



REVIEW

Why do species exist? Insights from sexuals and asexuals**

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Summary

Why does life diversify into the more or less discrete entities we recognise as species? Two main explanations have been proposed: i) species are a consequence of adaptation to different ecological niches, ii) species are a consequence of sexual reproduction and reproductive isolation. Phylogenetic studies of case-study groups can provide insights into the relative importance of divergent selection and isolation for speciation, but it can be difficult to infer causes of speciation unambiguously. The example of North American tiger beetles from the genus *Cicindela* is discussed. An alternative approach is to compare diversification between related sexual and asexual taxa to infer the relative importance of the two explanations. We outline expected patterns of diversification in sexual and asexual lineages under different scenarios using coalescent theory. Whether sexuals or asexuals diversify to a greater extent depends on the balance among various stages of diversification, particularly on the effects of sexual reproduction on rates of adaptive evolution. Rotifers offer a unique system to test these ideas, allowing comparison of patterns of genetic and functional morphological diversification in sexual (bdelloid) and asexual (monogont) clades.

Key words: bdelloids, evolution, phylogenetics, speciation theory

Introduction

Species are a fundamental phenomenon of life. Although details may vary among different organisms, all life is made up of the more or less discrete and diverse entities we call species. But why do species exist? Why does life evolve into discrete packages rather than comprising a continuous range of genetic and morphological forms? Previous discussions have identified two main explanations (Maynard Smith and Szathmáry, 1995; Coyne and Orr, 1998).

(1) Species reflect the varied environment found on our planet: species are adapted to different ecological niches and arise as a consequence of divergent natural

selection. There is increasing experimental and theoretical support for ecological speciation in sexual organisms (Schluter, 2001) and it has frequently been invoked to explain speciation in asexual organisms (Fisher, 1930; Mayr, 1957; Hutchinson, 1968).

(2) Species are a consequence of sexual reproduction: interbreeding maintains coherence within populations, while reproductive isolation leads to genetic and phenotypic discontinuities between isolated populations. Maynard Smith and Szathmáry (1995) and Coyne and Orr (1998) regarded this hypothesis as most important, but they highlighted that empirical tests were scarce.

How can we test the relative importance of divergent selection and isolation explaining why species exist? Tra-

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ditionally, testing hypotheses about the causes of speciation and evolution of diversity has been difficult, because of the long time-scale of the processes involved. The fossil record can provide important data on speciation (Benton and Pearson, 2001), but for most groups it is too incomplete and unresolved to observe speciation events, and information on important biological characters such as habitat and ecology is typically lacking. Instead, for many questions we must use comparisons of extant organisms to infer past processes driving diversification. The development of phylogenetic analysis and the use of molecular markers are transforming our ability to answer general questions about speciation (Avice, 2000; Barraclough and Nee, 2001). Here, we discuss how phylogenetic approaches can be used to investigate why species exist. We discuss methods for inferring causes of speciation in case-study groups from species-level phylogenetic trees, before outlining one promising approach for disentangling the two fundamental explanations of species: comparisons of the pattern of diversification in related sexual and asexual clades.

Causes of speciation from species-level trees

Phylogenetic trees, particularly those including all the living species within a higher taxonomic group, such as a genus, provide an indirect record of speciation events that led to present-day species (Hennig, 1966). Along with information on geographical and ecological attributes of species, they can be used to test hypotheses about the causes of speciation in a group (Barraclough et al., 1998; Berlocher, 1998). In addition, trees derived from DNA data contain information about the relative timing of reconstructed speciation events, which can be used to focus on the most recently diverged species. For example, the idea that geographic isolation is a predominant cause of speciation in birds comes from evidence that the most closely related species tend to be found in different areas rather than sympatrically (Mayr, 1963; Chesser and Zink, 1994; Coyne and Price, 2000). Can we use this approach to test the relative importance of isolation and divergent selection in explaining the existence of species?

We illustrate this approach using the example of North American tiger beetles (genus: *Cicindela*). The genus *Cicindela* represents a spectacular radiation of insects, with around 130 species in North America and over 1000 species worldwide (Pearson, 1988; Pearson and Vogler, 2001). They are sleek, raptorial predators, relying on fast locomotion (both in flight and on foot) and large mandibles to chase down their arthropod prey. The genus has been well studied, particularly in North America, where their taxonomy, ecology and geographic distribution are better known than most other

insect groups. In addition, recent work has reconstructed a phylogenetic tree of 75% of the recognised North American species from mitochondrial DNA sequence data (Vogler and Welsh, 1997; Vogler et al., unpubl. data). These data provide a unique tool for exploring the causes of speciation and adaptive radiation. In particular, we used these data to investigate the importance of geographic isolation and divergent selection in tiger beetle speciation.

As outlined above, if geographic isolation is important for speciation we predict that closely related species should occupy different areas, and sympatric species will tend to be more distantly related (Barraclough et al., 1998; Berlocher, 1998). To test this, geographic range data were used to calculate the degree of sympatry between all sister clades in the tree, defined as the area of overlap divided by the range size of the clade with smaller range. This ranges from 0, signifying no overlap in ranges, to 1, signifying that one clade's range is entirely overlapped by the other. Ranges of higher clades were calculated as the total area occupied by any of the member species. Sympatry values were then plotted against the age of nodes on the tree. An important role for geographic isolation would be evident as a predominance of sympatry values near 0 for very recent nodes. An excess of values near 1 for recent nodes might indicate a high frequency of sympatric speciation, i.e. speciation without geographic isolation (Barraclough and Vogler, 2000).

The plot obtained for endemic radiations in the North American tiger beetle fauna is shown in Figure 1. The

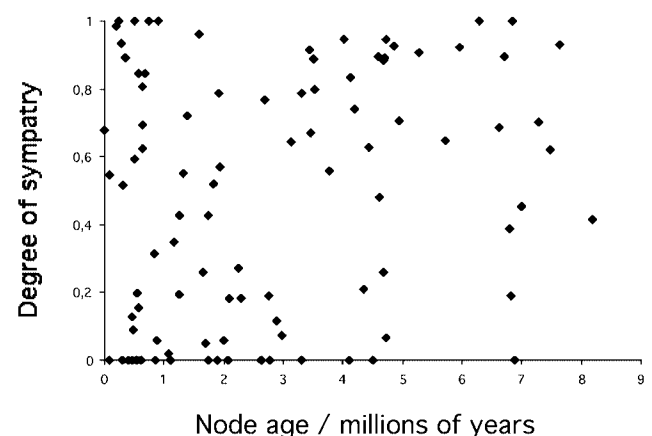


Fig. 1. Plot of the degree of sympatry against node age for endemic radiations of *Cicindela* tiger beetles in North America identified from a mtDNA phylogenetic tree. Values of 0 signify no range overlap between sister clades, 1 signifies that the range of one clade is entirely overlapped by the other. The tree was dated using biogeographic information for reconstructed speciation events associated with the emergence of Florida and the Isthmus of Panama (Barraclough and Vogler, 2002).

main feature is the spread of points between 0 and 1, even for recently diverged species. Intermediate values must be the result of species range movements: no mode of speciation produces values between 0 and 1. Although statistical analysis of range data is difficult because of difficulties in formulating a realistic null model, simulations confirm that this general pattern is produced when range movements have been so extensive that the signal of the geographic mode of speciation has been wiped out (Barracough and Vogler, 2000). This conclusion is unsurprising given the glacial history of the region. Analysis of the timing of speciation in the group provides some evidence of increased speciation rate during the Pleistocene (Barracough and Vogler, 2002), perhaps because of the impact of glaciation on population isolation, but there is no clear-cut record in present-day ranges.

A similar approach can be used to test whether divergent selection might have played a role in tiger beetle speciation, specifically, whether divergent selection between different habitat types has driven speciation. An alternative hypothesis would be that speciation is unrelated to habitat shifts, and that habitat preferences simply diverge eventually over time. The prediction of the habitat-shift speciation hypothesis is that closely related sister species should tend to occupy different habitats to a greater extent than expected under a null model of random accumulation of habitat differences over time (Barracough and Nee, 2001). A test of this prediction in the *Ellipsiptera* subgenus, an endemic clade of North American *Cicindela*, provided no evidence for habitat-shift speciation, but the sample size was too small for a powerful test (Barracough et al., 1999). Work on the entire fauna is providing some evidence for habitat changes linked to speciation events (T. Barracough, unpubl. data), but still the question remains whether this is due to divergent selection promoting speciation. One alternative is that habitat shifts tend to decouple the range movements of isolated populations during glacial changes, promoting isolation, whereas populations preferring similar habitats are more likely to re-establish contact before reproductive isolation is established. Field studies on tiger beetle populations might add further evidence on these possibilities.

In conclusion, there is some evidence for the role of divergent selection in tiger beetle speciation, but the existence of extensive range movements in North America appears to have wiped out any clear signal of the geographic mode of speciation, i.e. the role of isolation. It is possible that studies of tiger beetles in regions that have experienced less extreme climate changes could shed further light on these processes. Nonetheless, the examples show how it can be hard to infer causes of speciation unambiguously in a given group. An addi-

tional limitation is that most phylogenetic studies have necessarily relied on using described taxonomic species as surrogates for evolutionary species. Taxonomic species need not reflect underlying evolutionary species for several reasons, including subjectivity of taxonomic description, the possibility of cryptic species, and the effects of complex species histories involving hybridisation or species paraphyly (Avice, 2000; Morgan et al., 2000). Ideally, tests of the fundamental explanations for why species exist should incorporate individual-level sampling to delimit species boundaries more accurately.

Sexuals versus asexuals: theory

The idea that sexual reproduction might be necessary or important for the pattern of species to evolve led to suggestions that comparing the pattern of diversity in sexual and asexual organisms might help to answer fundamental explanations about why species exist (Maynard Smith and Szathmáry, 1995; Coyne and Orr, 1998). If asexuals display the same pattern of discrete clusters as found in sexual lineages, it would suggest that sexual reproduction is not a necessary condition for diversification. However, the theoretical basis for expected patterns of diversification in sexuals and asexuals has been unclear: there have been general statements but few detailed accounts. For example, recombination acts as a strong force against genetic divergence within sexual populations, because it breaks down polygenic differences (Felsenstein, 1981). This might reduce the tendency for diversification in sexuals in comparison to asexuals. Consideration of the effects of presence or absence of sex on each stage of diversification is needed to make reliable predictions. Also, comparisons between sexuals and asexuals are complicated by the difficulty of applying a consistent species concept to both lifestyles. The biological species concept does not apply to asexuals because all individuals are reproductively isolated entities. Attempts have been made to devise a universal species concept, but the many aspects and fuzzy boundaries of what people want 'species' to be has led to failure of these attempts to be widely accepted (Mishler and Donoghue, 1982; Hey, 2001). Clearly, we need a quantitative approach that tests for the entities produced by diversification, to circumvent problems of taxonomic nomenclature.

A useful framework for considering expected patterns of genetic and morphological diversification in sexuals and asexuals is to consider expected gene trees under different scenarios. This approach provides a link between pattern-based surveys of genetic and morphological diversity and underlying population processes that cause the observed patterns. A gene tree is a representa-

tion of the ancestry of alleles from the same locus sampled across a set of individuals. A large body of theory has established expected gene trees for a wide range of scenarios, including different types of selection and different population parameters (Hudson, 1991; Neuhauser and Krone, 1997; Fu and Li, 1999). We can use this to predict expected patterns of genetic variation in conditions promoting diversification, and thereby the expected variation in morphology and other traits. These expected patterns have been outlined in detail elsewhere (Barraclough et al., 2003): here we summarise the main conclusions before outlining general implications. For simplicity, we compare expected patterns in a strictly asexual case (i.e. no recombination) with a sexual case (with random mating). Cohan (1998; 2001) discusses the case of partial recombination in bacterial systems.

In the simplest case of a single population under a neutral model, the expected gene tree is given by the neutral coalescent (Kingman, 1982; Hudson, 1991). For a single locus, this is the same in sexuals and asexuals, if the sexuals are haploid. If sexuals are diploid, the time taken to coalesce back to the most recent common ancestor doubles, because a diploid population harbours twice as many gene copies. The main difference between sexuals and asexuals is in the pattern of variation across multiple loci and in polygenic traits. In asexuals, there is no recombination, therefore all loci display the same gene tree. This leads to hierarchical variation of

genetic and morphological variation within a single asexual population. In sexuals, because there is recombination, unlinked loci display different gene trees and the overall genotype comprises the average of a set of different random gene trees. The result is a unimodal distribution of neutral genetic or morphological variation based on multiple loci. This broad prediction is unaffected by purifying or directional selection acting in the populations (Barraclough et al., 2003). While the tree-like structure of asexual populations might give the appearance of discrete taxa, it is simply consistent with a neutral model in a single population. A more interesting question is to what extent do sexuals and asexuals diversify beyond levels expected in these simple null models, specifically in response to the two conditions favouring diversification: geographic isolation and divergent selection.

Both sexuals and asexuals are expected to split into two discrete clusters in the presence of a barrier to gene flow, i.e. geographic isolation. The reason is that a single limited population is split into two or more populations of smaller size, whose population sizes are limited independently of one another. After the split, the probability that an individual contributes to future generations depends only on the number of individuals within the same population, hence the two populations evolve independently and after a long enough time individuals will coalesce back to a different common ancestor in each population (Fig. 2). The average time taken for the

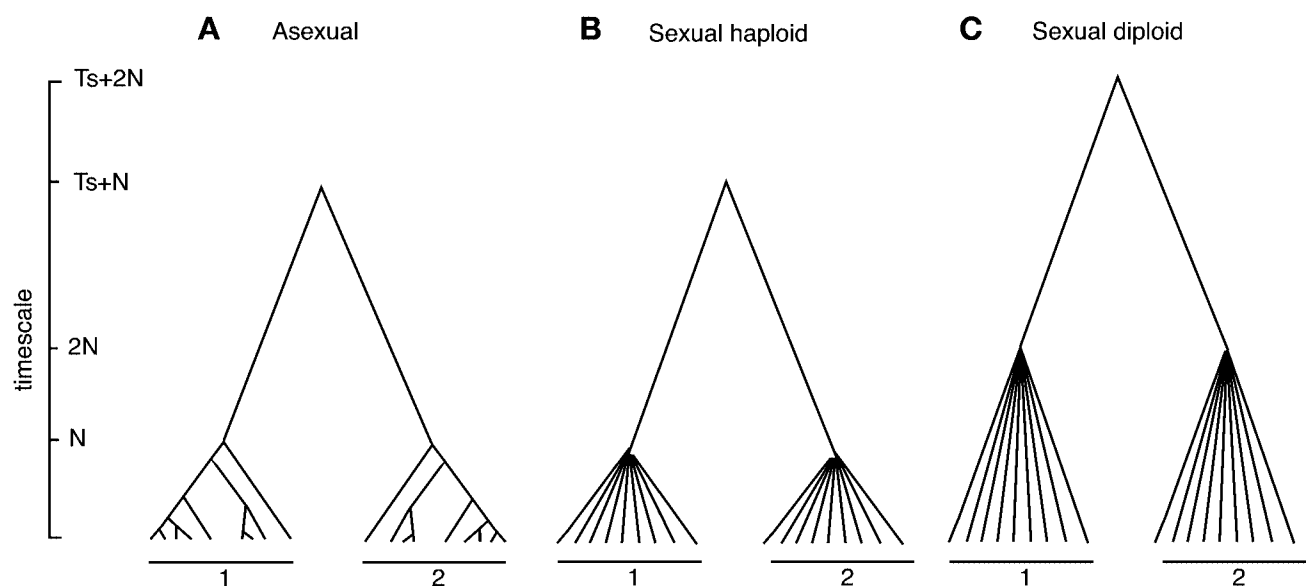


Fig. 2. Consensus gene trees from multiple loci sampled across two populations geographically isolated at time T_s , each with population size $N/2$ and under neutral coalescent model. (A) Asexual haploid population. (B) Sexual haploid population. Same as (A) except for lack of resolution in consensus trees. (C) Sexual diploid population. Note we assume that coalescence within populations is complete at time of sample.

clusters to form is the same in asexuals and haploid sexuals, but is twice as long for sexual diploids, assuming all other things are equal. Therefore, asexuals are just as likely to diversify into discrete clusters in different areas as sexuals, more so if sexuals are diploid. If divergent selection is acting on the two populations, for example due to local differences in the environment, this will speed up the formation of discrete clusters. Asexuals would display adaptive divergence of polygenic characters, but such changes can only accumulate in a stepwise fashion, because there is no recombination. Sexuals would be expected to diverge faster, assuming the rate of origin of beneficial alleles is not limiting, because recombination brings together favourable gene combinations (Burt, 2000). Hence, although the number of discrete clusters should be the same in each case, sexuals might display greater differences in adaptive traits, such as functional morphology, than asexuals (Fig. 3), perhaps making it easier to recognise sexual clusters than asexual clusters from morphological characters.

The extent of diversification in sexuals and asexuals will also depend on their relative abilities to evolve and maintain discrete clusters in sympatry. Divergent selection in a single population can lead to sympatric speciation in both sexuals and asexuals, but the chance of this happening is likely to differ. First, in asexuals, divergent selection at a single locus is sufficient to produce two clusters recognisable across the whole genome, whereas in sexuals it will only cause clustering at loci linked to the target locus (Hudson, 1991). Second, divergent selection acting on multiple loci can occur in asexual populations by stepwise accumulation of successive beneficial changes, but in sexuals it can only occur if assortative mating evolves concurrently, to prevent the break-down of favoured gene combinations by

recombination. If assortative mating did evolve, adaptive divergence might then proceed faster in the sexuals than the asexuals for reasons outlined above. Therefore, the relative tendency of sexuals and asexuals to undergo sympatric adaptive divergence depends on i) the rate of origin of reproductive isolation in sexuals and ii) relative rates of adaptive evolution in sexuals and asexuals. Sympatric speciation is widely believed to be much rarer than allopatric speciation in sexuals: if this is true, sympatric divergence will be easier in asexuals than sexuals. Similar arguments apply to consideration of whether clusters formed in allopatry will be able to coexist if contact is re-established: asexuals require functional differences between the clusters to prevent coalescence back to a single cluster, sexuals have the additional requirement that reproductive isolation must have evolved.

To synthesise our predictions, we can break down the process of diversification in both sexuals and asexuals into a series of stages (Fig. 4). In stage 1), an event occurs that creates conditions favouring the evolution of discrete clusters in a formally homogeneous population. This might be an event creating isolation, such as the formation of a barrier or establishment of a new population outside the ancestral range, or an environmental change leading to diversifying selection within the population, for example the appearance of a new host plant for a phytophagous insect. Such events depend on what is happening in the environment and how the organisms are affected by it. Assuming for now that recombination is the only difference between sexuals and asexuals, the frequency of these events will not depend on the sexuality of a lineage.

In stage 2), discrete clusters evolve in response to the changed conditions. We have outlined how asexuals are, if anything, likely to evolve discrete genotypic

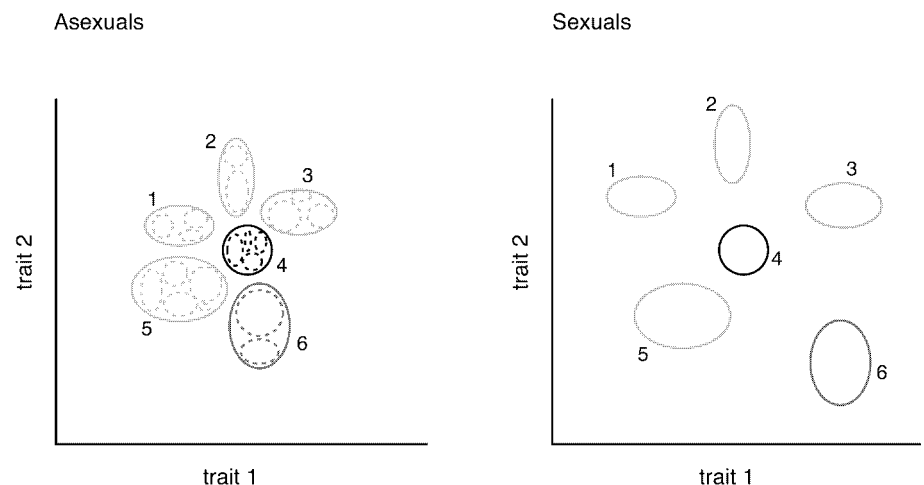


Fig. 3. Schematic figure showing the expected variation in functional morphological traits, such as those associated with feeding strategy, in sexual and asexual lineages. Both sexual and asexual lineages are expected to diversify into the same number of major clusters given a certain number of diversifying events, but i) sexuals display greater variation among clusters, ii) asexuals display minor clusters within each major cluster due to their tree-like ancestry.

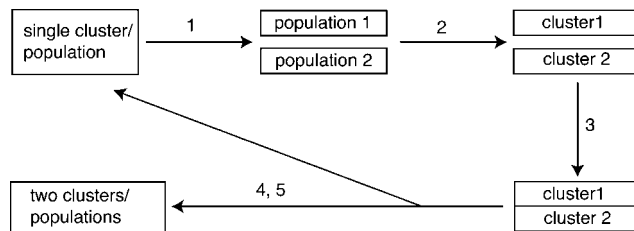


Fig. 4. Flowchart of stages in the formation of two discrete clusters from an ancestral population with a single cluster. 1) An event occurs that splits a single population/cluster into two independently limited populations (either geographically or within the same location). 2) Distinct clusters evolve in the two populations. 3) Secondary contact occurs between two clusters formed in isolation. 4) Adaptive differences have evolved that allow independent limitation (and therefore coexistence) of two clusters. 5) In sexual populations, reproductive isolation has evolved that prevents recombination of evolved differences. If these conditions are met (1–4 in asexuals, 1–5 in sexuals), the system will have changed from a single cluster to two clusters that are stable to contact between them. Stages 3–5 are not necessary for two clusters to be present if contact is never re-established.

clusters faster than sexuals, particularly in response to diversifying selection within a single location or if sexuals are diploid. Therefore, for a given series of events, asexuals are expected to evolve a stronger pattern of overall genetic clustering than sexuals. However, clusters in sexuals may be more distinct with respect to adaptive characters, such as functional morphology.

Finally, the extent of clustering observed in a lineage also depends on whether clusters formed in isolation are able to persist if contact is re-established. Stable coexistence requires sufficient adaptive/ecological differences between clusters that their populations are limited independently. In sexuals it also requires reproductive isolation. Clusters will be more likely to coexist stably in sexuals than asexuals if i) rates of adaptive divergence are limiting, and ii) reproductive isolation evolves fast enough to 'take advantage' of the sexuals' faster rates of adaptive change. This is the only scenario in which sexual lineages might exhibit greater genetic clustering than asexuals (Barraclough et al., 2003).

Overall, the extent of cluster formation within a lineage will depend on the balance among rates at each stage. For example, if environmental events that cause isolation and secondary contact occur rarely, then there might be time for both sexuals and asexuals to evolve into discrete clusters with sufficient adaptive (and reproductive) divergence to persist even if contact is re-established. However, if environmental events occur frequently, stable clusters might only have time to form in sexual lineages, and then only if reproductive isolation keeps step with rates of adaptive divergence. To complicate matters further, stable 'ecological' coexis-

tence might not be necessary for the sustained existence of clusters within lineages, if formation rates are fast enough relative to extinction (Hubbell, 2001). This might shift the balance back in favour of asexuals.

So what are the typical rates for each stage? It is difficult to know the frequency of environmental events that cause isolation, re-establishment of secondary contact or changes in selection pressures: fossil and phylogenetic evidence only shows us the events that successfully led to the formation of new taxa. However, we can say that reproductive isolation can evolve at least as quickly as adaptive divergence in principle, for example if reproductive isolation evolves as a pleiotropic outcome of adaptive change (Schluter, 2001), or geometrically by the accumulation of Dobzhansky-Muller incompatibilities (Orr, 1995). Therefore, the conditions needed for sexual lineages to display more clusters than asexuals are at least feasible.

Sexuals versus asexuals: evidence

The above account argues for the need for critical studies comparing the pattern of clustering in sexual and asexual clades. Although the predictions are more complicated than implied by the initial accounts of this problem, if sexuals are found to display a stronger pattern of diversification than asexuals this would provide evidence for the importance of adaptive divergence in the origin of diversity. To date, evidence on this topic has been largely anecdotal. Perhaps the most striking pattern of asexuality is its sparse phylogenetic distribution: most asexual lineages appear to be evolutionary dead-ends (Bell, 1982; Judson and Normark, 1996). For example, in land plants, asexuality has evolved in quite a large number of groups but nearly all cases appear to represent very recent, highly polyphyletic origins. This general lack of diversity in asexual lineages could be interpreted as evidence for a reduced tendency to form clusters, i.e. lower 'speciation' rates. However, the alternative is that asexuals have higher extinction rates, because of their well-documented failure to keep in step with changing environments and mutational loads (Burt, 2000), and simply do not last long enough to diversify.

Fortunately, a few apparently ancient asexual lineages with sexual relatives are known, the most famous being the bdelloid rotifers. No males or meiosis have ever been seen and recent genetic tests supported their asexual status (Mark Welch and Meselson, 2000), although the robustness of the genetic evidence remains to be confirmed (Gandolfi et al., 2003). Despite their apparent lack of sexual reproduction, bdelloids comprise around 360 recognised species that have been consistently recognised by rotifer taxonomists, and preliminary mtDNA data support the existence of genetic clusters in sympatry

(Birky, unpubl. data). The presumed sister group of bdelloids are the monogononts, which have a sexual stage in their lifecycle. Monogononts comprise around 1200 species, and appear to occupy a broader range of trophic niches and display a greater range of morphologies than bdelloid species (Donner, 1966; Ricci, 1987; Wallace and Snell, 1991). While this observation might be consistent with slower rates of adaptive divergence in bdelloids, phylogenetic tests are needed to control for possible confounding effects of taxon age on diversity.

Another group of putative ancient asexuals are the darwinulid ostracods, comprising 28 extant species. Darwinulids have apparently persisted without sex for over 100 million years. Species are recognised by slight but consistent morphological differences and appear to form genetic clusters (Martens, 1998). However, the status of Darwinulids is less certain than that of bdelloids: genetic work revealed unexpected differences in genetic variation between mitochondrial and nuclear DNA in one species (Schon, 1998; see also Gandolfi et al., 2001). Other clades of putative ancient and diverse asexuals exist (Judson and Normark, 1996; Burt, 2000), particularly among protists, fungi and mites, some with genetic evidence for prolonged asexuality (Kuhn, 2001).

These examples are suggestive of diversification in putatively ancient asexual clades, and possibly of low rates of adaptive radiation in asexuals, but at present the evidence is anecdotal. Statistical comparison of diversity between related sexuals and asexuals is needed to answer these questions. Current phylogenetic and phylogeographic methods are available to perform these tests (Avise, 2000; Barraclough and Nee, 2001). Together with others, we have embarked on a project to compare diversification in representative bdelloid and monogonont clades, which hopefully will provide some answers soon. Studies are also needed on recent asexual groups, which after all reflect the majority of asexual origins. Critically, all studies need to confirm or reject the asexual status of study clades before reliable conclusions can be drawn, for example by testing for allelic divergence (Mark Welch and Meselson, 2000) or for concordance of gene genealogies among loci (Avise, 2000).

Conclusions

The expansion of molecular methods and systematics over recent times has revolutionised studies of the evolution of diversity. For the first time the quantitative tools and statistical methods exist for uncovering the processes leading to the origin and diversification of the discrete entities we call species. Phylogenetic studies of case study clades can provide general information on the pattern and processes of speciation events leading to extant species. In tiger beetles, these have led

to greater understanding of the timing and possible causes of speciation, although major uncertainties and challenges remain. A principal limitation of many existing studies is the reliance on sampling just one exemplar from each recognised taxonomic species, necessary due to time requirements and expense of sequence analysis. Now that sequencing technology is becoming faster and cheaper, it is becoming feasible to test the evolutionary status of entities sampled in analyses, by sampling multiple individuals at multiple loci. Taking this work further, it is possible to perform quantitative surveys of the pattern of diversification within lineages, and to use higher order comparisons among lineages differing in key characteristics to test general ideas about the causes of speciation and diversification. We have outlined the theory and background behind one such comparison, namely between sexual and asexual clades. Comparisons of this kind should provide new insights into the existence of species and the macroevolutionary causes and consequences of sex.

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