

small rocks or rock flakes scattered among larger boulders on fine-grained soil.

The discovery of *Karsenia* has important biogeographical implications. Plethodontidae is now seen to have a Holarctic distribution, although it is impressively disjunct. The nearest plethodontids are found along the central coast of British Columbia, Canada (*P. vehiculum*, *Ensatina escholtzii*). To the west, no plethodontids are known until central Italy. Disjunctions between Asia and North America are recorded for many taxa, and for plants of eastern Asia and eastern North America. About 65 flowering plant genera¹² display such disjunctions, typically with Asian species in a given clade being more numerous¹³, a strong contrast with plethodontids, which are represented by many species in eastern North America. Phylogenetic relationships of the plants and estimated divergence times suggest multiple historical events at different periods in geological time, with disjunctions typically dating from the Miocene or earlier^{12–14}. Disjunct taxa usually are not cladistically close¹⁴. These conclusions apply to *Karsenia* as well. The species has no close relatives in North America, and the amount of genetic divergence suggests a long period of independent evolution, possibly pre-Tertiary^{8,10}. Plethodontids are known to be very old, with fossils of *Plethodon* and *Aneides* known from the Arikareean (Oligocene) of Montana^{15,16}.

Mammalian fossils indicate strong physical and phylogenetic connections between east Asia and North America at least until the Late Miocene¹⁷, and polar sea temperatures are estimated to have averaged about 15 °C 70 million years ago and more than 20 °C at 90 million years ago¹⁸. Such temperatures would have been favourable for plethodontid salamanders. Accordingly, there have been many opportunities for early migrations between the continents. An important implication of current distributions is that Old World plethodontids have had lower rates of speciation than those in the New World, but also may have been disproportionately subject to extinction. The discovery of plethodontids in Asia encourages further efforts to find more of these secretive animals. □

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An extant cichlid fish radiation emerged in an extinct Pleistocene lake

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The haplochromine cichlid fish of the East African Great Lakes represent some of the fastest and most species-rich adaptive radiations known¹, but rivers in most of Africa accommodate only a few morphologically similar species of haplochromine cichlid fish. This has been explained by the wealth of ecological opportunity in large lakes compared with rivers. It is therefore surprising that the rivers of southern Africa harbour many, ecologically diverse haplochromines. Here we present genetic, morphological and biogeographical evidence suggesting that these riverine cichlids are products of a recent adaptive radiation in a large lake that dried up in the Holocene. Haplochromine species richness peaks steeply in an area for which geological data reveal the historical existence of Lake palaeo-Makgadikgadi^{2,3}. The centre of this extinct lake is now a saltpan north of the Kalahari Desert, but it once hosted a rapidly evolving fish species radiation, comparable in morphological diversity to that in the extant African Great Lakes. Importantly, this lake seeded all major river systems of southern Africa with ecologically diverse cichlids. This discovery reveals how local evolutionary processes operating during a short window of ecological opportunity can have a major and lasting effect on biodiversity on a continental scale.

Lake Victoria (LV) and Lake Malawi (LM) are known for their extremely diverse flocks of haplochromine cichlid fish and have received much attention as model systems for explosive speciation and ecomorphological diversification^{1,4}. Together comprising more than 800 species, geological and mitochondrial (mt)DNA evidence suggests that these flocks evolved in a very short time period (Lake Victoria, 15,000–250,000 years^{5,6}; Lake Malawi, up to 5 million years⁷) from one or a few common ancestors^{8,9}. In contrast, the

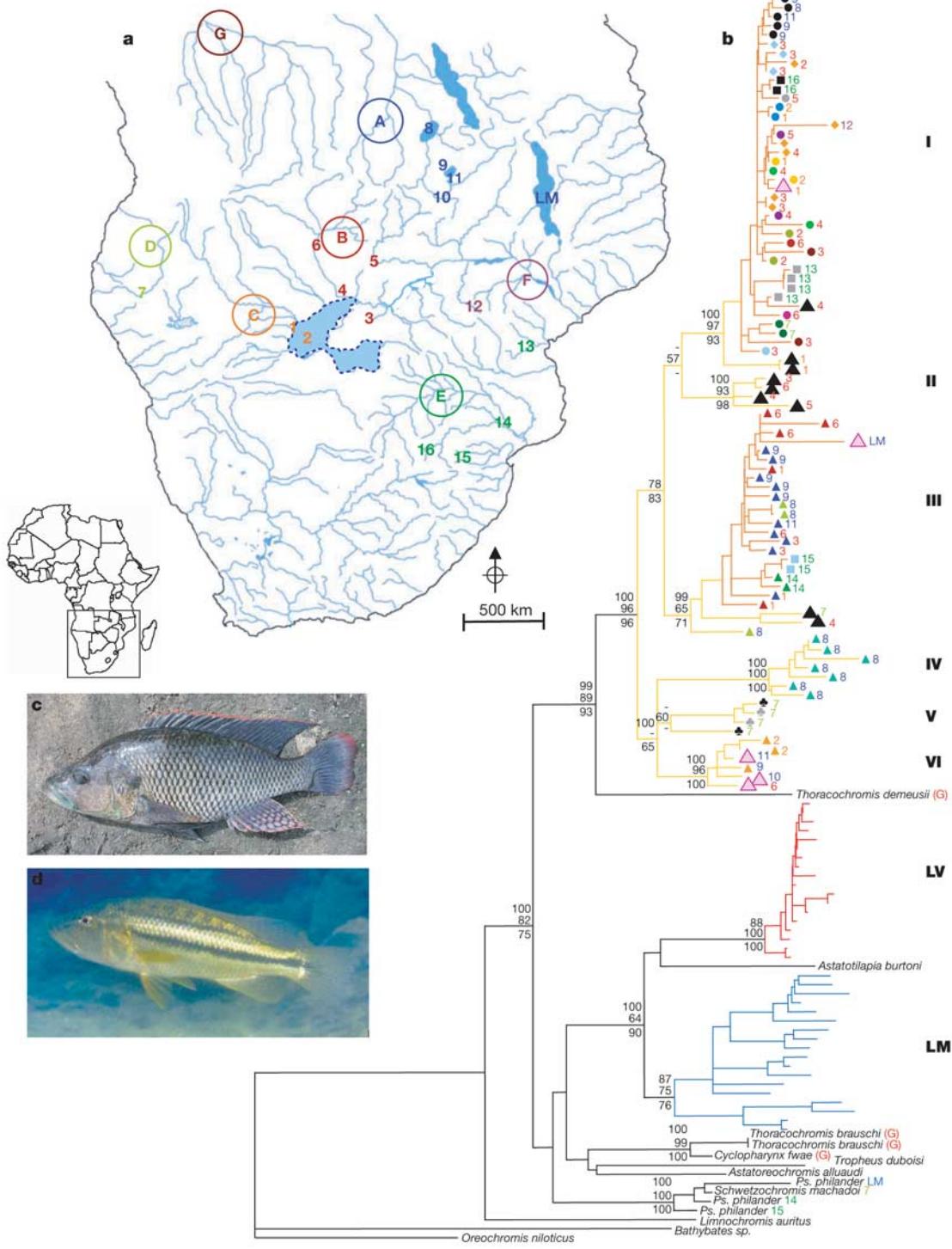


Figure 1 The serranochromine cichlids of southern Africa are a flock of closely related species that probably arose in Lake paleo-Makgadikgadi. **a**, The position and size of Lake paleo-Makgadikgadi at its Quaternary maximum. A, upper Congo (contains six serranochromine species, one *Thoracochromis* species); B, middle/upper Zambezi (12 serranochromine species); C, Okavango River system (11 serranochromine species); D, Cunene River (seven serranochromine species, two *Thoracochromis* species); E, Limpopo River (four serranochromine species); F, lower Zambezi including Lake Chivero (three serranochromine species); G, middle Kasai to lower Congo (no serranochromine species, but seven *Thoracochromis*-related species, of which four are endemic to Lake Fwa in the middle Kasai River). Numbers indicate sampling sites, and species are listed in the Supplementary Information. A dashed line represents the area covered by lacustrine and fluvio-lacustrine landforms representing Lake paleo-Makgadikgadi at its Quaternary maximum. **b**, Neighbour-joining (NJ) tree (GTR + I + Γ) with bayesian posterior probabilities and NJ bootstrap support above the branches, and bootstrap support for maximum likelihood shown below the branches. Only values >50% are shown. The

southern radiation is shown in orange, with rapidly radiating clades (that is, the Lake paleo-Makgadikgadi species flock) in darker orange, Lake Victoria (LV) clade in red and Lake Malawi (LM) clade in blue. Species are: *Sargochromis mellandi* (black circles), *Sarg. mortimeri* (grey circle), *Sarg. codringtonii* (blue circles), *Sarg. n. sp. 1* (purple circles), *Sarg. carlotta* (yellow circles), *Sarg. n. sp. 2* (bright green circles), *Sarg. giardi* (olive green circles), *Sarg. cf. mortimeri* (red circle), *Sarg. sp.* (brown circles), *Sarg. cf. giardi* (pink circle), *Sarg. coulteri* (dark green circles), *Sarg. cf. giard2* (light blue circle); *Serranochromis macrocephalus* (large black triangles), *S. robustus* (large pink triangles), *S. altus* (red triangles), *S. angusticeps* (blue triangles), *S. stappersi* (turquoise triangles), *S. meridianus* (bright green triangles), *S. n. sp. Mfimbo* (olive green triangles), *S. thumbergi* (light orange triangles); *Chetia flaviventris* (black squares), *C. brevicauda* (grey squares), *C. brevis* (blue squares); *Pharyngochromis n. sp.* (blue diamonds), *P. acuticeps* (orange diamonds); *Thoracochromis albolabris* (black cloverleafs), *T. buysi* (grey cloverleafs). **c**, **d**, *Serranochromis macrocephalus* (**c**) and *S. robustus* (**d**) appear in three of the six southern radiation clades.

rivers associated with Lake Victoria and Lake Malawi show very low haplochromine species richness and morphological diversity; this is believed to be because rivers lack the wealth of ecological opportunity thought to drive adaptive radiation in lakes^{4,10}. There are two widespread riverine lineages of haplochromine cichlids, *Pseudocrenilabrus* and *Astatotilapia/Thoracochromis*, and rivers in this and many other parts of Africa typically contain one species of each¹¹. Paradoxically, the rivers south and southwest of the East African rift valley are markedly different. The upper Congo, middle/upper Zambezi, Okavango, Cunene and Limpopo Rivers each harbour numerous sympatric haplochromines of diverse shape and size, classified as serranochromines¹² and *Thoracochromis* spp. (Fig. 1a).

We collected the majority of the described serranochromine species from these five river systems, as well as seven undescribed species. We also collected four *Thoracochromis* species (Fig. 1a, Supplementary Table S1 and Supplementary Fig. S1) and two other haplochromine cichlids that occur in the area (*Pseudocrenilabrus philander* and *Schwetzochromis machadoi*). To reconstruct the phylogenetic origins of the southern African riverine cichlid diversity, we amplified and sequenced the complete mitochondrial control region from 99 specimens and generated phylogenies using minimum evolution, maximum likelihood and bayesian analysis (Fig. 1b). The mtDNA haplotypes of all serranochromine and the two Cunene River *Thoracochromis* species form a well-supported (>95% bootstrap support, 100% bayesian posterior probability) lineage comprising six clades (I–VI, the ‘southern radiation’) to the exclusion of East African haplochromines, *Pseudocrenilabrus*, *Schwetzochromis* and Congolese *Thoracochromis*. A *Thoracochromis* species from the lower Congo is the sister taxon to this radiation.

Within this southern radiation, we recover a pattern typical of the adaptive radiations in the East African Great Lakes: emergence of ecological and phenotypic diversity in a rapidly multiplying lineage, characterized by low levels of neutral genetic divergence¹⁰. There are a large number of closely related haplotypes that have arisen from a small number of more divergent haplotypes (Fig. 1b). Clades I and III contain many different genera and species. Within these clades, haplotypes are not sorted by species, and branch lengths are short (mean pairwise genetic distance: clade I = 0.010, s.d. = 0.006; clade III = 0.019, s.d. = 0.010; Lake Victoria = 0.008, s.d. = 0.004; Lake Malawi = 0.042, s.d. = 0.016). Both patterns characterize the species flock of Lake Victoria and sections of the Lake Malawi species flock. The genera *Chetia* and *Serranochromis* contain divergent haplotypes and appear poly- and paraphyletic, respectively. More strikingly, we find deep divergence within *Serranochromis robustus* and *S. macrocephalus*, and even within a single population of *S. macrocephalus* (population 4; mean pairwise genetic distance = 0.036, s.d. = 0.001, across populations = 0.032, s.d. = 0.012). Conversely, several morphologically different and geographically distant species have very similar haplotypes. For example, one *Chetia brevicauda* from the Mussapa River, Mozambique (population 13) and *Serranochromis macrocephalus* from the upper Zambezi (population 4) differ by only 6 out of 887 base pairs (0.68%, genetic distance = 0.0072). At present, these rivers are separated by the middle Zambezi, the Victoria Falls, the lower Zambezi and about 100 km of Indian Ocean shoreline.

These phylogenetic relationships generate a second paradox: a species flock that emerged rapidly and simultaneously in many geographically distant river systems, in which very similar haplotypes occur thousands of kilometres apart, yet for which strongly differentiated haplotypes are found within single populations (Fig. 1b). We suggest a resolution of both paradoxes, based on geological records and biogeographic patterns. We mapped species richness of the serranochromine cichlids across southern Africa using geographical distribution records. We observed a single centre of serranochromine species richness in the area of the Okavango delta and the upper/middle Zambezi (Fig. 2a). Geological records

reveal the presence of a large lake in the Okavango delta region of northern Botswana until about 2,000 yr BP (Fig. 1a), Lake palaeo-Makgadikgadi³. The lake began to form when uplifting along the north-east trending Linyanti and Chobe faults severed the connection between the upper and middle Zambezi River sometime in the Pleistocene (315,000–460,000 yr BP), and diverted the flow of three large rivers, the Cuando, upper Zambezi and Kafue, into an inland drainage basin. This became Lake palaeo-Makgadikgadi¹³. Radio-carbon dating suggests a minimum age of 52,000 yr BP for the lake, and the area covered by lacustrine land forms in northern Botswana amounts to 60,000 km², an area larger than Switzerland.

We suggest that the riverine serranochromine species flock could have arisen in this palaeolake. Climate fluctuations throughout the Pleistocene led to alternating high and low stands, the duration and extent of which are still somewhat uncertain. It seems possible that the lake was largely dry during the last glacial maximum ~18,000 yr BP, when the deep rift Lakes Malawi and Tanganyika experienced 500–600 m lowering of their water levels. At its subsequent Holocene maximum, Lake palaeo-Makgadikgadi was >50 m deep and its potential components (areas of fluviolacustrine surface) amount to a vast 120,000 km² (ref. 14). During the course of the Holocene, however, the lake broke through the fault scarp to the northeast and began to empty into the middle Zambezi, eventually causing the headwaters of the Cuando, upper Zambezi and Kafue to become recaptured by the middle Zambezi. By 2,000 yr BP, the lake had become the seasonal lagoons of the Okavango delta, and further south, the Makgadikgadi salt pans.

To test the hypothesis that the riverine species flock arose in the lost palaeolake against the possibility that this region holds high

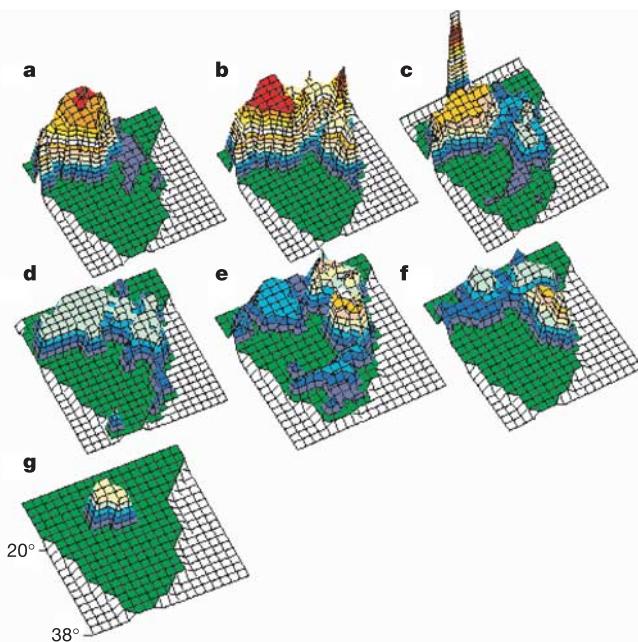


Figure 2 Geographic distribution of species richness in southern African fish groups. x and y axes represent grid squares overlaid on a map of southern Africa, in which occurrence of each species was marked for presence or absence (50% majority rule). The height shows the percentage species of that group found per grid square; gradations are 5% and range from green (0%) dark blue (5–10%) to white (40–45%) to dark red (80–85%). **a**, Southern radiation ($n = 16$ species that are included in our phylogenetic analysis and published distribution maps). **b**, Characidae ($n = 6$). **c**, *Barbus*, the most species-rich African fish group ($n = 43$). **d**, *Oreochromis*, the other main cichlid lineage in southern Africa ($n = 8$). **e**, **f**, Two characteristically riverine fish groups, *Labeo* (**e**, $n = 12$) and *Chiloglanis* (**f**, $n = 8$). A single peak of species richness in the Lake palaeo-Makgadikgadi region (**g**) can be seen only for the haplochromines; *Barbus* show a latitudinal gradient which increases into the Congo, represented by a single bar.

riverine fish diversity because of its biogeography and environmental characteristics, we mapped species richness of other fish groups, including three that, like the serranochromines, inhabit river, swamp and lake habitats (the family Characidae, the cyprinid genus *Barbus* and the cichlid genus *Oreochromis*, Fig. 2b–d) and two characteristically riverine groups (the cyprinid genus *Labeo* and the catfish genus *Chiloglanis*, Fig. 2e and f). No other taxon showed a single centre of species richness in the Okavango delta and upper/middle Zambezi, although many rivers now exist there.

Despite the lack of detailed distribution data within the Congo, it is clear that the genus *Barbus* (the most species-rich of all African riverine fish groups) does not peak in the Okavango/Zambezi, but reveals a latitudinal gradient of species richness, in which the percentage of African *Barbus* species increases northwards towards the Equator, from 9% in the Okavango and middle/upper Zambezi to 20% in the upper/middle Congo and even higher in the lower Congo. A similar pattern can be seen in the Characidae. In contrast, serranochromine richness decreases northwards towards the Equator: 43% are in the Okavango and middle/upper Zambezi area, 28% in the upper Congo, 20% in the upper Kasai (Congo system) and none in the lower Congo. Distribution patterns in all of these unrelated fish groups imply connections in the recent past between the Limpopo, middle Zambezi, Okavango, Cunene and upper Congo—the river systems that harbour the remnants of the Lake palaeo-Makgadikgadi cichlid radiation. However, only the species-richness landscape of the serranochromines implies dispersal from a single centre of diversification, the position of which coincides with that of Lake palaeo-Makgadikgadi.

To test the prediction that rapid diversification was driven by the ecological opportunity afforded by new niches associated with the formation of a large lake, we compared ecomorphological variation present in the serranochromine radiation with that in other riverine haplochromines and the Lake Victoria and Lake Malawi radiations. We measured ten linear morphometric distances that reflect ecologically relevant shape variation¹⁵ from between one and ten mature individuals from each species and also from representatives of all major trophic types of Lake Victoria and Lake Malawi cichlids. Principal component analysis was used to extract major axes of variation in the southern radiation, and shows that morphological diversity in the Lake palaeo-Makgadikgadi radiation is directly comparable to that in the haplochromine cichlid radiations of Lake Victoria and Lake Malawi, and is much larger than the variation in East African riverine haplochromines (Fig. 3a).

Ecological feeding types of all three lakes occupy similar positions in morphospace (Fig. 3b). For example, ambush piscivores from Lakes palaeo-Makgadikgadi, Victoria and Malawi all have relatively long heads and long, narrow jaws. Conversely, snail-crushing species have wider, deeper heads, which help to accommodate the hypertrophied pharyngeal jaw bones and muscles required for snail crushing. Absent from the Lake palaeo-Makgadikgadi morphospace are ecotypes that occupy niches now absent from the riverine habitats of the Lake palaeo-Makgadikgadi region: fish with relatively wide heads, shallow bodies and large eyes (represented by the pelagic zooplanktivores in Lake Malawi and Lake Victoria), the most shallow-bodied, long-headed fish (pelagic piscivores of Lake Malawi) and fish with short, wide heads and jaws, which tend to be epilithos-feeding rocky shore dwellers. This is consistent with the hypothesis that these ecological types might not have persisted when their habitats disappeared once the lake dried up.

In Fig. 3, we show the putative ancestors of each flock: the East African riverine haplochromines, some of which must have seeded Lake Malawi and Lake Victoria, and the basal members of the Lake palaeo-Makgadikgadi radiation, including those that possess haplotypes from more than one southern radiation clade (as these species might have originated before the recent radiation). Importantly, the Lake palaeo-Makgadikgadi flock differs from the Lake Malawi and Lake Victoria radiations in historical contingency

because its founders were likely to have been piscivores, rather than fish of a more generalist morphology that would feed on invertebrates and fish fry. Ecological types seem to have evolved in parallel directions along the axes of variation from different ancestral starting points. The ecologically relevant shape variation that accompanied the radiation in Lake palaeo-Makgadikgadi is comparable in magnitude to that of the classic examples of cichlid adaptive radiations⁴ (see Supplementary Fig. S1).

The genetic divergence that we observe between the most divergent mtDNA haplotypes within the southern radiation is comparable to that between the oldest lineages of Lake Malawi cichlids, estimated at a minimum of 1–2 Myr (ref. 16) but possibly up to 5 Myr old. Hence, these mutations cannot have accumulated in the ~50,000–400,000 yr since Lake palaeo-Makgadikgadi formed, but must have existed in the ancestral population. In order to retain the deep polymorphism observed (for example, across *S. robustus* and *S. macrocephalus*), this population would have had to maintain a substantial size, not only throughout the Lake palaeo-Makgadikgadi colonization and diversification period, and while the lake fluctuated in size, but throughout the much longer time between the occurrence of the deepest split in the southern

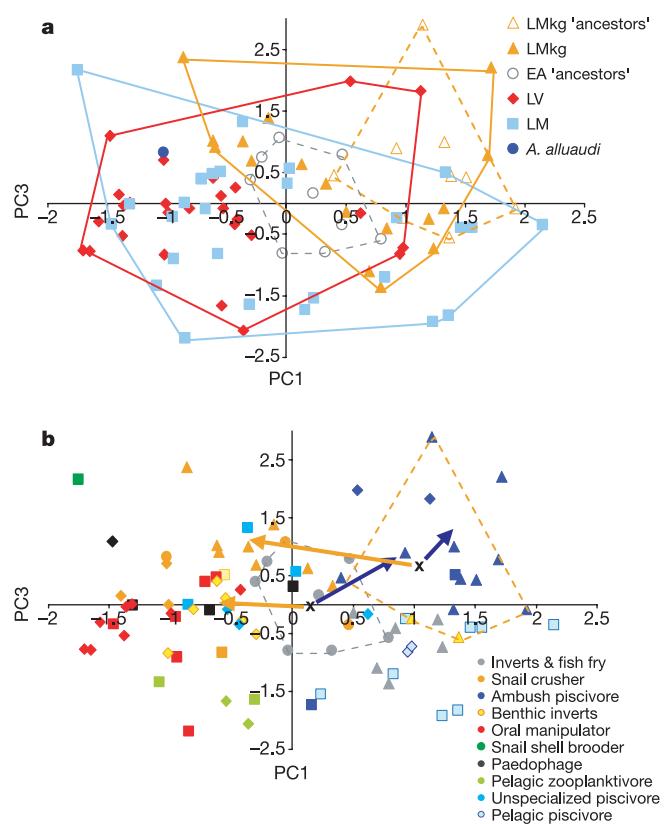


Figure 3 The area in morphospace occupied by the cichlid radiations. **a**, Most differentiation between the river and lake groups is along principal components (PC)1 and 3. PC1 increases with decreasing interorbital width, increasing head length and lower jaw length-to-width ratio. PC3 increases with an increase in cheek depth. The genetically diverse ancestral taxa (east African (EA) rivers and species of the southern radiation that contain the oldest haplotypes) occupy a smaller volume in morphospace than the genetically similar taxa that only emerged in the recent radiations. Species from Lake palaeo-Makgadikgadi (LMkg) occupy a volume comparable to species from Lake Victoria (LV) and Lake Malawi (LM). **b**, Colours represent trophic groups; symbols represent lakes (EA 'ancestors', circles; LMkg, triangles; LV, diamonds; LM, squares). Fish of similar feeding types from different lakes have diverged from their ancestors in similar directions, as expected if the radiation was driven by divergent ecological adaptation (snail-crushers and ambush piscivores are shown with appropriately coloured arrows).

haplotype clade and the formation of Lake palaeo-Makgadikgadi. Alternatively, the lake (which connected many drainage systems¹⁷) could have provided an opportunity for secondary contact, such that allopatrically divergent lineages of *Serranochromis* could meet and hybridize at the onset of the radiation¹⁸. The products of the radiation could then have dispersed through the Late Pleistocene and Holocene drainage systems to generate the present geographical distribution patterns of haplotype diversity. *In situ* radiation from several divergent ancestral haplotypes, followed by dispersal out of the radiation site, offers an explanation for why some geographically distant and morphologically divergent species have more similar haplotypes than some conspecific individuals from the same locality.

We conclude that the southern African riverine cichlids represent a case of explosive adaptive radiation into a functionally diverse species flock that emerged rapidly in a short, geologically transient window of opportunity. During the unstable history of Lake palaeo-Makgadikgadi and its drainage systems¹⁷, the members of this flock spread through the southern African rivers, where a substantial number of them have persisted and are now the only remnants of the radiation after the lake dried up ~2,000 yr BP. The origins of the functional diversity we now observe may have needed the ecological opportunities provided by a large lake, but the south African rivers seem to have maintained a significant portion of this diversity subsequently. Therefore, this appears to be an example of how a localized evolutionary process—an adaptive radiation in a geologically transient environment—can have a profound and lasting effect on the ecological and genetic diversity of a continental fauna. □

Methods

Taxon sampling

Sample taxa and site data can be found in Supplementary Table S1. We collected 17 out of 26 described serranochromines, and seven as yet undescribed serranochromine species from five river systems (Fig. 1a), as well as 4 out of the 8 *Thoracochromis* species of the Congo and Cunene, collecting the same species from more than one of these major river systems where they occur in multiple systems. We also collected the only two other haplochromine cichlids of southern Africa (*Pseudocrenilabrus philander* and *Schwertchromis machadoi*), as well as *Astatotilapia calliptera* from Lake Malawi. We used representatives of Lake Malawi and Lake Victoria haplochromines and other outgroups from GenBank (see Supplementary Table S1). Whole specimens of most of the southern African populations, except those from the Congo River, are held in the collection of SAIAB (South African Institute of Aquatic Biodiversity).

DNA extraction, amplification and sequencing

DNA was extracted from fin clips or muscle tissue using a standard phenol-chloroform extraction procedure, and the entire control region was amplified using forward primer HapThr-2 + 4 (5'-CCTACTCCAAAGCTAGGATC-3') and reverse primer Fish12s, (5'-TGGGAGACTTCATGTGAAG-3'). Polymerase chain reaction (PCR) products were cleaned using exonuclease and shrimp alkaline phosphatase, and a combination of the amplification primers and two internal primers (forward primer Dloopint 5'-AGCCAC CATCAGTTGATT-3' and reverse primer HapDloop 5'-GGTTGCAG AGTCTTAGAG-3') were used in cycle sequencing. This was carried out using DTCS quickstart (Beckman Coulter) according to the manufacturer's instructions, adding 1 M betaine to the sequencing reaction. Sequences were resolved using a Beckman capillary sequencer.

Sequence alignment and phylogenetic analysis

ClustalX¹⁹ was used to align the sequences (887 bp), with subsequent adjustments made by eye. We have submitted the alignment to GenBank (AY913844-AY913942). We used Modeltest²⁰ to estimate the likelihood parameters for our data set (general time reversible (GTR) substitution model with among site rate heterogeneity following a gamma plus invariant sites distribution, GTR + I + Γ, shape parameter 0.6799) and PAUP* (v4.b10, ref. 21) to build a neighbour-joining tree with 1,000 bootstrap pseudoreplicates. We obtained a tree and posterior probabilities for nodes with MrBayes²² using 2,000,000 Markov chain Monte Carlo generations. We plotted likelihood values against tree number to show that discarding the first 10% of trees ensured stationarity had been reached. A maximum likelihood tree was constructed using PHYML²³ with parameters from Modeltest; 100 pseudoreplicates gave bootstrap values for nodes.

Morphometric analysis

Standard length (SL), body depth (BD), head length (HL), snout length (SnL), pre-orbital depth (PoD), eye length (EyL), cheek depth (CD), inter-orbital width (IoW), lower jaw length (LJL) and lower jaw width (LJW) were measured from a total of 99 serranochromine specimens, comprising 23 species from 32 populations (mean of three fish per population), and also from 16 specimens (6 species) of East and North African riverine haplochromines, 6 specimens from one Lake Victoria species, and 15 species of

Lake Malawi cichlids. Data for 26 other species from Lake Victoria, 13 from Lake Malawi, and one from East African rivers were taken from other sources²⁴⁻²⁹. BD and HL were standardized with SL. SnL, PoD, EyL, and IoW were standardized with HL. The LJL/LJW ratio was used to enable the use of published data for Lake Victoria and Lake Malawi cichlids. HL/SL and LJL/LJW did not show any allometric trend. To remove allometry from the variation in the other distances, we regressed them against SL and used the residuals for further analysis. Because of significant deviation from normality, we log-transformed residual PoD/HL and LJL/LJW. Conservatively, missing data were replaced with means. Principal components 1 and 3 were plotted in Fig. 3.

Biogeographical analysis

We used published distribution data³⁰ for the species of southern Africa and overlaid grid rectangles (150 × 110 km), in which we recorded the presence or absence of each species using a 50% presence majority rule. We calculated the percentage species of each group that occurs per square. We plotted these values against longitude and latitude, assigning marine grids a minus number for clarity. Detailed published distribution data ends at the boundary between the upper Zambezi and the upper Congo, so to elucidate the species richness trends for serranochromines and barbs northwards of the Lake palaeo-Makgadikgadi region, we used records of each species from FishBase (<http://www.fishbase.org>) to calculate the percentage of species that occur in the Congo River, represented by a single bar for the barbs and hidden behind the richness peak for the serranochromines in Fig. 2.

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Epistasis and balanced polymorphism influencing complex trait variation

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Complex traits such as human disease, growth rate, or crop yield are polygenic, or determined by the contributions from numerous genes in a quantitative manner. Although progress has been made in identifying major quantitative trait loci (QTL), experimental constraints have limited our knowledge of small-effect QTL, which may be responsible for a large proportion of trait variation^{1–3}. Here, we identified and dissected a one-centimorgan chromosome interval in *Arabidopsis thaliana* without regard to its effect on growth rate, and examined the signature of historical sequence polymorphism among *Arabidopsis* accessions. We found that the interval contained two growth rate QTL within 210 kilobases. Both QTL showed epistasis; that is, their phenotypic effects depended on the genetic background. This amount of complexity in such a small area suggests a highly polygenic architecture of quantitative variation, much more than previously documented⁴. One QTL was limited to a single gene. The gene in question displayed a nucleotide signature indicative of balancing selection, and its phenotypic effects are reversed depending on genetic background. If this region typifies many complex trait loci, then non-neutral epistatic polymorphism may be an important contributor to genetic variation in complex traits.

Because of experimental constraints, our knowledge of complex trait variation is limited to QTL of relatively large effect. Conventional QTL mapping typically uses markers with an average spacing of several centimorgans, and uses sample sizes insufficient to identify minor QTL. Thus, although many minor QTL contribute to complex trait variation^{3,5}, we have little information on the magnitude, frequency, epistasis and fitness consequences of the genes that matter in quantitative genetics. Genes of large effect may not be representative of the majority of segregating polymorphisms, hence studies are needed that avoid the ascertainment bias that results from a focus on large-effect QTL. For this reason, we fine-mapped phenotypic effects segregating within a one-centimorgan chromosome interval for which lines with mapped recombination breakpoints were available, and examined the sequence signature of historical polymorphism.

Recently, we investigated 58 near-isogenic *Arabidopsis* lines that had recombined in a 210-kb interval on the upper arm of

chromosome 5 (refs 6, 7). These lines were developed from a cross between the *Arabidopsis* accession Col-0 and a Col-0/Ler-0 recombinant inbred line⁸, CL5. We tested for potential allocation costs associated with variation in herbivore resistance, and found no evidence for a growth rate QTL in the central portion of the interval. In the centre of this interval are located *MAM* genes, which encode methylthioalkylmalate synthases, enzymes involved in the synthesis of plant defence compounds^{6,7}. However, two regions several dozens of kilobases upstream and downstream of the *MAM* cluster displayed statistical support for growth rate QTL (Fig. 1). We investigated whether these regions indeed influence *Arabidopsis* biomass accumulation with a variety of advanced crosses that harboured recombination breakpoints in the vicinity of both presumptive QTL. We selected near-isogenic parental lines such that their F₂ progeny segregated in a very small interval but not in the flanking regions. We were thus able to investigate the effect of the segregating interval on growth rate.

We fine-mapped the downstream QTL in two series of crosses (Fig. 2 and Table 1). We examined a 30-kb region with progeny from five crosses (grLC1 to grLC5), each with Ler-0-like sequence 5' and Col-0 sequence 3' flanking the segregating interval. We also investigated a 13-kb subset of this region with four crosses (grCL6 to grCL9) in the opposite direction. In addition, we generated a control cross, grCL(6–9), in which the entire 13-kb sequence portion was segregating. Both series of crosses revealed the presence of a QTL. In the grLC crosses, the QTL mapped to a 6.7-kb interval segregating in cross grLC2 ($N = 273$; degrees of freedom (d.f.) = 1, 10; $F = 6.14$; $P < 0.05$). This interval contains two complete genes (At5g23160 and At5g23170) and 5' partial sequence from a third gene (At5g23180). In the grCL crosses, the QTL mapped to a 1.3-kb interval segregating in the grCL7 cross ($N = 391$; d.f. = 1, 9; $F = 6.24$; $P < 0.05$), which is contained within the 6.7-kb grLC2 interval. The grCL7 interval contains partial sequence of only one gene (At5g23170), indicating that this portion of the *Arabidopsis* genome constitutes the growth rate QTL. Only one-third of the 6.7-kb grLC2 interval is not included in any of the reciprocal grCL intervals. grCL6 and grCL8 bear no indication of a growth rate QTL. The remaining sequence between LJ01C and CJ10L (Fig. 2) harbours only three polymorphisms: a synonymous nucleotide substitution within the coding region of At5g23170, a 1-base pair

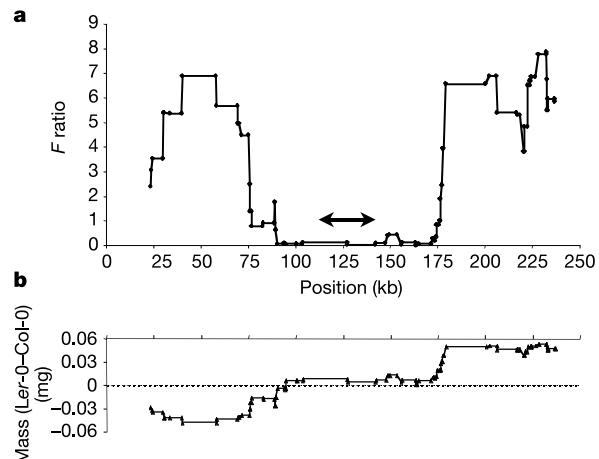


Figure 1 Growth rate differences between Col-0 and Ler-0 alleles in a 210-kb region of the *Arabidopsis* genome. Position (in kb) is based on an alignment of complete sequences in this region from both accessions. **a**, Statistical significance for the presence of growth rate QTL in near-isogenic lines^{6,7} (see also Supplementary Data and Supplementary Methods). The location of the *MAM* cluster is indicated by an arrow. **b**, Dry weight differences between Col-0 and Ler-0 genotypes, based on least square means. Note that in this experiment genotype performance is also influenced by uncharacterized growth rate QTL outside the 210-kb interval.