REVIEW

Twigs on the tree of life? Neutral and selective models for integrating macroevolutionary patterns with microevolutionary processes in the analysis of asexuality

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Abstract

Neutral models characterize evolutionary or ecological patterns expected in the absence of specific causal processes, such as natural selection or ecological interactions. In this study, we describe and evaluate three neutral models that can, in principle, help to explain the apparent 'twigginess' of asexual lineages on phylogenetic trees without involving the negative consequences predicted for the absence of recombination and genetic exchange between individuals. Previously, such phylogenetic twiggyness of asexual lineages has been uncritically interpreted as evidence that asexuality is associated with elevated extinction rates and thus represents an evolutionary dead end. Our first model uses simple phylogenetic simulations to illustrate that, with sexual reproduction as the ancestral state, low transition rates to stable asexuality, or low rates of ascertained 'speciation' in asexuals, can generate twiggy distributions of asexuality, in the absence of high extinction rates for asexual lineages. The second model, developed by Janko *et al.* (2008), shows that a dynamic equilibrium between origins and neutral losses of asexuals can, under some conditions, generate a relatively low mean age of asexual lineages. The third model posits that the risk of extinction for asexual lineages may be higher than that of sexuals simply because asexuals inhabit higher latitudes or altitudes, and not due to effects of their reproductive systems. Such neutral models are useful in that they allow quantitative evaluation of whether empirical data, such as phylogenetic and phylogeographic patterns of sex and asexuality, indeed support the idea that asexually reproducing lineages persist over shorter evolutionary periods than sexual lineages, due to such processes as mutation accumulation, slower rates of adaptive evolution, or relatively lower levels of genetic variability.

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Introduction

One of the greatest challenges for evolutionary biology is explaining the widespread occurrence of sexual reproduction throughout the animal and plant kingdoms. Sexual reproduction and eukaryotes probably evolved together (Cavalier-Smith 2002), about 2.0–3.5 billion years ago (Miyamoto & Fitch 1996; Gu 1997). Extant eukaryotes that reproduce asexually, that is, without recombination or genetic exchange between individuals, have therefore abandoned sex at some time in their evolution.

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The switch from sexual to asexual reproduction is theoretically associated with multiple advantages. The first and probably best-known is the twofold demographic advantage (Maynard Smith 1978). An asexual mutant arising in a sexual population, half of which comprises males that cannot themselves produce offspring, will double its representation in the population each generation. This demographic advantage should ultimately lead to the replacement of the sexual ancestor, all else being equal. The second most obvious benefit is the twofold fitness advantage of an asexual female (Williams 1975). For the same number of offspring produced, each asexual female transmits twice as many genes to each offspring as compared to a female producing offspring sexually. Other postulated advantages of asexuality

include reproductive insurance, the maintenance of co-adapted gene complexes (Fisher 1930), and the reduction of intra- and intergenomic conflicts (Bell 1982).

A large body of theoretical work has presented arguments designed to help explain the evolutionary maintenance of sexual reproduction and recombination despite the apparent advantages of asexuality. These theories can be broadly classified into mutational and ecological models (West *et al.* 1999). Mutational models propose that sexual reproduction reduces the accumulation of deleterious mutations and results in faster rates of adaptive evolution because genetic variation is reshuffled among individuals each generation. Several models have shown that the accumulation of deleterious mutations will decrease mean asexual lineage fitness to the point where extinction becomes inevitable (Muller 1964; Lynch & Gabriel 1990; Lynch *et al.* 1993; Kondrashov 1994; Hurst & Peck 1996). A lack of genetic diversity is also thought to hinder the evolutionary responses of asexual lineages to biotic and abiotic selective pressures, resulting in an increased probability of extinction (Jaenike 1978; Maynard Smith 1978; Hamilton *et al.* 1990; Barton & Charlesworth 1998). Mutational models thus provide theoretical support for a long-term advantage of sexual reproduction.

In contrast to mutational models, ecological models postulate a short-term advantage to sexual reproduction and hypothesize that sexual reproduction is selected for in fluctuating environments because offspring produced are genetically variable (Ghiselin 1974; Williams 1975; Bell 1982; Hakoyama & Iwasa 2004; Tagg *et al.* 2005). The most popular ecological hypothesis, the Red Queen, states that sex provides an advantage in biotic interactions (Bell 1982; Bell & Maynard Smith 1987). Red Queen models usually assume that selection generated by co-evolving parasites against common host genotypes provides the antagonistic driving force that gives sex an advantage (Jaenike 1978; Hamilton *et al.* 1990). The mechanisms that drive mutational and ecological models are not mutually exclusive and may even interact synergistically (West *et al.* 1999; Howard & Lively 2002; Bruvo *et al.* 2007). For example, it has been shown in microorganisms that a deleterious mutation load has a stronger negative impact on fitness in parasitized as compared to unparasitized hosts (Cooper *et al.* 2005) and that it increases the overall cost of parasite resistance (Buckling *et al.* 2006).

Based on the predictions of mutational and ecological models, eukaryotes reproducing exclusively asexually are presumed to represent evolutionary dead ends (e.g. Maynard Smith 1978; Lynch *et al.* 1993; Schultz & Lynch 1997). An evolutionary dead end can be defined as a lineage bearing a trait that may be initially selected for because of short-term advantages, but which is associated with elevated extinction rates. The main empirical argument used to support the idea of accelerated extinction of asexual lineages over evolutionary time is that asexuals appear to be concentrated at the tips of phylogenetic trees and hence that they originated recently. This postulated 'twiggy' phylogenetic distribution and young age of asexual lineages is ubiquitously cited in the literature. Surprisingly however, the question of whether a twiggy phylogenetic distribution actually provides evidence for the more rapid extinction of asexual lineages has yet to be investigated. More generally, in the macroevolutionary analysis of sexual vs. asexual reproduction, there appears to be a lack of appropriate 'null models', as first pointed out by Janko *et al.* (2008). Such models are fundamentally important in that they allow quantitative testing of whether empirical data, such as phylogenetic and phylogeographic patterns of sex and asexuality, indeed support the idea that asexual lineages cannot persist over evolutionary time because of such processes as mutation accumulation, slower rates of adaptive evolution, or relatively lower levels of genetic variability.

In this review, we present and evaluate three non-exclusive models, one previously proposed and two new ones, which predict a twiggy phylogenetic distribution of asexuality, overall younger ages of asexual as compared to sexual lineages, or both (Fig. 1). Each of the three models is 'neutral' with respect to reproductive mode, in that twiggyness and young age of the asexuals result from mechanisms independent of adaptive or maladaptive effects due to the lack of recombination and genetic exchange between individuals. For each model, we make predictions which allow comparison of the 'asexuality dead end' hypothesis with the neutral alternative. We then use the currently available phylogenetic and phylogeographic data to evaluate whether the neutral models can be rejected, and we suggest the type of data needed to thoroughly evaluate the different hypotheses.

Neutral models for analysing asexuality

The observation that asexuals tend to have a twiggy phylogenetic distribution was first made by Williams (1975) and Maynard Smith (1978). White (1978) and Bell (1982) provided lists of taxa comprising parthenogenetic species and showed that higher taxonomic levels with exclusively asexual lineages were very rare, suggesting that most asexual 'species' were of very recent origin and that any older lineages had presumably gone extinct.

Although a trait associated with elevated extinction rates is certainly expected to be rare and phylogenetically isolated (i.e. 'twiggy'), extinction is not the only mechanism that can generate such a pattern. In our first model, we illustrate three mutually non-exclusive mechanisms which could result in a twiggy distribution of a trait even if the trait is not associated with elevated extinction rates. The second and third neutral models show that even if asexuals are indeed shorter-lived than sexuals on average, their short phylogenetic lifespans could also be due to reasons unrelated to the selective consequences of asexual reproduction.
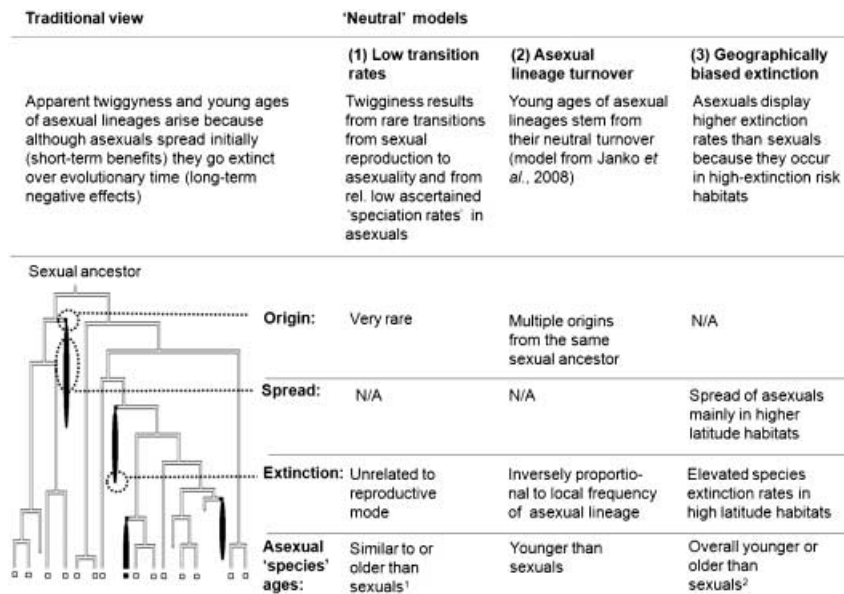
Traditional view	'Neutral' models		
	(1) Low transition rates	(2) Asexual lineage turnover	(3) Geographically biased extinction
Apparent twiggyness and young ages of asexual lineages arise because although asexuals spread initially (short-term benefits) they go extinct over evolutionary time (long-term negative effects)	Twigginess results from rare transitions from sexual reproduction to asexuality and from rel. low ascertained 'speciation rates' in asexuals	Young ages of asexual lineages stem from their neutral turnover (model from Janko et al., 2008)	Asexuals display higher extinction rates than sexuals because they occur in high-extinction risk habitats
Origin:	Very rare	Multiple origins from the same sexual ancestor	N/A
Spread:	N/A	N/A	Spread of asexuals mainly in higher latitude habitats
Extinction:	Unrelated to reproductive mode	Inversely proportional to local frequency of asexual lineage	Elevated species extinction rates in high latitude habitats
Asexual 'species' ages:	Similar to or older than sexuals[1]	Younger than sexuals	Overall younger or older than sexuals[2]

Sexual ancestor

Fig. 1 Comparison of the three models presented and the traditional view for the evolutionary trajectory of asexual lineages. Under the traditional view, asexuals go extinct faster than sexual species over evolutionary time. In contrast, each of the three models is 'neutral' with respect to reproductive mode, in that twiggyness and/or young ages of the asexuals result from mechanisms independent of adaptive or maladaptive effects due to the lack of recombination and genetic exchange between individuals.

[1]Relatively lower ascertained speciation rates in asexuals should result in relatively older asexual lineages.

[2]Dependent on extinction rates in higher latitude habitats; if relatively moderate, 'surviving' asexuals should be overall older than sexual species, if relatively high, they should be overall younger because most asexual clades go extinct very quickly.

Low 'net' transition rates from sexual to asexual reproduction

Theoretical studies in speciation and phylogenetics have examined expected distributions of quantitative traits on phylogenetic trees under different evolutionary scenarios (Maddison 2006; Maddison *et al.* 2007; Paradis 2008). Although the findings from such studies could be applied to generate predictions regarding the distribution of asexuals on specific phylogenetic trees, they have yet to be applied in macroevolutionary studies of asexuality. To illustrate how twiggy distributions can be produced in the absence of increased extinction rates, we simulated sets of phylogenetic trees under a model where taxa diversify depending on a character state (for example, sexual vs. asexual reproduction). We used the BiSSE ('binary-state speciation and extinction', Maddison *et al.* 2007) trees and characters simulator within the Mesquite system for phylogenetic computing (Maddison & Maddison 2007) to generate trees under three different evolutionary scenarios, each of which produces a twiggy distribution of a trait. The simulator generates trees by a speciation/extinction model in which a binary character controls rates of speciation/extinction. Thus, the model is specified by six parameters; character-specific speciation and extinction rates (four parameters), a transition rate from character 0 to 1 (for example, a transition from sexual to asexual reproduction) and a transition rate from character 1 to 0.

The first mechanism which results in a trait being rare and phylogenetically isolated is a low transition rate from the ancestral trait (Fig. 2A). Asexuality in modern eukaryotes is always a derived trait, given that sexual reproduction and eukaryotes probably evolved together (Cavalier-Smith 2002). There are several factors that might decrease the probability of a successful transition from sexual to obligate asexual reproduction:

1. The switch from sexual to asexual reproduction involves major cytological and physiological reorganizations in order to produce diploid eggs that can develop without fertilization (e.g. White 1964, 1973; Corley *et al.* 2001).
2. Offspring viability is typically extremely low in incipient asexual lineages (Bell 1982; Templeton 1982; Suomalainen *et al.* 1987) so that instead of the predicted twofold demographic advantage of asexuality, there is often a more than twofold demographic disadvantage.
3. If asexual reproduction is initially facultative, then gene flow from the sexual ancestor and extreme fitness benefits of male production in populations with highly female-biased sex ratios (Fisher 1930) may prevent asexuality from going to fixation.

Overall, the question of why sexual reproduction was extremely successful initially (i.e. at the origin of eukaryotes) thus appears to be at least partially distinct from the
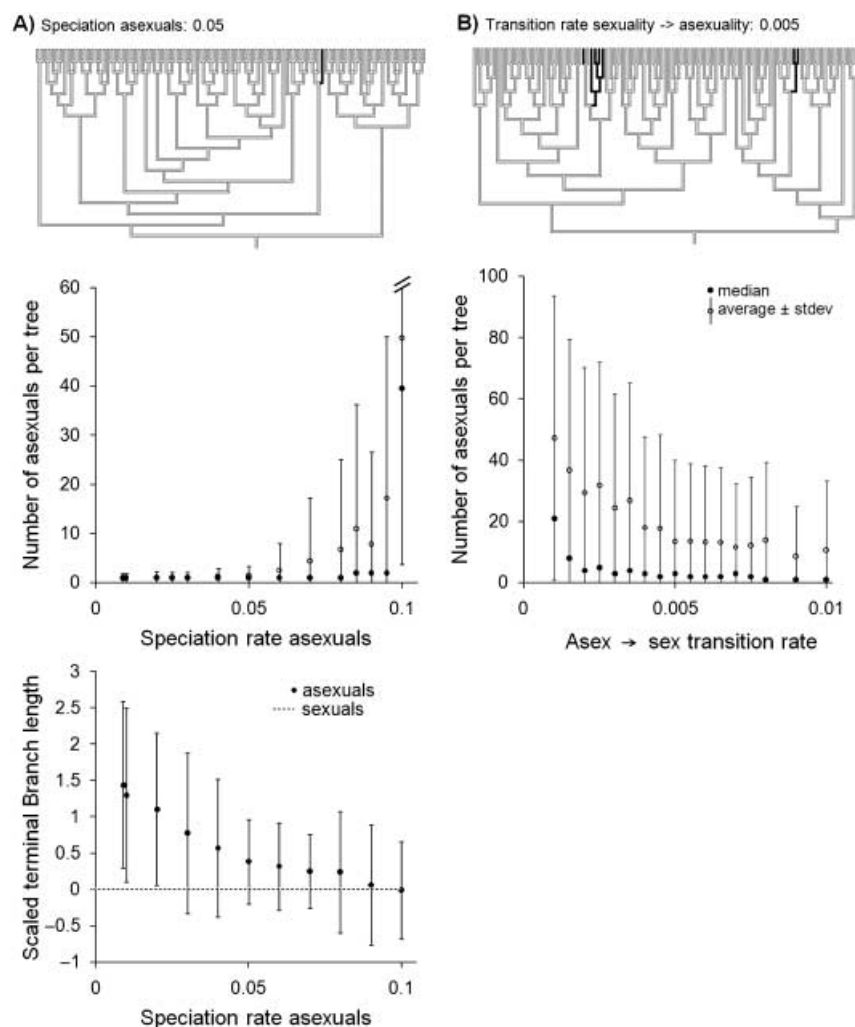
Fig. 2 Phylogenetic simulations illustrate how 'twigginess' of a trait such as asexuality can result from multiple processes that are independent of increased extinction rates. We generated random trees comprising 100 'species' under each of three different evolutionary scenarios using the BiSSE trees and characters simulator within the Mesquite system for phylogenetic computing (Maddison & Maddison 2007). Under each scenario, 'sexual reproduction' (i.e. character state 0) was the ancestral trait and the extinction rates were equal for both character states. Because we cannot display thousands of trees, we display the median and average ± SD number of asexuals on the phylogenies and terminal branch lengths for sexual and asexual 'species' across a small part of the parameter space. We further chose a representative tree for each scenario comprising the median number of asexual species for one parameter combination. Each point is estimated from 500 random trees and assumes constant extinction rates (0.05) in sexuals and asexuals, and constant speciation rates in sexual (0.1). Under the first scenario (A), we simply assumed that asexual lineages had a lower 'taxonomic' speciation rate than sexual lineages while transition rates were fixed (both 0.001). Under the second scenario (B), we assumed that the transition rate from sexuality to asexuality was lower than the reverse (fixed to 0.001). Branch lengths are not displayed because asymmetric transition rates do not interact with branch lengths for the two traits.

question of why asexuality is currently a rare trait (see also Maynard Smith 1978).

The second mechanism resulting in a twiggy distribution of a trait is a comparatively low speciation rate (Fig. 2B). A lineage which does not diversify is more likely to go extinct as a consequence of stochastic processes, because it pulls 'fewer tickets from the lottery' (Agnarsson *et al.* 2006). Thus, a lower speciation rate in asexuals than sexuals would support the asexual dead-end hypothesis, but the dead end

would be a consequence of the lack of ecological diversification rather than of relatively high extinction rates. With respect to speciation rates, two patterns need to be addressed in the macroevolutionary study of asexuality. First, large phylogenies comprising multiple asexual taxa should be used to test whether asexuals display lower 'speciation' rates than sexual taxa. Such a test would of course require the establishment of similar decision rules for distinguishing species in each group. Theoretically,

each mutation occurring in an asexual lineage leads to a new 'species' since reproductive isolation is at the individual level. Thus, most conventional species concepts apply by definition only to sexual organisms and named asexual 'species' are actually 'typological species' representing phenotypically ecologically uniform clusters of individuals (Maraun *et al.* 2004; Birky *et al.* 2005; Fontaneto *et al.* 2007, 2008). A second test would be required to ascertain that apparently lower speciation rates in asexuals as compared to sexual taxa are representative of a biological mechanism rather than a taxonomic artefact. On average, a diversifying asexual lineage is expected to display somewhat more continuous phenotypic variation than a diversifying sexually reproducing lineage of similar age (Templeton 1981, 1989; Hillis 2007). Because the variation is continuous in the asexual, the entire lineage will be considered as 'one asexual species', whereas it is more likely to be split into distinct species in the sexual lineage. As a consequence, asexuals may tend to be taxonomically underdefined relative to their sexual counterparts, and 'ascertained' speciation rates in asexuals should be lower as compared to sexual taxa. Support for the idea that asexual lineages tend to be taxonomically 'underdefined' stems from recent phylogenetic studies which have revealed that multiple clades with relatively high levels of genetic divergence are taxonomically considered as a single asexual 'species' (e.g. Heethoff *et al.* 2004, 2007; Laumann *et al.* 2007; Fontaneto *et al.* 2008). For example, within the named parthenogenetic oribatid mite *Platynothrus peltifer*, Heethoff *et al.* (2007) described seven clades which may have split more than 60 million years ago. Similar levels of 'cryptic diversification' were also documented in the asexual bdelloid rotifers (Birky *et al.* 2005; Fontaneto *et al.* 2007, 2008). In a sexually reproducing taxa, such levels typically correspond to distinct named species.

Third, twiggyness, considered as the phylogenetic isolation of a character state for a given trait, may result from asymmetrical transition rates between alternative character states, whereby the transition from one character state to the other is more likely to occur than the reverse (e.g. Maddison 2006). All else being equal, the character state which is relatively more likely to change will be rare (e.g. Maddison 2006; Paradis 2008; Fig. 2C). Although conceptually, asymmetrical transition rates are similar to an overall low transition rate from sexual to asexual reproduction, the two mechanisms may be quite different biologically. Low transition rates assume that it is possible to lose a complex trait (i.e. sexual reproduction) but impossible to re-evolve it (Dollo's law), for example, because genes involved in sexual reproduction degenerate. Asymmetric transition rates postulate that genes involved in sexual reproduction are maintained but that cytologically, one transition is more likely to occur than the reverse. Alternatively, asymmetric transition rates may stem from one reproductive mode

being selectively favoured over the other, in which case the mechanism would be non-neutral in the sense used here. It was initially considered by Williams (1975) that the relatively rare occurrence and apparent young age of asexual lineages may be due to frequent reversals from asexual to sexual reproduction. Although reversals from asexual back to sexual reproduction are considered unlikely (Bull & Charnov 1985), empirical data suggesting that such reversals may occur (such as the presence of sexually reproducing species within clades of exclusively asexual lineages) have been provided for multiple organisms, including *Daphnia* (Turgeon & Hebert 1994), oribatid mites (Domes *et al.* 2007), hybridogenetic fish (Cunha *et al.* 2008) and flatworms (Zietara *et al.* 2006). Unidirectional transitions and asymmetric rates also leave distinct patterns on trees; whereas rare transitions will result in purely asexual clades, reversals would result in a more interspersed phylogenetic position of the two reproductive strategies.

The tree patterns resulting under different evolutionary scenarios (Fig. 2A–C) illustrate that twiggyness per se does not indicate that asexual lineages go extinct faster than sexual lineages. The observed phylogenetic distribution of asexuality could arise simply as a consequence of sexuality being the ancestral character, and the transition from sexual to obligate and stable asexual reproduction occurring relatively rarely. Simon *et al.* (2003) state that approximately one out of a 1000 multicellular eukaryotes reproduces asexually, with large variation across phyla. Exactly how rare the transition to unisexuality has to be in a given phylum to produce a twiggy distribution will depend on characteristics of the group, such as their speciation rate relative to extinction rate.

Variation in speciation, trait transition and extinction rates can, in principle, be inferred from large phylogenies because the three processes differentially affect the internal and terminal branch lengths, as well as the distribution of internal nodes in taxon-wide phylogenies (Maddison 2006; Maddison *et al.* 2007; Paradis 2008). Recently, new methods have been developed which allow estimating trait-associated speciation and extinction rates from empirical phylogenies (Maddison *et al.* 2007; Paradis 2008). Currently, these methods assume that extinction rates are independent of lineage age, and it is not clear how the overall parameter estimates would be affected if extinction rates were age-dependent. Hence, these methods cannot yet be used to test for evidence of relatively high rates of asexual lineage extinction on phylogenies. A further limitation is the extent of available phylogenies, given that most of them comprise a single or only a few asexual taxa, or do not comprise all described species of a focal group.

Despite such limitations, the ages of extant species can be estimated relatively easily from phylogenies most of which are based on mitochondrial sequences. In such phylogenies, the age of a given species is approximately proportional to

Table 1 Studies used to estimate terminal branch lengths in sexual and parthenogenetic lineages with the number of included sexual and parthenogenetic 'species' for each study

Taxon	Sexual species	Parthenogens	Studies
Artemia shrimps	6	4	Baxevanis *et al.* 2006
Heterocypris ostracods	2	3	Rossi *et al.* 2007
Bacillus stick insects	3	4	Mantovani *et al.* 2001
Timema stick insects	13	5	Law & Crespi 2002a, 2002b
Adelgidae	12	6	Havill *et al.* 2007
Naryciinae bagworm moths	10	4	Grapputo *et al.* 2005
Microsporidia	3	11	Ironside 2007
Lasaea clams	2	10	O Foighil & Smith 1995
Otiorhynchus weevils	3	4	Stenberg & Lundmark 2004
Aramigus-group weevils	10	7	Normark 1996; Normark & Lanteri 1998
Calligrapha leaf-beetles	21	4	Gomez-Zurita *et al.* 2006
Oribatid mites	35	29	Maraun *et al.* 2004
Oribatid mites	10	16	Domes *et al.* 2007
Meloidogyne root-nod nematodes	2	5	Castagnone-Sereno *et al.* 1993; Tigano *et al.* 2005

its terminal branch length (DeSalle *et al.* 1987; Brower 1994). Although the age of current lineages does not provide information on extinction rates (because terminal branch lengths are affected by both speciation and extinction), the distribution of terminal branch lengths for sexual and asexual taxa can be used to test for evidence that current asexual 'species' are overall younger than their sexual counterparts.

In order to evaluate empirically if current asexual species are younger than their sexual counterparts, we determined the terminal branch lengths in 14 published phylogenies each comprising multiple asexual and sexual lineages (for 12 different groups or organisms including 133 sexual and 83 asexual species; see Table 1). The phylogenies were used as given by the authors (i.e. we did not evaluate the robustness of the phylogenies and indicated species definitions) and multiple lineages from a single species were collapsed into a single branch. Published phylogenies including branch lengths were recovered directly from the studies using TreeSnatcher1.0 (Laubach & von Haeseler 2007) and edited manually if necessary to correct for branch lengths errors introduced by the program. The terminal branch lengths in each tree were then determined with the Tuatara module (Maddison & Mooers 2007) implemented in Mesquite (Maddison & Maddison 2007). For trees without published branch lengths, we used the relevant sequences from GenBank and used the pairwise taxon divergences between sister taxa as an estimate for terminal branch length. In order to combine results across all phylogenies, we scaled, in each tree, the length of each individual branch by subtracting the average terminal branch value and by dividing by the standard deviation. These analyses revealed that, across the 14 phylogenies, there was no evidence that current asexual 'species' were younger than current sexual species (Kruskal–Wallis $X^2_1 = 1.4$, $P = 0.23$; Fig. 3).
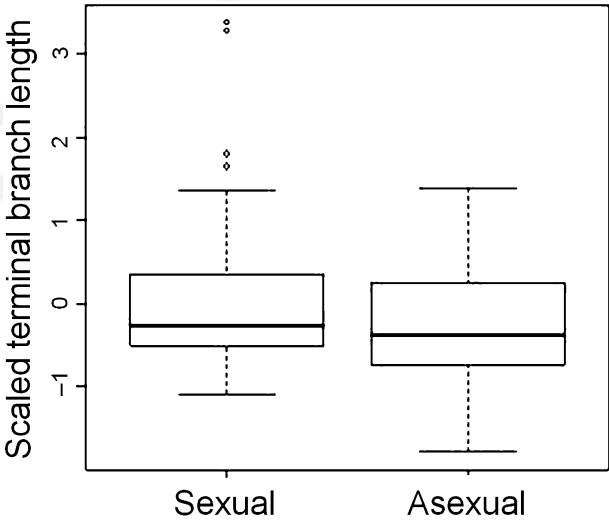

Fig. 3 Terminal branch lengths for sexual and asexual taxa determined from 14 published phylogenies (Table 1).

There are multiple caveats to comparing terminal branch lengths for sexual and asexual taxa. First, relatively taxon-rich phylogenies including asexual lineages might have been inferred selectively for taxa with potentially old or numerous asexuals, so the phylogenies used here would represent a biased sample. Second, factors such as increased rates of mutation accumulation in the asexuals, or a taxonomic underdefinition of asexuals, might lead to an overestimation of asexual lineage ages. By contrast, a more frequent hybrid origin for asexual than sexual species would result in lower ages for asexuals than for sexuals, because hybrids are necessarily younger than their parental species. Finally, with sexuality being the ancestral state, the theoretical maximum age for a sexual lineage will always be higher for

an asexual species. Independently of how valid or invalid the comparison is, it is important to note that there is currently no empirical evidence that existing asexual 'species' are young relative to sexual species.

Asexual lineage turnover

Janko *et al*. (2008) showed in a model of dynamic equilibrium between asexual lineage generation and loss that overall young ages of asexual lineages are the expected pattern if 'the influx of new asexual lineages into a community of related clones reduces the mean age reached by older clones as a result of neutral clonal turnover' (Janko *et al*. 2008). Hence, parental sexual species may continuously generate new asexual lineages, and the average age and genetic variability of what is considered a single asexual 'species' would be determined by a process analogous to the generation and maintenance of neutral genetic variation under mutation-drift equilibrium (Kimura & Crow 1964; Janko *et al*. 2008). The more frequently that new asexual clones split from the sexual ancestor, the lower is the average age of the independently derived asexuals and the more likely that the older asexuals are to disappear as a consequence of drift.

Within a phylogeny, such multiple, independent transitions to asexuality from the same sexual ancestor would often be considered parsimoniously as a single transition. Indeed, such a mechanism can only be detected by exhaustive and geographically broad sampling of the species concerned (see Delmotte *et al*. 2001 for an example) or if the asexuals derived from genetically divergent populations of the sexual ancestor. Although difficult to demonstrate, multiple, independent origins of asexuality from the same sexual ancestors have been reported in several taxa (Avise *et al*. 1992), including the ostracod *Eucypris virens* (Schon *et al*. 2000), the mite *Platynothrus peltifer* (Heethoff *et al*. 2006), the aphid *Rhopalosiphum padi* (Delmotte *et al*. 2001) and a *Poeciliopsis* fish (Quattro *et al*. 1991). Thus, in at least some groups, the generation of new clones may indeed be more or less continuous.

From their model, Janko *et al*. (2008) made four testable predictions regarding the geographical and age distribution patterns of asexual lineages and their sexual ancestors. First, asexual lineage ages should correlate negatively with the rate of sexuality to asexuality transitions in their sexual ancestor. Second, asexuals should be relatively younger if they live in sympatry or parapatry with their sexual ancestor rather than in allopatry, because new clones are more likely to enter the existing clonal pool. Third, clonal diversity should decrease with increasing distance from the sexual ancestor. Finally, older asexuals should have a wider geographical distribution than younger asexuals.

Although quantitative tests of the above predictions will require more complete phylogeographic information across many taxa, Janko *et al*. (2008) provided a list of studies that showed results consistent with the predictions. Thus, a hemiclonal *Poeciliopsis* fish with multiple origins appears to be younger than a single-origin hemiclone in the same genus (Quattro *et al*. 1991). In at least five groups [*Campeloma* snails (Johnson 2006), *Lasaea* clams (O Foighil & Smith 1995), *Timema* stick insects (Law & Crespi 2002a,b), *Squalius alburnoides* fish hybridogens (Cunha *et al*. 2004), and gynogenetic *Cobitis* loaches (Janko *et al*. 2005)], the asexuals geographically closer to their sexual ancestor appear to be younger than the geographically more separated ones. Clonal diversity does indeed decrease with increasing distance from the sexual ancestor in *Potamopyrgus* snails (Neiman & Lively 2004; Neiman *et al*. 2005), and older asexuals tend to have wider geographical distributions than younger asexuals in the *S. alburnoides* and *Poeciliopsis* fish (Quattro *et al*. 1991, 1992; Cunha *et al*. 2004), the *Cobitis* loaches (Janko *et al*. 2005) and *Campeloma* sails (Johnson 2006), but not in *S. polychroa* flatworms (Pongratz *et al*. 2003) or *Timema* stick insects (Law & Crespi 2002a,b). An additional line of evidence for the last prediction stems from the putatively 'ancient' asexual bdelloid rotifers, darwinulid ostracods and oribatid mites, many of which tend to be geographically widespread (see below).

Taken together, these lines of evidence suggest that a dynamic equilibrium between the origin of new clones and their extinction might contribute to the apparent young age of asexuals and help to explain patterns of geographical distribution of asexuals with variable ages, with respect to their sexual ancestors, in at least some groups.

Geographical parthenogenesis and geographically biased extinction

Asexuals may also be shorter-lived than sexuals for non-selective reasons under our third neutral model for the phylogenetic distribution of asexuality. In particular, this model posits that the risk of extinction for asexual lineages may be higher than that of sexuals simply due to the effects of geographical parthenogenesis, that is, the general pattern of higher-latitude Northern Hemisphere and higher-altitude distributions in asexual than related sexual taxa (Vandel 1928; Suomaleinen 1950; Glesener & Tilman 1978; Bierzychudek 1985; Kearney 2005; Lundmark & Saura 2006). Geographical parthenogenesis thus engenders a higher extinction rate among asexuals as a direct consequence of more extensive and rapid modifications of high latitude habitats at the onset of recent Pleistocene glacial periods or warm interglacials (Hewitt 1996), which differentially impact upon their ability to persist.

Patterns of geographical parthenogenesis may be generated and maintained by any of several processes, including: (i) a selective advantage of asexuals over sexuals in high-latitude habitats with low productivity, whereby in the sexuals, locally

co-adapted gene complexes are broken up and swamped by ecologically divergent alleles introduced from high-productivity low-latitude habitats (Peck *et al.* 1998); (ii) direct effects of asexual reproduction itself (Haag & Ebert 2004), such as presumed enhancement of colonizing ability in asexuals (but see Horne & Martens 1998; Pongratz *et al.* 2003) or a reduction in the efficacy of biotic selective forces in colder climates (Glesener & Tilman 1978); alternatively, such patterns may be caused by indirect, correlated effects of asexuality, such as: (iii) molecular-ecological consequences of polyploidy, which is relatively common in asexuals and in higher-latitude sexual animals and plants (Stebbins 1950; Brochmann *et al.* 2004), and which can result in broader ecological tolerances (e.g. Dufresne & Hebert 1998; Otto & Whitton 2000); (iv) more frequent hybridization at higher latitudes, due to re-contact of colonizing populations and species, separated during glaciations (Hewitt 1996), that results in enhanced generation of hybrid asexual lineages (e.g. Adamowicz *et al.* 2002); or (v) interactions between polyploidy and asexuality (Lynch 1984) or between hybridization and asexuality (Kearney 2005), whereby asexuality stabilizes newly generated lineages that are relatively well adapted.

Independent and joint effects of sexuality, polyploidy and hybridization are difficult to disentangle (Horandl 2006; Lundmark & Saura 2006), and need not be mutually exclusive, but determining their relative contributions to the processes that underlie geographical parthenogenesis is crucial for discerning the evolutionary factors that mediate the generation and maintenance of asexual taxa. In particular, polyploidy and hybridization commonly generate highly heterozygous genotypes that may facilitate the persistence of both asexual and sexual lineages.

By our geographical model, geographically biased extinction should affect both asexual and sexual lineages. Thus, sexual taxa occurring in higher-latitude (or higher-altitude) regions should exhibit higher extinction rates than sexual taxa inhabiting regions that were less affected by glacial cycling. Such extinctions should be concentrated near the beginnings of glacial or warm interglacial periods, and they should be functions of rapid habitat loss rather than inability to adapt. Historical extinction rates are difficult to infer from extant data, but some evidence exists for older ages of tropical than temperate species (Mittelbach *et al.* 2007), more-rapid species turnover in temperate regions (Weir & Schluter 2007), and increased species losses during periods of cooling (Hawkins *et al.* 2007); current patterns of habitat and species loss due to rapid warming, and analyses of the rapidity of Pleistocene glacial advances, may also provide insights into the processes underling these patterns.

Given that our geographically neutral model is predicated on geographical parthenogenesis in recent asexuals, it predicts that relatively old asexuals should not show higher-latitude (or higher-altitude) distributions than related sexuals. Relatively old asexuality has been supported or inferred for a small number of taxonomic groups (Judson & Normark 1996; Normark *et al.* 2003), and sufficient information is available for six of these groups to allow evaluation of latitudinal geographical range patterns in such asexuals and their sexual relatives. Several important caveats apply to these comparisons, including uncertainties concerning the presence and duration of exclusive asexuality (Normark *et al.* 2003; Smith *et al.* 2006; Gladyshev *et al.* 2008), limitations of geographical sampling and taxonomic or phylogenetic resolution (e.g. Rossetti & Martens 1998; Fontaneto *et al.* 2008), and increased difficulty of identifying close sexual relatives for older asexual lineages. Nevertheless, the available information on geographical distributions and age estimated for the relatively old asexuals are consistent with the idea that these groups do not display patterns of geographical parthenogenesis.

Bdelloid rotifers. Two of the best-known groups that include apparent old asexuals, rotifers and ostracods, are similar in that the small sizes of many species, under 1–2 mm, may permit effective long-distance dispersal (Finlay & Fenchel 2004; Fontaneto *et al.* 2008).

Rotifers comprise two main Classes, the fully asexual Bdelloidea, and the Monogononta, most of which show cyclical parthenogenesis (King *et al.* 2005). Robust inferences concerning the distribution patterns of bdelloid and monogonant rotifers are compromised by the fact that most species have been described only from their type localities (Segers & De Smet 2008). However, distribution data in the annotated checklist compiled by Segers (2007) permits a quantitative comparison of the latitudinal ranges of bdelloids vs. monogonants. By this compilation, 41% (184 of 448) of bdelloid rotifers are found in temperate regions (Nearctic, Palearctic, and/or Antarctic), 17% are from tropical or subtropical regions (Neotropical, African, Oriental, Pacific, and/or Australian), and 42% are reported from both regions. The respective proportions are virtually the same among monogonant rotifers, at 40% (590 of 1460) temperate, 17% mainly tropical or subtropical, and 42% across both regions.

Darwinulid ostracods. Ostracods comprise about 2000 species, about half of which are in the family Cyprididae which includes both sexual and apparently recent asexual taxa, while 29 species have been described in the related, fully asexual family Darwinulidae, which is putatively an ancient asexual group (Butlin *et al.* 1998; Rossetti & Martens 1998; Martens *et al.* 2008). Geographical parthenogenesis, polyploidy, and hybridization are common among non-darwinulid ostracods, especially among species of Cyprididae (Butlin *et al.* 1998; Horne & Martens 1998; Schon *et al.* 2000, 2003; Griffiths 2001). One species of ostracod in this family, *Eucypris virens*, exhibits a clear pattern of geographical parthenogenesis and an estimated maximum age of about

4.5 million years (Myr), but asexuality in this lineage is characterized by multiple origins and apparent periodic interbreeding of asexuals with males of related sexuals (Schon *et al.* 2000).

Darwinulidae do not show evidence of higher-latitude distributions than other ostracods, as their distributions across biogeographic and latitudinal zones are similar to those of Cyprididae (Martens *et al.* 2008, Table 1). Thus, 9 (31%) of 29 Darwinulidae species are reported from the Palearctic, Nearctic, and/or Antarctic, compared to 362 (36%) of 998 species of Cyprididae.

Oribatid mites. Ancient asexuality has been inferred in oribatid mites based on taxonomic studies showing the presence of a large number of wholly asexual higher taxa (Norton *et al.* 1993) and molecularphylogenetic analyses of select lineages (Maraun *et al.* 2004; Heethoff *et al.* 2006). Norton *et al.* (1993) and Behan-Pelletier (1998) report an absence of latitudinal or altitudinal geographical structuring to asexuality in oribatids. Cosmopolitan distributions are found in about 20% of asexual species of oribatid mites (Behan-Pelletier 1998), which apparently reflects continental drift rather than the ability to disperse over long distances (Stary & Block 1998; Heethoff *et al.* 2007).

Artemia *brine shrimp.* The genus *Artemia* comprises five described bisexual species and one asexual species, all of which inhabit inland saline ponds or lakes. The asexual species, *A. parthenogenetica*, exhibits ploidy levels of 2n, 4n and 5n and appears to be polyphyletic, with one of the asexual lineages inferred from mitochondrial and nuclear DNA data as having persisted for at least 3 Myr (Baxevanis *et al.* 2006). Of the sexual *Artemia* species, three are distributed on single continents (one in South America and two in Asia), one has been recorded from both Africa and Europe, and one is found across North and South America; by contrast, asexual populations have been reported from Africa, Asia, Europe and Australia (Triantaphyllidis *et al.* 1998).

Lasaea *clams.* Marine clams in the genus *Lasaea* comprise two diploid sexual species restricted to Australia, and a third species, *L. australis*, which is asexual and exhibits a cosmopolitan distribution (O Foighil & Smith 1995; Taylor & Foighil 2000). *L. australis* is comprised of multiple clonal lineages apparently derived via allopolyploidy, and although the species as a whole is distributed worldwide, it also exhibits notable levels of local genetic differentiation (Taylor & Foighil 2000).

Bacillus *and* Timema *walking-sticks.* The walking-stick species *Bacillus atticus* is reported as a candidate old asexual in Normark *et al.* (2003) with an age estimate of 15 Myr, and its distribution in the Mediterranean basin broadly overlaps with the distributions of its sexual relatives (Mantovani

et al. 2001). This species may thus represent an old asexual that does not exhibit geographical parthenogenesis, if its age and fully asexual status can be more accurately ascertained.

The genus *Timema* includes five independently derived asexual walking-stick species, in addition to about 15 sexual species (Law & Crespi 2002a,b; Schwander & Crespi in press). Geographical patterning to parthenogenesis in *Timema* is supported by the more-northerly distributions relative to their sexual ancestor of three of the asexuals, *T. douglasi*, *T. shepardi*, and *T. tahoe*, all of which have been inferred as relatively young (*T. tahoe*, 0.29 to 1.06 Myr) or very young (*T. douglasi* and *T. shepardi,* well under 0.5 Myr) (Law & Crespi 2002b). By contrast, the two asexuals (*T. genevieve* and *T. monikensis*) that are found at similar latitudes as their sexual ancestors may be older, with age estimates of 0.81 to 1.42 and 0.26 to 2.39 Myr, respectively (Law & Crespi 2002b). The degree to which these two asexuals can be considered as old, compared to asexual lineages in other taxa, is unclear, given the general paucity of firm quantitative evidence concerning asexual lineage ages and the integrity of their putative long-term exclusively asexual reproduction.

These data from six disparate taxa indicate that the distributional patterns of relatively old asexuals, in relation to their sexual relatives, demonstrate an apparent general absence of geographical parthenogenesis, in the context of higher-latitude distributions than related sexuals, for older asexual groups. This pattern is broadly consistent with an apparent short-lived nature of some asexual lineages being mediated in part by higher-latitude distributions that engender higher extinction rates due to recent glacial cycling rather than genomic effects of asexuality. This hypothesis can be evaluated further by determining whether a pattern of younger ages for temperate-zone species, compared to tropical species (Weir & Schluter 2007), applies broadly across taxonomic groups including invertebrates, and whether species generation and loss of asexuals, sexuals, or both, is notably intensified in regions most-subject to effects of glaciation.

The clearest biogeographic correlate of asexuality among the relatively old asexual taxa considered here is not latitude, but the common presence of cosmopolitan or otherwise intercontinental distributions, which are found in bdelloid rotifers, darwinulid ostracods, oribatid mites, *Lasaea* clams, and possibly *Artemia* shrimp. Among bdelloids, darwinulids, and *Artemia*, broad geographical distributions appear to be related to their long-distance dispersal abilities, which may be facilitated by the ability of asexuals to initiate populations with single females, and their capacity to survive unfavourable periods via the presence of dormant resting stages, which may help to maintain cosmopolitan metapopulations (Finlay & Fenchel 2004). These considerations regarding animal asexuality may help explain the apparent absence of old asexual lineages in plants (Whitton *et al.*

2008), to the degree that few plant taxa exhibit this combination of traits or these population structures.

Under a neutral model for the persistence of asexuals, asexual lineages with higher dispersal capabilities, better abilities to survive unfavourable periods, and broader geographical distributions should avoid extinction for longer periods of evolutionary time as a simple result of these properties — and these considerations should apply equally well to sexual lineages. By contrast, under a selective model, asexuals with intercontinental metapopulation structures may have the capacity to better delay extinction mediated by co-evolutionary biotic interactions, such as genetically based vulnerability to parasites (Jaenike 1978; Hamilton *et al*. 1990). Older asexuals may also exhibit mechanisms, such as horizontal gene transfer (Gladyshev *et al*. 2008), that allow them to successfully persist in regions of higher biotic complexity (Glesener & Tilman 1978), which could help to explain their apparent lack of latitudinal patterning to parthenogenesis.

Discussion

Neutral models characterize evolutionary or ecological patterns expected in the absence of specific causal processes, such as selection or competitive inequalities (e.g. Hubbell 2001). These models generate predictions, and provide explicit frameworks for comparison between alternative hypotheses, thus permitting robust tests based on strong inference. In this study, we have described and evaluated three models that can, in principle, help explain the apparent 'twigginess' of asexual lineages on phylogenetic trees, which has thus far been uncritically interpreted as evidence that asexuality is associated with elevated extinction rates and thus represents an evolutionary dead end (Fig. 1). Each of the three models is 'neutral' with respect to reproductive mode, in that twiggyness and young age of the asexuals result from mechanisms independent of adaptive or maladaptive effects due to the lack of recombination and genetic exchange between individuals. Neutral explanations for the phylogenetic distribution of asexuality need not be mutually exclusive with respect to mutational or ecological models for the long-term or short-term costs of asexuality compared to sex, and in some taxa, there is certainly convincing evidence that sex is maintained by selection (Lively 1987; Lively *et al*. 2004). However, to the extent that neutral models can parsimoniously explain the phylogenetic and phylogeographic distribution patterns of asexuality, these models should be evaluated in considerably more detail by assessing the accuracy of their assumptions and by generating quantitative neutral expectations that can be compared to expectations from selective models and the observed patterns themselves (see also Janko *et al*. 2008).

Our first neutral model uses simple phylogenetic simulations to demonstrate that low transition rates to stable asexuality, or low rates of ascertained 'speciation' in asexuals, can generate twiggy distributions of asexuality, in the absence of higher extinction rates for asexual lineages. We also analysed the relative terminal branch lengths of asexual vs. sexual lineages for 14 published phylogenies, and showed that there is no statistical support, from the available data, for a hypothesis that asexual species are shorter-lived. Taken together, these analyses indicate that there is a lack of theoretical expectation, or empirical evidence, that asexuals are actually short-lived compared to sexuals. This hypothesis can be evaluated further via the use of comparative, phylogenetic methods with larger data sets for any given clade, such that asexual and sexual species can be contrasted in a statistical framework.

By the second and third neutral models considered here, asexual lineages may indeed be younger on average than sexual lineages, but not due to deleterious genomic effects of asexual reproduction. The second model, developed by Janko *et al*. (2008), shows that a dynamic equilibrium between origins and neutral losses of asexuals can, under some conditions, generate a relatively low mean age of asexual lineages. This model is consistent with a considerable range of evidence regarding the recurrent origins of asexuals in some groups, their patterns of genetic diversity, and their geographical ranges, and it makes a suite of clear, falsifiable predictions.

The third neutral model is based on the common pattern of geographical parthenogenesis in asexuals, whereby they tend to inhabit regions, notably higher latitudes that have recently been subject to relatively strong climactic and biotic effects of glacial cycling. Geographical parthenogenesis has previously been interpreted in terms of selective or demographic processes, such as reduced complexity of biotic interactions at higher latitudes, local adaptation, differential effects of drift or gene flow in sexuals and asexuals, or presumed better dispersal ability in asexuals (Peck *et al*. 1998; Haag & Ebert 2004), but the efficacy of these processes has yet to be established. Our model posits that the selective or demographic basis of geographical parthenogenesis may be irrelevant to its primary effect: an elevation of extinction rates in asexuals due simply to their geographical positions being higher-latitude or higher-altitude than those of sexuals, and thus differentially affected by glaciations. This hypothesis is consistent with the apparent absence of geographical parthenogenesis in relatively old asexuals, and it can be evaluated further via combined biogeographic, phylogeographic and phylogenetic studies of asexuals and related sexuals.

The main evidence supporting selective models, in contrast to neutral models, for the evolution of asexual reproduction has been experimental and molecular–evolutionary studies on the accumulation of deleterious mutations in the absence of recombination, or faster adaptive evolution in sexual genetic systems with recombination (reviewed in Rice 2002; de Visser & Elena 2007). These studies have provided

clear evidence that a long-term lack of recombination engenders mutation accumulation (Rice 2002; de Visser & Elena 2007) often with negative fitness effects (Ajie *et al.* 2005; Cooper *et al.* 2005; Buckling *et al.* 2006; Bruvo *et al.* 2007). Most or all of these experimental systems have involved clonally transmitted elements in a usually sexual context, such that their relevance to the bulk of metazoans are unclear, especially for obligately asexual lineages that have been exposed to selection for long periods. Several studies have also revealed higher levels of nonsynonymous amino acid substitutions in asexually reproducing organisms as compared to their sexually reproducing sister species (Moran 1996; Normark & Moran 2000; Schon *et al.* 2003; Paland & Lynch 2006; Barraclough *et al.* 2007), which supports the idea of deleterious mutation accumulation, although the negative fitness effects of these mutations are generally only assumed (see Lambert & Moran 1998, for a notable exception). The same patterns (i.e. increased rates of nonsynonymous substitutions), however, are a major line of evidence for strong positive selection on genes in sexually reproducing organisms (e.g. Eyre-Walker 2006), which emphasizes the importance of discerning the causes and population-level effects of selection at the molecular level. More generally, despite the breadth and depth of evidence for mutation accumulation, the demographic and macroevolutionary consequences of these mutations in asexual lineages remain unknown. Similar considerations apply to ecological models for the benefits of sexuality, such as models based on increased vulnerability to parasitism in asexuals (e.g. Hamilton *et al.* 1990), which have thus far accumulated only a moderate degree of supporting empirical evidence salient to the costs and benefits of asexuality in natural populations (e.g. Busch *et al.* 2004; Kumpulainen *et al.* 2004).

Although there is little or no direct evidence showing that mutation accumulation, or reduced ability to adapt in the face of ecological selective pressures, cause increased extinction risk in obligately asexual lineages, asexual taxa and elements exhibit a range of unusual genomic characteristics that should alleviate the negative effects of their reproductive system. Thus, the origin of asexual reproduction commonly involves polyploidy and hybridization (e.g. Kearney 2005; Lundmark & Saura 2006; Whitton *et al.* 2008). Divergent evolution or expression of homologous alleles has been demonstrated following polyploidization in plants (Hegarty & Hiscock 2008) and bdelloid rotifers (Welch *et al.* 2008). Divergent evolution of homologous alleles in a diploid genome as well as horizontal gene transfer have been described in bdelloid rotifers (Pouchkina-Stantcheva *et al.* 2007; Gladyshev *et al.* 2008), and gene conversion apparently helps to prevent or delay mutational degeneration of the mammalian Y chromosome (Rozen *et al.* 2003); mechanisms with comparable effects presumably exist in asexual elements such as mitochondria (Loewe 2006; Loewe

& Lamatsch 2008). At the phenotypic level, relatively old asexuals, such as darwinulid ostracods, bdelloid rotifers, oribatid mites, and *Artemia* shrimp, appear to exhibit a relative preponderance of taxa with traits that may reduce extinction risk, including some combination of high dispersal abilities, dormant resting stages, broad geographical distributions, and large population sizes. The degree to which these characteristics compensate, on a macroevolutionary scale, for deleterious effects of the loss of recombination remains unknown, but their presence suggests that a range of mechanisms exist in asexuals that may potentially facilitate their long-term persistence.

Our analysis of neutral models for the evolution of asexuality motivates several specific recommendations for future work. First, macroevolutionary studies of asexuality will benefit from collecting phylogenetic data from clades that show multiple origins of asexuality, with concurrent analyses of the mechanisms whereby asexual reproduction originates, to evaluate whether different cytological routes to asexuality, such as automixis, hybridization, or polyploidy, engender differences in species or lineage longevity. Second, analyses at the level of microevolution need to more closely connect mechanisms with mutational and selective consequences, such as by testing for effects of mutation accumulation under apomixis vs. automixis or hybrid vs. nonhybrid asexual origin, and by testing for purifying selection and positive selection in ecologically important genes, such as genes involved in the immune system or plant–insect interactions. Finally, micro- and macroevolution can be connected by probing for ecological and demographic effects of functional genetic and genomic variation, in a phylogenetic context, which will ultimately allow partitioning of the roles of neutral and selective processes in the evolution of sex and asexuality.

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12

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