Morphological stasis in an ongoing gastropod radiation from Lake Malawi

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Evolutionary processes leading to adaptive radiation regularly occur too fast to be accurately recorded in the fossil record but too slowly to be readily observed in living biota. The study of evolutionary radiations is thereby confronted with an epistemological gap between the timescales and approaches used by neontologists and paleontologists. Here we report on an ongoing radiation of extant Bellamya species (n = 4) from the African Rift Lake Malawi that provides an unusual opportunity to bridge this gap. The substantial molecular differentiation in this monophyletic Bellamya clade has arisen since Late Pleistocene megadroughts in the Malawi Basin caused by climate change. Morphological time-series analysis of a high-resolution, radiocarbon-dated sequence of 22 faunas spanning the Holocene documents stasis up to the middle Holocene in all traits studied (shell height, number of whorls, and two variables obtained from geometric morphometrics). Between deposition of the last fossil fauna (~5 ka) and the present day, a drastic increase in morphological disparity was observed (3.7–5.8 times) associated with an increase in species diversity. Comparison of the rates of morphological evolution obtained from the paleontological time-series with phylogenetic rates indicates that the divergence in two traits could be reconstructed with the slow rates documented in the fossils, that one trait required a rate reduction (stabilizing selection), and the other faster rates (divergent selection). The combined paleontological and comparative approach taken here allows recognition that morphological stasis can be the dominant evolutionary pattern within species lineages, even in very young and radiating clades.

Evolutionary radiations are potentially responsible for much of the ecological and phenotypic diversity on earth (1, 2). Beyond rapid speciation through lineage splitting, radiations are often characterized by exceptional diversification into a variety of ecological niches, with divergence, at least in traits under selection, regularly occurring very rapidly (e.g., refs. 3 and 4). However, the controls on diversification may be complex and include historical contingencies, as well as ecological, genetic, and developmental factors (5, 6). One major challenge is that processes leading to evolutionary radiations occur regularly too fast to be accurately recorded in the fossil record where temporal resolutions finer than ~10^4 y are unusual, but perhaps still too crude to see speciation and radiation unfold. On the other hand, speciation generally occurs too slowly to be readily observed in living biota, resulting in an “epistemological gap” between the timescales and approaches used by neontologists and those that paleontologists adopt for the study of organismal evolution (7, 8). Moreover, it can be difficult to assess the adaptive significance of phenotypic variation among fossil taxa (e.g., ref. 9), partly because ecological, behavioral, physiological, and other data are lacking. Neontological approaches are more diverse, but they are usually limited to studying the subset of radiating species that is still alive, and with that offer only a partial view of the involved evolutionary processes, as adaptive radiations are unlikely to occur in the absence of extinction (4). Most recent studies have adopted phylogenetic and comparative approaches (e.g., refs. 10 and 11), whereas the evaluation of evolutionary patterns using time series remains underrepresented in the current literature (but see e.g., refs. 12 and 13).

Here we report on a unique and unusual study system of a young, ongoing radiation in Bellamya gastropods in the African Rift Lake Malawi (14). In addition to its extant diversity, this Bellamya clade left abundant, well-preserved fossil shells in a high-resolution, radiocarbon-dated sediment record that spans much of the Holocene (15). The study system presents a particularly promising opportunity to study radiations because some of the life-history traits that are thought to have led to the divergence of the modern descendants affect shell morphology and, hence, can be traced in fossils.

Below we analyze changes in morphological traits [shell size, the number of whorls (#whorls) and geometric shell shape] over a sequence of 22 Bellamya assemblages spanning the Holocene (i.e., 21 fossil faunas and the modern one). Upon fitting evolutionary models (stasis, random walk, and directional change), stasis received high support in all traits studied for the early and middle Holocene fossil assemblages. These results suggest that evolutionary trajectories within established lineages can be characterized best as fluctuations around a steady long-term mean, even in recently and rapidly radiating clades. Morphological divergences map well to molecular differentiation observed in the extant Bellamya, enabling us to integrate paleontological, phylogenetic, and natural history information to interpret the ecological importance of the patterns observed in this ongoing divergence.

Study System

Four taxonomically valid Bellamya species currently occur in Lake Malawi (16, 17): three inhabit shallow water environments (Bellamya capillata, Bellamya jeffreysi, and Bellamya robertsoni), whereas the fourth (Bellamya ecclesi) is rare and occupies substrates below the euphotic zone (16, 18). Molecular analysis using three gene fragments [mitochondrial cytochrome oxidase subunit I (COI), the mitochondrial large subunit of rRNA and the nuclear large subunit of rRNA] indicates that all Bellamya from the Malawi Basin form a monophyletic clade endemic to the basin, and although the species are young, great and highly significant genetic differentiation (FST = 0.234) exists among the three shallow-water species (14). Exact tests of differentiation demonstrate significant differences between B. capillata and B. jeffreysi (P = 0.0486), between B. capillata and B. robertsoni (P = 0.0003), but not between B. robertsoni and B. jeffreysi (P = 0.1105). (The restricted number of specimens from the deep-water species B. ecclesi prevented its inclusion in statistical tests.) Mismatch analyses using COI data
reveal a drastic spatial expansion of the entire Malawi group since 97 ka [95% confidence interval (CI): 0–215 ka], and taxa with stable mismatch distributions (B. capillata and B. robertsoni) also show remarkable demographic expansions since 200 ka (95% CI: 105–295 ka) and 102 ka (95% CI: 12–192 ka), respectively (14).

These estimates correspond well to two major megadrought phases ~135 and 75 ka ago, during which Lake Malawi’s water level (currently 706 m deep) dropped 600 m and 350 m below the current level, respectively (19). Over considerable periods during this 60,000-y interval Lake Malawi was a ~125-m-deep saline, alkaline, well-mixed lake (19). Since 55 ka, the Malawi Basin consistently experienced much wetter conditions and lake levels were high and similar to those at present, except for a drop of ~100 m below the current level at the last glacial maximum (19). These high lake levels have promoted organismal evolution in mollusks and cichlid fishes (20, 21), and the demographic history of Bellamya as inferred from the molecular analyses summarized above is consistent with this environmental history.

Results and Discussion

Principal component analysis (PCA) of morphological traits (shell height, #whorls, and geometric shell shape from semilandmark morphometrics) documents the morphospace occupation of the fossil and modern material (Fig. 1 and Fig. S1). We used model-based clustering to explore how many evolutionary lineages are present in our dataset (Fig. S2). Applying such clustering to the extant Bellamya directly in the 2D morphospace resulted in the highest support values for three clusters (=morpho taxa; ΔBIC ≥ 6.1 over other models; BIC, Bayesian Information Criterion). The major separation in the modern taxa is observed between the deep-water species B. ecclesi and the shallow-water taxa, and a secondary separation within this latter group between B. robertsoni and the group of B. capillata/B. jeffreysi. If models are forced to assign four clusters, then all four taxonomically valid species are recognized (ΔBIC ≥ 17.4 over other four cluster models, but ΔBIC = 11.7 below the best solution with three clusters) (Fig. 1B and Figs. S2 and S3).

The best-supported model was the same in both cases and has trait variances and covariances that are shared across species, which is realistic for close relatives (SI Text). Thus, model-based clustering without a priori assumptions (i.e., independent of the earlier identification of the specimens using the criteria established by previous researchers (16, 17)) recovered meaningful, morphologically delimited groups that correspond to taxonomically valid, extant Bellamya species from Lake Malawi. The abovementioned molecular analyses (14) used the same morphological identification system and demonstrated highly significant genetic differentiation between the morphospecies, except for the pair of B. robertsoni and B. jeffreysi. However, B. robertsoni and B. jeffreysi represent the only species pair with substantial overlap in morphospace occupation (Fig. 1B). This overlap may indicate that the divergence within this species pair is young compared with that in others (and perhaps incomplete).

Alternatively, the morphospace overlap of this species pair may explain the nonsignificant result of the molecular test of differentiation, as misidentifications would diminish apparent genetic separation. The significant genetic differentiation in other species pairs may indicate their reproductive isolation. Kat (22) reported informally that B. capillata and B. jeffreysi hybridize freely in the south of the lake but that the hybrids are sterile. However, haplotype networks including specimens of all Bellamya morphospecies from Lake Malawi indicate that speciation processes in this young radiation are still ongoing (14). More research is required, but the here-documented morphological differentiation and the concordant and highly significant molecular differentiation between morphospecies suggest that multiple Bellamya species currently exist in Lake Malawi, and that these correspond to the valid species in classic taxonomy.

Applying model-based clustering to each fossil assemblage resulted in the highest support values for a single cluster in most cases. For a few assemblages, multiple clusters were preferred, but these results represented biologically unlikely scenarios (SI Text, and Figs. S1, S2, and S4). In no case were bimodal distributions observed (Fig. S1), nor was any reasonable and consistent separation detectable by eye. If all fossils are lumped in one sample and model-based clustering is applied, a single component is highly supported (by 8 of the 10 models applied; ΔBIC ≥ 3.7 over suggestions with multiple components (Fig. L4). In addition, the
magnitude of morphological variation within the fossil samples is comparable to that seen in the living species (Fig. S5). These findings confirm the earlier qualitative result that all Holocene Bellamya fossils from Lake Malawi belong to a single morphospecies (23).

The existence of a single fossil Bellamya lineage in the Chipalamawamba Beds is somewhat surprising because these assemblages record three dispersal events (15) from a source region that is currently inhabited by three shallow-water Bellamya species, which regularly occur in sympathy (16, 22) (Table S1). No taphonomic biases exist that could explain the absence of multiple shallow-water Bellamya species in the fossil assemblages if they were existing locally at the time of deposition, and except for Bellamya, all other currently diversified mollusk genera show diversities in the Chipalamawamba Beds similar to modern levels (15) (Table S2). These observations are based on the identification of more than 40,000 fossil specimens from diverse shallow-water habitats and exclude inadequate sampling as explanation (11, 35, 36), because this habitat did not exist in the Malawi Basin during the Late Pleistocene megadroughts (19), nor perhaps at the last glacial maximum, it is unlikely that lineage splitting and the divergence toward B. ecclesi started considerably earlier than that in the shallow-water community.

The fossil specimens occupy a rather more restricted region of morphospace than the modern ones (Fig. 1 and Fig. S1). Although the fossil material is intermediate to the three extant shallow-water taxa in some morphological features, it also differs substantially in others: part of the morphospace occupied by the fossils is devoid of the shallow-water species (18). Because this habitat did not exist in the Malawi Basin during the Late Pleistocene megadroughts (19), nor perhaps at the last glacial maximum, it is unlikely that lineage splitting and the divergence toward B. ecclesi started considerably earlier than that in the shallow-water community.

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The large increase in disparity that we document here does not imply that selection pressures were always present, that they would have acted consistently in the same direction (the morphospace expansion is not unidirectional), or with equal strength for all traits studied. Nevertheless, considerable and rapid morphological differentiation occurred between the modern species, even though our fossil lineage documents ~5,000 y of morphological stasis for all traits studied.

Stasis does not imply that traits are invariant; some fluctuations around the long-term mean are expected (11, 35, 36), and indeed are observed in the traits studied. Under stasis, these fluctuations do not accumulate, and hence the bounds of stasis are independent of the time elapsed (95% probability interval in Fig. 2). Can the fossil stasis be reconciled with the divergence observed among the extant species? This question can be addressed by comparing the trait means of the extant species to the bounds of stasis, or by computing model support values for scenarios in which the extant species are added to the fossil series as end members (Fig. 2). Among the extant species, the trait values of only B. capillata fall close enough to the fluctuations observed in the fossils for the stasis model to remain best supported. All other species have one or more traits that lie well outside the confidence limits of the stasis observed in the fossils (Fig. 2) and are accordingly better accounted for by models, such as the random walk, that accumulate evolutionary change over time. Hence, the stasis documented for the fossil Bellamya sequence is not adequate to account for the larger divergences between the extant species, implying that morphological change departed from the normal dynamics of stasis to produce intervals of elevated change.

With data on the living species and a high-resolution fossil sequence, it becomes possible to compare the pace of trait evolution within and between evolutionary lineages. The exact phylogenetic relationship between the extant species in the Bellamya radiation from Lake Malawi is unresolved, so we used a large number of randomly resolved, time-scaled trees of the four modern species to calculate rates of morphological trait evolution according to the Brownian motion model (Materials and Methods). These phylogenetic rates were compared with paleontological rates obtained by fitting the equivalent model to the fossil time series. We report results across a wide range of divergence times (Fig. S6) but focus on a relatively literal reading of the fossil record (i.e., the scenario in which diversification of the lineages occurs after the last fossil sample at ~5.5 ka) (Fig. 3). For two traits (#whorls and morphoPC1) the paleontological rates from the fossil time series correspond well to the phylogenetic rates obtained when the depth of the root node was set to coincide with the last fossil sample (Fig. 3). Hence, the paleontological rates suffice to explain the divergence in these traits toward the modern taxa over 5,500 y, assuming that Brownian motion applied throughout. However, the two other characters, shell height and morphoPC2, behave differently. For morphoPC2, the paleontological rates were faster than phylogenetic rates over ~5.5 ka (negative log-ratio in Fig. 3), which remains true even if the depth of the root node is only 1.5 ka (Fig. S6). The difference in rates suggests stronger stabilizing selection for this trait when
lineages diversified than in the fossil lineage over 5,000 y, where fluctuations around the long-term mean were already limited (Fig. 2). Hence, this result testifies to the commonness of stabilizing selection in nature (24). For shell height, phylogenetic rates are faster than paleontological ones if the root node dates to 5.5 ka (Fig. 3). Upon increasing the depth of the root node, the rates become more similar, then equal each other for root node depths of ∼50 ka, after which they diverge again (Fig. S6). Two explanations are conceivable. The first is that divergence in shell height started considerably earlier than the deposition of the fossil material, but that this divergence remains unsampled in the fossil sequence. The second explanation is that divergence in shell height started more recently and that the evolutionary rates associated with it are, consistent with divergent selection, faster than the paleontological ones we documented. Regardless of the actual timing of divergence between these species, our results indicate that different traits have evolved differently during this young radiation (see also ref. 13).

External evidence for divergent selection is provided by the fact that the young Bellamya species from Lake Malawi occupy extreme positions for several traits among African Bellamya (18 species) (17). The southeastern arm of Lake Malawi is home to the most strongly calcified Bellamya species alive today [B. jeffreysi; adult specimens with a shell height of ∼38 mm weigh on average 5.8 g; that is, twice as much as any other African Bellamya of equal size (except for B. robertsoni)], and to the most weakly calcified one [B. ecclesi; adult specimens with a height of ∼52 mm weigh on average 3.4 g; shells of ∼42 mm are about 1.5 g, which is about 0.5 g less than equally sized B. phthinotropis, the thin-walled deep-water species from Lake Victoria; (16)]. Similarly, extant B. capillata from Lake Malawi are among the smallest African Bellamya together with B. costulata and B. constricta from Lake Victoria; they become mature at ∼17-mm and adults average ∼20-mm shell height (16, 17). B. ecclesi, with an average height of 54 mm, is by far the largest one (17). Selection on shell height in the Bellamya from Lake Malawi is particularly likely because females breed their young in a brood pouch and the size and volume of the brood pouch is directly and positively correlated with the size of the shell and the inflation of the body whorl. Differences in adult size are moreover correlated with marked differences in life history traits [e.g., the size at which juveniles are released in the environment (<6 mm in B. capillata, >8 mm in B. robertsoni and B. jeffreysi, and ∼14 mm in B. ecclesi) (16)].

The Bellamya from Lake Malawi represent an ongoing radiation (14), and may be undergoing “explosive” diversification, which has been observed regularly early in the process of radiation (37). The term hints to several lineage splits that are closely spaced in time—thousands to a few 10 thousands of generations. Yet, despite this active evolutionary milieu, all traits examined experienced morphological stasis within a densely sampled fossil lineage. This pattern is consistent with the long questioned (38, 39) suggestion of punctuated equilibrium proponents that morphological

![Fig. 2. Time-series plots of fossil Bellamya for the four univariate traits examined (shell height, number of whorls, morphoPC1, and morphoPC2) and the trait values of the extant species (symbols correspond to those in Fig. 1). Variation in the traits was standardized to within-sample SDs, three evolutionary models were fitted to these data [stasis (St), unbiased random walk (URW), and directional change (DIR)] and the model with the best fit is reported; AIC weights in subscript indicate model support. Values for the extant taxa are obtained by considering each of these taxa independently as a direct end member of the time series. Black horizontal lines indicate the trait means of the fossil specimens and shaded areas represent 95% probability intervals. Error bars represent 1 SE (±1 se).](image_url)
change occurs predominantly in association with events of branching speciation (35, 40), even though we cannot currently disentangle whether the elevated morphological changes occurred agagnostically before or after lineage splitting, or whether they coincided directly with cladogenesis. Either way, stasis appears to be a common pattern of trait evolution within species lineages, even during an ongoing and rapid radiation.

Materials and Methods

The paleontological material comes from abundant shell beds that were deposited in a wide variety of shallow-water habitats at the southern end of Lake Malawi during three high lake-level phases in the early to middle Holocene (15) (SI Text). Radiocarbon dating indicates minimal time averaging; a single bed spans a few decades maximum (15). The modern material was predominantly collected from a shoreline stretch of 50 km along the southwestern shores of the southeastern arm of Lake Malawi to sample the same diversity of shallow-water habitats in close proximity to the geo-graphically confined fossil sites (SI Text). A total of 912 fossil Bellamya specimens from a well-dated, high-resolution sequence of 21 fossil assemblages and 383 modern shells of the four extant species were digitized in apertural view conforming to the methods in ref. 41 for morphometric analyses. We used a semilandmark approach with 12 landmarks and four open curves of equally spaced semilandmarks (60, 20, 20, and 15, respectively) that were anchored by starting and ending landmarks (41). Landmarks and semilandmarks were digitized in TpsDig 2.16 (42), then subjected to Procrustes superimposition in CoordGen6h (43), after which the semilandmarks were slid along their respective curve in SemiLand6, using the minimum Procrustes distance criterion (43). Additionally, we included data on shell height and the #whorls, because these characters are considered taxonomically important (16). These measurements were log,

\[ t \] transformed, combined with the Procrustes superimposition coordinates and imported in R 2.15.0 (44) for further analyses.

PCA was applied to the final morphological dataset, after which we calculated variable loadings (shell height, #whorls, landmarks, and semilandmarks) and plotted their contribution to the morphospace occupation (ststs 2.15.0; vegan 2.0–4) (44, 45). A scree plot with the broken-stick expectation suggested that the first three PC axes contained significant information, but no clear separation in morphospace was observed on PC3, and only PCs 1 and 2 were used for subsequent analyses.

Model-based clustering was performed on the PC1 vs. PC2 coordinates using normal mixture models [mclust 3.5, (46); see ref. 47 for model descriptions]. The method allows identifying morphological clusters without a priori species designations and was applied to each of the 22 assemblages, and to the aggregated dataset of all fossils. This approach requires assumptions about the degree to which covariance patterns are shared across groups. We used the 10 default parameterizations, which differ in the degree of commonality of the shape, volume, and orientation of trait variances and covariances. Support for different models was compared with a BIC. Statistically valid cluster solutions are not necessarily realistic, a few such instances with high BIC support are discarded here but are discussed in SI Text.

Morphological disparity was calculated for each fossil population and for the modern species using the sum and the product of the variances on PC1 and PC2 (48, 49).

We performed time-series analyses of morphological evolution on the 21 fossil samples, and subsequently with each of the modern taxa as end member of the time sequence (paleoTS 0.4–4) (50, 51). These methods are designed for univariate variables and we performed a separate PCA on the multivariate morphometric dataset (landmarks, semilandmarks) from which the two first PCs were extracted and analyzed similar to the univariate characters in our dataset (shell height, #whorls). We standardized the variation in traits to within-sample SD units to facilitate comparisons among traits. Subsequently we fit three evolutionary models (stasis, an unbiased random walk, and directional change) to each univariate variable (shell height, #whorls, morphoPC1, morphoPC2). Stasis was modeled as uncorrelated, Gaussian variation around a stable mean trait value (50, 52). A random walk is a simple model in which evolutionary increments are independent of each other and equally likely to be increases and decreases. The directional evolution model is similar to the random walk, except that increases are more probable than decreases, or vice versa (50). Model support was determined via an AICc with AIC weights of 0 and 1 representing no and absolute support among the candidate models, respectively. In-depth explorations of the models, the AICc calculation, and their implementation are provided in ref. 50.

The phylogeny of the extant species remains unresolved (one polynomy that may be hard), but only (2n-3)!!-15 possible bifurcating trees with n = 4 terminal taxa are conceivable. To allow calculating rates of evolution along each randomly resolved tree, we set the depth of the root node to 50 equally spaced times between 1.5 ka and 200 ka, each separated by 3,970 y. For each, we randomly resolved the phylogeny 1,000 times into bifurcating trees using the packages ape 3.0-4, phytools 0.2-1 and paleotree 1.4 (53–57). Branch lengths were randomly assigned with a 500-y minimum for the period between two subsequent nodes and, hence, speciation events. A Brownian motion model (package geiger 1.3–1) (58) was fit to calculate phylogenetic rates of morphological evolution for each of the four variables along the randomly resolved phylogenies. These rates were then compared with the paleontological rates obtained by fitting an unbiased random walk to the same variables over the fossil time series. We made comparisons for all root-node depths (Fig. S5) but highlight a literal reading of the fossil record in which the root node was coeval with the youngest fossil assemblage sampled (~5.5 ka) (Fig. 3). Such comparisons are possible because Brownian motion and random walks are closely related models, with equivalent parameters (31), that are commonly used as a rate metric in comparative methods (10, 59) and paleontology (31). The advantage of using this metric is that it can provide a rate estimate that incorporates information on all populations of a time-series or phylogeny, and that it is not constrained to document trait change from the pairwise comparison of populations, as traditional rate metrics do (see e.g., ref. 60). When traits are scaled by within-sample SDs and time is measured in generations (i.e., ~1 y for Bellamya), the Brownian motion/random walk rate metric is equal to the a rate metric proposed by Lynch (29).

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