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Meiotic inheritance of a fungal supernumerary chromosome and its effect on sexual fertility in *Nectria haematococca*

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ABSTRACT

PDA1-conditionally dispensable chromosome (CDC) of *Nectria haematococca* MP VI has long served as a model of supernumerary chromosomes in plant pathogenic fungi because of pathogenicity-related genes located on it. In our previous study, we showed the dosage effects of *PDA1*-CDC on pathogenicity and homoserine utilization by exploiting tagged *PDA1*-CDC with a marker gene. CDC content of mating partners and progenies analyzed by PCR, PFGE combined with Southern analysis and chromosome painting via FISH. In this study, we analyzed mode of meiotic inheritance of *PDA1*-CDC in several mating patterns with regard to CDC content and found a correlation between CDC content of parental strains with fertility of crosses. The results showed non-Mendelian inheritance of this chromosome followed by duplication or loss of the CDC in haploid genome through meiosis that probably were due to premature centromere division, not by nondisjunction as reported for the supernumerary chromosomes in other species. Correlation of CDC with fertility is the first time to be examined in fungi in this study.

Introduction

B chromosomes (Bs) are known as supernumerary chromosomes existing as optional, dispensable extras in genomes of some individuals of a population. In plants and animals, Bs have been found in numerous species since they were first discovered in early 1900s: nearly 1300 plant species and well over 500 animal species have so far been reported to harbor Bs (Jones 1995; Camacho 2005). Because of the peculiar nature of Bs compared with the basic genome complement called A chromosomes (As), they have attracted interests from cytogeneticists and evolutionary biologists for almost 100 y.

As eukaryotes, fungi have also Bs (Mills & McCluskey 1990; Zolan 1995). However, existence of fungal Bs has been discovered far behind plants and animals mostly because of their tiny size and underdevelopment of cytological techniques suitable for fungal specimens. It was not until the beginning of 1990s that fungal Bs were discovered. At that time, pulsed field gel electrophoresis (PFGE) was introduced for karyotyping of fungi and detection of minute chromosomes was made

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possible. In addition, feasibility of Southern hybridization to PFGE gels enabled to prove the dispensability of fungal Bs in genome. Chronologically, fungal Bs was first reported for *Nectria haematococca* mating population (MP) VI (anamorph: *Fusarium solani* f.sp. *pisi*) in 1991 by Miao and her colleagues (Miao *et al.* 1991) and shortly after for *Cochliobolus heterostrophus* (Tzeng *et al.* 1992). Then, reports of the finding of Bs in a wide range of species, mainly plant pathogenic fungi, followed up (Kim *et al.* 1993; Masel *et al.* 1993; Xu *et al.* 1995; Ahn & Walton 1996; Orbach *et al.* 1996; Geiser *et al.* 1996; Leclair *et al.* 1996; Johnson *et al.* 2001; Hatta *et al.* 2002; Wang *et al.* 2003; Wittenberg *et al.* 2009). To our knowledge, at least 12 different fungal species are presently known to harbor Bs in their genomes.

Interestingly, most Bs found in plant pathogenic fungi carry functional genes that confer adaptive advantages to host fungi in certain habitats. For instance, genes involved in phytoalexin-detoxification (VanEtten *et al.* 2001) and biosynthesis of host-specific toxin (Hatta *et al.* 2002; Friesen *et al.* 2008) shown to locate on Bs. In this regard, fungal Bs are different from Bs of plants and animals that generally do not carry functional genes and hence it was proposed that Bs carrying functional gene(s) to confer adaptive merits to the host should be referred to as '**c**onditionally **d**ispensable' (abbreviated as CD) chromosome (Covert 1998). Currently, most researchers working on plant pathogenic fungi accept this proposed term. In the following, therefore, we also use CD chromosome (CDC) when referring to Bs with such features.

Nectria haematococca MP VI is a heterothallic ascomycete that causes root rot of garden pea. Owing to large contribution of H.D. VanEttens' lab (VanEtten *et al.* 2001), this fungus has been established as a leading model to study fungal CDC. In addition, this fungus got the honour of the first species for discovery of fungal Bs (Miao *et al.* 1991), also, cytological visualization (Taga *et al.* 1998) and complete sequencing (Coleman *et al.* 2009) of CDC were accomplished with this fungus ahead of other species. Among several kinds of CDCs found in this fungus, *PDA1*-CDC named after the gene *PDA1* (*pisatin demethylase 1*) residing on this chromosome has been intensively analyzed. This CDC is ca. 1.6 Mb in size and contains a gene cluster called *PEP* (**pe**a **p**athogenicity) that harbours pathogenicity-related genes including *PDA1* (Han *et al.* 2001; VanEtten *et al.* 2001). It also contains genes for utilizing homoserine as a sole carbon and nitrogen source (Rodriguez-Carres *et al.* 2008).

Since accumulation in genome is a typical feature of many Bs (Camacho 2005), we examined in previous study if *PDA1*-CDC can be accumulated and stably maintained in genome of *N. haematococca* MP VI (Garmaroodi & Taga 2007). In that study, intrastrain protoplast fusion technique was used to accumulate this chromosome and was shown that two or even four copies can be accumulated and maintained in a haploid genetic background. The dosage effects of *PDA1*-CDC were also analyzed in the same study, showing that pathogenicity to host plant and homoserine utilization ability were elevated in accordance with duplication of *PDA1*-CDC in genome. Accumulation of the same CDC had never been done before and the success of such study largely relied on the utilization of *PDA1*-CDC tagged with hygromycin resistance gene (Wassman & VanEtten 1996), introduction of cytological techniques such as chromosome painting fluorescence *in situ* hybridization (FISH) (Taga *et al.* 1999) that allowed exact counting number of *PDA1*-CDC in genome.

In this study, we aimed to analyze meiotic behavior of *PDA1*-CDC of *N. haematococca* MP VI. This subject was chosen because another important feature of Bs is their peculiar behavior during meiosis (Jones & Rees 1982). Also, we expected that the tagged *PDA1*-CDC and techniques established in the previous study could serve effectively to analyze this subject. Consequently, inheritance mode of *PDA1*-CDC during meiosis of *N. haematococca* MP VI was shown. Besides, negative effect of CDC on the sexual fertility was discovered for the first time in fungi.

Material methods

Fungal isolates

All parental isolates of *Nectria haematococca* MPVI used in crossing experiments are listed in Table 1. Strain 77-13-7 contained an intact copy of *PDA1*-CDC that is referred to as CDCi hereafter. Tr18.5, a transformant of 77-13-7, contained a single copy of *PDA1*-CDC tagged with hygromycin resistance gene by insertional mutagenesis on *PDA1* gene (Wassman & VanEtten 1996). This type of CDC is referred to as CDCm in this paper. Tetrad progenies obtained from crosses were denoted with three hyphenated numbers representing the cross number, ascus number, and ascospore number, respectively (e.g. 8-7-1). Randomly isolated ascospores were denoted with two hyphenated numbers indicating cross number and ascospore number (e.g. 2-14). Cultures were maintained on half-strength potato dextrose agar (PDA) slants at 4 °C in the dark.

Genomic DNA of fungal isolates was prepared by either PEX method described earlier (Garmaroodi & Taga 2007) or a method developed by Saitoh *et al.* (2006).

Sexual crossing

Crossings procedures and ascospore isolation were done according to Tegtmeier & VanEtten (1982). To assess fertility of each cross, the total number of mature perithecia and the ones with oozing ascospores per plate were recorded. For each combination of parental strains reciprocal crosses were performed. The notation convention of 'female × male' was adopted to specify sexuality of parental strains.

The transmission rate (k) of CDCi and CDCm was calculated as its frequency in the total number of CDCs contained in all the progenies. When both CDCi and CDCm involved in the sexual crossing, k was calculated separately and then the mean value was considered as transmission rate of CDC.

PCR

Detection of DNA marker for each of CDCi and CDCm was carried out as described before (Salamiah *et al.* 2001; Garmaroodi & Taga 2007). Identification of mating types was performed using degenerate primers developed by Kerenyi *et al.* (2004). PCR