica*. In some microtine rodents, there are polymorphisms for X chromosome mutations that cause XY individuals to develop as females. Polygenic variation affecting sexual phenotype occurs in fishes such as guppies and medaka.
Haplodiploidy	In a number of arthropods, including mites and several insect taxa, diploid individuals produced by fertilized eggs develop as females, and unfertilized eggs develop as haploid males. In several species of Hymenoptera, this is underlain by a single sex determining locus with many alleles, such that heterozygotes develop as females, and homozygous diploids develop as males.
Paternal genome loss	This is genetically very similar to haplodiploidy; all offspring result from fertilized eggs, but the entire paternal genome is eliminated in males. This occurs in mites, scale insects, and sciarid flies. In some cases, elimination takes place in germ cells only, in others early in development in somatic and germ cells, so that it is then equivalent to haplodiploidy. In most cases, it is not known if sex is determined first, and chromosomes are eliminated from males, or whether elimination leads to haploidy and maleness. In *Sciara*, sex is determined by the mother, some females (Aa) producing only females, others (aa) producing only males, so that all males are aa.
Cytoplasmic sex determination	There are several species of Crustacea in which offspring sex is affected by intracellular symbionts, such as *Wolbachia* bacteria, that override the normal sex determination system of males. These are maternally transmitted, and are polymorphic in natural populations. Gynodioecy in plants (polymorphism for females and cosexuals) often involves cytoplasmic male sterility factors (probably mitochondrial), and their nuclear gene suppressors.

Sources: Bull (1983, 1987), Rigaud (1997), Charlesworth & Guttman (1999) and Kraak & Pen (2001).

and Y), as in mammals and some plant species, or a complete suppression of crossing over in the heterogametic sex, as in *Drosophila* and Lepidoptera (Bull 1983, 1987). This is often accompanied by a dearth of active genes on the Y chromosome, whereas the X usually carries a normal complement of genes for its size. The lack of active Y-linked genes is often accompanied by mechanisms which ensure approximately equal amounts of gene products at X-linked loci in males and females: dosage compensation (Bull 1983, Marín et al 2000). The Y also often contains an unusual abundance of highly repetitive DNA sequences (Bull 1983, Charlesworth et al 1994).

The Y thus presents the bizarre phenomenon of a sizeable chromosome, which often consists almost entirely of 'junk' DNA. In some groups (such as *Drosophila*), it is not required for sex determination, and in others (such as *Caenorhaditis elegans*) it has even been completely lost (Bull 1983). These are examples that represent intermediate stages between apparently single gene inheritance and fully differentiated sex chromosomes (Table 1). Even in some advanced sex chromosome systems, there may still be some genes in common between X and Y (Lahn & Page 1999). This suggests that X and Y chromosomes have diverged from a pair of ancestral, largely homologous, chromosomes. The comparative evidence shows that this must have occurred independently in many lineages (Bull 1983). But what leads to the degeneration of the Y chromosome, and the other features of advanced sex chromosome systems?

This question was first posed by H. J. Muller (1918), who also discovered dosage compensation. As he noted, a lack of crossing over between X and Y is required for them to remain genetically distinct, and must have been the precondition for the evolution of the other features of the Y chromosomes. There is an advantage to suppressing crossing over only when there are two or more polymorphic genes which interact in their effects on fitness (Barton & Charlesworth 1998). Such genes are likely to have been present from the start of the evolution of separate sexes (dioecy). If dioecy evolves from cosexuality, the simplest hypothesis is that females are created by a mutation that suppresses male function, and males by a mutation that suppresses female function (Charlesworth 1996, Charlesworth & Guttman 1999). If dioecy evolves from ESD, the simplest path involves one mutation that causes individuals to develop as females, and another that causes maleness, independently of any environmental cues (Bull 1983, Charlesworth 1996). If such mutations involve separate loci, crossing over among them would produce selectively disadvantageous neuters (Fig. 1). Invasion of the ancestral population by two successive mutations creating males and females thus requires initial close linkage of the two loci, and reduced crossing over is favoured once they are both polymorphic for the sex-determining alleles (Charlesworth & Guttman 1999). Similar principles apply to more complex multi-gene models (Charlesworth & Guttman 1999, Kraak & Pen 2001).

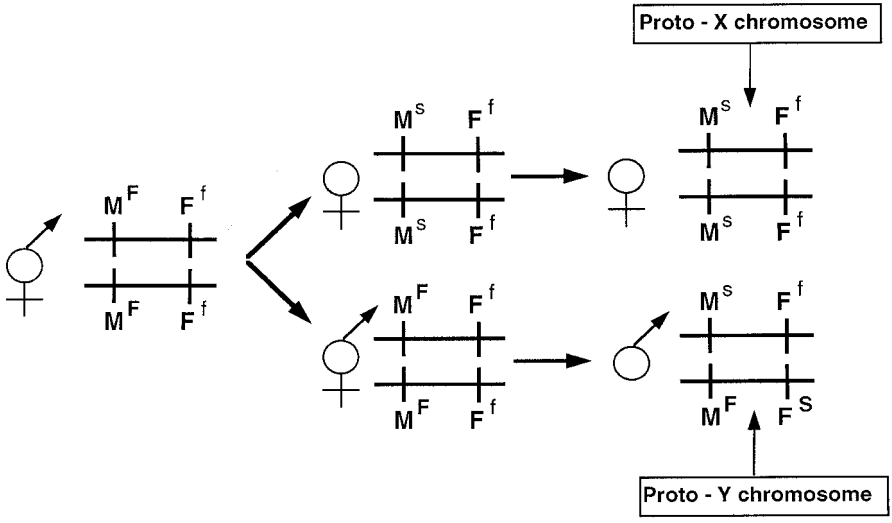

FIG. 1. The evolution of proto-X and proto-Y chromosomes from an initial cosexual state. M and F indicate loci controlling male and female fertility, respectively. Superscripts f and s indicate alleles conferring fertility and sterility, respectively. Dominant alleles are indicated by uppercase superscripts, recessive alleles by lowercase.

In addition, if there are loci with sex-dependent fitness effects, such that one allele is advantageous in males and disadvantageous in females, close linkage to the sex-determining genes is favoured by selection (Fisher 1931, Bull 1983, Rice 1996). The colour polymorphisms of the guppy, *Poecilia*, are a classic example of this: alleles conferring bright coloration are favoured by sexual selection on males, whereas alleles conferring dull colours are favoured in females, due to predation on brightly coloured individuals (Fisher 1931, Rice 1996). These genes are closely linked to the sex-determining region of the primitive sex chromosomes of this species, with alleles causing bright colours being closely associated with the male determinant. Cases where male fertility genes have apparently been transposed to the Y chromosome, such as *DAZ* in humans (Saxena et al 1996), are another possible example of this type of selection. If this process of accumulation of such 'sexually antagonistic' allelic effects is continued, it is easy to see how restricted recombination along the length of the proto-X and proto-Y chromosomes, or suppression of crossing over throughout the whole genome in the heterogametic sex, could evolve (Bull 1983, Rice 1996). Sequence comparisons of Y-linked genes in humans with their X-linked homologues do indeed provide evidence for a succession of steps towards suppressed crossing over, possibly as a result of a sequence of chromosomal inversions (Lahn & Page 1999). Of course, there is nothing inevitable about the establishment of complete crossover suppression,

consistent with the numerous intermediate stages between genetic and full chromosomal sex determination (Bull 1983, 1987).

Once crossing over has been suppressed, the proto-Y chromosome has the very unusual property of constituting a large, non-recombining haploid genome, which is permanently heterozygous. A deleterious mutation can therefore become fixed on the proto-Y chromosome without becoming homozygous. This process is facilitated by the fact that the number of Y chromosomes in the population is one-third of the number of X chromosomes, so that genes on the proto-Y chromosome are more vulnerable to genetic drift than their homologues on the proto-X. Sexual selection may further reduce the effective number of breeding males in systems with male heterogamety, thus enhancing this effect (Charlesworth & Charlesworth 2000).

The absence of genetic recombination also impairs the ability of natural selection to promote the fixation of adaptively favourable mutations and resist the fixation of deleterious ones (Barton & Charlesworth 1998). A variety of specific processes can lead to the faster accumulation of deleterious mutations, or slower accumulation of favourable mutations, on the proto-Y compared with the proto-X; these have recently been discussed (Charlesworth & Charlesworth 2000) and will not be reviewed in detail here. The majority depend on the 'Hill–Robertson' effect, which involves the fact that the increase in frequency of a favourable allele due to selection at one locus may cause an increase in frequency of a deleterious allele at a closely linked locus, so that the efficacy of selection is impaired in a non-recombining genome (Fig. 2 and Table 2). Collectively, these processes can be regarded as causing a reduction in the effective population size (N_e) of an evolving Y chromosome, thereby reducing the strength of selection relative to genetic drift. Given enough time, the functionality of genes on the proto-Y chromosome is expected to decline relative to that of genes on the proto-X chromosome (Charlesworth & Charlesworth 2000). The time-scale is likely to be long; a substantial decline in the fitness of the proto-Y may take more than a million generations, depending on the magnitude of the rate of mutation to deleterious alleles, the distribution of effects on fitness of such mutations and the population size of the species (Charlesworth & Charlesworth 2000). Y-linked genes that enhance male fitness, and whose functions cannot be supplied by their X-linked homologues, are likely to resist this process of erosion, since their loss would have drastic fitness consequences (Lahn & Page 1999).

The decline in fitness of the proto-Y relative to the proto-X in the heterogametic sex promotes the evolution of dosage compensation (Charlesworth 1996). This reflects the fact that most deleterious mutations have slight effects on fitness when heterozygous; in *Drosophila*, even so-called recessive lethals have been shown to reduce the viability of their heterozygous carriers by 1–2% (Crow 1993). Any accumulation of deleterious alleles on the proto-Y chromosome will

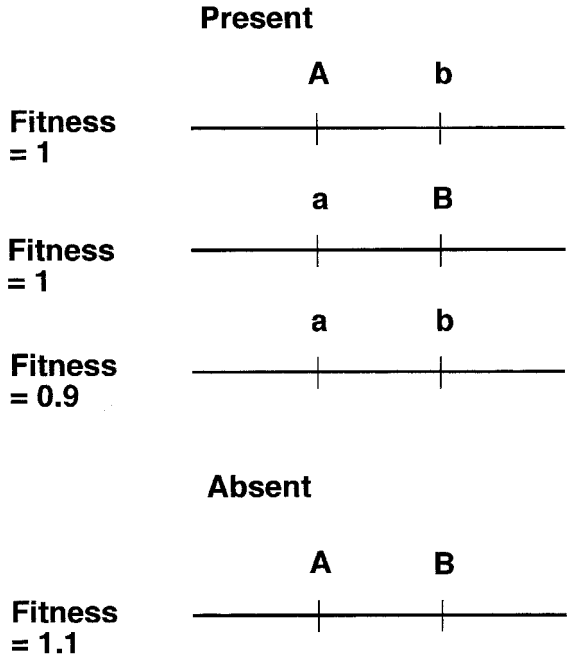

FIG. 2. The Hill–Robertson effect. A and B represent alleles at two different loci which are favoured by selection over their alternatives, a and b. If the initial state is ab, and A and B arise as independent mutations, the fittest combination AB cannot be produced in the absence of recombination.

therefore reduce the fitness of their carriers. There is thus an obvious advantage to enhancing the activity of genes transcribed from the proto-X, even at the expense of reducing the activity of their homologues on the proto-Y. Erosion of gene activity on the Y may therefore partly be an active process of down-regulation. If the up-regulation of X-linked genes were confined to the heterogametic sex, a dosage compensation system such as that of *Drosophila* would evolve, in which the end-product is a doubling of the rate of transcription of X-linked genes in males compared with females (Marín et al 2000). If, however, up-regulation is not sex-limited, X-linked activity would be doubled in both sexes, and no apparent dosage compensation would be observed, as is seemingly the case in Lepidoptera (Johnson & Turner 1979). This would in turn generate selection for restoring the level of activity in the homogametic sex to its previous, presumably optimal, level. This accounts for the systems of dosage compensation in mammals and *C. elegans*, which involve inactivation of the X and down-regulation of X-linked genes, respectively (Charlesworth 1996, Jegalian & Page 1998, Marín et al 2000).

TABLE 2 Evolutionary processes that can lead to reduced levels of adaptation and variation in a non-recombining genomic region

Hitchhiking by deleterious mutations (background selection)	A neutral or weakly selected mutation that arises in a large non-recombining population has a non-zero chance of survival only if it arises on a chromosome free of strongly deleterious mutations. The effective population size of a non-recombining chromosome can therefore be greatly reduced in a large population at equilibrium under selection and mutation. This accelerates the fixation of weakly deleterious mutations and retards the fixation of advantageous mutations.
Muller's ratchet	This involves the stochastic loss from a finite population of the class of chromosomes carrying the fewest deleterious mutations. In the absence of recombination and back mutation, this class of chromosome cannot be restored. The next best class then replaces it and is in turn lost, in a process of successive irreversible steps. Each such loss is quickly followed by fixation of a deleterious mutation on the chromosome.
Hitchhiking by favourable mutations	The spread of a favourable mutation in a non-recombining genome can drag to fixation any deleterious mutant alleles initially associated with it, so that successive adaptive substitutions on an evolving Y chromosome could lead to the fixation of deleterious mutations at many loci, contributing to its degeneration
Mutual interference among weakly selected sites	With a very large number of closely linked sites, subject to reversible mutation between favoured and disfavoured alleles and selection with a strength of the order of the reciprocal of effective population size, the mean level of adaptation is strongly reduced in non-recombining regions.

Source: Charlesworth & Charlesworth (2000).

Population genetic forces that can lead to the accumulation of repetitive DNA sequences in non-recombining genomic regions, including Y chromosomes, have been discussed elsewhere (Charlesworth et al 1994), and will not be considered further (see Table 3 for summary).

Testing the ideas

There are obvious difficulties in studying evolutionary forces that operate over very long time-scales, especially if more than one of these forces operate. In addition, advanced Y chromosomes have lost most of their active genes, so that the opportunity for detecting the signatures of Hill–Robertson effects is considerably reduced, since there is now a greatly reduced set of loci subject to selection (Charlesworth & Charlesworth 2000). Species where there are still many active genes on a predominantly non-recombining Y chromosome, as is likely to be true of some plant species (Charlesworth & Guttman 1999), are more

TABLE 3 Evolutionary processes which can lead to the accumulation of repetitive DNA in non-recombining genomic regions

Tandemly repeated non-coding sequences	Unequal crossing over among members of a tandem array can generate haplotypes with only one repeat; fixation of these by genetic drift results in loss of repeats. In consequence, long arrays can accumulate only in genomic regions with little crossing over
Transposable elements	Ectopic recombination can occur between pairs of homologous elements in different locations, generating deleterious chromosome rearrangements. This may contribute to the containment of the spread of elements; if this is less effective when meiotic recombination is infrequent, elements will accumulate in regions of reduced crossing over.
	Muller's ratchet and/or background selection can also cause the accumulation of elements in non-recombining regions, if they are associated with deleterious insertional mutations.
	Elements are also less likely to be eliminated by selection against insertional mutations in regions with low gene density, such as the Y chromosome or heterochromatin.

Source: Charlesworth et al (1994).

favourable examples for this purpose. Similarly, systems where a neo-Y/neo-X chromosome pair has been formed by fusion between an autosome and a sex chromosome (Fig. 3) offer excellent opportunities to study the processes involved in Y chromosome degeneration, especially in *Drosophila* where the absence of crossing over in males automatically ensures that a neo-Y chromosome is genetically isolated from its partner once it becomes fixed in a species (Charlesworth 1996, Charlesworth & Charlesworth 2000).

One prediction of the evolutionary models is that a newly-formed proto-Y or neo-Y chromosome which fails to cross over with its homologue X over most or all of its length will start to exhibit signs of reduced adaptation, which will become progressively more marked, the greater the age of the system. This prediction is met in the case of the neo-Y chromosomes that have evolved independently in different species of *Drosophila* (Table 4). The case of *D. miranda* shows that a high level of degeneration of Y-linked loci has been accomplished over a period of a few million generations, despite the fact that DNA sequence variation indicates an effective population size of approximately one million individuals (Yi & Charlesworth 2000a, Bachtrog & Charlesworth 2000). In birds, the rate of substitution of amino acid sequence variants at a locus with Z and W homologues is faster for the W than the Z copy, as expected if slightly deleterious variants are accumulating on the Z chromosome (Fridolfsson & Ellegren 2000). A lower rate of amino acid sequence evolution on the Y or W chromosome relative to X or Z would suggest that the rate of adaptive evolution has been slowed down

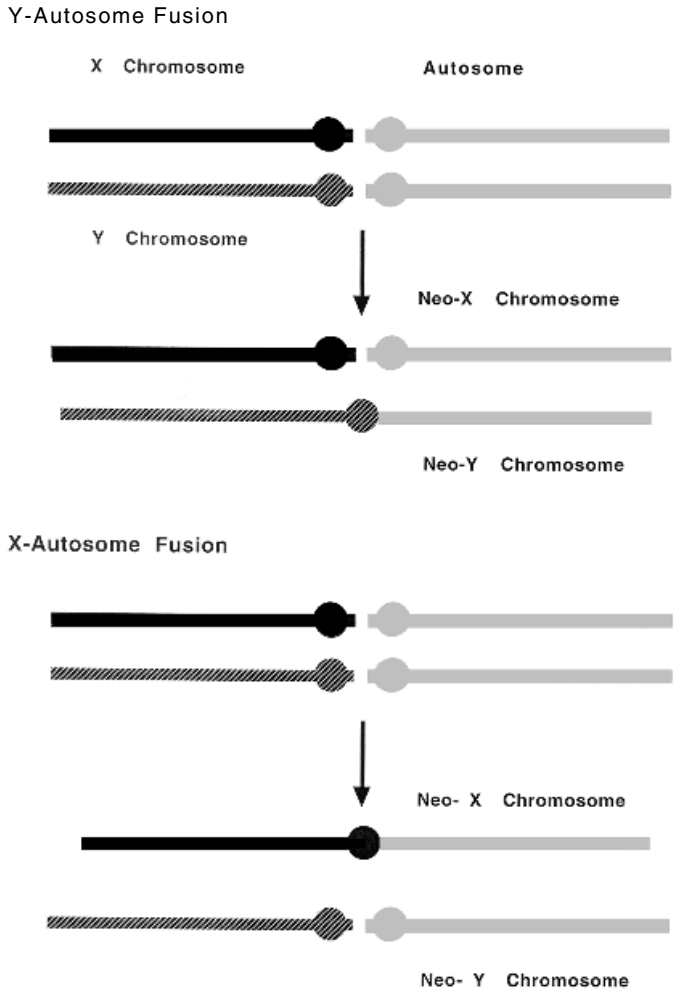

FIG. 3. Evolution of neo-X and neo-Y chromosomes by fusions between autosomes and sex chromosomes. Only males are indicated; females are homozygous for X and neo-X chromosomes.

because of its lower N_e (Orr & Kim 1998); the gene *cyclin B* in *D. miranda* shows this pattern for the neo-X and neo-Y chromosomes (D. Bachtrog & B. Charlesworth, unpublished results).

Another prediction is that the extent to which genes on the proto-X or neo-X chromosome are dosage compensated should parallel the extent of degeneration of their partner chromosomes, since dosage compensation is postulated to be an evolved response to Y chromosome degeneration. This is broadly confirmed by

TABLE 4 Properties of some neo-X/neo-Y sex chromosome systems in *Drosophila*

Species	*Age of system (Millions of years)*	*Extent of Y degeneration*	*Extent of dosage compensation*
D. pseudoobscura (X-autosome fusion)	13	Complete	Complete
D. miranda (Y-autosome fusion)	1	Partial	Partial
D. albomicans (X and Y autosome fusions)	$\ll 1$	None	Probably absent
D. americana americana	$\ll 1$	None	Absent

Sources: Bachtrog & Charlesworth (2000), Bone & Kuroda (1996), Charlesworth & Charlesworth (2000), Marín et al (2000), Mahesh et al (2001).

the *Drosophila* neo-Y chromosomes (Table 4). In particular, there is evidence that dosage compensation in *D. miranda* is patchily distributed along the neo-Y chromosome (Bone & Kuroda 1996, Marín et al 2000), as expected from the fact that only some of the neo-Y linked genes have degenerated. In at least one case, the evolutionary response to degeneration of a neo-Y-linked gene has involved duplication of the neo-X linked copy onto another chromosome (Yi & Charlesworth 2000b). In mammals, Jegalian & Page (1998) studied the inactivation status in females of a number of X-linked genes in different species of mammals, and found that it was closely associated with lack of a homologous copy on the Y chromosome, consistent with the idea that dosage compensation is an evolutionary response to a loss-of-function of Y-linked genes.

Since the standing level of neutral genetic variation is determined by the product of N_e and the mutation rate, comparisons of levels of silent polymorphism between X- and Y-linked homologues provide a test for the prediction of a reduced effective population size of the Y chromosome. In the case of *D. miranda*, there is clear evidence for such an effect from data on microsatellite loci and DNA sequence variation (Yi & Charlesworth 2000a, Bachtrog & Charlesworth 2000). Similarly, a locus on the Y chromosome of the white campion, *Silene latifolia*, shows about 20 times less variation that its homologue on the X chromosome (Filatov et al 2000). In contrast, the human Y chromosome, which has very few expressed genes, shows only a modest reduction in sequence variation (Shen et al 2000, Sachidanandam et al 2001). The observations on *D. miranda* and *S. latifolia* are consistent with the broader pattern of reduced DNA sequence variation in genomic regions with low levels of genetic recombination (Charlesworth & Charlesworth 1998).

While it is relatively easy to establish whether or not there is significantly reduced variation on evolving Y or neo-Y chromosomes, it is harder to

distinguish between different possible causes of such a reduction (Table 2). The recent spread of an adaptively favourable mutation is expected to eliminate all selectively neutral or nearly-neutral variation on a non-recombining chromosome; variants arising after such an event will on average be present at much lower frequencies than in an equilibrium situation, where genetic drift has had time to raise some of them to intermediate frequencies. There should, therefore, be significant departures from the frequency distribution expected for a population at statistical equilibrium between genetic drift and mutation if variability has been reduced by a recent hitchhiking event (Charlesworth & Charlesworth 2000). There are too few variants on the *D. miranda* neo-Y chromosome to allow such a test, but the data on *S. latifolia* show no indication of such an event (Filatov et al 2000). Although the other processes listed in Table 2 can produce a departure from neutrality, their expected effects are generally smaller than that of hitchhiking events. A consistent failure to detect departures from neutral frequency distributions would therefore seem to rule out hitchhiking as a cause of Y chromosome degeneration. Other features of DNA sequence variation that might help to discriminate between the various processes are discussed by Charlesworth & Charlesworth (2000). There is plenty of scope for further work in this area.

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