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Genetic diversity and differentiation in roses: A garden rose perspective

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ABSTRACT

For the first time genetic diversity among modern garden rose cultivars has been evaluated using a set of 24 microsatellite markers covering most chromosomes. A total of 518 different alleles were obtained in the set of 138 rose cultivars and this led to the conclusion that in terms of genetic diversity cut roses can be considered as a subgroup of the garden roses.

Genetic differentiation among types of garden roses (F_{st} = 0.022) was four times that among cut roses, and similar in magnitude to the differentiation among breeders, due to the fact that horticultural groups and breeders overlap largely in classification. Winter hardy Svejda's cultivars (Canadian Explorer roses) showed the least similarities to European roses, and introgression from wild species for winter hardiness was clearly visible. Roses of Harkness and Olesen shared a similar genepool. Comparison of the differentiation among linkage groups indicated that linkage group 5 is potentially a region containing important QTLs for winter hardiness. Linkage group 6 contains the largest amount of genetic diversity, while linkage group 2 is the most differentiated among types of garden roses.

1. Introduction

The genus *Rosa* consists of over 100 species, mostly from Asia but some native to North America, Europe and northwest Africa. Many of these species are thought to have arisen by hybridisation, often accompanied by polyploidization, either naturally or during cultivation (De Riek et al., 2013; Zhang et al., 2013). The wild ancestors of domesticated ornamental roses are found mainly in the sections (sect.) *Synstylae* (*R. moschata*, *R. wichurana* and *R. multiflora*), *Gallicanae* (*R. gallica*), *Indicae* (*R. chinensis* and *R. gigantea*) and *Pimpinellifoliae* (*R. foetida*) (Wylie 1954). Smaller contributions are from *R. spinosissima* in section *Pimpinellifoliae* and *R. cinnamomea* and *R. rugosa* in section *Cinnamomeae* (Smulders et al., 2011). This subset of wild species has enabled the enormous diversity of roses in shape, colour, and fragrance.

Variability of species and intraspecific hybridisations make genetic relationships within the genus *Rosa* complicated (Koopman et al., 2008), especially for cultivars. The most common grouping of ornamental roses is on the basis of usage into cut roses, garden roses and rootstocks (Shepherd, 1954; Gudin, 2000; Debener and Linde, 2009). Rootstock roses are wild or semi-wild genotypes, mostly *R. canina* (sect. *Caninae*, dogroses), which are pentaploid, and *R. laxa* (sect. *Cinnamomae*), which is tetraploid. Cut and garden roses belong to the hybrid tea roses; they are mostly tetraploid. Cut roses are under strict selection criteria such as absence of stem bending, production (high number of stems per m^2), thornlessness, and long vase life. At the same time various ornamental traits, including flower colour and shape, are bred to be quite diverse. In contrast garden roses are a varied group, as they are not bred and valued only for flowers, but also as potted plants, for hedging, for landscaping, for hip production and even for the production of components for food and cosmetic industry. In such a wide spectrum of cultivar uses it is not possible to implement a simple classification system. Traditionally garden rose cultivars are placed in one of three main groups: wild, old garden and modern garden roses (Table 1).

Hybridisation with and introgression from wild species is more common in garden rose breeding than it is in cut rose breeding. Specific traits, such as winter hardiness, are introduced from wild relatives (*R. rugosa, R. arkansana*, etc.). Each breeder uses a source for a trait of interest from wild species or cultivars with the preferred trait. In general, breeders are specialised for one or a few rose types and want to be recognisable by their cultivars so they use a set of germplasm that is different from other breeders. As a consequence it is possible to distinguish breeders on the basis of

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Table 1
Rose classification, morphological characteristics and origin of rose types.

Group	Circumscription	Cultivar group	Morphology	Information on ancestry
Wild	Natural species and hybrids		Low-maintenance shrubby, once flowering phenotypes tolerant to poor soil and shade.	–
Old Garden Roses	All roses that existed before the introduction of La France, first modern rose in 1867.	Alba	Strong growing shrubs with well-scented white to pale pink flowers and few thorns. Foliage and stems tend to be greyish.	An ancient groups of roses derived from *R. canina* and *R. gallica*, probably introduced by the Romans.
		Gallica	*R. gallica* is a species native to southern and central Europe eastwards to Turkey and the Caucasus. Cultivars of this species and hybrids close to appearance are considered as a cultivar group. It is an ancient group of short, compact shrubs with most commonly double or semi double once blooming flowers. The flower colour range from white (rare) to pink to the darkest purple.	The exact ancestry is unknown and other species may be involved.
		Damask	Once-blooming, thorny shrubs with intensely fragrant white to pink flowers. They are especially valued for their natural oils.	DNA analysis showed that damask roses evolved as a result of natural double crossing of *R. gallica* with *R. moschata* crossed again with *R. fedtschenkoana*. This hybridisation probably happened in Central Asia
		Centifolia or Provence	Known also as Cabbage rose thanks to the large number of petals. They are fragrant and extremely hardy roses with white or pink flowers.	It is a complex hybrid mainly derived from Gallica and Alba or Damask roses.
		Moss	The main characteristic of this rose group is mossy growth of sepals, calyx and stems. They can be once- or repeat-blooming.	Appeared as a mutation of Centifolia roses in 18th century. Later more compact and repeat-flowering hybrids evolved from the Damask roses.
		Portland	Small group of shorter, more compact shrubs with ability to repeat bloom in autumn. The flower colour range from white to pink and red.	It is a small group of hybrids derived from a rose named after plant collecting of Portland around 1780. DNA analysis showed that they are hybrids of Gallica and Damask roses.
		China	This is the class upon which modern roses are built. China roses are characterised by moderate fragrance and small blooms carried over twigs. They bloom repeatedly through summer and late autumn	The China roses, based on *R.chinensis*, have been cultivated in East Asia for centuries. From 18th century they have been cultivated in Western Europe.
		Tea	Tea roses are introduced in 19th century. They are repeat-blooming roses, named for their scent which reminds of Chinese black tea. The colour range includes pastel shades of white, pink and yellow apricot. They have individual flowers with petals that tend to roll back at the edge.	The Tea-scented China roses are hybrids of *R. chinensis* and *R. gigantea*.
		Burbon	This group originated from Bourbon on the coast of Madagascar. They are vigorous shrubs with glossy foliage that bloom repeatedly.	Probably they developed as a result of a cross between Damask and Old Blush China roses.
		Noisette	The first Noisettes were small-blossomed, winter-hardy climbers, but later introgression of Tea rose genes created a Tea-Noisette subclass with larger flowers, smaller clusters, and considerably reduced winterhardiness.	The first Noisette rose was bred by John Champneys as a seedling of China roses and *R. moschata.*
		Hybrid Perpetual (HP)	They are repeat- or once-blooming cultivars with tendency for massive spring blooming. The flower colour palette is limited to white, pink and red.	Represents a group of roses derived from Asian and European cultivars (Chinas, Bourbons, Noisette).
		Hybrid Musk	They arose when the era of Old Garden Roses was finished; still they are classed with them as their growth type is similar to Old Garden Roses. Hybrid musks are disease resistant cultivars characterised by repeat-blooming and clustered flowers. They are recognised by strong musk scent.	This group was mainly developed by Joseph Pemberton. *R. multiflora* is confirmed as a parent and R. moschata also figures in Hybrid Musk pedigrees.
		Hybrid Rugosa (HRG)	This is a group of vigorous, extremely disease resistant and fragrant cultivars characterised by recurrent blooming and double flat flowers.	Hybrid musk derived from R. rugosa from Japan and Korea in 1880s.

Table 1 (*Continued*)

Group	Circumscription	Cultivar group	Morphology	Information on ancestry
		Bermuda Mystery Rose	This group was discovered in Bermuda. The roses of this group have value and interest for breeders in tropical and semi-tropical regions, since they are highly resistant to nematodes and fungal disease. Additionally, they are capable to bloom during hot and humid seasons.	The parentage is unknown.
		Miscellaneous	This group includes miscellaneous climbing and shrub forms.	The parentage is unknown.
Modern Garden Rose	Once-blooming fragrant shrubs, European or Mediterranean by origin.	Hybrid Tea (HT)	Exhibit traits midway between both parents: hardier than Teas, but less hardy compared to Hybrid Perpetuals and more recurrent blooming than the Hybrid Perpetuals, but less so than Teas. This group of roses is characterised by large, well-formed flowers. The flowering stalk terminates in a single bloom.	Initially created by hybridising Hybrid Perpetuals with Tea roses.
		Pernetiana	Contain a new range of flower colours with shades from apricot, yellow, copper and orange to scarlet. Flower colour was introgressed together with disease susceptibility and scentlessness.	Initiated by Joseph Pernet-Ducher in 1900, included genes from *R. foetida*, also known as the old Austrian briar rose.
		Polyantha	Disease-resistant garden roses covered with tiny red, pink or white flowers of 2.5 cm in diameter on average. Polyanthas are the rose group characterised by prolific bloom from spring till late fall.	Developed in the late 19th century in France. Polyanthas were originally derived from crosses between *R. chinenses* and *R. multiflora.*
		Floribunda (F)	Roses characterised by blooming with Polyantha profusion and Hybrid Tea floral colour range and shape.	In 1907 Danish breeder Dines Poulsen introduced Floribunda roses as a result of crosses between Polyantha's and Hybrid Tea.
		Grandiflora	Grandifloras are typically larger than Hybrid Teas and Floribundas with flowers clustered in small groups of three to five.	In the mid-20th century a new rose group Grandiflora was introduced in order to designate back-crosses between Floribundas and Hybrid Tea roses.
		Miniature (Min)	They represent a group of twiggy, repeat-blooming shrubs ranging from 15 to 92 cm in height.	They are result of crosses between miniature Old Garden Roses and repeat-blooming Asian species to produce ever blooming miniature roses.
		Climbers (LCL)	Most climbing roses grow 20–56 cm in height. They are characterised by continuous blooming.	In many cases they are result of spontaneous mutations.
		Shrubs (S)	This is not precisely defined as a rose class, but is commonly used in books and catalogues. Roses of this class tend to be robust, what makes them suitable for borders or hedging.	As this class is defined on the base on their growth type their pedigree is not simple and unique.
		Modern English Rose (MOE)	The MOE group of roses that featured blooms with old-fashioned shapes and fragrances, evocative of classic *gallica*, *alba* and damask roses, with repeat-blooming characteristics and the larger colour range as well.	The MOE group was developed in 1960 by David Austin. His idea was to combine flower shape and fragrance of Old Garden roses, mainly from *R. gallica, R. alba* and *R. damascena* with new flower colour range and recurrent flowering of Floribundas and Hybrid Teas.
		Canadian Hardy (Can)	These cultivars are extremely tolerant to low temperature and can withstand temperature of −35 °C. Additionally, all Canadian roses share a similar growing type: they are bushy, scentless cultivars that remind a lot of wild species. Flowers are simple with poor colour range, mostly shades of pink.	As a response to extreme weather conditions in Canada at the Morden Research Station in Morden and Experimental Farm in Ottawa were created rose cultivars from Explorer (CE) and Parkland (CP) Series. Canadian roses derived mostly from crosses of wild species *R. rugosa* and *R. arkansana* with other species or cultivars.
		Landscape (Ground Cover)	This class is developed mainly for mass amenity planting. They are susceptible to pests and diseases. They are characterised by repeat flowering, lower growing habit, usually under 61 cm. Interestingly, they are grown on their own roots.	In the late 20th century they are involved in market. Their pedigrees are not known.

Table 1 (*Continued*)

Group	Circumscription	Cultivar group	Morphology	Information on ancestry
		Patio (PATIO)	Since 1970s attention of many breeders has been focused on compact rose development. This group of roses is suitable for small gardens and terraces, combines characteristics of miniature roses and Floribundas. The class of shrubs is not precisely defined garden rose class. It includes some single. and repeat flowering cultivars which tend to be robust, making them recommended for use as shrub borders or hedging.	As this class is defined on the base on their growth type their pedigree is not simple and unique.
		Renaissance (Ren)	Renaissance rose is a group of large flower and extremely scented cultivars created by Danish breeder Poulsen. This class is often marked as a class of Hybrid Tea roses. Renaissance roses remind of MOE roses. They are characterised by recurrent blooming and disease resistance.	The little data are available for the Renaissance rose pedigrees. According to the literature, in their pedigrees are involved Floribundas (Avignon, Radox Bouquet, Evening Star). They are crossed with other cultivars or seedlings from Poulsen breeding programme. Interestingly, in many pedigrees of Renaissances is involved Claire Renaissance. Additionally, climbers such as Jazz and Shrubs (Queen Margaret) are involved in their pedigree.

Sources: Kruissmann (1981), Hessayon (2004), Thomas (2004), Encyclopedia Britannica (2012); http://historicroses.org (accessed 17.04.13); http://www.oldroses.co.uk (accessed 17.04.13); www.wikipedia.com (accessed 17.04.13).

cultivar phenotype (e.g., Paulsen, Harkness, Austin, and Noack). At the same time, the sources for other traits, such as winter hardiness, thornlessness, recurrent blooming, and patio growth type are limited, so breeders may use the same or similar germplasm and gene donors.

In a number of studies the genetic diversity between different horticultural groups of roses has been studied. Esselink et al. (2003) concluded that rootstock roses were clearly distinguished from the Hybrid Tea varieties using 24 microsatellites markers. Scariot et al. (2006) used 6 microsatellite markers to analyse differences between wild species and old garden roses, and produced a classification similar to that based on morphology. Differentiation among modern rose cultivars mostly has been evaluated on the basis of morphological traits. Smulders et al. (2009) studied genetic differentiation among cut rose cultivars and found that the genetic differentiation among 17 breeding companies was less than 1%, which indicated that all companies basically used the same cut rose gene pool.

Only few studies have compared garden rose cultivars, and these studies included only a small set of cultivars (Vainstein et al., 1993; Ben-Meir and Vainstein, 1994; Debener et al., 1996). Debener et al. (1996) found that cultivars did not cluster according to the groups to which their parents belong to and, similarly as previously had been reported (Vainstein et al., 1993), that the Hybrid Tea and the Floribunda groups share the highest genetic similarity. Ben-Meir and Vainstein (1994) also observed that Hybrid Tea and Floribunda cultivars shared least similarity with Miniature roses.

In this study we have determined the genetic differentiation among eleven types of European garden roses and two Canadian garden rose programmes, and thus also among breeders, using a large set of 110 cultivars. For comparison we have also included a small set of cut rose cultivars and rootstocks (28 in total). In order to be able to identify the footprint of introgression from specific wild species into certain types of modern cultivars, which would increase the diversity in certain areas of the genome, and that of selection, which may decrease diversity locally, we employed a set of microsatellite markers that tagged most of the chromosome arms. As an example of functional trait introgression we used winter hardiness.

2. Materials and methods

2.1. Plant materials and DNA extraction

A set of 94 European and 16 Canadian garden rose cultivars was studied. For comparison we also included 19 cut rose cultivars and 9 rootstock roses (Table 2). Genomic DNA was extracted from freeze-dried young leaves using the DNeasy Plant Mini Kit (Westburg, The Netherlands) following the protocol of Esselink et al. (2003).

Twenty plants of population 97/7 (95/13 – 39 × 82/78 – 1; Linde et al., 2006; Spiller et al., 2011) were used to determine the linkage group (LG) of 13 previously unmapped microsatellite markers (RA044b, RA023b, RMS082, RMS080, RMS017, RMS097, RMS034, RMS008, Rog9, Rog18, Rog27, Rog3, and Rog5) in JoinMap 4 (Van Ooijen, 2006).

2.2. Microsatellite marker genotyping

Microsatellite markers were chosen on the basis of the level of polymorphism they revealed. In total, 25 microsatellite markers, covering most linkage groups except LG3, were used to genotype all cultivars (Table 3). Genotyping was performed on an ABI 3730 DNA analyser (Applied Biosystems, Foster City, California) or a Li-Cor 4300 analyser (Li-Cor Biosciences, Lincoln, NE, USA). Amplification reactions used for ABI were performed in 10 μl containing 8 ng DNA, 5 μl multiplex kit (QIAGEN, Germany) and 4 pmol of each forward (labelled) and reverse primer. Amplification was under the following condition: an initial denaturation at 95 °C for 15 min following with 30 cycles of 94 °C for 30 s, ramp 1 °C/s to 50 °C, 50 °C for 30 s, ramp 1 °C/s to 72 °C, 72 °C for 120 s and final extension at 72 °C for 10 min. One μl of 100× diluted PCR product was mixed with Hi-Di formamide (Applied Biosystems) containing GeneScan-500 LIZ size standard (Applied Biosystems) and run on an ABI 3730 DNA analyser. Output from the ABI platform was analysed with Genemapper 4.0 software (Applied Biosystems).

The microsatellite reaction mixtures used for Li-Cor contained 10 ng genomic DNA, 2 μl 10× Tag PCR buffer, 0.2 mM of dNTP, 10 pmol of each (labelled) forward and reverse primer, 0.5 U of Tag polymerase, in a final volume of 20 μl. PCR conditions were initial denaturation at 94 °C for 180 s, then 35 cycles of 94 °C for 30 s,

Table 2
Description of Rose material and origin.

Group	Code	Name	Type	Breeder	Code	Name	Type	Breeder	Code	Name	Type	Breeder	Code	Name	Type	Breeder	Code	Name	Type	Breeder
European Garden Rose	E-1	Abraham Darby	MOE	Austin	E-20	Cygne Noir	HT	Unknown	E-39	James Galway	MOE	Austin	E-58	Papagena	HT	McGready	E-77	Snowdon	HRG	Austin
	E-2	Alan Titchmarsh	MOE	Austin	E-21	Desinger Sunset	F	Pearce	E-40	Kings Mac	HT	Fryer	E-59	Pat Austin	MOE	Austin	E-78	Songs of Praise	F	Harkness
	E-3	Amber Queen	F	Harkness	E-22	Diamond Border	S	Olesen	E-41	L'aimant	MOE	Harkness	E-60	Patricia Kent	MOE	Harkness	E-79	St. Alban	MOE	Austin
	E-4	Amelia Renaissance	REN	Olesen	E-23	Double Terrazza	Patio	De Ruiter	E-42	Lavander Dream	S	Austin	E-61	Pink Terrazza	Patio	De Ruiter	E-80	Summer Song	MOE	Austin
	E-5	Anna Purna	HT	Dorieux	E-24	Eglantyne	MOE	Austin	E-43	LD Braithwaite	MOE	Austin	E-62	Pearl Ambudance	F	Harkness	E-81	Sun Hit	S	Olesen
	E-6	Apple Blossom	HRG	Noack	E-25	Escopade	F	Harkness	E-44	Lemon Coture	S	Pearce	E-63	Penny Lane	LCL	Harkness	E-82	Sunset Boulevard	F	Harkness
	E-7	Astrid Lingren	F	Olesen	E-26	Evelyn	MOE	Austin	E-45	Leonardo da Vinci	F	Meilland	E-64	Perception	HT	Harkness	E-83	Sweet Dreams	S	Fryer
	E-8	Betty Harkness	F	Harkness	E-27	Ferdinand Pitchard	HP	Tanne	E-46	Lilian Baylis	MOE	Harkness	E-65	Perpetually Yours	LCL	Harkness	E-84	Teasing Georgia	MOE	Austin
	E-9	Buttercup	MOE	Austin	E-28	FP/1	Patio	De Ruiter	E-47	Madrigal	MOE	Harkness	E-66	Peter Cottrell	F	Harkness	E-85	Tivoli	HT	Olesen
	E-10	Caribia	HT	Wheatcroft	E-29	FP/2	Patio	De Ruiter	E-48	Margareth Merril	F	Harkness	E-67	Piccolo	F	Tantau	E-86	Velvet Fragnance	HT	Fryer
	E-11	Charles Darwin	MOE	Austin	E-30	FP/3	Patio	De Ruiter	E-49	Marjorie Marshall	MOE	Harkness	E-68	Pink Tiara	S	Perace	E-87	Violet Parfume	HT	Tantau
	E-12	Charlotte	MOE	Austin	E-31	Gentle Hermione	MOE	Austin	E-50	Mary Rose	MOE	Austin	E-69	Princess Alexandra	MOE	Olesen	E-88	White Lace	S	Austin
	E-13	Christopher Marlowe	MOE	Austin	E-32	Gertrude Jackyll	MOE	Austin	E-51	Mayflower	MOE	Austin	E-70	Princess of Wales	F	Austin	E-89	Wild Edric	HRG	Austin
	E-14	City of London	F	Harkness	E-33	Glowing Pink	S	Pearce	E-52	Mullard Jubilee	HT	McGready	E-71	Queen of Sweden	MOE	Austin	E-90	Wild Eve	MOE	Austin
	E-15	Claire Rose	MOE	Austin	E-34	Graciously Pink	S	Unknown	E-53	Nadia Renaissance	REN	Olesen	E-72	Samaritian	MOE	Harkness	E-91	Winchester Cathedral	MOE	Austin
	E-16	Climbing Bonica	LCL	Unknown	E-35	Graham Thomas	MOE	Austin	E-54	Nipper	MIN	Harkness	E-73	Sharifa Asma	MOE	Austin				
	E-17	Compassion	LCL	Harkness	E-36	Helene Renaissance	REN	Olesen	E-55	Nostalgie	HT	Tantau	E-74	Shephardess	MOE	Austin				
	E-18	Cream Abundance	F	Harkness	E-37	Heritage	MOE	Austin	E-56	Orange Terrazza	Patio	De Ruiter	E-75	Shropshire Lass	MOE	Austin				
	E-19	Crown Princess Margareta	MOE	Austin	E-38	Irish Hope	MOE	Harkness	E-57	Othello	MOE	Austin	E-76	Snow Goose	HRG	Austin				
CP[a]	CP-1	Adelaide Hoodless	CanP	Marshall	CP-2	Cuthbert Grant	CanP	Marshall	CP-3	Hope for Humanity	CanP	Collicutt	CP-4	Morden Amorette	CanP	Marshall	CP-5	Morden Centennial	CanP	Marshall
	CP-6	Winnipeg Parks	CanP	Marshall																
CE[b]	CE-1	Alexander McKenzie	CanE	Svejda	CE-2	David Thompson	CanE	Svejda	CE-3	Henry Kelsey	CanE	Svejda	CE-4	Jens Munck	CanE	Svejda	CE-5	Johan Franklin	CanE	Svejda
	CE-6	John Cabot	CanE	Svejda	CE-7	John Davis	CanE	Svejda	CE-8	JP Connell	CanE	Svejda	CE-9	Therese Bugnet	CanE	Bugnet	CE-10	William Baffin	CanE	Svejda
Cut rose	Cut-1	Lexmei/Dolce Vita+			Cut-2	Olijredsp/El Toro			Cut-3	Meivildo/Yves Piaget			Cut-4	Pekcoujenny/First Red			Cut-5	Tanotika/Akito		
	Cut-6	Ruiy 5451/Wow			Cut-7	Seliron/Bull's Eye			Cut-8	Korflapei/Frisco			Cut-9	Predesplen/Splendid Surprise			Cut-10	Selaurum/Grand Prix		
	Cut-11	Ruirovingt/Prophyta			Cut-12	Schrazuid/Limonchello !			Cut-13	Avalanche+			Cut-14	Schremma/Femma			Cut-15	Presur/Surprise		
	Cut-16	Briroro/Valentino			Cut-17	Interlis/Lydia			Cut-18	Korcilmo/Escimo			Cut-19	Brigold/Helio						
Rootstock rose	R-1	Drora			R-2	Moerex/1001			R-3	Heinsohn's Rekord			R-4	Ivtamar/1568			R-5	R.inermis 2		
	R-6	R.rubiginosa			R-7	Kiese			R-8	Smit's Stekelloze			R-9	R.rubrifolia Glauca						

Rose types: MOE, Modern English roses; F, Floribunda; REN, Renaissance; HT, Hybrid Tea; HRG, Hybrid Rugosa; LCL, Climbing roses; S, Shrubs; Patio, Patio roses; MIN, Miniature roses; CanE, Canadian Explorer roses; CanP, Canadian Parkland roses.

[a] Canadian garden roses Parkland Group.

[b] Canadian garden roses Explorer Group.

Table 3
Characteristics of the 25 microsatellite markers used in study.

Marker name	Repeat sequence	LG	A			G			C			R		
			(*n* = 138)			(*n* = 110)			(*n* = 19)			(*n* = 9)		
			A	AP	H_e	A	AP	H_e	A	AP	H_e	A	AP	H_e
RMS015[a]	GA	1	32	94	0.89	29	80	0.89	7	11	0.76	15	9	0.95
RMS047[a]	GA	1	18	55	0.80	17	47	0.80	3	3	0.62	12	9	0.91
RhD201[b]	(TCT)33	1	15	45	0.75	22	38	0.75	4	6	0.71	12	8	0.83
RMS062[a]	GA&GT	2	24	93	0.89	24	75	0.88	9	13	0.83	13	9	0.93
RhB303[b]	(GA)11	2	20	58	0.86	19	37	0.81	6	15	0.81	9	8	0.82
RhO506[b]	(CAG)6(CAA)18–7(CAG)6	2	20	76	0.88	17	61	0.86	5	6	0.68	12	9	0.90
RMS082[a]	2xGA	2[e]	17	39	0.74	13	31	0.73	3	5	0.56	12	9	0.90
RMS080[a]	GT	4[e]	18	45	0.78	17	39	0.77	3	3	0.67	9	7	0.85
RhAB40[b]	(TC)14(AC)11–1	4	35	89	0.90	32	72	0.89	8	10	0.79	11	9	0.94
RhD221[b]	(TCT)21–1	4	27	52	0.77	14	38	0.76	5	12	0.76	7	6	0.80
RMS029[a]	GA	5	19	43	0.76	16	33	0.75	6	7	0.65	11	9	0.92
RA044b[d]	(AG)14	5[e]	22	43	0.76	17	36	0.76	3	4	0.53	11	9	0.92
RA023b[d]	(GA)20	5[e]	16	81	0.85	14	65	0.85	7	11	0.83	9	9	0.86
RMS017[a]	AT&GT	6[e]	32	103	0.90	30	91	0.90	8	13	0.82	7	5	0.71
RMS097[a]	GA&GT	6[e]	13	20	0.62	9	15	0.61	2	3	0.50	7	7	0.69
RhE2b[b]	(TGT)26	6	20	62	0.87	18	53	0.86	5	6	0.66	7	8	0.82
Rog9[c]	(AG)13	6[e]	17	58	0.84	15	43	0.82	9	14	0.85	9	6	0.84
Rog18[c]	(AG) 17	6[e]	14	78	0.87	14	62	0.86	9	16	0.85	8	6	0.85
RMS003[a]	GA	7	24	70	0.87	20	59	0.86	6	9	0.75	10	6	0.92
RMS008[a]	GA	*[f]	21	50	0.79	17	40	0.77	4	5	0.70	10	9	0.82
Rog3[c]	(CT)8	*[f]	23	70	0.87	18	57	0.85	7	12	0.81	13	8	0.91
Rog5[c]	(GA)10	*[f]	21	55	0.82	20	45	0.81	5	7	0.71	13	9	0.94
RMS034[a]	GA	*[g]	29	66	0.83	22	58	0.83	4	5	0.68	15	9	0.93
Rog27[c]	(TG)10	*[g]	21	70	0.86	19	55	0.85	8	11	0.80	14	8	0.91
Average			21.6	58.6	0.8	18.9	65.4	0.8	5.7	8.6	0.7	10.7	8.0	0.9

Marker name	Repeat sequence	LG	EG			CG			CP			CE		
			(*n* = 94)			(*n* = 16)			(*n* = 6)			(*n* = 10)		
			A	AP	H_e	A	AP	H_e	A	AP	H_e	A	AP	H_e
RMS015[a]	GA	1	26	71	0.89	12	15	0.890	11	6	0.904	10	10	0.891
RMS047[a]	GA	1	15	40	0.78	10	14	0.857	6	5	0.808	10	10	0.887
RhD201[b]	(TCT)33	1	20	30	0.73	12	12	0.857	6	5	0.900	10	8	0.826
RMS062[a]	GA&GT	2	18	64	0.88	18	14	0.915	7	5	0.858	15	10	0.931
RhB303[b]	(GA)11	2	19	34	0.83	7	10	0.709	5	4	0.664	5	7	0.728
RhO506[b]	(CAG)6(CAA)18–7(CAG)6	2	14	53	0.84	13	13	0.881	11	6	0.923	9	8	0.869
RMS082[a]	2xGA	2[e]	13	31	0.74	6	5	0.569	4	4	0.586	4	3	0.570
RMS080[a]	GT	4[e]	12	30	0.75	11	12	0.878	6	5	0.821	10	8	0.899
RhAB40[b]	(TC)14(AC)11–1	4	28	64	0.89	19	14	0.910	7	5	0.904	15	10	0.927
RhD221[b]	(TCT)21–1	4	14	31	0.74	6	11	0.784	6	6	0.795	6	9	0.798
RMS029[a]	GA	5	11	24	0.71	13	14	0.892	6	6	0.768	11	9	0.912
RA044b[d]	(AG)14	5[e]	10	26	0.72	15	14	0.920	8	6	0.876	15	9	0.948
RA023b[d]	(GA)20	5[e]	13	57	0.85	10	14	0.813	9	6	0.894	7	9	0.753
RMS017[a]	AT&GT	6e	26	80	0.89	20	16	0.943	10	6	0.905	18	10	0.958
RMS097[a]	GA&GT	6[e]	5	9	0.57	8	12	0.815	3	3	0.547	8	9	0.832
RhE2b[b]	(TGT)26	6	17	46	0.85	10	14	0.882	7	5	0.851	9	10	0.886
Rog9[c]	(AG)13	6[e]	14	40	0.82	9	10	0.831	5	5	0.727	9	6	0.879
Rog18[c]	(AG) 17	6[e]	13	52	0.85	9	14	0.852	6	5	0.814	9	10	0.878
RMS003[a]	GA	7	19	48	0.85	14	14	0.900	11	6	0.906	11	10	0.899
RMS008[a]	GA	*[f]	15	33	0.76	9	13	0.815	6	5	0.825	7	9	0.809
Rog3[c]	(CT)8	*[f]	14	47	0.84	13	14	0.882	7	5	0.777	12	9	0.875
Rog5[c]	(GA)10	*[f]	15	35	0.79	13	14	0.886	7	5	0.807	11	9	0.903
RMS034[a]	GA	*[g]	18	47	0.81	17	16	0.914	9	6	0.865	16	10	0.945
Rog27[c]	(TG)10	*[g]	16	43	0.83	15	14	0.910	8	6	0.891	13	10	0.916
Average			16.0	43.7	0.8	12.0	13.0	0.9	7.1	5.3	0.8	10.4	8.9	0.9

LG, Linkage Group, A, All rose samples; G, Garden roses; C, Cut roses; R, Rootstock roses; EG, European garden roses; CG, Canadian garden roses; CP, Canadian garden roses Parkland group; CE, Canadian garden roses Explore group.
n, number of rose samples.
A, number of alleles; AP, number of allelic phenotypes; H_e, expected heterozygosity.
[a] Microsatellite markers for genetic analyses and the differentation of roses.
[b] Esselink et al. (2003).
[c] Meng et al. (2009).
[d] Kimura et al. (2006).
[e] Mapped in 97/7 population.
[f] Could not be mapped in 97/7 population.
[g] Not polymorphic in 97.7 population.

ramp to 55–58 °C (1 °C/s), 55–58 °C for 30 s, ramp to 72 °C (1 °C/s), 72 °C for 60 s and final extension at 72 °C for 7 min. The 20x diluted amplification products were analysed on a Li-Cor 4200 or 4300 analyser.

2.3. Data analysis

Even though there are methods to score SSRs co-dominantly, such as MAC-PR (Esselink et al., 2004), obtaining reliable results in sets of unrelated genotypes is often not possible for the majority of tested markers. We therefore scored presence or absence of individual alleles for each microsatellite locus (dominant scoring). The data were recorded into a binary data matrix (1 for present and 0 for absent) and for each locus the "allelic phenotype" was taken (Esselink et al., 2003; Becher et al., 2000; Park et al., 2010). To assess and visualise genetic relationships among genotypes, we used NTSYS version 2.10 to perform a principal coordinate (PCO) analysis. A PCO can visualise data from various ploidy level data (De Riek et al., 2007). For the diversity estimation we used fixation index (F_{st}) and expected heterozygosity (H_e). F_{st} is a measure of population differentiation (genetic distance) based on allele frequency differences among populations (Holsinger and Weir, 2009). H_e, also referred to as gene diversity, is the probability that two randomly chosen alleles at a locus within a set of genotypes will be different under Hardy–Weinberg equilibrium (i.e., assuming random mating). For the genetic differentiation (F_{st}) and expected heterozygosity (H_e) we used SPAGeDi 1.3, which also can analyse various ploidy level data (Hardy and Vekemans, 2002).

3. Results

3.1. The microsatellite markers

A total of 25 microsatellite markers, which produced clear alleles and showed a high degree of polymorphism, were selected for this study (Table 3). Markers Rog 9 and Rog 10 (Meng et al., 2009) gave identical genetic results. Comparison of primer sequences showed that the forward primer of Rog 9 (TCCTGAAAACGAAGCCTCC) is largely the same (underlined) as the reverse primer of Rog 10 (TTCCTGAAAACGAAGCCT) but a few bp shifted. As some alleles of Rog 10 showed weaker amplification, only Rog 9 was used for further analysis, hence we used the data of 24 microsatellite markers.

Some markers used in the study had not been mapped previously. Using the 97/7 population, marker RMS082 was mapped on linkage group (LG) 2, RMS080 and RA044b were mapped on LG4, and RA023b was mapped on LG5 together with Rog 9, Rog18, RMS017 and RMS097. Markers Rog 27 and RMS034 were not polymorphic in the 97/7 population, and thus they could not be mapped. Although Rog 3, Rog 5 and RMS008 were polymorphic in the 97/7 population, they remained unmapped using only 20 plants.

A total of 518 different alleles were observed across the 24 markers (Table 3), with an average of 21.6 alleles per marker. RhAB40 had the highest number of alleles (35 alleles), while RMS097 had the lowest (13 alleles). In total, 1515 allelic phenotypes (Esselink et al., 2003) were identified among the rose samples. The most discriminating locus was RMS017 with 103 different allelic phenotypes in the 138 genotypes analysed, i.e., 75% of the genotypes could be distinguished using this locus alone.

Gene diversity (H_e) ranged from 0.618 to 0.902. Markers with fewer alleles generally had lower H_e values except Rog18, which had 14 alleles but a H_e value of 0.867. This H_e value is comparable to values of markers with much higher numbers of alleles. An exception was also marker Rhd201, it had 27 alleles but the H_e value was with 0.753 relatively low. In the garden rose group, cut rose group and rootstock rose group the H_e value ranged from 0.611 to 0.902, 0.503 to 0.852, and 0.693 to 0.947 respectively. On the basis of a Mann–Whitney U test (Supplementary Table 1), H_e value differences between rootstocks and garden roses and between rootstocks and cut rose cultivars were highly significant ($P < 0.001$, two-tailed test), while the difference between garden roses and rootstocks was significant ($P < 0.001$, two-tailed test).

3.2. Distinction of cut roses, rootstocks and garden roses

The PCO analysis showed that cut roses and rootstock roses were clearly separated from garden roses (Fig. 1). Genetic differentiation among cut, garden and rootstock roses was moderate ($F_{st} = 0.052$; Supplementary Table 2). Cut rose and rootstock rose were the most distinct groups ($F_{st} = 0.132$). Garden roses showed more similarity with cut roses ($F_{st} = 0.042$), while their differentiation from rootstocks was higher ($F_{st} = 0.081$).

The genetic differentiation between rose groups varied among different linkage groups (LGs). LG2 showed the highest differentiation ($F_{st} = 0.074$) and three of the four markers on this linkage group had the highest genetic distance in certain pairwise comparisons. Genetic differentiation between cut roses and rootstocks were similar for all LGs, ranging from 0.137 for LG2 to 0.120 for LG6. Garden roses showed the highest differentiation from cut roses for LG2 (0.080). The highest differentiation between garden roses and rootstocks was found for LG6 ($F_{st} = 0.106$).

3.3. Private alleles

We defined private alleles as those that were characteristic for one group or set of cultivars and did not appear in other groups. Private alleles are indicative for larger genetic variation. European garden roses had private alleles for each microsatellite marker. Similarly, rootstock roses had private alleles for all microsatellite markers except Rog18 and RMS062. Cut roses did not have any private alleles (Supplementary Table 3). The complete absence of private alleles in the set of cut roses cannot be ascribed to the small size of this group (only 19 cultivars). Although they had a lower number of alleles for all loci compared to garden roses, they still revealed 103 unique allelic phenotypes. In addition, there were only 9 rootstock roses, and these had as many as 63 private alleles (on average 7 per cultivar). Rather, the absence of private alleles in the cut roses may be an indication that they contain a subset of the variation present in the garden roses.

Partly owing to the large number of samples, European garden roses had the largest number of private alleles (97; on average 0.94 private alleles per cultivar). Of the two Canadians garden rose groups, the Explorer group had more private alleles (33, on average 3.3 alleles per cultivar) than the Parkland group (10, on average 1.67 alleles per cultivar). Some private alleles in the rootstock rose group, the European garden rose group and in the Canadian Explorer group occurred in more than one plant, but only the European garden rose group included samples with more than one private allele in the same cultivar.

Comparing linkage groups it was notable that rootstocks had the largest number of private alleles on LG5 (10), while for European garden roses this was on LG6 (21). For Canadian Explorer roses the same number of private alleles (6) was found on LG2 and LG6. Canadian Parkland roses had most on LG1 (3). Cultivars of cut roses, European and Canadian Explorer garden roses also showed the largest number of unique allelic phenotypes on LG6. Rootstock roses contained the largest number of unique allelic phenotypes on LG2 and Canadian Explorer roses on LG5 (Supplementary Table 3).

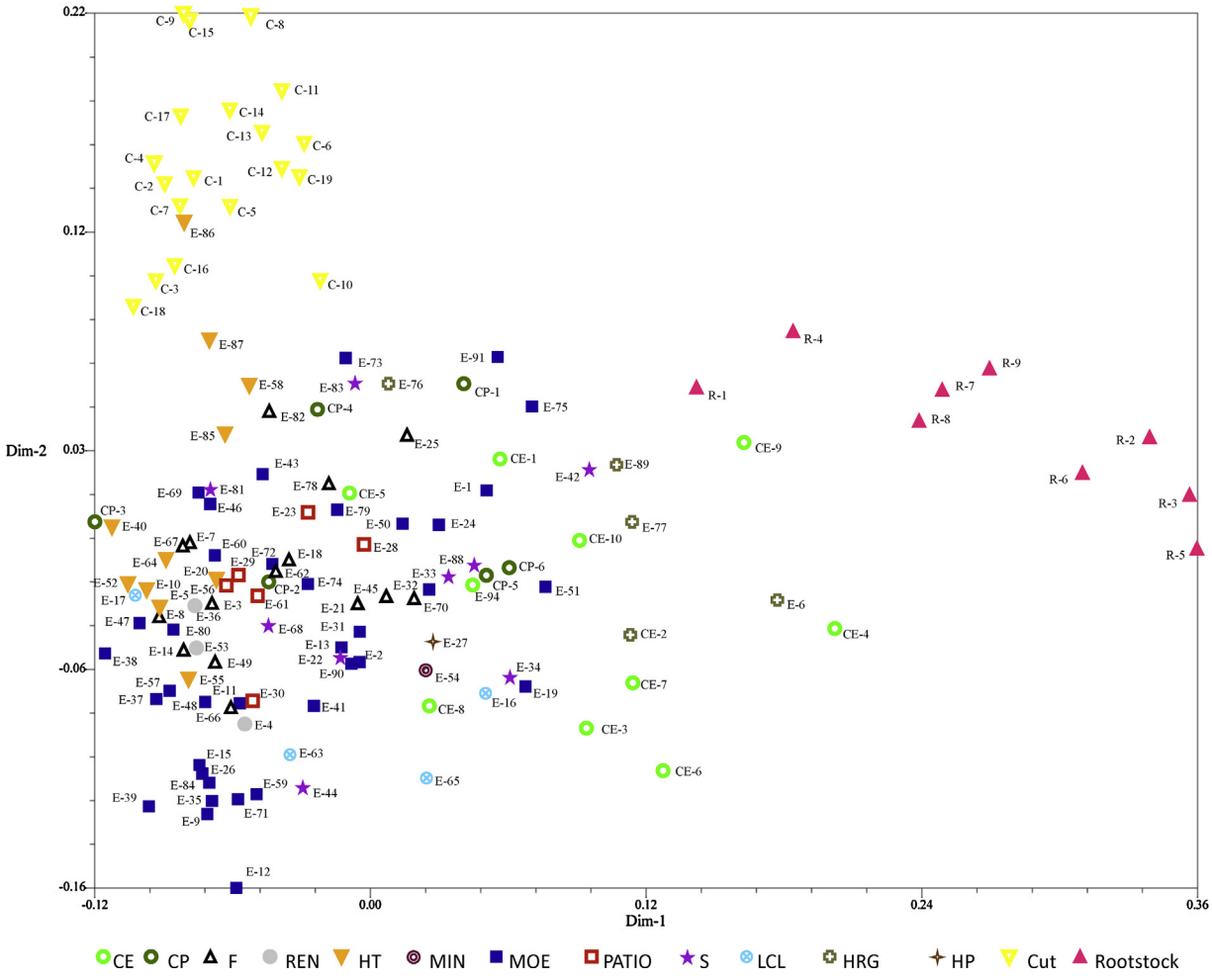

Fig. 1. PCO plot based on genetic distances among rose cultivars. PCO axes 1 and 2 explain 7.0% and 4.85% of the variation. CE, Canadian Explorer; CP, Canadian Parkland; F, Floribunda; REN, Renaissance; HT, Hybrid Tea; MIN, Miniature roses; MOE, Modern English roses; PATIO, Patio roses; S, Shrubs; LCL, Climbers; HRG, Hybrid Rugosa; HP, Hybrid Perpetual; Cut, Cut roses.

3.4. Distinction of different garden rose cultivars groups

For each garden rose type there are specific breeding goals. Usually, sources for those characteristics of interest are wild species or commonly used cultivars, which leads to the hypothesis that cultivars groups are also genetically differentiated from each other. The 110 garden rose cultivars used in this study belonged to seven different types: Canadian (CAN), Floribunda (F), Hybrid Tea (HT), Renaissance (REN), Hybrid Rugosa (HRG), English Modern Rose (MOE), and Shrubs (S). Cultivars of two Canadian breeding programmes, Canadian Explorer (CE) and Canadian Parkland (CP), are phenotypically similar and can be clearly distinguished from European garden roses on the basis of their pedigrees and characteristics. The main characteristic of Canadian roses is winter hardiness; phenotypically Canadian roses are similar to wild species. Due to the small number of cultivars, four groups of garden roses (Climbers (LCL), Miniature (MIN), Hybrid Perpetual (HP), and PATIO) have been excluded from the analysis per type (Fig. 2).

In the PCO analysis, cultivars of each garden rose type (MOE, CAN, HRG, HT, F, and S) clearly grouped together, but types largely overlapped (Fig. 1). Interestingly, 'Velvet Fragrance', one of the European garden roses cultivars, was positioned in the cut rose group, while the other European cultivars were distant from these. The Hybrid Tea's were the garden rose group that was closest to the Cut roses. The Canadian Parkland group overlapped with European cultivars, while the Canadian Explorer group was positioned close to the Rootstock roses, together with the Hybrid Rugosa's.

3.5. Genetic differentiation among garden roses

The differentiation among types of garden roses (F_{st} = 0.022, Table 4) was lower compared to the differentiation among cut, garden roses, and rootstocks. The largest F_{st} value (0.055) among garden rose types was found between Canadian Explorer and Hybrid Tea cultivars. In general, the Canadian Explorer group was the most differentiated from the rest of the groups, which is in agreement with the PCO. According to F_{st} values Renaissance roses fully overlap with Floribunda (F_{st} = 0.000), Modern English (F_{st} = 0.003), and Parkland roses (F_{st} = 0.007).

Comparative analysis of the two Canadian programmes (Table 4) showed that LG6 is most differentiated (F_{st} = 0.031). Overall, F_{st} values between Rootstock roses and each of the Canadian programmes was similar, except for LG5 and LG6, where Canadian Explorer showed respectively larger genetic differentiation from Rootstocks, while Rootstock rose and Canadian Parkland group had the highest differentiation for LG6 (F_{st} = 0.116). Interestingly, LG5 showed the lowest differentiation between Canadian Explorer and Rootstocks (F_{st} = 0.006). Canadian Explorer cultivars showed the most differentiation from European cultivars (Floribunda, Modern English roses, Hybrid Rugosasa, Renaissance, and Shrubs) for

Table 4
Genetic differentiation (F_{st}) among garden rose types.

Locus	LG	All[a]	Pairwise F_{st} values													
			CE-HT	CE-CP	CE-F	CE-MOE	CE-HRG	CE-REN	CE-S	CP-HT	CP-F	CP-MOE	CP-HRG	CP-REN	CP-S	F-HT
RhD201	1	0.004	−0.007	0.005	0.024	0.013	−0.022	−0.057	−0.008	0.041	0.061	0.055	−0.017	−0.097	−0.005	−0.001
RMS015	1	0.031	0.033	−0.008	0.016	0.012	0.017	−0.022	0.048	0.011	−0.004	0.013	−0.004	−0.014	0.0249	0.0047
RMS047	1	0.018	0.056	−0.006	0.023	0.010	−0.014	−0.026	0.033	0.026	0.003	0.006	−0.019	−0.001	0.0183	0.01
Average		0.018	0.027	−0.003	0.021	0.011	−0.006	−0.035	0.024	0.026	0.020	0.025	−0.014	−0.037	0.013	0.005
RMS082	2	0.017	0.023	0.048	0.063	0.065	0.020	0.065	−0.014	−0.022	−0.015	−0.006	−0.002	0.016	0.0222	0.0111
RhB303	2	0.027	0.043	0.067	0.050	0.075	0.076	0.045	0.013	0.008	−0.001	0.043	0.1964	−0.100	0.0434	−0.023
RhO506	2	0.028	0.030	−0.023	0.026	0.044	0.008	0.030	0.050	0.051	0.037	0.054	−0.037	0.040	0.0218	0.0008
RMS062	2	0.018	0.044	0.035	0.012	0.010	−0.004	0.009	−0.003	−0.003	0.028	0.008	0.0281	0.025	0.066	0.0154
Average		0.023	0.035	0.031	0.038	0.049	0.025	0.037	0.012	0.008	0.012	0.025	0.046	−0.005	0.038	0.001
RhD221	4	0.017	0.003	−0.028	0.052	0.040	−0.009	0.054	0.035	−0.002	0.052	0.032	−0.033	0.068	0.035	0.0139
RMS080	4	0.021	0.056	0.012	0.042	0.043	0.019	0.031	0.029	0.026	0.009	0.011	−0.011	0.015	0.0147	0.0044
RhAB40	4	−0.006	−0.022	−0.020	−0.002	0.000	0.009	0.011	0.001	−0.018	−0.035	−0.023	−0.038	0.024	−0.018	−0.01
Average		0.011	0.013	−0.012	0.031	0.028	0.007	0.032	0.022	0.002	0.009	0.007	−0.027	0.036	0.011	0.003
RA044b	5	0.028	0.093	−0.009	0.046	0.073	−0.011	0.026	0.075	0.040	−0.001	0.026	0.039	0.033	0.0201	−0.006
RA023b	5	0.021	0.077	0.015	0.041	0.029	0.102	0.101	0.066	0.044	0.017	0.019	−0.037	0.023	0.0186	−0.002
RMS029	5	0.043	0.180	0.061	0.112	0.068	0.043	0.095	0.078	0.049	−0.003	−0.012	0.0208	−0.029	−0.02	0.0091
Average		0.031	0.117	0.022	0.066	0.057	0.045	0.074	0.073	0.045	0.004	0.011	0.008	0.009	0.006	0.000
Rog9	6	0.020	0.029	0.035	0.016	0.022	0.034	0.075	0.021	−0.002	0.041	0.026	0.1328	0.142	0.042	0.0221
RMS097	6	0.048	0.163	0.081	0.180	0.112	−0.012	0.030	0.117	−0.005	−0.012	−0.007	−0.056	−0.109	0	−0.011
Rog18	6	0.031	0.054	0.016	0.024	0.038	0.041	0.059	0.032	0.070	0.051	0.065	0.0908	0.068	0.1194	0.008
RMS017	6	0.005	0.033	−0.009	0.023	0.017	0.002	−0.013	0.003	0.000	0.004	−0.002	0.0022	−0.008	−0.017	
RhE2b	6	0.011	0.002	0.033	0.016	0.023	0.048	−0.018	−0.015	0.020	0.019	−0.001	0.0108	−0.015	−0.001	0.0264
Average		0.023	0.056	0.031	0.052	0.042	0.023	0.027	0.031	0.017	0.021	0.016	0.036	0.016	0.029	0.009
RMS003	7	0.021	0.069	0.002	0.039	0.056	0.018	0.027	0.019	0.036	0.018	0.034	0.0458	0.004	0.011	0.0018
RMS008	–	0.051	0.073	0.021	0.060	0.029	−0.007	0.038	0.139	0.006	0.012	0.005	0.0223	−0.014	0.0789	0.0195
Rog3	–	0.024	0.106	0.012	0.059	0.041	0.012	0.065	0.013	0.015	−0.018	−0.010	0.0219	−0.003	0.0032	0.0382
Rog5	–	0.014	0.063	0.137	0.040	0.039	−0.016	0.031	0.014	0.019	−0.004	0.015	0.037	−0.019	−0.013	0.0022
RMS034	–	0.022	0.050	−0.005	0.034	0.043	−0.006	0.021	0.037	0.010	−0.009	0.010	−0.007	0.039	0.0083	−0.008
Rog27	–	0.018	0.047	0.031	0.033	0.023	−0.005	0.040	0.024	0.028	−0.005	0.010	0.0252	0.002	0.0401	0.0158
ALL LOCI		0.022	0.055	0.015	0.042	0.038	0.011	0.030	0.034	0.019	0.0109	0.016	0.0217	0.0073	0.0218	0.0063
Jackknifed estimators (over loci)																
Mean		0.022	0.055	0.015	0.042	0.038	0.011	0.030	0.034	0.019	0.011	0.016	0.0217	0.007	0.0218	0.0063
SE		0.003	0.010	0.006	0.007	0.005	0.0048	0.0078	0.0079	0.0048	0.0051	0.0048	0.0126	0.0103	0.007	0.0028

Locus	LG	All[a]	Pairwise F_{st} values													
			F-REN	F-MOE	F-HRG	F-S	HT-REN	HT-HRG	HT-MOE	HT-S	REN-HRG	REN-MOE	REN-S	HRG-MOE	HRG-S	MOE-S
RhD201	1	0.004	−0.039	−0.005	0.0866	−0.01	−0.047	0.0404	−0.003	−0.014	−0.023	−0.038	−0.09	0.0661	0.025	−0.007
RMS015	1	0.031	0.0156	0.0235	0.0351	0.0444	0.0296	0.0789	0.0426	0.0667	0.0206	0.0162	0.0329	0.0204	0.0531	0.0701
RMS047	1	0.018	0.0167	0.008	0.0395	0.0042	0.0733	0.0636	0.0366	0.0336	−0.008	−0.021	0.0193	0.029	0.0341	0.0211
Average		0.018	−0.002	0.009	0.054	0.013	0.019	0.061	0.026	0.029	−0.003	−0.014	−0.013	0.039	0.037	0.028
RMS082	2	0.017	−0.019	−0.004	0.0367	0.0425	0.0227	0.0639	0.0089	0.0082	−0.031	−0.028	0.0327	0.0291	0.0551	0.0432
RhB303	2	0.027	−0.008	0.012	0.051	0.013	0.0015	0.0194	0.0039	0.0017	0.0196	0.0262	0.0263	−0.019	0.033	0.0332
RhO506	2	0.028	−0.033	−0.003	0.032	0.0477	−0.024	0.0713	0.0109	0.0996	0.0248	−0.025	0.0494	0.0344	−0.01	0.052
RMS062	2	0.018	−0.01	0.0185	0.0112	−0.005	−0.005	0.05	0.0172	0.0596	0.0355	0.0066	0.0106	0.0003	−0.005	0.0286
Average		0.023	−0.017	0.006	0.033	0.025	−0.001	0.051	0.010	0.042	0.012	−0.005	0.030	0.011	0.018	0.039
RhD221	4	0.017	0.0003	0.0053	0.0509	0.0193	0.0001	−0.021	0.0073	0.0058	0.0718	−0.006	0.0222	0.0437	0.0163	0.0031
RMS080	4	0.021	−0.012	0.004	0.0283	0.0239	0.0127	0.0606	0.0097	0.0483	0.0404	−0.013	0.0129	0.037	0.0396	0.023
RhAB40	4	−0.006	0.0226	−0.01	0.0016	0.006	0.0044	−0.001	−0.008	−0.01	0.0605	0.0207	−0.026	0.006	0.0086	0.0077
Average		0.011	0.004	0.000	0.027	0.016	0.006	0.013	0.003	0.015	0.058	0.001	0.003	0.029	0.022	0.011
RA044b	5	0.028	0.0382	0.003	0.0538	0.0099	0.1278	0.1217	0.0036	−0.019	0.1019	0.0538	0.1123	0.1047	0.1169	−0.006
RA023b	5	0.021	−0.035	0.0076	−0.015	0.0228	−0.058	−0.03	0.0238	0.0205	−0.076	0.0155	−0.004	−0.018	−0.02	0.0125
RMS029	5	0.043	−0.035	0.0138	0.0195	0.0003	−0.025	0.0855	0.0366	0.0607	−0.019	−0.025	−0.021	0.0151	−0.004	0.0118
Average		0.031	−0.011	0.008	0.019	0.011	0.015	0.059	0.021	0.021	0.002	0.015	0.029	0.034	0.031	0.006
Rog9	6	0.020	0.0289	0.0064	0.031	0.0307	0.1232	0.0961	0.0324	0.0485	0.2291	0.0107	0.0051	0.0565	0.127	0.0064
RMS097	6	0.048	−0.057	0.011	0.038	0.0263	−0.034	0.0331	0.0136	0.0572	−0.117	−0.072	−0.083	−0.036	−0.014	0.005
Rog18	6	0.031	0.0621	0.0085	0.0523	0.0319	0.0643	0.0666	0.0138	0.068	0.096	0.0144	0.0818	0.0335	0.0792	0.0224
RMS017	6	0.005	0.0079	0.0094	0.0367	−0.005	0.0353	0.0332	0.0004	−0.006	0.0064	0.0257	−0.01	0.0242	0.0082	−0.004
RhE2b	6	0.011	−0.012	0.0034	0.0551	0.0012	−0.017	0.0186	0.026	−0.019	0.1065	−0.008	−0.04	0.047	0.0405	0.0013
Average		0.023	0.006	0.008	0.043	0.017	0.034	0.050	0.017	0.030	0.064	−0.006	−0.009	0.025	0.048	0.006
RMS003	7	0.021	−0.02	−0.006	0.0501	0.0008	0.0072	0.0896	0.0038	0.0332	0.0165	−0.005	−0.021	0.0692	0.0172	0.0231
RMS008	–	0.051	0.0002	0.0401	0.0374	0.052	−0.013	0.0525	0.0087	0.0814	0.016	−0.01	0.0783	0.0226	0.099	0.1132
Rog3	–	0.024	0.0131	0.005	0.0575	−0.003	0.0398	0.0732	0.0248	0.0463	−0.055	0.0042	0.0293	0.02	0.0188	0.0113
Rog5	–	0.014	−0.027	0.006	0.0286	−0.002	0.0091	0.0442	−0.004	0.0051	0.0247	0.0048	−0.011	0.0364	−0.007	0.0012
RMS034	–	0.022	0.0516	0.0005	0.032	0.0303	0.066	0.046	0.000	0.0528	0.0716	0.0705	0.0422	0.0337	0.039	0.0584
Rog27	–	0.018	0.0019	0.0014	0.0235	0.0193	−0.02	0.0598	0.0229	−0.004	0.0328	0.016	−0.001	0.0027	0.0207	0.0168
ALL LOCI		0.022	0.0001	0.0067	0.0363	0.0169	0.0175	0.0505	0.0141	0.0307	0.0317	0.0034	0.0122	0.0278	0.033	0.231
Jackknifed estimators (over loci)																
Mean		0.022	0.0002	0.0067	0.0363	0.0169	0.0175	0.0505	0.0141	0.0307	0.0319	0.0035	0.0123	0.0278	0.0331	0.0232
SE		0.003	0.006	0.0021	0.0042	0.0039	0.0099	0.0074	0.0029	0.0072	0.0144	0.0057	0.009	0.006	0.0082	0.0058

[a]All: all garden rose types; CE, Canadian Explorer; CP, Canadian Parkland; HT, Hybrid Tea; F, Floribunda; MOE, Modern English Roses; HRG, Hybrid Rugosa; REN, Renaissance Roses; S, Shrubs.

Fig. 2. Representatives of garden rose types used in study displaying variation in flower (colour, shape, number of petals, architecture) and leaf (number, shape, colour) characteristics, and growth type. REN, Renaissance rose; LCL, Climbers; CE, Canadian Explorer; MIN, Miniature rose; MOE, Modern English rose; HT, Hybrid Tea; F, Floribunda; Patio, Patio rose; S, Shrub; HRG, Hybrid Rugosa; CP, Canadian Parkland.

LG5 (F_{st} = 0.045–0.074), while LG4 had the lowest F_{st} value for comparisons between Parkland roses and Hybrid Rugosas (−0.0027), Modern English roses (0.007), and Floribundas (0.009). The LG1 of Modern English roses showed most differentiation among linkage groups in comparison with Canadian Parkland (F_{st} = 0.025), Floribunda (F_{st} = 0.009), Hybrid Tea (F_{st} = 0.026), and Hybrid Rugosa (F_{st} = 0.039), while Shrubs, with an exception of Canadian Parkland cultivars, which showed the most differentiation for LG2.

3.6. *Genetic differentiation (F_{st}) between breeders*

Most breeders are specialised in breeding of roses with specific characteristics. Usually they use a specific gene pool as donor of a

Table 5
Genetic differentiation (F_{st}) among breeders.

Locus	LG	Among all (Austin, Harkness, Svejda, Olesen)	Among Austin, Harkness, Olesen	Between Austin and Harkness	Between Austin and Olesen	Between Harkness and Olesen
RhD201	1	0.018	−0.002	0.003	−0.010	−0.021
RMS015	1	0.017	0.024	0.025	0.036	−0.003
RMS047	1	0.007	0.007	0.010	0.003	0.000
Average		0.014	0.010	0.013	0.010	−0.008
RMS082	2	0.017	0.006	0.011	−0.002	−0.010
RhB303	2	0.051	0.042	0.041	0.054	0.026
RhO506	2	0.024	0.002	0.005	−0.005	−0.007
RMS062	2	0.026	0.033	0.039	0.035	0.001
Average		0.029	0.021	0.024	0.020	0.003
RhD221	4	0.026	0.016	0.021	0.000	0.023
RMS080	4	0.016	0.006	0.009	0.001	0.000
RhAB40	4	−0.003	−0.004	−0.002	−0.012	0.000
Average		0.013	0.006	0.009	−0.004	0.008
RA044b	5	0.039	0.025	0.006	0.063	0.040
RA023b	5	0.023	0.009	0.009	0.014	0.003
RMS029	5	0.041	0.022	0.024	0.029	−0.010
Average		0.034	0.018	0.013	0.035	0.011
Rog9	6	0.020	0.005	−0.002	0.010	0.025
RMS097	6	0.062	0.031	0.041	0.006	0.018
Rog18	6	0.019	0.006	0.006	0.000	0.010
RMS017	6	0.008	0.007	0.010	0.009	−0.005
RhE2b	6	0.010	0.009	0.013	0.007	−0.005
Average		0.024	0.011	0.014	0.006	0.009
RMS003	7	0.023	0.005	0.008	−0.003	0.004
RMS008	–	0.018	0.015	0.022	0.010	−0.002
Rog3	–	0.033	0.024	0.020	0.021	0.050
Rog5	–	0.012	−0.001	−0.001	0.002	−0.004
RMS034	–	0.013	0.006	0.003	0.016	0.001
Rog27	–	0.016	0.013	0.015	0.013	−0.002
ALL LOCI		0.022	0.013	0.014	0.013	0.006
Jackknifed estimators (over loci)						
Mean		0.022	0.013	0.014	0.013	0.006
SE		0.003	0.003	0.003	0.004	0.004

specific trait, which may include wild species, existing cultivars and seedlings from their breeding programmes. As a result, cultivars from different breeders are well distinguished in morphology. We have quantified the genetic differentiation among breeders using 73 cultivars from the breeding programmes of Austin (A), Harkness (H), Olesen (O) and Svejda (S). Cultivars from the Austin breeding programme mostly included Modern English roses, while cultivars from Svejda involved Canadian roses of the Explorer series. Most of Harkness' roses belong to Modern English and Floribunda types, while roses from the Olesen breeding programme are of the Shrub, Renaissance, Modern English, and Hybrid Tea types. Only few cultivars in this study were from Marshall (M), Noack (N), and Pearce (P) and these were not included in this analysis.

Genetic differentiation among cultivars of different breeders was moderate with an overall F_{st} value of 0.022 (Table 5), which is the same value as the differentiation among types. The set of Svejda cultivars showed the least similarities with Austin roses (F_{st} = 0.035), while the level of differentiation between Svejda and Harkness (F_{st} = 0.05) and Svejda and Olesen roses (0.04) was at the same level. The largest differentiation among European cultivars (F_{st} = 0.014 between Harkness and Austin roses) was much lower than that between any of them and the Canadian' Svejda roses. Differentiation between Harkness' and Olesen's cultivars (F_{st} = 0.006) was almost zero, indicating that a similar gene pool was used for breeding. Comparing linkage groups, among all breeders by far the largest differentiation was present on LG5 (F_{st} = 0.034). Comparison of pairs of breeders showed that between Austin and Harkness cultivars LG2 was most differentiated (F_{st} = 0.024), while Olesen's cultivars are most differentiated from Austin's and Harkness's for LG5 (F_{st} = 0.035 and F_{st} = 0.011 respectively).

4. Discussion

4.1. Genetic diversity

In this study we have compared the genetic diversity in various types of garden roses with that of cut roses and rootstocks as outgroups. Of these three groups, the rootstocks showed the highest value of expected heterozygosity (or gene diversity) (H_e = 0.86), while it was somewhat lower in garden roses (H_e = 0.82) and considerably lower in cut roses (H_e = 0.73). Nybom (2004) showed that levels of heterozygosity can be compared across taxa, provided the markers are equally polymorphic and scored in the same way (dominantly or co-dominantly). We can add that the taxa should have the same ploidy level. This precludes a comparison of our study with studies on cultivated varieties of diploid Rosaceae, such as peach commercial varieties (H_e = 0.46; Aranzana et al., 2010), almond commercial varieties (H_e = 0.67; Rigoldi et al., 2011), and sweet cherry cultivars (H_e = 0.55; Marti et al., 2012). Peach is partly selfing, but the lower values found in almond and sweet cherry may be due to the lower ploidy level. We can compare with Esselink et al. (2003) who used 24 microsatellites to study the diversity among rootstock and cut roses and also found that rootstocks had a significantly higher gene diversity than cut roses.

Consistent with lower gene diversity, cut roses had the smallest number of alleles across all loci. Importantly, they contained only few alleles (6 out of 147) that were also not present in garden roses. This suggests that cut rose germplasm is a subset of the germplasm present in garden roses, even though as a group they are differentiated, and in the PCO plot (Fig. 1) they are clearly distinct from garden roses. The rootstock roses had many unique alleles, which

indicates that they form a separate gene pool. Indeed, it is known that their progenitors have not been involved in garden rose and cut rose breeding (Phillips and Rix, 2004).

4.2. Genetic differentiation of garden rose types

Based on our set of cultivars we found the highest similarity between Renaissance, Modern English, Floribunda, and the Canadian Parkland cultivars. These findings are in agreement with what is known about the pedigrees of these types of roses and confirms that the same genepool was used in breeding. For example, the small group of Renaissance roses is positioned in the PCO between Floribunda and Hybrid Tea roses. This position is not surprising as both Floribunda and Renaissance contain Hybrid Tea roses in their ancestry (Phillips and Rix, 2004). In only few studies cultivars from different rose types have been compared. The genetic similarity of Hybrid Tea and Floribunda had also been observed by Vainstein et al. (1993), Ben-Meir and Vainstein (1994), and Debener et al. (1996). They also observed that the Miniature roses were genetically most distant, but we did not include a sufficient number of miniature roses to be able to confirm this.

Our data showed that Hybrid Tea roses are close to cut rose cultivars. If we look more carefully to their pedigrees, Hybrid Tea roses were derived from crosses between Tea and Hybrid Perpetuals. The Hybrid Perpetuals combined Old European and Asian wild species and cultivars such as: Hybrid Chinas, Hybrid Bourbons, Hybrid Noisettes, *R. alba*, *R. centifolia*, *R. gallica*, and *R. chinenses* (Thomas, 2004). Modern cut roses were obtained by crossing Chinese roses with Bourbon and Hybrid Perpetuals (Zlesak, 2007). Thus, cut rose and Hybrid Tea varieties share a largely similar gene pool. Indeed, some Hybrid Tea roses are phenotypically close to cut roses and also used as cut roses. In our study this is exemplified by 'Velvet Fragrance', a Hybrid Tea cultivar that in the PCO has a position among the Cut Roses. This cultivar was used both for cut flower production and in gardens.

We included cultivars from two different Canadian breeding programmes, both bred with the purpose of creating cultivars that were very winter hardy Richer et al. (2000). The Canadian Explorer group used introgressions of germplasm from various wild species. It indeed had the largest gene diversity value (0.876) of all garden rose types, and it had many unique alleles (on average more than 3 per cultivar). The Parkland group cultivars were made using European founders, mostly Pernet-Ducher cultivars. Compared to European garden roses, Parkland roses had 15 unique alleles (out of 181); these alleles can present a species contribution/introgression. Indeed, the F_{st} between Parkland and the European garden rose types was small (0.010 overall) while that of the Explorer roses was 0.036. In the PCO the Explorer roses and Rugosa types are found closest to the Rootstocks, which may reflect the introgression of rootstocks (*R. arkansana*) and *R. rugosa* into the Explorer roses.

4.3. Differentiation among breeders

The results showed that the genetic differentiation among breeders is the same as that among garden rose types (F_{st} = 0.022). This is not unexpected if we keep in mind that each breeder is specialised in breeding of a specific type of roses with one or a few specific traits. Even if a breeder brings different rose types to the market he probably still used the same parents/genepool. Hence, basically, in garden roses horticultural groups and breeders overlap. The level of genetic differentiation is fourfold the value among breeders of cut roses (F_{st} = 0.0056, Smulders et al., 2009).

According to PCO plot Austin's Modern English cultivars represent the modern rose type characterised by the largest genepool. The set of Austin's cultivars may be divided into five subgroups based on their origin. The English old rose hybrids were the original English roses and they are characterised by pink, crimson or purple flower colour and strong fragrance, such as 'Eglantyne', 'Gentle Hermione', and 'Sharifa Asma'. In the pedigrees of the English Leander roses Old rose hybrids and Tea roses are involved, which enabled flower colour range improvement, while the fragrance is still strong (e.g., 'Alan Titchmarsh', 'James Galway', 'Pat Austin'). The English musk hybrids are the result of crosses between Old rose hybrids and Noisettes roses and they are characterised by soft fragrance and flower colour ('Heritage', 'Wildeve', 'Graham Thomas'). The English alba hybrids originated from crosses between English and Alba roses and their phenotype reminds of Wild rose growth type ('Shropshire lass', 'The Alexandra rose'). Finally, there is a group of Modern English cultivars that do not fit in some of the earlier mentioned subgroups, such as 'Princess Anne' and 'Wild Edric' (Austin, 2012). To sum up, mainly, there are five strategies in developing Modern English roses and for this purpose four sources of donors have been used. The selection for specific phenotype characteristics (flower architecture, fragrance, etc.) led to the similarity in phenotype of Modern English cultivars.

4.4. Evidence of introgression/functional variation

Twenty-four microsatellite markers were used in this study in order to be able to determine genetic diversity and differentiation for separate linkage groups. We were interested in this, as introgression would be expected to increase the genetic diversity in terms of number of alleles, while selection for specific phenotypes would be expected to reduce the number of alleles and increase the differentiation between garden rose types. Thus, differences between LGs may reflect introgression events and selection pressure during breeding.

The highest diversity (most alleles and most allelic phenotypes) for cut roses and garden roses were found on linkage group (LG) 6. So far it has been observed that on LG6 several QTLs are located for days to flowering (Dugo et al., 2005), leaf colour and growth rate (Yan et al., 2007). These are traits of general interest, and can be found in wide germplasm, not just in a single source. Additionally, breeders of pot roses are focused on developing rose genotypes characterised by earlier blooming. Until now breeders have been working on reducing numbers of days to flowering by combining different rootstocks during a process of budding and they succeeded to reduce the period to 2 weeks.

Overall, LG2 was by far the most differentiated among types of garden roses, which may indicate that during breeding selection for several traits has affected loci on this linkage group. QTLs for flower size, leaf size (Dugo et al., 2005), vigour and leaf colour (Yan et al., 2007), and inflorescence architecture (Kawamura et al., 2011) are all located on LG2. Indeed, beside fragrance, rose breeders are mainly focused on flower (size and shape/architecture) and leaf (looking for big, shiny, dark green leaf) characteristics.

The highest differentiation between the Canadian programmes and European garden roses that are different in winter hardiness was found on LG5. Interestingly, the Canadian Explorer series was most similar to the rootstock roses, which are also winter hardy roses, on LG5. Furthermore, Olesen is a breeder whose set of cultivars includes the few European winter hardy genotypes. The largest differentiation between Olesen's and the European cultivars of Austin's and Harkness, which are quite susceptible to low temperatures, was also found on LG5. These observations would suggest that LG5 may contain an important QTL for winter hardiness.

The largest differentiation between Austin's and Harkness' cultivars is also noted on LG5. This LG is also the location of various QTLs mainly related to plant vigour (flower and leaf size, number of shoots, nodes and internodes, shoot and internode length, leaf and stem dry weight and grow rate; Dugo et al., 2005; Yan et al.,

2007; Kawamura et al., 2011). The large differentiation observed for this LG would suggest that there are differences in growth vigour between cultivars of these two breeders. Indeed, Austin roses are shrubby genotypes and some are even climbers. In sharp contrast, cultivars of Harkness are shorter and more compact, with a few exceptions such as 'Madrigal' and 'Penny Lane'.

5. Conclusion

Genetic differentiation among all types of garden roses was four times that among cut roses, and similar in magnitude to the differentiation among breeders, due to the fact that horticultural groups and breeders overlap largely in classification. Our results indicate that, in terms of neutral genetic diversity, cut roses represent a subset of garden roses. Our study employed a larger number of markers (24) covering most linkage groups, and using this strategy we could assess that the differentiation varies between linkage groups. This leads us to suggest that LG5 is an important linkage group containing possible QTLs for winter hardiness. LG6 contains the largest amount of genetic diversity, while LG2 is the most differentiated among the garden rose types, which may be indicative of introgression from wild species and selection by breeders. We expect that future studies using denser marker maps or next generation sequencing will uncover more differences among linkage groups within the garden rose germplasm.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2013.08.015.

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