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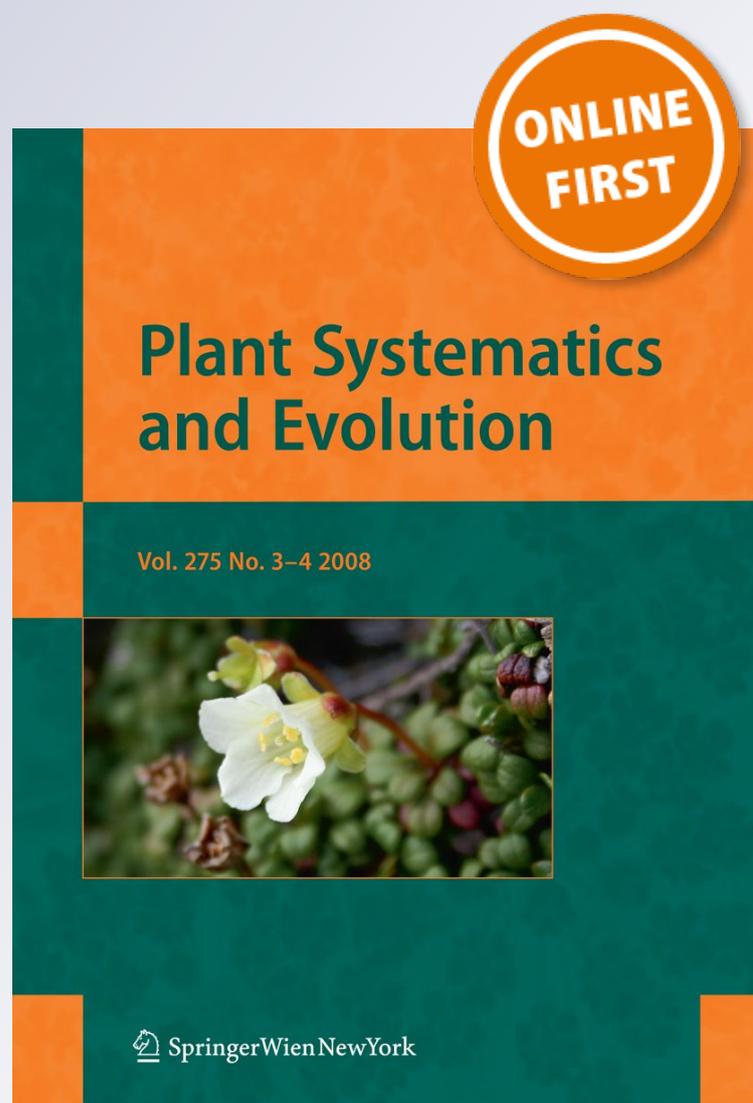
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Geographically differentiating morphology of genetically similar dogroses: consequences of canina meiosis

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Abstract Despite their genetic homogeneity, many taxonomic species are described as European dogroses (*Rosa* L. section *Caninae* (DC.) Ser.) with consistent morphological variability. Here we report a morphometric study of 27 hip and leaf characters of proximate dogrose populations that include *Rosa agrestis*, a member of the subsection *Rubigineae*, in Flanders. In principal components analysis one *R. agrestis* population in Kanne had intermediate morphology between *R. canina* and *R. corymbifera*, on the one hand, and three other *R. agrestis* populations, on the other, suggesting hybridogenic origin. Half-sib *R. agrestis* seedlings were grown under controlled conditions and their leaf characters were studied. A tendency toward deviating morphology in seedlings from the Kanne population, analogous to the mother plants in the field, and a correlation for specific leaf characters between mother plants and their corresponding seedlings reinforce a genetic basis for the observed divergence in the Kanne population. The assumed hybridogenic mother plants did not produce fewer seeds per hip than the others of the pure populations. In addition, for all sampled dogroses, which included six species, the diameter of the discus was correlated with the number of seeds per hip, whereas the

diameter of the orifice was not correlated with this character. This implies that only the diameter of the orifice and not the diameter of the discus might be decisive for species identification among European dogroses.

Keywords *Rosa agrestis* · Section *Caninae* · Subsection *Rubigineae* · Morphological divergence · Hybridisation · Orificium

Introduction

Wild roses have intrigued taxonomists ever since the species concept of Linnaeus (1753). The number of species within the genus *Rosa* L. (Rosaceae) varies among different taxonomic classifications. Henker (2000) describes 100–250 species world-wide, and 30–60 species are endemic in Europe (dominated by the section *Caninae* (DC.) Ser.). Interspecific hybridisation, apomixis, and polyploidy all contribute to this systematic complexity. Many rose species are polyploid, probably mostly allopolyploid (Wisseman 2002, 2007; Joly et al. 2006). Among angiosperms in general, genera with a higher proportion of polyploid species are more species-rich, and within the Rosaceae polyploidisation occurs more often in lineages that are already diversifying at greater rates (Vamosi and Dickinson 2006). In addition, polyploidy has been associated with self-compatibility and the ability to hybridise interspecifically (Vamosi and Dickinson 2006). Systematic classification of the subgenus *Rosa* into its five largest taxonomic sections *Pimpinellifoliae*, *Rosa*, *Caninae*, *Gallicanae*, and *Synstylae* is well supported by genetic studies despite regular overlap among the different taxa (De Cock 2008; Koopman et al. 2008).

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The section *Caninae* forms a well-defined group of polyploid taxa known as the dogroses. They evolved a unique mode of reproduction in plant kingdom—a distinctive unbalanced meiosis termed “canina-meiosis” (Täckholm 1920, 1922; Blackburn and Heslop-Harrison 1921). Dogroses are mostly pentaploids ($2n = 5x = 35$), although tetraploids or hexaploids occur ($2n = 4x$, $6x = 28, 42$). Only 14 chromosomes (two sets of 7 chromosomes) form bivalents during meiosis, leaving the others as univalents. The latter are included in egg cells, but not in pollen. Thus, seven bivalent forming chromosomes are transmitted through pollen grains, whereas egg cells contain 21, 28, or 35 chromosomes from seven bivalent forming chromosomes and 14, 21, or 28 univalents, depending on ploidy level. Cytological and molecular data suggest this allopolyploidy resulted from multiple hybridization between different members of non-dogroses and the extinct *ProtoCaninae* (Ritz et al. 2005; Kovarik et al. 2008).

In the Low Countries, the three subsections *Rubigineae*, *Vestitae*, and *Caninae* are indigenous (Table 1). De Cock et al. (2007, 2008) describe the subsection *Rubigineae* within the section *Caninae* as the most distinct genetic group, followed by the subsection *Vestitae* and, in its turn, by the subsection *Caninae*. The allopolyploid nature did not create a barrier to hybridisation among dogroses or between dogroses and non-dogroses. For instance, *R. stylosa* is believed to have originated as a cross between a member of the subsection *Caninae* and *R. arvensis*, a non-dogrose (De Cock 2008). Interspecific hybridisation among dogroses is assumed to be fertile if the bivalent forming chromosomes are sufficiently homologous to recombine and follow the canina meiosis (Nybom et al. 2004, 2006). Microsatellite allele-sharing between the maternally transmitted and highly conserved univalent forming chromosomes in controlled interspecific crosses reflected the taxonomic distance between different parental species. The frequently recombining bivalent forming chromosomes were taxonomically less informative (Nybom et al. 2006). In contrast with almost all diploid *Rosa* species, dogroses can produce seed by selfing, which, in general, will result in lower within-population variation and higher among-population differentiation than for strictly outcrossing species (Nybom et al. 2004, 2006). Many species are facultatively apomictic and usually also pseudogamous, i.e. pollination is necessary to trigger the parthenogenetic development of unreduced egg cells. Apomixis in dogroses has been demonstrated by Werlemark and Nybom (2001) and by Nybom et al. (2004, 2006). In summary, dogroses are genetically very similar. Still, great variability of morphological forms is widespread, with specific consistent characters that group populations into the large number of identified species. Relatively easy intercrossings may contribute to the peculiar location effect

observed in Flemish dogrose populations: the genetic similarity of geographically co-occurring but different dogrose species is higher than for distant populations of the same species (De Cock et al. 2007, 2008). This suggests a need for study of dogrose populations as a whole, including all species present on the study sites.

Orificium diameter and sepal position on the ripening hip (L/D type related characters described by Reichert 1998) have been shown to be transmitted through pollen in controlled crosses (Ritz and Wissemann 2003). L-type taxa have a loose growth habit (*laxus*) whereas D-type taxa have a dense one (*densus*). The L/D type variation occurs among all dogrose species (Table 1) including the subsection *Rubigineae*. The shape of the leaflets further divides this subsection in two groups: taxa with slender leaflets and wedge-shaped bases, for example *R. agrestis*, *R. inodora*, and *R. elliptica*, and taxa with broad leaflets and well-rounded bases: *R. rubiginosa*, *R. micrantha*, and *R. gremlii* (Table 1). As genetic differentiation among these species is very low (De Cock et al. 2007, 2008) and continuous transitions between species with rounded leaf base and those with a wedge shaped one are not described, it can be suggested that the form of the leaflet base is a character expressed by the univalents. Interspecific hybridisation between *R. micrantha*, *R. gremlii*, and *R. rubiginosa* may explain the continuous transition in the characters that constitute the L/D type, observed in populations where the three species occur together (De Cock et al. 2007, 2008). Here the typical characteristics of the putative parental species *R. micrantha* and *R. rubiginosa* tend to fade away. Thus hybrid swarms can be observed in several places in the Low Countries. Present day *R. agrestis* populations in the Low Countries, on the other hand, do not occur in hybrid swarms with *R. inodora* and *R. elliptica*.

Here we report an in-depth morphological study of the wild rose populations in Flanders that harbour *R. agrestis* ($2n = 5x$, $6x = 35, 42$ chromosomes; Ritz et al. 2005), an extremely rare member of the subsection *Rubigineae* in the Low Countries (Bakker et al. 2011). We study in detail:

1. the remarkable morphological differentiation of *R. agrestis* in geographically proximate populations;
2. the morphological characteristics of the *R. agrestis* populations in relation to those of the other dogroses present on the sites;
3. the relationship between leaf morphology of *R. agrestis* mother plants in the field and their half-sib descendants grown under controlled nursery conditions; and
4. the correlation of the number of seeds per hip with other morphological characters and the putative taxonomic consequences for dogroses in general.

We hypothesise on the origin of the morphological divergence in *R. agrestis* populations in the light of dogrose genetic structure and reproduction.

Table 1 Taxonomic classification of *Rosa* L. section *Caninae* (DC.) Ser. species according to Henker (2000), following the L/D system of Reichert (1998)

| L/D type, orificium diameter | Subsection <i>Rubigineae</i> Christ | | Subsection <i>Vestitae</i> Christ | Subsection <i>Caninae</i> Christ | | |
|------------------------------|--|--|---|---------------------------------------|--|-----------------------------------|
| | Leaflets glandular, apple odour, pubescent | | Leaflets glandular, turpentine odour, densely pubescent | Leaflets without odour | | |
| | Wedge-shaped leaflet base | Rounded leaflet base | | Rachis pubescent, glandular | Rachis pubescent, not glandular | Rachis not pubescent |
| L, <1 mm | <i>R. agrestis</i> Savi (a) | <i>R. micrantha</i> Borrer ex Sm. | <i>R. tomentosa</i> Sm. (t) | <i>R. balsamica</i> Besser (b) | <i>R. corymbifera</i> Borkh. (o) <i>R. stylosa</i> Desv. | <i>R. canina</i> L. (c) |
| L/D, ±1 mm | <i>R. inodora</i> Fries | <i>R. gremlii</i> (H.Christ) H.Christ (g) | <i>R. pseudoscabriuscula</i> (R. Keller) Henker et G. Schulze | | <i>R. subcollina</i> (Christ) Vuk. | <i>R. subcanina</i> (Christ) Vuk. |
| D, >1 mm | <i>R. elliptica</i> Tausch | <i>R. rubiginosa</i> L. | <i>R. sherardii</i> Davies | | <i>R. caesia</i> Sm. | <i>R. dumalis</i> Bechstein |

Only taxa indigenous to the Low Countries are included (De Cock 2008; Bakker et al. 2011). Essential diagnostic characters are included. Species and their abbreviations sampled for an in-depth morphological study are indicated in bold and in parentheses, respectively

Materials and methods

Rosa agrestis seedlings in the nursery

Based on an inventory of autochthonous woody plants (Vander Mijnsbrugge et al. 2005), all growth sites of *R. agrestis* in Flanders were prospected in the field in the autumn of 2009 (Fig. 1). Autochthony is interpreted here in accordance with Kleinschmit et al. (2004). Two to four fully developed hips were collected from 39 *R. agrestis* plants at the different growth sites (Table 2) and seedlings were grown for a living gene bank for this rare and endangered species. Seeds were stratified and five seedlings per mother plant were grown in pots in accordance with standard nursery procedures. From a random two-year-old seedling per mother plant three fully developed leaves were dried in accordance with standard herbarium methods. The seedlings were not yet fructifying in 2011.

Dogroses sampled in the field

All growth sites of *R. agrestis* were revisited in the field in the autumn of 2010 (Fig. 1). All *R. agrestis* plants present were sampled, together with all other dogrose species growing at the same site (Table 2). Identification followed Henker and Schulze (1993) and Henker (2000). A simplified identification key is shown in Table 1. As *R. canina* and *R. corymbifera* (counted together) were always more abundant than *R. agrestis*, no more than twice the number of these dogroses were sampled in comparison with the number of *R. agrestis* present on the site. Three hips and three leaves were sampled on fructifying twigs for each dogrose. Hips and leaves were fully developed and insect

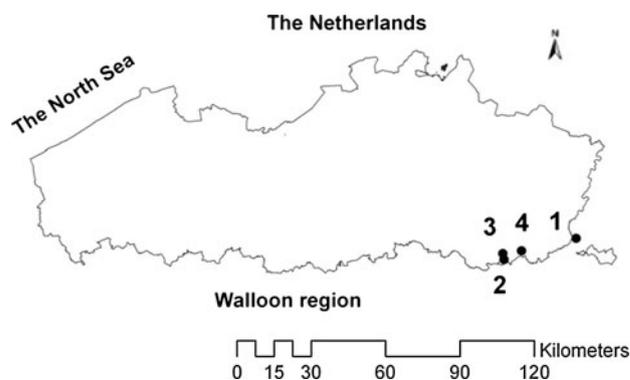


Fig. 1 Location of the sampled dogroses in Flanders. 1 Kanne, 2 Batsheers, 3 Gelinden, 4 Heks

and damage free. Leaves were dried in accordance with standard herbarium methods and hips were stored in ethanol (96 %). GPS coordinates of the exact growth sites of all sampled roses were registered in GIS using ArcGIS version 9.3 (ESRI). All growth sites were hedgerows along field trails and along sunken roads that contained a wide variety of indigenous shrubs and trees. They are relicts in an agriculturally dominated landscape that is strongly affected by scale enlargement. Soil type is in all cases loamy with sound physical properties.

Morphometric measurements, counts, observations, derived characters, and data analysis

Several characters were measured, counted, or observed on the leaves and hips (Table 3; Fig. 2). Selection of characters was based on previous studies (De Cock et al. 2007, 2008). The leaf characters LL, LW, LbL, and RL, and the hip characters HL, HbL, HW, PL, OD, and DD were

Table 2 Sampling of dogroses per growth site

| Location | Loc. no. | Seed | <i>R. agrestis</i> (a) | <i>R. canina</i> (c) | <i>R. corymbifera</i> (o) | <i>R. balsamica</i> (b) | <i>R. tomentosa</i> (t) | <i>R. gremlii</i> (g) | Total |
|-----------|----------|------|------------------------|----------------------|---------------------------|-------------------------|-------------------------|-----------------------|-------|
| Kanne | 1 | 15 | 17 | 31 | 0 | 1 | 0 | 8 | 57 |
| Batsheers | 2 | 12 | 11 | 8 | 9 | 0 | 0 | 0 | 28 |
| Gelinden | 3 | 8 | 15 | 21 | 4 | 0 | 6 | 0 | 46 |
| Heks | 4 | 4 | 3 | 2 | 3 | 0 | 1 | 0 | 9 |
| Total | | 39 | 46 | 62 | 16 | 1 | 7 | 8 | 140 |

Number of *R. agrestis* plants from which seeds were collected in 2009 (Seed) and number of different dogrose plants sampled in 2010 in the field for in-depth morphological analysis are indicated. Abbreviations of species are in parentheses

measured by use of a rod. The density of pubescence and glands were scored from 1 (absent) to 5 (densely pubescent or glandular) for the leaf characters RG, RPu, LmG and hip characters PG and PPU. The leaf character LmSe was scored from 1 (single toothed) to 3 (multiple toothed). The hip character SN was counted for 1 or 2 most representative hips. Of these 17 measured, counted, and observed

characters ten transformed variables were derived (Table 3). The data were studied by use of box and whisker plots, histograms, scatter plots, and summary statistics. Principal components analysis (PCA) and linear regression, available in S-Plus Professional (Tibco Software), were used for data analysis, using the mean values for three leaves and hips per shrub.

Table 3 Overview of characters used to describe dogrose species in this study

| Organ | Character | Ab. | Description |
|---------------------|--------------------------|------------|--|
| Leaf | Lamina length | LL | Length of leaflet lamina |
| | Lamina base length | LbL | Length of basal part of leaflet lamina till largest width |
| | Lamina width | LW | Largest width of leaflet lamina |
| | Rachis length | RL | Length of rachis |
| | Leaflet margin serration | LmSe | Serration of the leaflet margin scored from 1 (single toothed) to 3 (multiple toothed) |
| | Leaflet margin glands | LmG | Glands on the leaflet margin scored from 1 (no glands) to 5 (densely glandular) |
| | Rachis pubescence | RPu | Pubescence on the rachis scored from 1 (no pubescence) to 5 (dense hairiness) |
| | Rachis glands | RG | Glands on Rachis scored from 1 (no glands) to 5 (densely glandular) |
| | Lamina shape | LS | LL/LW |
| | Lamina base shape | LbS | LbL/LW |
| | Lamina length ratio | LLR | LL/RL \times 100 |
| | Lamina base length ratio | LbLR | LbL/RL \times 100 |
| | Lamina width ratio | LWR | LW/RL \times 100 |
| | Fruit | Hip length | HL |
| Hip base length | | HbL | Basal length of hip till largest width |
| Hip width | | HW | Largest width of hip |
| Pedicele length | | PL | Length of hip pedicel |
| Orificium diameter | | OD | Diameter of orifice |
| Discus diameter | | DD | Diameter of disc |
| Seed number | | SN | Number of well developed seeds per hip |
| Pedicele pubescence | | PPu | Pubescence of pedicel scored from 1 (no pubescence) to 5 (dense hairiness) |
| Pedicele glands | | PG | Glands on pedicel scored from 1 (no glands) to 5 (densely glandular) |
| Fruit length | | FL | HL + PL |
| Hip shape | | HS | HL/HW |
| Hip base shape | | HbS | HbL/HW |
| Hip length ratio | | HLR | HL/FL \times 100 |
| Orificium ratio | | OR | OD/DD \times 100 |

Ab., abbreviation

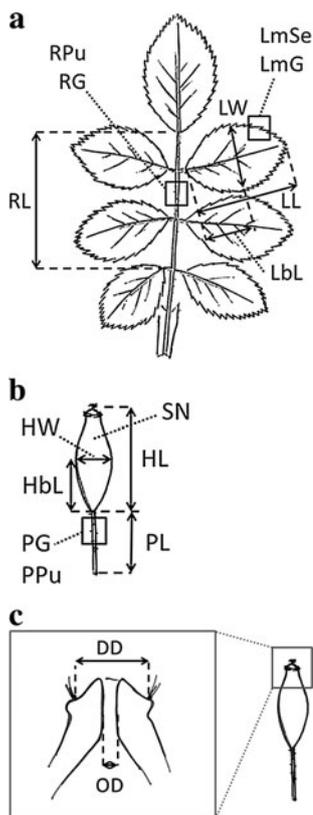


Fig. 2 Morphological leaf (a) and fruit (b, c) characters that were measured, counted, or observed. c Longitudinal section of the upper part of a hip. Abbreviations of characters are listed in Table 3

Results

Differentiating the morphology of geographically proximate *R. agrestis* populations

In a previous study, a tendency for geographical population differentiation in *R. agrestis* was observed in the morphology of a small number of sampled plants (De Cock et al. 2007, 2008). We examined this putative differentiation for whole populations, including other dogrose species present on the sites. A PCA biplot for all *R. agrestis* plants sampled in the field, including both primary characters and derived variables, reveals clear differentiation between the population in Kanne, on the one hand, and the populations of Batsheers, Gelinden, and Heks, on the other, along the first component axis (Fig. 3a). HS, LWR, HbS, LbS, LmG, OD, PG, LLR, and RPu are important explanatory characters, in decreasing order, according to the PCA loadings of the first axis. A bimodal distribution along this axis is visualised by grouping the *R. agrestis* plants in classes according to their first component scores in a histogram (Fig. 3b). PCA on only leaf or hip data does not separate the populations as obviously (Fig. 3c, d), confirming the contribution of both organs to the bimodal distribution.

Rosa agrestis compared with other dogroses present on the sites

As interspecific hybridisation among dogroses occurs, the population differentiation observed can be attributed to these events. To examine this hypothesis the morphology of the *R. agrestis* plants was compared with that of other dogroses present on the growth sites. PCA was performed on all the dogroses sampled in the field, including both primary characters and derived variables. In a biplot of the first two components no clear separation between the different dogrose species is visible (Fig. 4a), although grouping is evident. The *R. agrestis* populations of Kanne cluster closer to *R. gremlii*, another member of the subsection *Rubigineae*, along the first component axis in separate PCA in comparison with the other *R. agrestis* group (Fig. 4b). But, when compared with *R. canina* and *R. corymbifera*, the *R. agrestis* population in Kanne unmistakably inclines toward these members of the subsection *Caninae* (Fig. 4c). A box and whisker plot of the character hip shape (HS) reveals the deviating morphology of the *R. agrestis* populations in Batsheers, Gelinden, and Heks in comparison with all other dogroses present on the sites (Fig. 5).

Seed number

Hybrids or introgressed individuals may suffer from reduced reproductive capacity. Because the number of seeds per hip (SN) may be indicative of potential for reproductive success, this character was included in the analysis. Overall, the abundant species *R. canina* and *R. corymbifera* tend towards higher values for SN (Fig. 6a). No differentiation was observed between the sampled *R. agrestis* populations and *R. gremlii*, the only other member of the subsection *Rubigineae* present on the sampled sites, tends towards *R. agrestis* for this character (Fig. 6a). Remarkably, there is a significant correlation between SN and HW (correlation coefficient 75 %) but much less with HL (28 %). SN is correlated further with DD (62 %) but much less with OD (13 %). Linear regression statistics are shown in Table 4. In other words, the wider the hip, the wider becomes the discus and the more seeds it can contain, comparatively irrespective of the length of the hip or the diameter of the orificium.

Nursery seedlings versus mother plants sampled in the field

To examine the weight of environmental factors versus heritability in the geographically differentiating morphology of the *R. agrestis* populations, half-sib seedlings descending from the sampled *R. agrestis* mother plants were grown under controlled nursery conditions (Table 2).

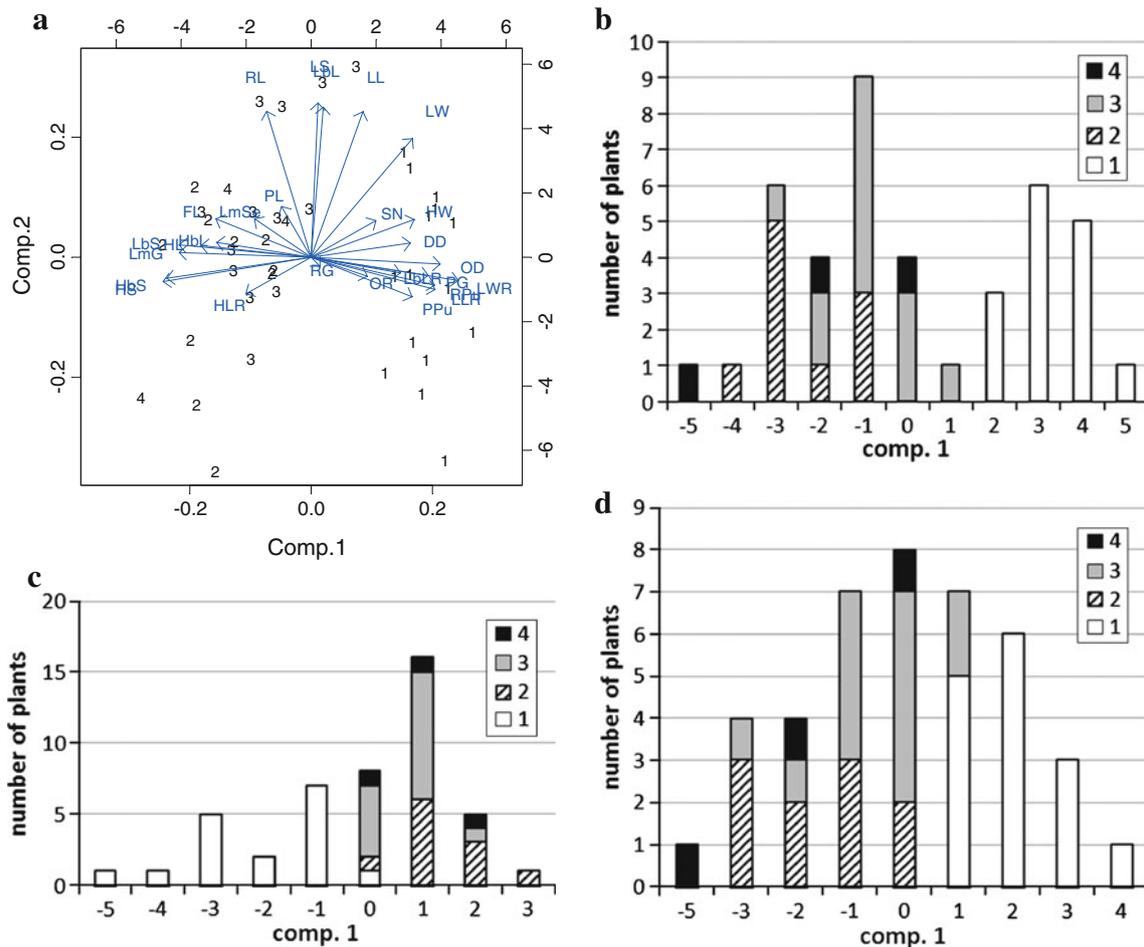


Fig. 3 PCA of morphological leaf and hip data of *Rosa agrestis* sampled in the field, indicating the different locations sampled (location nos are given in Table 2 and character abbreviations in Table 3). **a** Biplot of PCA with morphological characters of both leaf and hip. The first two components explain 36 and 17 % of the

variation. **b** Histogram of the first component from the PCA in Fig. 3a. **c** Histogram of the first component from PCA of leaf characters only. The first component explains 36 % of the variation. **d** Histogram of the first component from PCA of hip characters only. The first component explains 42 % of the variation

PCA of all leaf characters of this offspring was performed (biplot not shown). Loadings of the first two components explained 32 and 24 % of the variation, indicating the importance of the characters LLR, LbLR, LWR, and, to a lesser extent, RPu and RL for differentiating the Kanne population from the others (Table 5). Linear regression analysis of these characters between mother plants and their corresponding seedling reinforces the heritable aspect of the two morphologically differentiating *R. agrestis* groups (Table 5; Fig. 7).

Discussion

Hybridisation and evolution by reticulation

In the course of evolution, most higher plants did not diversify by simple bifurcation but rather by a reticulate

pattern of hybridisation and introgression (Yang and Zhou 2010). Morphological variation among dogrose populations should be interpreted in the light of their polyploid nature and the genetic consequences of the unique, unbalanced canina meiosis. In outcrossing sympatric plant taxa with sexual reproduction driven by normal meiosis, one can expect theoretically that interspecific crossings, which lead to fertility in hybrids comparable with that in the parental taxa, may evolve during fading of morphological boundaries between parental species with, eventually, the creation of a new species (an essential aspect of reticulate evolution). Among woody plants, hybridisation between related sympatric species occurs in many genera (Petit and Hampe 2006) although the hybrid swarms did not evolve to morphologically single species. Lower fertility of hybrids and introgressed individuals may hamper this unifying evolution as was, e.g., suggested for the *Q. robur*–*Q. petraea* complex (Vander Mijnsbrugge et al. 2011). In our

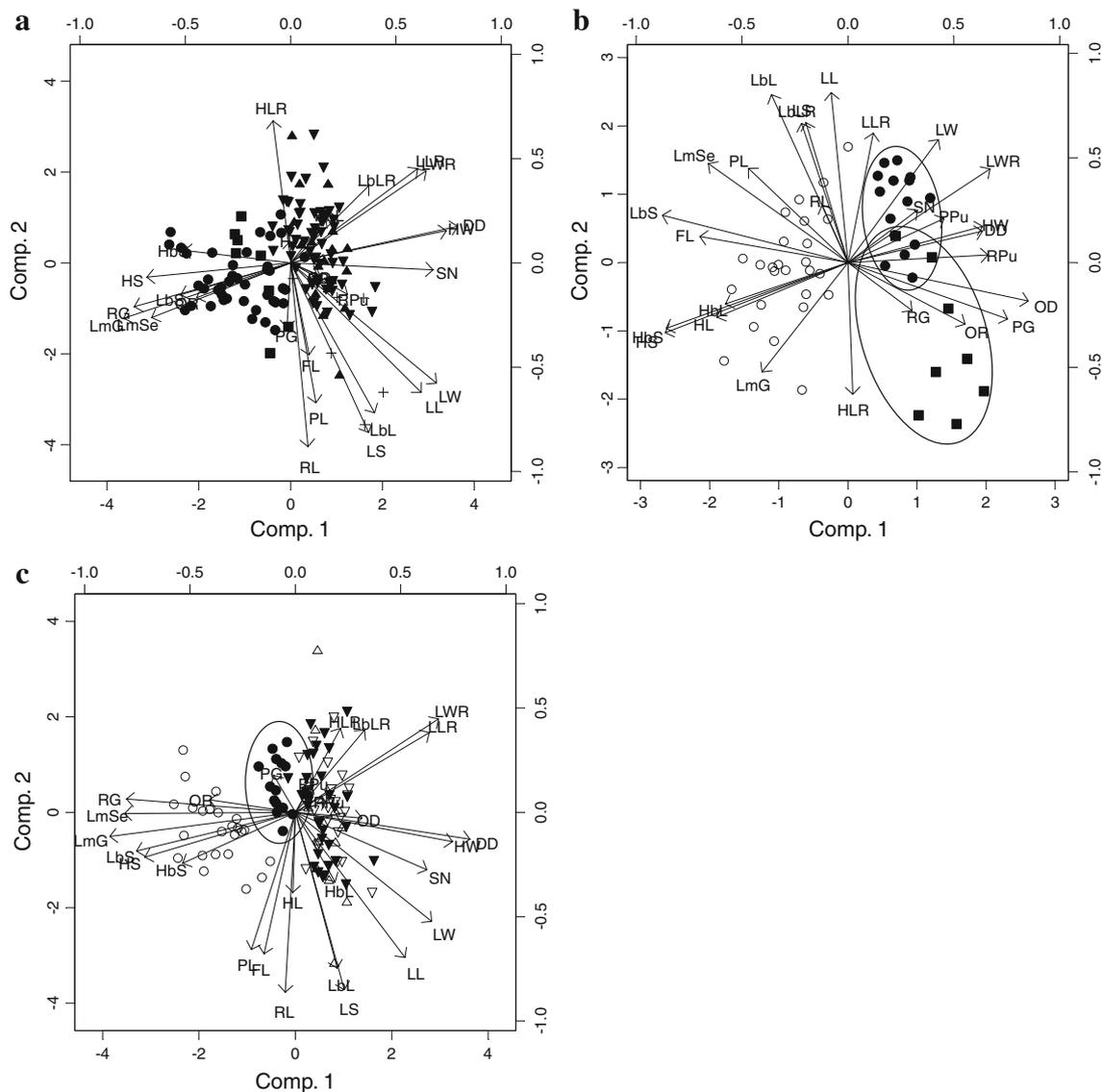


Fig. 4 Biplots from PCA of all morphological characters of the different sampled dogrose species in the field (character abbreviations are given in Table 3). Filled circles, *R. agrestis*; filled inverted triangles, *R. canina*; filled triangles, *R. corymbifera*; filled squares, *R. gremlii*; plus symbols, *R. tomentosa*. **a** PCA of all sampled dogroses. The first two components explain 25 and 18 % of the variation. **b** PCA of *R. agrestis* and *R. gremlii*. The first two components explain 31 and 19 % of the variation. Filled and unfilled

symbols indicate plants sampled in Kanne and in the other locations respectively. Ellipses include *R. agrestis* (filled circles) and *R. gremlii* (filled squares) from Kanne. **c** PCA of *R. agrestis*, *R. canina*, and *R. corymbifera*. The first two components explain 27 and 18 % of the variation. Filled and unfilled symbols indicate plants sampled in Kanne and in the other locations, respectively. The ellipse indicates *R. agrestis* from Kanne (filled circles)

study the form of the leaflet base, distinguishing subsection *Rubigineae* members, with a rounded leaf base, from *R. agrestis* (Table 1), was unambiguously diagnostic during identification in the field, and can be suggested as being determined, at least partly, by loci on the univalents. Morphological differentiation can be hypothesised to arise when interspecific hybridisation results in deviating morphological characters within the *R. agrestis* group, via bivalent recombination and the creation of fertile offspring.

Bimodal distribution of *R. agrestis*

We observed two obvious clusters of *R. agrestis* with individuals from Kanne, on the one hand, and individuals from the proximate populations in Batsheers, Gelinden, and Heks, on the other, with no overlap when both (vegetative) leaf and (generative) hip data were processed together in PCA (Fig. 3a). This bimodal distribution along the first component axis of the PCA (Fig. 3b) was

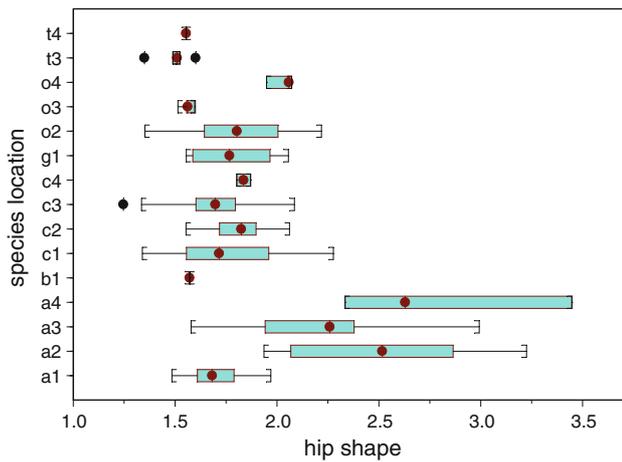


Fig. 5 Box and whisker plot of the character hip shape (HS) (species abbreviations and location nos are given in Table 2). *F*-statistic (one-way ANOVA) = 12.4 with *p* value = 0

unexpected, because all individuals were identified in the field without uncertainty as *R. agrestis*. Significant linear regression of leaflet length ratio (LLR), leaflet width ratio (LWR), and rachis pubescence (RPu) between mother plants in the field and their corresponding half-sib nursery descendants confirms a genetic cause of the observed differentiation of these characters in the two deviating *R. agrestis* groups (Fig. 7; Table 5). The putative hybrid origin of one of the deviating groups was examined by comparing its morphology with that of all the other dogrose species that co-occur on the growth sites. In PCA analysis (Fig. 4c), the *R. agrestis* population of Kanne clearly clusters closer to *R. canina* and *R. corymbifera* than the *R. agrestis* population of the Batsheers, Gelinden, and Heks group. Because of the unequal distribution of chromosomes in the gametes that fuse in canina meiosis, matroclinal inheritance is generally attributed to dogroses (Werlemark et al. 1999; Wissemann et al. 2007). Still, individual characters can deviate from this mode of inheritance, for example those that determine the L/D type (Ritz and Wissemann 2003) or growth form and mechanical characters (Wissemann et al. 2006). In addition, silencing of rDNA loci on the univalent has been demonstrated by Khaitová et al. (2010, 2006). We hypothesise a hybridogenic origin for the Kanne population, resulting from crossings between *R. agrestis* and a subsection *Caninae* member. This is exemplified by the character hip shape in Fig. 5. Values for this character for the Kanne *R. agrestis* plants are similar to those for all other sampled dogrose species at all the growth sites, whereas the values for the Batsheers, Gelinden, and Heks *R. agrestis* plants clearly deviate. If the Kanne population originated from interspecific crossings between a maternal *R. agrestis* population and a paternal one of an L-type member of subsection

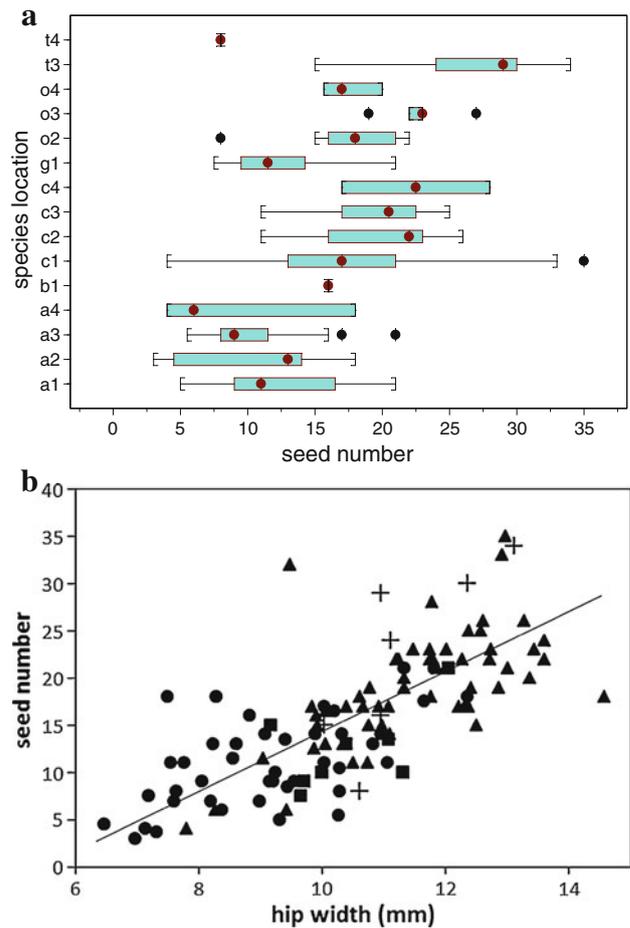


Fig. 6 Analysis of the character seed number (SN). **a** Box and whisker plot of SN across all sampled dogroses (species abbreviations and location nos are given in Table 2). *F*-statistic (one-way ANOVA) = 6.2 with *p* value = 0. **b** Scatter plot of SN and hip width (HW). Filled circles, *R. agrestis*; filled triangles, *R. corymbifera* and *R. canina*; filled squares, *R. gremlii*; plus symbols, *R. tomentosa* and *R. balsamica*. The linear regression line is indicated (statistics in Table 4)

Caninae, a hybrid population can come into existence as *R. agrestis* (form of leaf base supposedly univalent-driven) but incorporating, to some extent, characteristics of the subsection *Caninae* member via bivalent recombination, thus expressing characters that can follow Mendelian segregation. The Kanne population would thus cluster closer to the original *R. agrestis* mother group (supposedly more similar to the Batsheers, Gelinden, and Heks group) than the paternal subsection *Caninae* group. But, remarkably, the opposite is true. It can be postulated that the involved subsection *Caninae* characters are more dominant than those of the original *R. agrestis* population. Interspecific crossing between a maternal *R. agrestis* population and a paternal *R. gremlii* seems less likely as the diameter of the orificium would follow the paternal line and would thus result in larger values in the Kanne population.

Table 4 Linear regression of SN (seed number) with selected characters

| Character | df | R ² | F | p |
|-----------|-----|----------------|-------|---------|
| HW | 132 | 0.55 | 161.9 | 0* |
| DD | 132 | 0.37 | 76.54 | 0* |
| HL | 132 | 0.07 | 10.53 | 0.0015* |
| OD | 132 | 0.02 | 2.42 | 0.12 |

Character abbreviations are listed in Table 3

df, degrees of freedom; R², R-squared; F, F-statistic; and p values (p) are indicated

* p ≤ 0.05

Table 5 Analysis of the morphology of half-sib nursery seedlings of *R. agrestis*

| Character | Axis 1 | Axis 2 | df | R ² | F | p |
|-----------|--------|--------|----|----------------|------|-------|
| LLR | -0.38 | 0.33 | 25 | 0.20 | 6.20 | 0.02* |
| LWR | -0.36 | 0.33 | 25 | 0.15 | 4.56 | 0.04* |
| RPu | -0.23 | 0.44 | 25 | 0.14 | 4.06 | 0.05* |
| LbLR | -0.17 | 0.12 | 25 | 0.07 | 1.89 | 0.18 |
| RL | 0 | -0.54 | 25 | 0.01 | 0.18 | 0.66 |

Loadings for differentiating leaf characters of the first two components from PCA of all leaf characters of half-sib nursery seedlings. Statistics from linear regression of these leaf characters between mother plants in the field and their corresponding half-sib nursery seedlings. Character abbreviations are listed in Table 3

df, degrees of freedom; R², R-squared; F, F-statistic; and p values (p) are indicated

* p ≤ 0.05

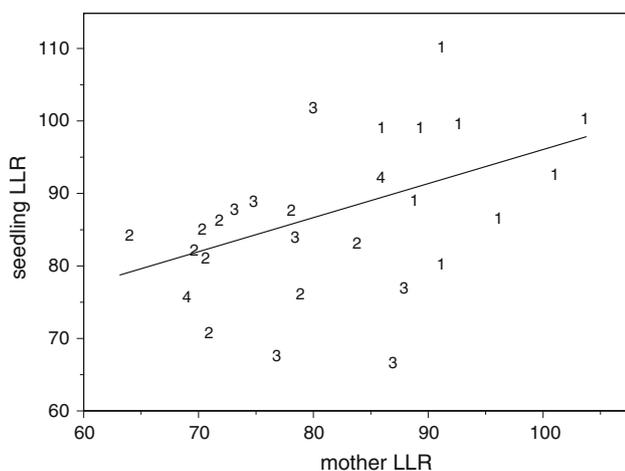


Fig. 7 Morphology of half-sib nursery seedlings of *Rosa agrestis*. Scatter plot of the character lamina length ratio (LLR) for the mother plants compared with LLR for the seedlings. The linear regression line is indicated (statistics in Table 5)

SN and the taxonomically diagnostic OD

Number of well developed seeds per hip (SN), a character potentially expressing plant fertility, did not differentiate the two deviating groups of *R. agrestis* (Fig. 6a). If the Kanne population is hybridogenic in origin, this may be

evidence of unaffected fertility. Interestingly, SN was strongly correlated with the width of the hip (HW) and the diameter of the discus (DD), although much less with the length of the hip (HL) or the diameter of the orificium (OD) (Table 4). The more seeds a hip should bear, the wider becomes hip and discus, relatively irrespective of the diameter of the orificium. The abundant *R. canina* and *R. corymbifera* on the studied sites harboured more seeds per hip than the rare *R. agrestis* and *R. gremlii*. Because OD, but not SN, determines the L/D type differences among the dogroses, and DD is more related to the number of seeds per hip than OD, the derived variable OR is likely to be less informative, although it is given diagnostic weight in the taxonomic work of Henker (2000). These results argue in favour of ignoring OR as a taxonomic division of L/D types among dogrose species.

In conclusion, the combination of a conservative univalent and a recombining bivalent genome both with putative differential expression patterns may result in morphological divergence, despite the reported preference of bivalent homology for successful gamete fusion and the overall genetic similarity among dogrose taxa using molecular marker techniques, for example AFLP and SSR. This morphological flexibility may have contributed to the evolutionary species radiation success in the subsection *Caninae*.

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