

Diet predicts intestine length in Lake Tanganyika's cichlid fishes

Catherine E. Wagner^{*,†,1,2,3}, Peter B. McIntyre^{†,1,2,4}, Kalmia S. Buels^{1,5}, Danielle M. Gilbert² and Ellinor Michel^{2,6}

¹Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853, USA; ²Nyanza Project, Department of Geosciences, University of Arizona, Tucson, Arizona 85721, USA; ³Fuller Evolutionary Biology Program, Lab of Ornithology, Cornell University, Ithaca, New York 14850, USA; ⁴School of Natural Resources and Environment, University of Michigan, Ann Arbor, Michigan 48109, USA; ⁵Program in Molecular and Cellular Biosciences, Oregon Health Sciences University, Portland, Oregon 97239, USA; and ⁶Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

Summary

1. Among vertebrates, herbivores have longer digestive tracts than animals at higher trophic levels, a pattern thought to reflect a trade-off between digestive efficiency and tissue maintenance costs. However, phylogenetic influences on this pattern have rarely been considered. Taxa that have undergone diversification accompanied by dietary shifts provide a powerful opportunity to examine the relationship between diet and intestine length while accounting for phylogeny.

2. In this paper we assess the relationship between diet and intestine length in the cichlid fishes of Lake Tanganyika, which are renowned for their diversity of species and trophic strategies.

3. First, we test the effect of trophic position on intestine length across 32 species, while controlling for phylogeny. Trophic position was inferred from nitrogen stable isotopes, which provide a temporally integrated, quantitative perspective on the complex diets of tropical fish. Second, we examine patterns of intraspecific variation in intestine length of an algivorous cichlid (*Tropheus brichardi*) along a natural spatial gradient in algal nitrogen content.

4. Trophic position explains 51% of size-standardized variation in intestine length after accounting for phylogeny. Accounting for phylogeny does not substantially alter the relationship between trophic position and intestine length, despite the existence of phylogenetic signal in both traits. Thus, diet is a strong predictor of variation at the interspecific level.

5. There is a striking inverse relationship between intestine length and algal nutrient content among populations of *T. brichardi*, suggesting substantial plasticity in response to food quality, and thus a strong dietary influence on patterns of intraspecific variation.

6. Diet is a strong predictor of intestine length at both intra- and interspecific scales, indicating that fish adjust their phenotype to balance nutritional needs against energetic costs. Furthermore, functional explanations for trophic diversification of cichlid fishes in the African Great Lakes have long focused on jaw structures, but our results indicate that intestinal plasticity in response to diet quality may also be an important mechanism for accommodating trophic shifts during evolutionary radiations.

Key-words: comparative analysis, phylogenetic generalized least squares, gut length, stable isotope, phenotypic plasticity, trophic differentiation, ecological stoichiometry, adaptive radiation.

Introduction

An inverse relationship between intestine length and trophic level has been well documented in birds (e.g. Ricklefs 1996; Battley & Piersma 2005), reptiles and amphibians (e.g. Ste-

vens & Hume 1995; O'Grady *et al.* 2005), mammals (e.g. Schieck & Millar 1985; Korn 1992) and fishes (e.g. Kapoor, Smith & Verighina 1975; Ribble & Smith 1983; Kramer & Bryant 1995b; German & Horn 2006). This pattern is believed to reflect the greater digestive processing time required by primary consumers due to the lower nutrient content and greater resilience to digestion of plant tissues compared with animal tissues (Horn 1989). Digestive tissues

*Correspondence author. E-mail: cew35@cornell.edu

†These authors contributed equally to this work.

are among the most energetically expensive tissues to maintain (Cant, McBride & Croom 1996; Hume 2005). Therefore, digestive tract length should reflect a balance between maximizing the capacity to extract nutrients and energy from the diet, and minimizing the energetic cost of maintaining digestive tissues (Sibly 1981). Under this trade-off, intestine length should be the minimum necessary for the sufficient uptake of dietary nutrients. However, phylogenetic relationships could also affect intestine characteristics; taxa may exhibit similar morphological traits simply as a result of shared ancestry (Felsenstein 1985; Harvey & Pagel 1991). Thus, resolving the influence of diet on intestine length also requires consideration of phylogeny.

Digestive tissues are notoriously plastic in their responses to dietary change (Starck 1999). Plasticity in gut morphology has been observed in response to fasting, increases in food intake and changes in diet (e.g. Starck 1999; Naya, Karasov & Bozinovic 2007; Olsson *et al.* 2007). In fluctuating environments, the capacity for phenotypic plasticity can itself be a target of selection (West-Eberhard 2003; Pigliucci 2005; Ghahambor *et al.* 2007), and patterns consistent with adaptive plasticity in intestine morphology have been demonstrated in rodents (Naya, Bozinovic & Karasov 2008). At the macro-evolutionary level, plasticity has important consequences when considering patterns of trait divergence. From one perspective, plasticity could retard evolution by shielding genotypes from selection. Alternatively, plasticity could enable the persistence of populations when exposed to novel environmental stimuli, thereby facilitating new opportunities for selection. In the latter scenario, natural selection could amplify initially small morphological changes initiated by plasticity (West-Eberhard 2003; Crispo 2007). Although the basic ideas underlying this concept are old (Baldwin 1902; Waddington 1942), it has received increasing recent interest, particularly in the context of explaining divergence and adaptive radiation (Losos *et al.* 2000; West-Eberhard 2003; Gomez-Mestre & Buchholz 2006; Wund *et al.* 2008), including changes in gut morphology associated with dietary shifts (Ledon-Rettig, Pfennig & Nascone-Yoder 2008). Plasticity in intestine morphology may, therefore, be a key factor influencing the capacity for a lineage to undergo trophic shifts.

East African cichlid fishes offer many classic examples of rapid radiation accompanied by trophic diversification (Fryer & Iles 1972; Liem 1973; Genner, Turner & Hawkins 1999; Ruber, Verheyen & Meyer 1999; Danley & Kocher 2001; Clabaut *et al.* 2007). Changes in diet are made possible by a suite of morphological changes that accommodate novel ways of capturing and processing food resources. Although changes in jaw morphology have been the primary focus of previous work on dietary shifts in cichlids (e.g. Liem 1973; Meyer 1987; Wimberger 1991), alterations to digestive tract morphology are likely critical for dietary changes and resource differentiation. Previous work has documented an inverse relationship between trophic level and intestine length in Lake Malawi's 'mbuna' cichlids (Reinthal 1989), in Lake Tanganyika's cichlids (Fryer & Iles 1972) and in Cuatro Ciénegas cichlid morphs (Kornfield *et al.* 1982). Intestine length and

coiling pattern have also been proposed as a useful character for phylogenetic reconstruction in several cichlid groups (Zihler 1982; Yamaoka 1985).

Lake Tanganyika's cichlids are an appealing focal group for studying the phylogenetic and ecological influences on phenotypic traits. Tanganyikan cichlids comprise ~200 described species (Poll 1986) stemming from within-lake radiations of multiple lineages (Salzburger *et al.* 2005) during the lake's long history (9–12 million years; Cohen, Soreghan & Scholz 1993). Ecological divergence during these radiations has yielded numerous trophic strategies that vary both within and among lineages of Tanganyikan cichlids. Thus, interspecific differences in traits associated with feeding, such as intestine length, could be influenced by phylogenetic affinities, current diet and other factors.

Two major obstacles have prevented rigorous combined analysis of dietary and phylogenetic influences on gut length in animal taxa. First, it has been difficult to account for the true complexity of animal diets using categorical characterizations. Previous studies have assigned species to discrete groups (e.g. algivores, invertivores, piscivores) based on temporal snapshots of gut contents, but many species consume multiple types of food either concurrently or in response to changing resource availability (Fryer *et al.* 1972; McKaye & Marsh 1982). These food types differ substantially in their nutrient and energy content, and hence in their nutritional value to consumers (Bowen, Lutz & Ahlgren 1995; Sterner & Elser 2002). Thus, gut content-based trophic designations may be poor reflections of long-term diet type and quality. Here we use nitrogen isotope ratios to infer each species' trophic position in the food web. The heavy nitrogen isotope (^{15}N) is differentially retained during waste elimination in animals, resulting in an enrichment in $\delta^{15}\text{N}$ of 3–4‰ in the consumer's tissues relative to its prey (Post 2002). This consistent shift in $\delta^{15}\text{N}$ with each trophic transfer makes $\delta^{15}\text{N}$ a continuous index of trophic position. Furthermore, nitrogen isotopes reflect assimilation over weeks or months (McIntyre & Flecker 2006), account for feeding at multiple trophic levels, and can be compared among all taxa in the food web (Post 2002). As a continuous metric, isotopic trophic position is also well suited for phylogenetically explicit analytical techniques requiring continuous variables.

The second major obstacle to combined analysis of dietary and phylogenetic influences on intestine length has been the lack of well-resolved phylogenies for relevant groups. Evolutionary relationships within the clades surveyed in previous studies are poorly known, thereby limiting phylogenetic comparisons of gut length patterns to higher taxonomic groups or comparisons between sister taxa (e.g. De Groot 1971; German & Horn 2006). To assess the effect of diet on cichlid intestine length while accounting for shared evolutionary history, we developed a phylogeny for our focal species using mitochondrial DNA sequences, and analysed the relationship between intestine length and trophic position using a phylogenetic generalized least-squares (PGLS) approach (Martins & Hansen 1997; Pagel 1998, 1999).

We also examined the influence of diet on intraspecific variation in intestine length. We quantified differences in intestine length among populations of algivorous *Tropheus brichardi* (Nelissen & Thys van der Audenaerde 1975) across a natural spatial gradient in algal nitrogen content. Algal nutrient content is an important aspect of diet quality for algivorous tropical fish, whose growth may be limited by nutrient intake rather than energy (Hood, Vanni & Flecker 2005). Given that the widely observed inverse relationship between trophic level and intestine length also represents a gradient of dietary nutrient content (Sturner & Elser 2002), we predicted that fish from sites with low-nutrient algae would have longer intestines than those from sites with high-nutrient algae.

The goals of this study were twofold: to examine the influence of trophic position on intestine length in a broad phylogenetic context and to examine the influence of diet on intraspecific variation in intestine length. The aims of these approaches overlap in that they both seek to test the influence of diet on variation in intestine length. If diet emerges as a strong predictor of intestine length, this would indicate that phenotypes reflect a balance between nutritional needs and energetic demands. Alternatively, if either the interspecific or intraspecific comparisons failed to demonstrate a link between diet and intestine length, then we would infer that evolutionary history or unmeasured variables are responsible for variation in intestine length within this radiation. Intraspecific comparisons are particularly valuable as a complement to our broader interspecific survey because genetic distance is minimized while diet quality varies substantially. By examining effects at both intraspecific and interspecific levels, we can assess whether diet influences patterns of variation at both micro- and macroevolutionary scales.

Material and methods

INTESTINE LENGTH, NITROGEN ISOTOPE AND GENETIC DATA

We collected specimens of 32 Tanganyikan cichlid species from a rocky shore near Kigoma, Tanzania during August 2002 (Table S1, Supporting Information; Jakobsen's Beach, Table S3). These species were selected to represent all trophic and phylogenetic groups of rock-dwelling cichlids at this site. Species sampled encompass the five major trophic guilds of Tanganyikan cichlids: algivores, benthic invertivores, zooplanktivores, piscivores and scale-eaters (Table S1). After recording the wet mass of each specimen ($n = 3$ per species in most cases; Table S1), muscle samples were collected for genetic and stable isotope analysis, the ventral body wall was cut open and specimens were preserved in 10% formalin and stored in ethanol. Intestine length was measured by removing the entire alimentary canal from stored specimens, unwinding the intestine with care to minimize stretching, and measuring the distance from the anus to the posterior end of the stomach using vernier callipers.

Nitrogen (N) stable isotope ratios were determined from dorsal white muscle from each fish analysed for gut length. Muscle was oven-dried at the field laboratory (60 °C, 48 h), stored in glass vials, ground to a fine powder and subsampled (1 mg) for analysis. Stable isotope analyses were conducted using the Cornell Isotope Labora-

tory using a (Thermo Finnigan, San Jose, CA, USA) MAT Delta Plus mass spectrometer calibrated against reference standards (trout tissue, methionine, atmospheric N₂). Results were expressed using the standard $\delta^{15}\text{N}$ notation (see Post 2002), and analytical precision was 0.2‰ $\delta^{15}\text{N}$ (SD). Nitrogen isotope ratios serve as a proxy for consumer trophic position because animals retain relatively more ^{15}N than ^{14}N from their diet. Our analyses use $\delta^{15}\text{N}$ itself as a continuous index of trophic position; we did not convert $\delta^{15}\text{N}$ into estimates of trophic level because the exact magnitude of ^{15}N enrichment between consumer and resource remains unresolved (Post 2002; McCutchan *et al.* 2003).

Following total DNA extraction from tissue samples using DNeasy tissue extraction kits (Qiagen, Valencia, CA, USA), we PCR-amplified and sequenced two mitochondrial genes from each species: cytochrome *b* oxidase (*cytb*, 1149 bp), and nitrogen dehydrogenase subunit 2 (ND2, 1047 bp). New external and internal primers (Table S2) were designed for *cytb* amplification and sequencing. External primers 1, 2, 6 and 7 (Kocher *et al.* 1995) and new internal primers (see Table S2) were used in the amplification and sequencing of ND2. PCR amplification followed standard procedures, and PCR products were prepared for sequencing using BigDye sequencing chemistry (Applied Biosystems, Foster City, CA, USA), and analysed on an ABI3100 automatic sequencer (Applied Biosystems).

To examine the influence of diet quality on intraspecific variation in intestine length, eight *T. brichardi* were collected from each of six sites in July to August 2003 (Table S2). After recording wet mass, intestines were removed and measured immediately (without preservation). The nutrient content of algae at these sites was assessed using the carbon : nitrogen ratio (C : N, molar) of purified algal samples collected in August 2002 ($n = 3\text{--}4$ per site). Periphyton was scrubbed from the upward surface of a rock, then centrifuged in a colloidal silica column (density $\sim 1.2\text{ g mL}^{-1}$) to separate filamentous algae from diatoms, detritus and microinvertebrates (e.g. Hamilton, Sippel & Bunn 2005). Filamentous algae, the main diet of *T. brichardi*, was collected on precombusted glass fibre filters (Gelman A/E, Ann Arbor, MI, USA), rinsed well, dried and analysed using a Carlo Erba NC2500 elemental analyser (Thermo Finnigan, San Jose, CA, USA). We compared fish intestine lengths to algal data collected the previous year because a diet manipulation experiment indicates that *Tropheus* require months to alter their intestine length in response to dietary change (P. McIntyre & Y. Vadeboncoer, unpublished data).

DATA ANALYSIS

Because intestine length varies with body size, data must be standardized for size effects before making comparisons among individuals or species (Kramer & Bryant 1995a). The traditional approach to this issue is a simple linear standardization, describing relative gut length as the ratio of intestine length to body length, but this method fails to account for the well-known allometry of gut length (Zihler 1982; Kramer & Bryant 1995a). We assessed allometric patterns among trophic guilds and removed the effects of body size prior to comparing gut lengths among species using a modification of the method developed by Kramer & Bryant (1995b). Allometric growth creates a power function relationship between gut length and body size, and this relationship can be linearized by \log_{10} transformation of both size (wet mass) and intestine length. The intercept of the linearized equation represents the coefficient of the power function, which summarizes the size-independent component of the growth trajectory of gut length. Therefore, when the slope of the linearized equation is equivalent among groups (representing equivalent exponents describing the scaling of gut length with mass), the intercept serves as an index of

size-standardized gut length that can be compared among individuals or groups (Fig. S1, Supporting Information).

We used ANCOVA to test for the effects of trophic guild (designations based on gut contents; Hori *et al.* 1993), log-transformed body mass and guild-mass interactions on log-transformed intestine lengths. The lack of significant differences in slope among trophic guilds (size-guild interaction; $F_{4,83} = 1.83$, $P = 0.131$; Fig. S1) indicates that size-scaling of intestine lengths did not differ among trophic guilds. Two outlier species were excluded from this analysis: *Boulengerochromis microlepis* (Boulenger 1899) and *Cyphotilapia frontosa* (Boulenger 1906). Both are considered piscivores (Hori *et al.* 1993), but this could not be verified because our specimens had empty stomachs. However, the sampled *B. microlepis* were small individuals (19.2–23.1 g) whose nitrogen isotope values were indicative of invertivory rather than piscivory. *Cyphotilapia frontosa* (23.9–266.0 g) showed high variability in the mass–gut length relationship, likely due to development of a large nuchal hump as adults.

Given that growth trajectories of gut length with body mass did not differ significantly among trophic guilds, we used the mean of the five guild-specific slopes (0.46; calculated after excluding outlier species) to represent the typical scaling of intestine length (IL) with body mass (M) across our focal species: $IL \propto M^{0.46}$. We used the mean allometric relationship across guilds rather than a single overall allometric relationship across all individuals because the number of species studied is uneven across guilds and guild representation is

uneven across the range of body sizes; both types of heterogeneity would bias the estimated allometric relationship across the entire data set, but not allometry within each guild. The linearized relationship was rearranged to derive an intestine length index (ILI) from measurements of intestine length (IL) and mass (M) for each individual ($ILI = \log IL - 0.46 \log M$). Variation in ILI among individual fish reflects their residual deviation from the allometric regression equation, hence we use ILI as a size-standardized index for evaluating patterns of intestine length across species. We used ANOVA to test for differences in ILI among trophic guilds, followed by Tukey's Honestly Significant Difference comparisons.

After editing and aligning sequence data, we used Bayesian phylogenetic methods implemented in MR.BAYES 3.1.2 (Huelsenbeck *et al.* 2001; Ronquist & Huelsenbeck 2003) to construct a phylogeny using the complete ND2 and *cytb* gene sequences for each species (Fig. 1). We ran MR.BAYES for two runs of 2 million generations each, assessing convergence by monitoring the standard deviation of split frequencies throughout the runs. We discarded 10% of trees to account for burn-in, leaving the posterior distribution of trees with standard deviations of split frequencies below 0.01. To ensure that independent runs had converged upon equivalent posterior distributions, we used the 'compare' function in the program AWTY (Nylander *et al.* 2008) to compare posterior probabilities of clades between runs. We also analysed the data set using parsimony and maximum likelihood (ML; GTR + I + G model estimated in MODELTEST 3.7; Posada & Cran-

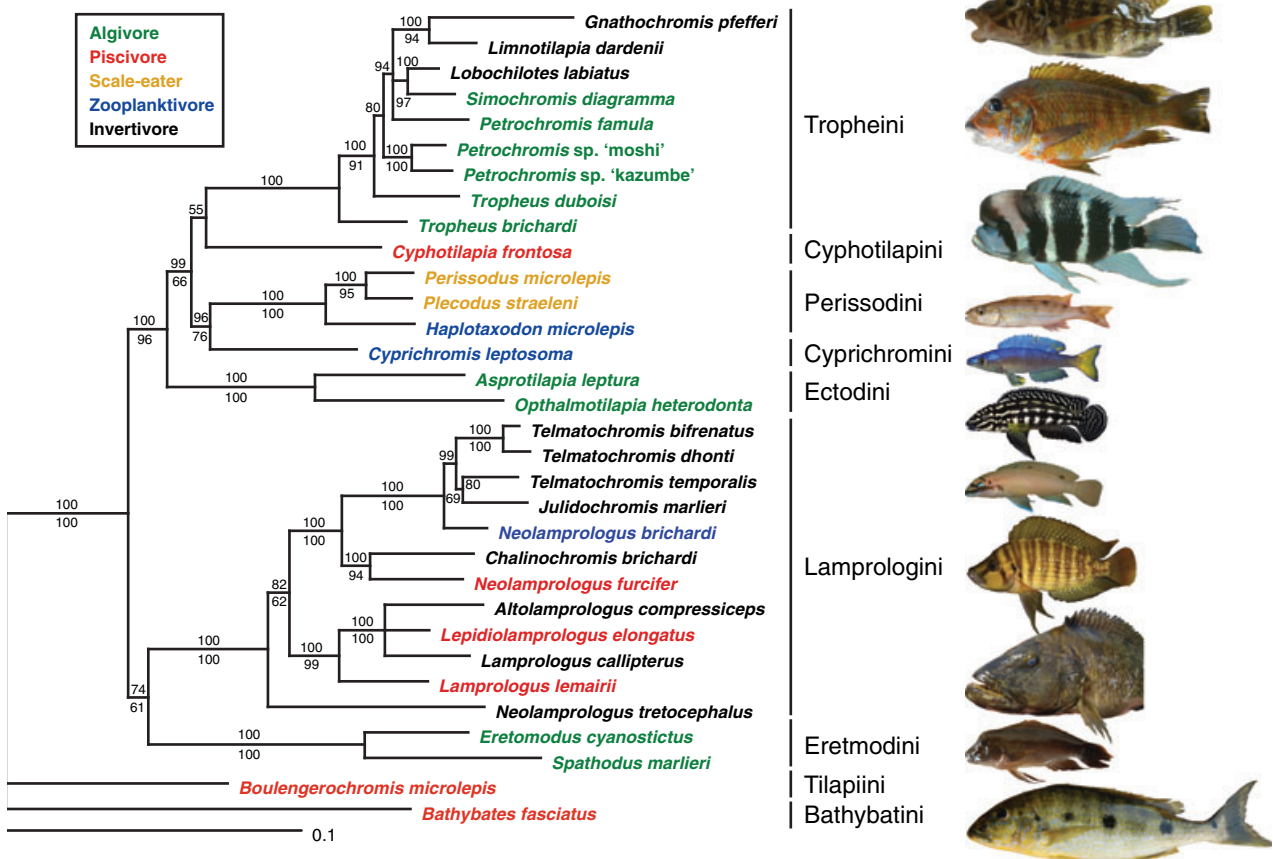


Fig. 1. Bayesian phylogram of Lake Tanganyikan cichlids based on cytochrome *b* (1149 bp) and nitrogen dehydrogenase subunit 2 (1047 bp) mitochondrial gene sequence. Numbers above the branches are Bayesian posterior probability percentages; numbers below the branches are maximum likelihood bootstrap scores (100 replicates). Values below 50% are not shown. Taxa are colour-coded by trophic guild inferred from gut contents (Hori *et al.* 1993; P. McIntyre, unpublished data).

dall 1998) methods implemented in PAUP* 4.010b (Swofford 2003) to assess the robustness of the topology obtained through Bayesian methods. We used results from the Bayesian analysis in subsequent phylogenetic comparative analyses. As we would expect traits evolving under a Brownian motion model to vary with time and not with the rate of molecular evolution, we ultrametricized all trees used in comparative analyses (without time calibration) using Sanderson's nonparametric rate-smoothing approach implemented in the function *chronogram* in the package APE for the R programming environment (Sanderson 1997; Paradis, Claude & Strimmer 2004).

To account for covariation in intestine length among species due to their phylogenetic histories, we used a PGLS approach (Martins & Hansen 1997; Pagel 1998, 1999). We first estimated the parameter λ for intestine length and for trophic position in an ML framework using the program BAYESTRAITS, and used likelihood ratio tests to test for significant difference from 0.0 to 1.0 (Pagel 1999). λ is an estimate of the phylogenetic signal derived through examination of the phylogenetic dependence of the residuals of a linear model. A λ -value of 0.0 indicates that trait variation is independent of the phylogeny, whereas a λ -value of 1.0 indicates that trait variation is as predicted by Brownian motion evolution along the phylogeny (Freckleton, Harvey & Pagel 2002). The λ -value can be applied to PGLS regression as a scaling factor (Pagel 1998, 1999). PGLS uses the variance-covariance matrix of shared branch lengths among species and an expected evolutionary model to transform the error term in the linear model describing the relationship between traits, thereby influencing the model's slope (Pagel 1998, 1999). We used a joint ML estimate of λ for intestine length and trophic position as the scaling factor in PGLS analyses, and we performed λ estimation and PGLS analyses in an ML framework using the program BAYESTRAITS (Pagel 1998, 1999). We examined the effect of λ -scaling on the PGLS analysis by constraining λ to 1.0 (indicating complete phylogenetic dependence; equivalent to Felsenstein's independent contrast) and to 0.0 (indicating no effect of phylogeny; equivalent to ordinary least squares regression) as null models. We used likelihood ratio tests to test for significant differences between the ML-estimated value of λ and the two null models (Freckleton, Harvey & Pagel 2002).

For PGLS analyses and the calculation of lambda values we used two approaches. First, we used a consensus topology generated from the posterior distribution of trees in the Bayesian analysis (Fig. 1). As these analyses require completely bifurcating topologies, we randomly resolved polytomies in the consensus tree using the function *multi2di* from the package APE in the R programming environment (Paradis, Claude & Strimmer 2004), and replaced branches of zero length with very short branches (length 10^{-6}). Second, to examine the effect of topological uncertainty on the analyses, we repeated analyses over 1000 trees from the Bayesian posterior distribution.

To assess the effect of spatial variation in food quality on gut length within *T. brichardi*, we used ordinary least squares regression

to examine the relationship between size-standardized ILI and algal C : N across sites. C : N data were averaged within each site to represent the typical algal nitrogen content experienced by each fish population, whereas individual ILI data were used to incorporate intrapopulation variation into the hypothesis test. We also regressed the site mean ILI against algal C : N to examine the influence of using site mean vs. individual ILI data in the hypothesis test. ILI was calculated by the method outlined earlier, but using the relationship between intestine length and mass observed across all *T. brichardi* populations in 2003 ($ILI = \log IL - 0.53 \log M$).

Results

Intestine lengths of Lake Tanganyika cichlids *Neolamprologue furcifer* (Boulenger 1898) varied from 0.43 to 9.34 (*Petrochromis* sp. 'kazumbe') times body standard length. $\delta^{15}N$ ranged from 2.79 to 9.25, suggesting that our study species spanned roughly three trophic levels (Post 2002). ANOVA indicated that there were significant differences in ILI among trophic guilds ($F_{4,95} = 63.36$, $P < 0.001$). Algivores had the longest intestines ($P < 0.001$ in all pairwise comparisons), and piscivores had shorter intestines than benthic invertivores or zooplanktivores ($P < 0.001$) but not scale-eaters.

Bayesian, ML and parsimony analyses of mitochondrial sequence data produced congruent phylogenetic topologies that are consistent with published Lake Tanganyikan cichlid phylogenies (Salzburger *et al.* 2002, 2005; Clabaut, Salzburger & Meyer 2005). Independent runs of the Bayesian analysis converged upon equivalent posterior distributions of topologies. Results of PGLS analyses using a consensus topology and computed over 1000 trees from the posterior distribution of the Bayesian runs were very similar to those calculated using a consensus tree. All results reported in the text are from the consensus topology, and Table 1 summarizes results from both the consensus tree-based approach and the analyses over the posterior distribution of trees.

The estimated λ for size-standardized intestine length (ILI) was 0.995, a value significantly greater than 0 ($P < 0.0001$) but not significantly less than 1 ($P = 0.945$). In contrast, the λ for trophic position (as $\delta^{15}N$), was 0.596, which is marginally greater than 0.0 ($P = 0.091$) and significantly less than 1.0 ($P < 0.0005$). The joint λ estimate for the data set was 0.718, a value significantly greater than 0 ($P < 0.006$) but not significantly less than 1 ($P = 0.066$).

	Con	PD	PD 2.5%	PD 97.5%	SD 0	SD 1
Lambda						
Trophic position	0.596*	0.596	0.548	0.636	0.051	1
Gut length	0.995†	0.984	0.85	0.999	1	0.026
Joint	0.718†	0.722	0.605	0.807	1	0.252
PGLS						
Slope	-0.129†	-0.128	-0.133	-0.121	1	-
Intercept	2.257	2.251	2.213	2.282	-	-

*Significantly different from 1.

†Significantly different from 0.

Table 1. Summarized results of analyses using a single consensus phylogeny (Con) and 1000 trees from the posterior distribution of the Bayesian phylogenetic analysis (PD). For analyses computed for the set of probable trees, mean values of the estimated parameters are given, as well as the 95% confidence intervals of the distribution of values (PD 2.5% and PD 97.5%), and the proportion of values significantly different from 0 (SD 0) and significantly different from 1 (SD 1)

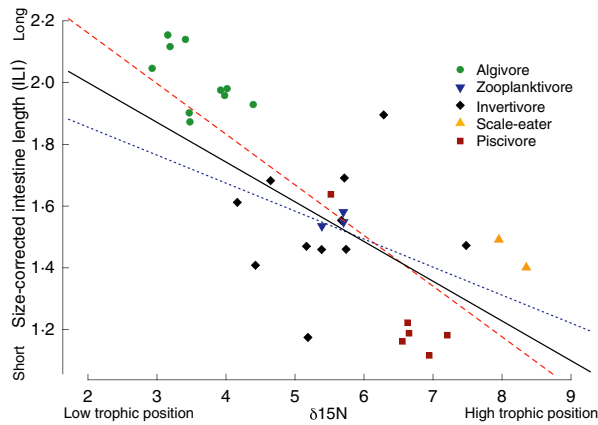


Fig. 2. Mean size-corrected intestine length (ILI) and trophic position in the food web (based on mean $\delta^{15}\text{N}$) for 32 species of Lake Tanganyikan cichlids. Regression lines are derived from generalized least squares regression where (a) $\lambda = 0$ (red dashed line; $y = 2.4890 \pm 0.1640x$; $r^2 = 0.5902$), equivalent to ordinary least squares regression without phylogenetic correction, (b) $\lambda = 0.7180$ (ML estimate; black solid line; $y = 2.2572 \pm 0.1287x$; $r^2 = 0.5093$) and (c) $\lambda = 1$ (blue dotted line; $y = 2.0371 \pm 0.0907x$; $r^2 = 0.4036$). Species are colour-coded by trophic guild inferred from gut contents (Hori *et al.* 1993; P. McIntyre, unpublished data).

In PGLS comparative analysis with ML estimation of λ , the correlation between trophic position and gut length is strong and negative ($r^2 = 0.509$, $\lambda = 0.718$; Fig. 2). Without accounting for phylogeny ($\lambda = 0$), the correlation is likewise strong and negative ($r^2 = 0.590$), as it is in the case of phylogenetic overcorrection ($\lambda = 1$; $r^2 = 0.404$) (Fig. 2 and Fig. S2 for individual data). Likelihood ratio tests indicate that using phylogenetic information improves the regression model ($P = 0.006$, $\lambda = 0.718$; $P = 0.039$, $\lambda = 1$), but there is not a significant difference between the λ -estimated model and the $\lambda = 1$ model ($P = 0.066$). The slopes of all models ($\lambda = 0.718$, 1 and 0) are highly significant ($P < 0.0001$).

Tropheus brichardi showed substantial variation in intestine length among the six study sites; intestine length ranged from 3.13 to 5.70 times body length (mean 4.37). Across sites, there was a significant positive relationship between algal C : N and *T. brichardi* ILI ($F_{1,46} = 8.55$, $P < 0.006$; $y = 0.02x + 0.545$; $r^2 = 0.157$). Regression of site mean ILI against algal C : N produced a nearly identical relationship to that of the regression using individual ILI values ($F_{1,4} = 63.47$, $P < 0.002$; $y = 0.02x + 0.540$; $r^2 = 0.941$). As algal C : N increased (indicating declining nitrogen content), we observed a relative increase in the size-standardized intestine length (Fig. 3).

Discussion

We found a strong negative relationship between trophic level and intestine length across the radiation of Tanganyikan cichlids. Trophic position explained 51% of the total body-size standardized variation in intestine length among species after accounting for phylogeny. This result is consistent with previous studies that have used categorical diet assignments to

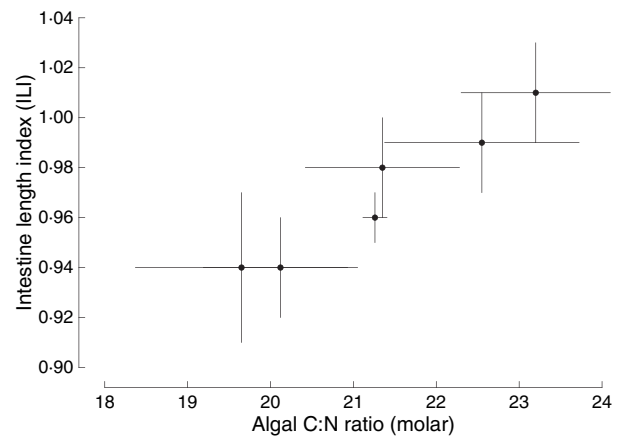


Fig. 3. Mean algal C : N ratio vs. mean size-standardized intestine length index (ILI) for *Tropheus brichardi* populations at six sites (± 1 SE of ILI and algal C : N ratio). Ordinary least squares regression produces a significant positive relationship between algal C : N ratio and ILI ($y = 0.02x + 0.54$; $r^2 = 0.941$).

study this pattern in cichlids (Fryer *et al.* 1972; Reinthal 1989) and in other fishes (e.g. Kapoor, Smith & Verighina 1975; Ribble & Smith 1983; Kramer & Bryant 1995b; German *et al.* 2006). Our use of nitrogen isotope data to distill the complexity of tropical fish diets into a single axis of trophic position provides the first quantitative evidence that gut length varies in a continuous fashion as a function of diet. These results suggest that intestine length in cichlids reflects a trade-off between maximizing nutrient and energy absorption and minimizing the energetic demands of digestive tissues.

The nutrient content, energy content and digestibility of food resources all influence the overall quality of an animal's diet. As these components of food quality are often correlated (e.g. low-nutrient, low-energy, high-fibre plant material vs. high-nutrient, high-energy, easily-digestible animal tissue), their relative influences on intestine length are difficult to separate. Our use of stable isotopes as a proxy for diet focuses on nitrogen that is assimilated, thereby emphasizing dietary material that is digestible. However, there may be considerable variation in the ease of digestion of items that have similar nitrogen isotope ratios. For example, although scale-eaters and piscivores both feed high on the food chain, scales are presumably more difficult to digest than muscle, and scale-eaters may therefore require a longer gut for the extraction of necessary nutrients and energy from their diet. Such differential digestibility of food resources may have contributed to variance in intestine length that was not explained by isotopic trophic position. Indeed, scale-eating fishes appear to have longer intestines than do piscivores of roughly equivalent trophic position (see Fig. 2). Although challenges remain in the full interpretation of stable isotope signatures (Gannes, O'Brian & Martinez Del Rio 1997; McCutchan *et al.* 2003), they offer a valuable, integrative index of trophic position for studies such as ours (Post 2002).

There were only minor differences in the results of the three PGLS analyses that we performed, i.e. overcorrecting for phylogeny ($\lambda = 1$), using an ML-scaled phylogenetic correction ($\lambda = 0.718$), and without phylogenetic correction ($\lambda = 0$). Trophic position explained 51% of variation in body-size standardized intestine length using the ML-scaled λ -value, and 59% without accounting for phylogeny. Although many authors have interpreted decreases in correlation after phylogenetic correction as the expected outcome when phylogeny has influenced the relationship between the traits in question, accounting for phylogeny should instead be viewed as a statistical correction only, and one that can increase or decrease observed correlations (Rohlf 2006). Conclusions about the relative roles of phylogeny vs. the predictor variable cannot be inferred from these analyses. However, the fact that the correlation between trophic position and intestine length is strong both with and without accounting for phylogeny provides clear evidence for an ecologically rooted correlation between these traits. In addition, significantly non-zero λ -values for intestine length and for the data set indicate that the evolutionary history of these species has played a role in structuring variation in these traits.

Although our ability to resolve patterns of trait evolution using this data set is limited by incomplete sampling of the Tanganyikan cichlid radiation, the observed phylogenetic signal in both trophic position and intestine length is intriguing. In particular, the substantially higher phylogenetic signal shown by intestine length alone ($\lambda = 0.995$) compared with trophic position alone ($\lambda = 0.596$) suggests that phylogeny predicts patterns of intestine length variation better than it does patterns of variation in trophic position. A number of studies have suggested that phylogenetic signal in ecological traits tends to be less than that of morphological characters, typically attributing these patterns to differences in the lability of these different kinds of traits (e.g. Blomberg, Garland & Ives 2003; Losos 2008). However, the processes underlying differences in phylogenetic signal are varied, complex and difficult to distinguish (Revell, Harmon & Collar 2008). Our detection of phylogenetic signal also speaks to a broader point. Given the rampant ecological divergence and convergent evolution observed within the African cichlid radiations, one might expect traits in this group to show comparatively little phylogenetic signal. Yet our study documents signal in both of the traits we investigated, underscoring the fact that phylogeny cannot be ignored by functional and comparative studies.

Further evidence for a substantial role of diet in determining intestine length is provided by intraspecific spatial variation in the specialized algae-browser *T. brichardi*. The dramatic differences in intestine length among populations are well correlated with algal nitrogen content, implicating food quality as a key determinant of intestine length. Although differential ingestion of animal material could also give rise to such patterns, *T. brichardi* is known to be a strict algivore from gut content analyses (designations based on gut contents; Hori *et al.* 1993), our extensive field observations (P. McIntyre and C. Wagner, unpublished data) and our sta-

ble isotope analyses at these and other sites (Fig. 2 and P. McIntyre, unpublished data). To illustrate the magnitude of intraspecific variation observed, we can estimate intestine length for adults (20 g wet mass) based on the ILI–algal C : N relationship (see Fig. 3). Mean intestine lengths at the six sites we studied would range from 42.6 to 49.8 cm, while extrapolation to the full range of algal C : N (18.8–29.4 M) observed across 12 sites in 2002 predicts intestine lengths from 40.3 to 65.7 cm. Although such a broad intraspecific range of variation remains to be demonstrated, substantial intraspecific variation in intestine length is not unexpected in herbivores because plants are highly variable in nutrient content compared with the animal prey of carnivorous species (Sterner *et al.* 2002). Thus, in Lake Tanganyika we expect that the observed variation in *T. brichardi* gut length is likely to apply to other algivorous species, but not to carnivores whose animal prey regulate their tissue stoichiometry in a homeostatic fashion.

Two non-exclusive mechanisms could contribute to spatial variation in the intestine length of *T. brichardi* in response to site-specific food quality: local adaptation and phenotypic plasticity. East African cichlid fishes are known for their highly spatially structured populations (e.g. Taylor, Ruber & Verheyen 2001; Sefc *et al.* 2007; Wagner & McCune 2009), and fine-scale population structure could allow local adaptation to sustained differences in algal nutrient content. However, we consider phenotypic plasticity to be a more likely explanation for two reasons. First, evidence of considerable gene flow within two close relatives of *Tropheus* in our study region suggests that gene flow is also likely among the populations studied here (Wagner & McCune 2009). Selection would have to be very strong to maintain differences among them in the face of gene flow. Second, intestinal plasticity has been demonstrated experimentally in perch (Olsson *et al.* 2007), prickleback fish (German *et al.* 2006) and *Tropheus* (P. McIntyre and Y. Vadeboncoeur, unpublished data), and observations in Lake Malawi cichlids suggest shortening of the gut in mouthbrooding females that are unable to feed regularly (Reinthal 1989). More generally, plasticity in internal organs in response to environmental stimuli has been documented in many vertebrates (reviewed in Piersma & Lindstrom 1997; Starck 1999), including fasting snakes (Starck & Beese 2002), migrating birds (Karasov *et al.* 2004) and rodents in fluctuating environments (Naya, Bozinovic & Karasov 2008), and the physiological mechanisms underlying gastrointestinal plasticity are well understood in several taxa (Starck 2003). Thus, we believe that the observed variation in *T. brichardi* intestine length is a largely plastic response to differences in the nutrient content of their algal diet.

Both our broad phylogenetic survey and our intraspecific comparisons suggest that the intestine length of Tanganyikan cichlids is determined in large part by diet quality. Ecological stoichiometry theory provides a framework for evaluating diet quality based on the degree of imbalance between the demand for a nutrient by a consumer and the nutrient content of its food resources (Sterner *et al.* 2002). *Tropheus brichardi* consumes algae that is very low in nitrogen (C : N > 18;

Fig. 3) compared with its own body ($C : N < 6, n = 4, P.$ McIntyre, unpublished data). In the light of this imbalance, the inverse relationship between algal nitrogen content and algal nitrogen content suggests that longer intestines aid in extracting nutrients from low-quality foods. The overall inverse relationship between trophic position in the food web and gut length among 32 species of Tanganyikan cichlids (Fig. 2) also matches broad predictions from ecological stoichiometry. The nutrient imbalance between fishes and their food resources increases from piscivores (no imbalance) to invertivores (moderate imbalance) to algivores (extreme imbalance), and we found that cichlid intestine lengths increase in the same order.

The inverse relationship between intestine length and diet quality matches expectations from the general trade-off between maximizing the extraction of nutrients and energy from the diet and minimizing the maintenance costs of digestive tissues. The presence of parallel patterns both among and within species highlights the functional significance of intestine length. However, the magnitude of intraspecific variation is small compared to that observed in the phylogenetic comparison. The observed range of ILI across species yields a prediction of 11-fold variation in intestine length for a 20 g fish (5.18 cm in piscivores; 56.6 cm in algivores). This is seven times the observed range of variation among *T. brichardi* population means (42.6–49.8 cm for a 20 g fish). For the 10 species of algivores included in this study, the observed range of ILI across species yields a predicted range of 29.6–56.6 cm in intestine length for a 20 g fish, nearly four times the observed range of *T. brichardi* variation. Thus, for both algivorous species only and for the entire trophic range, variation in intestine length among species is far greater than that observed among populations of *T. brichardi*.

Although diet predicts the observed patterns in intestine length at both inter- and intraspecific levels, considerable variation in this relationship remains unexplained. In addition to measurement error in both traits, unexplained variation could arise from the diversity of other intestinal features that influence the efficiency of digestion and assimilation. For instance, surface area, volume, villus area and other features are known to affect intestinal function (e.g. Ricklefs 1996; Lavin *et al.* 2008), and we did not evaluate these aspects of intestinal phenotype. Regardless of its source, the magnitude of the observed variation, and the correlation of this variation with diet, suggest that both plasticity and natural selection have influenced intestine length, although work examining the limits of plasticity in intestine length has not been conducted in any taxon (Starck 1999).

Plasticity is not only an intriguing proximate mechanism for the observed morphological response to differences in diet quality within species, but may also be an important mechanism for accommodating trophic shifts during diversification. The capacity to respond plastically to novel food sources could allow initial divergence in trophic morphology, thereby creating an opportunity for selection to further extend the phenotypic response (e.g. West-Eberhard 2003; Ledon-Retting, Pfennig & Nascone-Yoder 2008; Wund *et al.* 2008). The

role of plasticity in the evolution of mouth morphology, a trait frequently associated with trophic shifts, has been discussed repeatedly in cichlids (e.g. Meyer 1987; Wimberger 1991; Stauffer & Gray 2004). However, changes in diet require a broad suite of morphological changes to fully accommodate the use of novel food resources. The strong relationship between diet and intestine length observed among taxa in this and previous studies indicates that changes in intestine morphology are necessary in order for individuals to successfully undergo dietary shifts. Thus, intestinal plasticity could provide a mechanism for accommodating dietary differentiation over ecological time scales, thereby potentially facilitating diversification, and enabling the remarkable convergence in morphology associated with parallel trophic shifts among East African cichlid fishes.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. The relationship between intestine length and body mass among sampled Tanganyikan cichlid species.

Fig. S2. Mean size-corrected intestine length (ILI) and trophic position in the food web (based on mean $\delta^{15}\text{N}$) for each individual in the data set of Lake Tanganyikan cichlids.

Table S1. Specimen information, intestine length and mass data, and GenBank accession numbers.

Table S2. Primers for amplification and sequencing of the complete cytochrome *b* and for internal sequencing of ND2.

Table S3. Latitude and longitude for six sampling sites near Kigoma, Tanzania, where *Tropheus brichardi* and algal nutrient content data were collected.

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