Population-genomic insights into the evolutionary origin and fate of obligately asexual *Daphnia pulex*

Abraham E. Tucker, Matthew S. Ackerman, Brian D. Eads, Sen Xu, and Michael Lynch[1]

Department of Biology, Indiana University, Bloomington, IN 47405

Contributed by Michael Lynch, July 19, 2013 (sent for review May 13, 2013)

Despite much theoretical work, the molecular-genetic causes and evolutionary consequences of asexuality remain largely undetermined. Asexual animal species are rare, evolutionarily short-lived, and thought to suffer mutational meltdown as a result of lack of recombination. Whole-genome analysis of 11 sexual and 11 asexual genotypes of *Daphnia pulex* indicates that current asexual lineages are in fact very young, exhibit no signs of purifying selection against accumulating mutations, and have extremely high rates of gene conversion and deletion. The reconstruction of chromosomal haplotypes in regions containing SNP markers associated with asexuality (chromosomes VIII and IX) indicates that introgression from a sister species, *Daphnia pulicaria*, underlies the origin of the asexual phenotype. Silent-site divergence of the shared chromosomal haplotypes of asexuals indicates that the spread of asexuality is as recent as 1,250 y, although the origin of the meiosis-suppressing element or elements could be substantially older. In addition, using previous estimates of the gene conversion rate from *Daphnia* mutation accumulation lines, we are able to age each asexual lineage. Although asexual lineages originate from wide crosses that introduce elevated individual heterozygosities on clone foundation, they also appear to be constrained by the inbreeding-like effect of loss of heterozygosity that accrues as gene conversion and hemizygous deletion expose preexisting recessive deleterious alleles of asexuals, limiting their evolutionary longevity. Our study implies that the buildup of newly introduced deleterious mutations (i.e., Muller's ratchet) may not be the dominant force imperiling nonrecombining populations of *D. pulex*, as previously proposed.

evolution of sex | parthenogenesis

Obligately asexual lineages are thought to suffer from elevated deleterious mutation accumulation resulting from permanent linkage at selected sites, which reduces the efficiency of selection against newly arising deleterious alleles (1–5). However, although substantial theory suggests that the diminished ability to purge new deleterious mutations will constrain the longevity of asexual populations (6–8), little is known about the genetic mechanisms responsible for a shift in reproductive mode, the consequences of asexuality for genome evolution, or the fate of asexual lineages in nature. To examine the evolution of asexual genomes, we carried out a population-genomic analysis of an asexual animal, one made interpretable by the inclusion of a parallel set of related sexual genotypes.

Although most lineages of the microcrustacean *Daphnia pulex* are cyclically parthenogenetic, alternating between sexual and asexual phases, obligately asexual lineages have arisen polyphyletically across North America (9–11). The spread of obligate asexuality results from the proliferation of sex-limited, meiosis-suppressing genetic elements via males produced by asexual females (11). Unlike their female clone mates, these males are often capable of haploid gamete production, providing a path for transmission of the meiosis-suppressing elements to sexual populations via backcrossing. Each such event results in the production of a new asexual genotype.

We investigated the genetic basis and consequences of asexuality by sequencing the entire genomes of 11 cyclically parthenogenetic isolates (hereafter sexuals) and 11 obligately asexual isolates (hereafter asexuals) from small ponds across North America (Table S1) and found that all asexual genotypes of *D. pulex* studied share common haplotypes of chromosomes VIII and IX, which are apparently transmitted through asexual males without recombination (unlike the remaining 10 chromosomes). Here, we describe patterns of variation across the genomes of both reproductive types, identify features unique to asexuals, and present evidence that asexual populations are quite young ($\ll$1,000 y) but nevertheless show signs of deleterious-mutation accumulation. In addition, we show that the asexual-linked chromosome VIII/IX haplotypes arose by introgression from *Daphnia pulicaria*, the sister taxon to *D. pulex*, and that although initially harboring higher heterozygosity levels than sexuals, asexuals lose heterozygosity rapidly via deletion, gene conversion, and/or other internal homogenizing effects, which exposes preexisting, deleterious recessive alleles.

Results

In a genome-wide association survey for markers, we found 33,575 SNPs to be 100% associated with obligately asexual genotypes and exclusive of sexuals. In contrast, there are no markers unique to sexuals. More than 95% of these asexual markers are contained within 14.5 Mb of 13 marker-rich scaffolds (ref. 12 and Table S2), eight of which map to chromosome VIII or IX (Fig. S1), both of which were previously thought to be associated with obligate asexuality (11). The large numbers of SNPs in linkage disequilibrium over many megabases on asexual-associated chromosomes VIII/IX indicate that during the asexual radiation, a single large haplotype has spread across vast geographic distances while undergoing essentially no recombination. Obligately asexual populations of *D. pulex* have been estimated to be as young as 1,000 y (11) and as old as 172,000 y (10). Using silent-site divergence of asexual-linked chromosome VIII/IX haplotypes, we find the age of the entire asexual radiation to be about 1,250 y, with current lineages being substantially younger.

It has been proposed that lack of recombination and segregation in diploid, asexual populations encourages within-individual allelic divergence, thereby promoting elevated heterozygosity in

Significance

Drawing from whole-genome sequences of multiple genotypes, this study documents the molecular-genetic consequences of the loss of recombination in a microcrustacean, demonstrating that loss of heterozygosity via gene conversion-like processes is a dramatically more powerful force than accumulation of new mutations.

Author contributions: A.E.T. and M.L. designed research; A.E.T., M.S.A., B.D.E., S.X., and M.L. performed research; M.L. contributed new reagents/analytic tools; A.E.T., M.S.A., and M.L. analyzed data; and A.E.T., M.S.A., and M.L. wrote the paper.

The authors declare no conflict of interest.

Data deposition: The data reported in this paper have been deposited in the GenBank database (accession nos. SAMN02252729–SAMN02252752).

See Commentary on page 15518.

[1]To whom correspondence should be addressed. E-mail: milynch@indiana.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1313388110/-/DCSupplemental.

asexual genotypes (13). To test for this effect, we compared total heterozygosities (π_t) in asexual vs. sexual genomes. Although genome-wide π_t is elevated in asexual *D. pulex* by more than 31% (Fig. 1), the inflated allelic divergence is not uniform over all chromosomes. Instead, particularly high heterozygosity is concentrated on the chromosome VIII/IX scaffolds as a simple historical consequence of introgression from a sister taxon, *D. pulicaria* (Fig. 2), which introduced excess heterozygosity at the time of origin of obligate asexuality. Each recurrent passage of these nonrecombining *D. pulicaria*-derived chromosomes VIII/IX, via males produced by *D. pulex* asexuals, creates a new asexual lineage, trapping a background haploid genome from the recipient sexual genotype. The introgressed VIII/IX chromosomes, which harbor almost all (>95%) of the diagnostic SNPs noted earlier, result in a doubling of the silent-site heterozygosity (π_s) within these regions in asexuals relative to the situation at orthologous sites in sexual genotypes ($\pi_s = 0.0442$ vs. 0.0223 per site; Table S3).

Even when averaging over genomic regions exclusive of the asexual-marker scaffolds, asexual genotypes are still more heterozygous than are sexual genotypes, using total sites ($\pi_t = 0.0162$ vs. 0.0141 per site; Fig. 1) or coding sites (Fig. 3). This inflation could be a consequence of residual heterozygosity from the initial hybridization that apparently spawned the asexual lineage, or of mutation accumulation subsequent to asexual lineage production. The first explanation is ruled out by the observation that genomic regions outside of chromosomes VIII/IX in asexuals (>85% of the total genome) are devoid of *pulicaria*-specific markers (Fig. S2), implying a lack of association with meiosis suppression and loss of *pulicaria*-specific DNA by repeated backcrossing to sexual populations. With respect to the second possibility, given the young age of the asexual lineages, mutation accumulation resulting from the absence of recombination is expected to contribute a tiny fraction of the elevation in π_t in asexuals. The divergence among the total pool of 11 asexual linked haplotypes

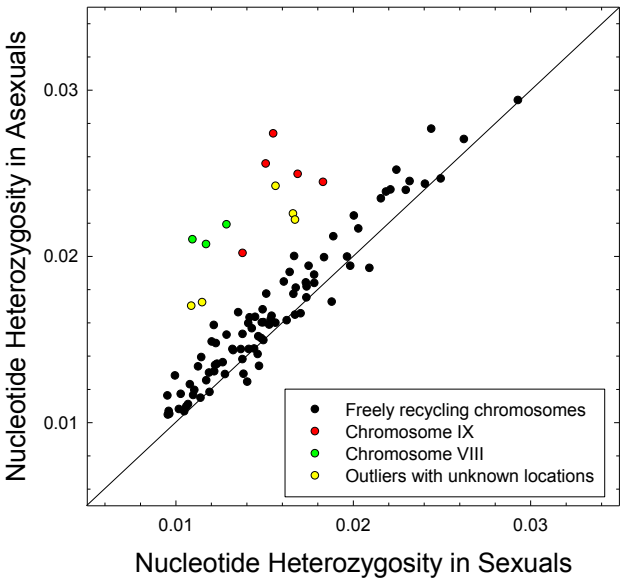

Fig. 1. Total within-clone heterozygosity (π_t) per nucleotide site for scaffolds (>0.45 Mb in length) averaged over 11 sexual and 11 asexual genotypes. Scaffolds containing SNP markers for asexuality are colored and contain regions that are on average 87.4% more heterozygous in asexuals than in sexuals resulting from their *D. pulicaria* origin. Nonmarker scaffolds (black) show an average 14.7% higher heterozygosity in asexuals, not caused by introgression but likely by the introduction of divergent sequences from the latest immigrant asexual male.

VIII/IX (residing in different asexual lineages) provides an upper limit to the level of excess heterozygosity that could have arisen by mutation accumulation within such genomes of just 1.04×10^{-4} mutations per site (Table S3), which is ~5% of the (0.0162–0.0141) = 0.0021 observed excess heterozygosity found in asexuals. As discussed later, mutational divergence will be further mitigated by cumulative deletions and chromosome homogenizing mechanisms. Thus, we conclude that the increased background levels of heterozygosity in asexuals are simply a result of the admixture that necessarily arises between recipient sexual haplotypes and males produced by recently immigrating asexual genotypes (i.e., wide crosses), who themselves would carry more distantly related sexual backgrounds acquired via previous asexual invasions into other populations.

The topology of the asexual-haplotype clade implies a stepping-stone-like radiation (Fig. 2, inset), in accordance with earlier findings suggesting a westward expansion from a northeast glacial refugium (10). Previous work (11) proposed a scenario in which asexuality in *Daphnia* displaces sexuality through repeated backcrossing of asexual males with resident sexual females. When sexual offspring are produced at the end of each season, males produced by asexual clones can spread the genes for asexuality to sexual clones, whereas asexual females cannot be fertilized by sexual males. This asymmetrical transmission of asexuality was thought to create a "contagious" process spreading asexuality through the proliferation of a driving meiosis-suppressing chromosome (11). However, the maintenance of "between pond" levels of heterozygosity in nonintrogressed regions of asexuals (Fig. 3) implies that little, if any, secondary backcrossing into resident sexual populations occurs in the course of asexual conquests. Instead, our data are consistent with the view that asexual clones displace sexuals because the admixed genotypes of the outcrossed asexuals confer an intrinsic competitive advantage, such as simply being an ecologically superior genotype or providing a means to avoid inbreeding (14, 15). Under this view, although opportunities for backcrossing into the resident sexual populations continue to exist in the short term, successful asexual clones maintain the admixed genotype (16) and secondarily backcrossed clones lose the advantageous genotype. The long-term prospects for any particular asexual lineage, however, are ultimately doomed by mutational and gene-conversion processes that reverse the advantage provided by the initial admixture of newly formed nonrecombining lineages.

To assess the genetic relationships among asexual lineages, we constructed phased haplotypes of chromosome VIII/IX marker-containing regions for each of the 11 diploid asexuals, producing 22 distinct sequences. We also placed 11 sexual *D. pulex*, one *D. pulicaria*, and two *Daphnia arenata* genome sequences in the tree (Fig. 2). The asexual-linked haplotypes form a shallow, monophyletic clade that is distantly related to the much more diverse sexual-derived haplotypes, including those captured by asexuals (Fig. 2), but pairs with *D. pulicaria*, supporting a *pulicaria* origin of the meiosis-suppressing genetic elements on these chromosomes in *D. pulex* asexuals. Consistent with the hypothesis that asexual lineages are derived from repeated invasions of the meiosis-suppressing haplotype into independent sexual backgrounds, and with the young age of extant asexuals, silent-site diversity among asexually captured sexual haplotypes is equivalent to that within sexual individuals (π_s ~0.023 vs. 0.024; Table S3), and sexual and asexually captured haplotypes are phylogenetically interspersed (Fig. 2).

The extant asexual genotypes are necessarily younger than the age of the entire asexual clade (< 1,250 y), as individual asexual chromosome VIII\IX haplotypes may have been transmitted to new sexual backgrounds at any point on the lineage (Fig. 2). To estimate the ages of individual asexual genotypes, we counted the numbers of sites at which each of the 11 captured sexual haplotypes were converted to the "asexual" SNP. Such gene-

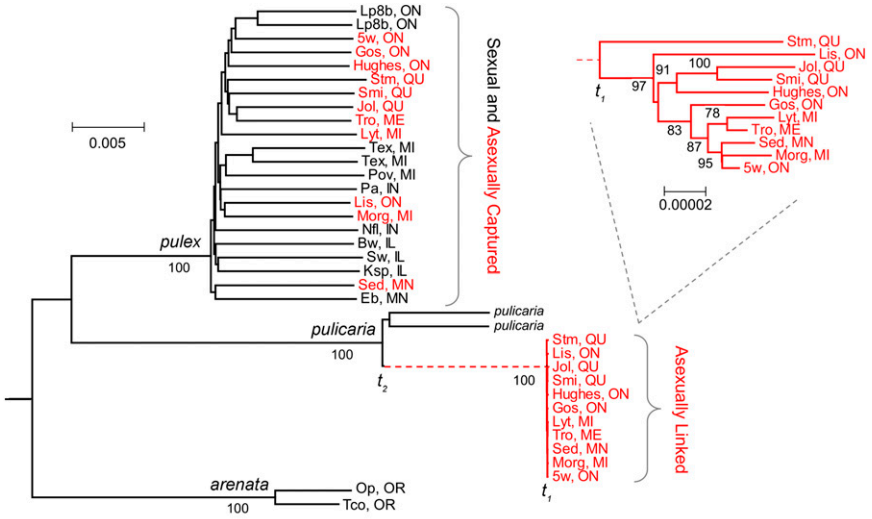

Fig. 2. Phylogeny of phased, concatenated haplotype sequences from 13 scaffolds of 11 sexual and 11 asexual genomes. The asexual-linked haplotype, which never recombines, forms a shallow clade that pairs with *D. pulicaria*, a sister species to *D. pulex*, whereas the "captured" (sexually derived) haplotypes of asexuals (red in the *pulex* clade) are interspersed with typical sexual haplotypes. Thus, each obligately asexual clone contains both a *D. pulex* sexually derived haplotype and a *D. pulicaria*-derived haplotype. *Inset* shows the phylogeny of the obligate asexual haplotypes in more detail. Bootstrap values greater than 75% are shown. Although 585,442 coding sites were aligned, 144,065 silent sites were used for phylogenetic inference. Both scales are in divergence per silent site. The overall tree is rooted with *D. obtusa* (not shown), and the basal clade consists of a geographically isolated sister species, *D. arenata* (12). The age of the entire asexual haplotype radiation (t_1) is estimated to be ~1,250 y, whereas the individual asexual genotypes are on average much younger (measured from rates of gene conversion), averaging ~0.02 t_1. The maximum age of the meiosis-suppressing haplotype (t_2) is equal to the time since divergence of the asexual clade and *D. pulicaria* (~187,000 y).

conversion events (or other homogenizing events, such as break-induced repair) must occur after sexual and asexual haplotypes are paired during the formation of each new asexual lineage. Because hemizygous deletions can mimic heterozygous to homozygous changes, sequence coverage was used to limit the inclusion of deletions in the analysis (Fig. S4). Using a total of 894 inferred one-way conversion events across ~14.5 Mb of the asexual marker haplotype, only a 2.5-fold difference was found in the number of inferred conversion events among asexual lineages (Fig. S3), consistent with the idea that the sampled asexual lineages are only moderately different in age. Moreover, using an estimated gene conversion rate of 3.3×10^{-5} per site/generation (17) and assuming minimal selection (see following), the average age of each asexual lineage is estimated to be only 22 y, about 50× younger than the entire asexual haplotype radiation. Consistent with our predicted timescale of recent asexual conquests, asexual *D. pulex* clones have been shown to displace native sexual lineages in only a few decades across a series of African lakes (18). If the high heterozygosity in the introgressed regions of asexuals suppresses gene conversion, we may be underestimating the number of gene-converted loci in the rest of the genome, thereby underestimating the age of asexuals. However, the ratio of deletions to conversions found in this study are consistent with genome-wide rates found in mutation accumulation lines of *D. pulex* (17).

The relatively long *D. pulicaria* branch on the chromosome VIII/IX haplotype tree (Fig. 2) indicates a higher rate of substitution relative to *D. pulex*, which may reflect a higher mutation rate and/or a difference in the number of generations per year. Because *D. pulicaria* populations typically inhabit lakes, whereas *D. pulex* populations are generally found in temporary ponds (19–21), the former almost certainly undergo more generations per year than the latter, leading to more opportunities for mutation associated with germ line replication. However, recent work on *Daphnia* mutation accumulation lines found that generation times may positively correlate with deleterious mutation rate (22), which would minimize any generation time effect, although the study did not consider the effects of dormancy. Although the branch leading to the asexual haplotype clade is even longer than those leading to lineages of both *D. pulex* and *D. pulicaria*, indicating a surplus of substitutions relative to sexuals of both species, it remains to be seen whether this is a result of typical branch-length variation among *D. pulicaria* chromosomes or an elevation in the mutation rate unique to asexual genomes.

Although the current asexual genotypes are young, the marker chromosomes VIII/IX carried by such clones will have been confined to ancestral asexual lineages since the time of origin of asexuality, surviving by periodic introgression into clean sexual backgrounds. To quantify the influence of purifying selection on mutation accumulation in a strictly asexual context (i.e., mutations accrued in the asexual genotypes rather than those present at the time of introgression), we examined the subset of mutations that have specifically accumulated within asexual chromosome VIII/IX segments by inferring derived SNPs along the haplotype phylogeny (Fig. 2, *Inset*). The ratio of the numbers of derived replacement mutations per replacement site (dN) to that of derived silent mutations per silent site (dS) is not significantly different from unity (dN/dS = 0.83; $P > 0.26$; Table S4), implying a near absence of purifying selection on amino acid-changing mutations on chromosomal segments that have been entirely confined to asexuals. However, because of the extremely recent origin of these mutations and the fact that we cannot compare them to an equivalent set of young mutations accumulated in sexual genotypes, it is uncertain whether the signature of relaxed selection found using asexual-hosted mutations is related to the loss of recombination in asexual lineages. The issue here is that young mutations largely behave in an effectively neutral manner because of fewer opportunities for selection (23, 24).

In contrast to the hypothesis of heterozygosity accumulation in asexual lineages (13), we found many more events of gene conversion and loss-of-heterozygosity via deletion on the asexual-associated chromosomes (1,616) than new point mutations in the same regions (316 point mutations). As the latter may have

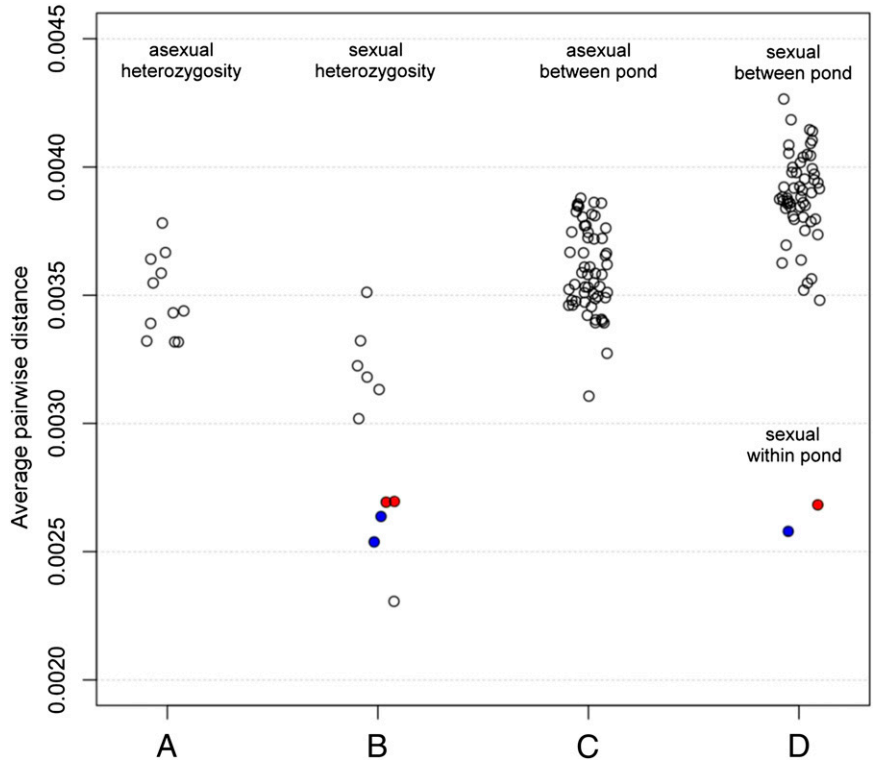

Fig. 3. Pairwise genetic distances (substitutions/site at coding sites) of genomes used in this study, excluding asexual marker regions (chromosomes VIII and IX). Genome-wide individual heterozygosities of 11 asexual genotypes (*A*) are on average higher than those of 11 sexual (*B*) genotypes. Two pairs of sexual genotypes sampled from the same pond (*D*, red and blue) show significantly reduced genetic distance relative to sexual genomes compared between ponds (*D*). Although sexual individuals have less variation than between-pond comparisons (*B* vs. *D*), asexuals harbor between-pond levels of variation (*A* vs. *C*). Individual heterozygosities are calculated from proportion of coding sites found in a heterozygous state within each diploid individual; between-lake genetic distances were calculated by the probability that two random nucleotides taken from two individuals are different.

accumulated during the entire asexual genealogy (including periods of residency in ancestral asexuals), whereas the former events must only occur in the specific genotypes sampled (the age of which spans an approximate fraction 0.02 of the entire genealogy of the asexual-marker scaffolds), the ratio of rates of loss to gains of heterozygosity is on the order of $1{,}616/(0.02 \times 316) = 256$. We used sequence-coverage data to determine that ~46% of loss-of-heterozygosity events are a consequence of gene conversion-like processes, with the remainder resulting from deletions that produce hemizygous regions (Fig. S4). As in the case of newly arising mutations, the converted regions can be evaluated for the ratio of rates of affected replacement and silent sites, and again we found no evidence of purifying selection against loss-of-heterozygosity events involving replacement sites ($dN/dS = 0.031/0.033 = 0.94$; $P > 0.60$; Table S5). Assuming similar net loss of heterozygosity for the entire genome (not just chromosome VIII and IX), asexual genotypes appear to lose heterozygosity at tens of thousands of sites in only a few hundred generations.

Discussion

We find that the chromosomal haplotypes associated with obligate asexuality in *D. pulex* originated in *D. pulicaria*. This observation could be explained either by the transmission of a meiosis-suppressing element previously segregating in *D. pulicaria* or by the generation of a novel change in reproductive mode caused by hybridization itself. Given that there are no reported cases of obligate asexuality in *D. pulicaria*, it has been suggested that a historical hybridization/introgression event between these sister species underlies the origin of asexuality (25). Although hybridization is thought to play a role in the origin of asexuality in other species (reviewed in ref. 26), crossing experiments between *D. pulex* and *D. pulicaria* generating hybrids that are cyclically parthenogenetic (19) imply that specific *D. pulicaria* parental genotypes may be necessary (although not necessarily sufficient) to generate obligate asexuals. In addition, the presence of a shared common chromosomal haplotype in all sampled asexuals is indicative of a single ancestral hybridization event leading to all extant asexual *D. pulex* populations, consistent with a rare genotype being responsible for the hybrid origin and rapid spread of obligate asexuality. However, we cannot rule out multiple origins of obligate asexuality in *D. pulex*, as we may have only sampled the most common of multiple possible asexuality-conferring haplotypes.

Our results suggest that the exposure of preexisting, deleterious recessive alleles by loss of heterozygosity may be a much more substantial contributor to the genetic deterioration of obligately asexual lineages than the gradual accumulation of new mutations (27). Nonbiased gene conversion does not lead to a net loss of variation in randomly mating populations, as each converted allele is placed in a new genetic background at each sexual event. However, gene-conversion-like events are permanently trapped in the progeny of asexual lineages, leaving a signature equivalent to inbreeding. Although it has been suggested that biased gene conversion in asexuals may actually slow the rate of clonal deterioration by correcting deleterious mutations (28), as well as speed up the fixation of beneficial mutations (29), given the extremely short longevity of obligately asexual *D. pulex* lineages, we suggest that the stochastic accumulation of loss-of-heterozygosity events (particularly those involving deletions) ultimately exposes deleterious recessive alleles in asexuals through loss of complementation (30, 31). Prior work on inbreeding depression shows a severe compromise in a number of life-history traits in *Daphnia*

when clones are selfed (32), and although loss-of-heterozygosity events are functionally equivalent to partial selfing, they cumulatively sum up to substantial inbreeding. By our estimate, the asexual genotypes in this study have lost ∼1.5% of their initial heterozygosity, and an average asexual clone will lose half of its heterozygosity (the equivalent of a round of selfing) in ∼500 y. In the case of the *D. pulex* system, obligate asexuality can nevertheless persist because the haplotype regions conferring asexuality are periodically rescued by introgression into new sexual backgrounds, which restores complementation in the relevant chromosomal regions and completely replaces all other unlinked regions with sexually derived chromosomes after an average of just a few backcrossing events. Depending on the birthrate of newly formed asexual lineages from wide crosses, migration rates of asexuals, and declines in fitness of asexual genotypes over time, we expect the turnover of asexual populations to be high and longevities of any particular asexual genotype to be low.

These observations allow us to revisit a previous analysis of deleterious mutation accumulation in asexual *D. pulex* (33). Although that study found elevated dN/dS along the external branches of mitochondrial haplotypes in asexuals vs. sexuals, implicating relaxed efficiency of selection in the former, our study makes it clear that most of the variants used in the prior analysis were likely present at the times of origin of individual asexual lineages, suggesting that the observed elevation of dN/dS was not a consequence of being in permanent linkage with a nuclear genome that no longer recombines or segregates (i.e., Muller's ratchet). For instance, using the estimated mutation rate in the *D. pulex* mitochondrial genome (2.0×10^{-8} nucleotide/generation; ref. 34), the average time since the recent common ancestor of the mitochondrial haplotypes, using silent-site divergence among all isolates sampled in Paland and Lynch (33), is estimated to be ∼70,000 y, with external branches at ∼40,000 y, many orders of magnitude older than what our data from the nuclear genome tell us about the age of the current asexual lineages (∼22 y). Therefore, assuming stochastic accumulation of silent-site mutations over time, $\ll$1% of the external-branch mitochondrial mutations in asexuals used in the prior study would have arisen after the transition to asexuality, providing $\ll$1 asexual-hosted mitochondrial mutation per lineage. In addition, when the PAML method (35) used by Paland and Lynch (33) is used to compare relative rates of dN/dS in the complete set of mitochondrial protein-coding genes in the 22 isolates described in this study, no significant difference between sexuals and asexuals is found. It is still possible that obligately asexual lineages are more likely to capture and retain mitochondria containing a higher load of deleterious mutations; for instance, if asexuals are more likely to displace sexual populations with extremely low effective population size. However, our results suggest that although mutations accumulated in asexual genotypes of nuclear-encoded genes are not under purifying selection, genes that are trapped on clone foundation do not have elevated dN/dS compared with sexuals. Therefore, we remain cautious about interpreting the observed elevation of dN/dS on the asexual-associated nuclear haplotypes (even when using mutations clearly accrued in the absence of recombination) as evidence for Muller's ratchet, especially because these mutations are few and are young.

Materials and Methods

***Daphnia* Clone Collection.** All *D. pulex* genotypes in this study were collected and maintained as clonal lineages by the Lynch Lab and have been analyzed for reproductive mode (ref. 10 and Table S1) and male production.

Sequencing and Bioinformatics. In total, 24 isolates (11 sexual *D. pulex*, 11 obligately asexual *D. pulex*, 1 *D. pulex-arenata,* and 1 *Daphnia obtusa*) were sequenced using Illumina paired-end short-read technology (library preparation and sequencing done by Beijing Genome Institute). In each case, high-molecular-weight DNA was purified from a clonal population (started from single diploid individual) using cetyltrimethylammonium bromide lysis buffer and standard phenol/chloroform extraction. The *D. pulicaria* genotype was sequenced independently and made available by the Shaw Lab (Indiana University). Illumina reads were mapped to the *Daphnia pulex* reference genome (12), using Novoalign (Novocraft Technologies), with settings optimized to limit repeat mapping (-r None). After applying coverage filters masking highly repetitive regions, SNPs were analyzed using Samtools (36) and custom scripts. Asexual-specific SNPs were defined as site-specific markers in which all 11 asexuals were heterozygous for same two bases and all 11 sexuals shared the same homozygous genotype for the nonmarker. Haplotype phasing of asexual marker regions was done using custom Python script and SAM files by binning Illumina reads containing the asexual and sexual SNP alleles. Loss-of-heterozygosity within introgressed regions of asexual clones was inferred from genotypes in which all but one of the 11 asexuals were heterozygous at a marker site, but in which the homozygous clone had both upstream and downstream heterozygous markers shared with other asexuals. Coverage was used to distinguish between gene conversion and deletion at loss-of-heterozygosity sites (Fig. S4). Annotation of all SNPs was based on the published gene models found in file FrozenGeneCatalog20110204.gff.gz at (http://genome.jgi-psf.org/Dappu1/Dappu1.download.ftp.html) (12).

Sequence Analysis. Genome-wide and scaffold-wide heterozygosities (π) for each clone were estimated for nonrepetitive regions of the genome contained in scaffolds 1–200 of the *D. pulex* assembly V1.1 (12) from sites with consensus-genotype quality scores exceeding 99.9% (Phred score >39) from pileup files (36) generated from SAM-formatted alignments. Silent-site heterozygosities were inferred from the published gene models (V1.1) for *D. pulex* (12), using KaKs Calculator (37).

Phased, concatenated sequences of 44 haplotypes of *D. pulex* (11 asexual and 11 sexual diploid genotypes), as well as two *D. arenata* and one *D. pulicaria*, were aligned using Molecular Evolutionary Genetics Analysis 5 (MEGA5) (38). MEGA5 was used for phylogenetic analysis using neighbor-joining with bootstrapping (500 replicates) on 144,065 silent sites contained within the alignment of 585,442 coding positions. Average pairwise distances at silent sites (π_s) and replacement sites (π_a) were calculated for three classes of phased chromosome VIII/IX haplotypes (asexual, sexual, and asexually captured), all calculated using MEGA5, using the Kimura 2-parameter method. The average pairwise distances between other chromosomes (non-VIII/IX, Fig. 3) were calculated on the basis of the probability that two random nucleotides, one from each of two individuals, are different. Heterozygosity is the fraction of sites in the sequence that are heterozygous.

The age estimate for the base of the asexual haplotype clade (Fig. 1; t_1) was calculated based on $t = k/\mu$, where t is generations, k is the ratio of silent-site changes per silent site from the node to each external branch, and μ is the mutation rate per site per generation. Generations (t) were converted to years, assuming seven generations per year. We estimated an average of 5.2×10^{-5} mutations per silent site from node to external branch (k) for the 11 asexual haplotypes and calculated a mutation rate of 5.95×10^{-9} mutations per site/generation (μ) from four fully sequenced *D. pulex* mutation-accumulation lines (39, 40) that were run in parallel with this project. The mutations accumulation (MA) process was designed in the following way: A diploid *D. arenata* individual (TCO) was grown up into a clonal population and split into multiple lineages, each of which was bottlenecked to a single individual for more than 100 generations. Bottlenecking to a single individual ensures that selection on accumulating mutations is practically eliminated (except for lethal mutations) and that mutations accruing in the bottlenecked individual are fixed. Because these lineages were maintained clonally (parthenogenetically), and barring subsequent hemizygous deletion and/or gene conversion, derived point mutations remained in a heterozygous state to the end of the experiment. We sequenced four independently maintained MA lineages of TCO to ∼20× coverage each and detected point mutations by mapping genomic reads to the reference genome of TCO (12), using Novoalign (Novocraft Technologies), and detected derived heterozygous mutations using two different methods: maximum likelihood and a quality score-based consensus method (36). Using the rate estimated from the MA experiment, the time since *D. pulicaria*/asexual haplotype divergence was estimated on the basis of the pairwise distance at silent sites between *D. pulicaria* and the asexual chromosome VIII/IX haplotype (k = 0.0227). Pairwise distance was calculated as the sum of net divergence and half the *D. pulicaria* heterozygosity (asexual haplotype has negligible polymorphism), where $t = 2k/\mu$, and assuming an average of 10 generations per year.

The ages of the extant asexual genotypes were estimated using the inferred number of gene-conversion events at asexual marker sites within

each asexual clone, where the sexual allele was converted to the asexual marker, as such events must have occurred since the pairing of the asexual haplotype and the captured sexual haplotype (the asexual haplotype may have been converted to sexual alleles during previous sexual invasions). Using an estimated conversion rate of 3.3×10^{-5} per site/generation (17) and assuming minimal selection (Table S5), the average age of the asexual lineages is estimated using $t = 2k_c/\mu_c$, where t is generations, k_c is the ratio of converted sites to total marker sites per clone [894 one-way conversions/(31,849 sites × 11 clones)] and μ_c is the rate of gene conversion per site per generation, giving an estimate of 155 generations (~22 y) for the average age of the asexual genotypes. SEs were calculated from the variance in estimated loss-of-heterozygosity rates from Xu et al. (17), giving a SE of our average age estimate of ±12.3 y.

ACKNOWLEDGMENTS. We thank T. Doak, D. Ebert, M. Hahn, D. Mark Welch, M. Pfrender, J. Colbourne, and S. Schaack for helpful discussions; E. Williams and M. Westphal for maintaining *Daphnia* clones; J. Shaw, S. Glaholt, N. Keith, and E. Turner for access to *D. pulicaria* sequences; and I. Lucas-Lledo, S. Michael, R. Henschel, L. Shin, W. Pfeiffer, Indiana University's National Center for Genome Analysis Support, and Beijing Genomics Institute for technical support. This project was supported by National Science Foundation Frontiers in Integrative Biological Research Grant EF-0328516 (to M.L.) and by National Institutes of Health Grant GM101672.

1. Muller HJ (1964) The relation of recombination to mutational advance. *Mutat Res* 106:2–9.
2. Hill WG, Robertson A (1966) The effect of linkage on limits to artificial selection. *Genet Res* 8(3):269–294.
3. Birky CW, Jr., Walsh JB (1988) Effects of linkage on rates of molecular evolution. *Proc Natl Acad Sci USA* 85(17):6414–6418.
4. Lynch M, Gabriel W (1990) Mutation load and survival of small populations. *Evolution* 44:1725–1737.
5. Comeron JM, Williford A, Kliman RM (2008) The Hill-Robertson effect: Evolutionary consequences of weak selection and linkage in finite populations. *Heredity (Edinb)* 100(1):19–31.
6. Maynard Smith J (1978) *The Evolution of Sex* (Cambridge Univ Press, Cambridge).
7. Barton NH, Charlesworth B (1998) Why sex and recombination? *Science* 281(5385): 1986–1990.
8. Lynch M, Bürger R, Butcher D, Gabriel W (1993) The mutational meltdown in asexual populations. *J Hered* 84(5):339–344.
9. Innes DJ, Hebert PDN (1988) The origin and genetic basis of obligate parthenogenesis in *Daphnia pulex*. *Evolution* 42:1024–1035.
10. Paland S, Colbourne JK, Lynch M (2005) Evolutionary history of contagious asexuality in *Daphnia pulex*. *Evolution* 59(4):800–813.
11. Lynch M, Seyfert A, Eads B, Williams E (2008) Localization of the genetic determinants of meiosis suppression in *Daphnia pulex*. *Genetics* 180(1):317–327.
12. Colbourne JK, et al. (2011) The ecoresponsive genome of *Daphnia pulex*. *Science* 331(6017):555–561.
13. Mark Welch D, Meselson M (2000) Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science* 288(5469):1211–1215.
14. Haag CR, Hottinger JW, Riek M, Ebert D (2002) Strong inbreeding depression in a *Daphnia* metapopulation. *Evolution* 56(3):518–526.
15. Ebert D, et al. (2002) A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295(5554):485–488.
16. Haag C, Ebert D (2004) A new hypothesis to explain geographic parthenogenesis. *Ann Zool Fenn* 41:539–544.
17. Xu S, Omilian AR, Cristescu ME (2011) High rate of large-scale hemizygous deletions in asexually propagating *Daphnia*: Implications for the evolution of sex. *Mol Biol Evol* 28(1):335–342.
18. Mergeay J, Verschuren D, De Meester L (2006) Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. *Proc Biol Sci* 273(1603):2839–2844.
19. Heier CR, Dudycha JL (2009) Ecological speciation in a cyclic parthenogen: Sexual capability of experimental hybrids between *Daphnia pulex* and *Daphnia pulicaria*. *Limnol Oceanogr* 54:492–502.
20. Vergilino R, Markova S, Ventura M, Manca M, Dufresne F (2011) Reticulate evolution of the *Daphnia pulex* complex as revealed by nuclear markers. *Mol Ecol* 20(6): 1191–1207.
21. Cristescu ME, Constantin A, Bock DG, Cáceres CE, Crease TJ (2012) Speciation with gene flow and the genetics of habitat transitions. *Mol Ecol* 21(6):1411–1422.
22. Latta LC, 4th, et al. (2013) Genomic background and generation time influence deleterious mutation rates in *Daphnia*. *Genetics* 193(2):539–544.
23. Gillespie JH (2004) *Population Genetics: A Concise Guide* (Johns Hopkins Univ Press, Baltimore, MD).
24. 1001 Genomes Consortium, et al. (2012) An integrated map of genetic variation from 1,092 human genomes. *Nature* 491(7422):56–65.
25. Xu S, Innes DJ, Lynch M, Cristescu ME (2013) The role of hybridization in the evolution of meiosis suppression in *Daphnia*. *Mol Ecol*, in press.
26. Simon JC, Delmotte F, Rispe C, Crease T (2003) Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. *Biol J Linn Soc Lond* 79:151–163.
27. Omilian AR, Cristescu ME, Dudycha JL, Lynch M (2006) Ameiotic recombination in asexual lineages of *Daphnia*. *Proc Natl Acad Sci USA* 103(49):18638–18643.
28. Khakhlova O, Bock R (2006) Elimination of deleterious mutations in plastid genomes by gene conversion. *Plant J* 46(1):85–94.
29. Mandegar MA, Otto SP (2007) Mitotic recombination counteracts the benefits of genetic segregation. *Proc Biol Sci* 274(1615):1301–1307.
30. Archetti M (2004) Recombination and loss of complementation: A more than two-fold cost for parthenogenesis. *J Evol Biol* 17(5):1084–1097.
31. Archetti M (2010) Complementation, genetic conflict, and the evolution of sex and recombination. *J Hered* 101(Suppl 1):S21–S33.
32. Deng HW, Lynch M (1997) Inbreeding depression and inferred deleterious-mutation parameters in *Daphnia*. *Genetics* 147(1):147–155.
33. Paland S, Lynch M (2006) Transitions to asexuality result in excess amino acid substitutions. *Science* 311(5763):990–992.
34. Xu S, et al. (2012) High mutation rates in the mitochondrial genomes of *Daphnia pulex*. *Mol Biol Evol* 29(2):763–769.
35. Yang Z (2007) PAML 4: Phylogenetic analysis by maximum likelihood. *Mol Biol Evol* 24(8):1586–1591.
36. Li H, et al.; 1000 Genome Project Data Processing Subgroup (2009) The sequence alignment/map (SAM) format and SAMtools. *Bioinformatics* 25(16):2078–2079.
37. Zhang Z, et al. (2006) KaKs_Calculator: Calculating Ka and Ks through model selection and model averaging. *Genomics Proteomics Bioinformatics* 4(4):259–263.
38. Tamura K, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28(10):2731–2739.
39. Lynch M, Latta L, Hicks J, Giorgianni M (1998) Mutation, selection, and the maintenance of life-history variation in a natural population. *Evolution* 52:727–733.
40. Lynch M (1985) Spontaneous mutations for life history characters in an obligate parthenogen. *Evolution* 39:804–818.