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Nuclear differentiation for mating types in the ciliate *Dileptus anser*: A hypothesis

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Abstract

We have obtained the first data on inheritance and genetic determination of mating types (MTs) in the lower ciliate *Dileptus anser* (= *D. margaritifer*). Observation of MT instability in young, just matured exconjugant clones obtained from some (not all) crosses appears to be the key finding in our study. In a clone of this kind, the states of maturity and immaturity (or adolescence) often alternate and/or one MT changes to another, sometimes repeatedly, during the period of several weeks after the clone's maturation. On occasion, all three MTs found in this species can be expressed consecutively. All cells of the culture are synchronously involved in such changes of their sexual state — no spontaneous selfing (intraclonal conjugation) was ever observed in such cultures. These observations suggest that the *mat* locus in dilepti is a compound integral one; it is inherited as a whole and can specify expression of any one of possible MTs (much as it occurs in *Tetrahymena thermophila*). Some other mechanisms, supposedly epigenetic ones among them, control what MT will be expressed in a given exconjugant clone in particular. Steady functioning of these mechanisms provides stable, unambiguous differentiation of the compound *mat* locus to one and only one MT and subsequent Mendelian behavior of the character over sexual generations. If, for some still unknown reasons the control of such differentiation is disturbed, MT expressed in a given exconjugant clone becomes unstable and its Mendelian behavior can be violated.

Keywords: Ciliates; *Dileptus anser*; Mating types; Macronucleus; Micronucleus; Differentiation; Epigenetic inheritance and variation

1. Introduction

Mating types (MTs) have been found in scores of species which represent all three ciliate classes (reviewed by Miyake, 1996). MT systems, their genetic control and modes of inheritance (where investigated) appeared to be widely diversified. To simplify matters, there are systems where MTs are directly and uniquely determined by genes and their alleles. On the other side of the range, one finds systems where the complex multipotential MT locus goes through epigenetic (according to Nanney, 1958; Jablonka and Lamb, 1999) differentiation which results in phenotypic realization of only one of several inherited genotypic potentialities (reviewed by Siegel, 1970; Sonneborn, 1977; Afon'kin, 1991; Bleyman, 1996; Miyake, 1996). So far, specific mechanisms of epigenetic differentiation for MTs and other characters still remain largely unknown. To take one example, the first detailed model of structure and differentiation of MT locus in *Tetrahymena thermophila* (the ciliate genetically explored much better than other ones) was suggested in 1981 (Orias, 1981) as the result of 25 year period of intensive experimentation. Nowadays, however, after 25 more years, this model still remains neither confirmed nor disproved.

The lower ciliate *Dileptus anser* (= *D. margaritifer*; Wirnsberger et al., 1984) becomes more and more common subject of laboratory experimentation (Yudin and Uspenskaya, in press for references). This raises the necessity to investigate the mode of inheritance and genetic control of MTs in dilepti to have an opportunity of controlled crossing and studying into genetics of the species. There is an additional reason for exploring MT genetics in dilepti. They belong to those not numerous ciliates that secrete inducers of sexual processes — specific

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metabolites referred to as mating pheromones or gamones — into a surrounding medium (Yudin et al., 1990). In this case, MT of a cell is specified by kinds of pheromones and pheromone receptors synthesized by the cell.

Since the discovery of MTs in *D. anser* in the course of the cultivation and pair wise mixing of various clones isolated from natural reservoirs (Nikolaeva, 1968), in the last few years we are studying the inheritance of the character during vegetative reproduction and in crosses (Yudin and Uspenskaya, 2006). Some unusual data were obtained when we initiated systematic studies of maturing exconjugant clones and their repeated testing for MT over a reasonable prolonged period of time. We summarize here these observations and propose some suggestions concerning structure and functioning of MT locus (*mat*) in dilepti.

2. Mating types, conjugation and maturation of exconjugant clones in dilepti

Only three MTs have been found in *D. anser* so far (Tavrovskaya, 1976, 1989, and others). Thus, the MT system in dilepti seems to belong to what is termed closed MT systems. These three MTs — MT I, MT II and MT III — are apparently inherited without any changes during vegetative (agamous) reproduction of the ciliates.

Individuals differing in their MTs are capable of conjugation (sexual process). Some initial studies in biology and cytology of conjugation in dilepti have been made in our laboratory (Vinnikova and Tavrovskaya, 1973; Vinnikova, 1975, 1976, and others) but we are still unaware of the cytogenetic processes during conjugation in this species. It remains to assume that the basic scheme of the processes is similar in function to those in the majority of ciliates (Raikov, 1972). In other words, it is suggested for dilepti that only one of numerous micronuclei (MIC) enters into meiosis and further only one of the meiotic products divides mitotically and produces one migratory (“male”) and one stationary (“female”) pronucleus. Thereafter the exchange of migratory pronuclei between conjugating cells and subsequent fusion of the “foreign” migratory pronucleus with “own” stationary one (i.e., fertilization) occurs. The zygotic nucleus (synkaryon) thus produced divides mitotically and some products of these mitotic divisions become MIC whereas other ones develop into macronuclei (MAC) which are also numerous in dilepti. MAC development in most ciliates is the complicated process involving precise fragmentation of their genomes, deletion of some fragments and amplification of others. Mature MAC is an ampliploid nucleus (Raikov, 1996). It is MAC with its structurally new-made genome determines the whole phenotype of a ciliate. However, MAC (the “somatic” nucleus) is mortal: it disintegrates after each conjugation and is substituted with a new one (according to the above scheme). As to MIC, it carries out only generative functions.

After conjugation, the two conjugants separate from each other, begin to feed and multiply by binary fission (agamous, or vegetative reproduction) and produce exconjugant clones, two from each pair of conjugants. For some time, dilepti from an exconjugant clone cannot pair with cells of complementary MTs and do not enter into next conjugation. As in many other ciliates, there is a so-called immaturity period in dilepti. For this reason, no MT can be attributed to the young clone — the latter does not express this character. Only after a large number of cell divisions (>100) exconjugant dilepti become mature and can pair with mature cells from other clones. Only then can their own MT be determined according to which MTs appear to be complementary to it.

Unfortunately, the survival of exconjugants in our experiments with dilepti turned out to be low (sometimes very low). As a rule, we managed to obtain only one clone from each pair of conjugants, although synclones (two exconjugant clones from each pair of conjugants) were also obtained from some crosses (Yudin and Afon’kin, 1987).

3. Mendelian and non-Mendelian inheritance of MTs at conjugation

When two randomly chosen *Dileptus* clones were crossed with each other, one out of two possible results were obtained. In some cases, the conventional Mendelian behavior of the MT was observed: the character was inherited over sexual generations synclonally and as though it were determined by a single genetic factor. The results of such crosses suggest that three MTs are controlled by one locus (*mat*) with three alleles which demonstrate pecking-order dominance (Yudin and Afon’kin, 1987; Yudin and Uspenskaya, 2006). Accordingly, dilepti with mat^1/mat^2 or mat^1/mat^3 genotype have MT I, those with mat^2/mat^2 or mat^2/mat^3 genotype, MT II, and those with mat^3/mat^3, MT III. Mature clones never change their MTs (in any case, such changes were never registered). Curiously, exactly the same system of MT genetic determination and inheritance has been described earlier (Orias, 1963; Simon, 1980) in *Tetrahymena pigmentosa*.

In some other crosses, clones with anomalous behavior for MT character were obtained, quite often along with “common” exconjugant clones. Anomalous features of such clones were as follows: (a) maturation of the clones was more or less delayed as compared with that in the “common” clones; (b) transient returns from mature to immature or adolescent state (i.e. instability of the mature state) were observed; (c) there appeared MTs that were unpredicted in a given cross by suggested model of genetic control of the character (Yudin and Afon’kin, 1987) — for instance, all three MTs in F_1 from the cross which was the test cross, according to genetic terminology (Uspenskaya and Yudin, 2003); and (d) non-Mendelian MT ratios were observed in exconjugant progeny.

Especially interesting observations were made when we repeatedly tested such “anomalous” clones at 1–2-week intervals for a long periods of time. MT of many just matured clones appeared to be unstable: when expressed for the first time, it was not uniquely determined and varied with time in one and the same clone. For reasons unknown to us, such change of MT encompassed all cells in a given culture synchronously and unidirectionally. At any rate, in no case was spontaneous selfing (i.e. intraclonal conjugation) observed, which would be expected under asynchronous and/or unlike