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SEX EXPRESSION IN SERBIAN DENDROFLORA – A CASE STUDY OF *FRAXINUS ORNUS* VAR. *ANGUSTIFOLIA*

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Abstract - Out of a total number of tree species in Serbian flora, 28% are hermaphrodites, 43% are monoecious and 29% are dioecious. The dendroflora appears to have a larger proportion of unisexual than hermaphrodite species. The monoecious system is the dominant unisexual system within the tree species in Serbia. The possible sex differentiation by RAPD markers and Correspondence Analysis was examined in a population of *Fraxinus ornus* var. *angustifolia*. On the basis of 6 selected RAPD bands which contribute to the differentiation in frequency between male and hermaphrodite individuals, Correspondence Analysis visualized the following tendency: 20 trees of each variety were separated into two groups that mainly correspond to sexual types. Our results showed that RAPD markers assisted by Correspondence Analysis could be used in identifying male and hermaphrodite individuals in *F. ornus* varieties.

Key words: Sexual types, trees, monoecy, dioecy, RAPD

INTRODUCTION

Interest in tree sex expression has a long and venerable history in biology. Despite the controversy that surrounds the ecology and evolution of unisexuality (monoecy and dioecy), there are only a few studies dealing with sex expression in regional floras or the ecological correlates of sex expression. Apart from the need to expand this area of research, the possibility of separating the sexes can be of great practical importance (Hormaza, 1994; Bannerjee et al., 1999). In an attempt to separate sexual types, molecular markers are increasingly used (Reamon-Buttner et al., 1999). Although less informative than codominant markers (e.g. RFLP, AFLP or SSR), RAPD markers can be useful in the first stage, even when it comes to gender division (Khasa and Dancik 1996; Schierenbeck et al., 1997; Gallois et al., 1998; Jordano and Godoy 2000). Preliminary investigations of Bojović et al. (2000) showed that RAPD markers could be used in differentiating sexual types of *Fraxinus ornus* var. *genuina*.

The aims of this work were to: (1) analyze sexual expression in the dendroflora in Serbia, and (2) to evaluate how RAPD markers with statistically assisted protocol can be useful for sexuality diagnosis in *F. ornus* var. *angustifolia* and challenges for future work.

MATERIALS AND METHODS

Sex system information was collected for native tree species in Serbia (according to Jovanović, 2007). Introduced species were considered only if they are spontaneous in the region. All examined species (108 tree species) were classified into one of three sex systems: hermaphroditic (all flowers on a plant bi-

Table 1. The percentage of hermaphroditic, monoecious and dioecious tree species in different biomes (forests).

Sex strategies	Our study (n=108)	Tropical forest (Bawa, 1974) (n=130)	Temperate forest (Curtis, 1959 after Bawa, 1974) (n=27)
Hermaphrodite[a]	28	68	7
Monoecious[b]	43	10	19
Dioecious[c]	29	22	74

Note: [a] Hermaphrodite = all flowers on a plant hermaphroditic (bisexual), [b] Monoecious = male and female flowers found as separate flowers on the same individual, [c] Dioecious = male and female flowers found on separate individuals, n = number of examined tree species.

Table 2. Attributes of the five selected RAPD primers and 6 of 15 selected bands which differ significantly in frequency of fragments between the hermaphrodite (H) and male (M) individuals in *F. ornus* var. *angustifolia*. *,** Significant at 0.05 and 0.01 probability levels, respectively; ns = not significant

primer	nucleotide sequence 5' to 3'	number of polymor-phic bands	band $_{Kb}$ showing differences between the sex	differences between H and M χ_1^2
OPG07	GAACCTGCGG	14	OPG07 $_{0.73}$	0.00 ns
			OPG07 $_{0.88}$	0.22 ns
			OPG07 $_{0.97}$	0.20 ns
			OPG07 $_{1.01}$	0.00 ns
			OPG07 $_{1.13}$	6.67**
OPG11	TGCCCGTCGT	16	OPG11 $_{0.74}$	0.00 ns
OPG20	TCTCCCTCAG	17	OPG20 $_{0.79}$	0.00ns
OPH11	CTTCCGCAGT	24	OPH11 $_{0.64}$	13.33**
			OPH11 $_{1.02}$	5.49*
			OPH11 $_{1.18}$	5.05*
			OPH11 $_{1.22}$	5.00*
			OPH11 $_{1.40}$	1.05 ns
			OPH11 $_{1.45}$	5.49*
			OPH11 $_{1.55}$	1.98 ns
OPH15	AATGGCGCAG	12	OPH15 $_{0.48}$	0.00 ns

sexual), monoecious (male and female flowers found as separate flowers on the same individual) or dioecious (male and female flowers found on separate individuals). A chi-square test was used to explore differences among different data sets and to compare the ratios of sex systems (Table 1). Correspondence Analysis (CA) – one of the descriptive multivariational methods capable of suggesting the structure and tendency of a set of categorical data, was carried out using the STATOSCOPE program (1997, version 1.6, Prof. M Roux, Laboratoire de Biomathématiques, Faculté des Sciences St-Jérôme, Marseille, France). The plant material (*F. ornus* var. *angustifolia*, Serbia, Košutnjak 44° 46'N, 20° 27'E) for DNA analysis consisted of 20 adult trees (10 hermaphrodite and 10 male). DNA extraction, amplification conditions and data analysis (CA) have been described earlier for *F. ornus* var. *genuina* (Bojović et al., 2000)

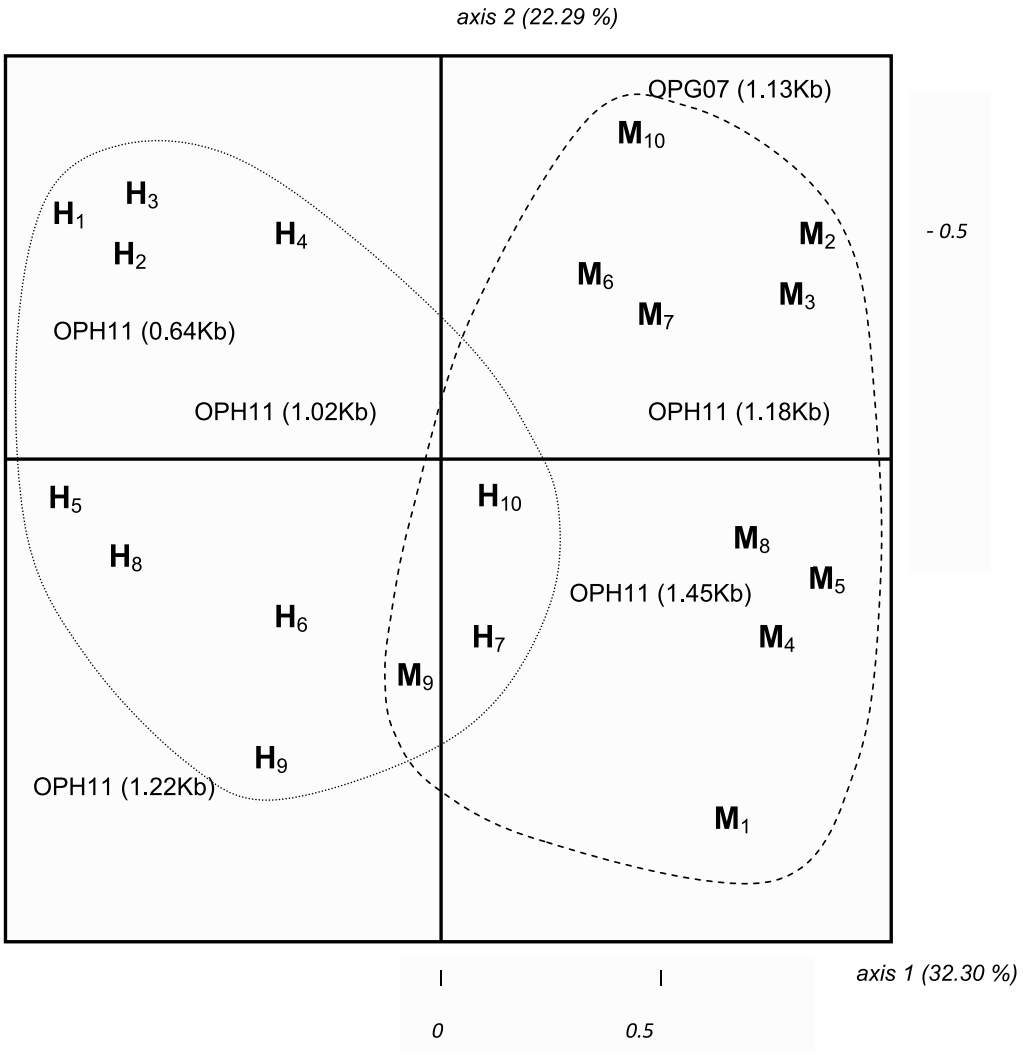

Fig. 1. Correspondence Analysis of the RAPD data. *Fraxinus ornus* var. *angustifolia*. Samples identified by their sex: H_1 to H_{10} = hermaphrodite individuals; M_1 to M_{10} = male individuals.

RESULTS

The type of sexual system was determined for 108 tree species that are present in Serbian forests. Out of a total number of tree species, 28% are hermaphrodites, 43% are monoecious and 29% are dioecious (Table 1). Serbian dendroflora appears to have a larger proportion of unisexual than hermaphrodite species. The proportion of sexual types is different between different biomes (χ_2^2 = from 19.08 to 45.47; P<0.05). Primers that reveal individual variation of *F. ornus* var. *angustifolia* were found (Table 2). Among 41 oligonucleotides, five informative ones were found (OPG-07, OPG-11, OPG-20, OPH-11 and OPH-15). Each of the 6 selected bands contributed to the differentiation only partially, and none completely (Fig. 1) and the sexual types partially overlapped. The first axis (CA) allowed the division of the trees into two separated groups that almost correspond to sexual phenotypes. 17 of 20 individuals (85%) were identified with regard to the sexual phenotypes for *F. ornus* var. *genuina*.

DISCUSSION

Serbian dendroflora appears to have a larger proportion of unisexual (monoecious and dioecious) than hermaphrodite species. The sexual systems of tree species from Serbia were compared with published data from tropical and temperate forests (Table 1). Hermaphroditic systems dominated in tropical flora, dioecy in temperate flora (deciduous forest of northeast USA) and monoecy in Serbian flora. Dioecy prevents intra-individual self-pollination absolutely, while monoecy merely prevents intra-flower self-pollination but not intra-individual self-pollination. Monoecy is very often studied as an evolutionary step towards dioecy (e.g. Freeman et al., 1997; Renner and Won, 2001; Dorken et al., 2002). There is considerable disagreement about the selective forces that have led to the evolution of dioecy in plants (Senarath, 2008). According to Ainsworth (2000) it is clear that there is no common underlying mechanism and that sex determination systems leading to unisexuality have originated independently many times in evolution.

Despite increasing research efforts on a number of different plant species, there is relatively little information available on the molecular basis of sex determination and it is difficult even to estimate the numbers of genes involved.

Identification of sex is complex; there is no universal method or protocol even for related species or subspecies within the species. Although molecular approaches have not yet identified primary sex-determining genes in any dioecious plant species, a range of molecular markers linked to sex have been generated for agronomically important dioecious species. In a minority of dioecious plants, the males are agronomically superior to the females, being higher yielding than the females (*Populus* species, Tschaplinski and Tuskan, 1994; *Fraxinus excelsior*, Jovanović, 2007). In dioecious plants cultivated for fruit or seed it is often difficult to identify females at an early stage of growth (Hormaza et al., 1994). Our results show that RAPD bands which are sex specific in *genuina* (Bojović et al., 2000) are not the same as RAPD bands which are specific for *angustifolia* (and *vice versa*). The presented results also revealed that dominant RAPD markers in combination with Correspondence Analysis could be used in identification and distinguishing between male and hermaphrodite individuals of *F. ornus* var. *angustifolia*, thereby opening up the "research runway" for other more informative markers.

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