Annex I. – Description of genetic entries for the second breeding cycle of *Larix* x *marschlinsii* in Québec, Can.

European larch (EL) Source[1]	No. of HSF[2]	No. of ST[3]	Japanese larch (JL) Source	No. of HSF	No. of ST
EL natural stand			***JL natural stand***		
Blizyn, Poland	1	4	Gunma prefecture, Japan	--	2
Kowary-Sniezka, Poland	1	4	Hokkaido, Japan	--	4
Secondary source			Honshu, Japan	--	4
Québec natural stand			Honshu Naganoken, Japan	--	2
Berthierville, Canada	2	8	Honshu Yatsugata, Japan	--	2
Drummondville, Canada	2	6	1500 m, Nagano Pref., Japan	--	4
Seed orchard			1650 m, Nagano Pref., Japan	--	4
Clone 205, Germany	1	3	1700 m, Nagano Pref., Japan	--	4
Two provenance tests (Exp. 202-G-1 and 209-B-1)			Kuriyama, Japan	1	1
Blizyn, Poland	3	4	***Secondary source***		
Farum, Denmark	7	9	***Québec, natural stand***		
Grojec, Poland	4	6	Chatham, Canada	1	2
Krakow, Poland	5	10	***Seed orchard***		
Kroscienko, Poland	3	3	Ganø, Lind., Denmark	--	5
Rundforbi, Denmark	2	2	Kongenhus Flen., Denmark	--	6
Schlitz, Germany	3	3	Morayshire, New., Scotland	3	5
Sckarzysko, Poland	2	6	***MRNF-Q seed orchard***		
Wroclaw, Poland	5	9	Harrington, Canada	--	2
MRNF-Q[4] clonal bank			Lotbinière, Canada	1	2
Clone 158, Wisconsin, USA	1	3	***Plantation***		
			Ganø, Lind., Denmark	--	5
			Ross-shire, Scotland	--	6
			Tokachi Hokk., Japan	6	20
EL total	42	80	**JL total**	12 and 13 prov.	80

[1] Origin of the mother trees.
[2] HSF = half-sib family.
[3] ST = selected tree. All ST are of MRNF-Québec provenance and progeny tests and seed orchards (4 JL elite-trees; based on 5 years results from two progeny tests).
[4] MRNF-Q = ministère des Ressources naturelles et de la Faune du Québec.

Low Chloroplast DNA Diversity in Red Dogwood (*Cornus sanguinea* L.)

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(Received 20th February 2008)

Abstract

The red dogwood *Cornus sanguinea* L. is a deciduous shrub of the temperate and Mediterranean zones. It is often used in landscape gardening for miscellaneous purposes.

Chloroplast DNA markers, the so-called cpDNA haplotypes, are a very potential marker type to characterise the large scale variation pattern within the natural range of a species.

In this study, a total of 86 populations and 673 individuals were sampled all over Europe. Eight different haplotypes were recognised by combinations of several PCR-RFLP patterns. They are divided into 3 groups of related types. There is no association between these 3 groups and their geographic occurrence within the tested material.

One haplotype strongly dominates in the whole distribution area. It takes nearly 90 percent whereas the remaining seven haplotypes together reach to approximately 10 percent. Besides the low number of haplotypes, the total genetic variation H_T = 0.15 is much lower in *Cornus sanguinea* compared to other European tree and shrub species. Despite the low level of variation, several cases of introduced populations could be detected. Other haplotypes than the common type are found only in narrow areas. This result indicates that after the

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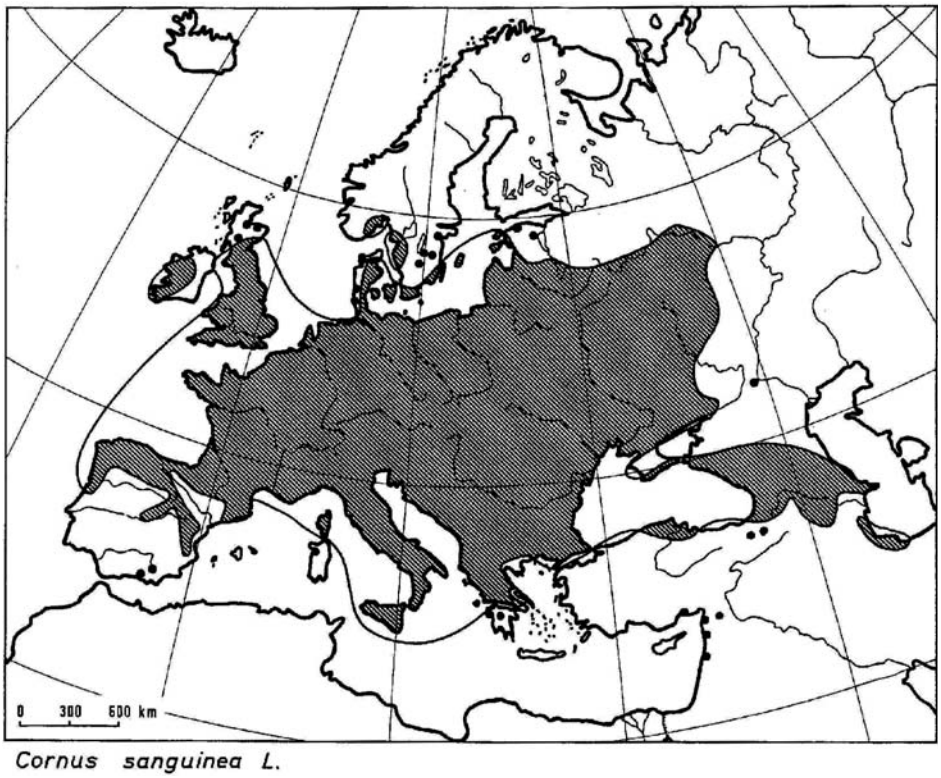

Figure 1. – Natural distribution area of *Cornus sanguinea*, adapted from MEUSEL et al. (1978), HEGI (1975), ERHARDT at al. (2000) and ROLOFF and BÄRTELS (2006).

colonisation of the European continent only a very restricted gene flow could have taken place.

Key words: Cornus sanguinea L., red dogwood, genetic variation, chloroplast DNA, PCR-RFLP, haplotypes, gene flow, distribution area, introduced populations.

Introduction

The red dogwood (syn. bloodtwig dogwood), *Cornus sanguinea* L., is a deciduous shrub of the temperate and Mediterranean zones belonging to the Cornaceae family. Its wide distribution range covers nearly the complete European continent and the Caucasian region including the northern part of Iran. It is absent in Scandinavia with exception of the southernmost regions, in the north-eastern part of the British Islands, and the southern parts of the Iberian Peninsula and of Greece (*Figure 1*).

The red dogwood is a monoecious plant with a mostly outcrossing breeding system. Additionally it can propagate vegetatively by adventitious rooting. A partially clonal structure within a *Cornus sanguinea* population was detected by isozyme analysis (LEINEMANN et al., 2002). The species can adapt their reproductive behaviour to habitat conditions by changing the relative importance of reproduction by seed and vegetative clonal growth (KRÜSI and DEBUSSCHE, 1988). Its ecological significance is associated with the pollination of flowers by insects like beetles, flies, hover flies, wasps and bees and with the black-violet seeds that were eaten and dispersed by birds.

Red dogwood is often used in landscape gardening for miscellaneous purposes. It is of particular importance for soil rooting at slopes and for the cultivation of embankments.

In general, there is an increasing interest in cultivation of shrubs originating from local adapted populations. Reproductive material from indigenous sources is recommended because of its better adaptability to local environmental conditions in comparison to material of the same species from far distant geographic regions and other climatic conditions.

Genetic markers with a geographic variation pattern in a respective species would be suitable to identify the geographic region of origin of plant material. Chloroplasts were maternally inherited in Angiosperms. The chloroplast DNA markers, the so-called cpDNA haplotypes, are a very potential marker type to characterize the large scale variation pattern within the natural range of a species, because these markers reflect the gene flow by seeds only and not by pollen.

Chloroplast DNA haplotypes were firstly applied for oak species in Europe in an extensive phylogeographic study (DUMOLIN-LAPEGUE et al., 1997a; PETIT et al., 2002). Three large and additional three small groups of genetically related cpDNA haplotypes were identified. The three large lineages correspond to the three large refugia during ice age, the Iberian, Italian, and Balkan peninsulas and reflect the postglacial recolonisation routes throughout Europe.

Consensus primers derived from the tobacco chloroplast DNA sequence are suitable to amplify noncoding regions of chloroplast DNA. These noncoding regions have the potential to detect variation within species associated with geographic areas. The method was successfully adapted to several other European forest tree species, i.e. *Fagus sylvatica* (DEMESURE et al., 1996), *Betula pendula* (PALMÉ et al., 2004), *Tilia cordata* (FINESCHI et al., 2003), *Fraxinus excelsior* (HEUERTZ et al., 2004) and *Populus nigra* (COTTRELL et al., 2005). Within the "Cytofor" project (PETIT et al., 2003) funded by the Commission of the European Communities, several shrub species were investigated, i.e. *Corylus avellana* (PALME and VENDRAMIN, 2002), *Hedera* sp. (GRIVET and PETIT, 2002), *Prunus spinosa* (MOHANTY et al.,

2002) and *Ilex aquifolium* (RENDELL and ENNOS, 2003). Despite of some statistical significant phylogeographic structure detected for several species in 25 populations (PETIT et al., 2003) an attribution of samples to geographic regions needs much more populations for an inventory in the distribution area.

Materials and Methods

Plant material

Representative material was collected to cover large parts of the natural range of red dogwood in Europe with emphasis on Germany and south-eastern Europe (*Tab. 1*). A differentiation between the subspecies *Cornus sanguinea* ssp. *sanguinea* and occurring *C. s.* ssp. *australis* has not been made, because of less morphological differences and no observed crossing barrier (ROLOFF and BÄRTELS, 2006; MEYER et al., 2007). Natural stands with typical plant communities were selected as far as possible to harvest the material. Red dogwood understory in riverside forests seemed to be most convenient, even when cultivated forests were found at the riverside the dogwood understory was assumed not to be planted. Locations nearby settlements were avoided to exclude sampling of plants originating from cultured forms. Within Germany, the material was mainly collected from Federal states gene preservation populations.

The aim was to save leaves from 10 individuals at each site with a minimum distance of approximately 10 to 15 meters between the specimens to avoid sampling of vegetative ramets.

Table 1. – Geographic location of *Cornus sanguinea* populations sampled.

Pop ID	Country	Location	Longitude E	Latitude N	Number of samples
1	Albania	Tirana	19° 48'	41° 18'	3
2	Austria	Ponigl/Graz	15° 27'	46° 55'	10
3	Austria	Untere Lobau/Wien	16° 42'	48° 09'	10
4	Austria	Nussbach	14° 06'	48° 00'	9
5	Austria	Schlierbach/Au 1	14° 06'	47° 54'	4
6	Austria	Schlierbach/Au 2	14° 06'	47° 54'	10
7	Austria	Raxalpe/Reichenau	15° 48'	47° 42'	4
8	Austria	Linz	14° 17'	48° 18'	3
9	Bulgaria	Sofia	23° 18'	42° 42'	11
10	Bulgaria	Stara Planina	25° 19'	42° 47'	8
11	Czech Republic	Lovos/Lovosice	14° 06'	50° 30'	10
12	Czech Republic	So-Hong Oblik/Louny	13° 48'	50° 24'	10
13	Czech Republic	Velky Vrch/Louny	13° 48'	50° 24'	8
14	Denmark	Arhus	10° 13'	56° 09'	4
15	France	Bordeaux	-0° 42'	44° 48'	11
16	France	La Brede/Bordeaux	-0° 36'	44° 42'	10
17	France	La Teste	-1° 12'	44° 36'	2
18	France	Lussac Les Chateau/Vienne	0° 48'	46° 24'	2
19	France	Avignon	5° 05'	43° 56'	6
20	Georgian Republic	Bantsurtkari/Dusheti Region	44° 36'	42° 06'	4
21	Georgian Republic	Lagodekhi Region	46° 18'	41° 48'	5
22	Georgian Republic	Zestaponi Region	43° 00'	42° 06'	5
23	Germany	Seidewitztal/Liebstadt	13° 52'	50° 54'	10
24	Germany	Tharandter Burgberg	13° 35'	50° 57'	9
25	Germany	Bad Driburg	9° 03'	51° 45'	9
26	Germany	Hagen/Rügen	13° 34'	54° 34'	10
27	Germany	Schönberg	11° 08'	53° 52'	7
28	Germany	Strelitz/Düsterförde	13° 07'	53° 15'	10
29	Germany	Kleve	6° 09'	51° 48'	9
30	Germany	Kinding/Altmühltal	11° 23'	49° 00'	9
31	Germany	Heyda	10° 54'	50° 45'	5
32	Germany	Mühlberg	10° 48'	50° 51'	5
33	Germany	Mettmann	6° 57'	51° 15'	9
34	Germany	Dankerode	11° 09'	51° 36'	3
35	Germany	Bad Münstereifel	6° 45'	50° 36'	10
36	Germany	Jessen/Meißner Elbtal	13° 30'	51° 12'	10
37	Germany	Bad Kösen	11° 42'	51° 12'	9
38	Germany	Vockerode	12° 21'	51° 48'	10
39	Germany	Pegnitz/Laufamholz	11° 12'	49° 30'	9
40	Germany	Freising/Isar	11° 47'	48° 24'	10
41	Germany	Elmstein	7° 54'	49° 21'	10
42	Germany	Brieskow Finkenheerd	14° 36'	52° 18'	8
43	Germany	Frankfurt (Oder)	14° 33'	52° 18'	10
44	Germany	Hann. Münden	9° 42'	51° 24'	9
45	Germany	Freiburg	7° 48'	48° 00'	5

Table 1. – Continued.

46	Germany	Bremen/Aumund	8° 49'	53° 05'	4
47	Germany	Werl, Kreis Soest	7° 55'	51° 33'	3
48	Germany	Menden, Oesbern	7° 48'	51° 26'	9
49	Germany	Beckum, Hellbach	8° 02'	51° 45'	5
50	Germany	Essen	7° 00'	51° 24'	10
51	Greece	Filakion	26° 17'	41° 42'	5
52	Hungary	Kapuwar/Vitnied	17° 00'	47° 35'	9
53	Hungary	Gerecse Mountains	18° 26'	47° 39'	10
54	Hungary	Tokaj/Tisza	21° 23'	48° 09'	9
55	Hungary	Pecs	18° 13'	46° 05'	2
56	Iran	Teheran/Elbrus	51° 18'	35° 36'	9
57	Italy	Meran	11° 06'	46° 42'	23
58	Italy	Tuscania	11° 48'	42° 24'	9
59	Italy	Cilento National Park	15° 18'	40° 03'	9
60	Italy	Vesuv	14° 24'	40° 48'	9
61	Italy	Lustra	15° 00'	41° 12'	3
62	Italy	Milano	8° 48'	45° 30'	9
63	Kosovo	Grebno/Pristina	21° 11'	42° 20'	11
64	Macedonia	Ohrid	20° 55'	41° 12'	10
65	Moldova	Schepte-Ban	27° 19'	47° 52'	9
66	Moldova	Prisacani	27° 57'	47° 04'	10
67	Poland	Krapkowice	17° 55'	50° 29'	10
68	Poland	Rogozno Zamek	18° 57'	53° 31'	8
69	Romania	Pesteana	22° 48'	45° 32'	10
70	Romania	Bukarest/Pietrele	26° 06'	44° 06'	6
71	Romania	Fägäras	24° 35'	45° 41'	5
72	Romania	Bahlui	26° 54'	47° 05'	9
73	Romania	Galgau	23° 44'	47° 15'	11
74	Serbia	Novi Sad	19° 22'	45° 16'	10
75	Serbia	Belgrad	20° 27'	44° 09'	8
76	Slovakia	Hrabusice/Slovakian Paradise	20° 24'	48° 57'	10
77	Slovakia	Donovaly/Lower Tatra	19° 12'	49° 03'	6
78	Slovakia	Poprad	20° 18'	49° 12'	6
79	Slovakia	Bratislava/Ostrove	17° 29'	47° 56'	10
80	Slovakia	Kosice	21° 09'	48° 41'	4
81	Slovenia	Ljubljana/Vir	14° 37'	46° 08'	9
82	Slovenia	Maribor/Starse	15° 43'	46° 30'	10
83	Spain	Sales telos Inantes	-4° 00'	39° 48'	2
84	Spain	Perales de Alfambra	-1° 00'	40° 42'	5
85	Switzerland	Uetliberg Adliswil/Zürich	8° 30'	47° 18'	5
86	Switzerland	Lägeren Spreitenbach	8° 21'	47° 24'	8

The intended number of 10 individuals could not be realised in every case. A total of 86 populations and 673 individuals were sampled (average 7.8 individuals per population, range 2…23). Fresh leave samples were frozen at –80 °C or dried at 40 °C until DNA extraction.

DNA extraction and PCR-RFLP analysis

The isolation of total DNA from leaves followed a modified CTAB protocol (DUMOLIN et al., 1995). The amplification of non-coding regions of chloroplast DNA based on a selection of eight consensus primer pairs (*Table 2*). These primer pairs were successfully applied on 20 other woody species (GRIVET et al., 2001).

Amplification reactions contained 1 x PCR buffer with 2.5 mM $MgCl_2$ (AppliChem Darmstadt), 250 µM dNTP-mix, 0.5 µM of each primer, 1 U Taq Polymerase (AppliChem Darmstadt) and 20–100 ng template DNA in a volume of 25 µl. Polymerase chain reactions (PCR) were carried out in a Biometra thermal cycler using the following conditions: 1 cycle with 95 °C/5 min, 30 cycles with 95 °C/45 sec denaturation, annealing temperature/45 sec, and 72 °C/elongation time (*Table 2*), and 7 min final elongation at 72 °C. The presence of a PCR product of expected length was checked with agarose gel electrophoresis. Amplification products were digested with AfaI, HhaI, HindIII, HinfI, MboI, MspI, SspI, TaqI and XbaI in according to manufacture's instructions to find out variation in restriction sites and/or fragment length. RFLP fragments were separated with agarose gel electrophoresis and stained with ethidium bromide. Gels were photographed with the Kodak EDAS 290 system.

A preliminary screening of samples representing all geographic regions was carried out to find combinations of primers and restriction enzymes revealing polymorphisms. This subset of 30 samples from 30 locations in 14 countries resulted in 6 haplotypes. The informative combinations were then used to screen all Cornus sanguinea populations. Two additional haplotypes appeared later by further combinations of known polymorphic sites.

Table 2. – Primer pairs tested for red dogwood.

Primer pair abbreviation	Chloroplast DNA region	Reference	Annealing temperature (°C)	Elongation time (min)	Length of PCR product in tobacco (bp)
AS	PsbA – trnS	DEMESURE et al. (1995)	58	4	3681
B2B3	PsbB - petB	GRIVET et al. (2001)	53	3	2781
CD	trnC - trnD	DEMESURE et al. (1995)	58	4	3167
DT	trnD - trnT	DEMESURE et al. (1995)	55	2	1213
HK	trnH - trnK	DEMESURE et al. (1995)	62	2	1831
K1K2	trnK - trnK	DEMESURE et al. (1995)	53	3	2585
TF	trnT - trnF	TABERLET et al. (1991)	58	2	1754
VL	trnV - rbcL	DUMOLIN-LAPEGUE et al. (1997b)	57.5	4	3850

Data evaluation

All combinations of primers and restriction enzymes with variation in RFLP patterns were scored for their polymorphic sites, which are the presence or absence of a restriction site or length variations of DNA fragments. Haplotypes were defined as certain combinations of polymorphic sites.

Two methods were applied to visualise the genetic relationships between haplotypes. Firstly, a dendrogram was constructed from genetic distances (SAS Institute Inc., 2003). Secondly, a minimum spanning tree was created with help of the software Arlequin (SCHNEIDER et al., 2000).

The parameters H_T (total diversity) and H_S (mean value of levels of diversity within populations) characterise the genetic variation of haplotypes within the range between 0 and 1. Parameters for genetic differentiation among populations of haploid data were described by PONS and PETIT (1995). G_{ST} is a parameter between 0 in case of total identity of all populations and 1 in case of lack of consistencies and measures the differentiation between populations from haplotype frequency data. NST additionally includes the genetic distances between haplotypes available from the coded polymorphic sites. The software PermutCpSSR (Version 2.0, http://www.pierroton.inra.fr/genetics/labo/Software/) calculates G_{ST} and N_{ST} for populations with at least 3 individuals. Additionally it tests whether G_{ST} and N_{ST} significantly differ to detect a phylogeographic structure by a permutation procedure (PONS and PETIT, 1996; BURBAN et al., 1999). These parameters are common used in numerous plant organelle studies and allowed a comparison of variation levels between species.

Results

Identification of cpDNA haplotypes

Seven of eight tested primer pairs resulted in amplification products of the expected length. No or faint bands were observed for the primer pair TF. PCR products of the two primer pairs DT and HK exhibit length variations between genetic variants. Eight different haplotypes were recognized by combinations of PCR-RFLP patterns. Fragments were described as F1, F2, … with descending length. Variations in fragment length were coded by 1 for the fragment with the maximum length followed by 2 for the next shorter fragment and so on. Examples are given in *Figure 2*. The presence resp.

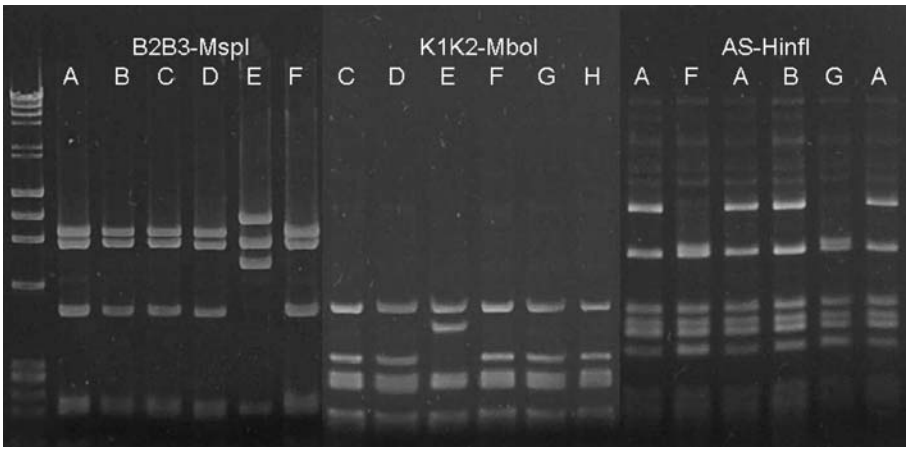

Figure 2. – Examples of PCR-RFLP patterns.

absence of a restriction site was coded with 1 and 0. *Table 3* displays 17 PCR-RFLP patterns with together 21 non-redundant polymorphic sites.

The genetic relationships among the eight haplotypes are displayed in *Figure 3*. Their genealogy inferred from 21 polymorphic sites is very similar with both methods, the minimum spanning tree (*Figure 3a*) and the UPGMA cluster dendrogram (*Figure 3b*). The common European haplotype A is closely related to haplotypes B and C. The only differences exist in the total length of the trnH-trnK fragment and one restriction site in the trnC-trnD region. Another group of closely related haplotypes is formed from type F, G and H. These three haplotypes distinguish in 3 to 4 polymorphic sites in several regions. Haplotype D from the Caucasian region is related to both mentioned groups with at least two different sites and could be their ancestor. Haplotype E from the Balkan Peninsula is derived from haplotype D by a high number of mutation steps and is genetically far distant from all other haplotypes.

Haplotype variation in Europe

A total of 673 samples from 86 populations belonging to 22 countries were analysed for their cpDNA haplotype. Haplotype A strongly dominates in the whole distribution area. It takes nearly 90 percent whereas the remaining seven haplotypes together come to approx. 10 percent (*Table 4*). The total genetic variation H_T is low (H_T = 0.190). The mean within population diversity H_S is low as well (H_S = 0.051). Based on this low level of genetic variation, the portion of differentiation among populations amounts to G_{ST} = 0.732. The parameter N_{ST}, which takes into account the genetic relatedness of single haplotypes, is slightly lower (N_{ST} = 0.730), but not significantly different from G_{ST}.

Five of seven rare haplotypes exist only in one geographic place (*Figure 4*): haplotype D (dark blue) in a Caucasian population, haplotype B (red) in one Slovakian population, haplotype H (black) in one place in Austria, haplotype C (pink) in two neighbouring locations in the Czech Republic, and haplotype G (brown) in South West France. In most populations these private haplotypes appear together with the common haplotype A (yellow).

The two remaining rare haplotypes initially differ from this pattern. Haplotype E (light blue) occurs in two neighbouring locations on the Balkan Peninsula, in two populations in central Germany and in one population in Italy. Haplotype F (green) was detected in two neighbouring Slovakian populations and two locations in the northern part of Germany. The geographic distribution of these two haplotypes induced the assumption that the mentioned German populations are completely or partially introduced from southern regions because there is no indication for stepping stones of a natural migration. In case of haplotype F (green) this suspicion was validated by enquiries of the local responsibility for gene preservation.

Table 3. – Coding *Cornus sanguinea* cpDNA haplotypes by 21 polymorphic sites.

Primer pair	AS			B2B3			CD						DT		HK			K1K2		VL	
Restriction enzyme	HinfI	MspI	SspI	HinfI		MspI	HhaI	HinfI	MspI		SspI		HinfI	MspI	PCR Product	HindIII	MboI	AfaI	MboI	HhaI	
Fragment	F1	F1	F2	F3	F5	F1	F3	F6	F1	F3	F1	F2	F1	F1		F2	F2	F3	F2	F1	F2
A	1	1	1	1	1	2	1	2	1	1	2	1	2	2	2	1	1	1	2	1	2
B	1	1	1	1	1	2	1	2	1	1	2	1	2	2	3	1	1	1	2	1	2
C	1	1	1	1	1	2	0	2	1	1	2	1	2	2	4	1	1	1	2	1	2
D	1	1	1	1	1	2	1	2	1	1	2	1	1	2	2	1	0	1	2	1	2
E	1	?	2	2	0	1	0	2	1	0	2	2	3	1	2	0	0	0	1	0	1
F	2	2	1	1	1	2	0	1	2	1	1	0	1	2	2	0	1	1	2	1	2
G	2	1	1	1	1	2	0	3	2	1	1	0	1	2	2	0	0	1	2	1	2
H	1	1	1	1	0	2	0	3	2	1	1	0	?	?	1	0	1	1	2	1	2

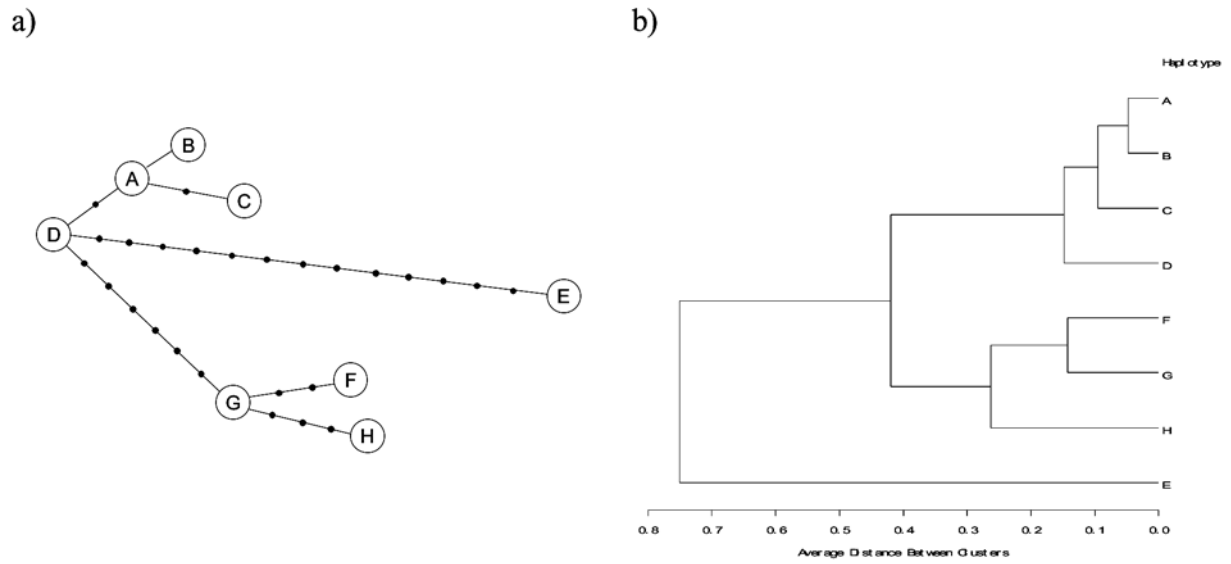

Figure 3. – a) Minimum spanning tree and b) UPGMA cluster dendrogram based on genetic distance.

Table 4. – Absolute frequencies of cpDNA haplotypes in European *Cornus sanguinea* populations (71 populations with exclusively haplotype A are pooled, see *Table 1* for their description).

Pop-ID	Country	Location	Total number	cpDNA haplotypes							
				A	B	C	D	E	F	G	H
7	Austria	Raxalpe/Reichenau	4	3							1
11	Czech Republic	Lovos/Lovosice	10	7		3					
12	Czech Republic	So-Hong Oblik/Louny	10	5		5					
15	France	Bordeaux	11	8						3	
20	Georgian Republic	Bantsurtkari/Dusheti Region	4	1			3				
27	Germany	Schönberg	7						7		
28	Germany	Strelitz/Düsterförde	10						10		
32	Germany	Mühlberg	5	4				1			
38	Germany	Vockerode	10	9				1			
58	Italy	Tuscania	9	8				1			
63	Kosovo	Grebno/Pristina	11	5				6			
64	Macedonia	Ohrid	10					10			
76	Slovakia	Hrabusice/Slovakian Paradise	10	8					2		
78	Slovakia	Poprad	6						6		
79	Slovakia	Bratislava/Ostrove	10		10						
Sum of all other 71 populations			546	546							
	Sum total		673	604	10	8	3	19	25	3	1
	Percentage %		100	89.7	1.5	1.2	0.4	2.8	3.7	0.4	0.1

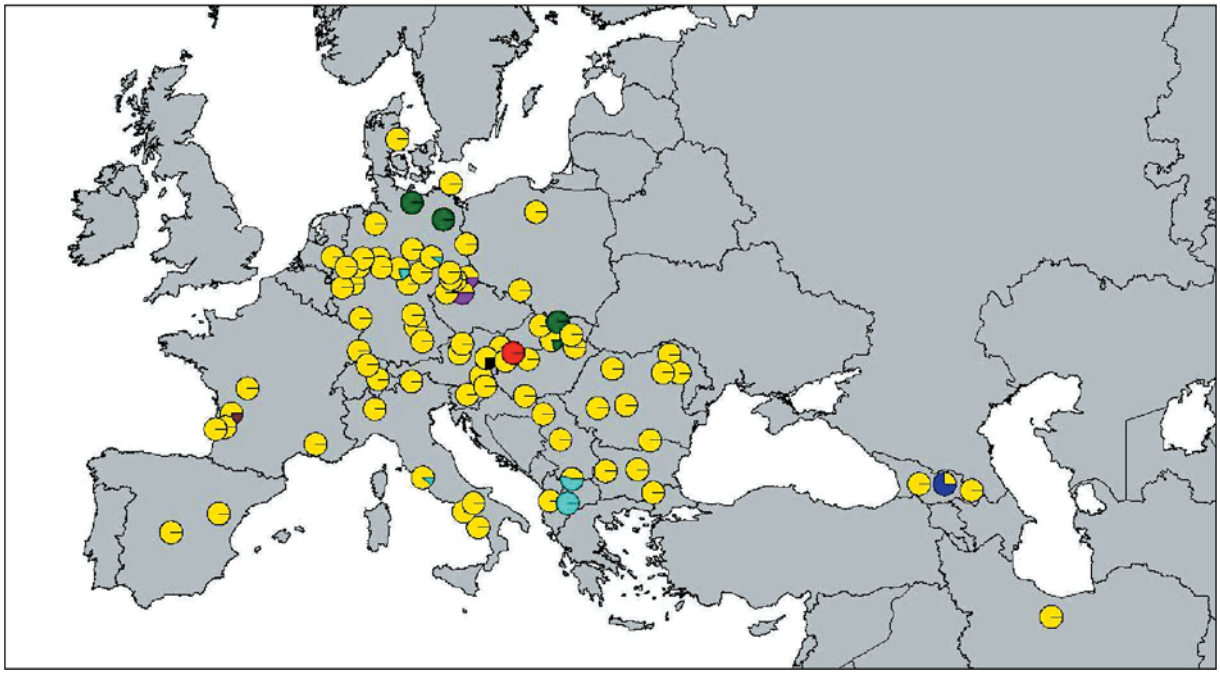

Figure 4. – Geographic distribution of eight chloroplast haplotypes identified in *Cornus sanguinea* (for populations see *Table 1*, haplotype A = yellow, B = red, C = pink, D = dark blue, E = light blue, F = green, G = brown, H = black).

Both populations in northern Germany were established in the 1960s with plant material from Slovakia (personal communication, W. VOTH, Mecklenburg-Western Pomerania). For the populations Mühlberg and Vockerode with haplotype E (light blue) it is strongly assumed that they originate at least partially from introduced material from the Balkan Peninsula. No explanation could be given for the existence of haplotype E in one population in Italy.

A recalculation of variation parameters without the four German populations that were obviously introduced resulted in a reduced total variation of H_T = 0.147. The values of G_{ST} and N_{ST} amount to 0.687 and 0.704, respectively.

Discussion

The cpDNA variation was assessed to be low in *Cornus sanguinea*. Regardless of the detected variation in 7 noncoding regions of chloroplast DNA evenly spread over the large single copy region, a low number of 8 cpDNA haplotypes was identified. This is in contrast to for example *Quercus sp.* with 33 haplotypes found with 4 noncoding regions (PETIT et al., 2002),

Prunus spinosa with 32 haplotypes found with 3 noncoding regions (MOHANTY et al., 2002) or *Populus nigra* with 83 haplotypes detected in 5 noncoding regions (COTTRELL et al., 2005) by the PCR-RFLP method. One example, *Carpinus betulus*, could be found with a similar low number of haplotypes even by testing many noncoding regions (GRIVET and PETIT, 2003).

The only other European species in the genus *Cornus*, *C. mas*, is unable to cross with *Cornus sanguinea*, therefore an interspecific hybridisation for *Cornus* can not contribute to variation. Likewise no other species is present in the genus *Carpinus*. More chances exist to distribute organelle haplotypes by hybridisation events for other genera with crossable species (i. e. *Quercus*: PETIT et al., 2002; *Betula*: PALMÉ et al., 2004) with different ecological demands or only partially overlapping distribution areas.

The *Cornus sanguinea* haplotypes are divided into 3 groups of related types. The first group consisting of haplotypes A, B, C and D (see *Figure 3*) cover the putative ancestor D and the most abundant haplotype A. The second group is composed of haplotypes F, G and H. Haplotype E is single and exhibits the largest genetic distance to all other haplotypes. There is no association between these 3 groups and their geographic occurrence within the tested material.

Besides the low number of haplotypes the total genetic variation H_T = 0.15 is lower in *Cornus sanguinea* than in 11 European tree species, where H_T ranges from 0.46 to 0.86 (reviewed in FINKELDEY et al., 2005) or in 8 other European shrub species, where H_T ranges from 0.26 to 0.97 (reviewed in LIESEBACH et al., 2007).

Life history traits of 29 tree and shrub species (without *Cornus*) and their chloroplast DNA variation levels between populations (G_{ST} and N_{ST}) were reviewed by AGUINAGALDE et al. (2005). For several species the PCR-RFLP method was completed by chloroplast microsatellite loci. *Cornus sanguinea* is a shrub with a mixed sexual and vegetative reproduction, with biotic pollination and its seeds are dispersed by animal ingestion. For species with these traits the average G_{ST} ranges from 0.38 to 0.43 and the average N_{ST} from 0.42 to 0.47 (AGUINAGALDE et al., 2005). The observed values of G_{ST} = 0.69 and N_{ST} = 0.70 for *Cornus sanguinea* in this study are more in accordance to species of opposite traits with exclusively sexual reproduction, wind pollination and a seed dispersal by wind or animal caching (average G_{ST} 0.56…0.83, average N_{ST} 0.62…0.83). A possible explanation for this atypical behaviour of *Cornus sanguinea* could be its absolute lowest H_T in the entire tree and shrub species collection, so that the scale to compare G_{ST} and N_{ST} could be skewed.

Other species with low H_T are *Crataegus monogyna* (H_T = 0.26) and *Fagus sylvatica* (H_T = 0.27). In both cases only 3 microsatellite sites and 21 to 23 populations were included to identify 4 resp. 6 haplotypes (PETIT et al., 2003). There can only be speculations about, if more polymorphic sites would have resulted in more total variation. In case of *Fagus sylvatica* a former survey with PCR-RFLPs in 85 populations yielded in 11 haplotypes, but the total diversity was low as well (H_T = 0.24) (DEMESURE et al., 1996).

Thus, the greatest analogies of *Cornus sanguinea* were observed to *Fagus sylvatica* and also to *Frangula alnus* (HAMPE et al., 2003) regarding the nearly uniform chloroplast haplotype structure in the temperate Europe, even though *Fagus* and *Frangula* possess very divergent reproduction and pollen and seed dispersal traits.

A higher number of chloroplast haplotypes in common refugia of European plant species (Iberian, Apennine and Balkan Peninsulas, Caucasus region) were reported for several trees and shrubs (PETIT et al., 2003). The lack of a number of haplotypes for *Cornus sanguinea* in at least one of these refugia could suppose a low variation before the ice age and/or a more or less strong bottleneck during recolonisation.

To get some more information on possible migration histories of *Cornus sanguinea*, a search in the European pollen database (http://www.ncdc.noaa.gov/paleo/epd/epd_main.html) was carried out additionally. It resulted in only a small number of detections of pollen in sediments in Syria, Turkey, Greece, Slovakia, the Czech Republic, Poland, Switzerland and Sweden and no relationship between geographic location and date. This is not surprising, because red dogwood is an insect pollinated species that releases only few pollen into the atmosphere. The earliest evidence is from Poland 9400 years ago (NORYAKIEWICZ and RALSKA-JASIEWICZOWA, 1989). Fruits of *Cornus sanguinea* were gathered from humans as it was detected for the Mesolithic period 5600–4000 BC in Denmark (KUBIAK-MARTENS, 1999), the late Mesolithic in Sweden (REGNELL et al., 1995) and in middle Neolithic in France (DIETSCH, 1996). But this species was never cultivated like *Cornus mas* L. In general, fossil verifications are very rare and could not really contribute to clarify the location of glacial refugia and the subsequent postglacial routes of this species after the ice age. *Cornus sp.* in the wild state is widespread in the Caucasus and Crimea and thus ZHUKOVSKY (1965) assumed a gene centre here.

The presence of the putative ancestor haplotype D in the Caucasian region, the derived common haplotype A in the whole European area and rare types B and C in Czech Republic and Slovakia are in concordance with this hypothesis. Other haplotypes, as the group G-F-H and the most exceptional haplotype E, accumulated many mutation steps and are far distant from the dominating group. Their geographic origin could not be clearly identified with the available data.

No phylogeographic structure could be detected due to the extreme dominance of the common haplotype A in the whole tested area and the punctiform variation structure of only 11 out of 82 populations excluding the 4 obviously introduced German populations. Likewise no structure could be noticed by pairwise G_{ST} that fluctuate between 0.6 and 0.8 for all classes of pairwise geographic distance up to 3000 km. Pairwise G_{ST} decreases to 0.5 for distance classes between 3000 and 4000 km (DistoN, http://www.pierroton.inra.fr/genetics/labo/Software, data not shown).

Other haplotypes than the common type A were detected only in locally restricted areas. Those findings indicate that after the colonisation of the European continent only a very restricted gene flow could have happened. At least populations with rare haplotypes, independently from belonging to one of the 3 groups, persist relatively isolated since no dissemination into surrounding areas with only haplotype A was observed in the sampled material. Deviations from this punctiform structure of variation of present-day populations are mostly explained by recent human influence as it was suggested for the 4 German populations.

Actually, guidelines for conservation and management of *Cornus sanguinea* in landscape cultivation should consider the putative low gene flow by seed between locations. More detailed information on the amount of gene flow within populations and between neighbouring occurrences are not available at present. This aspect has to be explored in another investigation. Recent studies by APLPs on the second European *Cornus* species, *Cornus mas* L., as well support a low and restricted gene flow (WISSEMANN et al., 2007) and a low distance of fruit dispersal (MÜLLER and WISSEMANN, 2007).

Acknowledgments

We would like to thank all colleagues in foreign institutes collaborating and helping with sampling their local dogwood populations: L. Blascó (Zvolen, Slovakia), D. Cogalniceanu (Romania), C. Fraissl and J. Guttenberger (Vienna, Austria), O. Holdenrieder (Zurich, Switzerland), M. Kereselidze (Tbilisi, Georgia), F. Kroiher (Linz, Austria), D. Pilarska (Sofia, Bulgaria), A. Proko (Albania), J. Reque Kilchenmann and B. Herrero (Madrid, Spain) and E. Teissier du Cros (Avignon, France). Additionally we thank our colleagues in Germany: G. Aas and M. Lauerer (Bayreuth), Mr. Arndt (Hofbieber), R. Baron, W. Kramer, K. Meinhardt, E. Müller, S. Rogall, O. Weigenand and B. Witt (Eberswalde), M. Forstreuther (Berlin), K. Lautenschlager (Enzdorf), H.-P. Schmitt and L. Schulze (Arnsberg), R. Tamm (Hassloch), W. Voth and Mr. Reituff (Schwerin) H. Weiss (Tharandt), C. Wessel (Freiburg) and G. von Wühlisch (Großhansdorf). We are grateful to Ms. Hannelore Enkisch for their engaged and reliable technical assistance in the laboratory. The study was financially supported by the Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft, Germany.

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Herausgeber: Johann Heinrich von Thünen-Institut. Bundesforschungsinstitut für Ländliche Räume, Wald und Fischerei.
Schriftleitung: Institut für Forstgenetik, Sieker Landstraße 2, D-22927 Großhansdorf
Verlag: J. D. Sauerländer's Verlag, Finkenhofstraße 21, D-60322 Frankfurt a. M.
Anzeigenverwaltung: J. D. Sauerländer's Verlag, Frankfurt am Main.
Satz und Druck: ADN Offsetdruck, Battenberg — Printed in Germany.

ISSN 0037-5349