LETTERS

Interference among deleterious mutations favours sex and recombination in finite populations

Peter D. Keightley[1] & Sarah P. Otto[2]

Sex and recombination are widespread, but explaining these phenomena has been one of the most difficult problems in evolutionary biology. Recombination is advantageous when different individuals in a population carry different advantageous alleles[1,2]. By bringing together advantageous alleles onto the same chromosome, recombination speeds up the process of adaptation[1,3–5] and opposes the fixation of harmful mutations by means of Muller's ratchet[4,5]. Nevertheless, adaptive substitutions favour sex and recombination only if the rate of adaptive mutation is high[1,6], and Muller's ratchet operates only in small or asexual populations[7]. Here, by tracking the fate of modifier alleles that alter the frequency of sex and recombination, we show that background selection against deleterious mutant alleles provides a stochastic advantage to sex and recombination that increases with population size. The advantage arises because, with low levels of recombination, selection at other loci severely reduces the effective population size and genetic variance in fitness at a focal locus[8] (the Hill–Robertson effect), making a population less able to respond to selection and to rid itself of deleterious mutations. Sex and recombination reveal the hidden genetic variance in fitness by combining chromosomes of intermediate fitness to create chromosomes that are relatively free of (or are loaded with) deleterious mutations. This increase in genetic variance within finite populations improves the response to selection and generates a substantial advantage to sex and recombination that is fairly insensitive to the form of epistatic interactions between deleterious alleles. The mechanism supported by our results offers a robust and broadly applicable explanation for the evolutionary advantage of recombination and can explain the spread of costly sex.

Sex and recombination break apart co-adapted gene combinations, thereby reducing fitness. Yet sexual reproduction is extremely widespread, and most eukaryotes have at least one chiasma per chromosome per meiosis[9]. One of the most promising evolutionary explanations for sex and recombination is that they break down associations between deleterious and beneficial alleles at different loci (negative disequilibria). By bringing together favourable alleles from different chromosomes, sex and recombination increase the additive genetic variance for fitness, and, by Fisher's fundamental theorem of natural selection, can increase the rate of adaptation[1,3,4,10,11]. Nevertheless, current evolutionary models have difficulties in explaining widespread sex and recombination by this mechanism. Directional selection acting on favourable alleles in the presence of drift generates negative disequilibrium, on average, because selection rapidly eliminates positive disequilibrium whenever it arises by chance[6]. This negative disequilibrium favours the spread of a modifier allele that increases recombination, because the modifier allele is more likely to occur on chromosomes containing multiple beneficial alleles that have been brought together by past recombination[2,6]. This advantage to a recombination modifier can be substantial in small populations[12] or in large populations that are spatially structured[13] and/or subject to directional selection at multiple loci[14], but it requires a high rate of beneficial sweeps, for which evidence is equivocal[15,16]. Other mechanisms that can favour recombination in large populations require certain forms of epistasis or fluctuating epistasis. For these alternative mechanisms to work, epistasis must be weak, synergistic, and similar between pairs of loci[17,18] or it must fluctuate rapidly[17,19]. There is currently little empirical support for such forms of epistasis[19,20].

Sex and recombination can also be favoured because they increase the rate of elimination of deleterious mutations. Deleterious mutations of small effect are a ubiquitous feature of living systems, and comparative molecular evolutionary analysis[21] and experiments involving the molecular analysis of mutation accumulation lines[22,23] suggest that there is at least one deleterious mutation per diploid per generation in taxa as diverse as *Caenorhabditis elegans*, *Drosophila*, mammals and birds. In the face of such recurrent deleterious mutations, sex and recombination can be advantageous for two distinct reasons. First, there is a deterministic advantage of recombination that eliminates the negative linkage disequilibrium generated by synergistic epistasis[17,18,24,25], but, as mentioned above, there is little evidence for such epistasis[20]. Second, there is a stochastic advantage in that recombination can reduce the fixation of harmful mutations by means of Muller's ratchet[4]. Yet harmful mutations are unlikely to become fixed in sexual populations unless the effective population size is very small[7]. Even if harmful alleles do not become fixed, they can still reduce the efficacy of selection on neighbouring loci through a process called Hill–Robertson interference[8]. This effect occurs because individuals bearing deleterious mutations are less likely to survive and reproduce, reducing the number of individuals that contribute genetically to the future population. This reduces the effective population size witnessed by a focal locus, thereby increasing the importance of random genetic drift at the locus relative to selection. The extent to which the Hill–Robertson effect favours the evolution of sex and recombination in the presence of recurrent deleterious mutations is not well understood. Although previous simulations[2,26] indicate that modifier alleles that increase the frequency of recombination can be favoured in small populations (1,000 or fewer individuals), whether this effect is appreciable in larger populations or in the presence of epistasis is unknown. To address this issue, we quantified rates of fixation of modifier alleles that increase recombination in chromosomes subject to recurrent deleterious mutations at many loci, exploring a range of population sizes and forms of epistasis.

In simulations, we allowed frequencies of deleterious mutant alleles and linkage disequilibria among them to approach mutation–selection–drift balance in a population of N haploids. We then introduced, at a random position and in a single copy, a

[1]Institute of Evolutionary Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. [2]Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia V6T 1Z4, Canada.

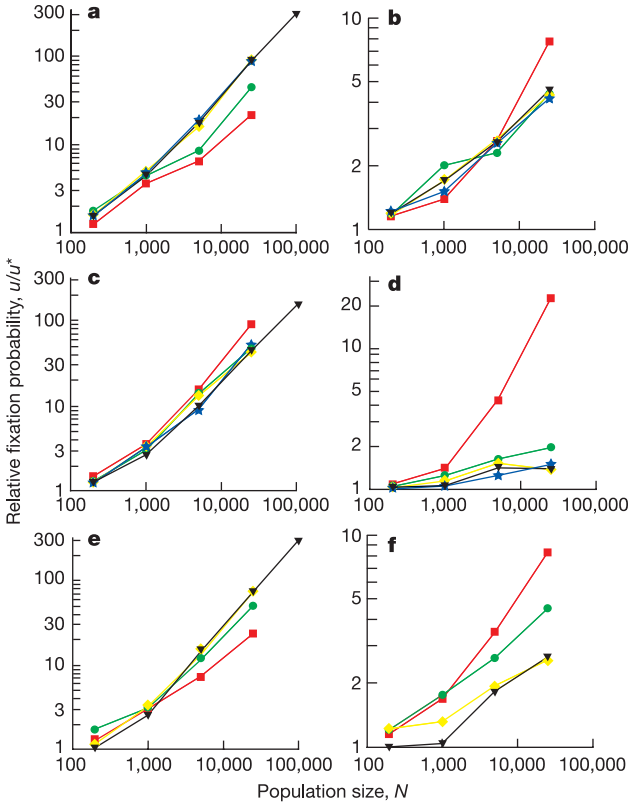

Figure 1 | Effect of model parameters on flux of modifiers of recombination. The fixation probability, u, of a modifier mutation that increases the length of a chromosome by 0.1 M, relative to the fixation probability of a neutral locus, u^*, is shown as a function of population size N, for cases of chromosomes of initial map length 0 M (**a**, **c**, **e**) or 0.1 M (**b**, **d**, **f**). Results are shown for simulations with five different values of the epistasis parameter β: 0.000001 (weak antagonistic epistasis; blue stars), 0 (black triangles), −0.000001 (yellow diamonds), −0.0001 (green circles) and −0.01 (very strong synergistic epistasis; red squares). We excluded cases in which antagonistic epistasis led to individuals with a fitness of more than 1, implying that advantageous mutations were present. The chromosomal mutation rate and fitness parameters were $U = 1$, $\alpha = 0.01$ (**a**, **b**); $U = 0.1$, $\alpha = 0.01$ (**c**, **d**); $U = 1$, $\alpha = 0.001$ (**e**, **f**). The s.e.m. of each point, measured as a percentage of the point's value, was less than or equal to 13%.

recombination modifier allele that uniformly stretched the genetic map length of a chromosome from an initial length L ($L = 0$, 0.1, or 1 morgan) to $L + 0.1$ morgan (M). The fixation probability, u, of the modifier allele was measured relative to the fixation probability, $u^* = 1/N$, of a neutral mutation. The quantity u/u^* when multiplied by the mutation rate to new modifier alleles, μ, also describes the expected rate of substitution (or 'flux') at modifier loci ($\mu N u = \mu u/u^*$). The effect of population size on u/u^* is shown in Fig. 1 for values of the chromosomal mutation rate, U, and deleterious mutational effect of single mutations, α, that span the range of empirical estimates for several eukaryotes[27]. We explored whether curvature in the fitness surface, as measured by the epistasis parameter β, had a major effect on the spread of the modifier (see Methods). In all simulations in which recombination started at a low level (that is, $L \leq 0.1$ M), u/u^* was greater than unity, implying that the recombination modifier is favoured. Even with chromosomes 1 M in length, the modifier of recombination tends to be favoured (Supplementary Table 1). Epistasis did not have a substantial effect on the outcome unless it was very strong and negative. For the cases considered in Fig. 1, the input of mutational variation for fitness per generation ranges from $V_M \approx U\alpha^2 = 10^{-6}$ to 10^{-4}, assuming that each mutation has an independent effect on fitness ($\beta = 0$). These values are comparable to empirical estimates of V_M per chromosome in

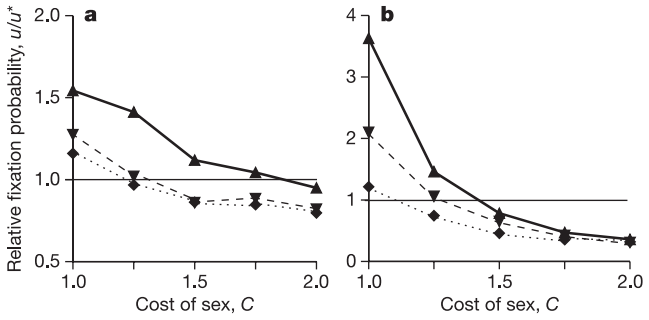

Figure 2 | Effect of model parameters on flux of modifiers of sex. The fixation probability of a modifier that causes an individual to undergo sexual reproduction with probability p_{sex} is plotted against the cost of sex, C, relative to the case of a mutation that has no effect on sex. The advantages of sex are greater than the costs for points above the horizontal line (u/u^*=1). Results for two values of p_{sex} are shown: 0.01 (**a**) and 0.05 (**b**). Each simulated individual carried a single chromosome of map length 1 M with $U = 1$, $\alpha = 0.01$, no epistasis, and no sex among the remaining individuals. Solid lines, $N = 25{,}000$; dashed lines, $N = 5{,}000$; dotted lines, $N = 1{,}000$. The s.e.m. of each point, measured as a percentage of the point's value, was less than or equal to 9% in **a** and less than or equal to 14% in **b**.

Drosophila—typically of the order of 2×10^{-5} (ref. 28). We also investigated simulations in which a modifier mutation arose at a random location in a genome containing five chromosomes. The advantage of the recombination modifier is only slightly smaller than that observed in a single chromosome genome (Supplementary Table 2).

The flux of modifier alleles increases with the population size over the range of parameters considered (Fig. 1, and Supplementary Fig. 1), showing that Hill–Robertson interference is relevant to the evolution of sex and recombination even in large populations (for example, u/u^* can exceed 100 in populations of size 100,000). It is striking that the stochastic advantage to sex and recombination is stronger in larger populations; this surprising observation stems from the fact that such populations maintain more polymorphic loci, increasing the strength of Hill–Robertson interference.

To investigate whether Hill–Robertson interference is strong enough to favour sexual reproduction, we asked whether sex could invade an asexual population, allowing for substantial costs of sex. After the allele frequency distribution had reached steady state in the asexual population, a mutation causing individuals to undergo sexual reproduction with probability p_{sex} was introduced into the population. The resulting zygotes had one recombination event, on average, per chromosome. We introduced a cost of sex, C, reducing the number of offspring per parent when reproduction was sexual rather than asexual, namely $w_{sex} = w_{asex}/C$; we explored values of C between 1 (no cost) and 2 (a twofold cost of sex). The results (Fig. 2) indicated that selection against deleterious mutations can favour costly sex (u/u^* exceeded 1 for C values ranging from 1 to 1.75), especially when the modifier causes only a small increase in the probability of sexual reproduction. In particular, costly sex is more likely to spread in large populations.

Theory for infinite populations predicts no advantage to a modifier of recombination when genes affect fitness independently ($\beta = 0$), because such selection does not generate disequilibria[10]. Over the range of population sizes explored, however, our simulations show that the relative fixation probability of a modifier of recombination increases with population size under a range of models of gene action, regardless of the presence or sign of epistasis. That the Hill–Robertson effect is behind this result was confirmed by a marked decrease in the effective population size (N_e) in our simulations in comparison with the actual population size. To estimate N_e, we measured the steady-state variance at a linked locus subject to recurrent neutral mutation (see Methods). The

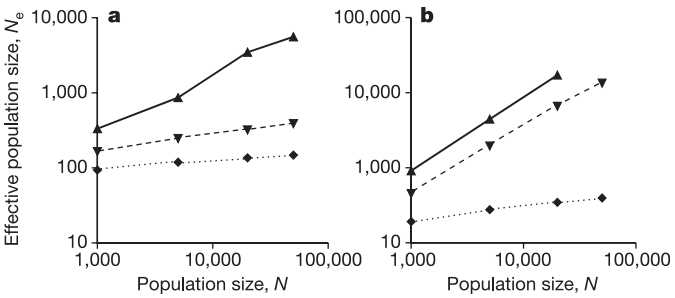

Figure 3 | Effect of background selection on effective population size. Effective population size was estimated in our simulations with $U = 1$ (**a**) and $U = 0.1$ (**b**), both with $\alpha = 0.01$ and $\beta = 0$ (no epistasis). Individuals carried only one chromosome, with the neutral locus situated at the very end. Even greater decreases in N_e are observed at centrally located neutral loci (see Supplementary Table 3). Solid lines, $L = 1$; dashed lines, $L = 0.1$; dotted lines, $L = 0$. The s.e.m. of each point, measured as a percentage of the point's value, was less than or equal to 7% in **a** and less than or equal to 21% in **b**.

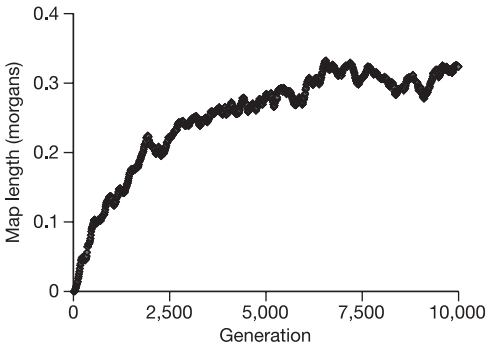

Figure 4 | The long-term evolution of recombination allowing recurrent mutation at modifier loci. The mean map length from 10 replicate simulations is plotted against generation number. Background selection was simulated on a single chromosome without epistasis ($U = 1$, $\alpha = 0.01$, $\beta = 0$). Recurrent recombination modifier mutations arose at 100 random loci on the chromosome at a rate of 0.001 per chromosome. Their effects were +0.1 M or −0.1 M, with equal probability. The population size was 1,000.

results (Fig. 3, and Supplementary Table 3) show that N_e remains far below the actual population size, especially when linkage is tight. For example, in the case of complete linkage, the effective population size remains below 200 even as the census size rises to 50,000 (Fig. 3a). The decrease in effective population size observed in our simulations matched theoretical predictions[29] (Supplementary Table 3), and the effect of recombination on N_e was a good predictor of the fixation probability of modifier alleles (Supplementary Fig. 2). As a result of small effective population sizes, deleterious mutations became fixed in several of the simulations (even in the presence of recombination). However, such fixation events were not solely responsible for our results, because sex and recombination was also favoured in cases in which deleterious mutations rarely, if ever, became fixed (for example, $L = 0.1$ M, $U = 0.1$).

Although not directly under selection, modifiers increasing the amount of recombination can experience substantial indirect selection through associations with fitter alleles at linked loci. The strength of selection, s, can be estimated from the fixation probability by using the standard diffusion result[30]

$$u \approx (1 - \exp[-2sN_e/N])/(1 - \exp[-2sN_e])$$

For example, in Fig. 1a ($L = 0$ M, $U = 1$, $\alpha = 0.01$, no epistasis), selection acting on the modifier equals $s \approx 0.02$ when $N = 1{,}000$ and rises to $s \approx 0.5$ when $N = 50{,}000$, whereas in Fig. 1b ($L = 0.1$ M), selection rises from $s \approx 0.004$ when $N = 1{,}000$ to $s \approx 0.008$ when $N = 50{,}000$ (using values of N_e estimated in our simulations). Drift hinders the fixation of even very beneficial modifier alleles because of the drastic decrease in the effective size relative to the census size of the population.

There are two principal conclusions from our results. First, theory developed for infinite populations to predict the fate of a modifier does not apply, because random genetic drift in the presence of selection generates disequilibria that can favour a modifier, even in large populations. Second, over the range of parameters explored, Hill–Robertson effects overwhelm the effects of epistasis as a force generating disequilibria among alleles at different loci. Consequently, we find that the form of epistasis is not critical to the advantage of sex and recombination in finite populations, in contrast to theoretical predictions from infinite populations at mutation–selection balance[17,18,24,25]. These conclusions apply over a wide range of plausible values for the genomic deleterious mutation rate and mean effect of deleterious mutations.

Although we have focused on the fixation probability of a single modifier mutation, simulations allowing recurrent modifier mutations show that chromosomal recombination rates can rise to the order of 1 M (Fig. 4). Multi-locus Hill–Robertson interference therefore provides a general and robust explanation for the evolution of recombination in any genome subject to recurrent deleterious mutations and can even contribute to the evolution of costly sex.

METHODS

We simulated haploid populations of N individuals with genomes of c independently segregating chromosomes, each containing 100 equally spaced loci affecting fitness. In each generation, deleterious mutations affecting fitness occurred before mating and zygote formation. The number of mutations per generation per individual was sampled from a Poisson distribution with a mean of U. Each mutation was assigned to a random locus in the genome. We kept track of the number of mutations carried at each locus, so that we could allow for multiple mutations. The fitness of an individual i was $w_i = \exp[-\alpha n_i + \beta n_i^2]$, where α is the independent fitness effect of a mutation, β its epistatic effect, and n_i the number of deleterious mutations carried by the individual. To ensure that mutations were always deleterious, we could explore only a narrow range of positive values of β. To form a mating pair, individuals were sampled with replacement with probability proportional to w_i. Having selected a mating pair, a zygote was formed by allowing n_c recombination events to occur between the pair's chromosomes, where n_c was sampled from a Poisson distribution with mean L to simulate a chromosome of initial map length L morgans. These n_c recombination events were then randomly and uniformly distributed between the 100 loci. This process was repeated until N offspring were produced, leading to a nearly Poisson distribution of offspring per parent.

To simulate the fate of a recombination modifier, the allele frequencies at the loci affecting fitness were allowed to approach their steady-state frequencies by allowing a long 'burn-in' period of at least N generations of mutation, selection, drift and recombination. This burn-in period was set to many times the effective population size measured in the simulations (see below). After the burn-in, the state of the population was saved. A recombination modifier mutation was introduced to a random individual of the burn-in population at a random position on a chromosome, coinciding with one of the fitness-altering mutations. The recombination modifier increased the expected number of recombination events in zygotes heterozygous (homozygous) for the modifier to $L + 0.05$ M ($L + 0.1$ M). For each burn-in population, the fates of between N and $5N$ modifier mutations were tracked. At least five replicate burn-in populations were simulated for each parameter combination. The fraction of recombination modifiers that became fixed per burn-in population was recorded, and the mean and s.e.m. of this fraction were calculated over independent burn-ins.

The simulation of the fate of a modifier of sex was similar to that for a modifier of recombination. Asexual burn-in populations were simulated, and then a mutation was introduced in a single copy that caused its haploid carrier to produce all of its offspring sexually with probability p_{sex} but at a cost, C.

To estimate effective population size (N_e) in the presence of deleterious mutations at linked loci, a neutral, linked locus was incorporated in the simulation. This locus was either telomeric or centrally located on the chromosome. In each generation, the value of each individual's neutral locus was altered by adding a normally distributed mutational effect of mean 0 and variance

$V_M = 1$. In the absence of selection, the equilibrium variance at the locus is expected to be NV_M, and this was confirmed in simulations. With selection at linked loci, the mean equilibrium variance at the neutral locus was then defined as the effective population size, N_e. A burn-in period of at least seven times the equilibrium N_e computed from theory was allowed; N_e was then computed in each generation for at least a further $10N$ generations. Average N_e estimates from independent burn-ins were used to calculate an overall estimate of the mean and standard error of N_e.

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1. Fisher, R. A. *The Genetical Theory of Natural Selection* (Oxford Univ. Press, Oxford, 1930).
2. Felsenstein, J. & Yokoyama, S. The evolutionary advantage of recombination. II. Individual selection for recombination. *Genetics* **83,** 845–859 (1976).
3. Morgan, T. H. *Heredity and Sex* (Columbia Univ. Press, New York, 1913).
4. Muller, H. J. Some genetic aspects of sex. *Am. Nat.* **66,** 118–138 (1932).
5. Felsenstein, J. The evolutionary advantage of recombination. *Genetics* **78,** 737–756 (1974).
6. Barton, N. H. & Otto, S. P. Evolution of recombination due to random drift. *Genetics* **169,** 2353–2370 (2005).
7. Lynch, M., Conery, J. & Bürger, R. Mutational meltdowns in sexual populations. *Evolution Int. J. Org. Evolution* **49,** 1067–1080 (1995).
8. Hill, W. G. & Robertson, A. The effects of linkage on limits to artificial selection. *Genet. Res.* **8,** 269–294 (1966).
9. Bell, G. *The Masterpiece of Nature: The Evolution and Genetics of Sexuality* (Univ. of California Press, Berkeley, 1982).
10. Felsenstein, J. The effect of linkage on directional selection. *Genetics* **52,** 349–363 (1965).
11. Bürger, R. Evolution of genetic variability and the advantage of sex and recombination in changing environments. *Genetics* **153,** 1055–1069 (1999).
12. Otto, S. P. & Barton, N. H. Selection for recombination in small populations. *Evolution Int. J. Org. Evolution* **55,** 1921–1931 (2001).
13. Martin, G., Otto, S. P. & Lenormand, T. Selection for recombination in structured populations. *Genetics* **172,** 593–609 (2006).
14. Iles, M. M., Walters, K. & Cannings, C. Recombination can evolve in large finite populations given selection on sufficient loci. *Genetics* **165,** 333–337 (2003).
15. Bierne, N. & Eyre-Walker, A. The genomic rate of adaptive amino acid substitution in *Drosophila*. *Mol. Biol. Evol.* **21,** 1350–1360 (2004).
16. Chimpanzee Sequencing and Analysis Consortium. Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* **437,** 69–87 (2005).
17. Barton, N. H. A general model for the evolution of recombination. *Genet. Res.* **65,** 123–144 (1995).
18. Otto, S. P. & Feldman, M. W. Deleterious mutations, variable epistatic interactions, and the evolution of recombination. *Theor. Popul. Biol.* **51,** 134–147 (1997).
19. Otto, S. P. & Nuismer, S. Species interactions and the evolution of sex. *Science* **304,** 1018–1020 (2004).
20. Rice, W. R. Experimental tests of the adaptive significance of sexual recombination. *Nature Rev. Genet.* **3,** 241–251 (2002).
21. Keightley, P. D. & Eyre-Walker, A. Deleterious mutations and the evolution of sex. *Science* **290,** 331–333 (2000).
22. Denver, D. R., Morris, K., Lynch, M. & Thomas, W. K. High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature* **430,** 679–682 (2004).
23. Halligan, D. L. & Keightley, P. D. Ubiquitous selective constraints in the *Drosophila* genome revealed by a genome-wide interspecies comparison. *Genome Res.* **16,** 875–884 (2006).
24. Kondrashov, A. S. Deleterious mutations as an evolutionary factor. I. The advantage of recombination. *Genet. Res.* **44,** 199–217 (1984).
25. Charlesworth, B. Mutation selection balance and the evolutionary advantage of sex and recombination. *Genet. Res.* **55,** 199–221 (1990).
26. Pálsson, S. Selection on a modifier of recombination rate due to linked deleterious mutations. *J. Hered.* **93,** 22–26 (2002).
27. Charlesworth, B. & Charlesworth, D. Some evolutionary consequences of deleterious mutations. *Genetica* **103,** 3–19 (1998).
28. Caballero, A. & Keightley, P. D. A pleiotropic nonadditive model of variation in quantitative traits. *Genetics* **138,** 883–900 (1994).
29. Santiago, E. & Caballero, A. Effective size and polymorphism of linked neutral loci in populations under directional selection. *Genetics* **149,** 2105–2117 (1998).
30. Kimura, M. Diffusion models in population genetics. *J. Appl. Probab.* **1,** 177–232 (1964).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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