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Madagascar as a model region of species diversification

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Tropical biotas provide excellent settings in which to explore mechanisms of evolutionary diversification, yet these processes remain poorly understood. Pioneering work on biodiversity patterns and diversification processes in other tropical regions has recently been complemented by studies in Madagascar. Here we review diversity models and diversification mechanisms proposed for the fauna of this island and the perspectives for testing them. Madagascar has a diverse biota that has evolved in isolation, and is characterised by regionally pronounced and locally steep environmental gradients, common patterns of microendemism across taxa and numerous evolutionary radiations. These characteristics establish Madagascar as a promising system for the study of pattern and process in species diversification.

Species diversification in the tropics

The past 15 years have seen significant progress in defining what species are [1], supported by a wealth of case studies on species formation. However, the mechanisms by which species arise are still among the least understood biological phenomena [2]. A variety of speciation modes are thought to generate biotic diversity: species formation can occur allopatrically or sympatrically, be gradual or instantaneous, and nonadaptive as well as driven by processes of sexual selection or ecological adaptation (see Glossary). Yet the relative frequency of these speciation modes and the principal drivers of the diversification process remain disputed [2–5].

Ongoing research on the diversification of tropical faunas, particularly in Amazonia, Central Africa and the Australian wet tropics [6,7], is beginning to highlight principal modes of speciation and diversification. The tropics house most of the world's biodiversity, possibly owing to high rates of speciation and/or slow rates of extinction [7–10]. This makes them appropriate regions in which to investigate general speciation patterns and processes. Here we review recently published hypotheses that contribute to explaining species diversity and diversification in Madagascar [11–23]. We summarise the speciation processes inherent to each of the newly proposed diversification mechanisms and the major predictions for process and pattern under each of the mechanisms, and propose ways

to test these predictions comparatively. Taken together, these studies indicate that Madagascar provides a unique opportunity to help understand evolutionary patterns and processes with the possibility of extrapolating the results to other tropical regions. Several other prominent diversification mechanisms [8,24,25] also merit extensive

Glossary

Allopatric speciation: formation of species by the separation of populations into two or more different geographical areas in which they undergo phenotypic or genetic divergence (equivalent to vicariant speciation, but with no need for a physical barrier, i.e. can be due to dispersal or ecology).

(Phylogenetic) Biome conservatism: the tendency of phylogenetic lineages to remain with their original biomes rather than colonising new ones.

Centres of endemism: areas within a biome with an increased number of species occurring nowhere else in the domain; in the watershed mechanism, centres of endemism are those areas where rivers have headwaters at low elevation.

Generalised dissimilarity modeling: a statistical technique for analysing and predicting spatial patterns of turnover in community composition (beta diversity) across large regions.

Gradient diversification or speciation: formation of species by adaptation of populations to different conditions along an environmental (e.g. elevational) gradient, rather than by vicariance; usually, the process is assumed to occur under parapatric conditions.

Latitudinal gradient in species richness: the generally observed increase of number of species per unit area toward low latitudes or the equator.

Microendemism: a high proportion of species with narrow range sizes.

Mid-domain effect (MDE): the increasing overlap of species ranges toward the centre of a shared geographical domain owing to geometric boundary constraints in relation to the distribution of species' ranges and midpoints. This leads to a peak of species richness in the centre of the domain.

(Phylogenetic) Niche conservatism: the tendency of species to retain ancestral ecological characteristics, such as aspects of their fundamental niche.

Orography: the effect of montane topography on weather (e.g. an aspect-specific precipitation pattern).

Parapatric speciation: the formation of species in adjacent areas (usually with a hybrid or contact zone).

Phylogenetic independent contrasts: a method for exploring cross-taxon relationships between traits by correcting for autocorrelation with phylogeny.

Phylogenetic species concept: a concept of species delimitation in which individuals belonging to a species are defined by the presence of one or more fixed, apomorphic characters.

Species turnover: the spatial (geographical, discussed here) or temporal (stratigraphic or phenological) change in species composition (beta diversity). A high spatial species turnover is often caused by a high proportion of microendemic species.

Taxonomic inflation: the rapid accumulation of scientific names owing to processes other than new discoveries of taxa (i.e. 'splitting,' the unwarranted elevation of taxa to a higher level such as species, or other taxonomic errors, such as overreliance on a particular species concept).

Vicariance, vicariant speciation or vicariant divergence: the formation of different populations and eventually different species by the formation of a physical divide between them (equivalent to allopatric speciation, but with a physical barrier needed); assumed to be nonadaptive (i.e. it does not require either of the new species to adapt to different environmental conditions).

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investigation in Madagascar. We argue here that the combination of these factors, together with several practical advantages, make Madagascar an ideal model region for testing the patterns of species richness, spatial species turnover and the processes of species diversification itself.

Microendemism and species richness in Madagascar

Its rich endemic flora and fauna places Madagascar as one of the top hotspots worldwide for biodiversity conservation [26,27]. For example, 100% of the native Malagasy amphibian and terrestrial mammal species, 92% of reptiles, 44% of birds, 74% of butterflies and >90% of plants occur nowhere else [28–30]. The current consensus is that many endemic lineages of the island resulted from the overseas dispersal of African founder individuals during the Cenozoic, from 65.5 million years ago (Ma) to the present [31], whereas others are relicts of the late Jurassic to upper Cretaceous fragmentation of Gondwana, during which Madagascar separated from Africa 183–158 Ma, from India 96–65 Ma and from Antarctica ∼130 Ma, with land bridges to this continent and thus connections to South America via Antarctica probably persisting until ∼90–80 Ma [32–35]. Radiations of these Malagasy lineages resulted in a wealth of endemic species, many of which are also microendemic to small ranges within the landmass [11,12,36]. Such a pattern of microendemism leads to high spatial species turnover, as in many other tropical regions [7]. However, what makes Madagascar unique is the high concentration of biotic endemism, not only at the species level but also at higher clades: for example, in native amphibians, all species, 23 out of 24 genera, and 1 out of 4 families are endemic to Madagascar, and most species are known from only one or a few localities within the island [37].

Many inventories over the past two decades have revealed high numbers of undescribed species in numerous Malagasy animal and plant groups, sometimes outnumbering those already described (see online Supplementary Material Table S1). In a few cases, this might partly reflect taxonomic inflation [38] as, for example, in lemurs, where the recent sharp rise in species numbers can be explained by the promotion of known populations or subspecies to full species status. This is not the case in other Malagasy animal groups where, owing mainly to intensified fieldwork, numerous previously unknown species have been uncovered [39]. For instance, species numbers in amphibians have increased from 133 species in 1991 to currently 244 species, while an additional 221 candidate species have been newly discovered [37].

Madagascar is not unique in this respect, as high proportions of undescribed and unrecognised species are also likely to be prevalent globally [40]. This often encumbers studies of species diversification for which dense sampling of taxa is essential and, therefore, comprehensive taxonomic inventories and revisions are a prerequisite. Such taxonomic research is greatly facilitated by fresh comparative material from type localities of described species, but the internationally scattered nature of these localities usually presents a serious logistic impediment. This restriction, however, does not apply to Madagascar, which offers the opportunity to study entire evolutionary radiations not only on a single island but also within the political boundaries of a single country. Consequently, the island has attracted intensive research programs that integrate interdisciplinary field surveys with subsequent taxonomic revisionary work (e.g. [37,39,41,42]). The distributional, morphological and molecular data sets available from these studies are remarkably complete compared with most other biodiverse tropical regions and provide an outstanding basis for evolutionary studies.

Madagascar as a testing ground for patterns of spatial species richness and turnover

Its evolutionary and biogeographical isolation has recently led biologists to examine Madagascar as a model system for explaining stochastic and deterministic influences on diversity patterns. Madagascar has had no mainland connections during the past 80–90 Ma, unlike many major tropical islands, such as Borneo or New Guinea and Sri Lanka, which had such connections during the Pleistocene to Neogene up to 23 Ma [43]; it also lacks the tectonic complexity of other islands such as Sulawesi [22,44]. Along its latitudinal axis, a central highland plateau rises to elevations of at least 1000 m and comprises three major massifs in the north, centre and south, which reach up to ∼2900 m (Figure 1). Humidity transported by the eastern trade winds precipitates on the eastern slopes of these mountain chains, forming a rainfall gradient from the tropical humid northeast and east to the subarid southwest of the island. An unusual feature in comparison with large tropical islands such as Borneo is that the major biomes of Madagascar exhibit sharp borders even in the lowlands: they grade from tropical humid to subarid bioclimatic extremes, including the eastern rainforests, western dry deciduous forests and southwestern subarid spiny forests. Isolated massifs within the drier regions harbour minute humid forest relicts, and monsoon rainforests reach the northwestern coast. Contrary to earlier suppositions (e.g. [45]), endemic, nonanthropogenic grasslands probably formed part of a mosaic landscape of woodland and open areas during the past 8 million years, although their extent remains debatable [44,46,47]. The vegetational composition is unique to each biome, and the biomes, such as spiny forest compared to rainforest, are thought to vary greatly in age [20]. This situation resembles major continental landmasses such as Africa as opposed to large islands such as Borneo [44,48]. Not only the range of biomes but also highly unpredictable rainfall patterns compared with other Afrotropical regions might have had a significant influence on evolutionary adaptations on the island, possibly accounting for the evolution of particular life histories and the rareness of frugivores [18].

Diversity patterns of most animal and plant groups in Madagascar relate to the primary biomes (rainforest, deciduous forest, spiny forest and grasslands) and are uneven, with species richness being on average highest in the eastern rainforest and low in present-day grasslands [16,28,49]. However, even in the rainforest band, species richness is not equally distributed, with maxima observed in many groups toward intermediate elevations and latitudes (see online Supplementary Material Table S2).
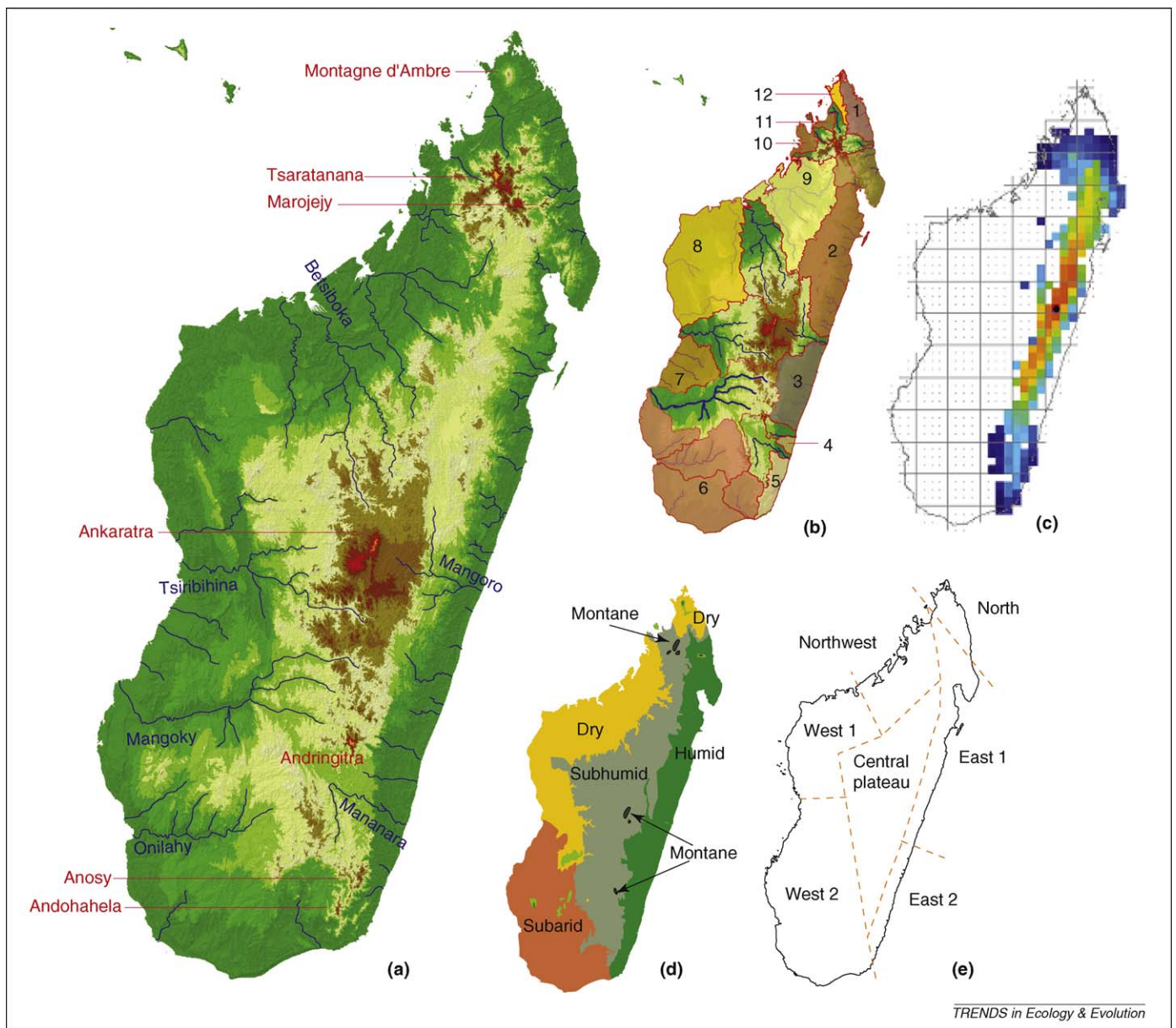
Figure 1. Geography, species richness and biogeographic zonation of Madagascar. **(a)** Topography of Madagascar, showing major rivers (width >10 m) (blue font) and mountain massifs (red font) as mentioned in the main text. **(b)** Centres of endemism (numbered 1–12) identified on the basis of the watershed mechanism [11] and overlaid on topography (in shades of brown). **(c)** Empirical evidence for the latitudinal MDE with increasing colours of warmth for increasing species richness based on distribution ranges of 637 rainforest endemic animals [51]. **(d)** Simplified bioclimatic zonation: 'humid' and 'montane' correspond approximately to rainforest biome, 'subhumid' either to rainforest relicts or grasslands, 'dry' to deciduous forest and 'subarid' to spiny forest [75]. **(e)** Main biogeographic areas of lemur distribution; dashed line represents approximate borders of biogeographic regions as originally drawn [79].

Such data contradict predictions that biodiversity should increase monotonically toward the equator, or toward areas of higher primary productivity, which usually correspond to low elevations [8,50].

These diversity observations in relation to the geography of Madagascar inspired the development of a stochastic null model for spatial variation in species richness, applicable in principle to any area or volume, known as the mid-domain effect (MDE) [19,51]. In its basic formulation, the MDE excludes environmental influence on species richness and further assumes independence of species location and evolutionary history, which is often violated in radiations that are endemic to a certain area [52].

Although this model has therefore been debated [19,49,50,53], methods are now available to test the

MDE rigorously. These tests can be applied in one spatial dimension using randomly selected range limits [19], in two dimensions using the 'spreading dye' algorithm that randomly 'grows' simulated ranges from a random starting grid cell [54], and also simultaneously for latitude, longitude and elevation, by randomly selecting species' range limits in all three dimensions and placing them within the geographical domain [55]. Moreover, the MDE has been extended to process-based simulations of environmentally constrained or patchily distributed species ranges and/or their expansion, dispersal or extinction, while randomising their initial locations (e.g. [53,56]). Because Madagascar has been suggested as a geographical region where the MDE pattern is particularly evident [19], it is an especially

suited locality to which to apply stringent tests using these novel methods.

As well as the MDE, a plethora of alternative models exists to explain the spatial distribution, assembly and maintenance of species richness [57], relating to climate and its stability and solar energy input (e.g. [58]), topographic and habitat complexity (e.g. [59]), phylogeny (e.g. [48,52,60]) or the effects of area, such as whole-island or biome area (e.g. [61]), habitat size and habitat fragmentation (e.g. [62,63]). Madagascar provides a useful setting in which to determine their relative influence comparatively, over and above other major tropical land areas [22], owing to its geographical setting and isolation, endemic radiations and the different extents and ages of its biomes. Such tests can also benefit from a wealth of high-resolution distributional and GIS data sets that are specific to Madagascar, coupled with ecological niche-modelling techniques [6,17,23,24,64], together with new evolutionary information from molecular as well as palaeoecological data [13–17,39,42,46,65–70]. To these data sets need to be added detailed reconstructions of the historical extent of biomes, which at global resolutions have suggested a northern rainforest refuge in Madagascar during the Pleistocene [71].

Further testing is also needed to understand the origin of the high degree of microendemism in Madagascar. There is some evidence that the restriction of taxa to small distributional areas might be influenced by the evolution of specialisation to particular environments (and thus reduced dispersal ability in areas with heterogeneous environments) that might be typical of tropical as opposed to temperate regions [10]. Recently, generalised dissimilarity modelling techniques [72] have produced an explicit model for spatial turnover in Madagascar [21]. Such models might help in defining and explaining biogeographical regions in Madagascar more objectively, as opposed to the available zonations [73,74] that were historically proposed on the basis of expert opinion and were not backed by explicit spatial analyses.

Mechanisms of species diversification in Madagascar

Madagascar also constitutes a natural laboratory for the study of species diversification mechanisms. The climatic and biotic disparities within Madagascar led to proposals to subdivide the island into discrete subunits corresponding broadly to its major biomes, defined by either bioclimatic factors [75] or by vegetation and elevation [45,73], or by faunal composition [74]. Inherent to these proposals is a fundamental subdivision between the humid east and the dry west, with a further emphasis on the region north of ~16°S, which consists of a fine-scale, topographically variable mosaic of dry and humid habitats.

This bioclimatic zonation was often simplified into biogeographical regions assumed to reflect patterns that are common to different taxonomic groups [45,74]. On this basis, several publications have recently introduced mechanisms of species diversification to explain the high degree of microendemism in Madagascar. We review these here and comparatively illustrate the underlying processes (Table 1); we then discuss them in the context of previous work in other tropical regions, and review their support for Madagascar as a superior model region for study of species diversification.

Ecogeographic constraint and montane refugial diversification

Species formation in Madagascar might have been influenced by bioclimatic disparities between the arid west and humid east. This mechanism has been referred to as 'ecogeographic constraint' [13], but has yet to be formulated in detail. It can be assumed to be predominantly adaptive, in that populations of a species that were originally widespread throughout eastern and western Madagascar adapt to dry versus humid conditions and then diverge into two separate species. Although this can occur under allopatric conditions, the eastern and western bioclimatic zones in some areas directly abut and form a steep ecotone [25] that might trigger parapatric species formation. Alternatively, a nonadaptive mechanism involving western rainforest refugia could also be related to the bioclimatic east–west differences: populations of a widespread species adapted to humid conditions remain isolated in humid forest relicts in predominantly dry areas during periods of cool and dry climate and, over time, diverge to become separate species, without adapting to different bioclimatic conditions. Various amphibian and reptile sister species pairs show distributions that could be explained by such east–west divergence scenarios [65] (see examples in online Supplementary Material Table S2).

By contrast, a molecular analysis of *Microcebus* mouse lemurs suggested a primary north–south split between phylogenetic lineages [13], which is also found at various phylogeographic levels in widespread reptile lineages or species [65] (see also online Supplementary Material Table S2). A possible cause for this is the separation of the northern Malagasy mountain massifs from the central highlands by ~100 km of largely low elevations. These northern massifs (Tsaratanana, Manongarivo, Marojejy and Montagne d'Ambre) could have acted as refugia for humid forests during periods of drier climate [76], when the interspersed lowlands became arid and grassy, as they partly are today [46]. Because their steep slopes start near sea level, with peaks above 1400–2900 m, these northern mountains stand out from other mountainous areas in Madagascar as an ideal topographic location for allopatric speciation of populations that remained isolated in such montane refugia [16]. These massifs would have also offered opportunities for adaptive speciation along elevational bands. Such adaptive speciation has been observed in other parts of the world [7,77], but explicit studies in Madagascar are still lacking.

Although the predominant speciation mechanisms in this area need further research, the northern tip of Madagascar and, to a lower degree, the southeastern massifs culminating in the Andohahela and Anosy Chains have a role in species diversification in Madagascar. They might act not only as species pumps [60], where new species and phylogeographic lineages originate and disperse southward or northward, respectively, but also as centres of diversification that, once reached by a dispersing lineage, trigger a new regional radiation [16].

Table 1. Predictions and tests for species diversification mechanisms proposed for Madagascar[a]

Species diversification mechanism				
Ecogeographic constraint	**Western rainforest refugia**	**Montane refugia**	**Riverine barrier**	**River catchment**
Pattern				
Phylogenetic splits separate sister species or clades occurring in eastern and western Madagascar, corresponding to humid and dry forest, respectively → Using phylogenetically independent contrasts, test whether east–west phylogenetic splits occur more often than do splits within either the western or eastern biome (compare number of east–west versus north–south sister lineages)	Phylogenetic splits separate sister species or sister clades occurring in eastern Madagascar and in humid forest relics in the west → Using phylogenetically independent contrasts, test whether east–west phylogenetic splits occur more often than do splits within either the western or eastern biome (compare number of east–west versus north–south sister lineages)	Higher endemism in species adapted to higher elevations, as these are more likely to remain isolated on mountains during climatic shifts → Test whether highland species are more strongly microendemic than are lowland species	Barrier effect is stronger in wider rivers → Search for positive correlation of species turnover with river width	Higher endemism in lowland-adapted species because lowland catchments remain more isolated during dry periods → Test whether lowland species are more strongly microendemic than are highland species
Youngest sister lineages or species are generalists occurring in different bioclimatic niches in eastern and western Madagascar; older sister lineages can be more specialized → Assess bioclimatic niche specialisation (and possibly morphological specialisation) in young sister lineages	Youngest sister lineages or species occurring in eastern versus western Madagascar occupy similar environmental niches (rainforest) → Assess bioclimatic niche specialisation in young sister lineages	Higher species diversity and endemism in areas with high elevational variation → Test whether elevational variation per unit surface area is positively correlated with species diversity and endemism	Higher local endemism in lowlands, where rivers are wider and stronger barriers → Test whether lowland species are more strongly microendemic than are highland species	Basins of smaller rivers, with headwaters at lower elevations, will be centres of endemism → Test whether transition zones based on distribution areas of extant taxa correspond to river catchment boundaries
Presumed refugia correspond to centres of endemism and of historical habitat stability → Test through analysis of species turnover and palaeoclimatic modelling		Presumed refugia correspond to centres of endemism and of historical habitat stability → Test through analysis of species turnover and palaeoclimatic modelling	More phylogeographic subdivision in lowland species, where rivers are stronger barriers → Search for a positive correlation between gene flow and mean elevational occurrence of species More species in lowlands because speciation primarily occurs here → Test for higher species richness in lowlands compared to highlands	Sister species or sister lineages occur in neighbouring catchments mainly if their headwaters are at low elevations → Compare numbers of neighbouring lineages in catchments with different elevation of headwaters
Process				
In the case of gradient speciation: gene flow between populations distributed at ecologically similar sites along the gradient is higher than between those distributed at ecologically different sites across the gradient → Assess and compare gene flow	Populations of species in rainforest refugia in the west show no gene flow to related populations in the main eastern rainforest block → Assess gene flow	Sister species (or sister lineages within species) occur on and often are endemic to neighbouring massifs → Test by comparing distribution of sister species in a phylogeny	In the case of primary barrier divergence, phylogeographic lineages (or species) on each side of the barrier are sisters to each other; in the case of divergence after dispersal across the river, lineages on one side of the river are nested among those on the other side → Test for phylogeography of lineages on either side of the river and neighbouring areas that could have acted as refugia; apply statistical phylogeography to exclude that divergence is due to secondary contact at river after expansion from refugia	Phylogeographic lineages in one river catchment (on both sides or one side of the river) differ more strongly from those in the neighbouring river catchment than from those on the other side of the river → Assess distribution of phylogeographic lineages across river catchments and rivers, in areas where headwaters are at low elevation

[a]For an overview of the mechanisms, see the main text and Box 1. We summarise the main predictions and tests to assess whether (i) the respective mechanism is predominant in a larger taxonomic group and will, thus, be reflected in common patterns of these organisms, and (ii) it reflects the process of lineage diversification within a particular species or a closely related group of species.

Box 1. Diversification mechanisms in Madagascar

Speciation, in general, is the process by which gene flow between population-level lineages is severed so that they then diverge to become evolutionarily independent. Each of the diversification mechanisms proposed for Madagascar (Figure I) is based on a particular but general process of speciation that also has relevance elsewhere.

Ecogeographic constraint

An ecologically tolerant species originates in arid western Madagascar (Figure Iai) and colonises humid eastern Madagascar (Figure Iaii) (or vice versa). Over time, populations in each biome adapt to the respective bioclimatic conditions and, thus, diverge to become distinct species. This can either occur allopatrically, as in Figure Iaiii, with no gene flow between populations in either biome, or parapatrically under the maintenance of gene flow at a steep gradient between the two biomes.

Western rainforest refugia

During more humid periods of time, rainforests and their associated species spread into western Madagascar (Figure Ibi), to become subsequently isolated in rainforest relict areas (Figure Ibii). This leads to vicariant speciation (Figure Ibiii).

Riverine barrier

A continuous distribution range (Figure Ici) is separated by a river (Figure Icii), leading to vicariant divergence (Figure Iciii). As a variant (Figure Id), a secondary contact at a river barrier can occur after primary differentiation in refugia.

Montane refugia

A widely distributed species (Figure Iei) remains isolated in relict forests on mountains during dry periods (Figure Ieii). This leads to vicariant divergence (Figure Ieiii).

Watershed

A species is distributed in lowland forests (Figure Ifi). Along rivers with headwaters at higher elevations, during geological periods of drier climate, connections remain or are newly established (Figure Ifiii), enabling dispersal and range extensions (Figure Ifiv), thus defining so-called retreat–dispersion watersheds (RDW) with low endemicity (Figure Ifv).

In rivers with low-elevation headwaters (Figure Igi), the forest and the forest-dependent species remain isolated in forest remains along the rivers (Figure Igii), which leads to vicariant divergence (Figure Igiii) resulting in centres of endemism (CE) separated by the ridges between the watersheds (Figure Igv).
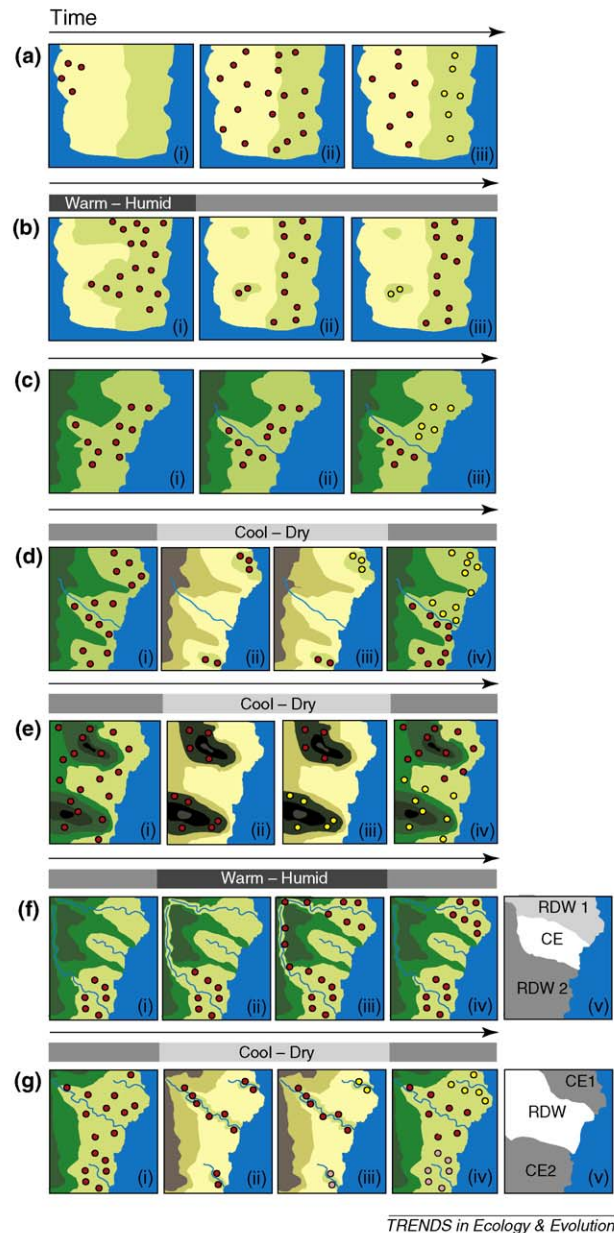

Figure I. Speciation processes assumed in diversification mechanisms proposed for Madagascar. For explanations of the mechanism, see box text. Each series of figures shows, from left to right, a time series of events. Several but not all mechanisms require climatic oscillations with either periods of dryer and probably cooler climate, such as during glaciations (light grey bars), or periods of more humid and probably warmer climate (dark grey) compared with current conditions. Green shades represent humid habitat, such as rainforest, and yellow shades represent drier habitat, such as grassland or deciduous forest. Blue represents water (the sea or rivers). Dots represent individuals that belong either to one species (with the same colour) or to two species (with different colour, after completing speciation). CE = centres of endemism; RDW = retreat–dispersion watershed.

The role of rivers and watersheds in diversification

For any region, large rivers can form semipermanent geographical barriers that some species are unable to cross. The strength of such riverine barriers decreases toward their narrow headwaters, which many species can frequently bridge. Therefore, rivers can act as the primary barrier in speciation especially in lowlands, where they are at their widest [78].

Such a riverine barrier mechanism was invoked for the diversification of lemurs in Madagascar based largely on subspecies limits [79], although even the largest Malagasy rivers do not constitute absolute limits for these primates

[12,14,15]. Most Malagasy rivers flow roughly eastward or westward from the central highlands, and the western Betsiboka and Tsiribihina and the eastern Mangoro and Mananara Rivers have been identified as important barriers for lemur dispersal [12,66,79].

As an alternative to the riverine barrier mechanism, a recent prominent study invoked a role for river basins as biotic refugia in Madagascar [11,80]. According to this watershed mechanism, climatic changes caused forest to contract to the surroundings of rivers. In watersheds with sources at low elevations, the forests became separated by intervening arid areas that constituted barriers to gene flow. These watersheds thus served as zones of isolation and speciation. By contrast, watersheds with their sources higher up served as zones of retreat and dispersal, which are expected to contain proportionally lower levels of endemism (Box 1). This mechanism particularly targets speciation in taxa adapted to lowland forests, whereas, as expected from the MDE, the highest diversity is at mid-elevations in many groups. For example, higher levels of endemism in narrow-mouthed frogs occur in the retreat–dispersal watersheds than in coastal lowlands [16]. Lemurs provide convincing examples for dispersal along retreat–dispersal watersheds [12], and of the effect of some riverine barriers [14,15,67], but evidence for differentiation within or among low-elevation watersheds has so far only been detected in some groups of reptiles [23] and lemurs [12] and is often also compatible with the riverine barrier mechanism. This suggests the need for detailed phylogeographic case studies that can distinguish between these mechanisms.

Establishing a new model region

None of the species diversification mechanisms proposed for Madagascar is completely novel in comparison to those developed for other tropical regions. Riverine barrier and montane refugia mechanisms, in particular, have been extensively discussed and studied elsewhere [59,78,81,82]. The ecogeographical constraint mechanism is comparable to adaptive gradient diversification with either parapatric or allopatric species formation [7] and depends on phylogenetic biome conservatism [48]. The watershed mechanism [11] relies on the possible isolation of forests in river basins and, thus, most closely resembles the river refugia mechanism [7,82]. Although each of these mechanisms could also be tested in other tropical and adjoining regions, various arguments characterise Madagascar as a model region that is particularly suited to test these alternative diversification mechanisms.

The first and major argument is the paucity of confounding factors that often impede studies of diversification patterns and processes elsewhere. The north–south orientation of the island, the fact that it does not straddle the equator and the sharp transitions among its biomes in many cases enable simplification of geographical analyses (i.e. the east–west axis can be disregarded and the eastern rainforest band can be considered as a one-dimensional, linear structure). This advantage holds for latitudinal analyses of species richness, tests of riverine barriers and the recognition of range expansions from northern or southern refugia, and is especially clear if compared

with the complex biogeography of the Amazon basin, where surrounding potential refugia [81,82] are each connected to other regions of South or Central America.

The second simplifying factor is that much of the species diversity of Madagascar comprises endemic radiations. This helps to understand the general historical influences on diversity patterns across taxa, such as phylogenetic niche and biome conservatism [24,48], and largely excludes confounding factors, such as diversification outside of the study region followed by multiple immigrations.

The third factor is the large number of species and endemic clades in Madagascar. These offer more opportunities to search for recurring patterns and the general underlying processes of evolution. For example, Madagascar contains almost ten times more species than do the Australian wet tropics, a region that is comparable in geographical linearity to Madagascar (e.g. for vertebrates, excluding fish, >1100 versus 181 species) [28,83].

With its recent and intense wave of anthropogenic deforestation over the past ∼500–1500 years, and given the pre-anthropogenic charcoal record and fossil data demonstrating Pleistocene oscillation in extent of biomes [46,47], Madagascar is also a potentially useful system for studies of the causes and time course of extinction. Various pioneering publications have documented higher degrees of faunal extinction and genetic isolation in older or smaller forest fragments in Madagascar [62,63,68], indicating the great potential of the region for such studies.

These scientific reasons for studying Madagascar are supplemented by the practical advantage of a complete biogeographic region within the political borders of one country whose institutions support long-term research collaborations. This offers unique opportunities to translate species diversity data directly into conservation recommendations [36,84].

Future research perspectives

Given the various diversification mechanisms hypothesised for Madagascar (Box 1; Table 1), the time is now overdue for applying explicit hypothesis testing to this system, using methods developed for other tropical regions [7,78,85] (Table 1; online Supplementary Material Figure S1). So far, such studies are scarce [14–16,23]. Future analyses should use novel statistical approaches such as approximate Bayesian computation [86] combined with spatial modelling, which, along with other applications of statistical phylogeography [87], enable the directions and extents of range expansions to be traced, the relative and absolute ages of phylogeographic splits to be estimated and alternative processes of demographic expansion to be explored. Furthermore, each diversification mechanism predicts specific patterns of the geographical distribution of endemism and diversity that can be addressed using explicit analyses of species turnover [88]. These methods offer the opportunity to address three severe gaps in the study of species diversification in Madagascar:

(i) The temporal component of the diversification mechanisms. The age of lineages should be concordant with the palaeoclimatic and palaeoecological

events on which a given mechanism relies. This is a shortcoming of the watershed mechanism, which has been hypothesised to be linked to Pleistocene and Pliocene climatic cycles (5 Ma to the present) [11]. The few available data indicate that splits between sister species in various Malagasy animal groups frequently pre-date this period [17,89]. Conversely, to relate mechanisms with empirical observations, a robust historical framework for Madagascar is needed and should include statistically validated palaeoclimatic modelling of past biome extent and assessment of the local effect of global-scale climatic perturbations.

(ii) The diversification of plants. In spite of major survey efforts of botanists, and large georeferenced databases of plant distribution available for conservation planning [36], there is a paucity of studies on plant phylogeography [70,90] or diversification [69].

(iii) Parapatric and sympatric modes of speciation. Most studies in Madagascar have so far assumed vicariant speciation, although the island offers ideal conditions for parapatric speciation and the formation of hybrid zones along environmental gradients (e.g. in the northern mountains and along humid–dry ecotones).

Research on Madagascar has already contributed significantly to the resurrection of oceanic dispersal hypotheses [91]. Testing the models and mechanisms reviewed here now provides the opportunity to look for exciting insights into the nature of globally observed patterns, such as the latitudinal gradient in species richness [10], and into diversification processes which have long been a matter of debate, such as the roles of refugia and adaptation. We thus flag Madagascar as a model research arena for species diversification studies at a scale encompassing islands as well as continents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.tree.2009.03.011.

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Madagascar as a model region of species diversification

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Table S1. Recent studies revealing high proportions of cryptic diversity and microendemism in Malagasy animals and plants

| Taxon	Microendemism	Cryptic diversity	Refs
Lemurs		19 new species described or resurrected since 1994; at least 10 additional species awaiting description; up to 100 species to be expected	S01
Tenrecs	species previously thought widespread species now more restricted, occurring along narrow elevational bands	newly discovered cryptic species double the number of known long-tailed shrew tenrecs (*Microgale*)	S02
Amphibians		235 species described in 2007, expected total number up to 400	S03
Freshwater fish	in many groups, e.g., bedotiids, most river drainages of eastern Madagascar may harbour endemic species	increase of 60%	S04, S05
Land snails	exceptional microendemism	685 spp. in 2003; many undescribed species remain to be named	S61,S63,S64
Spiders		470 spp.; 400 reported from single site; could be over 3000 spp.	S06
Scorpions	100% endemic; ranges not assessed	40 spp., 27 described since 1995, excluding one introduced sp.	S07
Springtails (Collembola)	93% endemic; Symphypleona, especially explosively speciating *Temeritas* and *Anjavidiella*, much more microendemic than Pooduromorpha based on known distribution and bioclimatic niche plots	69 species, expected to be small fraction	S08
Mayflies, (Ephemeroptera)		15-->~200 estimated species	S09
Caddisflies (Trichoptera)	98-100% endemic to Madagascar; exceptional levels of microendemism especially among Philopotamidae in rainforest	52 in 1994 --> >500 estimated species; exceptionally rich compared to Africa	S09
Ants (Formicidae)	91% (96% excluding recent invasives) of described native taxa endemic to Madagascar; exceptional microendemism; extreme degree of locally restricted Molecular Operational Taxonomic Units	418 known spp. including 25 tramps; increase of species numbers by 200% to be expected; High degree of cryptic (genetic) diversity detected by DNA barcoding e.g. in trap-jaw ants, 5 new *Anochetus* and 3 new *Odontomachus* were recognised	S10–S12
Tiger beetles (Cicindelidae)	Exceptional levels of microendemism		S68–S70
True butterflies (Papilionoidea) and Skippers (Hesperiidae)	211 (70%) of described species endemic to Madagascar; expected to rise to 74% including undescribed species; exceptional microendemism among Hesperiidae (93% endemic) and Satyrinae (98% endemic)	300 described spp. and 341 estimated species by end of 2001; two major radiations with 37 (49) to 46 (70) spp. 31% of 42 sequenced *Heteropsis* were undescribed; expected to rise to about 400 spp.	S13–S15
Other Lepidoptera	88% endemic; varies from 66.7% in Pyralidae: Spilomelinae to over 98% in Elachistidae, Cosmopterigidae and Oecophoridae, Gelechiidae, Ethmiidae, Arctiidae, Syntominae, Limacodidae, Notodontidae, Lithosiinae, Lymantriidae, Lasiocampidae and Saturniidae	4219 in 2000-> 4289 known spp in 2007; expected to rise by 25%	S13–S16
Ferns (Pteridophyta)	45% endemic (among highest in world; 47% with new species); a high number of species	676 spp. and varieties; >700 expected, more than Africa; revision of tree ferns in 2006-7 increases species number	S17,S18, S61,S62

	endemic to central domain of island (138, compared to 19 to East); 96% of 47 spp. *Cyathea* are endemic with high level of microendemism	by 12	
Euphorbiaceae	?> 90% *Euphorbia* has 170 taxa (~130 spp.) many with very limited ranges, high microendemism likely also in *Croton*, another megadiverse genus undergoing extreme speciation in Madagascar (~150 spp.) and *Phyllanthus* (60 spp.)		S19,S20
Rubiaceae		661 spp. in 2003 with 839 (up to 950) expected; 25-50% increase expected in large genera	S21
Bignoniaceae	Most are endemic	75 spp. (2nd richest flora after S. America); 46% of sequenced species were undescribed	S22,S23
Balsaminaceae	Approx. 97% endemic, with a large radiation of Dypsis (~56 spp.)	About 120 spp. in 2002, global hotspot	S65,S66
Arecaceae		Last major revision in 1995 increased number of species by 70 spp. to 175 spp.	S60

Table S2. Examples consistent with various species diversification mechanisms or diversity models. N – North, E – East, S – South, W – West

Taxa	Taxonomic level	Evidence	Refs
Ecogeographic constraint			
Geckos, Ebenavia inunguis (E) vs. E. maintimainty (W)	Species	Sister species distributed in East vs. West	S24
Geckos, Matoatoa spannringi (E) vs. M. brevipes (W)	Species	Sister species distributed in East vs. West	S25
Geckos, Paragehyra gabriellae (SE) vs. P. petiti (SW)	Species	Sister species distributed in East vs. West	S26
Day geckos, Phelsuma dubia (W and NW) vs. P. ravenala (SW)	Species	Sister species distributed in East vs. West (but species status of P. ravenala requires confirmation)	S27
Tree boas, Sanzinia m. madagascariensis (E) vs. Sanzinia m. volontany (W)	Subspecies	Sister (sub)species distributed in East vs. West	S28
Frogs, Boophis tephraeomystax (E) vs. Boophis doulioti (W)	Species	Sister species distributed in East vs. West	S30
Coffees, Coffea spp., subgenus Baracoffea (W) vs. others (E)	Species	entirely western, arid adapted clade, likely derived from humid adapted species	S53, S59
Western rainforest refugia			
Frogs, Boophis albilabris (E) vs. Boophis occidentalis (W)	Species	Sister species distributed in East vs. Western forest relicts	S29
Frogs, Boophis luteus (E) vs. Boophis tampoka (W)	Species	Sister species distributed in East vs. Western forest relicts	S31
Frogs, Heterixalus betsileo (E) / Heterixalus carbonei (W)	Species	Sister species distributed in East vs. Western forest relicts	S32
Butterflies, Charaxes a. andranodorus (E) vs. Charaxes a. andrefana (W)	Subspecies	Sister (subspecies) distributed in East vs. Western forest relicts	S14
North–South divergence (possibly related to mesic forest refugia or montane refugia in the north or south)			
Mouse lemurs, *Microcebus*	All species in the genus	Primary phylogenetic split among northern and southern groups (but "northern" clade extends far south)	S33
Frogs, *Anodonthyla*	All species of a clade	Increased endemism in northern Madagascar	S34,S35
Reptiles, Phelsuma, Hemidactylus, Trachylepis	Intraspecific lineages	Strongly divergent lineages in the north, possibly phylogenetically basal	S36
Snakes, Madagascarophis colubrinus	Intraspecific lineages	Strongly divergent lineages in the North	S37
Butterflies, Heteropsis subsimilis and its sister species	Sister species	The sister species is restricted to northern rainforests, H. subsimilis to more southern and central rainforests (two lineages abut at latitude of Makira)	S58
Palms, Beccariophoenix madagascariensis	Intraspecific lineages	Strong phylogeographic split between central eastern and southeastern populations adapted to different bioclimates and soil types Sister to a "Northeast" humid forest clade of 5 species	S55
Aralias, Polyscias tennantii (East) and its sister group	All species of a clade	Southern, central, northern and centre-northern clusters; higher genetic diversity at latitudes around 18°S suggested to be result of Pleistocene humid forest refuge	S56, S58
Rosewood, Dalbergia monticola	intraspecific lineages		
Montane refugia			
Frogs, Microhylidae, subfamily Cophylinae	All species of a clade	species richness and endemism correlates with elevational heterogeneity	S35
Leaf chameleons, *Brookesia*	All species of a clade	High species richness and regional endemism in massifs of northern Madagascar	S38
Riverine barriers			
Sportive lemurs, *Lepilemur*	One genus with 22 species	distribution areas of multiple species combined with molecular phylogeny and phylogeography: distribution areas agree with inter-river systems in the North West of Madagascar - some sister lineages occur in neighbouring IRS	S39,S40
Mouse lemurs, *Microcebus*	Genus	distribution areas of multiple species combined with molecular phylogeny and phylogeography: distribution areas agree with inter-river systems in the Norh West of Madagascar - some sister lineages occur in neighbouring IRS	S41

Eulemur collaris and *E. albocollaris*	Two species	Mananara river is northern limit for *collaris*; uncertain if the two are sister species since *Eulemur* phylogeny still unresolved	S70
Varecia variegata and *V. rubra*	Two sister species	Antainambala river may constitute the divide between the ranges of the two species; Lokoho river seems to form the northern barrier to *V. rubra*.	S70
Tree boas: *Sanzinia m. madagascariensis*	Lineages within subspecies	depleted haplotype variability indicates possible range expansion after crossing of Mangoro river	S28
Tree boas: *Sanzinia madagascariensis volontany*	Lineages within subspecies	phylogeographic break detected between lineages north and south of Maevarano river	S28
Radiated tortoise, *Astrochelys radiata*	Lineages within species	Rivers in south-western Madagascar separate genetically distinct lineages although riverbeds may temporarily dry out	S42
Retreat-Dispersion watersheds			
Brown lemur, *Eulemur fulvus*	Populations of one species	distribution areas: occurrence in the Central East and North West of Madagascar - dispersal route coincides with RDW	Distribution maps in S01
Rufous lemur, *Eulemur rufus*	Populations of one species	distribution areas: occurrence in the Southern Central East and West of Madagascar - dispersal route coincides with RDW	Distribution maps in S01
Mid-domain effects (selection of case studies)			
Frogs, butterflies, tenrecs	Multiple species	Latitudinal MDE; elevational MDE in mycalesine butterflies	S13
Ants	Multiple species	Species diversity peaks at mid-elevation	S43-S45
Birds	Multiple species	Species diversity peaks at mid-elevation in Andohahela reserve (weak effect)	S46
Ferns	Multiple species	Species diversity weakly peaks at mid-elevation	S47,S48
Vascular plants	Multiple species	Species diversity peaks at mid-elevation (1200 m)	S49
Fruit flies	Multiple species	Species diversity lowest at low and high elevations	S50
Amphibians and reptiles	Multiple species	Species diversity peaks at 700 m, but possibly related to differences in sampling effort	S51
Rodents	Multiple species	Species diversity peaks at 1250 m	S52

Figure S1. Phylogeographic methods to test diversification hypotheses [#48]. The watershed mechanism **(a)** predicts that distinct sister species (or sister haplotype lineages), as represented by circles of different colour, are found in neighbouring watersheds that have headwaters at low elevations, although the rivers themselves do not necessarily delineate lineages. The riverine barrier mechanism **(b)** predicts sister lineages on either side of major rivers, especially in species restricted to low elevations where rivers are widest. In the case of differentiation in refugia and secondary contact at rivers after range expansion **(c)**, the lineages on either side of the river are not sisters but each is more closely related to those in the refugia than they are to each other Dispersal across the river shown by arrows **(d)** leaves the signature of a nested lineage on one side of the river. In the montane refugia differentiation, vicariant speciation **(e)** can be distinguished from gradient speciation (f) by the monophyly versus non-monophyly of species or haplotype lineages endemic to each mountain massif.
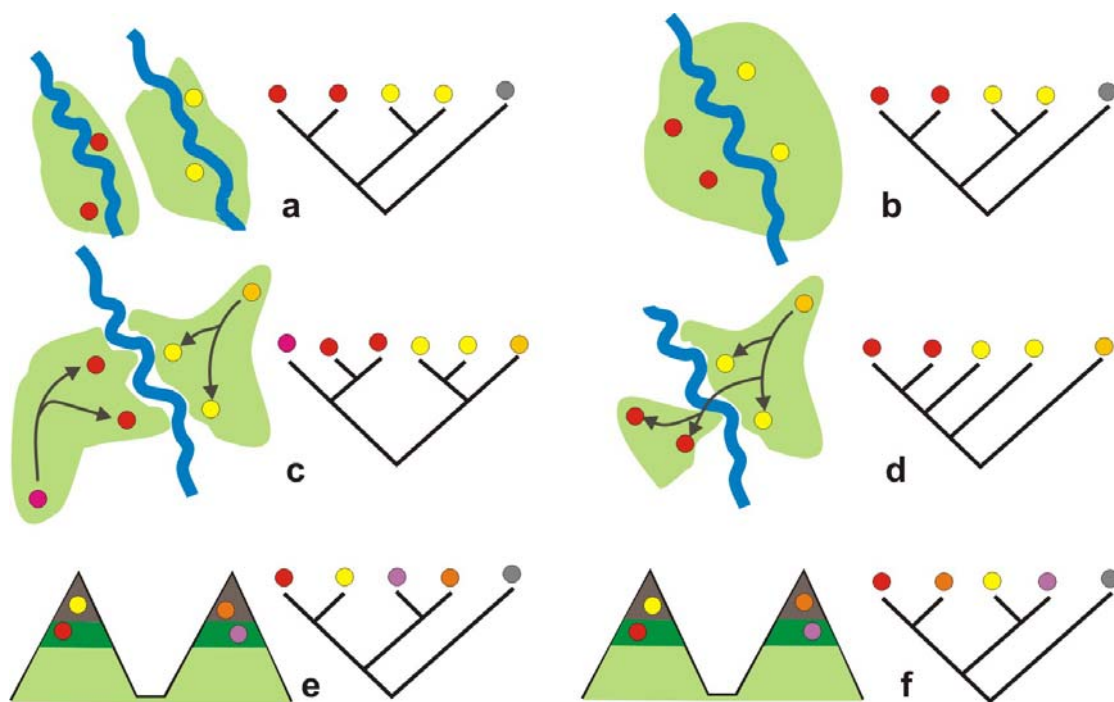
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