

Strategies of the Macronuclear Endocytobionts of *Paramecium* During the Sexual Process of the Host

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Abstract

Paramecia infected with endonucleobiotic bacteria were traditionally thought to lose the capacity to undergo the sexual process as a result of the infection. On the other hand, successful conjugation with newly generated macronuclei should lead to a loss of bacteria from the macronuclear compartment. Certain types of the nucleobiosis between *Paramecium* and bacteria namely *P. caudatum*-*Holospora obtusa*, *P. caudatum*-*Nonospora macronucleata*, *P. duboscqui*-*Caedibacter macronucleorum* and *P. biaurelia*-*H. caryophila* were studied with regard to the strategies of these bacteria during the host's conjugation. The enumerated bacteria were found to be able to survive during the host's conjugation in two different ways: modification (mainly) of this sexual process or prevention of its completion. As a result of the modifications of the nuclear reorganization, the infected exconjugant's progeny maintained the old macronucleus (macronuclear regeneration). For the first time it was demonstrated that intranuclear bacteria (*N. macronucleata*) induce the fusion of old nuclear fragments and new macronuclear anlagen with the forming macronuclear heterokaryon. Only for *H. caryophila* an alternative way – infection of the new macronuclear anlagen by a few of the bacteria from the old macronuclear fragments – was shown.

Keywords: *Paramecium*, bacteria, endonucleobiosis, nuclear reorganization

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1. Introduction

Cells of *Paramecium* (Ciliophora, Protista) may be habitats for more than 40 species of bacteria (Heckmann and Görtz, 1991; Fokin, 1993). Some of the bacteria have been found in the nuclei of paramecia, especially, in the macronucleus (Hafkine, 1890; Preer and Preer, 1984; Heckmann and Görtz, 1991; Fokin, 1993; Fokin et al., 1996). Usually endonucleobionts can be maintained in the host nuclei for a long time by the multiplication and distribution between progeny in every vegetative division of the infected cell. It was traditionally believed that a host-cell infected with endonucleobiotic bacteria loses the capacity to realize conjugation (sexual process) as a result of the infection (OSSIPOV, 1981). Usually, in the process of the nuclear reorganization during and after conjugation in *Paramecium*, new macronuclei are generated by the micronuclei and the old macronucleus transforms into fragments which degenerate and are resorbed (Hiwatashi and Mikami, 1989). This means that all bacterial endonucleobionts which are maintained in the old macronucleus should be lost as a result of this nuclear reorganization in *Paramecium* species (even if the infected host can conjugate). After detailed cytological investigations of certain types of the bacteria infecting the macronuclei of *P. caudatum*, *P. biaurelia* and *P. duboscqui* I have detected different strategies of these microorganisms during the host's sexual process.

2. Material and Methods

Cells and culture conditions

The following cultures were used for cytological observation of the nuclear reorganization: strains PK42-1 and PK42-3 of *Paramecium caudatum* (complementary mating types, syngen unknown) infected with *Holospora obtusa*; strain AP-8 and AP-18 of *P. caudatum* (complementary mating types, syngen unknown) infected with *Nonospora macronucleata* and the uninfected strains R20-8 and PP21-5 (complementary mating types, the same syngen as the infected ones); strains Ku4-8 and Ku4-11 of *P. duboscqui* (complementary types, syngen unknown) infected with *Caedibacter macronucleorum*; strains SK2-10 and MB3-3 of *P. biaurelia* (complementary mating types) infected with *H. caryophila* and BK10-7 (the same species) without macronuclear infection. The cultures were isolated from different places of the former Soviet Union and Germany (Table 1). Cells were grown at 20°C on lettuce medium inoculated with a non-pathogenic strain of *Enterobacter aerogenes*.

Table 1. Origin of cultures of *Paramecium*

Host species/ Clone	Place of isolation	Bacterium species
<i>P. caudatum</i>		
PK42-1	Old Peterhof, St. Petersburg, Russia	<i>Holospora obtusa</i>
PK42-3	Old Peterhof, St. Petersburg, Russia	<i>Holospora obtusa</i>
AP-8	Parzlich lake, Armenia	<i>Nonospora macronucleata</i>
AP-18	Parzlich lake, Armenia	<i>Nonospora macronucleata</i>
R20-8	Ropsha, St. Petersburg, Russia	–
PP21-5	Newa river, St. Petersburg, Russia	–
<i>P. duboscqui</i>		
Ku4-8	Pacific Ocean coast, Kunashir Isl., Russia	<i>Caedibacter macronucleorum</i>
Ku4-11	Pacific Ocean coast, Kunashir Isl., Russia	<i>Caedibacter macronucleorum</i>
<i>P. biaurelia</i>		
SK2-10	Stuttgart, Germany	<i>H. caryophila</i>
MB3-3	Münster, Germany	<i>H. caryophila</i>
BK10-7	Baikal lake, Russia	<i>H. caryophila</i>

Cytology

For the detection of the nuclear reorganization steps, infected and uninfected paramecia were immobilized with the help of a pressure control slide (a device for controlling the pressure of the cover slide, Skovorodkin, 1990). By this device it was possible to observe the same living cell and its progeny several times. Micrographs of unfixed and unstained living infected paramecia were taken with differential interference contrast (DIC) optics using a Polyvar microscope (Reichert, Austria) and Axioskop (Zeiss, Germany). Permanent preparations stained by the Feulgen technique were made for *P. duboscqui* only. Infected paramecia were fixed for electron microscopy with 1.5% glutaraldehyde in phosphate buffer, pH 7.2, and postfixed in 1% OsO₄. Sections were stained with uranyl acetate and lead citrate.

Crosses

Mating reactive cells obtained according to Hiwatashi (1968) were mixed together in the depression slides (2 ml) at 20°C. The conjugations with homotypical pairs (infected-infected or uninfected-uninfected) and heterotypical pairs (infected-uninfected) were performed (Figs. 1 and 2).

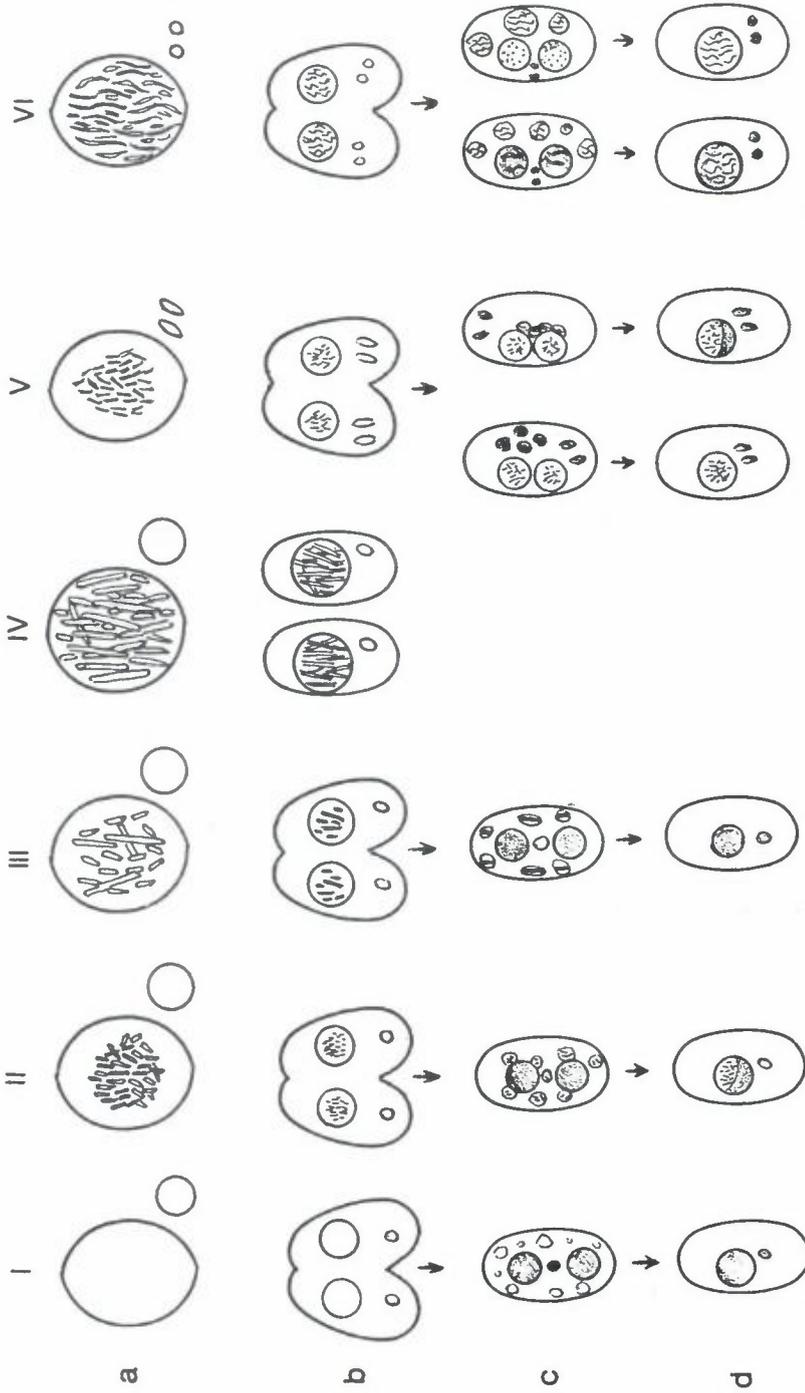


Figure 1. Scheme of the experimental crosses. I = uninfected cells (control), II = cells infected with *N. macronucleata*, III = cells infected with *H. obtusa* (low rate of the infectious forms), IV = cells infected with *H. obtusa* (high rate of the infectious forms), V = cells of *P. duboscqui* infected with *C. macronucleorum*, VI = cells of *P. biaurelia* infected with *H. caryophila*. I-IV = *P. caudatum*. a = nuclear apparatus of the paramecia, b = conjugated pairs, c = exconjugants (Ic-IIIc, VIc = first exconjugants cell cycle; Vc = before first exconjugant's division), d = result of the nuclear reorganization. o = old nuclei, ● = new nuclei, ⊖ = aborted anlagen.

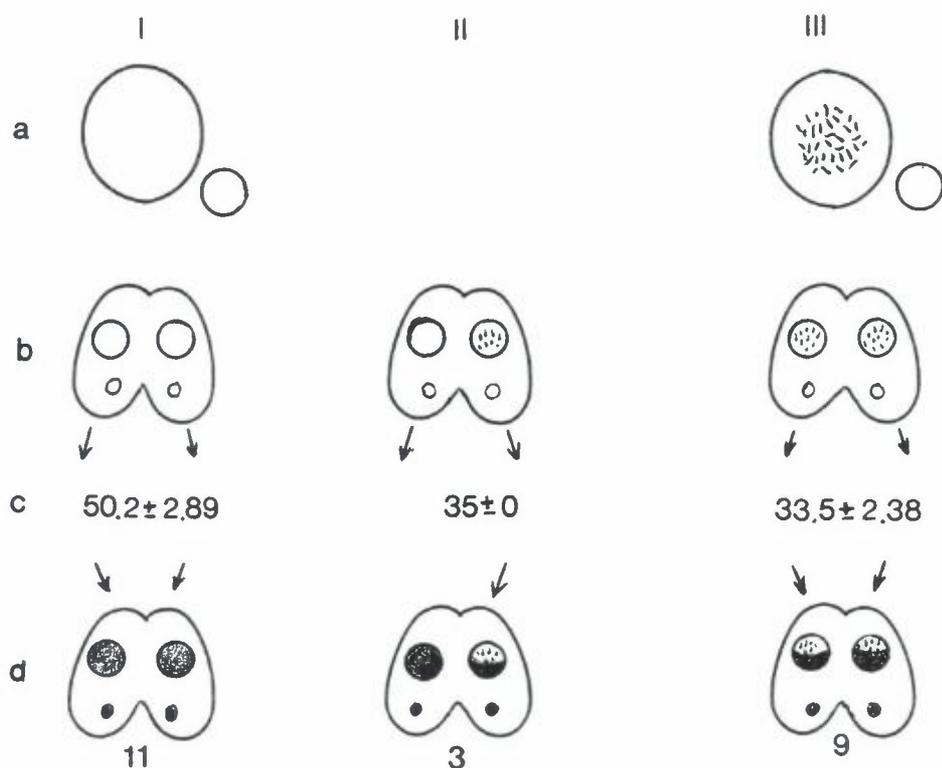


Figure 2. Scheme of the experimental crosses of *P. caudatum* with and without *N. macronucleata* infection. I = uninfected cells (control), II = heterotypical cross (infected-uninfected cells), homotypical cross (infected-infected cells). a = nuclear apparatus, b = conjugants, c = immaturity period (x divisions \pm SD), d = reconjugation, number of tested subclones.

Autogamy

The infected autogamonts of *P. biaurelia* were selected from the culture SK2-10 by the device mentioned above at the time of the second synkaryon division. The condition of the nuclear apparatus was tested in their progeny during I-III cell cycles.

3. Results

The infection of *P. caudatum* with *Holospora obtusa*, a macronuclear-specific infectious bacterium, blocked the sexual process of the host-cell in cases of high

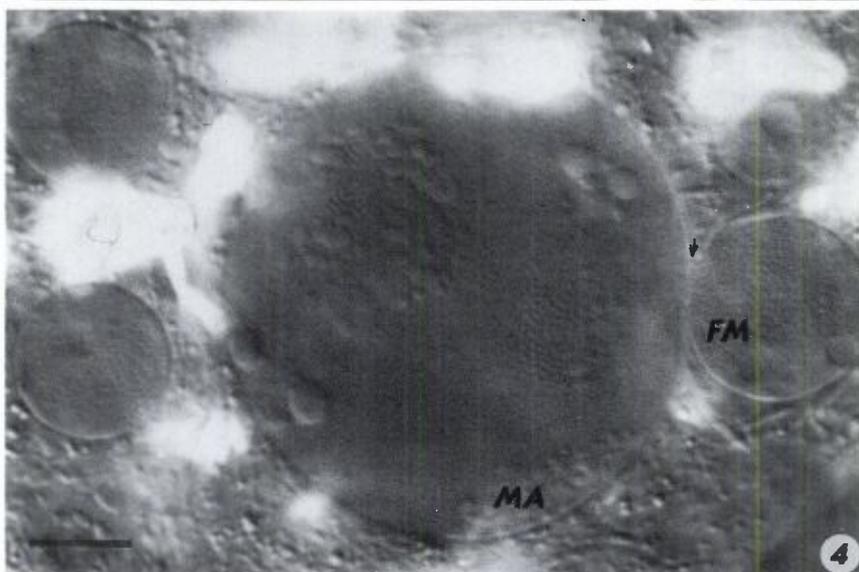
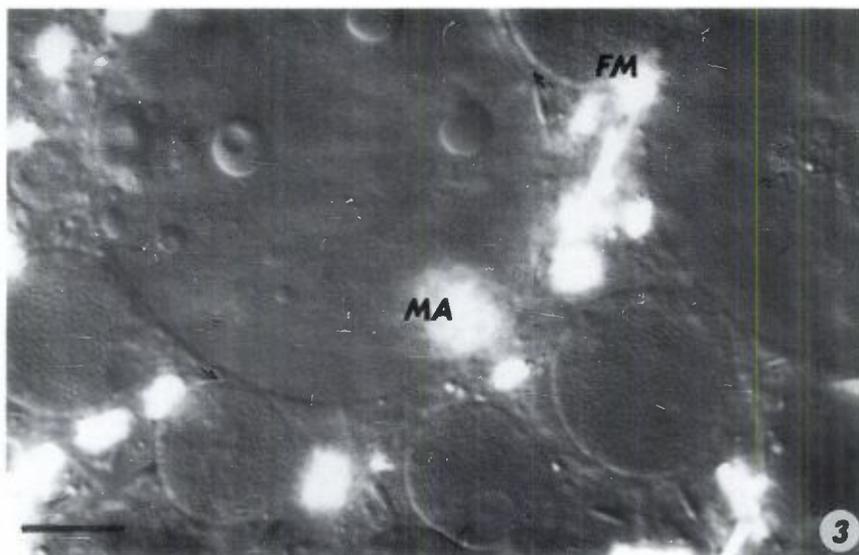


Figure 3. Macronuclear anlagen and fragments in *P. caudatum* infected with *N. macronucleata* after 1st exconjugant division. Some of the infected fragments are located very close to the anlagen envelop (arrows). MA = anlagen of the new macronucleus, FM = fragments of the old infected macronucleus. Living cell, DIC. Bar = 10 μ m.

Figure 4. Macronuclear anlagen and fragments in *P. caudatum* infected with *N. macronucleata* after 2nd exconjugant division. The anlage contains two clusters of bacteria which are the result of the fusion of two fragments with the anlage. Living cell, DIC. Bar = 10 μ m.

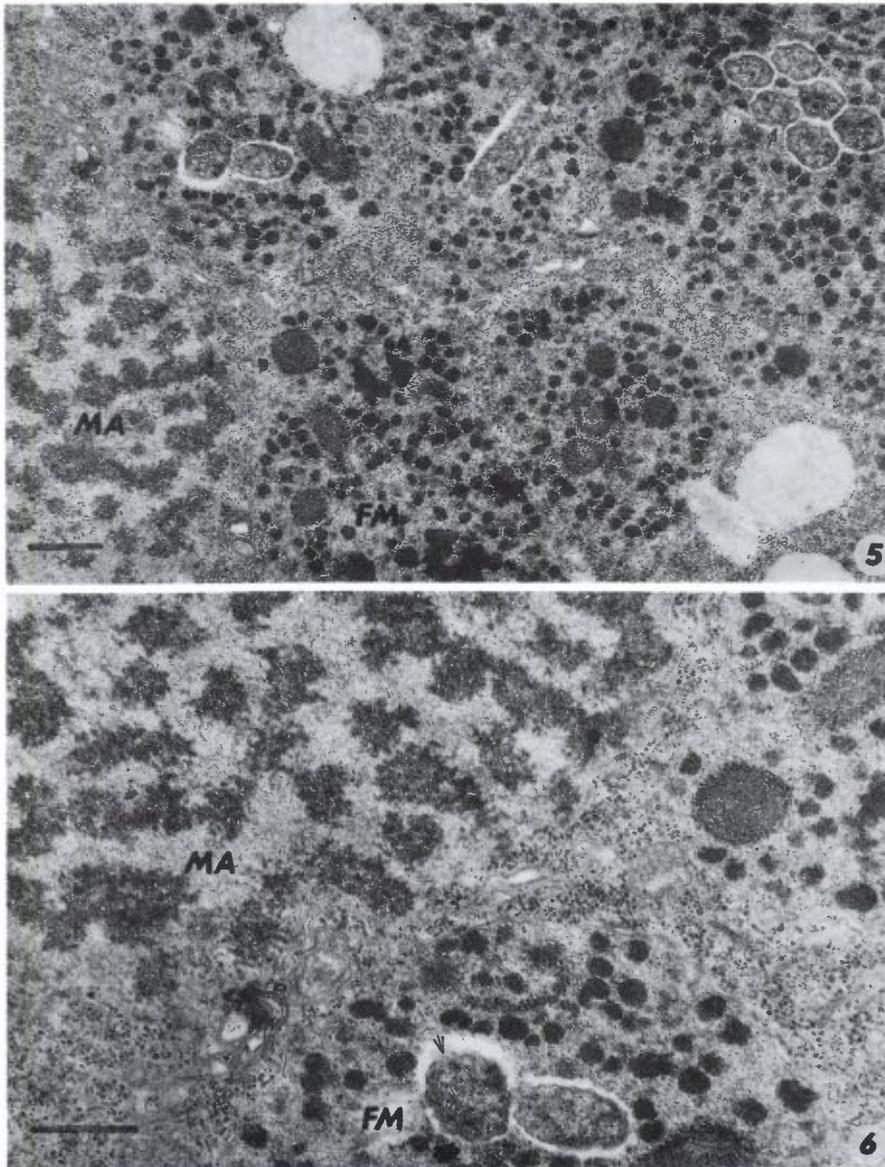


Figure 5. Electron micrograph of old macronuclear fragments infected with *N. macronucleata* and the new macronuclear anlage after 2nd exconjugant division. Arrows indicate bacteria. Bar = 1 μ m.

Figure 6. Electron micrograph of the part of new macronuclear anlage and infected fragment of the old macronucleus. The envelopes of the nuclei are located very close to each other. Bar = 1 μ m.

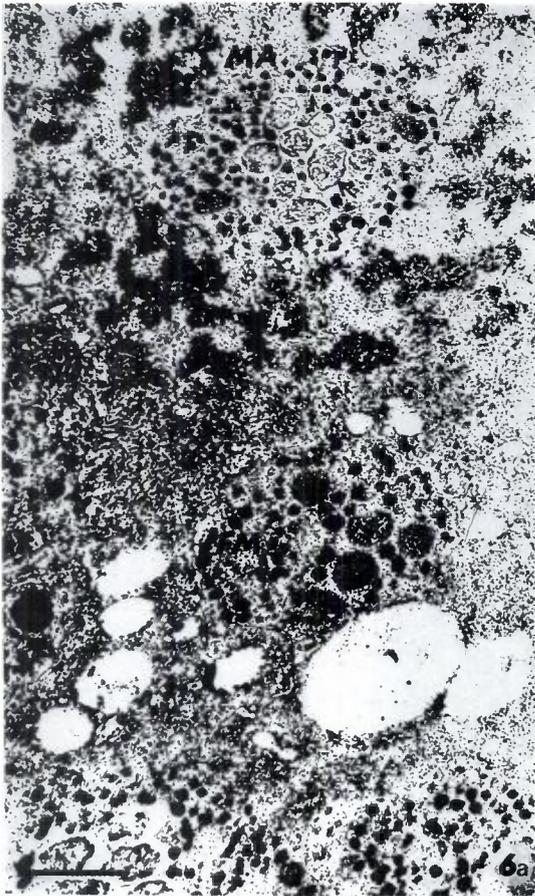


Figure 6a. Electron micrograph of the new macronuclear anlage fused with a fragment of the old macronucleus infected with *N. macronucleata*. Chromatin of the "fragment type" with bacteria surrounded by chromatin of the "anlagen type". Bar = 1 μ m.

infection rate (crosses between PK42-1 and PK42-3). In such cells the macronucleus maintained many of the infectious and reproductive forms of the bacterium (Fig. 1, IV) These cells never manifested the reaction of agglutination (mating reaction) and the nuclear reorganization process did not occur. If the infection was not so strong (a cross between the same strains at the beginning of their laboratory cultivation when the rate of the infectious forms was lower), the infected paramecia were able to undergo conjugation (Fig. 1, III).

The fate of the old infected macronucleus in this case was the same as in uninfected control cells (Fig. 1, I). It transformed into a thick ribbon-like structure ("skein") and then fragmented into many pieces. These fragments with the bacteria were resorbed in the cytoplasm of exconjugants during several first divisions of the exconjugant's progeny. The exconjugate clones appeared to be apoeudocytobiotic (Fig. 1, I, III).

The infection of *P. caudatum* with *N. macronucleata*, a macronucleus-specific bacterium which had a weak infectivity (Fokin et al., 1987), did not block the conjugation of the host-cells even in cases of strong infection. All nuclear reorganization events up to the second exconjugate cycle proceeded as in uninfected control exconjugants. The bacteria were retained in the fragments of the old macronucleus (Figs. 1, II, 3-5). The infection of the new macronuclear anlagen with a few bacteria did not occur in the way described for the usual infection process (Fokin et al., 1987). Some of the fragments were located very close to the macronuclear anlagen (Figs. 3 and 6). During the second and, especially, third exconjugate cell cycles several of these fragments fused with the new macronuclear anlagen (Figs. 4 and 6a).

As a result, several clusters of the bacteria were detected in different parts of the macronuclear karyolymph. Therefore, exconjugate clones had the heterokaryon as a macronucleus. This conclusion was confirmed by a reduction of the immaturity period for such exconjugate clones.

Several conjugate experiments with different partner conditions (infected or uninfected) were carried out (Fig. 2). In control experiments with bacteria-free cells (strains R20-8, PP21-5), 90% of the mates survived. A very similar rate of viability was found after heterotypic crosses (strains AP-8 and P20-8): 82% for infected mates and 87% for uninfected. From the crosses between bacteria-bearing cells (strains AP-8 and AP-18) 85% viable exconjugants were isolated. All infected exconjugate clones manifested a short immaturity period: 30-35 divisions (33.5 ± 2.38) instead of 45-55 (50.2 ± 2.89) as in uninfected progeny (Fig. 2).

The infection of *P. duboscqui* with *C. macronucleorum*, a macronucleus-specific bacterium without infectivity (Fokin and Görtz, 1993), also did not prevent the conjugation of the host cell. Up to the third synkaryon division all nuclear events were the same (Figs. 7 and 8) as described recently (Watanabe et al., 1996). However, the number of nuclei in infected cells after the III synkaryon division may be quite different than in uninfected exconjugants (Watanabe et al., 1996). The 4 macronuclear anlagen, 4 micronuclear anlagen and 2 fragments of the old macronucleus of uninfected cells (Watanabe et al., 1996) were found in just 31% of mates. Mates (28%) had more and the other 41% had less than 4 anlagen of both nuclear types.

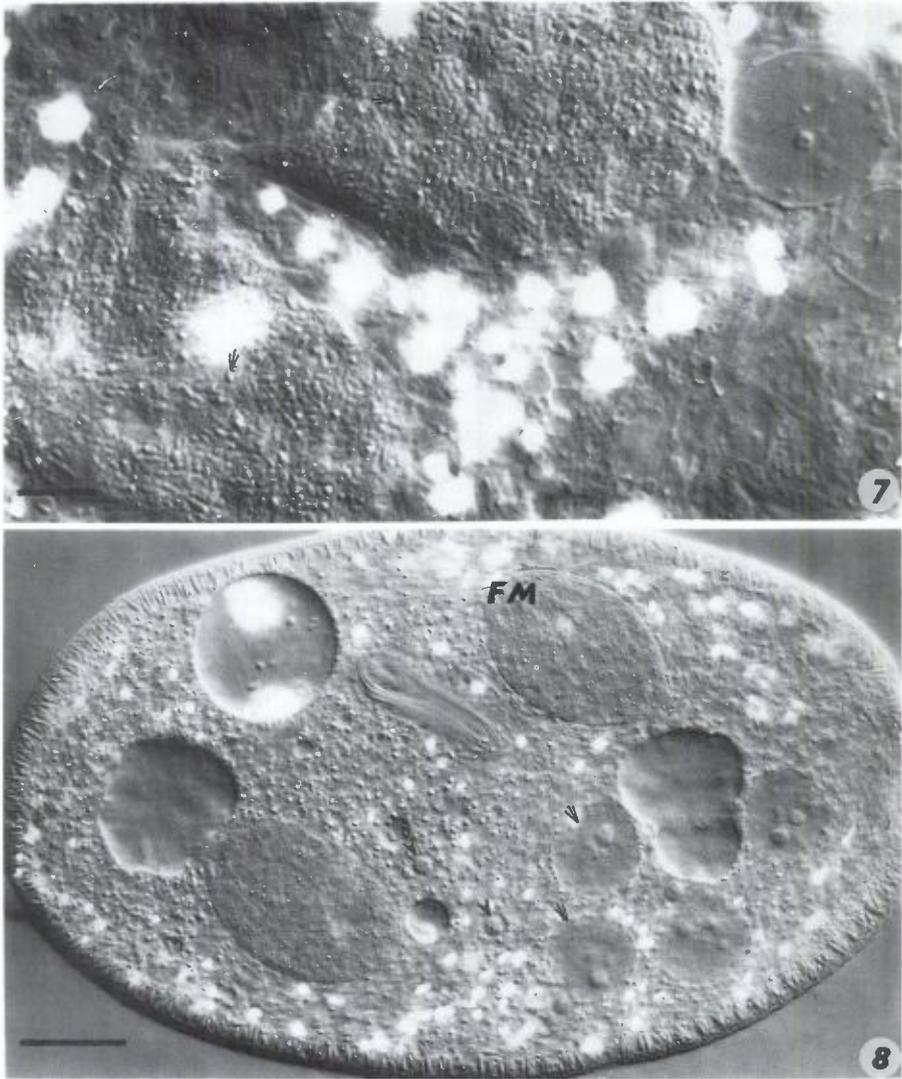


Figure 7. Dividing old macronucleus of *P. duboscqui* with *C. macronucleorum* in the process of 3rd synkaryon division. Arrows indicate the bacteria with R-bodies. Living cell, DIC. Bar = 10 μ m.

Figure 8. Infected exconjugant of *P. duboscqui* after 3rd synkaryon division. Arrows = macronuclear anlagen, arrowheads = micronuclear anlagen. Living cell, DIC. Bar = 20 μ m.

However, during the period before the first exconjugats division, two fragments of the old macronucleus with the bacteria did not resorb and fuse

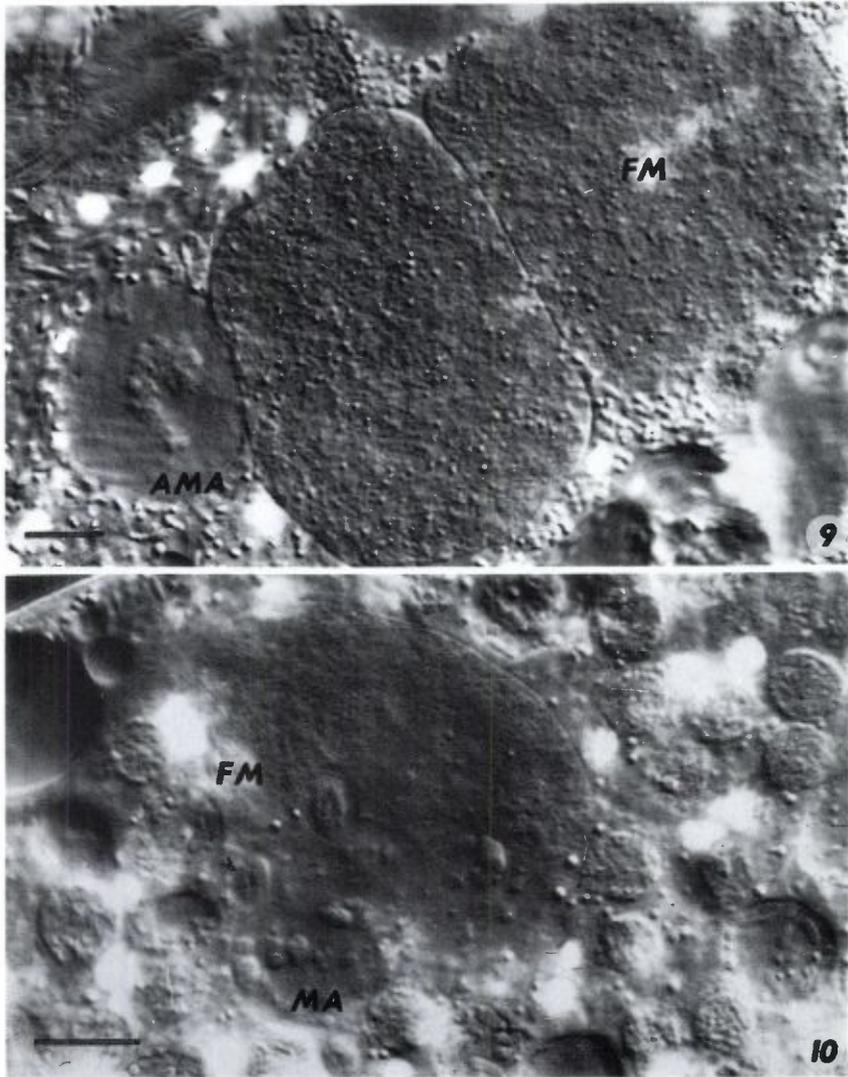


Figure 9. Two fragments of the old macronucleus infected with *C. macronucleorum* before its fusion. AMA = aborted macronuclear anlage. Living cell, DIC. Bar = 10 μ m.

Figure 10. Old macronucleus of *P. duboscqui* before fusion with the new macronuclear anlagen. Living cell, DIC. Bar = 10 μ m.

with each other (Fig. 9). This means that macronuclear retention occurred. At the same time, the new macronuclear anlagen apparently aborted. Most

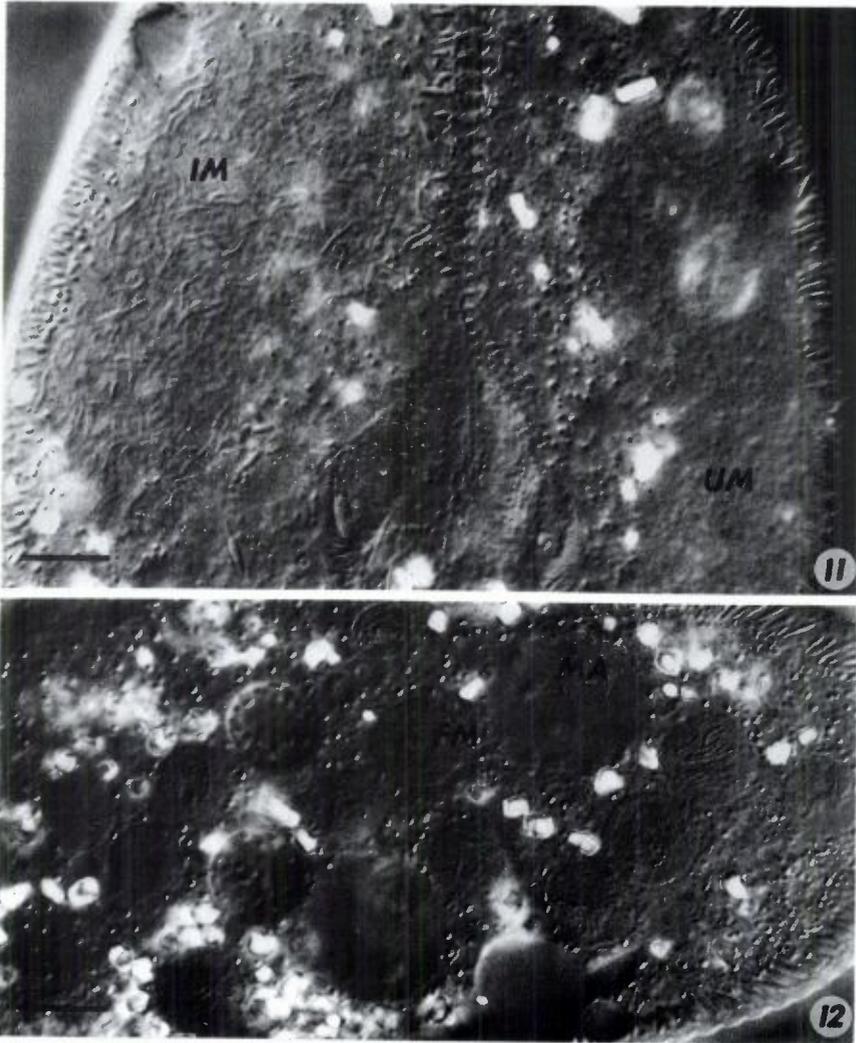
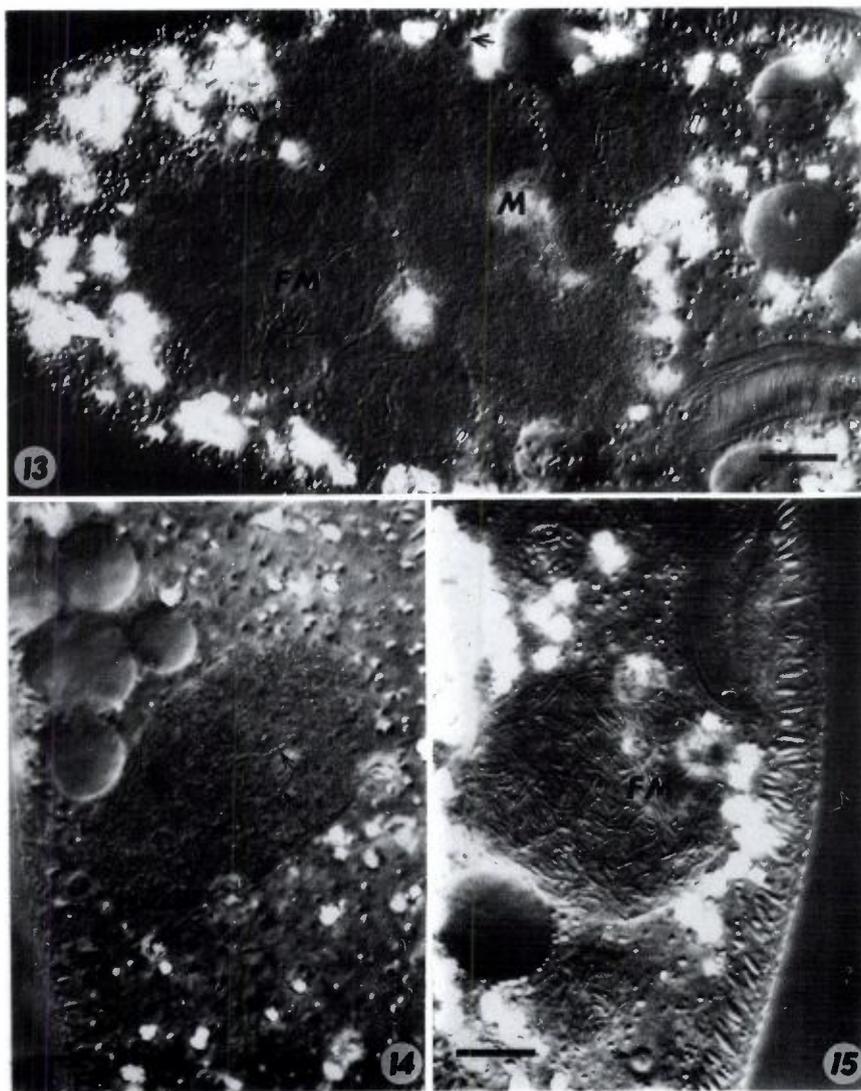


Figure 11. Conjugation of heterotypical pair of *P. biaurelia*. IM = macronucleus infected with *H. caryophila*, UM = uninfected macronucleus. Living cells, DIC. Bar = 10 μm

Figure 12. Exconjugant of *P. biaurelia* before 1st division. The macronuclear fragments maintain a lot of bacteria, the macronuclear anlagen are "clean". Living cell, DIC. Bar = 10 μm .

(74%) of exconjugats (n=111) which have been tested after the first division did not have the new macronuclear anlagen at all. On the other hand, some



- Figure 13. Exconjugant of *P. biaurelia* in IV cell cycle. The macronucleus (M) and the micronuclei (arrows) appear to be vegetative. Three large infected fragments of the old macronucleus still exist. Living cell, DIC. Bar = 10 μ m.
- Figure 14. Exconjugant of *P. biaurelia* in IV cell cycle. The fragments of the old macronucleus are absent. The new macronucleus is infected with a few bacteria (arrows). Living cell, DIC. Bar = 10 μ m.
- Figure 15. Exconjugant of *P. biaurelia* in IV cell cycle. The new macronucleus is absent. Two fragments of the old macronucleus (one of them is very large) contain a lot of bacteria. The micronucleus appear to be vegetative one (arrow). Living cell, DIC. Bar = 10 μ m.

exconjugants (26%) could not divide but survived for 5–8 days. In all of them the appearance of both the old macronuclear fragments and the new macronuclear anlagen were the same as after the third synkaryon division and the micronuclei appeared to be vegetative spindle-shaped.

The exconjugants without the macronuclear anlagen (after the I division) did not manifest the immaturity period at all and could reconstitute after 72 h. Probably, in some infected exconjugants the new macronuclear anlagen can fuse with the old macronuclear fragments (Fig. 10). An immaturity period for these cells was not found.

The infection of *P. biaurelia* with *H. caryophila*, a macronucleus-specific infectious bacterium, also did not prevent the host cell conjugation (Fig. 11). The infected macronucleus was fragmented in the usual way (Jurand and Selman, 1969), but the number of the fragments per cell was significantly less than in apocytobiotic cells (Table 2, Fig. 12). As a rule, the macronuclear fragmentation in the infected mate (heterotypical cross) started later than in the uninfected one. As early as the first exconjugate cell cycle, a few bacteria can pass, apparently, from the old macronucleus fragments into the new anlagen and infect it (Fig. 14). The portion of the cells infected in this way increased in 2–4-d exconjugate cycles (Table 2). Usually, 3–8 infectious forms of the bacterium could be found in the macronuclear anlagen. Fusion of the infected fragments with the new macronuclear anlagen was not observed.

Table 2. Morphological features of *P. biaurelia* cells with and without infection by *Holospira caryophila* (progeny of heterotypical pairs) during the first 4 exconjugate cell cycles

Cell cycle	Cells without infection			Infected cells		
	No.	No. of old Ma fragments per cell $\bar{x} \pm \text{S.D.}$	No. of Ma anlagen (total)	No.	No. of old Ma fragments per cell $\bar{x} \pm \text{S.D.}$	No. of Ma anlagen (total/infected)
I	10	33.1 \pm 3.0	2(20)	21	23.4 \pm 7.1	2(42/2)
II	20	15.3 \pm 1.5	1(20)	40	10.1 \pm 3.1	1(38*/17)
III	35	6.9 \pm 0.8	1(33*)	46	4.5 \pm 2.0	1(32*/15)
IV	66	3.3 \pm 0.7	1(62*)	88	2.5 \pm 1.3	1(70*/52)

Ma = Macronucleus, * = in other cells the Ma anlagen were not be found or were aborted.

Sometimes, a normal macronucleus was not formed in exconjugants. The proportion of such cells was higher in the infected progeny (Table 2). However, in these cases the old macronucleus regeneration occurred by the fusion of infected fragments with each other (Figs. 13 and 15). Only some of these exconjugate clones were tested and they did not manifest an immaturity period.

Similar peculiarities of the nuclear events in *P. biaurelia* infected with *H. caryophila* were found in case of autogamy (strain SK2-10). Twenty three autogamonts were tested step by step during I-III cell cycles. The old infected macronuclear fragments were observed up to the third division. The fusion of the fragments with the new macronuclear anlagen was not observed. The infection of the new macronuclear anlagen has been discovered in living cells in the second exautogamont cycle. The number of infectious forms detected in one anlage varied from 2 to 12 bacteria.

4. Discussion

It should be part of the life strategy of any endocytobiont to ensure continuation of its presence in the host-cell. Endonucleobionts of *Paramecium* can persist in their host-cells and fit into this ecological niche, apparently as a result of a number of adaptations (Görtz, 1986). A regulated distribution during host-cell division by a special mechanism (for some of *Holospora* bacteria) or a high number of bacteria per nucleus might be best guaranties of continued presence in the host-nuclei (Görtz, 1988; Fokin, 1993; Fokin et al., 1996).

The sexual part of the host's life cycle, therefore, is a crucial event for the nuclear bacteria because the nuclear reorganization may be fatal for poorly-adapted endonucleobionts. During conjugation of different *Paramecium* species the old macronucleus fragments into many pieces which usually degenerate and are resorbed (Hiwatashi and Mikami, 1989). We have just two exceptions from this rule: *P. bursaria* and *P. duboscqui*. In the first species the process of the macronuclear fragmentation in exconjugants is absent. The nucleus degenerates as a whole structure during the first two exconjugant divisions (Wichterman, 1986). In *P. duboscqui* the old macronucleus divides once after mates' separation and these two nuclei disappear during the first exconjugant cell cycle (Watanabe et al., 1996). Therefore, the endonucleobionts have to prevent the conjugation of the host cell or modify the nuclear reorganization in *Paramecium* for their survival.

Both of these types of adaptation have been found in the present study. Of all of the macronuclear infections investigated, only *H. obtusa* can block the conjugation of its host. *H. caryophila*, *N. macronucleata* and *C. macronucleorum* cause modification of the host cell's nuclear reorganization. For *H. caryophila*,

another method of survival during *Paramecium* conjugation is also possible: infection of the new macronuclear anlagen with a few bacteria from the old macronuclear fragments.

The investigated bacteria belong to different phylogenetic groups. The infectivity which is a very important feature for endocytobiosis, is completely absent in *C. macronucleorum* and is present in high and weak degree in *H. caryophila* and *N. macronucleata*, respectively. Probably, the type of strategy of these bacteria during the host conjugation is connected with this feature.

Transmission of *H. caryophila* during autogamy has already been studied by Preer (1969). She found that eventually a few bacteria pass from the old macronuclear fragments into the new macronucleus. *H. caryophila* (at that time "alpha") appears in the new macronucleus, usually after 2–4-d post-autogamonts fissions (Preer, 1969). Nobody has studied the mechanism of this invasion. The regeneration of the old infected macronucleus was not mentioned by this author.

The infection process has been investigated in several *Holospira* species (Ossipov, 1981; Görtz and Wiemann, 1989; Skovorodkin, 1993; Skoblo et al. 1995). Apparently, for all of these bacteria (*H. obtusa*, *H. undulata*, *H. elegans* and *H. acuminata*) the acidification in the primary food vacuole might be necessary to trigger the development of the infectious form (activation) of these bacteria (Görtz, 1988; Skovorodkin and Rautian, 1990; Fokin, 1993). In the old macronuclear fragments with *H. caryophila* acidification may not be expected.

Acid phosphatase might play the role of a trigger for *H. caryophila* to leave the old macronuclear fragments. Unfortunately, this proposal has not been investigated. Acid phosphatase was detected in the macronuclear fragments of exconjugants but autogamous cells did not show any reaction in the fragments (Saxena and Jurand, 1973). I did not find such enzyme activity in the old macronuclear fragments of either exconjugants or exautogamonts during I–III cell cycles (unpublished). It is still unknown how *H. caryophila* leaves the old macronuclear fragments before the infection of the anlagen.

Another way for *H. caryophila* to survive during host's sexual process is regeneration of the old macronucleus. In this study the cells with an regenerated old macronucleus have been found in high proportion (20%) among the progeny of the IV exconjugate cycle. In *P. caudatum* this phenomenon never occurs when exconjugats contain "healthy" macronuclear anlagen (Hiwatashi and Mikami, 1989). It is known that normal macronuclear anlagen in *P. aurelia* produce an inhibitor which selectively suppresses DNA synthesis in macronuclear fragments (Berger, 1973). Sonneborn (1947) observed that if the macronucleus or macronuclear anlage was removed, the macronuclear

fragments would begin to grow and would develop into a separate macronucleus in the course of several cell cycles.

Apparently, the old macronuclear fragments infected with *H. caryophila* in some cases (up to 20%) may suppress the development of new macronuclear anlagen. Such cells in my experiments did not lose the anlagen immediately, but these nuclei cannot grow. Nevertheless, some apocytobiotic stocks (16%) were obtained from the exconjugats.

In fact, vegetative cells of *P. biaurelia* with a large infected fragment of the old macronucleus were detected sometimes after conjugation and autogamy (Fig. 13). This means that within infected cells the new macronucleus cannot suppress the infected fragment's activity in any case and, sometimes, both nuclei are maintained simultaneously.

The behaviour of *N. macronucleata* during the host's sexual process was briefly described earlier (Fokin et al., 1987). The crossing experiments with more detailed cytological observations which have been performed by now, made clear this situation.

The fusion of the old macronuclear infected fragments with the new anlagen took place mainly in the third exconjugant cell cycle. This is a very precise process: all tested exconjugant clones were infected in this way. Usually, a young stock shows mating reactivity of about 50 fissions after conjugation (Itoh and Mikami, 1995) which is consistent with the results of the control crosses in this work.

The shortening of the immaturity time for the infected cultures indicates that the cells have a heterokaryon (mixture between the old and new macronuclei) as a somatic nucleus. Some examples of this phenomenon exist in the literature (Rao, 1964; Mikami and Hiwatashi, 1975; Alonso, 1982) but until now it has not been shown for any ciliates infected with intranuclear bacteria. It is clear that in our case the fusion was induced by *N. macronucleata*.

The regeneration of the old macronucleus infected with *C. macronucleorum* in *P. duboscqui* was shown in my experiments by cytological investigations of living and fixed cells before and after the first division of the exconjugants. The ability of the exconjugant's progeny to reconjugate after 72 h suggests the same conclusion.

Apparently, the sexual process of the infected cells shows several abnormalities in the nuclear reorganization in addition to the macronuclear regeneration. Only about 70% of the exconjugants were viable. Unfortunately, the heterotypical pairs for this *Paramecium* species were not tested because of the absence of available cultures. This endocytobiotic system (*P. duboscqui*-*C. macronucleorum*) is very stable. Infected cultures have been maintained since 1990 (Fokin and Görtz, 1993) under different conditions (5–20°C) without loss of the bacteria.

Cells of two other species of *Paramecium* infected with endomacronuclear bacteria – *H. curvata* in *P. calkinsi* (Fokin and Sabaneyeva, 1993) and *Holospora* sp. in *P. putrinum* (Fokin et al., 1996) – can undergo conjugation as well (unpublished).

The only investigation of conjugation of *Paramecium* infected with micronucleus-specific bacteria (*H. elegans*) was performed by Görtz and Fujishima (1983). Contrary to the results obtained by Ossipov et al. (1975) that cells of *P. caudatum* infected with the micronucleus-specific *H. undulata* did not conjugate, the authors found that paramecia with *H. elegans* can conjugate and undergo meiosis. From cytological observation it can be concluded that *H. elegans* does not inhibit nuclear reorganization and all stages known from bacteria-free cells can be observed (Görtz and Fujishima, 1983). Nevertheless, it was more likely that synkaryon in these cases did not give rise to a new functional macronucleus and that macronuclear regeneration occurred. The capacity of *P. caudatum* infected with *H. recta* to undergo conjugation was not shown as well (Fokin, 1991).

The investigation revealed evidence that the following strategies can be realized for the investigated bacteria: Continuous blocking of the sexual process of host cells with high infection rates (*P. caudatum*-*H. obtusa*; *P. caudatum*-*H. undulata*, *P. caudatum*-*H. recta*). Normal conjugation with the loss of the bacteria (*P. caudatum*-*H. obtusa*, weak rate of infection; *P. caudatum*-*H. elegans*). Fusion of the macronuclear fragments containing bacteria with the new macronuclear anlagen after which an infected heterokaryon was formed (*P. caudatum*-*N. macronucleata*; *P. duboscqui*-*C. macronucleorum* [probably]). Blocking of the development of the new macronuclear anlagen and the retention of the old infected macronucleus (*P. duboscqui*-*C. macronucleorum*). Infection of the new macronuclear anlagen by a few bacteria from the old macronuclear fragments (*P. biaurelia*-*H. caryophila*). Fusion of the infected macronuclear fragments which leads to regeneration of the old macronucleus and prevention of the development of the new macronucleus anlagen (*P. biaurelia*-*H. caryophila*).

It is now clear that nearly all of the endonuclear bacteria can survive during the host-cell's conjugation in two different ways: preventing or modification of this sexual process.

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