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ELECTROPHORETIC COMPARISON BETWEEN *EUPLOTES VANNUS* AND *E. CRASSUS* STRAINS (**)

Riassunto — Euplotes riferiti al morfotipo *E. vannus* (ceppo TM1) si sono dimostrati riproduttivamente isolati da euplotes riferiti al morfotipo *E. crassus* (ceppi LIV 1, POR 3, MN 3 e PB 1).

L'analisi della mobilità elettroforetica di sei sistemi enzimatici (enzima malico, malico deidrogenasi, fosfatasi acida, tetrazolio ossidasi, isocitrato deidrogenasi ed esterasi aspecifiche) ha dimostrato che il coefficiente di somiglianza tra gli euplotes del ceppo *E. vannus* e quelli dei ceppi *E. crassus* è 0,18-0,19, mentre tale coefficiente varia da 0,68 a 0,85 nei confronti tra ceppi di *E. crassus*.

Abstract — Euplotes morphologically identified as *E. vannus* (strain TM 1) have been found to be reproductively isolated from euplotes identified as *E. crassus* (strains LIV 1, POR 3, MN 3 and PB 1).

The analysis of the electrophoretic mobilities of six enzyme systems (malic enzyme, malic dehydrogenase, acid phosphatases, tetrazolium oxidases, isocitrate dehydrogenase and nonspecific esterases) have shown that the *E. vannus-E. crassus* similarity coefficient ranges 0.18-0.19, whereas it varies from 0.68 to 0.85 within the group of *E. crassus* strains.

Key words — Ciliates - *Euplotes* - isozymes.

INTRODUCTION

An ambiguous situation exists in the taxonomy of *E. vannus* and *E. crassus*, two of the most studied bottom-dwelling marine hypotrichs, both showing a dargyrome of the single-type (TUFFRAU, 1960; CURDS, 1975), as well as very similar cirral patterns (GATES,

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1978), and mating types genetically controlled by a multiple allele-single locus system with peck-order dominance (HECKMANN, 1963, 1964). Currently, the same euplotes that have an oval profile averaging 80 μ in length and carry a standard number of ten kineties are called *E. vannus* by some researchers and *E. crassus* by others.

TABLE 1 - Main morphological parameters provided by different authors to distinguish between *E. vannus* and *E. crassus* morphotypes.

Authors	Length (μ) *E.c. / E.v.*	Body shape *E.c. / E.v.*	Kineties (no.) *E.c. / E.v.*
Kahl (1932)	70-100 / 130-200	oval / reniform	n.r.
Tuffrau (1960)	100-127 / 75-97	oval / reniform	10 / 9
Heckmann (1963-64)	n.r. / 84-116	n.r. / reniform	10 / 8
Curds (1975)	100-130 / 75-100	oval / reniform	10 / 9
Gates (1978)	124 ($\bar{x}$) / 104 ($\bar{x}$)	n.r.	9 / 9

n.r., not reported; *E.c., E. crassus; E.v., E. vannus.*

As shown in Table 1, such ambiguity might have originally stemmed from an inversion of the parameters of *E. vannus-E. crassus* body length in TUFFRAU's (1960) revision of the genus *Euplotes* with respect to KAHL's (1932) key to the ciliates. *E. vannus* originally identified as having nearly double the dimensions than *E. crassus* later became the minor, elongated, and slightly reniform species carrying nine kineties (eight, in Heckmann's *E. vannus* description (1963)). Shortage of both living reference strains for breeding tests and deposited holotypes for structural comparison, besides the extensive intrinsic variability of diagnostic traits (MACHELON et al., 1984), have so far prevented resolution of this question.

Here we provide evidence (although preliminary because obtained from a survey of a limited number of strains) which suggests a close correspondence between morphological, genetic, and biochemical criteria in establishing the species status of *E. vannus* and *E. crassus*.

MATERIALS AND METHODS

Cells were grown at room temperature (ranging 22-24 °C) and fed with *Dunaliella tertiolecta* grown in Walne seawater medium. All experiments were performed on morphostatic, 2-day starved cells (DINI and LUPORINI, 1979).

Mating tests were carried out on pairwise mixtures of 0.5 ml strain samples containing about 10^3 cells that were examined for mate pairs at 12 h after preparation.

Measurements and morphological observations were performed on at least 50 silver-stained specimens processed according to Corliss (1953) except that cells were fixed for 60 min at 4 °C in 2.5% gluteraldehyde in seawater.

Cells for scanning electron microscope were prepared as reported elsewhere (Luporini and Dallai, 1980).

Enzyme extracts were prepared starting from cell cultures grown at a density of approximately 5 x 10^3 cells/ml in 2-liter buffled flasks and separed from debris by repeated washing with pure seawater before being eventually concentrated by centrifugation at 700 g for 5 min. Concentrated cells were resuspended in a corresponding volume of a stabilizing solution (0.18 M EDTA, 1 mM dithiothreitol, 0.2 M Tris, pH 7.4) at 4 °C and disrupted by sonication. After membrane residues were removed by centrifugation at 27,000 g for 40 min, supernatants were recovered as enzyme extracts and stored for maximum 1 month at —80 °C (with no appreciable loss of enzyme activity). Fourty-50 µl supernatant samples, containing a total amount of protein corresponding to 320-400 µg bovine serum albumin, were normally used in electrophoresis carried out in a vertical polyacrylamide slab gel (stacking gel 5%, pH 8, running gel 15%, pH 8.6) under constant voltage (200 V), for 5 h at 4° C. Run was performed in glycine 0.4 M and Tris 5 x 10^{-2} M buffer at pH 8.4. Staining and others electrophoresis conditions are reported in Table 2.

Results and preliminary conclusions

We consider the strains used in this study (TM 1, LIV 1, POR 3, MN 3, and PB 1) to be representative of the morphotypes *E. vannus* (TM 1) and *E. crassus* (the other four strains) on the basis of the complex of morphological characteristics summarized in Table 3 and shown in Fig. 1.

In pairwise strain combinations, the strain TM 1 shows to consist of euplotes incapable of cross-mating with those of *E. crassus* strains, while being fully capable of mating with morphological corresponding euplotes of strain TM 2 (only recently isolated from Torre

TABLE 2 - Staining solutions and conditions.

Enzyme	Substrate	Co-enzyme	Other additions	Staining buffer	Staining conditions
Tetrazolium oxidases	none	NAD 33.2 mg	PMS 4.9 mg NBT 40.8 mg	0.2 M Tris-HCl pH 9, 100 ml	5 h, 20 °C
Malic dehydrogenase	L-malic acid 75 mg	NAD 10 mg	EDTA 10 mg NBT 10 mg PMS 1 mg	0.1 M Tris-HCl pH 8.5, 50 ml	5 h, 20 °C
Malic enzyme	L-malic acid 50 mg	NADP 10 mg	$MgCl_2$ 50 mg EDTA 10 mg NBT 10 mg PMS 1 mg	0.1 M Tris-HCl pH 8.5, 50 ml	12 h, 20 °C
Isocitrate dehydrogenase	Na_3-isocitrate 20 mg	NADP 5 mg	$MgCl_2$ 200 mg NBT 5 mg PMS 1 mg	0.1 M Tris-HCl pH 8, 50 ml	5 h, 20 °C
Esterases	α-naphtyl acetate 26 mg	none	Fast Garnet GBC 30 mg	0.2 M Acetate buffer pH 5, 50 ml	3 h, 37 °C
Acid phosphatase	α-naphtyl phosphate 30 mg	none	Fast Garnet GBC 30 mg	0.2 M Acetate buffer pH 5, 50 ml	1 h, 37 °C

TABLE 3 - Morphological characterization of five different euplotes strains assigned to *E. vannus* and *E. crassus* morphotypes. (Dimensions do not include ciliary projections; $\bar{x}$, average, s, standard deviation).

Strain denomination	Geographic origin	Body length (μ)				Body width (μ)				Peristomial length (μ)			
		$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.
TM_1 (*E. vannus*)	Torre Mileto	101.65	6.23	92.1	115.8	52.75	5.64	42.1	63.1	73.18	3.16	63.1	78.9
POR_3 (*E. crassus*)	Porto Recanati	83.91	6.96	65.8	94.7	53.60	5.87	44.7	65.8	59.18	3.66	47.3	65.8
MN_3 (*E. crassus*)	Montecristo	80.45	5.04	71	92.1	58.89	5.43	50	71	58.27	3.47	50	65.8
LIV_1 (*E. crassus*)	Livorno	78.73	4.65	68.4	86.8	41.67	3.33	39.4	50	55.61	2.98	50	60.5
PB_1 (*E. crassus*)	Piombino	77.26	4.98	68.4	86.8	50.65	5.92	39.4	60.5	56.01	3.71	47.3	60.5

Number of kineties				Number of kinetosomes of the mid-dorsal row				Number of meshes of the mid-dorsal row			
$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.
8.02	0.16	8	9	16.11	0.94	14	19	10.77	0.76	9	12
10.81	0.42	10	11	14.33	1.08	13	17	15.69	1.34	14	18
10.65	0.48	10	11	14.86	1.54	12	18	12.38	1.54	10	16
9.66	0.48	9	10	13.62	0.96	12	15	13.70	1.13	12	16
10.30	0.47	10	11	15.42	1.13	13	17	13.76	1.44	11	18

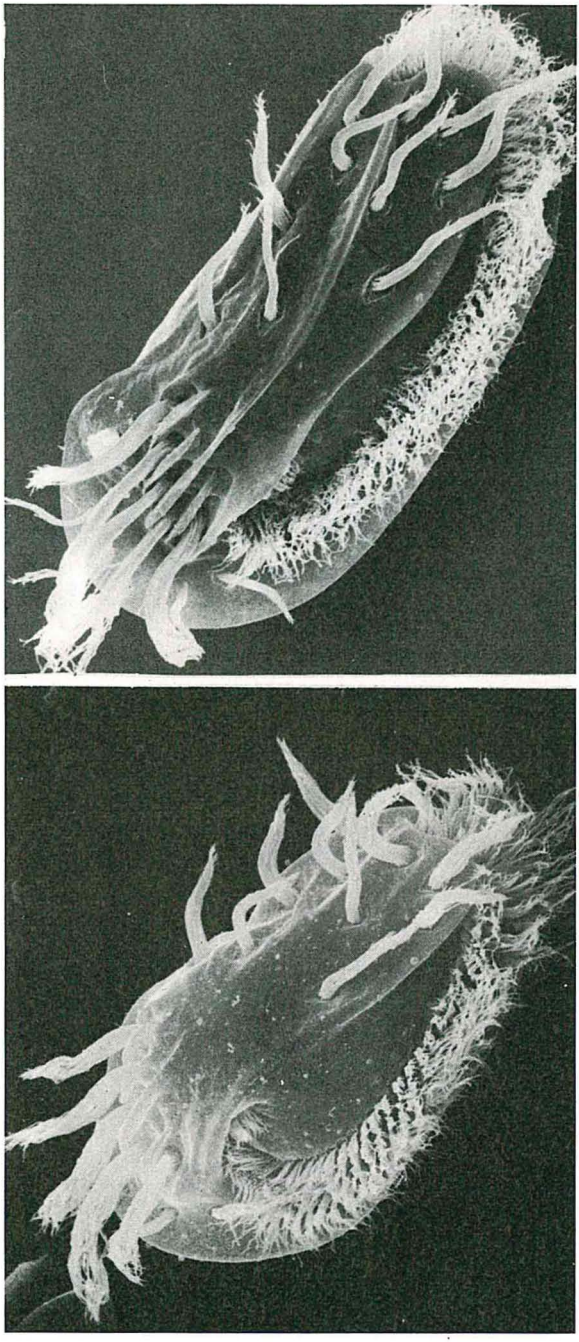

Fig. 1 - On the left, scanning electron micrographs of ventral surface of euplotes of strain TM 1 (*E. vannus*) (above) and strain POR 3 (*E. crassus*) (bottom). Magnification x = 1100. On the right, diagrams of the dorsal surface of a «median» specimen of the two strains.

Mileto collecting area and not considered in this report). The *E. crassus* strains represent four different mating types that are, however, interfertile only in combinations LIV 1-POR 3 and MN 3-PB 1.

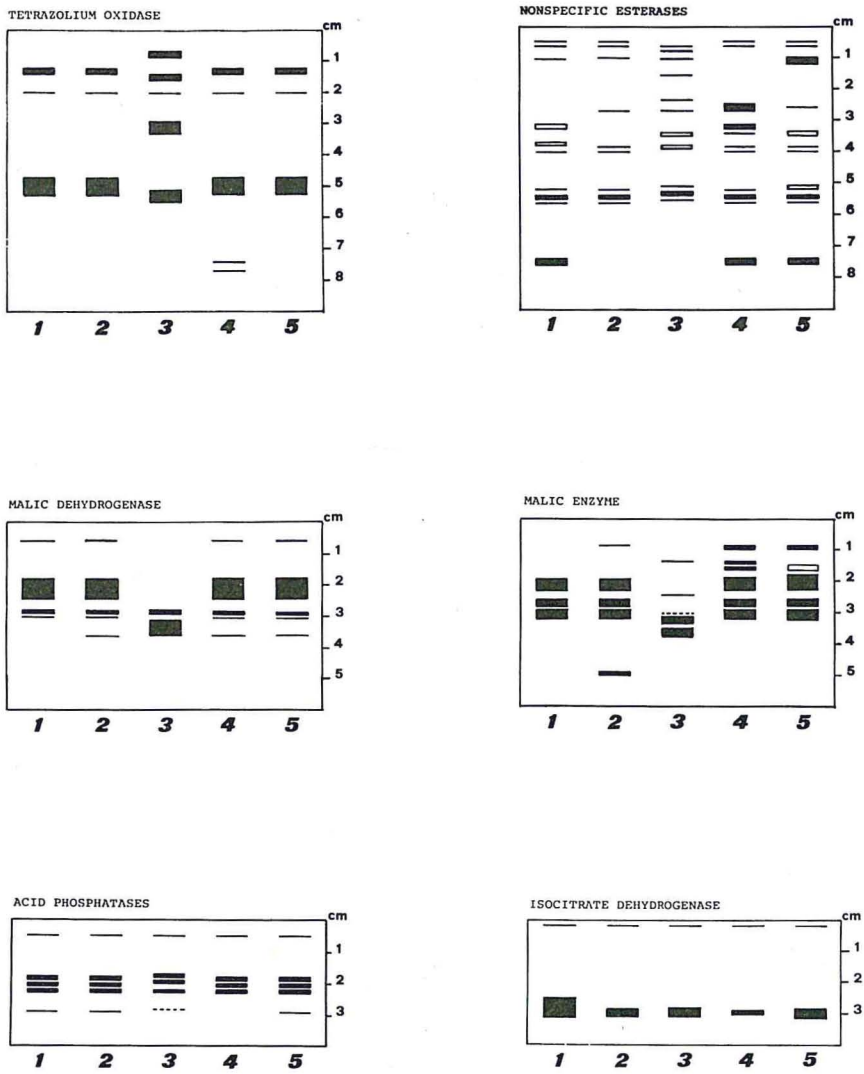

Fig. 2 - Diagrams of the electrophoretic mobilities of six different enzyme patterns. Lanes *1-5* are strains LIV 1, POR 3, TM 1, MN 3, and PB 1, respectively. Black boxes and lines indicate heavily stained bands; light boxes indicate faint bands; dashed lines indicate occasionally visible faint bands.

The typical patterns of electrophoretic mobilities of six different enzyme activities in the analysed strains are shown in Fig. 2. The *E. vannus* strain differs from all the *E. crassus* strains in the relative electrophoretic mobilities of malic enzyme, malic dehydrogenase, acid phosphatases, tetrazolium oxidases, and nonspecific esterases, while there is no difference in the mobility of isocitrate dehydrogenase. On the other hand, the *E. crassus* strains show identical or very similar electrophoretic patterns except that the nonspecific esterases that seems to be strain specific.

The interstrain similarity coefficients, computed in paired comparison of the electrophoretic patterns, are reported in Table 4. It appears that the degree of similarity between *E. vannus* and *E. crassus* strains is as low as 18-19%, whereas the interstrain similarities within *E. crassus* are more than three times higher (68-85%).

TABLE 4 - Similarity coefficient matrix obtained by the comparison of electrophoretic patterns according to the formula: S = no. positive matches / no. total comparisons.

	LIV_1	POR_3	MN_3	PB_1	TM_1
LIV_1	—	0.75	0.68	0.81	0.19
POR_3		—	0.72	0.85	0.19
MN_3			—	0.83	0.18
PB_1				—	0.18
TM_1					—

The temptative conclusion that can be drawn from the data shown is that *E. vannus* and *E. crassus* are true species, possibly hardly distinguishable from each other in surface anatomy but sharply separated at the molecular level. Critical evidence pointing to this same conclusion derives also from results of breeding tests early performed by NOBILI (1965) on other *E. crassus* and *E. vannus* strains, some of which were the same used by HECKMANN (1963) in his study of the mechanisms of mating type inheritance. It was shown, in fact, that gene exchange is regularly aborted between *E. vannus* and *E. crassus* individuals even if interspecific mate pairs may occasionally form in certain mating type combinations.

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