

Original Article

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Shell variations in the gastropod, *Monodonta labio*, in the North-western Pacific: the important role of temperature in the evolution process

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Abstract

Molluscan shells showing phenotypic variations are ideal models for studying evolution and plasticity. In north-eastern Asia, genetic and morphological diversity of the gastropod, *Monodonta labio*, were assumed to be influenced by both palaeoclimatic changes and current ecological factors. In this study, we examined spatial variations in shell shape of *M. labio* using general measurement and geometric morphometric analysis. We also investigated whether shell shape variation is best explained by environmental gradients or by genetic structuring, based on our prior molecular phylogeographic study. Two common morphological forms were observed among Chinese populations and in the adjacent Asian areas. Both the analyses revealed separation patterns in morphological variations of shell shape among the clades and populations. Environmental modelling analysis showed a significant correlation between shape variations and local maximum temperatures of the warmest month, indicating the role of natural selection in the evolution of this species. Data obtained in this study, combined with the cytochrome oxidase subunit I (COI) molecular phylogenetic data from the prior study, showed that morphological variations in *M. labio* were constrained by both local adaptation and phenotypic plasticity. We hypothesized that geographic separation by the Dongshan Landbridge was the first step towards its diversification, and that the temperature gradient between the East China Sea and South China Sea probably was the selective force driving the divergence of its morphological variations.

Introduction

The study of evolution and plasticity can provide insights into the roles of ecology and evolution in adaptation and speciation processes (Daniel *et al.*, 2002; Adams & Collyer, 2009). Adaptation of populations to different habitats, regarded as the first step towards ecological speciation (Rundle & Nosil, 2005; Schluter & Conte, 2009), can be induced by natural selection in two ways: first, by direct action on the additive genetic component, and second, by phenotypic plasticity (Johnson & Black, 2008; Hollander & Butlin, 2010). Differing mechanisms associated with local genetic adaptation and phenotypic plasticity may ultimately produce similarly high levels of phenotypic differentiation among populations. However, differentiation in morphological traits does not always indicate the occurrence of adaptation, because genetic drift may also influence patterns of variation (Dowle *et al.*, 2015). Additionally, environmental factors exerting selection, both biological (parasites, predators and competitors) and non-biological (physical factors, for example, wave splash and temperature), may vary with the geographic distribution of a species (Avaca *et al.*, 2013). Thus, it is difficult to determine the role of natural selection in shaping geographically partitioned variation. Understanding the role of divergent ecological selection requires quantification of genetic and plastic variation, particularly in response to environmental variables (Dowle *et al.*, 2015).

Quantifying phenotypic variation is the crucial first step towards understanding the evolution and ecology of species (Adams & Collyer, 2009). Phenotypic variation may be attributed to genetic differences, phenotypic plasticity, or both. These causes are not mutually exclusive, because evolution of adaptive phenotypic plasticity may lead to the success of organisms in novel habitats, and potentially contributes to genetic differentiation and speciation (Agrawal, 2001). Habitat-specific convergent evolution and local phenotypic adaptations provide feasible means for testing adaptive hypotheses (Dowle *et al.*, 2015; Yeaman *et al.*, 2016). In both cases, phenotypic traits would be in response to environmental factors rather than neutral genetic drift. Based on information on genetic variation, we can test the hypothesis that traits are adaptations resulting from local selection, through quantification of phenotypic variation.

Among the phenotypic traits, morphology is a general feature related to fitness, and is therefore under strong selective pressure (Johannesson *et al.*, 1993). Marine gastropods can act as useful models for studying fitness, due to the evolutionary and ecological significance



and to superimpose the coordinates into a common system. The above procedures were implemented using TpsRelw, version 1.69 ([Morphometrics at SUNY Stony Brook](#)). The new Cartesian coordinates generated from superimposition were imported into Morphology 1.06d (Klingenberg, [2011](#))

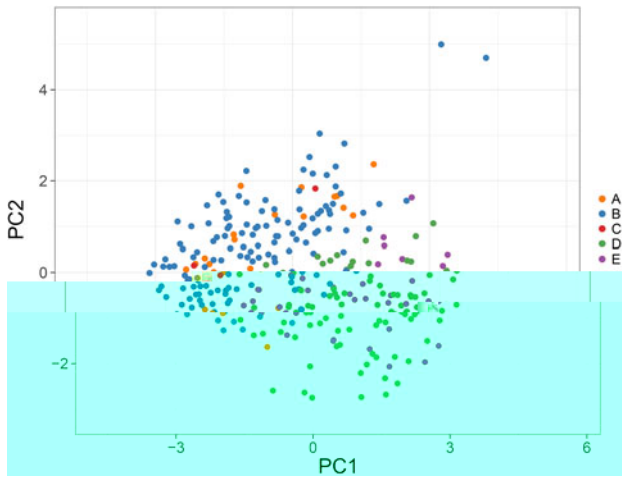


Fig. 4. Shell shape variation of *M. labio* as a function of the first two principal components, based on general measurement analysis of the five measured characters (except for the roughness of granules on the spiral ribs above the suture near the aperture).

Asia was influenced by local environmental factors or by neutral genetic drift.

Morphometric distances among geographic populations were based on Procrustes distances calculated from the CVA. The function earth.dist of the FOSSIL package (Vavrek, 2011) in R was used to determine geographic distance between each pair of sites. Due to the intertidal habitat of *M. labio*, we collected environmental data for each sampling site from WORLDCLIM 1.3 (Hijmans et al., 2005) and WORLD OCEAN ATLAS 2013 (Climate Data Guide) databases, using DIVA-GIS 7.4.01 (Hijmans et al., 2001) and Ocean Data View, respectively. Finally, 19 available Bioclim variables (WorldClim-Global Climate Data; Table S2) of climatic conditions and yearly averages of sea surface temperature and salinity of oceanic conditions were downloaded as the environmental attributes. F_{ST} values were calculated using DnaSP 5.10 (Librado & Rozas, 2009) based on the mitochondrial cytochrome oxidase subunit I (COI) dataset corresponding to the samples of prior study. F_{ST} values were chosen as proxies for the pairwise genetic distance.

The relationships among morphometric distance, pairwise genetic distance and geographic distance were examined by mixed linear modelling using the R package LME4 (Bates et al., 2014), due to the non-independence of the pairwise geographic distance data and pairwise genetic distance. According to the Akaike Information Criterion (AIC) scores, a mixed linear model with random intercepts (AIC = -320.244) was more appropriate to model the morphological, genetic and geographic data, relative to a mixed linear model with random intercepts and slopes (AIC = -316.596).

A general linear model was used to resolve the relationship between shell shape (Procrustes distances), shell size (CSs) and environmental attributes, because the data were independent.

Results

In this study, 287 individuals from 13 sampling sites (Figure 2) were used. Excluding one partially damaged sample, 286 shells were used for geometric morphometric analysis, and 287 shells for the general measurement analysis. The number of samples in each population ranged from 1 to 47 individuals (Table 1).

Descriptive statistics of all six shell variables are shown in Table S1. The shells of *Monodonta labio* populations showed wide variability within the sampling sites. Two distinct morphological forms of *M. labio* were observed in our samples: (1)

globular form with a lower apical acuteness and flat spiral ribs and (2) high-apex form with a higher apical acuteness and granular spiral ribs (Figure 3). Each of them dominates different regions along the Chinese coast, but both of them occurred in the southern areas. Although the globular form was more common in the northern part of the Chinese coast, the high-apex form dominated the southern areas.

In the general measurement method, principal component analysis (PCA) was successful in transforming the original five shell parameters into five components that could decipher most of the variation. Contributions of each of the five shell parameters to the five principal components (PCs) are shown in Table 2. The first two components accounted for as much as 79.9% of total variance (axis 1: 53.1%, axis 2: 26.7%). Ordinations of the first two principal components reveal an obvious segregation pattern between the two genetic clades (Clade A + B + C and Clade C + D; Figure 4), although slight overlap was detected. Linear discriminant analysis (LDA) of the first two principal components correctly classified 86.7% of the training data set and 95.1% of the test data set. According to the logistic regression model, shell height ($P = 0.690$) and width ($P = 0.674$) did not have a significant relationship ($P > 0.05$) with genetic grouping. However, the remaining independent variables, including roughness of granules on the spiral ribs, were statistically significant ($P < 0.05$). The classification rate was 97.2% based on the test dataset.

In the geometric morphometric method, 28 principal components were transformed from the 16 landmarks. The first four components cumulatively explained 68.3% of overall variance (axis 1: 27.4%, axis 2: 21.9%, axis 3: 10.4% and axis 4: 8.6%). However, individuals belonging to different genetic groups or geographic populations are mixed up along the first two PC axes (Figure 5A); none of the differences reflected distinctness among genetic groups and sites. In contrast, the degree of separation between the two genetic clades is evident along the PC5 (6.7%) and PC6 axes (5.7%), although they slightly overlap with each other (Figure 5B). The first four components summarized substantial variations, which correlated with characters around the aperture (reflected by the displacement of landmarks, 4–7 and 11–16; Figure 6A, B). Landmarks related to thickness of callus at the uppermost end of the aperture (LM 11 and LM 12; Figure 1A) exhibited the highest variation among the first four components (Figure 6A, B). PC5 and PC6 (Figure 6C, D) mainly reflected the distinctions between height and/or width of the apical whorls (LM 2 and LM 10) and the upper side of the last whorl (LM 3, LM 4 and LM 9), which is probably associated with the apical acuteness of shells (Figure 6C, D).

Concordance of shell shape variation among genetic groups was revealed in both canonical variate analysis (CVA; Figure 5C) and discriminant function analysis. Additionally, discriminant function analysis of geometric data revealed a significant morphological differentiation among both geographic populations and genetic clades. Specifically, among the genetic groups, the first canonical axis (CV1) explained 74.58% of the shell shape variation. In contrast, only 35.49% of between-population shape variation was explained by CV1.

The importance of specific characters in discriminating different groups for *M. labio* can be seen in Figure 6. Recognizable differences among genetic clades represented by the deformation grids in CV1 (Figure 6E) included height of the upper and lower sides of the last whorl (LM1, LM2, LM3 and LM10), apical acuteness (LM2 and LM 10) and width of the aperture (LM13). Among geographic populations (Figure 6F), thickness of callus at the uppermost end of the aperture (LM 11 and LM 12), shape of the aperture (LM5 and LM 16) and apical acuteness (LM2, LM4 and LM10) contributed majorly to the variation represented in CV1.

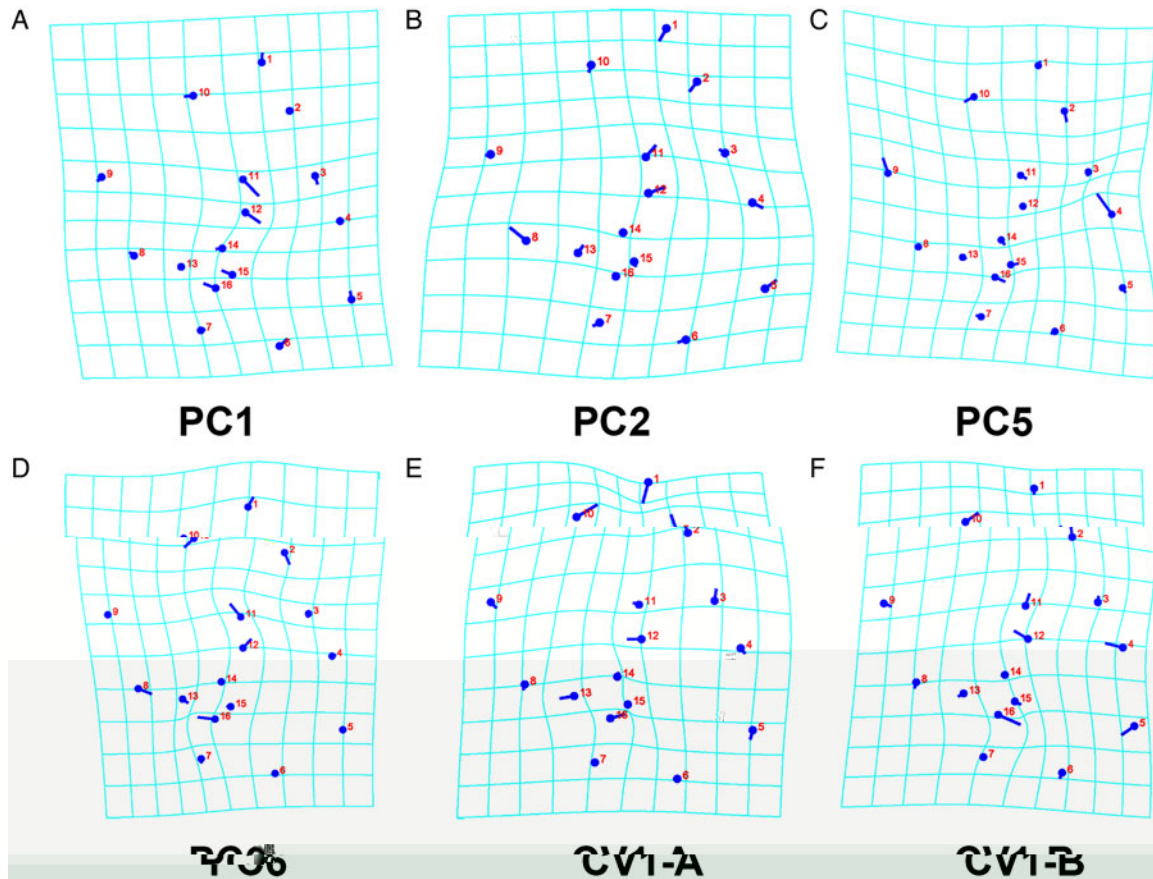


Fig. 6. Shape variations shown by deformation grids in the geometric morphometric analysis. (A) Shape deformation associated with principal component (PC) 1; (B) Shape deformation associated with PC2; (C) Shape deformation associated with PC5; (D) Shape deformation associated with PC6; (E) Shape deformation associated with the first canonical variate axis (CV1) in canonical variate analysis (CVA) among genetic clades; (F) Shape deformation associated with CV1 in CVA among geographic populations.

area, tend to be advantageous, because these characters can reduce the risk of dislodgement by waves (Trussell *et al.*, 1993; Forrester *et al.*, 2016). In contrast, in the case of protected shores with reduced wave splash, i.e. locations such as Bohai Bay in China, thick shells and small apertures are favoured (Wang *et al.*, 2013). However, hydrodynamic conditions were not recorded at sampling sites. Generally, plastic variations are related to local ecological factors, such as wave action, desiccation and predation risk, and their interactions (Vermeij, 1973). It is also important to note that allometry detected in this study may also result from differing microhabitat environments. These morphological differences were probably associated with sites with different environmental variables, so that at the local scale, those environmental variables may play a strong role. This suggestion is supported by the morphological difference among sites.

In conclusion, the morphological variations in *M. labio* are due to local adaptation, phenotypic plasticity, as well as allometry, and these factors would maximize fitness under the variable environmental conditions along the Chinese coast and possibly also in other areas in Asia.

Palaeoclimate background

Investigating the processes that produce divergent forms and diversity is vital in understanding the origins of new species in evolutionary biology.

In this study, different distribution patterns of the two forms may indicate the crucial differences in their evolution. As local adaptation led to the morphological differences with genetic background, it is important to identify the selection pressure. So far,

information is limited on how selection favours the maintenance of dimorphism in populations of *M. labio* within the studied geographic area. However, divergence time estimates in a prior study (Zhao *et al.*, 2017) may provide some clues for investigating this aspect in a palaeoclimatic context. The presence of two distinct forms is in agreement with the emergence of the Dongshan Landbridge during the early Pleistocene (Zhao *et al.*, 2017). Obviously, the geographic separation between the two forms may have been the first step towards their divergence. As morphological variation did not result from neutral genetic drift, temperature gradient-mediated selection between the two sides of the Dongshan Landbridge would have resulted in subsequent genetic adaptation of populations either side to different local environmental conditions.

Because of the north–south orientation of the Chinese coastline, some structural variations in shell shape could correspond to different temperatures (Irie, 2005; Watson *et al.*, 2012). During the early Pleistocene glacial epoch (2.5 million years ago), the sea temperature gradient between the ancient East China Sea and the ancient South China Sea was steepened due to the strengthened monsoon (Wang, 1994). The difference in upper temperature limits may have acted as a selective force promoting the divergence between the two morphological forms. This idea was partially confirmed by significant correlation between PC5 and the maximum temperature of the warmest month in environmental modelling.

Individuals inhabiting the northern temperate region, in the present study, tended to show smoother spiral ribs and more globular shell. In contrast, the typical southern form exhibited higher apical acuteness, which suggests a higher spire and smaller

aperture. This pattern of shape variation is in accordance with the model proposed by Vermeij (1973), suggesting that the increase in heat stress on gastropods at lower latitudes tends to favour slimmer shells with smaller apertures (thus reducing the contact area with substrate and enabling more efficient cooling) as adaptations to heat stress. The individuals in southern areas tend to possess granular spiral ribs (Table S1). This trait can maximize the influence of convection by reflecting heat from the shell surface to further reduce thermal stress (Wong & Lim, 2017).

The phenotypic traits and population genetics support the hypothesis that the initial genetic isolation from the geographic separation by the Dongshan Landbridge and subsequent temperature gradient-mediated selection resulted in continued morphological and genetic divergence.

Taxonomic status

Monodonta labio and closely related species (see below) are widely distributed in the Indo-West Pacific. Although its taxonomy has been established and stabilized for a long time, the results of recent studies (Yamazaki *et al.*, 2017; Zhao *et al.*, 2017) and this study suggest that *M. labio* diversified regionally both in genetic and phenotypic traits. Among the eight valid species or subspecies recognized in Asian *Monodonta*

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