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Fisheries Research 67 (2004) 93–98

FISHERIES RESEARCH

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Short communication

Discriminating between geographical groups of a Mediterranean commercial clam (*Chamelea gallina* (L.): Veneridae) by shape analysis

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Received 4 October 2002; received in revised form 28 May 2003; accepted 24 July 2003

Abstract

The venerid clam *Chamelea gallina* is a popular food item around the Mediterranean countries, with clams differing in market price according to their origin. On Mallorca, autochthonous clams are 50–100% more valuable than those coming from the Adriatic Sea, the more important commercial fishery of the western Mediterranean. In order to promote the consumption of autochthonous clams and avoid fraud, the local government of the Balearic Islands inspects the source labelling of clams for sale. However, it is difficult to distinguish the Mallorcan clams from those coming from other sites only by visual inspection. Shape analysis is a possible discriminating procedure to be applied, but *C. gallina* is a rounded object apparently poorly suitable to be morphometrically analysed. Here we explore the discrimination power of a morphometric analysis that combines elliptic Fourier decomposition of the shell perimeter and canonical variate analysis. Specimens from Mallorca were well differentiated from those from Italy and NE Iberia (rate of erroneous assignment <1%), and were more similar to those from Andalucia (S. Iberia) and Formentera (Balearic Islands). Moreover, the analytical approach applied permits visual and intuitive interpretation of inter-group differences in shell outline.

Keywords: Inter-group discrimination; Image analysis; Outlines; Elliptic Fourier transformation; Canonical variate analysis; Biogeography; *Chamelea gallina* (L.); Mollusca; Veneridae; Mediterranean

1. Introduction

The venerid clam *Chamelea gallina* is a popular food item around the Mediterranean countries, with clams differing in market price according to their origin. On Mallorca, autochthonous clams are 50–100% more valuable than those coming from the Adriatic Sea, the more important commercial fishery of the western Mediterranean. In order to promote the consumption of autochthonous clams and avoid fraud, the local government of the Balearic Islands inspects the source labelling of clams for sale.

However, it is difficult to distinguish the Mallorcan clams from those coming from other commercially exploitable geographical groups (this term is preferred against *population* because there are no data on the stock genetic structure) found along the western Mediterranean. Morphometric analysis of shell shape and size seems a priori to be a realistic alternative for inter-group discrimination because the variability displayed by this species is noticeable (shell size and sculpture pattern have been used to describe some taxonomically independent entities). Among different

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doi:10.1016/j.fishres.2003.07.006

morphometric approaches, conventional analyses are not suitable as discriminating tools because a very small number of distances can be measured from such type of "rounded" objects, but interesting developments have been done recently in the so-called "geometric" methods (Cadrin, 2000; Rohlf and Marcus, 1993). Concerning the geometric methods that focus on the contour of rounded objects (Marcus et al., 1996), the widely applied elliptic Fourier analysis (EFA) fits a Fourier series (a mere increasingly long combination of sine and cosine functions) to the points defining a contour.

Geometric morphometric methods differ from conventional methods in the way that the results are displayed because the former are intended to facilitate visual and intuitive interpretation (http://life.bio.sunysb.edu/morph/). Therefore, here we not only evaluate the success in between-group discrimination, but also describe visually the differences between seven geographical groups of *C. gallina*.

2. Methods

2.1. From clams to images

We analysed 668 individual clams belonging to seven geographical groups from the central and western Mediterranean (Fig. 1). After removing the soft parts, right valve was placed (concave side upwards) under a videocamera (Microm-ECV with a Cosina 50 mm objective). Optimas 6.0 was used for image pre-treatment (increasing contrast, automatic generation of outline along shell perimeter, and determination of area inside).

2.2. Obtaining morphometric variables from outlines

A series of 100 equidistant points was generated following the outline (clockwise). The starting point was always placed at a specific location of the *lunula*. The *xy* coordinates of these 100 points were recorded using TPSdig (Rohlf, 2001).

Adjustment for size and orientation was needed at this step to allow proper between-clam comparisons (Rohlf, 1990; Monti et al., 2001). Otherwise, the biological interpretation of the results would be doubtful (Bookstein et al., 1982). Alignment involved two steps: Firstly, the entire outline was translated until the *centroid* (i.e. the centre of the 100 points; Slice et al., 1996) was placed at the coordinate origin. Secondly, the axis determined by the starting point and the centroid was rotated until overlaying the abscissas axis. The outlines were then scaled so that all had the same area.

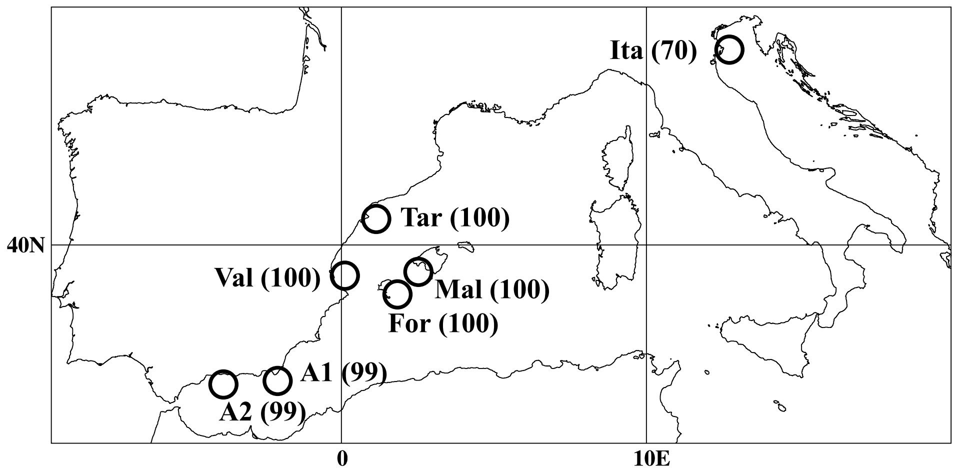

Fig. 1. Location of the seven geographical groups studied. Sample sizes (number of clams analysed) are indicated in brackets. Codes: A1 (Cabo de Gata, Almería), A2 (Málaga), For (Formentera, Balearic Islands), Ita (Venice, Italy, Adriatic Sea), Mal (Palma de Mallorca, Balearic Islands), Tar (Tarragona) and Val (Valencia).

After alignment and scaling, the 100-point series were subjected to an EFA that approximated the original outline by progressive addition of harmonics, each of them defined by a specific number of morphometric variables (Ferson et al., 1985). The EFA output for 10 harmonics was composed of 42 morphometric variables. These variables are homologous to the measurements of a conventional morphometric analysis. This step was completed using NTSYS.

2.3. Statistical analyses

The significance of between-group differences found in shape was evaluated using discriminant analysis (=canonical variate analysis, CVA). Moreover, the "average" shape of each group was placed in a point of a new space (the canonical space), accordingly to its resemblance to the shape of the other groups. Each axis of this canonical space is a shape gradient between two extreme configurations. Visual interpretation of the shape changes experienced along these canonical axes (and at any point in the canonical space) is possible by means of a simple and elegant approach (Monti et al., 2001). This procedure involves three steps: (1) calculating the multivariate regression of the morphometric variables (dependent variables) on the canonical variables; (2) using the regression model to predict the values of the morphometric variables corresponding to any point within the canonical space; (3) recovering the shape corresponding to these values using the inverse elliptic Fourier transformation (implemented in NTSYS).

As the objects were scaled at the first steps of the analysis, the Fourier parameters could include an allometric component. Therefore, it is possible that the differences found in shape could be size-dependent if the samples differed significantly in size (as they did, see below). This is not a trivial point because the abundance of clams belonging to specific size classes is probably related with specific management procedures. Therefore, inter-group shape differences could be simply the result of some biases in the distribution of size classes. We explored the relationship between size and shape in a number of ways. First, we evaluated the existence of an allometric pattern at intra-group level (multivariate regression of size on the harmonic coefficients). Second, we compared the allometric patterns found in the different groups (testing the significance of interactions between size and group). Finally, we regressed the scores derived from the CVA on size and group, looking for the percentage of the variance in shape explained by size only (variance decomposition were completed as suggested by Legendre and Legendre, 1998). Multiple regressions were done using CANOCO 4 (ter Braak and Smilauer, 1998).

2.4. Cross-validation

A total of 20 re-sampling simulations were carried out to check the feasibility of the method used to classify correctly the samples. At each simulation, 20 clams were selected randomly from each of seven sources or 140 clams. Twenty successive canonical variate analyses were completed on the training data set (i.e., the remaining 528 clams), and the resulting discriminant functions were used to predict the putative source of the clams from the testing data set (140).

3. Results

Geographical groups were successfully discriminated using CVA on the morphometric variables obtained from EFA. Shape differences between groups were highly significant (Wilk's $\lambda = 0.011$, approximate $F = 16.5$, d.f.1 $= 252$; d.f.2 $= 3696.4$, Prob. $< 10^{-100}$).

The between-group pattern of shape similarities was displayed by means of the position of "average" shape of each group in the canonical space. Shape differences accounted for the two first canonical axes (explaining 68.2% of the variance) are shown in Fig. 2. One key advantage of geometric methods is that the canonical axes are interpreted as shape gradients between two extreme configurations. These extreme configurations have been visualised using the inverse elliptic Fourier transformation, and are also shown in Fig. 2. The geometric interpretation of the first canonical axis is that the clams from Mallorca are more dorsally peaked than the other groups. Similarly, the second axis is visually interpreted as a gradient of relative depth shell.

Another example of the ability of geometric methods for a visual description of the differences in shape is the comparison between pairs of groups (Fig. 3).

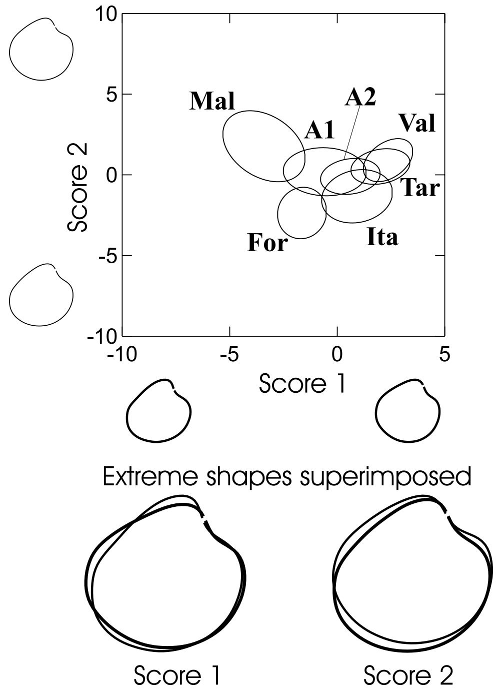

Fig. 2. CVA of the Fourier coefficients. Bivariate ellipses for the geographical groups centred on the group-averaged values for the two first canonical axes (47 and 22% of variance) are indicated. The discriminant axes could also be viewed as gradients of shape between two extreme configurations. These extreme configurations are also indicated on the axis, and they are superimposed at the bottom of the figure to enable the visual interpretation of the shape change involved at each case. Codes as in Fig. 1.

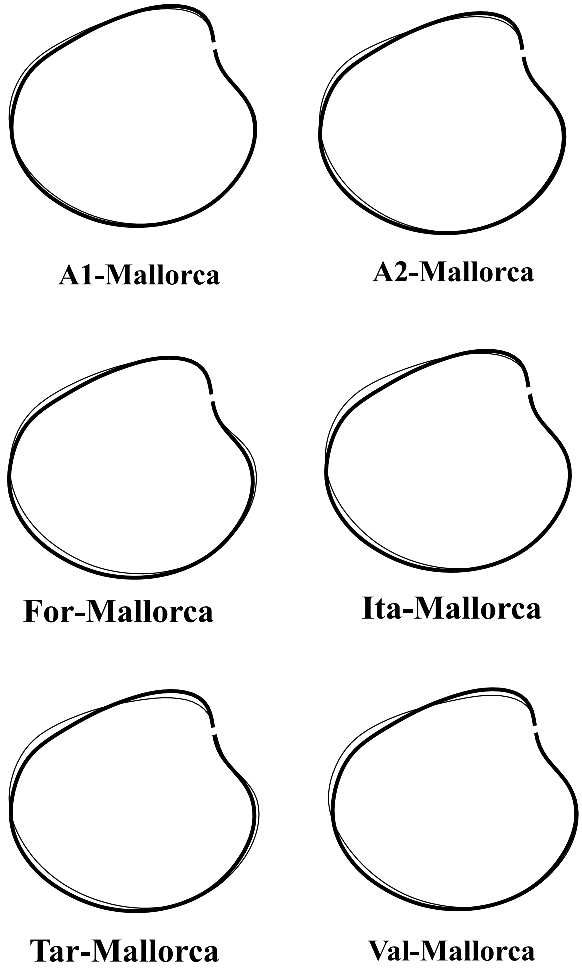

Fig. 3. Visual comparison of the "averaged" shape in the Mallorca group versus all others. Codes as in Fig. 1.

Clams from Tarragona and Valencia are clearly less dorsally peaked compared to those from Mallorca. Differences concentrate along the entire dorsal edge. Shape differences are smaller but still identifiable between Mallorcan clams those from Italy, Málaga (A2) and Formentera. In these cases, however, even though clams are also more dorsally peaked, they differ in the apex. Finally, clams from Mallorca and A1 (Cabo de Gata) are visually indistinguishable to each other. These geometric interpretations are not possible using conventional morphometric approaches.

The cross-validation confirms the usefulness (and the limits) of the method applied here. From the 20 simulations, the smallest number of clams from Mallorca that have been correctly assigned was 13 out of 20 (65%). The averaged (from the 20 simulations) rate of correct classification was 16.6 out of 20 (82.8%). The largest rate of incorrect classifications corresponds to A1 (Cabo de Gata; as expected from the results displayed in Figs. 2 and 3), with 5 out of 20 (25%) clams from this source incorrectly attributed as Mallorcan. Note, however, that this is a very extreme figure corresponding to a single simulation, and that the average (from the 20 simulations) of incorrect classifications (clams from A1 attributed to Mallorca) is 2.4 out of 20 (11.8%). Specimens from Formentera showed an intermediate averaged rate of incorrect classifications (4.5%), and the figure corresponding to the other groups was lower than 0.5% of incorrect classifications.

Groups significantly differ in size (ANOVA results: $F = 81.4$, d.f. = 6, Prob. = 9.2×10^{-12}), and the relationship between size and shape is complex. Focusing first on the intra-group level, clams from A1 (Cabo de Gata) and Italy show a clear significant effect of size on shape (14.2 and 9.4% of the shape variability is explained by size, respectively; Prob. < 0.01 in both cases). Other two groups (Mallorca and A2, Málaga) show a significant but smaller effect of size (less that 5% of shape variability explained by size; 0.05 > Prob. > 0.01), and the remaining groups (Formentera, Tarragona and Valencia) show no significant effects. Focussing on the groups that show a significant effect of size on shape, the interaction between source region and size is highly significant ($F = 5.7$; Prob. < 0.001), suggesting that there are differences between the allometric trajectories depicted by one or more groups. This fact potentially obscures the results of the discrimination analysis because the differences it suggests to occur may be related to size differences. However, focussing on the case of Mallorca and A1 (Cabo de Gata; the most closely related groups in shape), the maximum shape similarity would be achieved in a implausible scenario where clams from A1 (Cabo de Gata) were three times larger than those from Mallorca (675 versus 225 mm^2).

Moreover, additional results point against the possibility that the observed inter-group shape differences were mainly size-dependent. First, at the intra-group level, a multivariate regression of size on shape (estimated by the three first canonical variables, accounting for 80% of the shape variability) shows no significant effect. Second, considering all the clams studied, the percentage of shape variability (estimated by the morphometric variables derived from Fourier analysis) explained by size alone represents a significant ($P < 0.005$) but very small fraction of the total variation. Variance decomposition determined by partial multivariate analysis reveals that this size-related fraction is only 1.4% (compare this figure with 35.3% corresponding to the fraction explained by the geographical source). The results obtained using the scores of the clams within the canonical space are similar (0.3% of the shape variability explained by size, versus 51% explained by geographical source), suggesting that most of the discrimination power between groups is size-independent.

4. Discussion

Preliminary alignment (re-orientation) of the series of points that defines the contour of a clam would be sufficient to perform shape analyses comparable to a conventional morphometric analysis or to a landmark-based analysis (Monti et al., 2001). The alignment procedure used here was done using a single fixed landmark and the centroid of the object. The empirical result achieved by cross-validation randomisation suggests that this approach permits the accurate prediction of the source region of doubtful commercial samples of *C. gallina*. The power of the method is sustained by the large gap existing between the averaged rate of correct classification (82.8% clams from Mallorca were correctly attributed) and the averaged rate of incorrect classification exhibited by clams from other geographical groups. The shape of the clams from Mallorca and A1 (Cabo de Gata) are visually indistinguishable to each other (Fig. 3). In spite of this, only an average 11.8% of the clams from A1 were incorrectly attributed to Mallorca.

The method is specially powerful (less than 1% of incorrect assignments) in the case of the Adriatic clams, the main fishery from the western and central Mediterranean, and some fraud in labelling is expected due to the difference in market prizes with other regions (the Mallorcan clams are 50–100% more expensive than the Italian ones).

Acknowledgements

We are indebted to Michel Baylac for his suggestions on shape analysis, to Antoni Grau for providing the material studied and the data on market prices, to Beatriz Morales for allowing us to use an image analysis system at IMEDEA, and to Enric Descals for improving the manuscript. This study was supported by the Conselleria d'Agricultura i Pesca del Govern de les Illes Balears.

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