

## Genomic clues to an ancient asexual scandal

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### Abstract

Despite abandoning meiosis, the bdelloid rotifers have persisted for millions of years and given rise to hundreds of species. Several mechanisms - allelic variants with different functions, high effective population size, and resistance to radiation - may contribute to their success.

Bdelloid rotifers are diploid aquatic microinvertebrates that live in fresh or brackish water, especially in ephemeral habitats prone to periodic desiccation. They are the only well documented lineage that has eliminated meiosis yet has persisted for many millions of years (more than 35 million years [1]) and undergone an adaptive radiation - nearly 400 species in three families. Maynard Smith [2] referred to them as an “evolutionary scandal” because they are the exception to the usual pattern that asexual lineages die out before undergoing extensive speciation.

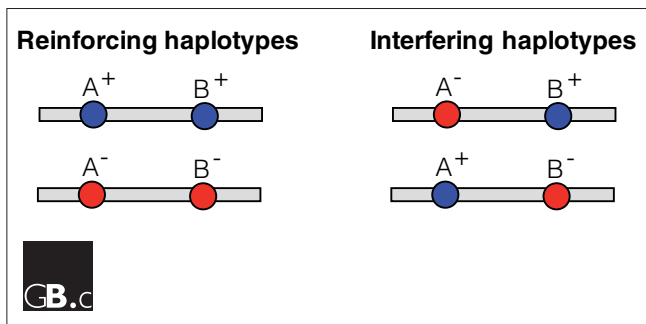
The fact that asexuals are composed entirely of offspring-producing females gives them an intrinsic demographic advantage over sexual competitors whenever males do not help to produce offspring (referred to as the ‘two-fold cost of sex’ or the ‘cost of producing males’). Evolutionary theory predicts, however, that obligate asexuals have a long-term evolutionary disadvantage, compared with sexuals, owing to a more pronounced ‘Hill-Robertson effect’, a reduction in the efficacy of natural selection that occurs because finite populations accumulate associations of linked genes (haplotypes) that interfere with selection [3,4] (Figure 1).

The Hill-Robertson effect arises when selection acts simultaneously at multiple linked sites (Figure 1). In this case, the fate of a mutation depends not only on its own selective value but also on that of its genetic backgrounds. Selection on genetic backgrounds introduces ‘noise’, which makes selection on a mutation less efficient. A similar interaction occurs between selection and random genetic drift, with smaller population sizes increasing the noise generated

by drift. The cost of the Hill-Robertson effect in asexuals can be expressed as a reduced effective population size ( $N_e$ , the size of an idealized, random-mating population with only chance fluctuations in family sizes) compared with the actual population size (census size,  $N$ ; see Box 1 for further details). Because the strength of the Hill-Robertson effect increases with tighter linkage, non-meiotic species like bdelloid rotifers, in which ‘interfering’ haplotypes cannot be routinely broken up, should have a much reduced  $N_e$  compared with their sexual competitors with similar census sizes, and should hence experience less effective selection. All else being equal, the bdelloids’ ability to compete with sexuals should erode over time, leading to their eventual extinction. This has not happened, so the bdelloids must have one or more compensating advantages. Several recent studies indicate how bdelloids may have achieved their “scandalous” status.

### Functional divergence among alleles

Pouchkina-Stantcheva *et al.* [5] have uncovered one possible counter-advantage - distinct functional divergence in the alleles of a gene. They showed recently that the bdelloid rotifer *Adineta ricciae* carries two alternative versions of a gene that is associated in other organisms with desiccation tolerance (a late embryogenesis abundant gene, with forms *Ar-lea-1A* and *Ar-lea-1B*). The two copies, which appear to be allelic, have diverged in sequence by 13.5% of their synonymous sites (that is, sites with ‘silent’ nucleotide substitutions), 12 of 136 aligned amino-acid positions, and by 132-bp indel (insertion/deletion) within an exon. Southern hybridization and fluorescence *in situ* hybridization



**Figure 1**  
 The effect of genetic linkage on the effectiveness of selection. Consider two closely linked single nucleotide polymorphisms (SNPs A and B) with one of the 'alleles' at each site favored by selection (denoted by a superscript +). Selection acts more weakly on the 'interfering' haplotypes (A<sup>-</sup>B<sup>+</sup> and A<sup>+</sup>B<sup>-</sup>), where positive selection on one SNP is counterbalanced by negative selection on the other, compared with the 'reinforcing' haplotypes (A<sup>+</sup>B<sup>+</sup> and A<sup>-</sup>B<sup>-</sup>), where selection on the two SNPs is complementary. This disparity causes interfering haplotypes to persist longer after they have accumulated by chance in finite populations. See Box 1 for further details.

indicated that only two recognizable copies of the *Ar-lea* gene were present in the genome. Marked sequence divergence among presumed alleles in bdelloids had been found previously in two other genes [6]. What distinguishes the gene investigated by Pouchkina-Stantcheva *et al.* [5] is the functional divergence between the gene copies. One copy codes for a protein that prevents desiccation-sensitive enzymes from aggregating during drying, whereas the other copy encodes a protein that lacks this activity but instead associates with phospholipid bilayers and may protect their integrity during desiccation. Pouchkina-Stantcheva *et al.* [5] interpret these results as divergence of a pair of alleles that have now taken on new functions - although they cannot unequivocally rule out other possibilities. They point out an unappreciated advantage of diploid asexuals: the lack of meiotic segregation permits alleles - at all gene loci simultaneously - to diverge in function in a manner that only occurs after genome duplication in sexual organisms. This potential may help to explain the paradox of the bdelloids' unprecedented adaptive radiation. It cannot be explained by this advantage alone, however, because many other microinvertebrates have clonal lineages that are diploid (for example, *Daphnia*, aphids and other types of rotifer), yet no similar adaptive radiation is known to have occurred in these taxa.

**High evolutionary potential**

A second relatively recent discovery is the surprisingly high genetic diversity and efficiency of selection in bdelloids. Birky *et al.* [7] measured the rate of divergence among mitochondrial genes in a sample of independently evolving bdelloid clades. Mitochondrial and nuclear genes are completely linked in clonally reproducing organisms, so both

**Box 1 The Hill-Robertson effect**

The cost of the Hill-Robertson effect in asexuals can be expressed as a reduced effective population size ( $N_e$ , the size of an idealized, random-mating population with only chance fluctuations in family sizes) compared with the actual population size (census size,  $N$ ). In natural populations, allele frequencies change deterministically owing to selection, and stochastically owing to genetic drift. The capacity for selection to be effectual despite drift is quantified by the simple metric  $Sel_{w/drift} = 4N_e|s|$  [21], where  $Sel_{w/drift}$  is an index of the efficacy of selection after accounting for drift, and  $s$  is the selection coefficient of a mutation (for example, if a mutation decreases fitness by 1% then  $s = -0.01$ ). To a close approximation, when  $Sel_{w/drift} < 1$ , drift overpowers selection, making it ineffectual, and when  $Sel_{w/drift} > 1$ , selection overpowers drift. Smaller  $N_e$  increases the contribution of drift relative to selection, so the reduction in the efficacy of selection due to asexuality (the Hill-Robertson effect) can be expressed as a reduction in  $N_e$ . Meiosis shuffles nonallelic polymorphisms each generation and thereby increases the efficiency of natural selection by breaking down interfering haplotypes, which persist in ameiotic species like bdelloids. The Hill-Robertson effect can be generated by large-effect mutations that are deleterious (background selection) or beneficial (selective sweeps) or by a mixture of favored and disfavored mutations with small effects (Hill-Robertson interference). See also Figure 1.

have the same  $N_e$ . Birky *et al.* [7] found levels of nucleotide diversity to be about 1% - a value similar to most sexual species. They also compared DNA divergence rates at synonymous and nonsynonymous sites (the latter are those at which there are amino-acid substitutions) and found no evidence for weaker purifying selection in the asexual bdelloid rotifers compared with the sexual monogonant rotifers (the bdelloids' closest sexual relatives). Using this information, Dolgin and Charlesworth [8] estimated  $N_e$  for these bdelloids to be  $10^5$ - $10^7$ , assuming a range of possible mitochondrial mutation rates per base pair of  $10^{-9}$ - $10^{-7}$ . For comparison, the  $N_e$  for humans is  $10^4$ .

Is this estimated  $N_e$  of bdelloid rotifers compatible with their antiquity and taxonomic diversification? Persistence and diversification of a lineage lacking meiosis is only hindered when the Hill-Robertson effect causes selection to be overwhelmed by genetic drift (Box 1). Using the extensive human HapMap dataset [9], it was estimated that only about 20% of nonsynonymous mutations in humans will contribute to decay (via drift overpowering selection) in our gene pool [10,11]. As bdelloids appear to have an  $N_e$  at least an order of magnitude greater than humans, their adaptive evolution is far less constrained than ours, and is

more than sufficient to permit them to persist and give rise to an adaptive radiation.

To succeed, bdelloids must be able to avoid competitive displacement by other microinvertebrates. Paland and Lynch [12] compared values of  $K_a/K_s$  (ratio of nonsynonymous mutations ( $K_a$ ) to synonymous mutations ( $K_s$ )) among sexual and clonal lineages of the microinvertebrate *Daphnia*. From this analysis they estimated that the clonal lineages were accumulating deleterious amino-acid substitutions at four times the rate of the sexual lineages, indicating that  $N_e$  was substantially smaller in the asexual lineages, and, therefore, that meiosis plays an important role in reducing mutational load. Barraclough *et al.* [13] extended this approach to bdelloids, finding evidence for weaker selection on nonsynonymous codons in bdelloids compared with sexual rotifers. These two studies indicate that lack of meiosis does have a substantial cost, and this may explain why most microinvertebrates have not produced clonal adaptive radiations. Bdelloids, however, have no close sexual relatives. If early in their evolution asexual bdelloids fortuitously eliminated all close sexual relatives, and if sexual competitors have been too poorly adapted to the bdelloids' ecological niche to compete with them, then the bdelloids' large  $N_e$  (despite an elevated Hill-Robertson effect) is compatible with their observed persistence and adaptive radiation.

### Anhydrobiosis and cryptic recombination

Why is the  $N_e$  of ameiotic bdelloids so large? One factor is the enormous census size of this microinvertebrate, but another less obvious factor may be their use of anhydrobiosis to accommodate to the periodic desiccation of their environment. Anhydrobiosis is a non-metabolizing state of life induced by extreme dehydration that enables the organism to survive such conditions, and bdelloid rotifers share this adaptation with many other small animals, and with some plant seeds and bacteria. Studies in such bacteria have shown that anhydrobiosis is associated with the production of numerous double-strand-breaks (DSBs) in the DNA, with a longer duration of anhydrobiosis leading to more numerous DSBs. In response, the bacteria have evolved an increased capacity for DSB repair. Interestingly, these adaptations to anhydrobiosis have fortuitously produced high resistance (more than 100 times normal levels) to ionizing radiation, which also produces DSBs [14]. Although detailed molecular studies are lacking, the fact that anhydrobiotic microinvertebrates such as tardigrades [15] and bdelloid rotifers (as briefly described in [16]) are also highly resistant to ionizing radiation indicates that they too are capable of repairing the numerous DSBs that would be generated during anhydrobiosis.

From bacteria to vertebrates DSBs are repaired primarily by a pathway that includes genetic exchange (via formation

of heteroduplex DNA and gene conversion) between homologous chromosomes [17]. An episode of anhydrobiosis in bdelloids is expected to lead to many DSBs and hence to many mitotic gene-conversion events that will sometimes lead to mitotic crossing-over. Bdelloids may be unusually susceptible to DSBs during anhydrobiosis because, unlike most other organisms known to be capable of anhydrobiosis, they do not produce the disaccharide trehalose, which promotes tolerance to desiccation [18]. Because of anhydrobiosis, bdelloid rotifers are expected to have a limited form of 'selfing' due to far higher levels of mitotic recombination than in organisms lacking anhydrobiosis. Such mitotic recombination must be rare, however, as pairs of alleles with nearly identical sequences have not yet been found in bdelloids.

On a more speculative note, the ecology of bdelloids may also cause them to experience limited amounts of out-crossing via transformation. Bdelloid rotifers are filter feeders that consume substantial amounts of dead or decaying material. As a consequence, DNA will be transferred between dead and living individuals, and this can potentially lead to recombination via transformation, especially when one considers the degradation of cell membranes that occurs during anhydrobiosis. This possibility is supported by recent comparisons of alleles shared by different bdelloid lineages [19] in which one author concluded [20] that "some virtually identical alleles are shared between otherwise divergent species [of bdelloids], suggesting that bdelloids may have some unusual means of occasionally sharing genetic material."

Interestingly, the need to repair DSBs after anhydrobiosis will partially offset the advantage of allelic divergence reported by Pouchkina-Stancheva *et al.* [5] because divergence of homologs will interfere with homology-dependent repair [17] and thus other, more error-prone, repair pathways will be used. Bdelloids could solve, and might have already solved [20], this problem on an evolutionary time scale by increasing their ploidy [19], as has occurred in anhydrobiotic bacteria, and hence generating new targets for homology-dependent DSB repair.

So do the recent studies of bdelloids indicate that they are an "evolutionary scandal"? Their high radiation tolerance [16] and the sharing of nearly identical alleles between otherwise distantly related lineages [20] indicate that bdelloids are not devoid of all recombination. So the scandal may be more gossip than fact. Nonetheless, the high divergence among presumed alleles [5,6] indicates that bdelloids are almost completely asexual, so their success does indicate that a life without meiosis is not precluded by the reduction in  $N_e$  due to Hill-Robertson interference, at least in abundant organisms like bdelloids that lack closely related sexual lineages. At present, our window into the bdelloid genome is limited by the small number of genes (about a half dozen) that have

been sequenced. Genome projects on bdelloids and their sexual relatives are critically needed to provide broader comparisons for evaluating the scandal status of this unique taxon.

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