

DISTINCT EVOLUTIONARY PATTERNS OF BRAIN AND BODY SIZE DURING ADAPTIVE RADIATION

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Morphological traits are often genetically and/or phenotypically correlated with each other and such covariation can have an important influence on the evolution of individual traits. The strong positive relationship between brain size and body size in vertebrates has attracted a lot of interest, and much debate has surrounded the study of the factors responsible for the allometric relationship between these two traits. Here, we use comparative analyses of the Tanganyikan cichlid adaptive radiation to investigate the patterns of evolution for brain size and body size separately. We found that body size exhibited recent bursts of rapid evolution, a pattern that is consistent with divergence linked to ecological specialization. Brain weight on the other hand, showed no bursts of divergence but rather evolved in a gradual manner. Our results thus show that even highly genetically correlated traits can present markedly different patterns of evolution, hence interpreting patterns of evolution of traits from correlations in extant taxa can be misleading. Furthermore, our results suggest, contrary to expectations from theory, that brain size does not play a key role during adaptive radiation.

KEY WORDS: Adaptive radiation, body size, brain size, coevolution, comparative analysis, phenotypic evolution, Tanganyikan cichlid.

Covariation of morphological traits reflects the influence of shared developmental pathways and mechanisms coordinating their development (Price and Langen 1992). As a consequence, development can bias evolution, because certain morphological changes may be more readily achieved than others (Lande 1979; Allen et al. 2008). Such covariance between traits could affect rates of evolutionary change, potentially limiting the independent evolution of one trait (Price and Langen 1992). For example, mechanistic or morphological constraints could make it unlikely that genetic variation in one direction will be gener-

ated by mutation. Allometric relationships between traits could act as mechanistic constraints limiting evolutionary divergence (Blows and Hoffmann 2005). However, covariation of two morphological traits may not necessarily involve parallel coevolution, as one trait could simply impose boundaries on the phenotypic space that the other correlated trait can occupy all the while allowing that trait to vary independently within the bounded morphospace.

Brain size and body size present an excellent example of two highly positively correlated traits across various taxonomic levels

(Striedter 2005). The reasons for how this strong relationship may have evolved, and why the allometric slope differs across taxonomic levels, has been the subject of great interest (e.g., Lande 1979; Riska and Atchley 1985; Pagel and Harvey 1989; Harvey and Krebs 1990; Price and Langen 1992). Several hypotheses have been proposed to explain the strong correlation between the two traits, such as a scaling relationship of body surface area to brain volume (Jerison 1973), metabolic constraints limiting brain size (Martin 1981), or strong genetic correlations between brain and body size resulting in brain size evolving simply through a correlated response to changes in body size (Lande 1979; Riska and Atchley 1985). Recently, however, brain size has been suggested to evolve in a more independent fashion, under less constraint from body size (Pagel and Harvey 1989; Harvey and Krebs 1990; Safi et al. 2005; Striedter 2005). The underlying assumption in these analyses is that any variance in brain size explained by ecological, behavioral, or life-history traits, after controlling for the effect of body size, reflects selection pressures acting specifically on cognitive abilities favoring an increase or decrease in relative brain size (Harvey and Krebs 1990; Safi et al. 2005). Hence, although highly correlated, brain and body size are expected to be responding to distinct selection pressures. Recent advances in comparative analyses enable detailed descriptions of patterns of evolution (e.g., Pagel 1999; Harmon et al. 2003; Freckleton and Harvey 2006), thus by comparing evolutionary patterns between traits it is possible to study the evolution of correlated traits and describe how the correlation between them may have originated.

Adaptive radiation provides an excellent framework within which to analyze patterns of brain and body size evolution as both traits are expected to be under strong selection leading to high levels of phenotypic divergence among species (Jerison 1973; Schluter 2000). Adaptive radiations are generally associated with high rates of divergence in body size (Schluter 2000; Kozak et al. 2005; Clabaut et al. 2007) whereas brain evolution is thought to be influenced by the biotic and abiotic environment of species. Indeed, brain size has been shown to be related to ecological factors, such as habitat (Safi and Dechmann 2005) and diet (Gonzalez-Voyer et al. 2009), social structure (Byrne and Bates 2007), and behavior such as parental care (Gittleman 1994; Gonzalez-Voyer et al. 2009). Larger relative brain size is generally associated with increased cognitive abilities, and recent comparative analyses have shown associations between relative brain size and survival (Sol et al. 2007), invasion success (Sol et al. 2002; Sol et al. 2008), and innovative behavior (Lefebvre et al. 2004). These results suggest that brain size could be a key trait, under strong selection, during adaptive radiations because of the potential advantages associated with larger brain size and selection acting on brains to adapt to recently colonized niches. For example, in caniform carnivorans relative brain size and vari-

ance in relative brain size increased through time, and this increase coincides with the period of rapid canid diversification during the Miocene (Finarelli and Flynn 2007). Further, selection for brains to adapt to different niches may involve both evolutionary increases and decreases in whole brain size (Safi et al. 2005).

Here, we analyzed patterns of evolution of brain and body size to test, first, whether high correlations between two traits necessarily arise through coevolution—defined here as when changes in one trait trigger a proportional, directional change in the other—or whether such correlations may conceal distinct patterns of evolution among traits. Second, we tested whether brain size exhibits the high rates of divergence expected for traits under strong selection during adaptive radiation. We combined recently developed phylogenetic comparative methods (Pagel 1999; Harmon et al. 2003; Freckleton and Harvey 2006) using Tanganyikan cichlid fish as our model. Recent comparative methods can detect departures from the basic Brownian motion model of evolution, where divergence is accumulated gradually with time in a stochastic manner, and incorporate alternative models of evolution. This is necessary because during adaptive radiations, speciation and phenotypic divergence may be closely linked if ecological specialization promotes genetic divergence between populations (Schluter 2000; Freckleton and Harvey 2006). Hence, traditional statistical comparative methods that assume constant rates of evolution may be severely compromised (Freckleton and Harvey 2006). Our analyses included body size, measured as standard length and body weight, and whole brain size, measured as brain weight. We were thus able to establish whether there were concordant patterns of evolution between both body size measures and, using these as our standard for phenotypic evolution, compare them with the evolutionary patterns for brain weight. Tanganyikan cichlids are an excellent model for the analysis of rates of phenotypic evolution as they are the most diverse phenotypically, morphologically, and behaviorally of the African cichlids and recent morphological analyses have demonstrated the adaptive nature of their radiation (Salzburger et al. 2005; Clabaut et al. 2007). For most of the species sampled, we had sexually mature individuals of both sexes, and because selection pressures influencing brain evolution can act differently on males and females (Gittleman 1994; Gonzalez-Voyer et al. 2009), we repeated the analyses for each sex separately to determine whether there were sex-specific patterns.

Methods

DATA

Standard length, body weight, and whole brain weight were collected from wild-caught, sexually mature individuals. Fish were anesthetized with benzocaine. Following anesthesia, fish were

measured (standard length [SL] = ± 1 mm), weighed (± 0.01 g; whole body mass), and the head was swiftly severed and preserved in 4% paraformaldehyde in a phosphate buffer for tissue fixation and preservation. Whole brain weight (± 0.001 g) was obtained from dissected brains following fixation. All weights were obtained using a Precisa 125A electronic scale (precision = 10^{-5} g) (Precisa Instruments AG, Dietikon, Switzerland). All cranial nerves, optic nerves, and meningeal membranes were removed before weighing and the brain was severed from the spinal cord 2–3 mm posterior of the dorsal medulla. The number of days samples spent in paraformaldehyde prior to dissection had no effect on brain weight even when controlling for body weight ($t = -0.83$, $P = 0.41$, $n = 194$). Individuals were sexed by observing the gonads. Intraspecific sample size was three to eight individuals, except for two species for which we only had one sample. For 37 species, we had both male and female individuals ($n = 1$ –6 individuals per sex). All measures were log transformed.

PHYLOGENY

We reconstructed a molecular phylogeny for the 43 species included in the analyses using three mitochondrial genes—NADH 2 (1047 bp), cytochrome b (402 bp), and the more variable control region (369 bp), downloaded from GenBank (see Table S1 for sequence accession numbers). Phylogenetic relationships were reconstructed using Bayesian analysis (Huelsenbeck et al. 2001) under a GTR plus Γ model of sequence evolution (Salzburger et al. 2002). We specified *Boulengerochromis microlepis* and *Bathybates fasciatus* as the outgroup clade. A consensus tree was constructed using Bayesian analyses employing Metropolis coupled Markov chain Monte Carlo (MCMC) methods in MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). We ran two independent parallel analyses with four simultaneous chains in each for 5,000,000 generations, sampling once every 1000th generation, and burnin at 1,000,000 generations. We used a uniform prior on topologies and exponential priors (10) on branch lengths. Our phylogeny (see Fig. S1) is in accord with another recent molecular phylogeny of Tanganyikan cichlids (Salzburger et al. 2002). For the sex-specific analyses, the phylogeny was cropped to include only the species for which we had data on both male and female individuals. Branch lengths were scaled proportional to time using nonparametric rate smoothing as implemented in the program APE (Paradis et al. 2004) in R. Our sample included most Tanganyikan cichlid species for which detailed phylogenetic information is available, and provides a representative sample of natural variation in the lake, including seven of the 12 tribes into which Tanganyikan cichlids have been grouped (Salzburger et al. 2002). Furthermore, our aim was to compare between patterns of phenotypic evolution within a single lineage rather than establish general patterns of phenotypic evolution for Tanganyikan cichlids.

COMPARATIVE ANALYSES

Because adaptive radiations are characterized by rapid rates of cladogenesis and our analyses involved a subsample of all Tanganyikan species; we first ensured that the molecular phylogeny accurately reflected such rapid cladogenesis. Departure from a null model of constant rates of diversification was tested using the γ statistic as implemented in APE (Pybus and Harvey 2000; Paradis et al. 2004). A significantly negative value of γ indicates a slowdown of the rate of cladogenesis over the history of a taxon (Pybus and Harvey 2000).

Correlations between brain weight and SL and brain and body weight were calculated with phylogenetic generalized least squares analyses in the Continuous package of Bayestrans (Pagel 1999). To analyze patterns of evolution, we applied three complementary methods: the morphological diversity index (MDI, Harmon et al. 2003), a maximum-likelihood estimate of the delta, and lambda parameters (Pagel 1999) and a node height test (Purvis and Rambaut 1995). All three methods test for departure from Brownian motion but differ in the approach used to test for this departure, the additional information they provide on rates of character evolution as well as in their limitations. Below we present the models describing the differences between them and what their potential biases may be. By combining the three methods, a result suggesting departure from Brownian motion will be robust if all three methods present patterns supporting departure from Brownian motion.

First, to examine the patterns of morphological evolution, we calculated disparity through time plots (Harmon et al. 2003) for SL, body weight, and brain weight, using the package GEIGER (Harmon et al. 2008) in R following Harmon et al. (2003). Morphological differences (disparity) were calculated from average pairwise Euclidean distances between species. Disparity through time was calculated as the average relative disparity of each subclade by dividing the average disparity of all subclades whose ancestral lineages were present at that time by the average disparity of the clade as a whole, and repeating this at each divergence event (i.e., each node) moving up the phylogeny from root to tip. A null hypothesis was constructed by simulating morphological divergence of each trait along the phylogeny under an unconstrained Brownian motion model. The MDI was calculated as the sum of the areas between the curve describing the morphological disparity of the trait and the curve describing the disparity under the null hypothesis of Brownian motion. Areas in which observed values were above expected were assigned positive values, whereas those below expected were assigned negative values. The MDI thus describes how morphological diversity is partitioned within the clade: values above 0 indicate that most morphological disparity is distributed within clades, suggesting recent phenotypic divergence, negative values suggest disparity is distributed among clades, suggesting early divergence, whereas

values near 0 indicate evolution has followed Brownian motion. For ease of interpretation, in the disparity through time plots we present the time scale as million of years to the present, using 10 million years ago (mya) as an estimate for the origin of the Tanganyikan cichlid radiation (Salzburger et al. 2002). The advantage of the MDI is that it avoids reconstruction of ancestral states and it provides a graphical representation, as well as a numerical index (MDI), of the pattern of morphological divergence along the phylogeny. A limitation of the MDI is that there is no statistical test of departure from the null Brownian motion model of evolution. It is worth noting that the MDI combines information on the pattern of cladogenesis and rate of phenotypic evolution to estimate diversity, hence its power to detect departure from Brownian motion could potentially be compromised by the branching pattern (Freckleton and Harvey 2006). However, because the estimate of morphological divergence under the null hypothesis is simulated on the same phylogeny, and in this study we were comparing across traits within a single lineage, we suggest that the method will be robust to biases resulting from the rate of cladogenesis.

Second, we calculated maximum-likelihood values for the δ parameter, which tests for acceleration ($\delta > 1$) or deceleration ($\delta < 1$) of morphological change through time, and for the λ parameter that tests whether traits evolve according to the null Brownian motion model ($\lambda = 1$) (Pagel 1999) using the package GEIGER (Harmon et al. 2008). The advantage of GEIGER is that it can incorporate an estimate of intraspecific variance into the analyses, in this case the standard error of SL, body weight, and brain weight for each species or sex in the sex-specific analyses (in the few cases in which a single sample was available for a species the error was set to 0). A *P*-value was obtained by comparing the models with the parameters to a null model of unconstrained Brownian motion with the log-likelihood statistic. Estimates of δ and λ do not require estimation of ancestral states, however it has been suggested that the δ parameter, which is similar to Grafen's ρ , could potentially be biased (Freckleton et al. 2002).

Third, to analyze whether patterns of phenotypic divergence fit a niche-filling model, as might be expected during evolutionary radiations (Freckleton and Harvey 2006), we applied a node height test. We calculated independent contrasts (Felsenstein 1985) for each trait and correlated the absolute value of the contrasts to the height of the nodes from which they were generated (Purvis and Rambaut 1995). If evolution follows a niche-filling model a negative correlation is predicted between the absolute value of independent contrasts and the height of the node, because as niches become filled with increasing species number, niche partitioning will become increasingly finer (Freckleton and Harvey 2006). The node height test assumes that the order of origin of nodes in the tree is correct and that all species in a clade are included in the analysis, if either condition is not met the test could be biased

(Freckleton and Harvey 2006). For example, if species are missing at the tips the contrasts could be overestimating the amount of divergence at the end of the radiation. However, the node height test provides a direct test of whether traits are evolving under a niche-filling model and simulations have shown the method is robust against variations in the branching pattern (Freckleton and Harvey 2006).

We also estimated the maximum-likelihood estimates of δ and λ for the correlation between SL and brain size, as well as for the correlation between body weight and brain size for the species-specific measures. When these parameters are estimated for the covariance of traits they describe whether the residual variance follows Brownian motion; the maximum-likelihood estimate of the parameters is the required adjustment to the variance-covariance matrix for it to fit a Brownian motion model (Rohlf 2001; Freckleton et al. 2002). Thus, the maximum-likelihood values of these parameters may differ from those estimated for each trait individually and can provide information regarding how the covariance between pairs of traits has evolved.

Results

Lineage accumulation showed significant departure from the null constant rates model and the rate of diversification slowed down with time ($\gamma = -2.82$, $P = 0.005$). These results indicate that rapid diversification occurred early in the evolutionary history of the clade and cladogenesis rates slowed down thereafter (Seehausen 2006).

BODY SIZE EVOLUTION

Evolution of both body size measures (SL and body weight) showed marked departure from Brownian motion as indicated by the diversity through time plots (Fig. 1). The positive MDI values (Table 1) show that most phenotypic disparity in body size was distributed within subclades, indicating independent divergence in body size within subclades possibly as a result of niche partitioning. This suggestion is supported by the results of the node-height test. SL and body weight showed a significant negative correlation between the absolute value of independent contrasts and the height of the node from which they were generated ($r = -0.40$, $t = -2.75$, $df = 40$, $P = 0.01$; $r = -0.35$, $t = -2.38$, $df = 40$, $P = 0.02$, respectively). The high values of δ for both body size measures indicate recent, high rates of phenotypic divergence in these two traits, whereas the low values of λ confirm that these traits have not evolved following Brownian motion (Table 1).

BRAIN SIZE EVOLUTION

Although highly correlated with body size ($R^2 = 0.77$ and $R^2 = 0.89$, respectively for SL and body weight, both $P < 0.001$) brain weight showed markedly different evolutionary patterns. Brain

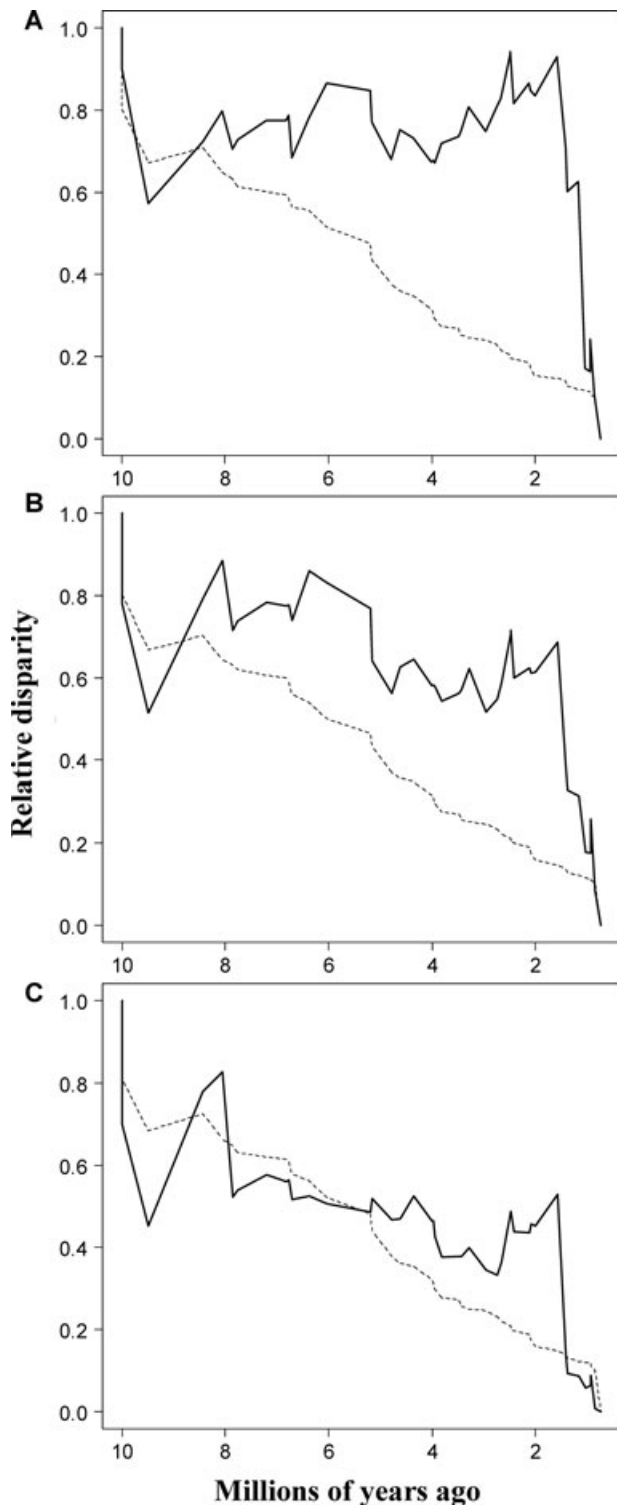


Figure 1. Morphological disparity through time plots for (A) standard length, (B) body weight, and (C) brain weight for the species-specific samples. The solid line shows the actual morphological disparity of the trait whereas the broken line shows the median disparity when trait evolution is modeled on the phylogeny following Brownian motion. Time is expressed as millions of years from the present, based on estimated dates for the Tanganyikan cichlid radiation (see Methods).

weight evolution did not depart from the null Brownian motion model, as shown by the disparity through time plot (Fig. 1) and the value of the MDI, which was 77% lower than that of SL and 68% lower than that of body weight (Table 1). The result of the morphological disparity analysis is supported by the value of δ , which did not differ significantly from unity indicating a constant rate of morphological divergence of brain size, and the value of λ , which also did not differ from unity indicating that the evolution of brain weight followed Brownian motion (Table 1). The node height test suggests there might be a trend for decreased divergence in brain size with increasing number of species ($r = -0.29$, $t = -1.93$, $df = 40$, $P = 0.06$), however because our sample included a subsample of all Tanganyikan cichlid species, the trend must be interpreted with caution as it could be influenced by incomplete sampling.

SEX-SPECIFIC ANALYSES

The sex-specific analyses showed similar patterns as above, with notable differences between the evolutionary divergence of body size measures and brain weight in both males and females; as shown by the diversity through time plots (See Fig. S2) and by the values of the MDI, δ and λ (Table 2). A pattern worth highlighting is that the MDI for male brain weight is 57% larger than the MDI for female brain weight and the species-specific MDI. This suggests that a higher proportion of the overall disparity in brain size in males is distributed within subclades, indicating more recent phenotypic divergence. The value of δ for male brain size was also higher than that for females (Table 2), however the results of the log-likelihood analyses do not support a departure from Brownian motion.

COVARIANCE BETWEEN TRAITS

The maximum-likelihood value of the δ parameter was 2.29 for the correlation between SL and brain weight and 2.31 for the correlation between body weight and brain size. However, the estimates did not differ significantly from 1 (log-likelihood ratio test, $\chi^2 = 2.67$, $P = 0.10$ and $\chi^2 = 2.58$, $P = 0.10$, respectively). The maximum-likelihood value of λ was 0.73 for the correlation between SL and brain weight and 0.71 for the correlation between body weight and brain size. Both estimates differed significantly from 1 (log-likelihood ratio test, $\chi^2 = 5.32$, $P = 0.02$ and $\chi^2 = 4.24$, $P = 0.04$, respectively). Thus, the values of λ suggest that the covariances between brain size and both measures of body size have not evolved following Brownian motion, but the values of δ do not support a recent acceleration in the rate of evolution of the covariances.

Discussion

The results of the rate of lineage accumulation analysis showed that our phylogeny is an accurate reflection of the Tanganyikan

Table 1. Morphological disparity index (MDI) and maximum-likelihood estimators for the δ and λ statistics of standard length, body weight, and whole brain weight. The ln likelihoods of the null Brownian motion model and those of the two alternative models are shown for comparison. *P* values for the δ and λ parameters were determined from likelihood-ratio tests against a model with constant rates of evolution (unconstrained Brownian motion). *The *P*-value for the δ parameter for brain weight is marked as nonsignificant (NS) because the likelihood value is actually lower than that for the null Brownian motion model, and because the log-likelihood test is designed to compare models that are nested, a *P*-value could not be calculated. The difference in AIC_c values between the two models (difference \approx 13) also strongly suggests that Brownian motion provides a more adequate description of brain size evolution.

Traits	MDI	Brownian			Delta			Lambda		
		Ln Likelihood	δ	Ln Likelihood	<i>P</i>	λ	Ln Likelihood	<i>P</i>		
Standard length	0.30	22.41	3.00	26.09	0.007	0.23	27.28	0.002		
Body weight	0.22	-20.26	3.00	-17.09	0.01	0.31	-16.96	0.01		
Brain Weight	0.07	115.65	3.00	110.34	NS*	0.71	116.23	0.28		

cichlid radiation. Rapid diversification occurred early in the history of the clade and rates then decreased significantly with time (Seehausen 2006). This early radiation in the Tanganyikan clade is in accord with the diversification process in the African Great Lakes, the Tanganyikan radiation being earliest of the three (Salzburger et al. 2005).

Both measures of body size showed concordant patterns of evolution. Both traits showed rapid rates of phenotypic divergence, as would be expected during adaptive radiation (Schluter 2000; Kozak et al. 2005; Clabaut et al. 2007), with significant departure from Brownian motion. A high proportion of the overall phenotypic variation was concentrated within subclades, as shown by the high MDI values. These results further suggest that subclades have diversified greatly in standard length and body weight presenting notable overlap between lineages, in line with the evolutionary convergence in body shape in Tanganyikan cichlids (Rüber and Adams 2001). The results of the node height test indicate that phenotypic divergence decreases as the number of species increased, suggesting increasingly finer niche parti-

tioning as available niches become filled during the radiation. In sum, the evolutionary patterns of both measures of body size are in accord with what would be expected if phenotypic divergence between emerging species was promoted by ecological specialization (Schluter 2000), and thus are in accord with the adaptive nature of this radiation (Clabaut et al. 2007).

On the other hand, evolutionary patterns of brain size were markedly different from those of both body size measures. Brain size evolution did not differ from a Brownian gradual model, with divergence accumulating with time rather than fuelled by ecological opportunity. The low MDI values also indicate that evolution of this trait has not departed from Brownian motion, a result that was confirmed by the values of λ and δ , in a stark contrast with both body size measures. Such gradual evolution of brain size, with divergence accumulating with time, suggests an absence of strong selection pressures acting on brain size during radiation. Hence, our results suggest that brain size is not a key trait under strong selection during adaptive radiations, at least not in Tanganyikan cichlids, in line with the recent finding that parental

Table 2. Morphological disparity index (MDI) and maximum-likelihood estimators for the δ and λ statistics of the two body size measures (standard length and body weight) and whole brain weight analyzed separately for males and females. The ln likelihoods of the null Brownian motion model and those of the two alternative models are shown for comparison. *P* values for the δ and λ parameters were determined from likelihood-ratio tests against a model with constant rates of evolution (unconstrained Brownian motion).

Traits	MDI	Brownian			Delta			Lambda		
		Ln Likelihood	δ	Ln Likelihood	<i>P</i>	λ	Ln Likelihood	<i>P</i>		
Males										
Standard length	0.30	17.52	3.00	20.78	0.01	0.15	21.33	0.006		
Body weight	0.28	-20.35	3.00	-17.35	0.01	0.22	-17.37	0.01		
Brain Weight	0.10	94.36	2.89	95.99	0.07	0.60	95.91	0.08		
Females										
Standard length	0.33	17.82	3.00	20.71	0.02	0.19	20.82	0.01		
Body weight	0.27	-16.13	3.00	-13.52	0.02	0.27	-13.84	0.03		
Brain Weight	0.07	103.25	2.39	104.31	0.15	0.70	103.66	0.37		

investment and fecundity, but not brain size, were associated with establishment success in introduced fish (Drake 2007). As such, our results contrast with previous studies that have suggested an association between invasion success and brain size in birds and mammals (Sol et al. 2002, 2008) and may point to possible taxon-specific effects. It is worth pointing out that some of the observed variation in brain size across species may result from phenotypic plasticity, which could potentially bias our analyses. For example, the early rearing environment can induce plastic variation in brain size (Kihslinger and Nevitt 2006; Chapman et al. 2008). However, in our study such a bias would result in an overestimation of between species divergence that would tend to reduce the probability of detecting a Brownian motion pattern of evolution for brain size.

The maximum-likelihood estimates of the λ parameter for the covariance between SL and brain size and body weight and brain size further suggest that brain size and body size have not covaried in a Brownian fashion. The fact that the maximum-likelihood estimate of λ differed significantly from Brownian motion suggests that closely related species show less similarity in the covariance between pairs of traits than would be predicted based on gradual, time-dependent divergence from a common ancestor (Freckleton et al. 2002). These results suggest that the distinct evolutionary patterns for body size and brain size have also influenced the covariance between these traits.

The sex-specific analyses showed similar patterns as the species-specific analyses, with notable differences between the evolutionary divergence of both body size measures and that of brain weight in both males and females. However, the MDI value for male brain weight was 57% larger than the MDI for female brain weight and the species-specific MDI. These results suggest there could be sex-specific effects governing brain evolution (Gittleman 1994; Gonzalez-Voyer et al. 2009). Males did not show higher variance in either body size measures or in brain weight than females, thus the differences could be due to the evolutionary patterns of phenotypic disparity. However, neither the δ nor λ parameters support significant departure from Brownian motion. Several Tanganyikan species present sexual size dimorphism, indeed the most pronounced example of size dimorphism among vertebrate species in which males are larger than females was found in the Tanganyikan cichlid *Lamprologus callipterus* (Schütz and Taborsky 2000). We cannot reject the hypothesis that larger evolutionary increases in body size in males than in females have influenced the rate of evolution of brain size in males. However, the results of the species-specific analyses present strong evidence in favor of different rates of evolution between body and brain size. Furthermore, the MDI values for both body size measures are nearly identical between males and females, which argue against higher evolutionary divergence in body size in one sex. It is also interesting to note that in females most phenotypic

divergence appears to have taken place at about mid-point in their evolutionary history (between 6 and 4 mya, See Fig. S2) with relative disparity within subclades rapidly decreasing thereafter. In males, on the other hand, most divergence appears to be concentrated later in their evolutionary history (between 5 and 2 mya, See Fig. S2), suggesting later divergence with relative disparity within subclades decreasing later than in females (at about 2 mya). This also suggests there might be sex-specific patterns in phenotypic evolution.

All three comparative methods gave consistent results: for both measures of body size all methods suggested departure from Brownian motion whereas none suggested so for rates of brain evolution. All three methods could potentially be influenced by incomplete coverage of taxa within the clade because this would result in an overestimation of the amount of phenotypic divergence at the tips of the phylogeny. For example, it is possible that the disparity through time plots slightly overestimate within subclade disparity in the later portion of the phylogeny—closer to the tips—because of missing species. Hence, if the analysis were repeated with a complete coverage of all available species, the decrease in relative disparity might occur earlier than our results suggest. However, because our interest lay in comparing between traits within the same lineage this slight overestimation of diversity at the tips is unlikely to bias our results as it is probably proportional across all traits. Finally, the low values of λ , and high values of δ , have two possible interpretations. They could result from low phylogenetic dependence, caused by high intraspecific variation about the mean or because of high error in the data. Alternatively, they could reflect actual departure from a Brownian motion model of evolution (Freckleton et al. 2002). Because interspecific variance was higher than intraspecific variance for all traits ($F_{1,41} = 21.19$, $F_{1,41} = 25.88$, and $F_{1,41} = 25.89$ all $P < 0.001$, respectively for SL, body weight, and brain weight) and because the magnitude of intraspecific variance was similar across all three traits it is unlikely that the differences in the parameter values between the traits are due to differences in the amount of variance or error in the data.

Our results show that even highly correlated traits, such as body and brain size, can present markedly different patterns of evolution. This suggests that the high correlation between these two traits notable in extant species is not the result of parallel coevolution, at least not throughout the entire phylogeny, nor that one trait has evolved simply as a correlated response to changes in the other (Riska and Atchley 1985; Price and Langen 1992). Rather, body size appears to be imposing boundaries on the maximal and minimal size a brain can have. In accord with this hypothesis, a previous study comparing brain size across cichlid species from the three African Great Lakes suggested that brain size is not significantly constrained by the space available within the skull (van Staaden et al. 1995). Van Staaden et al. (1995) further note

that the brain sometimes occupied less than half of the available space within the cranium and the balance was filled with fatty tissue, a trend that was more pronounced in larger species, this was also the case during our dissections. The difference in patterns of evolution between body and brain size, as well as the rapid and high divergence in body size, therefore suggest that the allometric relationship is not acting as a mechanistic constraint limiting evolutionary divergence (Lande 1979; Blows and Hoffmann 2005), at least not in body size. Brain size, on the other hand, appears to be constrained to evolve gradually, this may in part result from the elevated costs associated with larger brains (Aiello and Wheeler 1995). The different patterns of evolution between brain and body size further suggest that interpreting a strong correlation between traits in contemporary species as suggesting that genetic correlations may limit independent evolution of the traits or bias evolution as a result of concerted change (Price and Langen 1992) can be erroneous. A recent experimental study showed that two characters, which initially showed strikingly similar patterns of variation in all directions present in the population, responded to artificial selection in strikingly different ways (Allen et al. 2008). Interestingly, although the response of the traits to this artificial selection experiment demonstrated that the boundaries of phenotype space were very different, the genetic correlations for both traits suggest that the patterns of genetic covariation was similar for both (Allen et al. 2008). Further, our results are in accord with the recent consensus that brain and body size are influenced by different selection pressures and that these two traits may evolve, to a certain extent, independently of each other (e.g., Pagel and Harvey 1989; Harvey and Krebs 1990; Striedter 2005; Dunbar and Shultz 2007).

In conclusion, our results suggest that adaptive radiation, at least in Tanganyikan cichlids, was influenced by ecological factors, for example availability of multiple niches, rather than by the cognitive abilities of individuals. Furthermore, our results suggest that brain size is responding to different selection pressures than those acting on body size. Although selection may favor marked divergence in body size during adaptive radiation, adaptation of the brain to a species' biotic and abiotic environment possibly involves more modest changes in total brain size. Indeed, gradual evolution of brain size could result from selection pressures favoring a more efficient packaging arrangement, for example involving more extensive dendritic organization and novel complex structures such as laminar organization, so that more elaborate neuronal systems have not resulted in an excessively enlarged cranium (e.g., Butler and Hodos 2005). Finally, our results clearly show that even highly correlated traits can present markedly different patterns of phenotypic evolution. Therefore, a strong correlation between two traits in contemporary species cannot be interpreted as coevolution, generally defined as changes in one trait causing a proportional change in another trait, because

such a correlation could come about through very different evolutionary patterns in each trait. Thus, inferring evolutionary patterns from correlations between traits measured in contemporary species without analyzing evolutionary patterns can be misleading. We suggest future studies should incorporate this type of analysis to study patterns of evolution of other pairs of correlated traits across different taxa to test the generality of our results. Such analyses could provide important insight to determine whether trait correlations in contemporary species necessarily arise through a pattern of coevolution or whether they can involve more independent evolution of each trait.

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Supporting information

The following supporting information is available for this article:

Figure S1. Consensus mitochondrial DNA phylogenetic tree of the 43 species included in the analyses with *Boulengerochromis microlepis* and *Bathybates fasciatus* as outgroup clade.

Figure S2. Morphological disparity through time plots for male and female individuals analyzed separately in species for which data on both sexes were available.

Table S1: Accession numbers for the three mitochondrial genes used to construct the phylogeny.

Supporting Information may be found in the online version of this article.

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