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Photosynthetic innovation broadens the niche within a single species

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Abstract

Adaptation to changing environments often requires novel traits, but how such traits directly affect the ecological niche remains poorly understood. Multiple plant lineages have evolved C_4 photosynthesis, a combination of anatomical and biochemical novelties predicted to increase productivity in warm and arid conditions. Here, we infer the dispersal history across geographical and environmental space in the only known species with both C_4 and non-C_4 genotypes, the grass *Alloteropsis semialata*. While non-C_4 individuals remained confined to a limited geographic area and restricted ecological conditions, C_4 individuals dispersed across three continents and into an expanded range of environments, encompassing the ancestral one. This first intraspecific investigation of C_4 evolutionary ecology shows that, in otherwise similar plants, C_4 photosynthesis does not shift the ecological niche, but broadens it, allowing dispersal into diverse conditions and over long distances. Over macroevolutionary timescales, this immediate effect can be blurred by specialization toward more extreme niches.

Introduction

The ecological niche of organisms is shaped by the metabolic and morphological adaptations acquired during their evolutionary history (Kellermann et al. 2012; Araújo et al. 2013; Hertz et al. 2013). However, the relationships between adaptive traits and ecological niches are still poorly understood. Some traits can evolve *in situ*, for example, as a response to changes in the surrounding environment following migration or external modification of the local habitat, which leads to a shift in the ecological niche (Simon et al. 2009). Other traits can modify the niche breadth to facilitate the colonization of novel habitats, as well as persistence in the ancestral ones, with possible subsequent specialization to the new habitats (Ackerly 2004; Cacho & Strauss 2014). In plants, one important determinant of the ecological niche is the efficiency of photosynthesis in different environments. Photosynthetic efficiency can be lowered by photorespiration, which occurs when O_2 is fixed instead of CO_2 and requires energy to recycle the resulting metabolites (Ogren 1984). This phenomenon can retard net carbon-fixation in the ancestral C_3 photosynthetic type by more than one third (Skillman 2008), and increases under all conditions that limit the availability of CO_2 at the active site of the carbon-fixing enzyme Rubisco. Intercellular CO_2 decreases at low atmospheric CO_2 concentrations, but also at high temperatures, where the solubility of CO_2 decreases faster than the solubility of O_2, and Rubisco becomes less able to discriminate between CO_2 and O_2 (Ehleringer & Bjorkman 19774). In addition, arid and saline conditions promote stomatal closure and thereby reduce CO_2 input from the atmosphere (Sage et al. 2012).

Several lineages of plants have evolved novel trait complexes that decrease photorespiration. These include CO_2-concentrating mechanisms like C_4 photosynthesis, which evolved independently as an addition to the C_3 pathway in more than 60 lineages of flowering plants in response to past decreases in atmospheric CO_2 (Sage et al. 2011; Christin & Osborne 2014). C_4 physiology is assembled from a combination of anatomical and biochemical components that increases CO_2 concentration at the active site of Rubisco (Hatch 1987). The C_4 pathway nearly eliminates photorespiration (Skillman 2008), but requires extra energy such that the maximum efficiency of photosynthetic light-use in C_4 photosynthesis surpasses C_3 photosynthesis only when photorespiration is high (Ehleringer & Bjorkman 1977). C_4 photosynthesis is therefore predicted to provide an advantage in any environment that promotes photorespiration (Sage et al. 2012; Christin and Osborne 2014). Accounting for one quarter of terrestrial primary production (Still et al. 2003), plants using C_4 photosynthesis are globally ecologically important. In particular, the productive C_4 grasses dominate savannas and grasslands of warm regions, novel environments that expanded during the Miocene, and in which grazing ungulates and other groups, including humans, diversified (Lehmann et al. 2011; Sage & Stata 2014). The consequences of C_4 photosynthesis for the ecological niche have primarily been investigated through comparisons of species distributions, which show an important effect of temperature on the distribution of C_4 grasses (Teeri & Stowe 1976; Ehleringer et al. 1997). However, these investigations are biased by differences among phylogenetic groups (Taub 2000), and recent interspecific comparisons accounting for phylogenetic structure have revolutionized our understanding of C_4 evolutionary ecology (reviewed in Christin & Osborne 2014). In particular, phylogeny-based analyses have shown that C_4 photosynthesis evolved in groups of grasses inhabiting warm regions and facilitated shifts into drier and more saline habitats (Osborne & Freckleton 2009; Edwards & Smith 2010; Bromham & Bennett 2014). However, the photosynthetic transitions investigated in these analyses occurred tens of millions of years ago and there is often a gap of several million years between C_3 and C_4 nodes in species phylogenetic trees (Christin et al. 2011). These vast timescales make it difficult to confidently reconstruct the conditions under which C_4 photosynthesis evolved or the events that occurred immediately after this physiological divergence.

Identifying the selective factors that promoted the gradual assembly of C_4 photosynthesis within populations requires investigations within species complexes that vary in photosynthetic phenotype. Groups with such variation are rare, and the grass *Alloteropsis semialata* is the only known species that encompasses both C_4 and non-C_4 individuals (Ellis 1974). This taxon is spread throughout a diversity of habitats across multiple continents and therefore constitutes an excellent system to investigate the evolutionary ecology of C_4 photosynthesis. The history of photosynthetic transitions within the *Alloteropsis* genus is not resolved with confidence. Indeed, the reconstruction of photosynthetic types as binary characters on the species phylogeny would lead to the most parsimonious hypothesis of a single C_4 origin followed by a reversal to an ancestral non-C_4 type in *A. semialata* (Ibrahim et al. 2009). Such an approach, however, would fail to acknowledge the complexity of the C_4 trait and, when individual components are analyzed independently, a more complex scenario emerges (Christin et al. 2010). Indeed, the various C_4 species within the *Alloteropsis* genus use different tissue types for the segregation of photosynthetic reactions and different C_4 biochemical subtypes (Christin et al. 2010), and the genetic determinism for key C_4 enzymes differs among *A. cimicina*, *A. angusta,* and C_4 populations of *A. semialata* (Christin et al. 2012). The most likely scenario given current data therefore involves multiple C_4 optimizations from an ancestor with C_4-like or C_3-C_4 intermediate characters (Christin et al. 2012).

Here, we capitalize on the photosynthetic diversity within *A. semialata* to reconstruct the environments in which photosynthetic types diverged, and examine the consequences of photosynthetic innovation for the ecological niche. We sample individuals spread across the whole geographic range, and characterize their phenotype as well as their habitat. We then apply phylogenetic methods to markers from the chloroplast genome, which are maternally inherited, to reconstruct the history of expansion into new geographic areas and environmental conditions via seed dispersal. Based on this time-calibrated phylogeographic hypothesis, we quantify the rates of dispersal across geographical and environmental spaces, and compare these among clades that differ in their photosynthetic phenotype, and are also supported by nuclear markers. This first intraspecific investigation of C_4 evolutionary ecology demonstrates that C_4 photosynthesis does not shift the ecological niche but broadens it, leading to the rapid colonization of diverse habitats and dispersal over large geographic distances.

Materials and methods

Plant sampling, photosynthetic pathway, and habitat

Collection locations for 309 *A. semialata* specimens were collated from several sources, as described in the Supplementary Methods online (Table S1). Photosynthetic type was determined using stable carbon isotopes, which unambiguously differentiate individuals that grew using C_4 photosynthesis from those that grew without fixing the majority of carbon via phosphoenolpyruvate carboxylase (PEPC; Supplementary Methods). This latter category can include C_3 individuals as well as several types of C_3-C_4 intermediates (von Caemmerer 1992; Sage et al. 2012). In addition to photosynthetic type, ploidy level, seed size, culm height, and flowering phenology data were collected for several accessions (Supplementary Methods).

Characterization of the environment

Information on the environmental conditions at the collection location of the 309 *A. semialata* accessions was obtained by overlaying geographic coordinates onto high resolution raster layers of environmental variables predicted to potentially affect the sorting of C_3 and C_4 plants (reviewed in Christin & Osborne 2014; Table S2; see Supplementary Methods). As multivariate analyses on distribution data provide an estimate of the abiotic component of the ecological niche (Petitpierre et al. 2012), a principal component analysis (PCA) was performed to summarize the environmental variation among the collection localities of *A. semialata* using eight environmental variables (Table S2) with the FACTOMINER package (Lê et al. 2008) in R. In addition, localities were classified as being open or wooded habitats, based on descriptions provided on herbarium sheets, when available.

Sequencing and phylogenetic analyses

Besides the two congeners *A. cimicina* (one accession) and *A. angusta* (two accessions), a total of 66 accessions assigned to *A. semialata* and representing 55 different populations were sampled for phylogenetic analyses (Table S1). These were selected to encompass the largest possible diversity of geographical origins and photosynthetic types. Five plastid regions (*trnK-matK*, *rpl16*, *ndhF*, *rpoC2* and *trnL-trnF*) were isolated via PCR, or retrieved from previous studies (Ibrahim et al. 2009; Grass Phylogeny Working Group II 2012). In addition, the nuclear-encoded ITS marker was isolated from a subset of accessions (Supplementary Methods).

The complete chloroplast genomes of thirteen of these samples were subsequently obtained through genome skimming (Supplementary Methods). These samples were selected because they represent different lineages, as determined from preliminary analyses of the chloroplast markers. Genomic DNA was isolated from silica-gel dried material and sequenced using Illumina technology. Complete chloroplast genomes were assembled and aligned using in-house Perl scripts. The same approach was used to assemble the complete nuclear ribosomal DNA units (rDNA encompassing the ITS; Supplementary Methods).

The thirteen complete chloroplast genomes were added to an alignment of grass genomes covering the whole family, and the trimmed alignment was used to compute a time-calibrated phylogenetic tree through Bayesian inference (Supplementary Methods). A second phylogenetic analysis was conducted on *A. semialata* and *A. angusta* accessions only. All markers obtained via PCR were aligned with the complete chloroplast genomes obtained for these two species and a time-calibrated phylogenetic tree was inferred using Bayesian approaches, using relative divergence times in the absence of fossils for the group. The ITS sequences isolated by PCR were similarly added to the complete rDNA units, and a phylogenetic tree was inferred on these nuclear markers (Supplementary Methods).

Rates of ecological and geographical dispersal

The rates of dispersal across environmental and geographical spaces were estimated for *A. semialata* by regressing geographic and environmental pairwise distances to divergence times. Only one individual per population was selected, which resulted in 55 *A. semialata* samples for which both phylogenetic and environmental information was available. The geographic distance across the Earth's surface was calculated for each pair of locations using the latitude and longitude coordinates and the *earth.dist* function in the FOSSIL package (Vavrek 2011). The environmental distances among these 55 accessions were calculated as Euclidian distances in the space formed by the first four axes of the PCA produced on all accessions (see above). Finally, the divergence time between each pair of accessions was extracted from the phylogeographic tree, using the APE package (Paradis et al. 2004). Environmental distances are potentially correlated to geographical distances (spatial autocorrelation) and, as such, partial Mantel permutation tests, as implemented in the APE package, were used to test for statistical associations between the three matrices, and to correct for such spurious correlations. These tests were conducted separately on the ABC and DE sister groups, which were retrieved on both plastid and nuclear marker trees, and differ in their photosynthetic type (see results). Linear regressions were subsequently used to calculate the slope for significant relationships. In cases where all relationships were significant, the relationship between the part of environmental distances not explained by geographical distances (that is, the residuals of the regression) and divergence times was tested.

For illustration purposes, the history of seed dispersal across the PCA space was inferred by mapping changes in the scores along the first two axes onto the phylogenetic tree, using ancestral state reconstructions as implemented in APE. The same approach was used to reconstruct dispersal across environments differing in their mean annual temperature (MAT) and mean annual precipitation (MAP), two variables commonly used to characterize global climate space and selected in the past to compare C_3 and C_4 distributions (Teeri & Stowe 1976; Edwards & Smith

2010).

Results

Phylogenetic relationships and dispersal through geographical space

In the plastid phylogeny, all accessions assigned to the species *A. semialata* based on morphological characters formed a strongly supported monophyletic group, sister to the C_4 *A. angusta* (Figs S1 and S2), confirming previous investigations with fewer samples (Ibrahim et al. 2009; Grass Phylogeny Working Group II 2012). The first split within *A. semialata* separates some Tanzanian accessions, with carbon isotopes ratios indicative of C_4 photosynthesis (Clade F), from all other individuals (Fig. 1). The remaining accessions form two sister clades (ABC and DE; Fig. 1). The DE clade contains all accessions identified as C_4 outside of the F clade, while the ABC clade contains all the accessions for which a non-C_4 isotopic signature was measured (Fig. 1; Table S1). Some members of clade ABC have carbon isotope ratios between the classical C_3 and C_4 ranges (Table S1), which might indicate the occurrence of a weak C_4 cycle, although this requires further investigation. Based on complete chloroplast genomes of *A. semialata* incorporated within a grass-wide dataset, the divergence of clades ABC and DE is estimated at 2.42 Ma (95% CI = 1.42 – 3.77), the first split within clade ABC at 1.53 Ma (95% CI = 0.71 – 2.7) and the first split within clade DE at 1.25 Ma (95% CI = 0.7 – 1.98; Figs S2 and S3). The split between C_4 and non-C_4 lineages of *A. semialata* is consequently more recent than all other origins of monophyletic C_4 groups (Christin et al. 2011). This divergence occurred after the Miocene emergence of the C_4 grassy savanna biome (Edwards et al. 2010), but falls within the Pliocene interval when C_4 grasses became increasingly dominant in African savannas (Hoetzel et al. 2013). The phylogenetic tree based on complete nuclear rDNAs for *A. semialata* supports similar relationships, although the E clade is paraphyletic (Fig. S4). The ITS marker contained few informative sites, and the nuclear phylogenetic tree based on 37 *A. semialata* accessions was poorly resolved (Fig. S5), which might be partially caused by recurrent pollen-mediated gene flow after the habitat expansion via seed dispersal. The C_4 and non-C_4 accessions however still sort into two distinct clades (Fig. S5), which suggests that gene flow between clades ABC and DE was limited over the last million years, and the photosynthetic types remained tightly associated with the plastid lineages, despite overlapping geographic distributions and flowering periods (Fig. S6).

While nuclear markers are important to detect pollen-mediated gene movements, the colonization of new habitats by plants is caused by seed movements and consequently, better inferred from plastid markers. With the exception of the widespread *A. cimicina*, the three remaining congeners are of central African origin, where members of the early diverging clade F were also found, leading to the inference of a central African origin for *A. semialata* (Fig. 2). All members of clades B and C are also from central Africa, suggesting limited dispersal. However, all members of clade A are from southern Africa, which implies a single migration to southern latitudes at the base of clade A (Fig. 2). This strongly contrasts with clade DE, which, despite a more recent common ancestor, covers the tropical and subtropical regions of Africa, Asia, and Oceania (Fig. 2). In this group, clade E is endemic to mainland Africa, with early splits separating central African accessions and more recent splits leading to southern, western, and eastern African accessions (Figs 1 and 2). The first split in clade D separates Madagascan from Asian and Oceania accessions, suggesting a single migration outside of mainland Africa (Figs 1 and 2). Long distance dispersal across the Indian Ocean is often observed and might have occurred via previously emerged islands (Warren et al. 2010).

Statistical comparisons among pairwise geographic distances and divergence times revealed patterns of isolation by distance in both the C_4 clade DE ($p < 0.00001$) and the non-C_4 clade ABC ($p < 0.00001$). However, the slope of the regression of geographic distances against divergence times is nearly six times steeper in clade DE than in clade ABC (9,992 km per time unit versus 1,617 km per time unit; Fig. 3), which indicates that, while dispersal is limited in both clades, the limitation is stronger in the non-C_4 clade ABC. All analyses were repeated with topologies sampled from the posterior distribution, and the results remained unaltered (Fig. S7).

Dispersal through the environmental space

The distribution of C_4 individuals in the four first PCA axes, which together explain 87.69% of the environmental variation in the dataset, overlaps with that of non-C_4 individuals. However, the habitat space of non-C_4 accessions is smaller and represents a subset of the conditions inhabited by C_4 accessions (Figs 4 and S8). The subset of accessions included in the phylogeny covers most of the diversity seen in the sample of 309 populations (Fig. S8), and therefore constitutes an accurate representation of the ecological diversity of the species. Focusing on the accessions included in the phylogeny, non-C_4 individuals from central Africa (clades B and C) are clustered near the center of the PCA, together with the early-diverging C_4 clade F (Fig. 4). On the other hand, the southern African non-C_4 clade A spread toward negative values on the first axis, into cool and dry atmospheric environments (Figs 4 and S8; Table S3). The broad habitat of the C_4 clade DE encompasses the extremes along both PCA dimensions, without clear distinction between geographical regions, as C_4 accessions from different continents can be found in environments with similar abiotic characteristics (Fig. 4). Similar patterns are observed for the commonly used MAP and MAT variables (Fig. S9).

According to reconstructions based on the phylogeographic tree, the ancestors of all *A. semialata* accessions and of clade ABCDE occurred near the center of the PCA space, where members of the clades B, C, and F are still located (Figs 4 and 5). The ancestors of each of the C_4 clades D and E and non-C_4 clades B and C are inferred in the same location in environmental space (Fig. 5), suggesting that the divergence of photosynthetic types was not immediately followed by significant changes on the PCA axes, or for MAT or MAP (Fig. S9). Most of the environmental diversification therefore occurred after the divergence of the C_4 and non-C_4 clades. Members of the non-C_4 clades B and C remained in the same area of the PCA, in relatively warm areas (Figs 4 and 5). However, a strong departure from this type of environment occurred in the ancestor of clade A (Fig. 5), corresponding with a migration to temperate grasslands (Fig. S9; Table S3). The progressive changes within the non-C_4 clade ABC contrast strongly with those observed within the C_4 clade DE. Indeed, extreme values along both axes are randomly spread in clade DE (Fig. 5), indicating repeated migrations across a wide range of precipitation, temperature, fire, and light environments that can be tolerated by these C_4 plants (Fig. 4), in addition to different tree covers (Table S4).

Mantel tests confirm that rates of dispersal across the environmental spaces differ statistically between the C_4 and non-C_4 clades. Environmental distances are significantly correlated to divergence times within the non-C_4 clade ABC ($p < 0.001$), indicating a gradual migration into different conditions (Fig. 3). However, these environmental distances are also correlated to geographic distances ($p < 0.00001$). The relationship between environmental distances and divergence times remains significant once this spatial autocorrelation is taken into account ($p < 0.005$), which shows that lineages within clade ABC transitioned gradually into different environments as they adapted to slightly different conditions through natural selection. The results are very different in the C_4 clade DE, for which environmental distances are not correlated to divergence times ($p = 0.77$; Fig. 3). This shows that the migration of C_4 accessions to diverse environments happened rapidly, from their early diversification (Figs 2 and 3). Their ecology is neither explained by the timing of dispersal nor by their geographical proximity, which strongly supports the hypothesis of a broad ecological niche from the outset. These conclusions are not affected by phylogenetic uncertainty, as the results of Mantel tests are confirmed across trees from the posterior distribution (Fig. S7).

Discussion

Photosynthetic diversification within A. semialata

Chloroplast markers retain the signature of seed dispersal, and the phylogeographic hypothesis produced here indicates the successive seed-mediated dispersal across geographical and environmental spaces (Figs 2 and 5). Nuclear gene flow is likely to differ, being more frequent and occurring across longer distances in wind-pollinated species. There is however a tight association between the photosynthetic phenotype and the plastid lineages, and the nuclear-encoded ITS also supports monophyletic C_4 and non-C_4 clades (Figs S4 and S5). This suggests that gene flow was limited following the divergence of clades ABC and DE, despite overlapping geographical distributions and flowering periods (Fig. 2). The split of the sister groups ABC and DE consequently represents the physiological divergence between non-C_4 and C_4 plants.

The common ancestor of *A. semialata* clades ABC and DE identified here indisputably represents the last ancestor with both C_4 and non-C_4 descendants in the group. Variation other than photosynthetic types exists in *A. semialata* as within any species, and phenotypic variation was observed in both the ABC and DE clades (Figs S6, S10, and S11). However, no character other than C_4 photosynthesis consistently differed among the clades. All individuals are perennial, and similar plant height, gross morphology, flowering phenology, and seed size are present in the different chloroplast lineages (Figs S6, S10, and S11). Earlier work suggested that C_3 *A. semialata* are diploid while C_4 individuals are polyploid (Liebenberg & Fossey 2001). However, these studies included only South African accessions. The geographically diverse accessions presented here and in Ellis (1981) demonstrate that C_4 populations from Asia, Australia, and regions of Africa are diploid, with polyploidy only detected in southern African C_4 accessions (Fig. S1; Table S5), and the results of the Mantel tests remain unchanged if the five individuals from the clade that contains polyploids are removed. The divergence of clades ABC and DE is therefore mainly characterized by a switch between photosynthetic types. Based on dating analyses, the earliest divergences identified within each of the C_4 and non-C_4 clades happened shortly after their split and were followed in each case by continued dispersals through geographical and environmental spaces (Figs 1, 2, and 5). This short evolutionary history, together with the diversity of ecological conditions covered (Fig. 4), therefore provides a unique opportunity to investigate the ecological causes and consequences of physiological innovation.

Divergence of photosynthetic types is not followed by major ecological shifts

Based on the phylogenetic relationships inferred here, the common ancestor of the ABCDE clade originated from wooded savannas in central Africa, and the early members of clades ABC and DE persisted in this area for a considerable length of time. The initial divergence of clades ABC and DE might have been caused by geographic isolation, in a tectonically active region where mountain ranges, lakes, and rifts provide barriers to dispersal. Interestingly, the divergence of photosynthetic types did not directly lead to obvious modifications of the ecological niche, as assessed by climatic and fire variables (Figs S8 and S9). Representatives of the different clades and photosynthetic types can still be found in habitats within central eastern Africa that match those inferred for their common ancestor (Figs 4, 5, and S9). Indeed, some C_4 and non-C_4 members of clades B, C, E, and F are found in densely wooded savannas of Tanzania, Congo, and Cameroon, and individuals of clade D occur in similar habitats throughout Asia and Madagascar (Table S4). In these savannas with a high cover of deciduous trees, photorespiration is predicted to vary throughout the year as leaf fall drastically increases sunlight, temperature, and aridity at ground level. The range of open and wooded savannas in central Africa varied as a function of the glacial cycles, but wooded savannas were constantly present in this region from the Mioecene (Hoetzel et al. 2013; Pound et al. 2014). Mutations providing a more C_4-like physiology might have been selected for in these habitats where the persistence of more C_3-like or intermediate phenotypes is still possible. Based on these investigations, we speculate that C_4 physiology initially emerged in environments that advantage different photosynthetic types across the seasons or across small-scale ecological variations (e.g. densely versus lightly wooded habitats), where isolated populations could explore different parts of the phenotypic landscape as a function of random mutations.

… but C_4 photosynthesis enlarges the ecological niche and increases dispersal success

The ecological similarity between the early members of the non-C_4 and C_4 groups contrasts with the current distribution of the two photosynthetic types. Indeed, extant accessions of the C_4 clade DE inhabit environments ranging from the tropics to southern latitudes and cover a broad range of temperatures, precipitations, light intensities, and fire regimes, as well as open and wooded habitats (Figs 2, 4, S8 and S9; Table S4). Elucidation of the phylogeographic history shows that these varied habitats were colonized rapidly after the divergence of photosynthetic types, while the otherwise similar non-C_4 members of clade ABC remained confined to a narrower set of environmental conditions over the same period (Figs 1, 3, and 5). Moreover, members of clades D and E recurrently migrated across the environmental space (Figs 3 and 5), indicating that present distribution patterns are not due to specific groups of C_4 accessions specializing to different habitats, but to a constant movement across habitats, as attested by the lack of correlation between environmental distances and divergence times (Fig. 3). These results indicate that when other factors affecting the ecology of individual plant species remain similar, C_4 photosynthesis acts as a niche opener, and does not simply shift the ecological niche (Fig. 4). The main consequence of C_4 photosynthesis is to decrease photorespiration, and thus increase the amount of CO_2 fixed per absorbed photon in condition promoting photorespiration (Ehleringer & Bjorkman 1977). This enhances water- and nitrogen-use efficiencies (Ehleringer & Bjorkman 1977; Pearcy & Ehleringer 1984), which could facilitate the colonization of drier and less fertile habitats. However, it does not necessarily decrease success in fertile and wetter environments, where it can provide a competitive advantage by enabling faster growth (Monteith 1978; Long 1999). In addition, the combination of different C_4 biochemical subtypes observed in *A. semialata* might contribute to enlarging the ecological niche (Wang et al. 2014). The diversity of ecological conditions tolerated by the C_4 accessions of *A. semialata* probably explains the more efficient dispersal of these plants, as has been found across multiple species of plants and animals (Slatyer et al. 2013). Indeed, the capacity to survive in a broad range of environments following long distance dispersal events likely facilitated the colonization of distant regions, leading to the spread of these plants across three different continents (Fig. 2).

Other adaptations lead to ecological diversification

While the C_4 clade DE was quickly dispersing across geographical and environmental spaces (Fig. 3), members of the non-C_4 clade ABC continued evolving, emphasizing the importance of considering the variation within each photosynthetic type when inferring evolutionary processes. Indeed, non-C_4 lineages gradually came to colonize distinct environments independently of geography (Fig. 3). The gradual migration toward distinct habitats implies a continuous process of adaptation through natural selection. While clades B and C remained in central Africa, in habitats that broadly resemble those where the common ancestor of *A. semialata* grew, members of clade A strongly deviated from these conditions and colonized colder regions in southern Africa (Figs 2, 4, 5, and S9). This southern dispersal also involved the migration from wooded savanna habitats to open temperate grasslands with leached, acidic soils, where non-C_4 *A. semialata* are very successful, as attested by their local abundance (Ellis 1981). The South African non-C_4 *A. semialata* have acquired a cold adaptation mechanism for leaves to resist freezing, enabling a leaf canopy to persist throughout the winter (Osborne et al. 2008), and are able to maintain photosynthetic capacity under drought conditions (Ripley et al. 2007; Ibrahim et al. 2008). In addition, the non-C_4 *A. semialata* completes its growing period during the cooler periods in South African grasslands (Wand et al. 2002). These adaptations may have contributed toward their successful colonization of southern latitudes. C_4 photosynthesis, adopted by members of clade DE, and cold tolerance, present in clade A, might represent alternative novelties that allow the ecological expansion of tropical lineages. This pattern is already evidenced for the grass family as a whole, where distinct groups have evolved either C_4 photosynthesis or cold tolerance, both of which strongly increased diversification rates (Spriggs et al. 2014). Our intraspecific investigations show that, while C_4 photosynthesis broadens the niche and allows rapid dispersal across environmental space, cold adaptation might be an alternative but slower process that leads to a narrower realised niche in otherwise similar plants.

Conclusions

Capitalizing on the variation that exists within a single species complex, this study is the first to characterize the ecological changes that directly follow the emergence of different photosynthetic types. The joint analysis of geographical and environmental dispersal histories within a phylogenetic context shows that C_4 photosynthesis does not initially result in a shift of the ancestral niche, but broadens this niche to cover a wider range of conditions that encompass the ancestral ones (Fig. 4), enhancing the success of occasional long distance dispersal events, and therefore increasing the geographic range. The variety of environments available to C_4 plants is also reflected in the ecological diversity observed among C_4 species, with different C_4 taxa found in very distinct environments that promote photorespiration in different ways (Sage et al. 2012). Interspecific phylogeny-based analyses suggest that species using C_4 photosynthesis diversify across a wider range of environments than closely related C_3 species (Christin & Osborne 2014). However, individual taxa likely specialize in different environments after the initial evolution of C_4 physiology, through differential integration of the C_4 machinery with their growth and life-history traits (Christin & Osborne 2014). Over time, this process leads to some C_4 taxa becoming specialized to environments that differ strongly from those in which they evolved, inflating the ecological differences between C_3 and C_4 photosynthesis and blurring the initial effects resulting from differences in photosynthetic types.

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Figure captions

Figure 1: Phylogenetic relationships among *A. semialata* accessions. This tree was obtained through Bayesian inference on chloroplast markers, and branch lengths are proportional to estimated divergence time, in arbitrary time units. Branches leading to monophyletic C_4 groups are in red. Geographic regions are delimited next to the tips. The main clades are delimited on the right, and colored according to photosynthetic type with red denoting C_4, and black non-C_4, clades. Asterisks indicate nodes with Bayesian support values above 0.95. The phylogenetic tree is detailed in Fig. S1.

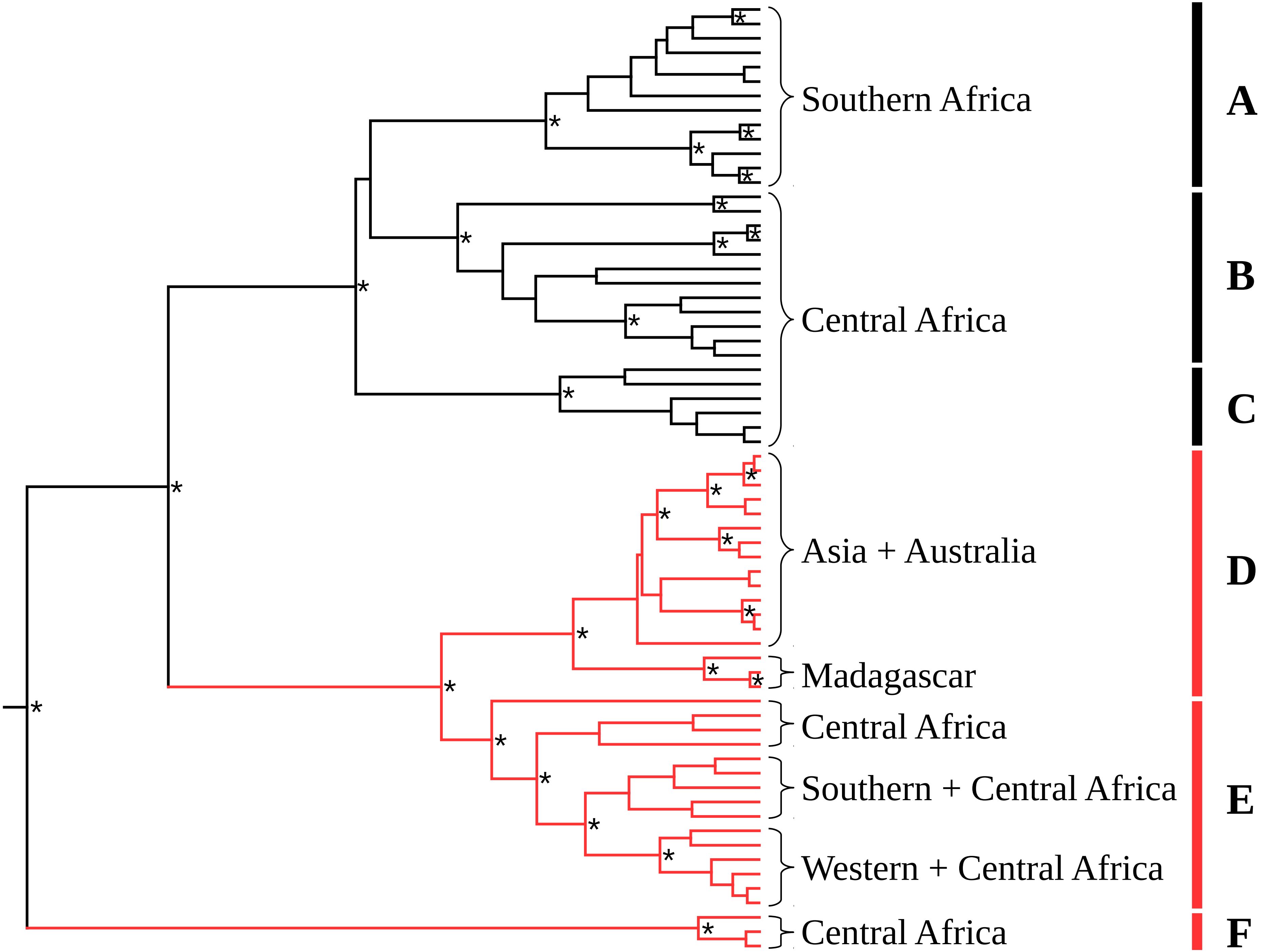
Figure 2: Distribution of sampled *Alloteropsis* individuals and inferred dispersal events. (A) The six main clades are represented by different symbols, with the C_4 accessions in red and the non-C_4 accessions in black. (B) The phylogeographic tree is approximately projected on the geographical space, with dispersal indicated by arrows (tips of arrows as in panel A). The branch from the root is in grey, and other branches are colored by photosynthetic type (C_4 in red and non-C_4 in black).

Figure 3: Comparison of geographical and environmental distances and divergence times. These analyses are based on distances between pairs of non-C_4 individuals from clade ABC (black) and between pairs of C_4 individuals from clade DE (red). Regression lines forced to the origin are shown for significant relationships, identified by Mantel tests.

Figure 4: Ecological niche as inferred by principal component analysis (PCA). In the left panel, dashed lines indicate the approximate distribution of C_4 (red) and non-C_4 (black) accessions in the PCA space (see Fig. S8 for the distribution of all points). The distribution of individuals included in the phylogeny is shown with circles, squares, and triangles colored by photosynthetic type. The location of the common ancestor of clades ABC and DE as inferred along the phylogeny is

indicated by a grey circle. The right panel indicates the inferred changes in the PCA space, with an environmental shift for the non-C_4 clade A (black arrow) and extension of the C_4 niche in multiple directions (red arrows).

Figure 5: Movements across the environmental space inferred along the phylogeographic tree. Dot size is proportional to the absolute values along the first two dimensions of the PCA, as observed for tips and inferred for ancestral nodes. Negative values are in black and positive values in pink. The main clades are indicated on the right.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Asia + Australia
D
Madagascar
Central Africa
Southern + Central Africa
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Western + Central Africa
Central Africa
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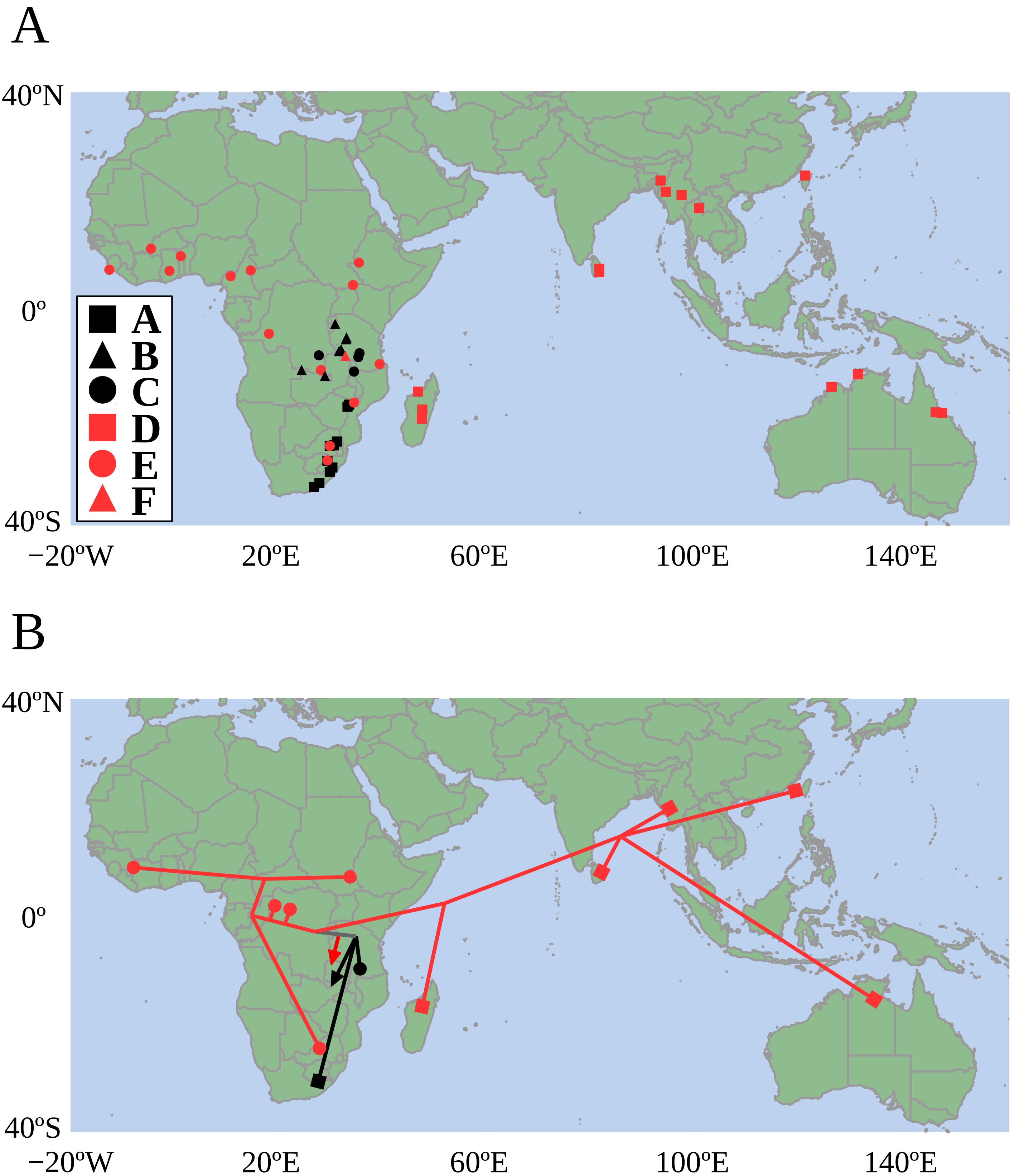
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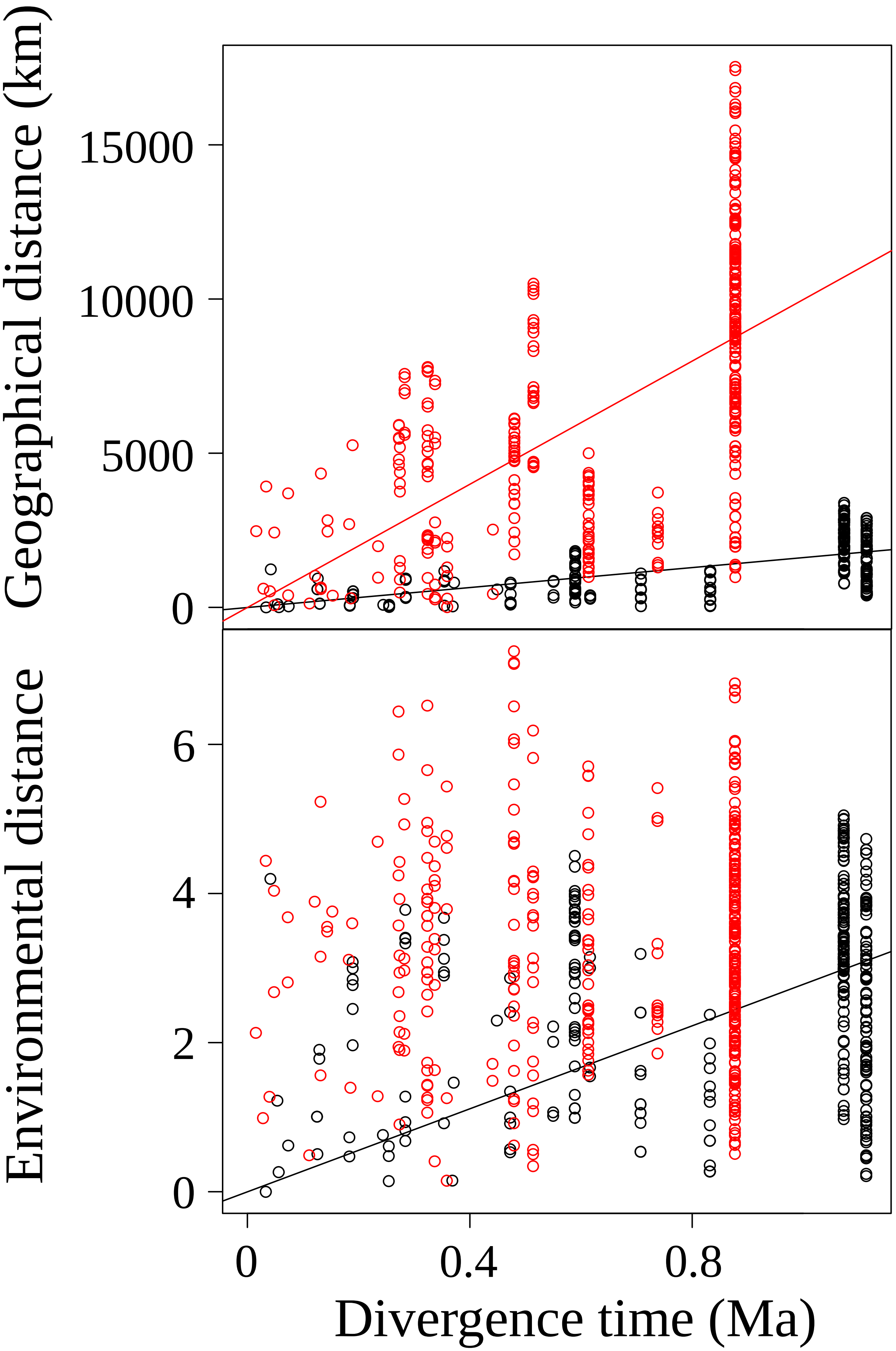
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Environmental distance
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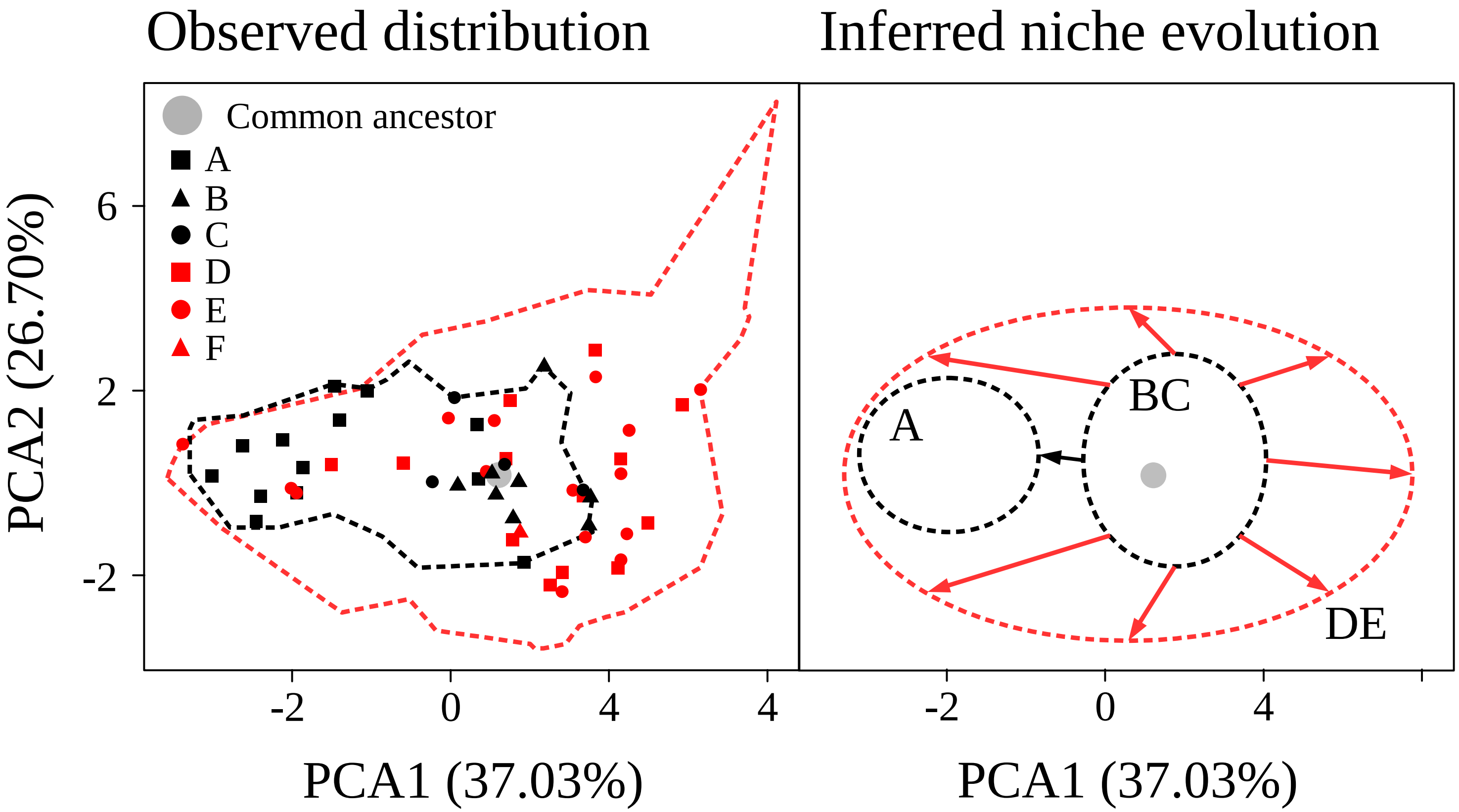
0.4
0.8
Divergence time (Ma)


Observed distribution
Inferred niche evolution
Common ancestor
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B
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D
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PCA2 (26.70%)
6
2
-2
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4
PCA1 (37.03%)
A
BC
DE
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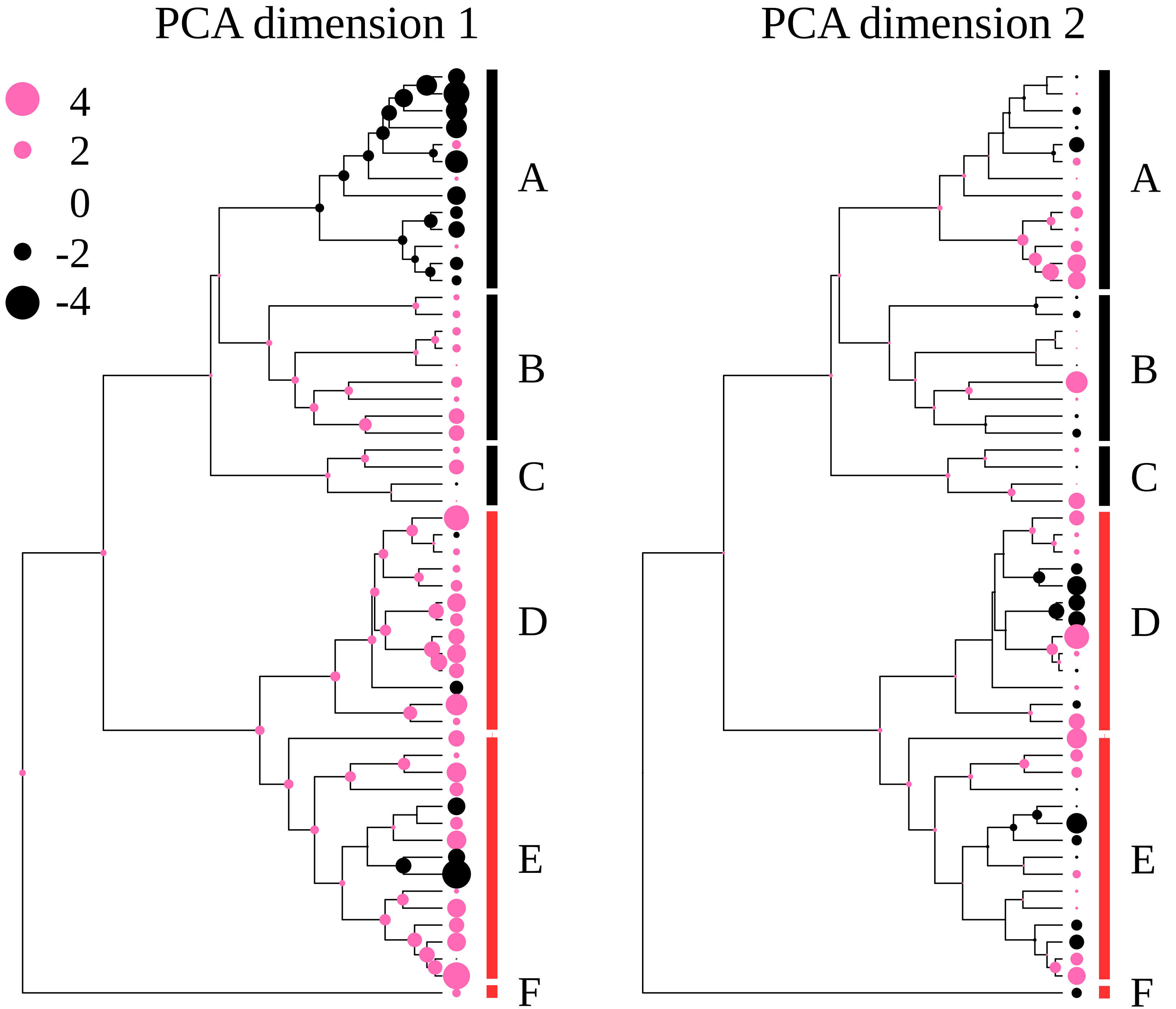
4
PCA1 (37.03%)


PCA dimension 1
PCA dimension 2
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