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## BREEDING SYSTEMS IN THE *EUPLOTES VANNUS-CRASSUS-MINUTA* GROUP

**Riassunto** — Esistono differenze nel sistema riproduttivo tra le popolazioni appartenenti alle morfospecie di *Euplotes* marini del gruppo *E. vannus-crassus-minuta*. Nel presente lavoro è valutata l'influenza che i diversi sistemi riproduttivi hanno sulla variabilità genetica, la struttura di popolazione e l'evoluzione di questi ciliati.

**Abstract** — Differences in the breeding system occur among populations comprising the marine *Euplotes* morphospecies of the *E. vannus-crassus-minuta* group. The bearing of the different breeding systems on the genetic variability, population structure, and evolutionary change in these ciliates is assessed.

**Key words** — Ciliate / *Euplotes* / breeding system / speciation.

The term «breeding system» is used to cover all those factors that determine the degree of genetic difference of the zygote-forming gametes. The breeding system controls genetic variability, population structure, and, ultimately, evolutionary change. «Outbreeding systems» are defined by combinations of factors favoring crossing with genetically different, not closely related individuals, particularly with members of different populations. On the contrary, combinations of factors that abet crossing with genetically similar individuals, particularly with close relatives, characterize «inbreeding systems». The closest form of inbreeding is self-fertilization. Since inbreeders mate preferentially with «close relatives», they are more likely to produce homozygotes and expose mutations. The greater the degree of inbreeding the greater the possibility of bringing readily new mutations to expression; the significance of the frequency of inbreeding

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in the performance of this task must not be overlooked (NYBERG, 1971). The inbreeding genetic economy may be thought of as an ecogenetic strategy for using mutational variety at hand to adjust to environmental changes. Among outbreeders, matings are preferentially between «strangers», that is, between individuals of different genetic origin. If on one hand this circumstance greatly reduces the probability of expressing mutations, on the other hand it favors the production of new combinations of genetic elements. As the genetic distance between mating partners increases, the number of different interactional combinations of genetic elements in their progeny increases vastly. The recombinant genotypes yielded have a wide range of contributions to viability, potentially ranging from lethal to highly fit. As compared to genotypes produced by deployment of the mutational variety, however, recombinants are less likely to manifest dramatically different phenotypes and are more likely to be viable for the genetic components to be recombined are the product of an already occurred selective process. New interactional combinations having a positive biological significance are differentially transmitted. Mutational pressure as a determinant of evolutionary change thus appears to be of negligible importance in outbreeders. This is not to say that they avoid mutations, but in fact require mutations as raw material to recombine. The outbreeding genetic economy may be viewed as an ecogenetic strategy for using recombinational variety to adapt to a changing environment as a substitute for mutational diversity. By no means must outbreeding and inbreeding be considered as simple alternatives; the organisms' breeding systems actually form a continuum on an inbreeding-outbreeding scale. «The precise level of inbreeding or outbreeding that is optimal for a species is affected by the diversity of the environment in which it lives, by the genetic diversity of the species as a whole, and by the usefulness of mutational variety as opposed to recombinational variety in meeting the special kinds of challenges to which the species is exposed» (NANNEY, 1980).

To begin a search for breeding systems in a group of organisms, it is reasonable to identify the factors that are generally most instrumental in evoking distinctive systems. In sexually reproducing ciliate protozoa, SONNEBORN (1957) recognized a number of biological features that bear upon the breeding system and assessed their consequences, for example, (1) the length of the period of immaturity following mating, (2) the total life span, (3) the occurrence of selfing

or autogamy, (4) the number of mating types, and (5) the mode of mating type determination. Taking all these biological features into account, it appears that the various species of ciliates can be arranged in a linear series running from extreme outbreeding through various degrees of out — and inbreeding to extreme inbreeding. As NANNEY (1980) stressed, since ciliate species otherwise closely similar may diversify in their breeding systems, they provide a rich source of comparative material for the study of ecogenetic strategies.

The present report deals with breeding systems of populations of marine ciliate protozoa of the genus *Euplotes*, the *E. vannus-crassus-minuta* group. These organisms usually reproduce asexually by binary fission and this is their mode of increasing in number. Sexual processes taking the forms of conjugation or autogamy — that do not lead to an increase in the number of the population — may occur, however, when proper conditions are achieved.

#### *Nonautogamous populations*

The bulk of populations of the above-mentioned group of *Euplotes* morphospecies (hereafter referred to as «nonautogamous, A<sup>-</sup>, populations») reproduce sexually only by conjugation (reciprocal-fertilization type of sexual phenomenon occurring between temporarily paired, unicellular organisms ordinarily of different mating types, which are thus in a sense cross-fertilizing hermaphrodites). Many A<sup>-</sup> populations have been collected all over the world and a consistent number of them have been sufficiently studied by various researchers. There do exist strong indications that these populations are generally committed to outbreeding, that is, they are adjusted for generating some degree of recombinational variety (see NOBILI et al., 1978, and DINI, 1984). First, A<sup>-</sup> populations show as a rule a sufficiently long immaturity period in number of fissions and time. If following karyogamy the new clone maintains sexual incapacity for a long time, it has the opportunity to move away from the parents and siblings and to come to interact with strangers, that is, with genetically more distant individuals. Second, immaturity is followed by long maturity and senility periods lasting for several years, as is necessary for organisms that have to await the discovery of a suitable stranger. Third, selfing (i.e. mating between individuals of the same clone) does not usually occur throughout the life cycle of A<sup>-</sup> populations, except that in some clones which have performed a large number of cell divisions. Nonautogamous populations show



finite life cycles initiated by conjugation. After a certain number of asexual generations since the last conjugation, clones enter a stage of senescence characterized by a gradual decline in growth rate and eventually die. Selfing occurring late in the life cycle may be understood as adaptive in outbreeders because it allows recombinations to diversify clones, and restores vigor to them that have almost reached the end of the life cycle without finding opportunities for outcrossing. Fourth, the mating-type system is a high-multiple system. A multiple system characterized by the existence of more than two mating types, as contrasted with a binary system with only two mating types, enhances the likelihood that a chance encounter between strangers will lead to mating. Finally, the bearing of the method of mating-type inheritance on the breeding system of *A<sup>-</sup>* populations deserves comment. The set of nuclear events occurring at conjugation (Fig. 1) accounts for the possibilities that the gamete nuclei which form in each conjugant of each mated pair can either be genotypically identical (postmeiotic-division sisters) or different (postmeiotic-division nonsisters) nuclei; (postmeiotic-division nonsisters can arise from the same or different premeiotic-division diploid nuclei). The situation arises from the fact that in *Euplotes* two of the eight products of meiosis persist (six degenerating) and the two gamete nuclei can arise from one or two different meiotic products. If genotypically identical (sisters) gamete nuclei occur in both partners of a mated pair then the two resulting exconjugants are isogenic. On the contrary, the consequence of at least one mate's gamete nuclei being genotypically different (nonsisters) is the appearance of genotypically different exconjugants from the same mated pair. Since the mating-type determination is under direct genetic control of multiple alleles at one locus (mating-type, *mat*, locus) with a dominance hierarchy, the exconjugant clones from the same mated pair (synclone) may express either the same (synclonal inheritance) or different (clonal inheritance) mating types. These situations *per se* have quite different reverberative effects on the breeding system; synclonal mating-type inheritance would favor matings between non-siblings (i.e. outbreeding), whereas clonal inheritance would favor those between siblings (i.e. inbreeding).

In two populations of the morphospecies *E. vannus* (HECKMANN, 1963) and *E. crassus* (HECKMANN, 1964), collected from the Adriatic Sea and the North Sea respectively, the observed ratio between pairs showing a synclonal and clonal mating-type inheritance agrees with

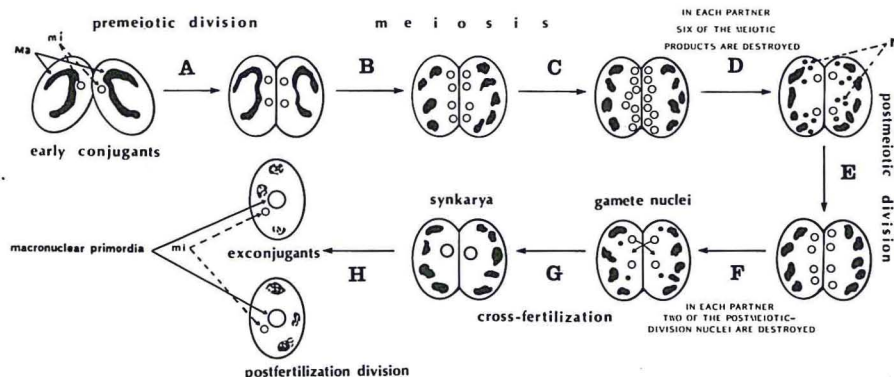


Fig. 1 - Nuclear events at conjugation in marine *Euplotes*. The nuclear apparatus of early conjugants consists of a typical, horseshoe-shaped, DNA-rich macronucleus (Ma) and a spherical, diploid micronucleus (mi). A - in each partner of a conjugation pair, the micronucleus divides mitotically («premeiotic division»); B-C - the premeiotic-division, diploid, nuclei undergo meiosis while the macronucleus loses its characteristic form and becomes fragmented into various subspherical lobes; D - six out of the eight meiotic (haploid) products yielded become relicts (r) and finally resorbed; E - the remaining two meiotic products find shelter and enter the «postmeiotic division» which is a mitotic division; F - two out of the four postmeiotic-division (haploid) nuclei yielded are again resorbed, while the two survivors become the functional «gamete nuclei»; G - the mating partners reciprocally exchange one gamete nucleus, and then karyogamy ensues within each mating partner leading to the formation of the diploid zygote nucleus («synkaryon» or «fertilization nucleus»); H - the mating partners separate. In each exconjugant the synkaryon divides mitotically one (or two) time(s) («postfertilization division s»). Only one of these postfertilization-division nuclei begins to enlarge since it continues replicating its DNA; it is the «macronuclear primordium» or «anlage». In the meantime, the lobes of the old (parental) macronucleus begin a process of autolysis. Finally, the anlage changes into the typical horseshoe shape of the functional macronucleus, before the asexual reproduction is resumed.

the expected values (see NOBILI and LUPORINI, 1967 a, and DINI and LUPORINI, 1980) assuming a complete random selection of nuclei for persistence and for gamete nucleus production during conjugation. On the contrary, two populations of the morphospecies *E. minuta* (NOBILI, 1966, 1967) and three populations of *E. crassus* (collected from the Indian Ocean, LUPORINI and DINI, 1977) showed a significant higher frequency of pairs with synclonal and clonal mating-type inheritance, respectively. This indicates that in the populations of *E. minuta* there was a real likelihood, above random expectation, for the two gamete nuclei to derive from the same meiotic product or for two nuclei carrying the same allele to persist, or both. The reverse occurred in the three populations of *E. crassus*. LUEKEN (1973) and MACHELON (1983) reported the occurrence of three (instead of



the four usually recorded) «prezygotic» micronuclear divisions (one premeiotic mitotic and two meiotic divisions) during conjugation between stocks of some  $A^-$  populations of the *E. vannus-crassus* group. In these stocks the postmeiotic (mitotic) division is lacking, and the two persisting meiotic products (out of the eight yielded) become the functional gamete nuclei. Hence, gamete nuclei arise always from different products of meiosis. Nothing has been reported in these populations as far as the ratio of clonal and synclonal mating-type inheritance is concerned. However, if we assume a completely random selection of meiotic products for gamete nucleus production, then this variation in the set of prezygotic micronuclear divisions increases the probabilities for pairs with two conjugants producing clones of different mating types (clonal inheritance). The foregoing conjugal, cytogenetic variations may be rationalized as means exploited by  $A^-$  populations to differentiate their breeding systems.

The breeding system actually determined depends on the particular combination of various life features, and all the life features, except one, shown by  $A^-$  populations are outbreeding stigmata. If on one hand  $A^-$  populations manifest some degree of synclonal nonuniformity of mating types thus providing potential mates among close relatives, on the other hand they are regularly endowed with a sufficiently long immaturity period following conjugation which permits individuals the time to spread and so renders more likely the possibility of an individual being among strangers when it is ready to mate. Hence, the presence of such an immaturity period plays down the importance of the method of mating-type inheritance in affecting the degree of genetic difference of mating partners. In conclusion, looking over the life features in their entirety, the bulk of  $A^-$  populations studied so far seem to be primarily outbreeders; if adjustments in their breeding system occur, these are variations upon the same outbreeding theme. Nevertheless, the above-mentioned details of the conjugal, cytogenetic pattern providing potential mates among sibling may be reckoned as traces of how populations which are customarily outbreeders contain seeds of inbreeding evolutionary descendants. In connection with this, it is worth mentioning the recent finding (LUPORINI, unpublished data) of stocks assigned to the morphospecies *E. crassus* that regularly undergo selfing just after the attainment of sexual maturity. We have recently reported on a population of the same morphospecies comprising stocks lacking an immaturity period (DINI et al., 1984). The existence of such «ex-

ceptional» A<sup>-</sup> populations which conform more to the pattern of inbreeders should not come as a surprise. Nonautogamous populations comprising each member of the *E. vannus-crassus-minuta* group are worldwide in distribution, and somewhere in response to a particular environment or situation genetical modifications may have become established determining an accommodation toward inbreeding. There should be little doubt, however, that among A<sup>-</sup> populations of these marine ciliates the outbreeding strategy is the basic one. Their regular organization in sets of interbreeding local populations having no apparently definite boundary lines of their own and distributed on a longitudinally and latitudinally wide range (NOBILI, 1966; NOBILI et al., 1978; DINI and LUPORINI, 1979; DINI, 1981 a, 1981 b; MACHELON, 1982) further support this statement. The range of distribution of a species may be taken both as a measure of the number of populations available for interbreeding and an index of the genetic heterogeneity of the species. Outbreeders generally have a wide range of distribution both in latitude and longitude.

The heterogeneity in geographical origin associated with environmental differences of the collecting areas — albeit in a number of physical and chemical parameters — indicate a large niche size of the foregoing sets of A<sup>-</sup> populations; a feature apparently related to their nature as outbreeders. Quantified measures of niche boundaries are available in the tolerance limits of physical or chemical stresses potentially important in the environment, hence providing concrete information about the niche size. NYBERG (1974) analyzed species of *Paramecium* and *Tetrahymena* ranging from extreme inbreeders to obligatory outcrosses and brought out an overall consistency of the relationship of greater tolerance to stress in outbreeders. Our findings in the *E. vannus-crassus-minuta* group point to the same kind of relationship (DINI, 1981 a, and unpublished data). Stocks of outbreeding A<sup>-</sup> populations seeking recombinational variety withstand various environmental stresses better than stocks of non-recombining populations (see below). Thus, there does exist conclusive experimental evidence for a relationship between breeding systems and niche breadth: outbreeding species shown a general pattern of greater tolerance to stress, hence tend to have a larger niche size.

According to a conventional view, physiological homeostasis is associated with heterozygosity. Since outbreeding favors heterozygosity, it could be argued that the greater tolerance of outbreeders is



the consequence of their heterozygous state. The non-recombining populations of *Euplotes* we analyzed somehow preserve heterozygosity (see below), yet they have reduced capabilities with respect to  $A^-$  (recombining) populations to withstand environmental stress. This supports NYBERG's (1974) hypothesis which attributes to recombination, and not to heterozygosity *per se*, an important role in the development of efficient physiological mechanisms for tracking environmental changes. In view of all this, outbreeders should not simply to be contrasted to inbreeders because they rely on recombinational vs. mutational variety as a means of meeting environmental changes, but in the performance of this task they seem also to be endowed with a greater physiological tolerance.

The basic outbreeding nature of the breeding system brings to focus the problem of speciation among  $A^-$  populations comprising the *Euplotes* marine morphospecies of the *E. vannus-crassus-minuta* group. In organisms with a developed complex of adaptations to outbreeding variations from population to population may occur, but populations are prevented from progressive divergence by the forced interbreeding. The maintenance of a common gene pool over a wide range ensues. There are, however, definite limits to the amount of genetic variability that can be arranged in a single gene pool without producing too high a proportion of poorly fit recombinants. Such an argument implies that (1) the marine littoral zone inhabited by the foregoing *Euplotes* contains more potential habitats — although this zone is in many ways simpler than terrestrial and fresh — water environments — and (2) the gene flow in these organisms is not great enough to overwhelm ecological forces favoring habitat adaptation throughout their distribution range. Should this be the case, when the above-mentioned limits are overstepped, according to a widely appreciated theory, natural selection pays a high premium for the development of means to avert matings determining fitness losses, and then the species begins to disintegrate into «subspecies». The selection is quite opportunistic; whichever kind of reproductive isolating mechanism is workable between a pair of diverging populations it is likely to become established. Among isolating mechanisms the prezygotic ones are the most economical.

Given the existence of well developed homeostatic mechanisms in  $A^-$  populations of marine *Euplotes* and the characteristics of the marine littoral zone — which may be hardly thought to hinder the dispersal hence the interchange of individuals between populations



of different geographical and ecological origins — there are no theoretical reasons to exclude some degree of continuous sympatry (biotic sympatry) of different gene pools. Collection data other than providing evidence for wide geographical distributions (see above) indicate also an extensive overlapping of geographical ranges of the different sets of interbreeding A<sup>-</sup> populations (those of the *E. vannus-crassus* group referred to as sibling species of the *E. vannus* complex by GENERMONT et al., 1976, and MACHELON, 1982); stocks belonging to two or more different sets have been repeatedly isolated from the same location or even from the same collecting jar, indicating that the geographical overlap involves also some a kind of sympatry. Very few reproductive isolating mechanisms are foolproof, and most of them are imperfect and incomplete in incipient species. Moreover, the perfecting of isolating mechanisms may proceed in different populations of a «polytypic» species, as well as between pairs of sibling species of the same complex, at different rates. Taking also into account the high multiple mating-type system and the simple system of genic inheritance of mating type shown by the organisms we are dealing with (see above), «erratic» interactions leading to heterotypic pairings between mating types of different sets should be frequent; an assumption strongly supported by laboratory observations (NOBILI, 1964; MACHELON, 1983). Under these circumstances, hybridizations to a greater or lesser extent should be anything but rare. The hybrid individuals may be ecologically or behaviorally inviable or sterile. On the other hand, they may be more or less fertile and able to backcross to some extent with one or both the parental extremes (introgressive hybridization). The success of the hybrids determines whether such hybridization lead to a strengthening of isolating mechanisms or to the development of zones of intergradation (primary intergradation; MAYR, 1942). We feel that the breeding behavior and the spatial occurrence of the single or groups of naturally occurring stocks reported by DINI (1981 b) and MACHELON (1982; 1983) could be interpreted as evidence that introgressing hybrids may actually occur in the *E. vannus* complex. In this context, it is interesting to note that, at least under laboratory conditions, stocks of the five sibling species of the *E. vannus* complex characterized by the French authors mate successfully to a greater or lesser extent in some interspecific combinations (MACHELON, 1983), suggesting that the possibility of gene exchange between them is not eliminated altogether. Given this state of affairs, the organiza-

tion of A<sup>-</sup> populations (at least of those assigned to *E. vannus* by the French authors) pursuing an outbreeding economy is viewed as a complex of semispecies (according to GRANT, 1971), each having a wide geographic distribution area basically different from that of every other member of the complex. Nevertheless, an extensive overlapping of the different geographic ranges occurs, and in some regions the members of the complex exist side by side and retain their identity, while in other regions they hybridize bridging somehow the gap between different gene pools, thereby providing opportunity for introgression. A leakage of genes from one gene pool to another could smooth out sharp differences between the members of the complex. Efforts made for distinguishing semispecies within the *E. vannus-crassus* group on the basis of electrophoretic characters have been up to now fruitless (see GENERMONT et al., and VALBONESI et al., in this issue). A plausible rationale for such a difficulty could be the occurrence of reticulate introgressive hybridizations between members of the complex leading to an enlargement and overlapping of their variation ranges. In some regions, however, pure, diversified populations of the single members should occur.

We do not wish to imply by the statements above that members of the complex are necessarily of recent origin or species *in statu nascendi*. Among plants, conditions like that outlined in *Euplotes* are all but rare, and in some instances there does exist evidence that their establishment goes back to as many as 10-15 million years (see DOBZHANSKY et al., 1977). One could regard this parallelism unfair, in view of the essential animal nature customarily awarded to ciliate protozoa, and in animals, at variance with plants, the evolutionary importance of hybridization seems small. The possibility that the biological attributes of ciliates we are dealing with are in many ways more similar to plants than to animals cannot be excluded by the available evidence (see also DINI and LUPORINI, 1985).

The (mechanical) preference for different kinds of food has been comparatively assessed in different sets of interbreeding A<sup>-</sup> populations committed to outbreeding and assigned to the *E. vannus-crassus* group (the distinguishability between these two morphospecies is still an unsettled problem) and *E. minuta*. There are no reasons derivable from the reported data (Fig. 2) not to believe that the ecological requirements of the two *Euplotes* forms are quite different. On the contrary, within the *E. vannus-crassus* group populations of different sets do not show significant differences in food preferences.



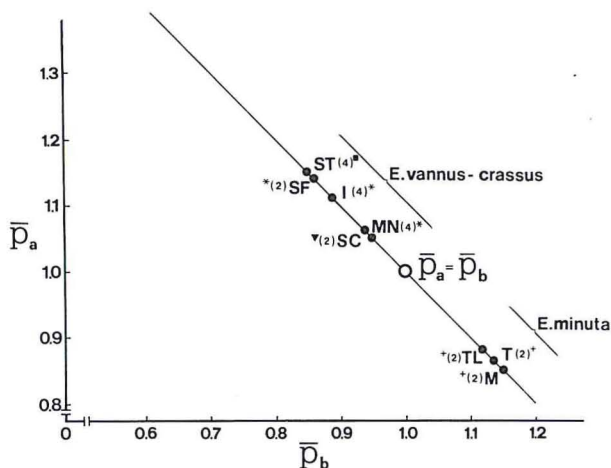


Fig. 2 - Preference of A<sup>-</sup> stocks assigned to the *E. vannus-crassus* group and *E. minuta* for marine algae and bacteria as food sources. Stocks are representatives of eight local outbreeding populations designated by capital letters, whereas the numbers in brackets indicate the stocks analyzed for each population. Populations belong to different sets showing a total genetic isolation; populations of the same set are labeled with the same mark. Three strains of green algae (*Dunaliella salina*; *D. tertiolecta*; *Chlorella* sp.) and three strains of bacteria (*Proteus mirabilis*; *Enterobacter aerogenes*; *Escherichia coli*) served as food organisms. Six lines of each stock were established and fed a different food organism for one week. Then, the daily fission rate of six sublines from each line was recorded over a time span of six days. On the abscissa is reported the preference for bacteria ( $P_b$ ) and on the ordinate the preference for algae ( $P_a$ ). The point  $P_a = P_b$  represents no preference for either algae or bacteria. Populations of the *E. vannus-crassus* group constitute a rather homogeneous group; they prefer more algae than bacteria. The reverse is true as far as the population of *E. minuta* are concerned; they prefer more bacteria than algae.

By no means does this imply that ecological specializations of the sets do not exist. If this were the case, then competition between them would be severe in the localities of contact. No sets would be in danger of extinction, however, provided that they have sufficiently large zones of ecological nonoverlap (areas of reduced or absent competition).

### Autogamous populations

Within the morphospecies of the *E. vannus-crassus minuta* group, including A<sup>-</sup> populations, there exist other populations (referred to as «autogamous, A<sup>+</sup>, populations»), much fewer in number, which manage the compromise between adaptive conservatism and wasteful innovation in a quite different way. These can exploit both conjuga-

tion and autogamy (self-fertilization occurring in unpaired individuals). There are, however, various lines of evidence indicating that  $A^+$  stocks are strongly committed to undergo autogamy rather than conjugation (see DINI, 1984). Whether conjugation does actually occur in nature or not is unknown. It is worth mentioning, however, that the crosses between *wild* stocks of the unique  $A^+$  population of *E. crassus* (in that two complementary mating types were found) resulted in a remarkable increase in mortality as compared to intrapopulation crosses of  $A^-$  stocks (LUPORINI and DINI, 1977). This weights for some degree of genomic incompatibility between these  $A^+$  stocks, suggesting they probably do not interbreed in nature.

The sequence of micronuclear divisions and selections of nuclei for persistence in autogamy of most stocks analyzed so far is identical to that occurring in conjugation of  $A^-$  stocks (cf. Figs. 1 and 3), except that it occurs in unpaired individuals, and the pronuclei uniting at fertilization are products of the immediate preceding nuclear division (SIEGEL and HECKMANN, 1966; NOBILI and LUPORINI, 1967 a; DINI and LUPORINI, 1980). (A minor variation in the pattern of micronuclear divisions, namely the lack of the postmeiotic mitotic division, has recently been reported by FLEURY and FRY-VERSAVEL 1981 in a stock of *E. vannus*. Such a variation, however, does not challenge the validity of the arguments made below). If also the set of cytogenetic events in autogamy were exactly the same as that in conjugation, then autogamy should be associated with an enforced homozygosity. As pointed out earlier, in each conjugating partner the possibility exists that gamete nuclei may be postmeiotic-division nonsisters. In autogamy, this circumstance would delay but would not prevent the attainment of a complete homozygosity in an heterozygous  $A^+$  stock. In others words, the stock should pass through a number of autogamies to be exclusively comprised of homozygous individuals. Yet, genetic evidence has been produced indicating that the appearance of a nonparental genotype is an extremely rare or nonexistent event in the *wild*, heterozygous  $A^+$  stocks that have been passed throught many autogamies (NOBILI and LUPORINI, 1967 a; LUPORINI and DINI, 1977; DINI, 1981 a). The mortality rate recorded at reorganization following autogamy cannot usually account by itself for the virtual nonappearance of genotypically diversified genotypes, even if its significance in the performance of this task must not be overlooked. It follows that peculiar cytogenetic events must occur during the autogamic nuclear processes leading



to the maintenance of the parental genotype. The nature of these events remains open to speculation. Everything that we know so far is that in autogamy chromosomal reduction occurs during meiosis and the diploid number is restored by the fusion of the two gamete nuclei from the same individual (DINI and BRACCHI, 1982). A cytogenetic model that could be exploited to enable  $A^+$  stocks of the marine *Euplotes* to maintain heterozygosity is suggested (Fig. 3).

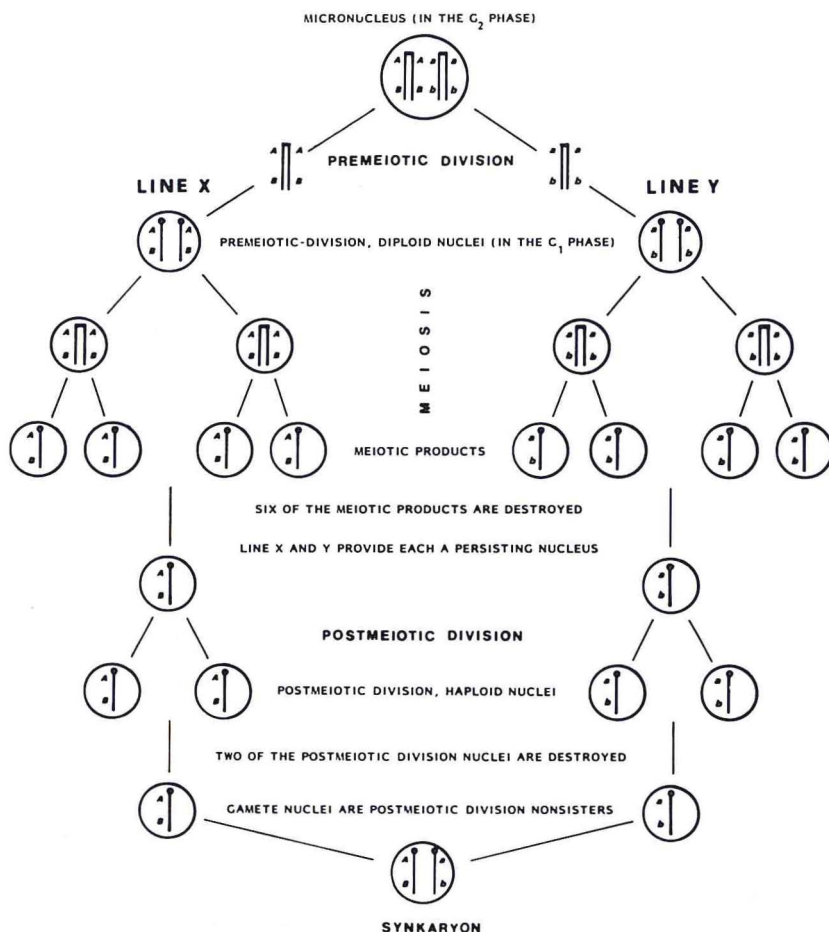


Fig. 3 - Cytogenetic model accounting for the maintenance of heterozygosity at autogamy in marine *Euplotes*. The sequence of micronuclear divisions and selections of nuclei for persistence is identical to that reported in Fig. 1. See text for details.

The model assumes that (1) for each pair of homologues, the two couples of sister chromatids move as units to different poles of the premeiotic-division spindle (crossing-over and chiasmata are absent), (2) the separation of the sister chromatids restores the diploid, chromosome number in the premeiotic-division nuclei, and (3) the two persisting meiotic products and the two gamete nuclei each derive from a different premeiotic-division nucleus. Nevertheless, casual dissociations from the suggested model cannot be excluded resulting in the production of some genotypically diversified exautogamous individuals. If we assume that  $A^+$  populations of marine *Euplotes* evolved toward a genetic system aiming at the maintenance of a given coadapted gene complex characterized by some degree of heterozygosity, it is highly likely that genotypes made homozygous at all loci are inviable. Instead, exautogamous individuals made homozygous at genes which have no important reverberating negative effects on the complex biochemical network of interacting protein sequences coded by the coadapted gene complex could have a larger likelihood of persisting. In this context, the homozygous condition at one or few marker loci (primarily controlling sexual reproductive features) found in several wild  $A^+$  stocks of *E. minuta* (SIEGEL and HECKMANN, 1966; HECKMANN and FRANKEL, 1968; K. HECKMANN, personal communication) and *E. crassus* (LUPORINI and DINI, 1977) — as well as in few  $F_2$  clones obtained in laboratory by autogamy from heterozygous  $F_1$  hybrids of crosses of  $A^+ \times A^-$  wild stocks — deserves comment. Available evidence does not exclude the possibility that  $A^+$  populations are recent derivatives of outbreeding  $A^-$  ancestors (see DINI, 1984). Should this assumption be correct, the homozygous locus or loci disclosed in the wild  $A^+$  stocks in question could be merely an heritage from their  $A^-$  ancestors. Granted that such a homozygosity is the result of an act of autogamic reproduction occurring sometime during their past life history, it by no means reflects a generalized homozygosity. As far as the appearance of exautogamous  $F_2$  segregants is concerned, it may be worth remembering the hybrid nature of the heterozygous  $A^+$  clones producing them. Given the existence of some degree of genomic incompatibility between  $A^+$  and  $A^-$  stocks (see below), the  $A^+$  genome may be incapable of responding regularly to normal developmental stimuli when brought together with the  $A^-$  genome. Finally in making considerations dealing with the relationship between genotype and fitness, one should make allowance for the dif-



ferences between laboratory conditions used in growing lines and those which probably exist in nature. Laboratory conditions are usually adjusted to favor growth of lines; natural conditions in their entirety operate surely a far more stringent selection. In view of this, the establishment in the laboratory of few nonparental genotypes by autogamy may be rationalized as a consequence of the sheltering nature of the laboratory conditions.

Whatever way matters stand, autogamy in marine *Euplotes* seems an unpromising starting point for an enforced homozygosity at all loci. On the contrary, autogamy in the species of the *Paramecium aurelia* complex causes a transition of all genes from the heterozygous to the homozygous condition in a single generation (SONNEBORN, 1947). The genetic consequences of autogamy in the two organisms are too contrasting for evolutionary significance to be the same. It has been argued that autogamy enables *Paramecium* to adapt genetically (by bringing new mutations to expression) to environmental change (NYBERG, 1974). In *Euplotes*, autogamy may be construed as a genetic system ensuring the preservation of «coadapted» genotypes, hence facilitating the maintenance of adaptation to particular environments. However, like in *Paramecium* (SONNEBORN, 1954), autogamy in *Euplotes* is significant in rejuvenating clones (DINI and BERTINI, 1978). Adequate reasons exist for drawing an analogy between *Euplotes*' autogamy and the automictic parthenogenesis of certain groups of higher organisms.

As has been previously stressed, A<sup>+</sup> stocks show reduced capabilities of meeting environmental stresses by physiological devices as compared to A<sup>-</sup> stocks. Considering the poor genetic and physiological flexibility of A<sup>+</sup> populations, grounds exist for suspecting that the range of their adaptive tactics is extremely reduced.

Genetic evidence has been provided indicating that autogamy in *E. minuta* (NOBILI and LUPORINI, 1967a) and *E. crassus* (DINI and LUPORINI, 1980) is determined by the dominant allele of a single locus with a pair of alleles. If autogamy depends on a fortuitous mutation then it may be conceivably argued that this event could have occurred repeatedly during the life history of the A<sup>-</sup> populations. Consequently, the occurrence of A<sup>+</sup> populations in nature should be anything but rare. At variance with the expectation, A<sup>+</sup> populations (considering even a single stock as representative of a local population) have been collected rarely and from widely scattered locations. Their occurrence in small sporadic patches is strikingly in contrast

with the widespread contiguous distribution and abundance of the  $A^-$  populations. Such a spotty distribution and rarity of the  $A^+$  populations permit the proposal that autogamy is a deleterious mutation in most microhabitats colonized by the  $A^-$  populations. This is the consequence of the fact that autogamy breaks the laws governing the outbreeding economy that enabled  $A^-$  populations to exploit such microhabitats. Occasionally, however, the newly arisen autogamic mutation may have an adaptive value. It is likely that this event occurs in some part of the geographical or ecological range of the  $A^-$  species populations where evolutionary lineages may break away from a reliance on flexibility in order to exist. The shift from an outcrossing to a self-fertilization system, which entails a virtually nonexistent recombination system, plays an important role in the development of reproductive isolation of the  $A^+$  lineages, and promotes a rapid fixation of the adaptive gene combinations. Indeed, there are various lines of evidence indicating some degree of evolutionary divergence between  $A^+$  and  $A^-$  populations studied so far (NOBILI and LUPORINI, 1967 b; LUPORINI and DINI, 1977; DINI, 1981 a; LUPORINI and SEYFERT, 1981; DINI and GIORGI, 1982). Notwithstanding severe restrictions to the gene flow between  $A^+$  and  $A^-$  populations, they demonstrate in the laboratory some mutual fertility, suggesting  $A^+$  populations are probably recent derivatives of the  $A^-$  ones.

#### CONCLUSIVE REMARKS

The marine ciliates of the genus *Euplotes*, the *E. vannus-crassus minuta* group, reproduce asexually by binary fission but must periodically undergo sexual processes to avoid genetic and eventually physical death. The bulk of populations comprising these morphospecies is represented by  $A^-$  populations in which sex takes the form of conjugation. Outbreeding  $A^-$  populations provide the main line of evolution of these marine *Euplotes*. If on one hand binary fission secures immediate survival by producing standardized progenies of genotypes that have proved their fitness, on the other hand conjugation, preferably between individuals of different genetic origin, provides by recombination genetic variety and physiological plasticity to adapt to a changing environment. Such a strategy seems to be successful in view of the fact that outbreeding  $A^-$  populations have colonized scores of environments around the world. These



populations are organized in sets of interbreeding local populations having no definite boundary lines of their own and distributed on a longitudinally and latitudinally wide range. Considering the characteristics of the inhabited marine littoral zone and the highly developed complex of adaptation to outbreeding in the foregoing sets of A<sup>-</sup> populations, the occurrence of (1) an overlapping of their geographic ranges, (2) a poor speciation, and (3) a blurring of the «biological» species borders should not come as a surprise.

The low number of «biological» species recorded so far within the *Euplotes* morphospecies we are dealing with is however expected to increase in view of a secondary adoption of other kinds of ecogenetic strategies, also among the A<sup>-</sup> populations themselves. Particularly noteworthy is the strategy pursued by the A<sup>+</sup> populations in which fertilization occurs by conjugation or autogamy. As compared to the outbreeding A<sup>-</sup> populations they are far fewer in number, spottily distributed, and manage the dilemma between adaptive conservatism and wasteful innovation in a quite different way. Autogamous populations seem to rely on autogamy as the only option to binary fission, and autogamy appears to mimic the advantages of asexuality more than those ascribed to sex. However, autogamy maintains the capacity of setting back the age clock of the parental generation, thereby overcoming the aging phenomena related to asexual reproduction. The combination between autogamy and binary fission may be thought of as «evolutionary opportunism carried to its limits» promoting maximum stability at the expense of flexibility, thus facilitating the maintenance of adaptation to particular discrete microhabitats. It follows that the genetic system of A<sup>+</sup> populations is ill-adapted to their evolutionary progress; such populations are doomed to conservatism and tied to the originally colonized microhabitats.

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