Chapter 10. Asexual Speciation

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The Importance of Asexual Species

There is a general consensus among biologists that species are real and important units of biological diversity, and understanding the mechanisms of speciation is a hot research topic (Coyne and Orr 2004). Nearly all of that work is focused on species and speciation in animals and plants that reproduce sexually. But it is also critically important to understand species and their origins in asexual organisms such as parthenogenetic animals and plants. A substantial part of the earth’s biological diversity consists of organisms that reproduce only asexually so far as we know, or reproduce sexually very rarely or with extreme inbreeding so that they are effectively asexual or clonal. Asexual organisms include not only the parthenogenetic animals and plants that are the subject of this book, but also many fungi; some green algae, including the large and ancient genus *Chlorella*; and many other eukaryotic protists, including possibly all of the members of one of the five major groups of eukaryotes, the Excavates. In addition to eukaryotes, many bacteria are largely asexual, although levels of homologous recombination vary and may be high enough to confer evolutionary advantages of sex in many groups (Cohan 2004; Ochman, Lerat et al. 2005). Many asexual eukaryotes and prokaryotes are
medically important parasites (see chapter $$ of this book by deMeeus et al.; also Ayala 1998), agricultural pests, or invasive species (Mergeay et al. 2006).

A deep understanding of what asexual species are and how to find them is a prerequisite for testing theories about the evolutionary advantage of sex, i.e. about the evolutionary consequences of losing sex. For example, theory predicts that asexual organisms should accumulate a higher load of detrimental mutations (Muller’s ratchet) than do sexual organisms, resulting in a higher extinction rate (Otto and Lenormand 2002). It is useful to test for the accumulation of detrimental mutations on evolutionary lineages between species as well as within species. Theory also predicts that asexuals should be less able to fix advantageous mutations (Orr 2000), perhaps resulting in both higher rates of extinction in changing environments and lower rates of speciation. Testing this theory obviously requires models of species (species concepts) and operational definitions (species criteria) that identify comparable evolutionary units in asexuals and sexuals.

Even determining whether a group of organisms is asexual requires knowing species boundaries. Some methods use population genetic analyses to look for linkage disequilibrium, while others look for incongruence in the phylogenetic trees of different genes within a species. Rare cases where genes are shared between individuals could be due to infrequent outcrossing and recombination within a species, or to horizontal transfer between species; only the former is sexual reproduction, although horizontal transfer may play an important role in ecological speciation in bacteria (Cohan 2004).

Clearly the use of population genetic methods to test whether a species is sexual or asexual is facilitated by having a species concept that is compatible with both asexuals and sexuals.

In spite of the importance of asexual organisms and species, the nature of species is even more controversial in asexual organisms than it is in sexuals; even the existence of species in asexual organisms has been questioned (Maynard Smith and Szathmary 1995). In this review we focus on the question of when and how speciation can occur in parthenogenetic animals and plants and other asexuals and on how species can be identified. We will show that asexual organisms are similar to sexual organisms in that they can undergo allopatric speciation due to physical isolation or sympatric speciation as a result of divergent selection for adaptation to different habitats. We also describe two different criteria for assigning individuals to species, and compare the resulting species to sexual species defined by reproductive isolation or other criteria.

**General theory of speciation and species in asexuals**

Theoretical studies of the evolutionary consequences of losing sex (aka the evolutionary advantage of sex) have shown that asexual organisms should accumulate more detrimental mutations than do sexual species and in consequence should have a higher rate of extinction. Also asexual organisms should be less able to fix advantageous mutations, which can also contribute to extinction in a changing environment, as well as making it more difficult to speciate by adapting to different niches. To test these theories we need to compare speciation rates in asexual and sexual organisms. This requires a
species concept or concepts that identify comparable evolutionary units in asexual and
sexual organisms. This is especially difficult because the very existence of species in
asexuals is controversial. Many people believe that asexual (and clonal) organisms will
form a continuum of genetic variation with no clearly distinguishable species.

To address this problem, we (Barraclough et al. 2003) developed a general theory of
speciation for asexual and sexual organisms. We focused on one commonly used
criterion, namely the very general observation that many, if not all, organisms fall into
discrete clusters of individuals with very similar genotypes and phenotypes, well-
separated from other such clusters. Our theory of the nature and origin of these clusters
can be developed as follows:

We begin by considering speciation in an established asexual lineage or inclusive
population descended from a single asexual individual. That individual could have arisen
either by loss of sexual reproduction in an organism that alternates asexual and sexual
reproduction, or by hybridization between two obligatory sexual species. Because all the
genes in an asexual organism are completely linked and behave as a unit, the origin and
ploidy of the asexual lineage are irrelevant (with exceptions described below). We further
assume that the asexual lineage is evolving as an independent evolutionary arena,
separated physically or by adapting to a different niche from its parent lineage(s).

The diagrams in Figure 1 show phylogenies of a gene in an asexual organism under
several different scenarios. Note that because there is no outcrossing and recombination,
the phylogeny of one gene is also the phylogeny of all the genes in the organism, and the
phylogeny of the individuals as well. This model assumes that all the copies of the gene
are orthologous, not the products of gene duplications and losses that can distort
phylogenies. Similar problems can arise in diploids that show the Meselson effect or in
polyploids (Birky 1996). These problems can be avoided by using mitochondrial or
chloroplast genes.

Figure 1a is a naïve model of the phylogeny (or genealogy) of a gene in a clone. For
simplicity it is assumed that the organism is reproducing by simple binary fission without
limit: each individual leaves two offspring in the next generation. In this model,
different lineages acquire different mutations so that different copies of the gene
gradually diverge from each other. At any one time the individuals in a clone show a
continuous distribution of sequence differences. If this model accurately reflected the
genealogy of a clone, there would be no species by any definition. But the model is
completely unrealistic, because it assumes that all individuals divide in synchrony and
there is no limit to the growth of the clone.

In real life, growth of the clone is limited by finite resources that set an upper limit on the
total size of the population (the census size N). Moreover, some individuals reproduce
more often than others. This is partly because of stochastic effects, which causes random

genetic drift of allele frequencies in population genetic theory, and partly because of
natural selection. Figure 1b shows a more realistic model in which the population size is
limited by the carrying capacity of the organism’s habitat. The stochastic loss of lineages
has created clusters separated by shallow gaps. These gaps will have a depth of
approximately $2N_e$ generations on average, and the clusters they form will be transient
with an average lifetime of approximately $2N_e$ generations. The clusters would not be
considered different species by population geneticists. They do form clades and might
have morphological differences that would allow them to be distinguished in the field.
However, they are not adapted to different ecological niches and are not evolving
independently. They are not comparable to ecological or sexual species, which can
coexist indefinitely even in sympathy.

**Figure 1 here**

Longer-lasting clusters can be formed by allopatry or by divergent selection, as illustrated
in Figure 1c. In this example, two individuals in the first generation were physically
isolated, e.g. by dispersal to distant locations. In the second generation two individuals in
one location were selected for adaptation to different ecological niches while remaining
sympatric. Consequently the offspring of these three individuals formed independently
evolving populations. Each one forms an independent arena for mutation, random drift,
and selection. Each one shows transient clusters separated by shallow gaps less than $2N_e$
generations deep. However, they are separated by long-lasting gaps with depths
significantly greater than $2N_e$ generations. Figure 1d shows the tree with extinct lineages
pruned; this is the realized tree that is actually observed using sequence data from extant
individuals.

The resulting populations show several characteristics that are widely, although not
universally, considered to be desirable traits in species: (i) they are independently
evolving lineages, as in the Evolutionary Species Concept (EvSC, Simpson 1951; Hey
2006); (ii) they are reciprocally monophyletic, so that every individual is more closely
related to another member of the same species than to any member of another species;
and (iii) assuming that phenotypic evolution is neutral or under divergent selection, they
will form distinct phenotypic clusters reflecting their significant differences in genotype.
In addition, the two populations on the left are adapted to different niches will be
different species by the Ecological Species Concept (EcSC, Van Valen 1976); the
population on the right is a different ecological species from at least one of these and over
time is likely to diverge ecologically from both.

At least two of the clusters meet an important criterion under the Biological Species
Concept (BSC), namely persistence in sympatry. It is unlikely that the two species on the
left in Figure 1c can ever merge again, as this would require reversal of the evolutionary
steps that adapted them to different niches. In this sense they are equivalent to BSC
species, which will not fuse even in sympathy because they are reproductively isolated.
Hey (2006) argued that most or all species concepts are really variants on the EvSC, or
are species criteria for the EvSC, working definitions for assigning individuals to
evolutionary species. The model of Barraclough et al. (2003) can also be considered a
variant of the EvSC. However, unlike Simpson’s original description of the EvSC and
subsequent versions, our model has an explicit theoretical basis in population and
evolutionary genetics. Moreover it generates explicit criteria and associated statistics for
identifying species from finite samples of specimens, as described below. In view of these differences, one of us has proposed that it be considered a new model called the Evolutionary Genetic Species Concept (EGSC, Birky in preparation).

Barraclough et al. (2003) justified the above theory in terms of coalescent theory, but the same theory can be derived from classical population genetic theory where $2N_e$ generations is the approximate expected time to the most recent common ancestor of the entire population. Note that although coalescent theory is frequently considered to be a neutral theory, one can define $N_e$ so that it takes selection, including hitchhiking, into account; this $N_e$ is smaller and the time to the most recent common ancestor is reduced accordingly. The coalescent approach provides an intuitive conceptual linkage to phylogenetic analysis of gene sequences.

**Species criteria for assigning individuals to species**

Models of the nature of species do not directly address the practical problem of assigning individuals to species. The task of identifying species in a collection of individual organisms, and of assigning newly collected individuals to those species, is called a species criterion. Consideration of expected patterns of clustering outlined above suggests two different approaches to assigning individual asexual organisms to evolutionary genetic species.

**The 4X rule**

The first species criterion, called the “4X rule” (Birky et al. 2005), has two characteristics that are uncommon among species criteria: (i) it explicitly recognizes that species are identified on the basis of samples, usually very small samples, of very large populations of individuals; and (ii) the probability that a species assignment is incorrect is set in advance at $\leq 0.05$. The 95% confidence limit is widely used in biology but more or less stringent intervals can be used if desired.

The 4X rule works as follows:

1. DNA is extracted and one or more genes are amplified and sequenced from each of a sample of individuals collected from nature. Mitochondrial or chloroplast genes are used because they are nearly always homoplasmic (only one allele in high frequency in each individual); consequently one can sequence amplified mitochondrial genes directly without having to clone them.
2. Phylogenetic trees are made of the resulting sequences and used to identify reciprocally monophyletic groups of sequences. These clades are samples from putative species.
3. The matrix of pairwise sequence differences is examined to find putative species (clades) that obey the 4X rule: the sequence differences between individuals in different clades are $\geq 4X$ greater than the mean pairwise sequence differences within the clades. More specifically, the mean pairwise sequence difference
within a putative species is used as an estimate of the nucleotide diversity in the species.

The theoretical rationale for the rule is illustrated in Figure 2. As (Barraclough et al. 2003) noted, gaps formed within asexual species by stochastic processes will be $2N_e$ generations deep on average; the 95% confidence interval is $4N_e$ generations. Consequently, one can infer with 95% confidence that gaps deeper than $4N_e$ generations separate independently evolving populations, i.e. EG species. The sequence difference between individuals in two such populations, corrected for multiple hits, will be $K = 8N_eu$ where $u$ is the mutation rate per site per generation. At the same time the mean sequence difference between individuals within each population, called the nucleotide diversity, will be $\pi \approx 2N_eu$. The ratio of the sequence difference between populations to the difference between individuals within a population is $K/\pi \geq 8N_eu/2N_eu = 4$.

Of course we cannot get sequences from every individual in a species; species are identified on the basis of samples, usually very small, from populations that are usually very large. Fortunately, sampling theory by Rosenberg (2003) shows that if samples of two or more individuals from two different populations separated by $\geq 4N_e$ generations are reciprocally monophyletic, then the entire populations are reciprocally monophyletic with probability $\geq 95\%$. If one population is represented by two individuals and the other by only one, the probability is still approximately 94%. This remarkable statistical power is possible because the probabilities are conditioned on the samples being reciprocally monophyletic, and because after $4N_e$ generations it is very likely that the species as a whole consists of a single clade which is necessarily the same as the sample clade.

This criterion will fail to identify populations that have recently begun to evolve independently but have not had time to show the phylogenetic signatures of independent evolution. The proportion of species missed will depend on the ratio of the speciation rate to $2N_e$ generations. Of course very rapid speciation events can confound species recognition with any species concept or criterion.

An alternative framework for considering whether a clade has diversified into distinct genetic clusters is to consider branching models (Pons et al. 2006). This approach is more complex than the 4X rule, but allows for a global test of whether the study clade has diversified into separate species or not. Under a null model that the entire sample derives from just a single asexual population, i.e. without divergence into independently evolving or ecologically distinct species, then the pattern of branching is expected to conform to a standard coalescent for a single population (Fig. 1b). A large body of theory is available to specify the likelihood of a given pattern of branching under a particular model (Hudson 1991), ranging from a neutral coalescent in a population of constant size, through to populations that have changed size through time or experienced different forms of selection (Rosenberg and Nordborg 2002).
Under the alternative model that the clade has diversified into species, as defined above, then we expect to observe distinct genetic clusters (Fig. 1d). Branching events within each cluster will reflect coalescence within populations of the same kind considered in the null model, but in this case with each cluster representing a separate, independently evolving population. Branches connecting the different clusters now represent the timing of divergence events, for example events causing geographic isolation or the onset of adaptive divergence into different niches. In other words, branching within clusters reflects population processes, whereas branching between clusters reflects diversification. Again, a large body of theory is available to specify the likelihood of branching times in a phylogeny depending on different models of speciation, extinction and sampling of species (Nee et al. 1994; Barraclough and Savolainen 2001; Nee 2001).

Pons et al. (2006) combined previous approaches for considering population coalescence and branching times within phylogenies to derive a general expression for the likelihood of branching times under the alternative model that the sample derives from a set of independently evolving populations. The model relies on the waiting intervals between successive branching events as input data (Fig. 2, Pons et al. 2006). Under a relatively broad set of circumstances, the net observed branching rate within species is expected to exceed that between species, hence the signature of clusters can be detected as a transition from slow to fast branching rates occurring near the tips of the tree (Fig. 1d). The likelihood of models with and without a transition in branching rate can be used to test whether the alternative model provides a better fit to the data than a null model in which the entire sample conforms to a single branching process. The models were modified to allow a range of qualitative departures from standard assumptions, such as constant population size, neutrality or constant speciation rates. (Pons et al. 2006) applied the approach to a sample of tiger beetles from salt lakes in Australia and showed that this lineage had diversified into a set of independently evolving species. The method is particularly suitable for asexual clades in which any single locus is expected to reveal the species history, not just the history of that gene.

In principle the model of speciation and the criterion for finding species that we have described can be applied to any asexual organism. However, in keeping with the theme of this volume, we will focus on parthenogenesis, which is limited to animals and plants. Moreover, our criteria can only be applied to lineages that (i) are thought to have been reproducing without sex long enough to have completed speciation, and (ii) have a suitable data base of gene sequence data. This limits us to two groups of animals, the bdelloid rotifers and oribatid mites.

**Application to bdelloid rotifers**

The bdelloid rotifers are an ancient asexual group that includes more than 374 species defined by phenotype, principally morphology (see chapter $$ of this book by Mark Welch et al.). Birky et al. (2005) applied the 4X rule to 110 specimens of bdelloid rotifers. These specimens were obtained from more than 34 sites in 7 states in the U.S. and 1 site in Italy. Morphological study revealed that they included members of six
genera. The collecting sites included permanent and temporary springs, streams, ponds, lakes, soil and moss from a wide range of ecosystems between sea level and 3660 meters. A 591 bp segment of the mitochondrial *cox1* (aka *COI*) gene was amplified and sequenced from clones of females descended from each specimen. Phylogenetic analysis (Figure 3) revealed the presence of a number of clades, including 21 terminal clades that obey the 4X rule and are simple, i.e they contain no such clades within them. The mean sequence difference among individuals in a clade ranged 0 – 2%, similar to other invertebrates (Avise 1994). These are samples from inclusive populations that are evolving independently because they are adapted to different niches or are geographically isolated. Moreover they have been separated long enough to become reciprocally monophyletic; they are species under the evolutionary and evolutionary genetic species concepts. There are also 14 singlets, individuals that are not part of any of these species; most or all of these singlets are separated from each other and from the species by such large sequence differences that they almost certainly represent samples of size one from additional species.

*Figure 3 here*

We also analyzed branching rates to find species in this sequence data set. The maximum likelihood solution for the independently evolving clusters model infers the presence of twenty-four clusters, with the remaining individuals inferred to be singletons (Fig. 3). Confidence limits within 2 log likelihood units of the maximum likelihood solution range from 22 to 25 clusters. The pattern of clustering is significantly greater than expected under the null model that the sample derives from a single population (likelihood ratio test, $X^2 = 108.5$, $p<0.0001$). Overall, the branching rate method resolves clusters more finely than the 4X rule approach: 8 clusters found in the latter are split into more clusters by the former. One possible explanation is that if a few populations harbor greater genetic diversity than the predominant trend, then they may tend to be split by the branching rate model because it assumes a single threshold for the transition from between- to within-cluster branching. Future implementations that allow for different genealogical branching rates within different clusters are in development (TGB unpublished data). An alternative explanation is that, by treating each case individually, the 4X rule may be more conservative: knowing the typical level of within cluster diversity across the sample may allow the branching rate method to split clusters that haven't diverged by 4X the intraspecific variation. Simulations of known scenarios could be used to compare the power of the two methods. Power aside, the branching rate method has the advantage of adopting a global analysis of the data, which facilitates hypothesis testing of different scenarios, whereas the 4X rule is simple to apply while still founded on robust population genetic theory.

Evidence for genetic clusters is consistent with independently evolving entities, but the nature of those entities remains in question. One possibility is that some or all of them evolve independently solely as a result of geographic isolation. Traditional accounts have tended to exclude such cases from consideration as species, because they cannot explain the existence of alpha-diversity, i.e. coexistence of species in a single area. Our analyses provide clear evidence of distinct clusters present in sympatry over successive years, but
it remains possible that their coexistence is transient or a feature of ongoing invasion from source areas. A more direct approach is to look for evidence of ecological divergence between clusters. Birky et al. 2005) found that Pha2 and Pha3 differ in temperature tolerance range; clones of Pha2 survive and reproduce at 36° while clones of Pha3 do not. However, it is not clear whether this difference detected in the lab reflects differences in their habitat.

Fontaneto et al. (submitted) performed a direct test for divergent selection on presumed eco-morphological traits in the genus Rotaria. If morphology has evolved neutrally or under constant selection among all lineages, we expect levels of intraspecific and interspecific variation to mirror levels of neutral genetic variation (McDonald and Kreitman 1991). However, if there has been divergent selection on morphology, for example as a result of divergence to occupy different niches, then we expect more eco-morphological divergence between species than within them. This pattern was indeed found in Rotaria, consistent with their adaptive radiation rather than simply neutral divergence in geographic isolation. Interestingly, morphological coherent clades experiencing divergent selection often contained two or more genetic clusters: these could either be solely the result of geographic isolation, or they may have diverged in unmeasured phenotypic traits, such as behaviour, life-history or other morphological traits. Now that the molecular systematics of bdelloids is being established, more work is needed to explore the biological significance of the genetically distinct clusters. All but one of the traditional species in the genus were found to comprise monophyletic clades, but many of them subsumed several genetic clusters: more resolved morphological techniques may be needed to provide non-genetic means for cluster identification.

**Application to oribatid mites**

Besides the bdelloid rotifers, sequence data suitable for detecting species are available for only one other ancient obligate parthenogenetic lineage, the oribatid mites. This is a diverse and widespread group of soil-dwelling mites that includes a number of apparently obligate parthenogenetic lineages (see chapter $\S$ of this book by Heethoff et al. for a detailed discussion). Some of the parthenogenetic lineages are quite ancient and speciose. Heethoff et al. (2007) obtained cox1 sequences of 65 specimens of the morphological species Platynothrus peltifer collected from Europe, Russia, Japan, and the United States. Phylogenetic analyses revealed seven clades within this species; sequence differences within these clades were less than 2%, while the differences between clades averaged 56% after correction for multiple hits. Using their data kindly provided by Michael Heethoff, we found that all seven clades qualify as species under the 4X rule. The branching rate recognizes 7 clusters plus one for the outgroup (Fig. 4). At present it is not clear if the seven clades of P. peltifer can be distinguished by morphology. However, there is some evidence that they have been physically isolated by continental drift and mountain uplifts (Heethoff et al. 2007 and chapter $\S$).

*Figure 4 here*
Predictions about relative speciation rates in sexuals and parthenogens

We have argued that asexual organisms should diversify into independently evolving and biologically distinct species broadly equivalent to sexual species. Evidence from bdelloid rotifers and oribatid mites supports this claim. But the question remains whether asexuals should diversify to a greater or lesser extent than sexuals. On the one hand, sexuals need to evolve reproductive isolation before diversity can evolve and be maintained in sympatry (Futuyma 1987; Eldredge 1989). Because asexuals have no such requirements, we might expect them to evolve sympatric diversity more easily. However, sexual organisms are expected to adapt more quickly to changing environments: if the rate of adaptation to new conditions were the limiting step in diversification, sexuals might have the edge over asexuals. Finally, if the events leading to geographic isolation or the onset of divergent selection were actually the limiting step in diversification, rather than rates of adaptation or the origin of reproductive isolation, then both sexuals and asexuals might have similar expected rates of diversification, all other things being equal (Barraclough et al. 2003). Therefore, the answer to the question could provide insights into which steps in the process of diversification are most important for determining speciation rates within clades.

Unfortunately, there are a number of difficulties in performing a rigorous test of these ideas. By far most origins of asexuality appear to be evolutionary dead-ends. Most asexuals originated from their sexual ancestors very recently and may have had insufficient time to diversify into distinct species. Moreover, in most groups with frequent origins of asexuality, such as land plants, there appears to be repeated origins of asexuality from a single sexual ancestor, meaning that genetic and phenotypic patterns of variation result more from gene flow from the sexual ancestor than as an outcome of asexuality.

Ancient asexual groups such as bdelloids offer the opportunity to study diversification in a group that has been asexual long enough for diversification to occur. Patterns of variation could be compared quantitatively with those in their nearest and most comparable facultatively sexual relatives, the monogonont rotifers. One difficulty here is that other traits aside from obligate versus facultatively asexuality could differ between the clades, for example bdelloids often live in more desiccating habitats than monogononts and differ in several key aspects of life-history (e.g. anhydrobiosis). However, bdelloids and monogononts do co-occur in some habitats, and therefore clades could be compared at least in comparable environments.

Ideally, we would have repeated comparisons of sexual and asexual sister clades, but other cases of ancient asexuals are few and far between. Oribatid mites or darwinulid ostracods offer some potential, but again may suffer from being so ancient that their nearest sexual relatives live very different lives. Perhaps more promising, although even less well known, would be fungi, single-celled eukaryotes, or prokaryotes. In fact species can be detected by applying the 4X rule to sequence data from several groups of unicellular eukaryotes (Birky, in preparation). However, a large amount of basic
knowledge and direct evidence for strict asexuality would need to be accumulated before
sexual versus asexual comparisons could be made in any of these groups. Ultimately, a
survey of patterns of diversification among clades with differing frequencies of
recombination may prove most feasible and most satisfying, rather than striving for the
difficult goal of finding unambiguous asexual versus sexual comparisons.

Conclusions

Population and evolutionary genetic theory shows that asexual organisms can form
species that share a number of properties with other species concepts. In particular, they
are inclusive populations that are independent arenas for the evolutionary processes of
mutation, selection, and random drift. As a result they form genotypic clusters separated
by long-lasting gaps due to physical isolation and/or divergent selection, as opposed to
transient gaps due to random genetic drift. The genotypic clusters will often be
phenotypic clusters as well, but they may not be readily detectable, in which case the
clusters will be cryptic species. This theoretical model of the nature and origin of species
suggests two different methods of distinguishing species using gene sequence data; one
detects the difference in branching rates of lineages between species and within species,
while the other identifies clusters that are separated by gaps too deep to be caused by
drift. These species criteria have been used to demonstrate that bdelloid rotifers and
oribatid mites have undergone speciation. Some of the resulting species are cryptic, not
presently distinguishable by phenotype.

Theory also shows that under some conditions, either asexuals or sexuals may show a
higher rate of speciation. Which is true is an empirical question, and the answer may
differ in different groups of organisms. Our species criteria provide the first step toward
answering this question.

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**FIGURES AND LEGENDS**

Figure 1. Four models of asexual growth. Each one is a phylogenetic tree of genes or individuals reproducing by binary fission. 1a: Unlimited growth, no variation in offspring number. 1b: Stochastic model with finite population size limited by carrying capacity of niche, stochastic variation in offspring number (random drift). 1c: Stochastic model, with two speciation events (arrows) producing the species shown in heavier lines. Each event could be allopatric speciation by physical isolation of two populations, or sympatric speciation by divergent selection. 1d: The tree that would be observed if one could sample all individuals in each species.
Figure 2. Rationale for the 4X rule. In this imaginary example, samples of 4-6 individuals from each of 3 independently evolving populations will be separated by $K \geq 8N_e u$ sequence differences per site. The mean sequence difference among individuals in a sample $\pi$ estimates $2N_e u$, so the ratio of $K$ to $\pi$ is 4.
Figure 3. Phylogenetic tree of the bdelloid rotifers sampled by Birky et al. (2005), showing clusters inferred using their 4X rule and by the branching rate method by Pons et al. (2006). Names of the specimens are not shown in order to save space, but are available from CWB on request. Triangles indicate clusters identified by the 4X rule. Black circles indicate clusters recovered in the maximum likelihood solution, grey dots indicate those appearing within confidence limits defined by 2 log likelihood units. The tree was reconstructed by neighbour-joining using GTR maximum likelihood distances. An ultrametric tree was obtained using penalized likelihood implemented in the software r8s by Sanderson (2002) with a smoothing parameter of 1. The root was arbitrarily scaled to have an age of 1 unit. The branching rate method was implemented using software in the R statistical programming language (Team 2006) by TGB.
Figure 4. Phylogenetic tree of the oribatid mites sampled by (Heethoff et al. 2007) showing clusters inferred using the 4X rule of Birky et al. (2005) (triangles) and the branching rate method of Pons et al. (2006) (black circles). The open circle indicates the outgroup. All of the branching rate clusters were recovered in the maximum likelihood solution: there were no other solutions within the standard 2 log likelihood units. The tree and clustering analyses were obtained following identical methods to those described for the bdelloid rotifers.