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Morphological and AFLP-based Differentiation within the Taxonomical Complex Section *Caninae* (subgenus *Rosa*)

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• *Background and Aims* The taxonomical structure of the polymorphic subgenus *Rosa* section *Caninae* is highly complex due to the combination of some unusual features: the unique polyploid chromosomal constitution, the heterogamic canina meiosis, the ability to hybridize interspecifically, and the predominantly matroclinal inheritance. Although most taxonomists agree on the subdivision of the section into three morphologically well-defined groups (*Rubigineae*, *Vestitae*, and *Caninae*), they disagree on the existence of smaller groups such as *Tomentellae*. The aim was to gain insight in the taxonomical structure and investigate the interpopulation differentiation of the polymorphic section *Caninae* by analysing morphological and AFLP-based characters of the seven most common Belgian dog-rose taxa.
• *Methods* The intersubsectional and -specific relationships within the dog-roses were examined using morphological and molecular-genetic markers. AFLP data were analysed with basic descriptive genetic statistics because of the lack of Hardy–Weinberg equilibrium due to the polyploid genetic structure and heterogamic meiosis.
• *Key Results* Both the morphological and AFLP-based analyses supported the subdivision of the dog-roses in three well-defined though partly overlapping groups, *Rubigineae*, *Vestitae* and *Caninae*. However, it was not possible to distinguish between the morphologically well-defined taxa within the same subsection using AFLP-based data. In addition, the results suggested a high similarity of *Rosa balsamica* with subsection *Caninae* taxa. Small-scale geographical AFLP-based differentiation was observed within several dog-rose taxa. Surprisingly, individuals sampled at one locality and belonging to morphologically distinct dog-rose taxa displayed higher genetic similarities in comparison to their congeners sampled at different localities.
• *Conclusions* The hybridogenic character of the dog-roses was reflected in the vague boundaries between the subsections and on the species level within the subsections. Indications were found for current or historical hybridization on the genetic structure of the population. No morphological or AFLP-based evidence was obtained to support the existence of the separate subsection *Tomentellae*.

Key words: Subgenus *Rosa*, *Caninae*, *Rubigineae*, *Vestitae*, *Tomentellae*, *Rosa balsamica*, dog-rose, polyploidy, interspecific hybridization, intraspecific morphological variation, AFLP-based diversity, taxonomy.

INTRODUCTION

The genus *Rosa* L. (Rosaceae) is naturally distributed throughout the temperate and subtropical regions of the northern hemisphere (Rehder, 1940). Worldwide, the genus comprises between 100 and 250 botanical species, while about 30–60 endemic species are located in Europe (Henker, 2000). The genus *Rosa* consists of four subgenera of which the largest subgenus *Rosa* L. is subdivided into ten sections. The most numerous and complex section in Europe is the section *Caninae* (DC.) Ser. 1825, from this point on referred to as dog-roses.

Wild roses have a base chromosome set that consists of seven chromosomes (Täckholm, 1920, 1922; Blackburn and Heslop-Harrison, 1921) and the ploidy levels range from diploid (2*x*) to octoploid (8*x*). The pentaploid (5*x*) chromosomal constitution observed in the dog-roses is unique and reflects the original genomes of the allopolypoid hybridogenic origin. Two of the five chromosome sets, the so-called bivalent-forming chromosomes, are highly homologous and exchangeable among all section *Caninae* taxa (Nybom *et al.*, 2006). Probably, they originate from a common Protocanina ancestor (Wissemann, 1999). The remaining three chromosome sets, the so-called univalent-forming chromosomes, are inherited apomictically through the seed parent (Nybom *et al.*, 2006) and originate from different non-*Caninae* species through multiple hybridizations (Wissemann, 1999). These non-recombining haploid genomes can be assumed to act as different and independent gene pools and are proposed to reflect the taxonomical distance among the taxa (Nybom *et al.*, 2006). In contrast to the large polymorphisms observed within the dog-roses, all species share the atypical chromosomal constitution and the heterogamous canina meiosis in which the progeny receives one of the bivalent- and all the univalent-forming chromosome sets from the seed parent and only one of the bivalent-forming chromosome sets of the pollen parent (Täckholm, 1920, 1922; Blackburn and Heslop-Harrison, 1921). This uneven allocation of maternal and paternal chromosomes to the progeny, also called hemisexuality, has a significant impact on the species. First, there is a tendency to a skewed maternal inheritance (Ritz *et al.*, 2005; Wissemann, 2005; Wissemann and Ritz, 2005). Secondly,

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genetic recombination is restricted to the highly homologous bivalent-forming chromosomes (Nybom *et al.*, 2004). In addition, dog-roses have the ability to hybridize among sections and subsections, to produce both sterile and fertile hybrids, and to reproduce through sexual and asexual strategies. These features all lead to a hybridogenic and highly polymorphic species-complex in which spontaneous hybrids are disguised and well-defined species groups are delimited (Ritz and Wissemann, 2003; Wissemann and Hellwig, 1997). The apparent morphological similarities allow the taxa to be merged into fewer but more diverse species-groups according to the preferences of the taxonomists. Nilsson (1999) divided the dog-rose species into three groups (*Rosa rubiginosa*, *Rosa villosa* and *Rosa canina*). Graham and Primavesi (1993) described four subsections (*Rubiginosae*, *Villosae*, *Caninae* and *Stylosae*), and both Henker (2000) and Wissemann (2003) agreed on six subsections (*Rubigineae*, *Vestitae*, *Tomentellae*, *Caninae*, *Rubrifoliae* and *Trachyphyllae*). Previous morphometrical and molecular-genetic (RAPD, STMS) research on wild dog-rose parents and their interspecific progeny (Nybom *et al.*, 1996, 1997, 2006; Werlemark *et al.*, 1999; Olsson *et al.*, 2000; Werlemark and Nybom, 2001; Nybom *et al.*, 2004) supported the subdivision of the analysed dog-rose species as was proposed by Nilsson (1999), and Graham and Primavesi (1993) excluding the *Stylosae*. Unless mentioned otherwise, the groups or subsections within the dog-roses are referred to using the nomenclature of Henker (2000).

A few distinct morphological characters delimit the three major groups within the section *Caninae* (Table 1). The first subsection *Rubigineae* is characterized by numerous, sticky glands on the leaflets spreading a typical strong scent of apples or vines. The taxa of the second subsection *Vestitae* display tomentose leaflets, and carry persistent stipitate glands on hips and pedicels spreading a turpentine odour. The taxa of the third group show a variation in pubescence and presence of odourless glands on the leaflets. According to Graham and Primavesi (1993) and Nilsson (1999), these taxa belong to the subsection *Caninae*, whereas both Henker (2000) and Wissemann (2003) split this group into two subsections: *Caninae* and *Tomentellae*. According to these latter authors, *Rosa balsamica* belongs to the subsection *Tomentellae* (Table 2).

The present study reports on the morphological and AFLP-based variation within and among dog-rose populations in Belgium. We assessed (*a*) the differentiation of the dog-rose species in Belgium and correlate it to their taxonomical subdivision, and (*b*) the within- and between-population diversity in several Belgian dog-rose taxa.

TABLE 1. *The section* Caninae *species classified according to the L/D system of Reichert (1998)*

			Rubigineae			Vestitae	Tomentellae*	Caninae	
Type‡	Receptacle	Diameter orifice	Position, persistence of sepals	Leaflets glabrous or pilose, glandular	Odour of apples	Leaflets tomentose and glandular Odour of turpentine	Glandular leaflets Odourless	Eglandular or glandular leaflets Odourless	Odourless
				Leaflets slender wedge-shaped base; pedicel mostly eglandular	Leaflets broad, well-rounded base; Pedicel glandular	Pedicel with glands smelling like turpentine	Leaflets pubescent	Leaflets glabrous	Leaflets pubescent
L	Bouquet	<1 mm	Reflexed, deciduous after anthesis	***R. agrestis* Savi**	***R. micrantha* Borrer ex Sm.**	***R. tomentosa* Sm.**	***R. balsamica* Besser**†	***R. canina* L.**	***R. corymbifera* Borkh.**
L/D	± Head	±1 mm	Patent, partly deciduous	*R. inodora* Fries	***R. henkeri-schulzei***	*R. pseudoscabriuscula* (R. Keller) Henker et G. Schulze	Hybrids with the subsections Caninae and Rubigineae*	*R. subcanina* (H. Christ) R. Keller	***R. stylosa* Desv.** *R. subcollina* (H. Christ) R. Keller
D	Head	>1 mm	Erect, persistent after anthesis	*R. elliptica* Tausch	***R. rubiginosa* L.**	*R. sherardii* Davies	Hybrids with the subsections Caninae and Rubigineae*	*R. dumalis* Bechstein	*R. caesia* Sm.

The species included in this study are indicated in bold.
* Adaptations according to Henker (2000), and Henker and Schulze (1993).
† Synonym of *R. tomentella* according to Kurtto *et al.* (2004).
‡ L, *canina* type; D, *dumalis* type (Nilsson, 1999).

MATERIALS AND METHODS

Sampling wild rose shrubs

In total, 723 wild rose shrubs, belonging to seven dog-rose species – *R. canina*, *Rosa corymbifera*, *R. balsamica*, *R. rubiginosa*, *Rosa agrestis*, *Rosa micrantha* and *Rosa tomentosa* (Table 2) – were sampled in Belgium (Fig. 1). The shrubs were identified and classified according to the taxonomy of Henker (2000). Leaves were dried according

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TABLE 2. *The taxonomical hierarchy of the section* Caninae *and the position of the most representative species according to Henker (2000) and Wissemann (2003) (= H&W), Graham and Primavesi (1993) (= G&P) and Nilsson (1999) (= Nilsson group)*

H&W subsection	G&P subsection	Nilsson group	Species	#L	#H	#AFLP
Rubrifoliae	n/a	*Rubrifoliae*	*R. glauca* Pourret (syn. *R. rubrifolia*†)	–	–	–
Rubigineae	*Rubiginosae*	*R. rubiginosa*	*R. rubiginosa*	49	32	114
			R. micrantha	16	8	38
			R. agrestis	14	13	65
Vestitae	*Villosae*	*R. villosa*	*R. tomentosa*	25	22	103
			R. pseudoscabriuscula	–	–	–
Tomentellae	*Caninae*	*R. canina*	*R. balsamica* (syn: *R. tomentella* Léman‡, *R. obtusifolia* Desv.*,†)	31	31	68
Caninae	*Caninae*	*R. canina*	*R. canina*	82	68	233
Caninae	*Caninae*	n/a	*R. corymbifera*	55	55	102

For the species analysed, the number of individuals for leaflet (#L) and hip (#H) characters and number of individuals for AFLP analysis (#AFLP) is indicated.

n/a, Not mentioned by the author(s).

*, †, ‡, Synonyms used by Graham and Primavesi, Nilsson, and Henker and Wissemann, respectively.

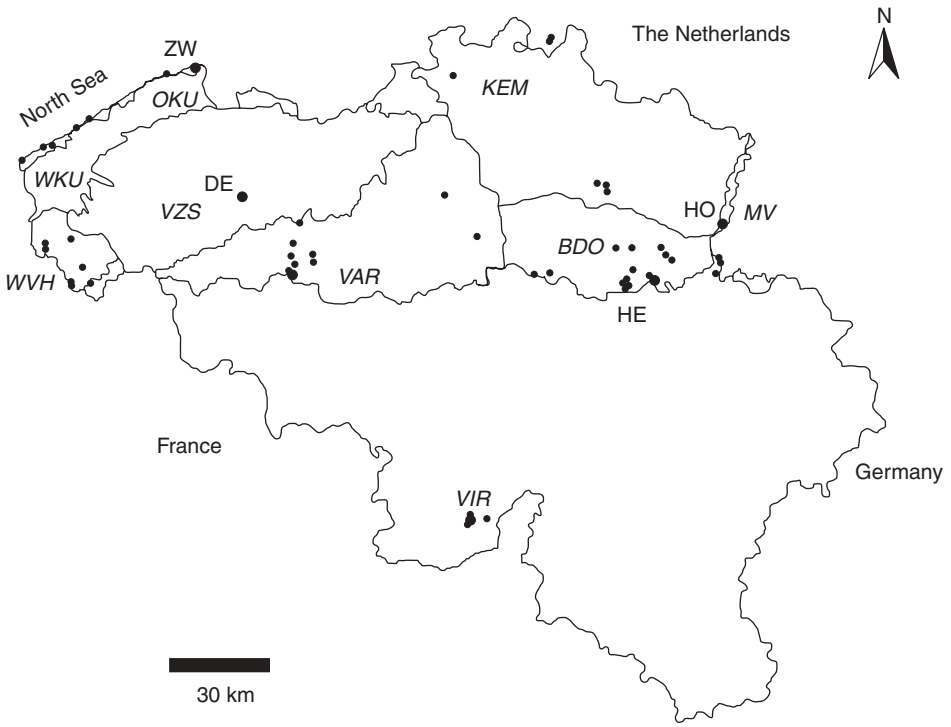

FIG. 1. Sampling localities of wild rose shrubs in Belgium. With regions of provenance indicated in italics: WVH, West-Vlaams Heuvelland; WKU, Westkust; OKU, Oostkust; VZS, Vlaamse Zandstreek; VAR, Vlaamse Ardennen; KEM, Kempen; BDO, Brabants District Oost; MV, Maasvallei; VIR, Viroin; and the localities: ZW, Het Zwin; DE, Deinze; HE, Heers; HO, Hochter Bampd. Populations referred to in this paper are indicated with larger dots.

to the standard herbarium protocols and hips were stored in ethanol (96 %). For the AFLP-based analyses, young and fresh leaflets were frozen in liquid nitrogen, lyophilized, and stored at −18 °C until DNA extraction.

Morphological analysis

In a previous study, 17 diagnostic characters were determined with principal component analyses (PCA; De Cock *et al.*, 2007; De Cock, 2008). The morphometric data, e.g. length and width of leaflets and hips, were explored in box and whisker plots, and the descriptive characters, such as frequency of glands on the leaflets' upper side, on hips or pedicels, were visualized in histograms. Additional principal components and canonical discriminating analyses were performed on these 17 factors in order to select the independent characters. All analyses were performed using S-Plus 6·2 Professional (Insightful Corporation). The detailed procedure is described in De Cock *et al.* (2007).

AFLP analysis

Three hundred nanograms of DNA were produced from 25 mg dried leaf material following the instructions of the

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Qiagen DNeasy Plant Mini Kit (Westburg, The Netherlands). The AFLP procedure was performed according to Vos *et al.* (1995). The restriction–ligation reaction was performed in one step. The amplification of the fragments was performed in two steps using the primer combinations *Eco*RI–AAG/*Mse*I–CAT, *Eco*RI–AAG/*Mse*I–CAG and *Eco*RI–ATC/*Mse*I–CTA. The amplified fragments were separated on the Global Edition IR2 system of LI-COR (LI-COR) and visualized in automatically generated TIFF-files. Fragments were automatically scored using SAGA-MX version 3·0 (LI-COR). Additional manual controls and corrections were performed. In total, 150 fragments with a size range of 75–652 bp were evaluated and transformed into a binary matrix (presence, 1; absence, 0) that was used as an input file for several statistical programs.

AFLP-based data analysis

Distance matrices were computed with the Jaccard similarity coefficient, and visualized in biplots of principal co-ordinate analyses (PCO) using Splus 6·2 Professional. TREECON version 1·3b (Van de Peer and De Wachter, 1994) calculated pair-wise genetic distances by the simple matching algorithm (100 bootstraps). The dendrograms were drawn with UPGMA (unweighted pair group method with arithmetic mean) cluster analyses, repeated $\times 100$. The most probable number of gene pools was estimated and individuals were assigned to the inferred populations using the Bayesian approach (Structure; Pritchard *et al.*, 2000), with the adaptations suggested by Evanno *et al.* (2005). RAPDDIV (Whitkus *et al.*, 1998) determined the partitioning of the diversity within and among different populations of *R. canina* and *R. corymbifera*. The diversity was calculated with the Shannon–Weaver diversity index using the Brillouin formula to eliminate the bias of finite sample sizes. This approach is based on band phenotypes, and does not rely on the Hardy–Weinberg assumptions. Only one of the representative congruent results is shown.

RESULTS

Morphological evaluation

Selection of independent diagnostic characters. The 17 morphometric and descriptive diagnostic characters explained at least 50 % of the variation in the three principal components. Nine independent characters were identified based on the loadings of the PCA (Table 3): the number of glands on the leaflet margin and pedicel, the length of the leaflet and pedicel, the serration of the leaflet margin, the pubescence on the upper and lower side of the leaflet, and the diameters of disc and orifice. They were analysed separately in box and whisker plots (Fig. 2) or histograms (Fig. 3) in order to assess inter- and intraspecific variation within the dog-roses. The impact of these characters on the hierarchical structure within the dog-roses was visualized in a PCA biplot (Table 3 and Fig. 4).

Interspecific morphometrical variation. Based on the measured characters, several interspecific tendencies were observed (Fig. 2). The leaflet dimensions of *R. tomentosa* were highly variable in comparison to the other taxa examined. The taxa *R. rubiginosa* and *R. micrantha* were characterized by smaller and shorter leaflets, whereas the leaflets of *R. canina*, *R. corymbifera* and *R. balsamica* were clearly wider and longer. *Rosa agrestis* was characterized by intermediate leaflet dimensions. Concerning the hip characters, the diameter of the orifice did not display consistent differentiations among the taxa analysed except for *R. rubiginosa* where it was much larger. The diameter of the disc divided the taxa into two overlapping groups. *Rosa canina*, *R. corymbifera* and *R. balsamica* were the taxa with a larger disc, and *R. agrestis*, *R. rubiginosa*, *R. micrantha* and *R. tomentosa* displayed smaller disc diameters. The pedicels of *R. tomentosa* were longer compared with the other taxa.

TABLE 3. *Loadings of the nine independent diagnostic morphometric and descriptive characters*

Diagnostic characters		Loadings		
Independent	Correlated with	Comp. 1	Comp. 2	Comp. 3
Density of glands on leaflet margin (MG)	Density of glands on rachis (RG)	**0·445**	−0·162	−0·180
Pedicel length (PL)			**0·660**	−0·317
Serration of leaflet margin (MS)	Density of glands on leaflet lower side (LlG)	**0·387**	−0·280	−0·349
No. of glands on pedicel (PG)	No. of glands on hip (HG) Shape of hip (HS)	**0·346**	0·250	0·117
Pubescence leaflet upper side (LuP)		**0·410**	0·230	
Pubescence leaflet lower side (LlP)		**0·319**	0·438	0·188
Diameter of disc (D)	Hip length (HL)	**−0·330**	0·254	0·411
Leaflet length (LL)	Leaflet base (LB) Leaflet width (LW) Rachis length (RL)	**−0·285**	0·273	−0·257
Diameter of orifice (O)		0·258	−0·116	**0·676**

The correlated characters are indicated, and the components in which each character explains the majority of the variation are marked in bold. Abbreviations used in Fig. 4 are indicated.

Variation within descriptive characters. The pubescence and presence of the glands on the leaflets, hips and pedicels were highly variable within the dog-roses and formed the basis of the subdivision in subsections (Fig. 3). The leaflets of the subsection *Rubigineae* were densely glandular and mostly pubescent. *Rosa tomentosa* (subsection *Vestitae*) was characterized by densely pubescent and tomentose leaflets, and displayed persistent glandular hips and pedicels. *Rosa canina*, *R. corymbifera* (both subsection *Caninae*) and *R. balsamica* (subsection *Tomentellae*) all showed mainly eglandular pedicels and hips. *Rosa corymbifera*

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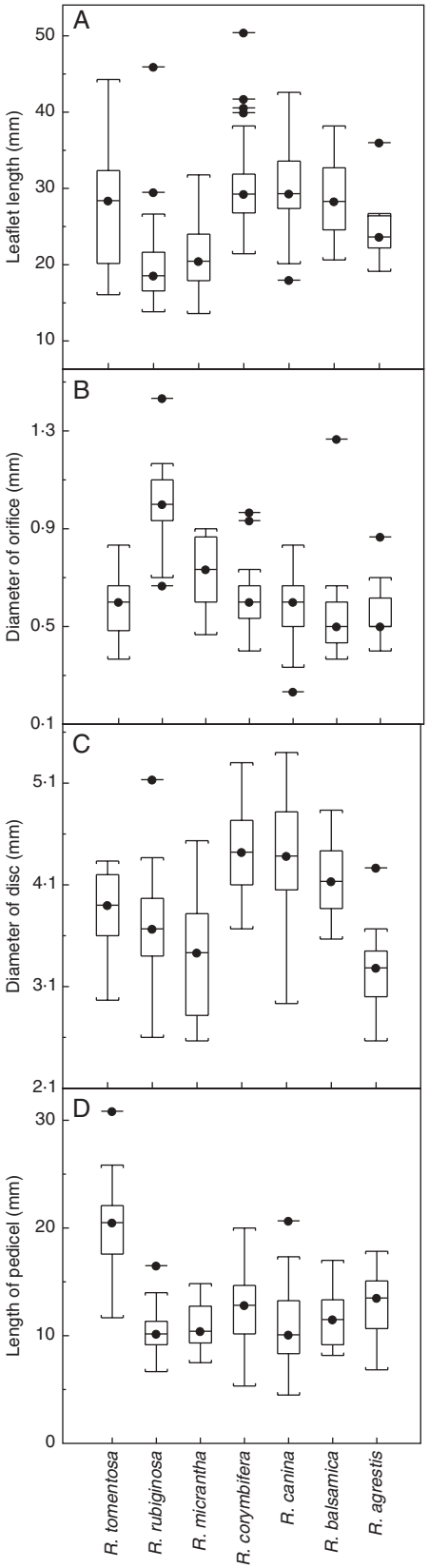

FIG. 2. Box- and whisker plots showing interspecific differentiation among the seven taxa of the section *Caninae* analysed for (A) leaflet length; (B) diameter of the orifice; (C) diameter of the disc; (D) pedicel length.

was characterized by eglandular rachides, leaflet margins and lower sides of the leaflet. For *R. canina*, they could be both eglandular or sparsely glandular. Glabrous and (irregular) uni- or biserrated (occasionally multiserrated) leaflets were typical for *R. canina*, whereas *R. corymbifera* had glabrous upper sides of the leaflet but the veins on the lower side were pubescent. The leaflets were (irregular) uniserrated. The rachides and leaflet margins of *R. balsamica* varied from sparsely to densely glandular and the veins at the lower side of the leaflet were moderately glandular. The upper sides of the leaflet were glabrous or sparsely pubescent, whereas the veins on the lower side were sparsely to moderately pubescent. The leaflet margins were mostly bi- to multiserrated or multiserrated.

Integration of diagnostic characters. In the PCA biplot that combined the four morphometric and five descriptive independent diagnostic characters, the taxa analysed grouped in three partly overlapping clusters (Fig. 4). The grouping reflected the taxonomical subsections. The subsection *Rubigineae* appeared as the most distinct group based on the pubescence on the leaflets, multiserrated and densely glandular leaflet margins, and the densely glandular rachides. *Rosa rubiginosa* displayed smaller leaflets and larger diameters of orifice compared with the other taxa analysed. The taxa of the subsection *Rubigineae* overlapped partly with *R. balsamica* (subsection *Tomentellae*) based on similar pubescence on the leaflets, presence of glands and serration of the leaflet margins (Fig. 4A). *Rosa tomentosa* of the subsection *Vestitae* displayed longer pedicels compared with the other dog-rose taxa and was characterized by densely pubescent or tomentose leaflets, strikingly glandular pedicels and hips (Fig. 4B). The taxa of the subsections *Caninae* and *Tomentellae* showed large overlap and formed a rather compact sphere. Yet, interspecific differentiation was present as each of these three taxa constituted the majority of the different parts of the sphere (Fig. 4C).

Intraspecific variation among pure and mixed populations. Tendencies towards intraspecific geographical differentiation were observed for several dog-rose taxa. A striking differentiation was observed between *R. rubiginosa* populations from the coastal region and the Maasvallei, situated about 250 km apart (Fig. 1). The coastal populations from the Westkust (WKU) consisted of over 60 *R. rubiginosa* shrubs and five *R. canina* or *R. tomentosa* shrubs. In contrast, the coastal population of Het Zwin (OKU) contained up to 100 *R. canina* and *R. corymbifera* individuals and about ten *R. rubiginosa*. *Rosa micrantha* was absent in the coastal region. At the Maasvallei, the population had a mixed presence with 19 *R. rubiginosa*, 11 *R. micrantha*, only four *R. canina* and one *R. tomentosa*. The *R. rubiginosa* of the Maasvallei population showed larger rachides and leaflets, and slightly smaller diameters of the orifice compared to those of the coastal populations (Fig. 5A, B). In addition, the *R. micrantha* individuals of the Maasvallei showed broader diameters of the orifice and disc (Fig. 5C, D), and the pedicels (data not shown) appeared to be shorter compared with their congeners from Brabants District Oost (BDO, with MV and BDO

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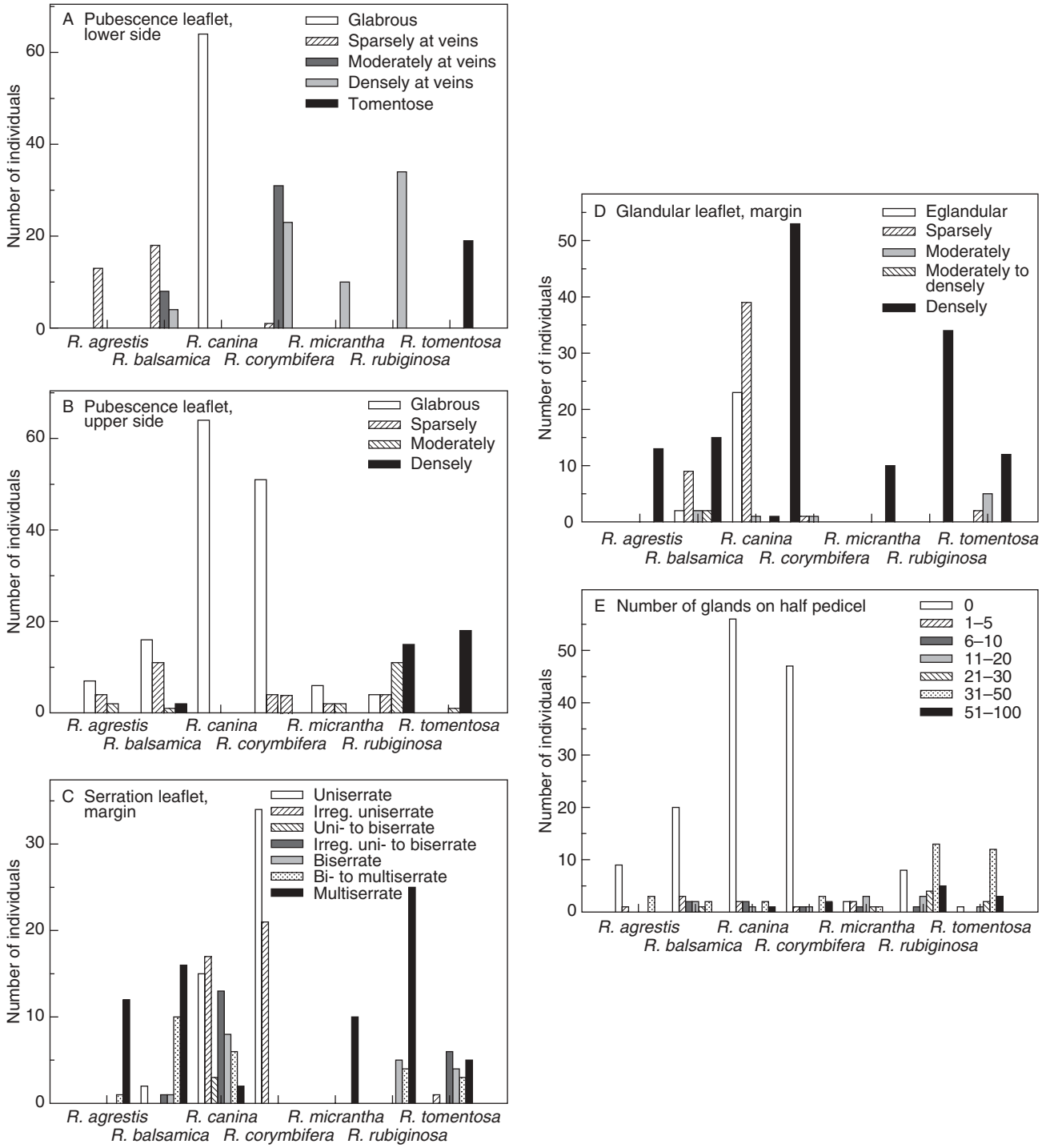

FIG. 3. Histograms showing interspecific differentiation among the seven taxa of the section *Caninae* studied for (A) pubescence of the leaflet lower side; (B) pubescence of the leaflet upper side; (C) serration of the leaflet margin; (D) glandular leaflet margin; (E) number of glands on half of the pedicel.

about 25 km apart) where *R. rubiginosa* is absent. The pedicels of *R. rubiginosa* Het Zwin (OKU) displayed a lower frequency in glands compared with their congeners at Maasvallei and Westkust (Fig. 6A). Similarly, the individuals at Het Zwin (OKU) that were morphologically defined as *R. canina*, *R. corymbifera* and *R. balsamica*, all showed a higher tendency to the presence of glands on the leaflets or pedicels compared with their congeners of populations lacking *Rubigineae* (Fig. 6B–F).

AFLP-based interspecific variation

Differentiation within the dog-roses. None of the applied approaches used to analyse the AFLP markers was able to

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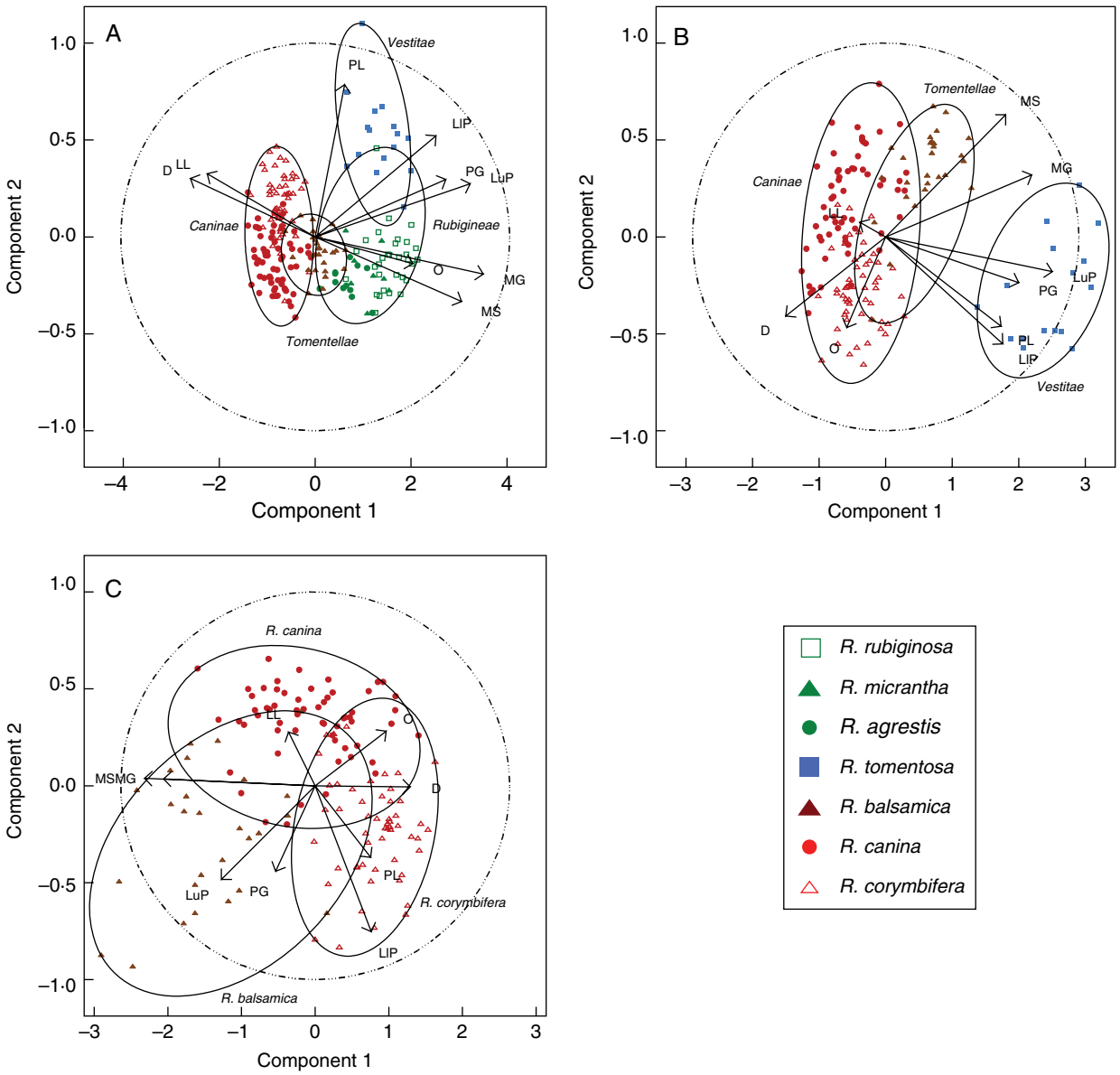

FIG. 4. Biplot of the principal components based on the nine independent morphological characters of: (A) the section *Caninae*, the cumulative percentages of the variation explained in the first three components (cum%) are 42, 58 and 70 %; (B) the subsections *Vestitae*, *Tomentellae* and *Caninae*, with cum% of 36, 53 and 64 %; (C) the subsections *Tomentellae* and *Caninae* with cum% of 25, 39 and 52 %. The individuals are labelled as indicated and morphological characters according to Table 3. The ellipses indicate the subsections in (A) and (B); and the species in (C) according to Henker (2000).

support the subdivision of the dog-rose species into a few distinct and well-defined groups. For instance, the similarity coefficients within section *Caninae* equalled 66 %, whereas the similarity among the subsections *Caninae* and *Tomentellae* was 68 % (Table 4). However, intersectional differentiation was observed. The subsection *Rubigineae* was the most differentiated, and formed a well-defined subcluster (Fig. 7A). When the subsection *Rubigineae* was excluded from the data set, the subsection *Vestitae* showed a tendency to form a separate cluster (Fig. 7B). Finally, PCO analyses performed on the two remaining subsections *Caninae* and *Tomentellae* could not detect any interspecific AFLP-based differentiation (Fig. 7C).

AFLP-based variation in pure and mixed populations.

The different approaches used to analyse the AFLP polymorphisms indicated a remarkable higher similarity among morphologically different species all originating from the mixed growth site compared with the similarity among congeners sampled at other localities (Fig. 7D, Table 5). For instance, the similarity coefficient among the *R. canina*, *R. corymbifera* and *R. balsamica* of Het Zwin (OKU) varied between 79 % and 100 %, whereas the similarity among different *R. canina* populations ranged between 69 % and 77 %. The partitioning of the diversity was assessed by comparing the within- and among-population variation for the two most common taxa: *R. canina* and

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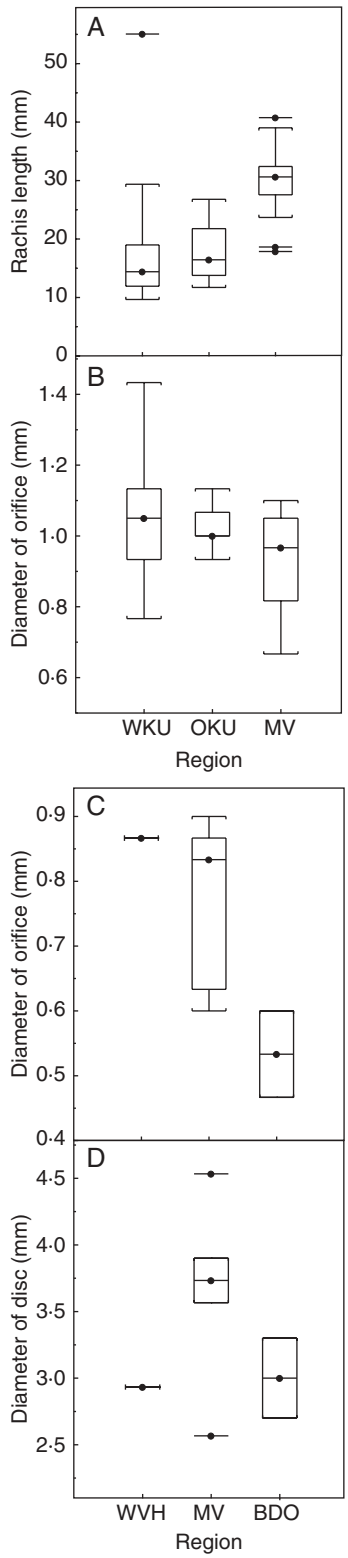

FIG. 5. Box and whisker plots showing the interpopulation differentiation within *R. rubiginosa* for: (A) rachis length and (B) diameter of orifice; and within *R. micrantha* for (C) diameter of orifice and (D) diameter of the disc. Abbreviations used are given in the legend to Fig. 1.

R. corymbifera (Table 6). The genetic variation (H_S) within *R. canina* was higher when more localities were taken into account (Table 6A, bold). Little to no difference was observed within (H_S) or among (D_{ST}) *R. canina* or *R. corymbifera* sampled at the same locality. For instance, the within-differentiation of *R. canina* Het Zwin equalled that of *R. corymbifera* Het Zwin and *R. corymbifera* Heers ($H_S = 0{\cdot}16$ for all populations; Table 6A, underlined). In addition, the differentiation between the two Het Zwin populations (D_{ST}) was set on 0·18 (Table 6A). Comparing the partitioning of the differentiation among the two taxa sampled at five localities with the differentiation among the five localities, the genetic variation among the localities was clearly higher ($D_{ST} = 0{\cdot}34$) than the variation among the two taxa sampled at these localities ($D_{ST} = 0{\cdot}16$; Table 6A, B, italics).

DISCUSSION

The observed inter- and intraspecific variation in the dog-rose taxa is influenced by large phenotypic and genetic plasticity, the ability to hybridize, the unique polyploid chromosomal constitution, the heterogamous canina meiosis, and the predominantly matroclinal inheritance. The combination of these factors leads to a complex and ambiguous taxonomical hierarchy which is subject to discussion. The hierarchical structure of the dog-roses into three groups as proposed by Graham and Primavesi (1993) and Nilsson (1999) and supported by an extensive study of the morphometrical and genetic characters of wild individuals and the interspecific progeny (Nybom *et al.*, 1996, 1997, 2004, 2006; Werlemark *et al.*, 1999; Olsson *et al.*, 2000; Werlemark and Nybom, 2001) was confirmed by the present morphological and AFLP-based analyses. However, the boundaries among the observed groups were vague and indicated the presence of intermediate morphological states and the hybridogenic character of the dog-roses.

Within the dog-roses, the subsection *Rubigineae* was the most differentiated in both the present morphological and AFLP-based analyses. This confirmed the results of the morphometrical study (Nybom *et al.*, 1996), the RAPD (Olsson *et al.*, 2000) and STMS analyses (Nybom *et al.*, 2004) in which *R. rubiginosa* was used to represent the subsection *Rubigineae*. Similarly, the AFLP analysis of Koopman *et al.* (2008) describes the subsection *Rubigineae* as a derived and genetically defined group within the dog-roses. The interspecific differentiation may be due to one or more distinct non-*Caninae* parent(s) in the historical hybridization event leading to the origin of the subsection *Rubigineae* taxa. Most likely, this putative historical parental species passed on some distinct univalent-forming chromosomes. Alternatively, the bivalent-forming chromosomes may be less homologous compared with the other *Caninae* subsections; however, this should impose a barrier for the reproducibility of interspecific hybrids (Nybom *et al.*, 2006). This barrier is in conflict with the observations of successful controlled interspecific crossings (e.g. Werlemark *et al.*, 1999) and of spontaneous interspecific progeny (Graham and Primavesi, 1993; Feuerhahn and Spethmann, 1995) including a subsection *Rubigineae* parent. The fertility of the interspecific progeny depends on

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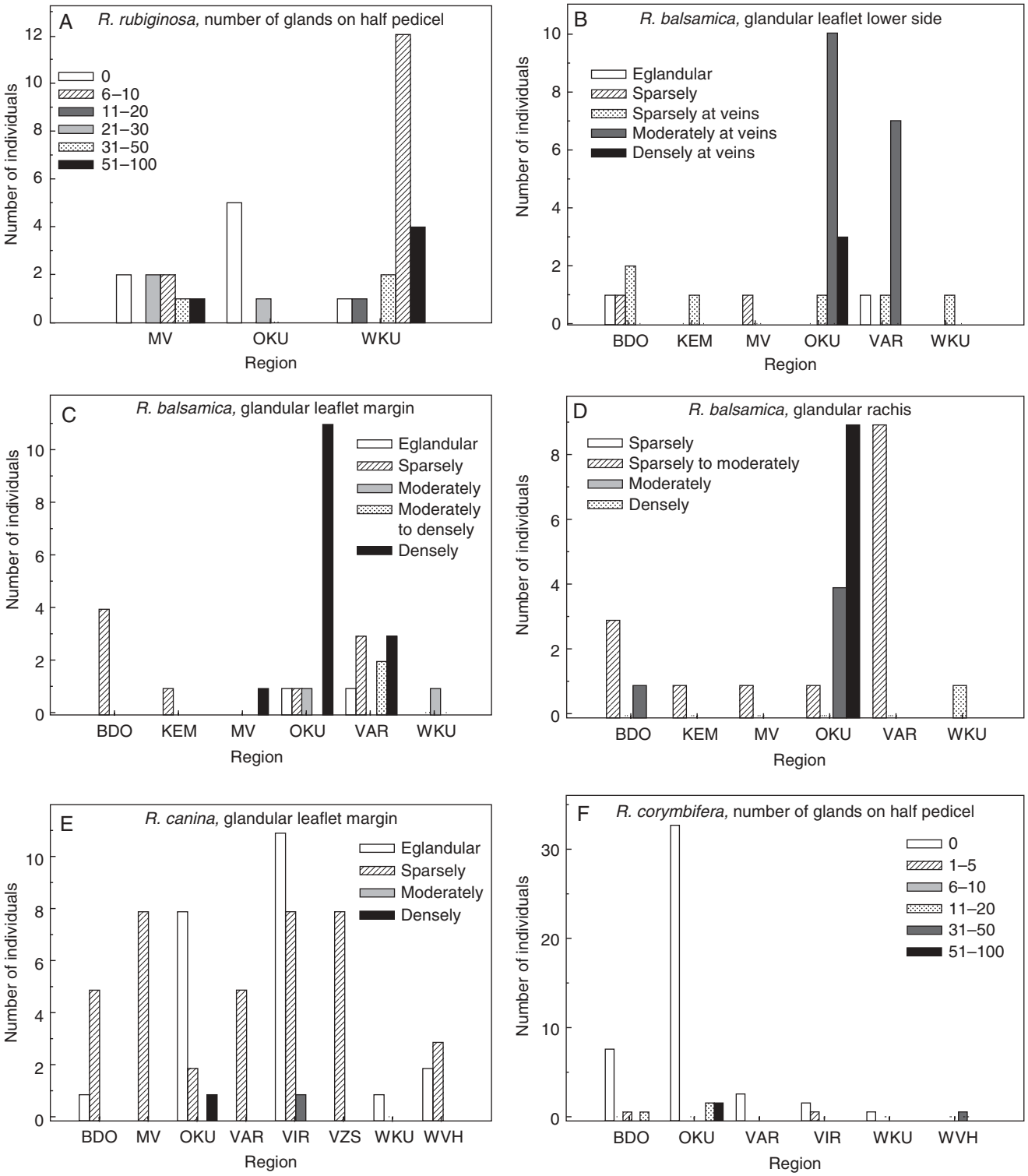

FIG. 6. Histograms showing the interpopulation differentiation within (A) *R. rubiginosa* for number of glands on half of the pedicel; *R. balsamica* for (B) density of glands on leaflet lower side, (C) glands on leaflet margin and (D) glands on rachis; and (E) within *R. canina* for glands on leaflet margin; and (F) *R. corymbifera* for number of glands on half of the pedicel. Abbreviations used are given in the legend to Fig. 1.

the homology of the bivalent-forming chromosomes of the parents (Nybom *et al.*, 2006). Therefore, the differentiation of the *Rubigineae* is most likely caused by distinct univalent-forming chromosome sets.

Excluding the most distinct subsection *Rubigineae*, a morphological and genetic differentiation of the subsection *Vestitae* with the subsections *Tomentellae* and *Caninae* was observed. The boundaries of this subdivision were more

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TABLE 4. *Mean Jaccard similarity coefficients calculated within and between the subsections of the section* Caninae

	Caninae	Rubigineae	Tomentellae	Vestitae
Caninae	0·66			
Rubigineae	0·63	0·71		
Tomentellae	0·68	0·66	0·80	
Vestitae	0·67	0·65	0·70	0·79

vague compared with the differentiation of the subsection *Rubigineae*. Previous cpDNA analyses have divided the dog-roses into the odorant glandular taxa (cf. *Rubigineae* and *Vestitae*) and the eglandular or non-odorant glandular taxa (cf. subsections *Caninae*, *Tomentellae*; Wissemann and Ritz, 2005) supporting the assignment of the subsection *Vestitae* to a separate cluster. Koopman *et al.* (2008) were not able to distinguish the subsection *Vestitae* from the subsections *Caninae* and *Tomentellae* in their phylogenetic analyses. Since they emphasize the phylogenetic relationships within the whole subgenus, their selected markers may not be sufficiently discriminatory at the lower taxonomical structures within the dog-roses.

In contrast to the classifications of Henker (2000) and Wissemann (2003), neither the present morphological nor the present AFLP-based approach was able to differentiate among the subsections *Caninae* and *Tomentellae*. In our morphological analyses, *R. balsamica* overlapped with the subsections *Caninae* and *Rubigineae*, which confirmed the morphological similarity of *R. balsamica* with both subsections as was mentioned by Wissemann (2000). The previously proven matroclinal inheritance of leaflet dimensions (Werlemark *et al.*, 1999) and epicuticular wax structures

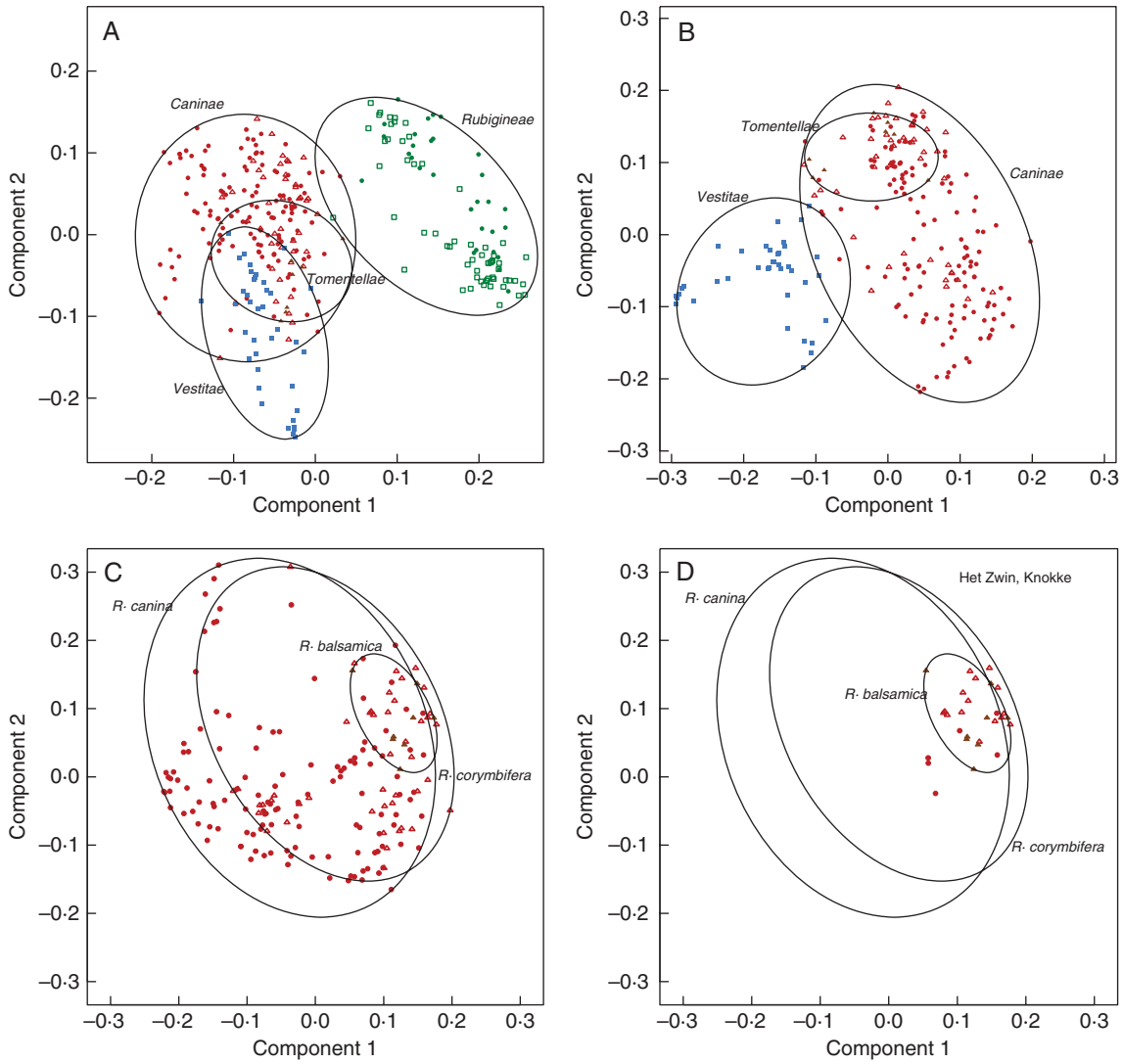

FIG. 7. Based on the AFLP data, PCO plots of the first two components of: (A) the section *Caninae*, based on 316 individuals, and cumulative percentage of three principal components (cum%) of 18, 28 and 37 %; (B) the subsections *Vestitae*, *Caninae* and *Tomentellae*, based on 224 individuals, and cum% of 14 %, 25 % and 34 %; (C) the subsections *Caninae* and *Tomentellae* with indication of species; and (D) highlighting the individuals of the mixed population at Het Zwin (OKU), based on 177 individuals, and cum% of 14, 25 and 33 %. The species labels used are given in Fig. 4.

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TABLE 5. *Mean Jaccard similarity coefficients calculated within and between the populations of the subsections* Caninae *and* Tomentellae *sampled*

			R. canina					*R. corymbifera*		*R. balsamica*
Taxon	Region	Locality	DE	HE	HO	VIR	ZW	HE	ZW	ZW
R. canina	VZS	DE	*0·79*							
R. canina	BDO	HE	*0·77*	*0·80*						
R. canina	MV	HO	*0·73*	*0·76*	*0·77*					
R. canina	VIR	VIR	*0·70*	*0·71*	*0·69*	*0·78*				
R. canina	OKU	ZW	*0·72*	*0·72*	*0·71*	*0·73*	***1·00***			
R. corymbifera	BDO	HE	*0·68*	0·70	0·70	0·67	0·70	*0·72*		
R. corymbifera	OKU	ZW	0·71	0·72	0·71	0·73	**0·87**	*0·71*	***0·87***	
R balsamica	OKU	ZW	0·69	0·69	0·69	0·69	**0·87**	0·68	**0·80**	***0·79***

Intraspecific (italics) and high interspecific (bold) similarity coefficients are indicated.
Abbreviations used are given in the legend to Fig. 1.

TABLE 6. *Results of RAPDDIV analyses: (A) the within and among taxa differentiation of the* R. canina *and* R. corymbifera *populations Het Zwin and Heers separately, and both mixed populations grouped with the* R. canina *populations Deinze, Hochter Bampd and Viroin; (B) the within and among locality differentiation of the five populations*

(A) Within (H_S) and among (D_{ST}) taxa differentiation

	Localities (# PM)								
	Het Zwin (48 PM)			Heers (103 PM)			ZW, DE, HE, HO, VIR (131 PM)		
Taxon	Ind	H_S	D_{ST}	Ind	H_S	D_{ST}	Ind	H_S	D_{ST}
R. canina	13	**0·16**		28	**0·18**		126	**0·21**	
R. corymbifera	16	0·16		30	0·16		46	0·15	
			0·18			*0·17*			*0·16*

(B) Within (H_S) and among (D_{ST}) locality differentiation

		Taxa (# PM) *R. canina* and *R. corymbifera* (131 PM)		
Region	Locality	Ind	H_S	D_{ST}
OKU	Het Zwin (ZW)	29	0·09	
VZS	Deinze (DE)	27	0·13	
BDO	Heers (HE)	58	0·16	
MV	Hochter Bampd (HO)	31	0·12	
VIR	Viroin (VIR)	27	0·22	
				0·34

PM, Number of polymorphic AFLP markers; Ind, number of individuals included; H_S, diversity within taxon or locality. Variation among taxa or localities, $D_{ST} = (H_T - \text{average } H_S)/H_T$).
Genetic variation within different *R. canina* populations is marked in bold, variation within different *R. corymbifera* populations is underlined, and the differentiation among taxa and among localities is marked in italics.
Abbreviations used are given in the legend to Fig. 1.

(Wissemann, 2000) both indicate the historical influence of a subsection *Caninae* taxon in *R. balsamica*. Alternatively, the presence of glands on the leaflets may originate from a subsection *Rubigineae* ancestor. Additional PCA analyses restricted to the subsections *Caninae* and *Tomentellae* indicated that the pubescence and glands on the *R. balsamica* leaflets supplemented the observed variation in *R. canina* and *R. corymbifera*. In addition, it was not possible to observe any intersubsectional or -specific AFLP-based differentiation for the analysed taxa of these two subsections. Moreover, the observed similarity among *R. canina*, *R. corymbifera* and *R. balsamica* equalled the similarity within each of these three taxa. Consequently, it was assumed that these taxa share highly related univalent-forming chromosome sets. The strong similarity of *R. balsamica* with the subsection *Caninae* has already been indicated by independent research methods and tools such as the chemical composition of the epicuticular waxes (Wissemann, 2000), nrITS-sequences and cpDNA analyses (Wissemann and Ritz, 2005). Until now, no indications were found that support the subdivision of *R. balsamica* from the subsection *Caninae* taxa.

Even within the small geographical area of Belgium, intraspecific geographic variation was observed among populations that indicate past or present-day hybridization. The values of the well-defined morphometric species-specific characters of *R. rubiginosa* from the coastal populations are congruent with those described in literature.

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In the mixed *R. rubiginosa*–*R. micrantha* population of the Maasvallei, intermediate values between those of the parental species have been observed. The orifice diameter is described as >1 mm for *R. rubiginosa*, whereas it is <1 mm for *R. micrantha* (Henker, 2000). *Rosa rubiginosa* sampled at the mixed Maasvallei-population displayed smaller diameters compared with the literature and to the congeners of the putative pure populations; whereas for *R. micrantha*, larger diameters were observed. Also for the rachis length of *R. rubiginosa* and the disc diameter of *R. micrantha* intermediate values were measured. Within the taxa *R. canina*, *R. corymbifera* and *R. balsamica*, intraspecific geographical variations were observed at the morphological level. The individuals of the mixed population Het Zwin all displayed a higher frequency of glandular leaflets compared to their congeners sampled at the other localities. This may indicate the past hybridization influence of *R. rubiginosa*. In addition, *R. rubiginosa* in Het Zwin (OKU) displayed fewer glands on both hips and pedicels compared with their congeners in Maasvallei. Moreover, the morphological intraspecific variation observed in *R. canina*, *R. corymbifera* and *R. balsamica* was supported by the higher genetic similarity among morphologically different taxa that were sampled at the same locality, compared with the similarity among congeners sampled at localities with a different composition of taxa. In other words, the genetic structure of these individuals reflected the common growth site instead of the taxonomical position. It can be hypothesized that past introgression events (interspecific hybridizations and backcrossing) in a mixed population such as Het Zwin resulted in more similar genetic constitutions and that genes responsible for the morphological differentiation between the taxa are limited and not highlighted with the applied molecular techniques. This may indicate that the differentiation among these taxa is a relative young phenomenon and is still in progress.

In conclusion, the taxa and subsections of the dog-roses are distinguishable by a set of well-defined morphological characters. However, the large and consistent overlap of the morphologically defined groups indicates the occurrence of intersubsectional and -specific hybridization that results in a combination of intermediate, transgressive and species-specific characters. In addition, these morphologically separable taxa are only to a certain extent distinguishable using routinely applied molecular-marker techniques. The allopolyploid origin of the non-recombining univalent-forming chromosomes can be responsible for the variable genetic similarities among the taxa. Even more, when different closely related dog-rose species are present at the same growth site, the genetic structure may show more differentiation between localities than between taxa. From an evolutionary point of view, the canina meiotic system has probably developed fairly recently (Lim *et al.*, 2005), supporting the idea that the dog-roses are rather young (Atienza *et al.*, 2005). Consequently, the intersubsectional boundaries are still being created, or are disappearing. Possibly, the partly apomictic character of the canina genome hampers quick evolutionary species formations and prevents the large-scale species differentiation in the dog-roses.

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