Research Article

PHENOTYPIC PLASTICITY OF MICROALGAL CULTURES IN CULTURE COLLECTIONS: A GEOMETRIC MORPHOMETRIC APPROACH

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Abstract

The algal culture collections include numerous old strains whose morphological characteristics may have changed after decades of subculturing. This case study of the fifty-year-old Pringsheim CAUP H 3301 strain of *Keratococcus bicaudatus* was conducted using geometric morphometric analysis of phenotypic plasticity to detect possible shifts in morphology. The results of relative warp analysis indicate that the young cultured population corresponds well with literature data regarding the morphology and phenotypic plasticity of the species. However, the culture expresses more phenotypic plasticity in both young and old populations.

INTRODUCTION

Microalgal cultivation has a long tradition in Europe. M. W. Beijerinck (1851-1931)of the Netherlands was probably the first to successfully isolate and culture an algal strain. Currently, there are still about eleven of Beijerinck's original strains kept in several algal collections. These include ten cultures of the genus *Chlorella* sensu lato, the *Trebouxia* sp. culture, and the culture of *Oocystis marssonii*. The British Culture Collection of Algae and Protozoa (CCAP) (http://www.windermere.ceh.ac.uk/ccap/) holds most of them – ten strains. However, probably the oldest algal strain in the world, that of *Chlorella vulgaris* isolated by Beijerinck in 1889 from an eutrophic pond near Delft, is kept in four algal collections (CCAP strain no. 211-11b; Culture Collection of Algae of Charles University of Prague – CAUP, no. H 1955; Sammlung von Algenkulturen Göttingen – SAG, no. 211-11b; Culture Collection of Algae at the University of Texas at Austin – UTEX, no. 259).

Robert Chodat (1865-1934) of Geneva was an another important pioneer of microalgal cultivation, and about twenty-five strains he isolated in the early twentieth century are currently held in algal collections. However, the most prominent achievements in the field of algal cultivation in the first half of the twentieth century were made by Ernst Georg Pringsheim (1881-1970), who established four important algal collections during his lifetime (Mollenhauer 2003, Day *et al.* 2004). Today there are five collections whose origin can be traced to Pringsheim; they are the CCAP in the UK, the SAG in Germany, UTEX in the USA, and the CCALA and the CAUP in the Czech Republic (the original Pringsheim algal collection was based in Prague, but it was split later and a portion of it was transferred to Třeboň, where it became the Culture Collection of Autotrophic Organisms - CCALA) (Day *et al.* 2004).

Today, there are still about 438 algal strains kept in these five collections, and they represent a valuable legacy that is still available for scientific research (Day *et al.* 2004). Thus, the current situation provides access to a number of very old living cultures. For example, in CAUP, more than 53% of the strains are more than 40 years old, more than 18% are older than 60 years, and four of them exceed 100 years. Many of these decades-old strains have been used as the basis for taxonomic descriptions, which have been made largely on the basis of the morphological characteristics of the organisms. However, these strains have been regularly subcultured since being isolated. Thus, they have become very "domesticated" organisms, whose countless generations spend their lives in the completely artificial environment of the algal collections. The process of algal domestication in culture was discussed in detail by Ettl & Popovský (1987). They recommended that conclusions

concerning the phenotypic plasticity of the investigated organisms should be drawn as soon as possible after isolation, due to possible changes after longer periods of cultivation. However, what happens to decades-old cultures in culture collections with regard to their natural phenotypic plasticity remains largely unexplored.

Geometric morphometrics (Bookstein 1991, Marcus *et al.* 1993, Dryden and Mardia 1998) is currently considered to be one of the most powerful tools in biological shape analysis (Bookstein 1996a, Bookstein 1996b, Rohlf 2000). The method allows for detailed investigations of shape dynamics (*e.g.*, in the characterization of phenotypic plasticity of organisms).

At the core of the method lies the thin-plate spline function (for algorithms see, *e.g.*, Bookstein 1991, Rohlf 1993). The first step of 2-D GM analysis is the detection and digitalization of homologous landmark points as x and y coordinates. The homology of the landmarks can vary from purely geometrical correspondence to biologically homologous structures according to the nature of the investigated shapes. Each individual in the set of objects is then described by a landmark configuration. In the next step, landmarks are superimposed according to the generalized least squares (or Procrustes) method (GLS) (Rohlf 1990), which involves the translation and rotation of objects and minimizes the sum of squared inter-landmark differences. The residual Procrustes distance is the square root of the sum of squared differences between the positions of the landmarks in superimposed configurations at the size measure known as centroid size (CS). Centroid size is defined as the square root of the sum of squared distances from each landmark to the specimen's centroid. Centroid size is removed from further shape analysis so that the relation of shape variables and size information can be analyzed separately. From the superimposed configuration, a mean configuration is obtained and used as a reference. The residuals of landmark position with respect to consensus configuration are further processed so that the representation of overall shape changes can be displayed as deformation grids (splines) inspired by the classic work of d`Arcy Thompson (1917) (Bookstein 1996a, Dryden & Mardia 1998, Loy *et al.* 2000).

Relative warp analysis, a modification of principal component analysis for shape variables, is the standard and is possibly the most frequently employed technique for processing GM data (*e.g.*, Rohlf 1993, de León and Zollikofer 2001, Bookstein *et al.* 2003). The relative warps represent the principal directions of shape change around the mean form, and the resulting transformation can be visualized as a deformation grid. The relative warps spanning shape dynamics can be thus employed to delimit the morphospace of the investigated set of objects.

The wide availability of suitable software (*e.g.*, the Tps series by F. J. Rohlf, Hammer *et al.* 2001, *etc*.; the exhaustive list on http://life.bio.sunysb.edu/morph/) means that GM techniques are currently employed in solving numerous biological problems. These include studies of phenotypic plasticity (*e.g.*, Singleton 2002, Langerhans *et al*. 2003, Trapani 2003) as well as taxonomic, evolutionary, and quantitative genetic studies (for a review see Adams *et al.*, 2004). Landmark methods have been successfully applied to the taxonomic analyses of certain vascular plant groups (*e.g.*, Jensen *et al.* 1993, Paler and Barrington 1995, Jensen *et al.* 2002), while the great potential of GM methods in botanical applications was reported in a recent review of this field (Jensen 2003). Within the scope of phycology, modern morphometric methods, namely outline analysis, have been applied several times in diatom taxonomy (Mou and Stoermer 1992, Pappas *et al.* 2001, Rhode *et al.* 2001, Pappas and Stoermer 2003) and in the taxonomy of coccal green algae (Neustupa 2004; Neustupa, submitted).

In the presented study, the author's aim was to characterize the phenotypic plasticity of the Pringsheim culture of *Keratococcus bicaudatus* in comparison with literature data on this species. The phenotypic plasticity of freshly isolated populations of this relatively frequently occurring freshwater and terrestrial green algal species was investigated carefully by Hindák (1970). He documented the principle morphs produced by the cultures cultivated under different conditions. Thus, it can be assumed that Hindák's figures provide a source of comparable information on the extent of natural phenotypic plasticity of natural populations and fresh cultures of the species. In addition, the shape dynamics of the author's strain will be compared with "typical" pictures of the species that are presented in Fott (1968) and later used in the monographs of Komárek & Fott (1983) and Ettl & Gärtner (1995).

In this way, an attempt will be made to identify possible shifts in the phenotypic plasticity of the investigated culture after about fifty years of cultivation.

MATERIAL AND METHODS

The strain of *Keratococcus bicaudatus*, CAUP H 3301, isolated by Pringsheim in 1955 from a pool near Kiel, Germany, was used in the investigation. The strain was cultivated at a constant illumination of about 2500 lux (light source Tungsram 36W F33, cool white) at a temperature of 14ºC on standard agar-solidified Bold Basal Medium (Bischoff & Bold 1963). The cultures were analyzed after three weeks and five months of cultivation. The populations were photographed using an Olympus BX51 light microscope with

Olympus U-CMAD3 microphotograph equipment. In total, 65 randomly selected horizontally positioned cells from both three-week- and five-month-old cultures were analyzed in subsequent GM analysis.
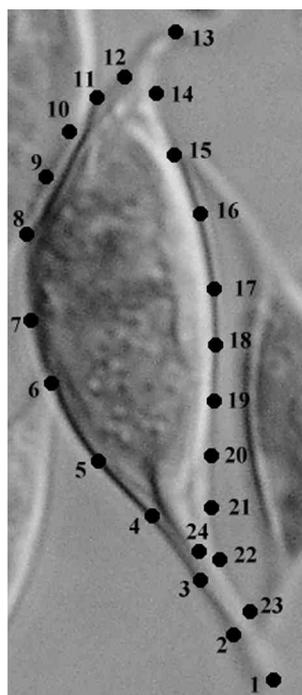
**Fig. 1.** A cell with 24 landmarks for analysis.

The cells were slightly heteropolar, so that distinction was possible between the thicker and usually longer basal part and the thin and sometimes rounded apical part and between the dorsal and ventral lateral parts (Fig. 1). Twenty-four landmarks were used for the analysis (Fig. 1). Of them, landmarks no.1 – the base of the stalk, no. 13 – the tip of the apical part, and no. 24 – the base of the protoplast, were considered to be fixed landmarks, whereas the other landmarks were allowed to slide along the outline of cells according to the Bookstein (1997) method. For the comparative shape analysis of literature data, the figures from Fott (1968) and Hindák (1970) were digitized and landmarks were depicted in the same manner as in the investigated populations. All the objects were Procrustes superimposed using the TpsRelw program (Rohlf 2004), and the subsequent relative warp analysis was conducted with parameter α set to zero, as recommended by Rohlf (1993) for exploratory studies of phenotypic plasticity.
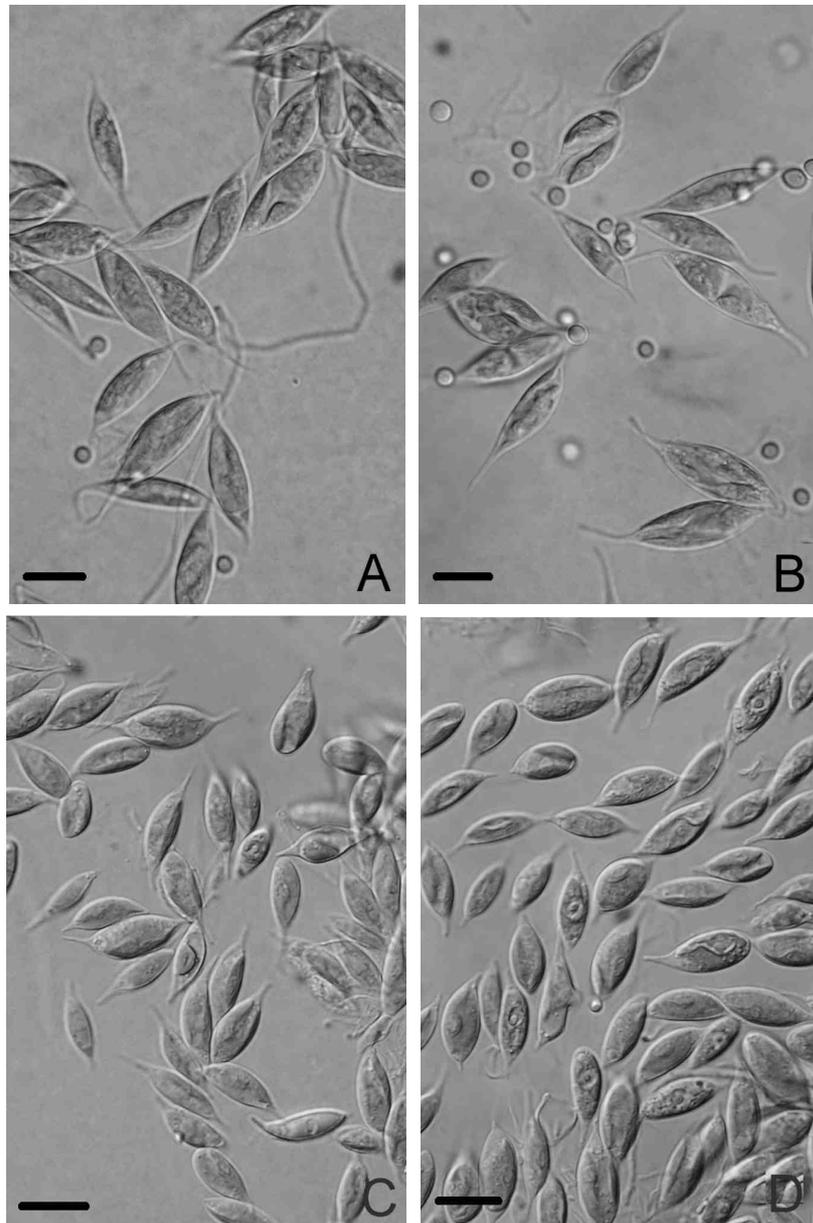
Fig. 2. A,B – *Keratococcus bicaudatus*, CAUP H 3301 strain, three-week-old culture. C,D – *Keratococcus bicaudatus*, CAUP H 3301 strain, five-month-old culture.

RESULTS AND DISCUSSION

The cells of the investigated populations (Fig. 2) corresponded well with the descriptions of *Keratococcus bicaudatus* in the taxonomic literature (Hindák 1970, Komárek & Fott 1983). Detailed shape dynamics were determined by relative warp analysis. The first relative warp axis explained 44.03% of the variability, while the second explained 23.16% of the variability in the investigated data (Fig. 6). On the deformation grids corresponding to marginal positions of individual relative warps, one can see the principle trends of shape dynamics in the investigated set. The first relative warp describes the change from a thin, almost isopolar cell in a negative position to a thicker cell with a less pronounced apical pole and the dorsal curvature shifted to the apical half of the cell in a positive position on RW1 (Fig. 6). The second relative warp clearly describes the shape change from a thin, almost isopolar cell in a negative position to thick cell with a rounded apical pole.
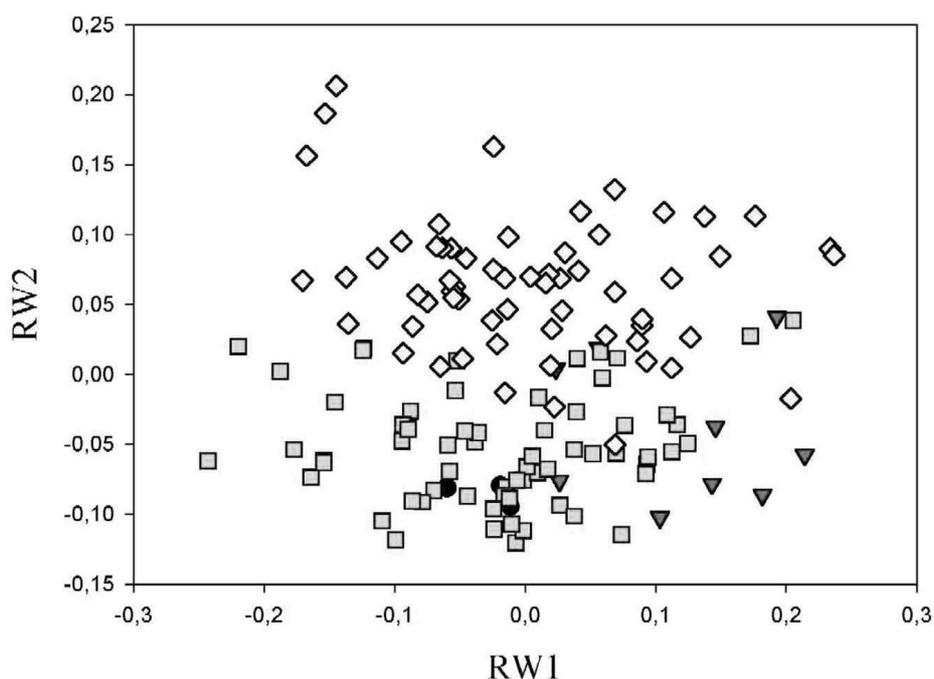
Fig. 6. Scatter plot of *Keratococcus bicaudatus* cells in the morphospace of the first and second relative warps. Squares – three-week-old CAUP H 3301 strain; rhombuses - five-month-old CAUP H 3301 strain; triangles – cells from Hindák (1970); circles – cells from Fott (1968).

Clear differences are apparent between the morphospace of young and old populations. This distinction is described by the second relative warp axis, where the older cells occupy the more positive positions with more thick, heteropolar cells, whereas the young population clearly has thinner and more isopolar cells (Fig. 6). As regards the literature data, the comparison of the position of cells in the morphospace of the first and second relative warps provides information on comparative phenotypic plasticity. Here, an interesting pattern is visible - both the Hindák (1970) and Fott (1968) cells fit well into the shape space occupied by the young population of the investigated Pringsheim strain. However, the cells of Fott (1968) and, even more surprisingly, those of Hindák (1970), which describe the overall phenotypic plasticity of the species, occupy only a small segment of the RW1 x RW2 shape space. Thus, it is apparent that the cultivated population expresses much more phenotypic plasticity than the populations investigated by Hindák (1970). The capacity of plastic morphology is frequently considered as an adaptive response of the organisms to the dynamic nature of the environment (*e.g.*, Schlichting & Pigliucci 1998, West-Eberhard 2003). At the same time, the developmental costs (*e.g.*, slower growth of certain morphotypes) limit the phenotypic plasticity of the individual populations (Pigliucci 2001). Thus, in the current case, it could be hypothesized that the amount of expressed phenotypic plasticity could be facilitated by the artificial environment of the unialgal culture thus almost entirely eliminating selection.

CONCLUSION

It has been demonstrated that the young population of fifty-year-old *Keratococcus bicaudatus* strain produces cells that correspond well to the natural population and to freshly isolated populations. In this respect, it can be said that despite decades of cultivation in the artificial environment of the culture collection, the culture did not lose its typical morphological characteristics. However, there seems to be a clear increase in the phenotypic plasticity expressed by the strain, which is not observed in the fresh isolates. Whether this feature could be considered as a more general phenomenon typical for microalgal strains cultivated for long periods should be investigated in future studies employing up-to-date, relevant morphometric methods.

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