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Unmatched tempo of evolution in Southern African semi-desert ice plants

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The Succulent Karoo is an arid region, situated along the west coast of southern Africa. Floristically this region is part of the Greater Cape Flora[1] and is considered one of the Earth's 25 biodiversity hotspots[2]. Of about 5,000 species occurring in this region, more than 40% are endemic[3]. Aizoaceae (ice plants) dominate the Succulent Karoo both in terms of species numbers (1,750 species in 127 genera) and density of coverage[3,4]. Here we show that a well-supported clade within the Aizoaceae, representing 1,563 species almost exclusively endemic to southern Africa, has diversified very recently and very rapidly. The estimated age for this radiation lies between 3.8 and 8.7 million years (Myr) ago, yielding a per-lineage diversification rate of 0.77–1.75 per million years. Both the number of species involved and the tempo of evolution far surpass those of any previously postulated continental or island plant radiation[5–7]. Diversification of the group is closely associated with the origin of several morphological features and one anatomical feature. Because species-poor clades lacking these features occur over a very similar distribution area, we propose that these characteristics are key innovations that facilitated this radiation.

Most species of Aizoaceae exhibit leaf succulence, whereas stem succulents are comparatively rare. The range of life forms, particularly in the highly succulent Aizoaceae, is remarkable. It includes annuals, perennials, trailing woody plants, shrubs and even small trees, as well as highly compact succulent forms (sometimes reduced to just two leaves), geophytes and a few stem succulents with deciduous leaves. The diversity of both life form and number of species has been largely attributed to a complex interaction between the availability of numerous and diverse niches[8] and strong genetic drift caused by very low rates of gene exchange between populations[4]. Because most species are found within the arid winter rainfall area, the onset of the winter rainfall regime (about 5 Myr ago) is also thought to have heralded the beginning of diversification in the Aizoaceae. In the past, the existing, although very scanty,

fossil evidence has been interpreted as providing support for the theory that between 10 and 5 Myr ago there was a major change in the composition of the flora in the region, from a moister to a much drier climate[9,10]. However, arid to semi-arid conditions interspersed with wetter periods dominated the climate of areas adjacent to the Succulent Karoo, such as the Namib, since the Cretaceous period about 80 Myr ago[11,12]. It is therefore possible that the Aizoaceae were well represented long before the onset of the winter rainfall regime.

In the absence of a good fossil record, molecular phylogenies are now used increasingly[5,6] to evaluate competing hypotheses about the timing of diversification. A key feature of all the DNA regions we investigated is the low level of divergence between representatives of the clade incorporating most members of the subfamily Ruschioideae. This clade represents a large group of about 1,563 species in 101 genera and thus constitutes about 85% of the Aizoaceae (Fig. 1). The small number of substitutions coupled with the high species diversity suggests very recent and very rapid diversification. The number of species involved in this radiation far exceeds that of any other group in which recent and rapid radiation has been postulated[5–7]. Contrary to an earlier hypothesis suggesting that all highly succulent Aizoaceae (subfamilies Mesembryanthemoideae and Ruschioideae) are of recent origin[4], we show here that only the core Ruschioideae has radiated recently, with an estimated age of between 3.8 and 8.7 Myr. Our estimates of the onset of the radiation therefore fall within the proposed time frame of increased aridification and opening of the winter-rainfall meganiche about 5 Myr ago[9,10].

The estimated per lineage diversification rate per million years lies between 0.77–1.75 (assuming zero extinction) and 0.58–1.32 (when incorporating a high rate of extinction[13]). Thus, the tempo of radiation in the core Ruschioideae is considerably higher than that for any other continental (angiosperm families, median of 0.12 and maximum of 0.39 species per Myr[14]; angiosperm orders, maximum of 0.76 species per Myr (ref. 13)) or island (Hawaiian silversword alliance, 0.56 ± 0.17 species per Myr (ref. 7)) plant radiation. This estimate also compares favourably with animal diversification rates (hexapods, 0.05 species per Myr (ref. 15); Neogene horses, 0.5–1.4 species per Myr (ref. 16); Hawaiian island drosophilids, 1.21 species per Myr; Lake Tanganyika cichlids, 0.75–1.49 species per Myr (ref. 17)). Even higher diversification rates have been reported for some cichlid radiations[17]. However, our study assumes a constant rate of diversification over a time interval of 3.8–8.7 Myr, and it is likely that the core Ruschioideae have undergone intervals of even higher diversification rates[18]. The question of features or conditions that might have facilitated this radiation is important. The two lineages basal to the core Ruschioideae, as well as the next closely related subfamily, the Mesembryanthemoideae, all contain far fewer species: the Mesembryanthemoideae consists of about 100 species, and the two lineages basal to the core Ruschioideae each consist of 11 species. This indicates a large imbalance in net speciation rates between these clades and the core Ruschioideae. Yet the species-poor lineages also consist exclusively of highly succulent plants. Furthermore, they share with the core Ruschioideae both a similar distribution area and a similar dispersal strategy. Therefore, climatic or ecological factors alone cannot explain the remarkable speciation burst. We propose that several morphological characters constitute key innovations that have facilitated the major radiation of the core Ruschioideae. One important invention seems to be the recently reported wide-band tracheids, which are found (with few exceptions) in the core Ruschioideae only and are absent from all taxa that are sister to this clade. These specialized cells are thought to prevent the collapse of the primary wall and have therefore been associated with adaptations to withstand water stress[19,20]. Taxa that possess these cells are likely to have an increased chance of survival in an arid environment over those taxa that lack them. A further major change in morphology associated with the node defining the
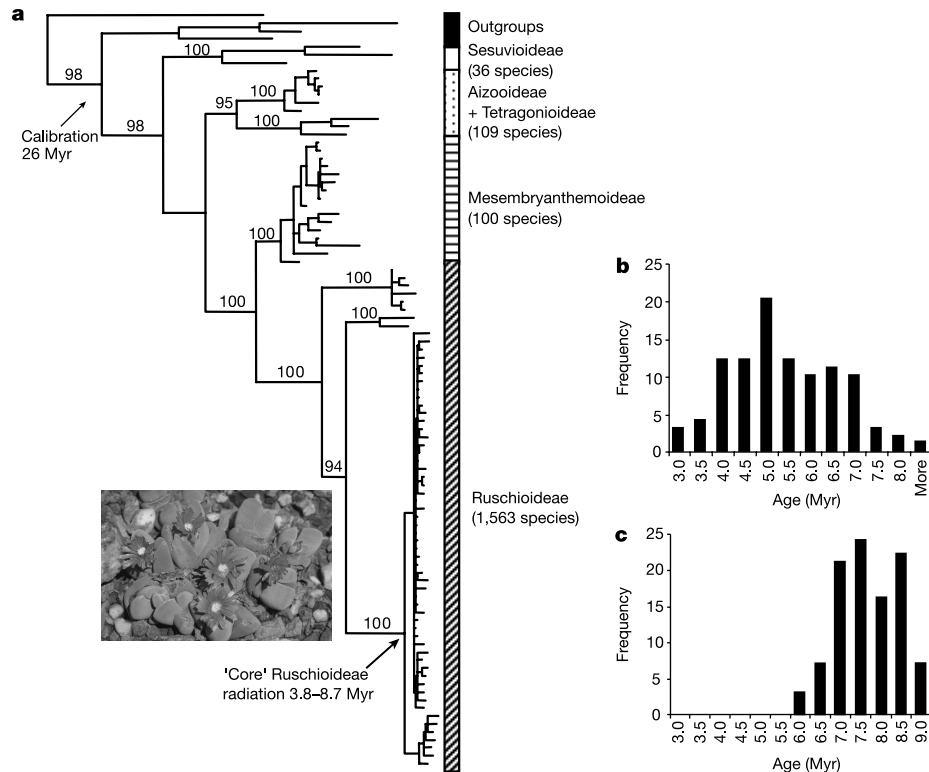
Figure 1 Parsimony analysis. **a**, One of the 5,000 equally parsimonious trees selected randomly from the parsimony analysis of the plastid data (tree length 1,413 steps) with bootstrap percentages indicated above the branches. Branch lengths are proportional to the changes in the plastid data. Calibration of the split between Nyctaginaceae and Aizoaceae + Phytolaccaceae at 26 Myr gave an estimated age for the 'core'

Ruschioideae clade of 3.8–8.7 Myr. The photograph shows a typical member of the 'core' Ruschioideae, *Gibbaeum haaglenii* H. E. K. Hartmann. **b**, ITS bootstrap distribution of age estimates. Mode = 3.8; s.d. = 3.2 Myr. **c**, Plastid bootstrap distribution of age estimates. Mode = 8.7; s.d. = 0.7 Myr.

species-rich clade is leaf shape. Flat or flattened leaves are common in all of the basal clades, whereas the leaves typically show a cylindrical or trigonous shape in the species-rich clade. This reduction in leaf surface area provides a further advantage in reducing water loss under dry conditions. Further xeromorphic modifications (particularly those of the leaves) are characteristic within the core Ruschioideae, but these are not directly associated with the onset of the radiation that we have dated in this study.

In addition, hygrochastic capsules, which open only when moistened to release a portion of their seeds in rain, might have had a function in the radiation of the core Ruschioideae. Although hygrochastic capsules are found in both the species-poor (such as the Mesembryanthemoideae) and the species-rich clades (core Ruschioideae), the morphology of the capsules found in the species-rich clade is far more specialized. Experimental removal of some or all of the specialized structures in Ruschioid capsules results in the retention of fewer seeds[21], suggesting that these structures spread seed dispersal through time, thus increasing the probability of seed release during rainfall events of adequate size and duration to ensure seed germination and the subsequent survival of seedlings. Members of the Mesembryanthemoideae always lack these structures and their seeds are consequently lost in a few rainfall events. In contrast, fruits found in members of the core Ruschioideae release only a few seeds at a time. This is considered to confer advantages because the presence of seeds for subsequent rains is an effective dispersal strategy in regions with mostly erratic rainfall. The coupling of both disperal and germination (although applicable to both the species-poor and species-rich clades) in turn reduces the possibility of secondary dispersal of seed, thus reducing gene-flow

distances and potentially promoting isolation of populations in space[21]. □

Methods

DNA sequencing

We used *rps16* intron and *trnL-F* sequences for 91 Aizoaceae species, representing all major lineages in the family, and four outgroup taxa (from the Molluginaceae, Phytolaccaceae and Nyctaginaceae)[22]. Internal transcribed spacer (ITS) sequences were also produced for 34 ingroup and 3 outgroup taxa.

Phylogenetic analyses

We analysed two matrices (the first consisting of combined *rps16* intron and *trnL-F* sequences and the second of ITS sequences) using the parsimony algorithm of PAUP* (ref. 23). Each data set was subjected to 1,000 random addition replicates using equal weights[24] with TBR branch swapping. Internal support was assessed using 1,000 bootstrap replicates[25]. The topologies retrieved in each analysis were similar with respect to the recovery of, and relationships between, the major lineages. Minor exceptions concerned the placement of *Tetragonia* and *Dorotheanthus* + *Cleretum* in the ITS analysis. Because we considered the more complete analysis including 95 taxa for two regions to be our best estimate of phylogenetic relationships, we used this as our best estimate of the phylogeny. High bootstrap percentages were recovered for all major lineages for both analyses, and differences in equally parsimonious trees for both data sets were restricted to terminal groupings mainly within the core Ruschioideae.

Age and diversification of core Ruschioideae

Maximum likelihood (ML) branch lengths were fitted using a three-parameter model; the transition–transversion ratio, base frequencies and gamma distribution of rate variability between sites were all estimated from the data. We chose this model over less complex alternatives with a likelihood ratio (LR) test in which the statistic was twice the difference in log likelihood scores between the simpler model and the more complex model. At each step we chose the more complex model if it provided a significantly better fit to the data. The hypothesis of rate constancy was rejected for both data sets using an LR test between a rate-constrained tree (forcing a molecular clock in PAUP*) and a rate-unconstrained tree. Ultrametric trees were therefore produced from both parsimony and ML branch lengths

with Sanderson's[26] method of non-parametric rate smoothing (NPRS) implemented in TreeEdit version 1.0 alpha 4-61 (ref. 27). In the absence of reliable fossil data[28,29], all trees were calibrated using an estimated age of 26 Myr for the split between Aizoaceae + Phytolaccaceae and its nearest relative, the Nyctaginaceae. We took this date from a DNA-sequence-based angiosperm phylogeny using ML branch lengths[30]. In addition, to account for the error in the calibration, we recalculated the age of the core Ruschioideae and diversification rate using the most conservative date estimated for the split between Aizoaceae + Phytolaccaceae and Nyctaginaceae (that is, 30 Myr using parsimony branch lengths and DELTRAN optimization)[30]. The standard error in age estimates for each data set with ML branch lengths was also estimated using 100 bootstrap matrices and one of the most equally parsimonious trees found from the initial heuristic searches (*Tetragonia* and *Dorotheanthus* + *Cleretum* once again constrained for the ITS analysis). With a calibration age of 26 Myr, the estimated age of the core Ruschioideae radiation from the plastid data set was 6.2 Myr with ML branch length (mode of bootstrap distribution 8.7 ± 0.7 Myr; Fig. 1) and 6.4 Myr with parsimony branch lengths; and from the ITS data set 3.1 Myr with ML branch lengths (mode of bootstrap distribution 3.8 ± 3.2 Myr; Fig. 1) and 6.6 Myr with parsimony branch lengths. With a calibration age of 30 Myr, the mode of the bootstrap distribution was slightly higher (plastid data 9.5 ± 0.8 Myr and ITS data 4.4 ± 3.7 Myr with ML branch lengths).

We estimated the per-lineage rate of diversification per million years for the core Ruschioideae radiation from both plastid and ITS data sets as $(\ln N - \ln N_0)/T$ (ref. 7), where initial diversity $N_0 = 2$, N is existing diversity and T is estimated clade age (we used the mode of the bootstrap distribution of age estimates as T). Thus, with a calibration of 26 Myr the estimated per-lineage diversification rate per million years based on the plastid data was 0.77 and for ITS 1.75 (0.70 and 1.5 respectively for the plastid and ITS data with a calibration point of 30 Myr). Taking extinction into account with a high value of $\epsilon = 0.9$ (ref. 13), we recalculated diversification rates as 0.58 lineages per million years for the plastid data and 1.32 lineages per million years for the ITS data.

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A euprimate skull from the early Eocene of China

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The debut of undoubted euprimates (primates of modern aspect[1,2]) was in the early Eocene, about 55 Myr ago. Since their first appearance, the earliest euprimates can be distinguished as *Cantius*, *Donrussellia* and *Teilhardina*[2–4]. Nonetheless, the earliest euprimates are primarily known from isolated teeth or fragmentary jaws. Here we describe a partially preserved euprimate skull with nearly complete upper and lower dentition, which represents a new species of *Teilhardina* and constitutes the first discovery of the genus in Asia. The new species is from the upper section of Lingcha Formation, Hunan Province, China, with an estimated age of 54.97 Myr ago[5]. Morphology and phylogeny analyses reveal that the new species is the most primitive species of *Teilhardina*, positioned near the root of euprimate radiation. This discovery of the earliest euprimate skull known to date casts new light on the debate[6–12] concerning the adaptive origin of euprimates, and suggests that the last common ancestor of euprimates was probably a small, diurnal, visually oriented predator.

Primates Linnaeus, 1758
Omomyidae Trouessart, 1879
Teilhardina Simpson, 1940
Teilhardina asiatica sp. nov.

Holotype. A partial skull with associated lower jaws (IVPP V12357, Figs 1, 2).
Included material. An isolated lower incisor (IVPP V12357-4) and two additional partial lower jaws (IVPP V12060, V13762).
Horizon and locality. Upper part of the Lingcha Formation, Hengyang Basin, China; earliest Eocene[5].
Diagnosis. Differs from *T. belgica* and *T. americana* in having a