High Rate of Large-Scale Hemizygous Deletions in Asexually Propagating *Daphnia*: Implications for the Evolution of Sex

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Abstract

The origin and maintenance of sex remains one of the most debated topics in evolutionary biology. Investigations of the molecular genetic consequences of asexuality, such as direct estimation of mutation and recombination rates in asexual lineages, are critical for explaining the prevalence of sex in nature. In this study, we use long-term mutation accumulation lines of asexually propagating *Daphnia pulex* and *D. obtusa* to examine the role of hemizygous deletion and ameiotic recombination (crossover and gene conversion) in the evolution of asexual taxa. Large-scale hemizygous deletions ranging from 2 to 30 kb are found to occur at a rate of 6.7×10^{-5} locus^{-1} generation^{-1} in *D. pulex*, which is one order of magnitude higher than the rate of large-scale deletions in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. A conservative estimate of gene conversion and crossing over in the apomictic germ line cells of *Daphnia* yields a rate of 3.3×10^{-5} locus^{-1} generation^{-1}. Our exceptionally high rate of large-scale segmental deletions suggests that the long-term survival of asexual *Daphnia* lineages is likely compromised by a high deleterious mutation pressure and that selection against deleterious mutations may play an important role in the evolution and maintenance of sex.

Key words: loss of heterozygosity, mutation accumulation, asexuality, hemizygosity, deletions.

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Introduction

Characterizing the evolutionary consequences of asexuality at the genetic level is important for understanding why sexual reproduction is predominant in nature. From a theoretical standpoint, asexual taxa are evolutionary dead ends because they are assumed to have evolutionarily negligible levels of homologous recombination (Maynard Smith 1978). Moreover, with the majority of mutations being deleterious, the mutation load for asexual taxa will irreversibly increase and lead to eventual extinction of populations (Lynch and Gabriel 1990; Lynch et al. 1993) because highly fit genotypes cannot be reconstituted without recombination (Muller 1964; Felsenstein 1974). Despite the central importance of the rates of mutation and recombination in understanding the consequences of asexuality, these basic parameters have rarely been directly estimated in asexual lineages (Omilian et al. 2006).

Mutation accumulation (MA) experiments facilitate the study of mutations (e.g., Morran et al. 2009) because the effects of natural selection are minimized. Replicate MA lines are propagated through regular bottlenecks each generation in a benign environment. Thus, the majority of spontaneous mutations accumulate in a neutral fashion (Keightley and Caballero 1997), thereby allowing for the direct estimation of mutation rates. In a recent study, Omilian et al. (2006) screened protein-coding and microsatellite loci across three major linkage groups in asexually reproducing *Daphnia* MA lines and found that the rate of ameiotic recombination inferred directly from the frequency of loss of heterozygosity (LOH) appeared to be orders of magnitude higher than the frequency of mutation (Denver et al. 2004, 2009; Keightley et al. 2009). This finding challenges the assumption that homologous recombination is evolutionarily negligible in asexual taxa. However, two important aspects of the findings of Omilian et al. (2006) need to be clarified before examining how ameiotic recombination influences the evolution of asexual taxa. First, it remains unclear whether the high rate of LOH is temporally stable or applicable to the entire *Daphnia* genome. Second, and more importantly, it is critical to distinguish LOH events resulting from ameiotic recombination (maintaining both DNA copies of a locus) from LOH events resulting from the segmental deletion of one DNA copy of a diploid locus (hemizygosity). Nonallelic homologous recombination (e.g., unequal crossover) and various pathways for repairing DNA double-strand breaks and/or stalled replication forks (e.g., single-strand annealing and breakage-induced repair) can produce hemizygosity at the affected loci (Helleday 2003; Hastings et al. 2009).

In this study, we estimate the genome-wide rate of LOH events in MA lines of asexually propagating *Daphnia pulex* and *D. obtusa* by screening microsatellite markers throughout the genome. Furthermore, we used quantitative polymerase chain reaction (qPCR) to determine the relative proportions of homozygous LOH events (gene conversion and crossovers) versus hemizygous LOH events (deletions). We find that the genome-wide LOH rate is consistent with the estimate of Omilian et al. (2006). However, a large proportion (67%) of our identified LOH events resulted from

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deletions leading to hemizygosity, yielding a deletion rate of 6.7×10^{-5} locus^{-1} generation^{-1} and a recombination rate of 3.3×10^{-5} locus^{-1} generation^{-1}. Lastly, we examine the physical length of deletion tracts and show that they are large (2–30 kb) and span open reading frames.

Materials and Methods

MA Lines

Single females of *D. pulex* (denoted as PX) and *D. obtusa* (OB) were isolated from temporary ponds located in Linwood, Ontario, Canada, and Trelease Woods, IL, respectively. Most *Daphnia* are cyclical parthenogens, capable of both sexual and apomictic reproduction. However, obligately asexual lineages do exist (Banta 1925; Innes and Hebert 1988), and asexual reproduction in several species of *Daphnia* has been shown to be ameiotic (Schrader 1925; Zaffagnini and Sabelli 1972). PX was determined to be an obligate parthenogen, whereas OB was determined to be a cyclical parthenogen, following established methods for breeding system determination (Innes et al. 1986).

Daphnia were maintained under standard conditions at 20 °C and fed ad libitum with a suspension of vitamin-fortified *Scenedesmus obliquus*. MA lines were initiated from 48 to 50 single progeny derived from a single stem mother for both PX and OB and maintained following previously described methods (Lynch 1985). Briefly, a single randomly chosen daughter was transferred to a new beaker every asexual generation (10–12 and 8–10 days for PX and OB, respectively), whereas two females of the same brood were transferred into separate vessels to serve as backups, in case the focal individual died without producing female offspring. Backups were used in 15–20% of the transfers, usually because the focal individual produced only resting eggs (PX) or males offspring (OB). The use of backups potentially leads to a downward bias in our LOH rate estimates because LOH that is lethal or substantially retards the production of immediately developing female offspring will be underrepresented. The MA lines screened in this study (20 lines for PX and 28 for OB) were propagated for an average of 116 and 190 generations, respectively (supplementary table S1, Supplementary Material online). These MA lines represented a subset of the lines used by Omilian et al. (2006) at about 75 (PX) and 107 (OB) generations but included all the lines that survived the long-term MA experiment.

DNA Extraction, PCR, and Genotyping

Genomic DNA of five to ten adult individuals for each MA line was extracted using a cetyltrimethylammonium bromide method (Doyle and Doyle 1987). We screened microsatellite markers that constitute the framework for the 12 linkage groups of *D. pulex* (Cristescu et al. 2006). We excluded microsatellite loci that were known to be homozygous in the stem mother because they are uninformative for revealing LOH events. A total of 141 microsatellite markers were genotyped for the PX (*D. pulex*) lines, whereas 95 of these 141 microsatellite markers were also screened for the OB (*D. obtusa*) lines. According to the *D. pulex* genome annotation (wfleabase.org), 65 of 141 microsatellite loci screened were located in protein-coding regions (48 in exons and 17 in introns). In order to avoid null alleles, markers that showed inconsistent amplification patterns across lines were excluded. All LOH events were confirmed with a second independent PCR using a different Taq polymerase. PCR reactions and genotyping followed methods in Cristescu et al. (2006).

Quantitative Microsatellite Analysis

Quantitative microsatellite analysis (QuMA) has been successfully applied to detect changes in DNA copy numbers in clinical genetic analyses (Ginzinger et al. 2000; Nigro et al. 2001; Suzuki et al. 2004). We quantified the DNA copy number of the 24 LOH events for 15 loci in the PX MA lines relative to heterozygous microsatellite loci using QuMA. Omilian et al. (2006) reported that one of their MA lines (LIN6, designated as PX6 herein) experienced a long tract of LOH that spanned more than half of chromosome three. Although PX6 was not part of our mutation and recombination rate estimates, we determined the DNA copy number of all 14 microsatellite loci located on this particularly long LOH tract. For QuMA, PCR reactions of a given LOH locus (referred to as the test locus) and a confirmed heterozygous locus across all MA lines (referred to as the reference locus) for the LOH MA line and two independent normal (calibrator) MA lines were performed in triplicate on the ABI 7500 Real-Time PCR system (Applied Biosystems). The microsatellite locus d088 was chosen as the reference locus because it appears to be heterozygous in all the PX MA lines (i.e., DNA copy number is 2), and its amplicon (~150 bp) is desirable for qPCR experiments.

The thermocycling regime consisted of a 10-min incubation at 95°, followed by 40 cycles of 15 s at 95°, and 1 min at 60° with a single fluorescent reading taken at the end of each cycle. Specificity of amplification in qPCR experiments was confirmed by a dissociation analysis at the end of each run. The 20 μl PCR reactions consisted of 10 μl SYBR Premix Ex Taq (Takara Bio), 0.2 μM of each primer, 0.4 μl Rox II dye, ddH$_2$O, and 1–5 μg DNA template. The amplification efficiency (E) for all the primer sets used in the actual qPCR experiments was calculated using the software LinRegPCR (Ruijter et al. 2009) to assure that the E values for the test and reference loci were approximately the same (>85%). The E value for each primer pair was also confirmed by performing qPCR experiments following standard procedures (user bulletin no. 2, ABI 7700 SDS; Applied Biosystems). We used the Sequence Detection Software (v.1.2.3, Applied Biosystems) to estimate the number of PCR cycles (C_T) required for the fluorescence to reach a threshold above background for the test and reference reactions that were performed in triplicate for each DNA sample.

For each DNA sample, the average of C_T values for the test locus and the reference locus was subtracted to obtain ΔC_T (ΔC_T = mean of C_T [test locus] − mean of C_T [reference locus]). Relative copy number at each test locus

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in the LOH sample was then calculated as $2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = \Delta C_T$ (LOH sample) − the average of ΔC_T for two calibrator samples. The relative copy number multiplied by 2 yields the copy number of the test locus in the LOH sample (copy number $= 2 \times 2^{-\Delta\Delta C_T}$) (Ginzinger et al. 2000).

To determine whether the relative copy number of a test locus was significantly different from that of the reference locus, a tolerance interval (TI) was calculated using the pooled standard deviation of ΔC_T values for the test and reference locus on 38 calibrator DNA samples using the following formula: TI $= 2 \times 2$ to the power $\pm \{2.46\times$ the square root of$[\Sigma_i(n_i-1)\times SD_i^2/\Sigma_i(n_i-1)]\}$, where n_i is the number of calibrators analyzed per microsatellite and 2.46 was the two-sided tolerance limit factor for the total degrees of freedom $(\Sigma_i(n_i-1)=38)$. The relative copy number of a normal sample will be within $2 \pm$ TI 95% of the time with 95% confidence. Based on this TI, DNA copy numbers less than 1.20 were considered deletions, whereas copy numbers greater than 3.33 were considered duplications.

Calculation of the Rates of LOH, Deletion, and Ameiotic Recombination

The rate of LOH (per locus per generation) was calculated following the method in Omilian et al. (2006) using the equation $\lambda = h/(L \times i \times T)$, where h is the number of observed LOH events, L is the number of MA lines, i is the number of total informative (heterozygous in the stem mother) loci, and T is the average number of generations for MA lines. The rates of ameiotic recombination and deletion were calculated with h representing the number of homozygous or hemizygous loci, respectively. To obtain a deletion/recombination rate per nucleotide, h is the number of base pairs that experienced hemizygosity or homozygosity, and i is the total number of informative base pairs (table 1). Given that all deletions and crossover/gene conversions span the entire length of the marker, the per locus and per base pair expressions are equivalent. However, we caution that the deletion rate per nucleotide is not directly comparable with point mutations rates; the deletion rate per nucleotide takes into account the physical length of the deleted genomic regions rather than the number of mutation events.

Characterization of Physical Lengths of Hemizygous Deletions

Physical lengths of hemizygous deletions were quantified for four loci, which included two loci (d050 and d083) that showed LOH in three or more PX MA lines (supplementary table S1, Supplementary Material online), and two loci (d078 and d054) that appeared to be on a long LOH tract in PX6 likely spanning one arm of chromosome three (fig. 2). A chromosome walking strategy was employed to estimate the physical lengths of hemizygous deletions. Genomic sequences of up to 100 kb surrounding focal LOH loci were downloaded from wFleaBase (wfleabase.org). qPCR primers (supplementary table S2, Supplementary Material online) were designed using PrimerQuest (www.idtdna.com/scitools/applications/primerquest) to amplify short fragments of DNA (~100 bp), which were 2–3 kb upstream or downstream of a focal LOH locus. QuMA analyses were performed to test the DNA copy number of these flanking markers. If these markers were found to be hemizygous, new markers distal to the previous markers were selected for further analysis until diploid markers were detected. The physical lengths of deletions were calculated as the distance (base pairs) between the two hemizygous markers at the ends of the deletion tracts, which are an underestimate of the true length of deletions.

Table 1. Summary of LOH Information in *D. pulex* (PX) and *D. obtusa* (OB) MA Lines. The Length of Loci Represents the Sum of Fragment Size for the Heterozygous/Homozygous LOH/Hemizygous LOH Loci.

Species	*D. pulex*	*D. obtusa*
Number of mutation lines	20	28
Average number of generations	116	190
Number of informative (heterozygous) loci	108	34
Total length of informative loci	28,159 bp	8,617 bp
Number of LOH events observed	24	41
LOH rate (locus^{-1} generation^{-1})	9.58×10^{-5}	2.27×10^{-4}
Number of homozygous LOH loci	8	N/A
Total length of homozygous LOH loci	2,519 bp	N/A
Number of hemizygous LOH loci	16	N/A
Total length of hemizygous LOH loci	3,565 bp	N/A
Segmental deletion rate (locus^{-1} generation^{-1})	6.7×10^{-5}	N/A
Segmental deletion rate (bp^{-1} generation^{-1})	5.5×10^{-5}	N/A
Ameiotic recombination rate (locus^{-1} generation^{-1})	3.3×10^{-5}	N/A
Ameiotic recombination rate (bp^{-1} generation^{-1})	3.9×10^{-5}	N/A

NOTE.—LOH, loss of heterozygosity; MA, mutation accumulation; N/A, not applicable.

Results

Genome-Wide LOH Rate

We screened 141 microsatellite markers in 20 MA lines of *D. pulex* (PX) and 95 microsatellite markers in 28 lines of *D. obtusa* (OB). One hundred and eight microsatellites in *D. pulex* and 34 microsatellites in *D. obtusa* were heterozygous in the founder of the MA lines and were therefore informative for detecting LOH events (table 1). In total, 24 LOH events were detected for 15 microsatellite markers in 20 PX MA lines over an average of 116 generations, whereas 41 LOH events for 15 microsatellite markers occurred in 28 OB MA lines over an average of 190 generations (table 1 and supplementary table S1, Supplementary Material online). The rate of LOH events was 9.58×10^{-5} (PX) and 2.27×10^{-4} (OB) events locus^{-1} generation^{-1}, respectively (table 1). Given that LOH rates from microsatellite loci and protein-coding loci (Omilian et al. 2006) are consistent (1.7×10^{-4} locus^{-1} generation^{-1}), we suggest that the microsatellite-based LOH rates are representative of the entire genome.

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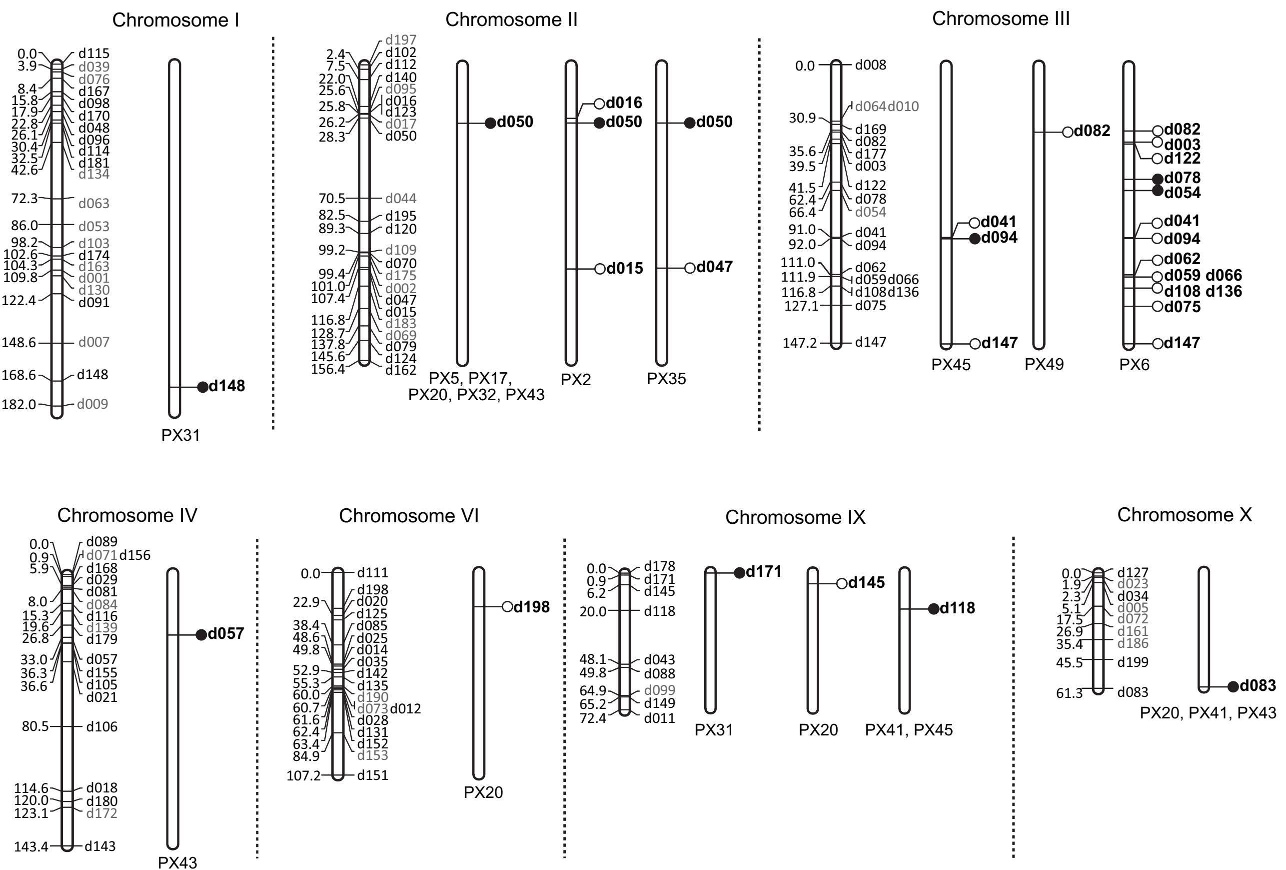

FIG. 1 Map locations for LOH loci (open circles) and hemizygous LOH loci (black circles) in the *D. pulex* MA lines. Only 7 of 12 screened chromosomes contain markers that underwent LOH. The ancestral diploid state for each chromosome is shown on the left of the map, with heterozygous loci in black type and homozygous loci in gray type. Numbers on the left indicate map distances (centimorgans), and numbers to the right indicate marker names. LOH profiles are shown to the right of the linkage map for each chromosome, with the MA line showing that profile indicated below.

The number of LOH events for each individual MA line ranged from zero to nine. A maximum of three nonconsecutive LOH events on the same chromosome occurred in the PX MA lines (fig. 1). The lack of a genetic linkage map for *D. obtusa* prevented us from determining the genomic location of LOH events in the OB MA lines. Hotspots for LOH events, defined as loci that displayed LOH in three or more individual MA lines, were also identified: d050 (7 lines) and d083 (3 lines) for PX and d024 (6 lines), d027 (3 lines), and d068 (17 lines) for OB.

Rate of Hemizygous Deletions in *D. pulex*

Following the TI for QuMA analysis, LOH loci with DNA copy numbers less than 1.20 should be regarded as hemizygous. In total, 16 of 24 LOH events appeared to be hemizygous. Seven microsatellite loci experienced hemizygosity, and the loci d050 and d083 were found to be hemizygous in multiple MA lines (fig. 1). The rate of hemizygous deletions in the *D. pulex* MA lines is 6.7×10^{-5} locus^{-1} generation^{-1} (table 1). Based on the *Daphnia* genome annotation (wfleabase.org), all hemizygous loci span open reading frames (supplementary table S4, Supplementary Material online), and thus, the deletions are likely deleterious. We also investigated an unusually long LOH tract spanning a string of 14 microsatellite loci. Of these, two internal loci (d054 and d078) were found to be hemizygous, whereas the rest maintained a copy number of two (fig. 1 and supplementary table S3, Supplementary Material online). This finding indicates that the long LOH tract is likely due to a crossover event followed by internal hemizygous deletions.

Physical Lengths of Hemizygous Deletions in *D. pulex*

qPCR experiments on linked markers in the regions located near focal hemizygous loci revealed that the physical lengths of deletion tracts for markers d050, d083, d054, and d078 ranged from ~2 to 30 kb (fig. 2 and table 2). Deletion tracts of dramatically different lengths were detected among individual MA lines at locus d050. For example, ~2 kb deletions were observed in the PX35 and PX43 lines, whereas PX2, PX5, and PX32 experienced deletion tracts of 25–30 kb. Deletions for locus d083 were similar in length (~23 kb) for both MA individuals, PX41 and PX43. Although markers d054 and d078 are adjacent in PX6, their deletion tracts were separated by an internal diploid segment; the physical lengths for d054 and d078 were ~9 and ~13 kb, respectively. All deletions span open reading frames (supplementary fig. S1, Supplementary Material online).

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Table 2. Summary of the Hemizygous Deletion Tracts in the *D. pulex* MA Lines, with Genomic Coordinates (wfleabase.org) and Lengths (base pairs) of the Affected Genomic Regions.

Locus	MA Line	Genomic Location	Length (bp)
d054	PX6	scaffold_62:48915-61803	12,889
d078	PX6	scaffold_62:121813-130992	9,180
d050	PX2	scaffold_63:448169-472883	24,715
d050	PX5, PX32	scaffold_63:448169-477951	29,782
d050	PX35, PX43	scaffold_63:448169-450291	2,123
d083	PX41, PX43	scaffold_17:891889-914491	22,603

NOTE. —MA, mutation accumulation.

Discussion

In this study, we used asexually reproducing *Daphnia* MA lines maintained for more than 100 generations to investigate the rate of hemizygous deletions and homologous recombination (ameiotic gene conversion and crossover) and their relative contribution to LOH in the *Daphnia* nuclear genome. The genome-wide rates of LOH at microsatellite loci in the *D. pulex* MA lines (9.58×10^{-5} locus^{-1} generation^{-1}) and the *D. obtusa* MA lines (2.27×10^{-4} locus^{-1} generation^{-1}) are consistent with the previously estimated LOH rate ($\sim 10^{-4}$) for microsatellites and protein-coding regions in lines from the same MA experiment but at a much earlier stage of MA (75 and 107 generations, respectively) (Omilian et al. 2006). Thus, the LOH rate appears to be temporally consistent in apomictic *Daphnia* germ line cells. The LOH rate in *D. obtusa* MA lines is approximately twice that in *D. pulex*. Because LOH can lead to the unmasking of deleterious alleles that have little phenotypic effect in a heterozygous state, a higher genetic load in the obligate asexual stem mother for *D. pulex* MA lines could lead to fewer viable offspring with LOH loci compared with the *D. obtusa* lines that were derived from a cyclical parthenogenetic mother. Our direct estimates of deletion and recombination rate via qPCR experiments show that only 33% of the observed LOH events were likely due to crossover and gene conversion events, whereas the remaining 67% of events were likely due to large-scale deletions.

High Rate of Hemizygous Deletions

We find a deletion rate of 6.7×10^{-5} locus^{-1} generation^{-1} in *D. pulex* MA lines (table 1). When deletion hotspots are excluded (i.e., d050), the deletion rate is 3.8×10^{-5} locus^{-1} generation^{-1}. The physical scale of genomic regions that experience deletions is roughly 10^3 or 10^4 higher than that for point mutations based on estimates (10^{-9} to 10^{-8})

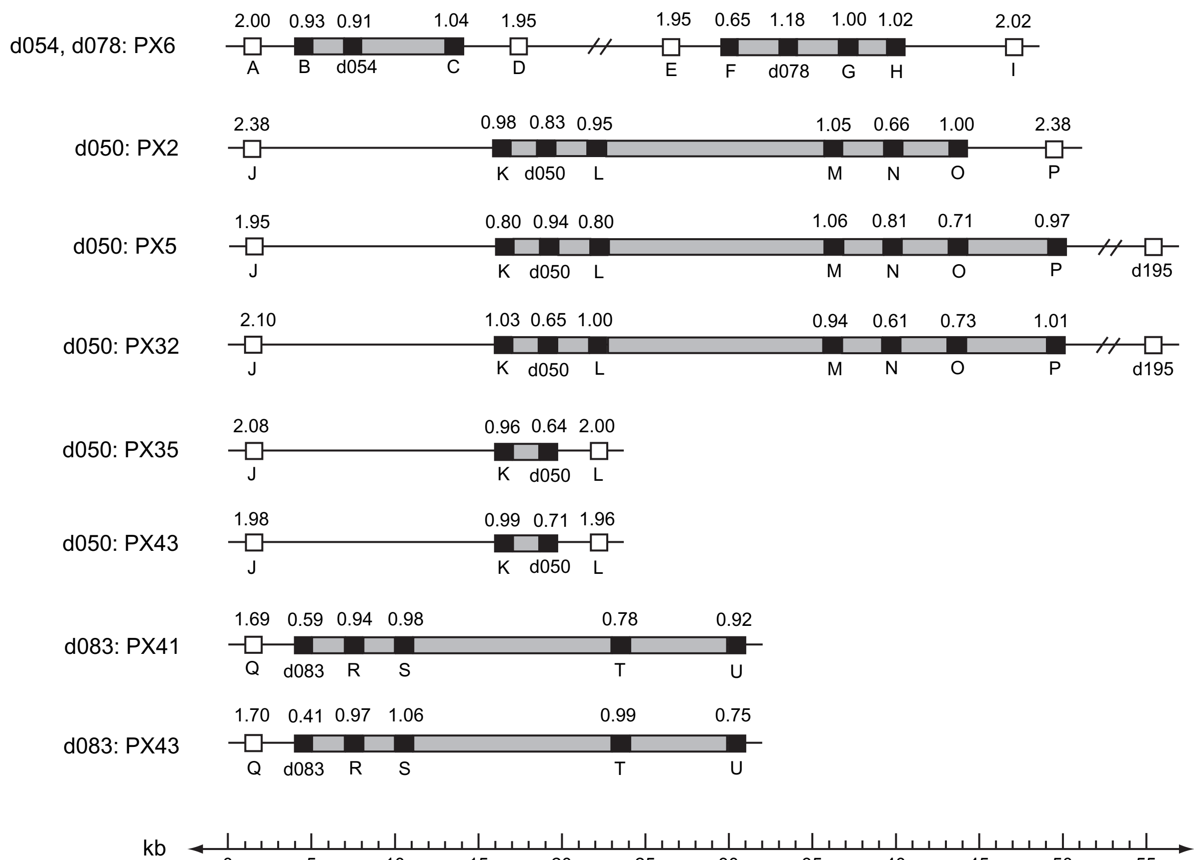

FIG. 2 Physical map of the hemizygous deletion tracts encompassing the loci d054, d078, d050, and d083 that experienced LOH in multiple MA lines. Letters denote the markers used in qPCR experiments (Supplementary table S2, Supplementary Material online). Black boxes represent hemizygous markers, whereas open boxes denote diploid markers. Gray areas indicate the inferred deletion tracts. Numbers above boxes indicate the DNA copy number for each marker tested. The marker d195 is a heterozygous marker (i.e., DNA copy number is 2) according to the genotyping results, although no qPCR experiments have been performed using this locus.

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from previous MA experiments of *Drosophila melanogaster* and *Caenorhabditis elegans* (Denver et al. 2004, 2009; Keightley et al. 2009). Because previous MA studies primarily use sequencing technologies that cannot reveal DNA copy number polymorphisms (but see Lynch et al. 2008), these estimates are based mainly on point mutation or indels of a few base pairs. Although our deletion rate is based on microsatellite markers, it is unlikely that the high deletion rate in *Daphnia* is due to the fragile nature of microsatellites as protein-coding loci sequenced in an earlier study were as equally likely to experience LOH events as microsatellite loci. Therefore, our results strongly suggest that segmental deletions represent an important component in the molecular spectrum of spontaneous mutations and deserve further attention.

The *Daphnia* deletion rate is about one order of magnitude higher than the rate of large-scale deletions per cell division in *Saccharomyces cerevisiae* (Lynch et al. 2008) and the rate of large-scale deletions per generation in *Drosophila melanogaster* (Watanabe et al. 2009). The extremely high rate of large-scale hemizygous deletions in the *Daphnia* nuclear genome is consistent with the high level of copy number polymorphisms (duplications and deletions) observed in natural populations of flies (Dopman and Hartl 2007) and mice (Graubert et al. 2007). Although detecting duplications is beyond the scope of our work, segmental duplications might also occur at a high rate in the *Daphnia* genome. About 20% of the 27,000 gene predictions obtained from the *Daphnia* genome sequence appear to be tandem duplicates (wfleabase.org). The joint impact of segmental duplications and deletions on the evolution of asexual taxa remains largely unknown, but the extraordinarily high rate of deletion in our data set suggests that these phenomena deserve further attention.

Our data suggest that some genomic regions are more vulnerable to deletions than others. For example, deletion tracts of three different lengths involving locus d050 in five different MA lines indicate that multiple deletion events affected the same chromosomal region across different MA lines (fig. 2). Given our rate of deletions per locus in *D. pulex* MA lines (6.7×10^{-5}) and assuming a Poisson distribution of deletions, the chance of observing seven deletions (supplementary table S3, Supplementary Material online) involving locus d050 is extremely small (2×10^{-19}). The nonrandom distribution of the observed deletions raises questions about the genomic background in which deletions occur. It is known that recombination between nonallelic homologues can produce genomic rearrangements, including deletions, duplications, inversions, and translocations (Mieczkowski et al. 2006). Furthermore, a number of studies have confirmed the role of repetitive genomic elements such as transfer RNA genes (Dunham et al. 2002), long terminal repeats (Diogo et al. 2009), transposable elements (Mieczkowski et al. 2006; Lynch et al. 2008; Watanabe et al. 2009), major repeat sequences (Lephart and Magee 2006), microsatellites (Bena et al. 2010), and segmental duplications (Sharp et al. 2005) in mediating chromosome rearrangements including deletions. We note here that microsatellites may facilitate deletions, but as we mentioned earlier, this is not likely to be the case with our data. Because the *D. pulex* genome is particularly rich in tandem gene duplications (see above), we suggest that they could mediate nonallelic homologous recombination that results in hemizygous deletions. Screening for flanking tandem genes in the 50-kb genomic regions surrounding the characterized deletion tracts in wFleaBase (wfleabase.org) revealed two tandem genes in a 10-kb window that flank the deletion tract observed in PX35 and PX43 (supplementary fig. S2, Supplementary Material online). Further work is necessary to elucidate the mechanism underlying the observed segmental deletion events.

Rate of Ameiotic Recombination

It is generally accepted that LOH is often the result of recombination such as crossover and/or gene conversion without the overall loss of genetic material (Luo et al. 2000; Blackburn et al. 2004; Andersen et al. 2008). However, only 33% of our observed LOH events are likely due to typical crossover and gene conversion events. Our estimate of the rate of ameiotic recombination in *Daphnia*, 3.3×10^{-5} locus^{-1}·generation^{-1}, is similar to the recombination rates in other asexually reproducing eukaryotes. For example, the rate of mitotic recombination is approximately 0.8×10^{-4} per generation in *S. cerevisiae* (Mandegar and Otto 2007), 9×10^{-4} per generation (2.82×10^{-6} events·kb^{-1}·generation^{-1} on a 325-kb interval) in *Candida albicans* (Lephart and Magee 2006), and 10^{-5} to 10^{-2} per generation in *Aspergillus niger* (Debets et al. 1993). However, our rate is much lower than the rate of recombination (2×10^{-2} to 6×10^{-2} events/generation) of the ribosomal DNA (rDNA) in the same *D. obtusa* MA lines, which is at the high end of rDNA recombination rate (10^{-5} to 10^{-2} events/generation) estimated from various species (McTaggart et al. 2007). This significant rate difference could reflect either our underestimated rate of ameiotic recombination as some hemizygous deletions are likely a result of unequal crossing over or a much higher recombination rate for rDNA than for other genomic regions. It is generally recognized that rDNA tends to experience high levels of unequal crossing-over and gene conversion due to its repetitive nature (McTaggart et al. 2007).

Implications for the Evolution of Sex

The high rates of ameiotic recombination and segmental deletions in the *Daphnia* nuclear genome have strong implications for the evolution and maintenance of sex. Using a diploid single-locus model, Mandegar and Otto (2007) showed that adaptation in asexual populations can be as rapid as that in sexual populations when the rate of mitotic/ameiotic recombination (10^{-4} or 10^{-5}) is much higher than the rate of mutations (e.g., 10^{-7}). Therefore, asexual taxa can gain most of the benefit of segregation via mitotic/ameiotic recombination while avoiding the costs associated with sex (Mandegar and Otto 2007). This hypothesis provides a plausible explanation for the existence of

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putative ancient asexual taxa such as the Darwinulid ostracods and the aphid tribe Tramini and could also explain why the Meselson effect (i.e., increased allelic divergence with age due to the lack of recombination) is absent in these lineages (Butlin et al. 1998; Normark 1999). However, because the *Daphnia* deletion rate is approximately twice as high as the ameiotic recombination rate, we suggest that the long-term survival of asexual *Daphnia* lineages is likely to be negatively affected by the high frequency of deletions. Moreover, the possibility that mitotic/ameiotic recombination could lead to large-scale deletions should also be considered when examining the role of recombination in the evolution of asexual taxa.

It is often argued that most mutations detected in MA lines are deleterious because the mean fitness of the lines declines over time (Bataillon 2000). Given the *Daphnia* genome size of ~200 Mb, the high segmental deletion rate (5.5×10^{-5} bp^{-1} generation^{-1}) indicates that on average one or two DNA segments spanning a total of 11 kb are deleted per genome per generation. Moreover, considering an average gene size of 2 kb and an intergenic size of 4 kb (wfleabase.org), we suggest that about one or two genes per generation could experience large-scale hemizygous deletions. Deleterious mutations are of central importance for several hypotheses on the evolution and maintenance of sex (Kondrashov 1988). For example, the deterministic mutation hypothesis predicts that, with a deleterious mutation rate greater than one per genome per generation, sex can be maintained by its capacity to purge deleterious mutations (Kondrashov 1988). However, comparative analyses of DNA base substitutions failed to detect a genome deleterious mutation rate higher than one per generation in a range of animal species (Keightley and Eyre-Walker 2000; Cutter and Payseur 2003). The high rate of deletions in our *Daphnia* MA lines strongly suggests that segmental deletions should be taken into account as an important type of mutation when evaluating the role of deleterious mutations in the evolution of sex. It is likely that selection against deleterious mutations can provide an advantage that offsets the inherent costs of sexual reproduction. Furthermore, our study clearly shows the dynamic nature of asexual genomes that experience unexpected high rates of mutation and recombination and the mutagenic consequences of ameiotic recombination.

Supplementary Material

Supplementary figures S1–S2 and tables S1–S4 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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