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Chloroplast DNA inheritance in the *Stellaria longipes* complex (Caryophyllaceae)

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Abstract Inheritance of chloroplast DNA (cpDNA) was examined in F_1 progenies derived from three crosses and three corresponding reciprocal crosses between *Stellaria porsildii* and *S. longifolia*. Chloroplast DNA restriction fragments were analyzed using methods of nonradioactive digoxigenin-11-dUTP labeling and chemiluminescent detection with Lumi-Phos 530. Distinct interspecific restriction fragment polymorphisms were identified and used to demonstrate the mode of cpDNA inheritance. Mode of cpDNA inheritance differed among crosses. Two crosses in which *S. porsildii*, SP2920-21, was the maternal parent exhibited three different types of plastids, maternal, paternal and biparental, among the F_1 hybrids, suggesting a biparental cpDNA inheritance and plastid sorting-out in *Stellaria*.

Key words *Stellaria* · Chloroplast DNA inheritance · Restriction fragment polymorphism · Digoxigenin · Lumi-Phos 530

Introduction

Stellaria longipes Goldie (Caryophyllaceae) is a polymorphic, polyploid species complex with circumpolar distribution (Chinnappa and Morton 1976). The species complex includes *S. longipes* subsp. *longipes* (2n = 4x = 52, 6x = 78, 8x = 104), *S. longipes* subsp. *arenicola* (2n = 4x = 52), *S. longifolia* (2n = 2x = 26) and *S. porsildii* (2n = 2x = 26) (Chinnappa 1992). Thus, members of this complex are represented by four cytotypes (2x, 4x, 6x and 8x), and these are interfertile (Chinnappa and Morton 1984). Early investigations (Chinnappa and Morton 1976, 1984) assumed a hybrid origin of *S. longipes* and attempted to search for the progenitors of this extremely plastic polyploid species. Evidence from interspecific hybridization (Chinnappa 1985) and enzyme electrophoresis (Cai et al. 1990) strongly suggested that the widely distributed diploid *S. longifolia* (2n = 26) is a progenitor of *S. longipes*. Preliminary studies of isozymes (Cai et al. 1990) and seed proteins (Chinnappa et al. 1992) have indicated that *S. porsildii* (2n = 26) may also have contributed in the evolution of *S. longipes*.

Chloroplast DNA (cpDNA) and ribosomal DNA (rDNA) markers are valuable tools for the analysis of polyploid species. Complementary use of cpDNA and rDNA not only can identify the progenitors of allopolyploids but can also illustrate the direction of hybridization in allopolyploid evolution (Soltis et al. 1992). Analyses of cpDNA rely on the assumptions concerning its mode of inheritance. In angiosperms, cpDNA is usually inherited maternally (Sears 1980), which permits the designation of the maternal parent of polyploids. However, exceptions of paternal or biparental inheritance of cpDNA have been reported in several angiosperm taxa (Metzlaff et al. 1981; Medgyesy et al. 1986; Lee et al. 1988; Schumann and Hancock 1989; Cruzen et al. 1993). On the basis of cytological and physiological mechanisms, which cause either uniparental and biparental plastid inheritance, Hagemann and Schröder (1989) defined four plant types: *Lycopersicon* type, *Solanum* type, *Triticum* type, and *Pelargonium* type. The *Pelargonium* type has biparental plastid inheritance while the other three types show uniparental plastid transmission. Therefore, inheritance of the cpDNA genome in angiosperms is not simply maternal and is thus a potential source of error in polyploidy and evolutionary studies (Harris and Ingram 1991). A lack of detailed information on the transmission genetics of the cpDNA genome limits the robustness of evolutionary studies of polyploidy. Therefore, it is necessary to examine the mode of cpDNA inheritance before cpDNA can serve as a useful evolutionary maker.

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As part of a major investigation on the origin and evolution of the *S. longipes* complex, cpDNA inheritance was examined in three interspecific crosses between *S. porsildii* and *S. longifolia* and their three reciprocals. We report here the molecular evidence of biparental cpDNA inheritance and plastid sorting-out in *Stellaria*.

Materials and methods

Plant material

Four accessions collected from *Stellaria* natural populations were used as hybridization parents in this study. Accession SP2920-21 and SP2920-9 of *Stellaria porsildii* was originally collected from Signal Mt., New Mexico. Accession SL3613-6 of *S. longifolia* was collected from Sangre de Cristo Mountains, New Mexico and SL3681 from Big Horn Mountains, Wyoming, USA. Three interspecific crosses, SP2920-21 × SL3613-6, SP2920-21 × SL3681 and SP2920-9 × SL3613-6, between *S. porsildii* and *S. longifolia*, and three reciprocals were made. Seeds from each cross produced 5–15 progenies. A total of 58 plants and four parental genotypes were analyzed for cpDNA inheritance.

DNA isolation and Southern blotting

Total cellular DNAs were extracted from 2–3 grams of liquid nitrogen-pulverized leaf tissue according to the method described by Doyle and Doyle (1987). The DNA samples were further purified by cesium chloride/ethidium bromide gradient ultracentrifugation (Maniatis et al. 1982). The concentration of DNA samples was estimated using a Lambda 3 UV/VIS Spectrophotometer from PerKin Elmer.

On the basis of results obtained from screening the four parental individuals with seven restriction enzymes and four probes, 3 µg of progeny DNA were digested with 15 units of the restriction enzymes *Eco*RI and *Bcl*I for 5 h according to the manufacturer's recommendations (Boehringer Mannheim). The digested DNA fragments were then separated on 0.8% agarose gel by electrophoresis overnight at 2 V/cm in TBE buffer (Maniatis et al. 1982). After electrophoresis, DNA fragments were transferred to positively charged nylon membranes (Boehringer Mannheim) using the alkaline transfer method of Reed and Mann (1985) and then baked at 120 °C for 20 min as recommended by Boehringer Mannheim.

Probe preparation and hybridization

Two *Petunia hybrida* cpDNA fragments cloned into plasmid pBR322 (Palmer et al. 1983) were used as hybridization probes in the analysis of cpDNA inheritance: P3, a 21-kb *Pst*I fragment from the large single-copy (LSC) region containing a part of the *rbcL* gene, and P10, a 9.0-kb *Pst*I fragment from the LSC region containing a part of the *PsbA* gene. Plasmid DNAs containing *Petunia* cpDNA inserts were isolated following the large-scale isolation method of Maniatis et al. (1982). Probes were labeled with digoxigenin-11-dUTP using the random primed method (Feinberg and Vogelstein 1983). About 3 µg of plasmid DNA was labeled overnight in a 50-µl reaction volume at 37 °C. This scaled-up labeling reaction produced larger amounts of DIG-labeled DNA. The yield of DIG-labeled probes was estimated using the labeled control pBR328 DNA from Boehringer Mannheim.

Membranes were placed in a hybridization bag and prehybridized for 4 h at 65 °C in hybridization solution: 5 × SSC, 1.0% blocking reagent for nucleic acid hybridization (Boehringer Mannheim), 0.1% N-lauroylsarcosine, and 0.02% sodium dodecyl sulfate (SDS). Hybridizations were performed overnight at 65 °C in hybridization solution containing 10 ng/ml DIG-labeled probe DNAs. Hybridization blots were then washed twice, 5 min per wash, in 2 × SSC, 0.1% SDS at room temperature, and twice, 15 min per wash, in 0.5 × SSC, 0.1% SDS at 65 °C. Chemiluminescent detection with Lumi-Phos 530 of DIG-labeled DNA was carried out following the manufacturer's procedure (Boehringer Mannheim). After application of Lumi-Phos (0.5 ml/100 cm^2), memberanes were incubated in the dark at 37 °C for 30 min and then exposed to X-ray films for 10 min.

Results

Interspecific restriction fragment polymorphisms

DNAs from four *Stellaria* parental plants were digested with seven restriction enzymes (*Bam*HI, *Bcl*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I) and hybridized with each of four Petunia cpDNA probes (P3, P6, P8, P10) to search for distinct polymorphisms. Two interspecific restriction fragment polymorphisms were detected (Table 1). By hybridizing with P10, *Eco*RI-digested DNAs revealed a 5.2-kb fragment in both *S. porsildii* accessions and a 3.9-kb fragment in both *S. longifolia* accessions. Physical mapping of cpDNA is underway to investigate the mutation causing this fragment length polymorphism. A second fragment polymorphism revealed by *Bcl*I digestion and hybridization with P3 was inferred to be caused by a restriction site difference between the two parental species. The absence of a *Bcl*I cutting site in *S. longifolia* resulted in only one 2.3-kb fragment compared with two fragments (1.6 + 0.7 = 2.3 kb) in *S. porsildii*. No intraspecific polymorphisms were observed. Plastids of *S. porsildii* will be referred to hereafter as "SP", while those of *S. longifolia* as "SL". These interspecific restriction fragment polymorphisms provided markers to distinguish between the parental cpDNA haplotypes and were used to demonstrate the mode of cpDNA inheritance in this study.

Inheritance of cpDNA in interspecific crosses

A total of 58 F_1 hybrid plants derived from three interspecific crosses between *S. porsildii* and *S. longifolia* and their three reciprocal crosses were screened for transmission of the distinct cpDNA polymorphisms. The results are summarized in Table 2. All progeny of three reciprocal crosses in which *S. longifolia* was the maternal parent had the SL plastid of the maternal parent. Inheritance of cpDNA was maternal. However,

Table 1 Interspecific restriction fragment polymorphisms used to demonstrate the mode of cpDNA inheritance in interspecific crosses in *Stellaria*

Enzyme/probe combination	*S. porsildii* (SP)		*S. longifolia* (SL)	
	SP2920-21	SP2920-9	SL3613-6	SL3681
*Eco*RI/P10	5.2	5.2	3.9	3.9
*Bcl*I/P3	1.6 + 0.7 = 2.3	1.6 + 0.7 = 2.3	2.3	2.3

Table 2 Inheritance of cpDNA in interspecific crosses between *S. porsildii* and *S. longifolia*

Cross	Parent (kb)[a]		Progeny[b]		
	♀	♂	♀	♂	♀/♂
SP2920-21 × SL3681	5.2	3.9	8	2	2
SL3681 × SP2920-21	3.9	5.2	9		
SL2920-21 × SL3613-6	5.2	3.9	12	2	1
SL3613-6 × SP2920-21	3.9	5.2	10		
SL2920-9 × SL3613-6	5.2	3.9	7		
SL3613-6 × SP2920-9	3.9	5.2	5		
Total			51	4	3
SP2920-21 × SL3681	1.6 + 0.7	2.3	8	2	2
SL3681 × SP2920-21	2.3	1.6 + 0.7	9		
SL2920-21 × SL3613-6	1.6 + 0.7	2.3	13	2	
SL3613-6 × SP2920-21	2.3	1.6 + 0.7	10		
SL2920-9 × SL3613-6	1.6 + 0.7	2.3	7		
SL3613-6 × SP2920-9	2.3	1.6 + 0.7	5		
Total			52	4	2

[a] See Table 1 for explanation of restriction fragment polymorphisms
[b] Number of progenies having the same cpDNA haplotype

cpDNA inheritance varied among three crosses in which *S. porsildii* was the maternal parent. Exceptions to maternal inheritance were observed in two crosses in which SP2920-21 was the maternal parent. In cross SP2920-21 × SL3681, 8 of 12 progenies had the maternal 5.2-kb, *Eco*RI/P10 fragment (SP plastid), 2 prossessed the paternal 3.9-kb fragment (SL plastid) and 2 plants had both parental polymorphic fragments, 5.2 and 3.9 kb, (SL/SP plastid) (Fig. 1). The same pattern was observed for the transmission of the *Bcl*I/P3 restriction fragment polymorphism (1.6 + 0.7 versus 2.3 kb). In cross SP2920-21 × SL3613-6, 15 progenies were screened. For the transmission of *Eco*RI/P10 fragment polymorphism, 12 plants were found to have the maternal SP 5.2-kb fragment, 2 had the paternal SL 3.9-kb fragment and 1 plant had both the 5.2- and 3.9-kb fragments. For transmission of the *Bcl*I/P3 fragment polymorphism (1.6 + 0.7 versus 2.3 kb), 13 of 15 plants were found to have the maternal 1.6 + 0.7-kb fragments and 2 had the paternal 2.3-kb fragment. No biparental combinations were observed (Fig. 2). In cross SP2920-9 × SL3613-6, 7 F_1 plants were screened, and all were found to contain only the maternal plastid.

Discussion

In this study, we have observed three types of plastid DNA, maternal, paternal and biparential, in F_1 hybrids from two crosses between *Stellaria porsildii* and *S. longifolia*. It is likely plastids from both parents are transmitted to the zygote and later vegetatively sorted out to separate cells during plant development. Such a biparental transmission of plastids necessitates us to postulate the existence of "mixed" cells or plants in the sexual progeny containing plastids from both parents. We have observed 3 such plants in this study. These plants may undergo a process of random sorting-out and eventually develop sectors having only maternal or paternal plastids. It is also possible that there is a bias in favor of retaining maternal plastids because maternal plastomes were predominant in our sampled progeny.

Fig. 1 Inheritance of cpDNA in an interspecific cross between *Stellaria porsildii* and *S. longifolia* revealed by *Eco*RI digestion and hybridization with probe P10. *Lane 1 S. porsildii* SP2920-21, *lane 2 S. Longifolia* SL3681, *lanes 3–14* progeny from cross SP2920-21 × SL3681 showing the paternal cpDNA type (*lane 12* and *13*) and biparental cpDNA type (*lanes 5* and *8*), *lanes 15–23* progeny from its reciprocal cross, SL3681 × SP2920-21, all having the cpDNA of the maternal parent

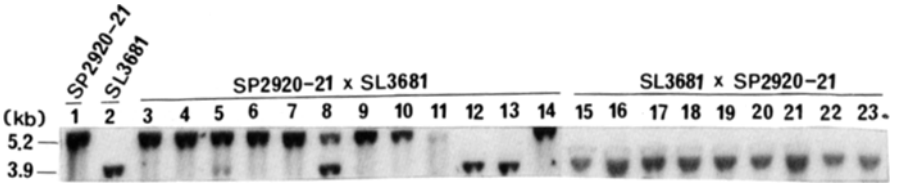
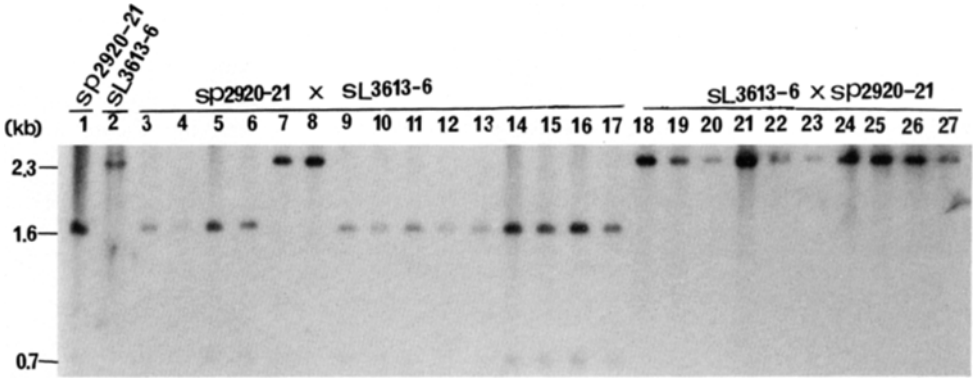


Fig. 2 Inheritance of cpDNA in an interspecific cross between *Stellaria porsildii* and *S. longifolia* revealed by *Bcl*I digestion and hybridization with probe P3. *Lane 1 S. porsildii* SP2920-21, *lane 2 S. longifolia* SL3613-6, *lanes 3–17* progeny from cross SP2920-21 × SL3613-6, *lanes 18–27* progeny from its reciprocal cross, SL3613-6 × SP2920-21. *Lanes 7* and *8* show F_1 plants with only the paternal plastid DNA type



We have found the presence of only paternal plastid DNA in only 4 plants of two crosses between *Stellaria porsildii* and *S. longifolia.*

When *S. longifolia* was the maternal parent all F_1 hybrids possessed the maternal SL plastid, but a mixture of maternal, paternal and biparental plastids was found in the F_1 generation of two crosses in which *S. porsildii* was the maternal parent. In fact, paternal and/or biparental plastids were observed in crosses only in which *S. porsildii*, SP2920-21 was the maternal parent. Although our sample size was not large enough to confidently draw conclusions about the differential strength of the two plastid types, it is possible that SL may have a competitive advantage over the SP plastid. The SL plastid may be transmitted both maternally and paternally, while the SP plastid may be transmitted only maternally. Vegetative sorting-out may be biased toward the SP plastid as well since no pure sectors or plants with only the SP plastid were observed in the sampled progeny when *S. porsildii* (SP plastid) was the paternal parent. Cases of plastid strength have been documented in *Oenothera* (Kirk and Tilney-Bassett 1978; Chiu et al. 1988). Since paternal and biparental plastids occurred only in coresses in which SP2920-21 was the maternal parent, it is possible that there is a genotypic effect that may permit or promote the paternal transmission of plastids to the F_1 generation. Genotypic effects of paternal transmission of cpDNA indeed have been observed in other analyses of cpDNA inheritance (Smith 1989; Tiley-Bassett and Almouslem 1989).

The use of both cpDNA and rDNA helps discern the parentage of both auto- and allopolyploids and contributes to a better understanding of the process of polyploidization in a number of cultivated plants (Soltis et al. 1992). The evolutionary analysis of cpDNA relies on understanding its mode of inheritance. Strict maternal inheritance facilitates the designation of the maternal polyploid ancestor provided that little or no divergence has occurred between progenitor and derivative species since the polyploidization event. In the present study we have observed the biparental inheritance of cpDNA in two experimental interspecific crosses. This finding leads us to be concerned with the impact of biparental transmission of cpDNA on cpDNA gene genealogies and phylogenetic inferences of the *Stellaria longipes* complex. The level of impact depends on how much cpDNA introgression through pollen exchange has really occurred in natural populations of *Stellaria.* Levels of interspecific cpDNA gene flow via pollen or seed movement in the natural populations of *Stellaria* are currently being investigated. Furthermore, the observations of genotypic effect and plastid differential strength in the present study must also be taken into consideration when estimating cpDNA introgression via the pollen parent. Further studies with large sample sizes to confirm and possibly extend the present results are needed.

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