

# Evolution by Reticulation: European Dogroses Originated by Multiple Hybridization Across the Genus *Rosa*

C. M. RITZ, H. SCHMUTHS, AND V. WISSEMANN

From the Institute of Systematic Botany, University of Jena, Philosophenweg 16, D-07743 Jena, Germany (Ritz, Wisseemann); and the Department of Experimental Taxonomy, IPK Gatersleben, Corrensstr. 3, D-06466 Gatersleben, Germany (Schmuths)

Address correspondence to C. M. Ritz at the address above, or e-mail: [christiane.ritz@uni-jena.de](mailto:christiane.ritz@uni-jena.de)

## Abstract

The European dogroses (*Rosa* sect. *Caninae* (DC.) Ser.) are characterized by a unique meiosis system (“canina-meiosis”), which controls the heterogamous development of tetraploid egg cells and haploid pollen grains resulting in a pentaploid somatic status. This permanent anorthoploidy is supposed to have originated by a hybridization event in the postglacial period. In this study we present molecular evidence by an analysis of nuclear ribosomal DNA data that dogroses are complex allopolyploids resulting from multiple hybridization events. As previously described, the nrITS-1 region does not undergo concerted evolution in dogroses. Thus, different ITS-1 sequences persist within single individuals. Secondary structure predictions do not point to the existence of pseudogenes within these ITS-1 types. Our data suggest that the pentaploid *Caninae* genome originated from different members of nondogroses and the now extinct *Protocaninae*.

The genus *Rosa* (Rosaceae) is one of the most important genus of ornamental plants in terms of economy and cultural history of humankind with about 200 species distributed in the Northern Hemisphere (Rehder 1949; Wisseemann 2003a). Conventional taxonomy (Wisseemann 2003a) divides the genus into four subgenera, three of which are monotypic: *Hulthemia* (Dumort.) Focke, *Platyrhodon* (Hurst) Rehder, and *Hesperbados* Cockerell. The fourth subgenus, *Rosa*, harbors about 95% of all species and is subdivided into 10 sections including *Caninae*, which is subject of this study. Phylogenetic investigations on the genus have been carried out, for example, by Wu et al. (2000, 2001) and Matsumoto et al. (1998, 2000). However, results of these studies remain contradictory. The natural distribution of the genus is separated into three major geographical areas: North America, East Asia, and Europe/West Asia. The European/West Asian region is dominated by members of section *Caninae* (DC.) Ser., the dogroses, which play an essential role in the production of root stocks for ornamental rose breeding. Dogroses have an exceptional position among plants due to their unique meiotic behaviour and breeding system (Grant 1971; Wisseemann 2000).

Contrary to normal meiosis, by which gametes of equal chromosome numbers are produced, canina-meiosis is a heterogamous system with haploid pollen grains and

tetraploid egg cells (Blackburn and Harrison 1921; Täckholm 1920, 1922). Outbreeding leads to permanent pentaploid organisms, which are matroclinal in characters due to the differential contribution of maternal (80%) and paternal genomes (20%) (Ritz and Wisseemann 2003; Wisseemann and Hellwig 1997). The evolutionary origin of this peculiar phenomenon in dogroses has been under intensive discussion since the beginning of the 20th century (Wisseemann 2000). Grant (1971) assumed that the system originated by hybridization leading to an allopolyploid status that enabled subsequent postglacial radiation.

To investigate the hybridogenic origin of the dogroses, we analyzed nuclear ribosomal DNA sequence data. We sequenced the internal transcribed spacer, ITS-1, which does not undergo concerted evolution in dogroses (Wisseemann 2000, 2003b) as is also described for a variety of other plant genera (reviewed in Alvarez and Wendel 2003; Bailey et al. 2003). The ITS region is located within the 18S-5.8S-26S rDNA at a single nucleolus organizer region (NOR) per haploid chromosome set in *Rosa* (Ma et al. 1997). Thus, up to five paralogous ITS sequences are expected to be found in the pentaploid dogroses under the assumption that ITS sequences are concerted within a NOR. Homogeneity within NORs was observed by Schlotterer and Tautz (1994), who showed that 35S rDNA repeats within the same NOR are

more similar than copies from different NORs. The potential of nrITS sequence data to prove historical hybridization and/or polyploidization events has been shown in Rosaceae for *Amelanchier* (Campbell et al. 1997) and in other plant taxa (Ainouche and Bayer 1997; Ritland et al. 1993; Sang et al. 1995; Soltis et al. 1995; Soltis and Soltis 1991; Suh et al. 1993; Vargas et al. 1999).

Additionally, we analyzed secondary structure predictions of the nonconcerted ITS-1 paralogs to detect the possible existence of pseudogenes, which may strongly bias phylogenetic hypotheses. The ITS region is subject to evolutionary constraints related to maintenance of secondary structures and functionality (reviewed in Alvarez and Wendel 2003; Baldwin et al. 1995). Secondary structure predictions and minimum free energy of the ITS region must be treated with caution, because ITS-1 does not exist as separate molecule but forms the 35S precursor rRNA together with ITS-2, parts of the IGS, 18S rDNA, 5.8S rDNA, and 28S rDNA (Volkov et al. 2004). However, although secondary structure predictions of ITS-1 cannot be considered true structure, they can help in the identification of pseudogenes (Bailey et al. 2003; Mayol and Rossello 2001) and can support phylogenetic hypotheses (Denduangboripant and Cronk 2001; Gottschling et al. 2001; Mayol and Rossello 2001).

Here we present molecular evidence for the multiple allopolyploid origin of dogroses. Dogroses contain a mixed set of different ITS sequence types. Several of these types are also found in nondogrose sections, but one ITS type, the *canina* type, is exclusively restricted to dogroses and thus might trace the former existence of extinct ancestors of the *Caninae*, which we call Protocaninae roses.

## Materials and Methods

### Plant Material

The plant material was collected from the field and from botanical gardens and rose nurseries. Voucher specimens were deposited in the author's herbarium (wis). Nomenclature of the taxa is according to Wissemann (2003a) and for members of sect. *Caninae* to Klášterský (1969) as well as Henker and Schulze (1993). The list of plant samples analyzed in the study, including classification, localities, and EMBL accession numbers, is shown in Table 1.

### DNA Extraction

Total DNA was extracted from silica gel-dried material of living plants or herbarium specimens using E.Z.N.A. Plant DNA Mini Kit (Peqlab Biotechnologie GmbH) following the manufacturer's instructions.

### Amplification

Amplification of double-stranded DNA was performed in 25 µl containing 2.5 µl 10-fold polymerase buffer, 2.5 µl 2 mM dNTP, 10 pmol/µl of each primer, 1 U of Taq polymerase (QBiogene), 1 µl DNA template, and 1 µl DMSO to

avoid the amplification of pseudogenes (Buckler and Holtsford 1996; Buckler et al. 1997).

Primers for ITS-1 regions were taken from White et al. (1990): "ITS5" 5'-GGAAGTAAAAGTCGTAACAAGG-3' and from Ochsmann (2000): "P2" 5'-CTCGATGGAA-CACGGGATTCTGC-3'. The standard polymerase chain reaction (PCR) conditions consist in a initial denaturation of 180 s at 95°C, 28 cycles of 30 s at 95°C, 1 min at 48°C, and 120 s at 72°C, with a final extension of 180 s at 72°C.

PCR products of nondogroses were directly sequenced in both directions with the same primers as for amplification with Amersham Bioscience Thermo Sequenase labeled Primer Cycle Sequencing kit with 7-deaza-dGTP. Samples of sect. *Caninae* and *Rosa* were subcloned before sequencing. PCR products were purified using Qiaquick PCR purification kit according to the manufacturer's instructions and subcloned with a *t*-tailed pBluescript II SK (+) cloning vector into the *Escherichia coli* strain JM13 via electroporation. Transformed *E. coli* cells were plated on LB agar with ampicillin (100 µg/ml), IPTG (0.2 mM), and X-Gal (40 µg/ml). White colonies were selected for growth, and these clones were picked and directly added to the amplification mix for ITS-1 and afterward sequenced (protocols and cycling profiles are identical to the ones described).

### Data Analyses

DNA sequences were aligned using ClustalX 1.83 (Thompson et al. 1997) and manually edited afterward. Additionally sequences of *R. gallica* L. were taken from GenBank (accession numbers AB035656, AB043835, AB043824). The complete alignment is deposited in GenBank. A haplotype network was calculated with TCS (Clement et al. 2000) using a parsimony algorithm (Templeton et al. 1992). Within the cladogram built by TCS, we detected five different nrITS-1 types, which consist of highly homogenic sequences. These ITS-1 types are also identified by eye on the base of a combination of 13 coupled diagnostic polymorphic sites in the alignment. Consensus sequences for the five different ITS-1 types were determined based on all sequences within the respective gray shaded box in the cladogram in Figure 1. To exclude the existence of pseudogenes secondary structure predictions of the five ITS-1 types were inferred with the program Alifold (Hofacker et al. 2002). The analysis was based on separate alignments of the sequences of the different ITS-1 types identified by the TCS analysis and the diagnostic polymorphic sites (a separate alignment of one ITS-1 type contains all sequences in the respective gray shaded box in Figure 1). Alifold was run with the partition function pair probabilities fold algorithm at 20°C using DNA parameters (SantaLucia 1998) and default options.

## Results

### ITS Analysis

The complete alignment (length = 259) of the ITS-1 sequence data of different species of *Rosa* contains 51

**Table 1.** Analyzed *Rosa* species with sources and EMBL accession numbers

Taxon	Sample origin and voucher no.	EMBL no.
Subgen. <i>Hesperobodos</i> Cockerell 1913		
<i>R. stellata</i> Wooton	Germany, Hessen, Kassel	AJ631842
$2n = 2x = 14$	VW163	
Subgen. <i>Hulthemia</i> (Dumort.) Focke 1888		
<i>R. persica</i> Michx. ex Juss.	Germany, Göttingen Botanic Garden, Section Ecology	AJ631841
$2n = 2x = 14$	C10	
Subgen. <i>Platyrhodon</i> (Hurst) Rehder 1940		
<i>R. roxburghii</i> Tratt.	Germany, Hessen, Kassel	AJ631843
$2n = 2x = 14$	VW72	
Subgen. <i>Rosa</i>		
Sect. <i>Banksianae</i> Lindl. 1820		
<i>R. banksiae</i> Ait.	USA, Texas, TAMU	AJ631853
$2n = 2x, 4x = 14, 28$	VW314	
Sect. <i>Bracteatae</i> Thory 1820		
<i>R. bracteata</i> Wendl.	USA, Texas, TAMU	AJ631863
$2n = 2x = 14$	VW315	
Sect. <i>Caninae</i> (DC.) Ser. 1825		
<i>R. abietina</i> Gren. ex Christ	Switzerland, Glarus, Braunwald	AJ631940
$2n = ?$	C9_1	AJ631941
unbalanced, heterogamous	C9_2	AJ631942
	C9_3	
<i>R. agrestis</i> Savi	Germany, Niedersachsen, Banenrode, VW150_1	AJ631899
$2n = 5x, 6x = 35, 42$	VW150_3	AJ631956
unbalanced, heterogamous	VW150_4	AJ631957
	VW150_5	AJ631959
	VW150_6	AJ631958
	VW150_7	AJ631904
<i>R. caesia</i> Sm.	Germany, Schleswig-Holstein, Fehmarn	AJ631954
$2n = 5x, 6x = 35, 42$	C6_1	
unbalanced, heterogamous		
<i>R. canina</i> L.	Germany, Niedersachsen, Bovenden, North of	AJ631886
$2n = 5x = 35$	Göttingen	AJ631923
unbalanced, heterogamous	VW355_1	
	VW355_2	
<i>R. rubiginosa</i> ssp. <i>columnifera</i>	Germany, Mecklenburg-Vorpommern,	AJ631934
Schwertschlag	Neubrandenburg, Lindenberg	
$2n = 5x = 35$	C3_1	
unbalanced, heterogamous		
<i>R. corymbifera</i> Borkh.	Germany, Niedersachsen, Gross Schneen near	AJ631931
$2n = 5x = 35$	Göttingen	
unbalanced, heterogamous	C5_1	
<i>R. glauca</i> Pourr.	Germany, Niedersachsen, Botanic Garden Göttingen,	AJ631895
$2n = 4x = 28$	Section Systematics	AJ631896
unbalanced, heterogamous	VW17_1	AJ631903
	VW17_2	
	VW17_3	
<i>R. jundzillii</i> Besser	Germany, Rheinland-Pfalz, Mertesdorf near	AJ631884
$2n = 6x = 42$	Trier	
unbalanced, heterogamous	VW356_1	AJ631924
	VW356_2	
<i>R. micrantha</i> Borrer ex Sm.	Germany, Mecklenburg-Vorpommern, Neustrelitz	AJ631840
$2n = 4x, 5x, 6x = 28, 35, 42$	C2_1	AJ631887
unbalanced, heterogamous	C2_2	AJ631929
	C2_3	AJ631930
	C2_4	AJ631888
	C2_5	AJ631933
	C2_6	

**Table 1.** Continued

Taxon	Sample origin and voucher no.	EMBL no.
<i>R. mollis</i> Sm. $2n = 4x, 5x, 6x = 28, 35, 42$ unbalanced, heterogamous	Germany, Schleswig-Holstein, Geltinger Birk, Flensburg VW152_1 VW152_2 VW152_3 VW152_4 VW152_5 VW152_6 VW152_7	AJ631901 AJ631907 AJ631960 AJ631905 AJ631906 AJ631961 AJ631949
<i>R. montana</i> Chaix $2n = 5x = 35$ unbalanced, heterogamous	Italy, Südtirol, Vinschgau, Sonnenberg near of Schlanders C8_1 C8_2	AJ631947 AJ631948
“ <i>R. mosqueta</i> ” = <i>R. rubiginosa</i> L. from South America $2n = ?$ unbalanced, heterogamous	Argentina, Provincia del Chubut, near of Parque Nacional Los Alerces, Puerto Limonoa C51_1 C51_2 C51_3 Argentina, Provincia de Neuquen, near of Parque Nacional Lanin, Hua-Hum C54_1 C54_2 C54_3 C54_4 C54_5 Argentina, Provincia de Neuquen, near of Parque Nacional Lanin, Hua-Hum C55_1	AJ631890 AJ631916 AJ631915 AJ631891 AJ631892 AJ631893 AJ631902 AJ631900 AJ631894
<i>R. pseudoscabriuscula</i> (R. Keller) Henker & G. Schulze $2n = 5x = 35$ unbalanced, heterogamous	Germany, Mecklenburg-Vorpommern, Burg Stargard C1_1 C1_2 C1_3	AJ631927 AJ631928 AJ631932
<i>R. rubiginosa</i> L. $2n = 5x = 35$ unbalanced, heterogamous	Germany, Schleswig-Holstein, Helgoland VW354_1	AJ631885
<i>R. sherardii</i> Davies $2n = 4x, 5x, 6x = 28, 35, 42$ unbalanced, heterogamous	Germany, Mecklenburg-Vorpommern, Neukloster VW309_1	AJ631925
<i>R. sicula</i> Tratt.	Germany, Sachsen-Anhalt, SGH VW161_1 VW161_2 VW161_3 VW161_4 VW161_5 VW161_6	AJ631937 AJ631938 AJ631939 AJ631889 AJ631955 AJ631946
<i>R. stylosa</i> Desvaux $2n = 5x, 6x = 35, 42$ unbalanced, heterogamous	Germany, Baden-Württemberg, Badenweiler C7_1	AJ631926
<i>R. subcanina</i> (H. Christ) R. Keller $2n = 5x = 35$ unbalanced, heterogamous	Germany, Mecklenburg-Vorpommern, Warin VW141_1 VW141_2	AJ631935 AJ631936
<i>R. subcollina</i> (H. Christ) R. Keller $2n = 5x = 35$ unbalanced, heterogamous	Germany, Niedersachsen, Westharz, Hohegeiss VW140_1	AJ631897
<i>R. tomentella</i> Léman $2n = 5x = 35$ unbalanced, heterogamous	Germany, Mecklenburg-Vorpommern, Poischendorf VW146_1	AJ631945

**Table 1.** Continued

Taxon	Sample origin and voucher no.	EMBL no.
<i>R. tomentosa</i> Sm. $2n = 5x = 35$ unbalanced, heterogamous	Germany, Mecklenburg-Vorpommern, Züsow VW142_1 VW142_2	AJ631943 AJ631944
<i>R. villosa</i> L. $2n = 4x, 8x = 28, 56$ unbalanced, heterogamous	Germany, Mecklenburg-Vorpommern, Lübz VW143_1 VW143_2 VW143_3	AJ631917 AJ631898 AJ631918
Sect. <i>Carolinae</i> Crép. 1891		
<i>R. carolina</i> Willd. I $2n = 4x = 28$	Germany, Hessen, Kassel C19	AJ631861
<i>R. carolina</i> Willd. II $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH C29	AJ631855
<i>R. nitida</i> Willd.	Germany, Niedersachsen, Göttingen, Leonard Nelson Strasse C18	AJ631860
<i>R. palustris</i> Marsh. $2n = 2x = 14$	Germany, Niedersachsen, Göttingen, Botanic Garden, Section Systematics C17	AJ631864
<i>R. virginiana</i> Herrm. $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH C28	AJ631857
Sect. <i>Cinnamomeae</i> (DC.) Ser. 1825		
<i>R. arkansana</i> I Porter ex. I.M. Coult $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH C30	AJ631858
<i>R. arkansana</i> II Porter ex. I.M. Coult $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH C35	AJ631862
<i>R. beggeriana</i> Schrenk $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH C24	AJ631866
<i>R. blanda</i> Ait. $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH C31	AJ631859
<i>R. cinnamomea</i> Linn. var. <i>glabra</i> $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH C21	AJ631854
<i>R. laxa</i> Retz $2n = 2x = 14$	China, Xinjiang, Kongur, Atoinak, 2750m, leg. M. Richter 1996-07-04 C36	AJ631881
<i>R. majalis</i> Herrm. $2n = 2x = 14$	Germany, Baden-Württemberg, Rottenburg/Neckar, Äuble C39	AJ631867
<i>R. multibracteata</i> Hemsl. et E.H. Wilson $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH C34	AJ631872
<i>R. pendulina</i> L. $2n = 2x = 14$	Switzerland, Engadin, Chua-Litschana C16	AJ631844
<i>R. rugosa</i> Thunb. $2n = 2x = 14$	Germany, Schleswig-Holstein, Sylt DL58	AJ631865
<i>R. sertata</i> Rolfe $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH C33	AJ631856
<i>R. suffulta</i> Greene $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH C32	AJ631851
<i>R. willmottiae</i> Hemsl. $2n = 2x = 14$	Germany, Niedersachsen, Botanic Garden, Göttingen, Section Systematics C14	AJ631871
<i>R. woodsii</i> Lindl. $2n = 2x = 14$	Germany, Hessen, Kassel C15	AJ631852
Sect. <i>Indicae</i> Thory 1820		
<i>R. chinensis</i> Jacq. $2n = 2x, 3x, 4x = 14, 21, 28$	Germany, Sachsen-Anhalt, SGH C38	AJ631847

**Table 1.** Continued

Taxon	Sample origin and voucher no.	EMBL no.
<i>R. odorata</i> (Andrews) Sweet $2n = ?$ Sect. <i>Laevigatae</i> Thory 1820	Germany, Sachsen-Anhalt, SGH C37	AJ631848
<i>R. laevigata</i> Michx. $2n = 2x = 14$ Sect. <i>Pimpinellifoliae</i> (DC.) Ser. 1825	USA, Texas, TAMU VW313	AJ631873
<i>R. altaica</i> Willd. $2n = 4x = 28$	Germany, Schleswig-Holstein, Fehmarn DL36	AJ631849
<i>R. ecae</i> Aitch. $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH DL7	AJ631878
<i>R. foetida</i> J. Herrm. $2n = 4x = 28$	Germany, Sachsen-Anhalt, SGH DL20	AJ631879
<i>R. hugonis</i> Hemsl. $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH DL6	AJ631882
<i>R. primula</i> Boul. $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH DL5	AJ631876
<i>R. sericea</i> Lindl. $2n = 2x = 14$	Germany, Sachsen-Anhalt, SGH DL18	AJ631874
<i>R. spinosissima</i> L. $2n = 4x = 28$	Austria, Senftenberg, Krems DL-V14 DL-V17 DL55 DL56 DL57	AJ631880 AJ631850 AJ631868 AJ631869 AJ631870
<i>R. xanthina</i> Lindl. $2n = 2x = 14$ Sect. <i>Rosa</i> ( <i>Gallicanae</i> DC.) Ser. 1825)	Germany, Sachsen-Anhalt, SGH DL62	AJ631875
<i>R. gallica</i> L. $2n = 4x = 28$	Germany, Baden-Württemberg, Rottenburg/Neckar, Seebronn VW101_1 VW101_2 sequence from EMBL database sequence from EMBL database sequence from EMBL database	AJ631908 AJ631922 AB035656 AB043824 AB043835
<i>R. alba</i> (= <i>gallica</i> × <i>dumetorum</i> )	Germany, Sachsen-Anhalt, SGH C25_2 C25_3 C25_4	AJ631919 AJ631951 AJ631952
<i>R. alba</i> var. <i>suaveolens</i>	Germany, Sachsen-Anhalt, SGH C26_1	AJ631920
<i>R. alba</i> “Mme. Plantier”	Germany, Sachsen-Anhalt, SGH C40_11 C40_12 C40_15	AJ631909 AJ631883 AJ631914
<i>R. alba</i> “Königin von Dänemark”	Germany, Niedersachsen, Göttingen, Botanic Garden, Section Systematics C41_6 C41_7 C41_8 C41_10	AJ631913 AJ631911 AJ631910 AJ631912
<i>R. alba</i> × <i>corymbifera</i>	Germany, Sachsen-Anhalt, SGH C44_1 C44_2 C44_3	AJ631921 AJ631953 AJ631950

**Table 1.** Continued

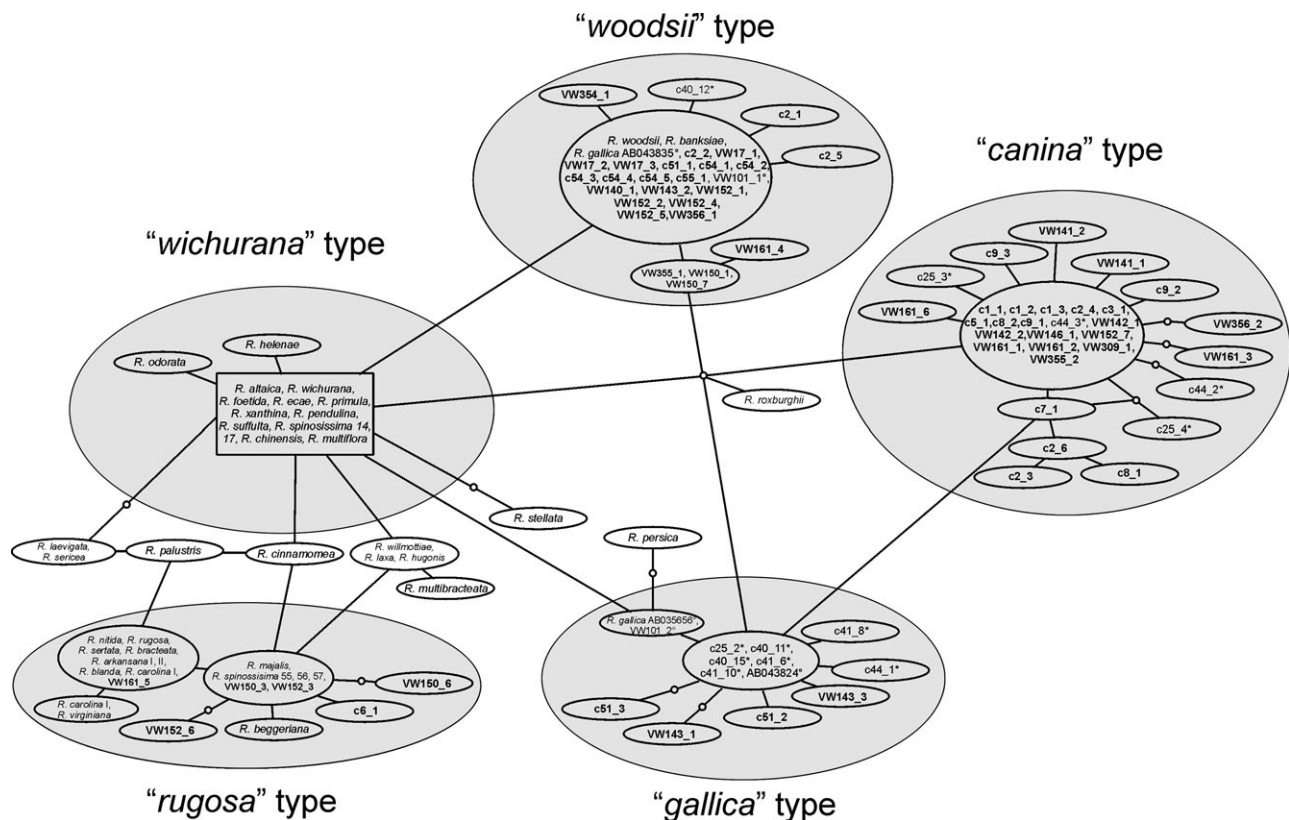
Taxon	Sample origin and voucher no.	EMBL no.
Sect. <i>Synstylae</i> DC. 1813		
<i>R. helenae</i> Rehd. & Wils. $2n = 2x = 14$	Germany, Hessen, Kassel C13	AJ631877
<i>R. multiflora</i> Thunb. ex. Murr. $2n = 2x, 3x = 14, 21$	Germany, Niedersachsen, Göttingen, Botanic Garden, Section Systematics C12	AJ631845
<i>R. wichurana</i> Crép. $2n = 2x = 14$	Germany, Niedersachsen, Göttingen, Botanic Garden, Section Systematics C11	AJ631846

Subgeneric classification, nomenclature and chromosome numbers are taken from Wissemann (2003a).

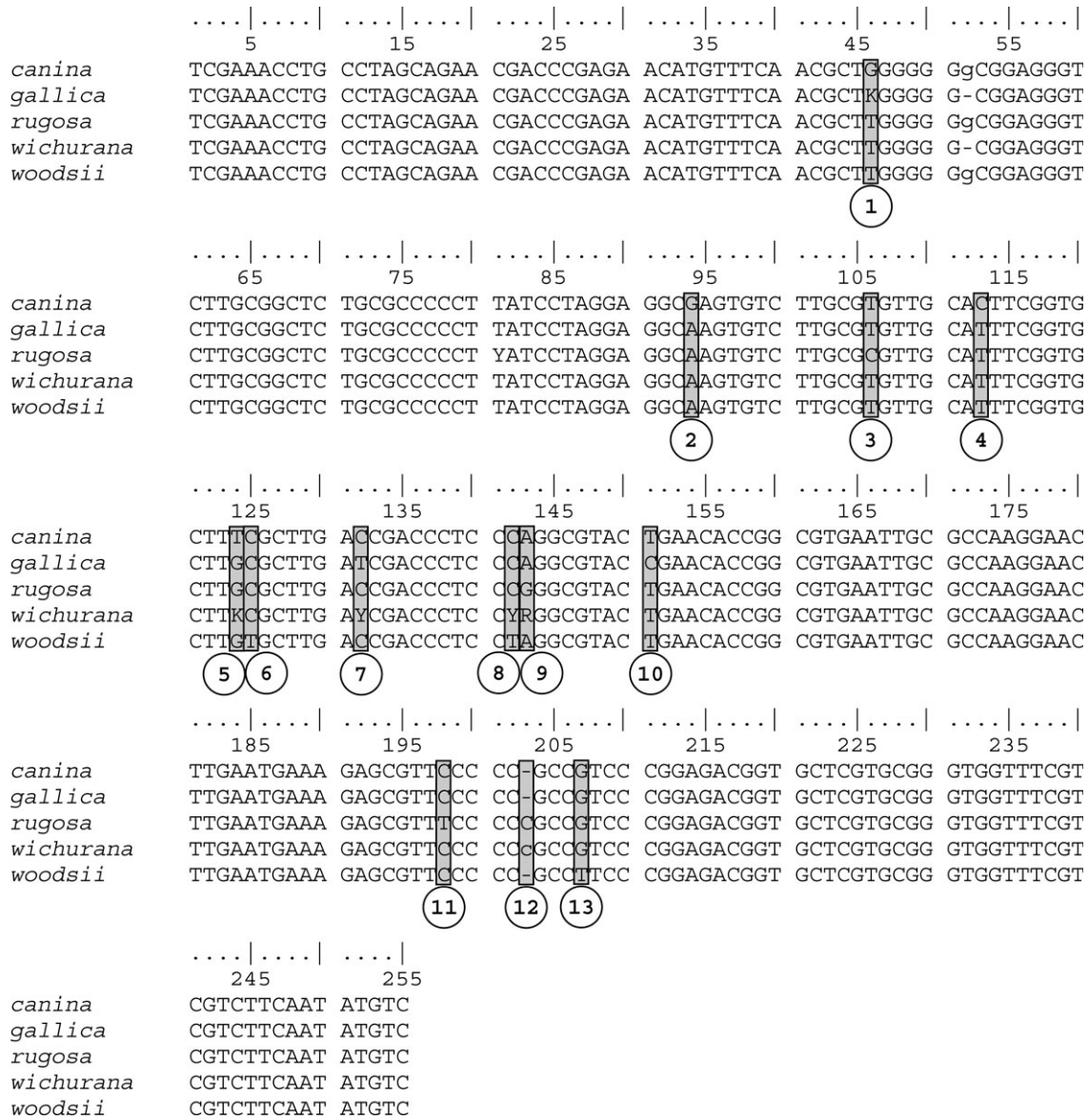
Abbreviations: SGH = Europa-Rosarium Sangerhausen, Kassel: Rose collection Kassel-Wilhelmshöhe, Germany; TAMU: Collection from Texas A&M University, Dept. of Horticultural Sciences.

polymorphic sites resulting in up to 4.6% sequence divergence. In a haplotype network, nrITS-1 sequences sampled from across the genus *Rosa* were confined to five major clusters: the *canina*, *gallica*, *rugosa*, *wichurana*, and *woodsii* types (Figure 1). These five ITS-1 types, with almost no sequence variation within the cluster, can be differentiated by a characteristic combination of 13 coupled single nucleotide

substitutions. The consensus sequences of the five different ITS-1 types with the diagnostic polymorphic sites are shown in Figure 2. ITS sequences of species of sect. *Caninae* and *Rosa* are nonconcerted. No double bands were detected when the ITS sequences of nondogroses were sequenced directly after PCR amplification and thus gave no hint for nonconcerted ITS evolution in these sections so far. With



**Figure 1.** Haplotype network of nrITS-1 sequences from the genus *Rosa*. Clonal sequences of sect. *Caninae*, sect. *Rosa* and "Alba" roses are named by a number code. Clonal sequences of sect. *Caninae* are presented in bold, sequences of sect. *Rosa* are marked with a circle and sequences of "Alba" roses are marked with an asterisk. The first number equals a specific individual of a dogrose (see Table 1), the second number corresponds to the number of the clone. (Example: C54\_2 = *Rosa rubiginosa* L., clone 2). All other rose taxa are indicated by their full species names.



**Figure 2.** Alignment of the consensus sequences of the *canina*, *gallica*, *rugosa*, *wichurana*, and *woodsii* ITS-1 types. Thirteen polymorphic sites diagnostic for the respective ITS-1 types are shown by numbers in circles and are marked by gray bars.

the exception of the *wichurana* type, the various ITS-1 sequences of dogroses occurred in all ITS-1 types. The *canina* type is restricted to dogroses (sect. *Caninae*) and clones of *R. × alba*, a group of putative *R. canina* × *gallica* hybrids. Within the *gallica* type ITS-1 sequences of *Rosa gallica* (sect. *Rosa*), *R. × alba* and one dogrose individual (“*R. mosqueta*,” c53) are found. Roses of other sections contain ITS-1 sequences, which are distributed within the *rugosa*, *wichurana*, and *woodsii* type. The *rugosa* type contains ITS-1 sequences of the monotypic sect. *Bracteatae* and species of sect. *Carolinae*, *Cinnamomeae*, and *Pimpinellifoliae*. The *wichurana* type occurs in species of the sections *Cinnamomeae*, *Indicae*, *Pimpinellifoliae*, and *Synstylae*. The ITS-1 sequences of *R. stellata* (subgen. *Hesperbodos*), *R. cinnamomea*, *R. multibracteata*, *R. palustris*, *R. willmottiae*, *R. laxa* (sect. *Cinnamomeae*), *R. laevigata* (sect.

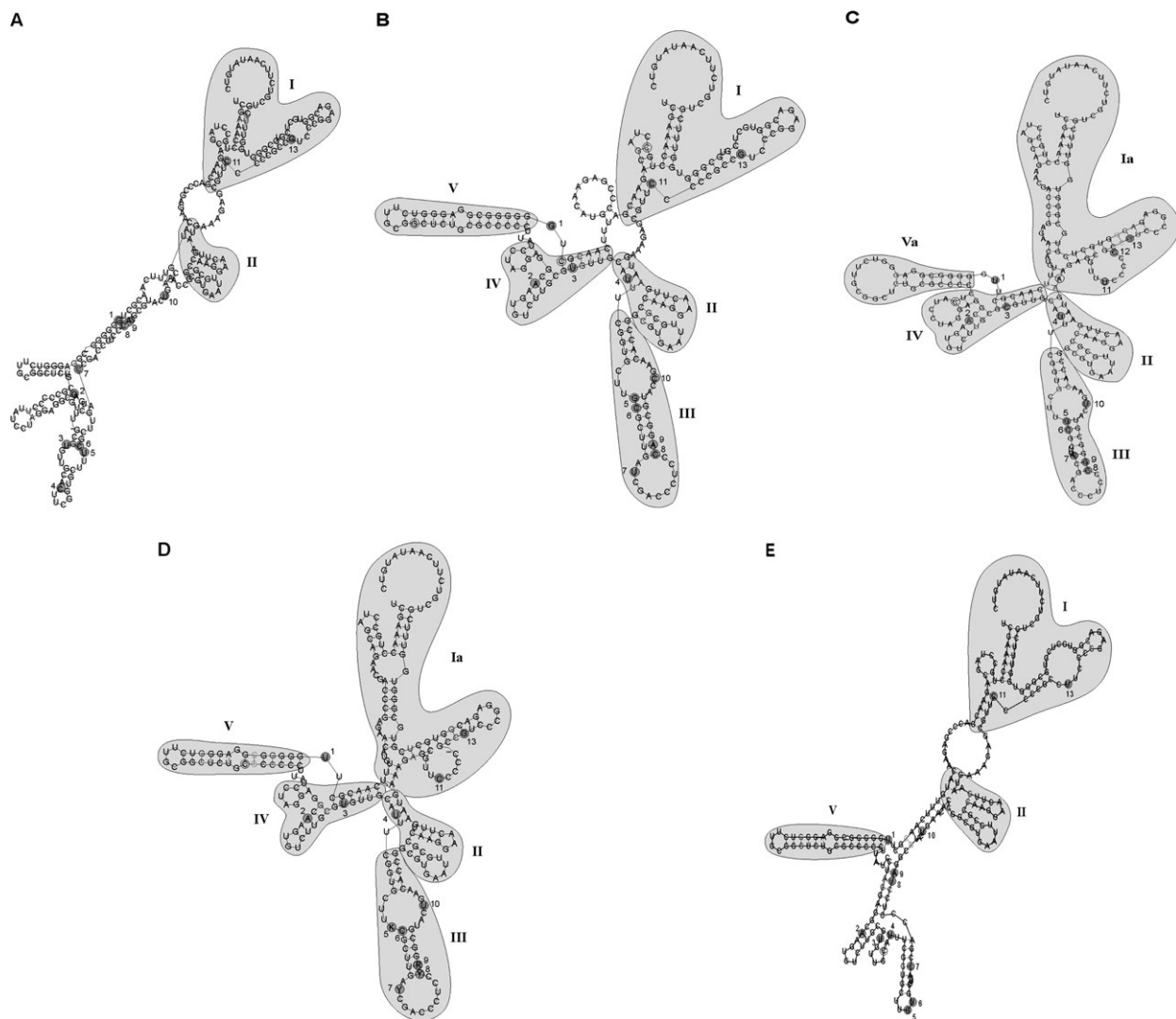
*Lavigatae*), *R. sericea*, and *R. hugonis* (sect. *Pimpinellifoliae*) are not directly recognized by the polymorphic sites diagnostic for the *wichurana* or *rugosa* type but have an intermediate position between these types. The *woodsii* type comprises in addition to ITS-1 sequences of sect. *Caninae* and *Rosa*, *R. banksiae* (sect. *Banksianae*) and *R. woodsii* (sect. *Cinnamomeae*).

## Secondary Structures

Secondary structure predictions of the nrITS-1 sequences for the alignments of the five different ITS-1 types are shown in Figure 3. G+C content is nearly identical between these alignments (Table 2).

We identified five different domains (or arms) within the secondary structures, which are shown as shaded areas in





**Figure 3.** Secondary structures of the different ITS-1 types predicted by the program Alifold. (A) *Canina* type, (B) *gallica* type, (C) *rugosa* type, (D) *wichurana* type, and (E) *woodsii* type. Diagnostic polymorphic sites are marked by gray circles and numbers (see alignment in Figure 2), helix domains are marked by shaded areas and Roman letters.

Figure 3. Domain I is found in all ITS-1 types (alignment position 1–22 and 194–255) but is slightly different in the *rugosa* and *wichurana* type (alignment position 1–38 and 188–255) (Ia). Secondary structures of all ITS-1 types contain domain II. This domain is identical for the *gallica*, *rugosa*, and *wichurana* type consisting of alignment positions 111–114 and 160–187. The first part of this domain of the *canina* and *woodsii* type includes the alignment positions 32–34 instead of 111–114. This domain contains the conserved angiosperm motif GGCYR-(4 to 7n)-GYGYCAAGGAA (Liu and Schardl 1994) in all ITS-1 types. The domain III is lacking in the *canina* and *woodsii* type but present in the *gallica*, *rugosa*, and *wichurana* type consisting of the alignment positions 116–159. Domain IV is found in the *gallica*, *rugosa*, and *wichurana* type (alignment positions 39–44 and 83–110) but lacking in the *canina* and *woodsii* type. Domain V (alignment position

46–79) is present in all ITS-1 types with the exception of the *canina* type.

Secondary structures of the *rugosa* and *wichurana* type are nearly identical, sharing all five domains. The *gallica* type is also very similar to the *rugosa* and *woodsii* type but contains the domain I instead of Ia. The *canina* type differs mostly from all

**Table 2.** Mean G+C content of the five nrITS-1 types and minimum free energy of the predicted secondary structures

Type	G+C [%]	$\Delta G$ (20°C) [kcal/mol]
<i>canina</i>	59.80	–72.43
<i>gallica</i>	59.30	–77.33
<i>rugosa</i>	59.68	–75.19
<i>wichurana</i>	58.13	–72.42
<i>woodsii</i>	57.72	–71.53

other ITS types because domains III, IV, and V are replaced by a unique structure. Alifold computed the secondary structures based on the most frequent base at ambiguous sites, but results did not differ when ambiguous sites (variable positions no. 5, 7, 8, and 9 in the *wichurana* type) were directly implemented.

## Discussion

ITS-1 sequences sampled from across the genus *Rosa* were confined to five major clusters: the *canina*, *gallica*, *rugosa*, *wichurana*, and *woodsii* types (Figure 1). The *woodsii*, *gallica*, and *canina* types are identical with the A-, B- and C-ITS types, respectively, detected in a previous study of dogrose species by Wissemann (2000). The apparent absence of concerted evolution of ribosomal DNA among various chromosome sets of different origin within dogroses, which putatively hybridize in nature (Ritz and Wissemann 2003) contrasts with the fast homogenization of ITS sequences in artificial *Armeria* hybrids (Aguilar et al. 1999) and with the elimination of parental rDNA in the putative hybrid *Nicotiana tabacum* (Volkov et al. 1999). On the other hand different ITS copies persist for long time periods in hybrids of *Amelanchier* (Campbell et al. 1997).

Minimum free energy values of the secondary structure predictions, the G+C content and the presence of the conserved angiosperm motif GGCRY-(4 to 7n)-GYGY-CAAGAA (Liu and Schardl 1994) in all ITS-1 types do not point to the existence of pseudogenes. Minimum free energy values and the G+C content differ only in up to 5.8 kcal/mol (2.6%), whereas Mayol and Rossello (2001) found differences of the G+C content of 12.7% and 43.6 kcal/mol of the minimum free energy of ITS-1 sequences of *Quercus rubra* L. and thus suggested the existence of pseudogenes. We assume that all detected ITS-1 types are potentially functional, because the *rugosa*, the *wichurana*, and the *woodsii* type occur also exclusively in diploid roses of nondogrose sections. The *canina* type and the *gallica* type always co-occur with other types (e.g., in *Rosa gallica* with the *woodsii* type and in dogroses with the *woodsii* type or the *rugosa* type), but the data are inconclusive as to whether several or only one ITS types are transcribed in the plants.

Relative similarity of secondary structures corresponds with the relationships reflected in the minimum spanning tree. Secondary structures of the closely related *rugosa* and *wichurana* type are most similar to each other sharing all five helix domains. The secondary structure of the *canina* type and the *woodsii* type are most distinct from all other types, which is also mirrored by their more isolated positions.

With the exception of the *wichurana* type, ITS-1 sequences in dogroses occur in all clusters detected by us. The *canina* type is restricted to dogroses and the Alba roses (putative *R. canina* × *R. gallica* hybrids), whereas the other four types contain ITS-1 sequences of various other wild roses of different sections of *Rosa*. The coexistence of identical ITS-1 sequences in the pentaploid genomes of the dogroses on one hand and in several sections of the genus *Rosa* on the other hand suggests that the *Caninae* genome arose by hybridiza-

tion. Thus our results based on molecular data confirm the hybridogenic origin of dogroses first postulated by Täckholm (1920, 1922) and also suggested by Blackburn and Harrison (1921), Gustafsson (1944), Gustafsson and Hakansson (1942), and Hurst (1925, 1928) based on cytological observations. Furthermore, our data implicate the multiple allopolyploid origin of dogroses between different ancestors of non-*Caninae* wild roses and Protocaninae. The restriction of the *canina* type sequences to dogroses implies the existence of a diploid ancestral Protocanina. By hybridogenic introgression into this diploid Protocanina the modern *Caninae* may have evolved, whereas ancient roses of the *canina* type cluster became extinct. However, the exact evolutionary process of the joining of the different nondogrose genomes and the Protocaninae genome remains unresolved. We hypothesize that the final pentaploid hybrid and its offspring became established by reproductive isolation via the *Canina* meiosis, which enabled subsequent radiation when an open landscape appeared after the end of the last glacial period in Europe.

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