

DNA Analysis as a Tool in Sea Buckthorn Breeding

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Sea buckthorn (*Hippophae rhamnoides* L., Elaeagnaceae) is a dioecious windpollinated shrub with nitrogen fixing ability. The berries have bright colors, varying from yellow, orange to red. Domestication of sea buckthorn started in Siberia in the 1930s (Kalinina and Panteleyeva 1987). Local germplasm (ssp. *mongolica*) from the Altai mountains was used in the onset of the breeding. Breeding projects have, later on, been initiated also in other countries such as Germany (Albrecht 1990), Finland (Yao and Tigerstedt 1994), China (Huang 1995), and Canada (Li and Schroeder 1996). At SLU-Balsgård, Sweden, breeding of sea buckthorn started in 1986. Conventional breeding methods, including germplasm evaluation, hybridization and selection, are used (Trajkovski and Jeppsson 1999). The Swedish food industry is very interested in the berries as a raw material for various food products as marmalade, beverages and as a flavor. Sea buckthorn is not yet grown at a commercial scale in Sweden. The main objective is to develop cultivars suitable for large scale production of berries in Sweden. We work mainly with adaptation, disease resistance, growth habit that will permit machine harvesting, and improvement of fruit quality and yield. The plant material used in our breeding program has been derived from domesticated Russian forms (ssp. *mongolica*) and native Scandinavian forms (ssp. *rhamnoides*). Initially, 28 genotypes were selected. Nine superior selections are now being tested in full scale orchards both in northern and southern Sweden. Recently we started to use the PCR-based method RAPD (Random Amplified Polymorphic DNA) to study population structure in the ssp. *rhamnoides* and to find a marker linked to gender determination.

RAPD METHODOLOGY

We use a standard method with the following steps (Weising et al. 1995):

1. DNA isolation and purification, using fresh or frozen leaves.
2. Enzymatic amplification of certain segments of DNA by use of thermostable DNA polymerase, deoxynucleotides and oligonucleotide primers. The DNA fragments are multiplied during 40 cycles of temperature shuttling between +94°C, +36°C and +72°C.
3. Electrophoretic separation of the fragments based on size followed by staining with ethidium bromide. Visual detection of bands under UV light.
4. The presence of a band is scored as 1 and the absence as 0. The resulting matrix is evaluated by statistical procedures, and parameters as e.g. genetic relatedness and heterozygosity are calculated.

GENETIC VARIABILITY

During the initial evaluation of germplasm, ssp. *mongolica* was shown to be susceptible to various diseases in Sweden. In comparison, native Scandinavian germplasm of ssp. *rhamnoides* seemed less susceptible. Progenies between these two taxa are now under evaluation at Balsgård.

The need for native germplasm from ssp. *rhamnoides* prompted the research on how the genetic variation is partitioned within and among native populations in northern Europe. RAPD variation was recently studied in ten populations (Fig. 1) from native stands (Bartish et al. 1999). Samples were collected from almost the whole distribution range, with the most southwestern sample from the Netherlands and the most northeastern sample from the arctic circle in Sweden. A population of seedlings derived from ssp. *mongolica* (population C) was used as an outgroup. Based on data from the RAPD analysis, a dendrogram showing genetic relatedness was calculated by use of cluster analysis (UPGMA). The population of ssp. *mongolica* was found to be the most remote population (Fig. 2). The ssp. *rhamnoides* populations grouped into two main clusters, one with populations Ea, Eb, Fb, Hb, Ib, and another with populations Fa, Ga, Gb, Ha. The population Ia clustered more distantly. The data was also analyzed with Principal Co-Ordinate analysis (PCO), and a plot of the first two principal co-ordinate axis was created which showed similar results, with the ssp. *mongolica* population separated from the ssp. *rhamnoides*. Using Analysis of Molecular Variance (AMOVA) to measure the partitioning of molecular variance within the ssp. *rhamnoides*, 85% of the variation was found within

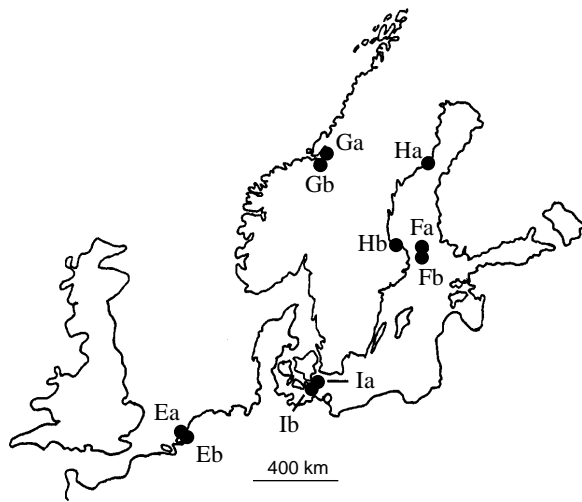


Fig. 1. Sampling sites for *Hippophae rhamnoides* ssp. *rhamnoides*.

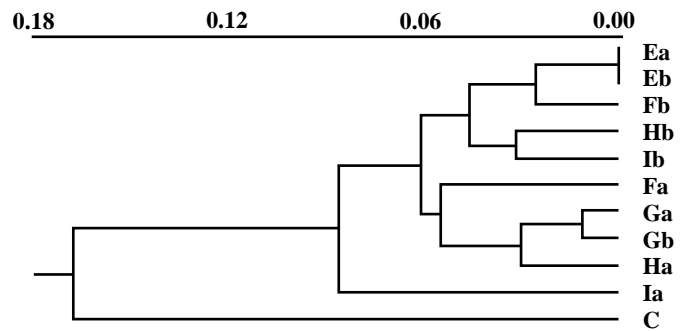


Fig. 2. UPGMA dendrogram of the genetic relationship among populations of *Hippophae rhamnoides*. (For population origins, see Fig. 1.)

populations and 15% among populations. That means that 85% of the RAPD variation can be captured by sampling within one or a few of the populations. If more than 85% of the variation is requested, sampling must be performed in more populations. However, to what extent RAPD variation is a good predictor for variation in important morphological and physiological characters is as yet unknown.

Since sea buckthorn is dioecious, wind pollinated, and obligately outcrossing, one would expect to find much variation within populations and very little variation between populations. In trembling aspen (*Populus tremuloides* Michx.), another dioecious species, 97% of the RAPD variability (Table 1) was found within the populations (Yeh et al. 1995). In Norway spruce (*Picea abies*), an outcrossing species, 95% of the isoenzyme variability was found within populations (Lagercrantz and Ryman 1990). Both trembling aspen and Norway spruce are widespread and continuously distributed. In Brazilwood (*Caesalpinia echinata* Lam.), another outbreeding species, RAPD variability within populations accounted for only 42% (Cardosos et al. 1998). This was suggested to be the result of discontinuous distribution that have lead to the isolation of small populations. Inbreeding and/or genetic drift has then increased the differentiation between populations.

Sea buckthorn appears to have differentiated more than trembling aspen and Norway spruce. The history of sea buckthorn in northern Europe actually supports this theory. Sea buckthorn of the ssp. *rhamnoides* has been extensively studied in Scandinavia. Fossil records of pollen have shown that it colonized the land shortly after the ice retreat in the late glacial period and showed a wide distribution. Later on, the distribution declined and became fragmented, resulting in the present discontinuous distribution (Sandegren 1943). During this time period differentiation due to isolation and restricted population size may have occurred.

A previous study based on isozymes showed differences in partitioning of genetic variation when ssp. *sinensis* and ssp. *rhamnoides* were compared, with the latter showing less similarity between populations (Yao and Tigerstedt 1993). Here too, the differences may be explained by differences in population sizes and/or the continuity in spatial distribution.

Table 1. Comparison of the within population variability component in different outcrossing species.

Species	Variability within populations (%)	Distribution	Population size
Trembling aspen <i>Populus tremuloides</i>	97	continuous	large
Norway spruce <i>Picea abies</i>	95	continuous	large
Sea buckthorn <i>Hippophae rhamnoides</i> ssp. <i>rhamnoides</i>	85	discontinuous	??
Brazilwood <i>Caesalpinia echinata</i>	42	discontinuous	small

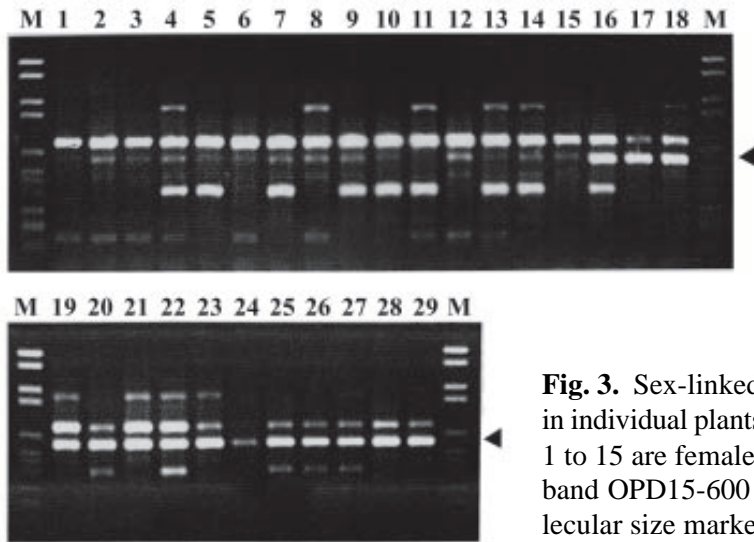


Fig. 3. Sex-linked RAPD marker amplified with primer OPD15 in individual plants from the cross 'Leikora' × 'Pollmix 1'. Lanes 1 to 15 are females, lanes 16 to 29 are males. The male specific band OPD15-600 is indicated with an arrow. M stands for molecular size marker.

GENDER DETERMINATION

Since sea buckthorn is dioecious, plant breeding projects aim at producing both female and male cultivars. However, breeding objectives for female and male cultivars differ and, generally, there are more quality criteria to be met in a female cultivar. Therefore, the selection pressure is higher on female cultivars and as a consequence, larger seedling population size is needed to obtain a certain number of female selections than to obtain the same number of male selections. Much of work and money could thus be saved if a large proportion of the males could be discarded already at an early stage in the evaluation process.

Gender is most often genetically determined in dioecious plants, either by distinguishable sex chromosomes or by alleles at one or several loci on non-distinguishable chromosomes (Irish and Nelson 1989; Durand and Durand 1990). The presence of distinguishable sex chromosomes in sea buckthorn was suggested by Shchapov (1979), although this has not been substantiated in other studies. Previously gender determination based on RAPD markers has been successful in *Silene latifolia* (Mulcahy et al. 1992), *Pistacia vera* (Hormaza et al. 1994), *Asparagus officinalis* (Jiang and Sink 1997), and *Atriplex garettii* (Ruas et al. 1998). Recently, we studied the usefulness of RAPD markers for gender determination in sea buckthorn (Persson and Nybom 1998). Two F_1 progenies were investigated (34 plants derived from the cross 'Leikora' and 'Pollmix 1' and 22 plants derived from the cross BHi 10224 and 2-24). When flowering, the gender of the plants were determined. The analyses were performed as a bulked segregant analysis (BSA). DNA was extracted from each individual and two bulks were produced in each cross, one from the males and the other from the females. Out of 78 primers tested, four seemed to yield partitioning between male and female bulks and these four primers were chosen for further amplification at individual plant level. The band OPD15-600 (Fig. 3) was present in all males in the offspring of 'Leikora' × 'Pollmix 1' as well as in the father, 'Pollmix 1' and absent in all female offspring of the same cross as well as in the mother, 'Leikora'. However, this band was present in only one of the males and in none of the females in the other cross. Unfortunately, this marker was not therefore universal and could be used for gender determination in only one of our progenies.

CONCLUSIONS

We used RAPD markers to determine genetic variation between and within native populations and to measure relatedness between populations. Such investigations can be used as guidelines for collection of germplasm in native stands but also to develop strategies for a breeding program. One marker was found to be present only in male genotypes of a progeny and in the father while it was absent in all females and the mother. This marker was useful only in one hybrid progeny out of two tested, and search for a more general marker will be undertaken, now that we have at least obtained evidence that gender is genetically determined in sea buckthorn. In a dioecious crop as sea buckthorn, molecular markers for fruit quality traits could also be

a powerful tool when selecting the male parent to be used in crosses. RAPD band patterns can serve as fingerprints for genotype identification in vegetatively propagated crops, and can therefore be useful also for gene bank management.

REFERENCES

- Albrecht, H.-J. 1990. Sortenentwicklung bei Sanddorn. *Gartenbauwissenschaft* 37:207–208.
- Bartish, I.V., N. Jeppsson, and H. Nybom. 1999. Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by RAPD markers. *Molecular Ecol.* 8:791–802.
- Cardosos, M.A., J. Provan, W. Powell, C.G. Ferreira, and D.E. De Oliveira. 1998. High genetic differentiation among remnant population of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinioideae). *Molecular Ecol.* 7:601–608.
- Durand, R. and B. Durand. 1990. Sexual determination and sexual differentiation. *Crit. Rev. Plant Sci.* 9:295–316.
- Hormaza, J.I., L. Dollo, and V.S. Polito. 1994. Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. *Theor. Appl. Genet.* 89:9–13.
- Huang, Q. 1995. A review of seabuckthorn breeding in China. *Proc. Int. Workshop on Seabuckthorn, Beijing, China.* p.111–117.
- Irish, E.E. and T. Nelson. 1989. Sex determination in monoecious and dioecious plants. *Plant Cell* 1:737–744.
- Jiang, C. and K.C. Sink. 1997. RAPD and SCAR markers linked to the sex expression locus *M* in asparagus. *Euphytica* 94: 329–333.
- Lagercrantz, U. and N. Ryman. 1990. Genetic structure of Norway spruce (*Picea abies*): Concordance of morphological and allozyme variation. *Evolution* 44:38–53.
- Li, T.S.C. and W.R. Schroeder. 1996. Sea buckthorn (*Hippophae rhamnoides* L.): A multipurpose plant. *HortTechnology* 6:370–380.
- Kalinina, I.P. and Y.I. Panteleyeva. 1987. Breeding of sea buckthorn in the Altai. In: *Advances in Agricultural Science.* Moscow, Russia.
- Mulcahy, D.L., N.F. Weeden, R. Kesseli, and S.B. Carroll. 1992. DNA probes for the Y-chromosome of *Silene latifolia*, a dioecious angiosperm. *Sexual Plant Reprod.* 5:86–88.
- Persson, H.A. and H. Nybom. 1998. Genetic sex determination and RAPD marker segregation in the dioecious species sea buckthorn (*Hippophae rhamnoides* L.). *Hereditas* 129:45–51.
- Ruas, C.F., D.J. Fairbanks, R.P. Evans, H.C. Stutz, W.R. Andersen, and P.M. Ruas. 1998. Male-specific DNA in the dioecious species *Atriplex garrettii* (Chenopodiaceae). *Am. J. Bot.* 85:162–167.
- Sandegren, R. 1943. *Hippophae rhamnoides* L. i Sverige under senkvartär tid. *Svensk Botanisk Tidskrift.* 37:1–26. (in Swedish with German summary).
- Shchapov, N.S. 1979. On the caryology of *Hippophae rhamnoides* L. *Tsitologiya i Genetika.* 13:45–47 (in Russian with English summary).
- Trajkovski, V. and N. Jeppsson. 1999. Domestication of sea buckthorn. *Botanica Lithuanica* Suppl. 2:37–46.
- Weising, K., H. Nybom, K. Wolff, and W. Meyer. 1995. *DNA fingerprinting in plants and fungi.* CRC Press, Boca Raton, FL.
- Yao, Y. and P.M.A. Tigerstedt. 1993. Isozyme studies of genetic diversity and evolution in *Hippophae*. *Genetic Resources Crop Evol.* 40:153–164.
- Yao, Y. and P. Tigerstedt. 1994. Genetic diversity in *Hippophae* L. and its use in plant breeding. *Euphytica* 77:165–169.
- Yeh, F.C., D.K.X. Chong, and R.C. Yang. 1995. RAPD variation within and among natural populations of trembling aspen (*Populus tremuloides* Michx.) from Alberta. *J. Hered.* 86:454–460.