Sex Identification in Dioecious Woody Landscape Plants

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INTRODUCTION

Dioecious plant species, in which individual plants are either male or female, are commonly used in horticulture. In the majority of these species, the earliest possible identification of sex occurs at the time of flowering, a stage that may not be reached for a number of growing seasons in woody trees and shrubs. Often, one sex is preferred over the other; for example, female hollies are favored because of their attractive fruits, whereas female ginkgos are essentially worthless for landscaping purposes because of their fruits. In those species in which fruit production is favored, it is advantageous to know the sex of individual plants in order to ensure that both sexes are represented in a newly established planting. Thus, it would be of considerable commercial interest to growers to be able to determine the sex of dioecious plants in the seedling stage, potentially reducing the amount of acreage and labor necessary to grow the plants to flowering.

The goals of this paper are twofold: (1) to provide a list of dioecious species used as landscape plants in the United States, and (2) to briefly describe the methods for sex identification that we are testing on dioecious plants.

DIOECY IN WOODY LANDSCAPE PLANTS

Dioecy occurs in a diverse array of taxa of flowering plants (Bawa, 1980; Meagher, 1988). This general pattern is mirrored in a survey of the breeding systems of woody landscape plants. Wyman (1990) lists 45 "genera with flowers dioecious." A complete survey of the plants described by Dirr (1990) increases the total to 51 genera, representing 36 families (Table 1). The overwhelming majority of plant families are represented by a single dioecious genus, but seven (Anacardiaceae, Aquifoliaceae, Elaeagnaceae, Moraceae, Rutaceae, Salicaceae, and Taxaceae) include two to four dioecious genera. The widespread nature of this phenomenon is evident in the fact that 15% of the genera described by Dirr (1990) contain at least one dioecious species. The most important of these genera for landscape purposes are *Ginkgo*, *Ilex*, *Celastrus*, and *Taxus*.

Also included in Table 1 is information on whether the plants are described in the literature as strictly dioecious. In 23 out of 51 genera, there is evidence that some degree of bisexuality occurs. This is probably an underestimate since most detailed studies of the reproductive biology of dioecious taxa reveal some lability in sex expression, as is documented for *Taxus* by Chadwick and Keen (1976). In three species (*Aucuba japonica*, *Ilex opaca*, and *Ruscus aculeatus*) bisexual cultivars have been selected (Dirr, 1990). Although this does not seem to be particularly well documented, in the overwhelming majority of the species listed, reliable fruit-set in females is contingent upon the proximity of male plants.

METHODS FOR SEX IDENTIFICATION

Every method of sex identification is based on inherent genetic differences between males and females. Since few dioecious plant species display sex-linked vegetative characters, one must rely on genomic or biochemical differences that are present in prereproductive plants. The methods that have been applied to plants or are currently being developed include: 1) DNA flow cytometry; 2) biochemical tests; 3) protein electrophoresis; and 4) sex-linked DNA-probe hybridization. We will describe each briefly.

Table 1. Genera of woody landscape plants that include at least one dioecious species. [List of genera compiled from Wyman (1990) and Dirr (1990); species names and breeding systems from Hortus Third (1976), Rehder (1986), and Dirr (1990) and current names and spelling from The New Royal Horticultural Society Dictionary of Gardening (1992).]

Genus	Species[a]	Strictly dioecious?[b]	Family
Acanthopanax	see *Eleutherococcus*		
Acer	*negundo, pensylvanicum*	Y	Aceraceae
Actinidia	*arguta*	N	Actinidiaceae
Ailanthus	*altissima*	N	Simaroubaceae
Aucuba	*japonica*	Y	Cornaceae
Baccharis	*halimifolia*	Y	Compositae
Broussonetia	*papyrifera*	Y	Moraceae
Carica	*papaya*	N	Caricaceae
Celastrus	*scandens*	N	Celastraceae
Cephalotaxus	*harringtonia*	Y	Cephalotaxaceae
Cercidiphyllum	*japonicum*	Y	Cercidiphyllaceae
Chionanthus	*virginicus, retusus*	N	Oleaceae
Cotinus	*obovatus, coggygria*	Y	Anacardiaceae
Diospyros	*virginiana, kaki*	N	Ebenaceae
Eleutherococcus	*sieboldianus, henryi*	N	Araliaceae
Eucommia	*ulmoides*	Y	Eucommiaceae
Fraxinus	*americana, pennsylvanica*	N	Oleaceae
Garrya	7 spp.	Y	Garryaceae
Ginkgo	*biloba*	Y	Ginkgoaceae
Gymnocladus	*dioicus*	N	Leguminosae
Helwingia	*japonica*	Y	Cornaceae
Hippophaë	*rhamnoides*	Y	Elaeagnaceae
Idesia	*polycarpa*	Y	Flacourtiaceae
Ilex	19 spp. + hybrids	N	Aquifoliaceae
Juniperus	10 spp.	N	Cupressaceae
Leitneria	*floridana*	Y	Leitneriaceae
Lindera	*benzoin, obtusiloba*	Y	Lauraceae
Maclura	*pomifera*	Y	Moraceae
Morus	*alba, rubra*	N	Moraceae
Myrica	*pensylvanica, cerifera, gale*	N	Myricaceae
Nemopanthus	*mucronatus*	N	Aquifoliaceae
Nyssa	*sylvatica*	N	Nyssaceae
Orixa	*japonica*	Y	Rutaceae

Table 1. (*Continued*)

Osmanthus	*heterophyllus*	N	Oleaceae
Phellodendron	*amurense*	Y	Rutaceae
Pistacia	*chinensis*	Y	Anacardiaceae
Podocarpus	*macrophyllus* var. *maki, nagi*	Y	Podocarpaceae
Populus	8 spp.	Y	Salicaceae
Rhamnus	*catharticus*	N	Rhamnaceae
Rhus	*aromatica, typhina*	N	Anacardiaceae
Ribes	*alpinum*	Y	Saxifragaceae
Ruscus	*aculeatus*	N	Liliaceae
Salix	*alba* (+ related spp.)	N	Salicaceae
Sassafras	*albidum*	N	Lauraceae
Schisandra	*chinensis, coccinea, Henryi, propinqua*	Y	Schisandraceae
Securinega	*suffruticosa*	Y	Euphorbiaceae
Shepherdia	*canadensis, argentea*	Y	Elaeagnaceae
Skimmia	*japonica, laureola*	Y	Rutaceae
Smilax	8 spp.	Y	Liliaceae
Taxus	*baccata, cuspidata,* × *media*	N	Taxaceae
Torreya	*californica, nucifera, taxifolia*	N	Taxaceae
Zanthoxylum	*americanum, piperitum, schinifolium, simulans*	Y	Rutaceae

[a]The majority of genera are represented by only one species. For genera with more than one species, the most important or the number of species is listed.

[b]Y = The species listed are always described as dioecious in the literature surveyed.

N = There is some indication that the sexes are not always separate: species descriptions include phrases such as "usually dioecious" or "polygamodioecious," or bisexual cultivars have been selected.

Flow cytometry was originally conceived to facilitate the counting and sizing of particles. One of the most common current applications of flow cytometry is analysis of DNA content in cells (Arumuganathan and Earle, 1991). Nuclei in suspension are stained with DNA-specific fluorescent dyes which emit light when passed through a laser beam. The amount of fluorescence is correlated with the amount of DNA in the nuclei. This technique has been useful for detecting differences in male and female genome size and composition in *Silene latifolia*, a dioecious herbaceous perennial (Costich et al., 1990). There is a 3% difference in total DNA content between males and females of this species, due to the large Y chromosome in males. Using a combination of fluorescent dyes that quantify total DNA and distinguish chemical composition of DNA, we will apply this technique to other dioecious plants. This technique has also shown promise in horticultural studies as a rapid means of determining ploidy level (Costich et al., in press).

Biochemical tests to assay sex-related differences in basic metabolic reactions were developed in the 1920s in the former Soviet Union (Dzhaparidze, 1965).

Unfortunately, Western scientists have not paid much attention to this line of research; however, the simple colorimetric assays that were developed, if their reliability were documented, could prove to be promising for use in the nursery industry.

Electrophoresis, the separation of proteins on the basis of electric charge, is a technique that has been applied with great success to assay genetic variation in plant populations, since allelic forms of enzymes can be identified (e.g. Costich and Meagher, 1992). One class of enzymes in particular, the peroxidases, shows promise for sex identification: there are a few published reports of sex-linked differences in the electrophoretic profiles of these proteins (see references in Meagher, 1988; Zhong, et al. 1982).

A final possibility for sex identification is the use of DNA-based molecular probes. Sex-linked DNA markers based on PCR techniques were recently reported for *Silene latifolia* (Mulcahy, et al., 1992), although the utility of these markers appears to be population-specific. As mentioned above, this species has sex chromosomes; these markers are presumably located on the Y chromosome. The use of molecular genetic techniques for sex identification is clearly in its infancy, and its utility in a horticultural context may be limited by the technical expertise and equipment required.

CONCLUSIONS

The goal of this project is to develop the sex-determination methods described above (particularly flow cytometry) for horticulturally important dioecious species. To achieve this goal we requested plant material from members of I.P.P.S. attending the meeting. Eleven propagators from nurseries and arboretums expressed interest in the project. With their help we will be expanding this survey to adequately sample the diversity of cultivated dioecious taxa.

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THURSDAY AFTERNOON 3 DECEMBER 1992

AWARDS LUNCHEON

The Awards Luncheon was held in Pavilion Rooms A-C of the St. Louis Marriott Pavilion Downtown, St. Louis, Missouri.

1992 RESEARCH GRANT AWARD

The Research Award was granted to **Dr. Brian K. Maynard**, Department of Plant Sciences, University of Rhode Island, Kingston, Rhode Island 02881-0804. The title of his grant proposal is "Dose Response Curves and Carrier Effects on Rooting."

CERTIFICATE OF APPRECIATION

President Still presented a Certificate of Appreciation on behalf of the I.P.P.S.—Eastern Region to **Peter Orum** for his effort in bringing about the establishment of the Denmark Region.

INTERNATIONAL AWARD OF HONOR

John Machen, Sr., Vice-President International Plant Propagators' Society, presented the fifth International Award of Honor to **Ralph Shugert**.

FELLOW RECIPIENTS—EASTERN REGION

The I.P.P.S.—Eastern Region named their third class of Fellows' at their 42nd Annual Meeting. Tom McCloud announced the following new Fellows':

Mr. William (Bill) Flemer III who was Eastern Region President in 1971-72 and received the Eastern Region's Plant Propagators' Award of Merit in 1973.