

Bdelloid rotifers revisited

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Sexual reproduction is one of the most striking features of the biological world. The great majority of the animals and plants with which we are most familiar reproduce at least part of the time by means of sexual reproduction with meiosis, recombination, and fertilization. But many eukaryotes reproduce asexually part of the time, and some are exclusively asexual (1, 2). Two articles in a recent issue of PNAS (3, 4) answered some questions about one such group, the bdelloid rotifers, and raised some new questions.

Rotifers are microscopic freshwater invertebrates. The class Bdelloidea (5, 6) consists entirely of females reproducing by apomixis, in which diploid eggs produced by mitotic division develop parthenogenetically into females. Their closest relatives are in the class Monogononta, which reproduce mostly by apomictic parthenogenesis, with an occasional one-generation sexual cycle. The bdelloids are believed to descend from a parthenogenetic female monogonont that lost the ability to enter a sexual cycle. Fossil evidence shows that the bdelloids are at least 35–40 million years old (7); the diversity of their gene sequences (8) suggests they are more than twice that age. In that time, the descendants of the first bdelloid diversified into >360 species and occupied nearly every freshwater habitat on every continent (5, 6).

Are Bdelloids Really Asexual?

The success of the bdelloids seems to contradict the population genetic theory and empirical evidence, which show that asexual animals have shorter evolutionary life spans and less ability to diversify than do sexual organisms (1, 2, 9). Consequently, the few old, diverse asexual groups have been called “ancient asexual scandals” (10, 11), and their asexuality has been questioned.

The two articles recently published in PNAS (3, 4) described the latest steps in an ongoing molecular evolutionary analysis that provides convincing evidence of bdelloid asexuality. One of the genetic criteria that can be used to verify the absence of sex (11, 12) is allelic sequence divergence: in a diploid apomictic lineage, the two alleles of a gene are expected to accumulate different mutations over time and gradually become more different in sequence (Fig. 1; refs. 8, 13, and 14). Allelic sequence diver-

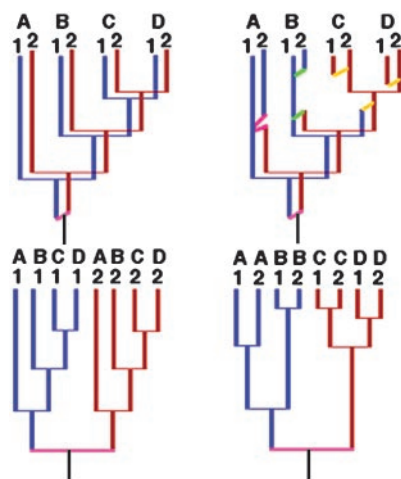


Fig. 1. After allelic sequence divergence has begun in an asexual species, the sequences of the two alleles of a gene follow separate paths during subsequent speciation events (Upper Left; A–D are species; 1 and 2 are alleles in each species, following blue and red paths, respectively; divergence begins at the pink line). Consequently, the alleles have separate evolutionary trees (Lower Left), each of which reflects the true evolutionary history of the organism in which species C and D form a clade. (Upper Right) The same tree as the Upper Left but with allelic sequence convergence events. In species A, mitotic recombination makes the alleles identical, after which they begin to diverge again (pink lines). In species B, the chromosome carrying one allele is lost by nondisjunction; subsequently a second nondisjunction restores diploidy for the chromosome (green). In the ancestor of C and D, one allele is deleted and is subsequently replaced by duplications in C and D (yellow). The resulting tree is incorrectly rooted.

gence is limited by natural selection favoring maintenance of two functional copies of a gene. It can be stopped temporarily by mitotic recombination in the germ line, which makes genes or segments of chromosomes homozygous (allelic sequence convergence). Another convergence mechanism is the loss of a chromosome, and one allele, by nondisjunction. This results in an aneuploid; a second nondisjunction event will restore diploidy, but all the genes on the chromosome will be homozygous.

Mark Welch and Meselson (8) realized that the best way to measure allelic sequence divergence is to count sequence differences that do not change amino acids; these synonymous sequence differences are expected to be selectively neutral, or nearly so, and free to accumulate within an asexual lineage.

Meselson’s insight was applied to four bdelloid species: *Adineta vaga* (family Adinetidae), *Habrotrocha constricta* (Habrotrochidae), and *Macrotrachela quadricornifera* and *Philodina roseola* (Philodinidae). Protein-coding nuclear genes were amplified from the descendants of single females, and the amplification products were cloned and sequenced. For each gene, the most similar copies differed at 36–73% of synonymous sites, much greater than the 0–2.4% difference between alleles seen in monogonont rotifers and other sexual animals. This is appropriately called the Meselson effect and is strong evidence for long-term asexual reproduction.

However, these studies left open the possibility that the two alleles of each gene were not detected because they are identical, as would happen if the bdelloids were sexual but strongly inbred or had low mutation rates. The highly divergent genes would be duplicates, not alleles. Now this possibility has been ruled out by one of the recent reports in PNAS (4). *P. roseola* has four copies of the *hsp82* gene; these were used as probes in fluorescent *in situ* hybridization to embryonic chromosomes. Each probe detected only one copy of the gene on one chromosome; because each chromosome contains only one DNA molecule (15, 16), all copies of the *hsp82* gene were detected, and two of them must be the alleles. Also, the karyotypes found in this article confirm earlier studies (17–20) showing that bdelloid chromosomes are heteromorphic, another hallmark of asexuality.

Sequence analysis of other asexual organisms, including the darwinulid ostracods (21), the parasitic diplomonad *Giardia lamblia* (22), and two species of the protist *Acanthamoeba* (Robert Rumpf, personal communication), failed to show the Meselson effect. The absence of high sequence divergence is most surprising in the darwinulids, where there is very convincing morphological evidence for ancient asexuality from the study of both living and fossil animals (23); presumably, they have frequent sequence convergence.

See companion articles on pages 1618 and 1622 in issue 6 of volume 101.

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Are Bdelloids Anciently Asexual?

Another intriguing result from the Meselson laboratory is that different alleles appeared to have separate phylogenetic trees (8). This separation is a consequence of allelic sequence divergence; once the two alleles of a gene begin diverging from each other, their most recent common ancestor will precede all subsequent speciation events (Fig. 1). Phylogenetic trees of the *hsp82* and *tbp* genes were interpreted as showing separate trees for two alleles, preceding the separation of three of the four bdelloid families. Now this conclusion is called into question by a reexamination of the *hsp82* and *tbp* trees that was reported (3). New phylogenetic trees were made by using more accurate methods, and more outgroups were included for the *hsp82* trees. These trees no longer clearly distinguish between models in which gene copies began to diverge before the bdelloid radiation or after the separation of the three families. Some caveats are required. Even if one tree were strongly supported, it would not be clear whether the earliest divergence represented the start of allelic sequence divergence or a duplication of the gene. Moreover, there could be any number of undetected episodes of allelic sequence divergence followed by convergence. Unfortunately, some combinations of these events can make it impossible to recover the correct topology (Fig. 1). How can these events be unraveled? Sequencing the regions

flanking the genes may help to determine which copies are alleles and which are duplicates. Mitochondrial genes can be used to find the correct tree structure, because stochastic processes ensure that all copies of the mitochondrial genome in an individual are nearly identical (24). Until this or other additional information is used to deduce the true tree and to differentiate between alleles and paralogues, we will not know exactly when and where sexual reproduction was lost, only that it was a long time ago.

Do we really need the Meselson effect to believe that darwinulids and bdelloids are ancient asexual organisms? In both groups, males or hermaphrodites would be easily recognized, but none have been seen among many hundreds or thousands of individuals. Negative evidence to be sure, but biologists routinely accept conclusions based on much smaller sample sizes. Whether bdelloids have always been asexual is a more difficult question. The phylogenetic analyses (8) allow the possibility that sexual reproduction was lost independently on at least three different occasions. It is more parsimonious to suppose that sexuality was lost only once in the bdelloids, but this argument would lose some of its force if their sexual ancestors were preadapted for asexual reproduction.

Are Bdelloids Really Scandalous?

A major barrier to accepting the asexuality of the bdelloids, darwinulids, and

other ancient asexual organisms is the perception that their great age and diversity contradict theory and empirical observation. It is true that population genetic theory shows that asexual organisms should have higher rates of extinction and lower rates of speciation than otherwise similar sexual organisms (9). However, this theory cannot be used to predict the fate of an asexual lineage because we lack good estimates of the numerous parameters such as population sizes, mutation rates, and intensity of natural selection. Empirically, one could say that an asexual group is scandalous if its age falls well outside the distribution of ages for similar asexual organisms. However, those distributions are unknown. Although asexual animals are mostly confined to single genera or families, this only shows that asexual organisms are not very diverse morphologically, and morphological diversity may not reflect the diversity of evolutionary units, especially in invertebrates. Because different groups of eukaryotes may differ in the extent to which the loss of sex is detrimental, the age and diversity of asexual organisms should be compared to that of closely related sexual organisms. In the case of the bdelloids, we lack a detailed phylogenetic tree of rotifers that would show whether their sister group is all of the monogononts or only one branch. There is still much to unravel in the evolutionary mystery of what happens when sexual reproduction is lost.

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