

1 **Lost Sex**  
2 **The Evolutionary Biology of Parthenogenesis**  
3 Edited by Peter Van Dijk, Koen Martens, Isa Schön  
4

5 **Chapter 10. Asexual Speciation**

6 C. William Birky, Jr.

7 *Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, AZ*  
8 *85745 USA*

9 Phone 520-626-6513

10 Fax 520-621-9190

11 Email [birky@u.arizona.edu](mailto:birky@u.arizona.edu)

12  
13 Timothy G. Barraclough

14 *Division of Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire*  
15 *SL5 7PY, UK*

16 *The Jodrell Laboratory, Royal Botanic Gardens, Kew TW9 3DS, UK*

17  
18 Phone: +44 (0)20 7594 2247

19 Fax: 44 (0)20 7594 2339

20 Email: [t.barracough@imperial.ac.uk](mailto:t.barracough@imperial.ac.uk)  
21

22 **The Importance of Asexual Species**  
23

24 There is a general consensus among biologists that species are real and important units of  
25 biological diversity, and understanding the mechanisms of speciation is a hot research  
26 topic (Coyne and Orr 2004). Nearly all of that work is focused on species and speciation  
27 in animals and plants that reproduce sexually. But it is also critically important to  
28 understand species and their origins in asexual organisms such as parthenogenetic  
29 animals and plants. A substantial part of the earth's biological diversity consists of  
30 organisms that reproduce only asexually so far as we know, or reproduce sexually very  
31 rarely or with extreme inbreeding so that they are effectively asexual or clonal. Asexual  
32 organisms include not only the parthenogenetic animals and plants that are the subject of  
33 this book, but also many fungi; some green algae, including the large and ancient genus  
34 *Chlorella*; and many other eukaryotic protists, including possibly all of the members of  
35 one of the five major groups of eukaryotes, the Excavates. In addition to eukaryotes,  
36 many bacteria are largely asexual, although levels of homologous recombination vary and  
37 may be high enough to confer evolutionary advantages of sex in many groups (Cohan  
38 2004; Ochman, Lerat et al. 2005). Many asexual eukaryotes and prokaryotes are

1 medically important parasites (see chapter \$\$ of this book by deMeeus et al.; also Ayala  
2 1998), agricultural pests, or invasive species (Mergeay et al. 2006).

3  
4 A deep understanding of what asexual species are and how to find them is a prerequisite  
5 for testing theories about the evolutionary advantage of sex, i.e. about the evolutionary  
6 consequences of losing sex. For example, theory predicts that asexual organisms should  
7 accumulate a higher load of detrimental mutations (Muller's ratchet) than do sexual  
8 organisms, resulting in a higher extinction rate (Otto and Lenormand 2002). It is useful to  
9 test for the accumulation of detrimental mutations on evolutionary lineages between  
10 species as well as within species. Theory also predicts that asexuals should be less able to  
11 fix advantageous mutations (Orr 2000), perhaps resulting in both higher rates of  
12 extinction in changing environments and lower rates of speciation. Testing this theory  
13 obviously requires models of species (species concepts) and operational definitions  
14 (species criteria) that identify comparable evolutionary units in asexuals and sexuals.

15  
16 Even determining whether a group of organisms is asexual requires knowing species  
17 boundaries. Some methods use population genetic analyses to look for linkage  
18 disequilibrium, while others look for incongruence in the phylogenetic trees of different  
19 genes within a species. Rare cases where genes are shared between individuals could be  
20 due to infrequent outcrossing and recombination within a species, or to horizontal  
21 transfer between species; only the former is sexual reproduction, although horizontal  
22 transfer may play an important role in ecological speciation in bacteria (Cohan 2004).  
23 Clearly the use of population genetic methods to test whether a species is sexual or  
24 asexual is facilitated by having a species concept that is compatible with both asexuals  
25 and sexuals.

26  
27 In spite of the importance of asexual organisms and species, the nature of species is even  
28 more controversial in asexual organisms than it is in sexuals; even the existence of  
29 species in asexual organisms has been questioned (Maynard Smith and Szathmary 1995).  
30 In this review we focus on the question of when and how speciation can occur in  
31 parthenogenetic animals and plants and other asexuals and on how species can be  
32 identified. We will show that asexual organisms are similar to sexual organisms in that  
33 they can undergo allopatric speciation due to physical isolation or sympatric speciation as  
34 a result of divergent selection for adaptation to different habitats. We also describe two  
35 different criteria for assigning individuals to species, and compare the resulting species to  
36 sexual species defined by reproductive isolation or other criteria.

## 37 38 **General theory of speciation and species in asexuals**

39  
40 Theoretical studies of the evolutionary consequences of losing sex (aka the evolutionary  
41 advantage of sex) have shown that asexual organisms should accumulate more  
42 detrimental mutations than do sexual species and in consequence should have a higher  
43 rate of extinction. Also asexual organisms should be less able to fix advantageous  
44 mutations, which can also contribute to extinction in a changing environment, as well as  
45 making it more difficult to speciate by adapting to different niches. To test these theories  
46 we need to compare speciation rates in asexual and sexual organisms. This requires a

1 species concept or concepts that identify comparable evolutionary units in asexual and  
2 sexual organisms. This is especially difficult because the very existence of species in  
3 asexuals is controversial. Many people believe that asexual (and clonal) organisms will  
4 form a continuum of genetic variation with no clearly distinguishable species.

5  
6 To address this problem, we (Barraclough et al. 2003) developed a general theory of  
7 speciation for asexual and sexual organisms. We focused on one commonly used  
8 criterion, namely the very general observation that many, if not all, organisms fall into  
9 discrete clusters of individuals with very similar genotypes and phenotypes, well-  
10 separated from other such clusters. Our theory of the nature and origin of these clusters  
11 can be developed as follows:

12  
13 We begin by considering speciation in an established asexual lineage or inclusive  
14 population descended from a single asexual individual. That individual could have arisen  
15 either by loss of sexual reproduction in an organism that alternates asexual and sexual  
16 reproduction, or by hybridization between two obligatory sexual species. Because all the  
17 genes in an asexual organism are completely linked and behave as a unit, the origin and  
18 ploidy of the asexual lineage are irrelevant (with exceptions described below). We further  
19 assume that the asexual lineage is evolving as an independent evolutionary arena,  
20 separated physically or by adapting to a different niche from its parent lineage(s).

21  
22 The diagrams in Figure 1 show phylogenies of a gene in an asexual organism under  
23 several different scenarios. Note that because there is no outcrossing and recombination,  
24 the phylogeny of one gene is also the phylogeny of all the genes in the organism, and the  
25 phylogeny of the individuals as well. This model assumes that all the copies of the gene  
26 are orthologous, not the products of gene duplications and losses that can distort  
27 phylogenies. Similar problems can arise in diploids that show the Meselson effect or in  
28 polyploids (Birky 1996). These problems can be avoided by using mitochondrial or  
29 chloroplast genes.

30  
31 Figure 1a is a naïve model of the phylogeny (or genealogy) of a gene in a clone. For  
32 simplicity it is assumed that the organism is reproducing by simple binary fission without  
33 limit: each individual leaves two offspring in the next generation. In this model,  
34 different lineages acquire different mutations so that different copies of the gene  
35 gradually diverge from each other. At any one time the individuals in a clone show a  
36 continuous distribution of sequence differences. If this model accurately reflected the  
37 genealogy of a clone, there would be no species by any definition. But the model is  
38 completely unrealistic, because it assumes that all individuals divide in synchrony and  
39 there is no limit to the growth of the clone.

40  
41 In real life, growth of the clone is limited by finite resources that set an upper limit on the  
42 total size of the population (the census size  $N$ ). Moreover, some individuals reproduce  
43 more often than others. This is partly because of stochastic effects, which causes random  
44 genetic drift of allele frequencies in population genetic theory, and partly because of  
45 natural selection. Figure 1b shows a more realistic model in which the population size is  
46 limited by the carrying capacity of the organism's habitat. The stochastic loss of lineages

1 has created clusters separated by shallow gaps. These gaps will have a depth of  
2 approximately  $2N_e$  generations on average, and the clusters they form will be transient  
3 with an average lifetime of approximately  $2N_e$  generations. The clusters would not be  
4 considered different species by population geneticists. They do form clades and might  
5 have morphological differences that would allow them to be distinguished in the field.  
6 However, they are not adapted to different ecological niches and are not evolving  
7 independently. They are not comparable to ecological or sexual species, which can  
8 coexist indefinitely even in sympatry.

9  
10 ***Figure 1 here***

11  
12 Longer-lasting clusters can be formed by allopatry or by divergent selection, as illustrated  
13 in Figure 1c. In this example, two individuals in the first generation were physically  
14 isolated, e.g. by dispersal to distant locations. In the second generation two individuals in  
15 one location were selected for adaptation to different ecological niches while remaining  
16 sympatric. Consequently the offspring of these three individuals formed independently  
17 evolving populations. Each one forms an independent arena for mutation, random drift,  
18 and selection. Each one shows transient clusters separated by shallow gaps less than  $2N_e$   
19 generations deep. However, they are separated by long-lasting gaps with depths  
20 significantly greater than  $2N_e$  generations. Figure 1d shows the tree with extinct lineages  
21 pruned; this is the realized tree that is actually observed using sequence data from extant  
22 individuals.

23  
24 The resulting populations show several characteristics that are widely, although not  
25 universally, considered to be desirable traits in species: (i) they are independently  
26 evolving lineages, as in the Evolutionary Species Concept (EvSC, Simpson 1951; Hey  
27 2006); (ii) they are reciprocally monophyletic, so that every individual is more closely  
28 related to another member of the same species than to any member of another species;  
29 and (iii) assuming that phenotypic evolution is neutral or under divergent selection, they  
30 will form distinct phenotypic clusters reflecting their significant differences in genotype.  
31 In addition, the two populations on the left are adapted to different niches will be  
32 different species by the Ecological Species Concept (EcSC, Van Valen 1976); the  
33 population on the right is a different ecological species from at least one of these and over  
34 time is likely to diverge ecologically from both.

35  
36 At least two of the clusters meet an important criterion under the Biological Species  
37 Concept (BSC), namely persistence in sympatry. It is unlikely that the two species on the  
38 left in Figure 1c can ever merge again, as this would require reversal of the evolutionary  
39 steps that adapted them to different niches. In this sense they are equivalent to BSC  
40 species, which will not fuse even in sympatry because they are reproductively isolated.  
41 Hey (2006) argued that most or all species concepts are really variants on the EvSC, or  
42 are species criteria for the EvSC, working definitions for assigning individuals to  
43 evolutionary species. The model of Barraclough et al. (2003) can also be considered a  
44 variant of the EvSC. However, unlike Simpson's original description of the EvSC and  
45 subsequent versions, our model has an explicit theoretical basis in population and  
46 evolutionary genetics. Moreover it generates explicit criteria and associated statistics for

1 identifying species from finite samples of specimens, as described below. In view of  
2 these differences, one of us has proposed that it be considered a new model called the  
3 Evolutionary Genetic Species Concept (EGSC, Birky in preparation).

4  
5 Barraclough et al. (2003) justified the above theory in terms of coalescent theory, but the  
6 same theory can be derived from classical population genetic theory where  $2N_e$   
7 generations is the approximate expected time to the most recent common ancestor of the  
8 entire population. Note that although coalescent theory is frequently considered to be a  
9 neutral theory, one can define  $N_e$  so that it takes selection, including hitchhiking, into  
10 account; this  $N_e$  is smaller and the time to the most recent common ancestor is reduced  
11 accordingly. The coalescent approach provides an intuitive conceptual linkage to  
12 phylogenetic analysis of gene sequences.

## 14 **Species criteria for assigning individuals to species**

15  
16 Models of the nature of species do not directly address the practical problem of assigning  
17 individuals to species. The task of identifying species in a collection of individual  
18 organisms, and of assigning newly collected individuals to those species, is called a  
19 species criterion. Consideration of expected patterns of clustering outlined above  
20 suggests two different approaches to assigning individual asexual organisms to  
21 evolutionary genetic species.

### 23 **The 4X rule**

24  
25 The first species criterion, called the “4X rule” (Birky et al. 2005), has two characteristics  
26 that are uncommon among species criteria: (i) it explicitly recognizes that species are  
27 identified on the basis of samples, usually very small samples, of very large populations  
28 of individuals; and (ii) the probability that a species assignment is incorrect is set in  
29 advance at  $\leq 0.05$ . The 95% confidence limit is widely used in biology but more or less  
30 stringent intervals can be used if desired.

31  
32 The 4X rule works as follows:

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

1           within a putative species is used as an estimate of the nucleotide diversity in the  
2           species.

3

4           The theoretical rationale for the rule is illustrated in Figure 2. As (Barraclough et al.  
5           2003) noted, gaps formed within asexual species by stochastic processes will be  $2N_e$   
6           generations deep on average; the 95% confidence interval is  $4N_e$  generations.

7           Consequently, one can infer with 95% confidence that gaps deeper than  $4N_e$  generations  
8           separate independently evolving populations, i.e. EG species. The sequence difference  
9           between individuals in two such populations, corrected for multiple hits, will be  $K =$   
10           $8N_e u$  where  $u$  is the mutation rate per site per generation. At the same time the mean  
11          sequence difference between individuals within each population, called the nucleotide  
12          diversity, will be  $\pi \approx 2N_e u$ . The ratio of the sequence difference between populations to  
13          the difference between individuals within a population is  $K/\pi \geq 8N_e u/2N_e u = 4$ .

14

15          ***Figure 2 here***

16

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

27

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

33

#### 34          **Analysis of branching rates**

35

36          An alternative framework for considering whether a clade has diversified into distinct  
37          genetic clusters is to consider branching models (Pons et al. 2006). This approach is more  
38          complex than the 4X rule, but allows for a global test of whether the study clade has  
39          diversified into separate species or not. Under a null model that the entire sample derives  
40          from just a single asexual population, i.e. without divergence into independently evolving  
41          or ecologically distinct species, then the pattern of branching is expected to conform to a  
42          standard coalescent for a single population (Fig. 1b). A large body of theory is available  
43          to specify the likelihood of a given pattern of branching under a particular model  
44          (Hudson 1991), ranging from a neutral coalescent in a population of constant size,  
45          through to populations that have changed size through time or experienced different  
46          forms of selection (Rosenberg and Nordborg 2002).

1  
2 Under the alternative model that the clade has diversified into species, as defined above,  
3 then we expect to observe distinct genetic clusters (Fig. 1d). Branching events within  
4 each cluster will reflect coalescence within populations of the same kind considered in  
5 the null model, but in this case with each cluster representing a separate, independently  
6 evolving population. Branches connecting the different clusters now represent the timing  
7 of divergence events, for example events causing geographic isolation or the onset of  
8 adaptive divergence into different niches. In other words, branching within clusters  
9 reflects population processes, whereas branching between clusters reflects diversification.  
10 Again, a large body of theory is available to specify the likelihood of branching times in a  
11 phylogeny depending on different models of speciation, extinction and sampling of  
12 species (Nee et al. 1994; Barraclough and Savolainen 2001; Nee 2001).

13  
14 Pons et al. (2006) combined previous approaches for considering population coalescence  
15 and branching times within phylogenies to derive a general expression for the likelihood  
16 of branching times under the alternative model that the sample derives from a set of  
17 independently evolving populations. The model relies on the waiting intervals between  
18 successive branching events as input data (Fig. 2, Pons et al. 2006). Under a relatively  
19 broad set of circumstances, the net observed branching rate within species is expected to  
20 exceed that between species, hence the signature of clusters can be detected as a  
21 transition from slow to fast branching rates occurring near the tips of the tree (Fig. 1d).  
22 The likelihood of models with and without a transition in branching rate can be used to  
23 test whether the alternative model provides a better fit to the data than a null model in  
24 which the entire sample conforms to a single branching process. The models were  
25 modified to allow a range of qualitative departures from standard assumptions, such  
26 constant population size, neutrality or constant speciation rates. (Pons et al. 2006) applied  
27 the approach to a sample of tiger beetles from salt lakes in Australia and showed that this  
28 lineage had diversified into a set of independently evolving species. The method is  
29 particularly suitable for asexual clades in which any single locus is expected to reveal the  
30 species history, not just the history of that gene.

31  
32 In principle the model of speciation and the criterion for finding species that we have  
33 described can be applied to any asexual organism. However, in keeping with the theme of  
34 this volume, we will focus on parthenogenesis, which is limited to animals and plants.  
35 Moreover, our criteria can only be applied to lineages that (i) are thought to have been  
36 reproducing without sex long enough to have completed speciation, and (ii) have a  
37 suitable data base of gene sequence data. This limits us to two groups of animals, the  
38 bdelloid rotifers and oribatid mites.

## 40 **Application to bdelloid rotifers**

41  
42 The bdelloid rotifers are an ancient asexual group that includes more than 374 species  
43 defined by phenotype, principally morphology (see chapter \$\$ of this book by Mark  
44 Welch et al.). Birky et al. (2005) applied the 4X rule to 110 specimens of bdelloid  
45 rotifers. These specimens were obtained from more than 34 sites in 7 states in the U.S.  
46 and 1 site in Italy. Morphological study revealed that they included members of six

1 genera. The collecting sites included permanent and temporary springs, streams, ponds,  
2 lakes, soil and moss from a wide range of ecosystems between sea level and 3660  
3 meters. A 591 bp segment of the mitochondrial *cox1*(aka *COI*) gene was amplified and  
4 sequenced from clones of females descended from each specimen. Phylogenetic analysis  
5 (Figure 3) revealed the presence of a number of clades, including 21 terminal clades that  
6 obey the 4X rule and are simple, i.e they contain no such clades within them. The mean  
7 sequence difference among individuals in a clade ranged 0 – 2%, similar to other  
8 invertebrates(Avise 1994). These are samples from inclusive populations that are  
9 evolving independently because they are adapted to different niches or are geographically  
10 isolated. Moreover they have been separated long enough to become reciprocally  
11 monophyletic; they are species under the evolutionary and evolutionary genetic species  
12 concepts. There are also 14 singlets, individuals that are not part of any of these species;  
13 most or all of these singlets are separated from each other and from the species by such  
14 large sequence differences that they almost certainly represent samples of size one from  
15 additional species.

16  
17 ***Figure 3 here***

18  
19 We also analyzed branching rates to find species in this sequence data set. The maximum  
20 likelihood solution for the independently evolving clusters model infers the presence of  
21 twenty-four clusters, with the remaining individuals inferred to be singletons (Fig. 3).  
22 Confidence limits within 2 log likelihood units of the maximum likelihood solution range  
23 from 22 to 25 clusters. The pattern of clustering is significantly greater than expected  
24 under the null model that the sample derives from a single population (likelihood ratio  
25 test,  $X^2 = 108.5$ ,  $p < 0.0001$ ). Overall, the branching rate method resolves clusters more  
26 finely than the 4X rule approach: 8 clusters found in the latter are split into more clusters  
27 by the former. One possible explanation is that if a few populations harbor greater genetic  
28 diversity than the predominant trend, then they may tend to be split by the branching rate  
29 model because it assumes a single threshold for the transition from between- to within-  
30 cluster branching. Future implementations that allow for different genealogical branching  
31 rates within different clusters are in development (TGB unpublished data). An alternative  
32 explanation is that, by treating each case individually, the 4X rule may be more  
33 conservative: knowing the typical level of within cluster diversity across the sample may  
34 allow the branching rate method to split clusters that haven't diverged by 4X the  
35 intraspecific variation. Simulations of known scenarios could be used to compare the  
36 power of the two methods. Power aside, the branching rate method has the advantage of  
37 adopting a global analysis of the data, which facilitates hypothesis testing of different  
38 scenarios, whereas the 4X rule is simple to apply while still founded on robust population  
39 genetic theory.

40  
41 Evidence for genetic clusters is consistent with independently evolving entities, but the  
42 nature of those entities remains in question. One possibility is that some or all of them  
43 evolve independently solely as a result of geographic isolation. Traditional accounts have  
44 tended to exclude such cases from consideration as species, because they cannot explain  
45 the existence of alpha-diversity, i.e. coexistence of species in a single area. Our analyses  
46 provide clear evidence of distinct clusters present in sympatry over successive years, but

1 it remains possible that their coexistence is transient or a feature of ongoing invasion  
2 from source areas. A more direct approach is to look for evidence of ecological  
3 divergence between clusters. Birky et al. 2005) found that Pha2 and Pha3 differ in  
4 temperature tolerance range; clones of Pha2 survive and reproduce at 36° while clones of  
5 Pha3 do not. However, it is not clear whether this difference detected in the lab reflects  
6 differences in their habitat.

7  
8 Fontaneto et al. (submitted) performed a direct test for divergent selection on presumed  
9 eco-morphological traits in the genus *Rotaria*. If morphology has evolved neutrally or  
10 under constant selection among all lineages, we expect levels of intraspecific and  
11 interspecific variation to mirror levels of neutral genetic variation (McDonald and  
12 Kreitman 1991). However, if there has been divergent selection on morphology, for  
13 example as a result of divergence to occupy different niches, then we expect more eco-  
14 morphological divergence between species than within them. This pattern was indeed  
15 found in *Rotaria*, consistent with their adaptive radiation rather than simply neutral  
16 divergence in geographic isolation. Interestingly, morphological coherent clades  
17 experiencing divergent selection often contained two or more genetic clusters: these  
18 could either be solely the result of geographic isolation, or they may have diverged in  
19 unmeasured phenotypic traits, such as behaviour, life-history or other morphological  
20 traits. Now that the molecular systematics of bdelloids is being established, more work is  
21 needed to explore the biological significance of the genetically distinct clusters. All but  
22 one of the traditional species in the genus were found to comprise monophyletic clades,  
23 but many of them subsumed several genetic clusters: more resolved morphological  
24 techniques may be needed to provide non-genetic means for cluster identification.

## 26 **Application to oribatid mites**

27  
28 Besides the bdelloid rotifers, sequence data suitable for detecting species are available for  
29 only one other ancient obligate parthenogenetic lineage, the oribatid mites. This is a  
30 diverse and widespread group of soil-dwelling mites that includes a number of apparently  
31 obligate parthenogenetic lineages (see chapter \$\$ of this book by Heathoff et al. for a  
32 detailed discussion). Some of the parthenogenetic lineages are quite ancient and speciose.  
33 Heathoff et al. (2007) obtained *cox1* sequences of 65 specimens of the morphological  
34 species *Platynothrus peltifer* collected from Europe, Russia, Japan, and the United States.  
35 Phylogenetic analyses revealed seven clades within this species; sequence differences  
36 within these clades were less than 2%, while the differences between clades averaged  
37 56% after correction for multiple hits. Using their data kindly provided by Michael  
38 Heathoff, we found that all seven clades qualify as species under the 4X rule. The  
39 branching rate recognizes 7 clusters plus one for the outgroup (Fig. 4). At present it is not  
40 clear if the seven clades of *P. peltifer* can be distinguished by morphology. However,  
41 there is some evidence that they have been physically isolated by continental drift and  
42 mountain uplifts (Heathoff et al. 2007 and chapter \$\$).

43  
44 **Figure 4 here**

## 1 **Predictions about relative speciation rates in sexuals and** 2 **parthenogens**

3  
4 We have argued that asexual organisms should diversify into independently evolving and  
5 biologically distinct species broadly equivalent to sexual species. Evidence from bdelloid  
6 rotifers and oribatid mites supports this claim. But the question remains whether asexuals  
7 should diversify to a greater or lesser extent than sexuals. On the one hand, sexuals need  
8 to evolve reproductive isolation before diversity can evolve and be maintained in  
9 sympatry (Futuyma 1987; Eldredge 1989). Because asexuals have no such requirements,  
10 we might expect them to evolve sympatric diversity more easily. However, sexual  
11 organisms are expected to adapt more quickly to changing environments: if the rate of  
12 adaptation to new conditions were the limiting step in diversification, sexuals might have  
13 the edge over asexuals. Finally, if the events leading to geographic isolation or the onset  
14 of divergent selection were actually the limiting step in diversification, rather than rates  
15 of adaptation or the origin of reproductive isolation, then both sexuals and asexuals  
16 might have similar expected rates of diversification, all other things being  
17 equal (Barraclough et al. 2003). Therefore, the answer to the question could provide  
18 insights into which steps in the process of diversification are most important for  
19 determining speciation rates within clades.

20  
21 Unfortunately, there are a number of difficulties in performing a rigorous test of these  
22 ideas. By far most origins of asexuality appear to be evolutionary dead-ends. Most  
23 asexuals originated from their sexual ancestors very recently and may have had  
24 insufficient time to diversify into distinct species. Moreover, in most groups with  
25 frequent origins of asexuality, such as land plants, there appears to be repeated origins of  
26 asexuality from a single sexual ancestor, meaning that genetic and phenotypic patterns of  
27 variation result more from gene flow from the sexual ancestor than as an outcome of  
28 asexuality.

29  
30 Ancient asexual groups such as bdelloids offer the opportunity to study diversification in  
31 a group that has been asexual long enough for diversification to occur. Patterns of  
32 variation could be compared quantitatively with those in their nearest and most  
33 comparable facultatively sexual relatives, the monogonont rotifers. One difficulty here is  
34 that other traits aside from obligate versus facultatively asexuality could differ between  
35 the clades, for example bdelloids often live in more desiccating habitats than  
36 monogononts and differ in several key aspects of life-history (e.g. anhydrobiosis).  
37 However, bdelloids and monogononts do co-occur in some habitats, and therefore clades  
38 could be compared at least in comparable environments.

39  
40 Ideally, we would have repeated comparisons of sexual and asexual sister clades, but  
41 other cases of ancient asexuals are few and far between. Oribatid mites or darwinulid  
42 ostracods offer some potential, but again may suffer from being so ancient that their  
43 nearest sexual relatives live very different lives. Perhaps more promising, although even  
44 less well known, would be fungi, single-celled eukaryotes, or prokaryotes. In fact species  
45 can be detected by applying the 4X rule to sequence data from several groups of  
46 unicellular eukaryotes (Birky, in preparation). However, a large amount of basic

1 knowledge and direct evidence for strict asexuality would need to be accumulated before  
2 sexual versus asexual comparisons could be made in any of these groups. Ultimately, a  
3 survey of patterns of diversification among clades with differing frequencies of  
4 recombination may prove most feasible and most satisfying, rather than striving for the  
5 difficult goal of finding unambiguous asexual versus sexual comparisons.  
6

## 7 **Conclusions**

8  
9 Population and evolutionary genetic theory shows that asexual organisms can form  
10 species that share a number of properties with other species concepts. In particular, they  
11 are inclusive populations that are independent arenas for the evolutionary processes of  
12 mutation, selection, and random drift. As a result they form genotypic clusters separated  
13 by long-lasting gaps due to physical isolation and/or divergent selection, as opposed to  
14 transient gaps due to random genetic drift. The genotypic clusters will often be  
15 phenotypic clusters as well, but they may not be readily detectable, in which case the  
16 clusters will be cryptic species. This theoretical model of the nature and origin of species  
17 suggests two different methods of distinguishing species using gene sequence data; one  
18 detects the difference in branching rates of lineages between species and within species,  
19 while the other identifies clusters that are separated by gaps too deep to be caused by  
20 drift. These species criteria have been used to demonstrate that bdelloid rotifers and  
21 oribatid mites have undergone speciation. Some of the resulting species are cryptic, not  
22 presently distinguishable by phenotype.  
23

24 Theory also shows that under some conditions, either asexuals or sexuals may show a  
25 higher rate of speciation. Which is true is an empirical question, and the answer may  
26 differ in different groups of organisms. Our species criteria provide the first step toward  
27 answering this question.  
28

## 29 **REFERENCES**

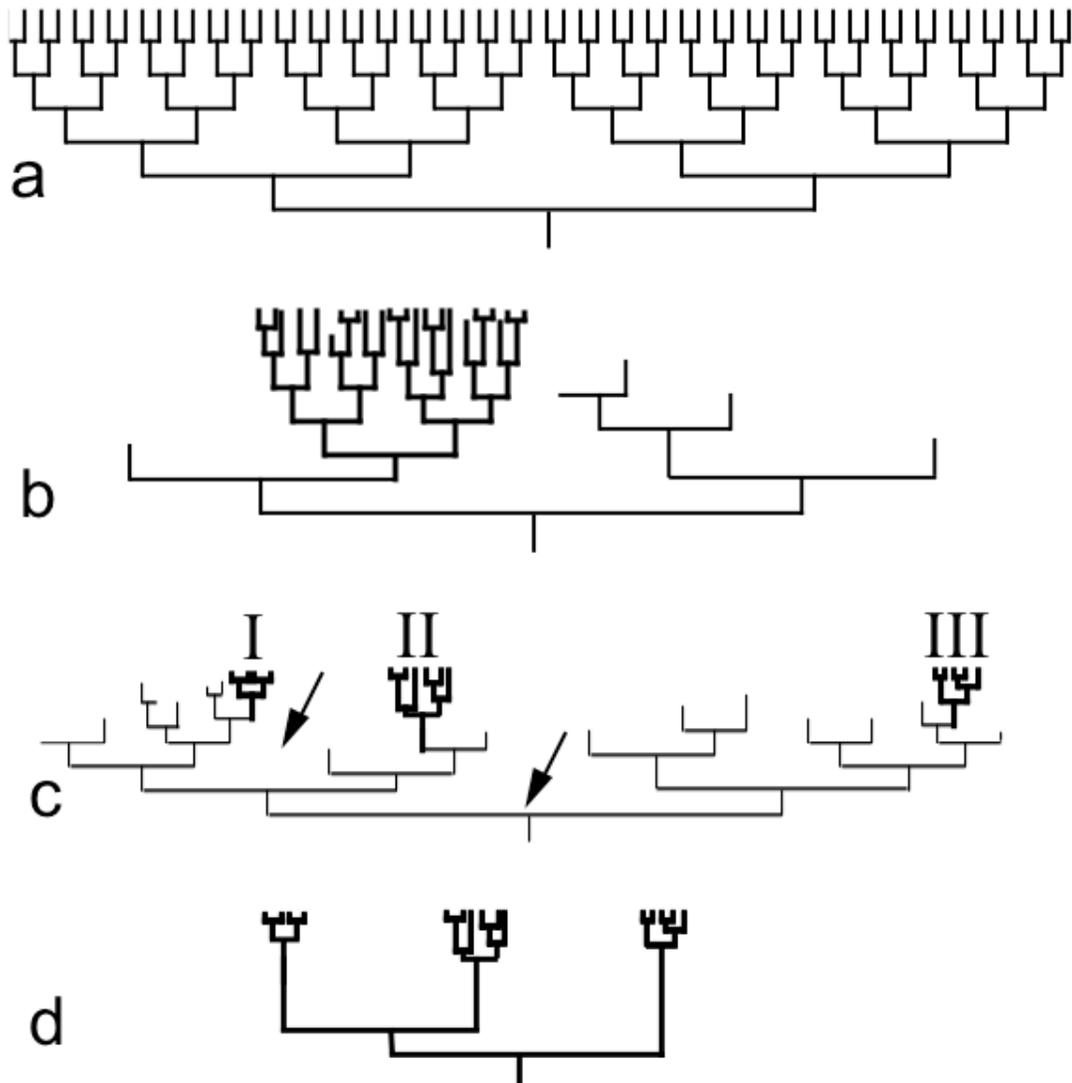
- 30  
31 Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall,  
32 Inc., New York  
33 Ayala FJ (1998) Is sex better? Parasites say no. *Proceedings of the National Academy of*  
34 *Sciences USA* 95: 3346-3348  
35 Barraclough TG, Birky CW, Jr. (2003) Diversification in sexual and asexual organisms.  
36 *Evolution* 57: 2166-2172.  
37 Barraclough TG, Savolainen V (2001) Evolutionary rates and species diversity in  
38 flowering plants. *Evolution* 55: 677-683.  
39 Birky CW Jr. (1996) Heterozygosity, heteromorphy, and phylogenetic trees in asexual  
40 eukaryotes. *Genetics* 144: 427-437.  
41 Birky CW Jr., Wolf C, Maughan H, Herbertson L, Henry E (2005) Speciation and  
42 selection without sex. *Hydrobiologia* 546: 29-45  
43 Cohan FM (2004) Concepts of bacterial biodiversity for the age of genomics. In: Fraser  
44 CM, Read T, Nelson KE. *Microbial Genomes*. Humana Press, Totowa, NJ, pp  
45 175-194.

- 1 Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Inc., Sunderland,  
2 Massachusetts
- 3 Eldredge N (1989) Macroevolutionary Dynamics. McGraw-Hill, New York.
- 4 Fontaneto DEA, Herniou EA, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough  
5 TG (submitted). Independently evolving species in asexual bdelloid rotifers. PLoS  
6 Biology
- 7 Futuyma DJ (1987) On the Role of Species in Anagenesis. American Naturalist 130: 465-  
8 473
- 9 Heethoff M, Domes K, Laumann M, Maraun M, Norton RA, Scheu S (2007) High  
10 genetic divergences indicate ancient separation of parthenogenetic lineages of the  
11 oribatid mite *Platynothrus peltifer* (Acari, Oribatida). Journal of Evolutionary  
12 Biology 20:392-402
- 13 Hey J (2006) On the failure of modern species concepts. Trends in Ecology and  
14 Evolution 21: 447-450
- 15 Hudson RR (1991) Gene genealogies and the coalescent process. Oxford Surveys in  
16 Evolutionary Biology 7: 1-44.
- 17 Maynard Smith J, Szathmary E (1995) The Major Transitions in Evolution W.H.  
18 Freeman, New York
- 19 McDonald JH, Kreitman M (1991) Adaptive protein evolution at the *adh* locus in  
20 *Drosophila*. Nature 351: 652-654.
- 21 Mergeay JD, Verschuren D, De Meester L (2006) Invasion of an asexual American water  
22 flea clone throughout Africa and rapid displacement of a native sibling species.  
23 Proceedings of the Royal Society of London. Series B, Biological Sciences 273:  
24 2839-2844
- 25 Nee S (2001) Inferring speciation rates from phylogenies. Evolution 55: 661-668.
- 26 Nee S, May RM, Harvey PH (1994) The reconstructed evolutionary process.  
27 Philosophical Transactions of the Royal Society of London. Series B, Biological  
28 Sciences 344: 305-311.
- 29 Ochman H, Lerat E, Daubin V (2005) Examining bacterial species under the specter of  
30 gene transfer and exchange. Proceedings of the National Academy of Sciences of  
31 the United States of America 102: 6595-6599.
- 32 Orr HA (2000) The rate of adaptation in asexuals. Genetics 155: 961-968.
- 33 Otto SP, Lenormand T (2002) Resolving the paradox of sex and recombination. Nature  
34 Reviews Genetics 2: 252-261.
- 35 Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S,  
36 Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA  
37 taxonomy of undescribed insects. Systematic Biology 55: 1-15.
- 38 Rosenberg NA (2003) The shapes of neutral gene genealogies in two species:  
39 Probabilities of monophyly, paraphyly, and polyphyly in a coalescent model.  
40 Evolution 57: 1465-1477.
- 41 Rosenberg NA, Nordborg M (2002) Genealogical trees, coalescent theory and the  
42 analysis of genetic polymorphisms. Nature Reviews Genetics 3: 380-390.
- 43 Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence  
44 times: A penalized likelihood approach. Molecular Biology and Evolution 19:  
45 101-109.
- 46 Simpson GG (1951) The species concept. Evolution 5: 285-298.

- 1 Team RDC (2006) R: A Language and Environment for Statistical Computing. Vienna,
- 2 Austria.
- 3 Van Valen L (1976) Ecological species, multispecies, and oaks. *Taxon* 25: 93-106.

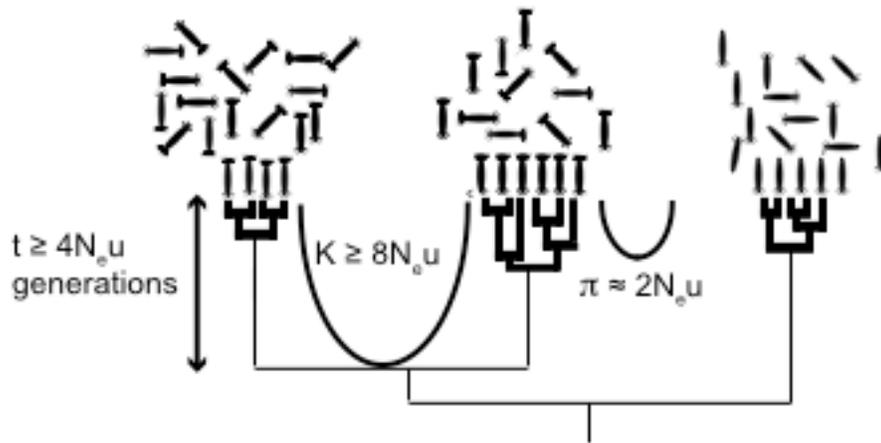
1  
2

## FIGURES AND LEGENDS

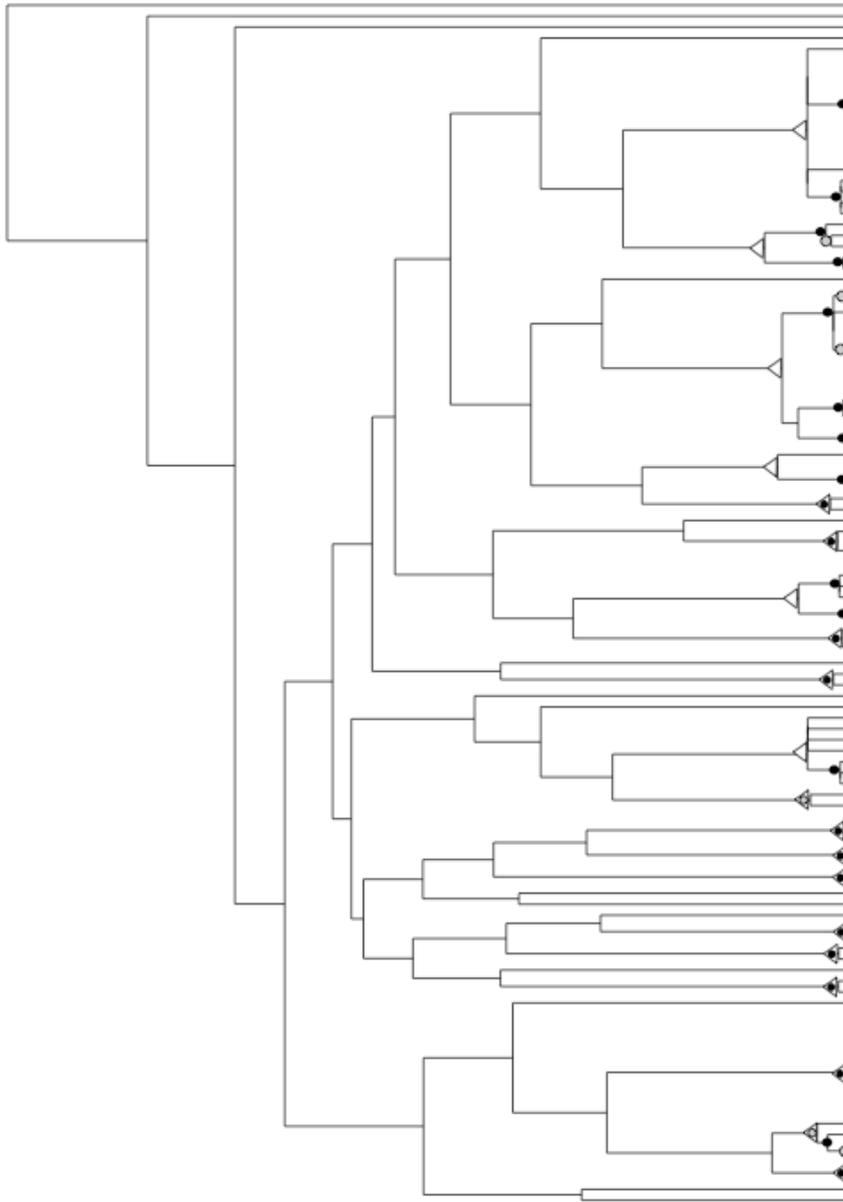


3  
4  
5  
6  
7  
8  
9  
10  
11  
12

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.



1  
 2 Figure 2. Rationale for the 4X rule. In this imaginary example, samples of 4-6 individuals  
 3 from each of 3 independently evolving populations will be separated by  $K \geq 8N_e u$   
 4 sequence differences per site. The mean sequence difference among individuals in a  
 5 sample  $\pi$  estimates  $2N_e u$ , so the ratio of  $K$  to  $\pi$  is 4.  
 6  
 7



1  
2  
3 Figure 3. Phylogenetic tree of the bdelloid rotifers sampled by Birky et al. (2005),  
4 showing clusters inferred using their 4X rule and by the branching rate method by Pons et  
5 al. (2006). Names of the specimens are not shown in order to save space, but are  
6 available from CWB on request. Triangles indicate clusters identified by the 4X rule.  
7 Black circles indicate clusters recovered in the maximum likelihood solution, grey dots  
8 indicate those appearing within confidence limits defined by 2 log likelihood units. The  
9 tree was reconstructed by neighbour-joining using GTR maximum likelihood distances.  
10 An ultrametric tree was obtained using penalized likelihood implemented in the software  
11 r8s by Sanderson (2002) with a smoothing parameter of 1. The root was arbitrarily scaled  
12 to have an age of 1 unit. The branching rate method was implemented using software in  
13 the R statistical programming language (Team 2006) by TGB.



1  
2