Nuclear behavior in exconjugants of *Paramecium bursaria*

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**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 University (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 50i 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).

During the conjugation of *P. bursaria*, there are three nuclear divisions before synkaryon (fertilized nucleus) formation (prezygotic divisions) and three after (postzytotic 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 postzygotic 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.

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**References**