Protistology 4 (4), 347-352 (2006/7)

Protistology

The effect of cultivation temperature on differentiation for mating type in exconjugant clones of the ciliate *Dileptus anser*

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Summary

The effect of cultivation temperature on differentiation of exconjugant clones for their mating types was studied in the ciliate *Dileptus anser*. Crosses of the clone # 7C (mating type I, MT I) with the clone # 155 (MT III) yielded 26 exconjugant F1 clones, one clone from each conjugant pair. Each young exconjugant clone was divided into two subclones. One of the subclones was further cultivated at 17 °C, the other one, at 25 °C. The clones were tested once a week with three test-clones for their mating ability, and their sexual immaturity vs. maturity, what permitted to determine their MTs. Of 26 clones cultivated at 17 °C, two clones remained immature throughout the experiment (23 to 25 weeks after crossing), at 17 °C as well as at 25 °C. 24 subclones cultivated at 17 °C appeared to be mature by the 20th to 23rd week after crossing. Eight of them had MT I, six - MT II, and ten - MT III. Of 24 subclones cultivated at 25 °C, five changed their MTs early in their maturity state, and three others did so even twice. In other words, these subclones showed unstable differentiation for their MT (cf. Yudin and Uspenskaya, 2006). Later, 23 to 25 weeks after crossing, 17 subclones had MT I, 2 - MT II, and 5 - MT III. The distributions for MTs at 17 °C and 25 °C differed significantly (χ^2 at α= 0.05), MT I prevailing at 25 °C and MT III, at 17 °C.

Key words: ciliates, *Dileptus anser*, mating types, temperature of cultivation, epigenetic inheritance

Introduction

Only three mating types (MT I, MT II and MT III) were found (Tavrovskaya, 1976, 1989, and others) in natural populations of the ciliate *Dileptus anser* (= *D. margaritifer*; Wirnsberger et al., 1984). Hybridological analysis of the character showed (Yudin and Uspenskaya, 2006) that some clones, when crossed, demonstrated synclonal inheritance and typical monofactorial Mendelian behaviour of the character through sexual generations. The data suggest the following genetic control of MTs in *D. anser*: this character is under control of a single *mat* locus; three alleles revealed in

the locus show peck-order dominance ($mat^1 > mat^2 > mat^3$). In some other crosses more or less substantial deviations from typical Mendelian behaviour of the character have been observed, including abnormal segregations for the character in sexual generations and instability of differentiation for the character in maturing exconjugant clones. Just after maturation, most clones changed their MT, sometimes more than once, and only later MT became stably inherited during vegetative reproduction. During the instability period, exconjugant clones could consecutively express two or even all three MTs characteristic of the species, MTs not permitted by the assumed genotypes of "parental" clones among them. These observations suggest a complex nature of *mat* locus in *D. anser*, which is inherited as a whole and can express any of possible MTs (in much the same way as it is in *Tetrahymena thermophila*, Sonneborn, 1977). Which one of possible MTs will be expressed by a given exconjugant clone is determined by some other mechanisms, including admittedly epigenetic ones. Stable operation of such mechanisms provides for stable and unique differentiation of the complex *mat* locus for a certain MT and for Mendelian behaviour of the character in sexual generations. When the normal differentiation is violated in one way or another, expression of a particular MT may be unstable in maturing exconjugant clones, and subsequent inheritance of the character becomes non-Mendelian.

Material and Methods

Dilepti were cultivated as mass cultures according to the procedure described earlier (Nikolaeva, 1968; Uspenskaya and Yudin, 2000) in plastic vials at 25 °C, using Prescott's salt solution as culture medium and *Tetrahymena pyriformis* as food. Under these culture conditions, dilepti usually divide once per day (one and a half to two divisions per day in individual cultures, Uspenskaya and Yudin, 1996).

The following clones were used in this study. Clone # 7C (MT I) is cultivated in the laboratory since 1998; it was isolated from Olginskie ponds, St. Petersburg. Clone # 2 (MT II) is maintained in the laboratory since 2000 (isolated from the same population). Clones # 153 and # 155 (both of MT III) were isolated by M.S. Rautian in 2002, Yakutia. These mature clones were used as standard testers of MT I, II and III; during their cultivation, they were regularly (approximately every half a year and in addition before each crossing) checked for complementarity and never showed any changes of their MTs.

Two of these clones, # 7C and # 155, were used for crossing (these clones have already been crossed earlier, Yudin and Uspenskaya, 2006). Dilepti of both clones were put together in one microaquarium. About 100 conjugant pairs were taken from the cell mixture. Such crosses were repeated 4 to 5 times. Since survival of exconjugants appeared to be extremely poor, only one progeny clone could be grown from most isolated conjugant pairs, 20 to 25 clones from each cross altogether. By the end of the first week after conjugation, the young exconjugant clones were tested for their maturity/immaturity state with tester clones. Most of the clones tested appeared to be mature, i.e. pseudoconjugants. Only 5 to 6 progeny clones in each sample were immature, i.e. genuine exconjugants. We managed to raise and test only 26 exconjugant clones altogether.

As soon as it became possible, each of the young exconjugant clones was split up into two subclones. One of the subclones was cultivated at 17 °C and the other one, at 25 °C. Every week all clones were tested with testers to determine their maturity/immaturity state and their MTs in matured ones.

Results

Table 1 shows maturation dynamics in clones obtained from # 7C (MT I) × # 155 (MT III) cross during their cultivation at 17 °C for the first 23-25 weeks after crossing. One can see from the table that (a) 24 of 26 exconjugant clones have become mature by the 23-25th week; (b) The clones of all three MTs (I:II:III) were among them, their frequencies being 33:25:42%, respectively; (c) No changes of MT have been observed in matured clones; (d) Predominantly MT I clones were the first to mature (by the 11-13th week), and MT II/MT III clones matured later (by the 14-16th week or later on).

Table 2 shows the maturation process in the same clones but cultivated at 25 °C. It can be seen that: (a) 24 of 26 exconjugant clones appeared to be mature by the 23-25th week; clones # 43-8 and # 44-7, i.e. the same as at 17 °C, remained immature; (b) Among matured clones, clones of all three MTs were found with frequencies of I:II:III = 71:8:21%, correspondingly; (c) In contrast to the 17 °C series, cases of MT changes in matured clones were not infrequent (in 8 clones altogether, 3 among them have changed their MTs even twice; other examples of the kind see in: Yudin and Uspenskaya, 2006); (d) Here too, clones with MT I were the first to mature (by the 5-7th week); (e) By and large, clones at 25 °C matured more rapidly than at 17 °C (the first 5 clones were already mature on the 5-7th week, and all 24 clones appeared mature on 14-16th week).

So, the maturation dynamics was different in clones cultivated at different temperatures (Table 3): (a) Maturation started more rapidly at 25 °C than at 17 °C; (b) In contrast to 17 °C, at 25 °C the matured clones rather frequently changed their MTs; (c) The segregation

Table 1. Maturation of exconjugant clones from # 7C (MT I) x # 155 (MT III) cross. Temperature of cultivation is 17 °C.

Clones	Mating types of clones tested for the character at specified age (weeks) after conjugation						
	5–7	8–10	11–13	14–16	17–19	20–22	23–25
42-2	-	-	-	III	-	III	III
42-7	-	-	-	II	-	II	II
42-9	-	-	I	I	I	I	I
42-10	-	-	-	I	I	I	I
42-11	-	-	-	II	II	II	II
42-14	-	-	-	III	-	III	III
43-3	-	-	I	I	I	I	I
43-4	-	-	-	II	II	II	II
43-8	-	-	-	-	-	-	-
43-9	-	-	-	III	III	III	III
43-11	-	-	-	III	III	III	III
43-17	-	-	I	I	I	I	I
43-21	-	-	-	-	III	III	III
44-1	-	-	-	-	III	III	III
44-4	-	-	-	-	III	III	III
44-7	-	-	-	-	-	-	-
44-10	-	-	I	I	I	I	I
44-13	-	-	I	-	I	I	I
44-18	-	-	-	II	-	II	II
44-25	-	-	-	I	I	I	I
45-3	-	-	II	-	II	II	II
45-4	-	-	-	III	III	III	III
45-9	-	-	-	III	III	III	III
45-13	-	-	I	I	I	I	I
45-19	-	-	-	III	III	III	III
45-23	-	-	-	II	II	II	II
Total number of clones: 26							
Among them	I : II : III	I : II : III	I : II : III	I : II : III	I : II : III	I : II : III	I : II : III
	0:0:0	0:0:0	6:1:0	7:5:7	8:4:8	8:6:10	8:6:10
Percentage of mature clones	0:0:0	0:0:0	86:14:0	37:26:37	40:20:40	33:25:42	33:25:42

The first number in a clone designation is the number of replica crossing. **Dash** signifies immaturity (mating-reaction with tester clones of MT I–III is absent).

ratios also differed at two temperatures of cultivation ($\chi^2 = 6.90$, which is significant at $\alpha = 0.05$; Urbakh, 1964, pp. 228-232): clones with MT II and MT III were more frequent at 25 °C, and those with MT I, at 17 °C.

Discussion

On the basis of hybridological analysis performed earlier, it was suggested that mating types (MTs) in *Dileptus anser* were controlled by a single *mat* locus with three alleles showing peck-order dominance: $mat^1 > mat^2 > mat^3$ (Yudin and Afon'kin, 1987). However, further studies showed that regular Mendelian behaviour of the character was violated in one way or another. Specifically, unstable expression of the character was observed in young, just matured exconjugant clones (e. g., changes from one MT to another with time) and violation of expected Mendelian ratios in crosses, including the appearance of all three MTs where it seemed to be impossible according to the initial hypothesis (Yudin and Uspenskaya, 2006).

Similar deviations from the initial scheme are also observed in the cross described in the present paper. According to earlier crosses involving these clones (Yudin and Uspenskaya, 2006), the clone # 7C seems to be a heterozygote mat^1/mat^3 and the clone # 155, a recessive homozygote mat^3/mat^3, that is, the present cross is a test one. Nevertheless, descendants of all three MTs appeared in the progeny from this cross, their ratio varying with cultivation temperature. Moreover, changes of MT were again observed in young, just matured exconjugant clones cultivated at 25 °C.

It was suggested that such deviations from common Mendelian behaviour of MT in *D. anser* are based on complex nature of the *mat* locus (Yudin and Uspenskaya, 2006). During maturation of an exconjugant clone, this complex locus goes through epigenetic differentiation (Jablonka and Lamb, 1999), which results in expression of one of the three genetic potentialities encoded in the locus: one of its possible epialleles determining the MT arises.

Characteristic features of this differentiation should be emphasized. Obviously, the whole complex locus (with all three potentialities) gets into MA of an exconjugant as demonstrated by consecutive appearance of all three MTs in some exconjugant clones, at least, in their early maturity life. Therefore, expression of any MT during this period must be caused by functional

Table 2. Maturation of exconjugant clones from # 7C (MT I) x # 155 (MT III) cross. Temperature of cultivation is 25 °C.

Clones	Mating types of clones tested for the character at specified age (weeks) after conjugation						
	5–7	8–10	11–13	14–16	17–19	20–22	23–25
42-2	-	II	-	I	I	I	I
42-7	-	-	II	II	II	II	II
42-9	-	I	I	I	I	I	I
42-10	-	I	I	I	I	I	I
42-11	-	-	II	-	I	I	I
42-14	-	-	III	-	III	III	III
43-3	-	I	I	I	I	I	I
43-4	-	II	II	II	II	II	II
43-8	-	-	-	-	-	-	-
43-9	I	I	-	III	-	I	I
43-11	I	-	I	I	I	I	I
43-17	-	I	I	I	I	I	I
43-21	-	I	-	III	I	I	I
44-1	-	II	II	III	III	III	III
44-4	I	II	-	I	I	I	I
44-7	-	-	II	-	-	-	-
44-10	-	I	I	I	I	I	I
44-13	I	I	I	I	I	I	I
44-18	-	-	II	II	II	-	III
44-25	-	-	I	I	I	I	I
45-3	-	I	I	I	I	I	I
45-4	-	II	-	I	I	I	I
45-9	-	-	III	III	III	III	III
45-13	I	I	I	I	I	I	I
45-19	-	-	III	III	III	III	III
45-23	-	-	I	I	I	I	I
Total number of clones: 26							
Among them	I : II : III	I : II : III	I : II : III	I : II : III	I : II : III	I : II : III	I : II : III
	5:0:0	10:5:0	11:6:3	14:3:5	16:3:4	17:2:4	17:2:5
Percentage of mature clones	100:0:0	66:33:0	55:30:15	64:14:22	70:13:17	74:9:17	71:8:21

The first number in a clone designation is the number of replica crossing. **Dash** signifies immaturity (mating-reaction with tester clones of MT I–III is absent).

inactivation of other two potentialities of the complex locus, and the consequent MT change must be due to the activity switch to another potentiality (in keeping with the mutual exclusion principle, Nanney, 1958, 1963). In this period at least, no mechanisms involving any DNA rearrangements in *mat* locus (see, e.g., Orias, 1981) seem to be relevant. It is well-known that the degree of stability of epialleles arising as a result of epigenetic differentiation may vary to a large extent (see for details: Jablonka and Lamb, 1999). In fact, stable epialleles behave like alleles arising mutationally, they are inherited in a normal Mendelian fashion and unequivocally determine MT in a maturing exconjugant clone. If the epiallele stability is reduced (such epialleles are also referred to as metastable ones, Rakyan et al., 2002) or disturbed for some reasons, transdifferentiation of *mat* locus is a possibility that results in MT change in clones just matured, as well as in various violations of Mendelian fashion of MT inheritance. For reasons yet unknown, such transdifferentiation proceeds similarly in all cells of a given maturing clone; there is a striking synchronism in all members of a clone with regard to expression of one or another MT potentiality and MT switches. So far it is unclear if there is some fundamental difference between mechanisms and states of nuclear differentiation for MT at the outset of the mature life of unstable exconjugant clones and in later periods of their cultivation. If, as we believe at present, some fixation (stabilization) of MT occurs in an initially unstable clone in the course of cultivation, it might be the result of either stable inactivation or even physical loss of all but one potentialities in the *mat* locus.

Our experiment also shows that the temperature affects direction and character of differentiation for MT in dilepti, its influence being exerted not during some special sensitive period when new macronuclei develop but, obviously, later (see Materials and Methods). In this respect, the MT differentiation in dilepti differs essentially from that in some other ciliates (Sonneborn, 1977), which may point to a difference in underlying mechanisms. The same clones can differentiate for MT at the early stage of their maturity both with MT changes (at 25 °C) and without them (at 17 °C). This difference, however, does not correlate with violations of Mendelian inheritance, such as appearance of all three MTs in the progeny and non-Mendelian segregation ratios, which were observed at both temperatures.

Table 3. Maturation of exconjugant clones from # 7C (MT I) x # 155 (MT III) cross at different cultivation temperatures.

Cultivation temperature	Ratios of clones with MT I, II and III in various periods after conjugation (weeks)						
	5–7	8–10	11–13	14–16	17–19	20–22	23–25
	Numbers						
17 °C	0:0:0	0:0:0	6:1:0	7:5:7	8:4:8	8:6:10	8:6:10
25 °C	5:0:0	10:5:0	11:6:3	14:3:5	16:3:4	17:2:4	17:2:5
	Percentage of matured clones						
17 °C	0:0:0	0:0:0	86:14:0	37:26:37	40:20:40	33:25:42	33:25:42
25 °C	100:0:0	66:33:0	55:30:15	64:14:22	70:13:17	74:9:17	71:8:21

The effect of cultivation temperature on relative frequencies of various MTs in the progeny was also observed in some other ciliate species with more or less significant epigenetic contribution to MT inheritance (see for review: Sonneborn, 1977). In *Paramecium primaurelia*, wild type locus *mat*$^{+}$ permits expression of two MTs and each macronucleus is determined for one or another MT independently and randomly . Determination events are temperature-sensitive (but only during the sensitive period, i.e. during the first cell cycle after conjugation). The lower the temperature is, the more O caryonides arise. Homozygotes for *mat-1* allele are restricted to MT O expression. Environmental factors, such as temperature (Nanney, 1960; Nanney et al., 1977; Portnoy and Nanney, 1980) or nutrition (Nanney et al., 1980; Orias and Baum, 1984, 1985) affect relative frequencies of various MTs in the progeny of *Tetrahymena thermophila*. Each genotype demonstrates a unique distribution of MT frequencies corresponding to the temperature applied during conjugation (18, 28 and 34 °C). In all genotypes, various MTs respond to temperature differently (Doerder et al., 1995; Arslanyolu and Doerder, 2000).

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