

Examination of the Cytoplasmic DNA in Male Reproductive Cells to Determine the Potential for Cytoplasmic Inheritance in 295 Angiosperm Species

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Mature pollen grains of 295 angiosperm species were screened by epifluorescence microscopy for a marker that denotes the mode of cytoplasmic inheritance. We used the DNA fluorochrome DAPI (4',6-diamidino-2-phenylindole) for pollen cell staining. The presence or absence of fluorescence of cytoplasmic DNA in the generative cell or sperm cells was examined in each species. The species examined represented 254 genera and 98 families, and 40 of these families had not been previously studied in this regard. The cytoplasmic DNA of the generative cell or sperm cells did not fluoresce in 81% of the species examined, from 83% of the genera and 87% of the families examined, indicating the potential for maternal cytoplasmic inheritance in these species. In contrast, the male reproductive cells of 19% of the species, from 17% of the genera and 26% of the families examined, displayed fluorescence of the cytoplasmic DNA, indicating the potential for biparental cytoplasmic inheritance in these species. The results revealed the potential for biparental cytoplasmic inheritance in several species in which the inheritance mode was previously unknown, including plants in the Bignoniaceae, Cornaceae, Cruciferae (Brassicaceae), Cyperaceae, Dipsacaceae, Hydrocharitaceae, Papaveraceae, Portulacaceae, Tiliaceae, Valerianaceae, and Zingiberaceae. Electron microscopy revealed that the sperm cells of *Portulaca grandiflora* contain both plastid and mitochondrial DNA. However, in the generative cells of *Musella lasiocarpa*, the mitochondria contain DNA, but the plastids do not. These data provide a foundation for further studies of cytoplasmic inheritance in angiosperms.

Keywords: Angiosperm — Biparental cytoplasmic inheritance — Cytoplasmic DNA — DAPI — Male reproductive cells — Maternal cytoplasmic inheritance.

Introduction

At the beginning of the twentieth century, maternal and biparental transmission of leaf color in angiosperms were reported (Correns 1909, Baur 1909). These non-Mendelian phenomena, termed cytoplasmic inheritance, have been studied in the intervening years (Sears 1980, Hagemann and

Schröder 1989, Kuroiwa 1991, Mogensen 1996). However, despite decades of research, the modes of cytoplasmic inheritance in angiosperms have been determined for only about 60 genera (Tilney-Bassett 1978, Smith 1988), and cytological examination to determine the mode of inheritance has been applied to only about 100 genera of flowering plants (Whatley 1982, Nagata et al. 1999). Plants whose patterns of cytoplasmic inheritance are known constitute the minority among all of the angiosperms, which number approximately 200,000 species in 10,000 genera and 300–400 families. By contrast, the development of molecular techniques has stimulated the application of plastid genome and mitochondrial genome studies on population genetics, systematics, and organismal evolution. However, many such studies routinely assume maternal cytoplasmic inheritance due to a lack of available information (Mogensen 1996). Therefore, the overall distribution of cytoplasmic inheritance patterns in angiosperms requires further investigation.

The method traditionally used for the cytological examination of cytoplasmic inheritance is electron microscopy. However, this method is inappropriate for the study of large numbers of species. Miyamura et al. (1987) used DAPI-staining fluorescence microscopy to study 16 species whose cytoplasmic inheritance patterns were known. The results demonstrated that cytoplasmic DNA is present in the generative cells or sperm cells of plants known to display biparental or paternal inheritance, but it is not present in species known to display maternal inheritance. Since this method allows rapid screening of large numbers of male reproductive cells, it is suitable for the examination of a large range of angiosperm species. Using the same method, Corriveau and Coleman (1988) examined 235 species within 80 families to determine their patterns of cytoplasmic inheritance. They found that biparental plastid inheritance occurs in about 14% of the genera and 19% of the families examined.

In the present study, we determined the potential for cytoplasmic inheritance in 295 additional flowering plants by detecting the presence or absence of cytoplasmic DNA in the generative cells or sperm cells of mature pollen. Our examination extended to 98 families, of which 40 had not been previously studied in this regard. Of the species examined, 81%, representing 83% of the genera and 87% of the families studied, displayed maternal inheritance, and the remaining 19% of

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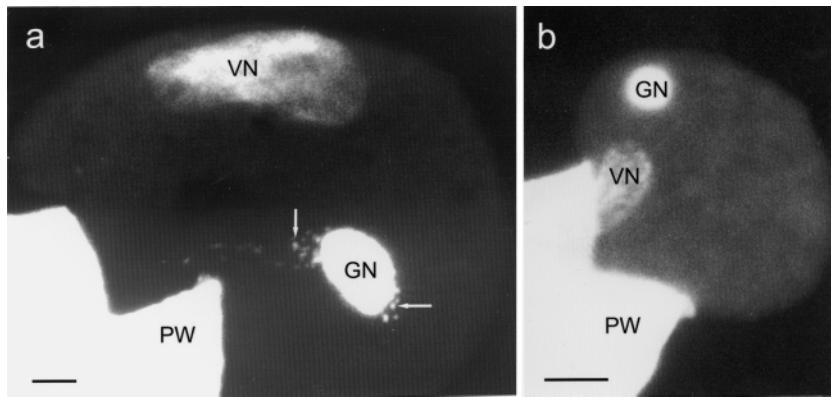


Fig. 1 DAPI-stained epifluorescence microphotographs showing squashed pollen grains of (a) *Actinidia deliciosa*, a species that displays biparental plastid inheritance, and (b) *Sophora japonica* f. *pendula*, a species that displays maternal cytoplasmic inheritance. Arrows indicate the fluorescent spots of cytoplasmic DNA. GN, generative nucleus; VN, vegetative nucleus, W, pollen wall. Bar = 5 µm.

the species, in 17% of the genera and 26% of the families examined, showed a potential for biparental inheritance. The results of the present study should contribute to a better understanding of the systematic distribution of cytoplasmic inheritance in angiosperms.

Results

Cytoplasmic DNA in generative cells and sperm cells

When mature pollen grains were squashed in buffer containing DAPI and glutaraldehyde and examined with an epifluorescence microscope, nuclear DNA and cytoplasmic DNA aggregates in the pollen cells were visible. In species with the potential for biparental cytoplasmic inheritance, the fluorescence of cytoplasmic DNA aggregates was clearly associated with the generative cell or sperm cells (Fig. 1a). In contrast, in species that display maternal inheritance, no fluorescence was detectable (Fig. 1b). The presence or absence of the fluorescence corresponded to species with the potential for biparental or maternal cytoplasmic inheritance, respectively. This trait was therefore used as a criterion in our screening of species for modes of cytoplasmic inheritance.

Distribution of maternal and biparental cytoplasmic inheritance

The generative cells or sperm cells of 295 species representing 254 genera in 98 families were examined. The results are summarized in Tables 1 and 2.

Maternal inheritance, the dominant mode of cytoplasmic inheritance in angiosperms, was detected in 238 species from 87 families. Cytoplasmic DNA was not detectable in the male reproductive cells of these species. Within the families containing species identified as displaying maternal inheritance, a single mode of maternal inheritance appeared in 72 families, including the two largest angiosperm families, the *Orchidaceae* (four species) and the *Compositae* (15 species in 14 genera).

Cytoplasmic DNA was detected in the male reproductive cells of 57 species, comprising 43 genera of 25 families, indicating the potential for biparental inheritance in these species. Within the families containing species identified as displaying

potential for biparental cytoplasmic inheritance, the potential for both maternal and biparental inheritance appeared in 14 families, including the *Aizoaceae*, *Araceae*, *Bignoniaceae*, *Caprifoliaceae*, *Cornaceae*, *Cruciferae*, *Leguminosae*, *Liliaceae*, *Musaceae*, *Oleaceae*, *Papaveraceae*, *Portulacaceae*, *Solanaceae*, and *Zingiberaceae*. Species with a single mode of biparental inheritance appeared in 11 families, including the *Actinidiaceae* (one species), *Cactaceae* (one species), *Campanulaceae* (four species in two genera), *Cyperaceae* (one species), *Dipsacaceae* (three species in three genera), *Hydrocharitaceae* (one species), *Linaceae* (two species in one genera), *Plumbaginaceae* (one species), *Tiliaceae* (two species in two genera), *Turneraceae* (one species), and *Valerianaceae* (three species in two genera). An unusual case was *Jasminum polyanthum* in the *Oleaceae*, which had dual features. Fluorescence of the cytoplasmic DNA was detected in the sperm cells of about 50% of the pollen grains, implying that the species is intermediate between biparental and maternal inheritance.

Although most genera were determined to have the same mode of cytoplasmic inheritance, interspecies variation appeared in three genera. In *Portulaca* (*Portulacaceae*), cytoplasmic DNA was detected in the sperm cells of *P. grandiflora* but not in *P. oleracea*, suggesting that the two species have different modes of cytoplasmic inheritance. Similar cases of intrageneric variation were found in *Mesembryanthemum* (*Aizoaceae*) and *Syringa* (*Oleaceae*).

Identification of plastid and mitochondrial DNA in male reproductive cells

The fluorescent spots of cytoplasmic DNA that appear in the generative or sperm cells can be emitted from either plastid DNA or mitochondrial DNA. In some cases, dimorphic fluorescent spots indicated that the male reproductive cells contained both plastid and mitochondrial DNA. However, in most species identified by us as having potential for biparental inheritance, it was impossible to discriminate between plastid and mitochondrial DNA aggregates with epifluorescence microscopy, since the fluorescent spots derived from this DNA had similar features (see examples in Fig. 2). In these cases, the

Table 1 The mode of cytoplasmic inheritance of 295 plant species determined using epifluorescence microscopy to reveal the presence or absence of cytoplasmic DNA in the generative or sperm cells

Taxon	Mode ^a	Pollen ^b		
Acanthaceae			<i>Pereskia aculeata</i>	B 3
<i>Adhatoda vasica</i>	M	2	Calycanthaceae	
<i>Thunbergia grandiflora</i>	M	2	<i>Chimonanthus praecox</i>	M 2
Actinidiaceae			Campanulaceae	
<i>Actinidia deliciosa</i>	B	2	<i>Adenophora divaricata</i>	B 2
Aizoaceae			<i>Adenophora paniculata</i>	B 2
<i>Mesembryanthemum cordifolium</i>	M	3	<i>Codonopsis lanceolata</i>	B 2
<i>Mesembryanthemum spectabile</i>	B	3	<i>Codonopsis pliosula</i>	B 2
Alismataceae			Cannabinaceae	
<i>Sagittaria subulata</i>	M	3	<i>Cannabis sativa</i>	M 2
Amaranthaceae			Cannaceae	
<i>Amaranthus lividus</i>	M	3	<i>Canna indica</i>	M 2
<i>Amaranthus tricolor</i>	M	3	Caprifoliaceae	
Amaryllidaceae			<i>Abelia biflora</i>	B 3
<i>Crinum aciaticum</i>	M	2	<i>Abelia chinensis</i>	B/M 3
<i>Crinum amabile</i>	M	2	<i>Kolkwitzia amabilis</i>	B 3
<i>Lycoris aurea</i>	M	2	<i>Lonicera maackii</i>	B 3
Apocynaceae			<i>Lonicera pekinensis</i>	B 3
<i>Allemanda cathartica</i>	M	2/3	<i>Lonicera X Tellmanniana</i>	M 3
<i>Allemanda nerifolia</i>	M	2	<i>Viburnum sargentii</i>	M 2
<i>Apocynum venetum</i>	M	2	<i>Weigela florida</i>	B 3
<i>Catharanthus roseus</i>	M	2	Caryophyllaceae	
<i>Rauvolfia verticillata</i>	M	2	<i>Arenaria friderica</i>	M 3
<i>Thevetia peruviana</i>	M	2	<i>Dianthus fragrans</i>	M 3
Araceae			<i>Lychnis fulgens</i>	M 3
<i>Pinellia ternata</i>	M	2	<i>Silene fortunei</i>	M 3
<i>Zantedeschia aethiopica</i>	B	3	Ceratophyllaceae	
Asclepiadaceae			<i>Ceratophyllum demersum</i>	M 3
<i>Cynanchum thesioides</i>	M	2	Commelinaceae	
Berberidaceae			<i>Commelina communis</i>	M 2
<i>Nandina domestica</i>	M	2	Compositae	
Bigoniaceae			<i>Adenocaulon himalaicum</i>	M 3
<i>Begonia argyrostigma</i>	M	2	<i>Anaphalis sinica</i>	M 3
Bigononiaceae			<i>Aster</i> sp.	M 3
<i>Catalpa bungei</i>	M	2	<i>Atractylodes lancea</i>	M 2
<i>Catalpa speciosa</i>	M	2	<i>Bidens bipinnata</i>	M 2
<i>Campsis radicans</i>	B	2	<i>Bupleurum chinense</i>	M 3
<i>Incarvillea</i> sp.	B	2	<i>Doellingeria scabra</i>	M 2
Bixaceae			<i>Eupatorium japonicum</i>	M 3
<i>Bixa orellana</i>	M	2	<i>Senecio rowleyanus</i>	M 3
Boraginaceae			<i>Galinsoga parviflora</i>	M 3
<i>Cynoglossum amabile</i>	M	2/3	<i>Hemistepta lyrata</i>	M 3
Bromeliaceae			<i>Ligularia lapathifolia</i>	M 3
<i>Anans</i> sp.	M	2	<i>Ligularia intermedia</i>	M 3
Buxaceae			<i>Myriopsis dioica</i>	M 3
<i>Buxus sinica</i>	M	2	<i>Syneilesis aconitifolia</i>	M 2
Cactaceae			Cornaceae	
			<i>Swida walteri</i> (= <i>Cornus walteri</i>)	B 2
			<i>Cornus officinalis</i> (= <i>Macrocarpium officinalis</i>)	M 2
			Crassulaceae	
			<i>Rhodiola rosea</i>	M 2
			<i>Sedum aizoon</i>	M 2

Table 1 Cont.

Taxon	Mode ^a	Pollen ^b		
			<i>Phlomis jeholensis</i>	M 2
			<i>Salvia japonica</i>	M 3
			<i>Salvia multiorrhiza</i>	M 3
Cruciferae			<i>Scutellaris baicalensis</i>	M 2/3
<i>Arabis pendula</i>	M	3	<i>Veronica linariaefolia</i>	M 2/3
<i>Hesperis trichosepala</i>	B	3	Leguminosae	
<i>Matthiola incana</i>	M	3	<i>Astragalus dahuricus</i>	B 2
<i>Nasturtium officinale</i>	M	2	<i>Astragalus strictus</i>	B 2
<i>Orychophragmus violaceus</i>	M	3	<i>Bauhinie feberi</i>	M 3
<i>Rorippa indica</i>	M	3	<i>Caesalpinia pulcherrima</i>	M 2
Cyperaceae			<i>Caesalpinia sappan</i>	M 2
<i>Carex baccans</i>	B	3	<i>Calliandra suriniaceae</i>	M 3
Dioscoreaceae			<i>Campylotropis macrocarpa</i>	M 2/3
<i>Dioscorea nipponica</i>	M	2	<i>Cassia surattensis</i>	M 2
Dipsacaceae			<i>Colutea arborescens</i>	B 2
<i>Dipsacus asperoides</i>	B	3	<i>Erythrina variegata</i>	M 2
<i>Morina nepalensis</i>	B	3	<i>Glycyrrhiza pallidiflora</i>	B 2
<i>Scabiosa tschiliensis</i>	B	3	<i>Lepedeza davurica</i>	M 3
Ebenaceae			<i>Mimosa pudica</i>	M 2
<i>Diospyros lotus</i>	M	3	<i>Phyllodium pulchellum</i>	M 2
Eucommiaceae			<i>Sophora flavescens</i>	M 2
<i>Eucommia ulmoides</i>	M	2	<i>Sophora japonica</i> f. <i>pendula</i>	M 2
Euphorbiaceae			<i>Trifolium repens</i>	B 2
<i>Euphobia milii</i>	M	2	<i>Vicia bungei</i>	B 2
<i>Leptopus chinensis</i>	M	2	<i>Vicia faba</i>	B 2
<i>Securinega suffruticosa</i>	M	2	<i>Vicia gigantea</i>	B 2
Gentianaceae			<i>Vicia pseudorobus</i>	B 2
<i>Halenia corniculata</i>	M	3	<i>Vicia unijuga</i>	B 2
<i>Nymphoides peltatum</i>	M	3	Liliaceae	
Gesneriaceae			<i>Allium odorum</i>	M 2
<i>Lysionotus paucilorus</i>	M	2	<i>Allium victorialis</i>	M 2
<i>Saintpaulia ionantha</i>	M	2	<i>Aloe</i> sp.	M 2
<i>Sinningia speciosa</i>	M	2	<i>Asparagus cochinchinensis</i>	M 2
Graminaceae			<i>Diurathera major</i>	B 2
<i>Melica scabrosa</i>	M	3	<i>Gagea</i> sp.	M 2
Hamamelidaceae			<i>Lloydia ixiolirioides</i>	B 3
<i>Loropetalum chinensis</i> var. <i>rubrum</i>	M	2	<i>Ornithogalum caudatum</i>	M 2
Hippocastansceae			<i>Ophiopogon japonicus</i>	M 2
<i>Aesculus chinensis</i>	M	2	<i>Polygonatum odoratum</i>	M 2
Hydrocharitaceae			<i>Zephyranthes grandiflora</i>	M 2
<i>Ottelia acuminata</i> var. <i>crispa</i>	B	2	Linaceae	
Hypoxidaceae			<i>Linum stelleroides</i>	B 3
<i>Cucurliigo capitullata</i>	M	2	<i>Linum usitatissimum</i>	B 3
Iridaceae			Loganiaceae	
<i>Belamcanda chinensis</i>	M	2	<i>Buddleja yunnanensis</i>	M 2
<i>Freesia refracta</i>	M	2	Lythraceae	
Juglandaceae			<i>Lagerstroemia indica</i>	M 2
<i>Juglans cathayansis</i>	M	2	<i>Lythrum salicaria</i>	M 2
Labiatae			Malpighiaceae	
<i>Agastache rugosa</i>	M	3	<i>Malpighia glavca</i>	M 2
<i>Ajuga lupulina</i>	M	2	Malvaceae	
<i>Clerodendranthus spicatus</i>	M	2	<i>Malva</i> sp.	M 2
<i>Dracocephalum rupstre</i>	M	3		
<i>Mentha haplocalyx</i> var. <i>piperascens</i>	M	2		

Table 1 Cont.

Taxon	Mode ^a	Pollen ^b		
Melastomataceae			Plumbaginaceae	
<i>Osbeckia opipara</i>	M	2	<i>Limonium sinuatum</i>	B 3
Menispermaceae			Polemoniaceae	
<i>Stephania epigaea</i>	M	2	<i>Phlox subulata</i>	M 2
Moraceae			Polygalaceae	
<i>Broussonetia papyrifera</i>	M	2	<i>Polygala arillata</i>	M 3
<i>Ficus elastica</i>	M	3	<i>Polygala sibirica</i>	M 3
<i>Humulus scandens</i>	M	2	<i>Polygala tenuifolia</i>	M 3
Musaceae			Polygonaceae	
<i>Heliconia aurantiaca</i>	M	2	<i>Fagopyrum esculentum</i>	M 3
<i>Heliconia pendula</i>	M	2	<i>Polygonum amplexicaule</i> var. <i>sinensis</i>	M 2
<i>Musa coccinea</i>	B	2	<i>Polygonum senticosum</i>	M 3
<i>Musa sapientum</i>	B	2	<i>Polygonum suffultum</i>	M 3
<i>Musella lasiocarpa</i>	B	2	<i>Rheum officinale</i>	M 3
Myrtaceae			Portulacaceae	
<i>Callistemon rigidus</i>	M	2	<i>Portulaca grandiflora</i>	B 3
<i>Syzygium cumini</i>	M	2	<i>Portulaca oleracea</i>	M 3
Nymphaeaceae			<i>Talinum patens</i>	M 3
<i>Nelumbo nucifera</i>	M	2	Pontederiaceae	
<i>Nymphaea tuberosa</i>	M	2	<i>Eichhornia crassipes</i>	M 2
Oleaceae			Primulaceae	
<i>Chionanthus retusus</i>	M	2	<i>Cyclamen persicum</i>	M 2
<i>Fontanesia fortunei</i>	M	3	<i>Lysimachia barystachys</i>	M 2
<i>Fraxinus excelsior</i> var. <i>aurea</i>	M	2	<i>Lysimachia clethroides</i>	M 2
<i>Jasminum polyanthum</i>	B/M	3	<i>Primula secundiflora</i>	M 2
<i>Syringa oblata</i>	M	2	<i>Primula sikkimensis</i>	M 2
<i>Syringa pekinensis</i>	B	2	Punicaceae	
Onagraceae			<i>Punica granatum</i>	M 2
<i>Circaea quadrisulcata</i>	M	2	Ranunculaceae	
<i>Fuchsia hybrida</i>	M	2	<i>Aconitum albobviolaceum</i>	M 2
Orchidaceae			<i>Anemone cathayensis</i>	M 2
<i>Arundina graminifolia</i>	M	2	<i>Anemone rivularis</i> var. <i>flore-minore</i>	M 2
<i>Calanthe discolor</i>	M	2	<i>Clematis hexapetala</i>	M 2
<i>Liparis yunnanensis</i>	M	2	<i>Paeonia lactiflora</i>	M 2
<i>Spiranthes lance</i>	M	2	<i>Ranunculus japonica</i>	M 2
Oxalidaceae			<i>Thalictrum aquilegifolium</i>	M 2
<i>Oxalis oregana</i>	M	2	<i>Trollius chinensis</i>	M 2
Palmae			Rhamnaceae	
<i>Archontophoeni alexandrae</i>	M	2	<i>Rhamnus florida</i>	M 2
Papaveraceae			Rosaceae	
<i>Chelidonium majus</i>	M	2	<i>Agrimonia pilosa</i>	M 2
<i>Corydalis bungeana</i>	B	2	<i>Chaenomeles speciosa</i>	M 2
<i>Dicentra spectabilis</i>	B	2	<i>Chamaerhodos erecta</i>	M 2
<i>Hepecoum leptocarpum</i>	M	2	<i>Dontostemon dentatus</i>	M 3
<i>Macleaya cordata</i>	M	2	<i>Erysimum bungei</i>	M 3
<i>Papver rhoeas</i>	M	2	<i>Geum aleppicum</i>	M 2
Phrymaceae			<i>Kerria japonica</i>	M 2
<i>Phryma leptostachya</i>	M	3	<i>Malus halliana</i>	M 2
Phytolacaceae			<i>Potentilla discolor</i>	M 2
<i>Rivina humilis</i>	M	3	<i>Rosa xanthina</i>	M 2
			<i>Sanguisorba officinalis</i>	M 2
			<i>Spiraea fritchiana</i>	M 2
			<i>Spiraea sahicifolia</i>	M 2
			<i>Sorbaria sorbifolia</i>	M 2

Table 1 Cont.

Taxon	Mode ^a	Pollen ^b		
<i>Taihangia rupestris</i>	M	2	<i>Sterculia nobilis</i>	M 2
Rubiaceae			Taccaceae	
<i>Galium aparine</i>	M	2	<i>Tacca chantrieri</i>	M 2
<i>Gardenia jasminoides</i>	M	2	Thymelaeaceae	
<i>Hamelia patens</i>	M	2	<i>Daphne</i> sp.	M 3
<i>Hymenodictyon flaccidum</i>	M	2	Tiliaceae	
<i>Ixora yunnanensis</i>	M	2	<i>Grewia bilobia</i> var. <i>parvifolia</i>	B 3
<i>Mussaenda erythrophylla</i>	M	2	<i>Tilia tuan</i>	B 3
<i>Mycetia gracilis</i>	M	2	Trapaceae	
<i>Serissa japonica</i>	M	2	<i>Trapa bispinosa</i>	M 3
Rutaceae			Turneraceae	
<i>Fortunella margarita</i>	M	2	<i>Turnera ulmifolia</i>	B 3
<i>Micromelum paniculata</i>	M	2/3	Umbelliferae	
Salicaceae			<i>Heracleum moellendorffii</i>	M 3
<i>Populus tomentosa</i>	M	2	<i>Saposhnikovia divaricata</i>	M 3
<i>Salix babylonica</i>	M	2	<i>Sphallerocarpus gracilis</i>	M 3
<i>Salix gracilistyla</i>	M	2	Urticaceae	
Sapindaceae			<i>Pilea paperomioides</i>	M 3
<i>Koelreuteria paniculata</i>	M	2	<i>Urtica angustifolia</i>	M 2
Saururaceae			Valerianaceae	
<i>Saururus chinensis</i>	M	2	<i>Patrinia heterophylla</i>	B 3
Saxifragaceae			<i>Patrinia scabiosaefolia</i>	B 3
<i>Astilbe chinensis</i>	M	2	<i>Valeriana officinalis</i>	B 3
<i>Deutzia grandiflora</i>	M	2	Verbenaceae	
<i>Ribes fasciculatum</i>	M	2	<i>Callicarpa arborea</i>	M 2
Scrophulariaceae			<i>Clerodendron thomsonae</i>	M 2
<i>Calceolaria crenatiflora</i>	M	2	<i>Clerodendron trichotomum</i>	M 2
<i>Digitalis purpurea</i>	M	2	<i>Stachytarpheta jamaicensis</i>	M 3
<i>Paulownia tomentosa</i>	M	2	<i>Tectona grandis</i>	M 3
<i>Pedicularis likiangensis</i>	M	2	<i>Verbena trifolia</i>	M 2
<i>Pedicularis siphonantha</i>	M	2	<i>Vitex negunda</i> var. <i>heterophylla</i>	M 2
<i>Pedicularis striata</i>	M	2	Zingiberaceae	
<i>Rehmannia glutinosa</i>	M	2	<i>Costus lacerus</i>	M 2
<i>Verbascum thapsus</i>	M	2	<i>Curcuma domestica</i>	M 2/3
<i>Veronica didyma</i>	M		<i>Etingera erythrophylla</i>	M 2
<i>Veronica linariaefolia</i>	M	2/3	<i>Globba barthri</i>	B 2
Simaroubaceae			<i>Globba racemosa</i>	B 2
<i>Ailanthus altissima</i>	M	2	<i>Hedychium spicatum</i>	M 2
Solanaceae			<i>Zingiber purpureum</i>	B 2
<i>Brunfelsia latifolia</i>	B	2	<i>Zingiber zerumbet</i>	B 2
<i>Datura</i> sp.	M	2	Zygophyllaceae	
<i>Lycium chinense</i>	M	2	<i>Tribulus terrestris</i>	M 3
<i>Solanum nigrum</i>	M	2		
Sterculiaceae				
<i>Firmiana simplex</i>	M	2		

^a M, maternal inheritance (cytoplasmic DNA was undetected in the male reproductive cells), B, biparental inheritance (cytoplasmic DNA was detected in the male reproductive cells), B/M, biparental or maternal inheritance (cytoplasmic DNA was detectable in sperm cells from about 50% pollen but undetectable in sperm cells from the other 50% pollen); ^b bi-cellular (2) or tri-cellular (3) pollen.

fluorescence derived from plastid and mitochondrial DNA needed to be distinguished in order to determine whether the plastids or mitochondria have the potential for biparental inheritance.

Electron microscopy of the sperm cells of *P. grandiflora* (Portulacaceae) indicated that the cells contained both plastids and mitochondria (Fig. 3c). In addition, Immunogold electron microscopy revealed that anti-DNA gold particles localized to

Table 2 Summary of results

	Families	Genera	Species
Total	98	254	295
Biparental	25 (11±14 ^a) (26%)	43 (40±3 ^b) (17%)	57 (19%)
Maternal	87 (73±14 ^a) (87%)	214 (211±3 ^b) (83%)	240 (81%)

^a Families in which both maternal and biparental cytoplasmic inheritance patterns were detected.

^b Genera in which the mode of cytoplasmic inheritance showed interspecies variation.

both of the organelles (Fig. 3b). These results suggest that the fluorescent spots in the sperm cells (as shown in Fig. 3a) must have been derived from both plastid and mitochondrial DNA, and therefore both the plastids and the mitochondria of *P. grandiflora* have the potential for biparental inheritance.

A similar examination was performed for *Musella lasiocarpa* (Musaceae). In plants of this species, there was a large ratio of cytoplasm to nucleus in the generative cell, which contained a large number of mitochondria but few plastids (Fig. 4a). In the case of *M. lasiocarpa*, however, clusters of gold particles were localized on the mitochondria but not on the plastids (Fig. 4c–f). These results showed that the fluorescent spots in the generative cell shown in Fig. 4b resulted only from mitochondrial DNA and provided evidence for the biparental inheritance of mitochondria and maternal inheritance of plastids in this species.

Discussion

The sexual crossing test is a traditional tool that gives an exact result for determining the mode of cytoplasmic inheritance. However, since the cytoplasmic phenotype mutants that are useful for crossing tests are exceedingly scarce, application of the method has been limited to about 60 angiosperm genera in crops and ornamental species (Tilney-Bassett 1978, Whatley 1982, Smith 1988, Reboud and Zeyl 1994). For the same reason, the use of molecular techniques such as restriction fragment length polymorphism is limited. This is why cytoplasmic inheritance has been less well studied in wild plants than in cultivated species. To investigate a broader range of angiosperms, we used fluorescence microscopy to detect the presence or absence of cytoplasmic DNA in the male reproductive cells and determine the potential mode of cytoplasmic inheritance. The

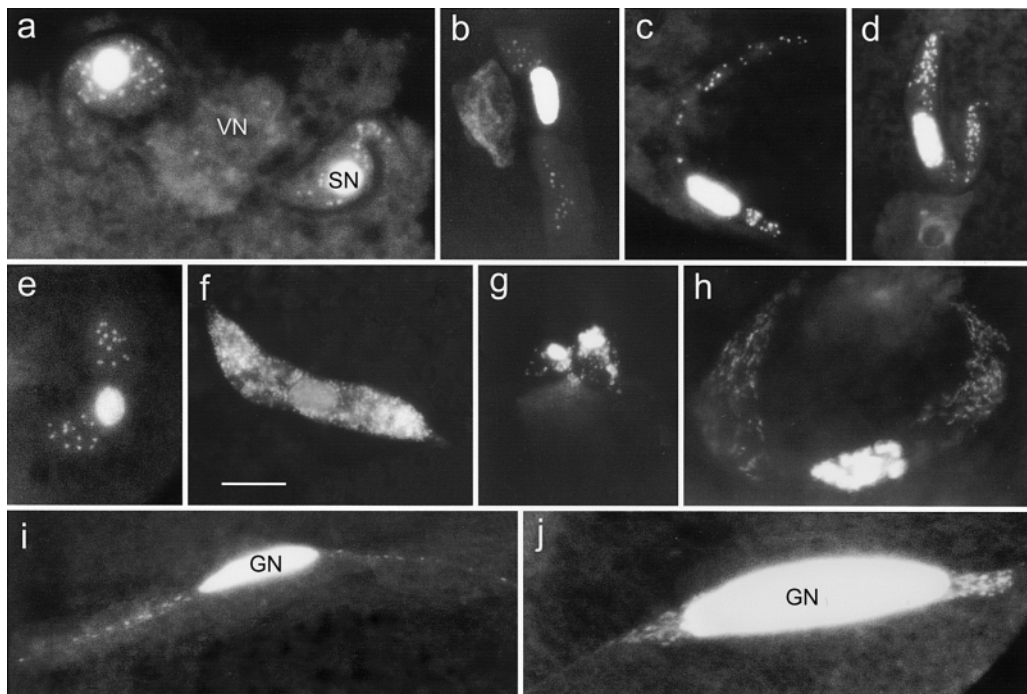


Fig. 2 DAPI-stained epifluorescence microphotograph showing the male reproductive cells squashed from pollen grains of (a) *Weigela florida*, (b) *Syringa pekinensis*, (c) *Codonopsis lanceolata*, (d) *Incarvillea* sp., (e) *Corydalis bungeana*, (f) *Musa coccinea*, (g) *Carex baccans*, (h) *Zingiber purpureum*, (i) *Trifolium repens*, and (j) *Vicia bungei*. Note the fluorescent spots in the cells. GN, generative nucleus; SN, sperm nucleus; VN, vegetative nucleus. Bar = 10 µm.

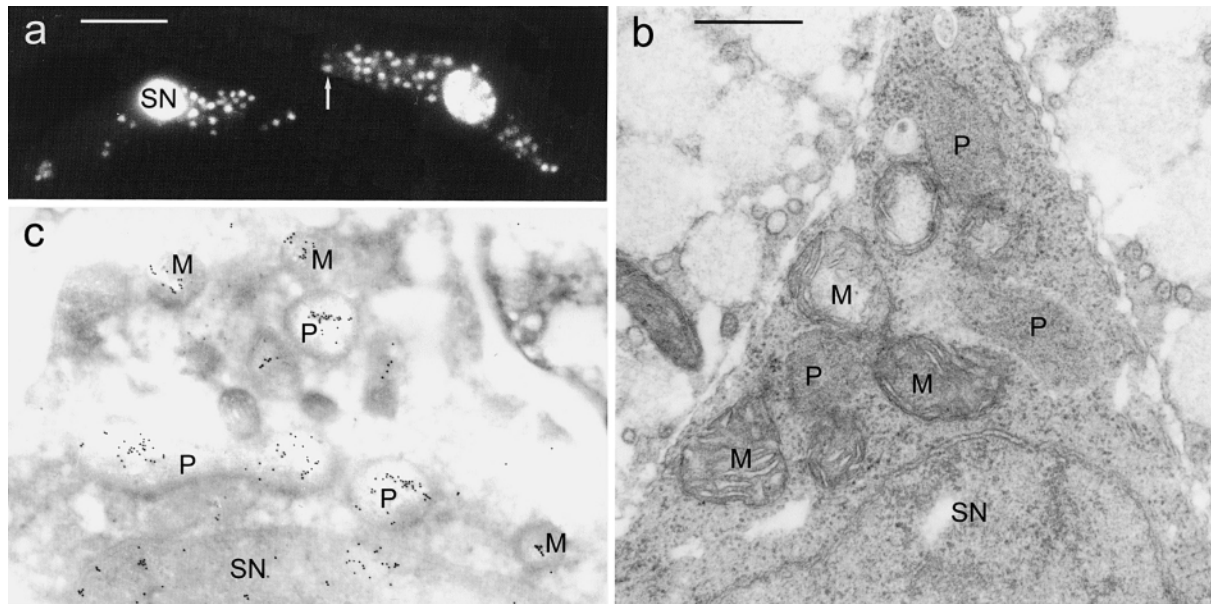


Fig. 3 DAPI-stained epifluorescence (a), electron (b) and immunoelectron (c) microphotographs showing sperm cells of *Portulaca grandiflora*. (a) Cytoplasmic DNA in the sperm cells emitted similar epifluorescent spots (arrow). (b) The cell contained both plastids and mitochondria. (c) Gold particles appeared on both of the organelles. *M*, mitochondrion; *P*, plastid; *SN*, sperm nucleus. Bars = 10 μm (a), 0.5 μm (b, c).

method allows rapid, large-scale screening of plants for the potential for different modes of cytoplasmic inheritance, with reliable results (Miyamura et al. 1987, Corriveau and Coleman 1988). In this study, cytoplasmic DNA was observed in the male reproductive cells of *Actinidia deliciosa*, *Zantedeschia aethiopica*, and *Turnera ulmifolia*, which were previously reported to display biparental plastid inheritance (Yao et al. 1994, Testolin and Cipriani 1997, Shore and Triassi 1998). This result may be thought as a positive control for the method. Based on the results of previous studies and this one, we conclude that the fluorescence of cytoplasmic DNA in male reproductive cells can be used as a reliable marker for our purposes.

Using fluorescence microscopy, Corriveau and Coleman (1988) examined 235 species of angiosperms representing 80 families. The results are important for understanding the distribution of different modes of cytoplasmic inheritance in angiosperms. We determined the potential for cytoplasmic inheritance in 295 additional angiosperm species using a strategy similar to that of Corriveau and Coleman (1988). The examination included 98 families, of which 40 had not been previously studied in this regard. Our results show that 81% of the species studied, in 83% of the genera and 87% of the families examined, display maternal inheritance, and 19% of the species studied, in 17% of the genera and 26% of the families examined, show a potential for biparental inheritance. These rates are similar to those determined by Corriveau and Coleman (1988), who found that approximately 80% of angiosperm species display maternal cytoplasmic inheritance. Furthermore, we discovered the potential for biparental inheritance in the Bignoniaceae, Cornaceae, Cruciferae (Brassi-

caceae), Cyperaceae, Dipsacaceae, Hydrocharitaceae, Papaveraceae, Portulacaceae, Tiliaceae, Valerianaceae, and Zingiberaceae. Biparental inheritance has not been reported previously in these families, which are distributed in quite distant orders. Four genera (seven species) in the Caprifoliaceae and all species studied in the Dipsacaceae and Valerianaceae have the potential for biparental inheritance in Dipsacales. Species with the potential for biparental inheritance were found in the Aizoaceae and Portulacaceae in the Aizoales. The distribution of families with biparental inheritance among the distant taxa examined in this study is consistent with the results of previous studies. Our results, therefore, support the suggestion that cytoplasmic inheritance evolved independently many times in response to different selective pressures (Sears 1980, Birky 1995).

An analysis of our results showed that the presence or absence of cytoplasmic DNA in male reproductive cells could be used as a trait for systematic studies. For example, cytoplasmic DNA was detected in *Corydalis* and *Dicentra* but not in other genera of the Papaveraceae. This result agrees with the suggestion of Lidén that *Corydalis* and *Dicentra* should be reclassified from the Papaveraceae to the new family Fumariaceae (Lidén 1993). In the Caprifoliaceae, four genera (*Abelia*, *Linnaea*, *Lonicera* and *Weigela*) showed a potential for biparental cytoplasmic inheritance, and two genera (*Sambucus* and *Viburnum*) displayed maternal cytoplasmic inheritance. These results coincide with Wilkinson's opinion that *Sambucus* and *Viburnum* (biparental genera) should be grouped into one lineage and others (maternal genera) should be grouped into another lineage (Wilkinson 1949). In addition, we detected

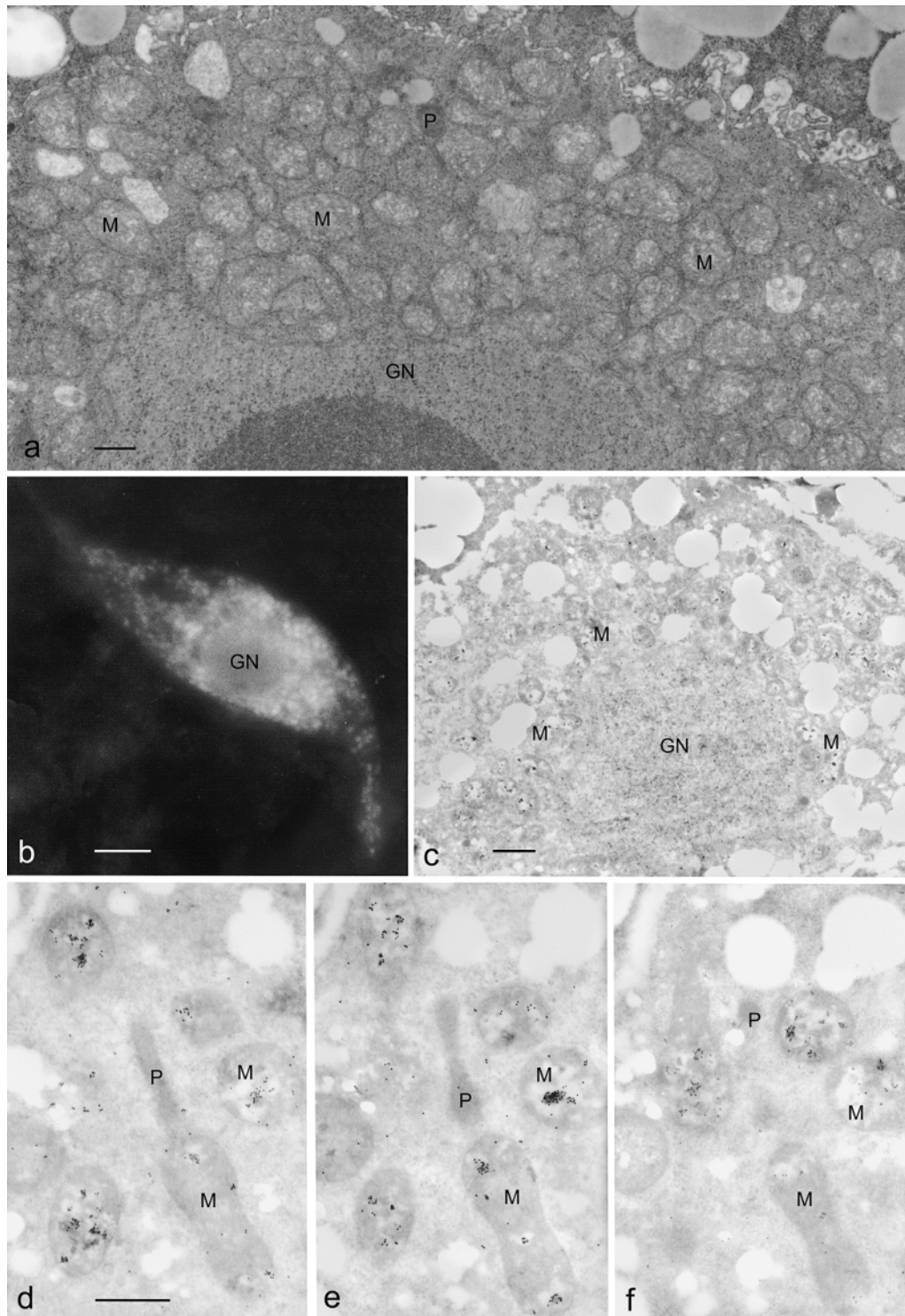


Fig. 4 Electron (a), DAPI-stained epifluorescence (b) and immunoelectron (c–f) microphotographs showing generative cells of *Musella lasiocarpa*. (a) Numerous mitochondria but few plastids were seen in the cell. (b) Fluorescence of cytoplasmic DNA was evident. (c–f) Gold particles appeared in clusters on mitochondria. (d, e, f) Serial sections through a plastid were sparsely labeled with gold particles. GN, generative nucleus; M, mitochondrion; P, plastid. Bars = 0.5 μ m (a, d–f), 5 μ m (b) and 1 μ m (c).

cytoplasmic DNA in the generative cells of *Swida walteri* (Cornaceae), also called *Cornus walteri*. However, no cytoplasmic DNA was detected in the generative cells of *Cornus florida* (Corriveau and Coleman 1988) or *Cornus officinalis*. These results provide evidence for the establishment of *Swida walteri* as a species.

Cytoplasmic inheritance includes the inheritance of both the plastid and the mitochondrial genomes. Recent studies have indicated that inheritance of these organelle genomes is controlled in different ways (Nagata et al. 1999, Sodmergen et al. 2002). Therefore, it will be necessary to determine whether the fluorescence in male reproductive cells following DAPI staining is derived from plastid or mitochondrial DNA. We have applied electron and immunoelectron microscopy for this purpose, as described previously (Sodmergen et al. 1997, Sodmergen et al. 1998, Zhang and Sodmergen 2003). The results show that both plastids and mitochondria of *P. grandiflora* have the potential for biparental inheritance, and that mitochondria, but not plastids, of *M. lasiocarpa* appear to result from biparental inheritance. A similar examination of the male reproductive cells of other species, using fluorescence of cytoplasmic DNA after DAPI staining, is underway.

Since the strategy used in this study was similar to that of Corriveau and Coleman (1988), our examination did not initially include the genera that were examined in their study. However, during the examination, several conflicts appeared. In the *Limonium*, *Astragalus*, *Trifolium*, *Vicia*, *Campsis*, *Linum*, and *Zingiber* genera, fluorescence of cytoplasmic DNA appeared in the generative or sperm cells in our study, but was not detected in these cells by Corriveau and Coleman (1988). In *Campsis radicans*, treatment of the cells with ethanol and glacial acetic acid as described by Corriveau and Coleman (1988) degraded the fluorescence. Therefore, the discrepancies may be due to differences in the methods used or intrageneric variation. For these genera, further confirmation of the mode of inheritance is required.

Materials and Methods

Mature pollen grains were collected from plants at sites in Beijing and Yunnan. The majority of the experimental materials were obtained from plants collected in three botanical gardens: Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences; Kunming Botanical Garden, Kunming, Yunnan; and Xishuangbanna Botanical Garden, Menglun, Yunnan. Pollen were sampled and examined from April to August.

Epifluorescence microscopy of cytoplasmic DNA was performed according to the method of Kuroiwa and Suzuki (1980). In brief, pollen grains were collected on a glass slide and immersed in a drop of 3% glutaraldehyde and 1 $\mu\text{g ml}^{-1}$ DAPI in Tan buffer (Nemoto et al. 1988). The pollen grains were covered with a cover slip, then squashed by pressing the cover slip against the slide. The samples were examined after 10 min with an Olympus BH-2 epifluorescence microscope.

Electron microscopy of pollen cells was performed using routine methods. For DNA Immunogold electron microscopy, pollen grains

were fixed with 3% glutaraldehyde in cacodylate buffer (pH 7.4) and embedded in LR White resin. The sections were labeled using the method of Johnson and Rosenbaum (1990). A mouse monoclonal antibody that recognizes single- and double-stranded DNA (Boehringer Mannheim, Germany) and a goat anti-mouse IgM antibody conjugated to 10-nm colloidal gold (British BioCell International, U.K.) were used for the detection. Sections were examined with a JOEL JEM-1010 electron microscope.

We routinely performed a DNase test to confirm the free localization of the Immunogold on the sample sections pretreated with DNase (data not shown).

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