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Factors affecting levels of genetic diversity in natural populations

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Genetic variability is the clay of evolution, providing the base material on which adaptation and speciation depend. It is often assumed that most interspecific differences in variability are due primarily to population size effects, with bottlenecked populations carrying less variability than those of stable size. However, we show that population bottlenecks are unlikely to be the only factor, even in classic case studies such as the northern elephant seal and the cheetah, where genetic polymorphism is virtually absent. Instead, we suggest that the low levels of variability observed in endangered populations are more likely to result from a combination of publication biases, which tend to inflate the level of variability which is considered 'normal', and inbreeding effects, which may hasten loss of variability due to drift. To account for species with large population sizes but low variability we advance three hypotheses. First, it is known that certain metapopulation structures can result in effective population sizes far below the census size. Second, there is increasing evidence that heterozygous sites mutate more frequently than equivalent homozygous sites, plausibly because mismatch repair between homologous chromosomes during meiosis provides extra opportunities to mutate. Such a mechanism would undermine the simple relationship between heterozygosity and effective population size. Third, the fact that related species that differ greatly in variability implies that large amounts of variability can be gained or lost rapidly. We argue that such cases are best explained by rapid loss through a genome-wide selective sweep, and suggest a mechanism by which this could come about, based on forced changes to a control gene inducing coevolution in the genes it controls. Our model, based on meiotic drive in mammals, but easily extended to other systems, would tend to facilitate population isolation by generating molecular incompatabilities. Circumstances can even be envisioned in which the process could provide intrinsic impetus to speciation.

Keywords: bottleneck; microsatellite; genetic variability; meiotic drive; heterozygosity

1. INTRODUCTION

Genetic diversity is the clay of evolution, the base material on which adaptation and speciation depend. High levels of variability are seen as healthy, conferring the ability to respond to threats such as disease, parasites and predators, and environmental change. Conversely, low levels of variability are seen as limiting a species' ability to respond to these threats in both the long and short term. It is therefore not surprising that there is widespread concern about the consequences of habitat fragmentation. Fragmentation will almost always lead to a reduction in population size and a consequent increase in the rate at which variability is lost through genetic drift. In extreme cases, the population may temporarily be reduced to very low numbers and suffer exaggerated loss, an event which is often referred to as a genetic bottleneck.

Despite almost universal recognition of the importance of genetic variability, there remain many instances where the level of variability, as measured by molecular genetic techniques, and apparent evolutionary success of a species differ markedly from expectations. Some species that are predicted to be genetically impoverished are not, whereas others harbour much less variability than expected. Similarly, there seem to be many exceptions to the rule that high levels of genetic variability equate to evolutionary health. Some species have low variability and exponentially increasing populations, whereas others appear to be declining despite great variability.

Ultimately, any given level of genetic variability carried by a species or population must be explicable in terms of a balance between the opposing processes of gain and loss. Thus, at or near mutation drift equilibrium, high levels of variability imply either high rates of gain or low rates of loss, just as low levels of diversity imply either low rates of gain or rapid loss. Diversity may be gained either through mutation or through gene flow from a neighbouring population. Loss of diversity occurs either passively through genetic drift or actively through natural selection, for example, when manifest as inbreeding depression. An important conclusion can be drawn from this simple summary. As replenishment of diversity is either slow (*de novo* mutations) or unlikely (influx from a neighbouring population or species), most large or rapid changes in diversity should be attributable to loss rather than gain.

In this paper we will briefly review the way in which population size, mutation, inbreeding, spatial distribution and social organization can affect genetic diversity. In the process we examine the evidence that these factors can account for observed levels of diversity. Where there are

important discrepancies, we suggest additional mechanisms which could help to explain the effects observed.

2. POPULATION SIZE AND VARIABILITY

The relationship between population size and genetic variability is well-known. All other things being equal, small populations carry less selectively neutral genetic diversity than equivalent larger ones. However, although this relationship gains abundant empirical support from a wide range of different measures of genetic variability (see Young *et al*. (1996) for a review of data from plants), variability in small and large populations is often less than would be expected simply as a result of drift (e.g. Prober & Brown 1994; Raijmann *et al*. 1994; Butlin, this volume). Some of this discrepancy may be because the genetic effective size of a fluctuating population is given by its harmonic mean, which lies closer to the minimum than the maximum value attained (Wright 1938). Hence a severe bottleneck in population size can exert a disproportionate effect on the long-term effective size of a population. However, in many cases where an undocumented bottleneck is invoked to explain low levels of genetic variability, the observed level of genetic loss is greater than predicted by classical population genetic theory. Consider the equation that describes the expected loss of heterozygosity over t generations from a population whose effective size is N_e:

$$H_t = H_0(1 - 1/2N_e)^t$$

where H_t and H_0 are heterozygosity at time t and time zero, respectively.

An endangered mammal with a generation length of 5 years and reduced to an effective population size of around 50 for the last 100 years would lose *ca*. 20% of its original heterozygosity. This degree of loss is small compared with many of the explicit or implicit levels of depletion one encounters in the conservation literature. For example, the cheetah (*Acinonyx jubatus*) is thought to have lost '90–99%' of its allozyme variability (O'Brien 1994), yet still exists at a population numbering several thousands. To lose 99% heterozygosity by drift alone would require on average 16 generations at $N_e = 2$. The extremely low variability observed at some loci in the cheetah may be explicable in terms of the low effective size that can, in principle, exist in some metapopulations (Gilpin 1991; Hedrick 1996; Hedrick & Gilpin 1997; and see §5). However, this explanation is a long-term equilibrium solution and does not require a bottleneck.

The northern elephant seal (*Mirounga angustirostris*) also shows a near absence of genetic variability at certain loci. In this case, the bottleneck was well-documented, but it was extremely brief. The species was heavily exploited in the 19th century, culminating in a final large catch of 153 animals in 1854 (Townsend 1885). Seven more (of eight seen) were killed in 1892, and small numbers were taken after this (Stewart *et al*. 1994). By 1922 the population had recovered to 350, and it currently exceeds 150 000 (Stewart *et al*. 1994). These figures make it highly unlikely that the population fell to below ten individuals for more than two generations. This bottleneck has been modelled and, although it appears to be possible to explain the near absence of mitochondrial DNA variability exhibited by the northern elephant seal (Hedrick 1995; Hoelzel *et al*. 1993), these models do not explain the extremely low levels of allozyme variability (Hedrick & Gilpin 1997).

All in all, there is rather little convincing evidence that significant losses occur as a result of genetic drift. In many instances the occurrence of low levels of variability can be explained more convincingly by other mechanisms. For example, highly significant differences in heterozygosity exist between populations of the guppy (*Poecilia reticulata*) in upstream and downstream sections of the same river systems in Trinidad (Shaw *et al*. 1994). It is tempting to attribute this to drift, as downstream populations have a higher effective population size than upstream ones. However, a systematic loss of alleles is observed as sampling progresses upstream (A. E. Magurran, B. H. Seghers, G. R. Carvalho and P. W. Shaw, unpublished results), so that all of the alleles that are recorded in an upstream area are also present further downstream. This is much more consistent with the erosion of diversity through unidirectional gene flow downstream than is drift.

Loss of allelic diversity tends to be a more sensitive indicator of historical bottlenecks than reduction in heterozygosity (Brookes *et al*. 1997; Leberg 1992). An extreme bottleneck of two individuals will reduce heterozygosity by 25%, but will leave a maximum of four alleles at any given locus. The consequences of this are particularly evident in DNA fingerprints, which contain information from many hypervariable minisatellite markers. Each hypervariable locus may carry 100 or more alleles and a reduction to just four in the population can therefore be visually striking. In a classic study of the Californian Channel Islands fox (*Urocyon littoralis*), Gilbert *et al*. (1990) showed that each island had its own unique fingerprint, a consensus banding pattern that was more or less shared by all individuals but which differed greatly between islands.

3. POTENTIAL PUBLICATION BIASES IN THE REPORTING OF LEVELS OF GENETIC VARIABILITY

Before trying to find biological explanations for discrepancies between the expected and observed effects of bottlenecks, it is worth considering how publication biases may distort our picture of what constitutes low variability. Several features of the decision-making process leading to the publication of scientific manuscripts can result in an inflated view of how much variability is likely to be present in an undepleted population.

Consider a study that sets out to examine the breeding behaviour of a species where a population decline is not suspected. If the first marker tested reveals exceptionally high levels of variability this may be deemed a publishable finding in its own right and is likely to reach the literature rapidly (Avise *et al*. 1989; Turner *et al*. 1992). Conversely, if the first marker proves monomorphic or has low variability, most researchers will probably turn to other markers until sufficient polymorphism is found. Persistent failure to find variability may never be reported, as has been the case for the European badger, *Meles meles*. The net result is an upward bias in the reported level of polymorphism, both at the level of individual markers, because monomorphic locus-species combinations are often ignored, and at the level of the species, because 'healthy' species with low variability are under-represented.

Now consider a study in which genetic tools are used to examine levels of variability in a species where a bottleneck is suspected to have occurred. Here, the opposite bias will accrue. Failure to find variability is now considered interesting and publishable because it 'confirms' prior expectations that the population or species will appear impoverished. As an illustration of this effect, 8 of the 11 studies published in the journal *Conservation Biology* in 1996 and 1997 that included genetic diversity or variability in their title report low levels of variability at some loci. In all cases this was attributed to an undocumented or poorly documented bottleneck in the population's history. Of the three studies that report 'normal' levels of variability, one concluded that this was because the population in question had not gone through a bottleneck. This latter study illustrates both a general preoccupation with the bottleneck concept and the ease with which a failure to find low variability can be attributed to other causes.

In addition, there are practical problems in the study of genetic drift in small populations. Essentially, such studies attempt to investigate a stochastic process by sampling with error from one or two replicate outcomes of that process. For the most part, samples will be available only from the current, depleted population. Such samples are likely to contain relatives, causing a downward bias in the estimate of variability and hence creating the impression that the population has lost more variability than it has, particularly in comparison with potentially inflated 'normal' levels (see above). Even if two samples from different time periods are available, there may still be problems. Many techniques for calculating rates of change of gene frequencies rely on estimates of genetic variance (Waples 1989). Estimates of variance based on the small samples that are likely to be available are likely to be biased upwards, and will therefore provide unreliable rates.

4. INBREEDING DEPRESSION

Even allowing for publication biases, it is clear that some species exhibit unusually low levels of variability. For example 4% of plants taxa (Hamrick & Godt 1989) and 4.7% of animal species examined (Nevo *et al.* 1984) show no allozyme polymorphism. In many cases, this depletion cannot be explained by drift alone, and other factors that have exacerbated the effects of a bottleneck and accelerated the rate of loss of variability above neutral expectations must have been involved. One such set of factors are those associated with inbreeding and its avoidance.

Inbreeding depression is the name given to the reduction in fitness or viability resulting from the increased expression of deleterious recessive alleles after a population crash (Saccheri *et al.* 1996). Large populations are able to carry disproportionately more deleterious recessive alleles than smaller populations, such that when a population declines, the excess tends to be purged by selection (Barrett & Charlesworth 1991). As a consequence, the offspring of those matings that involve the most closely related individuals and those parts of the genome responsible for the strongest effects will be under-represented in future generations. In many species, behavioural mechanisms minimize the effects of inbreeding, either by dispersal patterns that ensure that relatives tend not to meet when pairs are forming (Gilbert *et al.* 1991), or by some form of incest taboo (Amos *et al.* 1993). Such behaviours are referred to as inbreeding avoidance.

Inbreeding depression and inbreeding avoidance both filter the current gene pool as it passes into the next generation, but they act at different scales. Inbreeding depression acts on individual genes and gene complexes, and inbreeding avoidance acts on whole sets of chromosomes. The way in which these two processes affect levels of genetic diversity is complicated by two opposing effects. On the one hand, by reducing the representation of some parts of the gene pool in future generations, variability may be reduced. On the other hand, those individuals that do survive to reproduce will tend to be outbred, and hence will carry greater diversity than would be expected by chance. Which one of these effects will predominate is unclear and will probably vary considerably depending on what form of social organization operates and the magnitude of any population decline suffered.

As yet there is little evidence that either inbreeding depression or inbreeding avoidance play a major role in natural, bottlenecked populations. Inbreeding depression has been shown to occur in a number of controlled laboratory experiments and its effects may be quite strong. However, any purging is probably brief, and the consequences are probably rather slight if the inbreeding depression results from many genes, each of which exerts a small effect (Hedrick 1994).

The effects of inbreeding depression and inbreeding avoidance will both be strongest in long-lived, polygynous species and will exert disproportionately greater effects in small populations. Marine mammals are a group of long-lived, often highly polygynous species, many of which have undergone severe bottlenecks in their history as a result of overexploitation. Several northern species have congeneric equivalents in the southern hemisphere whose responses can be compared. Both northern and southern right whales (*Eubalaena glacialis* and *E. australis*) were reduced to very low numbers by commercial whaling in the 19th century (Klinowska 1991), but whereas the southern species has shown strong signs of recovery in the last two decades there has been little or no increase in northern right whale numbers (Knowlton *et al.* 1994). Similarly, the two subspecies of *Arctocephalus pusillus* (the Cape fur seal, *A. p. pusillus*, and the Australian fur seal, *A. c. doriferus*) were both reduced to low numbers by the beginning of the 19th century, but whereas there are now close to 2 million *A. p. pusillus*, *A. c. doriferus* has a much smaller population of less than 10 000 (Wickens & York 1997).

5. SOCIAL AND SPATIAL STRUCTURE

In theory, the rate at which heterozygosity is lost from a population is determined by the variance effective population size, and the effects of inbreeding are determined by the inbreeding effective population size. The expressions relating these two variables to the actual size of the population are only identical if the size of the population is constant (Kimura & Crow 1963).

Variance effective population size is affected by the sex ratio in the breeding population, interindividual variation in offspring number, generation time and the mating system. In certain special cases (for example, when all individuals contribute equally to the next generation), the

effective population size can be larger than the number of adult animals in the population. However, it is unusual for the variance effective population size to lie outside the range 0.25 to 1.0 times the number of adults (Nunney 1995). The effects of mating system and generation time interact so that, under many circumstances, effective population size is close to one half the number of adults (Nunney 1993). These calculations assume that matings occur at random between the individuals involved in reproduction.

Chesser and his colleagues (Chesser 1991; Sugg *et al.* 1996) have shown that the social organization of a species can result in non-random matings, for example, where there is strong philopatry by one sex and where the other sex disperses. As a result of this process, variance effective population size can exceed the number of adults in the population. Where the philopatric sex forms stable social groups (as is the case in many mammals), rare alleles can become fixed within individual groups (Sugg *et al.* 1996). As the same alleles are unlikely to become fixed in all groups, this process can increase the overall genetic diversity of the population and lead to local adaptation. Exactly the same kind of process can occur in spatially structured populations.

The spatial distribution of many species is fragmented, with patches of suitable habitat being separated by large areas of habitat that are either unsuitable or dangerous. The consequences of this spatial structure for genetic diversity depend on the rate at which individuals move between these patches of suitable habitat. If gene flow is sufficiently high, then the fragmented population behaves as a single population unit and the effect of fragmentation is negligible, but if gene flow is restricted, it is possible for specific rare alleles to become fixed in small local populations. In a manner identical to that proposed above for stable groups, this process can in theory result in an increase in total genetic variability (Harrison & Hastings 1996). However, the low level of migration that is necessary to maintain this distinctness means that individual local populations are likely to be prone to extinction from stochastic processes. This can result in a classic metapopulation structure with the periodic extinction of all individuals in a particular patch and subsequent recolonization of this patch from surrounding areas. Under these circumstances the variance effective size of the entire metapopulation can be very much lower than the number of mature individuals (Gilpin 1991; Hedrick & Gilpin 1997). Hedrick (1996) has calculated that the low genetic variability observed in cheetahs could be accounted for if this species has a metapopulation structure with a variance in effective population size of 200–2000 individuals. Barton & Whitlock (1997) have written an elegant review of the way in which metapopulation structure can affect the evolution of genetic diversity.

There is growing concern about the effects of human development on habitat fragmentation, which is often seen as imposing a metapopulation structure on species that formerly had a more continuous distribution. However, a number of authors (Harrison 1991, 1994; Thomas 1994) have questioned whether the classic metapopulation model is generally the most appropriate in these circumstances. There is also evidence that the rate of gene flow may actually increase as habitats become more fragmented (Young *et al.* 1993). The way in which organisms or propagules move between patches is of critical importance in determining how spatial structure affects the stability and dynamics of spatially structured populations (Rohani *et al.* 1997) as well as its effect on genetic diversity, and this is likely to be a particularly important area for research over the next decade.

6. HETEROZYGOTE INSTABILITY

An alternative explanation for why small populations might appear genetically impoverished arises from recent work on microsatellite DNA sequences. The possibility is that instead of smaller populations having less variability, larger populations might have disproportionately more. The basic model runs as follows (Amos & Rubinsztein 1996; Rubinsztein *et al.* 1995). Every base pair in the genome has a finite opportunity to mutate during DNA replication. However, during meiosis extensive regions of heteroduplex DNA are formed between paired homologous chromosomes. In this state, 'repair' of heterozygous sites could offer an extra opportunity to mutate, an opportunity which will be less available to homozygous sites. If true, then mutation rate will increase with increasing heterozygosity, which in turn increases with population size. Consequently, large populations would tend to evolve more rapidly than smaller populations.

Empirical support for this model can be divided into limited direct evidence and more extensive indirect evidence. Direct evidence comes from the small number of germline mutations that have been identified when large pedigrees have been typed for large numbers of microsatellite markers (Crawford & Cuthbertson 1996). In a human data set comprising 19 informative mutations, it was observed that significantly more mutations derived from the parent with the greater difference in length between his/her alleles (Amos & Rubinsztein 1996). A second study involving *Drosophila melanogaster* appears to demonstrate the converse of heterozygote instability, homozygote stability (Schug *et al.* 1997). In this experiment, microsatellite mutation rates were measured by looking for new mutations in homozygous inbred lines. Schug *et al.* (1997) found far fewer mutations than expected, estimating the average mutation rate to be 6.3×10^{-6}, compared with estimates from other species ranging from 10^{-5} to 10^{-3} (Banchs *et al.* 1994; Edwards *et al.* 1992; Weber & Wong 1993). This result was interpreted by the authors in terms of a length-dependent mutation rate, the short microsatellites they used being less mutable than the generally longer loci used in most mammalian studies, and an extremely large effective population size. However, their data are equally compatible with complete homozygosity causing a reduction in mutation rate.

Indirect evidence for heterozygote instability derives from a large body of molecular data which show that heterozygous sites may be both recognized and 'repaired' by gene conversion-like events during meiosis. Accepting that repair events do occur, it can then be argued that as no molecular processes are likely to be entirely error-free, any mutations that arise due to the gene conversion event itself will lie preferentially at or around heterozygous sites. Although the full process from heterozygous site to increased mutation rate has yet to be demonstrated, most of the key elements are now well-supported.

Extensive work on yeast has shown that large regions of heteroduplex DNA are formed during synapsis (Collins & Newlon 1996; Nag *et al.* 1995), and that within these regions heterozygous sites are repaired by gene conversion like events (Borts & Haber 1989; Borts *et al.* 1990; Szostak *et al.* 1983), a proportion of which result in genetic crossovers (Chambers *et al.* 1996; Manivasakam *et al.* 1996). Similar processes have been invoked as a general feature of eukaryotes that allows homologous regions to pair and undergo crossing over (Carpenter 1994*a*). During meiosis, homologous chromosomes pair extensively to form synaptonemal complexes in which so-called recombination nodules may form. Some of these nodules result in genetic crossing over whereas others appear to be associated with gene conversion events (Carpenter 1994*b*). The ubiquity of these processes is further emphasized by the fact that many of the enzymes involved in the yeast mismatch repair system have direct homologues in mammals (Baker *et al.* 1995, 1996).

Perhaps the clearest evidence for heterozygosity accelerating the rate of evolution is likely to come from studies of microsatellites and other tandemly repeated sequences, such as minisatellites. As increasing numbers of mutation events are being described, it is becoming apparent that most short, tandemly repeated DNA sequences tend to increase in length with time under biased mutation pressure favouring gains in length, due possibly to asymmetries in the topology of the replication fork (Gordenin *et al.* 1997). Biased mutation has been recorded in minisatellites (Jeffreys *et al.* 1994; Monckton *et al.* 1994) and microsatellites (Amos & Rubinsztein 1996; Amos *et al.* 1996; Crawford & Cuthbertson 1996; Primmer *et al.* 1996; Weber & Wong 1993), and also affects the tracts of triplet repeats responsible for a number of human diseases (Duyao *et al.* 1993; Rubinsztein *et al.* 1994). Given this directionality, variation in mutation rate due to population size differences should be manifest as consistent length differences between species and populations, with expanded populations harbouring longer loci relative to their homologues in smaller populations.

Early signs are that this prediction is fulfilled. Humans have expanded dramatically over the last 10 000 years, and human microsatellites (Meyer *et al.* 1995; Rubinsztein *et al.* 1995), minisatellites (Gray & Jeffreys 1991) and triplet repeat disease alleles (Djian *et al.* 1996) all show a clear tendency to be longer than their homologues in chimpanzees. Similarly, microsatellites in the highly abundant barn swallow are consistently longer than their homologues in related species (Ellegren *et al.* 1995). There is also the well-documented observation that compared with chimpanzees, humans have less mitochondrial DNA diversity but greater nuclear diversity (Wise *et al.* 1997). This puzzling observation could be explained in terms of human population expansion increasing the mutation rate at diploid nuclear loci but not at haploid mitochondrial loci, particularly as it appears that it is the human nuclear genes that are behaving unexpectedly (Wise *et al.* 1997).

It has been suggested that consistent microsatellite length differences between species are artefactual, being due to selection for length among marker loci (Ellegren *et al.* 1995), but this fear seems increasingly unfounded. Not only does the effect seem slight (Amos & Rubinsztein 1996), but also the key experiment—a reciprocal test between species—has now been performed. In an elegant and comprehensive study involving 542 markers from cattle and sheep, Crawford *et al.* (1998) showed that loci that are polymorphic in both species are twice as likely to be longer in sheep than cattle, regardless of the species from which they were cloned (termed the 'focal' species). Note, it is vital to exclude loci that are monomorphic in the non-focal species, as both models predict that the monomorphic homologue tend to be short. Indeed, Crawford *et al.* (1998) show that bovine loci that are monomorphic in sheep are approximately 7.5 times as likely to be longer in cattle than equivalent markers that are polymorphic in both species. Because of this effect, studies that are not careful to distinguish between monomorphic and polymorphic loci will often reveal a strong but (ironically) artefactual ascertainment bias (Ellegren *et al.* 1997; van Treuren *et al.* 1997).

Evidence that heterozygote instability may not be restricted to tandemly repeated DNA sequences comes from genetic studies of hybrid zones, where rare alleles not present in either pure population occur far more frequently than expected by chance. This observation is so universal that these rare alleles have earned their own name, hybrizymes (Barton *et al.* 1983; Woodruff 1989). There appear to be two possible sources of hybrizymes: either they arise by recombination between two pre-existing alleles, or they reflect an increased mutation rate in hybrids. Current evidence appears to support an increase in mutation rate (Hoffman & Brown 1995). This is exactly what would be predicted by the heterozygote instability model, because hybrid individuals should show markedly increased levels of heterozygosity.

7. SELECTIVE SWEEPS, MEIOTIC DRIVE AND Y CHROMOSOME EVOLUTION

Even after allowing for effects such as publication biases, accelerated effects of bottlenecks and increased mutability in large or expanded populations, there remain a number of species that appear to be much less variable than expected on the basis of their current population size and probable population histories. There appear to be two classes of general explanation that may account for these outliers: either the amount of variability being generated has been reduced or the rate at which variability is being lost has increased.

Ultimately, all genetic variability is generated by the process of mutation. Low levels of variability could result following a change in some critical enzyme involved with DNA replication, which either reduces the rate at which errors occur or improves the efficiency with which errors are corrected. Indeed there is a recent report demonstrating mutation rate evolution in bacteria (Sniegowski *et al.* 1997). However, a change in mutation rate explanation does not seem plausible in the context of exceptional mammals, most of which have close relatives with relatively 'normal' levels of variability. The problem is that even in the unlikely event of the mutation rate falling suddenly to zero, genetic drift would still take too long to eliminate the variability that was already present. Turning off the tap does not empty the bath.

If there has been insufficient time for a sudden lowering of mutation rate to account for the low levels of variability seen

in some species, the answer must lie with accelerated loss through an active process such as natural selection. What would be needed is for positive selection to act either simultaneously or in quick succession on many different parts of the genome. In this last section, we present a model based on meiotic drive, which is capable of generating such genome-wide selective sweeps. Although there are undoubtedly other possibilities, our model illustrates the sorts of processes involved and, incidentally, is itself capable of facilitating or even promoting speciation.

Meiotic drive is the name given to the theoretical battle between the sex chromosomes in species where one sex is heterogametic (Crow 1979). In the male mammalian germ line, the Y chromosome only ever has a future in a male foetus and the X chromosome only ever in a female foetus. Thus, each sex chromosome is only half as fit as it could be if all progeny were males for the Y or females for the X. Any mutation on the X chromosome which debilitates Y-bearing sperm (or vice versa) will be selected, and this selection process creates a constant state of tension between the two sex-determining chromosomes. As there are three X chromosomes for each Y, it seems reasonable to suppose that in any such battle, the X will tend to be on the offensive and the Y on the defensive.

A central question relating to the process of meiotic drive is that of how a gene on the X chromosome can recognize a Y-bearing cell (the process of recognition is not essential, as illustrated by antagonistic gene pairs such as *Stellate* and *Suppressor of Stellate* (Hurst 1992, 1996)). There seem to be three possible routes for recognition: through the protein products of Y-specific genes, through the RNA product of any Y-specific transcribed sequence (as in *Stellate*; Hurst 1996), or through recognition of Y-specific DNA sequences. Of these, the first two seem more probable than the last, as it is difficult to imagine a way by which a DNA sequence could be identified as coming from the Y rather than any other chromosome. Consequently, it seems reasonable to suppose that any expressed sequence on the Y has the potential to act as an identification flag that might attract adverse attention from the X.

The mammalian Y chromosome is thus likely to be engaged in a battle in which it is outgunned by its opponent. A logical consequence is that the Y should run away and hide, shedding any transcribed sequences that are not essential to its function. In line with this prediction is the well-known observation that the mammalian Y chromosome is degenerate (Graves 1995; Morell 1994), probably having fewer than twenty active genes. Traditional theory states that degeneration and loss of active genes from the Y is expected to occur to counteract the accumulation of deleterious mutations that begins as soon as the lack of recombination sets Muller's ratchet in motion (Charlesworth 1991; Fisher 1935). However, this process of degeneration, although detectable (Rice 1994), appears to progress rather slowly (Allen & Ostrer 1994; Charlesworth *et al.* 1997).

By contrast, the rate of loss of genes from the mammalian Y must have been extraordinary. Mammals evolved a little over 100 million years ago from an ancestor that presumably carried sex chromosomes of roughly equal size. A conservative estimate for the number of genes on an average mammalian X chromosome is 1000, and, with only a handful of active genes remaining on the non-pseudoautosomal region of the Y, this is approximately the number that must have been lost. Consequently, the average rate of loss is one gene per 100 000 years. However, as all major mammalian radiations have reached similar levels of Y degeneration, it seems likely that most of the gene loss occurred early (Bull 1983), suggesting that early mammals were shedding tens of genes per speciation event.

Consider how the battle might run. Most of the time the two sex chromosomes are in unstable equilibrium and the sex ratio is 1:1. Then a mutation occurs which disturbs the balance. If the battle is fought at the level of the RNA rather than the protein, most point mutation could, in principle, disrupt RNA:RNA pairing thereby conferring greater concealment to the Y or greater dominance to the X. Such a mutation will increase the fitness of the chromosome on which it arose and will tend to sweep through to fixation. As the sweep progresses, the sex ratio will become distorted and, according to Fisherian principles, this will increase the selection pressure for a compensatory mutation on the opposing chromosome, which redresses the balance. If and when the new mutation arises it may reduce the imbalance, eliminate the imbalance or even overcompensate. Potentially, a train of change and counter-change could follow.

A mutation of the type envisaged could as much as double the fitness of the chromosome on which it occurred. Consequently, even if the mutation is itself inherently slightly deleterious in terms of fitness at the level of the individual, it could be driven through to fixation. The interesting case to consider is a non-silent substitution in *SRY*, the sex-determining gene itself. *SRY* appears to be a cascade gene which controls either directly or indirectly the expression of many male traits, presumably including secondary sexual characteristics. Consequently, a slight change in the SRY protein could affect the expression of a range of genes associated with male behaviour and appearance. Although such changes would tend to be deleterious, they could still be driven to fixation if the net disadvantage was outweighed by the sex ratio distortion effect. Once fixed in the population, selection would then favour changes in the genes controlled by *SRY* so as to nullify or accommodate the detrimental effects of the original mutation. In other words, a genome-wide selective sweep.

Eventually, a new state of equilibrium will be found and the sex ratio will return to parity. In the aftermath, one or more selective sweeps will have left the Y chromosome with little or no variability. Also, there may now be an incompatibility between the sex chromosomes in the population/subspecies where these events took place and their homologues in other population units. There may even be changes in the appearance and behaviour of males. Together, these changes would at least facilitate the process of speciation, and might even actively promote it.

Although speculative, the above model does make a number of testable predictions, some of which enjoy limited empirical support. First, most evolution on the non-recombining portion of the mammalian Y chromosome should be extremely punctuated and occur at or around speciation. Second, within a species, levels of Y-chromosome variability should be extremely low. Third, assuming X–Y interactions can operate at the level of the

RNA, almost any change could give rise to selection, whether in an intron or an exon, synonymous or non-synonymous. Fourth, hybrid species should show evidence of meiotic drive, such as sex ratio distortion, lowered sperm counts and infertility.

The first three predictions are supported by recent work on primate Y chromosome evolution. Burrows & Ryder (1997) sequenced an intronic region from the *ZFY* gene from a number of primate species. In line with other studies of human Y chromosome sequences (Dorit *et al.* 1995; Hammer 1995), they found a complete lack of intraspecific variation, despite significant interspecific divergence. They concluded that this apparent contradiction could be rationalized only by invoking periodic selection on the Y. Other studies on *SRY* reveal a similar pattern. Within a species there is virtually no variability (Whitfield *et al.* 1995), yet between species there is divergence (Whitfield *et al.* 1993). This is despite the fact that (outside the conserved *HMG* box) *SRY* evolves extremely rapidly (Tucker & Lundrigan 1993; Whitfield *et al.* 1993). Furthermore, the ratio of non-synonymous to synonymous substitutions (K_a/K_s) in *SRY* is one of the highest yet recorded for an active gene (range for *SRY*, 0.32 to 1.88; average for 42 coding sequences, 0.189) a fact explained by the authors in terms of either a lack of function or the influence of strong selection (Whitfield *et al.* 1993).

With an increasingly consistent pattern emerging of monomorphism within populations units yet divergence between them, the question of when the interspecific changes arise becomes ever more important. The only direct evidence we have so far relates to humans. There is general agreement that human Y chromosomes have accumulated virtually no point mutation variability since their common ancestor (Dorit *et al.* 1995; Hammer 1995; Whitfield *et al.* 1995). At the same time, the widespread survival of ancient lineages predating movement out of Africa (Cooper *et al.* 1996; Jobling & Tyler-Smith 1995) shows that there has been plenty of time in which mutations could have occurred; this precludes a recent selective sweep. Together, the rapid rate of *SRY* evolution and the absence of differences between African and non-African lineages suggest that whenever a viable mutation affects S*RY* it has a high probability of being selected rapidly through to fixation. The appearance gained is one of a gene that is constantly fleeing in sequence space, just as would be expected of a gene which is 'trying' to hide yet remain functional.

The last prediction relates to sex ratio perturbation and loss of fitness. Such effects will undoubtedly be hard to track down as, by definition, they will be transient and require large sample sizes to detect. The best candidates for species affected by drive are perhaps the big cats. It is well known that felids have both relatively low levels of genetic variability and unusually high proportions of aberrant, non-functional sperm (see O'Brien (1994) and references therein). Most famous is the cheetah, a species with extremely low variability and 70% non-functional sperm.

Previously, low genetic variability and low sperm viability were linked by the assertion that spermatogenesis was being disrupted by inbreeding depression (O'Brien 1994). However, this explanation appears less and less convincing. Genetic purging through inbreeding depression is expected to be transient and to affect all phenotypic traits from zygote through to adult. By contrast, there is no evidence that the cheetahs have become much more inbred over the last few generations, their populations having remained approximately stable (Laurenson *et al.* 1995), and careful study of their life history table suggests that the primary threat faced by cubs is predation by lions and hyenas rather than congenital birth defects (Caro & Laurenson 1994). Therefore our alternative suggestion is that low variability and poor sperm viability are not causally linked, but instead both traits are byproducts of the same causative process, intragenomic conflict and its associated selective sweeps.

We would like to stress that the above model remains speculative and applies only to species such as mammals with single gene sex determination. However, it does show how periodic selective sweeps could result when an evolutionary arms race imposes change on a control gene, forcing downstream genes to coadapt. Thus, meiotic drive is presented as a specific example of a more general model. For example, sex determination in the fruit fly *Drosophila* differs fundamentally from the same process in mammals; fruit flies have not shed most of their genes from the Y chromosome and maleness is governed by the relative numbers of X and Y chromosomes. It is therefore perhaps not surprising that elegant studies of hybrid sterility in fruit flies have failed to find evidence of the sex ratio distortion that should exist if meiotic drive were a major factor causing postzygotic isolation (Johnson & Wu 1992). At the same time, other experiments, also aimed at dissecting the causes of hybrid sterility, show that the number of genetic factors that contribute to hybrid sterility between *D. simulans* and *D. mauritiana* probably exceeds 100 (Davis & Wu 1996; Wu *et al.* 1996). Although apparently not due to meiotic drive, this large number of factors is probably best explained in terms of coadaptation between a small number of control genes and a much larger number of 'effect' genes. To step back even further, it is not even necessary to postulate such a specific mechanism. If a significant proportion of these sterility factors were fixed by the action of natural selection (it is difficult to imagine how such changes could come about through drift alone), this would itself constitute a selective sweep(s) with the potential to denude large portions of the genome of variability.

8. CONCLUSIONS AND IMPLICATIONS FOR SPECIATION

In this paper we take a critical look at the measurement of genetic variability and the extent to which population size can explain interspecific variation in variability. We find that, although many cases of genetic impoverishment are attributed to population bottlenecks, in many cases the species in question have not spent long enough at low enough numbers. A number of alternative explanations are considered, including publication biases, the effects of inbreeding and inbreeding avoidance, metapopulation structure and the possibility that heterozygotes are more mutable than homozygotes. Instances of extreme impoverishment in species whose close relatives show 'normal' levels of variability appear to require active removal of variability through natural selection rather than passive loss through drift, in other words a selective sweep. We

present a model capable of generating such a sweep, based on forced coevolution between a control gene and the loci it regulates and exemplified by meiotic drive.

Our results have two primary implications for the process of speciation. First, they suggest that the relationship between population size and observed levels of neutral variability is not as straightforward as is often assumed. Some relatively abundant species have minimal variability and some persecuted and endangered species have no more or less variability than most other equivalent species. Second, any process that increases the rate of divergence between isolated or semi-isolated populations has the potential to facilitate or accelerate speciation. For the most part, the size of the effect is likely to be slight, for example, when inbreeding avoidance accentuates a founder effect following a bottleneck. However, the final scenario we present, meiotic drive, could have profound consequences, which warrant further discussion.

It has long been recognized that coevolution between genes within the genome has the potential to reduce hybrid fitness and hence to contribute to postzygotic isolation (Dobzhansky 1937; Muller 1942). However, previous models have tended to be passive, being based on the concept that mutations that become fixed in one genetic background may be detrimental in another. Although logically compelling, this model does not seem powerful enough to explain the large numbers of strong, independent incompatabilities that separate closely related species of *Drosophila* (Davis & Wu 1996).

Consider the implications. Incompatabilities that originate passively in the way envisaged by Dobzhansky (1937) and Muller (1942) should affect all genomes of equivalent complexity approximately equally. Consequently, the fact that many closely related species can form fertile hybrids suggests that passively acquired incompatabilities accrue rather slowly and tend to be weak. By implication, the *Drosophila* example probably involves a more driven process. The most obvious way to generate many profound incompatabilities rapidly would be through a much smaller number of changes in a key gene (or genes), which interact with many other genes distributed throughout the genome. In this way, most hybrids would contain multiple genetic conflicts.

On its own, this model does not work, because virtually all functional changes in a control gene would cause exactly the same sorts of incompatability as are seen in hybrids, and hence would be selectively eliminated. In general, control genes are unlikely to evolve rapidly unless influenced by a second force, a selective pressure that is powerful enough to force compensatory changes at all loci with which the control gene interacts. We speculate that meiotic drive could provide this force, though this is by no means the only possibility (Johnson & Wu 1992), and both sperm competition and runaway processes such as Fisher's model of sexual selection are two plausible alternatives which appear to offer adequate power. Interestingly, the genus *Drosophila* is characterized by extraordinarily long sperm, up to 20 times the length of the adult flies' body (Pitnick *et al.* 1995), an extreme trait whose mere existence is suggestive of the operation of powerful selection.

A control gene model of hybrid sterility has many interesting implications for the process of speciation. First, the process is powerful, offering a way to produce many strong incompatability factors rapidly. Second, the process is endogenous, a property of each independent lineage, and hence can operate both sympatrically and allopatrically with equal effectiveness. All that is required is some heterogeneity in the gene pool in either space or time, sufficient to allow a new variant control gene to become fixed. Third, by generating hybrid incompatabilities, incipient speciation events are at least facilitated, in that any pre-existing heterogeneity will tend to become exaggerated. Finally, in the specific case of the meiotic drive model, speciation could conceivably be driven by the genomic conflict itself. If a new functional variant of *SRY* were driven through to fixation in one gene pool, followed by compensatory changes in the rest of the genome, this could simultaneously create both a barrier to interbreeding and changes in male behaviour and or appearance.

In conclusion, we still seem some way from understanding the basis for many interspecific differences in variability. Part of the problem may be artefactual, and have more to do with the process of scientific publication. Elsewhere, it seems we need to learn more about how and when natural selection acts to accelerate the rate of loss of variability.

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