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Serotypes in the ciliate *Dileptus anser*: a case of non-Mendelian inheritance

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Summary

Two *Dileptus anser* clones, B and D, isolated from natural reservoirs, had different serotypes when cultured under identical conditions in the laboratory (the classical immobilization test of infusoria with rabbit polyclonal antisera). Conjugation between the B cells (the mating type I) and D (the mating type III) resulted in exconjugant F_1 clones. It turned out that all the F_1 clones react with the both anti-"parental" immune sera, i.e., they have an intermediate, "hybrid" phenotype. Each of five different F_1 clones was back-crossed with the both "parent" clones, B and D. Standard testing of F_2 (i.e., B_1) clones about in 5–10 weeks after the conjugation gave unexpected results: no segregation for "parental" serotypes was observed in this generation, and each of the 51 tested clones had the "hybrid" serotype that seemed to be the same as that of the F_1 clones. Such a non-Mendelian inheritance of the studied character is discussed in terms of the epigene hypothesis (Tchuraev, 1975) and considering data on epigenetic control of the serotype expression in the classic objects of ciliate genetics, species of the *Paramecium aurelia* complex and *Tetrahymena thermophila*.

Key words: serotypes, i-antigens, regulation of gene expression, ciliates, *Dileptus anser*, non-Mendelian heredity, epigenetic variation and inheritance

Introduction

It has been commonly accepted that in the serotype systems in ciliates, of the greatest interest are mechanisms of regulation of activity of genes of surface cell proteins both in norm and during the so-called serotype transformation. Numerous studies have been dealing with this problem (for the review of its current state and the main literature, see: Bleyman, 1996). Nevertheless, poorly understood so far remain mechanisms responsible for the principle of "mutual exclusion", a quite peculiar feature of these systems. This principle consists in that in the given cell at the given time only one gene is expressed out of the whole set of genes present in genome and coding alternative surface proteins ("interlocus exclusion") or that one of the two alleles of the gene is active ("interallelic exclusion"). Closely related to this principle is phenomenon of the serotype transformation, when either spontaneously or, more often, after various actions on the cell, the expression "switches over" from one gene to another and, as a result, one surface protein is replaced by another. This is often accompanied by the so-called "functional inertia", or "hysteresis" of serotypes, i.e., by their tendency to be inherited in cell generations, which results in that several different serotypes can be maintained within a clone under the same culture conditions (Nanney, 1980; Caron and Meyer, 1989). Thus, the serotype systems of ciliates are very interesting objects for studies on regulation of gene activity both at the cellular and at the molecular, biochemical, level. It is remarkable that despite the recent progress in molecular-biological studies of surface antigens and corresponding genes in ciliates (Preer, 1986; Forney et al., 1996; Meyer and Duharcourt, 1996; Schmidt, 1988, 1996), the above fundamental peculiarities of serotype systems have remained so far unexplained, although numerous hypotheses have been put forwards and checked experimentally (Capdeville, 1979; Finger et al., 1995, 1995/1996).

Numerous and different data on serotype systems, which have been long accumulated so far, are obtained on the higher ciliates, specifically, on 3 to 4 species of the *Paramecium aurelia* complex and some other *Paramecium* species, as well as on *Tetrahymena thermophila*. On one hand, this is what allows using these well developed model organisms for the current molecular-genetical studies, while, on the other hand, certainly makes it difficult to discriminate between general and specific phenomena and regularities. It stimulates a search for new ciliate species for such investigations, which provides, as a rule, for a new information on the studied phenomena.

We have recently obtained some data (Uspenskaya, 1988, 1990; Uspenskaya and Yudin, 1992, 1996, 1998a, 1998b) on serotypes, their experimentally induced transformation, and intraspecies polymorphism in the lower ciliate *Dileptus anser* (= *D. margaritifer* – Wirnsberger et al., 1984). Based on these data and on the above-described peculiarities of genetic control of serotypes in paramecia and tetrahymenas as well as on our long-standing interest in the problem of epigenetic heredity and variability (Yudin, 1982), we performed a hybridological analysis of the inheritance of serotypes in dilepti. We relied on our previous experience in hybridological analysis of another character, mating types, in this ciliate (Yudin and Afon'kin, 1987); we have managed to establish that three mating types occurring in this species are controlled by three alleles in the single locus *mat*, these alleles showing the peck-order dominance: $mat^1 > mat^2 > mat^3$.

Thus, the goal of this work was to study the mode of inheritance of serotypes in *Dileptus anser*, a ciliate that has not been previously studied in this respect.

Material and methods

The hybridological analysis of serotypes was performed on two *Dileptus anser* clones, B and D, obtained from individuals isolated at different time from natural reservoirs in the Leningrad District.

Dilepti were cultivated at 25°C in Prescott's inorganic medium and fed with the ciliate *Tetrahymena pyriformis* GL (Nikolayeva, 1968). When cultivated individually under these conditions, dilepti did daily from one and a half to two cell divisions (Uspenskaya and Yudin, 1996).

The B and D clones belonged to the complementary mating types I and III, correspondingly, and were active in conjugation.

Rabbit polyclonal immune sera (ISs) were raised against the B and D cells, using mass cultures of these clones. The procedure of raising the ISs against the *Dileptus* surface antigens was described in detail elsewhere (Uspenskaya, 1988). The B and D ciliates were tested with the ISs, using classic immobilization test (Beale, 1954). For further testing, such IS dilution and time of exposition (1:50, 4 hr) were chosen that no cross reactions were observed (one of the tests is presented in Table 1).

Crosses were made as follows. The ciliates of one of the clones to be crossed were pre-marked with Indian ink. For this purpose, tetrahymenas were put into the Indian ink suspension for 0.5 to 1 hr, then washed out and added to *Dileptus* culture as a food. After 15–20 min, numerous food vacuoles were observed in dilepti; they seemed black in transmitted light and were clearly seen during further manipulations with the cells. The dilepti thus marked were washed out again and mixed with complementary ciliates, 10 to 20 cells from each clone in one microaquarium. In many cases, ciliates began to divide before or already after pairing (the so-called "preconjugant" and "conjugant" cell divisions). A great diversity of the preconjugant and conjugant divisions were observed: there could be no divisions at all, divisions in both partners, or only in one of them, the daughter cells, in turn, being able to pair (Vinnikova, 1975). Usually the conjugating pairs were formed as early as in 2–4 hr, and heterotypic pairs, B + D, were easily discernible among them. After 16–18 hr, such pairs were isolated into separate microaquaria; by that time, all preconjugant and conjugant divisions usually were already completed, the conjugant pairs themselves becoming tight. The conjugant pairs remained in this state for 20–22 hr, then the exconjugant cells were isolated and grew separately. Unfortunately, in individual exconjugant cells, no Indian ink could be visualized by this time; therefore, their "cytoplasmic" parentage could not be determined.

For unknown reason, survival of exconjugant clones in all crosses was rather low: only from one third to one half of isolated heterotypic pairs yielded one viable miniclone each (i.e., the surviving exconjugant divided at least 3–4 times); almost no synclones were obtained (Table 2).

Each miniclone thus obtained was tested with standard clones of the I, II, and III mating types. The miniclones that started conjugation and, hence, turned out to be mature were considered to result from pseudoconjugation and

Table 1. Testing of B and D "parental" clones with homo- and heterologous immune sera (immobilization reaction)

IS dilution	Proportion of cells immobilized by immune sera (%) in different exposure time							
	Anti-B serum				Anti-D serum			
	B clone		D clone		B clone		D clone	
	40 min	4 hrs	40 min	4 hrs	40 min	4 hrs	40 min	4 hrs
1 : 10	98	100	0	8	0	2	100	100
1 : 20	72	100	0	6	0	0	84	100
1 : 50	76	100	0	4	0	0	80	100
1 : 100	44	90	0	0	0	0	58	64
1 : 500	0	0	0	0	0	0	0	0

Note: 50 cells were tested in each trial.

Table 2. Survival of exconjugant clones in different crosses of dilepti

Number of heterotypic pairs isolated	Number of miniclones obtained		
	Total	Mature (i.e., from pseudo-conjugation)	Immature (i.e., from true conjugation)
10	6	4	2
20	9	4	5
25	7	3	4
50	15	5	10
75	25	13	12
100	35	14	21
50	27	10	17
100	41	18	23
100	37	16	21
100	57	21	36
75	34	12	22
75	27	14	13
50	23	11	12
60	22	8	14
75	19	7	12
100	20	6	14
75	28	10	18
100	35	7	28
100	41	5	36
100	21	7	14

were discarded. Out of the total number of the exconjugant miniclones that were immature, we have managed to raise only 46 F_1 clones.

Serotypes of these clones were tested with original ISs, using the immobilization test and conventional procedure. Five cells of the tested clone were placed into each of 4–10 microaquaria containing the diluted (1:50) IS. In 4 hr, the number of cells that lost ability to move were counted. In some cases, indirect immunofluorescence by Coons' method was used (for the procedure detail, see: Uspenskaya, 1988).

Back-crosses of 5 different F_1(B x D) clones with the "parental" B and D clones were further performed. Correspondingly, 25 and 26 F_2 (B_1) clones were raised and tested for their serotypes.

Results

Exconjugant F_1 clones were tested for their serotypes, using anti-B and anti-D ISs, in 30 days (Table 3) and 4 months, on reaching their sexual maturity (Table 4), after conjugation. Ciliates from all F_1 clones tested at the age of 30 days reacted with the both ISs, i.e. they had hybrid phenotype, which, by the way, was another evidence for their origin from true conjugation. Judging from the reaction of immobilization, i-antigens of both "parental" types were often present on the surface of the F_1 cells in different amounts. Thus, in 33 out of 46 clones, proportions of cells immobilized under effect of one of the ISs differed significantly. Meanwhile, no essential predominance of one of the i-antigens was observed: 14 clones reacted stronger with the anti-B IS, while 19, with the anti-D IS.

The hybrid phenotype of the F_1 persisted during their further cultivation, up to their sexual maturation (Table 4). At that time, 12 out of 17 clones also showed quite different responses to the two ISs: in 6 clones, stronger was the effect of the anti-B IS, while in other 6 clones, that of the anti-D IS. Several clones were also analyzed with immunofluorescence technique and turned out to be homogenous for their serotypes. Thus, neither "maternal" inheritance of the studied character (like in the case, for instance, of the serotype inheritance in *Paramecium primaurelia*) nor the situation that might be described as the "allelic exclusion" of "parental" serotypes was observed in F_1. We also failed to detect anything like "vegetative assortment" of serotypes in heterozygous clones of *Tetrahymena thermophila*. On the contrary, the pattern of the serotype inheritance resembled that of allelic serotypes in heterozygous *P. tetraurelia* in which coexpression of both alleles takes

Table 3. Testing of immature F_1 clones with anti-"parental" immune sera (immobilization reaction) in roughly 1 month after conjugation

Clone	Proportion of cells immobilized by immune sera, %	
	Anti-B IS	Anti-D IS
5–2	44 (30–59)	54 (39–68)
5–3	36 (23–50)	36 (23–50)
5–5	24 (13–38)	72 (57–84)
5–6	16 (7–29)	64 (50–77)
5–8	20 (10–34)	60 (45–73)
5–9	28 (16–43)	58 (43–72)
5–10	32 (20–46)	56 (41–70)
5–14	60 (45–73)	34 (21–48)
6–4	44 (30–59)	48 (34–63)
6–5	24 (13–38)	76 (62–87)
6–10	30 (18–44)	58 (43–72)
6–12	36 (23–50)	60 (45–73)
7–1	58 (43–72)	24 (13–38)
7–3	66 (52–79)	26 (15–41)
7–4	64 (50–77)	30 (18–44)
7–6	60 (45–73)	12 (5–24)
7–8	54 (39–68)	36 (23–50)
7–10	42 (28–57)	48 (34–63)
7–12	68 (54–80)	32 (20–46)
8–2	50 (36–64)	56 (41–70)
8–4	44 (30–59)	46 (32–61)
8–5	40 (27–55)	50 (36–64)
8–7	40 (27–55)	40 (27–55)
8–10	36 (23–50)	54 (39–68)
8–12	52 (37–66)	38 (25–53)
8–14	56 (41–70)	40 (27–55)
9–2	46 (32–61)	44 (30–59)
9–7	68 (54–80)	26 (15–41)
9–11	36 (23–50)	48 (34–63)
9–13	36 (23–50)	50 (36–64)
9–15	24 (13–38)	54 (39–68)
9–16	26 (15–41)	60 (45–73)
9–19	30 (18–44)	64 (50–77)
9–20	72 (57–84)	20 (10–34)
10–1	21 (14–28)	61 (53–69)
10–2	38 (30–46)	51 (43–59)
10–3	53 (45–61)	43 (35–51)
10–5	41 (33–49)	32 (25–39)
10–6a	23 (16–30)	47 (39–55)
10–6b	30 (23–37)	43 (35–51)
10–20a	37 (29–45)	40 (32–48)
10–20b	26 (19–33)	33 (25–41)
10–21	48 (40–56)	40 (32–48)
10–22	53 (45–61)	31 (24–38)
10–23	30 (23–37)	51 (43–59)
10–24	63 (55–71)	28 (21–35)

Notes: a and b, sister exconjugant clones (from the same synclone). 50 or 150 (the 10-x series) cells of each clone were tested with each IS. In brackets, 95% confidence intervals for the proportions of immobilized cells, % (Snedecor, 1961; Plokhinsky, 1980). Bold face, clones with significantly different proportions of cells immobilized by each antiserum.

Table 4. Testing of immature F_1 clones with anti-"parental" immune sera (immobilization reaction) in roughly 4 months after conjugation

Clone	Proportion of cells immobilized by immune sera, %		Mating type
	Anti-B IS	Anti-D IS	
5–5*	34 (26–42)	28 (21–35)	II
5–8*	46 (38–54)	32 (25–39)	?
5–10*	26 (19–33)	51 (43–59)	II
5–14*	40 (32–48)	50 (42–58)	?
7–1	54 (46–62)	41 (33–49)	II
7–3	38 (30–46)	33 (25–41)	?
7–6	43 (35–51)	28 (21–35)	II
7–10	30 (23–37)	34 (26–42)	II
8–4	60 (52–68)	30 (23–37)	II
8–7	36 (28–44)	43 (35–51)	?
9–2	64 (56–72)	28 (21–35)	II
9–7	67 (59–75)	28 (21–35)	?
9–11	29 (22–36)	38 (30–46)	II
9–13	32 (25–39)	53 (45–61)	II
9–15	18 (12–24)	50 (42–58)	II
9–16	34 (26–42)	54 (46–62)	II
9–19	30 (23–37)	48 (40–56)	II

Notes: asterisks, clones whose homogeneity for serotype was proved with immunofluorescence analysis. 150 cells of each clone were tested. In brackets, 95% confidence intervals for the proportions of immobilized cells, % (Plokhinsky, 1980). In bold face, clones with significantly different proportions of cells immobilized by each antiserum. "?", adolescent phenotype.

place (for the data of comparison, see reviews: Beale, 1954, 1957; Bleyman, 1996; Finger, 1974; Nanney, 1980; Preer, 1968; Sommerville, 1970).

When the mature F_1 clones were tested for their mating types (MTs), most of the clones survived by that time turned out to be completely mature and belonging to the MT II. Five clones could conjugate only with the standard MT III clone, i.e., they had combined properties of the MT I and MT II. Most likely, these clones still were at the so-called "adolescent" stage during the tests (Table 4; see references in: Yudin et al., 1990). Surprisingly, they had remained adolescent even in 12–14 months after conjugation, up to the moment when the present paper was submitted for publication.

Partly for this reason, to subsequently obtain F_2 progeny, we crossed F_1 clones not with each other, but with each of the "parental" clones (the so-called back-crosses, Table 5). Some of the F_2 (or, more precisely, B_1) clones obtained were tested with anti-"parental" ISs as soon as possible after the conjugation. At that time, only 10–20 (maximum 50) cells could be taken from each miniclone. There were tested five clones from the F_1(6–7) x D cross, 7 clones from the F_1(7–10) x B cross, 7 clones from the F_1(7–10) x D cross, and 12 clones from the F_1(9–13) x D cross (the numbers of clones, see Table 4). All of them had the same "hybrid" phenotype as that of F_1 clones; no segregation for the tested character was observed. A part of these clones, as well as other similar clones obtained from other crosses, were tested much later. They turned out to retain steadily their "hybrid" phenotype (Tables 6 and 7), demonstrating non-Mendelian inheritance of the studied character. In some F_2 clones, like in F_1 clones, a significantly different reaction with the two testing anti-"parental" ISs was observed. But, unlike F_1, most of such F_2 clones reacted stronger with the anti-D IS (7 out of 9 clones in Table 6 and 10 out of 14, in Table 7).

Discussion

According to one of classifications of ciliates (Lee et al., 1985), *Dileptus anser* belongs to the phylum of Ciliophora, class Litostomatea, order Pharyngophorida and family Tracheliidae, i.e., to different subphylum than that of *Paramecium* or *Tetrahymena*.

In the *D. anser* life cycle, unlike the species of the *Paramecium aurelia* complex, there is no autogamy which provides homozygosity of laboratory paramecia strains for all their genes. Therefore, when working with *D. anser* clones isolated from nature, it is impossible, unfortunately, to meet the requirement of the classical genetic analysis for the obligatory homozygosity of "parental" forms for the studied characters (Aksenovich, 1999). For instance, it cannot be ruled out that either one of the original, "pa-

Table 5. Back-crosses of F_1 clones with "parental" clones

Cross	Clones crossed	F_2 progeny (clones)
11	F_1(7–6) x B	11–1, 11–3, 11–5, 11–7
12	F_1(7–6) x D	12–2, 12–3, 12–4, 12–5, 12–7
13	F_1(7–10) x B	13–2, 13–5, 13–7, 13–8, 13–10
14	F_1(7–10) x D	14–1, 14–2, 14–8, 14–9, 14–10
15	F_1(9–2) x B	15–1, 15–2, 15–4, 15–6, 15–9, 15–10
16	F_1(9–2) x D	16–1, 16–3, 16–5, 16–6
17	F_1(9–13) x B	17–3, 17–4, 17–6, 17–9
18	F_1(9–13) x D	18–1, 18–2, 18–3, 18–6, 18–8, 18–9, 18–10
19	F_1(9–19) x B	19–1, 19–3, 19–4, 19–6, 19–8, 19–9
20	F_1(9–19) x D	20–2, 20–3, 20–5, 20–9, 20–10

Table 6. Testing of F_2 clones from F_1 x B crosses with immune sera raised against "parental" B and D clones (immobilization reaction) in ca. 5 (the 17-x and 19-x series) or 9–10 (the 11-x, 13-x, and 15-x series) weeks after conjugation

Clone	Proportion of cells immobilized by immune sera, %	
	Anti-B IS	Anti-D IS
11–1	20 (13 – 29)	40 (30 – 50)
11–3	24 (16 – 33)	30 (21 – 40)
11–5	50 (40 – 60)	39 (29 – 49)
11–7	39 (29 – 49)	42 (32 – 52)
13–2	48 (38 – 58)	38 (28 – 48)
13–5	32 (23 – 42)	30 (21 – 40)
13–7	30 (21 – 40)	48 (38 – 58)
13–8	30 (21 – 40)	41 (31 – 51)
13–10	20 (13 – 29)	38 (28 – 48)
15–1	43 (33 – 53)	52 (42 – 62)
15–2	37 (28 – 47)	35 (26 – 45)
15–4	26 (18 – 36)	34 (25 – 44)
15–6	41 (31 – 51)	49 (39 – 59)
15–9	28 (19 – 38)	33 (24 – 43)
15–10	36 (27 – 46)	42 (32 – 52)
17–3	38 (28 – 48)	26 (18 – 36)
17–4	50 (40 – 60)	41 (31 – 51)
17–6	36 (27 – 46)	29 (20 – 39)
17–9	42 (32 – 52)	40 (30 – 50)
19–1	27 (19 – 37)	29 (20 – 39)
19–3	30 (21 – 40)	44 (34 – 54)
19–4	36 (27 – 46)	46 (36 – 56)
19–6	38 (28 – 48)	42 (32 – 52)
19–8	25 (17 – 35)	39 (29 – 49)
19–9	27 (19 – 37)	42 (32 – 52)

Notes: 100 cells of each clone were tested. In brackets, 95% confidence intervals for the proportions of immobilized cells, % (Snedecor, 1961). In bold face, clones with significantly different proportions of cells immobilized by each antiserum.

Table 7. Testing of F_2 clones from F_1 x D crosses with immune sera raised against "parental" B and D clones (immobilization reaction) in ca. 5 (the 18-x and 20-x series) or 9—10 (the 12-x, 14-x, and 16-x series) weeks after conjugation

Clone	Proportion of cells immobilized by immune sera, %	
	Anti-B IS	Anti-D IS
12–2	38 (28 – 48)	40 (30 – 50)
12–3	36 (27 – 46)	46 (36 – 56)
12–4	32 (23 – 42)	52 (42 – 62)
12–5	43 (33 – 53)	46 (36 – 56)
12–7	44 (34 -54)	42 (32 – 52)
14–1	52 (42 – 62)	30 (21 – 40)
14–2	20 (13 – 29)	37 (28 – 47)
14–8	28 (19 – 38)	48 (38 – 58)
14–9	40 (30 – 50)	51 (41 – 61)
14–10	22 (14 – 31)	54 (44 – 64)
16–1	49 (39 – 59)	32 (23 – 42)
16–3	30 (21 – 40)	48 (38 – 58)
16–5	50 (40 – 60)	23 (15 – 32)
16–6	33 (24 – 43)	40 (30 – 50)
18–1	49 (39 – 59)	42 (32 – 52)
18–2	38 (28 – 48)	28 (19 – 38)
18–3	36 (27 – 46)	32 (23 – 42)
18–6	29 (20 – 39)	40 (30 – 50)
18–8	37 (28 – 47)	31 (22 – 41)
18–9	26 (18 – 36)	42 (32 – 52)
18–10	51 (41 – 61)	33 (24 – 43)
20–2	27 (19 – 37)	41 (31 – 51)
20–3	46 (36 – 56)	42 (32 – 52)
20–5	28 (19 – 38)	37 (28 – 47)
20–9	29 (20 – 39)	44 (34 – 54)
20–10	33 (24 – 43)	35 (26 – 45)

Notes: 100 cells of each clone were tested. In brackets, 95% confidence intervals for the proportions of immobilized cells, % (Snedecor, 1961). In bold face, clones with significantly different proportions of cells immobilized by each antiserum.

rental" *D. anser* clones (B or D) or both of them were heterozygous for the locus controlling their serotype (e.g., B1/B2 or D1/D2). In this case, however, if even the heterozygous clone coexpresses both alleles to provide the presence at the cell surface of the both allelic variants of the corresponding i-antigen, the polyclonal IS against such heterozygous clone, naturally, cannot distinguish these variants and identifies serotype of such heterozygote as the single elementary character both in the original clone itself and in any other clones with the same serotype.

The initial genetic nature of the analyzed difference between the B and D serotypes is of course unknown: in the simplest case, it might be a difference for one locus (the allelic difference) or for two or several genetic loci. The absence of serological cross-reactions between the B and D clones indicates prominent immunological differences between the corresponding i-antigens to rather argue in favor of the the non-allelic nature of the corresponding serotypes.

All ciliates are diplonts with the gametic reduction of the chromosome number, and their meiosis is consistent with the classic scheme of meiosis in Metazoa (Raikov, 1972). Data on nuclear apparatus, conjugation, and conjugation cycle in *Dileptus anser* are rather scarce. Earlier publications are summarized by Dragesco (1963). Among more recent works, papers by Vinnikova (1974a, 1974b, 1975, 1976), Karadzhan (1985), and Golinska and Afon'kin (1993) are to be referred to. In terms of cytogenetics, of importance are observations by Vinnikova who found that 4–12 micronuclei started the first meiotic division, only 2–6 products of the 1st division, the 2nd meiotic division, and just the single nucleus completed the 3rd division. She observed pronuclei in joined probosci of conjugating dilepti and suggested in *D. anser* an exchange

of migrating pronuclei in agreement with the classical scheme (Raikov, 1972). According to Vinnikova's data, the number of divisions of synkaryon varies from 1 to 4, the number of the formed macronuclear anlagen, from 1 to 4 (more often, 4); in most cases, the set of new macronuclei of the exconjugant cell develops from several anlagen as a result of their multiple successive binary divisions, which was also noticed by Karadzhan (1985). Vinnikova has never observed metagamic divisions of exconjugant cells with distribution of anlagen into daughter cells in *Dileptus*, nor was there fusion of anlagen. Therefore, she admits that the macronuclei within a dileptus clone might be of different origin.

Judging by the prolonged maturation period and "hybrid" phenotype, the exconjugant F_1 clones selected for our analysis were products of the real, normal conjugation between ciliates of "parental" clones. It should again be emphasized that not all surviving exconjugant clones were of this kind. At the same time, all mature F_1 clones belonged to the mating type II. This fact does not contradict the genetic control pattern of mating types in *Dileptus anser* (Yudin, Afon'kin, 1987), but requires some assumptions, such, for instance, as that of differential survival of different genotypes in the progeny obtained from this cross.

Phenotype of F_1 clones is undoubtedly "hybrid". If the B and D serotypes are allelic, we deal here with coexpression of the corresponding alleles, whereas if they are not allelic, there is a violation of the principle of the "mutual exclusion" of different loci controlling the surface i-antigens in *Dileptus*. This alternative was to be resolved by obtaining F_2 (B_1) and analyzing segregation for the studied character.

However, the F_2 phenotype also has turned out to be "hybrid". There is no segregation within the second hybrid generation, which means the non-Mendelian inheritance of the character. The lack of the segregation in F_2 is an unexpected result. It can be considered a consequence of deviation from Bateson's principle of "gamete purity" in the first hybrid generation, which requires an explanation. This principle is well-known to be deviated in polyploids, as in these organisms, meiosis results, more or less frequently, in formation of heterozygous gametes (Inge-Vechtomov, 1989). However, this does not seem to have any relation to our case.

Interestingly, it is this result, the deviation from the normal Mendelizing of characters, that is predicted by the epigene hypothesis (Tchuraev, 1975). This hypothesis is one of numerous variants of the concept of the dynamic hereditary memory (Riggs and Porter, 1996). According to Tchuraev, one of possible results of crosses between the forms differing in epigene-controlled characters, is the "absorption effect" (or unification, to be more exact) when all epigenes switch to the same state in F_1. Thus, if the active, expressable state of the A locus is designated as A^1, while its inactive state, as A^0, the inactive allele in the A^1A^0 epiheterozygote can be activated by the active one, and the A^1A^0 epiheterozygote is converted to the A^1A^1 epihomozygote. As a result, the F_1 individuals will produce only one type of gametes (gametic nuclei, in our case), A^1. This will inevitably affect F_2 which will show no segregation. It is to be remembered that it is to account for specific features of genetic control and inheritance of serotypes in *Paramecium* that one of the first hypothetical schemes of the epigenetic control was suggested (Delbrück, 1949), this hypothesis actually becoming the basis for many of the subsequent concepts of this type (see reviews: Olenov, 1965; Riggs and Porter, 1996; Russo et al., 1996; Golubovsky, 1996; Golubovsky and Tchuraev, 1997; and others).

In the case of ciliates, however, the scheme is more complicated. These have been known to be characterized by nuclear heteromorphism (Ossipov, 1981). The reproducible changes in gene activity, which are described by the epigene hypothesis, are most likely to occur at the level of genes of the phenogenetically active ampliploid (Raikov, 1996) nucleus, the macronucleus. Meanwhile, the violation of the principle of "gamete purity", whose manifestation is the "blended" inheritance, is to occur at the level of phenogenetically inactive micronuclei and products of their meiosis (male and female pronuclei). The phenomena that might be designated a sort of predetermination of gametic nuclei in ciliates (specifically, during the serotype inheritance) were described by several authors (Sommerville, 1970).

Thus, due to the nontrivial genetic behavior of F_2 clones, the question has remained unanswered whether the serotypes of the "parental" B and D clones are controlled by alleles of the single locus or by different loci. The situation might have been clarified by experiments on temperature transformation of serotypes in the F_2 clones: thus, among these clones, a segregation according to capability for, or the character of, such transformation could be observed. In experiments on paramecia, the temperature transformation was an important auxiliary element of the hybridological analysis of serotypes (Beale, 1954).

The authors of the current work are aware that the performed analysis of the serotype inheritance in the ciliate *Dileptus anser* does not fit, in many aspects, the stringent requirements of the classic genetic analysis. Unfortunately, this is, to a degree, an inevitable payment for performing experiments on the species that has not yet become a laboratory model organism.

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