

The structure of morphological variation: Morphometric analysis of variation in four endemic Lake Tanganyika gastropod species

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Introduction

The study of morphology is a constant interplay between two sides of the same coin: it is both the study of essential defining characters and the study of morphological variation. Traditionally, morphologists studied “types”: phenotypic variability was seen as noise obscuring phylogenetic signal. It is more recently that morphological variation has become a quantity of interest in evolutionary studies (Debat and David, 2001). Phenotypic variation is the result of the interaction between sources of variation and self-regulatory, buffering processes. Variation in morphology is influenced by environmental effects, genetic mutations and developmental perturbations; buffering mechanisms include canalization, phenotypic plasticity, and developmental stability (Debat and David, 2001).

This project is based on the qualitative observation that some species of *Lavigeria* show much more morphological variation than others. The questions to be addressed are: what are the patterns of variation within and between populations of the same species? How do these patterns differ between species? Quantification and analysis of the structure of morphological variation, examined in light of ecology and life history, should produce a more complete picture of the natural history of these snails. Data on scarring and parasitism were collected to put the analysis of morphology in an ecological context.

Methods

The goal of this study is to quantify patterns of morphological variation in four species of *Lavigeria* using geometric morphometrics, a high resolution method to analyze size and shape of biological form.

Taxa & collection sites: Two pairs of gastropod species (*Lavigeria nassa* and *L. grandis*; *L. sp. J* and *L. sp. W*) were selected because they are phylogenetically paired (from two different subclades within the *Lavigeria* species flock) and largely sympatric. Snails were collected from six different sites around Kigoma: Jakobsen’s beach, Hilltop, Kalalangabo, Mzungu Beach, Ulambolo, and Nondwa between July 18 and August 4, 2005 (coordinates and map available in Smith, this volume). Fifty adult snails per species per site were collected along transects (length depending on the density of populations). Only adult specimens were used to keep ontogenetic comparisons consistent. The adults can be recognized because *Lavigeria* snails have determinate growth and adult modifications (Papadopoulos et al., 2004).

Shell Photography: Shells were scrubbed clean of algae, dried and mounted on a pin with ‘Blu-Tack’ putty to prevent shadows below a Nikon Coolpix 4500 digital camera fixed in horizontal orientation on a stand made from a cannibalized dissecting scope. The shell was oriented with the axis of coiling horizontal, and the plane of the aperture as horizontal as possible without altering the orientation of the axis of coiling. A millimeter scale bar was also mounted in the plane of aperture and profile focus. A side view is also taken, with the apertural plane perpendicular to the ‘face view’, such that the edge of the outer lip lined up with the inner edge of the aperture. Orientation is challenging in gastropods but critical for reliable morphometric data collection, thus we repeated the process until the error rate was minimal. An error series of repeated photos of the same shell was also taken to quantify orientation induced artifacts.

Vouchering & dissection: The shell was broken using a vice and the shell fragments vouchered, with collection specifics and individual number in a ‘whirl pack’ bag. Sex and parasite presence were recorded, and the complete brood was removed using a pin and tweezers and preserved in Eppendorf tube with 70% ethanol. The brood will be counted later in order to examine any correlation between brood size (number and size of babies) and maternal morphological features. Two tissue samples were archived for later DNA genetic analysis, one from the upper surface of the foot next to the operculum) and the other from the gonad.

Choice of landmarks: Morphometric landmarks were chosen that are likely to represent homologous points on the shell (Fig 1, left, middle). These were placed on spiral cords (identified by letters as in Papadopolous et al. 2004), apex, and points around the aperture. Twenty-two homologous landmarks were found on *L. grandis*, *L. nassa* and *L. sp. W.* Because *L. sp. J* does not produce spiral cord B, *L. sp. J* was analyzed with a reduced data set of 18 landmarks.

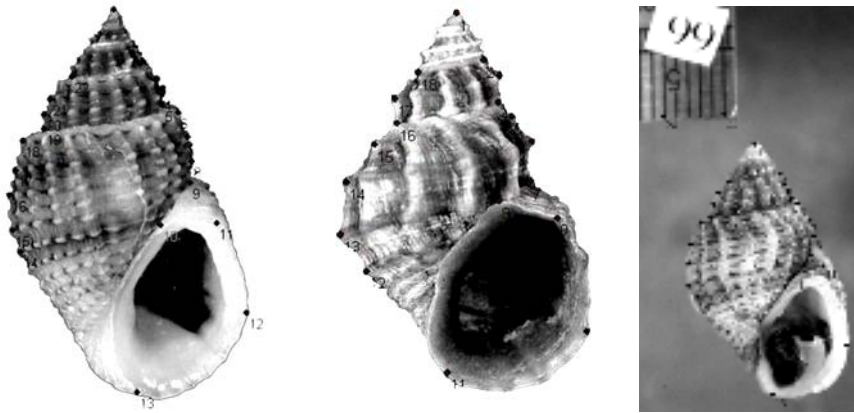


Figure 1. Landmark locations on spiral cords, sutures, apex, and repeatable points around the aperture. (Left) 22 points shown on *L. nassa* also used for *L. grandis* and *L. sp. W.*; (Middle) 18 landmark locations on *L. sp. J*. (Images adapted from Michel/vanArkel/IBED). (Right) *L. nassa* data photograph showing scale bar in plane of focus of aperture & shell profile, numbering system, mounting system to avoid shadows.

Geometric Morphometric Analysis:

The landmarks were digitized from photos using TpsDig (<http://life.bio.sunysb.edu/morph>). The file of digitized coordinates was opened in CoordGen, (from the Integrated Morphometrics Programs (IMP) series <http://www.canisius.edu/~sheets/morphsoft.html>), which was used to scale to unit centroid size, and rotate to minimize the summed squared distances between homologous landmarks - this is the Procrustes alignment (Zelditch et al., 2004). This standard alignment makes it possible to analyze shapes independently of size.

The shapes exist in a hyper-dimensional shape-space where all possible configurations of landmarks are found. Each specimen occupies a specific location in this shape-space, the coordinates of which are given by its partial warp scores. Principal components and canonical variate analyses were done on the partial warp scores to examine the structure of morphological variability. Partial warp scores were calculated in PCAGen6n (IMP). PCA and CVA were done on populations within a single species using PCAGen6n and CVAGen6j (IMP).

Pair-wise comparisons of populations of the same species were done using TwoGroup, an IMP program that allows statistical determination of (a) whether two populations are morphologically distinct or not and (b) the average distance between average shapes of two populations or species.

Results

Analysis of morphological variation

All four species showed morphological differences between sites.

L. nassa

The *L. nassa* populations from six sites were distinct but tightly structured. There were five distinct canonical axes, and the grouping assignment test grouped 84.5% of the snails to their correct site, however the graphical representation showed considerable overlap between the groups. The *L. nassa* individuals from Jakobsens had the most long and narrow shape, while those from Hilltop were the squattest.

There was no significant sexual dimorphism or parasite-induced morphological effect in this species. The CVA of the females, males, and parasitized individuals from all sites produced one distinct axis and correctly assigned only 60% of specimens to these three categories. It is notable that *L. nassa* females tended to be more morphologically distinct by site than the males: the CVA of the females by site produced four distinct canonical axes, while the CVA

of the males only one distinct axis. In addition, 82.9% of the females were correctly assigned to site by the assignment test (CVA Gen) whereas only 49.4% of the males were correctly assigned to site.

L. grandis

The *L. grandis* populations from five sites were distinct but with site-similarities by region, not tightly structured, and clearly sexually dimorphic. The assignment test grouped 93% of the specimens to the correct population, and correctly designated 81% of the specimens as either male or female (there were very few parasitized individuals, so they were excluded from the analysis). The graphical groupings of *L. grandis* are more spread out than the groupings of *L. nassa*, indicating a larger variance in morphologies. Along CV1 (which describes the most morphological variation), there was a grouping of populations from Nondwa & Kalalangabo on the one hand, and Jakobsens, Ulambola & Mzungu on the other hand. The morphometric gap matches the geographical separation between the coastline north of Kigoma bay (Nondwa and Kalalangabo) and south of Kigoma bay (Jakobsens, Mzungu, Ulambola). The *L. grandis* individuals from Jakobsens, Mzungu and Ulambola were relatively squatter than those from Nondwa and Kalalangabo, which were taller and narrower. The greatest axis of morphological variation in *L. grandis* seems to match geographical separation north/south of Kigoma Bay.

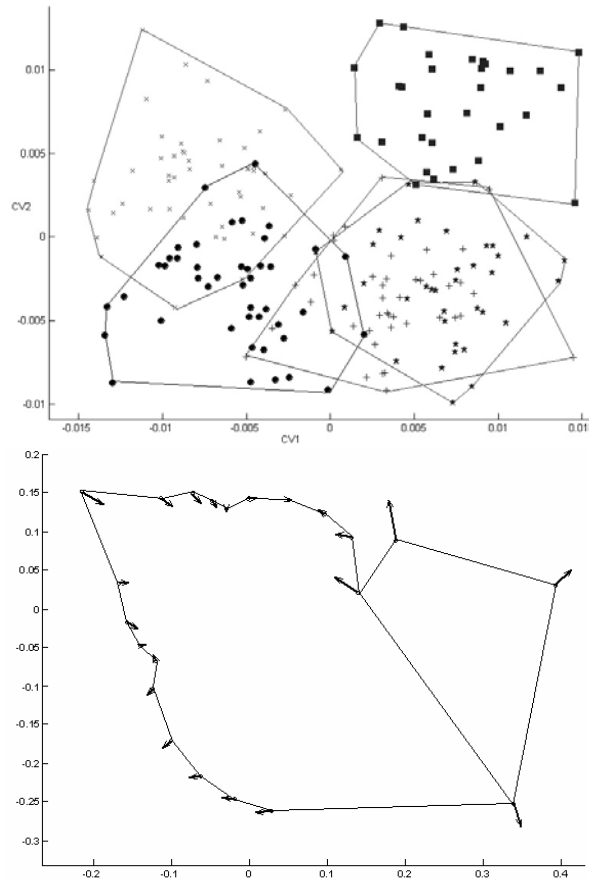


Figure 2. CVA of all *L. grandis* populations, and shape change associated with CV1. Kalalangabo (dot, bottom left); Nondwa (X, top left); Jakobsens (star, bottom right); Mzungu (square, top right); Ulambola (+, bottom right).

Lavigeria. sp. J

Individuals of *L. sp. J* were distinct by site and sex. The discriminant analysis produced two distinct canonical axes. The assignment test grouped 97.4% of the specimens simultaneously to the correct site and sex. The first canonical axis (accounting for the most variation) distinguished the snails by site and the second axis distinguished the females from the males. Nondwa *L. sp. J* had a shorter shell while Hilltop snails had a taller shape. In the Nondwa specimens, the cords on the lower whorl were relatively closer together and the whorl was horizontally expanded.

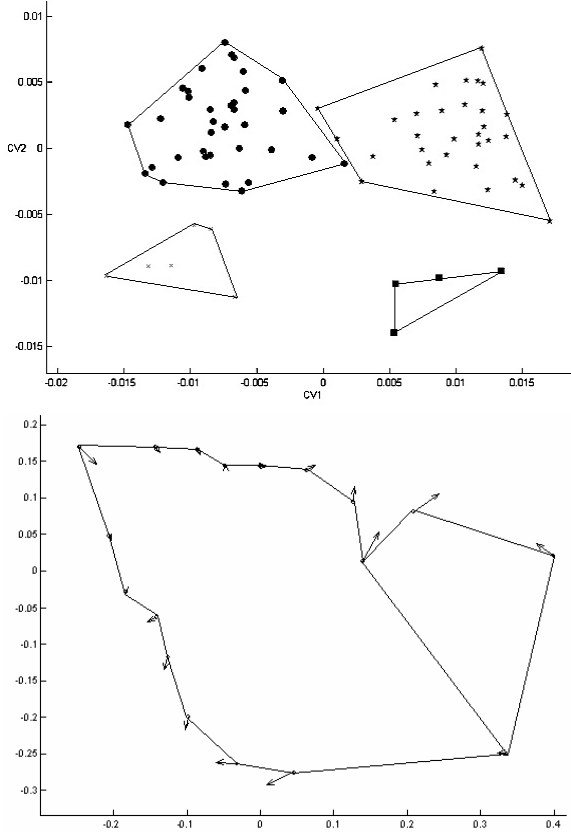


Figure 3. CVA of *L. sp. J* and shape change associated with CV1. Hilltop females (dots, top left); Hilltop males (X, bottom left); Nondwa females (stars, top right); Nondwa males (squares, bottom right).

L. sp. W

The individuals of *L. sp. W* were also morphologically distinct by site and sex. There were two distinct canonical axes and 97.3% of the snails were correctly assigned to site and sex categories. The first canonical axis distinguishes the individuals of species W by site, and the second axis distinguishes them by sex. The spiral cords on the Mzungu individuals are relatively farther down on the last whorl. The females and males differ in the last whorl, which is horizontally expanded in the females compared to the males.

Ecological patterns

Table 1. Sex ratios and parasitism prevalence.

Site	Species	Proportion of females	Proportion of males	Proportion parasitized (unknown sex)
Kalalangabo	<i>L. grandis</i>	0.82	0.18	0
Nondwa	<i>L. grandis</i>	0.88	0.12	0
Jakobsens	<i>L. grandis</i>	0.48	0.50	0.020
Mzungu	<i>L. grandis</i>	0.45	0.48	0
Ulambola	<i>L. grandis</i>	0.52	0.40	0.080
Kalalangabo	<i>L. nassa</i>	0.49	0.41	0.048
Nondwa	<i>L. nassa</i>	0.70	0.23	0.066
Hilltop	<i>L. nassa</i>	0.63	0.31	0.020
Jakobsens	<i>L. nassa</i>	0.32	0.42	0.260
Ulambola	<i>L. nassa</i>	0.66	0.24	0.080
Mzungu	<i>L. nassa</i>	0.43	0.30	0.033
Nondwa	<i>L. sp. J</i>	0.81	0.10	0.032
Hilltop	<i>L. sp. J</i>	0.84	0.14	0
Kalalangabo	<i>L. sp. W</i>	0.76	0.20	0.020
Mzungu	<i>L. sp. W</i>	0.75	0.22	0

The proportion of females, males and parasitized individuals varies from species to species and from site to site. *L. grandis* demonstrates a lot of variability, from 88% females at Nondwa to 48% females at Jakobsens's. *L. nassa* also shows quite some variability: from 70% females at Nondwa to only 32% females at Jakobsens. *L. sp. J* and *L. sp. W* do not show significant differences in proportions of females/males/parasitized individuals between sites although both species show a strong female bias. *L. sp. J* populations are on average 82% female while *L. sp. W* are, on average, 75.5% female.

Parasitism is unusually high for one population of *L. nassa* (26% at Jakobsens); otherwise the proportion of parasitized individuals is relatively low.

Lavigeria sp. J had the lowest scarring frequency (3.6%). The other three species had variable scarring frequencies; *L. grandis* and *L. nassa* at Jakobsen's Beach were frequently scarred (50-60% scarred individuals at Jakobsens) whereas the average scarring frequency elsewhere was roughly 20% for *L. grandis*, *L. nassa*, and *L. sp. W*.

Discussion

The discriminant analyses of *L. grandis* populations show clear morphological structuring between sites. The geographic division of populations north and south of Kigoma Bay match the morphological divergence. This suggests that Kigoma Bay represents a barrier to dispersal and that the populations north and south of the bay are genetically isolated. However, this does not seem to be the case: analysis of COI DNA sequence of populations from Kalalangabo and Jakobsens shows that populations of *L. grandis* share a number of common haplotypes, indicating gene flow between these regions (Michel et al. 2005). This presents a puzzle: the genetics argues that *L. grandis* appears to have good dispersal ability despite its relatively large babies and small broods (Michel et al. 2005), but the morphology indicates population differences. Phenotypic plasticity and habitat differences might be causing the different shapes of *L. grandis* populations or there might be a sampling phenomenon in that the genetics, with smaller sample sizes, is less representative of the population means than the morphological analyses. Because we have full photo documentation of all the specimens collected for further genetic analyses, we will eventually be able to address these alternatives. Two questions arise: (a) how does *L. grandis* disperse across Kigoma bay? (b) What physical and/or ecological factors might be inducing different morphologies in populations north and south of the bay?

The discriminant analysis of *L. nassa* populations shows tight morphological structuring—the populations of *L. nassa* are harder to distinguish morphologically than populations of *L. grandis*. Paradoxically, *L. nassa* populations seem to have more unique COI DNA haplotypes than *L. grandis* Michel et al. 2005), indicating less effective dispersal than its sister species *L. grandis*, despite its smaller babies and larger broods. A comparison of the sister species *L. grandis* and *L. nassa* produces an interesting pattern: the species with least genetic divergence is morphologically variable between sites while the species with a relatively conserved morphology appears to be genetically differentiated. One hypothesis might be that morphological variability does not arise from genetic variability—a reasonable hypothesis *a priori*—but instead from largely self-organizing epigenetic processes.

The discriminant analyses of *L. sp. J* and *L. sp. W* cleanly distinguish the individuals by site and sex. Qualitative observations indicate, however, that both of these species show quite a lot of variability within and between populations. In both cases, the first axis separates the populations by site and the second axis separates males from females, indicating that variability between sites is larger than sexual dimorphism. Both populations are strongly female biased. This reduces the “effective population size,” a theoretical measure of the number of individuals contributing to the gene pool, if the population is isolated from other populations, if the individuals mate at random and if there is no unbalanced mutation, selection or genetic drift (Hardy-Weinberg conditions).

While the probability of encountering predators at each site has not been quantified, explanations linking morphology with predation can be proposed. In general, scars can be interpreted as unsuccessful predation attempts (prey survival to predation attempt). The frequency of scarring should increase if (1) the probability of encountering a predator increases; (2) the probability of surviving an attack increases; (3) the age of the prey increases (the older the prey, the more likely it will have had an encounter with a predator) (Vermeij, 1987). Phifer et al. (2001) found that in *L. nassa*, individuals with fewer ribs and shorter apertures were more likely to bear scars. Possible explanations include directional selection by the predators or induced morphological responses in the prey. Strength of selection or frequency of induced phenotypes should vary with scarring frequency.

This is interesting in the context of large morphological variability in *L. sp. J*, which has an unusually low scarring frequency (3.6% individuals bear scars). Low scarring frequency may indicate weak selection on shell morphology, which permits a wider range of morphological variation. Additionally, cues for the presence of predators should induce snail defenses, but the absence of cues does not necessarily indicate absence of predators. Therefore, in environments where cues are absent, snails should produce average morphologies with greater variance than snails in the presence of predators (DeWitt and Scheiner, 2004). This strategy, “bet-hedging,” seems consistent with *L. sp. J*’s morphological and ecological traits. Several hypotheses can be proposed to explain the morphological variability in *L. sp. J*: (a) variability is permitted by weak selection on shell morphology, because predators are not present in high numbers where *L. sp. J* is found; (b) *L. sp. J* adopts a bet-hedging ecological strategy, because predators are present, but do not produce cues to which *L. sp. J* can detect and respond. Field data on the density of predators and frequency of encounters at various sites should allow us to support or reject these hypotheses.

Conclusion

The gastropod species of Lake Tanganyika are impressive not only because the many species are morphologically divergent, but also because each species’ patterns of morphological *variation* are so different. Patterns of variation are symptomatic of processes occurring on several levels: (1) different proportions of females and males in the populations imply different structures genetic diversity, which in turn has implications for phenotypic diversity; (2) varying strength of predation may induce different ecological and morphological strategies for survival; (3) autoregulatory developmental processes vary from species to species in their ability to buffer against environmental or genetic perturbations. Genetic, ecological and developmental sources of phenotypic variation may contribute to variation in these four species. Perhaps the most interesting pattern suggested by the results is the inverse relationship between genetic and phenotypic diversity: phenotypic variability does not follow from genetic diversity, and genetically close individuals produce large amounts of phenotypic variation. Specific morphological traits in *L. nassa* were linked to survival to predation attack (Phifer et al. 2001). However, morphological

variation failed to correlate with parasitism in *L. nassa* (D. Athuman, Nyanza 2004), and failed to correlate with wave energy in *L. grandis* (K. Hinkley, Nyanza 1999). The difficulty in linking snail morphology with environmental and genetic factors may lie in the fact that morphology is in large part the result of self-regulating epigenetic processes acting in the context of their (genetic and external) environments.

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References

- Athuman, D. Size and shape variation in *Lavigeria nassa* in relation to parasitism and site differences: a multivariate morphometric approach. Nyanza Report 2004.
- Debat V., David P., 2001. Mapping phenotypes: canalization, plasticity and developmental stability. *Trends in Ecology and Evolution*, 16 (10): 555- 561.
- DeWitt T. J., Scheiner S. M., 2004. Phenotypic variation from single genotypes. In Phenotypic Plasticity, Eds. DeWitt and Scheiner, Oxford University Press.
- Hinkley, K. Is variation in shell morphology of *Lavigeria grandis* an ecophenotypic response to wave energy? Nyanza reports 1999.
- Michel, E., J. Kuh & R. Thomas. 2005. Genetic structuring among sympatric sister species: correlates of diversification in a gastropod species flock. Systematics Association Biann. Mtg, Cardiff, UK.
- Papadopoulos L. N., Todd J. A., Michel E., 2004. Adulthood and phylogenetic analysis in gastropods: character recognition and coding in shells of *Lavigeria* (Cerithioidea, Thiaridae) from Lake Tanganyika. *Zoological Journal of the Linnean Society* 140: 223-240.
- Phifer M., McIntyre P. B., Michel M., 2001. The response of *Lavigeria nassa* shell armoring characters to crab attack, Published abstract, World Congress of Malacology, Vienna.
- Rohlf, J. TpsDig. <http://life.bio.sunysb.edu/morph/soft-dataacq.html>
- Sheets, H. D., Integrated Morphometrics Programs. <http://www2.canisius.edu/~sheets/morphsoft.html>
- Vermeij, G. 1987. Evolution and Escalation: an Ecological History of Life. Princeton University Press, Princeton, N.J.
- Zelditch, M. L., Swiderski D. L., Sheets H. D., Fink W. L., 2004. Geometric Morphometrics for Biologists: a Primer. Elsevier Academic Press.