does not preclude male territory quality being correlated with the number of deleterious alleles they bear and, as can be seen from Fig. 1 and equation (6), strong effects on load do not require strong correlations. Although the calculations that have been presented are based on best of n mate choice, mathematically the results are much more general. Any form of differential mating success that leads to the average father bearing fewer deleterious mutations than the average male will have similar effects.

Although sexual selection and differential male mating success is normally discussed in relation to animals, there is no reason why the mechanism outlined here cannot operate in plants. The preconditions are certainly satisfied[28].

Finally, from the perspective of viability, sexual selection often leads to the evolution of poorly adapted traits and destructive competition for mates. These are not a further cost of sex. Any asexual lineage arising from a sexual population would be very unlikely to lose costly female secondary sexual characteristics at the same time as becoming parthenogenetic. Moreover, males are already a sunk cost included in the twofold cost of sex so, unlike females, population fitness is unaffected by what happens to them individually. As illustrated above, this destructive competition and extravagant ornamental waste actually reduces the cost of sex by reducing the population's mutational genetic load. Consequently, sexual selection reduces mutational constraints on the size of genomes and the number of germline mitotic divisions. The fact that sexual organisms began to choose their mates may have had far-reaching consequences for the evolution of complex life. □

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Sexual selection and the maintenance of sexual reproduction

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The maintenance of sexual reproduction is a problem in evolutionary theory because, all else being equal, asexual populations have a twofold fitness advantage over their sexual counterparts[1,2] and should rapidly outnumber a sexual population because every individual has the potential to reproduce. The twofold cost of sex exists because of anisogamy or gamete dimorphism[2]—egg-producing females make a larger contribution to the zygote compared with the small contribution made by the sperm of males, but both males and females contribute 50% of the genes. Anisogamy also generates the conditions for sexual selection[3], a powerful evolutionary force that does not exist in asexual populations. The continued prevalence of sexual reproduction indicates that the 'all else being equal' assumption is incorrect. Here I show that sexual selection can mitigate or even eliminate the cost of sex. If sexual selection causes deleterious mutations to be more deleterious in males than females, then deleterious mutations are maintained at lower equilibrium frequency in sexual populations relative to asexual populations. The fitness of sexual females is higher than asexuals because there is no difference in the fecundity of sexual females and asexuals of the same genotype, but the equilibrium frequency of deleterious mutations is lower in sexual populations. The results are not altered by synergistic epistasis in males.

Sexual reproduction with anisogamy generates the conditions for sexual selection[3]. "Competition for mates usually characterizes males because males usually invest almost nothing in their offspring" (ref. 4). Sexual selection on males (through male–male competition and/or female choice) is realized as the variance in male mating success; this variance can be large, making sexual selection very strong[5]. Sexual selection is a powerful force responsible for driving marked morphological, physiological, and behavioural change in many species[3]. Sexual selection occurs in sexual populations, but does not affect asexual ones. This fundamental difference between sexual and asexual populations has been largely ignored (but see refs 6–8).

Several prominent theories for the evolution of sexual reproduction focus on differences between sexual and asexual populations in the equilibrium frequency of deleterious alleles at mutation–selection balance[9–13]. Because of recurrent mutation, neither sexual nor asexual populations can ever be completely free of deleterious mutations and, as a result, both suffer from mutation load[14]. Under some conditions, sexual reproduction allows mutations to be cleared more efficiently relative to asexual reproduction, and thus sexual populations can suffer substantially less mutation load than their asexual counterparts. These theories require specific forms of gene action such as epistasis[9–12] or dominance[13]. There is little empirical support for synergistic epistasis[15]. Although there is

considerable support for dominance[16], it is unlikely that deleterious alleles are sufficiently recessive to eliminate the cost of sex[13,17]. The impact of sexual selection on mutation load has received little attention even though sexual selection can reduce the mutation load contributed by a single locus[6].

Here I consider the effect of sexual selection on mutation load by applying different fitness functions to females and males. The fitness of a female (equation (1a)) and a male (equation (1b)) carrying i deleterious mutations is:

$$w_f(i) = \exp - (a_f i + b_f i^2) \quad (1a)$$

$$w_m(i) = \exp - (a_m i + b_m i^2) \quad (1b)$$

Synergistic epistasis[12] occurs when $b > 0$. When $b = 0$, there is no epistasis and the effects of loci are independent (that is, multiplicative effects on fitness).

Because synergistic epistasis can generate an advantage to sexual reproduction[10–12] even without sexual selection, I first consider the case where there is no epistasis, $b_f = b_m = 0$. With $a_m = \alpha a_f$, values of α greater than one indicate that sexual selection exacerbates the effects of deleterious alleles in males. When there is no epistasis, the equilibrium mean fitness of females can be solved analytically (Box 1):

$$W_{SEX} = \exp\left[\frac{4M(e^{-a_f} - 1)}{2 - e^{-a_f} - e^{-\alpha a_f}}\right] \quad (2)$$

where M is the average number of mutations per gamete per generation.

To determine when mutation load provides an advantage to sexual reproduction, I consider the ratio of mean fitness of sexual females to asexuals, $R = W_{SEX}/W_{ASEX}$. Regardless of the form of selection[9,10], $W_{ASEX} = e^{-2M}$. In the absence of sexual selection, ($\alpha = 1$), equation (2) reduces to $W_{SEX} = e^{-2M} = W_{ASEX}$. Thus, the standard result[12] is recovered: without sexual selection, there is no advantage to sex under multiplicative selection (that is, $R = 1$).

With sexual selection ($\alpha > 1$), R is greater than one. When $R \geq 2$, the reduced mutation load of sexual populations completely compensates for the twofold cost of sex. The maximum possible value of R occurs in the hypothetical situation of no mutation load in sexual populations ($W_{SEX} = 1$), in this case $R_{MAX} = e^{2M}$. Thus R_{MAX} can only be greater than or equal to two when $M > 0.346$. This mutation rate sets a lower bound required for any deterministic mutation-based hypothesis[9–13] to completely explain sex. Nonetheless, at lower mutation rates, mutation-based hypotheses can pay part of the cost of sex. Any value of R greater than one indicates that a sexual population has an advantage over an asexual population with respect to mutation load. Values of R are plotted in Fig. 1. The advantage to sex increases with both α and M.

Synergistic epistasis for fitness may be a common outcome of competition for mates[18]. Does synergistic epistasis in males alter the advantage of sex? It is possible to compare the advantage to sex with multiplicative selection males ($a_m \neq 0$, $b_m = 0$) to the advantage with synergistically epistatic selection males ($a_m = 0$, $b_m \neq 0$) by calculating the average selection coefficient against deleterious alleles (Box 1). When multiplicative and epistatic selection are of similar average strengths, both forms of selection produce similar R values (Fig. 2). Thus, synergistic epistasis in males alone does not provide an advantage to sex unless the average selection is stronger in males than in females. Notably, synergistic epistasis in females alone does provide an advantage to sex (Table 1).

For sexual selection to maintain sexual reproduction against asexuality, the mutation rate must be on the order of one, and selection against deleterious alleles must be about twice as strong in males as in females (Figs 1 and 2). Although there is consensus that mutation rates are much less than unity for prokaryotes and greater than unity for long-lived animals such as humans, the mutation rates for most eukaryotes are hotly contested[19,20]. Some studies[20] report values of the order of one but others argue that it is at least one order of magnitude lower[19,21].

There are no estimates of the extent to which selection is stronger on males than females. There is, however, no doubt that sexual selection on males is a powerful and ubiquitous force in sexual populations[3]. An analysis of selection studies suggests that sexual selection is usually stronger than natural selection[22]. Although studies of sexual selection tend to focus on gaudy display characters in males, sexual selection may well affect the entire genome[7]. Only a relatively small fraction of genes are responsible for how a male allocates his resources to different characteristics (including sexually selected characteristics). In contrast, most genes in the genome are involved, in some way, with the acquisition of resources. On average, a male with more resources will be better able to compete for females than a male with less resources.

Existing data shows that males that obtain more mating success have better than average genomes. For example, in house mice, offspring viability and performance is higher when mothers are allowed to choose their mates than when they are forced to mate randomly[23]. In a variety of other species, male secondary sexual

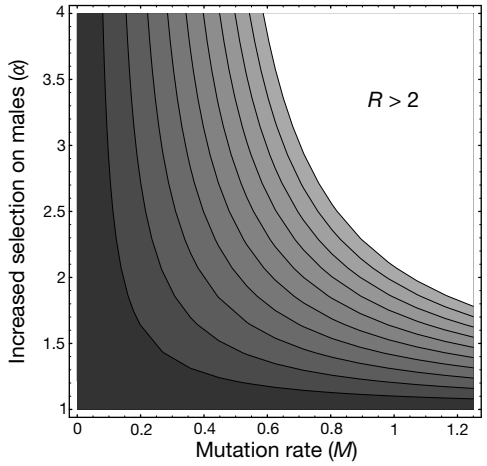

Figure 1 Effects of mutation rate and sexual selection on the cost of sex. A contour plot shows the mean fitness of sexual females relative to asexual individuals (R) as a function of the mutation rate (M) and the degree to which selection is stronger on males than females (α). The plot is generated using equation (2) with $a_f = 0.02$. Contour lines of $R = 1.1$, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2 are shown. R values increase from dark to light shading.

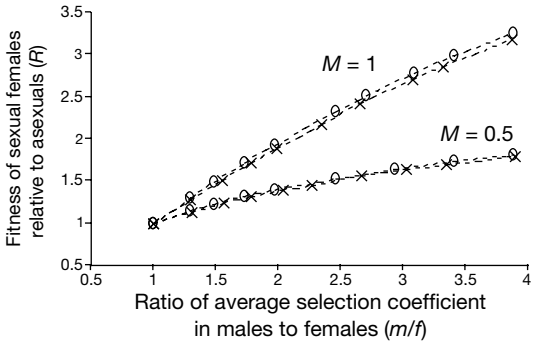

Figure 2 Multiplicative versus synergistically epistatic effects in males. The mean fitness of sexual females relative to asexual individuals (R) is plotted as a function of the average selection coefficient in males (m) relative to the average selection coefficient in females (f) (see Box 1). Circles, values under multiplicative fitness effects in males ($a_m \neq 0$, $b_m = 0$); crosses, values under epistatic effects in males ($a_m = 0$, $b_m > 0$). These values were generated using computer simulations with $a_f = 0.02$ and $b_f = 0$.

Box 1

The main mathematical details of the model

The multilocus approach in ref. 9 is followed closely. Unlike that study, I use an exponential fitness function[12] (equation (1)) and allow for different selection on males and females.

The analytical solution presented below assumes no epistasis ($b_f = b_m = 0$). Let p_i be the frequency of individuals with i mutations before selection. The average fitness of females (with respect to mutation load) is $W_f = \Sigma_{i=0}^{\infty} p_i w_f(i)$ and the average fitness of males is $W_m = \Sigma_{i=0}^{\infty} p_i w_m(i)$. The average selection coefficient[9] against each mutation in females (equation (3a)) and males (equation (3b)) is:

$$f = \sum_{i=0}^{\infty} p_i (w_f(i+1) - w_f(i))/W_f \quad (3a)$$

$$m = \sum_{i=0}^{\infty} p_i (w_m(i+1) - w_m(i))/W_m \quad (3b)$$

Because of sexual selection, the mean number of mutations in males after selection, λ'_m, is different from the mean number of mutations in females after selection, λ'_f. Because of free recombination, I assume that the number of mutations in male gametes and the number of mutations in female gametes will both follow Poisson distributions, but that they will have different means. Zygotes are produced by the fusion of randomly chosen male and female gametes. The probability of a zygote with i mutations is:

$$p_i = \sum_{x=0}^{i} \left(\frac{(0.5\lambda'_f + M)^x}{x!} e^{-(0.5\lambda'_f + M)} \right) \left(\frac{(0.5\lambda'_m + M)^{i-x}}{(i-x)!} e^{-(0.5\lambda'_m + M)} \right) \quad (4a)$$

$$= (e^{-0.5(\lambda'_m + \lambda'_f) + 2M}) \sum_{x=0}^{i} \left(\frac{(0.5\lambda'_m + M)^x (0.5\lambda'_f + M)^{i-x}}{x!(i-x)!} \right) \quad (4b)$$

$$= \frac{(0.5(\lambda'_m + \lambda'_f) + 2M)^i}{i!} e^{-(0.5(\lambda'_m + \lambda'_f) + 2M)} \quad (4c)$$

This equation shows that the number of mutations in each zygote of the next generation is Poisson-distributed with mean $\lambda = 0.5(\lambda'_m + \lambda'_f) + 2M$.

Using the Poisson distribution and setting $a_m = \alpha a_f$, I find:

$$W_f = \exp[-\lambda(1 - e^{-a_f})] \quad (5a)$$

$$W_m = \exp[-\lambda(1 - e^{-\alpha a_f})] \quad (5b)$$

The average selection coefficients (equation (3)) are $f = 1 - e^{-a_f}$ and $m = 1 - e^{-\alpha a_f}$.

Censusing after mutation and before selection, the mean number of mutations in the next generation is

$$\lambda(t+1) = 2M + 0.5(e^{-a_f} + e^{-\alpha a_f})\lambda(t) \quad (6)$$

At equilibrium there is no change to the mean number of mutations, so the equilibrium mean number of mutations, λ_{eq}, can be found by setting $\lambda(t+1) = \lambda(t)$ in equation (6). I find $\lambda_{eq} = 4M/(2 - e^{-a_f} - e^{-\alpha a_f})$. Substituting this λ_{eq} into equation (5a) yields the equilibrium fitness of females shown in equation (2).

These analytical solutions produce the same results as those found using deterministic computer simulations (as described in ref. 9). Briefly, zygotes are formed by the fusion of a randomly chosen male and female gamete. Selection is applied to females (equation (1a)) and males (equation (1b)). Females (or males) carrying different numbers of mutations produce eggs (or sperm) in proportion to their frequency after selection. Gametes are produced assuming free recombination; an individual with i mutant alleles produces gametes with various numbers of mutant alleles after a binomial distribution with i trials and a probability of success of 0.5. Equilibrium was usually reached in a few hundred generations (less than 500). Results of computer simulations were consistent with those reported in refs 10 and 13. All results involving epistasis (Fig. 2 and Table 1) were found using computer simulations.

Table 1 Synergistic epistasis in females versus males

Form of selection		Fitness of sexuals relative to asexuals (R)
Female	Male	
Mult	Mult	1.00
Mult	Epi	0.99
Epi	Mult	2.65
Epi	Epi	2.63

The effect of selection of different forms (but of equal strength) on females and males is shown with a representative example. Values were generated by computer simulations with $M = 1$. Multiplicative selection (Mult) was produced with $a = 0.02$ and $b = 0$; synergistically epistatic selection (Epi) was produced with $a = 0$ and $b = 0.0002$. These different forms of selection produce roughly equally strong average selection coefficients at equilibrium, $m/f \approx 1$. Synergistic epistasis only provides an advantage to sex when it occurs in females.

characteristics used in female choice are reliable predictors of offspring fitness (see ref. 24 and references therein). Even sperm competition, a cryptic form of sexual selection, results in the production of higher quality offspring[25]. Similarly, pollen competition in plants can result in the production of higher performance seedlings[26]. One study on *Drosophila melanogaster* found that a substantial portion of the selection against some mutations results from differential mating success[27].

Cases in which sexual selection is weak because males provide paternal care[3,4] do not challenge the theory presented here, as the twofold cost of sex is only expected if males contribute nothing beyond genes to their offspring[2]. One review notes that asexual reproduction is more common in isogamous than anisogomous lineages even though the cost of sex is expected to be greater in the latter[28]. I have shown that sexual selection on males (a result of anisogamy) can help to purge deleterious alleles from the entire sexual population. In this way, males can reduce and even eliminate the twofold cost of sex. In addition to removing deleterious alleles, theoretical work indicates that sexual selection can also increase the probability of fixation of new, beneficial mutations[29]. When the effects of sexual selection are combined to other advantages of sexual reproduction[30], the cost of sex could easily be overcome. □

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PCR amplification of the Irish potato famine pathogen from historic specimens

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Late blight, caused by the oomycete plant pathogen *Phytophthora infestans*, is a devastating disease of potato and was responsible for epidemics that led to the Irish potato famine in 1845 (refs 1–5). Before the 1980s, worldwide populations of *P. infestans* were dominated by a single clonal lineage, the US-1 genotype or Ib mitochondrial DNA (mtDNA) haplotype, and sexual reproduction was not documented outside Mexico, the centre of diversity of the pathogen[6,7]. Here we describe the amplification and sequencing of 100-base-pair fragments of DNA from the internal transcribed spacer region 2 from 28 historic herbarium samples including Irish and British samples collected between 1845 and 1847, confirming the identity of the pathogen. We amplified a variable region of mtDNA that is present in modern Ib haplotypes of *P. infestans*, but absent in the other known modern haplotypes (Ia, IIa and IIb)[8]. Lesions in samples tested were not caused by the Ib haplotype of *P. infestans*, and so theories that assume that the Ib haplotype is the ancestral strain need to be re-evaluated[4,7]. Our data emphasize the importance of using historic specimens when making inferences about historic populations.

During the nineteenth and early twentieth centuries, scientists collected and preserved potato and tomato leaves infected with *P. infestans* from the Irish potato famine, and these specimens can be used today to answer questions about the population biology of the pathogen[9] (Fig. 1). Disputes about nomenclature, phylogenetics, function and evolution of genes, and origins of populations can be addressed using herbarium collections[10–17]. We used polymerase chain reaction (PCR) amplification and sequencing of ribosomal DNA (rDNA) and mtDNA of *P. infestans* from herbarium specimens to determine whether the Ib mtDNA haplotype was the clonal lineage responsible for historic epidemics.

Dried potato and tomato lesions sampled from the Royal Botanic Gardens Mycological Herbarium, Kew, UK, and the US National Fungus Collection, Beltsville, Maryland, were aseptically removed from specimen envelopes and placed in sterile microfuge tubes. Initial DNA extractions from British and European samples were conducted in the Jodrell Laboratory at Kew in a laboratory without a history of work with the pathogen. Non-infected potato leaves and leaves infected with *P. infestans* from modern epidemics were pressed and dried in the Mycological Herbarium in the Department of Plant Pathology, North Carolina State University. All manipulations of DNA from modern samples were performed in our laboratory in the Department of Plant Pathology. Work with the herbarium specimens was performed in the North Carolina State University Phytotron Containment Facility Laboratory, which was equipped with separate supplies, reagents and equipment and has no history of research involving *P. infestans*.

DNA was extracted from herbarium samples according to a modification of a cetyltrimethylammonium bromide (CTAB) procedure[18]. DNA was diluted 1:10 and used with PCR primers PINF and HERB1 and PINF and ITS3 from 123 herbarium samples. Thirty-nine samples (31%) yielded the expected product (around 100 base pairs (bp) in size) when amplified with primers PINF and HERB1 (see Supplementary Information). Only three samples (2.4%), all from the 20th century, yielded a product approximately 300 bp in size when amplified with primers PINF and ITS3. These results are expected because DNA tends to degrade in ancient materials and primers that amplify smaller product sizes (<200 bp) are more effective in these types of specimen[13]. DNA of *P. infestans* was amplified from 39 samples, including four samples of *Solanum tuberosum* collected in Britain and France in 1845, four samples collected in Ireland and Britain in 1846, and one sample collected in Britain in 1847 (Fig. 2; and see Supplementary Information). We also amplified DNA of *P. infestans* from a sample of *Anthocercis ilicifolia,* a solanaceous evergreen shrub native to Western Australia

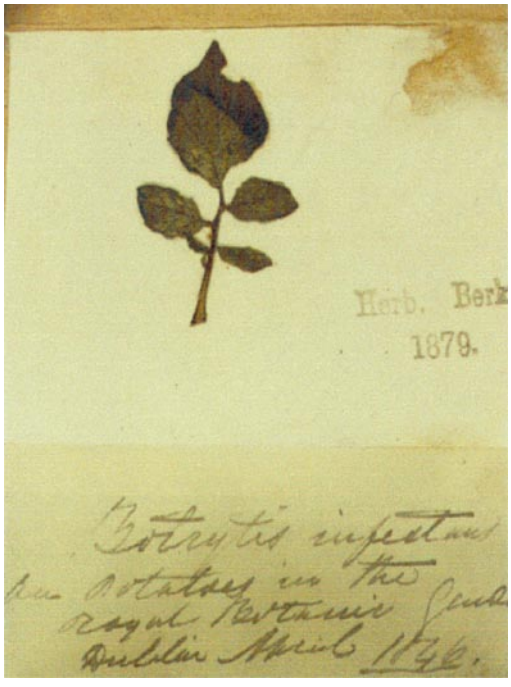

Figure 1 Specimen of potato infected with *P. infestans*. This sample was collected by J. Lindley in 1846 in the Royal Botanic Gardens, Dublin, Ireland and identified by M. J. Berkeley. It is deposited in the collections at the Royal Botanic Gardens, Kew, UK and is one of the oldest known specimens of potato from the potato famine epidemics.

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