common types of critical point are foci[23,27] (such as the point on the surface where the centre of a vortex touches down) and saddles[23,27] (which have two streamlines entering and two leaving the critical point from opposite directions). Saddle points are thought to be characteristic of separated flows[21–24]. The distribution of critical points, and of the streamlines joining them, defines the topology of the flow, and obeys topological rules[23,24]. For example, the number of nodes plus foci must equal the number of saddles plus 2 in the skin friction lines on a body surface, just as faces plus corners equals edges plus 2 for a solid body such as a cube.

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Sex releases the speed limit on evolution

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Explaining the evolutionary maintenance of sex remains a key problem in evolutionary biology[1–3]. One potential benefit of sex is that it may allow a more rapid adaptive response when environmental conditions change, by increasing the efficiency with which selection can fix beneficial mutations[4–7]. Here I show that sex can increase the rate of adaptation in the facultatively sexual single-celled chlorophyte *Chlamydomonas reinhardtii*, but that the benefits of sex depend crucially on the size of the population that is adapting: sex has a marked effect in large populations but little effect in small populations. Several mechanisms have been proposed to explain the benefits of sex in a novel environment, including stochastic effects in small populations, clonal interference and epistasis between beneficial alleles. These results indicate that clonal interference is important in this system.

As pointed out by Fisher[4], for sex to increase the rate of adaptation requires that the supply of beneficial mutations be abundant. If mutations arise only rarely, each is fixed before the next arises, and populations spend most of their time waiting for new mutations. Sex has little benefit under such conditions. If beneficial mutations arise more commonly, several different mutations are spreading through the population at the same time. Under such conditions, theoretical models show that clonal interference in asexual populations might place an important speed limit on adaptation, and sex should provide a significant benefit[8]. The robustness of this prediction depends to some extent on the unknown distribution of beneficial mutations, because current models of clonal interference ignore the possibility that multiple beneficial alleles arise in the same lineage[8,9]. If only a very small number of beneficial mutations (for example, two) are possible in the new environment, then the probability of both of these arising in a single asexual individual becomes non-trivial in very large populations. However, the probability that all mutations might arise simultaneously declines steeply and rapidly with the number of possible beneficial mutations. In this situation it seems likely that the genotypes carrying different subsets of beneficial alleles interfere in the same way as for a single mutation[9]. Furthermore, there is experimental evidence that clonal interference does occur in asexual populations of bacteria and viruses[9,10].

Because large populations have a larger number of mutations per generation than small populations, we might predict that the benefits of sex are greater in large populations. However, the effect of population size also depends on whether benefits of sex are based on stochastic associations between the beneficial mutations, which

Table 1 **Effective population size for the different experimental treatments**

Bottleneck size (N_0)	Approximate number of generations between transfers (g)	N_e
10^3	15	15,000
10^4	12	120,000
10^5	8.5	850,000
5×10^5	6.5	3,250,000
10^6	5.5	5,500,000

In common with other such microbial studies, I estimate the effective population size for beneficial substitutions (N_e) from an estimate of the number of divisions that occur in each tube between transfers (g), and the original inoculum size (N_0). $N_e = N_0 g$ (ref. 15).

are important in small populations[11], on clonal interference (which despite operating most strongly in large populations is based on stochastic processes) or on associations due to deterministic forces such as epistasis that occur at all population sizes[11]. Because the net effect of sex is affected by all of these factors, the actual way in which population size affects the benefits of sex is unclear and requires experimental investigation.

I allowed 20 asexual populations of *C. reinhardtii* to evolve for 250 generations in a simple laboratory environment containing a novel growth medium. Population size was manipulated by regularly bottlenecking populations to different degrees (to between 10^3 and 10^6 cells; see Table 1 for bottleneck and effective population sizes). I then examined the level of adaptation of these evolved populations by comparing their growth rate with the growth rate of their ancestor under the conditions of selection.

To examine the interaction between sex and population size, samples were taken from each of the asexual populations in three of the bottleneck treatments (10^3, 10^5 and 10^6) after 150 generations. Each population was passed through eight rounds of sexual reproduction and then allowed to evolve for a further 50 generations. During this subsequent evolution, all populations, both sexual and asexual, were bottlenecked to the same size (10^4 cells) at weekly intervals, to avoid confounding effects of sex with subsequent differences in population size. Unmated samples were also run under the same regime. The relative fitness of the sexual populations was then determined by comparing the growth rate of each sexual population with that of its asexual control.

In the asexual lines, bottleneck size affected the rate of adaptation, with a linear relationship between $\log_{10}$(adaptation) and $\log_{10}$(effective population size) ($F_{1,15} = 8.54$, $P = 0.010$; Fig. 1a). Moreover, there was no evidence for any difference in the effect of population size across lines ($F_{3,15} = 2.00$, $P = 0.157$), or of any overall difference between the rate at which the different lines adapted ($F_{3,12} = 0.65$, $P = 0.615$). The slope of the relationship was less than 1 (0.19 ± 0.06 (mean $\pm$ s.e.), $t = 13.5$, d.f. $= 15$, $P < 0.001$), implying a diminishing-returns relationship on the untransformed scale, consistent with clonal interference (Fig. 1b).

Sex produced a general increase in the rate of adaptation, with the overall relative fitness of the sexual lines being greater than 1 (Fig. 2; pooled relative fitness $= 1.10 \pm 0.02$ (mean $\pm$ s.e.), $t = 5$, d.f. $= 6$, $P < 0.01$) More importantly, the magnitude of the benefit of sex depends on the size of the population before the induction of sex. The largest population showed a substantial benefit of sex, whereas the smallest populations differed little from the asexual controls ($F_{2,6} = 5.92$, $P = 0.038$). Thus, in large populations sex seems to be able to release the speed limit on adaptation set by clonal interference.

Although the effect of population size on adaptation has been previously investigated in asexual populations of bacteria and viruses[9,10], and I have shown previously that sex can increase the rate of adaptation in *C. reinhardtii*[12], this is to my knowledge the first study that has examined experimentally the interaction between sex and population size on the rate of adaptation. The results of this experiment provide striking support for the conjecture that sex has the greatest effect in large populations where the supply of beneficial mutations is plentiful[2,4]. In a small population, the process of adaptation might be limited by the supply of novel mutations, and sex is of little benefit, whereas in large populations the limit might be imposed by the efficiency of selection, which can be markedly increased by sex.

The greater benefit of sex in the large population might at first seem counter to many people's intuition, and much theory, which shows that advantages of sex often depend on stochastic effects in small populations[1–3]. However, clonal interference, despite being based ultimately on stochastic forces (that is, the absence of some genotypes in finite populations), only becomes an important limit to the rate of adaptation in larger populations. The results of this study show that clonal interference can be important in providing a benefit to sex, in this system at least, and that the increased efficiency of selection might provide a benefit of sexual reproduction even in organisms with large population sizes. □

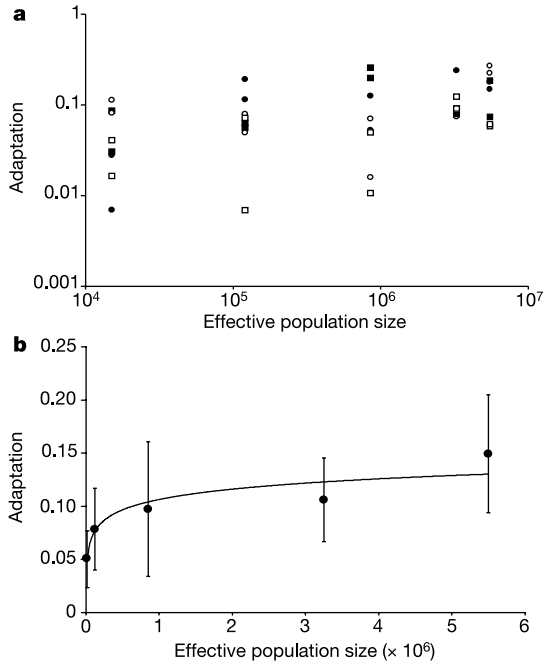

Figure 1 Effect of bottleneck size on adaptation in asexual populations. **a**, Individual points represent individual assays, and the different symbols indicate the lines from the initial four base populations. The relationship was examined statistically by fitting general linear models (GLM) to the data after log-transforming both variables. This has several advantages over using nonlinear regression on the untransformed data: the GLM framework means that line effects and line-by-population-size interactions can be fitted to the data as well as the population size term; and working with logarithms of population sizes reduces problems of leverage by the extreme population sizes. Initially I fitted both quadratic and linear population size terms, but the quadratic term did not significantly improve the fit, and so was dropped from the model ($F_{1,11} = 0.16$, $P = 0.69$). **b**, Overall pooled means and standard errors of the lines on an untransformed scale along with the fitted relationship from the GLM. On the untransformed scale the linear relationship takes the form adaptation $= b(N_e)^c$, a relationship that is potentially able to describe a variety of relationships from diminishing returns to linear to accelerating. The actual fitted relationship is adaptation $= 0.00575$ (effective population size)$^{0.19}$, which is clearly of the diminishing-returns form. I chose a relationship that passes through the origin because a population of size zero is not expected to adapt.

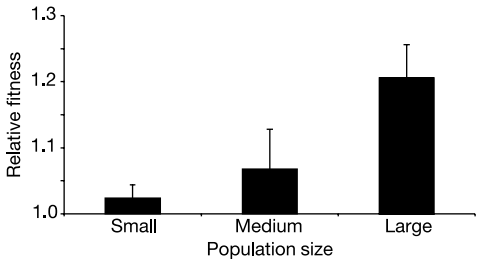

Figure 2 Interaction between sex and bottleneck size. Bars show the mean relative fitness ($\pm$s.e.) of the sexual lines compared with their asexual controls. Sex has a greater positive effect in the populations that were bottlenecked the least.

Methods

Selection lines were serially propagated in glass tubes containing 10 ml of Bold's medium supplemented with 500 mg l^{-1} sodium bicarbonate, a substance that reduces the growth rate of *C. reinhardtii*. Tubes were maintained under constant lighting and shaken continuously. Temperature was not controlled. The experiment was begun with four base populations each derived (about 30 generations previously) from a single different mt^+ spore drawn from a heterogeneous population. Each base population will have contained minimal genetic diversity. A sample of each base population was used in each of the five bottleneck treatments, giving a total of 20 experimental lines.

Bottlenecking was achieved by transferring a known number of cells to the new tube of medium after 7 days of growth. Cell density was estimated before transfer by measuring the absorbance of the culture at 665 nm (a measurement that is a good predictor of cell density over the range of densities used in this experiment (unpublished observations)). Using bottlenecks to vary population size is the method that has typically been used in studies of microbial population size. It has the disadvantage that it affects the selective environment as well as the population size. Such differences will be unimportant if the major response to selection is through an increase in exponential growth rate, as in other microbial systems[13]. I minimized any possible difference by choosing bottleneck sizes and volumes of medium where the populations were still growing at the time of transfer, and had not reached carrying capacity. Furthermore, there was a significant positive correlation between the fitness of the evolved lines from the different bottleneck treatments when assayed under the lowest and highest density conditions ($r = 0.89$, $n = 20$, $P < 0.001$) and also between the early growth rate of lines and their population size on day 7 ($r = 0.96$, $n = 20$, $P < 0.0001$).

To examine the effects of sex, samples of the populations of 10^6 cells were placed into nitrogen-free medium for 12 h to induce gamete production. Because all lines used in the experiment were of a single mating type it was then necessary to add cells of another clone of the opposite mating type for the first round of sex. All populations were mated with the same clone. Cells were placed into the light for 12 h to allow mating to occur, after which point mats of zygotes were clearly visible. Zygotes were matured in the dark for 5 days on solid medium and then germinated in the light for 5 days. The resulting populations, now containing a mixture of mating types, were then mated a further seven times in the same way (without the addition of any more cells). Such a procedure does involve the introgression of DNA from an unevolved line, which might affect adaptation, but it should do so to the same extent in all populations. Multiple matings were used because in the first mating recombination is only possible between mutations that have arisen within a selection line and mutations from the new genotype. Only by allowing multiple sexual cycles is it possible to allow recombination between the mutations that arose during selection. Asexual controls underwent the same sampling and bottlenecking regime as the sexual lines and were treated in the same way, with the exception that cells of the opposite mating type were never added, and cells were maintained in dim light rather than darkness for the development stage. Nitrogen starvation has been shown to produce a small increase in the mutation rate in *C. reinhardtii*[14], and although this led to no measurable effect on rates of adaptation[12] in an experiment similar to this, starving both the sexual and asexual populations to the same extent ensured that any such effect did not confound the effects of sex.

The level of adaptation of a line was estimated by using a paired design. Pairs of tubes containing the experimental growth medium were inoculated with a known number (corresponding to the appropriate bottleneck size) of either the evolved line or its ancestor. Thus, lines were assayed under the same conditions as those in which they had evolved. Two replicate pairs of tubes were set up for each evolved line. Pairs of tubes were allocated randomly to a position on the growth shelves, but the tubes in a pair were always placed next to one another. The fitness of a line was defined as the number of cells after 7 days of growth (again determined by absorbance at 665 nm), and the amount of adaptation of a line as (evolved fitness − ancestors' fitness)/ancestors' fitness

Assaying populations under their specific selection conditions meant that lines in different treatments were assayed under different conditions. However, similar results were obtained when populations were assayed under the same conditions (all inoculated with 10^4 cells; data not shown). To examine the effects of sex on rate of adaptation, the same paired assay was used, except that one tube of each pair contained the sexual line to be investigated, and the other its asexual control. Three replicate pairs were set up for each sexual line, but three of the replicates were lost owing to contamination.

All statistical analyses in this paper were performed on the means of the replicate measures for each selection line, to avoid problems of non-independence.

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Large clusters of co-expressed genes in the *Drosophila* genome

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Clustering of co-expressed, non-homologous genes on chromosomes implies their co-regulation. In lower eukaryotes, co-expressed genes are often found in pairs[1,2]. Clustering of genes that share aspects of transcriptional regulation has also been reported in higher eukaryotes[3,4]. To advance our understanding of the mode of coordinated gene regulation in multicellular organisms, we performed a genome-wide analysis of the chromosomal distribution of co-expressed genes in *Drosophila*. We identified a total of 1,661 testes-specific genes, one-third of which are clustered on chromosomes. The number of clusters of three or more genes is much higher than expected by chance. We observed a similar trend for genes upregulated in the embryo and in the adult head, although the expression pattern of individual genes cannot be predicted on the basis of chromosomal position alone. Our data suggest that the prevalent mechanism of transcriptional co-regulation in higher eukaryotes operates with extensive chromatin domains that comprise multiple genes.

We initially observed that five non-homologous, testes-specific genes (*Crtp*, *Yu, CK2βtes*, *Pros28.1B* and CG13581) form a cluster on chromosome 2 of *Drosophila melanogaster*, implying the presence of large clusters of co-expressed genes in *Drosophila* (A.I.K., D.I.N. & Y.Y.S., unpublished data). To assay the entire *D. melanogaster* genome for the presence of comparable clusters, we used a publicly available EST (expressed sequence tag) database as a source of gene expression data. The content of the EST pool for a given tissue type reflects the composition of original messenger RNA samples used for creation of the complementary DNA library. We generated the software that, through the BLAST homology search, assigns each EST from the database to a gene in the annotated *D. melanogaster* genome, and outputs a table with the numbers of ESTs found for

