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The degeneration of Y chromosomes

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Y chromosomes are genetically degenerate, having lost most of the active genes that were present in their ancestors. The causes of this degeneration have attracted much attention from evolutionary theorists. Four major theories are reviewed here: Muller's ratchet, background selection, the Hill–Robertson effect with weak selection, and the 'hitchhiking' of deleterious alleles by favourable mutations. All of these involve a reduction in effective population size as a result of selective events occurring in a non-recombining genome, and the consequent weakening of the efficacy of selection. We review the consequences of these processes for patterns of molecular evolution and variation at loci on Y chromosomes, and discuss the results of empirical studies of these patterns for some evolving Y-chromosome and neo-Y-chromosome systems. These results suggest that the effective population sizes of evolving Y or neo-Y chromosomes are severely reduced, as expected if some or all of the hypothesized processes leading to degeneration are operative. It is, however, currently unclear which of the various processes is most important; some directions for future work to help to resolve this question are discussed.

Keywords: Y chromosomes; Muller's ratchet; background selection; Hill–Robertson effect; hitchhiking

1. INTRODUCTION

Morphologically and genetically distinct X and Y chromosomes have evolved independently in many groups of animals and plants (Bull 1983; Charlesworth 1996*a*). A striking common feature is the almost complete erosion of genes from the Y chromosome (or W chromosome, in groups with female heterogamety: we shall use the term Y chromosome for both systems), except for some genes with functions specific to the heterogametic sex. The Y chromosomes of groups such as mammals or *Drosophila* were presumably originally homologous with the ancestral X chromosomes and have subsequently lost genetic activity, giving rise to the familiar phenomenon of sex-linked inheritance. The possession of genetically eroded Y chromosomes in many groups with differentiated sex chromosomes is also associated with dosage compensation, such that the activity of most X-linked genes is effectively the same in males and females (Bull 1983; Charlesworth 1996*a*).

Understanding the evolutionary causes of these phenomena is a challenge for both theoreticians and experimentalists. The first step in the evolution of sex chromosomes must have involved the establishment of restricted recombination between a pair of proto-X and proto-Y chromosomes (Muller 1918), probably in response to the need to prevent recombination between genes with primary sex-determining roles, which would produce neuters or hermaphrodites (Lewis 1942; Charlesworth & Charlesworth 1978; Charlesworth 1996*a*). Although sex determination in many contemporary organisms may only involve segregation at a single major switch gene (Bull 1983), the initial evolutionary transition from hermaphroditism or environmental sex determination to separate sexes probably often involved several loci with effects on sexual phenotype (Westergaard 1958; Charlesworth & Charlesworth 1978; Charlesworth 1996*a*).

Once genetic sex determination has evolved, selection for alleles that are advantageous in males but disadvantageous in females can lead to genetic differentiation between the two sex chromosomes at other loci, and to selection for the suppression of recombinational exchange over most or all of their length (Rice 1996). This sets the scene for the further evolution of an incipient Y chromosome. We shall be mainly concerned here with the question of why most genes that were originally present on both the proto-X and proto-Y chromosome have been lost from the Y chromosome. The answers must lie in the special situation of a large block of non-recombining genes, which are restricted to one sex and can never become homozygous.

Several models of the degeneration of proto-Y chromosomes have been proposed and are described below, but the models are only just starting to be tested and we are far from being able to decide definitively between them. Although other non-recombining systems are probably subject to the same evolutionary forces, relevant data are scarce for these too. However, there is some evidence for reduced levels of adaptation in clonally transmitted genomes in fishes (Leslie & Vrijenhoek 1978) and in organelle genomes (Lynch & Blanchard 1998), which probably reflect the action of one or more of the processes that we discuss in § 2.

One reason for the dearth of tests is that the most familiar and thoroughly investigated sex chromosome systems, such as those of *Drosophila* and mammals, are very ancient, and betray few signs of their evolutionary origins (but see Lahn & Page 1999). We currently know little about Y chromosomes of more recent origin, such as those in dioecious flowering plants (Westergaard 1958; Charlesworth & Guttman 1999), or systems of neo-Y

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chromosomes in which a fusion or translocation between an autosome and a sex chromosome has created a new chromosomal arm that must experience the same evolutionary forces as Y chromosomes (Lucchesi 1978; Charlesworth 1996*a*; Steinemann & Steinemann 1998). These situations are suitable for testing models of Y-chromosome degeneration, as we shall discuss.

2. MODELS OF Y-CHROMOSOME DEGENERATION

(a) *General features of models of Y-chromosome degeneration*

Once low recombination between proto-X and proto-Y chromosomes has evolved, any genes on the non-recombining part of the proto-Y with homologues on the proto-X (and which are not sex specific in function) are in a very peculiar genetic situation. Muller (1918) was the first to point out that the permanent heterozygosity of such genes implies reduced selection against deleterious mutations. Even mutations eliminating the functions of vital genes might have only minor effects on fitness when heterozygous: experiments on *Drosophila* suggest that recessive lethals reduce the fitness of heterozygotes by only 1–2% on average (Crow 1993).

Another difference is that there are one-third as many copies of Y-linked genes as X-linked genes in populations, and one-quarter as many copies as autosomal loci. Thus, compared with X-chromosomal genes, Y-linked genes experience a smaller effective population size, N_e. Y-linked deleterious mutations are therefore particularly likely to rise to high frequencies, or to replace wild-type alleles, by genetic drift (Nei 1970). In species with male heterogamety, the high variance in male reproductive success associated with sexual selection could further reduce the effective population size of the Y chromosome and (to a smaller degree) of the autosomes, relative to that of the X chromosome (Caballero 1995). N_e-values can be estimated from data on variation in silent DNA sequences, if mutation rates for neutral or nearly neutral changes are accurately known (Kimura 1983). Evidence for sex differences in the variance of reproductive success can therefore be obtained by measuring levels of silent variability; strong sexual selection on males should bring the X-linked value close to, or even higher than, the autosomal value (Charlesworth 1996*b*).

Initial explanations for degeneration were focused on these properties of Y chromosomes, ignoring other consequences of the lack of recombination between Y-linked loci. However, they encountered several difficulties (Charlesworth 1978, 1996*a*). Fixation probabilities for deleterious mutations are extremely low unless the product of N_e and the selection coefficient, s, is close to 1 (Fisher 1930, chapter 4). Accelerated fixation of Y-linked loss-of-function mutations thus requires very small population sizes, or a very high variance in male reproductive success. Mutations with extremely slight fitness effects, such that $N_e s$ is not much bigger than 1, could nevertheless accumulate. However, a reduction in N_e increases the proportion of loci with high frequencies of deleterious alleles only over a narrow range of $N_e s$ (see, for example, fig. 1 of McVean & Charlesworth 1999), such that the efficacy of drift is delicately balanced against selection at a large number of genomic sites, many of them polymorphic. Under these conditions, a lack of recombination leads to a reduction in the ability of the genome to increase its level of adaptation under natural selection, and to resist forces that reduce adaptation (Maynard Smith 1978; Kondrashov 1993; Barton & Charlesworth 1998).

(b) *Models of mutation and selection*

To understand the influence of the lack of recombination on Y-chromosome degeneration, it is necessary to examine some general features of the evolutionary dynamics of mutation and selection at many loci. Consider a large but finite random-mating population. For locus i, with deleterious mutation rate u_i, the equilibrium frequency of mutant alleles with selection coefficient $s_i \gg u_i$ is $q_i = u_i/s_i$ (Haldane 1927). In the context of the Y chromosome, s_i reflects the fitness reduction experienced by heterozygous carriers of the mutant allele. If different loci affect fitness independently, this generates a Poisson distribution of numbers of deleterious alleles carried on different Y chromosomes in the population (Kimura & Maruyama 1966; Johnson 1999), whose mean is the sum of u_i/s_i over all sites subject to mutation and selection. This is equal to the ratio of the net mutation rate, $u = \sum_i u_i$, for deleterious alleles at all relevant Y-chromosomal loci, to the harmonic mean selection coefficient, s_h. In a finite population with relatively free recombination and $N_e s_i \gg 1$ for all sites, this equilibrium is closely approached (see, for example, Charlesworth *et al*. 1993). The frequency of mutation-free individuals is then $f_0 = \exp(-u/s_h)$; this has a critical role in several of the processes discussed below.

(c) *Muller's ratchet*

Various processes can cause a non-recombining population, such as a proto-Y chromosome, to depart from this equilibrium and accumulate deleterious mutations. One such process is 'Muller's ratchet' (Muller 1964; Felsenstein 1974). This is the stochastic loss, from a population of finite size, of the class of chromosomes carrying the fewest deleterious mutations. In the absence of recombination and back mutation, this 'least-loaded' class of chromosome cannot be restored, once lost. The next best class then replaces it as the least-loaded class, and is in turn lost, and so on, in a fairly steady process of successive irreversible losses of the current least-loaded class. In a haploid genome (such as a proto-Y chromosome), each such loss is quickly followed by the fixation of one deleterious mutation on the chromosome (Higgs & Woodcock 1995; Charlesworth & Charlesworth 1997). This fixation process can be many orders of magnitude faster than in a freely recombining population. The ratchet could thus lead to the cumulative impairment of gene function at sites scattered over a proto-Y chromosome.

The speed at which the ratchet moves (reviewed in Gordo & Charlesworth 2000) is clearly critical for its plausibility in relation to the degeneration of the Y chromosome. Other things being equal, the ratchet should move faster as the population size becomes smaller. For mammal populations, whose N_e-values, estimated from variability in silent-site DNA sequences, are probably often only in the tens of thousands (Nachman 1997, 1998;

Wang *et al.* 1998), the ratchet might be plausible. For various species of *Drosophila* (Powell 1997) and flowering plants (Charlesworth & Charlesworth 1998), sequence variation data suggest effective population sizes in the millions, so that the potential ability of the ratchet to influence the degeneration of the Y in these taxa has been questioned (Charlesworth 1996*a*). However, recent work suggests that the ratchet can move rapidly in a very large population if there are many mutations with very small effects on fitness, so that f_0 is small (Gessler 1995; Gordo & Charlesworth 2000, 2001). To determine whether this is likely will require a better knowledge of the properties of spontaneous mutations (Keightley & Eyre-Walker 1999; Lynch *et al.* 1999).

Simulation results on very large populations with 500 000 haploid genomes (Gordo & Charlesworth 2001) indicate that the speed of the ratchet in large populations can be approximated by an explicit equation, assuming the same selection coefficient s for each site. With $u = 0.015$ and $s = 0.0015$, the mean and 95% confidence interval of the number of generations between successive clicks of the ratchet is 732 ± 66 from simulations; with $u = 0.03$ and $s = 0.005$, the estimate is $12\,819 \pm 5681$. These examples indicate that the ratchet can operate at a biologically relevant rate even in a very large population, provided that mutations with small selection coefficients occur sufficiently frequently.

How fast does mean fitness decline under Muller's ratchet? On a logarithmic scale, fitness decreases approximately with the product of the rate of the ratchet and the mean selection coefficient. A ratchet driven by the accumulation of mutations with very small selection coefficients (less than 0.001) will clearly produce a low rate of decline of mean fitness, even though the ratchet moves rapidly (Gessler 1995; Gessler & Xu 1999). With mutations of larger effect, the ratchet might be capable of explaining Y-chromosome degeneration over plausible time periods (see § 3(b)). The two numerical examples described above give expected mean fitnesses of the populations after five million generations of 3.6×10^{-5} and 0.14 of the initial value, reflecting the fixation of an average of 6830 and 390 mutations, respectively.

(d) *Background selection*

'Background selection' (Charlesworth *et al.* 1993; Charlesworth 1994), or 'background trapping' (Rice 1996), is closely related to Muller's ratchet. 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 (for which $N_e s \gg 1$) (Fisher 1930, chapter 4; Charlesworth 1994; Peck 1994; Barton 1995). Chromosomes carrying one or more strongly selected deleterious mutations are soon eliminated from the population, carrying any new variants with them. The effective population size of a non-recombining chromosome is therefore reduced to $f_0 N_e$. The fixation of mildly deleterious mutations with selection coefficients of the order of $1/f_0 N_e$ will therefore be accelerated (Charlesworth 1996*a*), and the fixation of mildly advantageous mutations will be retarded (Peck 1994; Barton 1995; Rice 1996; Orr & Kim 1998). Over long periods of evolutionary time, therefore, the mean fitness of the Y chromosome should decline, relative to that of the X. Background selection can operate with selection coefficients and population sizes where the ratchet is inoperative, and can lead to a significant reduction in the fitness of Y chromosomes in very large populations over a biologically plausible time-scale (Charlesworth 1996*a*).

In addition, the effective population size experienced by weakly selected sites will be reduced by the presence of mutations with selection coefficients sufficiently large that they will not accumulate by the ratchet. This could facilitate a ratchet at the more weakly selected sites. Simulations show that the rate of the ratchet for weakly selected mutations is well predicted by substituting $f_0 N_e$ for N_e in the relevant equations, where f_0 is determined by using the parameters of the the strongly selected mutations (I. Gordo and B. Charlesworth, unpublished data). The two processes might therefore operate in concert, with a considerably accelerated ratchet when strongly selected deleterious mutations are causing background selection.

Background selection also causes reduced genetic variability at neutral or very weakly selected sites (Charlesworth *et al.* 1993), because equilibrium diversity depends on the effective population size (Kimura 1983; McVean & Charlesworth 1999). When a ratchet is operating at a moderate rate, reduced neutral or nearly neutral variability will also probably result, because a ratcheting population has originated largely from the currently least-loaded class over a relatively short time-scale, compared with the coalescence time of a pair of alleles in a freely recombining population of comparable size (Rice 1987; Higgs & Woodcock 1995; Charlesworth & Charlesworth 1997). This remains to be studied quantitatively.

(e) *Weak selection Hill–Robertson effect*

A third, distinct but related, process is the 'weak selection Hill–Robertson' effect (McVean & Charlesworth 2000). Closely linked selected alleles interfere with each other, inhibiting the spread of favourable alleles and the elimination of deleterious ones (Fisher 1930, chapter 5; Muller 1932; Hill & Robertson 1966; Felsenstein 1974; Birky & Walsh 1988). Muller's ratchet and background selection can be viewed as examples of this principle for situations when the sites creating the effects have $N_e s \gg 1$, so that the loci subject to mutation and selection would be near their deterministic equilibria if recombination were not restricted. If, however, $N_e s$ is of the order of one or less, genetic drift and back-mutation from deleterious alleles to wild-type become more important. Models incorporating the joint effects of selection, forward and backward mutation and genetic drift are commonly used in the study of biased codon usage, in which alternative triplets coding for the same amino acid might be subject to weak but statistically detectable selection in insects, nematodes and plants (Li 1987; Bulmer 1991; Akashi 1994; Duret & Mouchiroud 1999; McVean & Charlesworth 1999). Some classes of mutations causing amino-acid changes might also fall into this category. Many sites will then segregate for favoured and disfavoured alleles at intermediate frequencies, so that the assumptions of the ratchet and background selection models break down.

Finite population-genetic models with large numbers of closely linked segregating sites are complex, and

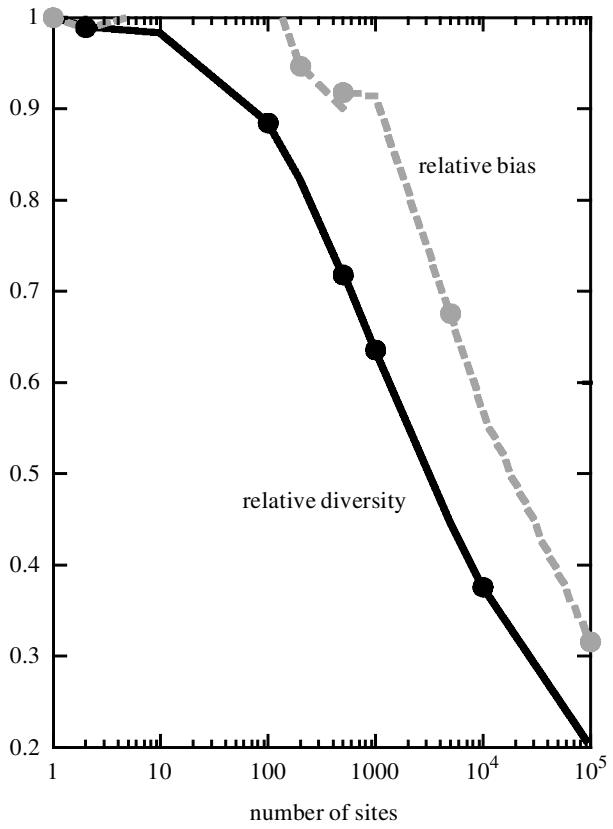

Figure 1. Weak selection Hill–Robertson effects in a non-recombining haploid genome. The mean diversity per site and mean proportion of sites with the selectively favoured allele are shown, relative to their values for a freely recombining genome, as functions of the number of sites in the genome. Reversible mutation at rate u between a favoured and a disfavoured allele at each site is assumed, such that $2Ns = 4$ and $2Nu = 0.04$.

investigation of this model requires Monte Carlo simulations (Li 1987; Comerón *et al.* 1999; McVean & Charlesworth 2000). With very large numbers of sites subject to mutation and selection, and assuming multiplicative selection, the mean level of adaptation (measured by the frequency of sites carrying favoured alleles among chromosomes sampled from the population) is strongly reduced when recombination is absent (see figure 1); genetic diversity is also reduced. Because the mean size of a eukaryote gene is about 500 codons, and a *Drosophila* or plant chromosome might have about 2000–3000 genes, the number of third coding positions at which silent mutations can occur on a proto-Y chromosome is of the order of 10^6, ten times the number for the most extreme example shown in figure 1. For a population size of 5×10^5, the parameters used in figure 1 imply a selection coefficient of 0.4×10^{-5}; with multiplicative fitnesses and only 10^5 sites, the fitness of the population at statistical equilibrium is *ca.* 5% of the value with free recombination.

Hill–Robertson effects with weak selection therefore have considerable power in the long term to erode the fitness of an evolving Y chromosome. However, the time taken for fitness to be substantially reduced could be considerable, because new mutations might have to approach fixation at numerous sites along the chromosome. The upper limit to the rate of fixation is given by the mutation rate (Kimura 1983); thus, if there are 10^6 sites with selection coefficients of 0.4×10^{-5}, and a mutation rate of 10^{-9} per site, the maximum per generation rate of decline of mean fitness would be of the order of 4×10^{-9}. With five generations per year, as might be expected for *Drosophila miranda* (see § 3(b)), it would take a duration of the order of five million years for fitness to be reduced by 10%. Weak Hill–Robertson effects therefore require a long period to reduce the fitness of an evolving Y chromosome significantly. This casts some doubt on their relevance to cases in which degeneration has occurred over a much shorter period, as with *D. miranda* discussed below.

(f) *Hitchhiking effects of favourable mutations*

The final class of model involves hitchhiking effects during the spread of selectively favourable mutations. Maynard Smith & Haigh (1974) were the first to study the effect of the spread of a selectively favourable mutation on variability at linked neutral sites. With complete linkage between the two loci, as for genes on a proto-Y chromosome, it is intuitively obvious that a 'selective sweep' of a favourable allele will eliminate all variation at the neutral locus; neutral or nearly neutral variability will recover only slowly, as new variants arise and drift to high frequencies. Selective sweeps in a non-recombining genome can also drag to fixation any deleterious mutant alleles associated with the favourable mutation, so that successive adaptive substitutions on a proto-Y chromosome could lead to the fixation of deleterious mutations at many loci, contributing to its degeneration (Rice 1987). However, the favourable mutations must be as strongly selected as the deleterious mutations segregating in the background if they are to have a good chance of fixation when there is no recombination (Peck 1994), so that the efficacy of this mechanism is questionable (Charlesworth 1996*a*).

3. TESTING THE MODELS

(a) *Detectable consequences of the different processes*

Some limits to the extent to which the above processes might operate could be established if we had accurate information on the effective population sizes of natural populations, together with estimates of mutation rates to deleterious alleles and selective effects of mutations. As noted in § 2, variation in silent DNA sequences can provide estimates of N_e, which must be corrected for the different effective population sizes experienced by autosomal, X-linked and Y-linked loci (Caballero 1995). However, population subdivision complicates the estimation of N_e because total neutral diversity values for alleles sampled species-wide will often be inflated over the value (N_e^P) for a panmictic population with the same total number of breeding individuals. N_e^P is probably the value most relevant to the processes discussed above; in simple migration models, such as the island or stepping-stone models, it can be estimated from the mean within-population diversity value, although this is not true in general (Nagylaki 1998). Estimates of mutational parameters for deleterious mutations are currently even more problematical, with different investigations yielding

Table 1. *Statistics of DNA sequence variation and evolution useful in testing for the action of processes involved in Y-chromosome degeneration*

(Symbols: + +, a strong effect; +, a weak effect; −, no effect; (?), a lack of rigorous theoretical analysis.)

parameter	ratchet	background selection	weak selection Hill–Robertson	hitchhiking
significant distortion of allele frequencies[a]	+ (?)	−	+ +	+ +
negative linkage disequilibrium[b]	−	−	+ +	−
increased fixation of slightly deleterious mutations[c]	+ +	+	+	+
increased fixation of strongly deleterious mutations[d]	+	+	−	−
increased *Q*-value[e]	+ + (?)	+ +	+ (?)	+ (?)

[a] This refers to the spectrum of frequencies of segregating changes at silent sites.
[b] This refers to negative linkage disequilibrium between pairs of synonymous variants representing preferred codons (Akashi 1994).
[c] These include amino-acid replacement changes at weakly constrained sites, and changes to synonymous variants representing unpreferred codons.
[d] These include amino-acid replacement changes at strongly constrained sites, deletions of parts of coding sequences, or frameshift mutations.
[e] *Q* is the ratio of interspecies divergence to intraspecies diversity value for slightly deleterious changes, divided by the ratio for neutral changes (Charlesworth 1994).

widely divergent values (Keightley & Eyre-Walker 1999; Lynch *et al*. 1999).

Given these difficulties, we can reach only very tentative conclusions about the possible roles of different mechanisms from this kind of approach. Another approach is to study patterns of molecular variation and evolution at loci on newly evolving Y chromosomes, and to relate them to the predictions of the models (Charlesworth 1996*a*). A starting point is to note that they all predict that the realized effective population size of a proto-Y or neo-Y chromosome should be greatly reduced below that predicted by demographic considerations. Estimates of silent variability at loci on such chromosomes should therefore be consistently lower than would be expected from measurements of variability at X-linked or autosomal loci, after the necessary adjustments for demographic factors and sexual selection (Nunney 1993; Caballero 1995).

A predicted consequence of a reduction in N_e is an accelerated rate of fixation of slightly deleterious substitutions, and a reduction in the rate of fixation of slightly advantageous mutations (Kimura 1983). Thus we might expect an increased rate of amino-acid replacement relative to silent substitutions on evolving Y chromosomes (provided that sufficient time has elapsed since the origin of the sex-chromosome system), and more fixations of non-preferred versus preferred synonymous substitutions at silent sites in exons (Charlesworth 1996*a*). If, however, Y chromosomes predominantly experience slower adaptive evolution relative to the X, as suggested by Rice (1996) and Orr & Kim (1998), the rate of replacement substitutions on the Y should be less than for the loci elsewhere in the genome. If both processes have a role, there should at least be increased variation between sites, some having faster substitution rates and others slower rates. Other tests for the signatures of the various processes are listed in table 1. Although in principle there are several criteria that could be useful, statistical power to discriminate between the different models requires extensive sequence information.

Very few studies have been conducted with the explicit aim of testing the observable consequences of different models of Y-chromosome degeneration. We review these below, together with relevant information from work performed for other purposes.

(b) Drosophila *neo-Y-chromosome systems*

Given the lack of crossing over in male *Drosophila*, an autosome that forms a neo-Y chromosome by fusion or translocation to a sex chromosome will immediately be exposed to forces that cause degeneration. Several such systems, of various ages, are known in the genus *Drosophila*. Given the highly conserved gene content of *Drosophila* chromosome arms (Muller's 'elements' (Muller 1940)), the opportunity exists to use data on the molecular variation and evolution of neo-X and neo-Y chromosomal genes to test the predictions of the models (Lucchesi 1978; Charlesworth 1996*a*; Steinemann & Steinemann 1998).

A prediction common to all models is that the process of evolution of a completely degenerate neo-Y chromosome, and of full dosage compensation of the genes on the neo-X chromosome, should be very slow. The comparative evidence from *Drosophila* supports this prediction (Charlesworth 1996*a*). The *D. pseudoobscura* neo-X chromosome was formed by the fusion of element D to the true X chromosome *ca*. 13 million years ago. There is apparently complete dosage compensation of genes on the neo-X, and loss of activity of all genes on the neo-Y. The related species, *D. miranda*, which diverged approximately two million years ago, has a neo-Y chromosome formed by the fusion of element C with the true Y chromosome. DNA sequence data indicate that the neo-X and neo-Y chromosomes have been diverging for approximately one million years (Yi & Charlesworth 2000). Although many of the genes on the neo-Y still retain their function, a substantial fraction have become non-functional or completely lost, and their homologues on the X have become dosage-compensated (Steinemann & Steinemann 1998, 1999).

The neo-Y of *D. americana americana* is a very recent fusion between element B and the X chromosome, which are unfused in its close relatives, *D. a. texana* and *D. virilis*. *D. a. americana* and *D. a. texana* are separated by

only very small genetic distances, and the *Adh* locus on the neo-Y (which is very close to the centromere of the fused chromosome) shows no fixed sequence differences from its neo-X counterpart (McAllister & Charlesworth 1999). These data suggest that the neo-Y is either only a few tens of thousands of years old or is still recombining with the neo-X chromosome (McAllister & Charlesworth 1999). Genetic experiments indicate that most loci on this chromosome are active (Charlesworth *et al.* 1997).

Another prediction, again common to all models for Y-chromosome degeneration, is a reduced effective population size for genes on the Y chromosome. Reduced Y-chromosomal silent-site variability is thus expected, and suggests the operation of some process or processes constantly reducing Y diversity. The ideal comparison would be between sequences of orthologous X- and Y-linked genes; autosomal data are also needed, to test whether diversity is reduced at Y-linked loci, or unusually elevated at X-linked loci. It is not necessary to study the loci at which the hypothesized mutational and/or selective processes are actually happening. All that is needed is a set of data from loci on the Y and X chromosomes, because (owing to the absence of recombination) all Y-linked loci will be affected by the processes outlined above. In *D. a. americana*, the neo-Y-linked *Adh* locus does indeed have significantly reduced variability compared with its neo-X counterpart, suggesting the action of one or more of the forces discussed in §2 (McAllister & Charlesworth 1999).

Among the potentially useful *Drosophila* systems for investigating the degeneration of neo-Y chromosomes, the *D. miranda* system seems the most promising, from the data on its age reviewed above. The *Lcp1* and *Lcp3* loci are present on both the neo-X and neo-Y chromosomes; the neo-Y copies apparently have reduced levels of expression (Steinemann & Steinemann 1998). Sequence comparisons indicate that silent variability at these loci on the neo-Y is *ca.* 29% of the neo-X value, which is similar to the null expectation of 33% (Yi & Charlesworth 2000). However, the confidence interval for this ratio is large, owing to the low polymorphism levels observed for both chromosomes; indeed, the neo-X *Lcp* loci seem to have lower silent diversity than loci on the true X chromosome, possibly because of hitchhiking effects associated with the evolution of dosage compensation (Yi & Charlesworth 2000). A survey of seven microsatellite loci that are present on both chromosomes indicates substantially reduced variability on the *D. miranda* neo-Y (*ca.* 3% of the neo-X value (Bachtrog & Charlesworth 2000)), which is consistent with a severely reduced effective size.

Because low mutation rates could also account for low Y-chromosomal variability, this must be tested for by comparing divergence between species, for Y-linked and other loci. Patterns of replacement and synonymous substitutions in the *D. miranda* lineage, with the use of *D. pseudoobscura* as an outgroup, show that amino-acid replacements have occurred on the neo-Y at the same rate as at silent sites, but at a much lower rate on the neo-X (Yi & Charlesworth 2000). This suggests relaxed selective constraints on the neo-Y, either because of reduced effective size or inactivation of neo-Y loci, and is the opposite of what would be expected if adaptive evolution were occurring more rapidly on the neo-X than the neo-Y (Rice 1996; Orr & Kim 1998).

Transposable elements have accumulated at a very high abundance on the *D. miranda* neo-Y chromosome, and might have been involved in causing a loss of gene activity (Steinemann & Steinemann 1998). Muller's ratchet is a possible mechanism for the accumulation of elements in non-recombining genomic regions (Charlesworth & Langley 1991), although other population-genetic processes can also cause the accumulation of transposable elements and other types of repetitive DNA in such regions (Charlesworth *et al.* 1994). To evaluate the ratchet's possible contribution to the degeneration of the *D. miranda* neo-Y chromosome, we would need to have estimates of the net rate of insertion of elements into this chromosome, and the mean selection coefficient on insertional mutations, neither of which is available. Estimates of the net rate of movement of elements in *D. melanogaster* are consistent with a minimum of around 0.005 insertions per generation into a genomic region of the size of the *D. miranda* neo-Y chromosome; the actual rate could well be three or four times this (Maside *et al.* 2000). As noted in §2(c), with a mutation rate of 0.015 the ratchet can cause a substantial decline in mean fitness over a realistic period, even in a very large population, provided that selection coefficients are small enough (Gordo & Charlesworth 2001). The role of the ratchet in causing the accumulation of deleterious transposable element insertions in this and other Y-chromosome systems (Bull 1983; Charlesworth *et al.* 1994) is therefore an important open question.

(c) *Comparisons of sequence diversity between homologous Y- and X-linked loci in plants*

A comparison of variability between single-copy Y- and X-linked loci in the dioecious flowering plant *Silene latifolia* (white campion) also suggests a reduced Y-chromosomal effective population size (Filatov *et al.* 2000). The sex chromosome system of this species probably evolved between eight and 20 million years ago, on the basis of estimates of the divergence times of dioecious species from their closest relatives (Desfeux *et al.* 1996). However, the Y chromosome retains some expressed genes, including the recently discovered *SLY1* locus with an X-linked homologue *SLX1* (Delichére *et al.* 1999). In a 2 kb region of these genes, sequenced from plants sampled from a number of natural populations, there were 54 fixed nucleotide differences, and no shared polymorphic sites, between the X and Y genes, showing that the *SLY1* gene must be in the non-recombining part of the Y chromosome. Net silent divergence between the coding regions of *SLX1* and *SLY1* is *ca.* 3%, suggesting a time of *ca.* 2.5 million years since the cessation of recombination between the two sets of alleles. This assumes a molecular clock of 0.6 silent-site differences per million years, which is widely used for plants (Gaut 1998); however, this calibration is quite uncertain (Bremer 2000).

When *SLY1* and *SLX1* sequences from 12 males were compared, the *SLY1* diversity was about one-twentieth of that for *SLX1* (Filatov *et al.* 2000). The X–Y difference is most probably due to low *SLY1* sequence diversity, not to unusually high variability between the X-linked alleles. Only one autosomal gene has so far been studied in this

species, but it shows significantly higher diversity than *SLY1* and does not differ significantly in diversity from *SLX1* (V. Laporte, D. Filatov, C. Vitte and D. Charlesworth, unpublished data). The low *SLY1* diversity cannot be explained by a low Y-chromosomal mutation rate, because comparisons with outgroup species in the genus suggest a higher divergence rate than for the X-linked gene; the low Y-chromosome diversity is thus even more striking than the raw data suggest (D. Filatov, V. Laporte, C. Vitte and D. Charlesworth, unpublished data).

A further interesting aspect of the results from the *S. latifolia* Y chromosome is that they do not readily fit a recent selective sweep. Such an event would eliminate all diversity, so that existing *SLY1* polymorphisms must have accumulated since the selective sweep and should thus mostly be at low frequencies (see table 1). However, all six *SLY1* polymorphic sites found are non-singletons, and no significant deviations from neutrality are detectable by standard test statistics (Fu & Li 1993). However, this does not take into account the probable population subdivision in this species. Although a selective sweep eliminating variability throughout the worldwide population of this species seems unlikely, effects of selective sweeps within local populations cannot be excluded.

(d) *Y-chromosome variability in humans and* Drosophila

In contrast with neo-Y and recently evolved chromosomes, Y chromosomes that have degenerated fully have few active loci and will therefore not be much affected by the processes we have described. Given enough time for variability to accumulate by mutation, sequences in old Y-chromosome systems should therefore have diversity levels similar to those expected from their relative effective population sizes. Disagreement with this prediction might suggest that the processes discussed in § 2 are not the only forces affecting Y chromosomes. In *Drosophila melanogaster* and *D. simulans*, a comparison of total sequence variability in a Y-linked flagellar dynein gene with several X-linked and autosomal loci indicated a reduction in diversity, even after correcting for the different effective population sizes for the different chromosomes (Zurovcova & Eanes 1999). However, so far only this Y-linked gene has been characterized in detail. If other Y-chromosomal genes are extremely large, as is true of the Y-linked flagellar dynein gene, they would present large mutational targets, and hence a role is possible for effects of background selection or selective sweeps.

From the few published results on human microsatellite and nucleotide sequence variability, it is not yet clear whether human populations have Y-linked diversity so low as to be inconsistent with expectations based on purely demographic considerations. In humans and mice, X-chromosomal microsatellite loci have lower diversity than autosomal ones (Jarne *et al.* 1998), but extensive comparisons of autosomes with the Y chromosome have not yet been published. The best available microsatellite comparison seems to be between a set of eight human Y-linked loci (Pritchard *et al.* 1999) and 20 other loci (18 autosomal and two X linked (Di Rienzo *et al.* 1998)). The loci are not directly comparable in terms of factors that affect mutability, such as repeat length and base composition (Estoup & Cornuet 1999). Nor are the population samples readily comparable; the non-Y-linked loci data came from three populations in different continents, whereas the Y-linked loci were sampled from much larger geographic units. However, both biases (narrower sampling region, and inclusion of X data in the non-Y loci) should reduce the difference between the Y and non-Y loci. The observed difference is nevertheless striking. The variance in repeat size for the Y-linked loci ranges from 0.50 (South America) to 1.18 (Africa), whereas the non-Y-linked values are 5.26 (South America), 7.49 and 8.30. Only one published comparison exists for a microsatellite locus that is present on both X and Y chromosomes, and it shows no consistent difference between them (Karafet *et al.* 1998). Because this gene has only recently been moved to the Y chromosome (Schwartz *et al.* 1998), there might have been too little time for diversity differences to develop.

The difference between the human Y-linked and other microsatellite loci is qualitatively consistent with data on other kinds of variability. It is rarely possible to compare levels of single nucleotide polymorphism in Y-chromosomal loci with homologous X-linked loci, simply because few such gene pairs are known (Lahn & Page 1997), so that the available data include different kinds of genes and sequence types. A large study of sequence-tagged site (STS) sequences from a worldwide Y-chromosome sample gave a nucleotide site diversity value of 3.6×10^{-4} (Underhill *et al.* 1997), and a DNA-chip-based study of a large European sample of autosomal and X-linked STSs gave a similar value (4.5×10^{-4} (Wang *et al.* 1998)). Data from several regions of the human Xq22 region, from a worldwide sample of 24 men, yielded a diversity value of 1.7×10^{-4} (Anagnostopoulos *et al.* 1999), whereas an 18.3 kb region of the Y chromosome from the same men had a diversity one-sixth as great (only three segregating sites). Human and chimpanzee divergence comparisons, obtained by sequencing regions that amplify from a chimpanzee by using human primers, suggest a Y-chromosome sequence divergence of *ca.* 1.33%, roughly twice that of X sequences. As for *Silene*, the estimated mutation rate is therefore certainly not lower in males than females (Anagnostopoulos *et al.* 1999).

Very few studies of Y- and X-linked sequence variability have compared homologous genes or regions. Much lower variability was found in a 676 bp sequence of the tenth intron of the human *ZFY* gene, compared with 1089 bp of the corresponding *ZFX* intron, on the basis of single-strand conformational polymorphism studies of large samples from several different populations (Jaruzelska *et al.* 1999). Although the probably higher mutation rate in males than in females makes this a striking difference, it might not be typical of X and Y genes, because the extreme *ZFX* diversity is due largely to the presence or absence of an *Alu* element. Excluding *ZFX* alleles containing *Alu*, the difference in variability levels was not significant. Overall, 22 pairwise comparisons between Y-linked sequences and other loci yielded only one significant test, after accounting for differences in interspecific divergence levels and multiple comparisons (Nachman 1998), so that the mean human XY difference in variability is perhaps no more than threefold.

4. DISCUSSION

Given the different processes that can operate in large non-recombining genomic regions, it is not surprising that Y chromosomes and neo-Y chromosomes of sufficient age have, in fact, degenerated. The processes described in § 2 are not, of course, mutually exclusive. Further exploration of the expected patterns of molecular variation and evolution produced by the various processes is needed to determine whether the conjectural predictions noted in table 1 are correct, and whether other testable properties of the models might exist. The overlapping predictions of the various models about patterns of molecular evolution and variation (see table 1) make empirical testing to assess the relative importance of the various processes a formidable task. However, it might be possible to answer some of the simplest questions, such as whether firm evidence exists for reduced genetic diversity in systems of evolving sex chromosomes (especially in comparison with established Y-chromosome systems) or for the distortions in allele frequency distributions associated with hitchhiking events.

It is important that several independent examples of evolving Y chromosomes should be studied, because the lack of recombination means that all genes on the Y of any given species share a common genealogy, which limits the amount of information that can be obtained from data on within-species variability. Both evolving Y chromosomes and neo-Y-chromosome systems need to be investigated; whereas the latter might have better-defined evolutionary histories, there is a possibility that their degeneration might be accelerated relative to that of truly newly evolved Y chromosomes, by co-opting pre-existing mechanisms for dosage compensation (Steinemann & Steinemann 1998).

This raises the further issue of whether Y-chromosome degeneration is driven mainly by the accumulation of deleterious mutations on the Y or is due to processes turning down the expression of genes on an evolving Y. We have focused on the first of these possibilities, but it might well not completely explain Y degeneration. If mutations of relatively minor fitness effects, with $N_e s$ not much bigger than 1, become fixed more rapidly on a degenerating Y chromosome than on the X, selection pressure would develop to reduce the transcription of Y genes, provided that this was compensated for in the heterogametic sex by an increased activity of their counterparts on the X. This could lead to the evolution of dosage compensation in parallel with the loss of gene activity on the Y chromosome (Charlesworth 1978, 1996*a*; Jegalian & Page 1998). More rapid adaptive evolution of X- relative to Y-chromosomal loci would also favour the silencing of Y-linked genes (Rice 1996; Orr & Kim 1998), as would the hypothesis due to Hamilton (1967) that segregation distortion by Y chromosomes has created selection to repress their genetic activity. Active evolutionary modification of patterns of gene activity (with fixation of the relevant DNA sequence changes at regulatory sites) should leave traces of hitchhiking events at sweeps at X-chromosomal loci whose Y counterparts are inactive (Yi & Charlesworth 2000). If their Y-linked homologues have been actively downregulated, we should also detect distorted allele frequency distributions on the Y chromosome. Thus, despite the difficulties, it seems possible to shed some light on the initially extremely puzzling process of genetic degeneration.

The degeneration of Y chromosomes illustrates the critical importance of recombination for maintaining the fitness of large genomic regions. With this view, it is one example of the wider pattern of a reduced ability of selection to maintain adaptation under an essentially asexual mode of reproduction (Maynard Smith 1978; Kondrashov 1993; Barton & Charlesworth 1998). As should be apparent from what we have written, John Maynard Smith has made many influential contributions to our understanding of the processes underlying this pattern.

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