1981–1983 to 2003–2005. The processes that control the timing and magnitude of glacier changes are, however, not completely characterized and understood at present. Glacier accelerations have been related to enhanced surface meltwater production penetrating to the bed to lubricate its motion (*20*), and ice-shelf removal (*13*), ice-front retreat, and glacier ungrounding (*21*, *22*) that reduce resistance to flow. The magnitude of the glacier response to changes in air temperature (surface melting) and ocean temperature (submarine melting at calving faces) also depends on the glacier-bed properties, geometry, and depth below sea level and the characteristics of the subglacial and englacial water-storage systems (*3*, *20*). Current models used to project the contribution to sea level from the Greenland Ice Sheet in a changing climate do not include such physical processes and hence do not account for the effect of glacier dynamics. As such, they only provide lower limits to the potential contribution of Greenland to sea-level rise. If more glaciers accelerate farther north, especially along the west coast, the mass loss from Greenland will continue to increase well above predictions.

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Transitions to Asexuality Result in Excess Amino Acid Substitutions

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Theory predicts that linkage between genetic loci reduces the efficiency of purifying selection. Because of the permanent linkage of all heritable genetic material, asexual lineages may be exceptionally prone to deleterious-mutation accumulation in both nuclear and organelle genes. Here, we show that the ratio of the rate of amino acid to silent substitution (K_a/K_s) in mitochondrial protein-coding genes is higher in obligately asexual lineages than in sexual lineages of the microcrustacean *Daphnia pulex*. Using a phylogeny-based approach to quantify the frequency of mutational-effect classes, we estimate that mitochondrial protein-coding genes in asexual lineages accumulate deleterious amino acid substitutions at four times the rate in sexual lineages. These results support the hypothesis that sexual reproduction plays a prominent role in reducing the mutational burden in populations.

Although sexual reproduction is costly when compared with asexual reproduction (*1*–*3*), it may accelerate the rate of adaptation and inhibit the accumulation of mildly deleterious mutations, because meiotic segregation and recombination facilitate the ability of natural selection to act independently on different genetic loci (*2*–*6*). These effects arise because the stochastic sampling variance associated with the interference between selection on linked loci reduces the genetic effective population size (N_e), which increases the power of random genetic drift (*7*–*9*). As the frequency of recombination is reduced, the fates of mutant alleles become increasingly dependent on the backgrounds in which they originate, and the buildup of repulsion disequilibrium reduces the fitness differential between chromosomes (the Hill-Robertson effect) (*7*), thereby diminishing the efficiency of selection. As a consequence, mildly deleterious mutations may accumulate through several population-genetic mechanisms (*10*–*15*), leading to a long-term decline in fitness. Depending on the distribution of mutational effects, epistatic interactions between consecutive mutations can either slow or accelerate this process (*16*). Although increased rates of nonadaptive evolution have been documented for genomic regions with low levels of recombination and for nonrecombining chromosomes (*17*, *18*), and it is thought that few asexual taxa persist for long periods of time (*19*), it remains to be determined whether mildly deleterious mutations play a critical role in their early demise (*20*).

To evaluate the degree to which sexual reproduction promotes the purging of deleterious mutations, we compared patterns of nucleotide substitution in the 13 protein-coding genes encoded by the mitochondrial genomes (supporting online text) of cyclically parthenogenetic ("sexual") *Daphnia pulex* with those in their obligately parthenogenetic ("asexual") derivatives (table S1). The latter represent independent lineages of recent origin resulting from a dominant sex-limited meiosis suppressor transmitted by male progeny of otherwise asexual lineages (*21*, *22*). We reconstructed a phylogeny by application of a Bayesian method (*23*). Because this species is ancestrally sexual and reversals of asexuality to sexuality are unlikely (and unknown), asexual evolution is represented by sequence changes on branches connecting current asexuals with their most recent sexual ancestors. However, because asexuality may have actually arisen part way down a given asexual branch, the true differences between sexual and asexual sequence evolution reported below will be underestimated, making our test conservative.

The predicted molecular signature of deleterious-mutation accumulation for genes mostly subject to purifying selection ($K_a/K_s < 1$) is an increased rate of evolution at the amino acid level, whereas genes predominantly under

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positive selection ($K_a/K_s > 1$) are expected to show decreased rates of protein evolution (*17*, *24*). The predicted acceleration in mutational decay in asexual lineages extends to the normally nonrecombining mitochondrial genes because the loss of segregation between nuclear and organelle genomes, analogous to the loss of recombination between nuclear loci, subjects such genes to selective interference from the entire nuclear genome. Because animal mitochondrial genomes generally have elevated mutation rates, the power to detect excess rates of mutation accumulation in short-lived lineages is enhanced, and the haploid nature of such genomes simplifies the acquisition of sequence data.

We applied a maximum-likelihood approach (*23*) to the concatenated coding sequences of all 13 mitochondrial protein-coding genes, using hierarchical models to evaluate the validity of alternative assumptions about the mode of evolution in asexual versus sexual lineages and internal versus external branches. Because high rates of adaptive evolution might represent a confounding factor in our analysis, we first evaluated the assumption that the mitochondrial protein-coding genes of *D. pulex* are mostly subject to purifying selection (i.e., $K_a/K_s < 1$). The one-ratio model estimates the average ability of amino acid–altering mutations to accumulate in mitochondrial protein-coding genes by constraining all branches of the phylogeny to have the same K_a/K_s ratio. The resultant overall K_a/K_s ratio of 0.159 (Table 1) reflects a significantly lower amino acid than silent substitution rate. The proportion of strongly deleterious spontaneous amino acid substitutions can then be expressed as $1 - (K_a/K_s) = 0.841$ (*25*), providing a clear indication that purifying selection rejects the majority of amino acid altering mutations in the mitochondrial protein-coding genes of *Daphnia*.

The two-ratio model tests for the presence of mildly deleterious mutations among the pool of observed amino acid substitutions by allowing different K_a/K_s ratios for internal and external branches of the phylogeny. External branches depict the most recent, and internal branches the more distant, evolutionary history of a sample of DNA sequences. If amino acid substitutions are either neutral or strongly deleterious, then K_a will approximate the mutation rate to neutral amino acid substitutions, and the K_a/K_s ratios of external and internal branches should remain constant, assuming that silent substitutions also accumulate at the neutral mutation rate. This hypothesis is rejected because the two-ratio model provides a significantly better fit to the data than the one-ratio model, yielding a K_a/K_s ratio about twice as high for external as for internal branches ($2\Delta\ell = 10.04$; df = 1; $P < 0.005$) (Table 1).

Although this pattern supports the existence of a class of mildly deleterious amino acid substitutions that persist in the short term but are removed by purifying selection in the longer term (*26*), to further evaluate whether purifying rather than positive selection is the predominant form of selection acting on intraspecific amino acid polymorphism in *D. pulex*, we used an extension of the McDonald-Kreitman test of neutral molecular evolution (*27*) to compare the ratio of the numbers of polymorphic amino acid (P_a) and silent (P_s) substitutions among sexual *D. pulex* with the ratio of the numbers of fixed amino acid (D_a) and silent (D_s) substitutions between sexual *D. pulex* and a closely related outgroup species, *D. melanica* (*23*). Predominantly adaptive evolution results in $P_a/P_s < D_a/D_s$, because advantageous amino acid substitutions are fixed relatively rapidly and contribute mainly to interspecific divergence, whereas $P_a/P_s > D_a/D_s$ reflects the signature

Table 1. Maximum-likelihood parameter estimates for the mitochondrial DNA data set and estimates of selective constraint under the three likelihood models.

	*p**	ℓ†	K_a/K_s‡	$1 - K_a/K_s$§
One-ratio model	1	−19,448.09	All branches = 0.159	0.841
Two-ratio model	2	−19,443.07	Internal branches = 0.098	0.902
			External branches = 0.192	0.808
Four-ratio model	4	−19,437.59	Internal sexual branches = 0.091	0.909
			Internal asexual branches = 0.143	0.857
			External sexual branches = 0.135	0.865
			External asexual branches = 0.268	0.732

*Number of free parameters in the likelihood analysis. †Log-likelihood values. ‡Maximum-likelihood estimates of the ratio of the rate of amino acid to silent substitution. §Estimates of selective constraint.

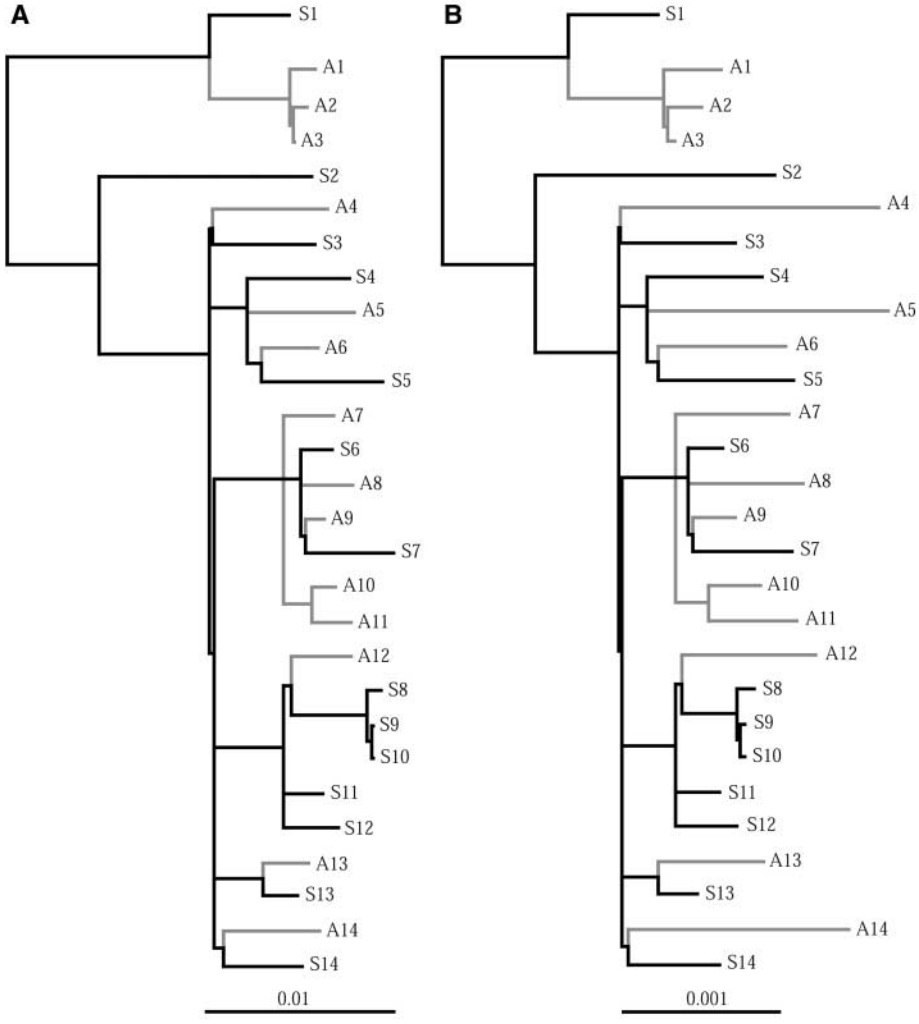

Fig. 1. Phylogenetic trees constructed using maximum-likelihood estimates of the expected number of silent (**A**) and amino acid (**B**) substitutions per site under the four-ratio model. Sexual (S1 to S14) and asexual (A1 to A14) lineages are denoted by black and gray branches, respectively.

of purifying selection against mildly deleterious amino acid substitutions that contribute mainly to intraspecific polymorphism (*28*). P_a/P_s is significantly larger than D_a/D_s ($P_a/P_s = 0.246$; $D_a/D_s = 0.150$; $P = 0.029$), confirming that purifying selection is the predominant form of selection acting on mitochondrial amino acid sequence variation in *D. pulex*. This type of pattern has been consistently reported in other animals (*26*).

A four-ratio model allowing different K_a/K_s ratios for sexual and asexual internal and external *D. pulex* branches fits the data significantly better than the two-ratio model ($2\Delta\ell = 10.96$, df = 2, $P < 0.005$) (Table 1). The estimated K_a/K_s for asexual external branches is twice as high as that for sexual external branches, and K_a/K_s for asexual internal branches is 1.6 times as high as that for sexual internal branches. Because of the recent origin of asexual lineages, there are only three internal asexual branches in our analysis, so the external-branch estimate provides a better indication of the disparity in patterns of sequence evolution between asexuals and sexuals. In any event, it is clear that excess amino acid substitutions occur in asexual lineages. Phylogenetic trees based on maximum-likelihood estimates of the expected number of silent versus amino acid substitutions per site under the four-ratio model have notably different shapes as a result of the elevated accumulation of amino acid substitutions on asexual branches (Fig. 1).

The degree to which the accumulation of deleterious mutations is accelerated in asexual lineages can be quantified by using a phylogeny-based method. This method assumes that (i) silent substitutions accumulate at the neutral rate, here validated by the lack of a significant difference in K_s between sexual and asexual branches (Wilcoxon two-sample test; external branches, $P = 0.505$; internal branches, $P = 0.422$) (see also Fig. 1); (ii) nearly all excess amino acid substitutions in asexual lineages are deleterious; and (iii) the frequency of adaptive amino acid substitutions is negligible. The observed K_a/K_s ratios for sexual and asexual branches of the phylogeny (Table 1) can then be used to estimate the frequencies of (i) strongly deleterious amino acid substitutions, subject to rapid purifying selection in both sexual and asexual populations, as $1 - (K_a/K_s)$ for external asexual branches; (ii) moderately deleterious amino acid substitutions maintained in asexual populations but subject to rapid purifying selection in sexual populations, as the difference between K_a/K_s on external asexual and sexual branches; (iii) mildly deleterious amino acid substitutions segregating in both sexual and asexual populations, as the difference between K_a/K_s on external and internal sexual branches; and (iv) effectively (but not necessarily absolutely) neutral substitutions, as K_a/K_s on internal sexual branches. Of the amino acid altering mutations arising in mitochondrial protein-coding genes of *D. pulex*, we estimate that 73.2% have strongly deleterious effects and are subject to purifying selection irrespective of the population's breeding system, 13.3% have moderately deleterious effects and persist only in asexual populations, 4.4% are mildly deleterious and allowed to persist in the short-term even in sexual populations, and 9.1% are effectively neutral. Thus, the rate of accumulation of deleterious amino acid–altering mutations in asexual lineages, 4.4 + 13.3 = 17.7%, is four times as high as that for sexual lineages (4.4%).

This difference is unlikely to be due mainly to ecological or demographic differences between sexual and asexual populations. Because new asexual lineages of *D. pulex* arise by the backcrossing of asexually produced males to females of the sexual species, not only do members of both lineages necessarily share a common recent biogeographic and ecological history (*22*), but also they contain the same background genomic content relevant to local adaptation. Newly invading asexuals often rapidly replace resident sexual populations, creating lineages with densities of many millions of individuals, so there is no evidence for prolonged demographic bottlenecks. Thus, our results indicate that sexual reproduction enhances the efficiency of purifying selection, supporting the theory that deleterious-mutation accumulation is a leading evolutionary force contributing to the short longevity of asexual lineages.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5763/990/DC1
Materials and Methods
SOM Text
Table S1
References

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Cdx2 Gene Expression and Trophectoderm Lineage Specification in Mouse Embryos

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Controversy exists as to whether individual blastomeres from two-cell-stage mouse embryos have identical developmental properties and fate. We show that the transcription factor Cdx2 is expressed in the nuclei of cells derived from the late-dividing but not the first-dividing blastomere of two-cell embryos and, by lineage tracing and RNA interference knock-down experiments, that this lagging cell is the precursor of trophectoderm. *Cdx2* mRNA is localized toward the vegetal pole of oocytes, reorients after fertilization, and becomes concentrated in the late-dividing, two-cell-stage blastomere. The asymmetrical distribution of *Cdx2* gene products in the oocyte and embryo defines the lineage to trophectoderm.

In most animals, the proper development of the embryo depends on the asymmetrical distribution of maternal transcripts and protein in the egg. In *Drosophila*, gradients of transcription factors are established that provide spatially restricted, *cis*-regulatory control over downstream