Nuclear behavior in exconjugants of *Paramecium bursaria*

Xin Gao[1], Yuanpu Song[2], Xianyu Yang[1]*

[1]College of Animal Science and Technology, Zhejiang Agricultural and Forestry University, 666 Wusu Street, Hangzhou, Zhejiang 311300, China; [2]School of Engineering, Zhejiang Agricultural and Forestry University, 666 Wusu Street, Hangzhou, Zhejiang 311300, China
*Corresponding author: Xianyu Yang, E-mail: yangxy78@zafu.edu.cn

Abstract: *Paramecium bursaria is the representative of "bursaria" group of Paramecium, whose nuclear behaviour during conjugation is quite different from other species. In its exconjugants, there are three types of nuclei, a parental macronucleus (pMac), two macronuclear anlagen (MA) and two presumptive micronuclei (Mic). According to the previous studies, both Mic and MA distribute equally during exconjugant division — each daughter cell receiving a Mic and a MA, which never happens in other Paramecium species studied so far. Meanwhile pMac sustains even in daughter cells. To clarify these issues, nuclear behavior in exconjugants of P. bursaria was studied by staining with carbol fuchsin solution. As the results, degeneration of one presumptive Mic as well as pMac in P. bursaria exconjugant was observed indicating different nuclear development pattern from previous reports.*

Keywords: *Paramecium bursaria; Exconjugant; Carbol fuchsin solution; Micronuclear degeneration; Parental macronuclear degeneration*

Introduction

According to the body shape, *Paramecium* divides into "aurelia" group and "bursaria" group. *P. caudatum* and *P. aurelia* complex are the members of "aurelia" group, and *P. bursaria* and *P. polycaryum* belong to "bursaria" group [1]. Over the last two decades, morphological studies on conjugation (sexual reproduction of unicellular ciliate) of *Paramecium* indicated some new important details [2-13]. *P. bursaria*, as the representative of "bursaria" group, few studies on its conjugation have been reported since last century [1, 14-17]. We collected *P. bursaria* occasionally and found many conjugating pairs in their mixed culture. Here we will report the nuclear behavior in exconjugants (single cells from separation of conjugating pairs) of *P. bursaria.*

Materials and Methods

Cells of *P. bursaria* were isolated from the water sample of a small pond on the East Lake Campus of Zhejiang Agricultural and Forestry Univesity (Hangzhou, China) and cultured with the filtered original pond water mixed with the same volume of mineral water of Nongfu Spring Company (Chun'an, China), into which half of a wheat granule was deposited. Many bacteria (from pond) grew up around the wheat granule soon, which became the food / nutrient of *P. bursaria*, meanwhile symbionic chlorella provides part of the nutrition through photosynthesis. When the cells encountered starvation, many conjugating pairs formed, which were collected by iron-dextran particles in current study [9, 18]. Temporary preparations

were made following "volume-fixing" method [19] and cells were stained with carbol fuchsin solution [20], observed and photographed under bright field of Nikon 50*i* fluorescence microscope.

Results and Discussions

Nuclear events during conjugation and nuclear types in exconjugant of *P. bursaria*

In the vegetative cells, *P. bursaria* has two kinds of nuclei, a micronucleus (Mic) and a macronucleus (Mac), whose characteristic is having large number of symbiotic chlorellas [1] (Figure 1).

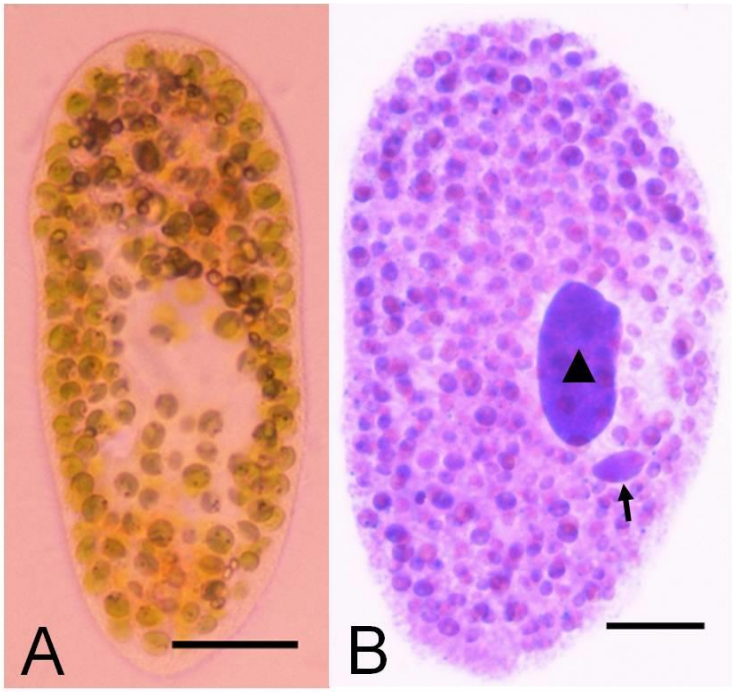

Figure 1. Vegetative *P. bursaria*

A: A living cell observed under bright field microscope, in which there are lots of chlorellas. B. *P. bursaria* stained with carbol fuchsin solution, in which a micronucleus (arrow) and a macronucleus (triangle) are observed besides many chlorellas. Scale bar : 20 μm.

During the conjugation of *P. bursaria*, there are three nuclear divisions before synkaryon (fertilized nucleus) formation (prezygotic divisions) and three after (postzytoic divisions), which happen in most of *Paramecium* [1]. Other characteristics different from other *Paramecium* are several times of Mic degeneration. The first time happens after the first prezygotic division, one product degenerates, and the remaining one accomplishes the second prezygotic division. The second and the third degeneration happen after the second prezygotic division and the first postzygotic division, respectively [1]. Therefore, 4 synkaryon division products exist in *P. bursaria* cells instead of 8 such as in *P. caudatum*, *P. multimicronucleatum*, *P. polycaryum* and *P. duboscqui* [1, 13]. Around the completion of the third postzygotc division of *P. bursaria*, a conjugating pair separates into two single cells, the so-called exconjugants in which three types of nuclei exist, two macronuclear anlagen (MA), a parental macronucleus (pMac) and two presumptive Mic[1](Figure 2A, 2B).

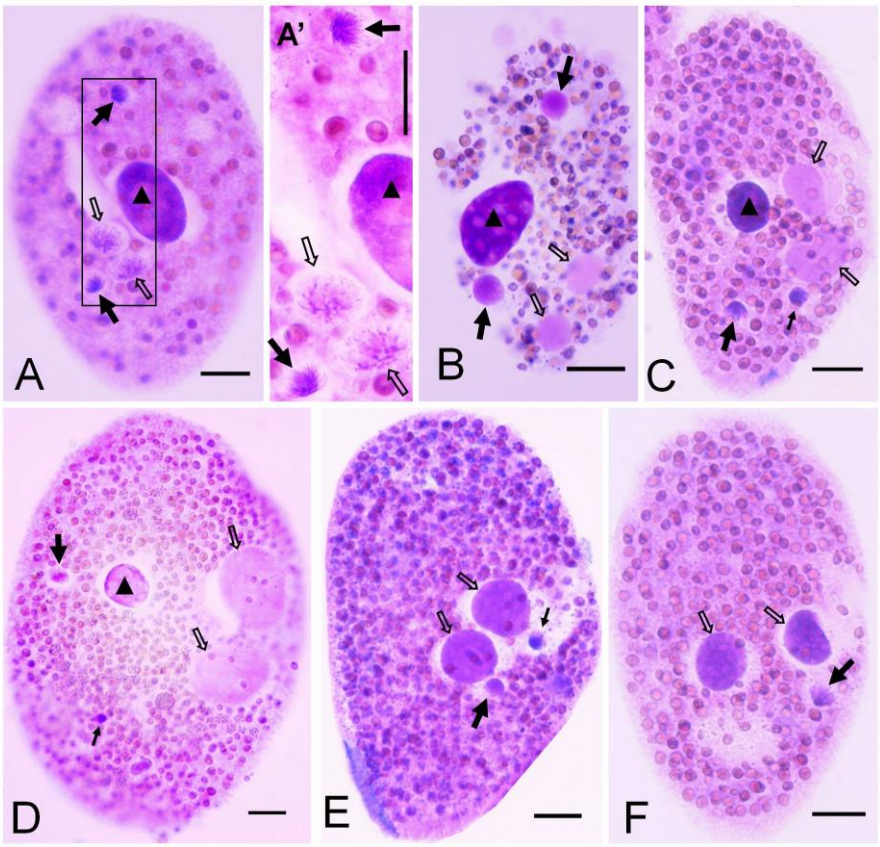

Figure 2. Carbol fuchsin stained *P. bursaria* exconjugants

Triangle: pMac; hollow arrow: MA; thick arrow: presumptive Mic; thin arrow: pycnotic Mic. Scale bar: 10 μm. A and B: An exconjugant with two MA, two presumptive Mic and one pMac. A': Framed portion in A is magnified. C, D: An exconjugant with two MA, a pMac, a survived Mic and a pycnotic Mic. E: An exconjugant with two MA, one survived Mic and one pycnotic Mic, without pMac. F: An exconjugant with two MA, one survived Mic, without pMac and pycnotic Mic.

MA behavior in exconjugant of *P. bursaria*

As described above, due to the degeneration of one product of the first postzygotic division, only one product survives and divides twice successively forming 4 synkaryon division products [1]. Two of them enlarge and heterochromatin appears in them indicating their differentiation into MA and DNA arrangement (hollow arrow in Figure 2A, 2A'). After the completion of DNA arrangement, MA shows homogenous morphology as observed in Figure 2C, which is followed by DNA replication and MA enlargement (Figure 2C-2E), finally replace pMac (Figure 2F).

Behavior of pMac and presumptive Mic in *P. bursaria* exconjugant

Accompanying with the development of MA, pMac become smaller and condenser (compare Figure 2B with 2C and 2D). Finally, pMac degenerates and completely disappears from exconjugants, and is substituted by MA (compare Figure 2E and 2F with 2D). Meanwhile, two Mics show the same morphological characteristics in early exconjugant (Figure 2A, 2B), then become smaller. However, one of them becomes pycnotic (thin arrow in Figure 2C-2E), and eventually disappears (Figure 2F). As the result, there are two MA and

one Mic in exconjugant before its division (Figure 2F), which is different from the previous study [1].

Nuclear behaviour in exconjugant of other ciliates

Conjugation produces new generations of ciliates, whose macronuclei and micronuclei both are derived from the synkaryon products. In other words, pMac degenerates and is substituted by a new developed macronucleus (the same meaning as MA), meanwhile the original micronucleus is substituted by a new synkaryon division product. Concerning the MA, they are distributed evenly into new individuals in all *Paramecium* species reported so far beyond all doubt [1] as well as in famous ciliate, *Tetrahymena thermophila* [21-24]. However, the behaviour of pMac and presumptive Mic is quite different depending on the species.

Concerning pMac degeneration, three types have been reported. The first is that pMac breaks into several tens of fragments through a skein-formed stage, which sustain for several cell cycles and are finally absorbed, such as in *P. caudatum* [1, 10]. The second is that pMac breaks into two pieces, which disappear gradually by the first exconjugant cell division being reported in *P. duboscqui* [13]. The third is degeneration without fragmentation, such as *P. bursaria* [1], pMac sustains in the daughter cells. In current study of *P. bursaria*, we observed no pMac fragmentation but degeneration (disappearance) in exconjugant before its division, which is different from the third type pMac degeneration [1], while consistent with that happens in *P. duboscqui* [13] and *T. thermophila* [23, 24]. Regarding to the fates of presumptive Mic, also are there three types reported so far. The first happens in multi-micronucleate species, all Mic divides mitotically in exconjugant division like *P. aurelia* complex [1], *P. multimicronucleatum* [1] and *P. dubosqui* [13]. The second happens in some uni-micronucleate species like *P. caudatum* [1, 10] and *T. thermophila* [23, 24], only one Mic divides during exconjugant division while the extra-Mic degenerate [10]. The third happens in *P. bursaria*, two Mic evenly distribute to daughter cells [1]. However, our current study indicated that one Mic remains and one Mic degeneration before *P. bursaria* exconjugant division. In fact, *P. bursaria* is a uni-micronucleate species, its Mic behaviour observed in current study is consistent with that in other uni-micronucleate species like *P. caudatum* [1, 10] and *T. thermophila* [23, 24]. These differences between our current observation and former studies might be due to the different strains, which are necessary to be clarified with more different strains.

Acknowledgements

This work was partially supported by the grant from National Natural Science Foundation of China (No. 31772409, 31372149).

References

1. *Wichterman R. The Biology of Paramecium. 2nd Ed. Plenum Press: New York and London. pp9-10, 263-266.*
2. *Gao X, Zhang X, Yang X. Morphological apoptotic characteristics of the post-meiotic micronuclei in Paramecium caudatum. Europ J Protistol 2010, 46: 243-250.*
3. *Gao X, Shi X, Yang X. Dynamic behaviour of stationary pronuclei during their positioning in Paramecium caudatum. Europ J Protistol 2011, 47(3): 235-237.*
4. *Gao X, Yang X, Zhu J, Yuan J, Wang Y, Song M. New details indicated by different stainings*

during conjugation of ciliated protozoa Paramecium. Zool Res 2011, 32: 651-656.

5. *Gao X, Zhu J, Yang X, Yuan J, Wang Y, Song M. Localization of stationary pronuclei during the conjugation of Paramecium indicated by immunofluorescence staining. Zool Res 2011, 32: 461-464.*
6. *Wang Y, Yuan J, Gao X, Yang X. Stage-specific appearance of cytoplasmic microtubules around the surviving nuclei during the third prezygotic division of Paramecium. Zool Res 2012, 33(E5-6): E97-E102.*
7. *Yang X, Shi X. Gametic nuclear exchange during the conjugation of Paramecium polycaryum. JPN J Protozool 2007, 40: 113-121.*
8. *Yang X, Takahashi M. Stage-related profiles of the reaction of the cells to heat shock during meiotic prophase in Parameicum caudatum. J Exp Zool 1996, 275: 53-60.*
9. *Yang X, Takahashi M. Disturbance of the determination of germinal and somatic nuclei by heat shock in Paramecium caudatum. J Eukaryot Microbiol 1999, 46: 49-55.*
10. *Yang X, Takahashi M. Nutrient supply induces germinal nuclear selection in exconjugants of Paramecium caudatum. Proc Japan Acad 2000, 76: Ser B, 87-91.*
11. *Yang X, Takahashi M. Nuclei may anchor at specific locations during nuclear determination in Paramecium caudatum. Europ J Protistol 2002, 38: 147-153.*
12. *Yang X, Gao X, Shi X. Detection of haploid nuclear death in living Paramecium caudatum. JPN J Protozool 2007, 40: 123-130.*
13. *Watanebe T, Shi X, Liu G, Jin M. Cytological studies of conjugation and nuclear processes in Paramecium duboscqui Chatton & Brachon 1933. Europ J Protistol 1996, Suppl 1: 175-182.*
14. *Wichterman R. Conjugation and fate of exconjugants of zoochlorellae-free Paramecium bursaria. Anat Rec 1943, 87: 50.*
15. *Wichterman R. Recent discoveries of nuclear processes and sexual phenomema in paramecium. Turtox News 1944, 22: 105-110.*
16. *Wichterman R. Direct observation of the transfer of pronuclei in living conjugants of Paramecium bursaria. Science 1946, 104: 505-506.*
17. *Wichterman R. The time schedule of mating and nuclear events in the conjugation of Paramecium bursaria. Turtox News 1948, 26: 2-10.*
18. *Sun X, Wu J, Gao X, Yang X. To enhance the production of magnetic iron-dextran particles by ultrasonic treatment and its applications in ciliate studies. Chn J Zool 2010, 45(1): 90-93. (in Chinese)*
19. *Lin Y, Gao X, Yang X. A method for observation of living Paramecium. Shichuan J Zool 2009, 28: 276. (in Chinese)*
20. *Carr DH, Walker JE. Carbol fuchsin as a stain for human chromosomes. Stain Technol 1961, 36: 233-9236*
21. *Blackburn EH. Structure and function of telomeres. Nature 1991, 350:569-573.*
22. *Cech TR. The chemistry of self-splicing RNA and RNA enzymes. Science 1987, 236:1532-1539.*
23. *Nanney DL. Nucleo - cytoplasmic interaction during conjugation in Tetrahymena. Biol Bull 1953, 105: 133-148.*
24. *Qiao J, Xu J, Bo T, Wang W. Micronucleus-specific histone H1 is required for micronuclear chromosome integrity in Tetrahymena thermophila. PLoS ONE 2017, 12(11): e0187475.*