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Paternal inheritance of plastids in *Medicago sativa*

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Summary. Plastids are plant cellular organelles that are generally inherited from the maternal parent in the angiosperms. Many species exhibit biparental inheritance of plastids, but usually with a predominantly maternal influence. In contrast to this, we report strong paternal inheritance of plastids in reciprocal crosses of alfalfa, *Medicago sativa,* by following restriction fragment length polymorphisms for plastid DNA in two normal green plastids. Mitochondrial inheritance remained exclusively maternal.

Key words: *Medicago* – Alfalfa – Plastid inheritance – Organelle inheritance

Introduction

Plastids are one of two plant cellular organelles which contain extra-nuclear DNA. Previous reports have indicated that they are inherited primarily from the maternal parent in the angiosperms, although a low level of biparental inheritance has been documented in many species (reviewed in Kirk and Tilney-Bassett 1978; Hagemann 1979; Sears 1980).

Biparental plastid inheritance has commonly been noted when green and white sectored tissue has appeared in progeny following crosses between a normal green plant and one having a plastome-encoded chlorophyll deficiency. *Oenothera* (Kirk and Tilney-Bassett 1978; Sears 1980; Chiu et al. 1988) and *Pelargonium* (Kirk and Tilney-Bassett 1978; Sears 1980; Tilney-Bassett 1973) are two well-known examples of genera exhibiting biparental plastid inheritance that have been documented in this way. A drug resistance marker has also been used to show that rare biparental plastid inheritance occurs in *Nicotiana* (Medgyesy et al. 1986), a taxa which was previously considered to have exclusively maternal inheritance of plastids (Kirk and Tilney-Bassett 1978; Corriveau and Coleman 1988).

In contrast to the angiosperms, plastid transmission in the gymnosperms is mostly paternal. Utilization of a plastid mutant has indicated greater than 95% paternal transmission of plastids in *Cryptomeria* (Ohba et al. 1971). Similarly, restriction fragment length polymorphism (RFLP) analyses have documented high levels of paternal inheritance in *Pinus* (Wagner et al. 1987), *Larix* (Szmidt et al. 1987), *Pseudotsuga* (Neale et al. 1986), and *Picea* (Stine 1988).

In this report, we describe the first evidence of predominantly paternal transmission of normal green plastids in an angiosperm, *Medicago sativa,* based on data from RFLPs. This technique allows one to follow the transmission of normal green plastids through sexual crosses, thus eliminating any differential survival bias which might exist against a mutant type.

Inheritance of mitochondria, the other DNA-containing plant organelle, was also determined.

Materials and methods

Plant material

Reciprocal populations were produced from crosses between two individuals from different subspecies of alfalfa, *Medicago sativa.* Subspecies freely intercross but are morphologically distinct. *M. sativa* ssp. *sativa* has an upright growth habit and purple flowers, while ssp. *falcata* has a more spreading growth habit and yellow flowers. Based on subspecies designation, the organelles of the *sativa* parent (an individual from population PI 299049, obtained from the USDA North Central Regional Plant Introduction Station at Ames, Iowa) will be referred to as "S", and those of the *falcata* parent (an individual from population W71-42, described by Bingham 1975) as "F".

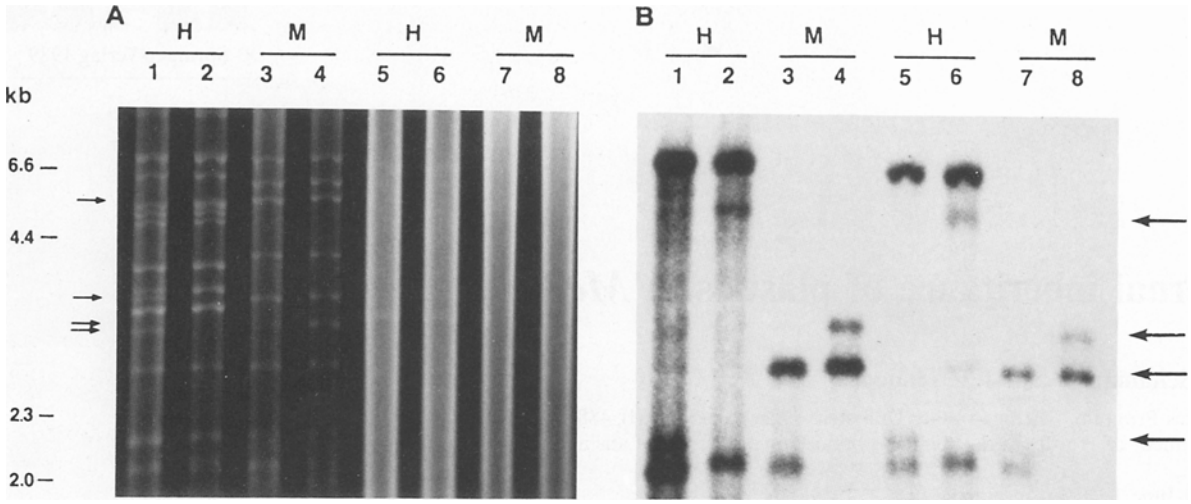

Fig. 1 A and B. Ethidium bromide-stained gel (**A**) and Southern blot (**B**) of purified chloroplast DNA (*lanes 1–4*) and total cell DNA (*lanes 5–8*) of parents. Unique fragments are indicated by **A** *fine arrows* and **B** *bold arrows*. *Lanes 1, 3, 5, and 7,* F; *lanes 2, 4, 6, and 8,* S. *Lanes 1–2, 5–6,* HindII (HincII) digest; *lanes 3–4, 7–8,* MspI digest. Size markers from HindIII digests of lambda DNA are indicated. DNA fragments were separated on a 1.25% agarose gel at 20 V. The probe used in B was pLecP4 (Phillips 1985)

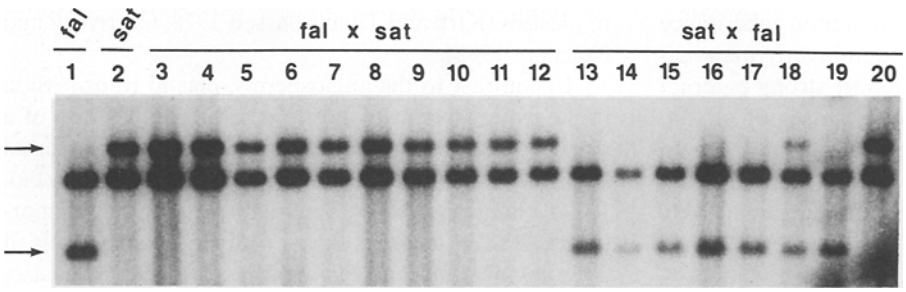

Fig. 2. Southern blot of MspI-digested DNA of parents and progeny from a reciprocal cross, showing plastid DNA polymorphism. *Lane 1,* F; *lane 2,* S; *lanes 3–12,* progeny from the cross F × S; *lanes 13–20,* progeny from the cross S × F (maternal parent is listed first in crosses). Four micrograms total cell DNA was separated on a 1% agarose gel at 25 V

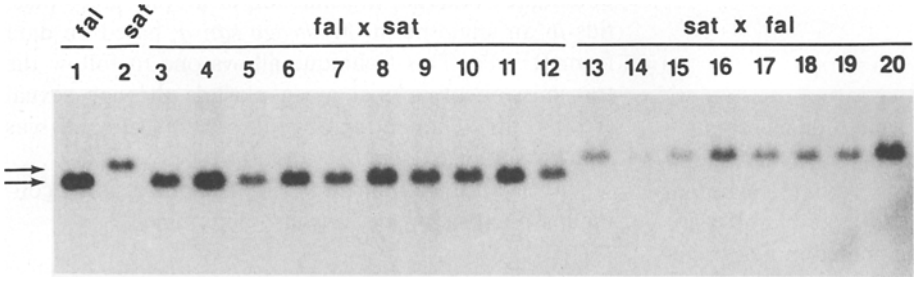

Fig. 3. Southern blot of MspI-digested DNA of parents and progeny from a reciprocal cross, showing mitochondrial polymorphism between parents and maternal inheritance among progeny. *Lane 1,* F; *lane 2,* S; *lanes 3–12,* progeny from the cross F × S; *lanes 13–20,* progeny from the cross S × F. Blot is from the same filter as Fig. 2, which has been stripped and re-probed with pmtSylSa8 (Aviv et al. 1984)

Organelle DNAs of both parents and a total of 30 progeny were characterized.

DNA isolation

Total cell DNA was isolated from leaf tissue by phenol/chloroform extraction and CsCl ultracentrifugation (Maniatis et al. 1982). Plastid DNA was isolated by sucrose step gradients and CsCl ultracentrifugation (Palmer 1986).

RFLP detection

DNA restriction reactions were according to the manufacturer's instructions (Boehringer Mannheim). Restriction fragments from 3 μg plastid DNA or 4 μg total cell DNA were separated on 1% or 1.25% agarose gels at 20 or 25 V, blotted to nitrocellulose, and probed with pLecP4, which contains a 19.4-kb fragment of tomato plastid DNA (Phillips 1985), or pmtSylSa8, which contains a 22.1-kb fragment of *Nicotiana sylvestris* mito-

chondrial DNA (Aviv et al. 1984). Probes were labeled through random priming (Boehringer Mannheim kit), and hybridization conditions were as described in Thomashow et al. (1981).

Results

A plastid DNA polymorphism was present in the parents that allowed us to follow plastid inheritance in the progeny. As seen in Fig. 1 B, HindII digests produced a 2.3-kb band unique to the F genome, and a 4.6-kb band unique to the S genome. The 4.6-kb fragment is visible in the gel as a double molar band (Fig. 1 A, lane 2). In MspI digests, a 2.1-kb band is unique to the F genome while a 3.5-kb band is unique to the S genome.

Thirty progeny were analyzed by restriction with both diagnostic enzymes. There was 100% agreement in inheritance data between enzymes; only MspI digests are shown. Eighteen progeny are shown in Fig. 2. Plastid inheritance was strongly paternal in both directions of this cross. When the *sativa* subspecies was the paternal parent, all progeny had exclusively S plastid DNA (Fig. 2, lanes 3–12; six progeny not shown). When the *falcata* subspecies was the paternal parent, inheritance was also strongly paternal, but some maternal plastids were transmitted (Fig. 2, lanes 13–20; six progeny not shown). Of the 14 *M. sativa* ssp. *sativa* × *M. sativa* ssp. *falcata* progeny analyzed, 12 had exclusively F plastids, 1 had exclusively S plastids, and 1 plant had both types of plastid DNA (lane 18 in Fig. 2).

Even though plastid inheritance was largely paternal in our population, mitochondrial inheritance was entirely maternal. Differences in mitochondrial genomes between the *falcata* and *sativa* parents allowed RFLP analysis of progeny, and can be seen in Fig. 3. Inheritance was strictly maternal in both directions of this cross, including the one progeny that was biparental for plastid type (lane 18).

Single-shoot analysis of the plant carrying both parental types of plastid DNA (lane 18, Figs. 2 and 3) can be seen in Fig. 4. Panel A indicates that individual shoots have sorted into pure tissue of one plastid type or the other, producing a fixed chimera. Vegetative sorting out has classically been observed via visible green and white sectors in leaves, and here is documented at the DNA level as well. Panel B indicates that mitochondrial type is strictly maternal in these shoots, with no trace of paternal input.

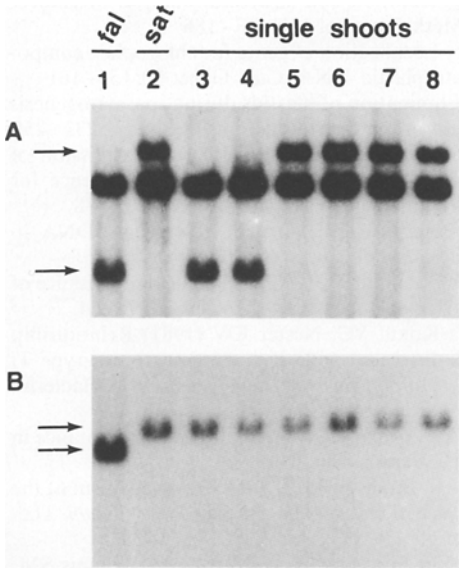

Fig. 4 A and B. Southern blot of MspI-digested DNA extracted from single shoots of one plant (lane 18 in Figs. 2 and 3) from the cross S × F. *Lane 1*, F; *lane 2*, S; *lanes 3–8*, individual shoots. **A** probed with labeled plastid DNA as in Fig. 2. **B** probed with labeled mitochondrial DNA as in Fig. 3

Discussion

Although there have been reports of uniparental paternal inheritance of plastids in the angiosperm *Pelargonium* (Tilney-Bassett and Birkey 1981), entirely paternal progeny have only predominated in a cross when the maternal plastids were white. It has been documented in *Pelargonium* that white plastids are less successfully transmitted than are green plastids (Kirk and Tilney-Bassett 1978; Hagemann 1979; Tilney-Bassett and Birky 1981), and paternal transmission of green plastids over green maternal plastids has not been reported. Thus, ours is the first report of paternal plastid inheritance in an angiosperm when both plastid types were phenotypically normal.

Our results of maternal mitochondrial inheritance are in contrast to a report by others of biparental inheritance of mitochondria in this species (Fairbanks et al. 1988). However, our results are similar to those observed in conifers and other plant species. Although paternal inheritance of plastids is common among gymnosperms, less data exists regarding the mode of mitochondrial inheritance. However, results of one study indicate that mitochondrial inheritance is maternal in *Pinis taeda*, loblolly pine (Neale and Sederoff 1989). Maternal inheritance of mitochondria has been extensively documented in angiosperms, mammals, and insects (Connett 1987; Lansman et al. 1981). Thus, there is little precedent for other than strict maternal inheritance of mitochondria, even when plastid inheritance is strongly paternal.

The independent inheritance of chloroplasts and mitochondria in this population may suggest that two separate organelle exclusion mechanisms were operating. Since progeny from one cross (*M. sativa* ssp. *sativa* × *M. sativa* ssp. *falcata*) show three distinct types of plastid inheritance (uniparental paternal, biparental, uniparental maternal), it is likely that plastids from both parents were transmitted to the zygote followed by vegetative

sorting out. The bias in favor of the paternal plastids may have occurred because there was an unequal input of plastids from the gametes of each parent. The zygotes may have initially contained more paternal plastids than maternal ones, and the maternal plastids may have been diluted out in subsequent cell divisions. It is also possible that paternal plastids were preferentially replicated, and that they ultimately out-competed maternal ones. No evidence exists for paternal input of mitochondria.

Examples of nuclear control of plastid transmission have been described in *Pelargonium* (Kirk and Tilney-Bassett 1978; Tilney-Bassett and Birky 1981) and *Petunia* (Cornu and Dulieu 1988), and variation for this trait may exist among *Medicago* genotypes as well. Such variation would reconcile our observations with those of others (Smith et al. 1986; Lee et al. 1988) who report biparental plastid inheritance in this species. These studies utilized mutant plastids, however, which may have introduced a bias similar to that observed in *Pelargonium*.

Our sample size was too small to draw conclusions about differential strengths of the two plastid types, but since the S plastid was transmitted both maternally and paternally, while the F plastid was only transmitted paternally, it is possible that S may have a competitive advantage over F. Such a system operates in *Oenothera* (Kirk and Tilney-Bassett 1978; Chiu et al. 1988).

The question of plastid strength in *Medicago* can be addressed by utilizing vegetative clones of the chimeric plant. This provides a system for comparing the transmission of the two plastid types in exactly the same nuclear background. Reciprocal crosses of these plastid types to a third plastid type in a different nuclear background will allow a direct comparison of plastid strength between the S and F types.

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