Ameiotic recombination in asexual lineages of *Daphnia*

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Despite the enormous theoretical attention given to the evolutionary consequences of sexual reproduction, the validity of the key assumptions on which the theory depends rarely has been evaluated. It is often argued that a reduced ability to purge deleterious mutations condemns asexual lineages to an early extinction. However, most well characterized asexual lineages fail to exhibit the high levels of neutral allelic divergence expected in the absence of recombination. With purely descriptive data, it is difficult to evaluate whether this pattern is a consequence of the rapid demise of asexual lineages, an unusual degree of mutational stability, or recombination. Here, we show in mutation-accumulation lines of asexual *Daphnia* that the rate of loss of nucleotide heterozygosity by ameiotic recombination is substantially greater than the rate of introduction of new variation by mutation. This suggests that the evolutionary potential of asexual diploid species is not only a matter of mutation accumulation and reduced efficiency of selection, but it underscores the limited utility of using neutral allelic divergence as an indicator of ancient asexuality.

allelic divergence | loss of heterozygosity | mutation accumulation

It has long been assumed that the absence of meiosis reduces rates of homologous recombination to evolutionarily unimportant levels in asexual eukaryotes. The resultant reduction in the efficiency of natural selection is expected to magnify the rate of deleterious mutation accumulation and reduce the rate of fixation of adaptive mutations, condemning asexual species to an early extinction (1–4). Yet, despite this bleak theoretical forecast, some lineages, including the bdelloid rotifers (5), oribatid mites (6), and darwinulid ostracods (7, 8), dispensed with sexual reproduction long ago, and the majority of animal phyla have some obligately asexual species (9).

A substantial body of theory has been developed to account for the evolutionary persistence of asexual species (or lack thereof), but only a few large-scale empirical surveys have been undertaken to characterize the molecular genetic consequences of asexual reproduction (e.g., refs. 10 and 11). It has been suggested that the absence of meiosis in asexual lineages causes the two alleles at any given locus to become progressively more divergent given that within-individual recombination (gene conversion and/or crossing over) and chromosomal deletions occur at negligible levels (5, 12, 13). However, with the exception of the bdelloid rotifers, most closely studied asexual lineages fail to exhibit high levels of neutral allelic divergence (e.g., refs. 5 and 14–16). These observations call attention to other processes that might erode allelic divergence in real biological systems, such as recombination, unusually effective DNA repair, automixis, or clandestine sexual reproduction (e.g., refs. 12–16). Direct experimental observations on the genomic stability of asexual lineages are necessary to shed light on this issue.

Although mitotic recombination has been studied in many organisms for decades (e.g., refs. 17–20), recombination in the germ line of asexual species has rarely been directly evaluated. Here, we define ameiotic recombination as either the reciprocal (crossover) or nonreciprocal (gene conversion) exchange of genetic information in apomictic germ-line cells, and we investigate this process in 96 mutation-accumulation (MA) lines of two microcrustaceans: *Daphnia pulex* and *Daphnia obtusa*. The MA individuals derived from *D. pulex* were determined to be obligately asexual, whereas *D. obtusa* is a cyclical parthenogen. The MA lines were established from three separate isolates (denoted *D. pulex*: LIN, OL3; *D. obtusa*: TRE), with replicate sublines being propagated asexually by single-progeny descent in a benign environment for an average of 83 generations. Genetic changes in the MA lines were investigated by genotyping 126 microsatellite loci and sequencing 16 nuclear protein-coding loci. Spontaneous loss of heterozygosity resulting from ameiotic recombination was discovered at many loci.

Results

Of an initial microsatellite survey of 2,917 informative assays, we detected 34 instances of a locus becoming homozygous, which we refer to as loss of heterozygosity (LOH) incidents (Table 1 and Table 2, which is published as supporting information on the PNAS web site). Eighteen LOH incidents occurred in the LIN lines, 16 in the TRE lines, and none in the OL3 lines. The allele that was shorter in length was lost in 67% of the LOH incidents for LIN and in 88% of the incidents for TRE. On average, 45% of informative microsatellite loci in LIN and TRE experienced LOH in at least one line. The number of LOH incidents per individual line varied from zero to eight, although if loci that experience LOH are linked physically, these events are not necessarily independent of each other.

Of the 16 protein-coding loci, 88% for LIN, 69% for OL3, and 31% for TRE were informative in that at least one heterozygous site was observed in the assayed fragment. We detected 13 LOH incidents for these loci: seven for LIN, six for TRE, and zero for OL3 (Table 1). With only one exception, all LOH tracts encompassed the entire length (500–1,000 bp) of the sequenced fragment (Table 2).

LOH incidents from the LIN lines were mapped in linkage groups I, II, and III of the *Daphnia pulex* genetic map (ref. 21; Fig. 1). If LOH is due to crossing over, it is expected that homozygosity would exist for all markers distal to a crossover event. Indeed, this phenomenon is observed in linkage group III, where one mutation-accumulation line has lost heterozygosity for 15 adjacent markers, spanning a distance of 115 centimorgans, and appearing to extend for an entire arm of a chromosome. All other LOH tracts observed in our data set were restricted to the most terminal marker of the linkage group or less than four centimorgans in length, so for these events, we

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Abbreviations: LOH, loss of heterozygosity; MA, mutation accumulation.

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Table 1. Summary of loss of heterozygosity information for each MA lineage

Measurement	LIN	OL3	TRE
Population			
No. of lines	40	21	35
Avg no. of generations	75	58	107
Protein-coding loci			
No. of informative loci	14	11	5
No. of LOH events observed	7	0	6
LOH rate, incidents per locus·generation^{-1}	0.00017 (0.00006)	0	0.00032 (0.00013)
LOH rate, per nucleotide site·generation^{-1}	0.00018 (0.00003)	0	0.00045 (0.00008)
Microsatellite loci: Genomewide survey			
No. of informative loci	36	32	23
No. of LOH events observed	18	0	16
LOH rate, incidents per locus·generation^{-1}	0.00017 (0.00004)	0	0.00019 (0.00005)
Microsatellite loci: Linkage groups I, II, III			
Avg no. of generations	82	NA	NA
No. of informative loci	46	NA	NA
No. of LOH events observed	48	NA	NA
LOH rate, incidents per locus·generation^{-1}	0.00032 (0.00005)	NA	NA
Minimum no. of exchanges	46	NA	NA
Recombination rate, per chromosome·generation^{-1}	0.00467 (0.00069)	NA	NA

Numbers in parentheses are standard errors.

cannot differentiate between gene conversion and a double crossover. Two markers underwent LOH in at least eight individuals. For linkage group III, two individuals had experienced three similar (but not the same) LOH events.

There are several ways to quantify the rate of LOH with these data. For the protein-coding loci, the rate of incidents per locus is almost identical to the rate of LOH on a per-nucleotide basis because almost all exchanges encompass the entire locus. These rates vary considerably among the sets of experimental lines and have an overall average (including the OL3 lines that did not exhibit LOH) of 0.00016 (0.00009) per locus and 0.00021 (0.00013) per nucleotide per generation (Table 1). Similar estimates were obtained for the rate of LOH in the genomewide survey of microsatellite loci, with an average of 0.00012 (0.00006) over all lines (Table 1). A somewhat higher microsatellite-based estimate is obtained from the survey of mapped loci in the LIN lines, 0.00032 (0.00005), and from these data, the minimum rate of exchange events per chromosome per generation is estimated to be 0.00467 (0.00069).

Discussion

Could our observed instances of LOH be artifacts of processes other than ameiotic recombination? PCR artifacts, such as the preferential amplification of one allele, are unlikely to explain our data. For each putative LOH incident in our protein-coding data set, we performed an additional PCR, and many of these

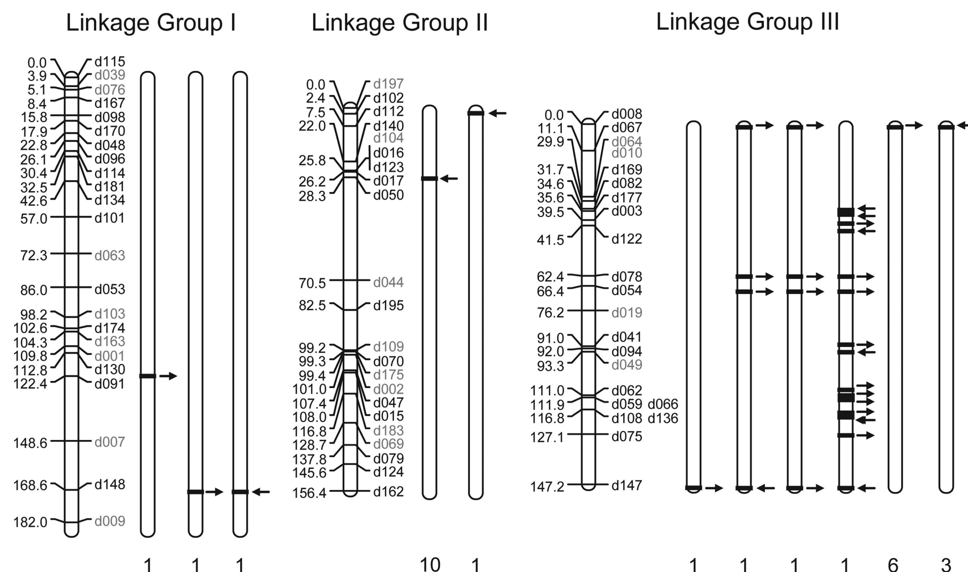

Fig. 1. Map locations of LOH in linkage groups I, II, and III for the LIN mutation-accumulation lines. The ancestral diploid state for each linkage group is shown on the left, with heterozygous (informative) loci denoted by black type and uninformative loci in gray type. Numbers to the left denote map distances (centimorgans), and numbers to the right indicate marker names. Loci revealing LOH are denoted with arrows, and numbers of individuals experiencing a particular LOH profile are given below each profile. Arrows pointing right denote loss of the shorter allele, and those pointing left denote loss of the longer allele.

confirmatory PCRs were performed with a different type of *Taq* polymerase and/or DNA extraction. Resequencing confirmed all LOH incidents included in this analysis. The occurrence of null alleles due to mutations in priming sites is a remote possibility. However, point mutations in the animal nuclear genome have been measured to occur at a frequency of $\approx 10^{-8}$ to 10^{-7} per site·generation^{-1} (22), and given an average primer length of 20 bp and the extremely conservative assumption that one mutation within a priming site causes amplification failure, the chance of a null allele at any given locus is not likely to exceed $\approx 8 \times 10^{-6}$ per locus·generation^{-1}. Because our observed rate for LOH is $\approx 10^{-4}$, it is unlikely that null alleles explain LOH in *Daphnia*. Moreover, our mapped data provides spatial evidence against null alleles. Most convincingly, in linkage group III, 15 independently amplified adjacent markers experience LOH; when linked loci show simultaneous transitions to homozygosity, ameiotic recombination is clearly a better explanation.

A general loss of heterozygosity also could arise from mating between male and female members of the same line. This is a very remote possibility for the cyclically parthenogenetic TRE lines because these lines are capable of sexual reproduction. However, the 50% reduction in genome heterozygosity that is expected after a bout of intraclonal mating was not observed in any of our MA lines. Only 3 of 35 TRE lines exhibited LOH at more than a single locus; the genomewide levels of LOH for these three lines were just 7%, 14%, and 36%. The possibility of intraclonal mating is even more improbable for the LIN lines because they are derived from an obligately asexual clone. Assays of 14 heterozygous microsatellite loci in 12 replicate hatchlings obtained from LIN dormant eggs were 100% consistent with the maternal genotype (probability = 2.7×10^{-51} under the assumption of random selfing). Moreover, most LIN individuals experiencing LOH did so for only one locus (Table 2 and Fig. 1). Thus, the possibility of clandestine sexual reproduction in our MA lines can be ruled out.

Nondisjunction or partial chromosome deletion can result in LOH, although it should be emphasized that deletions can be produced as a result of recombination (23), so their presence does not necessarily preclude the occurrence of recombination. In any event, several lines of evidence indicate that deletions are unlikely to explain our data. First, every mapped tract of LOH was linked physically with other markers that retained heterozygosity (Fig. 1), demonstrating the presence of two distinct chromosomes. Second, one mapped LOH event spanned nearly the entire mapped chromosome length, covering a distance of at least 100 centimorgans. Chromosomal deletions of this magnitude are unlikely to be tolerated by most animals because of the exposure of deleterious recessives or haploinsufficiency. Third, our observed instance of partial LOH is evidence against hemizygosity, because the cloning and subsequent sequencing of this locus revealed two distinct alleles. Although we cannot entirely rule out partial chromosomal deletions as a mechanism for LOH at some loci, very small deletions would be expected to yield unexpectedly short PCR products, which we never observed.

Studies of somatic tissues often report that LOH is due to mitotic recombination (e.g., refs. 24–27), and studies of mitotic recombination describe features of this process that we observed in our data. First, mitotic recombination has been reported to show hot spots of activity (e.g., 20, 24, 28). Hotspots of ameiotic recombination also may occur in *Daphnia*, because 21 of the 27 LIN chromosomes with mapped LOH events were associated with just two of the 46 informative markers (Fig. 1). Most LOH incidents were at the most terminal mapped marker for a given chromosome, indicating that LOH may occur more readily at distal chromosome regions in *Daphnia*. Alternatively, proximal crossovers would cause more loci to become homozygous, thereby increasing the likelihood of exposing lethal recessives, leaving the false impression of a higher recombination rate in distal regions. In either event, one prediction that should be addressed with future studies is that asexuals may be more homozygous at distal chromosome regions than their sexual counterparts, and ancient asexuals may have a greater percentage of homozygous chromosome tips than young asexuals.

Second, previous studies of mitotic recombination report negative interference such that one recombination event increases the chances that another event will occur on the same chromosome (20, 29). In our data set, two individuals had LOH profiles, suggesting that at least four exchange events resulted in two LOH tracts at the tips and one in the interior of linkage group III (Fig. 1). Excluding these two individuals from the analysis yields an estimated rate of exchange events for linkage group III of 0.00417, similar to the overall average noted above, which implies an ≈ 0.0005 probability of observing four or more events on one chromosome after 83 generations in the LIN lines under the assumption of no interference and an ≈ 0.0002 probability of observing two of 40 lines with such an extreme condition. Our data tentatively support the hypothesis of negative interference (reinforcement) between recombination events.

Thus, our direct observations are fully consistent with the frequent occurrence of recombination in asexual lineages of *Daphnia*. In our experimental lines, ameiotic recombination frequently causes LOH rates (λ) $>10^{-4}$ per site·generation^{-1}. In contrast, for animal species, the rate of base-substitution mutation (μ) is $\approx 10^{-8}$ to 10^{-7} per site·generation^{-1} (22). This implies that spontaneous LOH resulting from ameiotic recombination in *Daphnia* occurs $\approx 1{,}000\times$ faster than the rate of production of new nucleotide heterozygosity by mutational input. Although this recombination is internal and does not allow genetic exchange across lineages as in outcrossing sex, our observation of ameiotic recombination shows that one of the bedrock assumptions of "evolution-of-sex" theory, that asexual lineages acquire variation through mutational input only, is substantially violated in a real biological system.

These results challenge the view that the two alleles at any given locus will indefinitely accumulate independent mutations when sexual reproduction ceases within a diploid lineage, the so-called "Meselson effect." In the absence of any cross-talk between alleles, the expected rate of gain of sequence heterozygosity at neutral homozygous sites is 2μ, and with simultaneous occurrence of LOH, the expected equilibrium level of sequence divergence at neutral sites is $2\mu/[(8\mu/3) + \lambda]$, or between 0.0002 and 0.0020 at the rates noted above. Because the average (and SD) standing levels of silent-site heterozygosity in sexually reproducing vertebrates and invertebrates are 0.0041 (0.0030) and 0.0265 (0.0142), respectively (22), and similar values can be expected for newly arisen parthenogens, our results provide a simple explanation for the low levels of allelic divergence frequently encountered in asexual lineages. With ameiotic recombination eliminating heterozygosity so much faster than mutation replenishes it, clonal lineages are generally expected to experience losses of allelic variation over time except when initiated from unusually homozygous individuals, and even in the latter case, the increase is unlikely go beyond what is typically seen in outcrossing species.

There are two potential concerns with the generality of this conclusion. First, one set of lines in our study (OL3) yielded no evidence of ameiotic recombination. Using the average rates for the LIN and TRE lines (Table 1), the probabilities of observing no LOH incidents at protein-coding and microsatellite loci in the OL3 lines by chance are 0.0394 and 0.0009, respectively. Although we cannot rule out the possibility that the OL3 lineage has a substantially reduced recombination rate, as drastic rate differences have been reported in other systems (30), an alternative possibility is that the ancestral OL3 genotype harbors a substantial load of recessive deleterious mutations. Exposure of

inviability or sterility mutations by LOH events would prevent the propagation of lines that experienced ameiotic recombination, leading to the false impression of reduced recombinational activity. The OL3 lines were notably less hardy than either the LIN or TRE lines: We resorted to backup stocks twice as often in the propagation of OL3 lines (doubling the opportunity for selection), the number of lines surviving to the time of assay was reduced by ≈50%, and the average number of generations was reduced by ≈36% (Table 1). Sexual clones within the *D. pulex* complex often exhibit substantial inbreeding depression (31), and for this reason, even our estimated rates of recombination in the LIN and TRE lines may be downwardly biased.

A second issue of relevance is the impact of chromosomal location on the sensitivity of a locus to LOH. Although we are unable to formally distinguish between large tracts of gene conversion and crossover events for most chromosomal alterations, some regions appear to be much more vulnerable to ameiotic recombination than others (e.g., the tips of linkage-group III; Fig. 1). With a mutation rate of $\mu = 10^{-7}$, a 100-fold reduction of λ to 10^{-6} would lead to an expected silent-site heterozygosity of 0.158 under mutation-recombination balance. Because this level of divergence will act as a strong deterrent to homologous recombination (32, 33), regions with low λ can be expected to enter the domain of the Meselson effect, i.e., indefinite neutral allelic divergence. Thus, to a degree that depends on the regional constraints on recombination and the stochastic nature of the process, asexual genomes may progressively develop mosaic patterns of allelic divergence, as may be the case in bdelloid rotifers (5).

Nevertheless, our results are consistent with many other studies that have failed to find high levels of allelic divergence in asexual organisms. Recombination often has been suggested as one of several possible reasons for this observation (12–15, 34–37). However, previous studies have been unable to convincingly rule out other possible explanations for this phenomenon, such as a more recent transition to asexuality than expected (14), low mutation rate (15, 35), or sexual reproduction (14). Even studies that infer recombination based on phylogenetic or other statistical approaches suffer from the uncertainty that the detected recombination events occurred before the lineage transitioned to asexuality (37). By allowing the direct tracking of chromosomal alterations while also ensuring that sexual reproduction is not occurring, our *Daphnia* MA lines provide a convincing demonstration of substantial heritable recombination in the absence of meiosis.

Although a substantial body of theory has addressed the evolutionary advantages of sexual vs. asexual reproduction, nearly all such theory assumes that the loss of sexual reproduction is accompanied by the complete loss of recombination. Thus, our observations have three major implications for our understanding of the consequences of transitions to asexuality. First, the occurrence of ameiotic recombination helps explain the dearth of allelic divergence observed in many putatively ancient asexual lineages, underscoring the risks associated with the use of neutral allelic divergence to estimate the age of diploid asexuals and demonstrating that ancient asexuals are likely to be more widespread than previously thought.

Second, ameiotic recombination provides some evolutionary flexibility in asexual lineages. Depending on the location of recombination breakpoints and the manner in which heteroduplex DNA is resolved, asexually produced progeny may inherit alleles that are mosaics of the parental alleles, a substantial contrast to conventional wisdom that acknowledges mutational input as the only source of new variation.

Third, recombination will alter the expected extinction dynamics of an asexual population. Because 25% of the progeny experiencing an ameiotic recombination event will be homozygous for each parental chromosomal region within the tract, some offspring clones (those with a net exposure of deleterious recessive alleles) will be worse off than the parent clone, whereas a subset might experience improved fitness relative to the heterozygous parental state. Whether the latter condition will occur at an appreciable frequency will depend on the net distribution of heterozygous and homozygous fitness effects of mutations within a typical recombination tract. Deleterious mutations with the maximum effect on the viability of clonal populations are those with effects in the neighborhood of $1/N_e$, where N_e is the effective population size, because these mutations are quite vulnerable to fixation by random genetic drift while still having an appreciable effect on fitness (38). Because ameiotic recombination effectively reduces the rate of long-term inheritance of mutations by 50% while at least doubling their effects (depending on the degree of dominance) (39), the degree to which ameiotic recombination influences the viability of asexual lineages will depend critically on the extent to which the distributions of deleterious fitness effects of heterozygous vs. homozygous mutations reside within the vicinity of $1/N_e$. Because the details of these parameters remain to be determined, it is premature to say whether our results mean that the evolutionary prospects of asexuals are better or worse than previously believed, but the fact that LOH is occurring at a much higher rate than mutation indicates that conventional theoretical models for the evolutionary dynamics of asexual populations should be reevaluated.

Materials and Methods

***Daphnia* Mutation-Accumulation Line Maintenance.** Single female individuals of *Daphnia pulex* (designated LIN and OL3) and *D. obtusa* (TRE) were isolated from three temporary ponds (located in Linwood, ON, Canada; Barry County, MI; and Trelease Woods, IL, respectively). Most *Daphnia* are cyclical parthenogens, capable of both sexual and apomictic reproduction. However, obligately asexual lineages do exist (40–42), and asexual reproduction in several species of *Daphnia* has been shown to be ameiotic (e.g., refs. 43 and 44). LIN and OL3 were determined to be obligately ameiotic parthenogens, whereas TRE was determined to be a cyclical parthenogen by following established methods in ref. 45.

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 and, subsequently, maintained by following described methods in refs. 46 and 47. Our protocol for propagating lines was as follows: 10–12 (LIN), 12–14 (OL3), or 8–10 (TRE) days after the previous transfer, a single randomly chosen daughter was transferred to a new beaker, and two of her sisters were transferred into separate vessels to serve as back-ups, 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 dormant eggs (OL3 and LIN) or male (TRE) offspring. 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.

Microsatellite Loci. Protocols for microsatellite DNA amplification, genotyping, and primer design are described in Table 3, which is published as supporting information on the PNAS web site, and ref. 21. Initially, 59 microsatellite loci were genotyped in 96 individual lines (40 LIN, 21 OL3, and 35 TRE) for a total of 5,664 loci. Forty-three percent of these loci, previously mapped in *D. pulex* (21), revealed linkage groups in which LOH had occurred. An additional 67 markers were then analyzed on three of the longest linkage groups (I, II, and III) spanning a total genetic distance of 500 centimorgans and an average distance

between adjacent markers of ≈7.5 centimorgans. This linkage-group analysis only included the LIN lines (40 individuals), because the OL3 lines did not exhibit LOH, and the TRE lines are derived from an unmapped species.

Microsatellite loci were analyzed with GeneMapper Software v3.0 (Applied Biosystems). Individuals that showed evidence of LOH were reanalyzed with a different dye and an excess of *Taq* polymerase as a precautionary measure to avoid the differential amplification of one allele (48). Loci that could not be scored unambiguously or manifested a signature of differential amplification of one allele (i.e., pronounced difference in peak height between the two alleles) were excluded from the analysis.

Protein-Coding Loci. Sixteen protein-coding loci were sequenced for individuals from 96 MA lines (40 LIN, 21 OL3, and 35 TRE) for a total of 1,536 loci. Primers were designed from conserved regions in genes present in both *D. pulex* and *D. magna* cDNA libraries (Table 4, which is published as supporting information on the PNAS web site). Details about DNA extraction, PCR amplification, and sequencing of *Daphnia* nuclear loci are described in ref. 49, and we used similar protocols with the following major exceptions. One unit of *Taq* polymerase (Eppendorf, Hamburg, Germany) was used for each PCR, and PCR products were purified with solid-phase reversible immobilization (50). LOH incidents were identified as MA-line electropherograms that were devoid of polymorphisms at ancestrally heterozygous nucleotide sites. LOH tracts extended the length of the analyzed fragments except for one locus. This locus was cloned with an Invitrogen (Carlsbad, CA) TOPO TA kit, a QIAprep Spin miniprep kit (Qiagen, Valencia, CA) was used for plasmid purification, and a T7 primer was used to sequence the cloned inserts.

DNA sequence electropherograms were analyzed with CodonCode Aligner software v1.4.3. To rule out PCR and sequencing errors, LOH was confirmed by reamplification with a different *Taq* polymerase (Clontech, Palo Alto, CA) and resequencing. Only LOH events supported by data from two independent PCRs were used for our analysis. For the confirmatory PCRs, fresh DNA extractions were often used (50% of the time), and because these extractions were performed at a later date, the extracted individual was advanced in the number of generations of mutation accumulation. For all confirmatory PCRs (including microsatellite loci) that used individuals that were advanced in their number of generations of mutation accumulation, LOH events persisted across generations of asexual reproduction; this indicates that LOH occurs in the germ line and is heritable.

A BLAST search for each protein-coding locus recovered the following highly significant hits: ATP synthase epsilon chain, β-tubulin, calcium binding protein, cell division cycle protein, cleavage stimulation factor, engrailed, glyceraldehyde-3-phosphate dehydrogenase, Rab subfamily of small GTPases, myosin light chain, NADH dehydrogenase, rab11, translation initiation factor, ubiquitin conjugating enzyme, and vitellogenin fused with superoxide dismutase. BLAST did not yield a significant hit for an annotated gene for the following loci: C6C12 and G4G10.

Similar LOH Profiles in Multiple Individuals. Two individuals had similar LOH profiles that included three discontinuous LOH tracts in linkage group III. The probability of three LOH tracts being shared by two individuals should be prohibitively rare, and at first glance, this appears to be a contamination artifact of either *Daphnia* cultures or PCR. However, the direction of allele loss varies for one locus between these two individuals: one individual became homozygous for one allele, whereas the other individual became homozygous for the other allele (Fig. 1), and LOH incidents at five other loci differentiate these two individuals. For 66% of the other situations in which similar LOH profiles occurred in multiple individuals, clone contamination could be ruled out on the basis of the direction of allele loss or additional microsatellite mutations or LOH incidents elsewhere in the genome. This suggests that shared LOH profiles are a result of hotspots of recombination rather than contamination.

Calculation of LOH Rates. The LOH rate was calculated for each MA lineage (LIN, OL3, and TRE) with the equation $\lambda = h / (L \cdot i \cdot T)$, where λ is the LOH rate (per locus·generation^{-1}), h is the number of observed LOH events, L is the number of MA lines, i is the total number of informative loci, and T is the average number of generations for the MA lines. To obtain a rate per nucleotide site, h is the number of nucleotide sites that became homozygous, and i is the total number of heterozygous sites observed in the data set. The rate of ameiotic recombination per chromosome was estimated based on the linkage group analyses with the same equation. For this calculation, i is the number of informative chromosomes, and h is the number of recombination events. Adjacent loci that experienced LOH were considered to be part of the same recombination event.

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