J. Génermont (*), V. Machelon (*), C. Demar (**)

THE «*VANNUS*» GROUP OF GENUS *EUPLOTES*.
SIBLING SPECIES AND RELATED FORMS;
EVOLUTIONARY SIGNIFICANCE
AND TAXONOMICAL IMPLICATIONS

Riassunto — *Il gruppo «vannus» del genere Euplotes. Specie gemelle e forme affini; significato evolutivo e implicazioni tassonomiche.* Vengono descritte cinque species criptiche appartenenti al gruppo monofiletico del complesso *vannus* del genere *Euplotes*. L'entità di diversificazione evolutiva tra queste cinque specie è molto bassa sia a livello morfologico che a quello biologico e biochimico. Vengono discussi anche i rapporti filetici del complesso di specie qui descritte con altre forme del gruppo *vannus*.

Abstract — In the monophyletic «*vannus*» group of genus *Euplotes* a complex of five sibling species is described. The amount of evolutionary divergence between these five species is very low not only at the morphological level, but still at the biological and biochemical level. The relationships of this complex with other forms of the «*vannus*» group are discussed.

Key words — Ciliate / electrophoresis / morphometrics / speciation / taxonomy.

Sibling species are not exceptional among animals: they are found in all major taxa. The status of Ciliates seems however rather unusual, since several complexes are known each of which includes a very high number of species. In fact, the well known complexes are restricted to Holotrichs, «*Paramecium aurelia*» (SONNEBORN, 1975) and «*Tetrahymena pyriformis*» (NANNEY and Mc COY, 1976) for instance. In Hypotrichs, up to 1975, each morphotype was believed to include either a single or a few «biological species»: all the

(*) Laboratoire de Zoologie 2, Université Paris-Sud, F-91405 Orsay cedex.
(**) Laboratoire de Zoologie, E.N.S., 46 rue d'Ulm, F-75230 Paris cedex.

organisms referred to as *Euplotes vannus* by HECKMANN (1963) were fully fertile; in «*Stylonichia mytilus*», two biological species were recognized (AMMERMANN, 1965), but it was shown later that they were different by cell size and some minor morphological features (AMMERMANN and SCHLEGEL, 1983). It could be suspected that the morphological criterion of species was more powerful in Hypotrichs in connection with the highly organized and readily observable cortical patterns in this group, contrasting with the rather uniform distribution of cilia in Holotrichs.

A clue for testing this hypothesis was offered by a group of three closely related morphotypes which were designated as species by «conventional» taxonomists: *Euplotes vannus*, *E. crassus* and *E. mutabilis* (TUFFRAU, 1960; CURDS, 1975). The main discriminating character between them was the number of dorsal ciliary rows: 9, 10 and 11 or more, respectively. We have carried out an extensive breeding analysis of this group. The early results of this study indicated that the number of dorsal ciliary rows was not reliable for species discrimination according to the biological concept of species. Later, we were able to recognize within the *vannus-crassus-mutabilis* group at least five intrafertile intersterile classes making up together a complex of sibling species which has been provisionally referred to as the «*Euplotes vannus* complex» (GÉNERMONT, 1976). We shall summarize here the biological, morphological and biochemical features of this complex, emphasizing both variation within the complex and differentiation from related *Euplotes*, then discussing the implications of current data on infrageneric taxonomy and understanding of evolutionary dynamics.

BIOLOGICAL CHARACTERIZATION OF THE COMPLEX

Mating reactions

Mixing two sexually reactive samples of different clones results into one or another among a wide spectrum of sequences, from no reaction at all to: pairing, induction of meiosis and other nuclear phenomena, pronuclear exchange, caryogamy, separation of mates, development of a new nuclear apparatus, cortical morphogenesis and subsequent growth of flourishing exconjugant lines up to sexual maturity. The latter sequence indicates that the two clones are sex-

ually compatible, provided that at least some of the exconjugant lines are derived from heterotypic pairs (i.e. the two members of which belong to different clones). Any other sequence indicates sexual incompatibility of the two clones under study.

When two clones are sexually compatible, it can be concluded that they belong to different mating-types of a single species. When two clones do not react with each other but each one is sexually compatible with a third one, they belong to the same mating-type of a single species (and the third one to another mating-type of this species). In any other instance, the two clones under study belong to different species, with isolating mechanisms operating at some stages of the full mating sequence (Fig. 1).
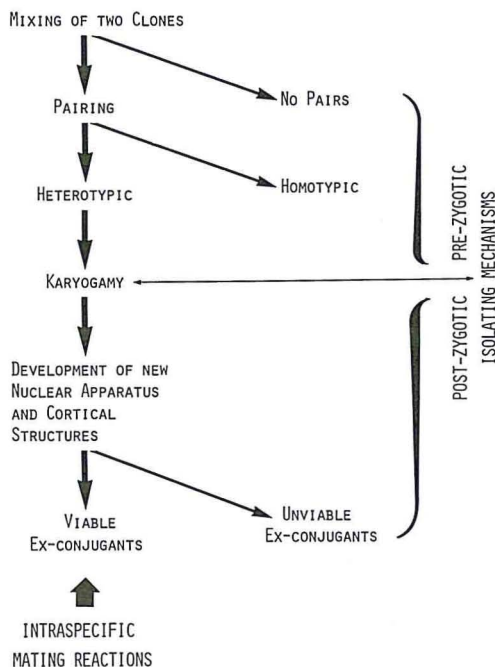

Fig. 1 - Standard sequence of intraspecific mating-reactions between sexually compatible clones and main shifts resulting in reproductive isolation between heterospecific clones.

According to these criteria, five species have been identified (GÉNERMONT et al., 1976; HALLET, 1978; MACHELON, 1978, 1982). These species are morphologically highly similar, as will be discussed later;

thus they are members of a complex of sibling species, the so-called *E. vannus* complex. Up to now, about 100 clones collected in various geographical areas (Europe, Northern Africa, Eastern America) have been identified as representative of this complex.

Within the complex, interspecific mating reactions are common. Nevertheless, as a rule, the isolating mechanisms, either prezygotic or post-zygotic, are quite efficient. Somewhat exceptional appears the isolation between species 1 and 2, since a few clones are able to mate successfully both with typical species 1 and typical species 2 clones, thus allowing, at least in laboratory conditions, some introgression between the two species. Isolation between these two species is exclusively prezygotic; they display no genomic incompatibility, a feature which reflects a very low level of evolutionary divergence.

Some of the clones we have studied have been classified, on the basis of mating tests, outside the group of five species referred to as *E. vannus* complex. Among them some exhibit a definitely smaller cell size and are assignated to the *minuta* morphotype. Another one is rather larger, the TM 1 clone, from Adriatic sea, kindly provided by Prof. LUPORINI. Another one, Frc 3, from french mediterranean coast, does not differ from the complex by cell size, but is not able to mate successfully with any clone belonging to the complex nor with *minuta* clones. All these clones are evolutionary close to the *E. vannus* complex, since in mating tests sexual reactions are frequently observed, involving in some instances the completion of homotypic conjugation; furthermore, their overall morphology is very similar.

Other biological features

Some clones have been found exhibiting more or less uncommon traits: autogamous, killers, mate-killers, monsters, etc. In the present state of knowledge, there is no reason to suspect that they are unequally distributed between the five species of the *E. vannus* complex.

On the basis of preliminary experimental results in laboratory conditions, the ecological requirements of the five species seem very similar (tolerance to high and low temperature, increase or decrease of salinity; thermal optimum; nutritional requirements). It cannot be excluded, however, that their ecological niches are different, since their geographical ranges, though widely overlapping, are not iden-

tical (species 5, for instance, is only known from american localities) and they seem not to be submitted to the «exclusion principle» (up to 3 species have been found simultaneously in very restricted areas, less than 1 meter wide). Some further investigations in the field are necessary in order to ascertain the ecological differentiation within the complex.

MORPHOLOGY AND MORPHOMETRY

In order to describe the morphometrical variation among all *Euplotes* related to *E. vannus*, i.e. the A section defined by CURDS (1975), we have submitted a sample of 34 clones to a biometrical analysis by means of univariate as well as multivariate procedures. The results of this study have been published recently (MACHELON *et al.*, 1984).

One of the most outstanding feature of this variation is that the sample is made up of two classes, on the basis of cell size, mean cell length ranging from 53 to 66 μm in the smaller one, from 73 to 92 μm in the larger. The gap between the two classes is statistically highly significant, and is much more prominent when a second attribute is simultaneously taken into consideration, the relative lenght of peristome (Fig. 2). The following formula allows unambiguous discrimination between the two classes:

$$\text{«larger»:} \quad \bar{P} + 0.006 \, \bar{L} > 1.21$$
$$\text{«smaller»:} \quad \bar{P} + 0.006 \, \bar{L} < 1.11$$

($\bar{P}$ = mean relative peristome lenght; $\bar{L}$ = mean cell length in μm; values obtained from silver-impregnated specimens). These two discrete classes have been referred to as «*vannus* morphotype» and «*minuta* morphotype».

So far we have not been able to establish subclasses within the *minuta* morphotype. Conversely, the clones belonging to the *vannus* morphotype are distributed between the five species of the *E. vannus* complex, with only one exception, the clone Frc 3 which is interfertile with no clone of our collection, so that it is not possible to know if it is a sterile clone or a representative of a new species.

Within the *E. vannus* complex itself, discrimination of the component species cannot be achieved by means of simple procedures using morphological or morphometrical data. Suggestive results of

discriminant analysis need further confirmation on the basis of more extensive sampling.

Much recently, the morphometrical analysis was extended to the clone TM 1. Its mean cell length is very high: 100.5 μm, significantly out of the range of our *vannus* morphotype. Another distinctive feature of this clone is its rather low relative width (Fig. 2). It seems likely that this clone is representative of a third morphotype. If this is true (i.e. if similar clones are obtained), our previous nomenclature of morphotypes must be reconsidered.
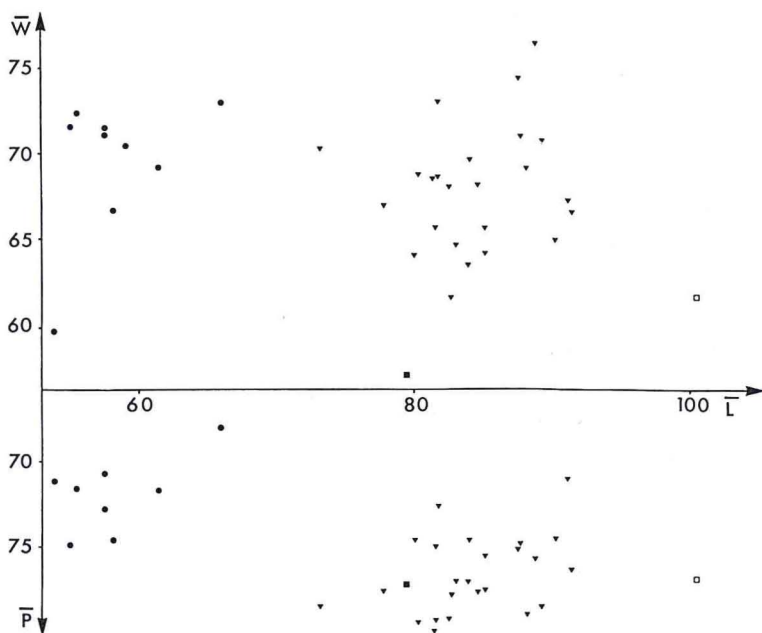

Fig. 2 - Joint variation of mean cell length, L (μm), mean relative cell width, W (in percent of L), and relative length of peristome, P, among a sample of clones. Circles: *minuta* morphotype, triangles: *vannus* complex; full squares: Frc 3; open squares: TM 1.

ELECTROPHORETICAL DATA

Since electrophoretical patterns have proven to be fruitful tools for species discrimination in some complexes of sibling species, especially in Ciliates (SONNEBORN, 1975; NANNEY and Mc COY, 1976),

we have undertaken the analysis of some enzyme systems: the most conclusive results have been obtained from amylases and acid phosphatases, while esterases and malate dehydrogenases provided some additional informations.

It must be stated first that, at the generic level, the electrophoregrams obtained in the section A are quite different from those obtained in other sections of genus *Euplotes* (for instance fresh water species with double-type dorsal argyrome).

Within section A, despite some degree of similarity, clear-cut differences are observed between the *E. vannus* complex and the *minuta* morphotype. For instance, in *minuta* all amylase patterns are strictly identical and single-banded, while in *vannus* two or three bands are observed, none of them being located similarly as the *minuta* one (Fig. 3). For acid phosphatases too, the *minuta* zymograms
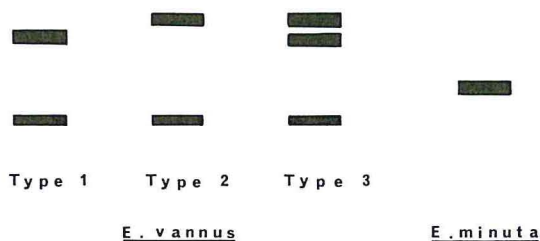

Fig. 3 - Amylase zymograms obtained for the *E. vannus* complex (three types) and for the *minuta* morphotype (one single-banded pattern).

are constant, single-banded, while in *vannus* there are several bands, one of which, referred to as the main band since it is more intensely stained than the others, occupies a constant site (among 50 clones examined) different from that of the *minuta* band (Fig. 4) (MACHELON and DEMAR, 1984).

Within the *E. vannus* complex, a considerable amount of variation is observed for acid phosphatases, since 9 phenograms have been described. Preliminary results indicated that these 9 types were unequally distributed between the five species. This idea was not supported by further analysis; moreover the genetic basis of intraspecific variation is obviously complex and could not be elucidated so far. As for amylases, every species exhibit intraspecific variation, determined by a single pair of alleles.

The status of clone Frc 3 seems peculiar: though belonging to the *vannus* morphotype, its electrophoretical patterns are those of the *minuta* one.

Fig. 4 - Acid phosphatase zymograms obtained for the *E. vannus* complex (three patterns are shown, out of nine presently known) and for the *minuta* morphotype (one single-banded pattern).

As for the TM 1 clone, it exhibits unique patterns.

In conclusion, there is a good agreement between morphometrical and electrophoretical classification within the section A of genus *Euplotes*, with three well differentiated groups: *minuta* morphotype, *vannus* complex and TM 1. Within the *vannus* complex, interspecific divergence is very low at the biochemical as well as at the morphological level. As for clone Frc 3, it appears in some manner as a link between the *minuta* morphotype and the *vannus* complex.

DISCUSSION

Three classes are recognized within the A section of genus *Euplotes* on the basis of morphometrical criteria; these classes are referred to as morphotypes; since the main discriminative feature between these morphotypes is cell size, it is convenient to designate them by *larger*, *medium* and *smaller* morphotypes. On another hand, three electrophoretical classes have been characterized. Despite a rather good correlation between these two classifications, they are not exactly congruent, since one of the *medium* clones is electrophoretically related to the *smaller* ones. Thus further investigations are needed in order to appreciate fully the relationships between biological, morphological-morphometrical and electrophoretical classifications. Moreover the gap between *larger* and *medium* mor-

photypes must be confirmed: it is necessary to know several clones of the *larger* type and to describe with some detail their biological and morphological features.

For these reasons, the conclusions which can be drawn from the above data are provisional and must be hold as working hypotheses.

Evolutionary status of the E. vannus *complex*

The *E. vannus* complex is correctly characterized as an assemblage of five sexually reproducing, intrafertile, intersterile and morphologically indistinguishable groups. Such groups are commonly referred to as sibling species (MAYR, 1942). These five species are very closely related, since interspecific sexual reactions are common and the morphological, morphometrical and electrophoretical features of all species are almost identical. Thus it seems likely that they are very recent products of a single ancestral species. Moreover, genomic compatibility of species 1 and 2 strongly suggests that in this case the interspecific divergence is practically restricted to some variation of mating-type substances, thus supporting the idea that speciation can be achieved through as few as one or two mutations of the genes which control the synthesis of these substances. Such a mode of speciation is a sympatric one, in agreement with the extensive overlapping of the different geographical ranges.

If this conclusion is correct, speciation may be a rather common event, the consequence of which is a tendancy to a rapid increase of the number of species. On another hand, the ecological valences of these species are expected to be very similar, if not identical, so that interspecific competition is likely to be extremely severe and species extinction rate rather high. Therefore the complex should include an approximately stable number of individually labile species and should undergo repetitive speciation without any significant consequence either on transspecific evolution or on dynamics of microbial ecosystems.

From this point of view, the evolutionary meaning of the *E. vannus* complex seems quite different from that of some other complexes of sibling species in Ciliates. «*Tetrahymena pyriformis*», for instance, displays considerable interspecific divergence at biochemical level (NANNEY, 1984). The component species of the «*Paramecium aurelia*» complex exhibit distinctive electrophoretical patterns (ALLEN

et al., 1983 a and b) and probably occupy different ecological niches (GILL and HAIRSTON, 1972). More similar to the E. vannus complex seems «Paramecium caudatum» where isolating mechanisms are almost exclusively prezygotic, at least between some of the so-called «syngens» (TSUKII and HIWATASHI, 1983).

These examples provide a good illustration to the idea that morphological similarity between sibling species may result from very different causes. For instance it may be a consequence of some morphological constraints and then is not exclusive of physiological and ecological divergence, so that it does not necessarily reflect close phylogenetic relationships. Conversely, it may be related to a rapid turn-over of species within a single adaptive zone under the pressure of ecological constraints, provided that efficient isolating mechanisms are able to arise through a low number of a mutational events. Between these two extremes lies a wide range of other possible situations.

Concerning the diversification of the whole section A of genus Euplotes, it may be suggested that the three morphotypes correspond to three different adaptive zones, so that any intermediate morphological state is likely not to be tolerated by natural selection. Whatever the mechanisms by which this present situation has been reached, its consequence is that two kinds of evolutionary processes are expected to occur according that they are associated or not with a jump from one adaptive zone to another. The turn-over of species within the E. vannus is obviously the result of repetitive speciation without such a jump. Conversely, if one assumes that the biochemical similarity of the Frc 3 clone to the minuta clones reflects a close phylogenetic relationship, it is likely that this clone belongs to a lineage in which a jump from the smaller to the medium morphotype occurred more or less recently. Of course this interpretation does not imply that the differentiation of the three morphotypes during the early evolution of the section was achieved by means of saltatory phenomena.

Taxonomical and nomenclatural implications

It is now clear that the morphological criteria do not allow species identification within the section A of genus Euplotes. However they allow some classification, the taxonomic value of which must be questionned. In the present state of knowledge, it is likely that

one of the three morphotypes is not monophyletic (the *medium* one), so that it cannot be considered as a «good» taxon. Conversely, the complex of sibling species which we have referred to as «*E. vannus* complex» is presumably monophyletic. Thus it is a good supraspecific taxon and may be designated either as a complex of sibling species or as an *ultraspecies* (for definition, see BERNARDI, 1980).

Another presumably monophyletic taxon is obtained by bringing together the *smaller* morphotype and clones such as Frc 3. This taxon too is probably supraspecific, but further investigations are necessary for unequivocal demonstration.

As for the taxonomic status of the *larger* morphotype, it will remain uncertain until several representative clones will be known.

Thus, as far as now, the only well characterized taxon is our «*E. vannus* complex». It must be remembered that the binomen *E. vannus* has been provisionally assigned to the complex (GÉNERMONT, 1976); at this time, the complex seemed to include all *Euplotes* previously named *vannus*, *crassus* and *mutabilis*. At present it seems likely that the original descriptions of *E. vannus* (MULLER, 1786) and *E. crassus* (DUJARDIN, 1841) apply respectively to the *larger* and the *medium* morphotype. This must be confirmed by an analysis of the morphological variation within the *larger* morphotype, but if it is true the complex will be correctly designated as *E.* ultrasp. *crassus*. It must be noticed that the binomen *E. crassus* has been for some years used by italian workers for a taxon which is presently known as identical to at least a part of our complex (see for instance LUPORINI and DINI, 1977). It may be expected that this problem will be solved in the next future.

On another hand it is clear that for unspecialized Ciliatologists the discrimination between the species of the complex, as well as between the complex itself and other forms belonging to the *medium* morphotype, will remain impracticable for a long time. For this reason, the use of the supraspecies concept (GÉNERMONT and LAMOTTE, 1980) is convenient, since it applies to morphologically homogeneous, but not necessarily monophyletic entities.

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