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SUGGESTIONS FOR REVISION OF THE CILIATE GENUS *EUPLOTES*

Riassunto — *Suggerimenti per una revisione del genere Euplotes (Ciliata).* Un riesame morfometrico delle variazioni dei ciliati del genere *Euplotes* permette una revisione della nomenclatura di questo genere.

Viene proposta una classificazione tassonomica conservativa basata sulla disposizione dei cirri locomotori ventrali e sul reticolo argentofilo dorsale.

Abstract — A morphometric appraisal of variation in ciliates of the genus *Euplotes* suggests a revision of the nomenclature of this genus. A conservative taxonomic classification is based on the positional patterns of the ventral locomotory cirri and on the dorsal argentophilic network.

Key words — *Euplotes* / taxonomy / morphometrics.

The resolution of taxonomic problems in the ciliate genus *Euplotes* can be approached from the general perspective of evolutionary theory (GATES, 1978; GENERMONT *et al.*, 1976; MACHELON *et al.*, 1984; NOBILI *et al.*, 1978). The two fundamental realities of the living world are 1) extensive and discontinuous variation among the different forms (or types) of organisms that humans happen to distinguish and 2) the fact of evolution.

We recognise and distinguish among different forms by comparing the variation which occurs within taxonomic categories with variation among our categories: individuals of one «species» are more similar to each other, by all kinds of criteria, than each is to any individual of another «species». But the fact of evolution guarantees that our «types» do not remain fixed; populations of all «species» necessarily change with time.

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One way to study evolution is to examine the processes by which naturally-occurring variation is partitioned among different, naturally-occurring, genetically-related populations of individuals, belonging to the several taxonomic «types» of interest.

Morphometrics

The quantitative study of structure, morphometrics, provides a powerful tool in undertaking such studies: 1) objective data, based on repeatable measurements, can be subjected to standard statistical treatment; 2) multivariate analyses eliminate the need to rely on only one or two, often biased, taxonomic attributes; 3) quantitative attributes are very sensitive indicators of population differences, yielding a higher resolving power than, for example, allele frequency distributions (Lewontin, 1984).

Many of the uncertainties of traditional taxonomy arise from a failure to adopt a populational viewpoint. One cannot say that two populations are the same or different by looking at only one or a few specimens of each. This is the notorious problem of the «type» concept and of idealized taxonomic illustrations (upon which ciliatologists routinely rely). Similarly, one should not make judgements of taxonomic similarity by only looking once (one time, one particular condition of culture growth, etc.) at «representative» samples of ciliates.

Finally, the matter of scale is important. Measurements should be appropriate to the organism, and they should be of sufficient detail to capture a large subset of the resolvable attributes. In comparing two populations of humans, total height is not sufficient. Nor is total length in the genus *Paramecium* an adequate measure of structure. But by looking carefully enough and closely enough, it is possible to distinguish morphometrically the syngens of the *P. aurelia* sibling species complex (Gates *et al.*, 1975), except for the two species most similar in their total biology (Gates and Berger, 1976; Sonneborn, 1975).

The genus *Euplotes*

Because of their complex morphological structure, ciliates of the genus *Euplotes* afford an ideal opportunity to examine the parti-

tioning of morphometric variation among natural populations and to explore the more general question of speciation in ciliates.

These organisms possess an elaborate cortical structure (see Fig. 1) presented on two morphogenetically independent surfaces of a

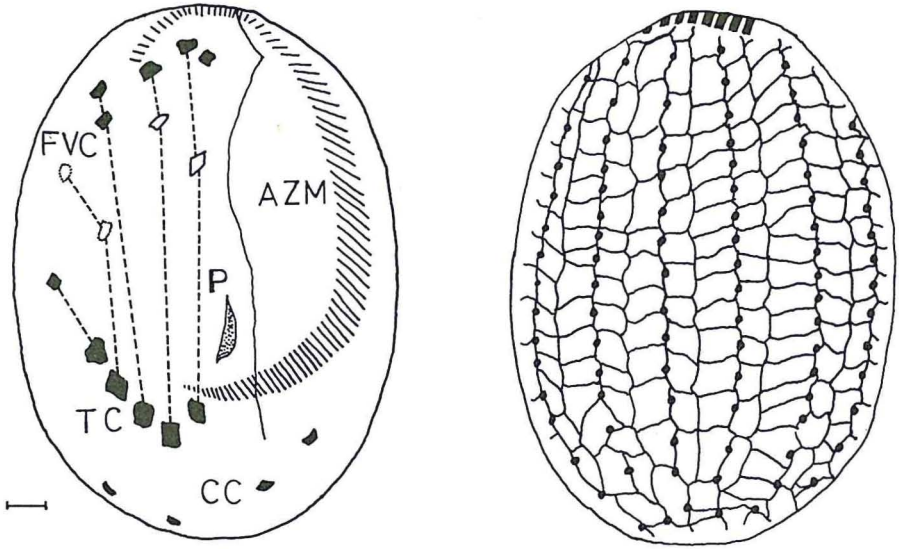

Fig. 1 - Schematic drawings of the ventral (left) and dorsal (right) surfaces of *Euplotes*. On the ventral surface, the five morphogenetic streaks are indicated, along which the frontoventral (FVC) and transverse (TC) cirri are formed during division. The non-solid cirri in the FVC group are those whose presence or absence determines the cirrotype. The ones on the left determine the two variants of the cirrotype 9 pattern. Also indicated are the caudal cirri (CC), the adoral zone of membranelles (AZM), and the paroral membrane (P). The pattern of a double dargyrome is shown on the dorsal drawing.

dorso-ventrally flattened body. They occur commonly in a wide range of marine, freshwater, and brackish habitats, under a variety of ecological conditions, and they are locally abundant while cosmopolitan in distribution. In the laboratory, they are grown easily, relatively easily stained, and easily measured. Variation in this genus is extensive: there are more than 50 described species (CURDS, 1975), and individuals range over an order of magnitude in size (20-200 μm) and through a variety of overall shapes.

On the ventral surface, there is variation in the number and positions of the large locomotory *cirri* (compound ciliary organelles); in the extent, conformation, and constituent number of the adoral

zone of membranelles (*AZM*); in the pattern of the silver-staining network of subpellicular alveolar boundaries (the *dargyrome*); and in details of the often extensive cortical sculpturing. Dorsally, the kinetosomal distribution varies, both in number of rows (the *corticotype*) and in the allocation of the total number of cilia (the *kinetotype*) among the rows (or *kineties*). There is variation, too, in the number, placement, and extent of dorsal ridging. As well, the dorsal argyrome (or *dargyrome*) varies, depending on the number of subpellicular silver-staining vacuolar boundaries that appear between adjacent kineties; this number is commonly one, but is often two and occasionally more (especially in freshwater «species»). A detailed morphometric analysis has shown the invalidity of one of the classic diagnostic criteria: the position of the central fibril in *Euplotes* with double dargyromes (GATES and CURDS, 1979).

The shape of the large macronucleus is highly variable. It has been reported to be of value in taxonomic discrimination, although no quantitative studies have been undertaken, and macronuclear shape is quite variable within a single clone (pers. obs.). Similar anecdotal information is available for a number of ultrastructural attributes. Genetic variation has been studied for only a few conjugation-related and electrophoretic loci. Both quantitative behavioural traits and precise ecological attributes are poorly characterized, except for the presence or absence of endosymbiotic prokaryotes (HECKMANN *et al.*, 1983).

VENTRAL CIRRAL PATTERNS

By concentrating on morphometric variation in the patterns presented by the large locomotory cirri on the ventral surface, it is possible to begin to assess objectively the ways in which evolutionary processes have partitioned populations belonging to this genus of ciliates. The ventral cirri classically are divided into three groups (see Fig. 1): the frontoventral cirri, whose number (the *cirrotype*) ranges from 7 to 10; the tranverse cirri, almost invariably 5 in number; and the more variable caudal cirri at the posterior of the cell (commonly 4 in number, but ranging from 3 to 6, or rarely more). The frontoventral and transverse cirri are intimately related morphogenetically; during division, these cirri arise fom the concomitant condensation, ciliation, and migration of five elongated,

subpellicular kinetosomal streaks. The anterior ciliary bundles become frontoventral cirri, while the posterior ones become transverse cirri. This morphogenetic process explains the constancy of the number of transverse cirri in this and related genera.

Significant variation occurs, therefore, in *cirrotype* (the number of frontoventral cirri) and in the relative positions of the frontoventral and transverse cirri. In order to analyse these cirral patterns, which are spatial point patterns on the relatively flat ventral surface, it was essential to develop a quantitative measure which captured the essence of any pattern (GATES, 1977, 1979). Then distributions of this measure could be formed for various sampled populations of *Euplotes* and their distributions compared (GATES, 1978 b, 1978 c).

It is possible to show that, among freshwater *Euplotes* populations having a cirrotype of 9, there are only two basic cirral patterns (Fig. 2), depending essentially on the relative location of the anterior cirrus in one of the five cirral streaks (see Fig. 1). Thus, there are cirrotype 9-«type 1» and 9-«type 2» patterns, represented by the classical species *E. patella* and *E. affinis*, respectively (GATES,

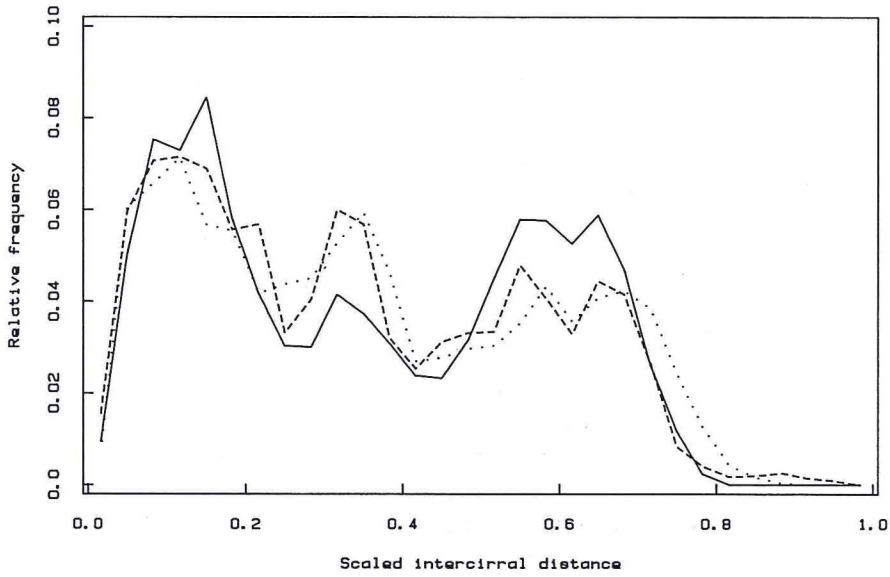

Fig. 2 - Ventral cirral patterns for three cirrotype 9 samples. The dotted and dashed lines denote samples of «type 1» stains from Denmark and from Ontario, Canada, respectively, while the solid line denotes a «type 2» strain from England.

1978 b). In contrast, cirrotype 10 *Euplotes* display a continuum of patterns, with few distinct groupings of strains (GATES, 1978 c).

DORSAL KINETOSOMAL PATTERNS

Because the dorsal and ventral surfaces of *Euplotes* are morphogenetically independent, an analysis of the patterns of allocation of kinetosomes across the dorsal surface provides a separate assessment of evolutionary partitioning in this organism.

Simply by counting cilia and forming the standardized, relative distribution of their numbers, it is possible to derive a useful measure of dorsal kinetosomal patterns (FRANKEL, 1975) which can be applied to a comparison of population samples from worldwide localities, analogous to the study undertaken for the ventral surface. The remarkable conclusion of such a study (GATES, 1984, and in prep.) is that, although the two surfaces look very different and develop differently and independently, yet the variation in kinetosomal patterns on the two surfaces has not evolved independently: the assortment of strains falls into the same general groupings. Thus, for example, there are only two basic dorsal kinetosomal distributions (see Fig. 3) among cirrotype 9 freshwater *Euplotes*, and these correspond precisely to the type 1 (*patella*) and type 2 (*affinis*) patterns of the ventral cirri. There has been a general correspondance between the ways variation in kinetosomal partitioning, whether in cirral streaks on the ventral surface or in rows of simple cilia on the dorsal surface, has been partitioned among populations of *Euplotes* in the course of evolution.

APPLICATION TO TAXONOMY

The aim of taxonomy is to describe the limits of observed variation. By quantitatively examining the variability of attributes in samples from natural populations of ciliates, and by examining such variability in laboratory strains derived from those populations, but which have been subjected to extremes of various environmental factors (such as temperature, pH, salinity), it is possible to infer the limits of possible variation in nature. Then, when such analyses are complete, it may be useful to attach binominal names to samples which represent the known limits of variation.

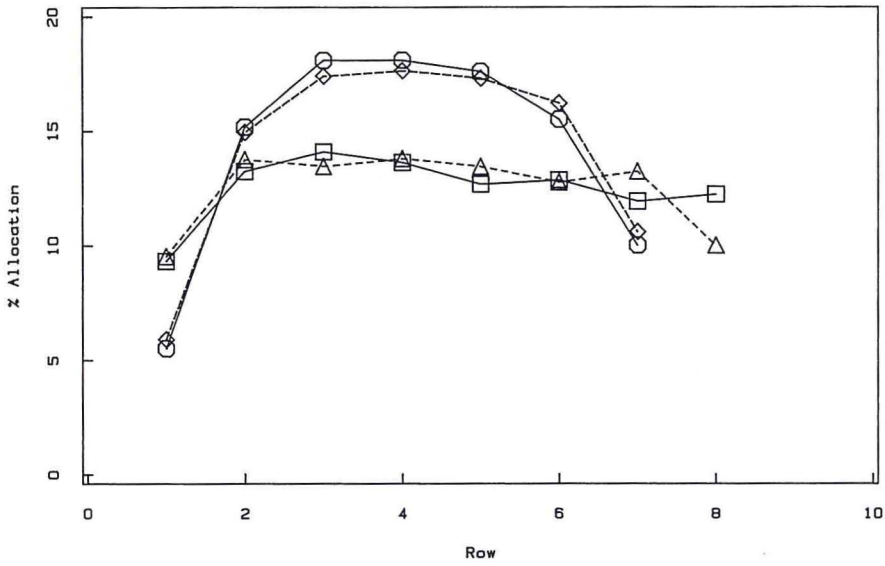

Fig. 3 - Dorsal kinetosomal distributions for four cirrotype 9 samples. Hexagons and diamonds denote «type 1» strains from Texas, U.S.A., and from the Northwest Territories, Canada, respectively, while squares and triangles denote «type 2» strains from England and from Florida, U.S.A., respectively.

For the genus *Euplotes*, even morphometric analyses of this nature are not complete. Still, it is possible tentatively to suggest a conservative scheme of taxonomy, based on one person's perception of the crucial and most stably varying attributes of populations of *Euplotes*. Such a classification is given in Table 1, where I have listed only the names having nomenclatural priority within each group. Elsewhere, I will present a list of synonomies and detailed systematic arguments.

In my view, a reasonable classification of the limits of morphological variation can be based on ventral cirral pattern and dorsal argyrome alone. Within each of the cells of Table 1, i.e., within such morphologically-defined, phenotypic limits, there has been and undoubtedly continues to be considerable genetic differentiation. Indeed, the mating type, autogamy, and some electrophoretic loci have been well studied in the *vannus* and *raikovi* groups of marine sibling species (Dini and Giorgi, 1982; Dini and Luporini, 1976, 1979, 1980, 1982; Heckmann, 1963, 1964; Heckmann and Frankel, 1968; Luporini and Dini, 1977; Machelon and Demar, 1984; Miceli *et al.*, 1981; Nobili, 1966; Siegel and Heckmann, 1966; Wichterman, 1967) and in the

patella group of freshwater sibling species (Ito, 1971; Kosaka, 1970, 1973).

My contention is, not that the forms listed in Table 1 are the only «biological species» in the genus *Euplotes*, but that only these morphological species can be defended on the basis of quantitative analyses of structural variation.

Table 1 - Described and possible *Euplotes* «species». Each cell indicates the number of well-described species of given cirrotype and dargyrome combination, as well as the appropriate name.

Cirrotype	Dargyrome		
	single	double	multiple
10	6 (*vannus*)	16 (*charon*)	2 (*indentatus*)
9-1	0	9 (*patella*)	1 (*tegulatus*)?
9-2	0	5 (*affinis*)	4 (*muscicola*)
8	0	3 (*parkei*)	0
7	0	1 (*raikovi*)	0

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