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GENETIC DIVERSITY OF WILD ROSES (*ROSA* SPP.) IN EUROPE, WITH AN IN-DEPTH MORPHOLOGICAL STUDY OF FLEMISH POPULATIONS

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:
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Summary

The taxonomical hierarchy within the subgenus *Rosa* and the section *Caninae* is known to be complex. This complexity is due to a combination of factors such as a large phenotypic and genetic plasticity, the possibility of interspecific and even intersectional hybridisation, and less straightforward modes of reproduction. Within the section *Caninae*, this complexity increases even more due to the unique chromosomal constitution, combining two types of genomes, and the heterogamous canina meiosis which causes predominant maternal inheritance.

The first goal of this thesis was to expand the knowledge of the taxonomical complexity within the section *Caninae* by analysing morphological characters and molecular-genetic markers. Secondly, we wanted to assess the intraspecific genetic differentiation of the European and Flemish wild roses. In addition, the eight most common Flemish rose species were analysed morphologically.

The polyploid and heterogamous section *Caninae* does not meet the Hardy-Weinberg assumptions required for generally applied population genetic analyses. Consequently, alternative and more descriptive strategies were used to analyse the molecular-genetic polymorphisms. The combination of these different approaches is assumed the best strategy to handle such a polyploid and hybridogenic species-complex as complementary outcomes are obtained.

TAXONOMICAL DIFFERENTIATION

Henkers' classification of the European subgenus *Rosa* into five sections *Pimpinellifoliae, Rosa, Cinnamomeae, Synstylae* and *Caninae* was supported. In addition, the unique and peculiar position of the section *Caninae* was confirmed as this polymorphic group forms a very dense and well-defined genetic unit within the subgenus *Rosa*.

Within the section *Caninae*, a hierarchical structure was observed reflecting the three groups described by Graham and Primavesi, and Nilsson: *R. rubiginosa*-, *R. villosa*-, and *R. canina*-group. In contrast, the subdivision in subsections according to Henker and Wissemann was only partly supported by our outcomes. Although each group is characterised by few well-distinguishable and consistent morphological characters, overlap between the groups is substantial, indicating the combined presence of species-specific characters and intermediate forms. In contrast to the subdivision of the groups *Rubigineae* and *Vestitae*, we did not find a morphological or molecular-genetic argument to support the subdivision of *R. balsamica* (subsection *Tomentellae*) nor of the taxa of the subsection *Caninae* as was proposed by Henker. We confirm the grouping of Nilsson who placed *R. balsamica* into the *R. canina*-group, or refer to *Caninae-Tomentellae*.

Within each subsection, few evident and parallel combinations of morphological characters distinguish the section *Caninae* taxa in L and D types. Two of the principal characters to determine these types are the diameter of the orifice and the persistence of the sepals. As both characters have been proven to be inherited

paternally, and additionally interspecific F1 hybrids can be fertile, these characters should not have a diagnostic value.

The influence of past hybridisation and/or the present-day occurrence of different taxa is clearly observed when comparing the morphological and molecular-genetic characters of "species-pure" and mixed populations. Accepting the loss of the species level in mixed populations, the spontaneous hybrids that are characterised by a range of variable transitional forms between the parental taxa should be assigned to a species-complex. Each species-complex consists of two considerably pure parental taxa and a range of intermediate individuals or hybrids. The parental taxa display well-defined species-specific characters in the "species-pure" populations. The pure individuals are mostly absent in the mixed population. One example is the status of *R. henkeri-schulzei*. Although it has a species status according to Henker, no consistent or detailed description is found in literature. Moreover, the molecular-genetic analyses were not able to discriminate between the two parental taxa. Therefore, the spontaneous hybrids, displaying both transitional and species-specific characters in mixed *R. micrantha* - *R. rubiginosa* populations, are suggested to be assigned to the *R. micrantha* - *R. henkeri-schulzei* - *R. rubiginosa* species-complex.

The morphological well-defined and distinguishable differences between *R. pseudoscabriuscula* and *R. tomentosa* are less obvious and consistent among the Flemish *Vestitae* individuals. In addition, no genetic differentiation was observed among the European *R. pseudoscabriuscula* and *R. tomentosa*. We suggest considering *R. pseudoscabriuscula* and *R. tomentosa* as only one species, or similar to the species complex *R. micrantha* - *R. henkeri-schulzei* - *R. rubiginosa* assign them to one species complex.

The hybridogenic origin of *R. stylosa* through intersectional hybridisation between *R. arvensis* and section *Caninae* taxa was confirmed by the morphological and molecular-genetic analyses. The paternal influence of *R. arvensis* was confirmed; however we could not identify the most probable seed parent. Among the three section *Caninae* taxa: *R. canina*, *R. corymbifera* and/or *R. balsamica* little morphological and no genetic differentiation is observed. No conclusions were drawn regarding the influence of the subsection *Rubigineae* through ancient hybridisation as suggested by the phylogenetic analyses.

GEOGRAPHICAL DIFFERENTIATION

At the European scale *R. spinosissima, R. gallica, R. majalis*, and *R. pendulina* displayed intraspecific geographical differentiation. In addition, at the small geographical scale within Belgium genetic differentiation was assessed among the inland and coastal populations of *R. spinosissima*. Moreover, *R. arvensis* displayed both genetic and morphological intraspecific differentiation at an even smaller geographical scale.

Within the section *Caninae*, no geographical genetic differentiation was observed among the European populations. Particularly in Flanders, few indications have been found towards intraspecific morphological and/or genetic differentiation

for e.g. *R. agrestis* and *R. tomentosa*. This difference might be the expression of local adaptation, or of a rare ancient and untraceable hybridisation event. The influence of these differences can only be validated in provenance trails.

Several indications were observed for the occurrence of (ancient) interspecific hybridisation events, stressing the far-reaching influence of the presence of multiple section *Caninae* taxa on the morphological and/or genetic variation of the taxa in particular and the population in general. The most striking example is the higher genetic similarity among morphologically distinguishable individuals, *R. canina*, *R. corymbifera* and *R. balsamica* all sampled at Het Zwin (Westkust), compared to the genetic similarity with their congeners sampled at other localities.

The expected intrapopulational clonality was presumed for the tetraploid *R. spinosissima* and validated for *R. arvensis*. However, the clonality within one population should not be overestimated, considering that different allelic phenotypes (*R. spinosissima*), or genotypes (*R. arvensis*) were observed within one densely grown population using STMS markers.

CONSERVATION

In the framework of conservation and use of autochthonous genetic resources, the observed intraspecific differentiation should be maintained if it is reflected in the genetic structure of the population and when it influences the fitness of that population or species. In addition, the conserved populations should contain sufficient genetic variation to allow them to adapt to changing environmental conditions. Each species is characterised by special life history features and populations are affected by different influences. Consequently, the conservation strategies of the different taxa are discussed separately.

Within *R. spinosissima* (section *Pimpinellifoliae*), *R. gallica* (section *Rosa*), *R. majalis*, and *R. pendulina* (both section *Cinnamomeae*), the observed genetic differentiation suggests the presence of local adaptation. For *R. arvensis* (section *Synstylae*), both morphological and genetic differentiation was observed within Flanders. As the assessment of interpopulational differentiation is only the first step, provenance trails should validate if the observed differentiations are worth conserving. Until then, the precautionary principle is followed and the deviating population will remain separate from the others.

Within the taxa of the section *Caninae*, the impact of introgression of non-adapted genes might be less threatening. First, the non-adapted genes have to be located on the bivalent-forming chromosomes. In addition, the homology with the maternal bivalent-forming chromosome has to be sufficiently high before the F1 hybrids are fertile and able to backcross. Moreover, the non-recombinant univalent-forming chromosome sets may serve as an additional buffer to compensate for the non-adapted genes. Secondly, the observed morphological intraspecific variation is probably caused by the introgression of other neighbouring section *Caninae* taxa, and is generally not reflected in the genetic structure of the individuals. At this moment, too many uncertainties remain regarding the canina meiosis. Therefore, populations displaying morphological and genetic interpopulational differentiation in certain

taxa or subsections will be conserved as a separate unit. Moreover, each population, species or species complex has to be evaluated separately.

The value of certain hotspot localities is already acknowledged, as they are a protected area by the Flemish decree of dunes.

Samenvatting

Het subgenus *Rosa*, en meer specifiek de sectie *Caninae*, heeft een complexe taxonomische structuur. Het gecombineerde voorkomen van enkele factoren zoals een grote feno- en genotypische plasticiteit, de mogelijkheid tot inter-specifieke en zelfs inter-sectionele hybridisatie, en minder voor de hand liggende voortplantingsstrategieën liggen aan de basis van deze complexiteit. Binnen de sectie *Caninae* wordt de complexiteit nog verhoogd door de unieke chromosoomsamenstelling, het voorkomen van twee genomentypes en door de heterogame meiose die aanleiding geeft tot predominante maternale overervingpatronen.

Onze eerste doelstelling was meer inzicht verwerven in de taxonomische structuur van de sectie *Caninae* aan de hand van morfologische kenmerken en moleculair-genetische merkers. Daarnaast wilden we de genetische intra-specifieke differentiatie bepalen van zowel Europese als Vlaamse wilde rozen door middel van moleculair-genetisch merker onderzoek. De acht meest voorkomende wilde rozen in Vlaanderen werden ook onderworpen aan een uitgebreid morfologisch onderzoek.

Omdat de polyploide en heterogame sectie *Caninae* taxa niet voldoen aan de Hardy-Weinberg voorwaarden, werden alternatieve en meer beschrijvende strategieën toegepast om de moleculair-genetische polymorfismen te analyseren. De resultaten van de verschillende methoden waren complementair. Bijgevolg kunnen we besluiten dat de combinatie van deze analysestrategieën een geschikte aanpak is om een polyploid en hybride soortencomplex te benaderen.

TAXONOMISCHE DIFFERENTATIE

De taxonomische opdeling van het Europese subgenus *Rosa* in de secties *Pimpinellifoliae, Rosa, Cinnamomeae, Synstylae* en *Caninae*, zoals voorgesteld door Henker, werd bevestigd in onze analyses. Daarenboven werd de unieke positie van de sectie *Caninae* onderstreept in de genetische analyses waar deze polymorfe groep een compacte en goed afgelijnde genetische eenheid vormt binnen het subgenus *Rosa*.

De hiërarchische structuur van de sectie *Caninae* zoals omschreven door Graham en Primavesi, en Nilsson werd bevestigd door de genetische en de morfologische analyses. Dit is in contrast met de taxonomische indeling volgens Henker en Wissemann welke maar tot zekere hoogte ondersteund wordt. Op basis van ons onderzoek aanvaarden wij het bestaan van drie groepen of subsecties *Rubigineae*, *Vestitae* en *Caninae* binnen de sectie *Caninae*. Elke groep wordt getypeerd door enkele typische en goed te onderscheiden morfologische kenmerken. Toch is de overlap tussen de groepen groot. In tegenstelling tot de afsplitsing van de *Rubigineae* en *Vestitae*, hebben we geen morfologische of moleculair-genetische argumenten gevonden die Henkers' afsplitsing van *R. balsamica* (subsectie *Tomentellae*) ondersteunt. Wij ondersteunen de opdeling van Nilsson en plaatsen *R. balsamica* in de subsectie *Caninae*, of verwijzen naar *Caninae-Tomentellae*.

Binnen elke subsectie verdelen duidelijk omschreven en gecorreleerde morfologische kenmerken, zoals de diameter van het stijlkanaal en de persistentie van de kelkblaadjes, de soorten in L en D types. Aangezien beide kenmerken via de pollenouder worden doorgegeven en hybriden tussen L en D type ouders fertiel kunnen zijn, zouden deze kenmerken geen taxonomische waarde mogen hebben.

De invloed van historische hybridisatie of het voorkomen van verschillende taxa op éénzelfde locatie is duidelijk waarneembaar bij het vergelijken van morfologische en moleculair-genetische kenmerken tussen "soortzuivere" en gemengde populaties. Wanneer we het verdwijnen van de zuivere soorten in een gemengde populatie aanvaarden, kunnen de spontane hybriden, gekenmerkt door een gradiënt van variabele overgangsvormen tussen de oudersoorten, toegewezen worden aan een soortencomplex. Elk soortencomplex bestaat uit twee eerder zuivere oudersoorten en hybriden gekenmerkt door verschillende gradaties van intermediaire vormen. In de zogenaamde "soortzuivere" populaties vertonen de individuen de typische soort-specifieke kenmerken. Echter deze zuivere individuen zijn meestal verdwenen in de gemengde populaties. Een typisch voorbeeld is de status van *R. henkeri-schulzei*. Ook al heeft Henker deze individuen als soort omschreven, er is tot nu toe geen consistente of gedetailleerde omschrijving gepubliceerd. Daarenboven was het in onze moleculair-genetische analyses niet mogelijk om de twee oudersoorten te onderscheiden. We stellen daarom voor om de spontane hybriden in een gemengde *R. micrantha* - *R. rubiginosa* populatie, die een combinatie van soort-specifieke en overgangskenmerken vertonen, toe te wijzen aan het soortencomplex *R. micrantha* - *R. henkeri-schulzei* - *R. rubiginosa*.

De duidelijke en goed beschreven morfologische verschillen die *R. pseudoscabriuscula* en *R. tomentosa* kenmerken, werden niet consistent waargenomen in de Vlaamse *Vestitae*. Daarenboven konden de Europese *R. pseudoscabriuscula* en *R. tomentosa* genetisch niet onderscheiden worden. Daarom stellen we voor om *R. pseudoscabriuscula* en *R. tomentosa* als één soort, of naar analogie met het soortencomplex *R. micrantha* - *R. henkeri-schulzei* - *R. rubiginosa* als een soortencomplex te beschouwen.

De hybridogene oorsprong van *R. stylosa* door inter-sectionele hybridisatie tussen *R. arvensis* en taxa van de subsecties *Caninae-Tomentellae* werd bevestigd in de morfologische en moleculair-genetische analyses. De invloed van *R. arvensis* werd als pollenouder bevestigd. We waren echter niet in staat om de meest waarschijnlijke *Caninae-Tomentellae* zaadoudersoort te selecteren. De drie mogelijke taxa *R. canina*, *R. corymbifera* en/of *R. balsamica* vertonen weinig morfologische en geen genetische inter-specifieke differentiatie. Verder kon er ook geen uitsluitsel gegeven worden in verband met de mogelijke historische invloed van de subsectie *Rubigineae* zoals werd gesuggereerd door fylogenetische analyses.

GEOGRAFISCHE DIFFERENTIATIE

Op Europese schaal vertonen de soorten *R. spinosissima, R. gallica, R. majalis* en *R. pendulina* intra-specifieke geografische differentiatie. Op kleinere geografische

schaal, meer bepaald binnen België, werd intra-specifieke genetische differentiatie vastgesteld voor *R. spinosissima* tussen de landinwaarts gelegen populaties en deze aan de kust. Tenslotte werd zowel genetische als morfologische differentiatie waargenomen voor *R. arvensis* op een nog beperktere geografische schaal.

De Europese sectie *Caninae* vertoonde geen geografische genetische differentiatie. Dit is in tegenstelling tot de Vlaamse soorten waar een aantal indicaties voor intra-specifieke morfologische en/of genetische differentiatie bij o.a. *R. agrestis* en *R. tomentosa* werden waargenomen. Deze differentiatie kan een aanwijzing zijn van lokale adaptatie, of van een zeldzame historische of ontraceerbare gebeurtenis. De invloed van deze variatie kan enkel via herkomstproeven achterhaald worden.

Het voorkomen van (historische) inter-specifieke hybridisatie werd op verschillende wijzen gesuggereerd. Daarenboven werd de invloed van het gezamenlijke voorkomen van verschillende sectie *Caninae* taxa op de morfologische en/of genetische structuur van de individuen en van de populatie bevestigd. Het meest opvallende voorbeeld is de hogere genetische similariteit tussen morfologisch onderscheidbare individuen uit eenzelfde gemengde populatie (bijv. *R. canina*, *R. corymbifera* en *R. balsamica* allemaal afkomstig uit Het Zwin (Westkust)) in vergelijking met de soortgenoten uit vermoedelijke zuivere populaties.

De verwachtte klonaliteit werd verondersteld bij de tetraploide *R. spinosissima* en bevestigd voor *R. arvensis*. De aanwezige klonaliteit binnen een zelfde populatie mag niet overschat worden aangezien er verschillende allelische fenotypes (*R. spinosissima*), of genotypen (*R. arvensis*) werden waargenomen met STMS merkers binnen een dichtbegroeide populatie.

BEHOUDSSTRATEGIEËN

In het kader van het behoud en gebruik van autochtone genenbronnen is het zinvol om de huidige intra-specifieke differentiatie te behouden als deze een genetische basis heeft en de fitness van de populatie of soort beïnvloedt. Het behoud van voldoende genetische variatie is noodzakelijk voor het overleven van de populatie bij veranderende omgevingsinvloeden. Elke soort wordt beïnvloed door kenmerken verbonden met zijn ontwikkelingsgeschiedenis, levenscyclus en dergelijke. Het is dan ook een must dat de behoudstrategieën voor elke soort of soortengroep afzonderlijk moet behandeld worden.

De waargenomen intra-specifieke genetische differentiatie bij *R. spinosissima* (sectie *Pimpinellifoliae*), *R. gallica* (sectie *Rosa*), *R. majalis* en *R. pendulina* (beiden sectie *Cinnamomeae*) suggereert de aanwezigheid van lokale adaptatie. De Vlaamse *R. arvensis* (sectie *Synstylae*) populaties vertoonden zowel morfologische als genetische differentiatie op een kleine geografische schaal. Het bepalen van intra-specifieke differentiatie is een eerste stap in het opstellen van behoudstrategieën. De impact en de waarde van de waargenomen variatie kan slechts gevalideerd worden aan de hand van herkomstproeven. In afwachting kan men het beste uitgaan van het voorzichtigheidsprincipe, en zullen de gedifferentieerde populaties niet gemengd worden.

Voor de taxa van de sectie *Canina* is de impact van de introgressie van niet-geadapteerde (intra-specifieke) genen waarschijnlijk minder bedreigend. Om invloed te hebben op de fitness van de sectie *Caninae* taxa moeten de niet- geadapteerde genen op de bivalent-vormende chromosomen zitten en moeten ze voldoende homoloog zijn met de bivalent-vormende chromosomen van de zaadouder om vruchtbare F1 hybriden te produceren. Deze vruchtbare hybriden zijn noodzakelijk om terugkruising in de wilde populatie mogelijk te maken. Wanneer deze voorwaarden voldaan zijn, zullen de niet-recombinerende univalent-vormende chromosoomsets de negatieve invloed van het niet- geadapteerde gen vermoedelijk bufferen. Daarenboven kan de geobserveerde morfologische intra-specifieke variatie ook het gevolg zijn van de introgressie (interspecifiek) met naburige sectie *Caninae* taxa in de omgeving. Deze morfologische variatie is niet noodzakelijk waarneembaar in de genetische structuur van de individuen. Op dit moment zijn er te veel onzekerheden betreffende de impact van de canina meiose. Bijgevolg zullen de morfologische en/of genetische gedifferentieerde populaties als aparte eenheden beschouwd worden, en moet de evaluatie voor iedere populatie, soort of soortencomplex bekeken worden.

De aanwezige diversiteit van wilde rozen op bepaalde locaties aan de kustzone, de zogenaamde hot-spots, werd al erkend door hun bescherming in het Vlaamse duinendecreet.

Abbreviations and Acronyms

33P	Isotope of phosphorus, used as radioactive tracer
AFLP	Amplified fragment length polymorphism
AP	Allelic phenotype
ATP	Adenosine triphosphate
bp	Base pair
BSA	Bovine serum albumine
cpDNA	Chloroplast DNA
cpIGS	Chloroplast intergenic spacer
DDT	Dichloro-Diphenyl-Trichloroethane
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
F1	First generation hybrid
FNG	Functional nuclear genes
Fst	Coefficient indicating population differentiation based on genetic polymorphism data (Wright)
Gst	Coefficient of genetic differentiation equivalent to Fst but generalised for any number of alleles (Nei)
Hp	Diversity within population
Ht	Total diversity
HW	Hardy Weinberg
MAC-PR	Microsatellite allele counting using peak ratios
MQ	Milli-Q ultrapure water
nrDNA	Nuclear DNA
nrITS	Nuclear internal transcribed spacer
PCA	Principal component analysis
PCO	Principal coordinate analysis
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
RL	Restriction-Ligation
SD	Standard Deviation
STMS	Sequence tagged microsatellites
Taq	Thermus aquaticus
UPGMA	Unweighted Pair Group Method with Arithmetic mean, clustering method

1. OBJECTIVES AND OUTLINE OF THIS THESIS

The data presented in this thesis fits in the framework of two research projects. "Population biology of the autochthonous roses (*Rosa* spp.) and hawthorns (*Crataegus* spp.) in Flanders (B&G/19/2001)" was funded by the Flemish Community (Agency for Nature and Forests). The emphasis was on the diversity of the wild roses in Flanders. In the European project "Genetic Evaluation of European Rose Resources for Conservation and Horticultural Use", in short GENEROSE, the wild rose species of Belgium, Germany, France, The Netherlands, and the Scandinavian countries were analysed (Van Huylenbroeck *et al.* 2005) (EU Research programme "Quality of Life and Management of Living Resources"). Both projects aimed to identify the most valuable wild populations and individuals of the subgenus *Rosa* in order to conserve the present-day gene-pool. For that purpose, an increased knowledge of the taxonomical structure of the subgenus *Rosa*, and more specifically of the section *Caninae,* was necessary. In addition, the geographical differentiation within and between the autochthonous rose populations needed to be assessed. At the Belgian level, the taxa were analysed with both morphological characters and molecular-genetic markers (AFLP and STMS). At the European scale, genetic diversity within and between the populations of the wild rose species was investigated with AFLP.

This thesis starts with a literature review concerning the taxonomy of the subgenus *Rosa*, followed by a historical overview of the taxonomical classification of the complex section *Caninae*. The different features, such as polyploidy, canina meiosis, hybridisation, different reproduction strategies and inheritance patterns, causing the complexity within this section *Caninae* are highlighted. A short overview is given of the published research regarding the section *Caninae*. Finally, the general principles regarding the autochthony and conservation of biodiversity are summarised with an emphasis on the conservation strategies that are followed in Flanders, Belgium.

Next, a description is given of the sampled plant material and the techniques and methods used to study the morphological characters and the molecular-genetic markers. The outcomes are summarised and described in the results.

The description of the results is divided in two major parts, the European versus the Flemish data set. Within the European subgenus *Rosa*, the genetic differentiation of the samples is described following the taxonomical classification in sections and subsections. For the Flemish wild roses, an additional subdivision was made based on the studied characters: the molecular-genetic markers and the morphology. Within the molecular-genetic analyses, the outcomes concerning the AFLP and STMS polymorphisms are split up once more. The results of the AFLP analyses are described according to the same taxonomical classification as was used in the European subgenus. The description of the STMS polymorphisms was restricted to specific questions concerning clonality within a population, the origin of *R. stylosa* and *R.* x *irregularis*, and the reproduction of isolated plants. The morphometric and descriptive characters were described separately in the

morphological evaluation and the diagnostic characters were identified. The inter- and intraspecific variation displayed by the combined diagnostic morphometric and descriptive characters was studied. Finally, the morphology of the Flemish individuals was compared to the species descriptions in literature.

In the discussion, the most remarkable outcomes are discussed. We started with an overview of the restrictions we had to face by analysing a hybridogenic and polyploid species-complex. The discussion regarding the population differentiation, taxonomical aspects and implications for conservation starts with some thoughts concerning the conservation of the section *Caninae*. Next, the classification of the subgenus *Rosa* and the observed polymorphisms within the section *Caninae* are discussed. The influence of hybridisation processes among the section *Caninae* taxa on the taxonomic structure, on the character of the present-day population (mixed presence of different section *Caninae* taxa) and on the conservation guidelines are discussed. Also the occurrence of intersectional hybridisation among *R. arvensis* and section *Caninae* taxa was handled.

Intraspecific geographical differentiation was observed at both the European and the Belgian level, and the within-population clonality of *R. spinosissima* and *R. arvensis* was discussed.

2. THEORETICAL BACKGROUND

2.1. Taxonomy

2.1.1. The genus *Rosa*

The natural distribution of the genus *Rosa* L. (Rosaceae) is situated 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 to 60 endemic species are situated in Europe (Henker 2000). Occasionally, these semi-woody perennial plants can live up to 100 years (Martin *et al.* 2001).

The taxonomic treatment of this highly diverse subgenus is complicated due to some biological phenomena in reproductive biology and insufficient morphological and anatomical characters to adequately discriminate between species (Wissemann and Ritz 2005). Consequently, the taxonomy was subject to many changes over the centuries depending on the opinions of the taxonomists of that time.

From the Renaissance to the eighteenth century, roses were subdivided into wild and gentle species, followed by a subdivision based on the petal colour. The recognition of the ability to hybridise and the acknowledgment of the existence of mixed species by Linnaeus (1753) was a major breakthrough. In addition, Linnaeus (1753) created a rose taxonomy exclusively based on the shape of the hips. In 1811, Willdenow suggested the existence of some species-specific characters, such as the form and presence of prickles, of hairs and of glands. As a consequence, the number of species and species-classification systems increased explosively. However, the uncertainty about the description of the European species was mainly restricted to the section *Caninae* (DC.) Ser. 1825, as the species boundaries are more straightforward in the other sections. It was only at the end of the 19th century that an artificial classification system was created, using preferable correlated characters to describe natural taxa instead of the previously created artificial entities. Consequently, the number of rose species was reduced to 30 (Christ 1873).

In 1940, Rehder subdivided the genus *Rosa* into four subgenera. The subgenera *Hulthemia* (Dumort.) Focke 1888, *Platyrhodon* (Hurst) and *Hesperhodos* Cockerell 1913 are monotypic or contain only three different species. This is in large contrast with the fourth subgenus *Rosa* L. in which about 115 species are classified in ten sections based on morphological and anatomical data such as the shape of the prickles, the number of leaflets, inflorescences, length of the styles and the attachment of the leafy stipules. Although Rehder's classification has been cited in many publications and is acclaimed for its excellence, many additional species have been described subsequently and new evidence of relationships has been published since 1940. For instance, results of phytochemical and molecular-genetic studies (e.g. Matsumoto *et al.* 1997, Matsumoto *et al.* 1998) do not support this classification. Consequently, a new monograph on the genus was necessary. In the Encyclopedia of Rose Science (Roberts 2003), Wissemann (2003) published addenda and corrections to the system

of Rehder (1940). His major adaptation was the subdivision of the section *Caninae* into six subsections (Roberts 2003, Wissemann 2003). Henker had accordingly classified the European rose species in his "Illustrierte Flora von Mitteleuropa" (2000), the reference work for roses on the European continent (Table 2.1). Additional analyses of the species variation with mitochondrial and chloroplast DNA probes (Matsumoto *et al.* 1997) and matK sequences (Matsumoto *et al.* 1998) confirmed the consistency of the present subdivisions of the genus. One of the smaller adjustments of Wissemann (2003) was the change of name of the subgenus *Eurosa* in the subgenus *Rosa*.

The lack of well-discriminating species-specific morphological, anatomical and phytochemical characters encouraged the search for molecular markers to get insight in the phylogenetic relationships within the genus (Wissemann and Ritz 2005). The comparison of nuclear internal transcribed spacer (nrITS) sequences improved the insight into the reliability and stability of sections within the subgenus. However these sequences were not suited to clarify intersectional patterns. For instance, the nrITS sequences indicate that section *Synstylae* is the direct sister group of the section *Caninae* (Wissemann and Ritz 2005), and the latter can be described as a natural allopolyploid group characterised by its autapomorphic nrITS-C-type and the heterogamous and unique canina meiosis. Analysing the chloroplast intergenic spacer (cpIGS) sequences, the section *Caninae* is divided into the eglandular or non-odorant glandular species and the odorant (turpentine and apple-scented) glandular species (Wissemann and Ritz 2005).

To conclude, Kurtto *et al.* (2004) have stated that it is highly improbable that a generally approved classification of the extremely complicated variation of the genus *Rosa* will ever be achieved. Nevertheless, they are convinced that several widely diverging classifications will continue, though they consider that these might become more uniform.

Given the European scale of this study, we focus on the European subgenus *Rosa*, and more specifically the section *Caninae*. The generally accepted taxonomical hierarchy of the European genus *Rosa* according to Henker (2000), confirmed by Wissemann (2003) is summarised in table 2.1.

2.1.2. The section *Caninae*

The current taxonomical position of the section *Caninae* in the subgenus *Rosa*, is supported by the common presence of the atypical and polyploid chromosome constitution, the unique meiotic behaviour (Täckholm 1920, 1922, Blackburn and Heslop-Harrison 1921), the predominant maternal inheritance and the presence of the autapomorphic nrITS-type, C-type, exclusively observed in the section *Caninae* (Wissemann and Ritz 2005, Ritz *et al.* 2005a, Wissemann 2005). However, the lack of a section *Caninae*-specific morphological character and the presence of large phenotypic plasticity within this section are in conflict with the unity of this group. In addition, these individuals are able to hybridise interspecifically. The combination of these unusual features (polyploidy, canina meiosis, hybridisation, inheritance) interferes with the detection of wild individuals and the delineation of well-defined

species groups within this hybridogenic and polymorphic group (Ritz and Wissemann 2003, Wissemann and Hellwig 1997). The characterisation of a species is only possible by combining a recognisable set of morphological characters (Ritz and Wissemann 2003). In addition, the morphological similarities allow the species to be merged into fewer but more diverse species groups, or subsections (Gustafsson 1944, Nilsson 1967).

During the last 15 years, different authors have attempted to compile a determination key for the European subgenus *Rosa* L. The most relevant works are outlined, described and compared to each other. Each publication is characterised by the (restricted) sampling area, the historical or current presence of other species in the neighbourhood, a different impact of founder or bottle neck effects, differences in adaptation to the local conditions, or isolation (e.g. Graham and Primavesi or Nilsson).

Nilsson (1967) studied and described the morphology of the Scandinavian species of the subgenus *Rosa* quite extensively. He focused on the taxonomically critical section *Caninae*, to which the majority of these Nordic rose species belong. In addition, several extended morphometrical and molecular marker (RAPD, STMS) studies were performed on wild section *Caninae* individuals and the progeny of interspecific crossings (Nybom *et al.* 1996, 1997, 2004, 2006, Olsson *et al.* 2000, Werlemark *et al.* 1999, Werlemark and Nybom 2001).

In 1999, Nilsson published his classification of the Nordic *Caninae* individuals, and divided the section into three fairly distinct groups: *R. canina* group, *R. rubiginosa* group, *R. villosa* group. These groups are defined by the presence of few common morphological characters (Nilsson 1967, Nybom *et al.* 1996, 1997), and are supported by molecular-genetic techniques such as RAPD (Olsson *et al.* 2000, Werlemark *et al.* 1999, Werlemark and Nybom 2001). In addition, Nilsson (1999) also acknowledged some evident parallel combinations of characters within each of these three groups, discriminating the species into the so-called "*canina*" and "*dumalis*" types. To conclude, Nilsson (1999) accepts that interspecific hybridisation may produce new biotypes able to survive and become stabilised, due to the predominant maternal inheritance. Consequently, a comparative wide phenotypic plasticity is displayed by the section *Caninae* (Nilsson 1999).

"Roses of Great Britain and Ireland", written by Graham and Primavesi (1993), describes the rose species present on both islands. In this work, the subgenus is partitioned in several sections. The section *Caninae* consists of four subsections: *Stylosae*, *Caninae*, *Villosae*, and *Rubiginosae*. Graham and Primavesi (1993) assume that interspecific variation is the result of hybridisation. Consequently, the intraspecific variation is limited and all possible interspecific hybrids are listed separately. Today, these plants are isolated from the roses on the European continent, and therefore they might be morphologically differentiated.

On the mainland of Western Europe, "Hegi Illustrierte Flora van Mitteleuropa" by Henker (2000) is the reference work by eminence. It was based on a study that improved the knowledge of the wild roses and their current distribution in the North German Plain (Henker and Schulze 1993). The proposed taxonomical

Table 2.1: The taxonomical position of the European subgenus *Rosa* based on Henker (2000) and Wissemann (2003). The relevant (sub-)sections and species for the European study are marked in bold; the species included in the Flemish study are underlined.

FAMILY	SUBFAMILY	GENUS	SUBGENUS	SECTION	SUBSECTION	SPECIES
Rosaceae	Rosoideae	***Rosa***				
			Hulthemia			*R. persica*
			Rosa			
				Pimpinellifoliae		*R. ecae, R. foetida, R. hemisphaerica, R. hugonis, R. koreana, R. myriacantha, R. omeiensis, R. primula, R. sericea,* ***R. spinosissima****, R. xanthina*
				Rosa		***R. gallica****, R. alba, R. centifolia, R. damascena, R. francofurtana, R. polliniana*
				Caninae		
					Trachyphyllae	***R. jundzilii***
					Rubrifoliae	***R. glauca***
					Rubigineae	***R. agrestis, R. rubiginosa, R. micrantha, R. inodora, R. elliptica, R. henkeri-schulzei***
					Vestitae	***R. tomentosa, R. pseudoscabriuscula, R. sherardii, R. mollis, R. villosa***
					Tomentellae	***R. balsamica*** (syn: ***R. tomentella*** and ***R. obtusifolia***)***, R. abietina***
					Caninae	***R. canina, R. corymbifera, R. caesia, R. subcollina, R. subcanina, R. dumalis, R. stylosa, R. montana***
				Carolinae		*R. carolina, R. foliolosa, R. nitida, R. palustris, R. virginiana*
				Cinnamomeae		*R. acicularis, R. amblyotis, R. arkansana, R. banksiopsis, R. beggeriana, R. bella, R. blanda, R. davurica, R. californica, R. causata, R. corymbusola, R. davidii, R. fedts-chenkoana, R. forrestiana, R. gymnocarpa, R. laxa,* ***R. majalis****, R. marretti, R. moyseii, R. multibracteata, R. nanothamnus, R. nutkana,* ***R. pendulina****, R. pisocarpa, R. prattii, R. rugosa, R. sertata, R. setipoda, R. suffulta, R. sweginzowii, R. webbiana, R. willmottiae, R. woodsii*
				Synstylae		*R. abyssinica, R. anemoniflora,* ***R. arvensis****, R. brunonii, R. filipes, R. helenae, R. henryi, R. longicuspis, R. luciae, R. maximowicziana, R. moschata, R. phoenica, R. multiflora, R. rubus,* ***R. sempervirens****, R. setigera, R. soulieana, R. wichurana*
				Indicae		*R. odorata, R. gigantea, R. chinensis*
				Banksianae		*R. banksiae, R. cymosa*
				Laevigatae		*R. laevigata*
				Bracteatae		*R. bracteata, R. clinophylla*
			Platyrhodon			*R. roxburghii*
			Hesperhodos			*R. stellata, R. minutifolia*

classification is congruent with that of Wissemann (2003), who adapted the illustrious system of Rehder (1940) with the present-day knowledge. The subgenus was divided into several sections, whereas the most complex section *Caninae* consisted of six subsections. The subsections *Trachyphyllae* and *Rubrifoliae* are monotypic, while the subsection *Tomentellae* contains only two species: *R. balsamica* and the rarer species, *R. abietina*. In contrast, the subsections *Vestitae*, *Rubigineae*, and *Caninae* are polytypic. A detailed overview of the taxonomical structure of the subgenus *Rosa* is given in (Table 2.1). Henker accepts large intraspecific variation; therefore little to no spontaneous hybrids were described as separate species.

The above mentioned reference works contain striking differences. The most remarkable are mentioned below and summarised in Table 2.2.

In each work, plant material originates from different countries. Nilsson (1967) focused on the representative species of the Scandinavian countries. Therefore, only a small subset of the European section *Caninae* was described. Henker and Schulze (1993), the work on which Henker (2000) was based, sampled the species-rich region of the North German Plain, while Graham and Primavesi (1993) described the species and hybrids of the islands of Great Britain and Ireland.

Furthermore, they apply a different hierarchical structure in their taxonomical systems. Compared to the other works, only Nilsson (1967, 1999) elaborates on the status subspecies within some taxa: e.g. *R. dumalis* subsp. *dumalis,* and subsp. *coriifolia*.

In addition, the interpretation of the species concept causes differences. Graham and Primavesi (1993) assume a very limited intraspecific variation, and therefore listed and described all possible, over 80, interspecific hybrids. In contrast, Nilsson (1967, 1999), Henker and Schulze (1993), Henker (2000) and Wissemann (2003) accept the presence of intraspecific variability as a consequence of interspecific hybridisation, so only the most common hybrids are mentioned.

The subsequent change of names and use of synonyms increases the complexity in taxonomy and classification of the wild roses. For instance, only in 2002, Wissemann concluded that *R. spinosissima* (described by Linnaeus in 1753) and *R. pimpinellifolia* (Linnaeus 1759), actually represent an identical taxon (Wissemann 2002b). The presence or absence of glands on the pedicels appeared not to be sufficient to consider them as two different species, as Wissemann observed both glandular and eglandular pedicels on the same herbarium specimen. Since *R. spinosissima* was described first, it is accepted as the official new name. In contrast, the presence of glands, the more robust habit and occasionally the occurrence of pink flowers are indications of introgression of cultivated genes (Graham and Primavesi 1993). An example within the section *Caninae* is the discussion about the correct name of *R. tomentella* Léman ex Cass. in Henker (2000), known as *R. obtusifolia* sensu auct. mult. non Desv. in Graham and Primavesi (1993) and Nilsson (1967, 1999). According to the Flora Europaea Orientalis 2001: 354-355 (Kurtto *et al.* 2004) the correct name is *R. balsamica* Besser 1815, whereas *R. obtusifolia* Desv. is accepted to be a synonym for *R. corymbifera* (Kurtto *et al.* 2004). The confusion in species names is a direct consequence of the use of different species concepts and the lack of a generally accepted taxonomy at the time of publication.

The taxonomical complexity of the section *Caninae* is increased by the different classifications described by each author. Nilsson (1999) divides the section *Caninae* in groups, whereas Graham and Primavesi (1993), Henker (2000) and Wissemann (2003) speak of subsections. In addition and more far-reaching, they have a different opinion on the number of subsections/groups: Nilsson (1999) mentions three groups, Graham and Primavesi (1993) have four subsections, while Henker (2000) and Wissemann (2003) agree on six subsections. Consequently, the assignment of the species to these subsections differs. For instance, *R. balsamica* belongs to the subsection *Tomentellae* according to Henker (2000) and Wissemann (2003). In contrast, Nilsson (1967, 1999) did not accept existence of the subsection *Tomentellae*. Although aware of the somewhat intermediate position of *R. obtusifolia* (cfr. *R. tomentella*, or *R. balsamica*), that shared characters of both *R. canina* and *R. rubiginosa* groups (Nilsson 1999), Nilsson placed this species within the *R. canina* group (Nilsson 1967). Similarly, Graham and Primavesi (1993) classified *R. obtusifolia* within the subsection *Caninae.* Also the position of *R. stylosa* differs enormously. Following Graham and Primavesi (1993) it forms a separate subsection *Stylosae*, whereas it is part of the subsection *Caninae* according to Henker (2000) and Wissemann (2003).

Table 2.2: The taxonomical hierarchy of the section *Caninae* and the position of the most representative taxa according to Graham and Primavesi (1993), Nilsson (1999), Henker (2000) and Wissemann (2003). Synonyms used by Graham and Primavesi°, Nilsson†, Henker and Wissemann*, /: not mentioned by this author.

Henker, Wissemann	Graham&Primavesi	Nilsson	
Subsection	Subsection	Group	Species
Rubrifoliae	/	*Rubrifoliae*	*R. glauca* (syn: *R. rubrifolia*†)
Rubigineae	*Rubiginosae*	*R. rubiginosa*	*R. rubiginosa* *R. micrantha* *R. agrestis* *R. elliptica* *R. inodora*
Vestitae	*Villosae*	*R. villosa*	*R. tomentosa* *R. pseudoscabriuscula* *R. sherardii* *R. mollis* *R. villosa*
Tomentellae	*Caninae*	*R. canina*	*R. balsamica* (syn: *R. tomentella**, *R. obtusifolia*°, †)
Caninae	*Caninae*	*R. canina*	*R. canina* (*R. canina* Group *Lutetiana*°) *R. caesia* *R. dumalis*
Caninae	*Caninae*	/	*R. corymbifera* (*R. canina* Group *Pubescentes*°)
Caninae	*Stylosae*	/	*R. stylosa*
Trachyphyllae	/	*R. canina*	*R. jundzilii*

The variations between the systems are caused by the very subtle taxonomical differences among the subsections/groups of the section *Caninae* (Table 2.2) (Atienza

et al. 2005), and the presence of a large intraspecific plasticity of the diagnostic characters (Ritz and Wissemann 2003).

Morphological differentiation within the section Caninae

Within the section *Caninae* three major groups could be distinguished based on some well-defined and clearly observable morphological characters. In general, they correspond to the subdivision in subsections (Graham and Primavesi 1993, Henker 2000, Wissemann 2003) or groups (Nilsson 1999). Moreover, within each group some evident parallel combinations of morphological characters distinguish the section *Caninae* taxa into the so-called L and D type (Henker and Schulze 1993, Reichert 1998, Henker 2000 and Wissemann 2003), or the "*canina*" and "*dumalis*" type (Nilsson 1999) respectively. A schematic overview of the morphological variation within and among the subsections of the section *Caninae* is given in table 2.3, and the figures 2.3 to 2.9 illustrate this morphological variation.

The taxa of the L type (Laxus, Loose) are characterised by an arching or loose habit, the flower stalks are nearly as long as the receptacle or longer, and hidden by the bracts only at their base. The petals are white or pale pink, after the fall of the petals the sepals are reflexed, and fall early before the colouring of the hips. The disc is wide and the orifice is narrow (varying between 0.4 - 0.8 mm). The styles are glabrous or hairy but generally not villous and form a little bouquet above the orifice. In general, the hips ripen in September (Reichert 1998). In contrast, the D type (Densus, Dense) taxa have an erect or dense habit, the flower stalks are usually shorter than the receptacle, and often half as long or even shorter, hidden by the bracts. The petals are deep pink. After the fall of the petals the sepals are spreading or erect, and persist until hips begin to redden, or even longer. The disc is narrow and the orifice wide (over 1.1 mm). The styles are villous, forming a low, wide head above the orifice. The hips ripen mostly in August (Reichert 1998). Despite the apparently obvious differences between the two types, numerous intermediate forms: the so-called L/D types, have been identified.

The taxa of the subsection *Rubigineae* are characterised by mainly hooked prickles. The leaflets are densely glandular, multiserrated and the veins on the lower sides are pubescent. The numerous glands on the leaflets are sticky and spread a typical strong scent of apples or vines. Within this subsection two major groups can be identified based on the leaflet shape: (a) taxa with slender leaflets, and a wedge-shaped base: such as *R. agrestis*, *R. inodora*, and *R. elliptica*, and (b) taxa with broad leaflets and a rounded leaflet base: such as *R. micrantha*, *R. henkeri-schulzei* and *R. rubiginosa*. In addition, in each group the taxa display the so-called L-D variation (Table 2.3). *R. micrantha* and *R. agrestis* are the so-called L type taxa displaying a loose habit, a narrow orifice (< 1 mm), the sepals are reflexed and deciduous early after anthesis, and the stigma is bouquet-shaped (Reichert 1998). These are in contrast with the D type taxa: *R. rubiginosa* and *R. elliptica*, which are characterised by a dense habit, a broad orifice (> 1 mm), erect and persistent sepals, and head-shaped stigmas. In-between, the L/D types: *R. inodora* and *R. henkeri-schulzei* have more intermediate diameters of the orifice (± 1 mm), sepals are spreading and more or less persistent

after flowering, the habit and the stigma vary (Henker and Schulze 1993, Reichert 1998, Nilsson 1999, Henker 2000)

The taxa of the subsection *Vestitae* are characterised by tomentose and glandular leaflets, smelling like turpentine. The hips and pedicels are glandular with persistent stipitate glands. *R. tomentosa* is the only L type taxon in this subsection (Table 2.3), with narrow orifice, reflexed and deciduous sepals, a bouquet-shaped stigma, and uni- to biserrated leaflet margins. *R. pseudoscabriuscula* is described as the L/D type by intermediate forms of orifice, sepals and stigma, and mostly bi- to multiserrated leaflet margins. The different sources in literature did not reach a consensus in the description of these two taxa**.** First of all, Graham and Primavesi (1993) only mentioned *R. tomentosa*, while Henker (2000) assumes the presence of glands and the serration of the leaflet margins, the diameter of the orifice, and consequently the disc index, the shape of the stigma, and the length of the pedicel to be different. In the Atlas Florae Europaea (Kurtto *et al.* 2004), *R. (pseudo)scabriuscula* is interpreted as *R. tomentosa* x *R. canina*, and others include this taxon in *R. tomentosa* or *R. sherardii*. An overview is given in Atlas Florae Europaea (Kurtto *et al.* 2004). The taxa *R. sherardii*, *R. villosa* and *R. mollis* are D types, having a broad orifice, erect and persistent sepals, and a head-shaped stigma. However, *R. villosa* and *R. mollis* are clearly distinguishable from *R. sherardii.* Both taxa have multiserrated leaflet margins, glandular petals and curved prickles while *R. sherardii* is characterised by irregular multiserrated margins, eglandular petals and erect prickles. In addition, the density and length of the prickles on the petals, the width of the hips, the diameter of the orifice, and the disc index should differentiate among *R. mollis* and *R. villosa*.

In the taxonomical classification according to Henker (2000), the subsections *Caninae* and *Tomentellae* are defined separately. In Europe, the subsection *Tomentellae* has two representatives: *R. balsamica* and the very rare *R. abietina.* The latter was not included in our data set, and therefore we can only refer to *R. balsamica* for this subsection. This taxon is morphological characterised by pubescent and glandular veins at the lower side of the leaflets, bi- to multiserrated leaflet margins, narrow orifice and reflexed and deciduous sepals. Within the subsection *Caninae*, the taxa *R. canina*, *R. subcanina, R. dumalis*, *R. corymbifera*, *R. stylosa*, *R. subcollina*, *R. caesia*, and *R. montana* are gathered. The subsection *Caninae* can be divided in two groups based on the pubescence on the leaflets (Table 2.3): (a) the taxa displaying glabrous leaflets: *R. canina*, *R. subcanina* and *R. dumalis*, and (b) those with pubescent leaflets: *R. corymbifera*, *R. stylosa*, *R. subcollina* and *R. caesia*. Within each group the L-D type variation (concerning the diameter of orifice, the position and persistence of the sepals) occurs.

The actual taxonomic classification of the section *Caninae* in L-, L/D-, and D types is highly artificial, as (a) it goes beyond the major morphological characters that define the subsections; (b) the inheritance of the "widening of the orifice" and the "persistence of the sepals" goes through the pollen parent (Ritz and Wissemann 2003); and (c) reciprocal hybrids of *R. canina* (L type) and *R. rubiginosa* (D type) always display the loose habit similar to *R. canina*, instead of a parentally skewed or

intermediate L/D habit (Wissemann *et al.* 2006). The system classifies morphospecies, however it does not reflect the phylogenetic relationships, or the evolutionary history of the section (Ritz and Wissemann 2003).

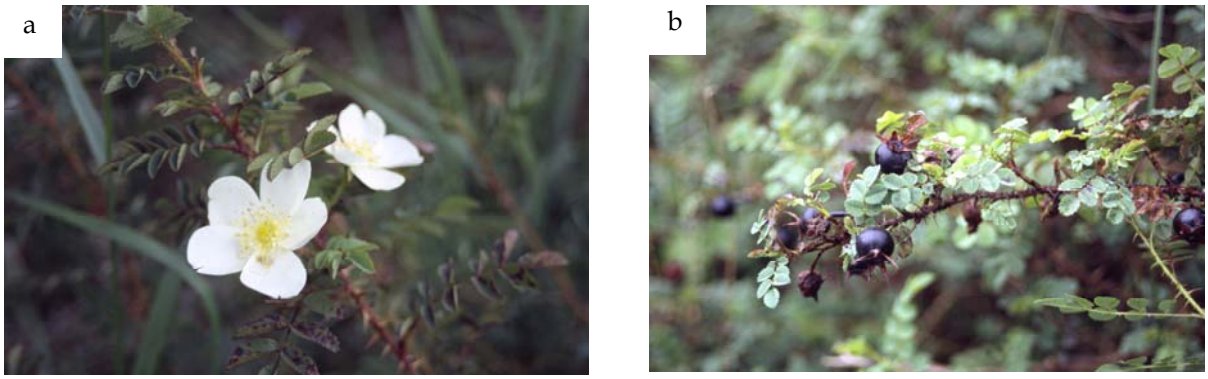


Figure 2.1: *R. spinosissima*: stems with numerous prickles and small ovate leaflets (a) the solitary flowers; (b) the purplish-black hips when ripe.

Figure 2.2: *R. arvensis*: (a) the typical accolade-shaped serrated leaflets; (b) hips with agglutinated and protruding styles and long pedicels

Figure 2.3: subsection *Rubigineae::* (a) the strong glandular lower sides of the leaflets, rachides, hips and pedicels of (a) *R. rubiginosa* and (b) *R. micrantha*

a

b

Figure 2.4: subsection *Rubigineae:* (a) the slender leaflets with wedge-shaped of *R. agrestis;* (b) the broad leaflets with well-rounded base of *R. micrantha*.

a

b

Figure 2.5: subsection *Rubigineae*: (a) the prickles on the stems of *R. rubiginosa* (left) and *R. micrantha* (right) (Maes *et al.* 2006); (b) the hips of *R. henkeri-schulzei* (Maes *et al.* 2006)

Figure 2.6: *R. tomentosa*: the tomentose leaflets and the densely stipitate glands on the pedicel and hips.

Figure 2.7: *R. balsamica*: the hips (Maes *et al.* 2006)

a

b

Figure 2.8: *R. canina*: (a) the glabrous and eglandular leaflets; (b) the eglandular pedicels and hips.

a

b

Figure 2.9: *R. stylosa*: (a) the back-folded lower leaflets and the delta-shaped prickles; (b) the conical discs with agglutinate and exserting styles.

Table 2.3: The section *Caninae* sorted according to the L-, L/D- and D type of the species based on Reichert (1998). With: †: L: *canina* type and D: *dumalis* type (Nilsson 1999).

				Subsection *Vestitae*	Subsection *Rubigineae*	
				Leaflets tomentose Leaflets glandular	Leaflets glabrous of pilose, odour of apple Leaflets glandular, prickles hooked Leaflets uni- to multiple serrated	
Typ†	Recept	O	Sepals	Pedicels with glands smelling like turpentine Prickles more or less erect	Leaflets slender, wedge-shaped base Pedicels mostly eglandular	Leaflets broad, well-rounded base Pedicels glandular
			Reflexed	*R. tomentosa*	*R. agrestis* Pedicels sometimes with glands	*R. micrantha*
L	Bouquet	< 1 mm	Deciduous after anthesis	Leaflets uni- to multiple serrated Pedicels long Styles slightly pilose Flowers white to pinkish	Pedicels long Styles glabrous Flowers white	Pedicels long Styles glabrous Flowers pinkish-white
L/D	± Head	± 1 mm	Patent Partly deciduous	*R. pseudoscabriuscula* Leaflets uni- to multiple serrated Pedicels short	*R. inodora* Styles slightly pilose	*R. henkeri-schulzei* (syn. *R. columnifera*) Styles slightly pilose
D	Head	> 1 mm	Erect Persistent after anthesis	*R. sherardii* Leaflets multiple serrated Pedicels short Styles woolly Flowers very pink	*R. elliptica* Pedicels short Styles pilose Flowers pinkish-white	*R. rubiginosa* Pedicels short Styles pilose Flowers pink

Table 2.3 continu: The section *Caninae* sorted according to the L-, L/D- and D type of the species based on Reichert (1998). With adaptations based on: *: Henker (2000) and Henker and Schulze (1993); °: Kurtto *et al.* (2004); †: L: *canina* type and D: *dumalis* type (Nilsson 1999).

			SUBSECTION TOMENTELLAE*	SUBSECTION *CANINAE*	
			Leaflets slightly to densely glandular Odourless Prickles hooked	Leaflets eglandular or glandular Odourless Prickles hooked	
TYP†	RECEPT	O	Leaflets pubescent	Leaflets glabrous	Leaflets pubescent
L	Bouquet	< 1 mm	***R. balsamica*°** (syn. *R. tomentella**) Leaflets multiserrated Flowers mostly white	***R. canina*** Leaflets uni- to multiserrated Leaflets eglandular or glandular	***R. corymbifera*** Leaflets uniserrated Leaflets eglandular to sparsely glandular ***R. stylosa*** Leaflets uni- to multiserrated Adhered styles Conical disc Pedicels (e)glandular
			Erect but hanging shrubs, with little to no stolons Flowers are pinkish to white		
L/D	± Head	± 1 mm	Hybrids with the subsections *Caninae* and *Rubigineae**	***R. subcanina*** Leaflets uni- to multiserrated	***R. subcollina*** Leaflets bi- to multiserrated
D	Head	> 1 mm	Hybrids with the subsections *Caninae* and *Rubigineae**	***R. dumalis*** Leaflets uni- to multiserrated	***R. caesia*** Leaflets uni-, or occasionally multiserrated

2.2. Complexity

The complexity of the subgenus *Rosa* is caused by an enormous phenotypic, genotypic and ecologic variability and plasticity due to some evolutionary processes, such as hybridisation, introgression, etc. Wissemann (2005) states that all these factors are related: hybridisation has caused asymmetric meiosis; asymmetric meiosis is the reason for heterogamy, whereas heterogamy results in asymmetrical, and mostly matroclinal inheritance of characters and character states.

In Europe, this complexity is mainly situated within the section *Caninae*, being caused by the ability to hybridise interspecifically, even among sections and subsections, to produce sterile or fertile hybrids, to reproduce through different sexual and asexual strategies. The section *Caninae* is characterised by the allopolyploid chromosomal status and the unusual heterogamous canina meiosis, which influences the inheritance patterns, disguises spontaneous hybrids, etc.

In North America, a similar taxonomic problem is known as the *R. carolina* complex (Lewis 1957). Although the diploid and putative parental species (sect. *Carolinae*: *R. foliolosa*, *R. nitida*, *R. palustris*, and sect. *Cinnamomeae*: *R. blanda*, *R. woodsii*) are relatively well-defined, the three tetraploid hybrid taxa: *R. arkansana* (sect. *Cinnamomeae*), *R. virginiana* and *R. carolina* (both sect. *Carolinae*) are characterised by an extensive continuous morphological variation fading the limits among each other and with their putative ancestors (Joly *et al.* 2006).

2.2.1. Polyploid chromosomal structure

Polyploidy is the possession of more than two complete sets of chromosomes, and can be seen as a major engine for diversification (Vamosi and Dickinson 2006) influencing the evolutionary history of plants (Leitch and Bennett 1997, Mable 2003). About 30 to 80% of all angiosperms are presumed to have a polyploid origin (Soltis and Soltis 2000). In the genus *Rosa* polyploidy occurs frequently, varying between 50%, and 75% (Vamosi and Dickinson 2006).

Several factors might influence the success of polyploids. They maintain higher levels of heterozygosity compared to their diploid progenitors and exhibit less inbreeding depression as they tolerate higher levels of selfing. Most polyploids are polyphyletic, having formed recurrently from genetically different diploid parents instead of a single origin (monophyletic). Populations of independent origins can come into contact and hybridise, generating new genotypes that display higher genetic diversity compared to polyploid taxa of single origin (Soltis and Soltis 2000). Moreover, they might have the ability to colonise unoccupied niches and/or outcompete their diploid progenitors (Vamosi and Dickinson 2006).

From a systematic viewpoint, the recurrent formation of polyploids may offer an important explanation for the taxonomic complexity in polyploid species, particularly where the probable diploid progenitor species have a wide geographical distribution (Leitch and Bennett 1997), or when the presumed diploid ancestral species became extinct as in the section *Caninae* (Wissemann and Ritz 2007).

2.2.1.1. Subgenus Rosa

The base chromosome set of the subgenus *Rosa* consists of seven chromosomes (Täckholm 1920, 1922, Blackburn and Heslop-Harrison 1921), and the ploidy levels range from diploid to octoploid (Henker 2000). Excluding the section *Caninae*, all the rose species have an even number of chromosome sets, the majority being 2x or 4x, and follow the regular type of meiosis, the Mendelian meiosis. In contrast, the chromosome constitution of the section *Caninae* individuals is quite uncommon, and therefore requires special attention.

2.2.1.2. Section Caninae

The species of the polymorphic and complex section *Caninae*, are mostly pentaploid (2n = 5x = 35), although tetra- (2n = 4x = 28) and hexaploid (2n = 6x = 42) shrubs have also been detected (e.g. Darlington and Wylie 1961, Henker 2000). Within one species, different ploidy levels might be present, e.g. for *R. sherardii*, *R. mollis*, and *R. micrantha* 4x, 5x, and 6x shrubs were described (Henker 2000). In table §4.2, the ploidy levels are summarised for each of the analysed and native species in Europe.

In a pentaploid *Caninae* shrub, each chromosome set is present in fivefold. Two of these sets pair, and are referred to as bivalent-forming chromosome(s) (sets) or bivalents, while the remaining three sets are called the univalent-forming chromosome(s) (sets) or univalents (Täckholm 1920, 1922, Blackburn and Heslop-Harrison 1921) (Figure 2.10). The number of univalent-forming chromosomes depends on the ploidy level of the individual. For instance, in a tetra- or hexaploid individual two or four univalent-forming chromosome sets are present, respectively (Nybom *et al.* 1997).

The actual genotype of polyploid parents and their reciprocal interspecific progeny can be assessed by a combination of STMS polymorphisms and MAC-PR approach (Microsatellite Allele Counting using Peak Ratios, Esselink *et al.* 2004). All analysed pentaploids displayed a maximum of four simultaneously occurring alleles for each locus, while the tetraploids displayed a maximum of three different alleles (Nybom *et al.* 2004) (Figure 2.10). Therefore, the bivalent-forming chromosomes must be highly homologous, whereas the remaining univalent-forming chromosomes are homeologous chromosomes. These univalents might have different alleles, both from one another as from the bivalents (Nybom *et al.* 2006). Through localising the 5S rDNA and 18S-28S rDNA loci in a pentaploid *R. canina*, Lim *et al.* (2005) confirmed this hypothesis. In addition, they suggest that the differences between the three univalent-forming chromosomes are possibly reinforced by genetic control mechanisms that prevent them from pairing. These chromosome sets can be assumed haploid genomes. In conclusion, the genetic constitution of the section *Caninae* species might be described as two different genomes: the bivalent-forming chromosomes may be regarded as being diploidized, while the three univalent-forming 'passenger' genomes are maternally inherited. Consequently, the loci

residing on the bivalent-forming chromosomes or on the univalent-forming chromosomes may originate from very dissimilar species in the original hybridisation event, and have thereafter experienced considerably different evolutionary processes.

The normal meiotic pairing behaviour and the capability for crossing-over of the bivalent-forming chromosomes suggest that they are influenced through sexual recombination, and appear to be shared among the genotypes almost regardless to which taxa they belong. The lack of species-specific alleles on the bivalents could be caused by interspecific hybridisation events. In contrast, the univalent-forming chromosomes, that have only evolved by mutation and selection as they lack sexual recombination, should contain the constant characters for the four subsections and thus reflect the taxonomic distance between the genotypes. Therefore, the assessment of the taxonomical relationships among and within taxa will depend on whether the bi- or univalent genome was analysed (Nybom *et al.* 2006).

Different percentages of similarities were assessed by comparing the percentage of shared alleles on the bivalent-, and univalent forming chromosomes among six section *Caninae* individuals (subsection *Rubigineae*: *R. rubiginosa*; subsection *Vestitae*: *R. villosa* subsp. *mollis*, and two *R. sherardii* individuals; subsection *Caninae*: *R. caesia* and *R. dumalis*). The similarities of the alleles on the univalent-forming chromosomes differ according to the taxonomical relationships. Their similarity is the highest comparing two genotypes of the same species, e.g. *R. sherardii*, 98%; whereas the similarity among species within the same subsection, e.g. subsection *Vestitae*: *R. sherardii* and *R. villosa*, equalling 87 – 89%, is higher than the similarity among subsections. For instance, the species of the subsections *Vestitae* versus *Caninae* share 52 – 68% of the alleles on the univalent-forming chromosomes. Finally, the similarity among the subsection *Rubigineae* and both subsections *Vestitae* and *Caninae* show the lowest similarity values (32 – 45%). In contrast, the similarity of the alleles on the bivalent-forming chromosomes varies between 47%, and 84%, irrespective the taxonomical relationship, e.g. the two *R. sherardii* genotypes share only 72% of the alleles on the bivalents, while the similarity of two *R. sherardii* genotypes with *R. villosa* varies largely: 58%, and 79% (Nybom *et al.* 2006).

All the subsections contain species of the two vegetative habits: L (lax growth habit) and D (compact growth habit) types; therefore the related characters are expected to be determined by the bivalent-forming chromosomes (Ritz and Wissemann 2003). The paternal inheritance of some fruit characters such as the persistence of sepals and widening of orifice was assessed through reciprocal crossings (Ritz and Wissemann 2003). In contrast to the expected parentally determined or intermediate state, Wissemann *et al.* (2006) observed the dominant presence of the L type growth habit (as found in *R. canina*) in reciprocal offspring (*R. canina* and *R. rubiginosa*). Therefore, growth is presumed to be a syndrome, being influenced on multiple levels. The growth habit will be the result of the sum of all the interactions.

In evolutionary terms, individuals from the section *Caninae* benefit from a combination of fitness (conservation of unpaired genomes), flexibility (recombination between the pairing genomes), and vigour (the presence of three, four or five different genomes) (Lim *et al.* 2005). However, presuming the three univalent-forming genomes will never –or only rarely- be involved in pairing (Nybom *et al.* 2006, Lim *et al.* 2005), their evolutionary fate will be genetic degradation through the accumulation of mutations, causing them to become redundant and ultimately disappear. This hypothesis is supported by the genetic divergence already present in the univalent-forming genomes. However, at this moment there is no divergence of the 5S rDNA loci in the univalent-forming chromosomes, pointing to the recent uni-, bivalent demarcation in the evolution of *R. canina* (Lim *et al.* 2005).

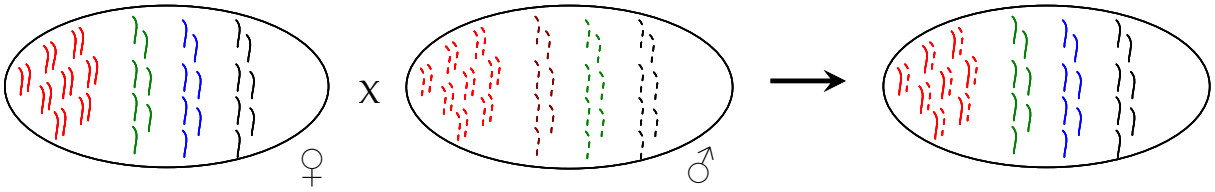
2.2.2. Canina meiosis

The pentaploid state and peculiar canina meiosis were discovered in the early twenties (Täckholm 1920, 1922, Blackburn and Heslop-Harrison 1921). The unusual chromosome constitution of bivalent- and univalent-forming chromosome sets in the section *Caninae* inhibits the Mendelian meiosis. Therefore, a new and unique type of meiosis emerged.

The heterogamous canina meiosis leads to hemisexuallity; this is the uneven allocation of maternal and paternal chromosomes to the progeny (Figure 2.10). During the female meiosis, the two bivalent-forming chromosome sets (these are fourteen chromosomes in total) are formed and line up on the equatorial plane of the embryo mother cell, while the univalent-forming chromosomes remain together at the micropylar end of the cell. The bivalent-forming chromosomes separate as usual and move towards the poles giving rise to two cells. The cell closest to the micropylar end contains one set of bivalent-forming chromosomes (seven chromosomes) together with all the univalent-forming chromosomes (21 chromosomes). The second cell only consists of the other bivalent-forming chromosome set. During the second meiotic division, the univalent-forming chromosomes divide normally along with the bivalent-forming chromosomes, resulting in tetrads that comprise two viable megaspores, each with 28 chromosomes (derived from seven bivalent-forming and 21 univalent-forming chromosomes), and two non-viable megaspores (each with only seven bivalent-forming chromosomes). The megaspore closest to the micropyle develops into an embryo sac. The viability of the egg cell depends on the chromosome constitution (Werlemark 2000a). During male meiosis, the univalent-forming chromosomes migrate more slowly than the bivalent-forming chromosomes towards the equatorial plane, and are left scattered about the dividing microspore mother cell. When the bivalent-forming chromosomes have separated in a normal manner, the univalent-forming chromosomes move to the region where the bivalent-forming chromosomes have been. Several univalent-forming chromosomes manage to reach the poles in time to be included in the daughter cells. At the next division they lag behind resulting in a tetrad of four cells, each containing one set of bivalent-forming chromosomes together with many micronuclei formed from the univalent-forming chromosomes. Therefore, each microspore mother cell forms numerous

microspores with a varying number of chromosomes (Täckholm 1920, Gustafsson 1944). However, only pollen grains with exactly seven chromosomes, derived from one set of bivalent-forming chromosomes, are functional. The percentage of morphologically good pollen as well as the percentage of viable pollen has been found to be markedly lower in the section *Caninae* individuals compared to species from the other sections of the subgenus *Rosa* (Jičínská *et al.* 1976). Analysing the allelic configuration of progeny of the reciprocal crossings (combining STMS markers and MAC-PR approach), Nybom *et al.* (2004) established that the same paternal allele was always inherited by the derived sexual offspring, occurring in at least two copies in the pollen parent, thus being located on the bivalent-forming chromosomes.

The fusion of a fertile pollen grain, containing one set of the bivalent-forming chromosomes, and a fertile egg cell, consisting of one set of the bivalent-forming and three sets of the univalent-forming chromosomes, restores the original pentaploid chromosome constitution in the descendants (Nybom *et al.* 1996). The progeny of interspecific hybridisation will only be fertile if the two bivalent-forming chromosomes sets are sufficiently homologous, so they are able to recombine during meiosis (Nybom *et al.* 1996).



NRITS-TYPES (Wissemann 2000a)

C C A D E	C C A D E	C C A D E

STMS POLYMORPHISMS (Nybom *et al.* 2006)

R. rubiginosa	*R. caesia*	*R. rubiginosa* x *R. caesia*
D D B B B	D D A B F	D D B B B

Figure 2.10: Schematic representation of the genetic constitution of a pentaploid section *Caninae* individual, and the inheritance of the chromosomes through canina meiosis. The seed parent (full line) contributes one of the bivalent-forming chromosome sets (full line; red), and the three univalent-forming chromosome sets (full line; green, blue and black), whereas the pollen parent (dotted line) contributes one of his bivalent-forming chromosome sets (dotted line; red). Similar colours indicate homologous chromosomes. In addition, for each chromosome set the nrITS-types (Wissemann 2000a) and polymorphisms of allele Rh_B303 (Nybom *et al.* 2006) are represented as illustration. The chromosome sets of the seed parent are underlined.

The regularity with which only seven bivalent and no multivalent associations occur on the equatorial plane during meiosis suggests that only two genomes out of five are homologous, while the other genomes are heterologous (Lim *et al.* 2005). Several investigations show evidence that genetic markers of two genomes, the bivalents, are similar to each other but distinct from the other genomes, the univalents (Figure 2.10). For instance, Wissemann (1999) described only four distinct alleles of nrITS of the ribosomal DNA unit in pentaploid species. Similarly, Nybom *et al.* (2004) found a maximum of four different STMS alleles at each of several loci in pentaploids, and three different STMS alleles at each of several loci in tetraploid species. Moreover, the progeny groups of interspecific reciprocal crossings proved that the same paternal allele out of four was always transmitted to the progeny,

suggesting the preferential pairing of two highly homologous genomes forming the bivalents in the pollen meiosis (Nybom *et al.* 2004).

In addition, the meiotic behaviour is supposed to be under strict genetic control. One part of the regulation consists of the precise formation of the seven bivalent sets regardless of the total chromosome number. The other part consists of the behaviour of the univalent-forming chromosomes, which apparently split into separate chromatides already during the first meiosis (Lim *et al.* 2005).

The heterogamous canina meiosis, through which each descendant inherits $4/5^{th}$ of the chromosomes from the seed parent, and only $1/5^{th}$ from the pollen parent, has a significant impact on the species. First, there is a tendency to a skewed uniparental inheritance. The predominant maternal inheritance fades the influence of the pollen parent and increases the complexity of the identification of the spontaneous and wild hybrids as they are highly similar to the mother species. Moreover, hybrids could be sterile, while others are able to cross once more with the parental or non-parental section *Caninae* species. In addition, genetic recombination is restricted to the highly homologous bivalent-forming chromosomes, whereas the non-recombinant univalent-forming chromosomes remain unchanged during inheritance through the seed parent (Nybom *et al.* 2004). Because the bivalent-forming chromosomes are highly homologous, and little recombination occurs, there is a resemblance with apomictic reproduction. Consequently, it is hard to distinguish between sexually and asexually derived offspring (Nybom *et al.* 2006). Finally, the absence of recombination on the univalent-forming chromosomes means that the majority, $3/5^{th}$ of the genome will stay unchanged. This might explain the stability of the section (Graham and Primavesi 1993), and the maintenance of the subsections or groups within the section *Caninae* (Olsson *et al.* 2000).

The occurrence of irregularities in the canina meiosis has been demonstrated; moreover unreduced gametes may result in viable gametes, leading to new ploidy levels (Nybom *et al.* 2006). Two seedlings of reciprocal interspecific crosses showed an elevated ploidy level when analysed with STMS markers. One of the seedlings of two different pentaploid parental species appeared to be a hexaploid, containing the five maternal alleles and one allele of the bivalent-forming chromosome set of the pollen parent. This descendent was most likely formed by the fertilisation of an unreduced egg cell by a normally formed haploid pollen grain. The second aberrant seedling was derived from a normally reduced egg cell fertilised by an unreduced pollen grain, which perhaps still contained some of the univalent-forming chromosomes (Nybom *et al.* 2006). The pollen viability decreases with aberrant meiosis, but the egg cell formation may be less sensitive to this (Werlemark 2000a).

2.2.3. Hybridisation

2.2.3.1. Concepts

Hybridisation is a common and unequally spread phenomenon in the plant kingdom. It is considered to be an important mechanism in plant evolution and speciation, providing a source of genetic variation upon which selection can act. This may result in the differentiation of ecotypes and the breakdown or reinforcement of isolating barriers (Rieseberg and Ellstrand 1993, Rieseberg 1995, Neuffer *et al.* 1999).

Depending on the species concept, hybridisation can be defined in several ways. The most widely accepted species concept defines a biological species as "a group of interbreeding natural populations which are reproductively isolated from all other such groups" (Mayr 1963). However, it denies the species status of hybridising taxa. Alternatively, a biological species can be described as "a group of interbreeding populations that are 'genetically isolated' from each other" (Rieseberg and Carney 1998). However, in taxa with promiscuous hybridisation like the subgenus *Rosa*, section *Caninae*, intraspecific variability must be accepted (Graham and Primavesi 1993). Therefore, natural hybridisation is described in a broad sense as "the cross-fertilisation between individuals from populations, which are distinguishable on the basis of one or more heritable characters" (Harrison 1990). Similarly, introgression refers to "the transfer of genes between genetically distinguishable populations" (Rieseberg and Carney 1998).

2.2.3.2. Section Caninae

The occurrence of interspecific hybridisation within the section *Caninae* is widely known (overview by Wissemann and Hellwig 1997). The members of the section *Caninae* show varying degrees of interfertility. It is assumed that they hybridise freely and that hybridisation is another reason for their complex patterns of variation (Melville 1975). However, there is little information on hybridisation in natural populations of dogroses.

The impact of hybridisation on dogroses must be viewed against the background of their pentaploid chromosomal structure and heterogamous canina meiosis. First, the canina meiosis may act as a chromosomal barrier that is extremely efficient in reducing and eliminating introgression by resisting gene flow selectively. Therefore species differences may be maintained even in the face of extensive introgression (Rieseberg 1995). In addition, the majority of the hybrids displays a mosaic of parental, intermediate, and transgressive or novel morphological characters rather than just intermediate ones (Rieseberg and Ellstrand 1993). They can be fertile or sterile, with continuous intermediates (Gustafsson 1944). However, due to the general lack of discriminating morphological characters between the different *Caninae* species and because of the predominant matroclinal inheritance resulting from the unequal canina meiosis, it is nearly impossible to detect spontaneous *Caninae* hybrids (Ritz and Wissemann 2003, Wissemann and Hellwig 1997).

The first assumptions about the allopolyploid constitution (polyploidy produced through hybridisation between different species) of the section *Caninae* were based on the morphology, the anatomy, the polyploid chromosomal constitution and the peculiar mode of heterogamous gamete formation, the canina meiosis (Blackburn and Heslop-Harrison 1921, Täckholm 1920, 1922, Gustafsson 1944). Only recently, several cytological and molecular analyses confirmed this allopolyploid hybridogenic origin of the section *Caninae*.

First, it was proven that the spontaneous development of a canina-like pentaploid pollen mother cell was possible through the production of haploid pollen grains in a diploid hybrid between *R. arvensis* and *R. chinensis* (Wulff 1954).

Secondly, the genomic integrity of the polyploid complex section *Caninae* was proven to be high because of the reduced levels of recombination (Nybom *et al.* 2004) as the individual chromosomal sets could be identified by nrITS sequences (Ritz *et al.* 2005, Wissemann 2000a). In 2002, Wissemann identified four different nrITS-types: A, B, C, and E-type (Wissemann 2002a). Whereas A, B, and E-type are present in all the sections of the genus *Rosa*, the C-type is restricted to the section *Caninae*. As each nrITS-type can be used to identify a specific chromosome set, it confirms the allopolyploid origin of the section *Caninae* and of the subsections *Vestitae*, *Rubigineae* and *Caninae* (Wissemann 2002a). For *R. canina*, the genomic constitution based on the nrITS sequences can be described as: ACCDE (Figure 2.10). Here, CC stands for the highly homologous bivalent-forming genomes, and ADE represents the three non-recombinant heterologous haploid genomes (Wissemann 1999). In addition, the phylogenetic trees of nrITS sequence data suggest that the sections *Synstylae* and *Caninae* are sister groups (Wissemann and Ritz 2005).

All these results indicate the multiple allopolyploid origin of the section *Caninae,* through hybridogenic introgression of several non-*Caninae* species with the common and probably extinct ancestral species of the section *Caninae*, the so-called Protocanina. The genomes of the non-*Caninae* species form the non-recombinant univalent genomes; whereas the Protocanina provided the diploid genome, the bivalent-forming chromosomes (Ritz *et al.* 2005). Moreover, it is presumed that the internal diploid genome might be responsible for the origin of the canina meiosis (Zielinski 1985, Lim *et al.* 2005). However, unless any such diploid species possessing the canina nrITS type is discovered, there is no conclusive evidence for the existence of the Protocanina. One cannot rule out the mutative origin of this diploid genome (Wissemann and Ritz 2007). The hybrid origin of the entire section *Caninae* and the consequently low interspecific genetic distances (Wissemann 2000a) may explain why hybrids of controlled crossings between L and D type parents do not suffer from hybrid depression (measured by e.g. number of seeds per hip, or number of fertile seeds per hip both in hybrids and in parental species) (Ritz and Wissemann 2003).

Species groups which permanently resort to a similar or identical allopolyploid background (sharing parts of the same gene pool) should suffer from a similar or identical parasite spectrum. Moreover, according to the hybrid bridge hypothesis (Floate and Whitham 1993), hybrids presumably act as connections

between species on which parasites can change from one host species to another, expanding their host spectrum. This general assumption can be observed in the section *Caninae*, where until now no specialisation of parasites was detected, neither with respect to the total fauna of rose bushes nor in studies concentrating on single organisms. In addition, the hybrid bridge hypothesis also described the unidirectional system in which interspecific hybridisation occurs. Hybrids backcross only with one of the parental species. This might be explained by the fact that the F-generations share the infection rates and the parasite spectrum with the parental species which either contributed the most to the hybrid offspring, or which is, for whatever reason, preferred by the parasite. In the section *Caninae*, this quasi-unidirectional inheritance was also observed and caused by the strongly asymmetric character inheritance due to the heterogamous reproduction mode. For instance, previous crossing experiments (Wissemann and Hellwig 1997) showed that *R. rubiginosa* acted as a good pollen donor, but was not as good as seed parent in the reciprocal crossings. The rare occurrence of maternal hybrids with *R. rubiginosa* might support the fact that the gall former, *Diplolepis rosae*, is not able to radiate outside *R. rubiginosa* (Wissemann and Ritz 2007).

The detection of matroclinal inheritance of chemical surface characters, such as the epicuticular wax morphology around the stomata and the chemical wax compounds at the ab- and adaxial leaflet surfaces, has consequences for the interpretation of possible evolutionary processes of hybridogenic taxa in *Rosa*, section *Caninae*. If the inheritance of characters subject to selection follows the maternal line, offspring will only be able to establish under conditions where the seed parents already exists. Thus there will be a negative selection against hybrids if they establish outside the natural potential range of the seed parent. Additionally, establishment of the hybridogenic offspring will be impeded if the seed parent has already successfully filled the ecological niches and is competitive. Both scenarios are controlled by the mechanism of matroclinal inheritance and prevent genetic drift and break-off of the seed parent species by controlling offspring radiation possibilities (Wissemann *et al.* 2007).

2.2.4. Reproduction strategies

In flowering plants, three fundamentally different modes of reproduction have been identified: (a) outcrossing sex or xenogamy; (b) selfing by auto- or geitonogamy; and (c) asexual strategies, such as vegetative reproduction or apomixis. Each mode influences the population structure and the evolutionary potential in different ways. Perennial plants, as the genus *Rosa*, commonly use multiple reproductive strategies to fine-tune their reproductive strategy to changing ecological circumstances (Richards 2003). A historical overview of the contradictory outcomes and uncertainties concerning the reproduction strategies in the section *Caninae* is given by Wissemann and Hellwig (1997). The occurrence and success of the modes of reproduction within the section *Caninae*, the lack of clearly defined species-boundaries, already indicated by Linnaeus (1753), the predominant maternal inheritance of the morphological characters, etc. prevent a proper insight into the

origin of spontaneous seedlings as they all show a high morphological resemblance to the maternal parent.

2.2.4.1. Xenogamy

The occurrence of cross-fertilisation within section *Caninae* species is very common. Moreover, interspecific hybridisation [i.e. cross-fertilisation across species boundaries or even across (sub-) sections] has been proven for certain species (e.g. Wissemann and Hellwig 1997, Werlemark *et al.* 1999, Nybom *et al.* 2004). However, the high morphological resemblance of the spontaneous hybrids to the maternal parent prevents the detection of spontaneous hybrids in the wild. Performing controlled interspecific crossings with *R. canina* and *R. rubiginosa*, the viable yield of the reciprocal crossings differed significantly, compared to the intraspecific hybrids of both parental species, and among the reciprocal crossings (Wissemann and Hellwig 1997). In §2.2.3. hybridisation is handled in more detail.

2.2.4.2. Self-fertilisation

Self-incompatibility is widespread in the genus *Rosa*, especially in the diploids. In contrast, the individuals of the pentaploid section *Caninae* are self-compatible as they can produce seed through selfing (Nybom *et al.* 2006). Ueda and Akimoto (2001) performed artificial pollinations and evaluated the self- and cross-compatibility in various species of the genus *Rosa* under field conditions. They concluded that the self-incompatibility system that widely exists in the genus *Rosa* breaks down as the polyploid level increases (Ueda and Akimoto 2001).

Self-fertilisation includes both auto- and geitonogamy. As autogamy can be defined as "the fertilisation by pollen of the same flower, but resulting from different meiosis", geitonogamy involves "the fertilisation by pollen of other flowers belonging to the same plant". Therefore, no qualitative difference would be expected between the progeny of both types of self-fertilisation (Wissemann and Hellwig 1997).

Although the occurrence of selfing was never questioned, several studies have shown that dogroses are capable of producing a high proportion of seed through self-fertilisation (Jičínská 1976a, Wissemann and Hellwig 1997, Ueda and Akimoto 2001). It is impossible to quantify the contribution of auto- and geitonogamy to the viable seeds in nature; moreover they will be hidden by xenogamy. For that reason, controlled crossings between wild parents were performed. In contrast to the presumed lack of genetic difference between the two types of reproduction, the production of viable seeds through geitonogamy appears to be significantly higher compared to autogamously produced seeds. Nevertheless, they could not find a good explanation for this outcome (Wissemann and Hellwig 1997). Compared to strictly outcrossing species, the self-compatible species have significantly lower within-population variation and a higher among-population differentiation (Nybom *et al.* 2004).

2.2.4.3. Apomixis

Apomixis is a way of asexual reproduction that can be defined as "the ability to set seed without meiotic reduction and fertilisation". Consequently, there is an exclusive transmission of the entire maternal genotype to the next generation, establishing a genetically stable, seed-propagated clone (Vielle Calzada *et al.* 1996).

The occurrence of apomixis in the plant kingdom is associated with some particular features. Most of the apomictic taxa (a) are polyploid (Asher and Jerling 1992); (b) are highly polymorphic with numerous microspecies leading to a difficult and controversial taxonomic treatment (Czapik 1994); (c) have peripheral or marginal habitats; (d) have a tendency to colonise; and (e) have a hybrid origin (Werlemark 2000a). The ability to produce seedlings asexually is widespread in Rosaceae (Nybom *et al.* 2006). Emphasising the section *Caninae*, (a) these species are mostly pentaploid, although tetra- and hexaploids were also observed; (b) the group has a common unique chromosomal constitution and an unbalanced meiosis, but lacks common morphological similarities; (d) *R. rubiginosa* has the ability to rapidly colonise new habitats (Hatton 1989 in Olsson 1999a); and (e) the allopolyploid origin of the section *Caninae* is supported by genetic analyses (e.g. nrITS, Wissemann 2002a).

Although there is some disagreement in literature (overview by Wissemann and Hellwig 1997), the occurrence of apomixis in the section *Caninae* has been experimentally assessed by several independent studies.

Wissemann and Hellwig (1997) performed crossing experiments using wild parental material in order to assess the importance of the different reproduction strategies through the assessment of the viability of the seeds. After emasculation of the flowers, they proved that seed production through apogamy is possible in the section *Caninae*, although only 5% of the seeds are fertile. Consequently, they did not presume apomixis to be the predominant form of reproduction. However, their conclusions about the low viability of the seeds are surprising since apomicts usually have a high seed viability, equal to the pure parental species (Werlemark 2000b).

Werlemark *et al.* (1999), Werlemark (2000a) and Werlemark and Nybom (2001) performed an extended study on the progeny of reciprocal crossings between two section *Caninae* species: *R. dumalis* and *R. rubiginosa*. The descendents showed strong maternal inheritance of both morphological and molecular markers. All species-specific markers of the mother plant were inherited by the descendants, while almost 10% of the offspring lacked the pollen parent specific RAPD markers. This pattern was confirmed using STMS analysis (Nybom *et al.* 2004, 2006). Moreover, two of the morphological characters, sepal length and ovary width, were correlated with the inheritance of the pollen-specific markers (Werlemark *et al.* 1999). In addition, the viability of the pollen grains of the presumed apomictical derived offspring was different compared to that of the pollen grains of the reciprocal hybrid offspring. The interspecific hybrids showed significantly lower pollen viability compared to that of the presumed apomicts, which resembled the viability of the pure parent species. Both the distribution of RAPD markers and the viability of the pollen grains indicated the occurrence of apomixis but did not exclude sexual reproduction. Therefore, it is referred to as "facultative apomictic reproduction" (i.e. combining sexual and apomictic reproduction, even within the same population) (Czapik 1994).

The ability to transmit the maternal genotype integrally into the following generation can be seen as an advantage, as it "reduces the cost of meiosis" (Marshall and Brown 1981). Although the apomictic reproduction leads to a restricted recombination of the genomes and contributes directly to a low intraspecific variability, this might not play a substantial role in the section *Caninae*. The large part of the genomic constitution of these pentaploids is already locked up in a permanent heterozygous condition as only the bivalents ($2/5^{th}$ of the genome) are available for recombination (Grant 1971).

Notwithstanding the fact that (facultative) apomixis in dogroses can occur (Wissemann and Hellwig 1997, Werlemark 2000b), the evidence of apomixis in wild *Rosa* L. is extremely limited and confined to the section *Caninae* (Dickinson *et al.* 2007). Moreover, no study has investigated the proportions of apomictically derived progeny in natural populations, or whether different taxa vary in their ability to produce seeds by apomixis (Olsson 1999b).

2.2.5. Patterns of inheritance

The unequal segregation of meiotic chromosomes is expected to result in a skewed distribution of inherited characters. Consequently, the mode of inheritance within the polyploid section *Caninae* has been the subject of several studies. Gustafsson (1944) was the first to make controlled crossings between well-defined L and D type parents (Table 2.3), and investigated the hybrids with respect to presence of hairs, odorant glands and sepal persistence. Later, Jičínská (1976b) observed a matroclinal inheritance of leaf characters on interspecific hybrids of section *Caninae* seed parents and *R. rugosa* as pollen parent. The prickles are inherited from the pollen parent and the flowers and hips are bigger than either those of the parents. However, neither Gustafsson (1944) and Jičínská (1976b) mentioned any statistical data evaluation in their publications. As their results are in conflict with later statistically well-performed studies, they will not be taken into further consideration.

To our knowledge, the research group at Balsgård (Sweden) was the first to analyse the inheritance of morphological characters with an in-depth and large-scale study. In order to minimise the influence of the environment, the so-called phenotypical plasticity, seeds were harvested of wild parental plants (e.g. Nybom *et al.* 1996, Olsson *et al.* 2000) or of descendents of controlled intraspecific crosses among wild parental plants (e.g. Werlemark and Nybom 2001, Nybom *et al.* 2006). The seedlings were grown in a randomised design in a controlled environment (Werlemark 2000a). Parallel to this research, studies were performed to reveal the patterns of inheritance among the species of the section *Caninae*, emphasising on the epicuticular wax morphology (Wissemann 2000b, Wissemann *et al.* 2007), on different leaflet and hip characters (Ritz and Wissemann 2003), and on the growth form (Wissemann *et al.* 2006). The morphological characters in hybrid plants usually display a mosaic of parental, intermediate, transgressive, or novel ones (Rieseberg and Ellstrand 1993, Werlemark *et al.* 1999, Wissemann and Ritz 2007). The majority of the investigated characters show a maternally-biased inheritance as expected in

heterogamous meiosis. However, a few characters show a more balanced, biparental inheritance while other characters even have a paternally-biased pattern of inheritance. Nevertheless, the discrimination between parental and intermediate inheritance of morphological characters may not be completely straightforward. The expression of the characters and the molecular marker inheritance in the hybrid offspring are dependent upon the direction of the cross, and the parental species involved (Werlemark 2000a). A summary of the experimentally observed modes of inheritance is given in table 2.4.

2.2.5.1. Matroclinal inheritance

The inheritance of maternal characters may be favoured, even in species following the Mendelian meiosis, (a) through the inheritance of the endosperm, which is larger in the seeds compared to the pollen grains; (b) through organelle inheritance, e.g. plastids and mitochondria; (c) through phenotypic effects mediated by environmental factors, such as stress during seed development. These maternal effects are most pronounced in seed size and in young plants, and usually decrease in older plants (Roach and Wulff 1987). In addition, they will seldom cause any major deviation from the phenotype that is expected from the Mendelian-inherited nuclear genes (Werlemark *et al.* 1999). However, the heterogamous meiosis of the section *Caninae*, in which the descendants inherit $4/5^{th}$ of the maternal genome and only $1/5^{th}$ of the paternal genome, is expected and proven to result in a skewed distribution of predominantly maternally inherited characters (Werlemark *et al.* 1999).

In Sweden, the progeny of wild parental species (*R. sherardii*, *R. villosa*, *R. dumalis*, and *R. rubiginosa*) and interspecific reciprocal hybrids among the wild parental plants were analysed profoundly. In a preliminary study, the parental species used could be distinguished based on the studied morphological characters (Nybom *et al.* 1996, 1997). Since then, the progeny has been intensively studied. The reciprocal crossings between *R. dumalis* and *R. rubiginosa* produced two hybrid groups that closely resemble the morphology of their seed parent. The two groups differed significantly from each other in sepal length and lobes, flowering peak and leaflet shape. In addition, offspring of the *R. dumalis* x *R. rubiginosa* cross appeared to be more heterogamous than the reciprocal progeny (Werlemark *et al.* 1999). The progeny of the reciprocal interspecific crossings overlapped, indicating partially matroclinal offspring (Werlemark *et al.* 1999, Werlemark 2000a, Werlemark and Nybom 2001). Consequently, discrimination between matroclinal and intermediate inheritance of morphological characters may not be completely straightforward (Werlemark *et al.* 1999). In addition, the genetic constitution of the used parents and the reciprocal progeny was analysed. The RAPD markers were able to discriminate between *R. rubiginosa* and the other two species. However, overlap was found between *R. sherardii* and *R. villosa* (Olsson *et al.* 2000), and a highly skewed chromosomal distribution was observed in the progeny. All but one of the seed-specific markers was transmitted to the progeny. As for the pollen-specific markers only half were inherited by the descendents. They were inherited by all the sexually

derived offspring. Moreover, about 35% of the pollen-specific markers were never transmitted to the progeny (Werlemark *et al.* 1999, Werlemark and Nybom 2001, Olsson *et al.* 2000). Comparing the STMS polymorphisms, almost all the seed parent-specific alleles were inherited from the maternal parent, whereas less then half of the pollen parent-specific alleles were transmitted to the progeny (Nybom *et al.* 2004, Nybom *et al.* 2006). They were inherited by all the sexually derived offspring (Nybom *et al.* 2006).

Examining the epicuticular wax morphology of the section *Caninae*, Wissemann (2000b) concluded that *R. rubiginosa* (subsection *Rubigineae*) is characterised by a granule type of epicuticular waxes, whereas *R. canina* (subsection *Caninae*) has triangular rodlets. Analysing the inheritance pattern through reciprocal crossings, they observed a matroclinal inheritance pattern (Wissemann *et al.* 2007).

Ritz and Wissemann (2003) investigated the expression of taxonomically important morphological characters on interspecific hybrids within the section *Caninae*. This study revealed that the hybrids are not distinguishable from the seed parent with respect to the presence of hairs or glands at the leaflet surface, rachis or pedicel. Unfortunately, the offspring of the interspecific crossings was not reciprocal.

2.2.5.2. Patroclinal inheritance

A study of the reciprocal hybrids of L and D type parents within the section *Caninae* revealed a significant correlation between two taxonomically important characters, the diameter of the orifice (L type: narrow; D type: wide diameter), and the persistence of the sepals during hip ripening (L type: deciduous; D type: persistent). In addition, they observed a high similarity in the offspring with the pollen parent, regardless of the character state expressed (Ritz and Wissemann 2003). Quite remarkable was the absence of an intermediate state (L/D type) for these characters. The correlation of both characters was already described for crosses within the section *Caninae* (Gustafsson 1944, Graham and Primavesi 1993) and for intersectional hybrids (Gustafsson 1944, Feuerhahn and Spethmann 1995). Henker (2000) noted that persistent sepals always occur with wide orifice, and vice versa. This might be explained by the influence of the same gene or gene-complex or coupled genes or gene-complexes (Ritz and Wissemann 2003). However, there is no acceptable reason to believe why both characters should be inherited paternally. These two characters have no clear evolutionary significance. Nevertheless, they might be linked to other unknown important characters. Ritz and Wissemann (2003) concluded that at least one allele for sepal persistence and the diameter of the orifice is located on the bivalent-forming chromosomes of the pollen grains.

2.2.5.3. Transgressive inheritance

The occurrence of transgressive characters in F1 generations is not unusual. Rieseberg and Ellstrand (1993) reported that 64% of the F1 hybrids exhibited extreme

characters, including both transgressive and novel ones. This might be caused by (a) an increased mutation rate in hybrids; (b) complementary action of normal alleles; (c) recessive genes present in heterogamous forms in the parents, becoming homogamous in the progeny; (d) a reduced developmental stability; or (e) any combination of these four (Rieseberg and Ellstrand 1993).

Compared to the progeny of the seed parent, Werlemark and Nybom (2001) observed an enhanced amount of glandular hairs on the ovaries and pedicels on the hybrid progeny of *R. sherardii* x *R. villosa* and its reciprocals. This phenomenon was already reported by Blackhurst (1948). Moreover, Blackhurst (1948) reported heavier and denser armature in hybrids of *R. rubiginosa* x *Caninae* spp.. Given the high genomic similarity of *R. sherardii* x *R. villosa*, the complementary action of the new homogamous status of the hybrids may be the most likely cause.

2.2.5.4. Syndrome or dominant inheritance

Within each subsection of the section *Caninae*, the species can be divided into L and D types, characterised by an arching (L type) or erect (D type) growth type. The difference in growth habit (distinguishing between self-supporting, non-self-supporting, and semi-self-supporting) can be assessed by measuring the flexural and torsional stiffness (Wissemann *et al.* 2006).

The species *R. canina*, *R. rubiginosa* and their reciprocal hybrids appear to be self-supporting species. Surprisingly, this is more pronounced in *R. canina* (L type) and the two reciprocal hybrids than in *R. rubiginosa* (D type). However, small stems of *R. rubiginosa* are markedly stiffer in bending and torsion than those of *R. canina* and the reciprocal hybrids. These differences in mechanical properties of young stems are interpreted as the functional reason for the formation of different growth habits in *R. rubiginosa* and *R. canina*. The growth habit is reflected in ecological niche differentiation. Most individuals of *R. rubiginosa* occur as free-standing plants in open areas. In contrast, *R. canina* grows very often in stands like thickets, leaning and arching over other shrubs or climbing into trees at forest waysides. However, when *R. canina* grows as a free-standing shrub, the individual stems often provide mutual internal support. Comparing the two species on free stands, the difference in growth habit is recognizable.

In contrast to the expected mode of unbalanced inheritance, the reciprocal hybrids of the parental species L type *R. canina* and D type *R. rubiginosa*, showed neither a parentally skewed nor intermediate habit. Irrespective of whether *R. canina* was used as seed or pollen parent, the hybrids always showed a loose habit. This resemblance to the L type, *R. canina*, might have several reasons. According to Wissemann *et al.* (2006), the most likely cause is that the growth in its phenotypical and functional emergence is a syndrome, influenced on multiple levels (intrinsic and extrinsic principles, environmental influence, etc.), and is realised as a sum of interactions, of which some are subject to inheritance and others are not. They also suggested that hybrids might show a considerable degree of heterosis acting on the united cell structure and leading to a more open, loose and taller growth. This is not seen in the actual plants, as they all reach the same height, but might have its effects

at the level of wood anatomy. A last reason might be that growth is a character dominantly inherited by *R. canina*. If so, this character would segregate in F2 and then emerge as a first character inherited according to Mendelian laws in dogroses. So far, no evidence has been published to support one of the postulated hypotheses. The latter two could be tested analysing further hybrid combinations (Wissemann *et al.* 2006).

Table 2.4: Summary of the experimentally assessed modes of inheritance. Indicated are the observed characters, the modes of inheritance and the reference of the publications. With °: both matroclinal and patroclinal, or majority patroclinal; *: offspring of interspecific crossings was not reciprocal.

Characters	Matroclinal	Patroclinal	Transgressive	Syndrome
Presence of glands and hairs on leaflet surface, rachis and pedicel	Ritz & Wissemann 2003*			
Growth form: L type				Wissemann *et al.* 2006
Heavier and more Dense armature			Blackhurst 1948	
Leaf shape	Werlemark *et al.* 1999			
Cuticular waxes	Wissemann 2000b, Wissemann *et al.* 2007			
Peak flowering	Werlemark *et al.* 1999			
Diameter of orifice		Ritz & Wissemann 2003		
Pedicel length	Werlemark *et al.* 1999°	Werlemark *et al.* 1999°		
Glandular hairs on ovary			Werlemark & Nybom 2001, Blackhurst 1948	
Glandular hairs on pedicel	Ritz & Wissemann 2003*			
Sepal length	Werlemark *et al.* 1999			
Sepal serration	Werlemark *et al.* 1999			
Sepal persistence		Ritz & Wissemann 2003		
% species-specific RAPD markers inherited by progeny	Almost 100% Werlemark *et al.* 1999, Werlemark & Nybom 2001, Olsson *et al.* 2000	About 50% Werlemark *et al.* 1999 Werlemark & Nybom 2001, Olsson *et al.*2000		
% species-specific STMS markers inherited by progeny	Almost 100% Nybom *et al.* 2004, Nybom *et al.* 2006	About 50% Nybom *et al.* 2004, Nybom *et al.* 2006		

2.3. Research on Roses

The morphological diversity, as it was observed by Linnaeus (1753), was not sufficient to describe and classify the polymorphic section *Caninae*. Today, it is common knowledge that the taxonomical classification of the section *Caninae*, based on the shared presence of polyploid chromosomal status and the canina meiosis, is highly artificial. Therefore a more integrated approach is required to expand the insight into this species-complex: e.g. biochemical, molecular-genetic and morphological studies.

The life history traits of a species might influence the genetic diversity within and among populations (Hamrick *et al.* 1992). For instance, an outcrossing woody species with a widespread distribution and widely dispersed seeds tends to have a higher within-populations diversity, and displays less variation among populations compared to selfing species. However, the evolutionary history of each species may also play an important role in determining the levels and distribution of genetic diversity (Hamrick *et al.* 1992).

Both the morphological differences and molecular marker polymorphisms indicate that the different taxa of the section *Caninae* have different amounts and patterns of interpopulational variation (Nybom *et al.* 1996, 1997, Olsson *et al.* 2000).

2.3.1. Morphometric analyses

The morphometric variation of wild individuals of the five most common section *Caninae* species in Sweden: *R. canina*, *R. dumalis*, *R. rubiginosa*, *R. villosa* and *R. sherardii* (Nybom *et al.* 1996) was assessed. A set of morphological characters (both vegetative and reproductive ones) divided these species into three groups. Following the subdivision of Henker (2000) these are: subsection *Caninae* (both *R. canina* and *R. dumalis*), subsection *Rubigineae* (*R. rubiginosa*), and subsection *Vestitae* (*R. villosa* and *R. sherardii*). Of these five species, *R. rubiginosa* seems to be the most distinct taxon, displaying the least intraspecific variation. However, using a classification test based on the investigated morphological characters, only half of the wild individuals were reassigned correctly (Nybom *et al.* 1996).

The morphometric diversity was used as a measure to estimate the genetic variability within and among taxa. Consequently, the genetic distance is assumed to be more or less proportional to the distance measure based on a sufficiently large number of phenotypical characters (Nybom *et al.* 1996). Nevertheless, the phenotypical differences might not be proportional to the number of underlying gene mutations; the expression of characters might be either uni-parental, or intermediate depending on the character, and on the mono- or polygenic control. Moreover, there is uncertainty about the extent of phenoplasticity of the environment, and the influence of the developmental stage of the plant (Werlemark 2000a). No geographical pattern could be detected while analysing individuals of a wider geographical scale (Nybom *et al.* 1996).

In order to assess the variability and diversity among and within the taxa of the section *Caninae*, there is a need to investigate the taxa with more enhanced

techniques or methods of analyses, such as molecular markers, complementary to the morphological study.

2.3.2. Chemotaxonomy and the quantification of mechanical characters

In 2000, Wissemann compared the chemical structure of the cuticular waxes on the leaflets (Wissemann 2000b). The wax characters have proven to be important taxonomical markers (Rafii and Dodd 1998), and are known to play a pivotal role in a wide range of interactions between plants, insects, phytopathogens and their environment, e.g. light intensity and water stress (Wissemann 2000b, Wissemann *et al.* 2007). Therefore, they may allow ecological niche differentiation (Wissemann *et al.* 2007). The correlation between the morphology and the corresponding chemical composition is generally accepted (Wissemann *et al.* 2007). All taxa of the subsection *Rubigineae* are characterised by a granule type of epicuticular waxes, whereas members of the other subsections have triangular rodlets, presumably formed by secondary alcohols (Wissemann *et al.* 2007). It has to be mentioned that *R. corymbifera*, *R. subcanina* and *R. stylosa*, all belonging to the subsection *Caninae*, display the *Rubigineae* granule type. This rather unexpected similarity might be explained by the polyphyletic origin of the section *Caninae* (Wissemann 2000b). The wax structure is determined by matroclinal inheritance (Wissemann 2000b).

Wissemann *et al.* (2006) performed quantitative analyses of mechanical characters, and proposed that growth form, the vegetative habit, might be a syndrome rather than a dominant inherited character, as the L type as in *R. canina* was expressed in the reciprocal hybrids with *R. rubiginosa*, a D type. A syndrome realises as an emergent functional property with underlying phenotypic structural differences in stem and wood anatomy.

2.3.3. Biochemical and molecular research

The conventional morphological study of the phenotypical variation of individuals does not enable us to resolve questions, uncertainties and problems concerning e.g. the phylogenetic relationships, the taxonomical structures, the genetic diversity of species or populations, the impact of gene flow on natural populations, or the origin of wild hybrids and of cultivars. Until this moment, a range of biochemical and molecular-genetic studies on the genus *Rosa* have been performed. The majority of these studies emphasised on the diversity and origin of the rose cultivars. In some studies, the relationship with the wild individuals was taken into account. In table 2.5, we have made an overview of the investigated topics and the performed studies.

The results of the earliest performed studies are inconsistent, probably due to a limited number of analysed samples, an insufficient resolution of the used markers, the interpretation of the output without a correlation with the morphology or the distribution of the individuals, or without taking into account the unique and

Table 2.5: Overview of the published researches on the subgenus *Rosa* and on the section *Caninae*. For each study the used method and research question is indicated. With: FNG: Functional nuclear genes; ORG.: Organelle.

	METHODS											
	ENZYME/ ISOZYME	RAPD	RFLP	AFLP	STMS	MINI-, MICROSAT. PROBES	NRITS	ATPB-RBCL IGS	FNG	ORG. DNA PROBES	CPDNA MATK	CPDNA TRNL/F	RDNA
Phylogenetic relationships													
Rosaceae											Potter *et al.* 2002		
Rosoideae							Eriksson *et al.* 2003					Eriksson *et al.* 2003	
Rosa		Millan *et al.* 1996, Debener *et al.* 1996, Atienza *et al.* 2005		Koopman *et al.* 2008			Wissemann, Ritz 2005				Matsumoto *et al.* 1998		
Caninae		Atienza *et al.* 2005											
Taxonomical position													
	Grossi *et al.* 1997, 1998, 1999	Bruneau *et al.* 2005	Hubbard *et al.* 1992, Rajapakse *et al.* 1992, Ballard *et al.* 1995	Debener *et al.* 2000, Zhang *et al.* 2000, Leus *et al.* 2004, Wen *et al.* 2004, De Cock *et al.* 2007a	Esselink *et al.* 2003, 2004	Ben-Meir, Vainstein 1994, Vainstein, Ben-Meir 1994	Leus *et al.* 2004, Wissemann, Ritz 2005		Joly *et al.* 2006	Matsumoto *et al.* 1997	Leus *et al.* 2004, Matsumoto *et al.* 1998		
		Torres *et al.* 1993, Cubero *et al.*1996		Bruneau *et al.* 2005									
Apomixis													
		Werlemark 2000b											
Inheritance strategy of section *Caninae* and genomic constitution													
					Nybom *et al.* 2006								Lim *et al.* 2005

Table 2.5 continu: Overview of the published researches on the subgenus *Rosa* and on the section *Caninae*. For each study the used method and research question is indicated. With: FNG: Functional nuclear genes; ORC.: Organelle.

					METHODS							
ENZYME/ ISOZYME	RAPD	RFLP	AFLP	STMS	MINI-, MICROSAT. PROBES	NRITS	ATPB-RBCL IGS	FNG	ORG. DNA PROBES	CPDNA MATK	CPDNA TRNL/F	RDNA
Genetic Diversity of species or populations												
Grossi *et al.* 1997, 1998	Reynders-Aloisi, Bollereau 1996, Werlemark *et al.* 1999, Olsson 1999, *et al.* 2000, Werlemark, Nybom 2001, Alienza *et al.* 2005		Crespel *et al.* 2001, De Cock *et al.* 2007a, 2007b									
Origin of Hybrids												
Kim, Byrne 1996						Wissemann 2000a,2002a						
Origin of Cultivars												
Grossi *et al.* 1997	Torres *et al.* 1993, Millan *et al.* 1996, Debener *et al.* 1996, Cubero *et al.*1996, Martin *et al.* 2001, Mohapatra, Rout 2006			Piola *et al.* 2002, Esselink *et al.* 2003, Nybom *et al.* 2004, 2006		Wissemann, Ritz 2005, Ritz *et al.* 2005						
Construction of Genetic Linkage Maps												
	Debener, Mattiesch 1999, Debener *et al.* 2001, Rajapakse *et al.* 1992, 2001	Ballard *et al.* 1995	Rajapakse *et al.* 1992, 2001, Debener, Mattiesch 1999, Debener *et al.* 2001									
Assessing impact of gene flow												
			Debener *et al.* 2003									

unequal canina meiosis or its expected effects on marker distribution (Wissemann 1999, Werlemark *et al.* 1999).

An integrated approach, combining biochemical and molecular techniques with a study of the morphological characters, is required. Molecular markers have the distinct advantage over biochemical and morphological characters, as they are independent of gene expression. They are thus insensitive to the influence of environment and genetic background, and are developmentally stable (Leitch and Bennett 1997).

2.3.4. The Nordic section *Caninae*

In addition to the morphological trait analyses, a selected number of wild Swedish individuals and reciprocal seedlings between wild parents were analysed with molecular markers, such as RAPD, STMS (Werlemark *et al.* 1999, Olsson 1999b, Olsson *et al.* 2000, Werlemark and Nybom 2001, Nybom *et al.* 2004). The investigated wild *R. canina*, *R. rubiginosa*, and *R. villosa* individuals, each representing a different subsection, were distinguishable using morphological and RAPD markers (Werlemark *et al.* 1999, Olsson *et al.* 2000). In contrast to the morphology, the RAPD markers were unable to differentiate between two species of the same subsection, e.g. *R. canina* and *R. dumalis*, or *R. villosa* and *R. sherardii* (Olsson *et al.* 2000). Also, the subdivision *R. dumalis* in the subspecies *dumalis* and *coriifolia*, was not confirmed by RAPD markers (Olsson 1999b, Olsson *et al.* 2000). All STMS markers observed in *R. villosa* were also found in *R. sherardii*. However, the latter displayed some additional markers. This might indicate a hybridogenic origin of *R. sherardii* from *R. villosa* or a close relative as seed parent and an unknown *Caninae* species as pollen donor (Nybom *et al.* 2004). The absence of marker differentiation among two species that clearly show morphological variation indicates the importance of including the morphological characters in the investigations.

The RAPD analysis confirmed the sparse intraspecific variation in the section *Caninae* that was observed with the morphometric analyses (Nybom *et al.* 1996, 1997, Olsson *et al.* 2000). Consequently, it is possible to use one individual as a representative for the whole species to predict intraspecific variability. Nevertheless, the amount appears to vary between the species. *R. dumalis* stands out as the most variable species, while *R. rubiginosa* displays the least intraspecific variation. *R. villosa* subsp. *mollis* shows a significant variability between populations comparable to *R. dumalis*, while the within-population variability is more similar to *R. rubiginosa* (Nybom *et al.* 1997). Moreover, *R. rubiginosa* is clearly recognisable from the other investigated species. Assessing the level of heterozygosity using STMS markers, the overall heterozygosity was similar among the analysed species. Still, there was a small decrease similar to the pattern based on the morphological analysis (Nybom *et al.* 2004). In addition to the clearly delimited *R. rubiginosa* group, relatively rare hybrids involving *R. rubiginosa* have been recorded in Sweden (Malmgren 1986). Both the low intraspecific variability and the genetic distinction with the other species might be explained by varying hybridisation and introgression events, due to differences in flowering phenology. *R. rubiginosa* blooms a few days after *R. dumalis*

and *R. villosa*, so little, if any, foreign pollen is available for interspecific hybridisation (Werlemark 2000a). In addition, the occurrence of interspecific pollen competition might cause the species to have different inclinations to hybridise in nature (Werlemark 2000a).

The population genetic structure within and between the seven most common section *Caninae* taxa in the Nordic countries, *R. canina*, *R. dumalis* subsp. *dumalis*, *R. dumalis* subsp. *coriifolia*, *R. rubiginosa*, *R. sherardii* var. *umbelliflora*, *R. sherardii* var. *venusta* and *R. villosa* subsp. *mollis*, was assessed combining a morphometric diversity study (including automated image analysis of leaflet shape and manually measured reproductive characters), and a molecular diversity study using RAPD markers (Olsson 1999a, b). The assessed molecular diversity can be partitioned in within- and between-population components (Whitkus *et al.* 1998). Partitioning of diversity may be similar for different character types; the characters may reveal different patterns of geographic differentiation. It is difficult to use geographic patterns of differentiation in one type of character to predict patterns of geographic differentiation in other types of characters, because different functional complexes of morphological characters may respond differently to different selection pressures (Prentice 1986). Combining the outcomes of several morphological descriptors and RAPD markers, Olsson concluded that:

(a) the between-taxon component of the diversity accounted for the majority of the total diversity (about 80%), and was followed by the between-population diversity within taxa (about 20%, Olsson 1999a, 1999b). This supported the division of the section *Caninae* into three major groups, subsections: *Caninae*, *Rubigineae*, and *Tomentosae/Villosae*. Moreover it confirmed the morphological study of Nilsson (1999);

(b) the majority of the within-taxon diversity was found between the populations, which is consistent with a predominant selfing or apomictic mode of reproduction (Olsson 1999b). In general, it is assumed that outcrossing species have the majority of total within-taxon diversity stored within population, while self-pollinators or apomictic species have the majority between the populations, and a high internal homogeneity within the populations. Therefore, the restricted recombination due to canina meiosis means that the observed partitions of diversity cannot simply be interpreted as indicators of apomixis or selfing. Even if dogroses were highly outcrossing, the predominant maternal inheritance of the chromosomes would cause a structure of diversity similar to that of selfing individuals (Gustafsson 1944, Werlemark *et al.* 1999). However, the effects of selfing or apomixis cannot be distinguished from the effects of the canina meiosis (Olsson 1999b);

(c) the section *Caninae* taxa were considered to be autoallopolyploid, and showed varying degrees of homology between their genomes (Olsson 1999a). *R. dumalis* subsp. *dumalis* showed the highest within- and between-family components of diversity, followed by *R. canina*, which might be a reflection of its heterogeneous genome or of a higher degree of outcrossing compared to the other taxa. In contrast, *R. rubiginosa* was characterised by low levels of intraspecific variation, and the diversity partitions should be interpreted with caution. The high intraspecific variation in *R. dumalis* subsp. *dumalis*, and low level in *R. rubiginosa* was already

suggested in previous studies (Gustafsson 1944, Nybom *et al.* 1996, 1997, Werlemark *et al.* 1999, Olsson *et al.* 2000), and might reflect differences in their genomic constitution and/or different levels of apomixis or selfing (Olsson 1999a). High levels of within-population differentiation and the overlap between families sampled at different sites suggest that there is, or has been, gene flow between the sites of *R. dumalis* subsp. *dumalis* and *R. villosa*. In contrast, *R. rubiginosa* showed extremely low within-population differentiation and almost no overlap between maternal families belonging to different sites. Both the low within-population differentiation of *R. rubiginosa* and the lack of overlap between the families belonging to different sites might suggest that the populations are the result of founder effects. Each population represents a different recombination event. Alternatively, it might be caused by historical episodes of small population size or reproductive isolation during the species' postglacial colonization of Northern Europe (Gustafsson 1944). The low level of intraspecific variation in *R. rubiginosa* may reflect higher levels of selfing or apomictic reproduction. Moreover, the conservative effect of the canina meiosis may have been reinforced by differences in flowering time. The later blooming period of *R. rubiginosa* may have led to a degree of phenological (reproductive) isolation and prevented crossings between *R. rubiginosa* and other section *Caninae* taxa (Olsson 1999a). Despite the low levels of variation, *R. rubiginosa* has an enormous ability to rapidly colonize new habitats. Soon after its introduction in the 19th century, it was declared as one of the worst invasive weeds in Australia (Hatton 1989 in Olsson 1999a);

(d) the present taxonomy may have placed too much emphasis on characters that display a somewhat mosaic pattern of geographic differentiation between populations of one species, e.g. *R. dumalis* (Olsson 1999a). The geographically distinct taxa might be classified as subspecies, as they are morphologically distinguishable, *R. dumalis* subsp. *dumalis* and subsp. *coriifolia* (McDade 1995). Based on the poor discriminating power of RAPD marker variation (Olsson *et al.* 2000), the lack of reliable discrimination through reproductive and vegetative descriptors (e.g. Nybom *et al.* 1997, Olsson 1999a), the similar population structures (Olsson 1999a) and the identical geographical distributions and ecological preferences (Nilsson 1967, 1999), Nilsson (1999) suggested that the observed variation within *R. dumalis* might be better described at the species level. Moreover, the leaflet pubescence discriminating between the two subspecies is controlled by only one or two genes (Gottlieb 1984).

2.4. Biodiversity

At the Convention on Biological Diversity (CBD article 2) in Rio de Janeiro (1992), Biological Diversity was defined as "the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part; including diversity within species, between species, and ecosystems". In short, biodiversity should be considered on the individual, the species, and the ecosystem level.

2.4.1. Autochthonous populations

A population is defined as autochthonous, when it has a continuous presence at a specific site under regular environmental conditions for a specified and "sufficiently" long time span, in most cases for woody perennials, since the post-glacial remigration (Kleinschmit *et al.* 2004). Thus, a population can be considered to be autochthonous within a defined geographical region. These so-called regions of provenance are defined according to the ecological growing conditions, and the genetic variability between the natural populations of the species. Information on adaptation to local conditions and adaptability to environmental change can be deduced in part from provenance trails. As only the ecological and genetic conditions are relevant, this means that provenance regions may be geographically discontinuous (Kleinschmit *et al.* 2004). Moreover, habitat matching may be critical for the success of the introduction, especially in an environmental mosaic (Krauss and Koch 2004).

Autochthonous populations are believed to be adapted to the local environmental conditions and to be genetically distinct compared to the non-local populations (Kleinschmit *et al.* 2004). The adaptability of a population is integrally related to the genetic variation present in that population, and is a prerequisite to the ability to respond to the changing environment. A population will only be evolutionary adapted if selection can act on a range of impairments caused by the variation, and if the selection will consistently assign lower fitness to individuals with a higher impairment. In addition, selection can be neutral when populations are at growth equilibrium, or alternatively it might be maladaptive if the populations are continuously reduced (Kleinschmit *et al.* 2004).

In general, one can assume that the local genotypes may be superior to the non-local genotypes if (a) the genetic differences between the two provenances are the result of local adaptation (van Andel 1998, Jones *et al.* 2001); or if (b) the maladaptation can be transferred from introduced non-local plants to the local populations, and is expressed through outbreeding depression (e.g. reduced seed production, reduced progeny fitness relative to within-population crosses) in subsequent generations (Keller *et al.* 2000); or if (c) the introduction of the non-local gene pool causes the genetic swamping of the local gene pool, meaning a loss of biodiversity (Sackville Hamilton 2001, Montalvo and Ellstrand 2001, Krauss and Koch, 2004).

Such adaptive genetic differentiation between populations has been found to increase with geographical distance, reflecting a correlation between distance and differences in environmental conditions to which populations are adapted (Joshi *et al.* 2001, Etterson 2004, Becker *et al.* 2006). Provenance trails of trees, such as *Pinus sylvestris*, *Betula pendula*, and shrubs, such as *Crataegus monogyna*, showed that stock of British origin is better adapted to British conditions when compared to continental stock (Worrell 1992, Jones *et al.* 2001). Even at small scales of 500 m or less, adaptation to different local habitat types has been reported (Waser and Price 1985). In the clonal species *Hydrocotyle bonariensis* and *Ranunculus reptans*, adaptations occurred even

within populations among plants at higher and lower elevations, displaying a different flooding frequency (Knight and Miller 2004, Lenssen *et al.* 2004).

The survival of indigenous species and local autochthonous populations is threatened by the increased anthropogenic impacts on landscapes. Ongoing processes such as habitat destruction and fragmentation, alteration of the distribution species strongly affect the ecosystems, populations and species (Lienert 2004, den Nijs *et al.* 1999).

The extent of the anthropogenic fragmentation far exceeds the natural fragmentation rates, and operates at a faster time-scale than many populations can adapt to. This results in smaller habitat patches with an increased isolation of the populations (Lienert 2004). The genetic constitution of these populations is influenced by the three-fold Allee effect: increased random genetic drift, elevated inbreeding, and reduced gene flow among populations. These factors lead to reduced fitness of individuals and populations with an increased probability of local extinction of demes within a metapopulation (Young *et al.* 1996, Willi and Fischer 2005). In the short term, the loss of heterozygosity (e.g. fixation of recessive detrimental mutations) can reduce the individuals' fitness and lower remnant population viability. In the longer term, the reduced allelic richness may limit a species' ability to respond to changing selection pressures. In general, there will be a loss of biodiversity with a reduced genetic variation within a population, and an increased genetic differentiation among populations (Young *et al.* 1996).

The increased global trade and travel frequencies of humans in combination with altered dispersal patterns of plants and/or animals and climate change allow alien species to expand their natural ranges, threaten the indigenous flora, change the character of the invaded locality, cause diseases, and behave as pest organisms (den Nijs *et al.* 1999). However, not only the introduction of alien species, but also the introgression of foreign genes of indigenous species may threaten the relict gene pools (Keller *et al.* 2000, Vander Mijnsbrugge *et al.* 2005).

Finally, the abiotic conditions of the surrounding landscape may be altered by habitat fragmentation, influencing the biotic interactions (Lienert 2004), such as the dysfunction of plant-animal interactions (e.g. competition, mutualism, herbivory, etc.) (Keller *et al.* 2000, van Andel 1998).

2.4.2. Restoration measures

All over Europe, ecological restoration projects are conducted in order to restore altered habitats back to more 'natural' ecosystems rich in native species (Vander Mijnsbrugge *et al.* manuscript) (e.g. The Netherlands: Maes *et al.* 1991, Denmark: Graudal *et al.* 1995, England: Ennos *et al.* 2000, Germany: Kowarik and Seitz 2002, 2003, and Seitz 2003, Flanders (Belgium): Vander Mijnsbrugge *et al.* 2005). The common goal of these restoration projects is to conserve and maintain the biological diversity of the individual, the population and the species. However, little is known about the long-term impact of such management actions on the genetic variation, on the survival of the population, and on the influence and position in the ecosystem.

The first point to take into consideration is that it is impossible to restore the original genetic variation of the vulnerable and endangered populations. Secondly, two different views concerning the expected environmental change need to be considered individually (Kleinschmit *et al.* 2004). (a) If the change is directional and predictable, plant material of a region with the predicted conditions should be transferred, to establish a new and adaptable population. However, a presumably large number of other adaptively relevant factors are not taken in consideration. Therefore, a genetic enrichment of the remaining local populations with material from the predicted regions might be less risky; (b) if the change is not predictable, non-specific enrichment of the genetic diversity, or the use of material with a proven adaptability to a wide range of environmental condition might be the more suitable action (Kleinschmit *et al.* 2004). The restoration of populations through the enrichment of the genetic variation (t.i. heterozygosity) requires enlarging the population with conspecific, non-identical genotypes. Several mind-bending topics have to be taken into consideration in every conservation management action: should the genotypes originate from the same local provenance or if they are absent, too small or genetically deteriorated, will non-local provenances also be sufficient? Is it better to use the genotypes present in nature, or should we introduce interpopulational outbreeding hybrids? How should we assess the local character of populations?

An alternative to the introduction of non-local genotypes, is the expansion of the genetic variation of a local population with F1 hybrids of an interpopulational cross involving the home population, also known as "gene flow management" or "interpopulational outbreeding crossing" (Erickson and Fenster 2006). The increase in heterozygosity through hybridisation and gene flow would be beneficial if recessive deleterious alleles are masked, or if heterozygosity is of a general fitness advantage, and leads to heterosis in the F1 hybrids. However, the magnitude of differential adaptation and differentiation in co-adapted gene complexes between target and source populations should be considered first in order to avoid outbreeding depression (Willi and Fischer 2005). Furthermore, one should investigate their impact on the performance of later-generation hybrids under field conditions. In contrast to the common similar or superior performance of the F1 generation compared to the local parent, explained by heterosis, later generations may suffer from reduced population stability (Keller *et al.* 2000).

Studies have shown that hybridisation and recombination between adaptively divergent populations can provide the necessary genetic variation for the adaptive evolution within the species, and therefore favour the fitness of the local population. This is especially true when the natural populations are threatened by genetic erosion and inbreeding depression, e.g. *Chamaecrista fasciculata* (Erickson and Fenster 2006), *Tympanuchus cupido* spp. *Pinnatus* (Westemeier *et al.* 1998). Moreover, in some cases when the differentiation among the source populations is not too large, heterosis can outweigh the loss of co-adaptation in interpopulational outbreeding (Fenster and Galloway 2000). In addition, hybrid performance is strongly influenced by population proximity (Galloway and Etterson 2005). The F1 hybrids of the interpopulational outbreeding of *Chamaecrista fasciculata* were universally superior to

the parents, while the F3 hybrids suffered a loss of fitness in comparison to the F1 generation. However, the fitness of the F3 generation was often, with exception for longest-distance crosses, equal to and sometimes even larger than that of one of the parents (Erickson and Fenster 2006). Similarly, the F1 hybrids between distant populations of *Campanula Americana* performed poorly relative to their parents, while hybrids between proximate populations outperformed their parents (Galloway and Etterson 2005).

In addition, when local adaptation is limited, restoring populations using genotypes of distant sites will have no deleterious consequences (Fenster and Galloway 2000). It could be argued that the decreased fitness due to a non-recurrent genetic disruption will be recovered over time by natural selection (Keller *et al.* 2000). It may be suggested that the risks of population extinction due to outbreeding depression (introgression of inadequate adapted alleles, disruption of co-adapted gene complexes) in some species may be much smaller than those due to inbreeding and environmental stochastic (Keller *et al.* 2000).

The final dilemma that will be discussed is the use of single or multiple seed source populations. The risk of introducing too little genetic variability versus the introduction of unwanted genotypes has to be considered. According to van Andel (1998), the presence of a population with a reduced fitness should be the better option compared to the absence at that site. However, the large-scale use of seed from a few sources presents a potential threat to biodiversity through homogenisation of the locally differentiated genetic diversity of the species (Kowarik and Seitz 2002). The uniform genetic material can reduce the genetic diversity and interfere with the genetic structure of locally differentiated populations (potentially "endemic" alleles may be swamped out by hybridisation with a larger introgression source) (Keller *et al.* 2000). A more secure solution is to introduce a mixed source displaying an adequate range of variability on which selection can act. The transplant experiments have shown that the introduction of non-local seeds can reveal a reduced fitness, but they have never shown harmful results (van Andel 1998).

2.4.3. Conservation in Flanders

The Flemish Community gouvernment authorised an inventory survey, which started in 1997 and ended in 2007, to locate the remaining autochthonous populations of Flanders (Maes and Rövekamp 1998, Rövekamp and Maes 1999, Rövekamp and Maes 2000, Maes and Rövekamp 2000, Rövekamp *et al.* 2000, Opstaele 2001, Maes *et al.* 2003, Maes *et al.* 2005, Rövekamp *et al.* 2005, Rövekamp *et al.* 2008). This was the first step in a large-scale project with the aim to establish and maintain the indispensable prerequisites for securing evolutionary adaptability of autochthonous trees and shrubs (Vander Mijnsbrugge *et al.* 2005).

During the inventories, the autochthony of a certain tree, shrub or locality was evaluated following the methods of Maes *et al.* (1991) and Maes (1993). Initially, woodlands (e.g. forests, thickets) were selected if they were indicated as forests on historical maps such as the Ferraris map of 1779. In addition, information on flora, soil conditions and geomorphologic data were used to refine the selection of

potentially relevant sites. In the field, the trees and shrubs were evaluated according to a set of criteria, all evaluated in relation to each other: (a) the tree or shrub is of a wild variety, not a cultivar, and does not show any signs of introgression (e.g. pubescence or glands); (b) the tree or shrub has an old appearance; (c) the locality does not show any signs of plantations; (d) the site is located within the natural geographic range of the species and the growing conditions correspond with the ecological requirements of the species; (e) the tree or shrub is also present on similar sites in the neighbourhood; (f) several plants on the locality are indicators of ancient undisturbed woodland (Vander Mijnsbrugge *et al.* 2005).

Using the inventory survey, conservation measures need to be taken for the most important, valuable and endangered populations and individuals. For that purpose, different strategies were evaluated. The preservation of the habitat, *in situ* conservation, is not applicable in Flanders as it requires populations large enough to regenerate naturally. In addition, the private ownership of many valuable sites also restricts conservation options. Consequently, the conservation action focuses on *ex situ* conservation, e.g. the creation of clonal banks of rare species, the production of autochthonous reproductive material, seed orchards (Vander Mijnsbrugge *et al.* 2005).

The creation of clonal banks of locally or regionally endangered autochthonous populations is absolutely necessary as these populations are too small, have a high risk of disappearance, and/or are seriously threatened by disease. In order to retain the local gene pool, these individuals are vegetatively propagated. These genotypes can be used in reintroduction projects, or to enrich reduced populations. It is impossible to restore the genetic diversity of the original populations, and the source plant material used for the relocation will influence the genetic variability of future populations. The authors aknowledge this disadvantage but it is not as bad as the risk of extinction of the populations, or the species. In Flanders, such clonal banks were established for *R. stylosa*, *R. micrantha*, *R. rubiginosa*, *R. agrestis*, *R. tomentosa*, and *R. balsamica* (Vander Mijnsbrugge *et al.* 2005).

The increasing demand for planting stock used to restore landscapes also requires to take action with species with populations large enough to regenerate naturally. This is dynamic *ex situ* conservation. It prevents the introduction of foreign provenances. For this purpose, *in situ* seed collection was conducted on surveyed sites. This practice is labour-intensive, and time-consuming as these sites are fragmented and have variable seed productions. Nevertheless, mixing the seeds collected at different sites, within one region of provenance, should guarantee a sufficient genetic variability in the planting stock. In contrast, the establishment of seed orchards would be a more efficient practice: the different populations are gathered in the same provenance-based orchard, and have a larger seed production. Moreover, such an orchard represents the genetic variability of the region of provenance, preserving the autochthonous gene pool through vegetative reproduction of the autochthonous plants and the inhibition of pollination of non-local gene sources. In Flanders, such measures have already been taken for *R. canina* and *R. arvensis* (Vander Mijnsbrugge *et al.* 2005).

3. DESCRIPTION OF USED PLANT MATERIAL AND METHODS

3.1. Plant material

The term "taxon" represents a taxonomical group at any hierarchical level: e.g. species, subsection or section. Given the complex taxonomical structure of the section *Caninae*, and especially the poorly defined boundaries between the species, the term "taxon" will be used in this thesis instead of "species". However, the commonly accepted terms "interspecific" and "intraspecific", as well as terms such as "species-concept" will still be used.

The term "population" is used for all samples belonging to a certain taxon collected at a specific locality.

As this thesis only refers to roses, the genus name *Rosa* is abbreviated to *R.* in all rose species or taxa.

Furthermore, the discussed plant material was gathered in the framework of two projects: the individuals sampled in the European project will be referred to as the "European taxa", whereas the samples analysed in the Flemish project are called the "Flemish taxa". Using the terms "European" and "Flemish", there is no intention to refer to the geographical meaning, they only indicate the wider scale in which these taxa were sampled.

3.1.1. Wild roses of Europe

Material from wild-growing plants in Europe was sampled within the framework of the EC-funded research project GENEROSE (Van Huylenbroeck *et al.* 2005). This project focussed on the genetic diversity within and between wild populations, species, and/or subsections present in Belgium, The Netherlands, Germany, France, and the Scandinavian countries. The criteria used for the sampled populations include e.g. the local and European distribution of the taxon, the presence of supposed autochthonous material, and the intriguing taxonomical position of the so-called "species". If available, inventories on the occurrence and distribution of indigenous rose species were used to select the sampled populations. Each partner involved in the project was responsible for the sampling in his/her country. For Belgium, a strategy similar to the one described in §3.1.2. "Wild roses of Flanders, Belgium" was used.

In each country up to five populations of the non-*Caninae* species were sampled. Given the poor species delimitations within subsections of the section *Caninae*, up to eight populations of each subsection were sampled. In total, the European data set contained 1140 individuals, representing 338 populations. An overview of the sampled populations is given in table 3.1.

AFLP polymorphisms were employed to study genetic diversity within and among taxa of the European populations.

Table 3.1: Taxonomical overview of the autochthonous species sampled for the European data set of the genus *Rosa* based on Henker (2000). The number of individuals sampled in Belgium (B), France (F), Germany (G), The Netherlands (N) and the Scandinavian countries (Sc) is indicated. Synonyms: °: *R. rubrifolia*; *: *R. tomentella*.

Genus *Rosa*	B	F	G	N	SC	TOTAL
Section *Pimpinellifoliae*						
R. spinosissima	37	43	97	58	65	300
Section *Rosa*						
R. gallica		90	10			100
Section *Caninae*						
Subsection *Trachyphyllae*						
R. jundzillii			10			10
Subsection *Rubrifoliae*						
R. glauca°	1	7	8			16
Subsection *Rubigineae*						
R. rubiginosa	25	5	18	36	40	124
R. micrantha	6		6	14	1	27
R. elliptica		3	5	2		10
R. agrestis	9	10		10	1	30
R. inodora					8	8
R. henkeri-schulzei				35	9	44
Subsection *Vestitae*						
R. tomentosa	26	1	1	56		74
R. pseudoscabriuscula			8	1		9
R. sherardii		1	28	10	6	45
R. mollis			7		37	44
R. villosa	2	24				26
Subsection *Tomentellae*						
*R. balsamica**	16	4	5	49	4	78
Subsection *Caninae*						
R. canina	109	63	99	100	128	499
R. corymbifera	10	7	32	62		111
R. dumalis		1	5	5	33	44
R. caesia	5	2	1	3	5	16
R. subcanina	2		2	6	4	14
R. subcollina				11	5	16
R. montana		10				10
R. stylosa	3					3
Section *Cinnamomeae*						
R. pendulina		2	10			12
R. majalis			21		8	29
Section *Synstylae*						
R. arvensis	91	37	115	60		303
R. sempervirens		8				8
Hybrids						
R. x *irregularis*	1			1		2
R. canina x *R. stylosa*	2					2
R. montana x *R. dumalis*		1				1

Table 3.2: Taxonomical overview of the autochthonous species sampled for the Flemish data set of the genus *Rosa* based on Henker (2000). Number of sampled individuals is indicated for regions of provenance: Westkust (WKU); Oostkust and Middenkust (OKU); West-Vlaams Heuvelland (WVH); Vlaamse Ardennen (VAR); Vlaamse Zandstreek (VZS); Brabants District Oost (BDO); Kempen (KEM); Voeren (VOE); Maasvallei (MV) and Viroin (VIR). Synonyms: °: *R. rubrifolia*; *: *R. tomentella*.

GENUS *Rosa*	WKU	OKU	WVH	VAR	VZS	BDO	KEM	VOE	MV	VIR	TOTAL
Section *Pimpinellifoliae*											
R. spinosissima	60	3								7	70
Section *Caninae*											
Subsection *Rubrifoliae*											
R. glauca°	1										1
Subsection *Rubigineae*											
R. rubiginosa	79	6							29		114
R. micrantha			13			4			20	1	38
R. agrestis				11		41			13		65
R. henkeri-schulzei						3		1			4
Subsection *Vestitae*											
R. tomentosa	51		6	6		37			2	1	103
R. villosa						1	1				2
Subsection *Tomentellae*											
*R. balsamica**	11	12	3	9		7	2	12	11	1	68
Subsection *Caninae*											
R. canina		28	5	5	73	41			50	31	233
R. corymbifera		38	16	3	2	42				1	102
R. caesia	4								1		5
R. subcanina							1		3		4
R. subcollina					1			1			2
R. stylosa	9		10								19
Section *Synstylae*											
R. arvensis			39	42				5		31	117
Hybrids											
R. x *irregularis*			1						2		3
R. agrestis x *R. canina*						1					1
R. canina x *R. corymbifera*								1			1
R. canina x *R. stylosa*	3		2								5

Table 3.3: Number of the morphologically analysed individuals per species and region of provenance in Flanders and Viroin. Number of analysed individuals is indicated (leaflet/hip data) for each taxon and region of provenance. Abbreviations in table 3.4; "-": no data available.

	VZS	WKU	OKU	KEM	WVH	VAR	BDO	MV	VIR	SUM
R. arvensis	-	-	-	-	23/10	12/8	-	-	30/1	65/19
R. rubiginosa	-	20/18	6/6	-	-	-	-	23/8	-	49/32
R. micrantha	-	-	-	-	1/1	-	2/2	13/5	-	16/8
R. agrestis	-	-	-	-	-	-	8/7	6/6	-	14/13
R. tomentosa	-	9/9	-	-	1/1	4/4	10/7	1/1	-	25/22
R. balsamica	-	1/1	14/14	2/2	-	9/9	4/4	1/1	-	31/31
R. canina	8/8	1/1	17/17	-	5/5	5/5	7/6	12/8	27/18	82/68
R. corymbifera	-	1/1	37/37	-	1/1	3/3	10/10	-	3/3	55/55

The distribution of each analysed taxon is presented in Europe (Kurtto *et al.* 2004), and in the Netherlands and Flanders (Maes *et al.* 2006) in figures A.1 to A.18. The sampled populations are indicated on a map of Western Europe in the figures A.19 to A.21.

3.1.2. Wild roses of Flanders, Belgium

In Flanders, the main goal was to compare the variation within and between species and/or subsections, and to perform an in-depth study of the within-population diversity. Several populations could be sampled in one region of provenance, and each population could contain up to 30-35 individuals. In addition, three species were also sampled at a Walloon region, the Viroin.

The set of populations was based on the inventories of autochthonous trees and shrubs in Flanders (Maes and Rövekamp 1998, Rövekamp and Maes 1999, Rövekamp and Maes 2000, Maes and Rövekamp 2000, Rövekamp *et al.* 2000, Opstaele 2001, Maes *et al.* 2003), and on personal recommendations of M. Leten and B. Opstaele. The probability of autochthony, the density of the population, and the distribution within and between the regions of provenance were taken into account when selecting populations. The regions of provenance used in Flanders were based on Vander Mijnsbrugge *et al.* (2005) (Figure 3.1). The adaptations are summarised in table 3.4.

In total, the Flemish data set consists of 1021 individuals, sampled in 124 different populations, representing the different regions of provenance in Flanders, and additionally the Viroin region (Table 3.2).

The samples were analysed with AFLP markers. Additionally, a small subset (289 samples, five individuals from each population) was analysed with STMS markers. An extensive morphological study was performed on 337 individuals, determined as *R. arvensis*, or one of the seven most frequent section *Caninae* species (*R. rubiginosa*, *R. agrestis*, *R. micrantha*, *R. tomentosa, R. balsamica*, *R. canina* and *R. corymbifera*) sampled in Flanders, and the Viroin (Table 3.3). The target was to analyse five individuals of each population, and at least one population per region of provenance, in total at least 25 individuals of each species. For *R. micrantha* and *R. agrestis*, number of samples is lower due to lack of suitable plant material in Flanders.

In order to assess the occurrence of spontaneous interspecific hybridisation or introgression in the field, hips of an isolated autochthonous *R. micrantha* plant were collected (West-Vlaams Heuvelland, Ploegsteert). At this locality, several autochthonous rose species were identified, e.g. *R. stylosa* and *R. arvensis*. However no additional *R. micrantha* shrubs were present. The harvested seeds were sown, and nine randomly chosen seedlings were sampled, and analysed with STMS markers.

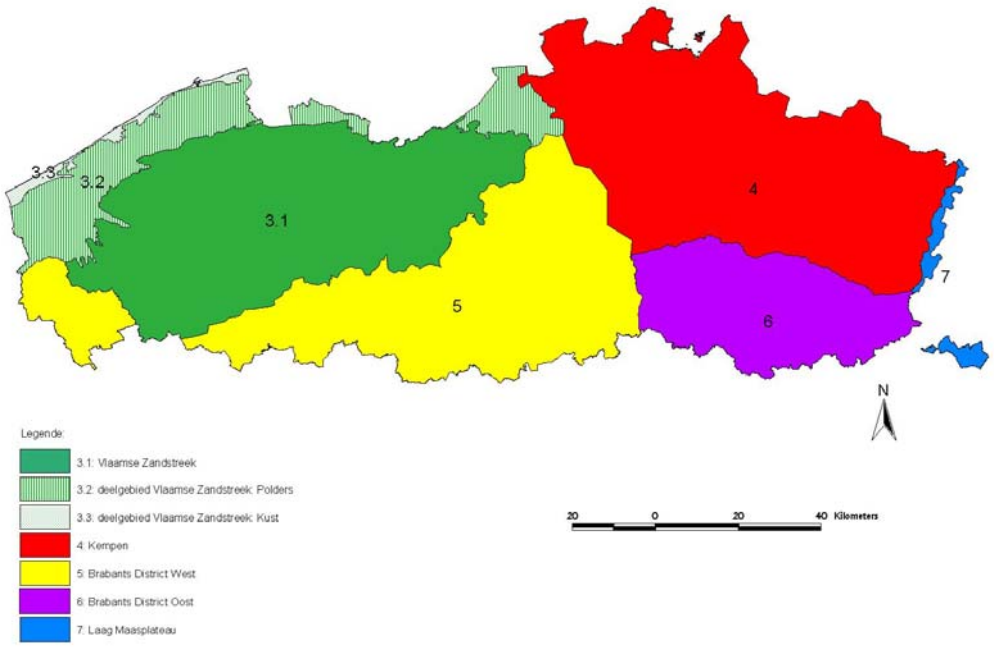


Figure 3.1: Map of Flanders with indication of regions of provenance (Vander Mijnsbrugge *et al.* 2005).

Table 3.4: Regions of provenance in Flanders, subdivided according to Vander Mijnsbrugge *et al.* (2005), and the adaptations used in this thesis. The abbreviations (ABBR.), and symbols (S) used in this thesis are mentioned.

REGION OF PROVENANCE	ADAPTED SUBDIVISION	ABBR	S
Vlaamse Zandstreek	Vlaamse Zandstreek	VZS	□
Polders	Polders		
Kust	Westkust	WKU	▼
	Oostkust/ Middenkust	OKU/ MKU	▲
Kempen	Kempen	KEM	■
Brabants District West	Brabants District West	BDW	
	West-Vlaams Heuvelland	WVH	▼
	Vlaamse Ardennen	VAR	▲
Brabants District Oost	Brabants District Oost	BDO	♦
Laag Maasplateau	Maasvallei	MV	□
	Voeren	VOE	●
Non-Flemish region	Viroin	VIR	●

3.2. Molecular techniques

3.2.1. DNA extraction

Young fresh leaflets were frozen in liquid nitrogen and lyophilised. The dried material was stored at -18 °C under vacuum conditions until DNA extraction. Following the instructions, the Qiagen DNeasy Plant Mini Kit (Westburg, The Netherlands) was used to yield 300 ng extracted DNA from 25 mg dried leaf material.

Agarose gel electrophoresis (1% agarose, 70 V, 40 min) was used to assess quality and quantity of the extracted DNA. Subsequently, each sample was diluted

to 300 ng DNA in 20 µl solution. If present, the remaining RNA was removed by adding another 3 µl RNAse, and incubating the mixture during 30 minutes.

This template DNA was used for the molecular-genetic analyses, i.e, AFLP and STMS.

3.2.2. Amplified Fragment Length Polymorphism (AFLP)

The AFLP procedure was performed according to Vos *et al.* (1995), however some adaptations were made.

The restriction-ligation (RL) of the template DNA was performed in a one-step reaction. The RL mix of each sample contained 15.25 µl MQ, 5 µl 10x One Phor All buffer [100 mM Tris-Ac (pH 7.5), 100 mM MgAc, 500 mM Kac], 5 µl DDT (50 mM), 1 µl ATP (10 mM), 1 µl *Eco*RI adapter (5 pmol), 1 µl *Mse*I adapter (50 pmol), 0.25 µl *Eco*RI (20 U/µl), 0.5 µl *Mse*I (10 U/µl), and 1 µl T4 DNA ligase (1 U/µl). For each DNA extract (30 ng DNA/20 µl), 30 µl RL mix was added. Samples were incubated at 37 °C during 4 hours, and afterwards stored at 4 °C. The success of the RL step was assessed by comparing the RL fragments with a digest of λ-*Pst*I on agarose gel (1.5%, 70 V, 60 min).

The amplification was performed in two steps. In the pre-amplification, *Eco*RI-A and *Mse*I-C primers were used. In the selective amplification step the primers contained two additional selective nucleotides. In total, sixteen primer combinations were tested on a subset of eight wild rose species. Based on a clear banding pattern and reproducibility, three primer combinations were selected for further use: *Eco*RI-AAG/*Mse*I-CAT, *Eco*RI-AAG/*Mse*I-CAG, and *Eco*RI-ATC/*Mse*I-CTA. In addition, at Plant Research International (Wageningen, The Netherlands), the European individuals were also analysed with *Eco*RI-ATC/*Mse*I-CCG, 33P-labelled.

For each RL sample the pre-amplification mix contained 32.8 µl MQ, 5 µl 10x PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 Mm $MgCl_2$, pH 8.3 at 20 °C), 2 µl $MgCl_2$ (25 mM), 2 µl dNTP (5 mM), 1.5 µl *Eco*RI-A primer (50 ng/µl), 1.5 µl *Mse*I-C primer (50 ng/µl) and 0.2 µl *Taq* DNA polymerase (5 U/µl). To this mix, 5 µl of the RL mix was added and PCR amplifications started using 28 cycles of 30 s at 94 °C, 60 s at 60 °C, and 60 s at 72 °C. After the last cycle, the samples were cooled down to 4 °C. The pre-amplified DNA fragments were compared with the size marker λ-*Pst*I performing agarose gel electrophoresis (1.5%, 150 V, 20 min).

The selective amplification mix consisted of 11.38 µl MQ, 2 µl 10x PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 Mm $MgCl_2$, pH 8.3 at 20 °C), 0.8 µl dNTP (5 mM), 0.1 µl *Eco*RI+AXX primer (50 ng/µl), 0.6 µl *Mse*I+CXX primer (50 ng/µl), and 0.12 µl Taq DNA polymerase (5 U/µl). Five µl of the preamp mix was added, and the following program was repeated 13 times: 10 s at 94 °C, 30 s at 65 °C performing a gradient towards 56 °C, and decreasing 0.7 °C per cycle, and remaining 1 minute at 72 °C. Next, 18 cycles with the following parameters was carried out: 10 s at 94 °C, 30 s at 56 °C, and 60 s at 72 °C. Finally, the temperature of the samples was kept at 72 °C for 2 minutes before decreasing to 4 °C.

After amplification, the DNA fragments were separated on the Global Edition IR² system of LI-COR (LI-COR) following the procedure of the Genetic Analysis Manual - Global Edition IR² system (LI-COR). The automatically generated TIFF-file can be imported and analysed in SAGA-MX version 3.0 (LI-COR) according to the standard procedure. The automatically generated scoring was checked carefully, and manual corrections were performed. In total, 150 fragments were scored on the Flemish individuals within a size range of 75 bp to 652 bp. For the European individuals, 137 bands were scored, between 90 bp and 352 bp. The scoring (presence: 1; absence: 0) was transformed into a binary matrix, and used as an input file for several statistical programs which are described in §3.3.1 "AFLP analyses in polyploids".

3.2.3. Simple sequence polymorphisms (STMS)

At Plant Research International (PRI, Wageningen, The Netherlands), a total of 24 STMS loci were developed for identification of rose cultivars (Esselink *et al.* 2003). Six of these loci (Table 3.5) were tested and chosen for use in the wild rose samples, based on their clear banding pattern, reproducibility, position of the loci in the genome, and number of polymorphisms.

Table 3.5: STMS loci used on the Flemish wild roses. Repeat motif, linkage group ([A]: according to Debener *et al.* 2001; [B]: Not determined), used labels are indicated.

LOCUS	REPEAT MOTIF	LINKAGE GROUP[A]	LABEL
RhAB15	$(GT)_{19\text{-}2}(GA)_{16}$	2	HEX
RhP519	$(TGA)_{11\text{-}1}$	n.d.[b]	FAM
RhM405	$(TCTGAT)_5$	n.d.[b]	NED
RhO517	$(GAC)_7$	1	NED
RhAB22	$(GT)_{13}(GA)_{13}$	6	FAM
RhB303	$(GA)_{11}$	n.d.[b]	HEX

Five out of the six loci were amplified using the same procedure. Five µl of genomic template DNA (2 ng DNA/µl) was used in a reaction volume of 15 µl containing 92 µl MQ, 2 µl 10x PCR buffer (Goldstar, 750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 1.2 µl $MgCl_2$ (25 mM), 0.2 µl dNTP (10 mM), 0.6 µl primers (20 pmol/µl), and 0.08 µl *Taq* DNA polymerase (Goldstar, 5 U/µl). For RhB303, 2.5 µl genomic DNA (2 ng DNA/µl) was added to a 17.5 µl reaction volume containing 13.22 µl MQ, 2 µl 10x PCR buffer with NH_4OH [Fermentas, 750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20], 1.2 µl $MgCl_2$ (25 mM), 0.6 µl 100x BSA (Biolabs, 10 mg/ml), 0.2 µl dNTP (10 mM), 0.2 µl primers (20 pmol/µl), and 0.08 µl *Taq* DNA polymerase (Roche, 5 U/µl). *Taq* DNA polymerase was added only in the final step.

The amplification of the loci was carried out using the following parameters: 3 min at 94 °C, followed by 30 cycles of 40 s at 94 °C, decreasing at 1 °C/s to 55 °C, and holding for 30 s (RhB303: 50 °C), next an increase of temperature (1° C/s) to 72 °C, and holding for 120 s. In the last cycle, the samples were kept at 72 °C for 10 min.

The amplification was carried out for each locus separately, and the quality was checked using gel electrophoresis. Next, the amplification products were

multiplexed as suggested by Esselink and analysed on ABI Prism 310 Genetic Analyser (Perkin-Elmer Applied Biosystems) according to the user's manual. Genotyper 2.5 (Perkin-Elmer Applied Biosystems) was used to score the alleles according to their molecular weight.

3.3. Analysis of molecular data

3.3.1. AFLP analyses in polyploids

A general pattern for the statistical analysis of the AFLP data was maintained for each analysed taxon. For the pentaploid section *Caninae* taxa, the majority of the analysed taxa, the Hardy-Weinberg assumptions required for using the F-statistics are not met since these species are polyploid, mostly pentaploid (HW requires diploidy), and have the heterogamous canina meiosis (HW requires a Mendelian meiosis). Therefore, an alternative approach was followed in order to gain insight in the within- and between-taxa differentiation. This set of analyses was repeated at different hierarchical levels such as section, subsection, species, or even populations.

The explorative analysis was performed by calculating distance matrices based on the Jaccard coefficient using Splus 6.2 Professional (Insightful Corp.), and Principle Co-ordinate analyses (PCO). In the PCO output, the components were determined that explain the majority of the variation present in the analysed data set. The relationships among the individuals were visualised in biplots along these components. The third component was displayed only when it explained additional variation among the individuals compared to the two major components.

For *R. spinosissima* (4x), and *R. arvensis* (2x), F_{ST}-values were calculated with AFLP-SURV 1.0 (Vekemans *et al.* 2002) according to Lynch and Milligan (1994). These two species were the only ones that followed the Mendelian meiosis being, or acting as, diploids, and therefore meeting the required HW assumptions for the standard population statistics.

The Jaccard similarity coefficients within and between relevant taxa were summarised in similarity matrices. Hereby, the similarity within- and among-taxa could be quantified.

Dendrograms were computed by TREECON version 1.3b (Van de Peer and De Wachter 1994). As an input file, the binary AFLP scoring table was used. Pair wise genetic distances were calculated by the algorithm of Simple Matching with 100 bootstraps. The trees were calculated with UPGMA cluster analyses, repeated 100x. In contrast to the PCO, where the variation of only two components is displayed, all the components are taken in account during the building of the tree.

A model-based clustering method, Structure 2.0 (Pritchard *et al.* 2000), was used to infer a population structure, and assign individuals to different populations or gene pools based on multilocus genotype data. This way, the commonly used

characters on which populations are defined were questioned: does the combination of geography (more specifically: locality or region) and morphology (more specifically: species, subsection, or section) represent the true population structure?

Given the AFLP data, the independent allele frequency model with haploid alleles was used assuming the no-admixture model. A total of 50,000 burnin lengths and 600,000 simulations were chosen to estimate the most probable number of populations or gene pools (K). The estimation of the most probable number of gene pools present in the data set by using an Bayesian approach to calculate the LnP(D), as suggested by Pritchard *et al.* (2000), was not straightforward in species with complex populations due to subgrouping, hybridisation or uneven migration patterns (Evanno *et al.* 2005). Instead of reaching a maximum for a certain number of gene pools (K), the LnP(D) slightly increases. Evanno *et al.* (2005) propose the calculation of the mean DeltaK, the second order rate of change of the likelihood function with respect to K, as a more suited predictor to infer the real number of clusters in a complex data set (Figure 3.2a). The mean DeltaK value was calculated using Structure-sum.R (Ehrich 2006). However, in the case that one or two gene pools might be present, this analysis did not solve the problem, e.g. for the European subgenus *Rosa* (Figure 3.2b). Another restriction of the program Structure is the identification of groups corresponding to the uppermost hierarchical level. Therefore, additional analyses were performed at lower hierarchical levels, e.g. sections, subsections, populations or even species, to detect the number of populations or gene pools in each taxon.

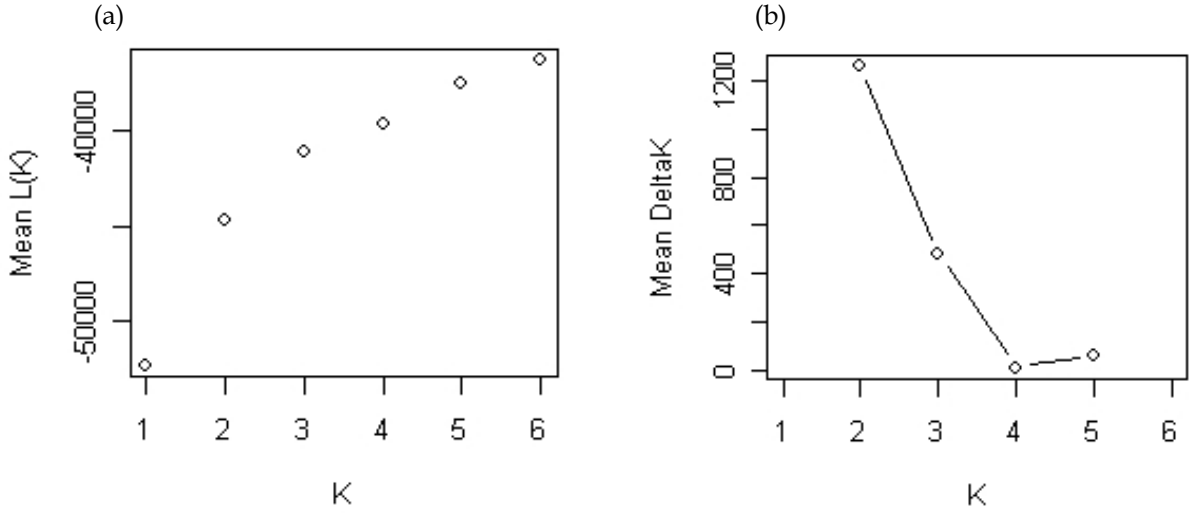


Figure 3.2: Assumption of the optimal number of gene pools (K) present in the European subgenus *Rosa*. (a) Calculation of mean LnP(D) according to Structure (Pritchard *et al.* 2000); (b) calculation of mean Delta(K) using the method of Evanno *et al.* (2005).

Additionally, the program RAPDDIV (Whitkus *et al.* 1998) was used to calculate the partitioning of the diversity within- and among-groups, e.g. taxa, populations or localities, using band phenotypes and not relying on the required HW assumptions. Originally, this program was designed to calculate the RAPD band diversity; however, it is also useful for AFLP fragments as both are dominant markers. The diversity is calculated with the Shannon-Weaver Diversity index using Brillouin formula to eliminate the bias of finite sample sizes.

The most common taxa in Flanders, *R. canina* and *R. corymbifera* were emphasised. Of both taxa, individuals sampled at the mixed localities Het Zwin (Westkust) and Heers (Brabants District Oost) were included, with in addition three well-sampled pure *R. canina* populations: Deinze (Vlaamse Zandstreek), Hochter Bampd (Maasvallei) and Viroin. Of these populations the variation within- (Hp) and among- [(Ht-aver.Hp)/Ht equals Gst] taxa and localities were calculated.

3.3.2. STMS analyses in polyploids

The reproducibility of the STMS analyses was checked by performing independent repeats and equalled 100%.

Of all the individuals analysed with STMS markers, the ploidy level was assessed (at ILVO, Dr. ir. T. Eeckhaut). Due to the pentaploid chromosome constitution of the section *Caninae*, up to five different alleles could be expected in each locus. However, in the majority of the individuals only three or four different alleles were visualised per locus. Sometimes this was restricted to only one or two different alleles (Figure 3.3). The used amplification technique did not allow the quantification of each visualised allele. Therefore, it was not possible to assess allele frequencies for the loci in polyploids. Alternatively, descriptions of differences and tendencies within- and between-taxa were possible when considering allelic phenotypes (after Becher *et al.* 2000), meaning that the presence of the alleles of a locus is used as one character (Esselink *et al.* 2003). Specific topics concerning clonality of species or populations, the presumed ancestral taxa of spontaneous hybrids could be addressed. Only for the diploid *R. arvensis*, the assessment and comparison of allelic frequencies was possible.

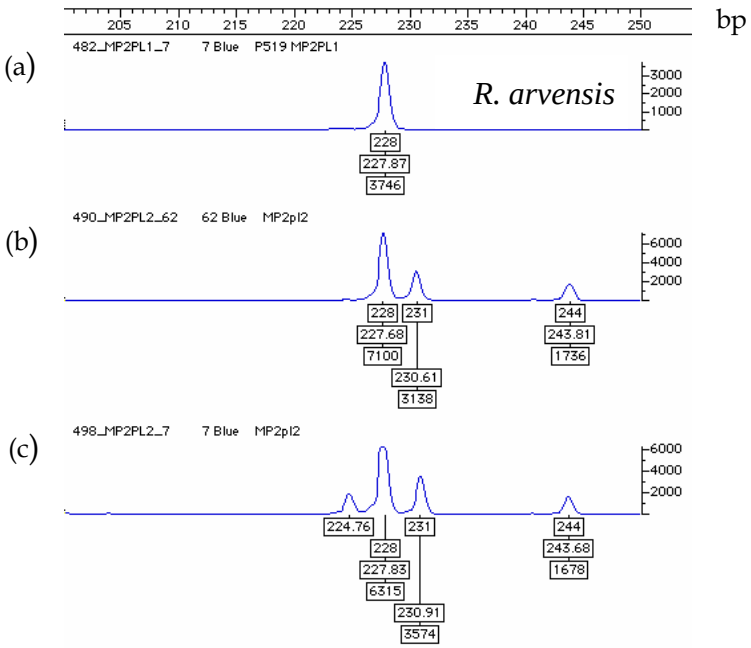


Figure 3.3: Output of STMS markers for *R. arvensis* (2x), and two section *Caninae* species (5x). The allelic phenotype of RhP519 of (a) *R. arvensis*; (b) *R. tomentosa*; (c) *R. canina* x *R. stylosa* is shown.

3.4. Morphological evaluation

The morphological evaluation consisted of analysing both morphometric and descriptive characters of leaflets and hips. Of each analysed shrub, at least five mature leaves of the more mature stems were collected during spring or summer. Since a second visit during late summer and autumn was planned in order to collect five well-developed hips, the locality and sampled shrubs were described in detail, coordinates were noted and the shrubs were labelled. Nevertheless, this system did not prevent the disappearing of labels, the dying of shrubs, etc. leading to the absence of morphological data. Moreover, not all individuals fructified at the time of the sampling.

The leaves were dried for the herbarium, while the hips were cut longitudinally and stored in ethanol (96%). When hips were clustered, the most representative hips were chosen; in addition the one in the central position was always avoided. The studied characters (Table 3.6) were based on previous published studies of White *et al.* (1988), Graham and Primavesi (1993), Nybom *et al.* (1996), Nybom *et al.* (1997), Werlemark *et al.* (1999), Henker (2000) and Werlemark and Nybom (2001). In order to include the variation within the individual, each measurement or observation was repeated three times on the leaflet or hip material of the same individual.

Table 3.6: The studied morphological characters of leaflets and hips. The used abbreviations (ABBR) of the diagnostic characters are indicated.

LEAFLET	ABBR	HIP	ABBR
MORPHOMETRIC			
Width of leaflet	LW	Orifice diameter	O
Length of leaflet	LL	Disc diameter	D
Base of leaflet	LB	Length of hip	HL
Length of rachis	RL	Length of pedicel	PL
DERIVATIVE CHARACTERS			
Width/Length of leaflet		Disc Index	
Length/Width of leaflet		Relative length of pedicel	
Base/Length of leaflet			
DESCRIPTIVE CHARACTERS			
Number of leaflets		Shape of disc	
Shape of leaflets		Shape of styles	
Overlap of leaflets		Receptacle	
Pubescence upper side	LuP	Shape of hip	HS
Pubescence upper side (detail)		Glands on hip	HG
Pubescence lower side	LlP	Number of glands on one half hip	
Pubescence lower side (detail)		Pubescence on hip	
Glands on lower side	LlG	Glands on pedicel	PG
Number of glands on ¼ cm^2		Number of glands on one half pedicel	
Glands on leaflet margin	MG	Pubescence on pedicel	
Serration leaflet margin	MS		
Number of teeth per cm margin			
Pubescence rachis			
Pubescence rachis (detail)			
Glands on rachis	RG		
Shape prickle on rachis			

3.4.1. Morphometry

The dried leaves were scanned at 300ppi using HPscanjet 3500cc and measured with the digital Imaging software Scion Image (Scion Corp.), with accuracy 0.2 mm. The measurements were performed on the leaflet positioned above to the left (Figure 3.4).

The hips were cut longitudinally before conservation in ethanol (96%). The most interesting hip characters were the diameter of the orifice and of the disc (Figure 3.5), the length of the hip, and of the pedicel. The disc is the thickened zone within the inner circle of stamens on top of the hip (~d1 in Figure 3.5). The centre of the disc is perforated by the orifice through which the styles emerge. The diameter of the orifice must be measured at the narrowest part (~d2 in Figure 3.5). Both diameters were assessed with an Eschenbach Achromat 10 x loupe with accuracy 0.1 mm. The length of the hip and the pedicel were assessed using a ruler (accuracy 0.5 mm).

3.4.2. Descriptive analyses

In total, 24 leaflet and hip characters (Table 3.6) were observed using a binocular stereoscope (Kyowa Model SZM, 0,6x-3x) and a cool light source (Euromex fiber optic light source EK-1). Of each character, discrete classes were defined, e.g. the presence or absence of glands varied from eglandular, sparsely, moderately, or densely glandular, sometimes with intermediate states. The pubescence on both sides of the leaflets, on the rachis, etc. was described as glabrous, sparsely, moderately, densely pubescent, sometimes only at the veins, or tomentose for *R. tomentosa*. The detailed classifications for the nine diagnostic descriptive characters (§4.3.2. Morphological evaluation) are summarised in table 3.7.

3.4.3. Statistical analyses

For each character and analysed shrub, the measurements and observations were repeated three times. After data-cleaning, the mean morphometric values were analysed in dot- and Box-and-Whisker plots, while the states of the descriptive characters were divided into discrete classes (for diagnostic characters: Table 3.7) and presented in histograms. In the Box-and-Whisker plots, the limits of the boxes indicated the lower and upper quartiles, while the whiskers represented the minimum and maximum values. Based on these preliminary analyses, some deviating individuals were identified compared to their presumed species descriptions. Of these individuals, the field determination was evaluated again and inaccurate field determinations were corrected.

Diagnostic characters were identified by calculating the cumulative percentages of the components of the Principle Components Analysis (PCA) based on the mean of the measurements or observations. Therefore, only completely analysed individuals (with both leaf and hip data) were included in the analyses, and separate PCA plots based on the morphometric and the descriptive data sets were performed (§4.3.2. Morphological evaluation).

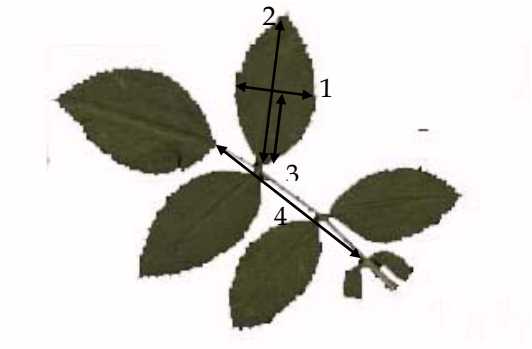


Figure 3.4: A dried and scanned leaf with indication of the morphometric characters: (1) leaflet width, (2) leaflet length, (3) leaflet base, and (4) rachis length.

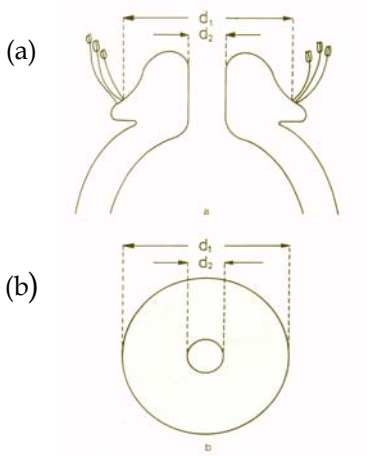


Figure 3.5: (a) Longitudinal section of the hip; (b) view of the hip from above. The diameter of the disc (d_1) and of the orifice (d_2) (Henker, 2000: 14) are indicated.

Table 3.7: Overview of the nine diagnostic descriptive characters and their subdivision in discrete classes.

Class	Pubescence Leaflet lowerside	Pubescence Leaflet upperside	Glandular Leaflet margin	Glandular Rachis	Glandular hip (# on ½ hip)
1	Glabrous	Glabrous	Eglandular	Eglandular	Eglandular (0)
2	Sparsely at veins	Sparsely	Sparsely	Sparsely	Sparsely (1-10)
3	Moderately at veins	Moderately	Moderately	Sparsely to moderately	Moderately (11-50)
4	Densely at veins	Densely	Moderately to densely	Moderately	Densely (51-100)
5	Tomentose		Densely	Moderately to densely	
6				Densely	

Class	Serration leaflet margin	Shape of hip	Glandular Leaflet lowerside	Glandular pedicel (# on ½ pedicel)
1	Uniserrated	Ovoid	Eglandular	Eglandular (0)
2	Irregular uniserrated	Ovoid to elliptical	Sparsely	Sparsely (1-5)
3	Uni- to biserrated	Elliptical	Sparsely at veins	Sparsely (6-10)
4	Irregular uni- to biserrated	Bottle-shaped	Moderately	Moderately (11-20)
5	Biserrated	Reversed ovoid	Moderately at veins	Moderately (21-30)
6	Bi- to multiserrated	Pear-shaped	Densely	Densely (31-50)
7	Multiserrated	Reversed pear-shaped	Densely at veins	Densely (51-100)
8		Globose		

4. RESULTS

4.1. AFLP polymorphisms in polyploids

In population genetics, F-statistics are the most commonly used method to assess the genetic differentiation within and between populations by calculating the allelic frequences based on e.g. AFLP polymorphisms. However these statistics assume that the organisms and populations meet the Hardy-Weinberg principles. Amongst other requirements the organism should be diploid, or at least act as diploids following a meiosis in which both parents contribute equally to the genetic constitution of the progeny. As already mentioned, the section *Caninae* taxa are polyploid following a heterogamous meiosis in which the pentaploid seed parent donates $4/5^{th}$ of the genome and the pollen parent only $1/5^{th}$. Therefore, an alternative approach was taken in order to gain insight in the within and between taxa differentiation.

A general pattern for the statistical analysis was maintained for each analysed taxon (sections, subsections, taxa or populations). In an explorative analysis, Principal Co-ordinate analyses (PCO) were performed based on the Jaccard coefficient. The first two (or if relevant three) principal components were visualised in biplots. Additionally, the Jaccard similarity coefficients within and between relevant taxa were summarised in similarity matrices. The pair wise genetic distances were visualised in dendrograms. For taxa meeting the HW requirements, i.e. *R. spinosissima* (4x) and *R. arvensis* (2x), F_{ST}-values were calculated. Finally, a model-based clustering method was performed to infer population structures and assign individuals to populations or gene pools. The followed method of analysis is mentioned as a subtitle.

Additionally, the partitioning of the diversity within and among populations of *R. canina* and *R. corymbifera* was calculated with the Shannon-Weaver Diversity index.

4.2. European subgenus *Rosa*

The European data set consisted of 1140 presumably wild roses from Belgium, France, Germany, The Netherlands, and the Scandinavian countries (Sweden, Norway, Finland and Denmark) (Table 3.1). Using four primer combinations (Table 4.1), 137 polymorphic AFLP markers were obtained. The individuals with missing information were excluded from further analyses, thus a total of 900 were analysed. The further use of the term "European" refers to this analysed data set and not to the whole European subgenus *Rosa*.

Table 4.1: The used AFLP primer combinations. The number of polymorphic markers (# PM), the scored fragment size range in base pairs (FRAG SIZE RANGE), and the used label (L) are indicated.

PRIMER COMBINATION	# PM	FRAG SIZE RANGE (BP)	L
*Eco*RI_AAG-*Mse*I_CAT	32	93-304	700 nm
*Eco*RI_AAG-*Mse*I_CAG	24	90-291	800 nm
*Eco*RI_ATC-*Mse*I_CTA	19	155-352	700 nm
*Eco*RI_AAG-*Mse*I_CCG	62		33P

4.2.1. The subgenus *Rosa*

PCO

A Principal CoOrdinate analysis of the individuals of the subgenus *Rosa* produced three larger clusters. The first two components explained 31% of the variation present in the AFLP-based data set (Figure 4.1, labels see Table 4.2). The individuals of the sections *Pimpinellifoliae* and *Cinnamomeae* were mingled in one cluster, the individuals of the sections *Synstylae* and *Rosa* formed a second cluster, in which each section could be regarded as a subcluster. Finally, the largest and most dense cluster consisted of all the individuals of the section *Caninae*. Along the third component, the subsection *Rubigineae* was clearly different from the other individuals of the section *Caninae*.

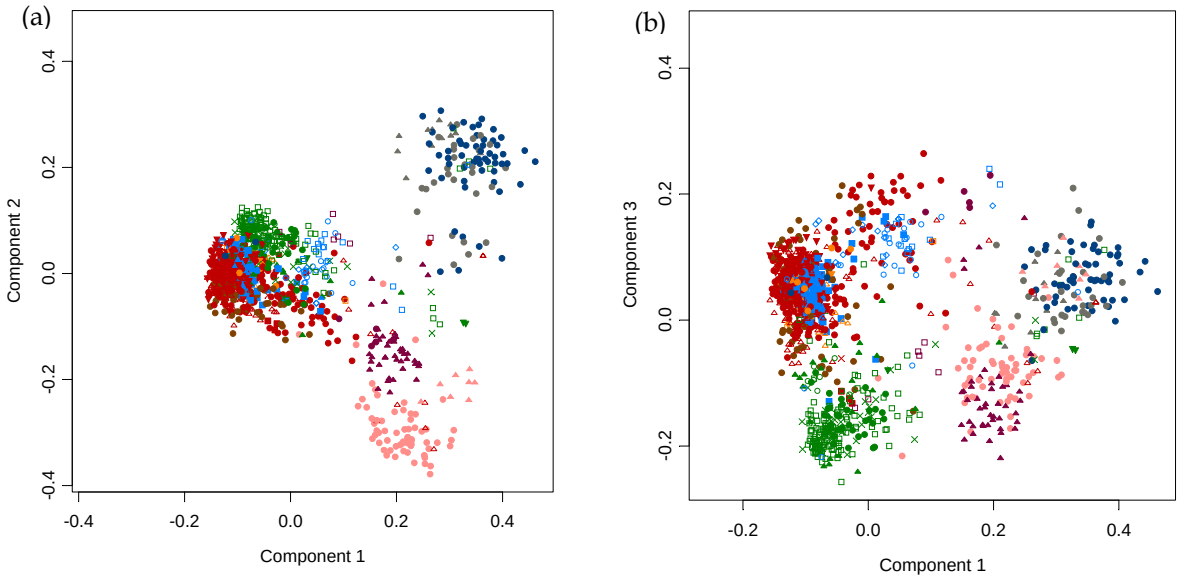


Figure 4.1: PCO plots of the European subgenus *Rosa* based on 137 polymorphic AFLP markers (a) the first two components; (b) the first and third component. The first three components explained 20%, 11%, and 10%, respectively, of the variation. Sections *Pimpinellifoliae* (Dark blue), *Cinnamomeae* (Grey), *Synstylae* (Pink), *Rosa* (Purple), and section *Caninae* with subsections *Rubigineae* (Green), *Vestitae* (Blue), *Tomentellae* (Brown) and *Caninae* (Red) are indicated; the individuals were labelled with the species determination (Table 4.2).

Table 4.2: Taxonomical structure of the subgenus *Rosa* according to Henker (2000), and °:Kurtto *et al.* (2004). The used abbreviations (ABBR), common name in Dutch/English (NAME); type of meiosis (M): R: regular; C: *canina*; ploidy level (PL), autochthony in Flanders (A) and used symbols (S) in following graphs are indicated. ‡: formerly known as *R. tomentella*; *: synonym to *R. columnifera.*

SUBGENUS *ROSA*	ABBR	NAME	M	PL(X)	A	S
Section *Pimpinellifoliae*						
R. spinosissima	R SPI /R PIM	Duinroos Burnet rose	R	4	X	●
Section *Rosa*						
R. gallica	R GAL	Franse roos French rose	R	4		▲
Section *Cinnamomeae*						
R. pendulina	R PEN	Alpine rose	R	4		▲
R. majalis	R MAJ	Kaneelroos Cinnamon rose	R	2,4,8		●
Section *Synstylae*						
R. arvensis	R ARV	Bosroos Field rose	R	2	X	●
R. sempervirens	R SEM	Evergreen rose	R	2,4°		▲
Section *Caninae*						
Subsection *Trachyphyllae*						
R. jundzillii	R JUN		C	6		●
Subsection *Rubrifoliae*						
R. glauca	R GLA	Bergroos Redleaf rose	C	4		□
Subsection *Rubigineae*						
R. rubiginosa	R RUB	Egelantier Sweetbriar, Eglantine	C	5	X	□
R. micrantha	R MIC	Kleinbloemige roos Small-flowered sweetbriar	C	4,5,6	X	▲
R. elliptica	R ELL	Wigbladige roos	C	5,6		▼
R. agrestis	R AGR	Kraagroos Small-leaved sweetbriar	C	5,6	X	●
R. inodora	R INO	Schijnkraagroos	C	5,6		○
*R. henkeri-schulzei**	R COL	Schijnegelantier	C	5	X	X
Subsection *Vestitae*						
R. tomentosa	R TOM	Viltroos Harsh downy-rose	C	5	X	■
R. pseudoscabriuscula	R PSE	Ruwe viltroos	C	5	X	▲
R. sherardii	R SHE	Berijpte viltroos Sherard's downy-rose	C	4,5,6		◊
R. mollis	R MOL		C	4,5,6		○
R. villosa	R VIL	Bottelroos Soft downy-rose	C	4		□
Subsection *Tomentellae*						
R. balsamica‡	R TON/ R BAL	Beklierde heggenroos Round-leaved dog-rose	C	5	X	▲

Table 4.2 continu: Taxonomical structure of the subgenus *Rosa* according to Henker (2000), and °:Kurtto *et al.* (2004). The used abbreviations (ABBR), common name in Dutch/English (NAME); type of meiosis (M): R: regular; C: *canina*; ploidy level (PL), autochthony in Flanders (A) and used symbols (S) in following graphs are indicated. ‡: formerly known as *R. tomentella*; *: synonym to *R. columnifera*.

SUBGENUS *ROSA*	ABBR	NAME	M	PL(X)	A	S
Subsection *Caninae*						
R. canina (*R. pouzinii*)	R CAN	Hondsroos Dog-rose	C	5	X	●
R. corymbifera	R COR	Heggenroos	C	5	X	Δ
R. dumalis	R DUM	Kale struweelroos Glaucous dogrose	C	5,6		▼
R. caesia	R CAE	Behaarde struweelroos Northern dog-rose	C	5,6	X	●
R. subcanina	R SCA	Schijnhondsroos	C	5	X	□
R. subcollina	R SCO	Schijnheggenroos	C	5		▼
R. montana	R MON		C	5		Δ
R. stylosa	R STY	Stijlroos Short-styled Field-rose	C	5,6	X	■

Jaccard matrix

Table 4.3: Mean Jaccard similarity coefficients (%) calculated within and between the sections of the subgenus *Rosa*.

SECTION	*CANINAE*	*CINNAMOMEAE*	*PIMPINELLIFOLIAE*	*ROSA*	*SYNSTYLAE*
Caninae	0.61				
Cinnamomeae	0.31	0.40			
Pimpinellifoliae	0.30	0.31	0.41		
Rosa	0.44	0.28	0.29	0.56	
Synstylae	0.41	0.23	0.24	0.43	0.45

The Jaccard coefficients suggested that the subgenus *Rosa* consisted of five well-defined units, corresponding to the sections (Table 4.3).

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals for the total data set to enhance the readability of the dendrogram.

The main clusters in the dendrogram corresponded to one of the five taxonomical sections (Figure 4.2). The sections *Pimpinellifoliae* and *Cinnamomeae* clustered in the most distinct group in the subgenus, the sections *Rosa* and *Synstylae* were grouped in a second cluster. The largest cluster contained all analysed individuals of the section *Caninae*. Within this latter, several subclusters could be identified as subsections. However the subsections *Caninae* and *Tomentellae* were mingled in the same subcluster. The subsections *Rubrifoliae* and *Rubigineae* each

formed one compact cluster, while the subsections *Vestitae* and *Caninae-Tomentellae* were split in two subclusters.

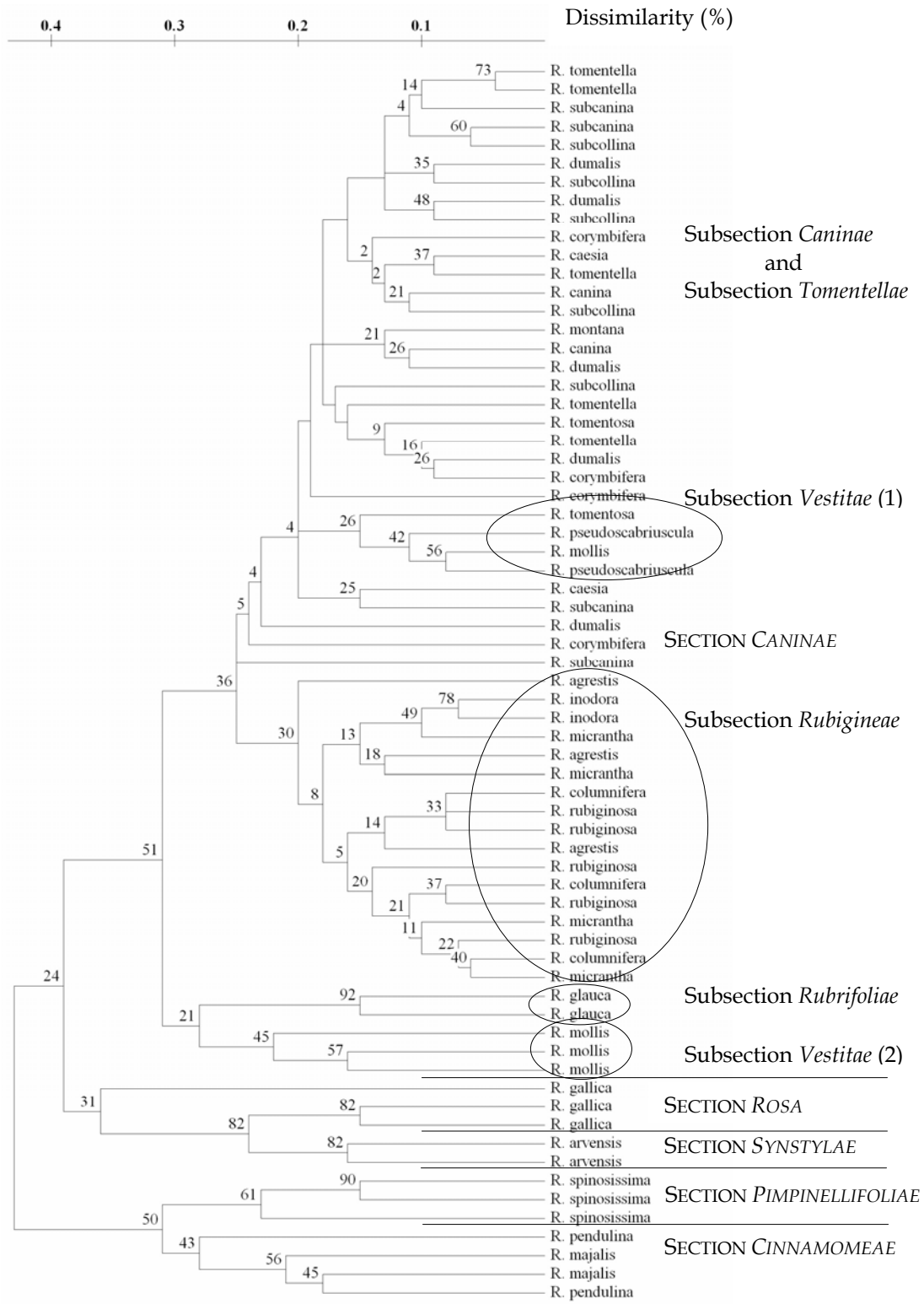


Figure 4.2: UPGMA cluster dendrogram of a subset of the sampled subgenus *Rosa* labelled with species determination. The distance scale and subdivision in sections and subsections are indicated.

Structure

Based on the calculation of the mean DeltaK, the optimal number of subclusters for the European subgenus *Rosa* could not be determined. Either one or two gene pools were possible. Assuming two gene pools, the percentage of species assignment to one of the two inferred gene pools was summarised in table 4.4.

Gene pool 1 comprised all the individuals of the sections *Rosa* (*R. gallica*), *Cinnamomeae* (*R. pendulina* and *R. majalis*), *Synstylae* (*R. arvensis* and *R. sempervirens*), and the majority of the sections *Pimpinellifoliae* (*R. spinosissima*, 95%). Some taxa of the section *Caninae* also showed a high genetic similarity to this gene pool: *R. villosa* (86%), *R. glauca*, and *R. mollis* (both 64%).

Gene pool 2 contained the majority of the taxa of the section *Caninae*: subsections *Rubigineae* (between 89% and 100%), *Vestitae,* excluding *R. mollis* and *R. villosa* (between 92% and 100%), *Tomentellae* (99%), and *Caninae* (between 97% and 100%). In addition, some hybrids, e.g. *R.* x *irregularis*, *R. henkeri-schulzei* and *R. canina* x *R. stylosa* were also attributed to this gene pool.

The AFLP polymorphisms divided the European wild roses into more or less well-defined groups. The peculiar position of the section *Caninae* within the subgenus *Rosa* is supported. The taxa belonging to the other sections within the subgenus *Rosa* are also grouped per section. The sections *Pimpinellifoliae* and *Cinnamomeae* appeared to be the most related as they show complete overlap. In addition, the sections *Rosa* and *Synstylae* also appear to have a closer link.

Within the well-defined section *Caninae*, the subsection *Rubigineae* separated from the other subsections. However, based on the wild individuals sampled in Europe, the assignment of the taxa *R. glauca*, *R. mollis*, and *R. villosa* to the section *Caninae* was not straightforward.

After analysing all the individuals belonging to the subgenus *Rosa*, different hierarchical levels (sections, subsections and, if relevant, species) were analysed separately following a similar strategy.

The European populations were coded as follows: the letter of the country of origin (Table 4.5) is followed by a three digit number. The combination of both is unique in our data set.

Table 4.4: Species distribution of the subgenus *Rosa* to each of the two inferred gene pools. The section, subsection and taxon determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are indicated in bold.

SECTION SUBSECTION	TAXON	GP1	GP2	IND
Pimpinellifoliae	*R. spinosissima*	**0.95**	0.05	44
Rosa	*R. gallica*	**1.00**	0.00	36
Cinnamomeae	*R. pendulina*	**1.00**	0.00	10
	R. majalis	**1.00**	0.00	22
Synstylae	*R. arvensis*	**1.00**	0.00	43
	R. sempervirens	**1.00**	0.00	8
Caninae				
Rubrifoliae	*R. glauca*	**0.67**	0.33	6
Rubigineae	*R. rubiginosa*	0.04	**0.96**	111
	R. micrantha	0.11	**0.89**	25
	R. elliptica	0.00	**1.00**	1
	R. agrestis	0.00	**1.00**	25
	R. inodora	0.00	**1.00**	7
Vestitae	*R. tomentosa*	0.00	**1.00**	93
	R. pseudoscabriuscula	0.00	**1.00**	5
	R. sherardii	0.08	**0.92**	10
	R. mollis	**0.67**	0.33	15
	R. villosa	**0.86**	0.14	14
Tomentellae	*R. balsamica*	0.01	**0.99**	45
Caninae	*R. canina*	0.02	**0.98**	145
	R. corymbifera	0.03	**0.97**	95
	R. dumalis	0.00	**1.00**	82
	R. caesia	0.00	**1.00**	7
	R. subcanina	0.00	**1.00**	6
	R. subcollina	0.00	**1.00**	10
	R. montana	0.00	**1.00**	11
	R. stylosa	0.00	**1.00**	3
Hybrids	*R.* x *irregularis*	0.00	**1.00**	1
	R. henkeri-schulzei	0.04	**0.96**	27
	R. canina x *R. stylosa*	0.00	**1.00**	1

Table 4.5: European countries of origin. The used abbreviations (ABBR) and symbols (S) are indicated.

COUNTRY	ABBR	S
Belgium	B	●
Germany	D	●
France	F	●
The Netherlands	N	●
Denmark	S	●
Sweden	S	●

4.2.2. The section *Pimpinellifoliae*

R. spinosissima, a representative of the section *Pimpinellifoliae*, was sampled in Belgium, Germany, France, The Netherlands, and Denmark. This species is able to reproduce vegetatively, forming spacious carpets in dunes. In total, 44 individuals were scored for 137 AFLP markers. Sixteen populations were defined based on the locality (Figure A.19).

PCO

The two Principal Components explained 28% of the variation present in the data set (Figure 4.3). Along the first component, a differentiation based on country of origin was observed, although some overlap was present. The Dutch individuals were grouped on the left side, while the individuals of both France and Belgium were situated on the right. The German and the Danish individuals were located between the two clusters. Along the second component, the French and Belgian populations were subdivided, while the Danish individuals were split off along the third axis.

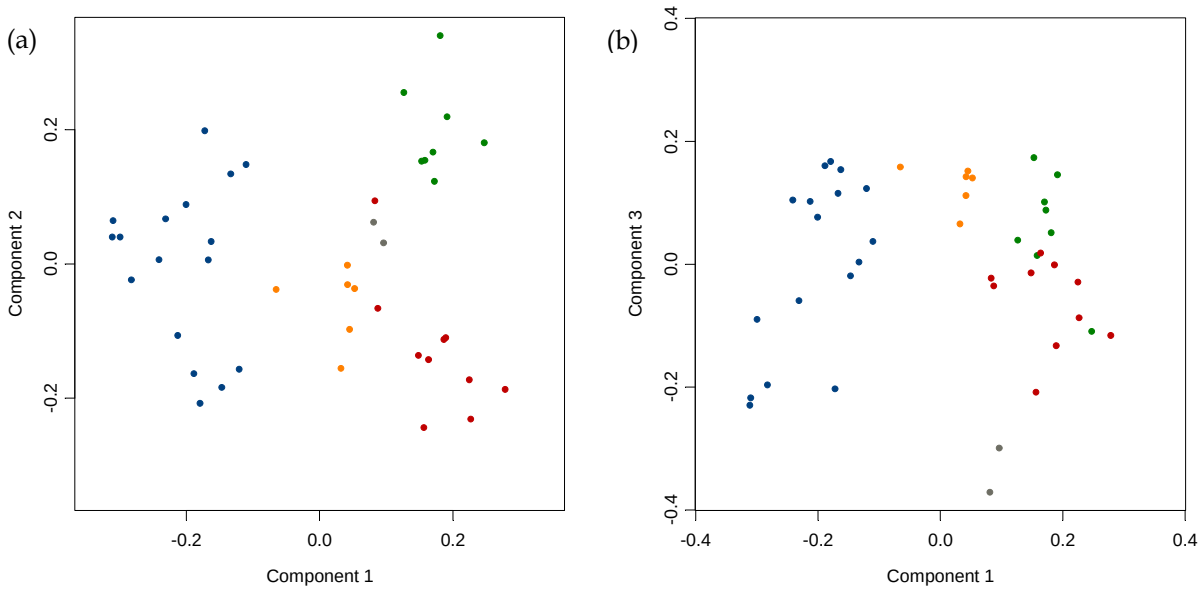


Figure 4.3: PCO plots of the European section *Pimpinellifoliae*. (a) the first two components; (b) the first and third component. The first three components explained 17%, 11%, and 11%, respectively, of the variation. Individuals are labelled with the country of origin (Belgium: ●; Germany: ●; France: ●; the Netherlands: ●; Denmark: ●).

Jaccard matrix

The two Danish populations, S058 and S059, showed a low similarity towards the other European *R. spinosissima* populations (Table 4.6). Among the other populations, the similarity appeared to be comparable.

Table 4.6: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of *R. spinosissima*. Population codes (POP) explained in Table 4.7, the most distinct populations are indicated in bold.

POP	B034	B043	D062	D064	F037	F038	F039	F040	F041	F042	N016	N032	N048	N066	S058	S059
B034	0.62															
B043	0.51	0.57														
D062	0.36	0.36	0.53													
D064	0.46	0.47	0.50	0.77												
F037	0.34	0.34	0.36	0.36	1.00											
F038	0.46	0.46	0.47	0.50	0.53	1.00										
F039	0.45	0.41	0.33	0.47	0.33	0.46	1.00									
F040	0.43	0.44	0.42	0.45	0.49	0.78	0.38	1.00								
F041	0.43	0.43	0.44	0.47	0.44	0.57	0.43	0.54	0.47							
F042	0.46	0.45	0.44	0.50	0.44	0.56	0.44	0.53	0.53	0.58						
N016	0.48	0.41	0.45	0.57	0.33	0.45	0.43	0.43	0.42	0.48	0.86					
N032	0.45	0.44	0.40	0.50	0.29	0.41	0.44	0.38	0.40	0.44	0.56	0.63				
N048	0.42	0.40	0.43	0.49	0.37	0.47	0.40	0.39	0.42	0.45	0.57	0.50	0.67			
N066	0.36	0.33	0.39	0.39	0.31	0.36	0.33	0.34	0.35	0.37	0.48	0.47	0.43	0.54		
S058	**0.31**	**0.35**	**0.31**	**0.37**	**0.32**	**0.36**	**0.35**	**0.33**	**0.34**	**0.31**	**0.34**	**0.35**	**0.32**	**0.25**	1.00	
S059	**0.28**	**0.33**	**0.31**	**0.34**	**0.30**	**0.35**	**0.34**	**0.30**	**0.30**	**0.28**	**0.28**	**0.36**	**0.28**	**0.25**	**0.72**	1.00

Dendrogram

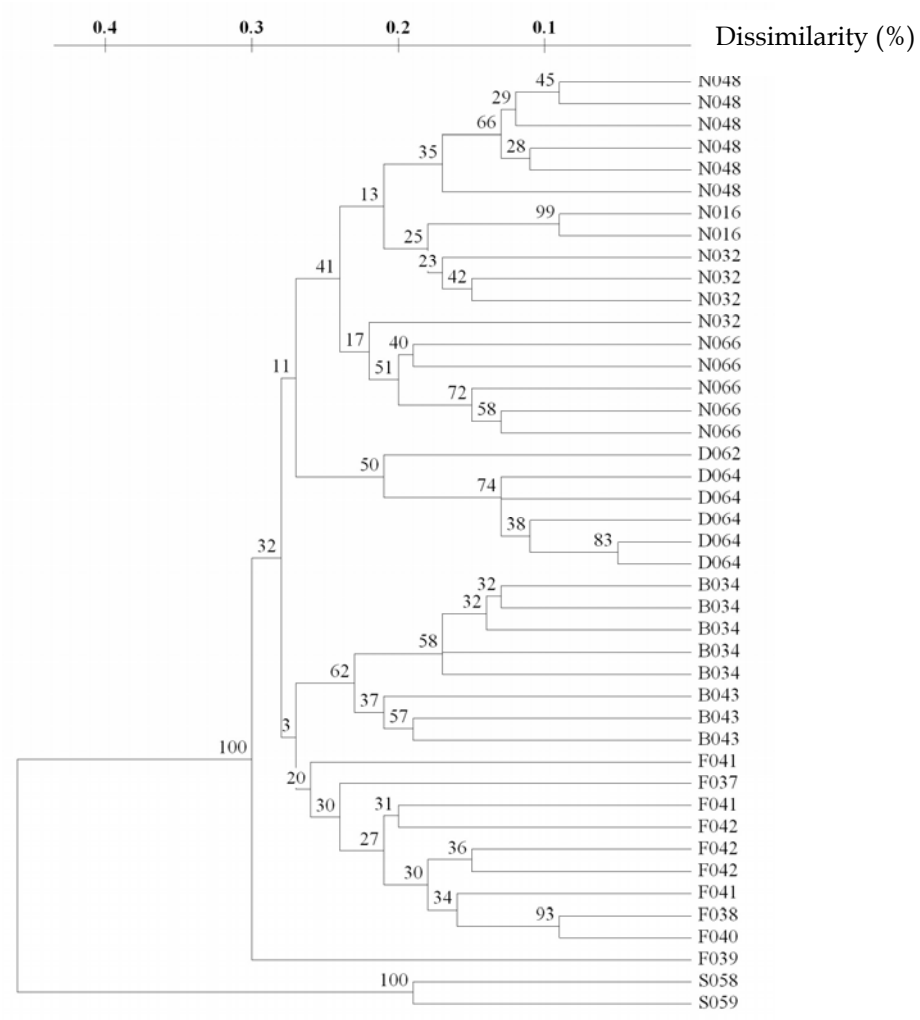


Figure 4.4: UPGMA cluster dendrogram of the sampled *R. spinosissima* individuals. The distance scale is indicated, individuals are labelled with population codes (Table 4.7).

The Danish populations, S058 and S059, differentiated the most from their congeners sampled in the other countries (Figure 4.4). For the other populations, the general pattern is a clustering according to the country of origin. The higher similarity suggested between the Dutch and German populations on the one hand and the Belgian and the French on the other is unexpected given the geographical proximity of the Belgian and the Dutch populations. Moreover this similarity was not well-supported by bootstrap analyses. Within each country cluster, locality patterns were observed.

Structure

Based on mean DeltaK, the optimal number of subclusters for the section *Pimpinellifoliae* could not be determined (Table 4.7). Either one or two gene pools were possible. Assuming that the sampled *R. spinosissima* populations could be assigned to two gene pools, gene pool 1 contained all Belgian, German, French and Dutch populations, while gene pool 2 consisted only of the two Danish populations.

Table 4.7: Population distribution of *R. spinosissima* to each of the inferred gene pools. Population codes (POP), region and locality, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are indicated in bold.

POP	REGION	LOCALITY	GP1	GP2	IND
B034	Westkust	Oostvoornduinen	**1.00**	0.00	5
B043	Kust	Middelkerke	**1.00**	0.00	3
D062	Baden-Wuerttemberg	Böllat	**1.00**	0.00	1
D064	R-P	Starkenburg, Mosel	**1.00**	0.00	5
F037	Hautes Alpes	CBNA	**1.00**	0.00	1
F038	Hautes Alpes	CBNA	**1.00**	0.00	1
F039	Hautes Alpes	CBNA	**1.00**	0.00	1
F040	Hautes Alpes	CBNA	**1.00**	0.00	1
F041	Hautes Alpes	Les Lunels	**1.00**	0.00	3
F042	Hautes Alpes	Col de Gleize	**1.00**	0.00	3
N016	Waddendistrict	Bospad, Schiermonnikoog	**1.00**	0.00	2
N032	Renodunaal district	Meijendel, Wassenaar	**1.00**	0.00	4
N048	Estuariëndistrict	Heveringen, Westvoorne	**1.00**	0.00	6
N066	Renodunaal district	Kokkendal/ Zuider Achterveld, Bergen	**1.00**	0.00	6
S058	Denmark, Jutland	Römö	0.00	**1.00**	1
S059	Denmark, Jutland	Sternbjerg	0.00	**1.00**	1

The European *R. spinosissima* populations showed strong geographical genetic differentiation.

The Danish populations were clearly the most distinct, but each of the two localities was only represented by one individual. The subdivision of the remaining countries in two larger groups was not well-supported; at a lower level locality patterns were observed.

4.2.3. The section *Rosa*

R. gallica, representing the European section *Rosa*, was sampled in Germany and France. In total, 36 individuals were scored for 137 AFLP markers. One German and eight French populations were defined based on the locality (Figure A.20b).

PCO

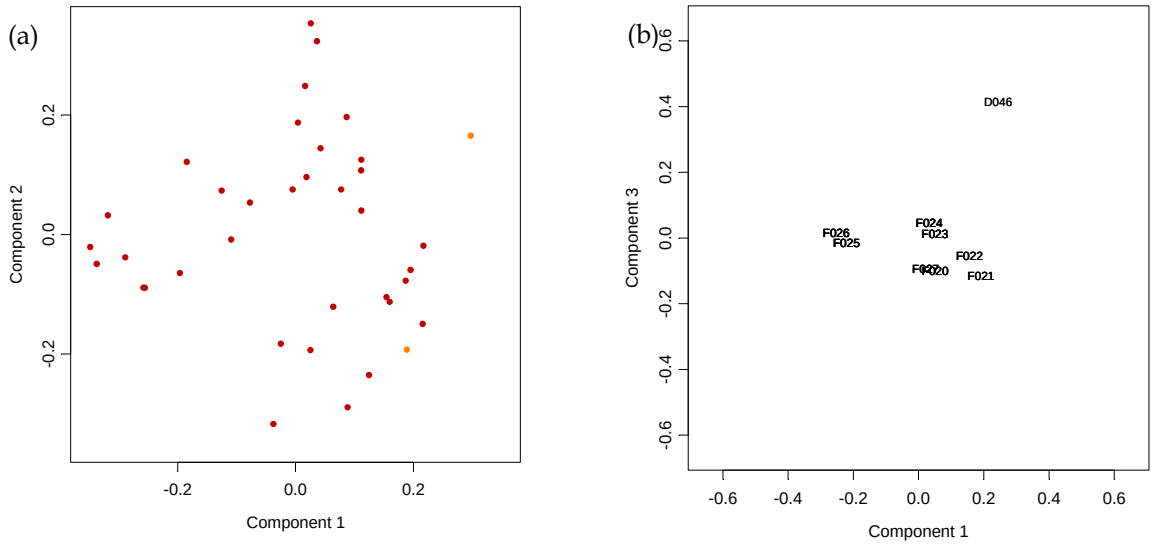


Figure 4.5: PCO plots of the European section *Rosa (R. gallica)*. The first three components explained 15%, 13%, and 11%, respectively, of the variation present. (a) Individuals labelled according to the country of origin (Germany: ●; France: ●); (b) indication of population mean (used population codes: Table 4.9).

The first two components of the section *Rosa (R. gallica)* explained 28% of the variation (Figure 4.5). In the third component, the German population separated clearly from the French populations.

Jaccard similarity

Table 4.8: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of *R. gallica*. Population codes (POP) explained in table 4.9; the most distinct population is indicated in bold, the most similar populations are marked in bold-Italics.

POP	D046	F020	F021	F022	F023	F024	F025	F026	F027
D046	0.51								
F020	**0.34**	0.82							
F021	**0.38**	0.69	0.80						
F022	**0.36**	0.69	0.68	0.72					
F023	**0.34**	0.64	0.66	0.65	0.69				
F024	**0.34**	0.65	0.64	0.63	0.67	0.70			
F025	**0.33**	0.67	0.65	0.63	0.63	0.65	0.75		
F026	**0.33**	0.65	0.63	0.62	0.65	0.65	***0.73***	0.77	
F027	**0.37**	0.63	0.67	0.64	0.68	0.66	0.67	0.69	0.82

The Jaccard similarity coefficients distinguished two groups in *R. gallica*: the German versus the French populations (Table 4.8). Within the French populations, F025 and F026 appeared to be more similar compared to the other populations.

Dendrogram

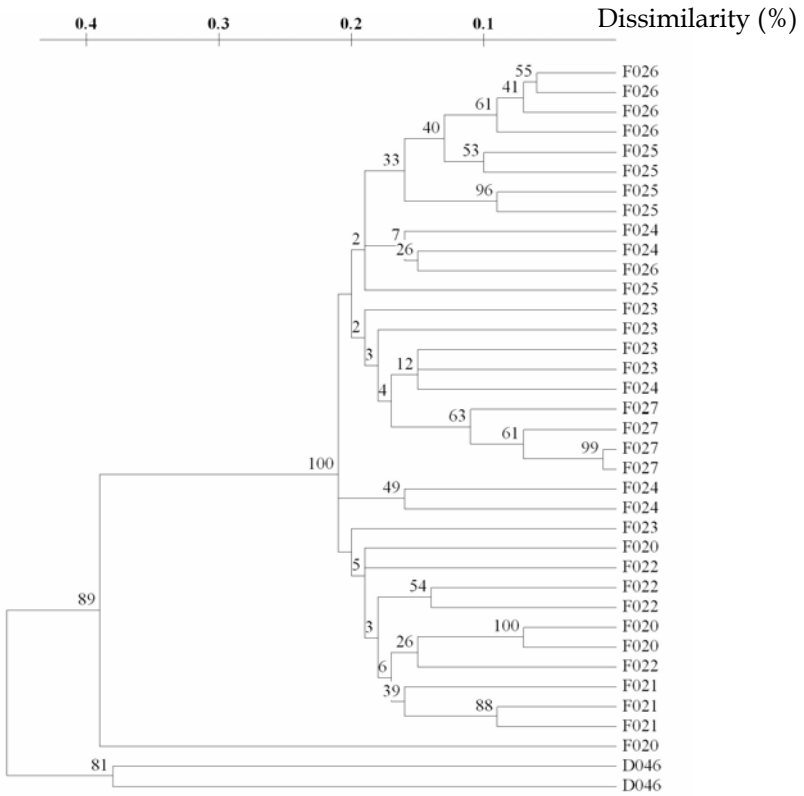


Figure 4.6: UPGMA cluster dendrogram of the sampled *R. gallica*. The distance scale is indicated, individuals are labelled with population codes (Table 4.9).

The German population was clearly differentiated from the eight French populations (Figure 4.6).

Structure

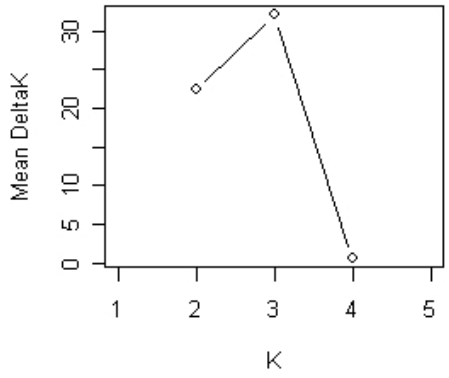


Figure 4.7: Assumption of the optimal number of gene pools present in the section *Rosa* (*R. gallica*), based on Structure (Pritchard *et al.* 2000) and adapted with the method of Evanno *et al.* (2005).

Table 4.9: Population distribution of section *Rosa* (*R. gallica*) to each of the inferred gene pools. Population codes (POP), region and locality, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	LOCALITY	GP1	GP2	GP3	IND
D046	Baden-Württemberg	Wendelsheim	0.00	0.00	**1.00**	2
F020	Hautes Alpes	Les Blayes	**1.00**	0.00	0.00	3
F021	Var	Grime	**1.00**	0.00	0.00	3
F022	Hautes Alpes	La Garenne-Trescléoux	**1.00**	0.00	0.00	4
F023	Hautes Alpes	La Grande Ste-Anne	**1.00**	0.00	0.00	5
F024	Hautes Alpes	Rosans	**1.00**	0.00	0.00	5
F025	Alpes maritimes	Saint-Antonin	0.19	**0.81**	0.00	5
F026	Alpes maritimes	Sigale	0.28	**0.72**	0.00	5
F027	Var	Le Val	**1.00**	0.00	0.00	4

Based on the mean DeltaK (Figure 4.7), the individuals of the section *Rosa (R. gallica)* were assigned to three gene pools (Table 4.9). Gene pool 1 consisted of six of the French populations, and between 19% and 28% of the two remaining French populations, F025 and F026, respectively. Gene pool 2 was characterised by the majority of these two French populations F025 (81%) and F026 (72%), both originating from Alpes maritimes. Gene pool 3 contained the only sampled population of Germany.

Within the European *R. gallica*, a clear geographical pattern was observed. Most of the differentiation occurred among individuals from different countries of origin, Germany versus France. Moreover, the populations sampled at Alpes maritimes were more similar to each other than to the other French populations.

4.2.4. The section *Cinnamomeae*

From this section, two species were analysed, *R. majalis* originating from Germany and Sweden, and *R. pendulina* sampled in Germany and France. In total, 32 individuals were analysed, grouped in 10 populations (Figure A.20a).

PCO

The three major components represented 32%, 13%, and 10% of the variation (Figure 4.8). The two analysed species of the section *Cinnamomeae*, *R. majalis* and *R. pendulina*, were separated along the first component. However, one German population of *R. majalis* (D025) grouped together with *R. pendulina* on the right side of the biplot. Along the first component, the French *R. pendulina* populations were split from their German congeners. Along the second component, a similar geographical differentiation was found between the Swedish and German *R. majalis* populations (not taking population D025 into account).

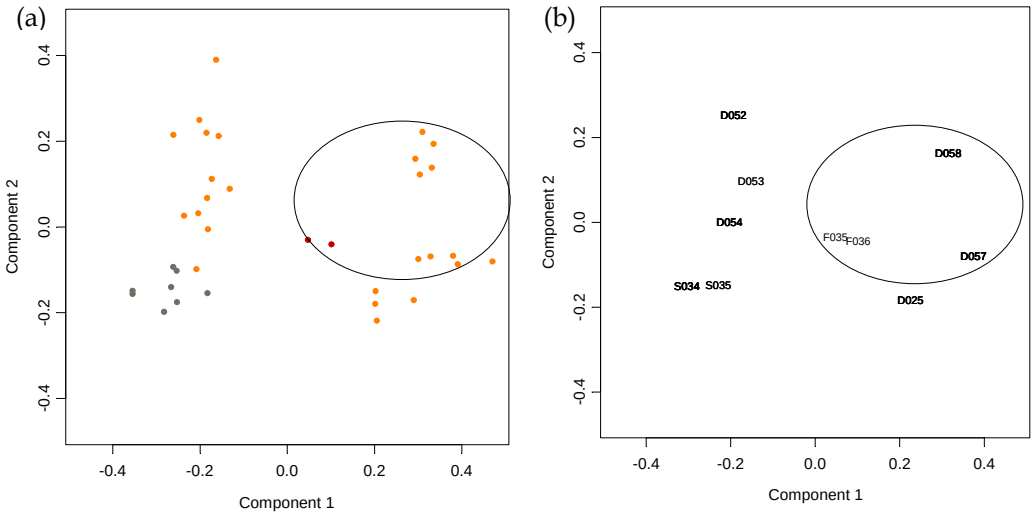


Figure 4.8: The first two principal components of the section *Cinnamomeae*. The three major components represented 33%, 11%, and 10% of the variation. *R. pendulina* indicated with circle. (a) Individuals labelled with country of origin (Germany: ●; France: ●; Sweden: ●); (b) indication of population mean. Population codes explained in table 4.11.

Jaccard matrix

Table 4.10: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of the section *Cinnamomeae*. Population codes (POP) explained in table 4.11; the most distinct population is indicated in bold.

TAXON	POP	D025	D052	D053	D054	D057	D058	F035	F036	S034	S035
R. majalis	D025	0.62									
R. majalis	D052	**0.32**	0.69								
R. majalis	D053	**0.36**	0.56	0.59							
R. majalis	D054	**0.34**	0.51	0.54	0.70						
R. pendulina	D057	**0.39**	0.32	0.36	0.36	0.73					
R. pendulina	D058	**0.40**	0.36	0.39	0.37	0.47	0.77				
R. pendulina	F035	**0.36**	0.42	0.40	0.39	0.42	0.41	1.00			
R. pendulina	F036	**0.40**	0.39	0.39	0.36	0.39	0.41	0.52	1.00		
R. majalis	S034	**0.31**	0.47	0.51	0.50	0.27	0.30	0.42	0.39	0.66	
R. majalis	S035	**0.33**	0.46	0.50	0.52	0.33	0.34	0.41	0.36	0.62	0.61

The German population D025 was the most differentiated of the sampled populations (Table 4.10). Within the section *Cinnamomeae*, the two species were separated. Differentiation among populations within species was comparable.

Dendrogram

Apart from population D025, the section *Cinnamomeae* was subdivided into four main clusters: *R. majalis* and *R. pendulina* each grouped in a taxon cluster (Figure 4.9). In addition to the species differentiation, also within-species variation was found. The Swedish *R. majalis* individuals divided from the German congeners, the *R. pendulina* populations originating from France and Germany were split from each other.

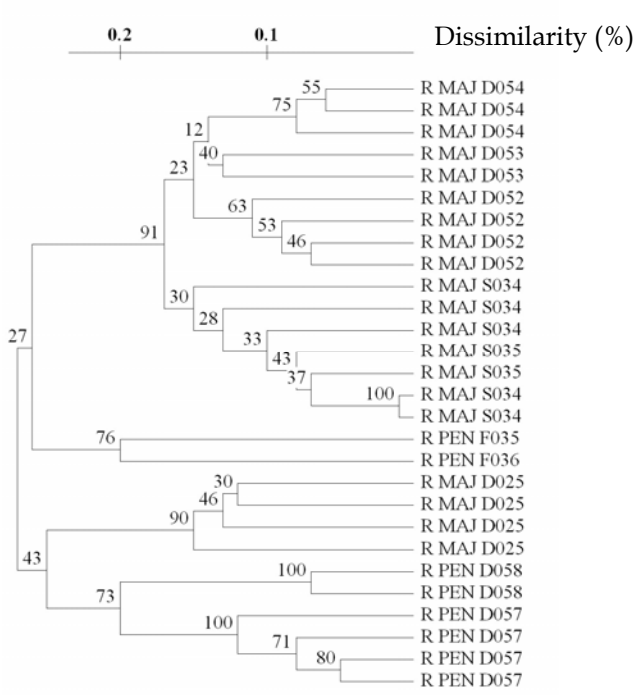


Figure 4.9: UPGMA cluster dendrogram of the section *Cinnamomeae* individuals. The distance scale is indicated, individuals are labelled with population codes (Table 4.11).

Structure

Based on mean DeltaK, the optimal number of subclusters for the section *Cinnamomeae* could not be determined. Either one or two gene pools were possible (Table 4.11). Assuming two gene pools, the assignment of the individuals was as follows:

Gene pool 1 consisted of all *R. pendulina* populations, sampled in Germany and France, and one *R. majalis* population, D025.

Gene pool 2 contained all *R. majalis* populations irrespective of their country of origin, except for D025.

Table 4.11: Population assignment of the section *Cinnamomeae* (*R. majalis* and *R. pendulina*) to each of the inferred gene pools. Population codes (POP), region and locality, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

TAXON	POP	REGION	LOCALITY	GP1	GP2	IND
R. majalis	D025	S-H Coast	Geltinger Birk	**1.00**	0.00	4
R. majalis	D052	Saksen-Anhalt	Kalktal, Kyff	0.00	**1.00**	5
R. majalis	D053	Saksen-Anhalt	Klocksberg, Kyff	0.00	**1.00**	2
R. majalis	D054	Baden-Wuerttemberg	Schlatt, Hechingen	0.00	**1.00**	3
R. majalis	S034	Umeå	Mårdsele	0.00	**1.00**	5
R. majalis	S035	Umeå	Brännland	0.00	**1.00**	3
R. pendulina	D057	Baden-Wuerttemberg	Dürrenwald	**1.00**	0.00	4
R pendulina	D058	Baden-Wuerttemberg	Dreifaltigkeitsberg	**1.00**	0.00	4
R. pendulina	F035	Hautes Alpes	CBNA	**1.00**	0.00	1
R. pendulina	F036	Hautes Alpes	CBNA	**1.00**	0.00	1

R. majalis and *R. pendulina,* two species of the section *Cinnamomeae* were analysed. Apart from the population D025 which proved to be an outlier in all analyses, the AFLP polymorphisms clearly differentiated between the two species. Based on the morphology, the deviation population was a *R. majalis* population. However, as these individuals were part of a hedge around a parking place, they were planted for ornamental purposes. Most likely, they might be cryptic hybrids of totally unknown origin. Therefore, their deviating position among the other populations is not taken in account.

Intraspecific variation was found between countries and between populations within countries.

4.2.5. The section *Synstylae*

Two European endemic species of the section *Synstylae* were investigated, *R. arvensis* and *R. sempervirens*. *R. arvensis* is exclusively diploid and has Mendelian meiosis. The ploidy level of *R. sempervirens* might vary from 2x to 4x (Kurtto *et al.* 2004). In total, 56 individuals, originating from thirteen *R. arvensis* populations (Figure A.21a), and four *R. sempervirens* populations were analysed.

PCO

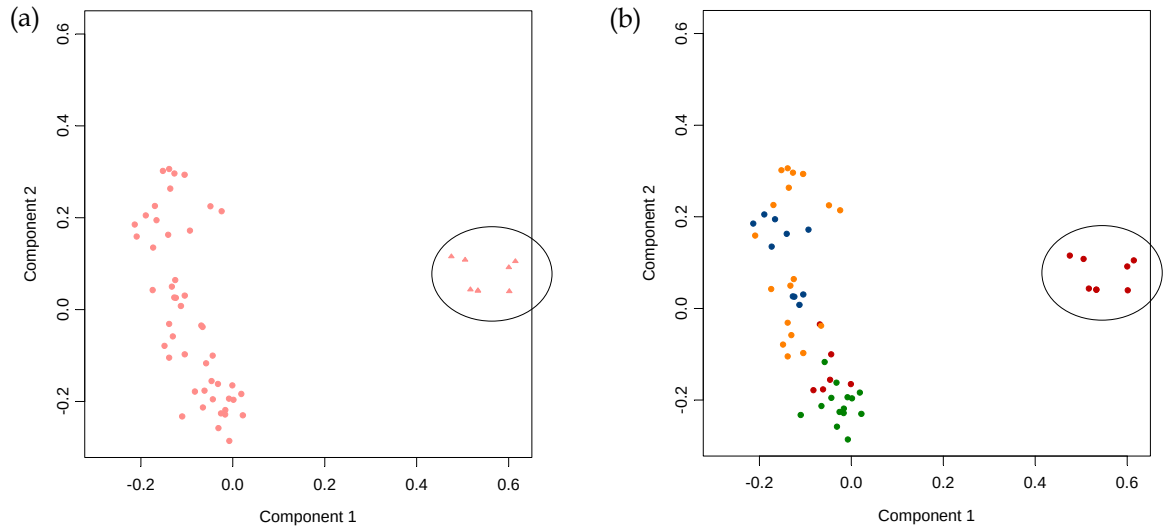


Figure 4.10: PCO plots of the European *R. arvensis* and *R. sempervirens* (section *Synstylae*). The three major components represented 32%, 18%, and 7% of the variation. *R. sempervirens* indicated with circle. (a) Individuals labelled with species codes (*R. arvensis*: ●; *R. sempervirens*: ▲); (b) individuals labelled with country codes (Belgium: ●; Germany: ●; France: ●; The Netherlands: ●).

Along the first component, a clear partition was present between the *R. sempervirens* and *R. arvensis* populations (Figure 4.10). *R. sempervirens* was only sampled in France and all the individuals grouped together in a compact cluster. Within *R. arvensis*, a differentiation was found based on the country of origin. The German and Dutch populations were clustered in the upper part of the *R. arvensis*

group, while the French and the Belgian were grouped in the lower part. Both subgroups showed overlap.

Jaccard matrix

Table 4.12: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of section *Synstylae*, *R. arvensis* and *R. sempervirens*. Population codes (Table 4.13); the most distinct populations are indicated in bold.

SPECIES	POP	B016	B017	B019	B005	D018	D022	D044	D045	F005	F050	F051	F052	F053	F006	N034	N041	N081
R. arvensis	B016	1.00																
R. arvensis	B017	0.70	0.77															
R. arvensis	B019	0.68	0.66	0.76														
R. arvensis	B005	0.65	0.61	0.62	0.59													
R. arvensis	D018	0.46	0.46	0.45	0.40	0.63												
R. arvensis	D022	0.46	0.47	0.47	0.43	0.50	0.62											
R. arvensis	D034	0.62	0.59	0.58	0.54	0.53	0.53	0.70										
R. arvensis	D035	0.61	0.59	0.57	0.54	0.48	0.53	0.64	0.68									
R. arvensis	F005	0.68	0.65	0.65	0.59	0.47	0.50	0.62	0.62	0.77								
R. sempervirens	F050	**0.34**	**0.33**	**0.32**	**0.31**	**0.32**	**0.26**	**0.31**	**0.31**	**0.34**	1.00							
R. sempervirens	F051	**0.46**	**0.43**	**0.39**	**0.36**	**0.39**	**0.31**	**0.39**	**0.37**	**0.42**	0.65	1.00						
R. sempervirens	F052	**0.39**	**0.34**	**0.32**	**0.32**	**0.30**	**0.24**	**0.31**	**0.31**	**0.34**	0.56	0.64	0.85					
R. sempervirens	F053	**0.47**	**0.41**	**0.38**	**0.36**	**0.35**	**0.27**	**0.36**	**0.36**	**0.39**	0.57	0.68	0.66	0.87				
R. arvensis	F006	0.51	0.48	0.50	0.46	0.48	0.45	0.49	0.51	0.58	**0.28**	**0.34**	**0.27**	**0.31**	1.00			
R. arvensis	N034	0.58	0.54	0.54	0.50	0.56	0.52	0.64	0.53	0.56	**0.33**	**0.39**	**0.29**	**0.33**	0.49	0.74		
R. arvensis	N041	0.61	0.60	0.59	0.53	0.57	0.54	0.69	0.61	0.60	**0.32**	**0.42**	**0.33**	**0.38**	0.48	0.65	0.74	
R. arvensis	N081	0.47	0.48	0.44	0.40	0.48	0.43	0.53	0.49	0.47	**0.27**	**0.34**	**0.27**	**0.32**	0.38	0.52	0.55	0.59

The species *R. arvensis* and *R. sempervirens* clearly showed a lower similarity coefficient towards each other (Table 4.12). The similarity coefficients within species and between countries did not show clear differences.

Dendrogram

The main subdivision in the tree was based on the species determination: *R. sempervirens* versus *R. arvensis* populations (Figure 4.11). Within the *R. arvensis* cluster, the Dutch and German populations clustered together in the upper part, while the Belgian and French populations clustered in the lower part.

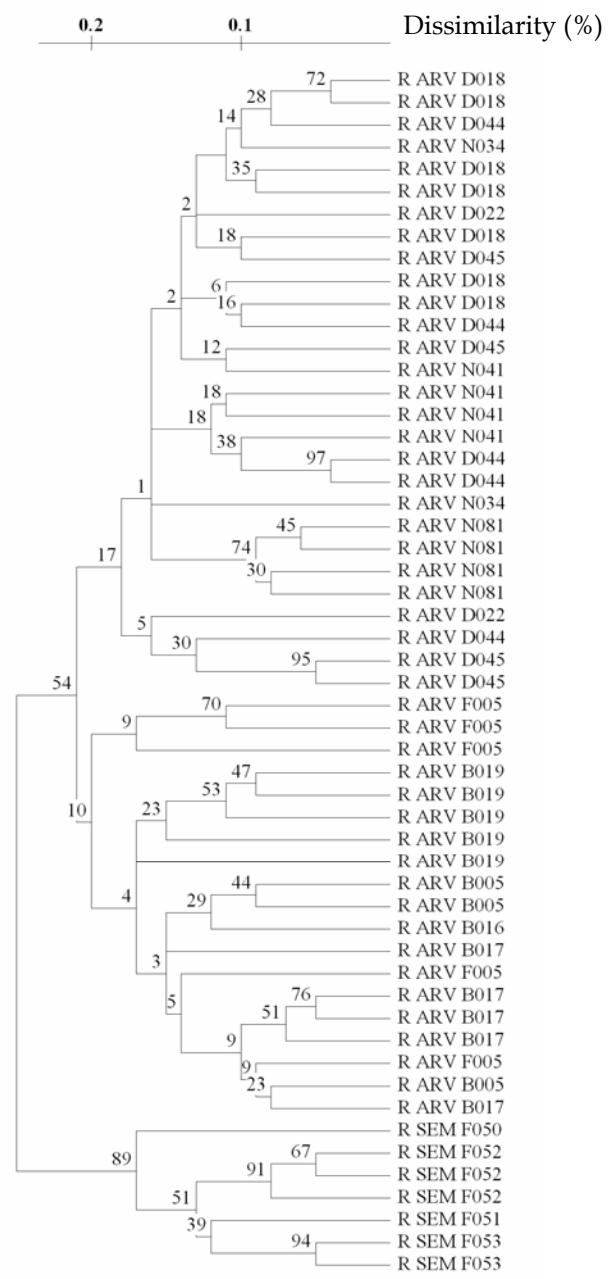


Figure 4.11: UPGMA cluster dendrogram of section *Synstylae*. The distance scale is indicated, individuals are labelled with species and population codes (Table 4.13).

Structure

Based on mean DeltaK, the optimal number of subclusters for this section could not be determined. Either one or two gene pools were possible (Table 4.13). Assuming two gene pools, the assignment of the individuals was mainly based on the species determination. Gene pool 1 consisted of all the *R. arvensis* populations, irrespective of their country of origin. Only the Dutch population, N081, was assigned for only 80%, the other populations were assigned for 100%. Gene pool 2 contained the four *R. sempervirens* populations and the remaining 20% of the Dutch *R. arvensis* population, N081.

Table 4.13: Population distribution of the section *Synstylae*, *R. arvensis* and *R. sempervirens*, to each of the inferred gene pools. Population codes (POP), region and locality, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	LOCALITY	GP1	GP2	IND
R. arvensis					
B005	Brabants District West	Brakel	**1.00**	0.00	3
B016	West-Vlaams Heuvelland	Belle	**1.00**	0.00	1
B017	West Vlaams Heuvelland	Kemmel	**1.00**	0.00	5
B019	West Vlaams Heuvelland	Ploegsteert	**1.00**	0.00	4
D018	Baden-Wuerttemberg	Eichelberg	**1.00**	0.00	3
D022	Baden-Wuerttemberg	Tief. Kreuzbergweg	**1.00**	0.00	2
D034	Baden-Wuerttemberg	Wendelsheim	**1.00**	0.00	5
D035	Baden-Wuerttemberg	Seebronn	**1.00**	0.00	4
F005	Hautes Alpes	La Garenne-Trescléoux	**1.00**	0.00	4
F006	Hautes Alpes	Rosans	**1.00**	0.00	1
N034	Subcentreuroop district	Doort, Echt	**1.00**	0.00	2
N041	Zuidlimburgs district	Gerendal	**1.00**	0.00	4
N081	Zuidlimburgs district	Onderste Bosch, Epen	**0.80**	0.20	5
R. sempervirens					
F050	Hautes Alpes	CBNA	0.00	**1.00**	1
F051	Alpes maritimes	Pierrefeu-La Colette	0.00	**1.00**	1
F052	Alpes maritimes	Pierrefeu-La Colette	0.00	**1.00**	3
F053	Alpes maritimes	Pierrefeu-La Colette	0.00	**1.00**	3

One of the restrictions of the program Structure (Pritchard *et al.*, 2000) is the assignment of the individuals in gene pools at the highest hierarchical level. Excluding *R. sempervirens*, the assignment analysis was repeated in order to assess possible gene pools within the European *R. arvensis* populations.

Table 4.14: Population distribution of *R. arvensis* to each of the inferred gene pools. Population codes (POP), region and locality, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	LOCALITY	GP1	GP2	IND
B005	Brabants District West	Brakel	0.00	**1.00**	3
B016	West-Vlaams Heuvelland	Belle	0.00	**1.00**	1
B017	West Vlaams Heuvelland	Kemmel	0.00	**1.00**	5
B019	West Vlaams Heuvelland	Ploegsteert	0.00	**1.00**	4
D018	Baden-Wuerttemberg	Eichelberg	**1.00**	0.00	3
D022	Baden-Wuerttemberg	Tief. Kreuzbergweg	**1.00**	0.00	2
D034	Baden-Wuerttemberg	Wendelsheim	**1.00**	0.00	5
D035	Baden-Wuerttemberg	Seebronn	**1.00**	0.00	4
F005	Hautes Alpes	La Garenne-Trescléoux	0.00	**1.00**	4
F006	Hautes Alpes	Rosans	0.00	**1.00**	1
N034	Subcentreuroop district	Doort, Echt	**1.00**	0.00	2
N041	Zuidlimburgs district	Gerendal, Valkenburg a/d Geul	**1.00**	0.00	4
N081	Zuidlimburgs district	Onderste Bosch, Epen	**1.00**	0.00	5

Based on mean DeltaK, the optimal number of subclusters for *R. arvensis* could not be determined. Either one or two gene pools were possible. Assuming two gene pools, the assignment of the individuals was as indicated in table 4.14. The first gene

pool contained the three German and three Dutch *R. arvensis* populations, while the second gene pool consisted of the four Belgian and the two French populations.

The two species in section *Synstylae*: *R. arvensis* and *R. sempervirens* were strongly differentiated.

Within *R. arvensis,* a geographical pattern was detected. Populations from Belgium and France showed a higher similarity, while the Dutch and German populations also clustered more closely together.

4.2.6. The section *Caninae*

PCO

The most differentiated and compact cluster in the subgenus *Rosa* (Figure 4.1) consisted of the polymorphic section *Caninae*. All the individuals of the section *Caninae* are polyploid, mostly pentaploid and follow the unique and heterogamous canina meiosis (§2.2.2.).

In the PCO analysis restricted to the section *Caninae*, the subsection *Rubigineae* was split off from a large and loose cluster consisting of subsections *Vestitae*, *Tomentellae*, and *Caninae*; however the two clusters showed overlap. The first two principal components explained 33% of the variation (Table 4.15, Figure 4.12a). The subsections *Rubrifoliae* and *Trachyphyllae* were represented by too few samples, so no conclusions could be drawn concerning their position within the section *Caninae*.

The PCO analysis was repeated, excluding individuals belonging to the subsection *Rubigineae*. The outcome showed a subdivision of the subsection *Vestitae* from the subsections *Caninae* and *Tomentellae* (Figure 4.12b, Table 4.15). Similar procedure was repeated without the individuals of the *Vestitae*. However, no differentiation was observed between the individuals of the subsections *Caninae* and *Tomentellae* (Figure 4.12c, Table 4.15).

Table 4.15: Principal components of the PCO analyses of the whole section *Caninae* and of the analyses of three and two subsections, respectively. The number of analysed individuals (#IND) for each subset and the percentage of variance explained by the first three components (COMP) are indicated.

ANALYSED TAXA	# IND	COMP 1	COMP 2	COMP 3
Section *Caninae*	731	18%	15%	8%
Subsections *Vestitae*, *Caninae* and *Tomentellae*	529	15%	11%	8%
Subsections *Caninae* and *Tomentellae*	397	17%	9%	8%

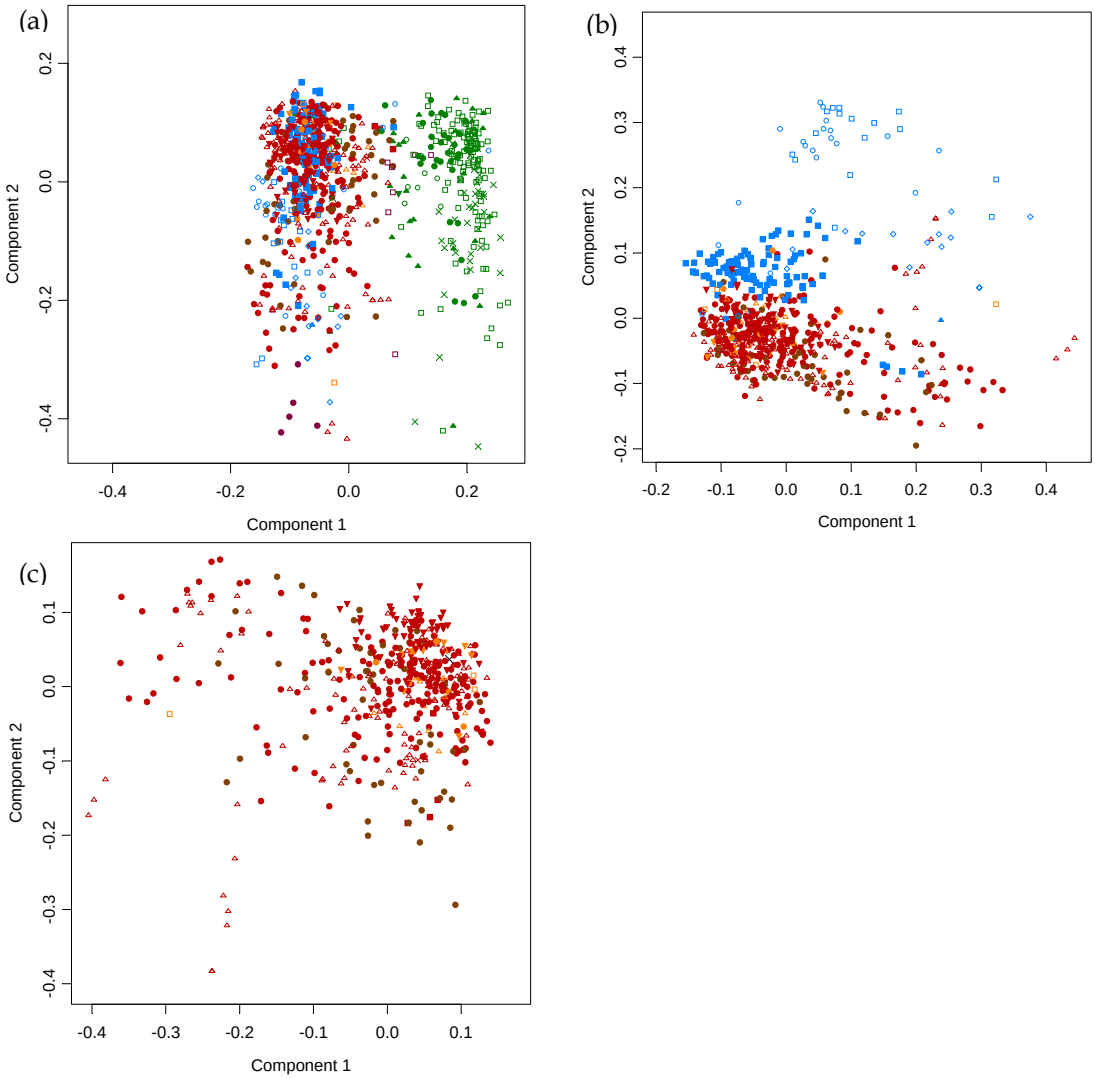


Figure 4.12: PCO plot of the European section *Caninae*. (a) PCO plot based on the subsections: *Rubigineae* (Green), *Vestitae* (Blue), *Tomentellae* (Brown) and *Caninae* (Red, Orange); (b) PCO plot based on the subsections *Vestitae*, *Tomentellae* and *Caninae*; (c) PCO plot based on the subsections *Tomentellae* and *Caninae*. Individuals were labelled with species determination (Table 4.2).

Jaccard matrix

Table 4.16: Mean Jaccard similarity coefficients (%) calculated within and between the sampled subsections of the section *Caninae*. The high similarities between the subsections *Caninae* and *Tomentellae* are indicated in bold.

SUBSECTION	*CANINAE*	*RUBIGINEAE*	*RUBRIFOLIAE*	*TOMENTELLAE*	*TRACHYPHYLLAE*	*VESTITAE*
Caninae	**0.65**					
Rubigineae	0.57	0.67				
Rubrifoliae	0.50	0.53	0.68			
Tomentellae	**0.64**	0.57	0.48	**0.66**		
Trachyphyllae	0.44	0.38	0.35	0.46	0.79	
Vestitae	0.61	0.55	0.52	0.60	0.40	0.65

The mean Jaccard similarities between the subsections of the section *Caninae* indicated that the subsections *Rubigineae*, *Rubrifoliae*, and *Trachyphyllae* showed the

largest differentiation among each other and towards the other three *Caninae* subsections (Table 4.16). In contrast, the similarity between the subsections *Caninae* and *Tomentellae* was high and equalled 64%. The similarity within these two subsections equalled 65% and 66%, respectively.

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals in order to improve the clarity of the dendrogram (Figure 4.13).

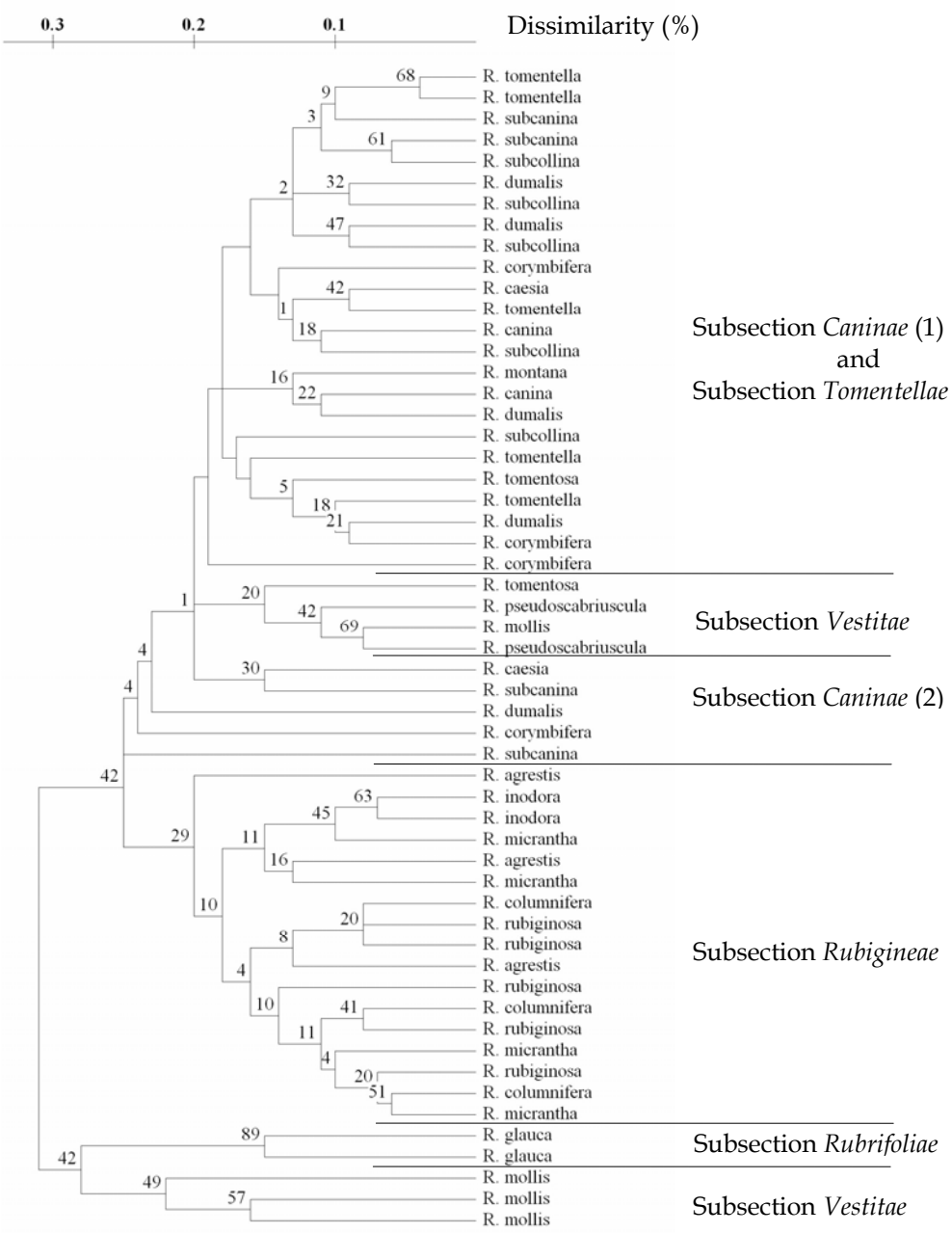


Figure 4.13: UPGMA cluster dendrogram of the section *Caninae*. The distance scale is indicated and individuals are labelled with species name and subdivided in subsections.

The two taxa *R. mollis* (three of the four analysed individuals) and *R. glauca* (subsection *Rubrifoliae*) split off first, followed by the individuals of the subsection *Rubigineae*. Within the remaining cluster, the spare individuals of the subsection *Vestitae* grouped together and were placed in-between two clusters formed by the subsections *Caninae* and *Tomentellae*. In both clusters the individuals of subsections *Caninae* and *Tomentellae* were completely mingled.

Structure

Table 4.17: Species assignment of the European section *Caninae* to each of the inferred gene pools. Subsection and species determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

SUBSECTION	TAXON	GP1	GP2	IND
Rubrifoliae	*R. glauca*	**1.00**	0.00	6
Rubigineae	*R. rubiginosa*	**0.97**	0.03	111
	R. micrantha	**0.92**	0.08	25
	R. elliptica	**1.00**	0.00	1
	R. agrestis	**1.00**	0.00	25
	R. inodora	**1.00**	0.00	7
Vestitae	*R. tomentosa*	0.01	**0.99**	93
	R. pseudoscabriuscula	0.00	**1.00**	5
	R. sherardii	0.00	**1.00**	10
	R. mollis	0.27	**0.73**	15
	R. villosa	0.00	**1.00**	14
Tomentellae	*R. balsamica*	0.00	**1.00**	44
Caninae	*R. canina*	0.02	**0.98**	145
	R. corymbifera	0.03	**0.97**	95
	R. dumalis	0.00	**1.00**	82
	R. caesia	0.00	**1.00**	7
	R. subcanina	0.00	**1.00**	7
	R. subcollina	0.00	**1.00**	10
	R. montana	0.01	**0.99**	11
	R. stylosa	**1.00**	0.00	3
Hybrids	*R.* x *irregularis*	0.00	**1.00**	1
	R. henkeri-schulzei	**0.96**	0.04	27
	R. canina x *R. stylosa*	**0.77**	0.23	1

The method of Evanno *et al.* (2005) was not able to confirm an optimal number of clusters within the section *Caninae* (one or two gene pools). Assuming two gene pools within the European section *Caninae*, the subdivision of the individuals was given in table 4.17.

Gene pool 1 contained between 92 and 100% of all the taxa belonging to the subsection *Rubigineae* and the hybrid *R. henkeri-schulzei*. In addition, *R. stylosa* and the

hybrid *R. canina* x *R. stylosa* (only 77% of one individual) were also assigned to this gene pool. Moreover, the only representative of the subsection *Rubrifoliae*, *R. glauca*, appeared to be related to this gene pool.

Gene pool 2 contained all the taxa of the subsections *Vestitae*, *Tomentellae*, and *Caninae* with in addition the hybrid *R.* x *irregularis (*based on one individual*)*. Finally, also 23% of the only analysed *R. canina* x *R. stylosa* individual was assigned to this gene pool.

Analysis of samples only from section *Caninae* revealed a relatively detailed hierarchical structure of this section.

The taxonomical subdivision of section *Caninae* into subsections is confirmed to a certain extent. The low number of sampled individuals in subsections *Trachyphyllae* and *Rubrifoliae* did not allow any conclusions about their position in the section.

Of the remaining subsections, the subsection *Rubigineae* was the most distinctive, followed by the subsection *Vestitae*.

Some of the hybrids showed high similarity with the subsection *Rubigineae*. *R. henkeri-schulzei* is presumably a hybrid between *R. rubiginosa* and *R. micrantha*. The assignment of these taxa to the same gene pool confirmed the relatedness among them. In addition, also *R. stylosa* and the hybrid *R. canina* x *R. stylosa* (77% of the analysed genome) were assigned to the subsection *Rubigineae* gene pool. Nevertheless, in the taxonomical structure of Henker, they are both placed within the subsection *Caninae*.

4.2.7. The subsection *Rubrifoliae*

R. glauca is a rare taxon of the subsection *Rubrifoliae*. In total, seven individuals were sampled belonging to one Belgian, one German, and three French populations (Figure A.21b).

PCO

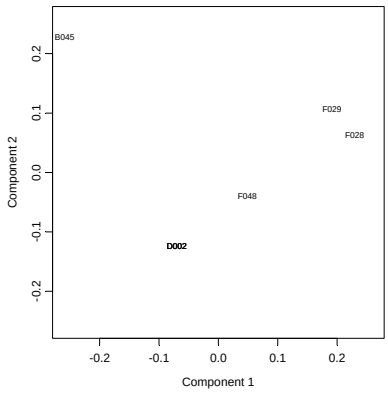


Figure 4.14: PCO plots of the European subsection *Rubrifoliae*. The first three components explained 50%, 23%, and 13%. Indication of the population mean labelled with population codes (Table 4.19).

Jaccard matrix

Table 4.18: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of the subsection *Rubrifoliae*. Population codes (POP) explained in table 4.19; the highest similarity is indicated in bold.

POP	B045	D002	F028	F029	F048
B045	1.00				
D002	0.59	0.87			
F028	0.48	0.63	1.00		
F029	0.53	0.64	**0.87**	1.00	
F048	0.55	0.73	0.69	0.69	1.00

A high similarity was indicated between the French populations, F028 and F029 (Table 4.18). The Belgian individual appeared to be the most distinguished from the sampled congeners.

Dendrogram

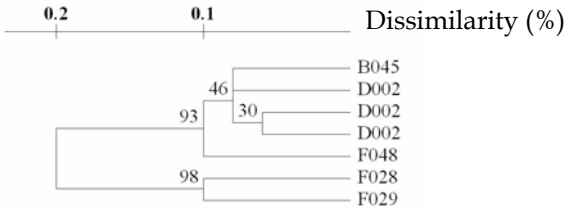


Figure 4.15: UPGMA cluster dendrogram of individuals of *R. glauca*, the subsection *Rubrifoliae*. The distance scale is indicated and individuals are labelled with population codes (Table 4.19).

The individuals were grouped according to their country or population of origin (Figure 4.15). The Belgian population contained only one individual and clustered with the German population. The two French populations F028 and F029 appeared to be very similar.

Structure

The outcome of the mean DeltaK calculation was not straightforward. Assuming that the optimal assignment was two gene pools, the assignment of the populations was summarised in table 4.19.

The two French populations, F028 and F029, were assigned to the same gene pool, while the other populations, sampled in Belgium, Germany and France, were assigned to the second gene pool.

Table 4.19: Population distribution of *R. glauca* (subsection *Rubrifoliae*) to each of the inferred gene pools. Population codes (POP), region and locality, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	LOCALITY	GP1	GP2	IND
B045	Westkust	Ter Yde	0.00	**1.00**	1
D002	S-H Coast	Hohwacht	0.00	**1.00**	3
F028	Hautes Alpes	CBNA	**1.00**	0.00	1
F029	Hautes Alpes	CBNA	**1.00**	0.00	1
F048	Hautes Alpes	CBNA	0.00	**1.00**	1

The low number of analysed individuals in the subsection *Rubrifoliae* precludes the drawing of conclusions about the hierarchical position in the section *Caninae*.

No clear within-species geographical pattern could be detected.

4.2.8. The subsection *Rubigineae*

The analysed taxa in subsection *Rubigineae* are *R. rubiginosa* (32 analysed populations), *R. micrantha* (7 pop), *R. inodora* (2 pop), *R. agrestis* (10 pop), *R. elliptica* (1 pop) ,and the presumed hybrid *R. henkeri-schulzei* (syn.: *R. columnifera*) (10 pops). In total, 191 individuals of this subsection were included.

PCO

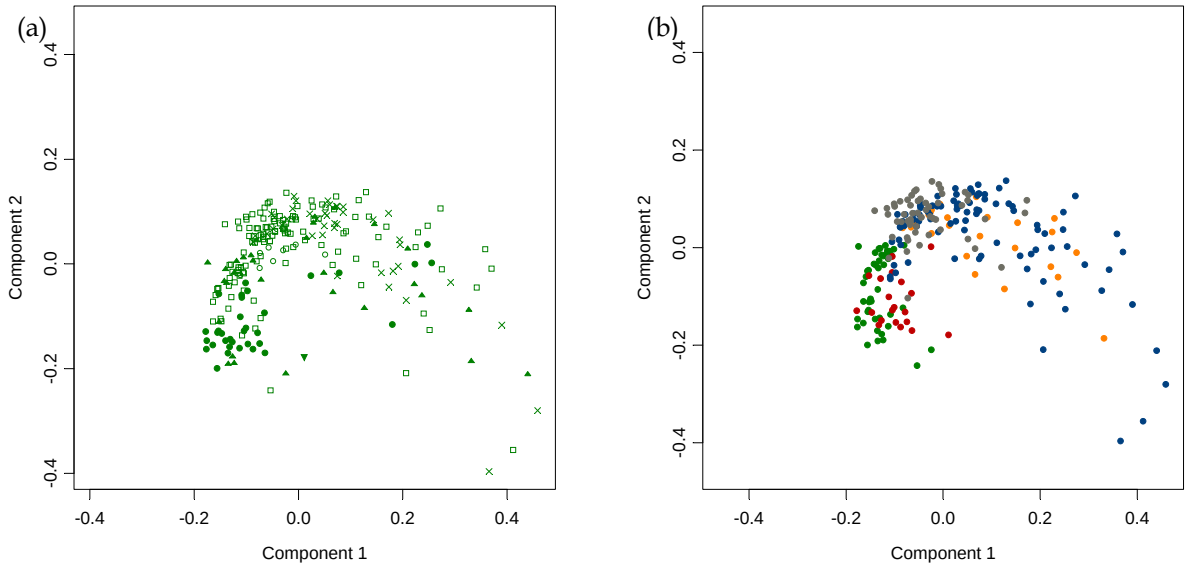


Figure 4.16: PCO plot of the subsection *Rubigineae*. The first two components explained 32% of the variation. (a) Individuals were labelled with species determination (*R. rubiginosa*: □; *R. micrantha*: ▲; *R. agrestis*: ●; *R. inodora*: ○; *R. elliptica*: ▼ and *R. henkeri-schulzei*: x); (b) Individuals were labelled with country of origin (Belgium: ●; The Netherlands: ●; France: ●; Germany: ●; The Scandinavian countries: ●).

The first three components explained 21%, 11%, and 9%, respectively, of the variation in the European subsection *Rubigineae*. Taxon differentiation along the second component was observed (Figure 4.16). *R rubiginosa*, *R. micrantha*, *R. inodora*, and *R. henkeri-schulzei* were clustered in the upper group, while *R. agrestis* and *R. elliptica* formed the smaller and lower group. Although the differentiation between the two groups was visually present, the boundaries were vague and a large overlap was present.

Two groups may be discerned; individuals sampled in Belgium and France were assigned to one group versus individuals from The Netherlands, Germany and the Scandinavian countries belonging to the second group.

Jaccard matrix

Table 4.20: Mean Jaccard similarity coefficients (%) calculated within and between the sampled taxa of the subsection *Rubigineae*.

TAXON	AGR	HEN	ELL	INO	MIC	RUB	RUBXHEN
R. agrestis	0.69						
R. henkeri-schulzei	0.63	0.72					
R. elliptica	0.62	0.58	1.00				
R. inodora	0.64	0.66	0.69	0.81			
R. micrantha	0.60	0.65	0.59	0.63	0.63		
R. rubiginosa	0.64	0.70	0.58	0.65	0.64	0.70	
R. rubiginosa x *R. henkeri-schulzei*	0.60	0.69	0.56	0.61	0.62	0.66	0.77

The similarity coefficients between the taxa of the subsection *Rubigineae* were remarkably high and no clear pattern could be detected (Table 4.20).

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to make the dendrogram better readable.

No clear grouping was found based on the taxonomical level, or based on the country of origin (Figure 4.17). However, few tendencies were observed: the sampled *R. elliptica*, *R. agrestis* and *R. inodora* individuals were gathered in the lower part of the dendrogram together with few populations of *R. rubiginosa*, *R. micrantha* and *R. henkeri-schulzei*, while the upper part only consisted of *R. rubiginosa*, *R. micrantha,* and their presumed hybrid *R. henkeri-schulzei*.

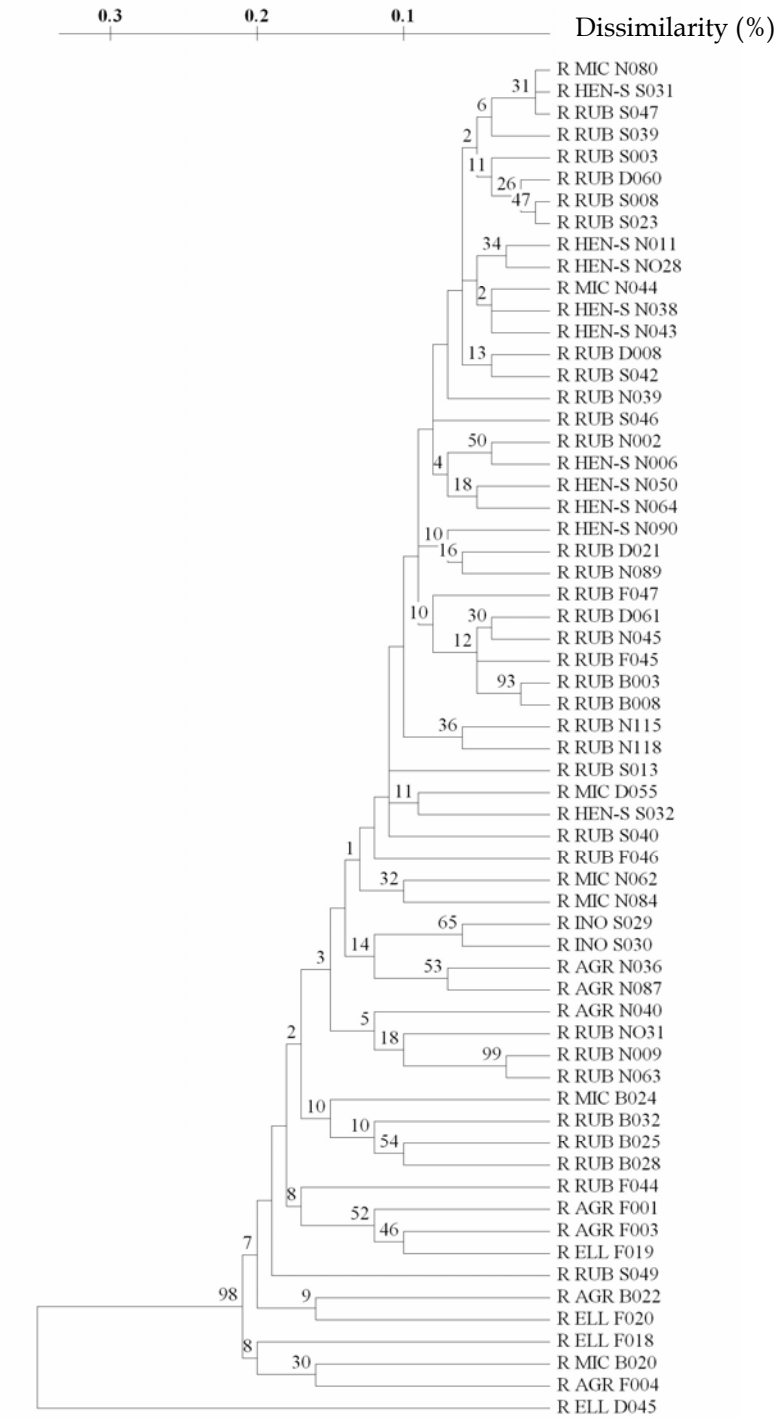


Figure 4.17: UPGMA cluster dendrogram of the subsection *Rubigineae* individuals. The distance scale is indicated, individuals are labelled with species names and population codes (Table 4.22).

Structure

Table 4.21: Species distribution of the subsection *Rubigineae* to each of the inferred gene pools. Species determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

TAXON	GP1	GP2	IND
R. rubiginosa	0.32	0.68	106
R. micrantha	0.36	0.64	25
R. agrestis	**0.86**	0.14	25
R. elliptica	**1.00**	0.00	1
R. inodora	0.00	**1.00**	7
R. henkeri-schulzei	0.00	**1.00**	27

Based on the mean DeltaK, one or two gene pools might be present in this data set. Taking two gene pools as an assumption, the division was summarised in (Tables 4.21 and 4.22). Assuming two gene pools, no consistent taxon, or geographical pattern was detected in the assignment of the individuals. Especially the assignment of both *R. micrantha* and *R. rubiginosa* in the two presumed gene pools supported the decision to treat the subsection *Rubigineae* as one single gene pool.

Within the subsection *Rubigineae*, no consistent differentiation was observed based on taxon or on geographical pattern. Moreover, *R. rubiginosa* and *R. micrantha* were assigned to the two inferred gene pools in a 65/35 ratio.

However, a tendency might be present towards two taxa clusters: the first containing *R. rubiginosa*, *R. micrantha*, and their presumed hybrid *R. henkeri-schulzei*; whereas the second consisted of *R. elliptica, R. inodora*, and *R. agrestis*.

Table 4.22: Population distribution of the subsection *Rubigineae* to each of the inferred gene pools. Population code (POP), region of provenance, species determination, the assignment (%) to each gene pool (GP), and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	TAXON	GP1	GP2	IND
B010	Brabants District Oost	*R. agrestis*	**1.00**	0.00	3
B022	Maasvallei	*R. agrestis*	**1.00**	0.00	4
F001	Var	*R. agrestis*	**1.00**	0.00	3
F002	Hautes Alpes	*R. agrestis*	**1.00**	0.00	1
F003	Hautes Alpes	*R. agrestis*	**1.00**	0.00	2
F004	Hautes Alpes	*R. agrestis*	**1.00**	0.00	1
F019	Hautes Alpes	*R. agrestis*	**1.00**	0.00	3
N036	Zuidlimburgs district	*R. agrestis*	**0.80**	0.20	5
N040	Zuidlimburgs district	*R. agrestis*	0.00	**1.00**	2
N087	Zuidlimburgs district	*R. agrestis*	0.57	0.44	1
F018	Hautes Alpes	*R. elliptica*	**1.00**	0.00	1
N006	Waddendistrict	*R. henkeri-schulzei*	0.00	**1.00**	1
N011	Waddendistrict	*R. henkeri-schulzei*	0.00	**1.00**	2
N028	Renodunaal district	*R. henkeri-schulzei*	0.00	**1.00**	2
N038	Zuidlimburgs district	*R. henkeri-schulzei*	0.00	**1.00**	8
N043	Estuariëndistrict	*R. henkeri-schulzei*	0.01	**1.00**	3
N050	Estuariëndistrict	*R. henkeri-schulzei*	0.00	**1.00**	1
N064	Renodunaal district	*R. henkeri-schulzei*	0.00	**1.00**	2
N088	Zuidlimburgs district	*R. henkeri-schulzei*	0.00	**1.00**	1
N090	Zuidlimburgs district	*R. henkeri-schulzei*	0.00	**1.00**	2
S032	Marstrand	*R. henkeri-schulzei*	0.00	**1.00**	5
S029	Tjörn	*R. inodora*	0.01	**1.00**	4
S030	Henån-Lövås	*R. inodora*	0.00	**1.00**	5
B007	Maasvallei	*R. micrantha*	**1.00**	0.00	3
B020	West-Vlaams Heuvelland	*R. micrantha*	**1.00**	0.00	1
B024	Maasvallei	*R. micrantha*	**1.00**	0.00	5
D055	Lower-Saxony	*R. micrantha*	0.00	**1.00**	4
N062	Fluviatiel district	*R. micrantha*	0.04	**0.96**	1
N080	Zuidlimburgs district	*R. micrantha*	0.01	**0.99**	4
N084	Zuidlimburgs district	*R. micrantha*	0.00	**1.00**	2
B003	Kust	*R. rubiginosa*	**1.00**	0.00	5
B008	Westkust	*R. rubiginosa*	**1.00**	0.00	3
B025	Maasvallei	*R. rubiginosa*	**1.00**	0.00	5
B028	Maasvallei	*R. rubiginosa*	**1.00**	0.00	3
B032	Westkust	*R. rubiginosa*	**1.00**	0.00	5
D008	M-V	*R. rubiginosa*	0.00	**1.00**	4
D021	Baden-Wuerttemberg	*R. rubiginosa*	0.00	**1.00**	1
D034	Baden-Wuerttemberg	*R. rubiginosa*	**1.00**	0.00	5
D060	Lower-Saxony	*R. rubiginosa*	0.00	**1.00**	3
D061	Lower-Saxony	*R. rubiginosa*	0.00	**1.00**	5
F044	Hautes Alpes	*R. rubiginosa*	**1.00**	0.00	1
F045	Hautes Alpes	*R. rubiginosa*	**1.00**	0.00	1
F046	Hautes Alpes	*R. rubiginosa*	**1.00**	0.00	1
F047	Hautes Alpes	*R. rubiginosa*	**1.00**	0.00	2
N002	Waddendistrict	*R. rubiginosa*	0.19	**0.81**	3
N009	Waddendistrict	*R. rubiginosa*	0.00	**1.00**	2
N031	Renodunaal district	*R. rubiginosa*	0.00	**1.00**	2
N039	Zuidlimburgs district	*R. rubiginosa*	0.00	**1.00**	1

Table 4.22 continu: Population distribution of the subsection *Rubigineae* to each of the inferred gene pools. Population code (POP), region of provenance, species determination, the assignment (%) to each gene pool (GP), and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	TAXON	GP1	GP2	IND
N063	Renodunaal district	*R. rubiginosa*	0.00	**1.00**	1
N089	Zuidlimburgs district	*R. rubiginosa*	0.00	**1.00**	2
N115	Estuariëndistrict	*R. rubiginosa*	0.00	**1.00**	8
N118	Estuariëndistrict	*R. rubiginosa*	0.00	**1.00**	3
S003	Denmark, Bornholm	*R. rubiginosa*	0.01	**0.99**	10
S013	Skivarp	*R. rubiginosa*	0.13	**0.87**	3
S023	Öland	*R. rubiginosa*	0.00	**1.00**	4
S039	Tosteberga	*R. rubiginosa*	0.00	**1.00**	2
S040	Kjugekull	*R. rubiginosa*	0.00	**1.00**	3
S042	Denmark, Hornbæk	*R. rubiginosa*	0.00	**1.00**	4
S046	Denmark, Fjellerup	*R. rubiginosa*	0.15	**0.85**	4
S047	Halls fiskeläger	*R. rubiginosa*	0.00	**1.00**	4
S049	Borgholm	*R. rubiginosa*	0.33	0.67	3
N045	Estuariëndistrict	*R. rubiginosa* var. *jenensis*	0.04	**0.97**	6

4.2.9. The origin of *R. henkeri-schulzei*

Based on morphological characters, *R. henkeri-schulzei* (synonym: *R. columnifera*) is supposed to be a descendant of *R. micrantha* and *R. rubiginosa.* In order to confirm or reject this hypothesis, a small subset of the two parental taxa and the hybrid was made and AFLP polymorphisms were compared.

In total, 143 individuals were compared representing 31 populations of *R. rubiginosa*, seven of *R. micrantha,* and ten of *R. henkeri-schulzei*.

PCO

In the AFLP-based biplot, *R. rubiginosa*, *R. micrantha,* and their presumed hybrid *R. henkeri-schulzei* are visualised (Figure 4.18). The first three components explained 23%, 11%, and 11%, respectively, of the variation. The two presumed parental taxa, *R. rubiginosa* and *R. micrantha*, clustered together. Moreover, their presumed descendants overlapped completely with the parental cluster.

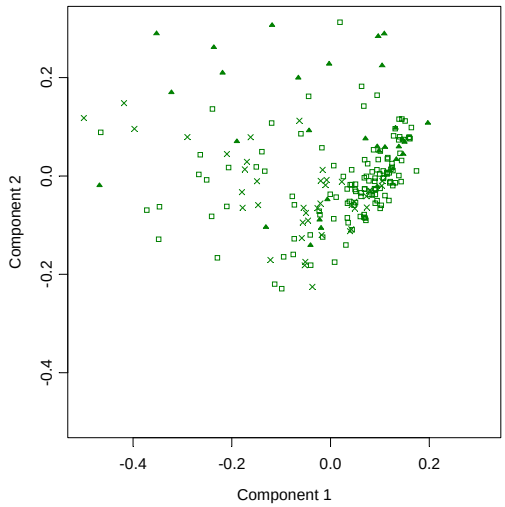


Figure 4.18: PCO plot of the European *R. rubiginosa*, *R. micrantha* and *R. henkeri-schulzei*. The first two components explained 34% of the variation present in the data set. Individuals labelled with species determination (*R. rubiginosa*: □; *R. micrantha*: ▲; *R. henkeri-schulzei*: X).

Jaccard matrix

Table 4.23: Mean Jaccard similarity coefficients (%) calculated within and between plants of the presumed hybrid *R. henkeri-schulzei* and the presumed parental taxa: *R. rubiginosa* and *R. micrantha*.

TAXON/HYBRIDS	*R. henkeri-schulzei*	*R. micrantha*	*R. rubiginosa*
R. henkeri-schulzei	0.72		
R. micrantha	0.65	0.63	
R. rubiginosa	0.70	0.64	0.70

The Jaccard similarity coefficients showed no difference between and within the parental taxa and the hybrid (Table 4.23).

Dendrogram

In the cluster analysis, the presumed parental taxa, *R. rubiginosa* and *R. micrantha*, were completely mingled with the hybrid, *R. henkeri-schulzei*. No pattern could be detected based on species determination or on country of origin (Figure 4.19).

Structure

Based on the mean DeltaK, one or two gene pools might be present in this data set. Taking two gene pools as an assumption, the division was summarised in (Table 4.24). However, assuming two gene pools, no taxon or geographical pattern was detected in the assignment of the individuals.

The AFLP polymorphisms could not distinguish between the two presumed parental taxa, *R. rubiginosa* and *R. micrantha,* and their hybrid *R. henkeri-schulzei*. Therefore, their close relationship is confirmed.

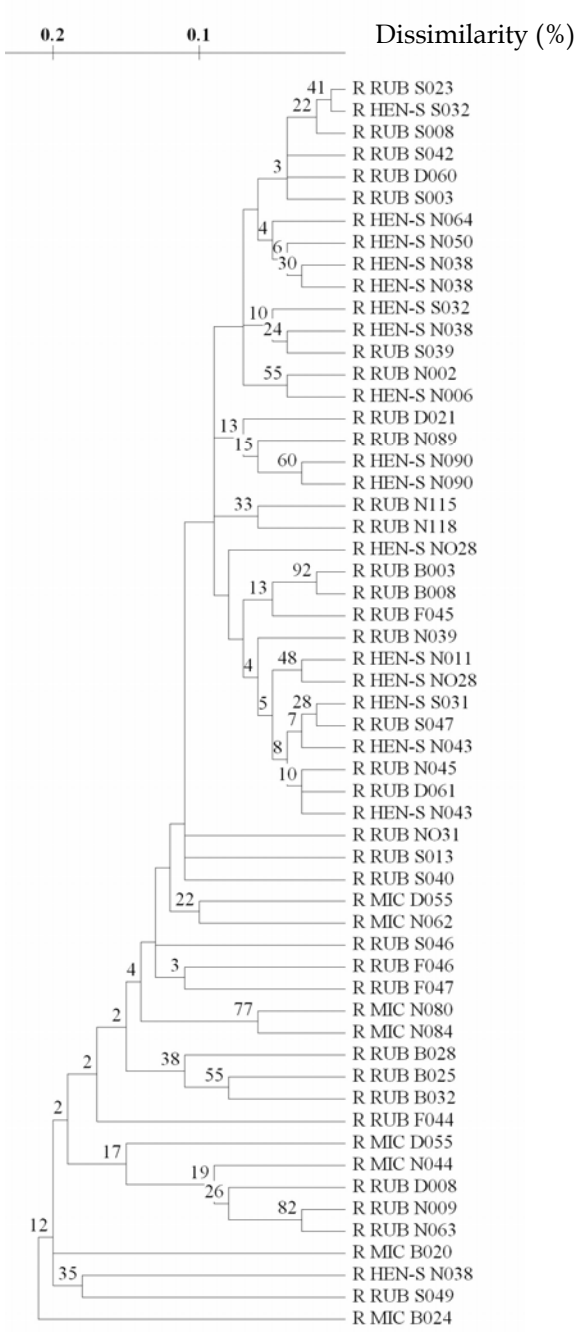


Figure 4.19: UPGMA cluster dendrogram of the hybrid *R. henkeri-schulzei* and the presumed parental taxa. The distance scale is indicated, individuals are labelled with species names and population codes (Table 4.24).

Table 4.24: Population distribution of *R. henkeri-schulzei* and the presumed parental taxa to each of the inferred gene pools. Population code (POP), region of provenance, species determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

POP	REGION	TAXON	GP1	GP2	IND
N011	Waddendistrict	*R. henkeri-schulzei*	**1.00**	0.00	2
N028	Renodunaal district	*R. henkeri-schulzei*	0.64	0.36	2
N038	Zuidlimburgs district	*R. henkeri-schulzei*	**0.86**	0.14	7
N043	Estuariëndistrict	*R. henkeri-schulzei*	**0.97**	0.03	3
N050	Estuariëndistrict	*R. henkeri-schulzei*	**1.00**	0.00	1
N064	Renodunaal district	*R. henkeri-schulzei*	**1.00**	0.00	1
N088	Zuidlimburgs district	*R. henkeri-schulzei*	**0.97**	0.03	1
N090	Zuidlimburgs district	*R. henkeri-schulzei*	**1.00**	0.00	2
S031	Tjuvkil/Marstrand	*R. henkeri-schulzei*	**1.00**	0.00	2
S032	Tjuvkil/Marstrand	*R. henkeri-schulzei*	**1.00**	0.00	4
B007	Maasvallei	*R. micrantha*	0.03	**0.98**	3
B020	West-Vlaams Heuvelland	*R. micrantha*	0.00	**1.00**	1
B024	Maasvallei	*R. micrantha*	0.00	**1.00**	5
D055	Lower-Saxony	*R. micrantha*	**0.99**	0.01	2
N044	Estuariëndistrict	*R. micrantha*	**1.00**	0.00	4
N062	Fluviatiel district	*R. micrantha*	0.00	**1.00**	1
N080	Zuidlimburgs district	*R. micrantha*	0.25	**0.75**	4
N084	Zuidlimburgs district	*R. micrantha*	0.03	**0.97**	2
B003	Kust	*R. rubiginosa*	0.00	**1.00**	5
B008	Westkust	*R. rubiginosa*	0.00	**1.00**	3
B025	Maasvallei	*R. rubiginosa*	0.00	**1.00**	5
B028	Maasvallei	*R. rubiginosa*	0.00	**1.00**	3
B032	Westkust	*R. rubiginosa*	0.00	**1.00**	5
D008	M-V	*R. rubiginosa*	**1.00**	0.00	4
D021	Baden-Wuerttemberg	*R. rubiginosa*	**1.00**	0.00	1
D034	Baden-Wuerttemberg	*R. rubiginosa*	0.00	**1.00**	5
D060	Lower-Saxony	*R. rubiginosa*	**1.00**	0.00	3
D061	Lower-Saxony	*R. rubiginosa*	**1.00**	0.00	5
F044	Hautes Alpes	*R. rubiginosa*	0.00	**1.00**	1
F046	Hautes Alpes	*R. rubiginosa*	0.01	**0.99**	1
F047	Hautes Alpes	*R. rubiginosa*	0.00	**1.00**	2
N002	Waddendistrict	*R. rubiginosa*	**1.00**	0.00	1
N009	Waddendistrict	*R. rubiginosa*	**1.00**	0.00	1
N031	Renodunaal district	*R. rubiginosa*	**1.00**	0.00	1
N039	Zuidlimburgs district	*R. rubiginosa*	**1.00**	0.00	1
N045	Estuariëndistrict	*R. rubiginosa*	**0.81**	0.19	1
N118	Estuariëndistrict	*R. rubiginosa*	**1.00**	0.00	3
S003	S Allinge/Bornholm/DK	*R. rubiginosa*	**1.00**	0.00	5
S008	Oppmanna	*R. rubiginosa*	**1.00**	0.00	5
S013	Skivarp	*R. rubiginosa*	0.37	0.63	3
S023	Räpplinge/Öland	*R. rubiginosa*	**1.00**	0.00	4
S039	Tosteberga	*R. rubiginosa*	**1.00**	0.00	2
S042	Hornbæk, DK	*R. rubiginosa*	**1.00**	0.00	4
S046	Fjellerup,DK	*R. rubiginosa*	**0.81**	0.19	4
S047	Halls fiskeläger	*R. rubiginosa*	**1.00**	0.00	4
S049	Borgholm	*R. rubiginosa*	0.67	0.33	3

4.2.10. The subsection *Vestitae*

The sampled taxa of the European subsection *Vestitae* are *R. tomentosa* (16 analysed populations), *R. pseudoscabriuscula* (2 pop), *R. villosa* (2 pop), *R. mollis* (7 pop), and *R. sherardii* (6 pop). In total, 127 individuals of this subsection were analysed.

PCO

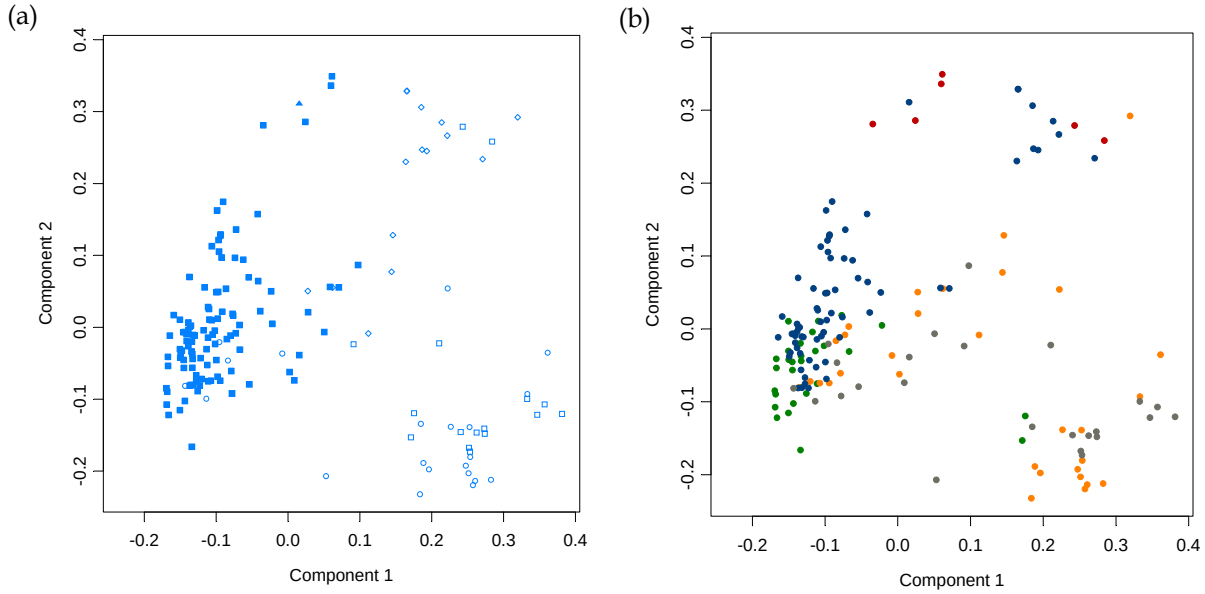


Figure 4.20: PCO plot of the European subsection *Vestitae*. The first two components explained 40% of the variation. (a) Individuals labelled with species determination (*R. tomentosa*: ■; *R. pseudoscabriuscula*: ▲; *R. mollis*: ○; *R. sherardii*: ◇; *R. villosa*: □); (b) individuals were labelled with country of origin (Belgium: ●; The Netherlands: ●; France: ●; Germany: ●; The Scandinavian countries: ●).

The first three components explained 24%, 16%, and 9%, respectively, of the variation present in the data set (Figure 4.20). Along the first component, differentiation between *R. tomentosa* and *R. pseudoscabriuscula* versus *R. mollis*, *R. sherardii,* and *R. villosa* was present. However, both clusters showed overlap. A tendency towards geographical differentiation might be present; however this seems to be linked with the species determination.

Jaccard matrix

Table 4.25: Mean Jaccard similarity coefficients (%) calculated within and between the sampled taxa of the subsection *Vestitae*. The highest similarities are indicated in bold.

TAXON	MOL	PSE	SHE	TOM	VIL
R. mollis	0.63				
R. pseudoscabriuscula	0.59	**0.79**			
R. sherardii	0.53	0.57	0.71		
R. tomentosa	0.58	**0.73**	0.58	**0.74**	
R. villosa	0.61	0.58	0.58	0.57	0.70

The Jaccard similarity coefficient indicated that the similarity among *R. tomentosa* and *R. pseudoscabriuscula* equalled the similarity within both taxa (Table 4.25). Among the other *Vestitae* taxa, the coefficients were comparable.

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to increase the readability of the dendrogram.

A global pattern was detected in the cluster analysis: the upper part of the tree consisted mainly of the taxa *R. tomentosa* and *R. pseudoscabriuscula* and in addition few individuals of *R. mollis* and *R. sherardii* (Figure 4.21). In the lower upper part of the tree, the majority of *R. villosa*, *R. mollis*, and *R. sherardii* were grouped, together with one additional *R. pseudoscabriuscula* individual.

Structure

Based on the mean DeltaK calculations, the most likely number of gene pools in the subsection *Vestitae* could not be inferred. Trying to get more insight in this subsection, the assignment of the samples in two gene pools was considered (Table 4.26).

Table 4.26: Species distribution of the subsection *Vestitae* to each of the inferred gene pools. Species determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

TAXON	GP1	GP2	IND
R. pseudoscabriuscula	**1.00**	0.00	5
R. tomentosa	**0.95**	0.05	93
R. mollis	0.33	**0.67**	15
R. sherardii	0.00	**1.00**	10
R. villosa	0.00	**1.00**	14

The majority of *R. pseudoscabriuscula* and *R. tomentosa* were assigned to gene pool 1, while the analysed individuals of *R. villosa* and *R. sherardii* were completely assigned to gene pool 2. *R. mollis* was the only taxon that was partly assigned to both gene pools, 33% to gene pool 1 and 67% to gene pool 2.

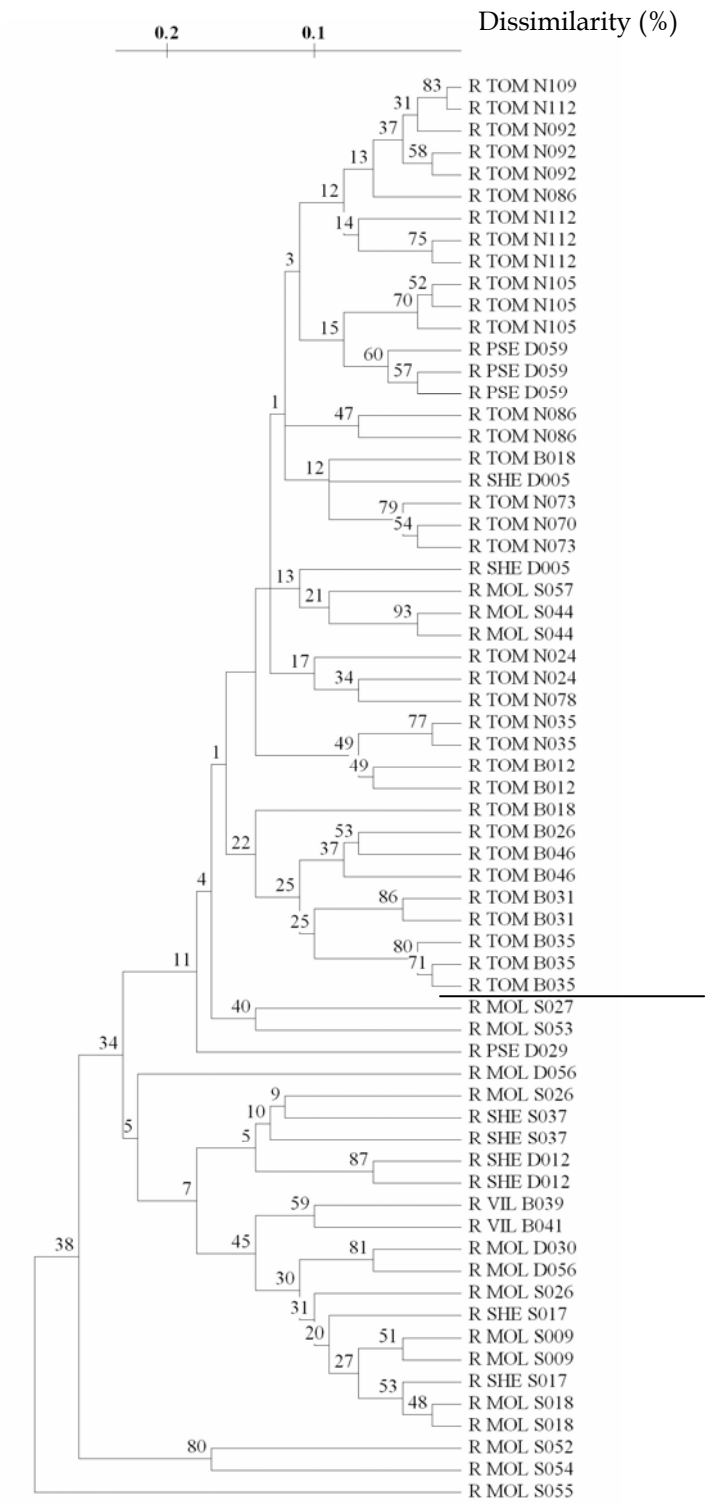


Figure 4.21: UPGMA cluster dendrogram of the subsection *Vestitae* individuals. The distance scale is indicated, individuals are labelled with species names (Table 4.26).

The analysed members of subsection *Vestitae* could be divided into two well-defined clusters: *R. tomentosa* and *R. pseudoscabriuscula*, originating from Belgium, Germany, the Netherlands and Sweden, appeared to have a high genetic similarity and differed clearly from the taxa *R. mollis*, *R. sherardii*, the majority originated from Sweden and Germany, and *R. villosa* that were assigned to the second group.

4.2.11. The subsections *Caninae* and *Tomentellae*

According to the analyses of the whole section *Caninae*, no differentiation could be observed between the two subsections *Caninae* and *Tomentellae*. These subsections were therefore treated together in the subsequent analyses. In total 394 individuals were analysed.

The analysed material of subsection *Caninae* contains the taxa *R. canina* (54 analysed populations), *R. corymbifera* (31 pop), *R. caesia* (6 pop), *R dumalis* (14 pop), *R. subcanina* (8 pop), *R. subcollina* (6 pop), *R. montana* (3 pop), *R. stylosa* (2 pop), and few hybrids *R. canina* x *R. stylosa* (1 individual) and *R. canina* x *R. montana* (1 ind).

The subsection *Tomentellae* consists of *R. balsamica* and *R. abietina*. Nineteen populations were sampled of the former taxon, whereas the latter is very rare and was therefore not included in our analyses.

PCO

The first three components explained 17%, 9%, and 8% respectively, of the variation (Figure 4.22). Although this data set consisted of the individuals of the two subsections *Caninae* and *Tomentellae*, no subdivision in one or more clusters was detected.

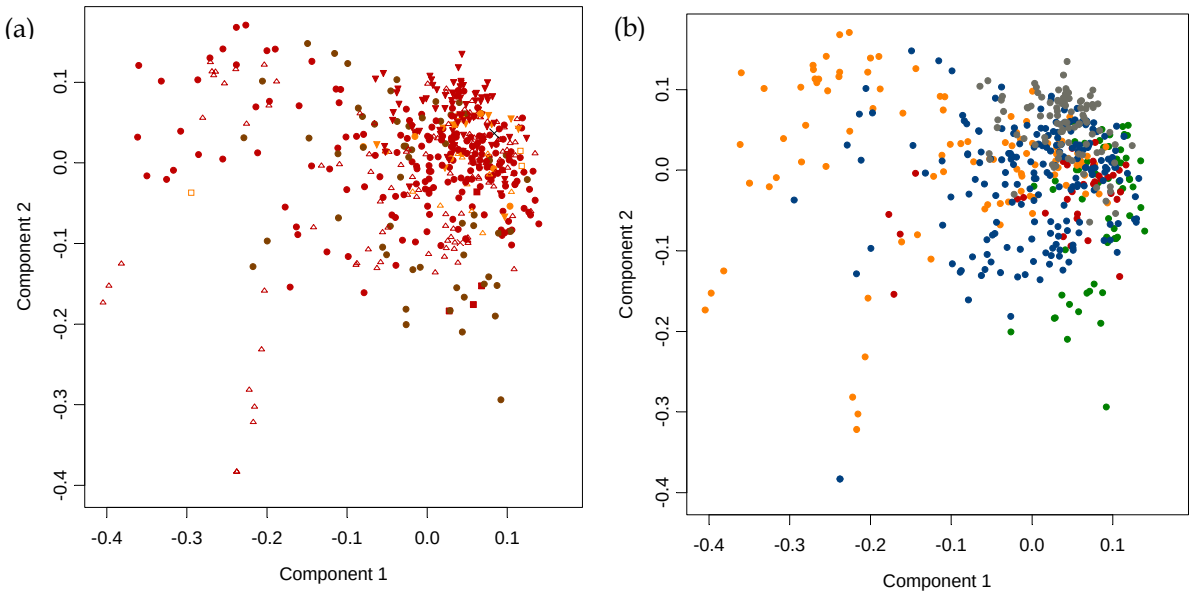


Figure 4.22: PCO plot of the European subsections *Caninae* and *Tomentellae*. The first two components explained 26% of the variation. (a) Individuals labelled with species determination (*R. canina*: ●; *R. corymbifera*: △; *R. dumalis*: ▼; *R. caesia*: ●; *R. subcanina*: □; *R. subcollina*: ▼; *R. montana*: △; *R. stylosa*: ■; *R. balsamica*: ▲; *R. canina* x *R. stylosa*: x; *R. canina* x *R. montana*: x); (b) individuals were labelled with country of origin (Belgium: ●;The Netherlands: ●; France: ●; Germany: ●; The Scandinavian countries: ●).

Jaccard matrix

Table 4.27: Mean Jaccard similarity coefficients (%) calculated within and between the sampled taxa of the subsections (SubS): *Caninae (*Can*)* and *Tomentellae* (Ton).

SUBS	TAXON	CAE	CAN	CANxSTY	COR	DUM	MON	STY	SCA	SCO	BAL
Can	*R. caesia*	0.71									
Can	*R. canina*	0.65	0.64								
Can	*R. canina* x *R. stylosa*	0.59	0.57	1.00							
Can	*R. corymbifera*	0.65	0.62	0.56	0.64						
Can	*R. dumalis*	0.70	0.66	0.60	0.65	0.74					
Can	*R. montana*	0.67	0.65	0.62	0.64	0.67	0.78				
Can	*R. stylosa*	0.58	0.57	0.64	0.57	0.58	0.57	0.76			
Can	*R. subcanina*	0.68	0.64	0.58	0.63	0.68	0.66	0.58	0.67		
Can	*R. subcollina*	0.73	0.67	0.61	0.67	0.72	0.69	0.60	0.72	0.80	
Ton	*R. balsamica*	0.66	0.63	0.58	0.64	0.65	0.64	0.58	0.64	0.67	0.66

Irrespective of the subsection to which the taxa belong, all these analysed taxa showed a high interspecific similarity towards the other taxa of the subsections *Caninae* and *Tomentellae* (Table 4.27).

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to make the dendrogram better readable.

The cluster analyses did not divide the two subsections in subclusters based on taxon, region or population (Figure 4.23). Remarkable was the grouping of the two analysed *R. stylosa* individuals and the possible *R. canina* x *R. stylosa* hybrid (marked with circle).

Structure

Table 4.28: Taxon distribution of sections *Caninae* and *Tomentellae* and some presumed hybrids to each of the inferred gene pools. Subsection and species determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

SUBSECTION	TAXON	GP1	GP2	IND
Tomentellae	*R. balsamica*	0.42	0.58	45
Caninae	*R. canina*	0.35	0.65	145
Caninae	*R. corymbifera*	0.22	**0.78**	95
Caninae	*R. caesia*	0.15	**0.85**	7
Caninae	*R. dumalis*	0.21	**0.79**	82
Caninae	*R. stylosa*	**1.00**	0.00	3
Caninae	*R. subcanina*	0.17	**0.83**	6
Caninae	*R. subcollina*	0.15	**0.85**	10
Caninae	*R. montana*	0.16	**0.84**	11
Caninae	*R. canina* x *R. stylosa*	**1.00**	0.00	1
Caninae	*R.* x *irregularis*	0.00	**1.00**	1

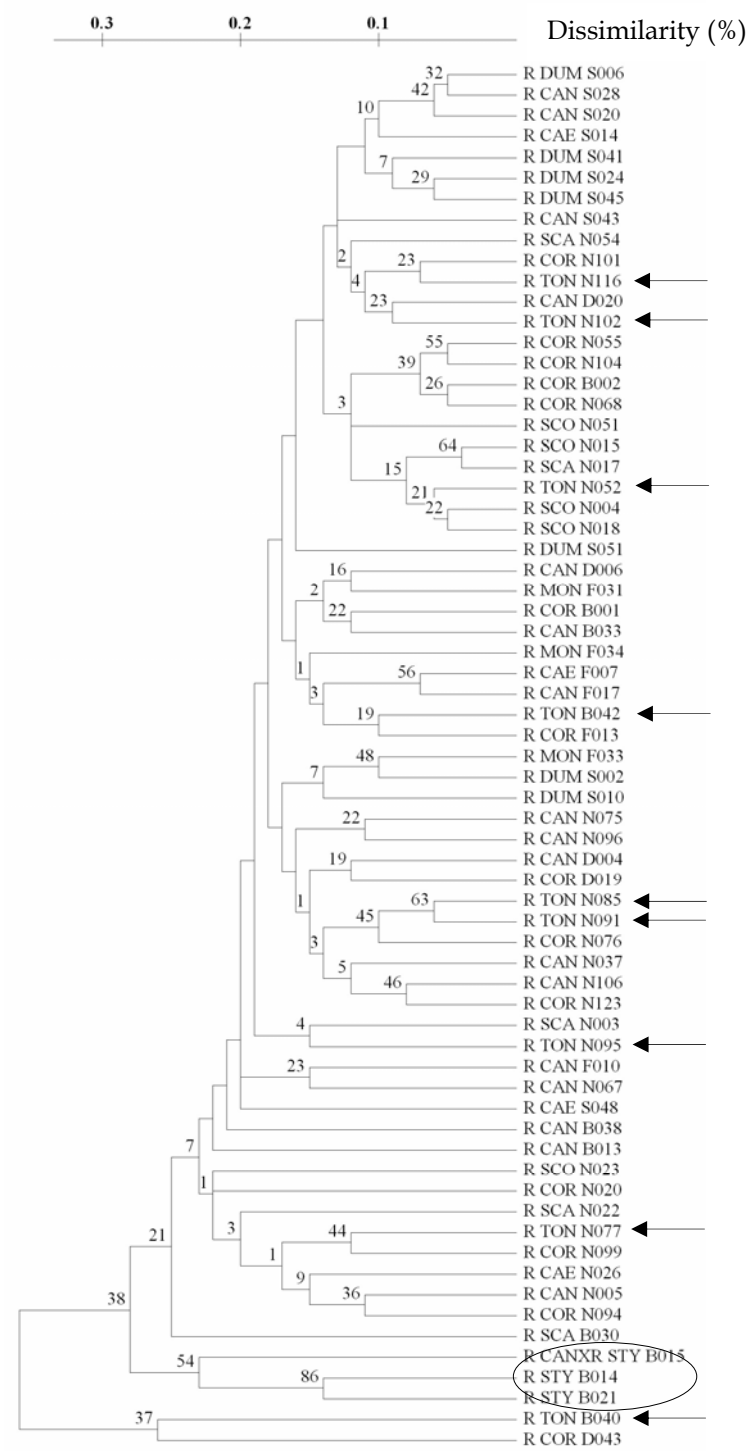


Figure 4.23: UPGMA cluster dendrogram of the subsections *Caninae* and *Tomentellae* individuals. The distance scale is indicated, individuals are labelled with species names and population codes (Country of origin is indicated, population code is not explained). *R. balsamica* (R TON) individuals are indicated with arrows; the *R. stylosa* individuals and hybrid *R. canina* x *R. stylosa* are marked with a circle.

For the subsections *Caninae* and *Tomentellae*, the mean DeltaK calculations did not indicate the number of gene pools present in these subsections (Table 4.28). Assuming two gene pools, the assignment of the samples was as follows: *R. stylosa* and the presumed hybrid *R. canina* x *R. stylosa* were completely assigned to gene pool

one, while the majority of the *R. corymbifera*, *R. caesia*, *R. dumalis*, *R. subcanina*, *R. subcollina,* and *R. montana* was assigned to gene pool two. The taxa *R. canina* and *R. balsamica* were assigned to both gene pools (about 40/60 ratio).

The distinction of subsections *Caninae* and *Tomentellae* as suggested by Henker (2000) and Wissemann (2003) was not supported by the AFLP polymorphisms.

Moreover, *R. canina* and *R. balsamica* were assigned to the two assumed gene pools in a 40/60 ratio. As the gene pools of the two subsections were not well-defined, proper taxa boundaries within subsection *Caninae* are lacking completely.

The hybrid *R. stylosa* and the individuals determined as *R. canina* x *R. stylosa* appeared to be the most distinct in this data set, thus confirming the unexpected grouping of *R. stylosa* with the subsection *Rubigineae* instead of within the subsection *Caninae*.

4.2.12. Origin of *R. stylosa* and *R.* x *irregularis*

Based on the morphological similarities, *R. stylosa* and *R.* x *irregularis* are presumed to be descendants of intersectional crossings between *R. canina* or *R. corymbifera* (section *Caninae*, subsection *Caninae*) and *R. arvensis* (section *Synstylae*). A genetic basis for this hypothesis was investigated by comparing the AFLP polymorphisms of the presumed parental taxa and the descendants. A data set containing the hybrids *R. stylosa* and *R.* x *irregularis* and the presumed parental taxa, *R. canina* or *R. corymbifera* and *R. arvensis* was thus analysed. In addition, *R. balsamica* individuals were also included since the gene pools of subsections *Caninae* and *Tomentellae* overlapped completely.

PCO

The first three components explained 30%, 11%, and 7%, respectively, of the variation (Figure 4.24). No differentiation was observed among the three *Caninae* parental taxa, *R. canina*, *R. corymbifera,* and *R. balsamica*. In contrast, the subdivision of *R. arvensis* on the one hand and the *Caninae* parent on the other hand was very clear. Nevertheless, few *R. arvensis* samples took a more intermediate position between the two clusters. Both the hybrids *R. stylosa* and *R.* x *irregularis* clustered with individuals of the section *Caninae*. Moreover, *R.* x *irregularis* was completely mingled in the *Caninae* cluster, while *R. stylosa* had a more intermediate position.

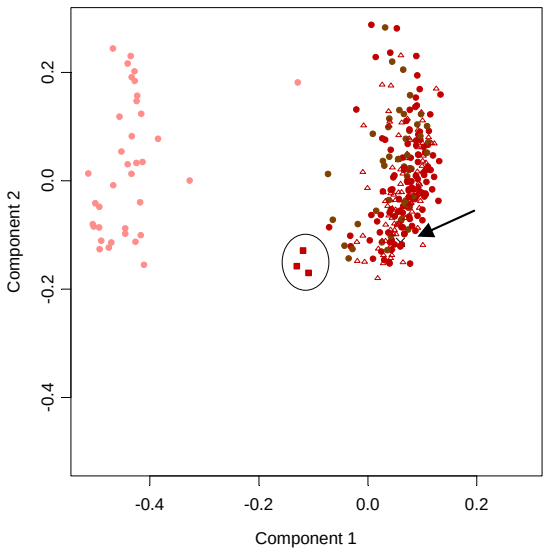


Figure 4.24: PCO plot of the hybrids *R. stylosa* and *R.* x *irregularis* and their presumed parental taxa. The first two components explained 41% of the variation. Individuals are indicated with species determination: *R. canina* (●); *R. corymbifera* (△); *R. balsamica* (●); *R. arvensis* (●); *R. stylosa* (■, with circle); *R.* x *irregularis* (X, with arrow).

Jaccard matrix

Table 4.29: Mean Jaccard similarity coefficients (%) calculated within and between the sampled hybrids *R.* x *irregularis* and *R. stylosa* and presumed parental taxa, the lowest similarities are indicated in bold.

TAXON	ARV	CAN	COR	STY	TON	XIRR
R. arvensis	0.54					
R. canina	**0.39**	0.64				
R. corymbifera	**0.40**	0.62	0.64			
R. stylosa	**0.44**	0.57	0.57	0.76		
R. balsamica	**0.40**	0.63	0.64	0.58	0.66	
R. x *irregularis*	**0.39**	0.67	0.66	0.66	0.66	1.00

Intraspecific similarity was lower in *R. arvensis* than in the other taxa and comparisons between *R. arvensis* and the other taxa yielded much lower similarities than comparisons among the *Caninae* taxa (Table 4.29). Compared to *R. stylosa*, *R.* x *irregularis* showed a higher similarity towards the *Caninae* taxa and a lower similarity towards *R. arvensis*.

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to make the dendrogram better readable (Figure 4.25).

In the dendrogram, the subcluster of *R. arvensis*, one of the presumed parents, was well-separated from the *Caninae* parent cluster [*R. canina*, *R. corymbifera*, *R. balsamica* (R TON)]. The *R.* x *irregularis* hybrid was mingled in the cluster of *R. canina*,

R. corymbifera and *R. balsamica,* while the analysed *R. stylosa* individuals were grouped at the edge of the *Caninae* parent group.

Structure

Based on the mean DeltaK, one or two gene pools might be present in this data set. Taking two gene pools as an assumption, the division was summarised in table 4.30.

Table 4.30: Taxa and hybrids assignment of *R. stylosa* and *R.* x *irregularis* and their presumed parental taxa to each of the inferred gene pools. Species determination, the assignment to each gene pool (GP) in percentage, and number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

SUBSECTION	TAXON	GP1	GP2	IND
Synstylae	*R. arvensis*	0.00	**1.00**	21
Caninae	*R. canina*	**1.00**	0.00	25
Caninae	*R. corymbifera*	**1.00**	0.00	21
Tomentellae	*R. balsamica*	**1.00**	0.00	13
Hybrid	*R. stylosa*	**1.00**	0.00	3
Hybrid	*R. canina* x *R. stylosa*	**1.00**	0.00	1
Hybrid	*R.* x *irregularis*	**1.00**	0.00	1

The smallest gene pool (GP2) contained all the *R. arvensis* individuals, while the other gene pool (GP1) consisted of *R. canina*, *R. corymbifera*, *R. balsamica,* and the two hybrids, *R. stylosa* and *R.* x *irregularis*.

The putative hybrids *R. stylosa* and *R.* x *irregularis* showed a high similarity with the presumed *Caninae* parental taxa. All three taxa, *R. canina*, *R. corymbifera,* and *R. balsamica* are candidates as parental taxa.

R. stylosa showed a higher similarity to *R. arvensis* then *R.* x *irregularis* did.

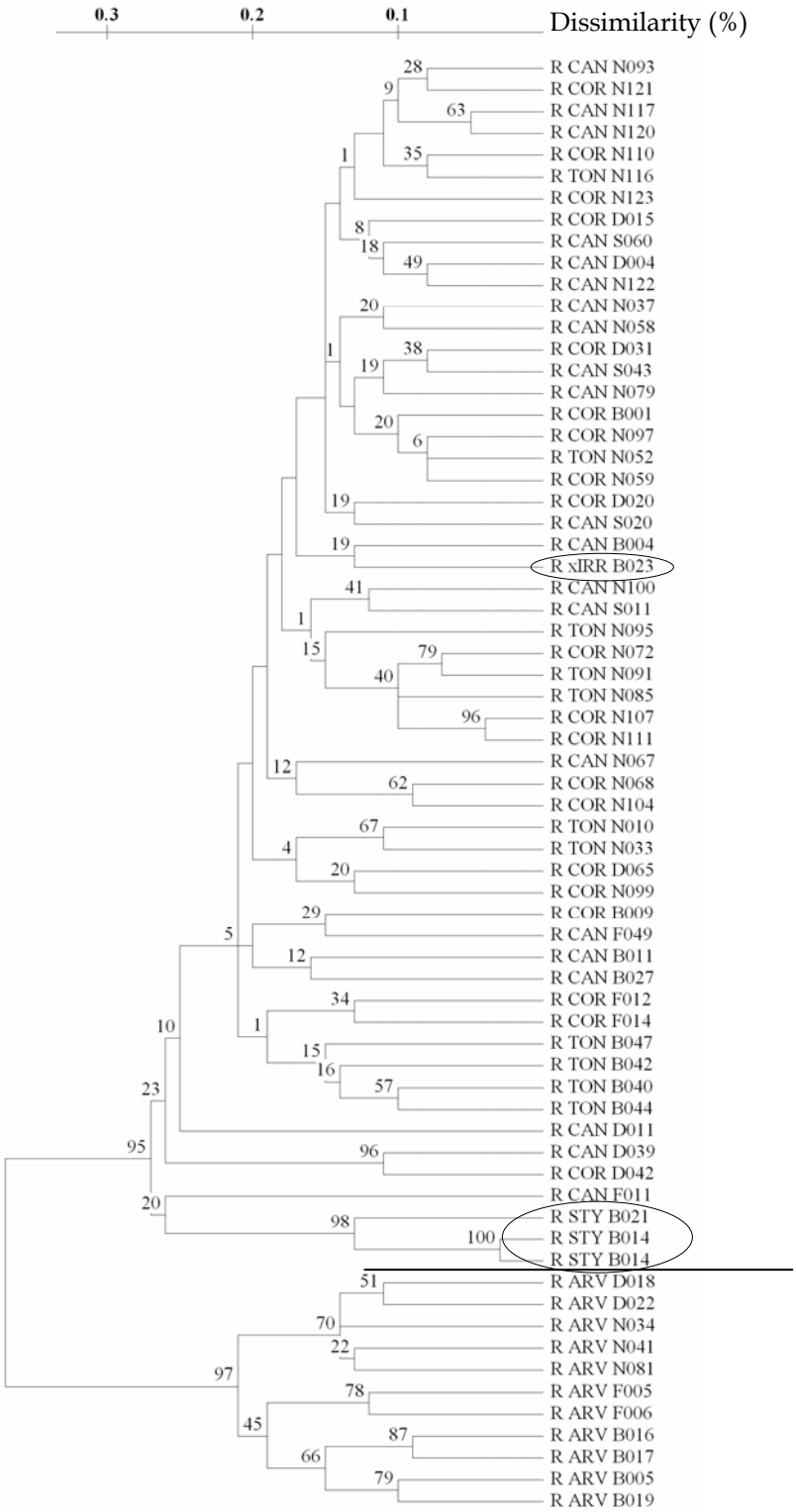


Figure 4.25: UPGMA cluster dendrogram of the hybrids *R. stylosa* and *R.* x *irregularis* and their presumed parental taxa. The distance scale is indicated, individuals are labelled with species names and population codes (Country of origin is indicated, population code is not explained), hybrids are circled.

4.3. The Flemish subgenus *Rosa*

Recent inventories for autochthonous gene sources of woody plants revealed that Flanders contains some unexpected rose species and a number of species-rich and valuable localities. Therefore an in-depth study was performed in order to assess if intraspecific population differentiation is present within and among the regions of provenance. Additional questions were tackled concerning the clonality of certain taxa, the influence of different taxa at a well-defined locality and the origin of presumed hybrids.

4.3.1. Molecular-genetic analyses

4.3.1.1. Amplified Fragment Length Polymorphism (AFLP)

The term "Flemish taxa" indicates the individuals sampled in the Flemish project, not all the individuals or populations that are present in this geographical area.

In the Flemish data set, 438 wild individuals (Table 3.2) were analysed with three polymorphic AFLP primer combinations (Table 4.31). Based on fragment density and resolution, three *Eco*RI-*Mse*I primer combinations were selected out of 16 tested on a subset of different taxa. These three resulted in 150 polymorphic markers in the subgenus *Rosa*.

Table 4.31: The used AFLP primer combinations. The number of polymorphic markers (# PM), the scored fragment size range in base pairs (Frag Size Range) and the used label (L) are indicated.

PRIMER COMBINATION	# PM	FRAG SIZE RANGE (BP)	L
*Eco*RI_AAG-*Mse*I_CAT	53	93-652	700 nm
*Eco*RI_AAG-*Mse*I_CAG	40	75-433	800 nm
*Eco*RI_ATC-*Mse*I_CTA	57	91-648	700 nm

This set of 150 AFLP fragments scored on the Flemish roses was well-suited for detecting interspecific differentiation in the subgenus *Rosa*. However, these markers were not appropriate for the detection of intraspecific variation, i.e. differentiation between populations of the same species. At the species level, the variation caused by the run appeared to be larger than the possible variation due to population differentiation. Therefore, a set of markers was selected showing a high differentiation between the populations, combined with a low variation among the runs (Figure 4.26). For each analysed taxon such a specific marker set was identified.

4.3.1.1.1. Identifying AFLP markers for assessing intraspecific population variation

Starting from the total set of 150 polymorphic fragments for the subgenus *Rosa*, the allelic frequency of the markers was calculated for each population (Pop) and run for the specific taxon. Next, the standard deviation (SD) of the two frequencies, SD_{pop} and SD_{run}, respectively, was assessed. Fragments with a SD equal to zero did not show any differentiation within the taxon and were excluded from

further analyses. In contrast, the most differentiating fragments showed a low SD_{run} combined with a high SD_{pop}. These fragments were identified with following formula:

$$SD_{run\text{-}pop} = -SD_{pop} * \text{Mean } SD_{run} / \text{Mean } SD_{pop} + SD_{run}$$

The frequency distributions of the $SD_{run\text{-}pop}$ were visualised by histograms (e.g. Figure 4.26). If normally distributed, the 150 scored fragments would form a Gauss-curve. In these analyses, only a few fragments were distributed according to a Gauss-curve. Fragments situated in the lower part of the histogram were characterised by a large population differentiation, hence no lower limit has to be defined. In contrast, fragments on the upper part of the distribution showed high SD_{run}, meaning large differentiation between runs. Consequently, the fragments situated in the upper part might represent variation caused by the run rather than caused by population differentiation. Therefore, an upper limit with an acceptable $SD_{run\text{-}pop}$ had to be defined on the upper part of the Gauss-curve. The assessment of the limits was subjective and depended on the distribution of the $SD_{run\text{-}pop}$; therefore subsets with different upper limits were compared. The variation explained by the three principal components differs slightly (Table 4.32).

As an illustration, the output for *R. arvensis* is given. The frequency distribution of the $SD_{run\text{-}pop}$ is displayed in figure 4.26. The presumed upper limits varied between 0.124 and 0.202. The two data sets were analysed with PCO analyses. The difference in cumulative percentage explaining the variation in the first three components, summarised in table 4.32, was negligible. Further analyses were based on the data set with upper limit 0.124.

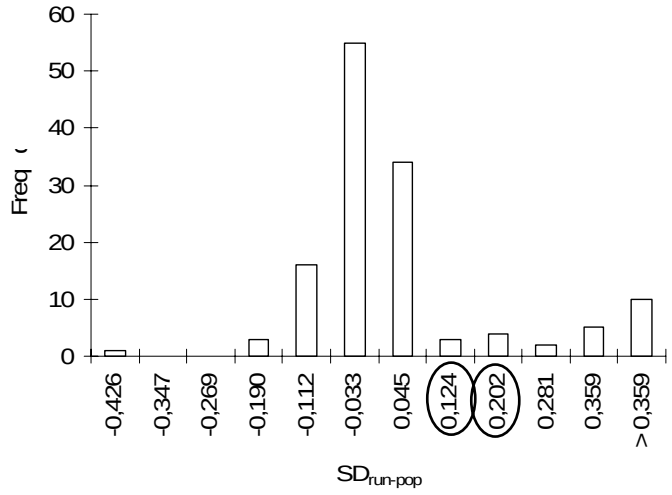


Figure 4.26: Frequency distribution of the Standard $Deviation_{run\text{-}pop}$ for *R. arvensis*. The acceptable upper limits for the most differentiating data set are circled.

Table 4.32: The output of PCO analyses of two data sets of *R. arvensis* based on different upper limits was compared. The upper limit, the number of polymorphic markers (#PM), and the cumulative percentages (CUM%) for the three main components are indicated.

		CUM%		
LIMIT	# PM	COMP. 1	COMP. 2	COMP. 3
0.124	110	20	38	48
0.202	114	20	37	47

4.3.1.1.2. The subgenus *Rosa*

PCO

Principal Co-Ordinate analysis calculated with Jaccard coefficients showed subdivision of the subgenus *Rosa* congruent with the taxonomical structure at the level of the different sections: *Pimpinellifoliae*, *Synstylae,* and *Caninae* (Figure 4.27). In total, the first two components explained 49% of the variation present in the AFLP-based data set. For this analysis, all the Flemish samples without missing data were included. In contrast to the section *Caninae*, the sections *Pimpinellifoliae* and *Synstylae* are monotypic in Flanders.

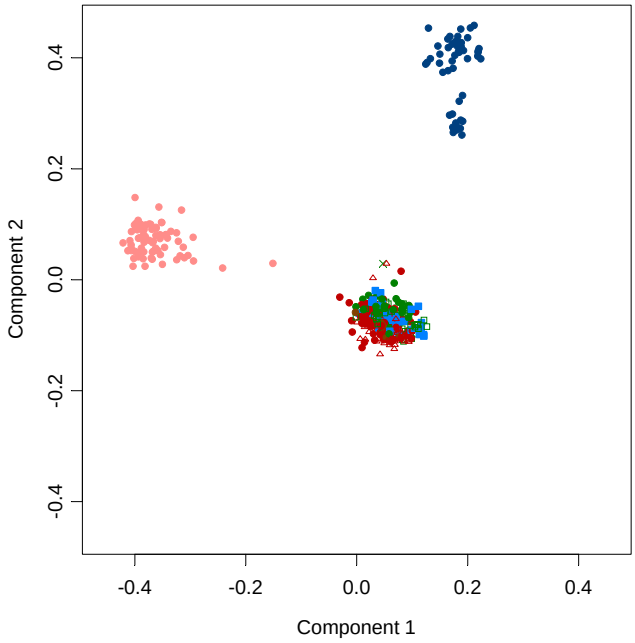


Figure 4.27: PCO plot of the subgenus *Rosa* based on AFLP markers. The first three components explained 28%, 21%, AND 7%, respectively, of the variation in the data set. With: section *Pimpinellifoliae*: ●; section *Synstylae*: ●; subsection *Rubigineae*: ●; subsection *Vestitae*: ■; subsection *Tomentellae*: ▲; subsection *Caninae*: ●. The detailed species labels can be found in table 4.2.

Jaccard similarity

Table 4.33: Mean Jaccard similarity coefficients (%) calculated within and between the sections of the Flemish subgenus *Rosa*.

SECTION	*Caninae*	*Pimpinellifoliae*	*Synstylae*
Caninae	0.66		
Pimpinellifoliae	0.42	0.62	
Synstylae	0.47	0.30	0.64

Based on the Jaccard coefficients and on only one representative species for the sections *Pimpinellifoliae* and *Synstylae*, the taxonomically described sections within the subgenus *Rosa* appeared to be valid since similarity among samples was considerably higher within sections than between sections (Table 4.33).

Table 4.34: Sampled regions and localities of origin in Flanders and one region in Walloon. Region of provenance with used abbreviations (R_ABBR) and symbols (R_S), and localities with used abbreviations (L_ABBR) and symbols (L_S) are indicated.

REGION OF PROVENANCE	R_ABBR	R_S	LOCALITY	L_ABBR	L_S
Vlaamse Zandstreek	VZS	□	De Pinte	DPI	■
			Deinze	DE	●
			Maldegem-Eeklo	MA	▲
			Nazareth	NA	○
			Pittem	PI	▼
			Temse	TE	◆
Westkust	WKU	▼	De Panne	DP	▲
			Koksijde	TY / DO	●
			Oostduinkerke	MO / OVD	□
Middenkust	MKU	●	Middelkerke	MI	◆
Oostkust	OKU	▲	Knokke, Het Zwin	ZW	○
Kempen	KEM	■			
West-Vlaams Heuvelland	WVH	▼	Belle	BE	●
			Galgebos	GB	▲
			Helleketelbos	HKB	■
			Nieuwkerke	NIE	◆
Vlaamse Ardennen	VAR	▲	Balegem	BA	○
			Brakel	BR	△
			Hemelveerdegem	HEM	□
			Ophasselt	OP	▼
			Zulzeke, Beiaardbos	BEI	◇
Brabants District Oost	BDO	♦	Heers	HE	●
			Hoegaarden	HOE	■
			Hoeselt	HT	▲
			Kortenberg	KO	▼
			Kortessem	KT	◆
			St-Truiden	ST	▽
			Tongeren	TO	△
			Wellen	WE	○
			Zemst	ZE	◇
Maasvallei	MV	□	Lanaken, Hochter Bampd	HO	●
			Riemst	RI / SPB	▲
Voeren	VOE	●			
Viroin	VIR	●	Nismes	VIR	○
			Olloy	VIR	□
			Tienne aux Pauquis	VIR	▲
			Vierves	VIR	▼

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to make the dendrogram better readable.

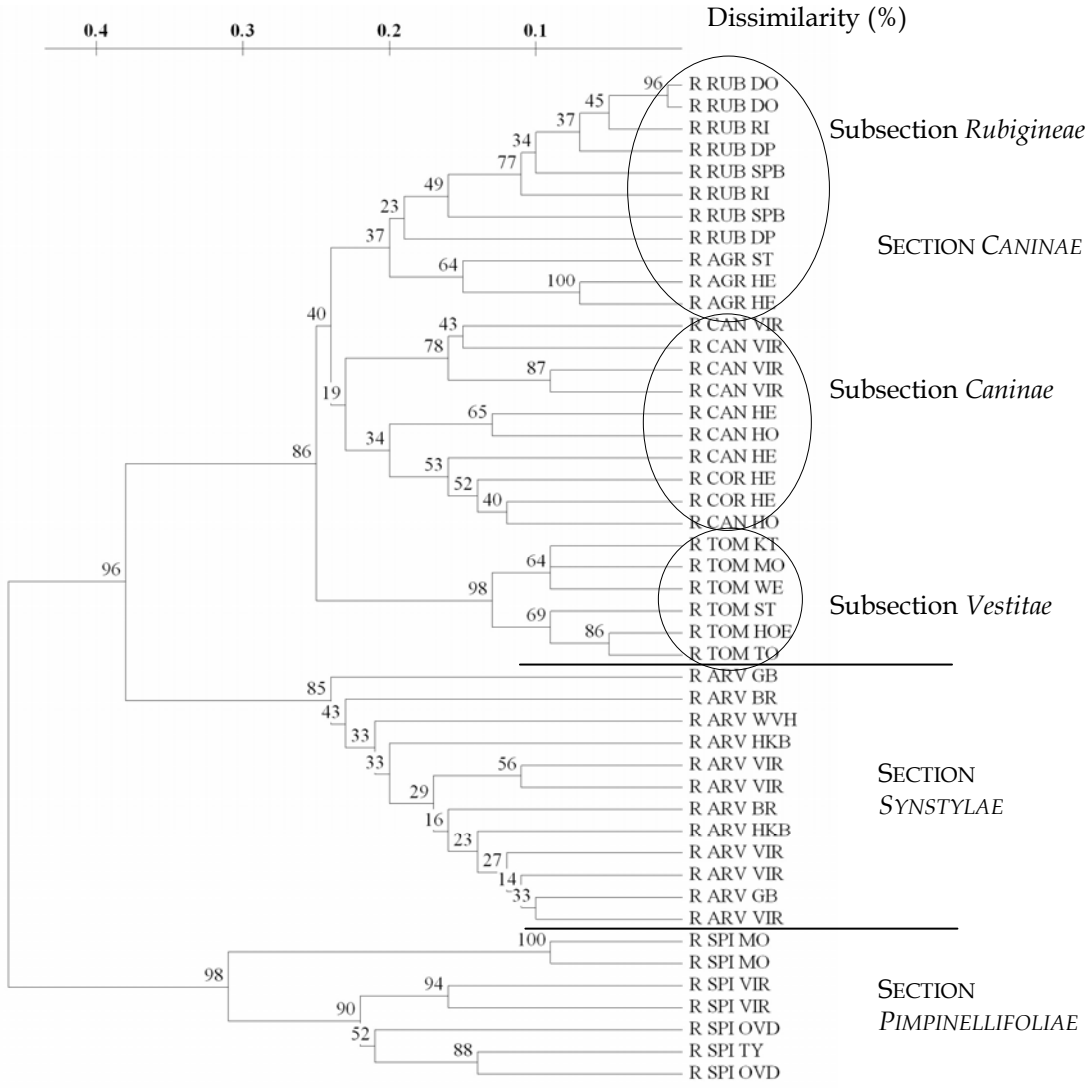


Figure 4.28: UPGMA cluster dendrogram of the subgenus *Rosa*. The distance scale is indicated, individuals are labelled with species names and population codes (Table 4.2 and Table 4.34).

The dendrogram consisted of three main subclusters according to the taxonomical sections: *Pimpinellifoliae*, *Synstylae,* and *Caninae* (Figure 4.28). Within the section *Caninae*, three subsections could be identified: subsections *Rubigineae*, *Vestitae,* and *Caninae.* Of the subsection *Tomentellae*, no representative was included in this cluster analysis.

Structure

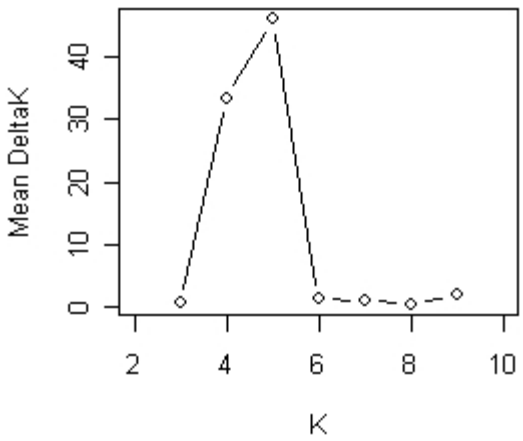


Figure 4.29: Assumption of the optimal number of gene pools present in the data set, based on Structure (Pritchard *et al.* 2000) and adapted with the method of Evanno *et al.* (2005).

Calculating the mean DeltaK, the best model given the population structure of the Flemish subgenus *Rosa* was attained for five gene pools (Figure 4.29). Table 4.35 gave an overview of the species assignment in to five inferred gene pools.

Table 4.35: Species assignment of the Flemish subgenus *Rosa* to each of the five inferred gene pools. Section, subsection and species determination, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

SECTION / SUBSECTION	TAXON	GP1	GP2	GP3	GP4	GP5	IND
Pimpinellifoliae	*R. spinosissima*	**1.00**	0.00	0.00	0.00	0.00	45
Synstylae	*R. arvensis*	0.00	**0.91**	0.07	0.01	0.01	76
Caninae							
Rubigineae	*R. rubiginosa*	0.00	0.00	0.00	0.08	**0.92**	62
	R. micrantha	0.00	0.00	0.00	**1.00**	0.00	1
	R. agrestis	0.00	0.00	0.07	0.00	**0.93**	32
Vestitae	*R. tomentosa*	0.00	0.00	**0.98**	0.00	0.02	47
Tomentellae	*R. balsamica*	0.00	0.00	**1.00**	0.00	0.00	8
Caninae	*R. canina*	0.00	0.00	**0.80**	0.20	0.00	146
	R. corymbifera	0.00	0.04	**0.88**	0.08	0.00	49
Hybrids	*R. henkeri-schulzei*	0.00	0.00	0.00	0.00	**1.00**	1

Gene pool 1 exclusively comprised *R. spinosissima* individuals (section *Pimpinellifoliae*). Gene pool 2 consisted of most *R. arvensis* (section *Synstylae*) individuals. Gene pool 3 comprised the majority (between 80-100%) of the subsections *Vestitae* (*R. tomentosa*), *Tomentellae* (*R. balsamica*), and *Caninae* (*R. canina*, *R. corymbifera*). Gene pool 4 contained the only *R. micrantha* individual analysed and a small proportion of *R. canina* (20%). The last gene pool, number 5, consisted of the majority of *R. rubiginosa* and *R. agrestis,* and the only analysed *R. henkeri-schulzei* (presumed hybrid of *R. rubiginosa* x *R. micrantha*). The latter taxa all belong to the subsection *Rubigineae*. The deviating position of *R. micrantha* is probably due to the fact that only one representative was included.

The AFLP marker analysis of the Flemish wild roses confirmed the subdivision of the subgenus *Rosa* in three sections: section *Pimpinellifoliae*, *Synstylae* and *Caninae*. In addition, within the section *Caninae*, the subsection *Rubigineae* appeared to be the most distinguished when compared to the other subsections.

4.3.1.1.3. Section *Pimpinellifoliae*

The ability of *R. spinosissima* to reproduce vegetatively and consequently form expansive carpets in the dunes hampers the assessment whether two branches belong to the same individual/shrub/genotype or not. Therefore, different sampling strategies were followed: (a) along 100m: every 5-10-15 or 20m, or (b) randomly within a population with some distance between two samples.

In total, 109 polymorphic AFLP markers were compared in 59 individuals belonging to Westkust (49 analysed individuals), Middenkust (3 ind), and Viroin (7 ind) (Figure A.22).

PCO

The first three components explained 20%, 14%, and 10%, respectively, of the variation (Figure 4.30). The intensive sampling at the Westkust (49 individuals) gave a large contrast with the small populations at the Middenkust and Viroin, i.e. three and seven individuals, respectively (Figure 4.30a). Along the third component, the Viroin population might be differentiated (Figure 4.30b). Within the Westkust, the populations of Ter Yde and the Monoblocduinen also appeared to differentiate (Figure 4.30c).

Based on the generally accepted threshold for clonality, in which at least 95% of the AFLP bands are identical, the eight samples from the Monoblocduinen (MO), numbered 2 to 9, could be assumed to be one genotype, whereas R SPI MO 10 and 11 also appeared to be the same clone. These two genotypes were sampled along the 100m haul, of the randomly sampled populations the majority of the samples differed more than 5% of the scored AFLP polymorphisms. Therefore, they were assumed to be individual genotypes. Based on the threshold, the samples R SPI TY 13, 15 and 16, R SPI OVD 23 and 25, R SPI OVD 28 and 29, R SPI VIR 3 and 4 and R SPI VIR 6 and 7 also were assumed to represent five different genotypes.

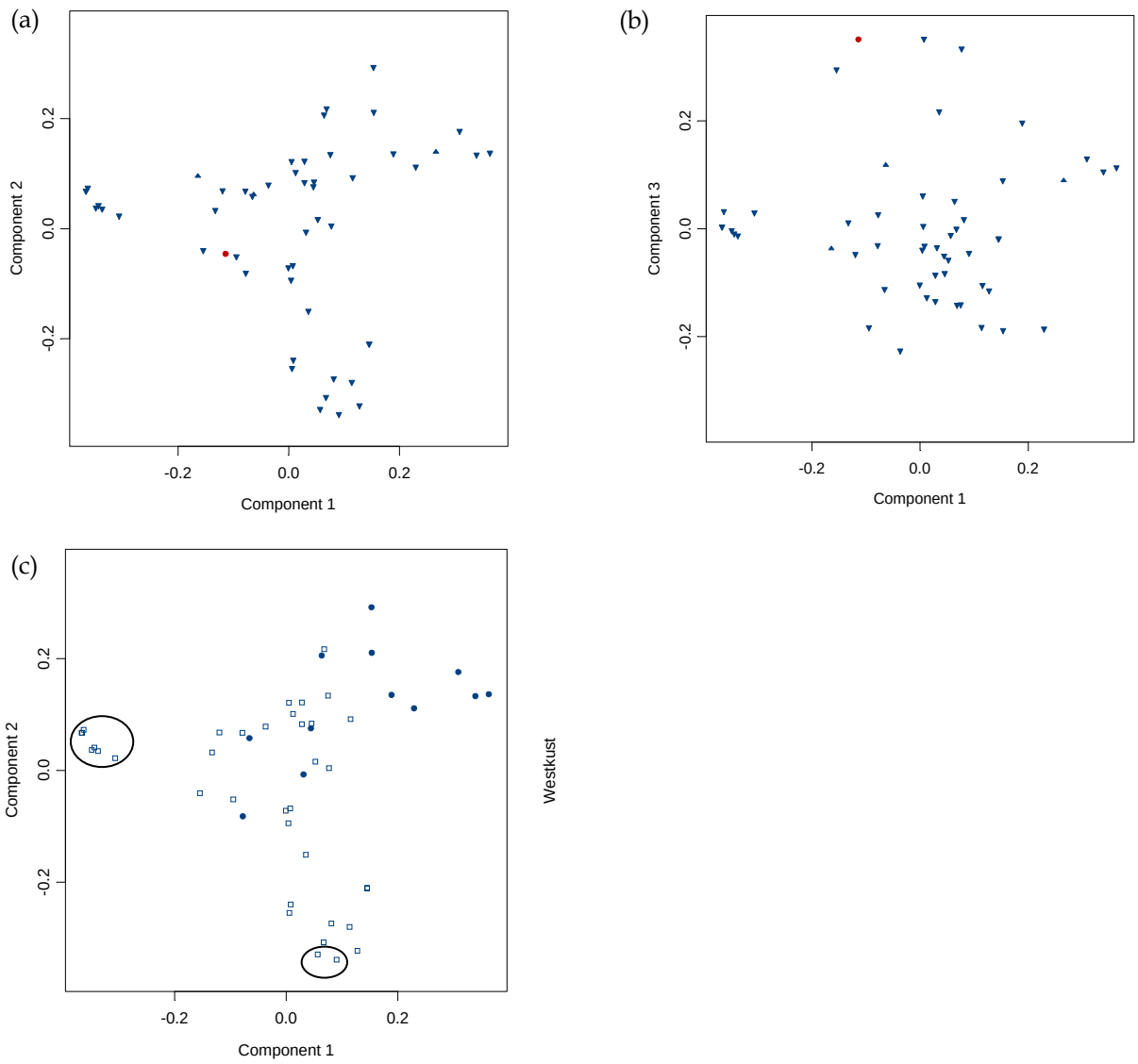


Figure 4.30: PCO plots of the section *Pimpinellifoliae* individuals (a) along the first two components labelled with region of provenance; (b) along the first and third component labelled with region of provenance (Westkust: ▼; Middenkust: ▲; Viroin: ●); (c) PCO plot of the individuals of the section *Pimpinellifoliae* sampled in the region Westkust, along the first two components. Individuals labelled with locality (Oostduinkerke: □; Ter Yde: ●), two clonal genotypes are circled.

AFLPsurv

R. spinosissima is a tetraploid taxon following the Mendelian meiosis. The calculation of the F_{ST} equalled 0.055, calculated according to Lynch and Milligan, suggesting a moderate genetic differentiation between the individuals of the sampled populations.

Jaccard similarity

The similarity within each of the four populations is high, however intrapopulational differentiation was present (Table 4.36). Between the sampled

populations, no large differentiation was observed within and between the regions of provenance or sampled localities.

Table 4.36: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of the section *Pimpinellifoliae*. Region and locality of origin are indicated.

REGION	LOCALITY	MI	OVD	TY	VIR
Middenkust	Middelkerke	0.79			
Westkust	Oostvoornduinen	0.62	0.71		
Westkust	Ter Yde	0.62	0.67	0.75	
Viroin	Viroin	0.61	0.65	0.64	0.86

Dendrogram

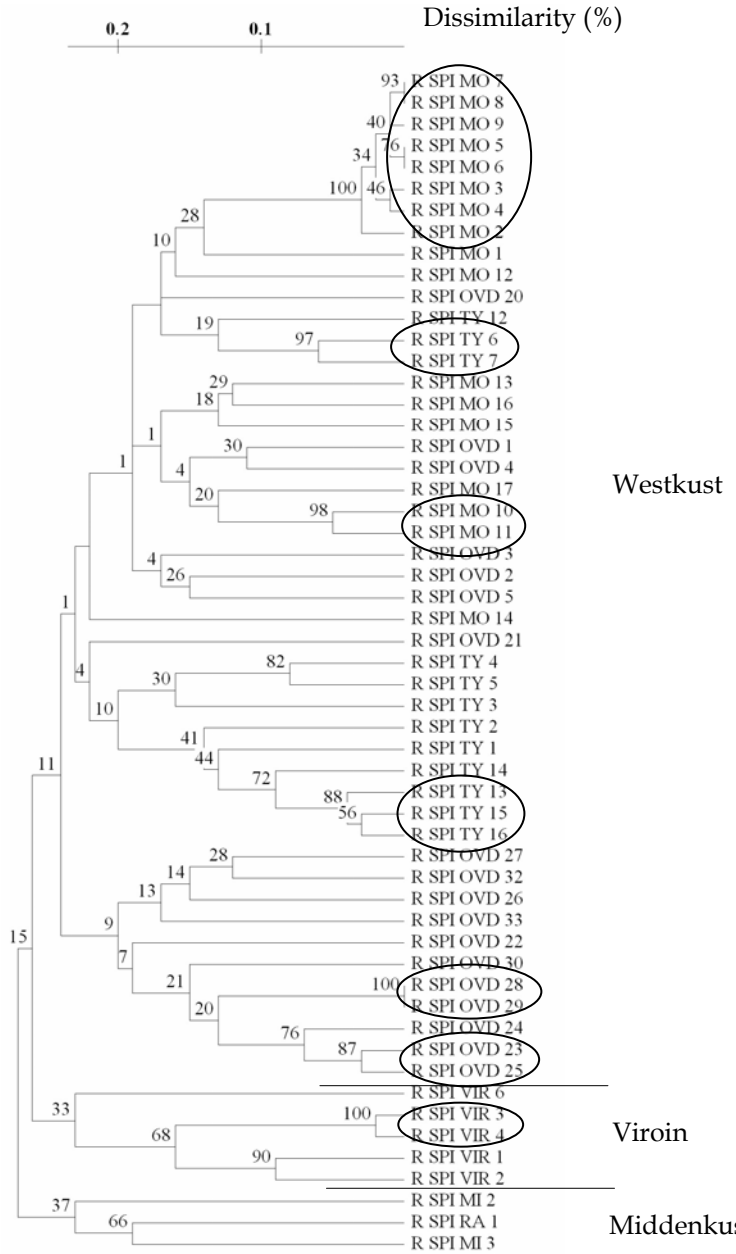


Figure 4.31: UPGMA cluster dendrogram of the section *Pimpinellifoliae*. The distance scale is indicated, clonal genotypes are circled, and individuals are labelled with species names and population codes (Table 4.34).

The sampled individuals were divided into three major clusters (Figure 4.31). Each cluster contained the individuals of one sampled region. The two localities Raverszijde (RA) and Middelkerke (MI) of the region of provenance Middenkust grouped together, the Viroin individuals (VIR) formed another cluster. All the individuals of the intensively sampled Westkust formed the largest cluster. The latter group was subdivided based on the locality. The population of Ter Yde (TY) was mainly separated from the populations of Oostduinkerke (MO and OVD). Individuals with a similarity of at least 95% are assumed to be clones, e.g. R SPI MO 2 to 9, R SPI TY 13, 15 and 16, R SPI OVD 23 and 25, R SPI OVD 28 and 29, and R SPI VIR 3 and 4.

Structure

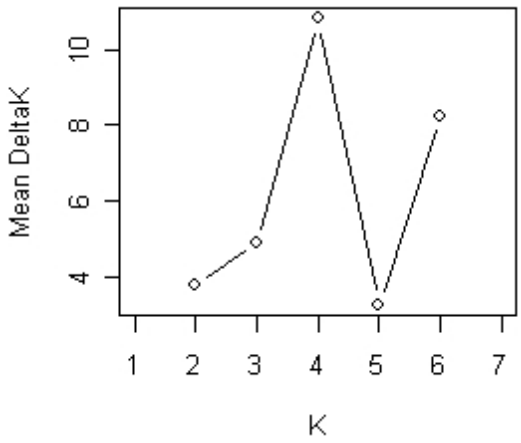


Figure 4.32: Assumption of the optimal number of gene pools present in the Flemish *R. spinosissima* populations, based on Structure (Pritchard *et al.* 2000) and adapted with the method of Evanno *et al.* (2005).

The calculation of the mean DeltaK assigned the individuals to four different gene pools (Figure 4.32). The assignment of the individuals was summarised in table 4.37. Gene pool 1 consisted of all the three individuals sampled at the Middenkust and the whole Ter Yde population (Westkust), with in addition 45% of the population Oostvoornduinen (Westkust). Gene pool 2 contained the completely sampled population of the Viroin, while the two remaining gene pools 3 and 4 each consisted of a smaller proportion of the population Oostvoornduinen (Westkust), 31%, and 22%, respectively.

Table 4.37: Population assignment of *R. spinosissima* to each of the inferred gene pools. Region and locality, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (Ind) are indicated. The presumed GP to which the populations are assigned are marked in bold.

REGION	LOCALITY	GP1	GP2	GP3	GP4	IND
Middenkust	Middelkerke	**1.00**	0.00	0.00	0.00	3
Westkust	Oostvoornduinen	**0.45**	0.02	**0.31**	**0.22**	37
Westkust	Ter Yde	**1.00**	0.00	0.00	0.00	12
Viroin	Viroin	0.00	**1.00**	0.00	0.00	7

Although a moderate genetic interpopulational differentiation was observed, clonality within *R. spinosissima* population was indicated.

The *R. spinosissima* samples originating from Viroin, the only sampled inland population, appeared to be genetically different from the coastal populations.

The most intense sampled population, Oostvoornduinen, also seemed to have the highest level of genetic variation, partly overlapping with the other two coastal populations.

4.3.1.1.4. Section *Synstylae*

R. arvensis is the only wild representative of the section *Synstylae* in Flanders and Belgium. Moreover, it is the only diploid wild rose in Flanders. This taxon is also known to reproduce vegetatively and therefore the clonality within a population was checked.

One hundred and twelve AFLP bands were compared in 69 individuals belonging to three Flemish populations (Brakel 18 individuals, Galgebossen 6 ind and Helleketelbos 19 ind) and one Walloon population (Viroin 26 ind) (Figure A.23).

PCO

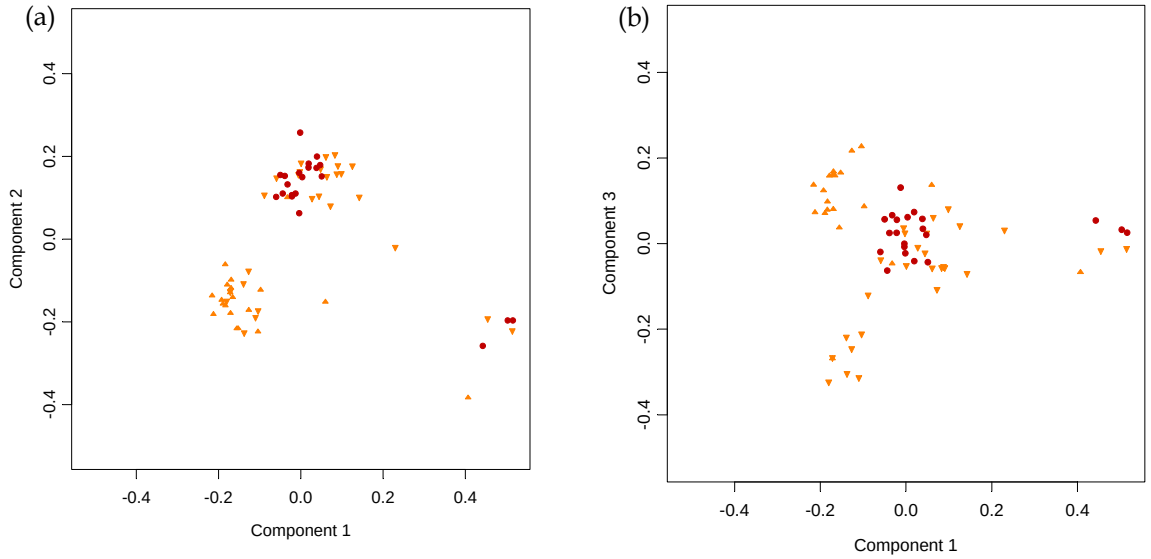


Figure 4.33: PCO plots of (a) the first two components; (b) the first and third component of *R. arvensis* (section *Synstylae*) labelled with region of provenance (West-Vlaams Heuvelland: ▼; Vlaamse Ardennen: ▲; Viroin: ●).

The first three components of the PCO biplot explained 20%, 18%, and 10%, respectively, of the variation and divided the 69 *R. arvensis* individuals into two large and one smaller cluster (Figure 4.33). The upper cluster contained individuals of the localities of West-Vlaams Heuvelland (Helleketelbos and Galgebossen) and the individuals from Viroin. The lower cluster contained all individuals sampled in Vlaamse Ardennen and part of the Helleketelbos (WVH) population. The smallest

cluster displayed a constitution similar to the large upper cluster. Along the third component, the individuals of Vlaamse Ardennen differed even more from the populations West-Vlaams Heuvelland and Viroin.

AFLPsurv

R. arvensis is the only autochthonous diploid taxon in Flanders following the Mendelian meiosis, which allows the calculation of the F_{ST}-value (according to Lynch and Milligan, 1994). The F_{ST} equalled 0.13, suggesting a moderate genetic differentiation between the individuals of the sampled populations.

Jaccard matrix

Table 4.38: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of the subsection *Synstylae*. The region of provenance and locality are indicated.

REGION	LOCALITY	BR	GB	HKB	VIR
Vlaamse Ardennen	Brakel	0.73			
West-Vlaams Heuvelland	Galgebossen	0.54	0.64		
West-Vlaams Heuvelland	Helleketelbos	0.58	0.58	0.61	
Viroin	Nismes	0.56	0.61	0.58	0.61

Within each of the sampled *R. arvensis* populations genetic diversity was observed, among the populations the assessed similarity was comparable (Table 4.38).

Dendrogram

In the dendrogram (Figure 4.34), several subclusters could be identified. In the largest and upper cluster, the individuals of different regions (Viroin and West-Vlaams Heuvelland: Galgebossen and Helleketelbos) were mingled. Apart from this major cluster, two smaller groups were formed. One contained part of the Helleketelbos population (WVH), while the other cluster consisted of all the individuals sampled at Brakel (Vlaamse Ardennen).

Based on the generally accepted threshold of clonality, few clones were observed, e.g. the samples R ARV HKB 4 and 6, R ARV HKB 10 and 11, R ARV HKB 7 and 9, R ARV BR 14 to 17 and R ARV BR 27 and 28 each displayed a similarity of at least 95%.

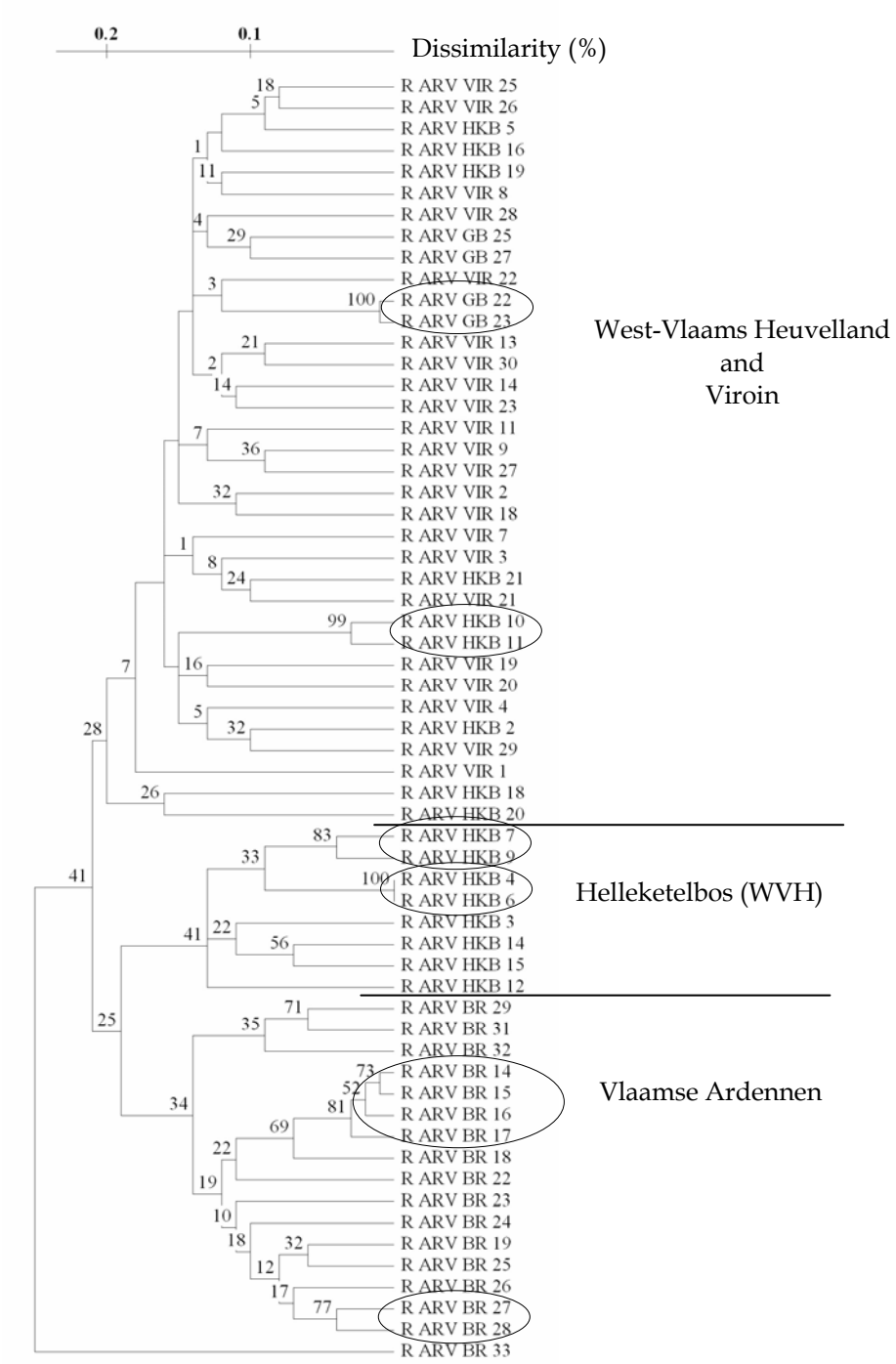


Figure 4.34: UPGMA cluster dendrogram of the section *Synstylae*, *R. arvensis*. The distance scale is indicated, clonal genotypes are circled, individuals are labelled with species names and population codes (Table 4.34).

Structure

The mean DeltaK calculations suggested the assignment of the sampled *R. arvensis* in three gene pools (Figure 4.35 and Table 4.39). Gene pool 1 consisted of 94% of the population from Brakel and about half of that of Helleketelbos. Gene pool 2 contained the majority of the genotypes of Galgebossen (83%) and Viroin (89%) and about half of the samples of Helleketelbos. The third gene pool comprised the

remaining individuals: 17% of Galgebossen, 6% of both Helleketelbos and Brakel and only 1% of Viroin.

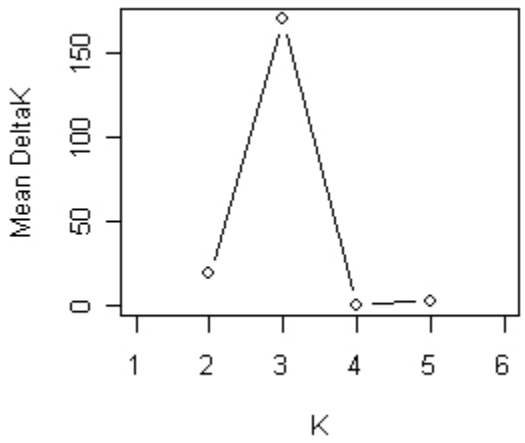


Figure 4.35: Assumption of the optimal number of gene pools present in the Flemish *R. arvensis* populations, based on Structure (Pritchard *et al.* 2000) and adapted with the method of Evanno *et al.* (2005).

Table 4.39: The population assignment of *R. arvensis* to each of the inferred gene pools. Region of provenance and locality, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (Ind) are indicated. The presumed GP to which the populations are assigned are marked in bold.

REGION	LOCALITY	GP1	GP2	GP3	IND
West-Vlaams Heuvelland	Galgebossen	0.00	**0.83**	0.17	6
West-Vlaams Heuvelland	Helleketelbos	**0.47**	**0.47**	0.06	19
Vlaamse Ardennen	Brakel	**0.94**	0.00	0.06	18
Viroin	Viroin	0.00	**0.89**	0.11	26

Comparing a set of AFLP polymorphisms, genetic differentiation was observed between the analysed *R. arvensis* populations. More specifically, *R. arvensis* from Brakel (Vlaamse Ardennen) and part of the Helleketelbos population (West-Vlaams Heuvelland) were clearly different from their analysed congeners.

The presence of clonality is confirmed, however in each of the sampled populations genetic differentiation was also observed.

4.3.1.1.5. The Flemish section *Caninae*

According to the taxonomical structure of Henker (2000), this section contains five subsections and numerous taxa and hybrids. The two subsections *Vestitae* and *Tomentellae* are monotypic in Flanders, only represented by *R. tomentosa* and *R. balsamica*, respectively. At the moment, the existence of the subsection *Tomentellae* is subject of discussion. The Flemish subsection *Rubigineae* contains three taxa: *R. rubiginosa*, *R. micrantha*, *R. agrestis,* and the hybrid: *R. henkeri-schulzei*. Finally, the subsection *Caninae* consists of *R. canina*, *R. corymbifera*, *R. caesia,* and *R. stylosa.* However, the latter two taxa were not included in the global analyses. However, *R.*

stylosa was analysed in a separate subset in order to assess the origin of this presumed hybrid species.

PCO

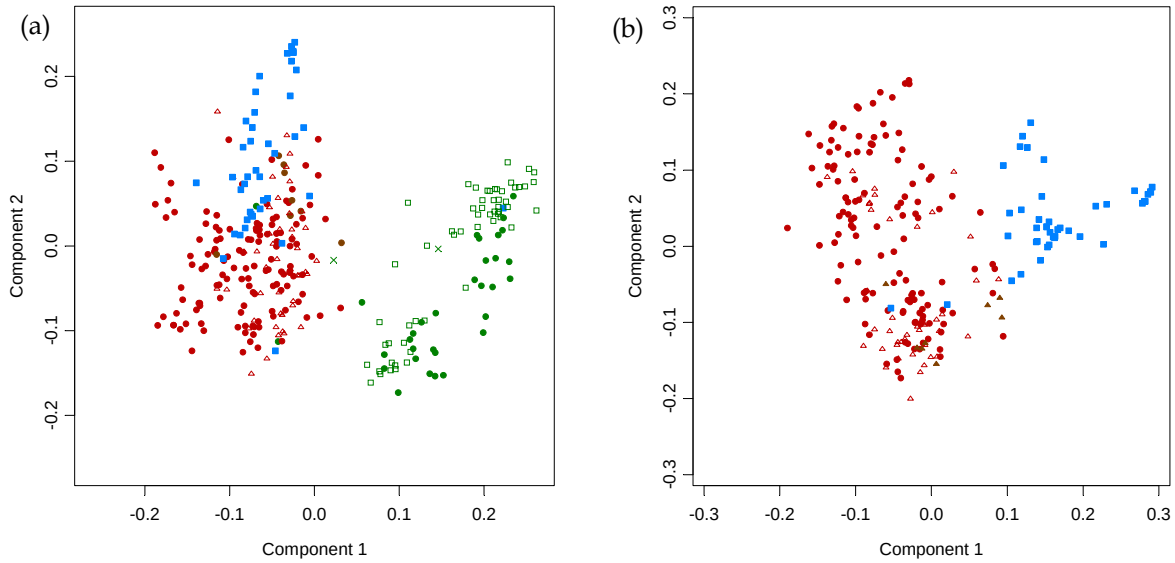


Figure 4.36: PCO plots of the first two components of (a) the section *Caninae*; (b) the subsections *Vestitae*, *Caninae* and *Tomentellae* based on AFLP markers. With: subsection *Rubigineae* (Green); subsection *Vestitae* (Blue); subsection *Tomentellae* (Brown); subsection *Caninae* (Red). The detailed species labels can be found in figure 4.2.

Table 4.40: Number of Flemish individuals for each analysed subset of the section *Caninae* and the percentage of variance explained by the first three components.

SUBSECTIONS ANALYSED	IND	COMP 1	COMP 2	COMP 3
Subsections *Rubigineae*, *Vestitae*, *Caninae* and *Tomentellae*	316	18%	10%	9%
Subsections *Vestitae*, *Caninae* and *Tomentellae*	224	14%	11%	9%
Subsections *Caninae* and *Tomentellae*	177	14%	11%	8%

Focussing on the compact cluster of the polymorphic section *Caninae*, the subsection *Rubigineae* was the most differentiated and formed a well-defined subcluster in the section *Caninae* (Figure 4.36 and Table 4.40). Excluding the subsection *Rubigineae*, similar analyses were performed subdividing the subsection *Vestitae* (Figure 4.36b and Table 4.40). Compared to the subdivision of the subsection *Rubigineae*, the subsection *Vestitae* showed more overlap with the remaining two subsections, but differentiation was confirmed. Finally, PCO analyses were performed on the two remaining subsections: *Caninae* and *Tomentellae*, but no subsection- or species-based pattern was detected (Table 4.40, biplot similar to Figure 4.12c).

Jaccard similarity

Given the discussion about the taxonomical structure within the section *Caninae*, the similarity analyses were performed at two hierarchical levels: the subsection and the taxon level.

Table 4.41: Mean Jaccard similarity coefficients (%) calculated within and between the subsections of the section *Caninae*.

SUBSECTION	*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

Table 4.42: Mean Jaccard similarity coefficients (%) calculated within and between the taxa of the section *Caninae*.

TAXON	AGR	CAN	CANand	HEN	COR	MIC	RUB	BAL	TOM
R. agrestis	0.78								
R. canina	0.61	0.66							
R. canina var. *andegavensis*	0.55	0.54	1.00						
R. henkeri-schulzei	0.63	0.57	0.49	0.60					
R. corymbifera	0.64	0.64	0.54	0.59	0.69				
R. micrantha	0.50	0.56	0.55	0.49	0.53	1.00			
R. rubiginosa	0.66	0.61	0.55	0.60	0.62	0.57	0.70		
R. balsamica	0.65	0.65	0.51	0.63	0.69	0.54	0.62	0.79	
R. tomentosa	0.66	0.66	0.55	0.60	0.67	0.53	0.64	0.67	0.81

Little to no difference in similarity was assessed between the different subsections (Table 4.41), or between the different taxa (Table 4.42) of the section *Caninae*.

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to make the dendrogram better readable.

The major subclusters of the dendrogram (Figure 4.37) could be identified as one of the subsections of the section *Caninae*. Three of these clusters were identified as the subsections *Rubigineae, Vestitae,* and *Caninae*. The fourth cluster consisted of individuals belonging to the subsections *Caninae* and *Tomentellae* originating from Het Zwin (Oostkust).

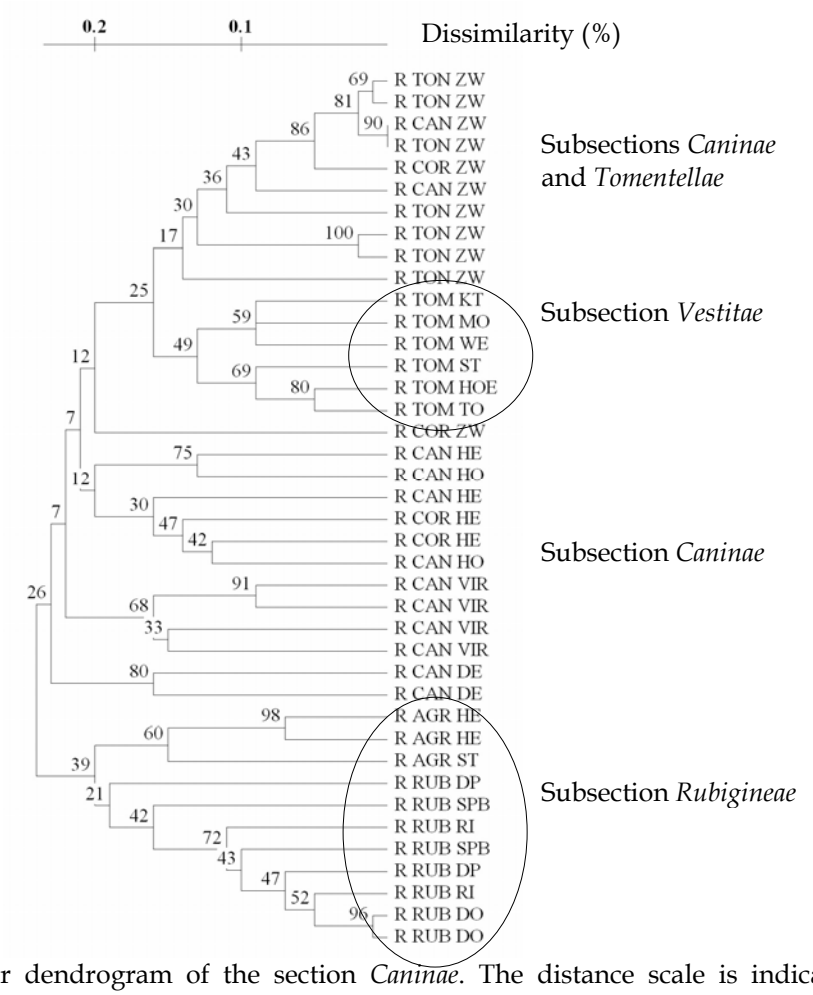


Figure 4.37: UPGMA cluster dendrogram of the section *Caninae*. The distance scale is indicated, individuals are labelled with species names and locality or region codes (Tables 4.2 and 4.34). The subsections *Rubigineae* and *Vestitae* are marked with a circle.

Structure

Table 4.43: The species assignment of the section *Caninae* to each of the inferred gene pools. Subsection and species determination, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

SUBSECTION	TAXON	GP1	GP2	IND
Rubigineae	*R. rubiginosa*	**0.86**	0.14	95
	R. micrantha	0.14	**0.86**	14
	R. agrestis	**0.98**	0.02	41
Vestitae	*R. tomentosa*	**0.91**	0.09	81
	R. villosa	0.57	0.43	7
Tomentellae	*R. balsamica*	**0.93**	0.07	15
Caninae	*R. canina*	**0.82**	0.18	218
	R. corymbifera	**0.93**	0.08	80
	R. stylosa	0.00	**1.00**	12
	R. subcollina	0.00	**1.00**	1
Hybrids	*R. canina* x *R. stylosa*	0.00	**1.00**	3
	R. henkeri-schulzei	**1.00**	0.00	3

Calculating the mean DeltaK, no optimal number of clusters could be assessed within the Flemish section *Caninae*. Both one and two gene pools could be present. Assuming two gene pools, the assignment of the individuals is shown in table 4.43. Gene pool 1 contained the majority (> 82%) of the individuals of the section *Caninae*, with exception of *R. villosa* (57%), *R. micrantha* (14%), and the complete absence of *R. stylosa*, *R. canina* x *R. stylosa*, and *R. subcollina*. The latter three were completely assigned to the second gene pool, with in addition 86% of *R. micrantha*, 43% of *R. villosa* and less than 20% of the other taxa.

In the Flemish section *Caninae*, different subsections might be identified. As mentioned in the part with the European section *Caninae* and in the analyses of the whole subgenus *Rosa*, the subsection *Rubigineae* was the most distinguished from the other subsections, followed by the subsection *Vestitae*. However, the latter showed overlap with the remaining subsections *Caninae* and *Tomentellae*. Within the latter two subsections no distinction could be made.

Remarkable was the lack of differentiation within the section *Caninae* based on the assignment test.

4.3.1.1.6. Subsection *Rubigineae*

Analyses of the subsection *Rubigineae* were performed with 122 polymorphic AFLP markers on a total of 151 Flemish individuals representing *R. rubiginosa* (4 analysed populations), *R. micrantha* (3 pop), *R. agrestis* (3 pop), and *R. henkeri-schulzei* (1 pop) (Figure A.24).

PCO

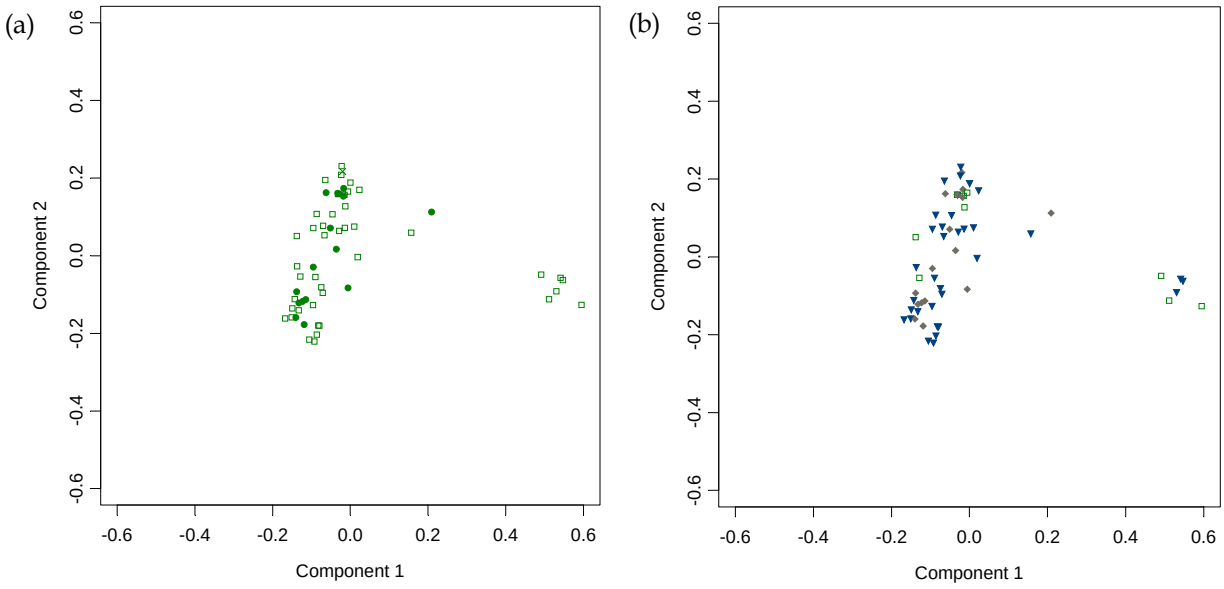


Figure 4.38: PCO plots of first two components of the Flemish subsection *Rubigineae*. (a) Individuals labelled with species determination (*R. rubiginosa*: □; *R. micrantha*: ▲; *R. agrestis*: ●; *R. henkeri-schulzei*: X).; (b) individuals labelled with region of provenance (Westkust: ▼; Brabants District Oost: ◆; Maasvallei: □).

The first three components explained 33%, 16%, and 12%, respectively, of the variation present in the Flemish *Rubigineae* (Figure 4.38). However, no differentiation patterns were found between the individuals, not based on taxonomical structure, or on locality or region of provenance.

Jaccard matrix

Table 4.44: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations (Taxon-Locality) of the subsection *Rubigineae*. The intraspecific similarity coefficients (Italics) and the lowest similarity coefficients (bold) are marked. For the locality see table 4.34.

TAXON		*R. AGRESTIS*			*R. HEN*	*R. MIC*		*R. RUBIGINOSA*			
	LOCALITY	HE	ST	VAR	HE	SPB	WVH	DO	DP	RI	SPB
R. agrestis	Heers	*0.79*									
R. agrestis	St-Truiden	*0.71*	*0.85*								
R. agrestis	Vlaamse Ardennen	*0.50*	*0.50*	*1.00*							
R. henkeri-schulzei	Heers	0.64	0.57	0.46	*0.82*						
R. micrantha	St-Pietersberg	**0.43**	**0.46**	**0.53**	**0.44**	*0.80*					
R. micrantha	WVl Heuvelland	**0.44**	**0.45**	**0.59**	**0.45**	*0.66*	*0.80*				
R. rubiginosa	Doornpanne	0.64	0.65	0.41	0.63	**0.39**	**0.37**	*0.86*			
R. rubiginosa	De Panne	0.70	0.70	0.53	0.66	**0.47**	**0.49**	*0.70*	*0.82*		
R. rubiginosa	Riemst	0.61	0.61	0.41	0.59	**0.36**	**0.35**	*0.84*	*0.65*	*0.94*	
R. rubiginosa	St-Pietersberg	**0.49**	**0.51**	**0.52**	**0.49**	0.64	0.59	***0.49***	***0.55***	***0.47***	*0.61*

As the similarity coefficients among the populations of *R. micrantha* and the other taxa appeared to be the lowest (Table 4.44), they indicated that the taxon *R. micrantha* tended towards a more distinct position within the subsection *Rubigineae*. This tendency might be confirmed by the higher similarity of *R. micrantha* with the population *R. rubiginosa* St-Pietersberg and in addition by a lower similarity of the *R. rubiginosa* St-Pietersberg populations compared to his congeners. The sampled locality, St-Pietersberg, contained the mixed presence of *R. micrantha*, *R. rubiginosa,* and their presumed hybrids.

Dendrogram

In the cluster analysis of the subsection *Rubigineae* and apart from some outliners, two well-defined subclusters were formed (Figure 4.39). The upper cluster only consisted of *R. rubiginosa*, while the lower was exclusively formed by *R. agrestis* (indicated with circle). The majority of the mixed population St-Pietersberg was the most distinct within the subsection *Rubigineae*.

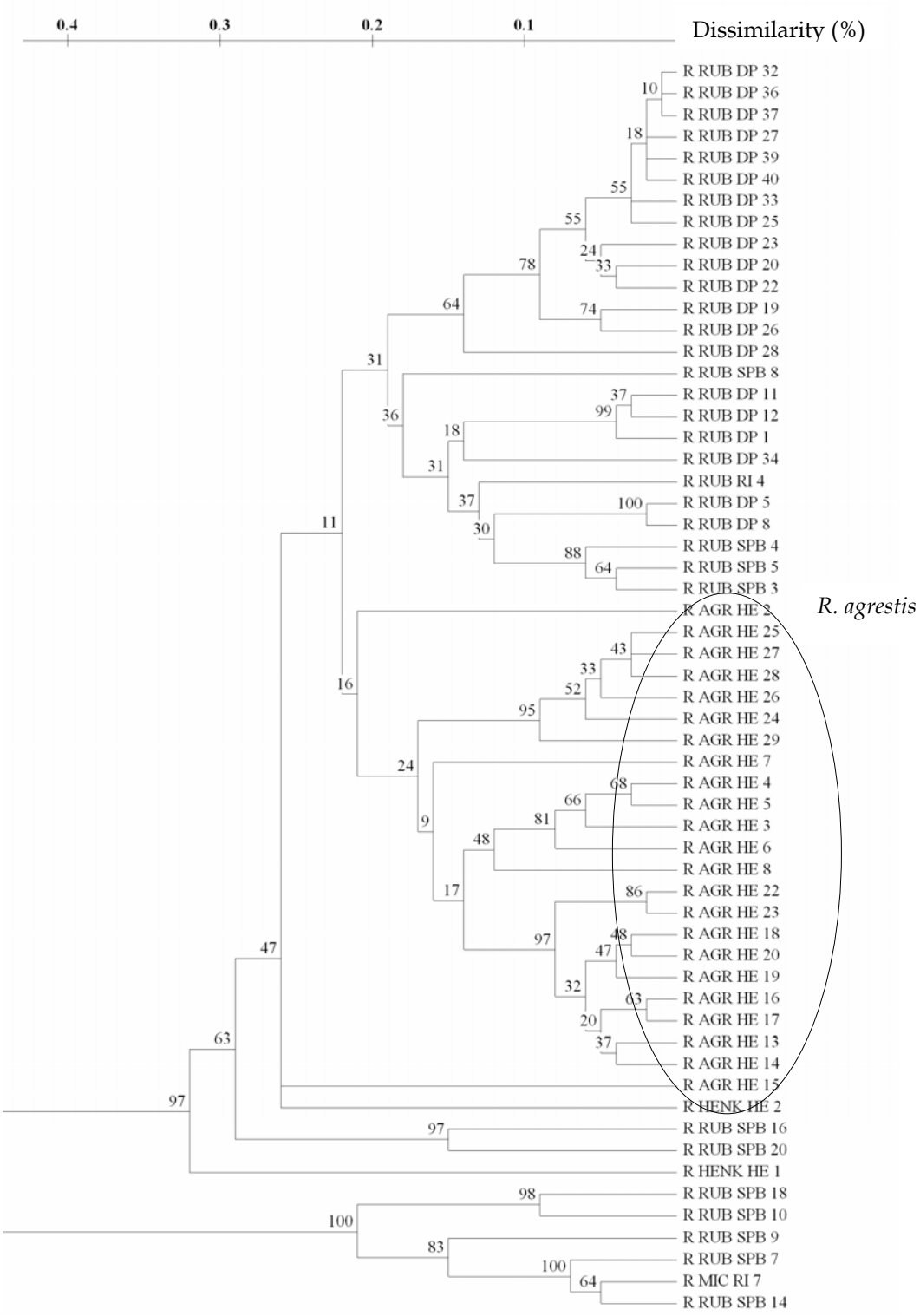


Figure 4.39: UPGMA cluster dendrogram of the subsection *Rubigineae*. The distance scale is indicated, individuals are labelled with species names, population codes and individual numbers (Tables 4.2 and 4.34).

Structure

Following the method of Evanno *et al.* (2005), it was not possible to decide if the subsection *Rubigineae* could be divided into one or two gene pools. Assuming

that there are two gene pools, table 4.45 showed the assignment of the populations. Gene pool 1 contained all three sampled *R. agrestis* populations (Heers, Riemst and St-Truiden), *R. micrantha* originating from Riemst and the two *R. rubiginosa* populations sampled at Westkust (Doornpanne and De Panne). In addition, 77% of the *R. rubiginosa* population of Riemst and 44% from St Pietersberg were also assigned to this gene pool. The second gene pool consisted of the other two *R. micrantha* populations (St-Pietersberg and West-Vlaams Heuvelland) and also 17%, AND 55% of *R. rubiginosa* originating from Riemst and St Pietersberg, respectively.

Interesting is the division of the population *R. rubiginosa* from St-Pietersberg into the two inferred gene pools.

Table 4.45: Population assignment of subsection *Rubigineae* to each of the inferred gene pools. Species determination, region of provenance and locality, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold, the population inferred to both GPs is marked in red.

TAXON	REGION	LOCALITY	GP1	GP2	IND
R. rubiginosa	Maasvallei	Riemst	**0.83**	0.17	4
R. rubiginosa	Maasvallei	St-Pietersberg	**0.45**	**0.55**	23
R. rubiginosa	Westkust	Doornpanne	**1.00**	0.00	30
R. rubiginosa	Westkust	De Panne	**1.00**	0.00	38
R. micrantha	Maasvallei	Riemst	**1.00**	0.00	2
R. micrantha	Maasvallei	St-Pietersberg	0.00	**1.00**	10
R. micrantha	West-Vlaams Heuvelland	West-Vlaams Heuvelland	0.00	**1.00**	1
R. agrestis	Brabants District Oost	Heers	**1.00**	0.00	29
R. agrestis	Maasvallei	Riemst	**1.00**	0.00	1
R. agrestis	Brabants District Oost	St-Truiden	**1.00**	0.00	10
R. henkeri-schulzei	Brabants District Oost	Heers	**1.00**	0.00	3

The AFLP results of the Flemish subsection *Rubigineae* indicated that *R. micrantha* should be the most distinct of the three *Rubigineae* taxa. However the results of the European subsection *Rubigineae* and the subtle morphological differences between *R. rubiginosa* and *R. micrantha* contradict this outcome.

Remarkable was also the high similarity of the individuals all belonging to the mixed population St-Pietersberg, irrespective of the species determination. Moreover, the *R. rubiginosa* of St-Pietersberg was almost equally divided into both gene pools.

In conclusion, we assumed that there is no taxon or geographical pattern within or among the populations of the Flemish subsection *Rubigineae*. Nevertheless, the individuals of the St-Pietersberg tended towards a more specific genetic position.

4.3.1.1.7. Subsection *Vestitae*

Within Flanders, *R. tomentosa* and *R. pseudoscabriuscula* were the two autochthonous representatives of the subsection *Vestitae*. However, based on the species descriptions of Henker (2000), the Flemish *Vestitae* could not be assigned to one of the two taxa consistently (§4.3.2.1.2. Intraspecific variation, *R. tomentosa*). Therefore, all 58 individuals were determined as *R. tomentosa* and 74 polymorphic

AFLP markers were compared. The sampled populations are indicated in figure A.25.

PCO

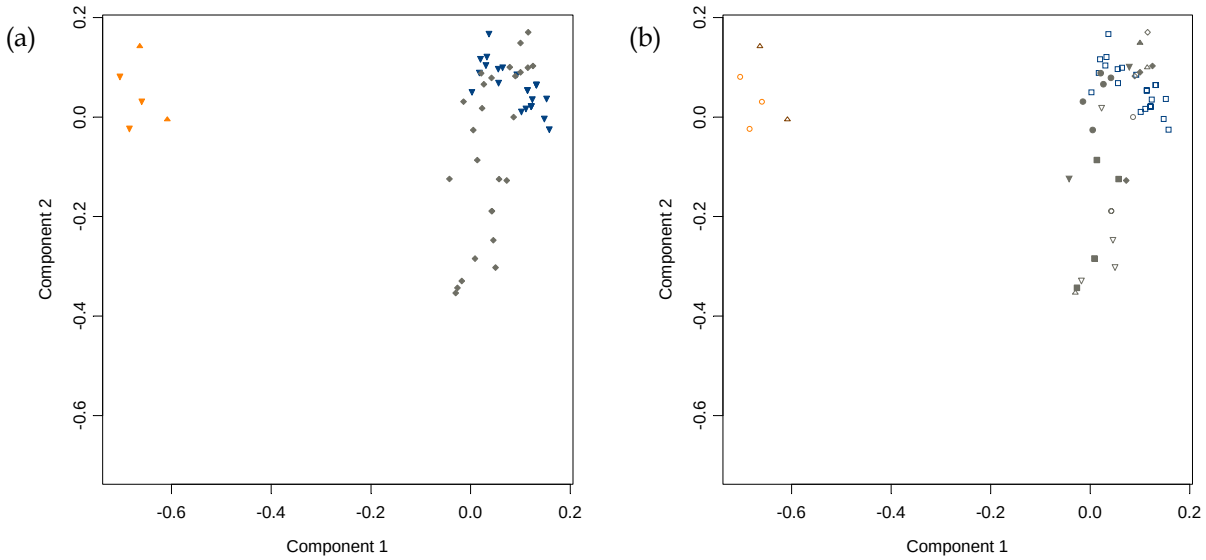


Figure 4.40: PCO plots of the first two components of *R. tomentosa*; these explained 57% of the variation. (a) Individuals labelled with region of provenance (Westkust: ▼; Brabants District Oost: ◆; West-Vlaams Heuvelland: ▼; Vlaamse Ardennen: ▲); (b) labelled with locality (West-Vlaams Heuvelland:○; Brakel: △; Monoblocduinen and Oostvoornduinen: □; Heers: ●; Kortessem: ◆; Kortenberg: ▼; Zemst: ◇; Hoeselt: ▲; Wellen: ○; Hoegaarden: ■; St-Truiden: ▽; Tongeren: △).

Based on 74 polymorphic AFLP markers, the first three components explained 42%, 15%, and 10%, respectively, of the variation. Along the first axis, two clusters were formed based on region of provenance (Figure 4.40). The individuals of West-Vlaams Heuvelland and Vlaamse Ardennen clustered together on the left, while those from the Westkust and Brabants District Oost also grouped together. The population of Brabants District Oost seemed to be more differentiated compared to the Westkust population. Within a region of provenance no locality differentiation was observed.

Jaccard matrix

The similarity between the populations of Vlaamse Ardennen and West-Vlaams Heuvelland was higher compared to the other sampled *R. tomentosa* populations. A high similarity was also observed among the populations of Brabants District Oost and Westkust, and among the different populations of Brabants District Oost (Table 4.46).

Table 4.46: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of *R. tomentosa*. The most distinct populations are indicated in bold.

REGION		VAR	BDO								WVH	WKU
	LOCALITY	BR	HE	HOE	HT	KO	KT	ST	TO	WE	ZE	WVH	OVD
VAR	Brakel	**0.87**											
BDO	Heers	0.44	0.85										
BDO	Hoegaarden	0.36	0.65	0.81									
BDO	Hoeselt	0.40	0.80	0.66	1.00								
BDO	Kortessem	0.42	0.76	0.68	0.79	0.84							
BDO	Kortenberg	0.39	0.77	0.69	0.85	0.77	0.90						
BDO	St-Truiden	0.39	0.77	0.67	0.84	0.75	0.83	0.85					
BDO	Tongeren	0.37	0.68	0.73	0.69	0.71	0.72	0.71	0.85				
BDO	Wellen	0.38	0.74	0.73	0.77	0.73	0.77	0.75	0.77	0.81			
BDO	Zemst	0.39	0.71	0.69	0.75	0.72	0.76	0.74	0.72	0.74	0.87		
WVH	WVl Heuvelland	**0.73**	0.41	0.40	0.39	0.41	0.39	0.39	0.42	0.40	0.38	**0.91**	
WKU	Oostvoornduinen	0.38	0.77	0.67	0.89	0.79	0.82	0.79	0.68	0.76	0.76	0.38	0.95

Dendrogram

The analysed *R. tomentosa* individuals were divided into two major clusters (Figure 4.41). The individuals sampled in West-Vlaams Heuvelland and Brakel (Vlaamse Ardennen) clustered together in the smallest (and lowest) group, in which the population of the West-Vlaams Heuvelland formed a subcluster. The second cluster was formed by the individuals from Brabants District Oost and Westkust. In the latter, no patterns of origin were detected.

Moreover, within the Flemish *Vestitae* a high degree of clonality was observed, e.g. R TOM MO 31, 32 and 38, R TOM WE 21 and 22.

Structure

Following the analyses of Structure, the Flemish *Vestitae* complex could consist of one or two major clusters. The assignment of the individuals considering two gene pools was summarised in table 4.47. When the sampled *R. tomentosa* individuals were divided into two different gene pools, the individuals sampled at Brakel (Vlaamse Ardennen) and West-Vlaams Heuvelland were assigned to one gene pool, while all the other *R. tomentosa* individuals originating from Brabants District Oost and Westkust were grouped in the other gene pool.

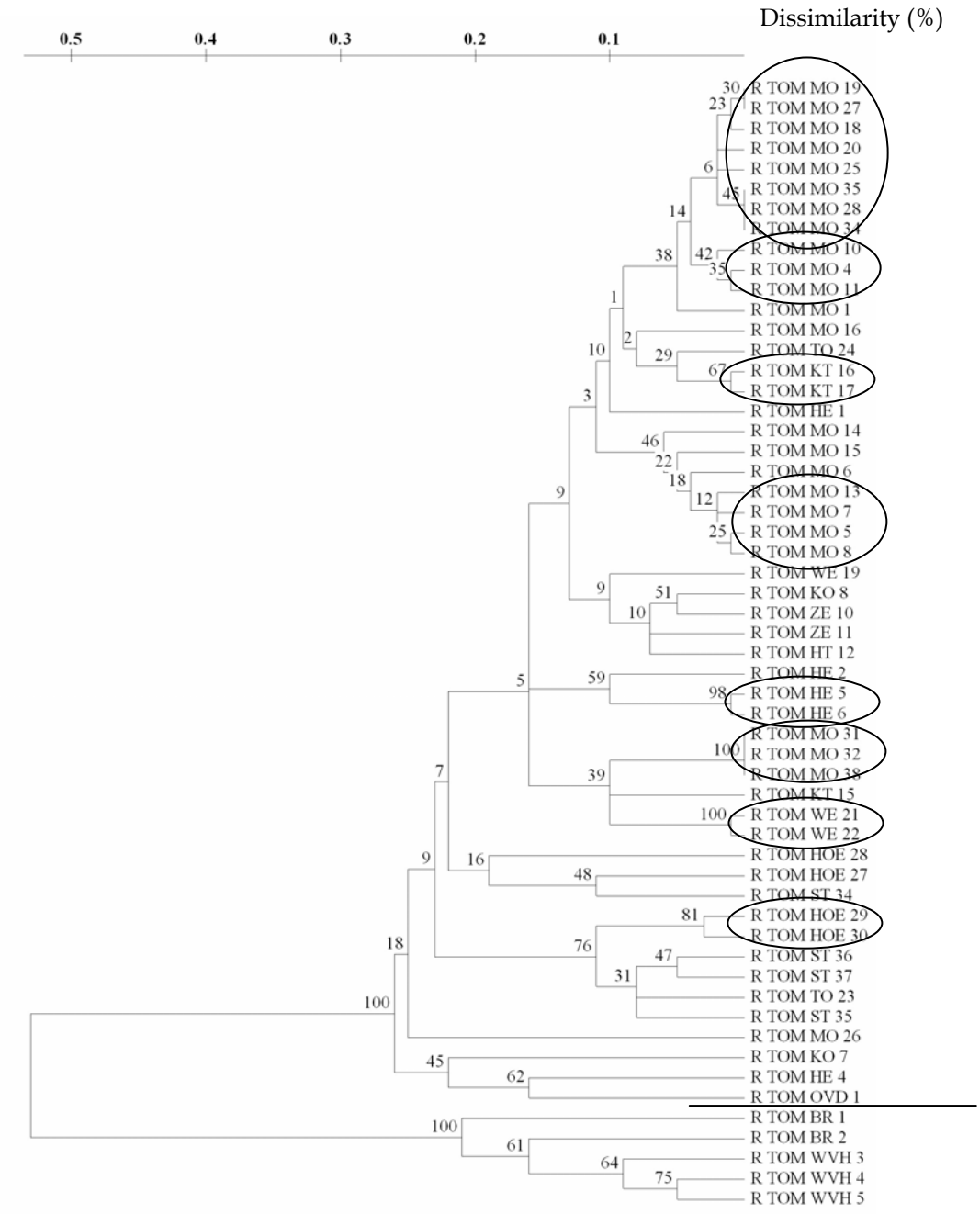


Figure 4.41: UPGMA cluster dendrogram of *R. tomentosa*. The distance scale is indicated, clonal genotypes are circled and individuals are labelled with species names, population codes and individual numbers (Tables 4.2 and 4.34).

Table 4.47: Population assignment of *R. tomentosa* to each of the inferred gene pools. Region of provenance and locality, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

REGION	LOCALITY	GP1	GP2	IND
Westkust	Monoblocduinen	0.00	**1.00**	24
	Oostvoornduinen	0.00	**1.00**	1
Vlaamse Ardennen	Brakel	**1.00**	0.00	2
West-Vlaams Heuvelland	WVH	**1.00**	0.00	3
Brabants District Oost	Heers	0.00	**1.00**	5
	Kortenberg	0.00	**1.00**	2
	Zemst	0.00	**1.00**	2
	Hoeselt	0.00	**1.00**	2
	Kortessem	0.00	**1.00**	4
	Wellen	0.00	**1.00**	3
	Tongeren	0.00	**1.00**	2
	Hoegaarden	0.00	**1.00**	4
	St-Truiden	0.00	**1.00**	4

All the analyses based on the AFLP polymorphisms indicated the presence of a geographical differentiation within the Flemish *Vestitae* according to the regions of provenance. The populations originating from West-Vlaams Heuvelland and Vlaamse Ardennen showed a high similarity, whereas the populations from Brabants District Oost and Westkust also tended to be genetically similar.

Moreover, the clonality with the sampled populations was unexpectedly high.

4.3.1.1.8. Subsections *Caninae* and *Tomentellae*

Based on the analyses of the European section *Caninae*, the subsections *Caninae* and *Tomentellae* did not differentiate. Consequently, these subsections were analysed together. Of the most common taxa of the Flemish subsection *Caninae*, *R. canina* (13 populations) and *R. corymbifera* (5 pop) were sampled. In addition, of *R. balsamica* (syn. *R. tomentella*), the only Flemish taxon of the subsection *Tomentellae*, only 1 population was included (Figure A.26).

PCO

The first three components, based on 106 polymorphic AFLP markers, explained 34%, 10%, and 8%, respectively, of the variation in the subsections *Caninae* and *Tomentellae*. The PCO analysis confirmed the overlap of both subsections, moreover the individuals were divided into three well-separated clusters (Figure 4.42a). Each cluster was characterised by a combination of species determination and locality (shown for each taxon in Figure 4.43).

The most dense cluster, above to the right (Figure 4.42a), consisted of all *R. canina* sampled at Maasvallei, all *R. canina* and *R. corymbifera* originating from

Brabants District Oost, part of the Oostkust population (*R. canina*, *R. corymbifera* and *R. balsamica*) and little *R. canina* individuals sampled at Vlaamse Zandstreek (Deinze).

The two other clusters were mainly characterised by only one population. The lowermost cluster was exclusively formed by *R. canina* from Vlaamse Zandstreek (Deinze), whereas most of *R. canina*, *R. corymbifera,* and *R. balsamica* sampled at the Oostkust and some of the Vlaamse Zandstreek (*R. canina*) grouped in the third cluster.

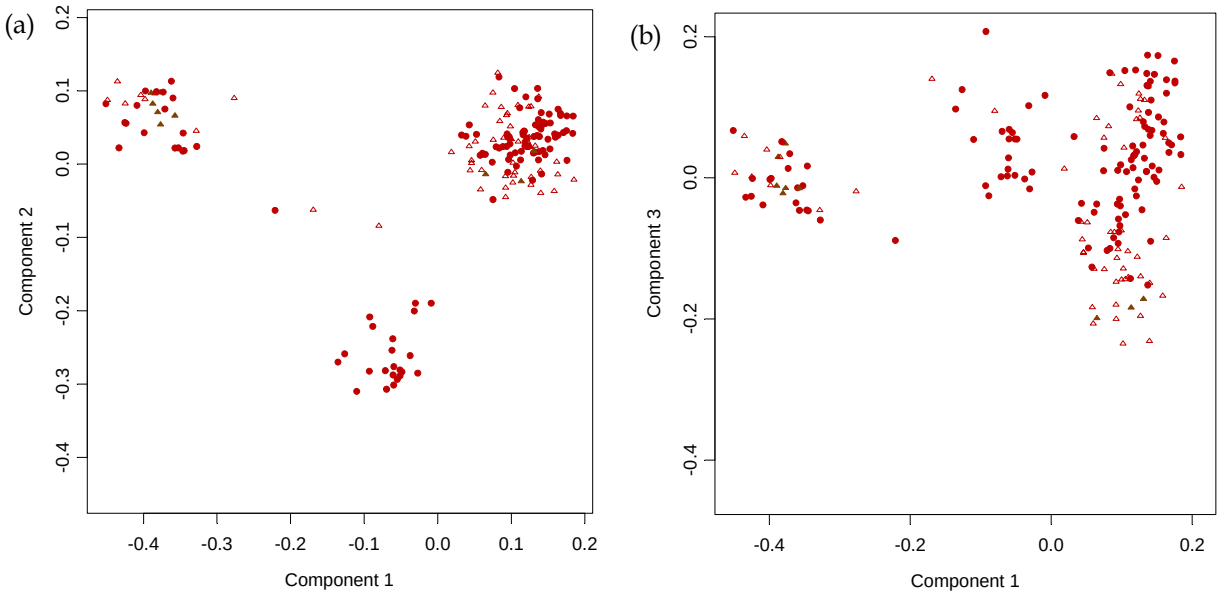


Figure 4.42: PCO plots of (a) the first two and (b) the first and third component of the subsections *Caninae* and *Tomentellae*. Individuals were labelled with species determination (*R. canina*: ●; *R. corymbifera*: △; *R. balsamica*: ▲).

Jaccard matrix

The similarity coefficients suggested that the localities of origin might be more important than the species determination based on the morphology. The similarity between the populations *R. canina*, *R. corymbifera,* and *R. balsamica* all sampled at Het Zwin was remarkably higher compared to their congeners sampled at other localities (Table 4.48, bold). For instance, the similarity between the populations *R. canina* Deinze and *R. canina* Zwin equalled 72%, while the populations *R. corymbifera* Zwin and *R. canina* Zwin were similar for 87%. The same was observed for *R. balsamica* Zwin. Moreover, this output confirmed the lack of boundaries between the Flemish subsections *Caninae* and *Tomentellae*.

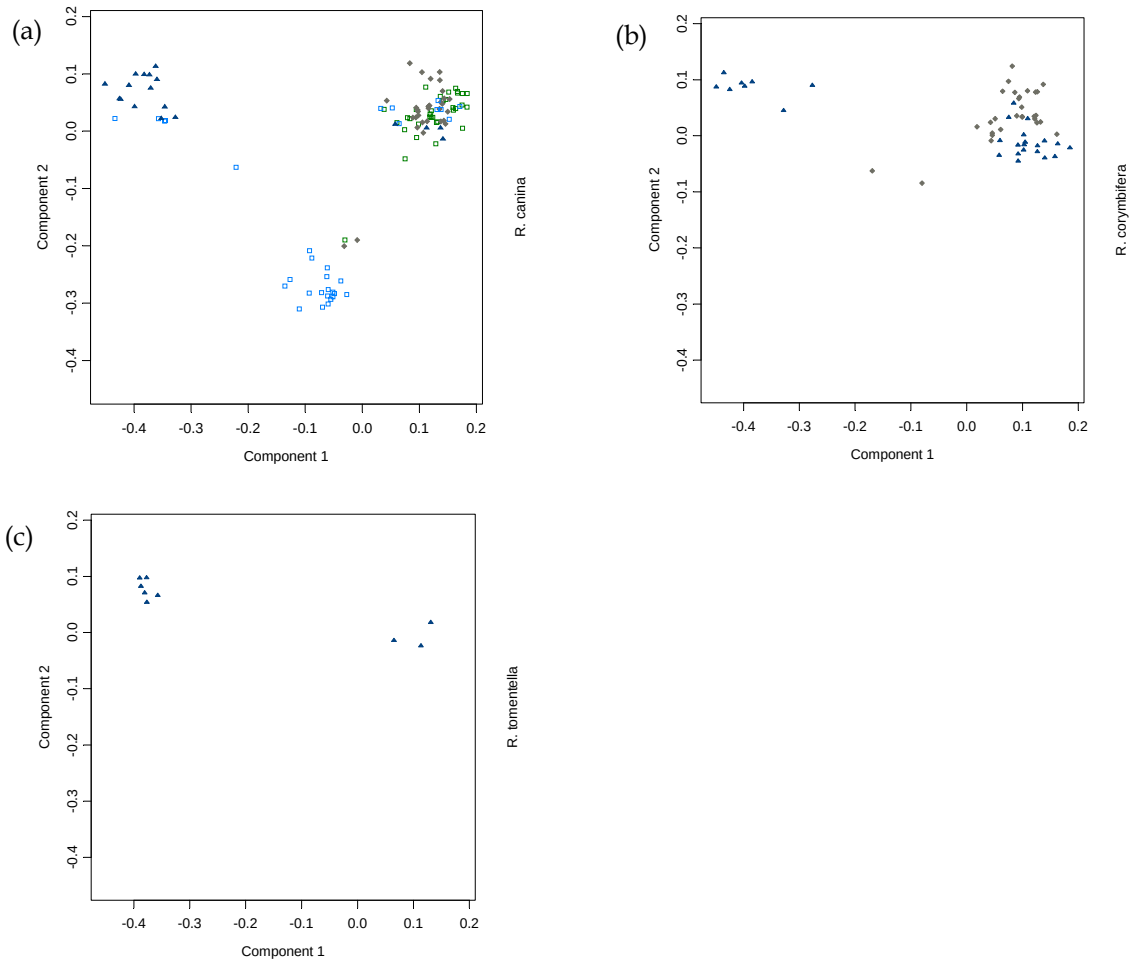


Figure 4.43: PCO plots of first two components of (a) *R. canina;* (b) *R. corymbifera*; and (c) *R. balsamica* (syn: *R. tomentella*). Individuals labelled with region of provenance (Oostkust: ▲; Vlaamse Zandstreek (Deinze): □; Maasvallei: □; Brabants District Oost: ◆).

Table 4.48: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations of the subsections *Caninae* and *Tomentellae*. Intraspecific (Italics) and high interspecific (bold) similarity coefficients are indicated. Used abbr.: WVH: West-Vlaams Heuvelland; VAR: Vlaamse Ardennen; BLG: Balegem; VZS: Vlaamse Zandstreek; BDO: Brabants District Oost; MV: Maasvallei; HO: Hochter Bampd; MA: Maldegem; SPB: St-Pietersberg; VIR: Viroin; OKU: Oostkust; HEM: Hemelveerdegem.

			R. canina													*R. corymbifera*					*R bal*
TAXON	REGION	LOCALITY	WVH	BLG	DE	DPI	HE	HO	MA	OH	PI	SPB	TE	VIR	ZW	HE	HEM	NA	OH	ZW	ZW
CAN	WVH	WVH	*1.00*																		
CAN	VAR	BLG	*0.54*	*0.76*																	
CAN	VZS	Deinze	*0.52*	*0.45*	*0.79*																
CAN	VZS	De Pinte	*0.52*	*0.66*	*0.47*	*0.75*															
CAN	BDO	Heers	*0.55*	*0.47*	*0.77*	*0.50*	*0.80*														
CAN	MV	HO	*0.54*	*0.45*	*0.73*	*0.49*	*0.76*	*0.77*													
CAN	VZS	MA	*0.53*	*0.61*	*0.54*	*0.65*	*0.57*	*0.56*	*0.63*												
CAN	VAR	Ophasselt	*0.55*	*0.82*	*0.48*	*0.71*	*0.50*	*0.49*	*0.66*	*1.00*											
CAN	VZS	Pittem	*0.53*	*0.68*	*0.49*	*0.72*	*0.53*	*0.51*	*0.69*	*0.68*	*0.89*										
CAN	MV	SPB	*0.50*	*0.66*	*0.46*	*0.71*	*0.50*	*0.48*	*0.66*	*0.67*	*0.78*	*1.00*									
CAN	VZS	Temse	*0.53*	*0.68*	*0.48*	*0.74*	*0.50*	*0.50*	*0.68*	*0.73*	*0.79*	*0.78*	*0.89*								
CAN	VIR	VIR	*0.55*	*0.45*	*0.70*	*0.50*	*0.71*	*0.69*	*0.56*	*0.49*	*0.52*	*0.49*	*0.50*	*0.78*							
CAN	OKU	Zwin	*0.52*	*0.47*	*0.72*	*0.51*	*0.72*	*0.71*	*0.58*	*0.51*	*0.52*	*0.50*	*0.51*	*0.73*	*1.00*						
COR	BDO	Heers	0.54	0.44	0.68	0.48	0.70	0.70	0.54	0.48	0.50	0.47	0.48	0.67	0.70	*0.72*					
COR	VAR	HEM	0.51	0.73	0.46	0.76	0.51	0.48	0.68	0.78	0.78	0.80	0.79	0.48	0.49	*0.47*	*1.00*				
COR	VZS	Nazareth	0.56	0.68	0.51	0.74	0.54	0.53	0.66	0.78	0.71	0.67	0.76	0.54	0.53	*0.52*	*0.79*	*0.96*			
COR	VAR	Ophasselt	0.58	0.75	0.51	0.75	0.55	0.53	0.69	0.82	0.77	0.75	0.77	0.53	0.54	*0.50*	*0.85*	*0.78*	*1.00*		
COR	OKU	Zwin	0.56	0.49	0.71	0.51	0.72	0.71	0.57	0.52	0.52	0.51	0.51	0.73	**0.87**	*0.71*	*0.49*	*0.53*	*0.54*	***0.87***	
BAL	OKU	Zwin	0.51	0.46	0.69	0.50	0.69	0.69	0.56	0.50	0.50	0.48	0.50	0.69	**0.87**	0.68	0.49	0.52	0.53	**0.80**	***0.79***

Dendrogram

In this cluster analysis, each taxon was represented by randomly chosen individuals to make the dendrogram better readable (Figure 4.44).

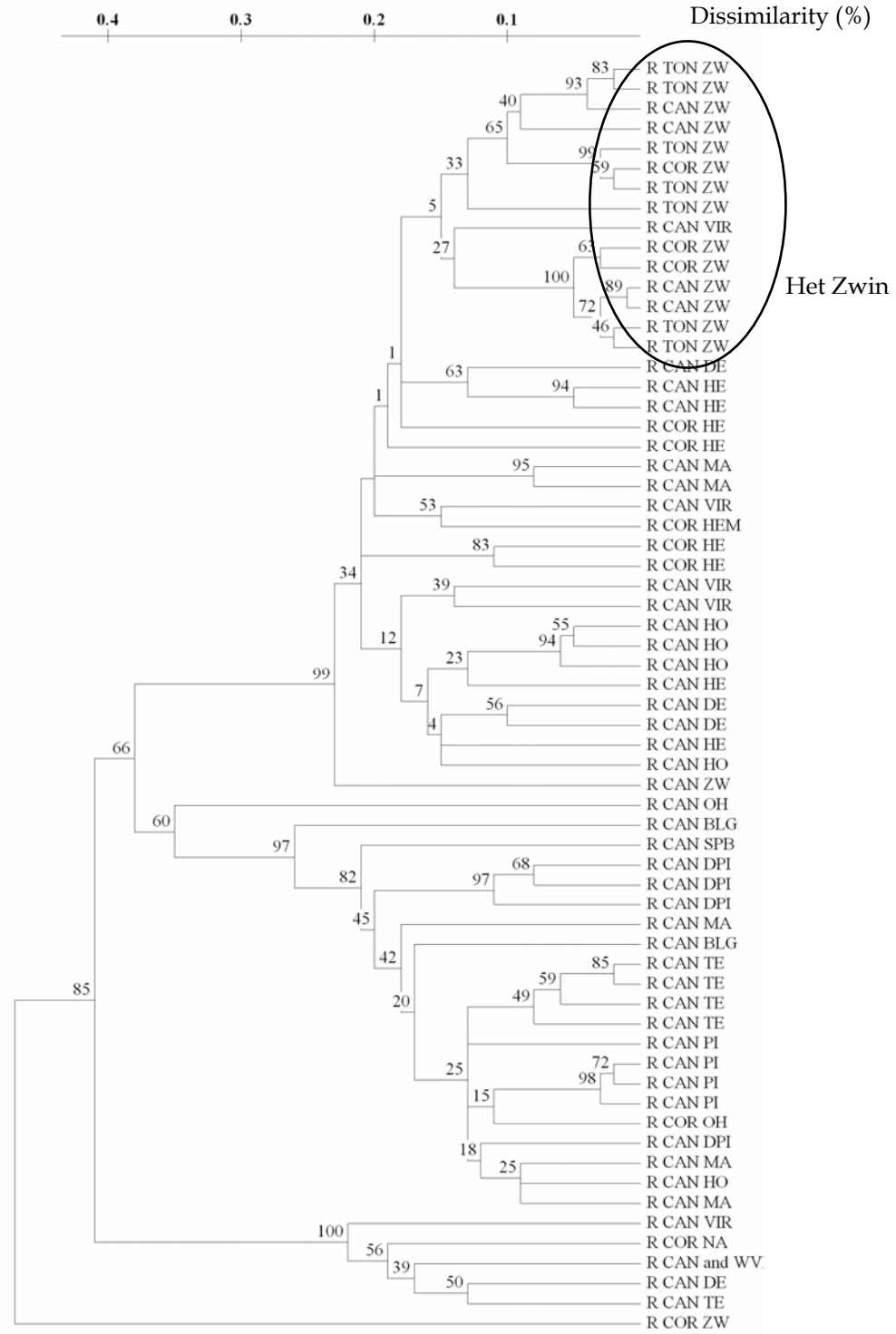


Figure 4.44: UPGMA cluster dendrogram of the subsections *Caninae* and *Tomentellae*. The distance scale is indicated, individuals are labelled with species names (*R. balsamica* is indicated as R TON) and population codes (Tables 4.2 and 4.34).

No strict taxon, populations, or region structure was present in the analysed subsections *Caninae* and *Tomentellae*. However, irrespective of the species

determination, the individuals sampled at Het Zwin (Oostkust) all clustered together in the upper part of the tree.

Structure

Following the analyses of Structure, this complex could consist of one or two major clusters. The assignment of the individuals considering two gene pools was summarised in table 4.49.

Table 4.49: Population assignment of subsections *Caninae* and *Tomentellae* to each of the inferred gene pools. Species determination, region of provenance and locality, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the populations are assigned are marked in bold.

TAXON	REGION	LOCALITY	GP1	GP2	IND
R. canina *R. corymbifera* *R. balsamica*	Westkust	Het Zwin	0.00	**1.00**	55
R. canina x *R. stylosa* *R. stylosa*	Westkust	Ter Yde	**1.00**	0.00	11
R. canina x *R. stylosa* *R. canina* var. *and*	West-Vlaams Heuvelland	West-Vlaams Heuvelland	**0.86**	0.14	7
R. canina	Vlaamse Zandstreek	Deinze	0.00	**1.00**	32
R. canina	Vlaamse Zandstreek	De Pinte	**1.00**	0.00	12
R. subcollina *R. canina*	Vlaamse Zandstreek	Maldegem	**0.86**	0.14	7
R. corymbifera	Vlaamse Zandstreek	Nazareth	**1.00**	0.00	2
R. canina	Vlaamse Zandstreek	Pittem	**1.00**	0.00	4
R. canina	Vlaamse Zandstreek	Temse	**1.00**	0.00	5
R. canina	Vlaamse Ardennen	Balegem	**1.00**	0.00	4
R. corymbifera	Vlaamse Ardennen	Hemelveerdegem	**1.00**	0.00	1
R. canina *R. corymbifera*	Vlaamse Ardennen	Ophasselt	**1.00**	0.00	2
R. corymbifera *R. canina*	Brabants District Oost	Heers	0.00	**1.00**	63
R. canina	Maasvallei	Hochter Bampd	0.00	**1.00**	34
R. canina	Maasvallei	St-Pietersberg	**0.75**	0.25	4
R. canina	Viroin	Viroin	0.00	**1.00**	29

The division in two gene pools was not related to species determination, nor was there a region- or locality-based pattern. The individuals of the mixed population of Het Zwin (Oostkust) are all assigned to the second gene pool, irrespective of the species determination. Moreover, these populations were assigned to the same gene pool as the pure *R. canina* populations from Vlaamse Zandstreek (Deinze), from Maasvallei (Hochter Bampd), from Viroin and *R. canina* and *R. corymbifera* both from Brabants District Oost (Heers). Another remarkable contrast was that the pure population *R. canina* Maasvallei was assigned to two gene pools, while *R. canina*, *R. corymbifera* and *R. balsamica*, all sampled at Het Zwin were assigned to the same gene pool.

The different methods of analyses came to different subdivisions of the subsection *Caninae* and *Tomentellae*. However, few important facts were confirmed in all outcomes: (a) the taxonomical subdivision of the subsections *Caninae* and *Tomentellae* lacks a genetic basis; (b) the locality aspect might be more important than the taxonomical determination, especially on localities were several taxa have a mixed presence, e.g. Het Zwin (Oostkust).

4.3.1.1.9. Genetic diversity in mixed populations

The sampled populations varied in the presence of taxa, the number of sampled localities, etc. At some localities, only one taxon occurs, whereas at other localities several taxa have a mixed presence. In order to get an idea about the impact of the mixed presence of taxa on the genetic structure of the individuals, two mixed localities were analysed. The main question is: Will morphologically different individuals, thus identified as different taxa, sampled at the one well-defined locality, belong to the same gene pool, or will they be assigned to different gene pools?

The mixed population at the South-orientated slope of St-Pietersberg (Riemst, Maasvallei) contains a mixture of *R. rubiginosa*, *R. micrantha*, *R. canina*, *R. tomentosa,* and presumed hybrids. In this subset, 23 *R. rubiginosa*, ten *R. micrantha,* and four *R. canina* individuals were analysed with 147 polymorphic AFLP markers. The only sampled *R. tomentosa* individual was not included in this analysis.

PCO

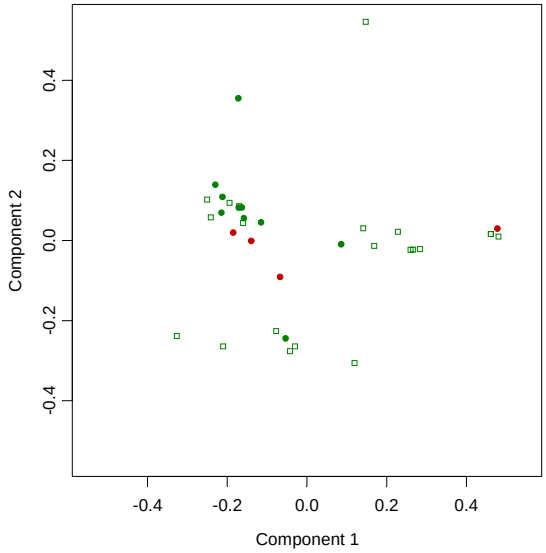


Figure 4.45: PCO plot of first two components of individuals of *R. canina* (●), *R. rubiginosa* (□), and *R. micrantha* (●) sampled at St-Pietersberg (Maasvallei). The first two components explained 62% of the variation.

In the PCO biplot (Figure 4.45), no section or species differentiation could be detected. The first three components explained 41%, 11%, and 11%, respectively, of the variation present in the data set.

Jaccard matrix

Table 4.50: Mean Jaccard similarity coefficients (%) calculated within and between the sampled populations in St-Pietersberg.

TAXON	LOCALITY	*R. canina*	*R. micrantha*	*R. rubiginosa*
R. canina	St-Pietersberg	1.00		
R. micrantha	St-Pietersberg	0.71	1.00	
R. rubiginosa	St-Pietersberg	0.56	0.69	0.63

Among *R. canina* and *R. rubiginosa* a tendency to a lower interspecific similarity was observed (Table 4.50). The similarity between *R. micrantha* and the two other taxa was comparable.

Dendrogram

In the cluster analysis, some additional individuals were included as reference samples. *R. micrantha* sampled at other localities in Riemst (Maasvallei) but nearby St-Pietersberg, *R. canina* and *R. corymbifera* individuals sampled in Het Zwin (Oostkust) and *R. canina* and *R. agrestis* individuals originating from Heers (Brabants District Oost).

The most differentiated individuals were sampled in Het Zwin, whereas the individuals originating from Heers and Riemst were completely mingled with the St-Pietersberg population, irrespective of their species determination (Figure 4.46). However, all the *R. agrestis* individuals from Heers formed a compact subcluster.

Structure

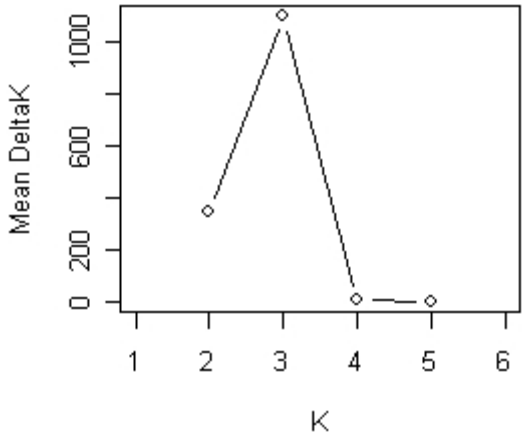


Figure 4.47: Assumption of the optimal number of gene pools present in the population St-Pietersberg, based on Structure (Pritchard *et al.* 2000) and adapted with the method of Evanno *et al.* (2005).

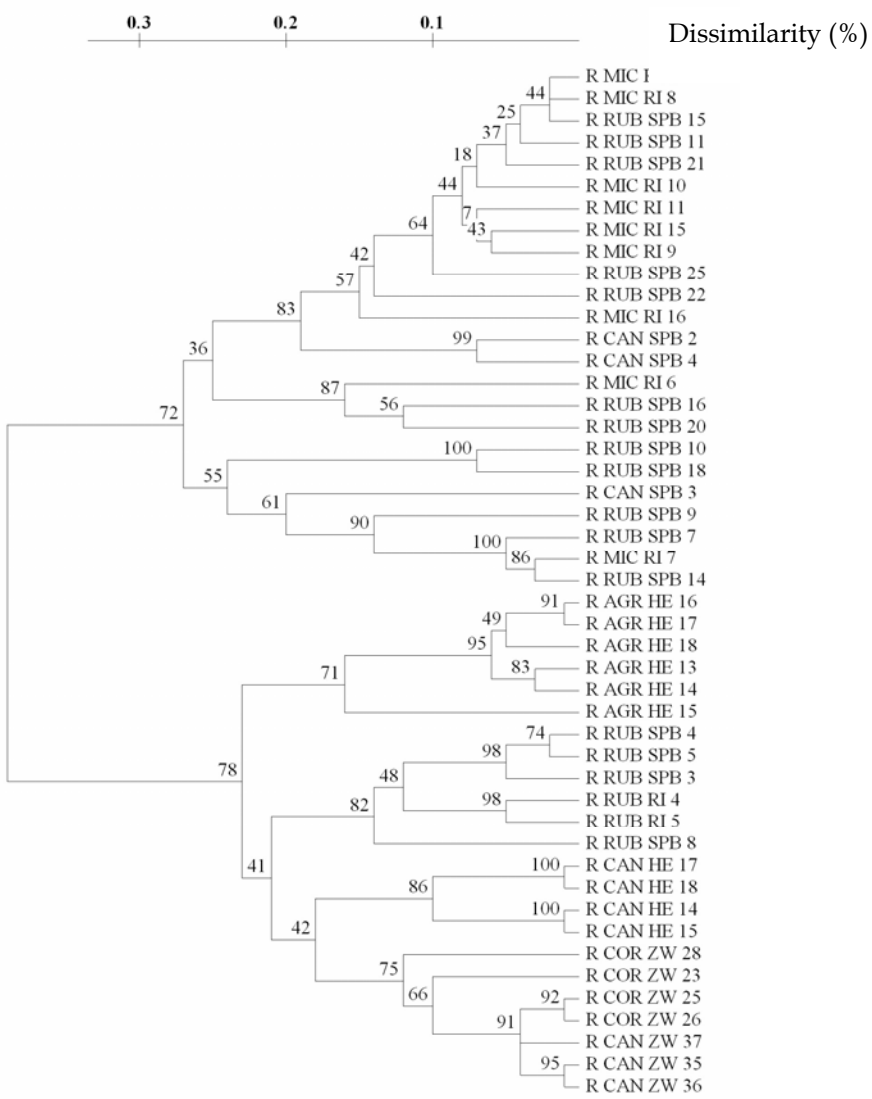


Figure 4.46: UPGMA cluster dendrogram of the populations of St-Pietersberg and reference samples. The distance scale is indicated, individuals are labelled with species names, population codes and individual numbers (Tables 4.2 and 4.34).

Based on the mean DeltaK calculations, the analysed individuals are assigned to three gene pools (Figure 4.47 and Table 4.51). Gene pool 1 consisted of 48% of *R. rubiginosa*, 10% of *R. micrantha,* and 25% of *R. canina* sampled at St-Pietersberg, while gene pool 2 contained 26% of *R. rubiginosa*, 10% of *R. micrantha,* and 25% of *R. canina* sampled at St-Pietersberg. The third gene pool contained the majority of *R. micrantha* and *R. canina*, 80%, AND 50%, respectively, and only 26% of *R. rubiginosa*.

Table 4.51: Species assignment of the individuals sampled at St-Pietersberg (Maasvallei) to each of the inferred gene pools. Species determination, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) are indicated. The presumed GP to which the taxa are assigned are marked in bold.

TAXON	GP1	GP2	GP3	IND
R. rubiginosa	**0.48**	0.26	0.26	23
R. micrantha	0.10	0.10	**0.80**	10
R. canina	0.25	0.25	**0.50**	4

Within the St-Pietersberg population, there was no clear species delimitation in the genetic background. Especially the fact that the *R. canina* individuals from St-Pietersberg were more similar to *R. rubiginosa* and *R. micrantha* of the same locality, than to the *R. canina* individuals from Heers, might indicate the occurrence of interspecific hybridisation.

The mixed population at Het Zwin (Oostkust) was sampled at one large and well-defined locality with a mixed occurrence of *R. canina*, *R. corymbifera*, *R. balsamica*, *R. rubiginosa,* and several varieties of *R. canina*. In the data set, 128 polymorphic AFLP markers were included and a total of 19 *R. canina*, 27 *R. corymbifera,* and ten *R. balsamica* individuals were analysed.

PCO

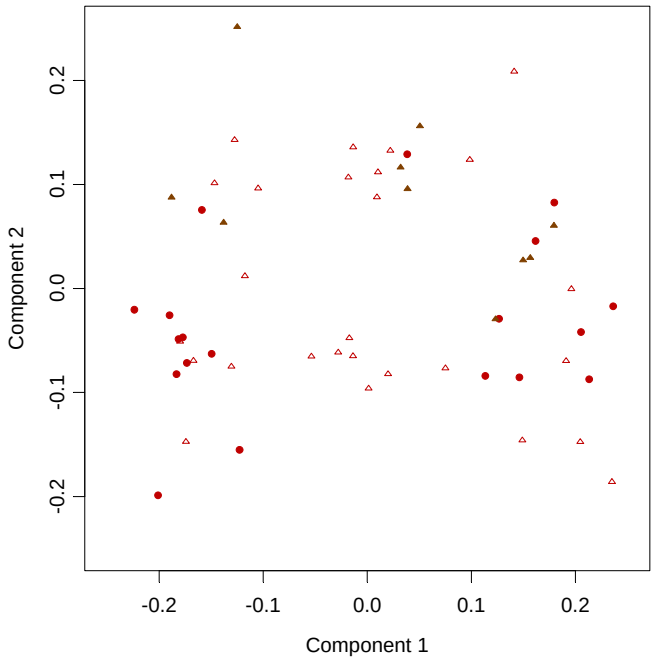


Figure 4.48: PCO plot of first two components of individuals of *R. canina* (●), *R. corymbifera* (△), and *R. balsamica* (▲) sampled at Het Zwin (Oostkust).

The PCO biplot did not show a taxon related clustering (Figure 4.48). The first three components explained 26%, 14%, and 12%, respectively, of the variation present at Het Zwin.

Jaccard matrix

The Jaccard similarity coefficients did not show any difference in similarity between the three taxa (Table 4.52). Moreover, the similarity between *R. canina* and *R. balsamica* was higher (87%) than within *R. balsamica* (79%).

Table 4.52: Mean Jaccard similarity coefficients (%) calculated within and between the sampled taxa of Het Zwin (Oostkust).

TAXON	*R. canina*	*R. corymbifera*	*R. balsamica*
R. canina	1.00		
R. corymbifera	0.87	0.87	
R. balsamica	0.87	0.81	0.79

Dendrogram

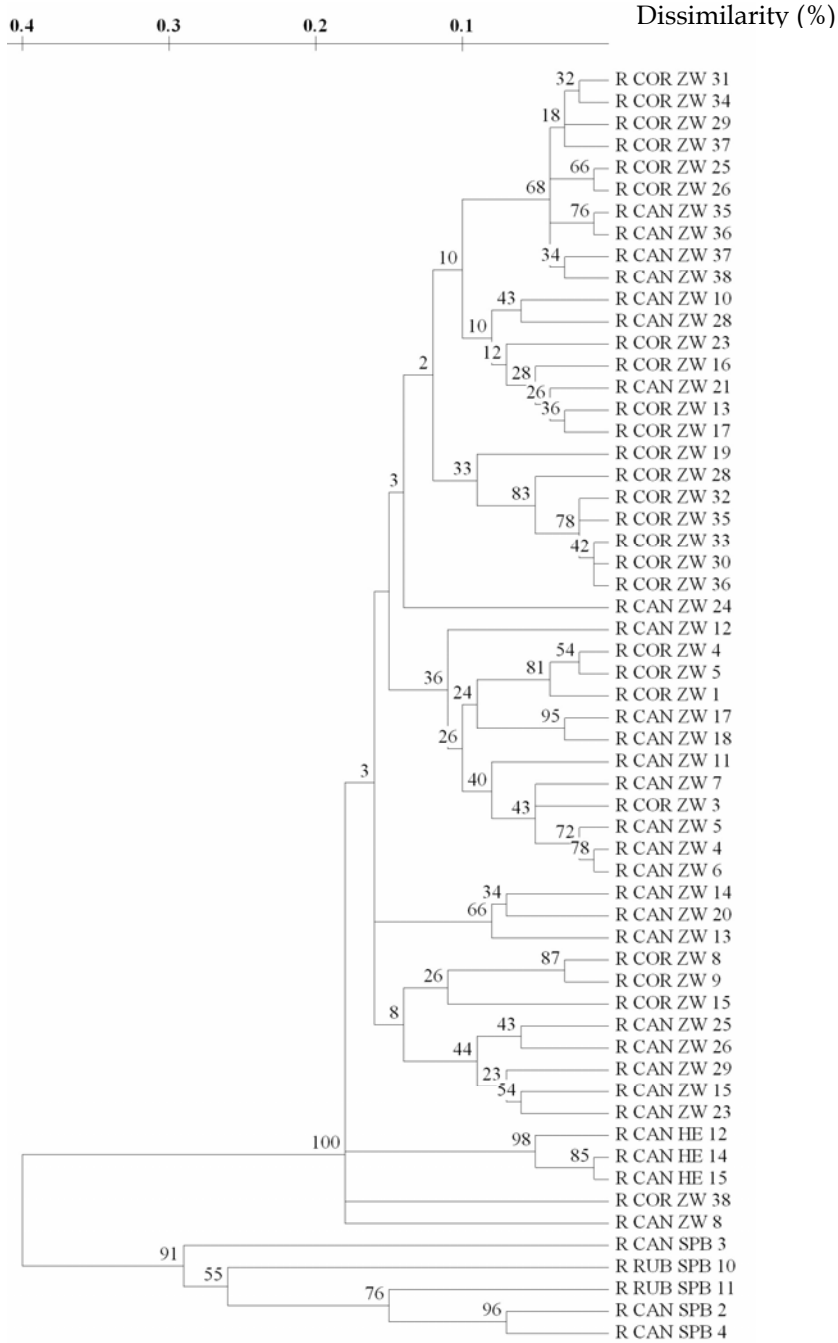


Figure 4.49: UPGMA cluster dendrogram of *R. canina*, *R. corymbifera* and *R. balsamica* from Het Zwin, with inclusion of some reference samples. The distance scale is indicated, individuals are labelled with species names, population codes and individual numbers (Tables 4.2 and 4.34).

In the cluster analysis, additional individuals were included as reference samples: *R. canina* and *R. rubiginosa* from St-Pietersberg (Maasvallei), *R. canina* from Heers (Brabants District Oost), and *R. micrantha* of Riemst (Maasvallei) (Figure 4.49).

In this dendrogram, the similarity between *R. canina* and *R. corymbifera*, both sampled at Het Zwin, was larger than between *R. canina* individuals sampled at Het Zwin and St-Pietersberg. In contrast, the individuals of *R. canina* Heers were completely mingled with *R. canina* and *R. corymbifera* of Het Zwin. Moreover, *R. canina* and *R. rubiginosa* both sampled at St-Pietersberg formed an out-group in this analysis.

Structure

Based on the calculation of the mean DeltaK, it was not possible to define the most probable number of gene pools present in the data set of Het Zwin. Assuming two gene pools, the majority of the three analysed taxa were assigned to the second gene pool (Table 4.53).

Table 4.53: Species assignment of the individuals sampled at Het Zwin (Oostkust) to each of the inferred gene pools. Species determination, the assignment to each gene pool (GP) in percentage, and the number of individuals on which assignment was based (IND) was indicated. The presumed GP to which the taxa are assigned are marked in bold.

TAXON	GP1	GP2	IND
R. balsamica	0.30	**0.70**	10
R. canina	0.37	**0.63**	19
R. corymbifera	0.19	**0.82**	27

4.3.1.1.10. Partitioning the diversity within and among taxa and localities

The within- and among-population variation was assessed for the two most common taxa in Flanders: *R. canina* and *R. corymbifera* both present at Het Zwin (OKU) and Heers (BDO) (Table 4.54). The following strategy was used: (a) the intra- and interspecific variation was calculated for each locality separately; (b) all the individuals of the two localities were analysed together, calculating both the partitioning of the differentiation among taxa and among localities; (c) the individuals of five well-sampled localities were grouped and the within- and among-diversity partitioning was assessed. The five different sampling localities each represent one region of provenance. In each of the selected localities a balanced number of individuals is present. Only the completely scored and polymorphic (PM) AFLP markers were included.

In general, the genetic variation (Hp) within *R. canina* was larger when more localities were taken in account. The variation within *R. canina* based on five localities equalled 0.21, while it was only around 0.15 for one or two localities. In contrast, there was no significant difference assessed for *R. corymbifera* as it was only present at Het Zwin and Heers.

The genetic variation among the two taxa: (Ht-aver.Hp)/Ht ~ Gst), sampled at Het Zwin (equalling 0.18) and Heers (equalling 0.17) appeared to be similar at the two localities.

Comparing the partitioning of the differentiation among the *R. canina* and *R. corymbifera* sampled at the five selected localities, the genetic variation among the five localities was clearly higher (Gst = 0.34) compared to the variation among the two taxa (Gst = 0.16). This suggests that the locality of provenance is more important than the species determination based on the morphological characters. However, comparing differentiation among the two mixed localities, little to no difference was found among the localities and the taxa sampled over there, 0.14 and 0.11, respectively.

Table 4.54: Results of RAPDDIV analyses. The within- and among-taxa differentiation at the two mixed localities, and the within- and among-taxa and -locality differentiation of two and five populations are indicated. With: number of individuals included (IND); diversity within taxon or locality (Hp); variation among taxa or localities [Gst = (Ht-aver.Hp)/Ht]

(A) WITHIN- AND AMONG-TAXA DIFFERENTIATION IN A MIXED POPULATION

Het Zwin (48 AFLP markers)				Heers (103 AFLP markers)			
Taxon	Ind	Hp	Gst	Taxon	Ind	Hp	Gst
R. canina	13	0.16	0.18	*R. canina*	28	0.18	0.17
R. corymbifera	16	0.16		*R. corymbifera*	30	0.16	

(B) WITHIN- AND AMONG- AND -LOCALITY DIFFERENTIATION OF TWO MIXED POPULATIONS (based on 107 AFLP markers)

Taxon differentiation				Locality differentiation				
Taxon	Ind	Hp	Gst	Region	Locality	Ind	Hp	Gst
R. canina	41	0.14	0.11	BDO	Heers	58	0.16	0.14
R. corymbifera	46	0.16		WKU	Het Zwin	29	0.10	

(C) WITHIN- AND AMONG-TAXA AND -LOCALITY DIFFERENTIATION OF FIVE MIXED POPULATIONS (based on 131 AFLP markers)

Taxon differentiation				Locality differentiation				
Taxon	Ind	Hp	Gst	Region	Locality	Ind	Hp	Gst
R. canina	126	0.21	0.16	WKU	Het Zwin	29	0.09	0.34
R. corymbifera	46	0.15		VZS	Deinze	27	0.13	
				BDO	Heers	58	0.16	
				MV	Hochter Bampd	31	0.12	
				VIR	Viroin	27	0.22	

The different methods all indicated that the three main taxa occurring in Het Zwin (Oostkust), *R. canina*, *R. corymbifera,* and *R. balsamica*, showed a higher interspecific similarity compared to their congeners sampled at other locality.

This supports the hypothesis that especially for taxa in mixed populations the locality might be more important than the taxonomical determination.

4.3.1.2. SSR-analysis

A subset of Flemish wild roses was additionally analysed with six STMS loci in order to get a global view of the allelic diversity within and among taxa, and to get an idea about the clonality in certain taxa and populations.

In general, about five individuals per population were analysed, with a maximum of 40 individuals per taxon. For the presumed clonal taxa, *R. arvensis* and *R. spinosissima*, one population was studied more profoundly.

The allelic diversity of each analysed locus was summarised in table 4.55. The direpeat locus RhAB15 appeared to be the most polymorphic with 20 different alleles. In contrast, the locus RhM405 showed only five different alleles.

Table 4.55: Allelic diversity at microsatellite loci scored in Flemish wild roses. The range size of the fragments (in base pairs) and the number of alleles are indicated for each locus.

LOCUS	FRAGMENT RANGE SIZES (BP)	NUMBER OF ALLELES
RhAB15	93-146	20
RhB303	112-145	14
RhAB22	151-198	13
RhP519	200-249	11
RhO517	240-264	6
RhM405	152-177	5

Given the polyploidy state of the section *Caninae*, the allelic frequencies of the STMS loci could not be calculated. However, an indication of the genetic constitution was given by describing the allelic phenotypes of the analysed taxa. The allelic phenotypes are defined as "using the presence of the alleles of a locus as one character" (Esselink *et al.* 2003), the frequency of the alleles is not taken into account.

4.3.1.2.1. Subgenus *Rosa*

Comparing allelic phenotypes of the six analysed STMS loci, the three analysed sections of the subgenus *Rosa* showed some section-related patterns (Tables 4.56 to 4.58). More specifically, the loci RhP519, RhB303, and RhAB15 showed different allelic phenotypes for each section. The locus RhP519 displayed one or more section-specific alleles in each section and is given as illustration. The alleles RhP519_200, RhP519_212, and RhP519_222 were only present in the section *Pimpinellifoliae*. The first two alleles were detected in 14% of the analysed individuals, whereas the allele RhP519_222 was observed in 40%. The allele RhP519_247 appeared in 25% of the analysed *R. arvensis* individuals (section *Synstylae*), and was completely absent in the other sections. Finally, RhP519_231 was only detected in the section *Caninae* and was observed in 81-100% of the individuals. To be complete, the presumed intersectional hybrids *R. stylosa* and *R.* x *irregularis* were not taken into account for this global view as they are handled in more detail later on.

Within the section *Caninae*, the loci RhP519 and RhAB15 displayed different patterns corresponding to the subdivision in subsections (Tables 4.56 to 4.58). The difference of the allele RhP519_244 is highlighted. This allele was observed in about

Table 4.56: Allelic phenotypes of the STMS loci RhP519 and RhAB22 for each taxon of the analysed subgenus *Rosa*. Deviating intersectional or intersubsectional patterns (bold); the alleles referring to the origin of hybrids *R. stylosa* and *R.* x *irregularis* (underlined) are indicated.

			RHP519											RHAB22											
SECTION-SUBSECTION	TAXON	SUM	200	212	216	222	225	228	231	237	244	247	249	151	153	155	159	165	167	169	171	178	180	182	190	198
Pimpinellifoliae	*R. spinosissima*	29	**0.14**	**0.14**	0.32	**0.41**	0.00	1.00	0.09	0.09	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Synstylae	*R. arvensis*	40	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.14	0.00	0.25	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.15	0.22	**0.56**	**0.59**	**0.04**	**0.07**
Caninae- Rubigineae	*R. rubiginosa*	25	0.00	0.00	0.08	0.00	0.00	1.00	**1.00**	0.00	0.08	0.00	0.04	0.00	0.04	0.04	0.00	0.00	0.92	0.04	0.88	0.08	0.00	0.00	0.00	0.00
	R. micrantha	14	0.00	0.00	0.00	0.00	0.00	1.00	**0.92**	0.00	0.46	0.00	0.00	0.00	0.15	0.08	0.00	0.00	0.85	0.08	0.92	0.00	0.00	0.00	0.00	0.00
	R. agrestis	16	0.00	0.00	0.70	0.00	0.00	1.00	**1.00**	0.00	0.20	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.95	0.00	0.90	0.10	0.00	0.00	0.00	0.00
	R. inodora	1	0.00	0.00	1.00	0.00	0.00	1.00	**1.00**	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
Caninae-Vestitae	*R. tomentosa*	29	0.00	0.00	0.17	0.00	0.00	1.00	**1.00**	0.00	**0.97**	0.00	0.00	0.00	0.24	0.69	0.00	0.00	0.76	0.03	0.07	0.00	0.00	0.00	0.00	0.00
	R. villosa	2	0.00	0.00	0.00	0.00	0.00	0.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. mollis	2	0.00	0.00	0.00	0.00	0.00	0.50	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Tomentellae	*R. balsamica*	46	0.00	0.00	0.38	0.00	0.00	1.00	**1.00**	0.00	**0.98**	0.00	0.00	0.00	0.55	0.48	0.02	0.00	0.34	0.36	0.80	0.05	0.00	0.02	0.00	0.00
Caninae- Caninae	*R. canina*	27	0.00	0.00	0.61	0.00	0.00	1.00	**1.00**	0.00	**0.91**	0.00	0.00	0.00	0.36	0.04	0.00	0.04	0.52	0.64	0.12	0.12	0.00	0.00	0.00	0.00
	R. canina var. *andegavensis*	5	0.00	0.00	0.00	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.60	0.00	0.00	0.00	1.00	0.40	0.60	0.00	0.00	0.00	0.00	0.00
	R. canina var. *dumalis*	5	0.00	0.00	0.60	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.40	0.80	0.00	0.00	0.00	0.00	0.00	0.00
	R. corymbifera	15	0.00	0.00	0.56	0.00	0.06	1.00	**0.81**	0.00	**0.81**	0.06	0.00	0.00	0.25	0.00	0.00	0.00	0.69	0.44	0.13	0.13	0.00	0.00	0.00	0.00
	R. corymbifera var. *deseglisei*	3	0.00	0.00	0.00	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. corymbifera var. *scabrata*	2	0.00	0.00	0.50	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50	0.50	1.00	0.00	0.00	0.00	0.00	0.00
	R. caesia	5	0.00	0.00	1.00	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. dumalis	1	0.00	0.00	0.00	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. stylosa	13	0.00	0.00	0.17	0.00	0.08	1.00	**0.58**	0.33	**0.67**	0.00	0.00	0.00	0.00	0.31	0.46	0.00	0.92	0.15	0.15	0.00	0.00	0.15	0.00	0.00
	R. subcanina	5	0.00	0.00	0.00	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.25	0.75	0.00	0.25	0.00	0.00	0.00	0.00
	R. subcollina	2	0.00	0.00	0.00	0.00	0.00	1.00	**1.00**	0.00	**1.00**	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Hybrid	*R.* x *irregularis*	3	0.00	0.00	0.33	0.00	0.00	1.00	**0.33**	0.00	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00

Table 4.57: Allelic phenotypes of the STMS loci RhO517, RhM405, and RhB303 for each taxon of the analysed subgenus *Rosa*. Deviating intersectional or intersubsectional patterns (bold); the alleles referring to the origin of hybrids *R. stylosa* and *R.* x *irregularis* (underlined) are indicated.

			RHO517						RHM405					RHB303												
SECTION-SUBSECTION	TAXON	SUM	240	252	255	257	258	264	152	158	164	171	177	112	114	120	122	125	127	131	133	135	137	139	141	143	145
Pimpinellifoliae	*R. spinosissima*	29	0.12	0.84	0.56	0.08	0.72	0.04	0.00	**0.50**	0.93	0.86	0.12	0.04	**0.23**	0.04	**0.85**	0.00	0.04	0.00	0.08	0.00	0.00	0.00	0.08	0.04	0.00
Synstylae	*R. arvensis*	40	0.00	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.51	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00	**0.15**	0.24	0.53	**0.15**	0.41	**0.03**	**0.26**	**0.09**
Caninae- Rubigineae	*R. rubiginosa*	25	0.00	1.00	1.00	0.00	0.04	0.00	**0.92**	0.00	0.83	1.00	0.00	0.04	0.96	0.00	0.96	0.08	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. micrantha	14	0.00	1.00	1.00	0.00	0.00	0.00	**1.00**	0.00	1.00	1.00	0.07	0.29	1.00	0.00	1.00	0.07	0.43	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. agrestis	16	0.00	1.00	1.00	0.00	0.05	0.00	**0.90**	0.00	0.95	0.45	0.60	0.33	1.00	0.00	1.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. inodora	1	0.00	1.00	1.00	0.00	0.00	0.00	-	-	-	-	-	-	1.00	0.00	-	-	-	-	-	-	-	-	-	-	-
Caninae-Vestitae	*R. tomentosa*	29	0.00	1.00	1.00	0.00	**0.93**	0.00	0.06	0.00	1.00	1.00	0.03	0.50	1.00	0.00	0.96	0.50	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. villosa	2	0.00	1.00	1.00	0.00	**1.00**	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. mollis	2	0.00	1.00	1.00	0.00	**1.00**	0.00	0.00	0.00	1.00	0.50	0.00	0.00	1.00	0.00	1.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Tomentellae	*R. balsamica*	46	0.00	1.00	1.00	0.00	0.05	0.00	**0.80**	0.00	0.91	1.00	0.20	0.47	1.00	0.00	1.00	0.40	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Caninae	*R. canina*	27	0.00	1.00	1.00	0.00	0.58	0.00	0.00	0.09	1.00	1.00	0.09	0.38	1.00	0.00	1.00	0.23	0.73	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. canina var. *andegavensis*	5	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.60	0.20	1.00	0.00	1.00	0.40	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. canina var. *dumalis*	5	0.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.80	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. corymbifera	15	0.00	0.93	1.00	0.00	0.00	0.00	0.18	0.00	1.00	0.94	0.06	0.53	0.93	0.00	0.93	0.73	0.20	0.00	0.07	0.00	0.00	0.07	0.00	0.00	0.00
	R. corymbifera var. *deseglisei*	3	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.50	1.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. corymbifera var. *scabrata*	2	0.00	1.00	1.00	0.00	0.00	0.00	0.50	0.00	1.00	1.00	0.00	0.50	1.00	0.00	1.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. caesia	5	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.20	0.80	1.00	0.00	1.00	0.00	0.20	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.00
	R. dumalis	1	0.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. stylosa	13	0.00	1.00	1.00	0.00	0.08	0.00	0.46	0.00	0.62	1.00	0.31	0.64	1.00	0.00	1.00	0.21	0.79	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00
	R. subcanina	5	0.00	1.00	1.00	0.00	0.40	0.00	0.00	0.00	1.00	1.00	0.20	0.60	1.00	0.00	1.00	0.40	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. subcollina	2	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.50	1.00	0.00	1.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Hybrid	*R.* x *irregularis*	3	0.00	0.33	1.00	0.00	0.00	0.00	0.00	0.00	0.67	0.33	0.67	0.67	0.67	0.00	0.67	0.67	0.00	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00

Table 4.58: Allelic phenotypes of the STMS locus RhAB15 for each taxon of the analysed subgenus *Rosa*. Deviating intersectional or intersubsectional patterns (bold); the alleles referring to the origin of hybrids *R. stylosa* and *R.* x *irregularis* (underlined) are indicated.

			RHAB15																			
SECTION-SUBSECTION	TAXON	SUM	93	97	105	107	109	111	113	115	117	122	124	126	128	130	132	134	136	138	142	146
Pimpinellifoliae	*R. spinosissima*	29	0.07	0.00	0.00	0.03	0.07	0.03	0.17	0.10	0.00	**0.45**	**0.31**	**0.07**	**0.17**	0.10	0.28	0.07	**0.52**	0.07	0.03	0.07
Synstylae	*R. arvensis*	40	0.00	0.00	0.00	0.00	0.00	0.55	0.53	**0.48**	**0.25**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Rubigineae	*R. rubiginosa*	25	0.00	0.08	0.04	**0.92**	0.76	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.76	0.00	0.00	0.00	0.00	0.00
	R. micrantha	14	0.00	0.07	0.00	**0.93**	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.50	0.36	0.00	0.00	0.00	0.00
	R. agrestis	16	0.13	0.19	0.00	**1.00**	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.44	0.00	0.00	0.00	0.00	0.13
	R. inodora	1	0.00	0.00	0.00	**1.00**	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Caninae-Vestitae	*R. tomentosa*	29	0.00	0.07	0.21	**0.93**	0.21	0.45	0.14	0.00	0.00	0.00	0.03	0.00	0.00	0.41	0.21	0.38	0.00	0.07	0.00	0.00
	R. villosa	2	0.00	0.00	0.00	**1.00**	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. mollis	2	0.00	0.00	0.00	**1.00**	0.00	1.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00
Caninae- Tomentellae	*R. balsamica*	46	0.02	**0.93**	0.52	0.07	0.43	0.02	0.00	0.00	0.00	0.00	0.48	0.00	0.00	0.63	0.20	0.20	0.00	0.02	0.02	0.00
Caninae- Caninae	*R. canina*	27	0.00	**0.74**	0.41	0.30	0.37	0.19	0.00	0.00	0.00	0.00	0.04	0.00	0.04	0.48	0.37	0.15	0.00	0.07	0.00	0.00
	R. canina var. *andegavensis*	5	0.40	**1.00**	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.40	0.00
	R. canina var. *dumalis*	5	0.00	**1.00**	0.80	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.20	0.00	0.00
	R. corymbifera	15	0.00	**0.93**	0.60	0.00	0.20	0.13	0.07	0.07	0.00	0.00	0.07	0.00	0.00	0.73	0.07	0.13	0.00	0.00	0.00	0.00
	R. corymbifera var. *deseglisei*	3	0.00	**1.00**	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	R. corymbifera var. *scabrata*	2	0.50	**1.00**	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.00
	R. caesia	5	0.00	**0.80**	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.80	0.20	0.00	0.00	0.00	0.00	0.00
	R. dumalis	1	0.00	**1.00**	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
	R. stylosa	13	0.08	**0.23**	0.00	0.00	0.85	0.08	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.38	0.46	0.23	0.00	0.00	0.08	0.00
	R. subcanina	5	0.00	**0.60**	0.00	0.40	0.60	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.20	0.00	0.00	0.00	0.00	0.00
	R. subcollina	2	0.00	**1.00**	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Caninae- Hybrid	*R.* x *irregularis*	3	0.00	0.00	0.00	0.33	0.33	0.00	0.67	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00

all of the analysed individuals of the subsections *Vestitae*, *Caninae*, and *Tomentellae*, varying between 81 and 100%. In contrast, it was only observed in few subsection *Rubigineae* individuals (*R. micrantha*: 46%; *R. agrestis*: 20%; *R. rubiginosa*: 8%) (Table 4.56).

The locus RhO517 displayed a remarkable allelic pattern. Of this tri-repeat, the alleles RhO517_257 and RhO517_258 were observed in the same samples (R SPI VIR 1 and VIR 2). Moreover, the allele RhO517_257 was only detected in these two individuals.

4.3.1.2.2. Section *Pimpinellifoliae*, *R. spinosissima*

The 29 analysed *R. spinosissima* samples originated from three different regions of provenance: Middenkust, Westkust, and Viroin. Within the West- and Middenkust, two localities were sampled. The observed allelic phenotypes were summarised (Figures 4.50 and 4.51) and the most remarkable results were highlighted.

The six investigated loci were polymorphic in the analysed samples. However, the locus RhAB22 is excluded from this point on, given the failure of the analyses for all but one sample. The loci RhAB15, RhB303, and RhP519 appeared to be the most polymorphic, in total 17, eight, and seven different alleles were observed, respectively (Table 4.60 and 61). Some population related alleles were detected. The small inland population of the Viroin displayed certain unique alleles for the taxon e.g. RhB303_133, RhM405_177, RhP519_237, and RhO517_257.

Within the most intensively sampled population, Oostvoornduinen (Westkust), a large genetic variation was assessed. This contradicts the hypothesis of intense clonal propagation within one *R. spinosissima* population (Tables 4.60 and 61). In addition, in a dense carpet of *R. spinosissima* every five to ten meters a sample was taken over a total distance of 105 m. A schematic overview of the sampling and a summary of the observed allelic phenotypes (AP) is given in table 4.59.

The samples R SPI MO 4 to 9 showed similar allelic phenotypes (refered to as AP1). The assessed distance between R SPI MO 4 and 9 is about 24 meters, with four samples (R SPI MO 5, 6, 7 and 8) located in-between. The samples R SPI MO 10 and 11, only 5 meters apart, also displayed an identical allelic phenotype (refered to as AP2). The combination of distance between the samples and the identical allelic phenotypes indicated that these 8 samples might represent only two different genotypes. In contrast, the samples R SPI MO 1 to 3, and R SPI MO 12 to 17 each displayed a unique allelic phenotype.

Table 4.59: Schematic representation of the sampled path at Oostvoornduinen, The distance to R SPI MO 1 is indicated in meters: D (m), allelic phenotype: (AP), similar allelic phenotypes (x).

R SPI MO 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D (m)	2	8	11	15	20	25	30	35	45	55	65	75	85	95	105	5
AP1			x	x	x	x	x	x								
AP2									x	x						

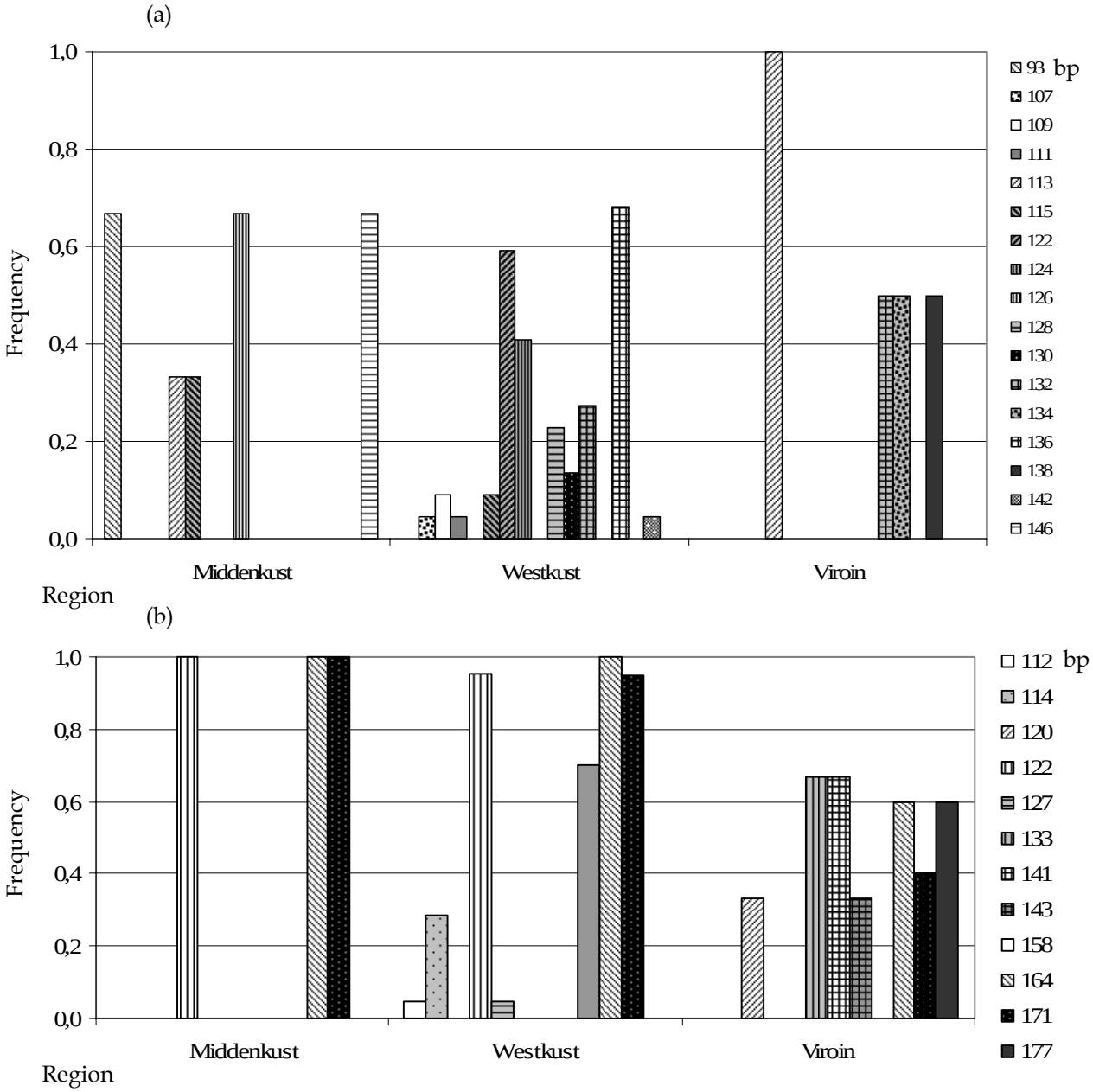


Figure 4.50: Polymorphisms of the loci (a) RhAB15; (b) RhB303 in the analysed *R. spinosissima* populations. The length of the observed alleles in base pairs (bp), the frequency of samples in which they were detected, and the regions of provenance are indicated.

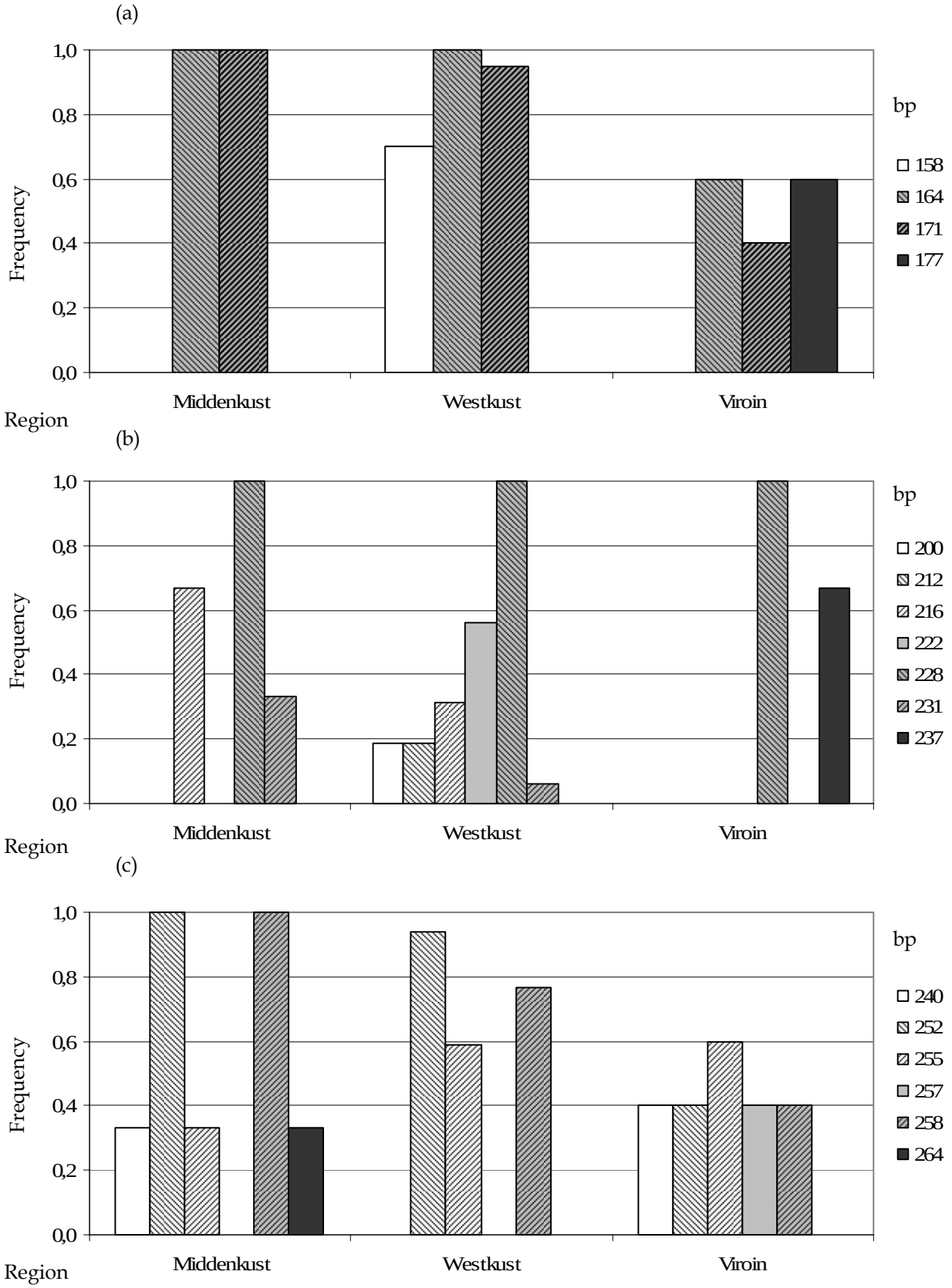


Figure 4.51: Polymorphisms of the loci (a) RhM405; (b) RhP519; (c) RhO517 in the analysed *R. spinosissima* populations. The length of the observed alleles in base pairs (bp), the frequency of samples in which they were detected, and the regions of provenance are indicated.

Table 4.60: Polymorphisms of the loci RhAB22, RhB303, RhM405, and RhP517 for the sampled and analysed *R. spinosissima* individuals. Region of provenance, the locality (abbr: see Table 4.34), and number of individuals analysed are indicated. Similar phenotypes are marked, by a combination of bold, Italics, and/or underlined. With: "-": absence of data; "1": presence of the allele; " ": absence of the allele.

			AB22		B303								M405				P519						
REGION	LOCALITY	NR	155	167	112	114	120	122	127	133	141	143	158	164	171	177	200	212	216	222	228	231	237
MKU	MI	2	-	-				1						1	1				1		1		
MKU	MI	3	-	-				1						1	1						1	1	
MKU	RA	1	-	-	-	-	-	-	-	-	-	-		1	1				1		1		
WKU	MO	13	-	-				1					1	1	1					1	1		
WKU	MO	1	-	-				1					-	-	-	-	-	-	-	-	-	-	-
WKU	MO	2	-	-				1					1	1			-	-	-	-	-	-	-
WKU	MO	3	-	-				1					-	-	-	-	-	-	-	-	-	-	-
WKU	**MO**	**4**	-	-				**1**					**1**	**1**	**1**				**1**		**1**		
WKU	**MO**	**5**	-	-				**1**					**1**	**1**	**1**		-	-	-	-	-	-	-
WKU	**MO**	**6**	-	-	-	-	-	-	-	-	-	-	**1**	**1**	**1**		-	-	-	-	-	-	-
WKU	**MO**	**7**	-	-				**1**					**1**	**1**	**1**		-	-	-	-	-	-	-
WKU	**MO**	**8**	-	-				**1**					**1**	**1**	**1**				**1**		**1**		
WKU	**MO**	**9**	-	-				**1**					**1**	**1**	**1**				**1**		**1**		
WKU	*MO*	*10*	-	-				*1*					*1*	*1*	*1*					*1*	*1*		
WKU	*MO*	*11*	-	-				*1*					*1*	*1*	*1*					*1*	*1*		
WKU	MO	12	-	-				1						1	1						1		
WKU	MO	14	-	-				1						1	1					1	1		
WKU	MO	15	-	-		1		1						1	1					1	1		
WKU	MO	16	-	-		1		1						1	1		1	1		1	1		
WKU	MO	17	-	-		1		1					1	1	1		1	1		1	1		
WKU	OVD	1	-	-		1		1					1	1	1					1	1	1	
WKU	OVD	2	-	-				1						1	1						1		
WKU	OVD	3	1	1	1	1		1	1				1	1	1		1	1		1	1		
WKU	OVD	4	-	-		1								1	1				1		1		
WKU	OVD	5	-	-				1					1	1	1				1		1		
VIR	*TP*	*1*	-	-	-	-	-	-	-	-	-	-		*1*	*1*		-	-	-	-	-	-	-
VIR	*TP*	*2*	-	-	-	-	-	-	-	-	-	-		*1*	*1*		-	-	-	-	-	-	-
VIR	**TP**	**3**	-	-						**1**	**1**					**1**					**1**		**1**
VIR	**TP**	**4**	-	-						**1**	**1**					**1**					**1**		**1**
VIR	TP	5	-	-			1					1		1		1					1		

Table 4.61: Polymorphisms of the loci RhAB15 and RhO519 for the sampled and analysed *R. spinosissima* individuals. Region of provenance, the locality (abbr: see Table 4.34), and number of individuals analysed are indicated. Similar phenotypes are marked, by a combination of bold, Italics, and/or underlined. With: "-": absence of data; "1": presence of the allele; " ": absence of the allele.

			AB15																	O517					
REGION	LOCALITY	NR	93	107	109	111	113	115	122	124	126	128	130	132	134	136	138	142	146	240	252	255	257	258	264
MKU	MI	2					1												1		1	1		1	
MKU	MI	3	1								1								1	1	1			1	
MKU	RA	1	1					1			1										1			1	1
WKU	MO	13							1	1						1					1	1			
WKU	MO	1							1	1										-	-	-	-	-	-
WKU	MO	2							1							1				-	-	-	-	-	-
WKU	MO	3							1							1				-	-	-	-	-	-
WKU	**MO**	**4**							**1**							**1**					**1**			**1**	
WKU	**MO**	**5**							**1**							**1**					**1**			**1**	
WKU	**MO**	**6**							**1**							**1**				**-**	**-**	**-**	**-**	**-**	**-**
WKU	**MO**	**7**							**1**							**1**				**-**	**-**	**-**	**-**	**-**	**-**
WKU	**MO**	**8**							**1**							**1**					**1**			**1**	
WKU	**MO**	**9**							**1**							**1**					**1**			**1**	
WKU	*MO*	*10*			*1*					*1*		*1*		*1*							*1*	*1*			
WKU	*MO*	*11*			*1*					*1*		*1*		*1*							*1*	*1*			
WKU	MO	12								1				1								1		1	
WKU	MO	14						1	1												1	1		1	
WKU	MO	15								1		1		1							1	1			
WKU	MO	16										1		1		1					1	1		1	
WKU	MO	17								1						1					1	1		1	
WKU	OVD	1						1								1		1			1	1		1	
WKU	OVD	2							1					1		1					1			1	
WKU	OVD	3		1		1				1			1								1	1		1	
WKU	OVD	4							1			1	1			1					1			1	
WKU	OVD	5								1			1			1					1			1	
VIR	*VIR*	*1*					*1*							*1*	*1*		*1*			*1*	*1*		*1*	*1*	
VIR	*VIR*	*2*					*1*							*1*	*1*		*1*			*1*	*1*		*1*	*1*	
VIR	**VIR**	**3**					**1**															**1**			
VIR	**VIR**	**4**					**1**															**1**			
VIR	VIR	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			1			

4.3.1.2.3. Section *Synstylae*, *R. arvensis*

R. arvensis is the only autochthonous diploid rose taxon in Belgium. Several populations were sampled and analysed originating from different localities in four regions of provenance. In total, STMS loci were compared among 31 *R. arvensis* shrubs. Seventeen individuals were sampled in West-Vlaams Heuvelland (Galgebossen, Helleketelbos, Ploegsteert, and Kemmel), 15 in Vlaamse Zandstreek (Brakel), five in Voeren (Remersdaal), and four in the Viroin (Nismes, Tienne aux Pauquis, and Olloy).

The six analysed STMS loci differed in level of polymorphisms; RhB303 was the most diverse locus with eight different alleles, while RhM405 displayed only two polymorphisms. Moreover, the locus RhO517 was monomorphic within this taxon (Tables 4.62 and 4.63). In some populations, unique alleles were detected; e.g. Helleketelbos: RhB303_141, Ploegsteert: RhAB22_190, Voeren: RhAB22_159 (Figures 4.52 and 4.53).

Tables 4.62 and 4.63 summarised the allelic phenotypes, given the diploid state of this taxon it was possible to assess the clones. For instance, the individuals R ARV BR 1 to 6, R ARV BR 8 to 9, R ARV RE 2 and 3, or R ARV RE 4 and 5 are presumed to represent one genotype each. The majority of the analysed samples displayed a unique genotype. The shrubs were sampled with the intention to reduce the number of clones, so the genetic variation within populations could be assessed. Based on the output of the STMS analyses, the sampling of different genotypes in one population was achieved.

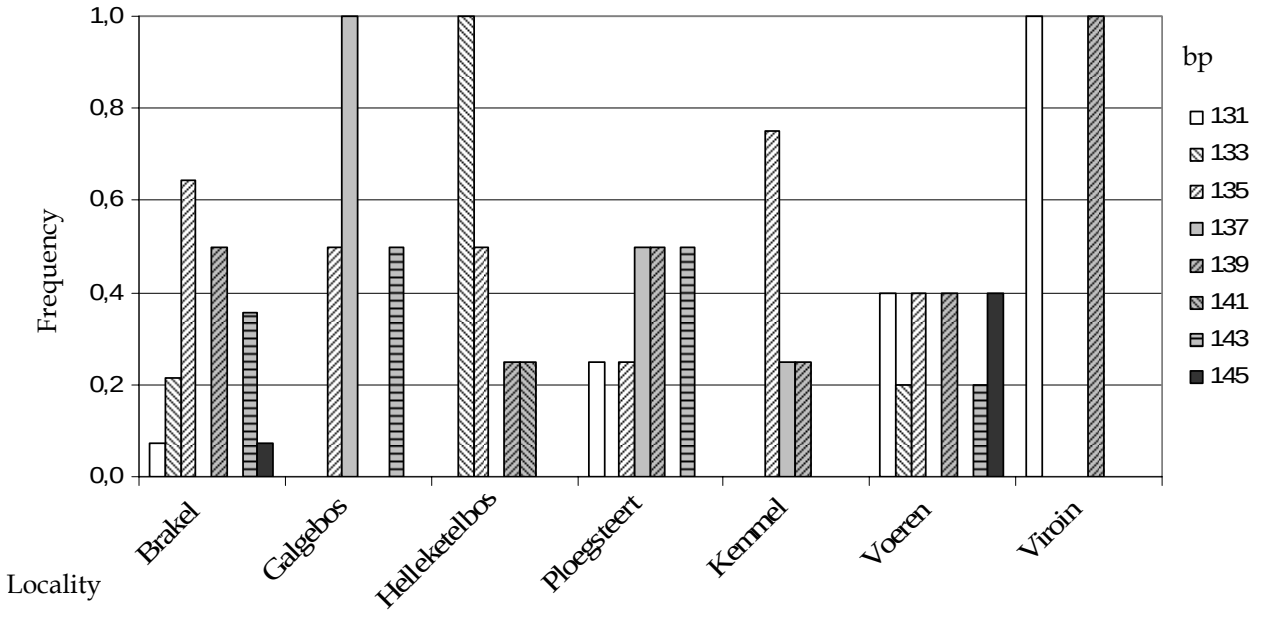


Figure 4.52: Polymorphisms of the locus RhB303 in the analysed *R. arvensis* populations. Length of the observed alleles in base pairs (bp), the frequency of samples in which they were detected, and the localities of origin are indicated.

Table 4.62: Polymorphisms of the loci RhAB15, RhO517, RhAB22, RhB303, RhM405, and RhP519 in the analysed *R. arvensis* populations of West-Vlaams Heuvelland and Voeren. Length of the alleles in bps, the region of provenance, the locality (abbr: see Table 4.34), and individual numbers are indicated. Similar phenotypes are marked by a combination of bold, Italics, and underlined. With: "-": absence of data; "1": presence of the allele; " ": absence of the allele.

	RhAB15				RhO517	RhAB22							RhB303								RhM405		RhP519		
Nr	111	113	115	117	255	159	171	178	180	182	190	198	131	133	135	137	139	141	143	145	164	177	228	237	247
West-Vlaams Heuvelland																									
GB22	1		1		1		1									1			1			1	1		
GB23	1		1		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	-	-	-
GB25	1			1	1				1				-	-	-	-	-	-	-	-	1	1	1		1
GB27			1	1	1		1		1						1	1					1	1	1		
HKB1		1		1	1	-	-	-	-	-	-	-		1			1					1	-	-	-
HKB2		1	1		1	-	-	-	-	-	-	-		1	1							1	-	-	-
HKB3		1	1		1	-	-	-	-	-	-	-		1	1						1	1	-	-	-
HKB5		1			1	-	-	-	-	-	-	-		1				1				1	-	-	-
PL1	-	-	-	-	-			1		1			1			1						1	1		
PL2			1		1			1				1			1				1			1	1		1
PL3		1	1		1			1		1							1		1			1	1		1
PL4		1	1		1				1				-	-	-	-	-	-	-	-	1	1	1		
PL5	1	1			1				1		1					1	1					1	1		1
1	-	-	-	-	-	-	-	-	-	-	-	-			1	1					-	-	-	-	-
2	1		1		1			1	1						1							1	1		
3	1		1		1		1	1							1						1	1	1		
5	1			1	1		1			1							1				1	1	1		
Voeren																									
RE1				1	1	1			1					1					1			1	1	1	
RE2	**1**		**1**		**1**				**1**	**1**					**1**					**1**		**1**	**1**		
RE3	**1**		**1**		**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**			**1**					**1**		**1**	**1**		
RE4			*1*		*1*					*1*			*1*				*1*					*1*			*1*
RE5			*1*		*1*	**-**	**-**	**-**	**-**	**-**	**-**	**-**	*1*				*1*					*1*	**-**	**-**	**-**

Table 4.63: Polymorphisms of RhAB15, RhO517, RhAB22, RhB303, RhM405, and RhP519 in the analysed *R. arvensis* populations of Vlaamse Ardennen and Viroin. Length of the alleles in bp, the region of provenance, the locality (abbr: see Table 4.34), and individual numbers are indicated. Similar phenotypes are marked by a combination of bold, Italics, and underlined. With: "Kw": sampled in the nursery of INBO; "-": absence of data; "1": presence of the allele; " ": absence of the allele.

	RHAB15				RHO517	RHAB22							RHB303								RHM405		RHP519		
NR	111	113	115	117	255	159	171	178	180	182	190	198	131	133	135	137	139	141	143	145	164	177	228	237	247
Vlaamse Ardennen, Brakel																									
1	-	-	-	-	-	-	-	-	-	-	-	-			1		1				1	1	-	-	-
2	1	1			1	-	-	-	-	-	-	-			1		1				-	-	-	-	-
3	1	1			1				1	1					1		1				1	1	1		
4	1	1			1				1	1					1		1				1	1	1		
5	1	1			1				1	1					1		1				1	1	1		
6	1	1			1				1	1					1		1				1	1	1		
7	1				1					1				1							1	1	1		
8	1	1			1				1	1				1					1			1	1	1	
9	1	1			1				1	1				1					1			1	1	1	
10	1	1			1					1			-	-	-	-	-	-	-	-	1	1	1		
449		1		1	1					1							1		1		1	1	1		
450			1	1	1					1			1								1	1	1		
457		1	1		1			1	1						1					1	1	1	1		1
458			1	1		-	-	-	-	-	-	-			1				1		-	-	-	-	-
467		1			1				1						1				1		1	1	1		1
Viroin																									
Kw		1			1					1		1	1				1				1		1	1	
3	1		1			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	-	-	-
4	1	1			1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	-	-	-
5			1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-

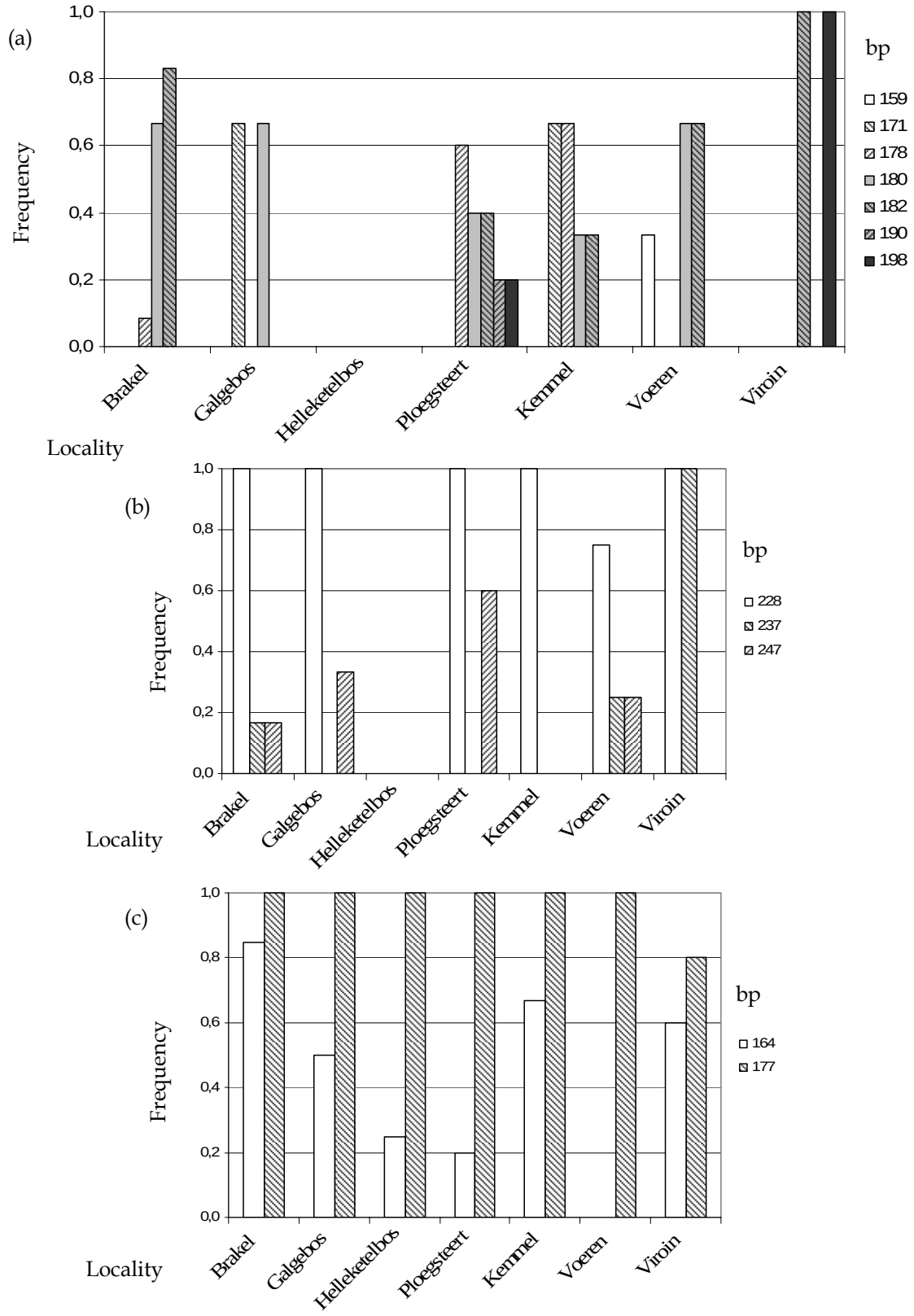


Figure 4.53: Polymorphisms of (a) RhAB22; (b) RhP519; (c) RhM405 in the analysed *R. arvensis* populations. Length of the observed alleles in base pairs (bp), the frequency of samples in which they were detected, and the localities of origin are indicated.

4.3.1.2.4. The origin of *R. stylosa* and *R.* x *irregularis*

Based on the morphological characters, *R. stylosa* and *R.* x *irregularis* are the presumed descendants of interspecific crosses between a *Caninae* mother parent and a paternal *R. arvensis*. The three candidate *Caninae* taxa, *R. canina*, *R. corymbifera,* and *R. balsamica*, mainly differ in pubescence, presence of glands, and serration of the leaflets. However, the AFLP and STMS polymorphisms did not display interspecific differences.

In order to confirm or reject the hypothesis of origin of both hybrids and reveal the true *Caninae* parent, the allelic phenotypes of both hybrids *R.* x *irregularis* and *R. stylosa* were compared to those of the putative parental taxa.

The *Caninae* parent on the one hand and *R. arvensis* on the other both displayed taxon-specific alleles (Tables 4.64 to 4.66 and Figures 4.54 and 4.55). The alleles RhM405_152, RhP519_225, RhO517_252, RhB303_112, RhB303_114, RhB303_122, RhB303_125, RhB303_127, RhAB15_93, RhAB15_97, RhAB15_107, and RhAB15_109 were completely absent in all randomly chosen *R. arvensis* shrubs, but were fixed or present in (one of) the *Caninae* parent taxa and in both hybrids. In contrast, the alleles RhP519_237, RhB303_135, and RhAB22_182 were only observed in *R. arvensis* and *R. stylosa*; whereas they were completely absent in the presumed *Caninae* parents.

Within the *R. stylosa* population sampled at Ter Yde (Westkust), six of the analysed samples could be assigned to only one genotype. They were located in each others neighbourhood and therefore can be assumed to be clones.

The allelic phenotypes of the presumed *Caninae* parent, of the *R. arvensis* paternal parent, and of the two hybrids: *R. stylosa* and *R.* x *irregularis* were compared. The analysed hybrids showed a mixture of *R. arvensis* and *Caninae* alleles. Moreover, the analysed *R. stylosa* individuals displayed species-specific alleles of the *Caninae* parent, and of the *R. arvensis* parent, whereas *R.* x *irregularis* only displayed species-specific alleles of the *Caninae* parent.

The hypothesis about the origin of the hybrids could not be rejected based on this output, however based on these polymorphisms no conclusion can be drawn concerning the *Caninae* parent.

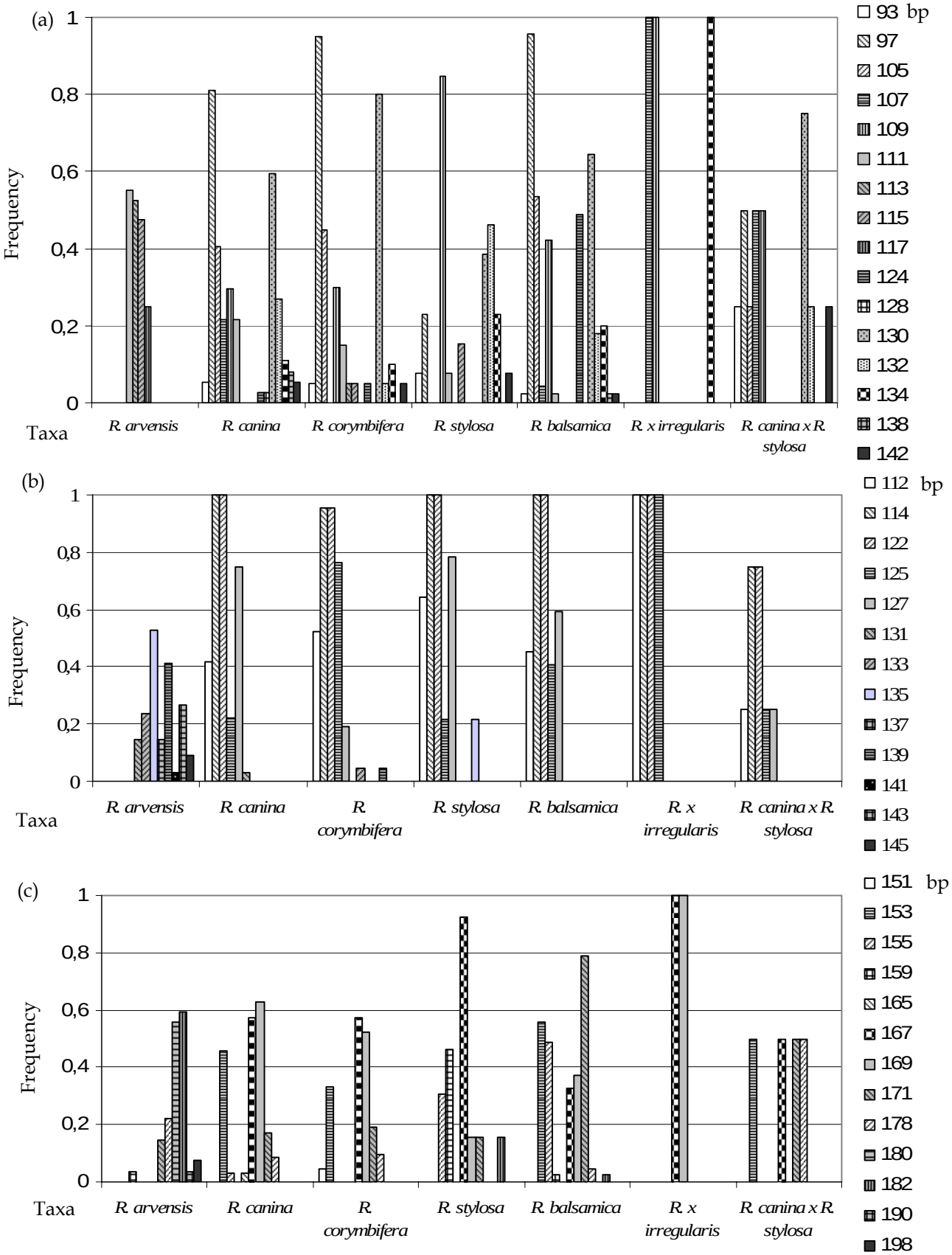


Figure 4.54: Polymorphisms of (a) RHAB15; (b) RhB303; (c) RhAB22 in *R. stylosa*, *R.* x *irregularis* and the putative parental taxa. Length of the observed alleles in base pairs (bp), the frequency of samples in which they were detected, and the presumed parental taxa are indicated.

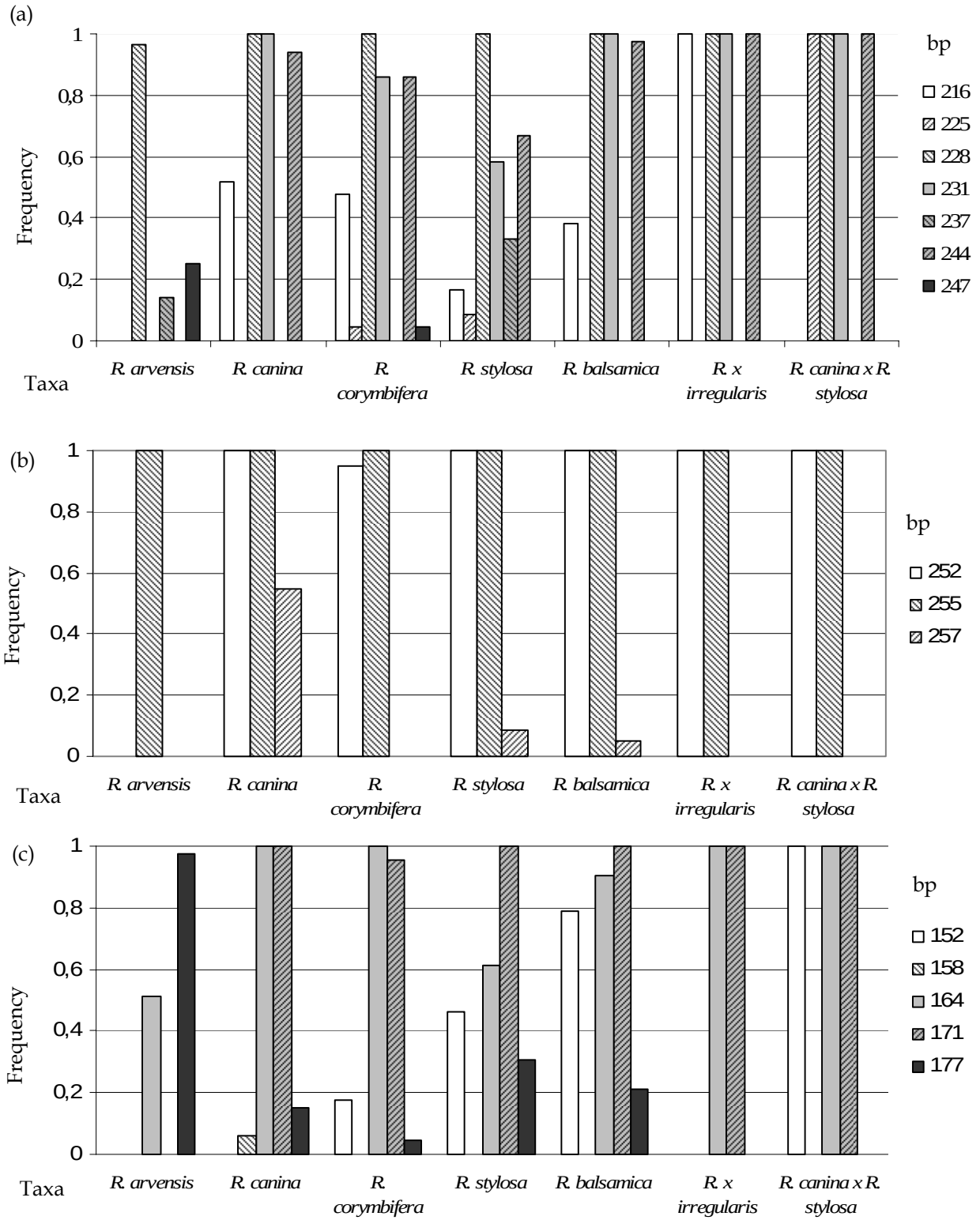


Figure 4.55: Polymorphisms of (a) RhP519; (b) O517; (c) RhM405 in *R. stylosa*, *R.* x *irregularis* and the putative parental taxa. Length of the observed alleles in base pairs (bp), the frequency of samples in which they were detected, and the presumed parental taxa are indicated.

Table 4.64: Polymorphisms of the loci RhAB15 and RhO517 for the analysed putative hybrids *R. stylosa* and *R.* x *irregularis,* and their parental taxa: *R. arvensis* and *Caninae* parent (*R. canina*, *R. corymbifera,* and *R. balsamica*). For each taxon* the allelic phenotype was indicated; for each individual° (ABBR. species name and locality –Table 4.34); the presence (1) or absence ( ) of that allele was noted. Individual codes consisted of species, locality or region, and number. Missing values (-); species-specific alleles (bold), and presumable clones (Italics) were indicated.

	RHAB15																RHO517		
TAXON*	**93**	**97**	105	**107**	**109**	111	113	**115**	117	124	128	**130**	**132**	**134**	138	**142**	**252**	255	258
R. arvensis	0.00	0.00	0.00	0.00	0.00	0.55	0.53	**0.48**	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
R. canina	**0.05**	**0.81**	0.41	**0.22**	**0.30**	0.22	0.00	0.00	0.00	0.03	0.03	**0.59**	**0.27**	**0.11**	0.08	**0.05**	**1.00**	1.00	**0.55**
R. corymbifera	**0.05**	**0.95**	0.45	0.00	**0.30**	0.15	0.05	0.05	0.00	0.05	0.00	**0.80**	**0.05**	**0.10**	0.00	**0.05**	**0.95**	1.00	0.00
R. balsamica	**0.02**	**0.93**	0.52	**0.07**	**0.43**	0.02	0.00	0.00	0.00	0.48	0.00	**0.63**	**0.20**	**0.20**	0.02	**0.02**	**1.00**	1.00	**0.05**
R. stylosa	**0.08**	**0.23**	0.00	0.00	**0.85**	0.08	0.00	**0.15**	0.00	0.00	0.00	**0.38**	**0.46**	**0.23**	0.00	**0.08**	**1.00**	1.00	**0.08**
R. x *irregularis*	0.00	0.00	0.00	**1.00**	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	**1.00**	1.00	0.00
INDIVIDUAL°																			
R STY TY 1					*1*								*1*				*1*	*1*	
R STY TY 6					*1*								*1*				*1*	*1*	
R STY TY 7					*1*								*1*				*1*	*1*	
R STY TY 8					*1*								*1*				*1*	*1*	
R STY TY 9					*1*								*1*				*1*	*1*	
R STY TY 10					*1*								*1*				-	-	-
R STY WVH 1					**1**							1					**1**	1	
R STY BE WVH 1					**1**			1						1			**1**	1	
R STY BE WVH 2					**1**			1						1			**1**	1	
R STY WVH 20					**1**							1					**1**	1	
R STY WVH 21		**1**				1						1					**1**	1	
R STY WVH 22	**1**	**1**										1				1	**1**	1	
R STY WVH 23		**1**			**1**							1		1			**1**	1	1
RxIRR RI 1				**1**	**1**									1			1	1	
R CANxR STY TY 1				**1**	**1**								1				-	-	-
R CANxR STY TY 2				**1**	**1**							1					**1**	1	
R CANxR STY WVH 1		**1**	1									1					**1**	1	

Table 4.65: Polymorphisms of the loci RhAB15 and RhO517 for the analysed putative hybrids *R. stylosa* and *R.* x *irregularis,* and their parental taxa: *R. arvensis* and *Caninae* parent (*R. canina, R. corymbifera,* and *R. balsamica*). For each taxon* the allelic phenotype was indicated; for each individual° (ABBR. species name and locality –Table 4.34); the presence (1) or absence ( ) of that allele. Individual codes consisted of species, locality or region, and number.Missing values (-); species-specific alleles (bold), and presumable clones (Italics) were indicated.

	RHAB22													RHM405				
TAXON *	151	153	**155**	**159**	165	**167**	169	171	178	180	**182**	190	198	**152**	158	164	**171**	177
R. arvensis	0.00	0.00	0.00	**0.04**	0.00	0.00	0.00	0.15	0.22	0.56	**0.59**	0.04	0.07	0.00	0.00	0.51	0.00	0.97
R. canina	0.00	0.46	**0.03**	0.00	0.03	**0.57**	**0.63**	0.17	0.09	0.00	0.00	0.00	0.00	0.00	0.06	1.00	**1.00**	0.15
R. corymbifera	0.05	0.33	0.00	0.00	0.00	**0.57**	**0.52**	0.19	0.10	0.00	0.00	0.00	0.00	**0.17**	0.00	1.00	**0.96**	0.04
R. balsamica	0.00	0.55	**0.48**	**0.02**	0.00	**0.34**	**0.36**	0.80	0.05	0.00	0.02	0.00	0.00	**0.80**	0.00	0.91	**1.00**	0.20
R. stylosa	0.00	0.00	**0.31**	**0.46**	0.00	**0.92**	**0.15**	0.15	0.00	0.00	**0.15**	0.00	0.00	**0.46**	0.00	0.62	**1.00**	0.31
R. x *irregularis*	0.00	0.00	0.00	0.00	0.00	**1.00**	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	**1.00**	0.00
INDIVIDUALS°																		
R STY TY 1				*1*		*1*										*1*	*1*	
R STY TY 6				*1*		*1*										*1*	*1*	
R STY TY 7				*1*		*1*										*1*	*1*	
R STY TY 8				*1*		*1*										*1*	*1*	
R STY TY 9				*1*		*1*										*1*	*1*	
R STY TY 10				*1*		*1*										*1*	*1*	
R STY WVH 1			1			1								**1**			1	1
R STY BE WVH 1			1			1					**1**			**1**			1	1
R STY BE WVH 2			1			1					**1**			**1**			1	1
R STY WVH 20			1			1								**1**			1	1
R STY WVH 21							1	1						**1**		1	1	
R STY WVH 22						1		1						**1**		1	1	
R STY WVH 23						1	1										1	
RxIRR RI 1						1	1									1	1	
R CANxR STY TY 1		1							1					-	-	-	-	-
R CANxR STY TY 2	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**		1	1	
R CANxR STY WVH 1						1		1						**1**		1	1	

Table 4.66: Polymorphisms of the loci RhAB15 and RhO517 for the analysed putative hybrids *R. stylosa* and *R.* x *irregularis*, and their parental taxa: *R. arvensis* and *Caninae* parent (*R. canina*, *R. corymbifera*, and *R. balsamica*). For each taxon* the allelic phenotype was indicated; for each individual° (ABBR. species name and locality –Table 4.34); the presence (1) or absence ( ) of that allele. Individual codes consisted of species, locality or region, and number. Missing values (-); species-specific alleles (bold), and presumable clones (Italics) were indicated.

	RHB303													RHP519						
TAXON*	**112**	**114**	**122**	**125**	**127**	131	133	**135**	137	139	141	143	145	**216**	**225**	228	**231**	**237**	**244**	247
R. arvensis	0.00	0.00	0.00	0.00	0.00	0.15	0.24	**0.53**	0.15	0.41	0.03	0.26	0.09	0.00	0.00	0.96	0.00	**0.14**	0.00	0.25
R. canina	**0.42**	**1.00**	**1.00**	**0.22**	**0.75**	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.52**	0.00	1.00	**1.00**	0.00	**0.94**	0.00
R. corymbifera	**0.52**	**0.95**	**0.95**	**0.76**	**0.19**	0.00	0.05	0.00	0.00	0.05	0.00	0.00	0.00	**0.48**	**0.05**	1.00	**0.86**	0.00	**0.86**	0.05
R. balsamica	**0.47**	**1.00**	**1.00**	**0.40**	**0.58**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.38**	0.00	1.00	**1.00**	0.00	**0.98**	0.00
R. stylosa	**0.64**	**1.00**	**1.00**	**0.21**	**0.79**	0.00	0.00	**0.21**	0.00	0.00	0.00	0.00	0.00	**0.17**	**0.08**	1.00	**0.58**	**0.33**	**0.67**	0.00
R. x *irregularis*	**1.00**	**1.00**	**1.00**	**1.00**	**0.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.00**	0.00	1.00	**1.00**	0.00	**1.00**	0.00
INDIVIDUALS°																				
R STY TY 1	*1*	*1*	*1*		*1*											*1*			*1*	
R STY TY 6	*1*	*1*	*1*		*1*											*1*			*1*	
R STY TY 7	*1*	*1*	*1*		*1*											*1*			*1*	
R STY TY 8	*1*	*1*	*1*		*1*											*1*			*1*	
R STY TY 9	*1*	*1*	*1*		*1*											*1*			*1*	
R STY TY 10	*1*	*1*	*1*		*1*									-	-	-	-	-	-	-
R STY WVH 1		1	1		1											1	1	**1**		
R STY BE WVH 1		1	1		1			**1**								1	1	**1**		
R STY BE WVH 2		1	1		1			**1**								1	1	**1**		
R STY WVH 20		1	1		1											1	1	**1**		
R STY WVH 21	1	1	1	1										1		1	1		1	
R STY WVH 22	1	1	1	1											**1**	1	1		1	
R STY WVH 23	1	1	1	1										1		1	1		1	
RxIRR RI 1	1	1	1	1										1		1	1		1	
R CANxR STY TY 1		1	1	1										-	-	-	-	-	-	-
R CANxR STY TY 2		1	1											-	-	-	-	-	-	-
R CANxR STY WVH 1	1	1	1		1										**1**	1	1		1	

4.3.1.2.5. Reproduction of isolated plants

Of an isolated *R. micrantha* mother plant, several hips were harvested and seeds were sown. Of nine randomly chosen seedlings, the allelic phenotypes were compared to that of the mother plant (Table 4.67). The few descendents showed allelic differentiation for the loci RhM405, RhP519, and RhAB22. Moreover, also among the seedlings genetic variation was observed. Some seedlings lacked one of the alleles detected in the mother plant, e.g. RhM405_152, RhM405_164, RhP519_244, and RhAB22_167. In contrast and more striking was the presence of allele RhM405_158 in one seedlingthat was absent in the mother plant. This suggests the presence of an external parent.

The occurrence of introgression in the wild is suggested by this small-scale experiment. Few seedlings were characterised by an allele that was absent in the isolated mother plant. In the close neighbourhood of this *R. micrantha* mother plant, other wild rose shrubs such as *R. stylosa* and *R. agrestis* were observed. However, these shrubs also lacked the presumed introgressed allele, RhM405_158. This allele was only detected in some *R. canina* individuals from De Pinte (Vlaamse Zandstreek) and *R. spinosissima*.

Table 4.67: Allelic phenotypes (STMS polymorphisms) of the isolated *R. micrantha* mother plant (presence: 1 or absence: 0) and all nine spontaneous descendants (%). The alleles present in the mother plant (M), but absent in few seedlings (S) are marked in Italics, the allele lacking in the mother plant but displayed by few seedlings is highlighted in bold.

	RHM405				RHP519			RH0517		RHAB15			RHAB22	
	152	**158**	*164*	171	228	231	*244*	252	255	107	109	134	*167*	171
M	*1*	**0**	*1*	1	1	1	*1*	1	1	1	1	1	*1*	1
S	*0.7*	**0.2**	*0.7*	1.0	1.0	1.0	*0.9*	1.0	1.0	1.0	1.0	1.0	*0.7*	1.0

4.3.2. Morphological evaluations

In our morphological analysIs, the emphasis was on *R. arvensis* (section *Synstylae*) and the most common Flemish section *Caninae* taxa. The set of analysed morphological characters was based on previously published research.

First, all characters were analysed exploratively, followed by the assessment of the diagnostic morphometric and descriptive characters. Therefore, Principal Component Analysis (PCA) was performed. The biplot visualises the correlations among the diagnostic characters, the relationships between the individuals, and the influence of these characters on the individuals. Each vector represents a character. The direction and length of each vector is quantified by loadings giving information on correlations with other characters and on the impact of that character on the individuals. Characters with comparable loadings are correlated. The percentage of the variation explained by each character in each component was calculated based on the loadings. A character was determined diagnostic for taxonomical identification if at least 50% of the variation was explained in the first two components, or if it explained the majority of variation in one of the other components. Only completely analysed individuals (with both leaf and hip data) were included. Based on these assumptions, the eight analysed morphometric and nine of the descriptive characters were identified to have a discriminating value among the analysed taxa (Table 4.68).

For each of these diagnostic characters, interspecific comparisons were made using Box-and-Whisker plots for the morphometric characters and histograms for the descriptive characters.

Next, different strategies were tested to obtain the most optimal selection of discriminative characters to distinguish between the analysed taxa. Two strategies displayed the most discriminating power on the analysed individuals and similar results were obtained. In both strategies, correlations between the diagnostic characters were identified and one representative character was chosen for each group of correlated characters (Table 4.69).

All the measured leaflet dimensions (length, width, and base of the leaflet, and the length of the rachis) appeared to be strongly correlated, although the leaflet base deviated little in the second component. Similar pattern was observed for leaflet width and rachis length in the third component. The length of the hip and the diameter of the disc also were correlated. In addition, both characters were inversely proportional with the length of the pedicel. Within the descriptive characters, the glands on the pedicel and on the hip, and the shape of the hip were correlated. In addition, they were independent from the correlated glands on the lower side of the leaflet and the serration of the leaflet margin. Finally, also the glands on the rachis and the leaflet margin were correlated.

In the first strategy, a PCA was based on these nine selected characters. In the second strategy, canonical discriminant analyses were performed on the same nine representative characters. All analyses and biplots were performed using S-Plus 6.2 Professional (Insightful Corporation).

Finally, species descriptions of Nilsson (1967, 1999), Graham and Primavesi (1993), Henker (2000) and Wissemann (2003) were compared among each other and with the observations on the Flemish roses.

Table 4.68: Loadings and cumulative percentage (CUM%) for each morphometric and descriptive character in the three Principal components. The used abbreviation for each character (ABBR) is indicated; the diagnostic characters are marked in bold; the components in which the majority of the variation is explained are underlined.

		LOADINGS			CUM%		
CHARACTER	ABBR	COMP.1	COMP.2	COMP.3	COMP.1	COMP.2	COMP.3
Leaflet Length	**LL**	0.49			90.7	**92.1**	92.4
Leaflet Base	**LB**	0.47	0.18		84.0	**88.3**	88.4
Leaflet Width	**LW**	0.46		-0.22	80.0	**80.9**	85.9
Rachis Length	**RL**	0.45		-0.14	75.6	**76.5**	78.5
Pedicel Length	**PL**		0.66	0.18	0.6	**62.4**	65.7
Hip Length	**HL**	0.17	-0.55	0.45	11.6	**53.6**	74.2
Diameter Disc	**D**	0.26	-0.43		25.3	**50.8**	51.6
Diameter Orifice	**O**	-0.19	-0.16	-0.83	13.0	16.3	85.3
Glandular Leaflet Margin	**MG**	0.39	0.17	0.12	75.8	**84.5**	86.7
Glandular Rachis	**RG**	0.40	0.12	0.13	79.3	**83.3**	85. 8
Glandular Pedicel	**PG**	0.22	-0.45		23.9	**82.8**	82.8
Glandular Leaflet lower side	**LlG**	0.34	0.25	0.23	59.4	**77.9**	86.0
Serration Leaflet Margin	**MS**	0.34	0.26	0.20	57.7	**76.9**	83.3
Pubescence Leaflet upper side	**LuP**	0.34		-0.18	59.7	**60.0**	65.0
Hip Shape	**HS**	0.20	-0.35	0.11	21.0	**56.5**	58.4
Pubescence Leaflet lower side	**LlP**	0.32	0.11	-0.33	52.5	**56.2**	73.7
Glandular Hip	**HG**	0.25	-0.29	-0.14	31.1	**54.8**	57.8
Styles	S		-0.38		1.8	43.9	44.2
Receptacle Shape	RS	-0.16	0.33		12.7	43.8	43.8
Shape Disc	DS	-0.15	0.19	-0.28	11.7	21.8	34.7
Pubescence Rachis	RP	0.19		-0.43	17.7	17. 7	47.9
Shape Prickle of Rachis	RPS		0.20	0.46	2.1	14.0	47.7
Pubescence Hip	HP		0.20	-0.24	0.0	11.0	20.3
Pubescence Pedicel	PP		0.19	-0.40	0.4	10.3	36.4
Shape Leaflet	LS				2.6	2.9	4.2

Table 4.69: The seventeen discriminating morphometric and descriptive characters, one character is chosen to represent a group of correlated characters.

REPRESENTATIVE CHARACTER	CORRELATED CHARACTERS
Diameter of disc	Length of hip
Diameter of orifice	
Length of pedicel	
Length of leaflet	Width and Base of leaflet, Length of rachis
Pubescence upper side of leaflet	
Pubescence lower side of leaflet	
Serration of leaflet margin	Glands on lower side of leaflet
Glands on leaflet margin	Glands on rachis
Glands on pedicel	Shape of hip, Glands on hip

4.3.2.1. Morphometrical characters

The interspecific differentiation within each of the eight presumed diagnostic morphometrical characters (Table 4.68) was visualised in Box-and-Whisker plots.

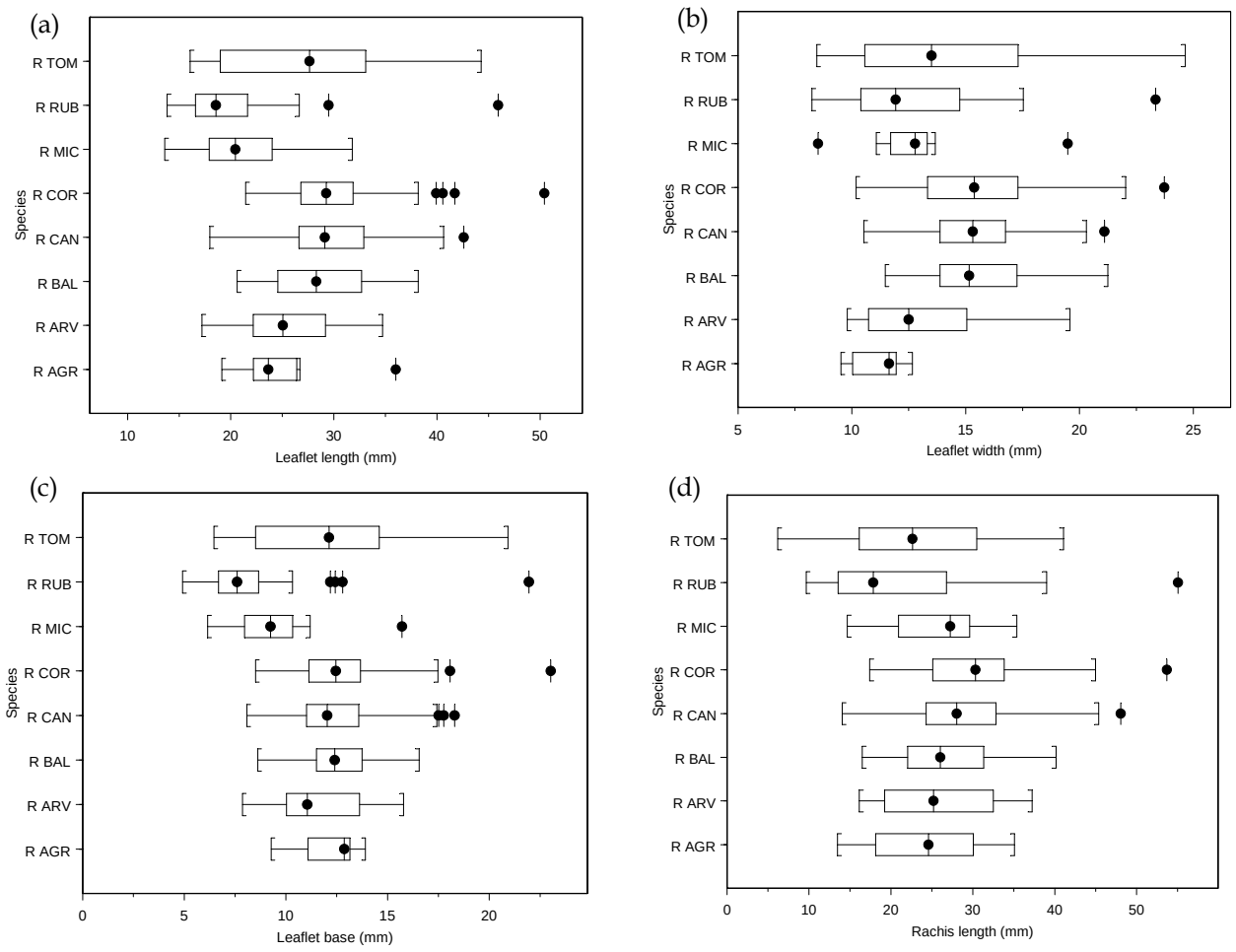


Figure 4.56: Box-and-Whisker plot of (a) leaflet length; (b) leaflet width; (c) leaflet base; (d) rachis length for each analysed taxon. For species codes see table 4.2.

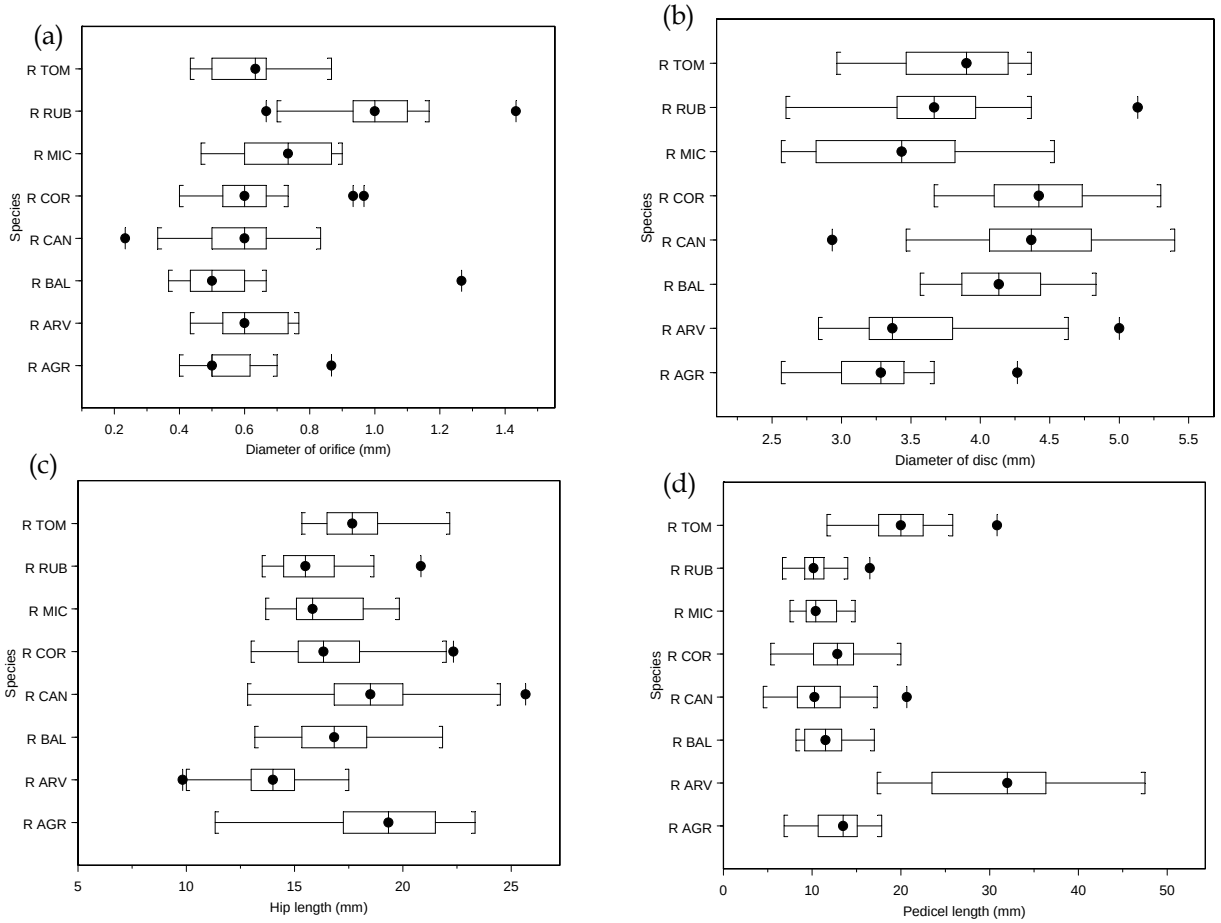


Figure 4.57: Box-and-Whisker plot of (a) diameter of the orifice; (b) diameter of the disc; (c) hip length; (d) pedicel length for each analysed taxon. For species codes see table 4.2.

The leaflet dimensions (length, width and base) and the length of the rachis did not show significant differentiations between the investigated taxa. However, few interspecific tendencies were observed (Figure 4.56). *R. tomentosa* displayed the most intraspecific variation in the leaflet dimensions. The other taxa could be assigned to two partly overlapping groups: a) *R. rubiginosa* and *R. micrantha* were characterised by small and short leaflets, with a smaller leaflet base; whereas b) the other taxa had wider and longer leaflets.

In contrast, the hip characters did reveal tendencies towards interspecific variation (Figure 4.57). Compared to the other taxa in which the diameter of the orifice is smaller than 0.9 mm, it was the largest in *R. rubiginosa* (> 0.7 mm). *R. micrantha* showed an overlapping and intermediate diameter of the orifice with both groups. The diameter of the disc divided the eight taxa into two, also overlapping, groups. The taxa with a larger disc were *R. canina*, *R. corymbifera,* and *R. balsamica*, in contrast to *R. agrestis*, *R. micrantha, R. arvensis, R. tomentosa,* and *R. rubiginosa* all having a more narrow disc. In literature, the disc index, this is the ratio of the diameter of the disc to the diameter of the orifice, is reported to be an important discriminating value (Henker 2000). In our analyses, this disc index divided the taxa in three groups: displaying a large (*R. balsamica* and the subsection *Caninae*), a small (*R. rubiginosa* and *R. micrantha*), and an intermediate disc index (*R. agrestis*, *R. tomentosa,* and *R. arvensis*). In addition, *R. arvensis* showed a tendency towards

smaller hips and significant longer pedicels. The hips of *R. agrestis* and the pedicels of *R. tomentosa* tended to be longer compared to the remaining taxa. The ratio of the length of the pedicel to the length of the hip, being the relative length of pedicel, is also assumed to have a discriminative value and was clearly higher in *R. arvensis* towards the other taxa. However, the relative length of the pedicel in *R. tomentosa* overlapped with both groups.

4.3.2.2. Descriptive characters

The interspecific variation of the nine diagnostic descriptive characters (Table 4.68) was visualised in histograms (Figures 4.58 to 4.60). In addition, the relevant interspecific differences of the non-diagnostic characters were summarised as they could be informative for only one taxon and not for all the taxa included in the data set.

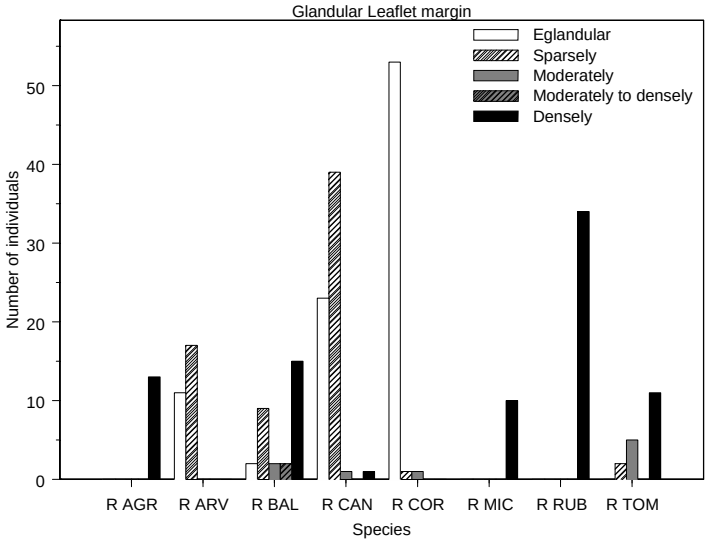


Figure 4.58: Histogram of glandular leaflet margin on the eight studied taxa. For species codes see table 4.2.

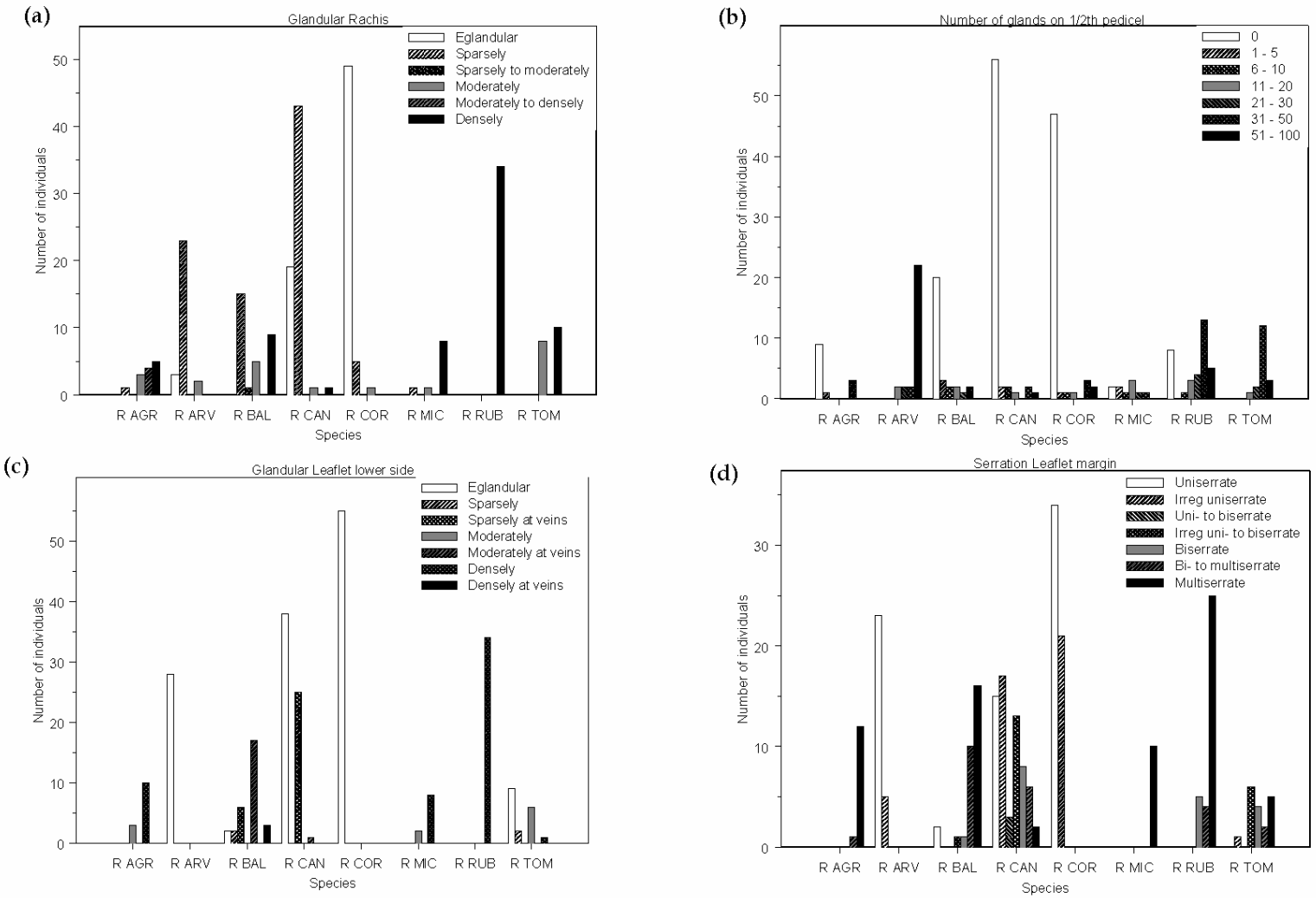


Figure 4.59: Histogram of (a) glandular rachis; (b) number of glands on 1/2th of the pedicel; (c) glandular leaflet lower side; (d) serration of the leaflet margin on the eight studied taxa. For species codes see Table 4.2.

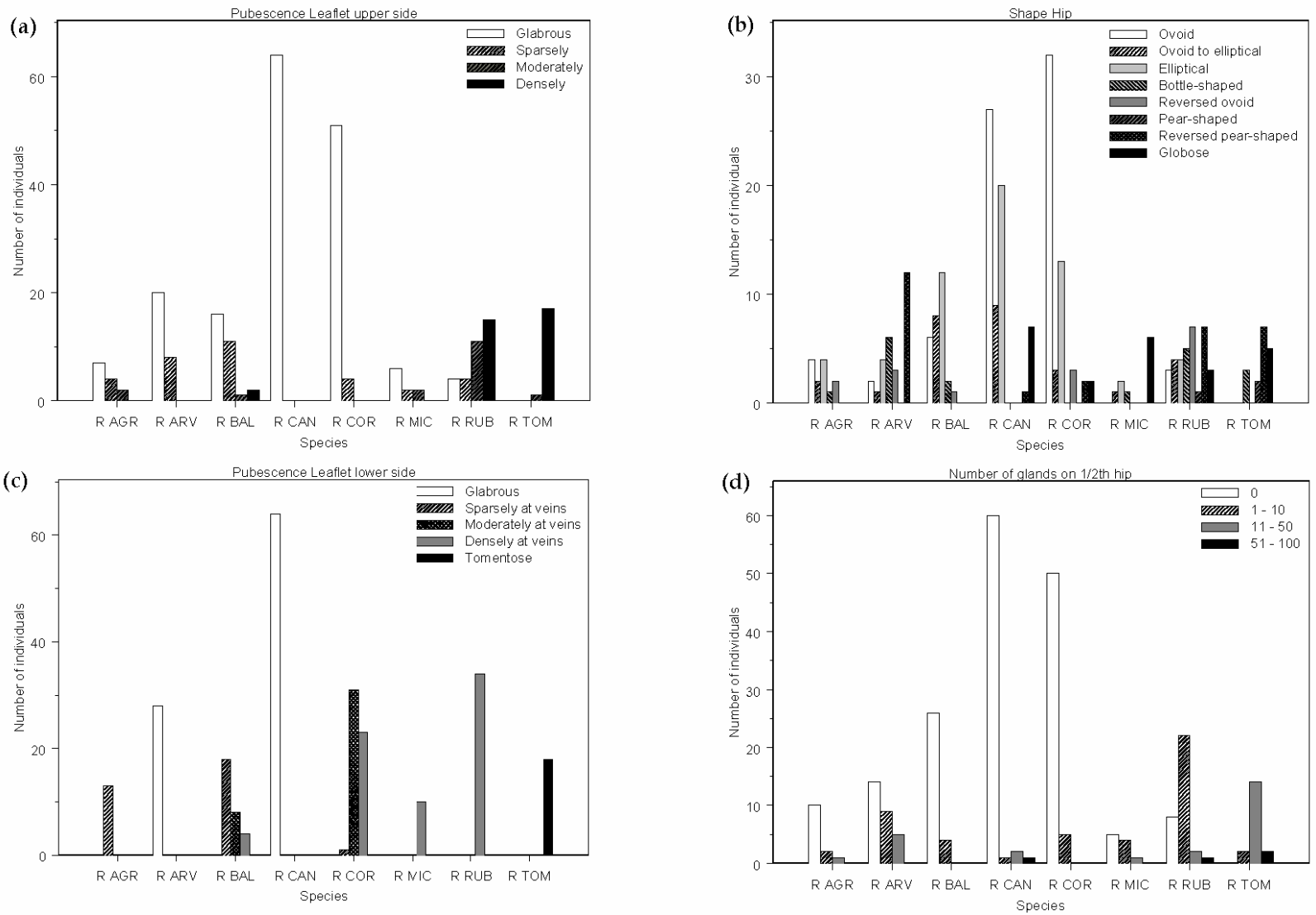


Figure 4.60: Histogram of (a) pubescence of the leaflet upper side; (b) shape of the hip; (c) pubescence of the leaflet lower side; (d) number of glands on 1/2th of the hip on the eight studied taxa. For species codes see Table 4.2.

R. arvensis (section *Synstylae*) displayed (irregular) uniserrated leaflet margins with typical bracket-shaped teeth. The leaflet margins and the rachides were eglandular or sparsely glandular, while the leaflet lower sides were always eglandular and glabrous. The upper side of the leaflets varied between glabrous and sparsely pubescent. The hips were mostly elliptical, bottle-shaped, or reversed pear-shaped, and sometimes (reversed) ovoid. They varied from mainly eglandular or sparsely to moderately glandular. The pedicels were mostly densely glandular. The agglutinated and elongated state of the styles is species-specific for *R. arvensis* as they form a loosely aggregated column in the other investigated taxa.

Within the section *Caninae*, the presence and type of the glands on the leaflets is strongly related with the grouping in subsections.

The taxa of the subsection *Rubigineae* showed densely glandular lower sides of the leaflets, leaflet margins, and rachides. However, for *R. agrestis* also moderately glandular rachides were observed. The hips of the *Rubigineae* were mainly eglandular or sparsely glandular, while the pedicels varied from eglandular to densely glandular with intermediate states. In the subsection *Vestitae*, only represented by *R. tomentosa*, the leaflet margins varied from densely to sparsely glandular, and the rachides from moderately to densely glandular. The lower sides of the leaflets were moderately glandular or even eglandular. Both the hips and pedicels were moderately or densely glandular with persistent and stipitated glands. The main difference between the glands of the subsections *Rubigineae* and *Vestitae* was the odour spread by the leaflet glands. The *Rubigineae* were characterised by a strong scent of apples, while the glands of *R. tomentosa* smelled like turpentine.

In contrast, *R. canina*, *R. corymbifera* (both subsection *Caninae*), and *R. balsamica* (subsection *Tomentellae*) were characterised by mainly eglandular pedicels and hips. These taxa displayed a difference in the presence of non-odourous glands on the leaflets. The rachides of *R. canina* and *R. corymbifera* were eglandular to sparsely glandular, while they varied for *R. balsamica* from sparsely to densely glandular. The leaflet margins and lower sides of the leaflets were mostly eglandular in *R. corymbifera*, and varied between eglandular or sparsely glandular (on the veins) in *R. canina*. In *R. balsamica* both sparsely and densely glandular margins, and sparsely to moderately glandular veins were observed.

Additional morphological differences between the *Caninae* taxa were the serration of the leaflet margins, the pubescence of both sides of the leaflets, and the shape of the hips. Within *R. tomentosa*, the serration varied from irregular uni- to biserrated, over biserrated and bi- to multiserrated, to multiserrated. Within the *Rubigineae,* all the taxa were mainly multiserrated, although biserration and intermediate forms were observed in *R. rubiginosa*. *R. corymbifera* displayed (irregular) uniserrated leaflet margins, while it was mostly bi- to multi-, and multiserrated in *R. balsamica*. *R. canina* showed the largest intraspecific variation concerning the serration of the leaflet margin, going from (irregular) uniserrated, bi- and occasionally to multiserrated margins, including intermediate forms.

R. tomentosa was the only taxon with a very densely pubescent upper side and tomentose lower side of the leaflets. This is in large contrast with *R. canina*, which had always glabrous lower and upper sides of the leaflets. Within *R. corymbifera*, the pubescence on the lower side varied from moderately to densely at the veins, while *R. balsamica* was characterised by sparsely pubescent veins, sometimes varying towards densely pubescent. On the upper side, the pubescence of the two taxa varied between glabrous and sparse. Within the subsection *Rubigineae*, a difference was observed between *R. agrestis* having a more sparse pubescence on the veins, and *R. rubiginosa* and *R. micrantha,* both displaying densely pubescent veins. Within the subsection *Rubigineae*, the lower sides were mainly densely pubescent on the three taxa. In contrast, *R. rubiginosa* had moderately and densely pubescent lower sides, while *R. agrestis* and *R. micrantha* were mostly glabrous, or sparsely pubescent. Additionally, *R. agrestis* was characterised by a typical elongated-elliptical leaflet with wedge-shaped bases.

The studied *R. rubiginosa* individuals showed the most variation in the shape of the hip, varying from ovoid to elliptical, with in addition (reversed) pear-and bottle-shaped and globose hips. Also, *R. tomentosa* showed a lot of variation in the hip shape. In contrast, the hips of *R. canina* and *R. micrantha* were ovoid- to elliptical and sometimes globose. *R. agrestis*, *R. corymbifera*, and *R. balsamica* had ovoid- to elliptical hips.

The shape of the disc and the shape of the prickle of the rachis showed little to no interspecific variation. This is remarkable for the shape of the disc which is described as an important diagnostic character in literature (Henker 2000).

4.3.2.3. Integration of diagnostic morphometric and descriptive characters

4.3.2.3.1. Interspecific variation

Principal Components Analyses (PCA)

The PCA based on the nine selected representative characters was the most discriminating method to combine the morphometric and descriptive diagnostic characters. The nine independent characters were glandular leaflet margin (MG), pedicel length (PL), serration leaflet margin (MS), glandular pedicel (PG), pubescence leaflet upper side and lower side (LuP and LlP), leaflet length (LL), and diameter of disc (D) and orifice (O).

In total, 223 individuals were analysed in a PCA. The first three components explained 39%, 16%, and 12%, respectively, of the variation (Table 4.70, Figure 4.61).

Table 4.70: Loadings and cumulative percentages (CUM%) of the morphometric and descriptive representative characters. The correlated characters are indicated and the components in which each character explains the majority of the variation are marked in bold. Abbreviations used see table 4. 68.

CHARACTERS		LOADINGS			CUM%		
REPR	CORRELATED WITH	COMP.1	COMP.2	COMP.3	COMP.1	COMP.2	COMP.3
MG	RG	0.456	-0.240	0.159	**72.8**	81.1	83.9
PL			0.728	0.110	0.8	**77.6**	78.9
MS	LlG	0.377	-0.368	0.341	**49.9**	69.5	82.0
PG	HG, HS	0.306	0.445	-0.263	**32.9**	61.6	69.1
LuP		0.408		-0.250	**58.2**	58.2	65.0
LlP		0.339	0.139		**40.4**	43.2	43.4
D	HL	-0.322	-0.192	-0.475	**36.4**	41.8	66.0
LL	LB, LW, RL	-0.319			**35.6**	35.6	35.8
O		0.258	-0.149	-0.697	23.2	26.5	**78.8**

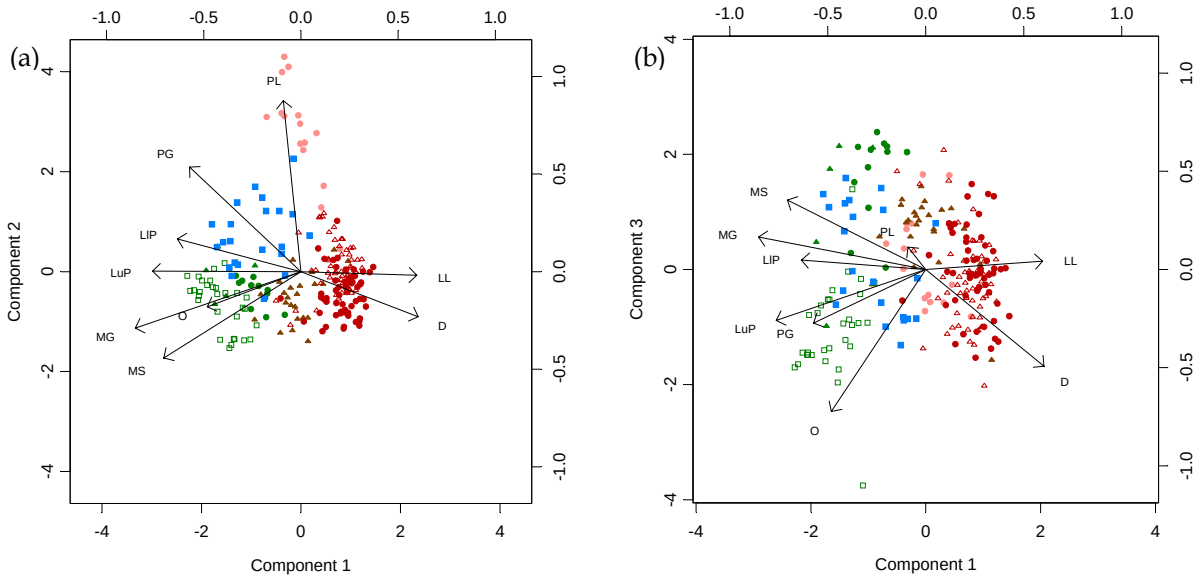


Figure 4.61: Biplots of the Principal components based on the nine selected morphological characters. (a) The first two components; (b) the first and third component. With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); leaflet length (LL); diameter orifice (O); diameter disc (D); pedicel length (PL); *R. arvensis* (●); *R. tomentosa* (■); *R. rubiginosa* (□); *R. micrantha* (▲); *R. agrestis* (●); *R. balsamica* (▲); *R. canina* (●); *R. corymbifera* (△).

The integration of the morphometric and descriptive characters stressed the morphological differentiation between the sections *Synstylae* and *Caninae*. Especially, the longer pedicels for *R. arvensis* branched off the section *Synstylae* along the second component from the more central and spherical cluster: the section *Caninae* (Figure 4.61a). Within the sphere formed by the section *Caninae* different groups could be identified, each was represented by one of the subsections. However, overlap between the different parts was still present.

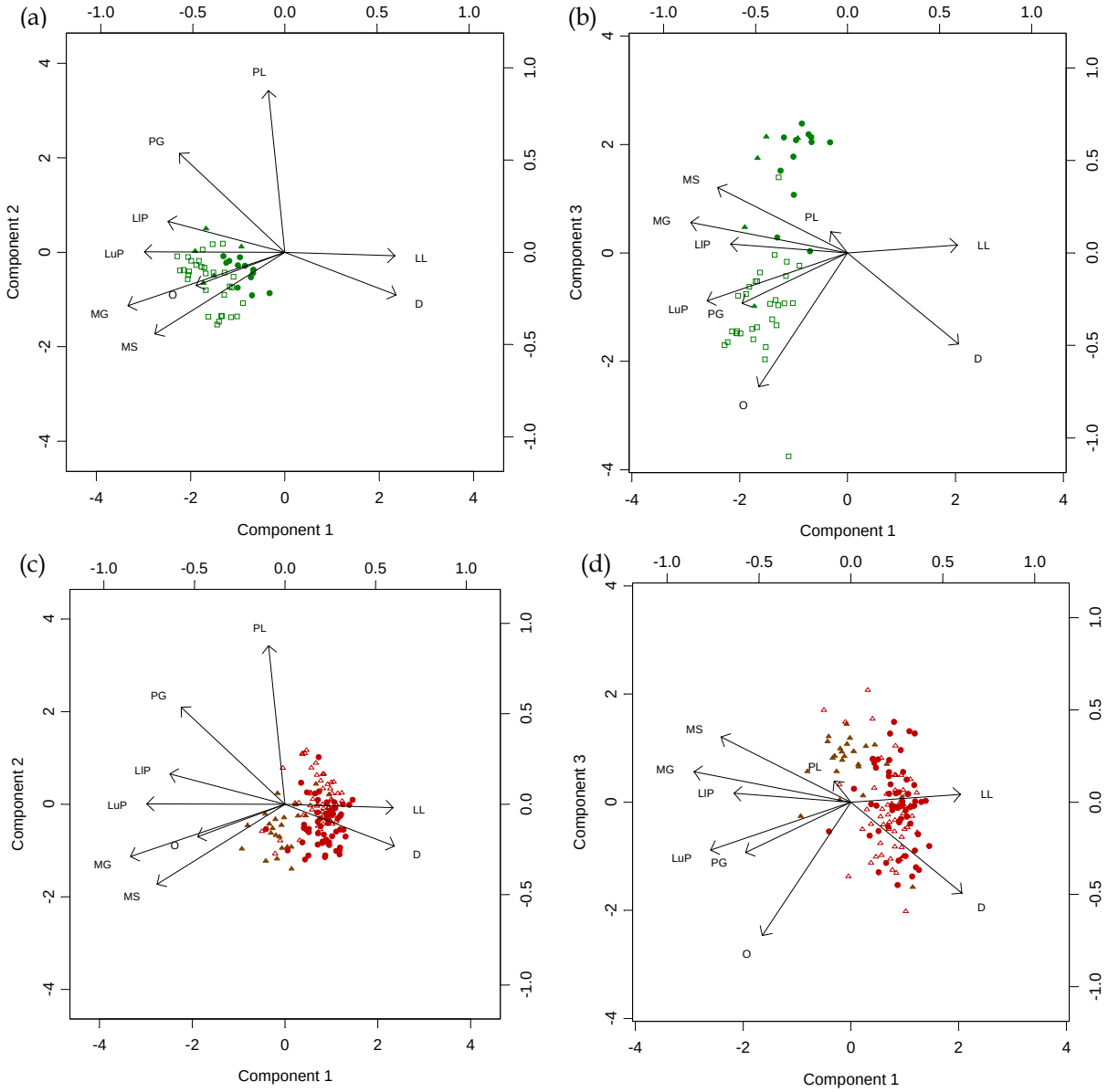


Figure 4.62: Biplots of the Principal components of (a; b) the subsection *Rubigineae;* (c; d) the subsections *Caninae* and *Tomentellae* based on the nine selected morphological characters. (a; c) the first two components; (b; d) the first and third component. With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); leaflet length (LL); diameter orifice (O); diameter disc (D); pedicel length (PL); *R. rubiginosa* (□); *R. micrantha* (▲); *R. agrestis* (●); *R. balsamica* (▲); *R. canina* (●); *R. corymbifera* (△).

In general, *R. tomentosa* (subsection *Vestitae*) was characterised by more narrow diameters of the disc, shorter hips, longer and more densely glandular pedicels and moderate but persistent stipitate glands on the reversed pear-shaped or globose hips (Figure 4.61a). In addition, the pubescence on the lower side of the leaflets was densely tomentose, while the densely pubescent upper side of the leaflets was less pronounced.

The subsection *Rubigineae* had densely glandular and multiserrated leaflet margins, densely glandular rachides, and leaflet lower sides. Within this subsection, *R. rubiginosa* distinguished from *R. agrestis* by a clearly larger diameter of the orifice,

a more frequent presence of multiserrated leaflet margins, and more densely glandular leaflet margins, rachides, and lower sides of the leaflet. The few *R. micrantha* individuals overlapped both groups (Figure 4.62a,b). Compared to the other taxa of the section *Caninae*, the orifice of *R. rubiginosa* was the largest (Figure 4.61b). In addition, the leaflets of the subsection *Rubigineae* were clearly smaller and shorter than those of *R. balsamica* and the subsection *Caninae*.

The third part of the sphere was represented by the subsection *Caninae* that displayed longer and wider leaflets, longer rachides, broader discs, longer hips, and eglandular and glabrous to sparsely glandular or pubescent hips, leaflets, and pedicels (Figure 4.61a). Finally, *R. balsamica* (subsection *Tomentellae*) had an intermediate position between the subsections *Caninae* and *Rubigineae*. This was mostly based on the glandular leaflet margins, rachides, and lower leaflet sides (eglandular or sparsely glandular for the *Caninae* versus densely glandular for the *Rubigineae*), the pubescence of the upper side of the leaflets (glabrous for the *Caninae* versus moderately or densely pubescent for the *Rubigineae*), and the bi- to multiserration of the leaflet margins [(irregular) uniserrated for the *Caninae* versus multiserration for the *Rubigineae*]. However, the morphometrical characters showed little to no difference among the subsections *Caninae* and *Tomentellae*. The leaflets were larger compared to the *Rubigineae*, the pedicels shorter compared to *R. tomentosa* and the hips and the diameter of the disc were larger compared to both taxa.

Canonical Discriminant Analyses

The outcome of the canonical discriminant analysis confirmed the results of the PCA. The most discriminating characters distinguishing between the sections, subsections and taxa appeared to be the glandular leaflet margin (MG), the length of the pedicel (PL) and the diameter of the orifice (O). However, in this approach the subsections *Caninae* and *Tomentellae* overlapped completely. Consequently, this approach was not elaborated.

4.3.2.3.2. Intraspecific variation

The Principal Component Analysis, based on the nine selected and independent morphometric and descriptive characters (Table 4.70), was used to investigate and identify the presence of intraspecific variation.

For each taxon a separate biplot was shown similar to figure 4.61, only highlighting the individuals of that taxon that were labelled with the region of provenance. Significant intraspecific differences or tendencies visualised in the biplots were verified in the Box-and-Whisker plots for the morphometric characters or in the histograms for the descriptive characters.

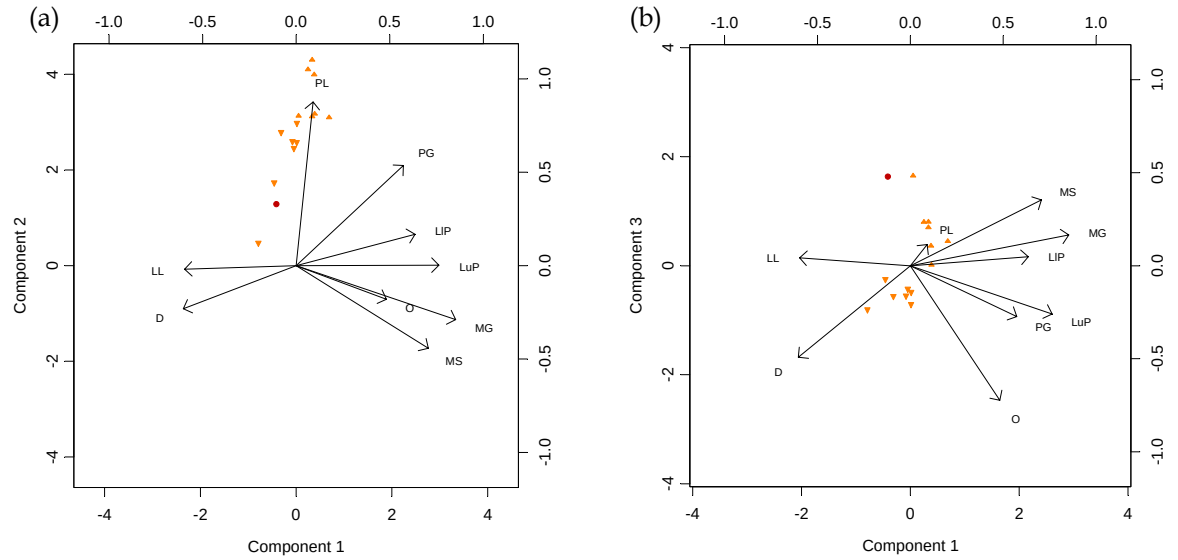


Figure 4.63: Biplot of the Principal components of *R. arvensis* based on the nine selected morphological characters. (a) The first two components; (b) the first and the third component. Individuals are labelled with region of provenance: West-Vlaams Heuvelland (▼); Vlaamse Ardennen (▲); Viroin (●). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

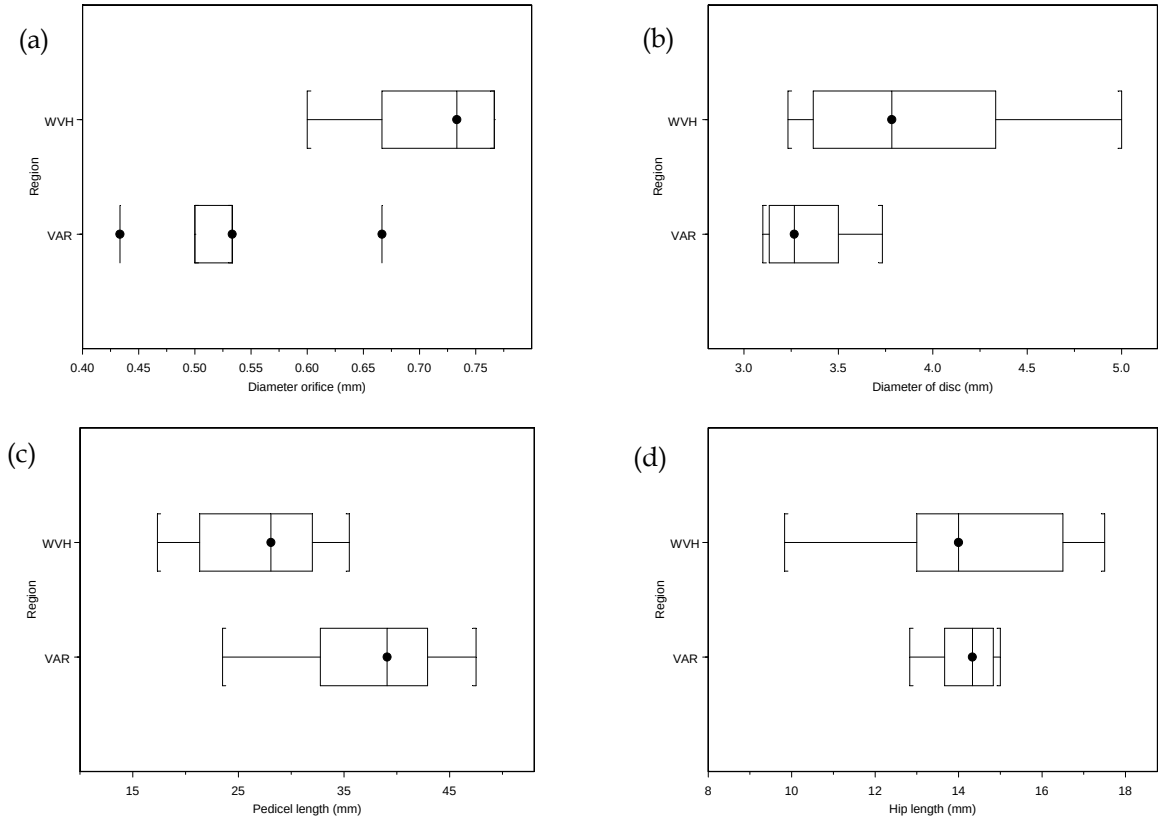


Figure 4.64: Box-and-Whisker plot of the intraspecific variation in *R. arvensis* based on (a) diameter of orifice; (b) diameter of disc; (c) pedicel length; (d) hip length. With West-Vlaams Heuvelland (WVH); Vlaamse Ardennen (VAR).

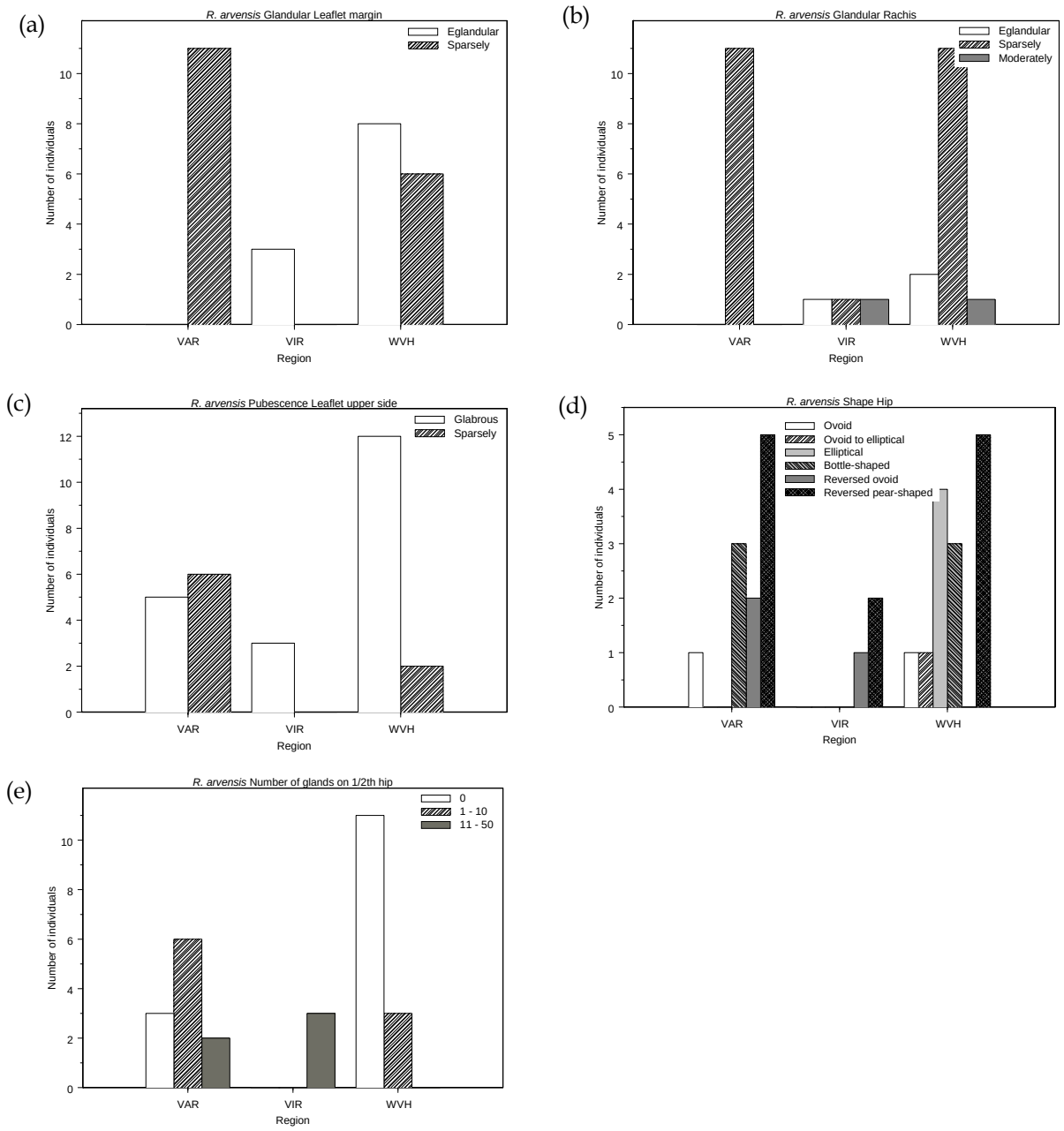


Figure 4.65: Histogram of intraspecific variation in *R. arvensis* based on (a) glandular leaflet margin; (b) glandular rachis; (c) pubescence leaflet upper side; (d) shape of hip; (e) number of glands on the 1/2th hip. With West-Vlaams Heuvelland (WVH); Vlaamse Ardennen (VAR); Viroin (VIR).

R. arvensis (Figures 4.63 to 4.65) was sampled in two Flemish regions, West-Vlaams Heuvelland and Vlaamse Ardennen, and in the Walloon region, Viroin. Only few shrubs from the Viroin carried hips, therefore this region was excluded for further morphological analyses. The two Flemish populations showed distinct intraspecific variation. The population originating from Vlaamse Ardennen showed significantly narrow diameters of the orifice. In addition, they displayed tendencies towards more narrow diameters of the disc and longer pedicels compared to their congeners from West-Vlaams Heuvelland. In contrast to the previously observed correlation between the hip length and the diameter of the disc (Table 4.70), no

intraspecific variation was observed for the hip length. In addition, the rachides of the individuals originating from Vlaamse Ardennen were always sparsely glandular, while they varied from eglandular to moderately glandular, the majority being sparsely glandular, in the population from West-Vlaams Heuvelland. Similarly, the leaflet margins were always sparsely glandular in Vlaamse Ardennen, while half the population from West-Vlaams Heuvelland had eglandular and the other half had sparsely glandular leaflet margins. In addition, the majority of the individuals from West-Vlaams Heuvelland had eglandular hips and glabrous upper sides of the leaflets, while there was variation observed from glabrous towards sparsely pubescent leaflets and from eglandular to sparsely glandular hips in the population Vlaamse Ardennen. The shape of the hips varied in the two populations. In contrast to the frequent presence of elliptical hips in West-Vlaams Heuvelland, there were none in the population Vlaamse Ardennen.

R. rubiginosa

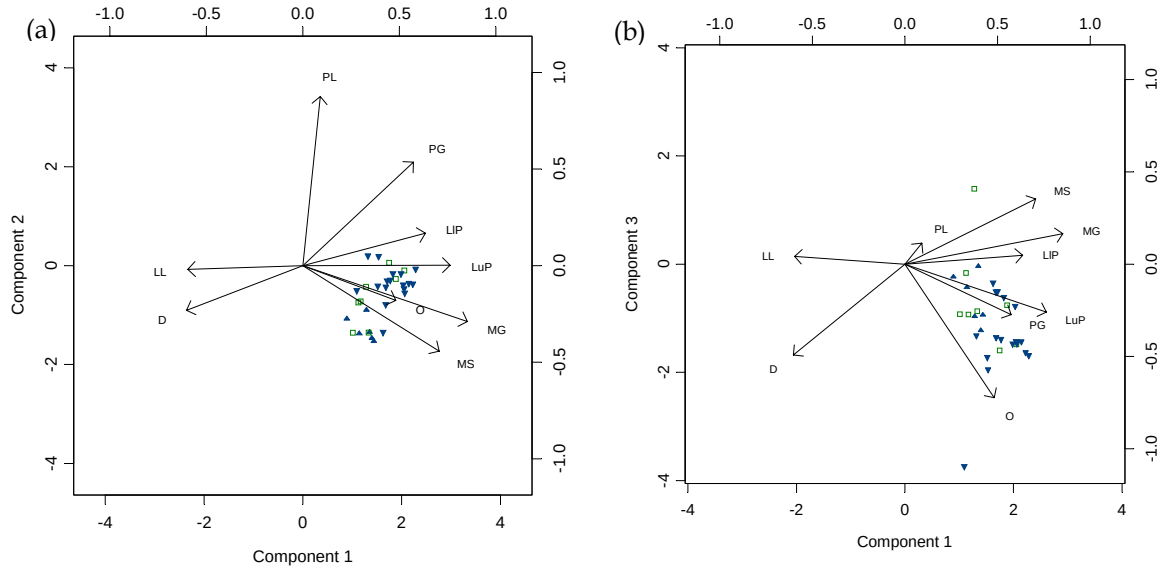


Figure 4.66: Biplot of the Principal components of *R. rubiginosa* based on the nine selected morphological characters. (a) The first two components; (b) the first and the third component. Individuals are labelled with region of provenance: Westkust (▼); Oostkust (▲); Maasvallei (□). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

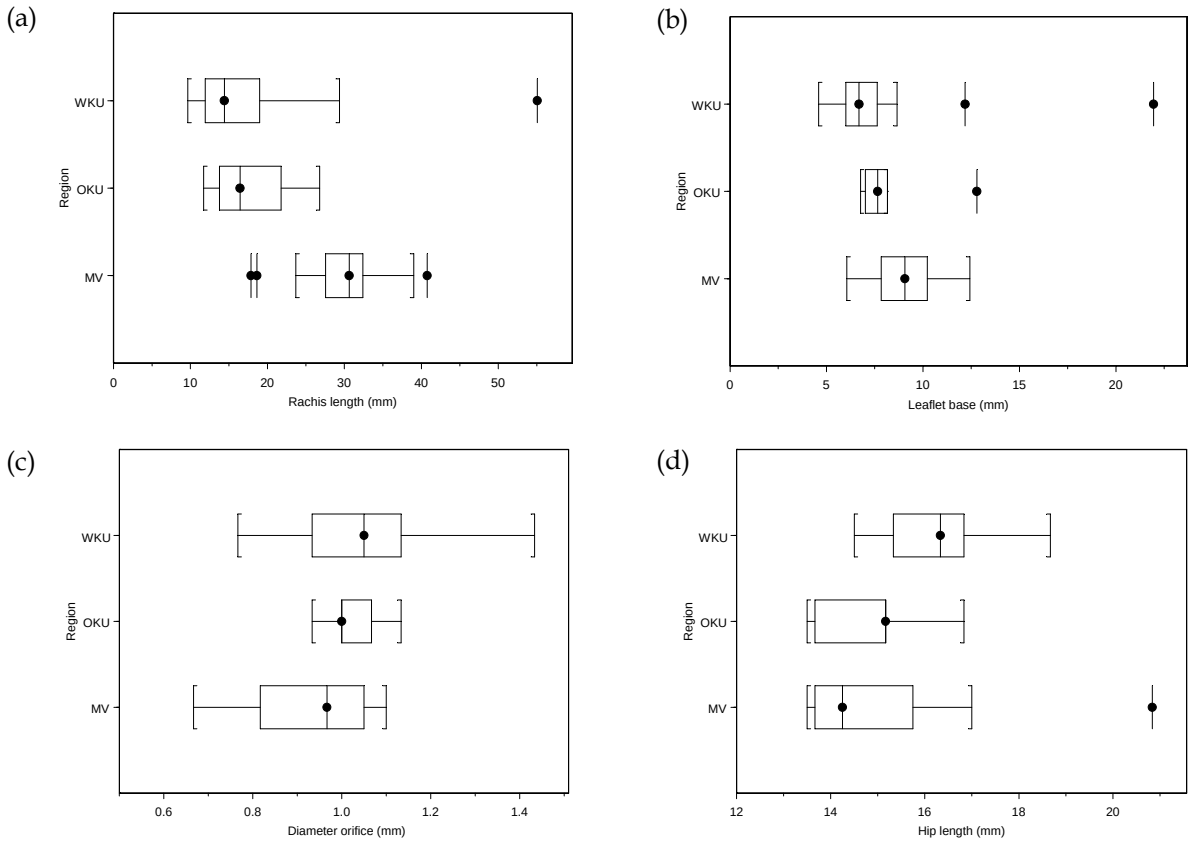


Figure 4.67: Box-and-Whisker plot of intraspecific variation in *R. rubiginosa* based on (a) rachis length; (b) leaflet base; (c) diameter of orifice; (d) hip length. With Westkust (WKU); Oostkust (OKU); Maasvallei (MV).

Based on the PCA-biplot, little to no intraspecific variation was assessed between the *R. rubiginosa* populations (Figures 4.66 to 4.68). However, a tendency in differentiation was observed between the West- and Oostkust populations on the one hand and the Maasvallei population on the other. The Box-and-Whisker plots and histograms showed that the individuals of the Maasvallei had longer rachides and a tendency towards longer leaflets compared to the two coastal populations (WKU and OKU). In addition, the hips appeared to be longer at the Westkust and the individuals of the Oostkust had mostly eglandular pedicels and ovoid or elliptical hips. The leaflets were always multiserrated and the upper sides varied from sparsely to densely pubescent. In contrast, the congeners from Maasvallei and Westkust were characterised by moderately to densely glandular pedicels, more reversed ovoid or globose hips, the leaflet margins varied from bi- to multiserrated and the upper sides were mostly densely pubescent and sometimes glabrous. Moreover, the hips were eglandular to sparsely glandular in the two coastal populations, while the population in the Maasvallei had sparsely to densely glandular hips.

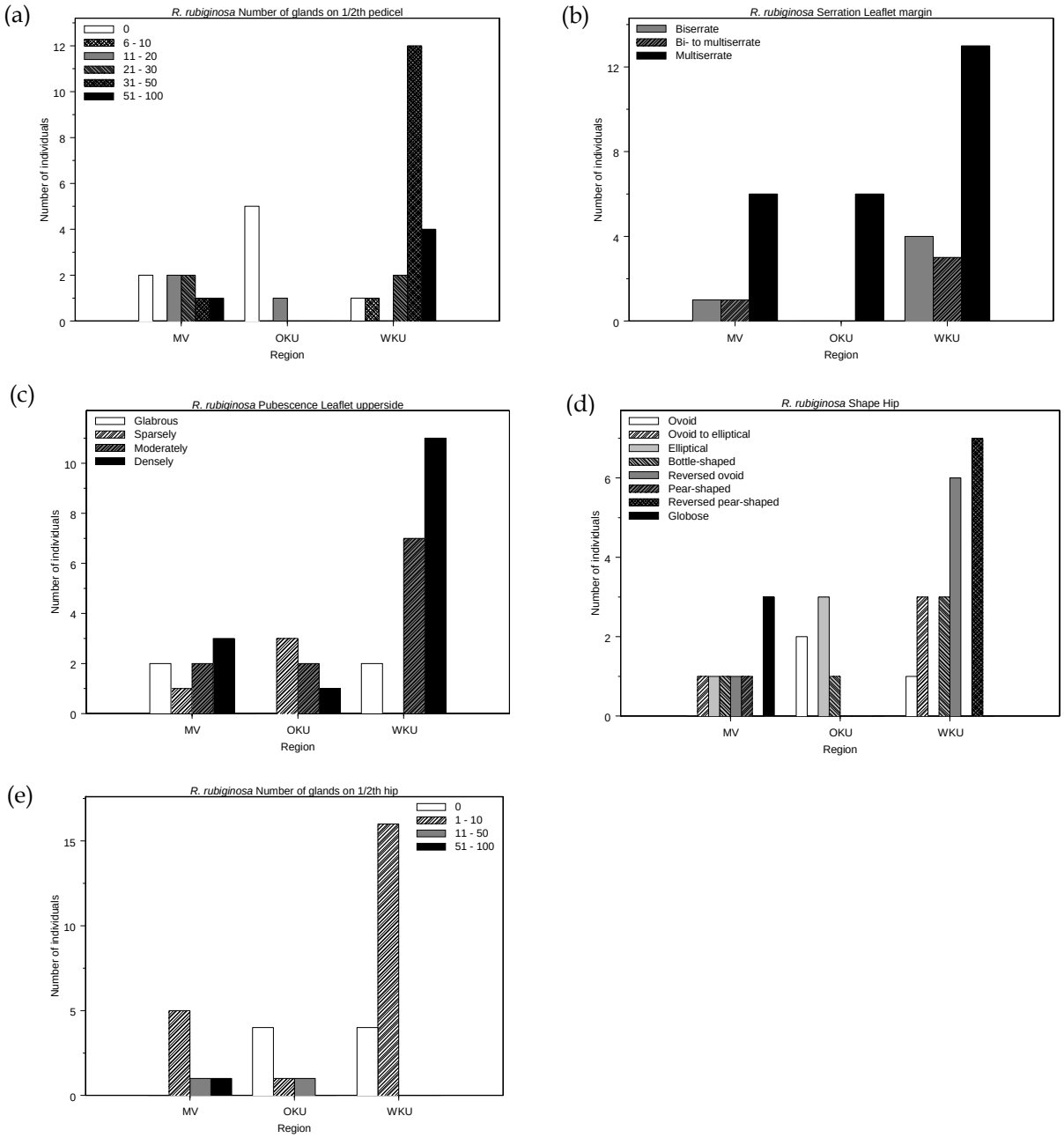


Figure 4.68: Histogram of intraspecific variation in *R. rubiginosa* based on (a) number of glands on 1/2th pedicel; (b) the serration leaflet margin; (c) pubescence leaflet upper side;, (d) shape of hip; (e) number of glands on 1/2th hip. With Maasvallei (MV); Oostkust (OKU); Westkust (WKU).

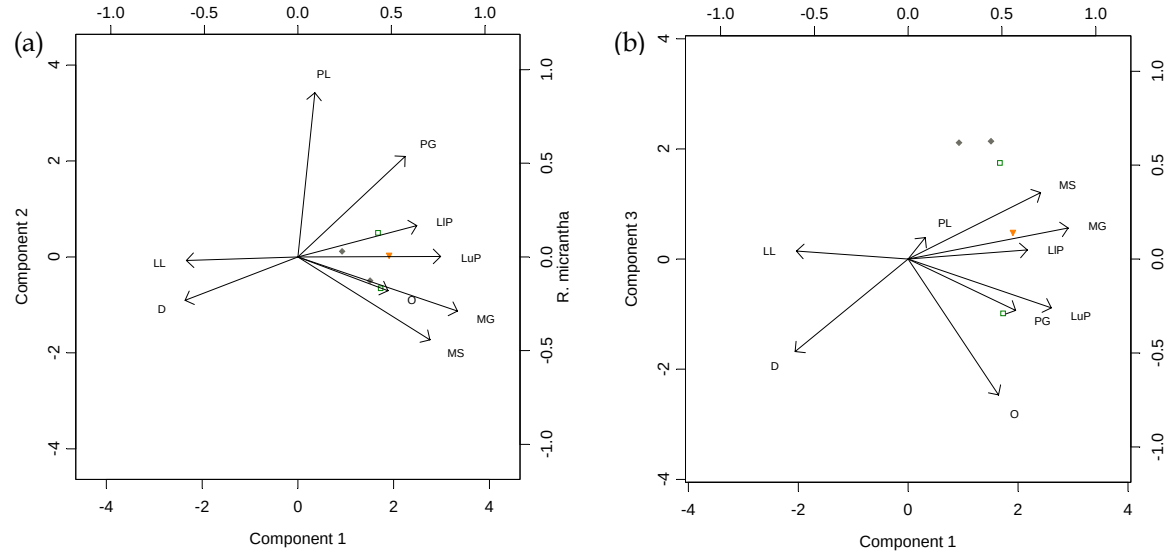


Figure 4.69: Biplot of the Principal Components of *R. micrantha* based on the nine selected morphological characters. (a) The first two components; (b) the first and the third component. Individuals are labelled with regions of origin: West-Vlaams Heuvelland (▼); Brabants District Oost (◆); Maasvallei (□). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

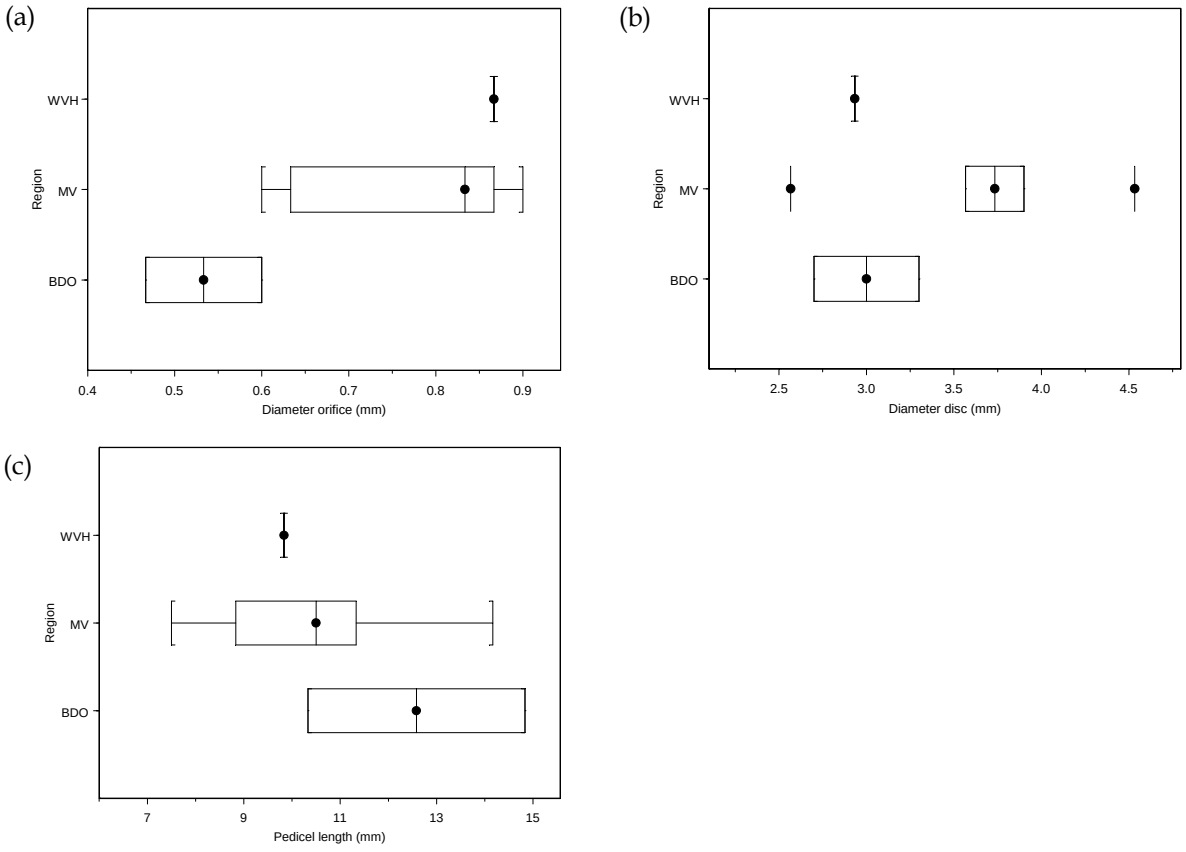


Figure 4.70: Box-and-Whisker plot of intraspecific variation in *R. micrantha* based on (a) diameter of the orifice; (b) diameter of the disc; (c) pedicel length. With West-Vlaams Heuvelland (WVH); Maasvallei (MV); Brabants District Oost (BDO).

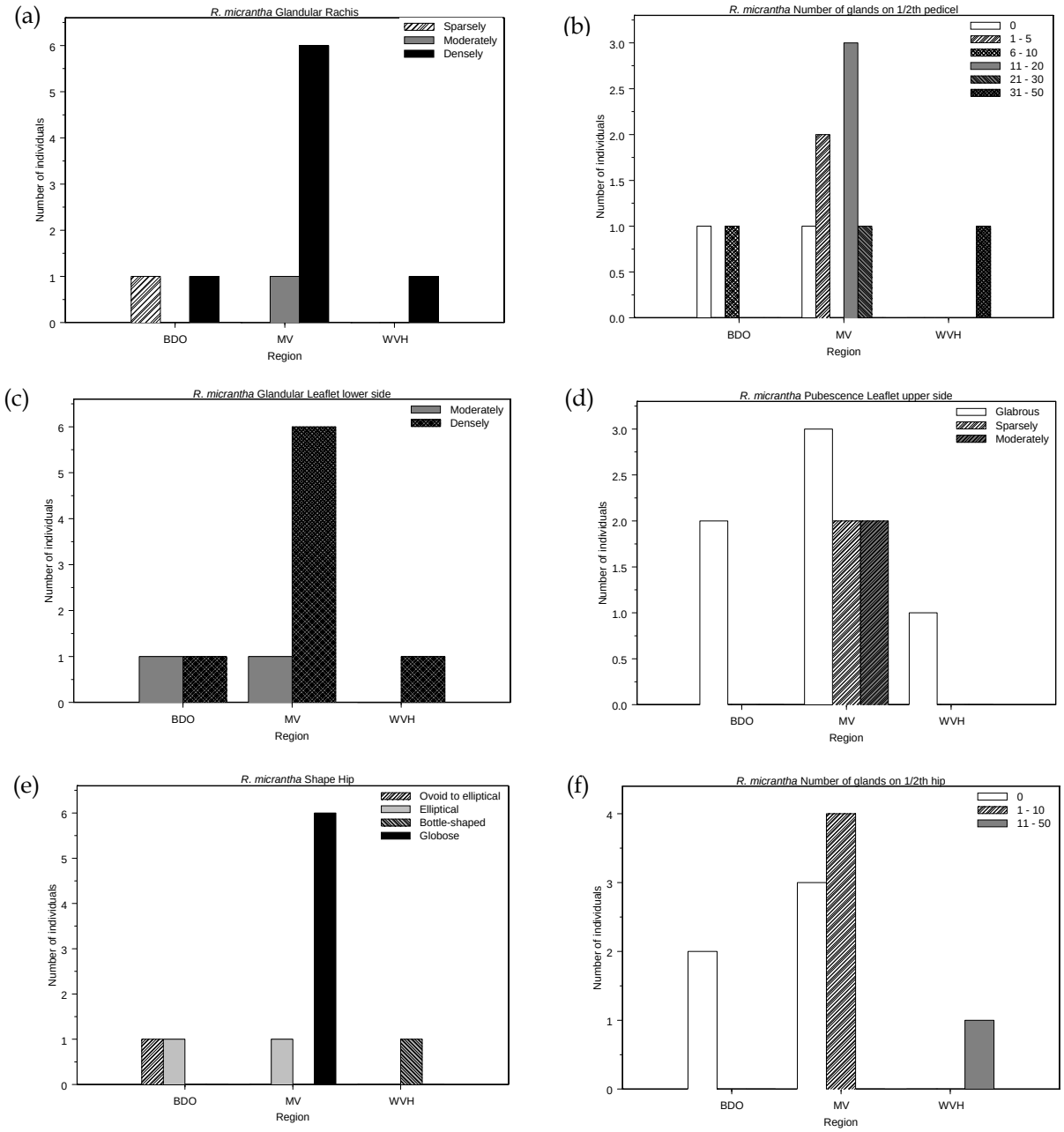


Figure 4.71: Histograms of intraspecific variation in *R. micrantha* based on (a) glandular rachis; (b) number of glands on 1/2th pedicel; (c) glandular leaflet lower side; (d) pubescence leaflet upper side; (e) shape of hip; (f) number of glands on 1/2th hip. With Brabants District Oost (BDO); Maasvallei (MV); West-Vlaams Heuvelland (WVH).

Too little individuals of *R. micrantha* (Figures 4.69 - 4.71) were sampled to observe significant intraspecific variation in the biplot, however few tendencies could be observed. The diameters of the orifice and the disc were clearly larger in the Maasvallei population compared to the population of Brabants District Oost. In contrast, the individuals sampled in Brabants District Oost tended towards longer pedicels than their congeners of Maasvallei and West-Vlaams Heuvelland. The pubescence on the upper side of the leaflets varied from glabrous to sparsely and moderately pubescent on the individuals from the Maasvallei, while individuals of the other populations had only glabrous leaflet upper sides. The shape of the hip was

frequently globose at the mixed Maasvallei population and ovoid to elliptical or bottle-shaped in Brabants District Oost and West-Vlaams Heuvelland. Moreover, only at West-Vlaams Heuvelland, moderately glandular hips were observed. In the other regions, they were mostly eglandular or sparsely glandular.

R. agrestis

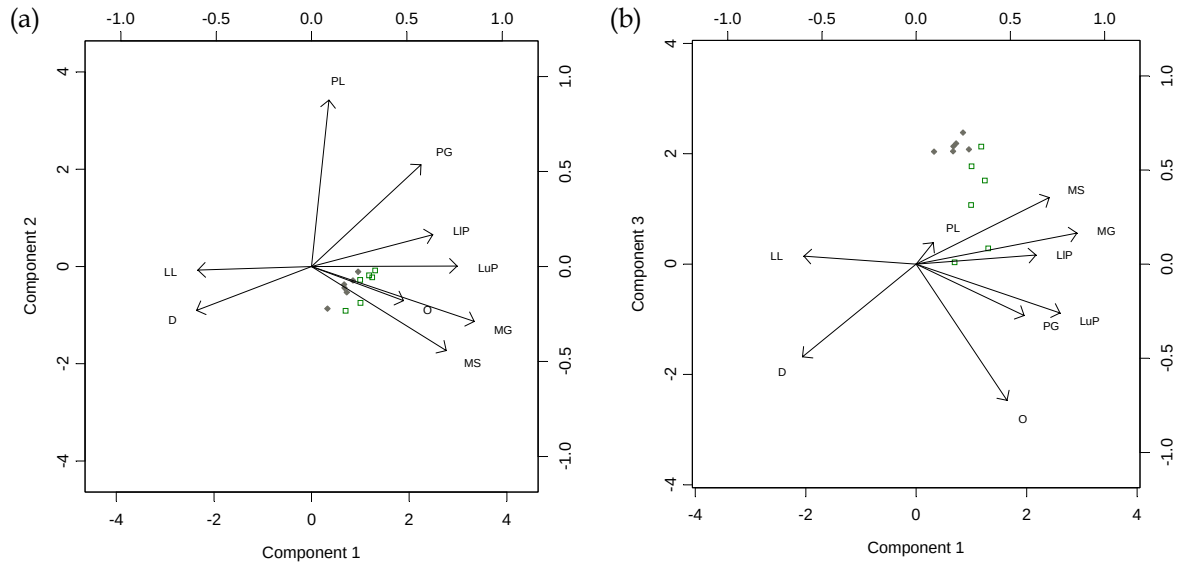


Figure 4.72: Biplot of the Principal components of *R. agrestis* based on the nine selected morphological characters. (a) The first two components; (b) the first and third component. Individuals are labelled with region of provenance: Brabants District Oost (◆); Maasvallei (□). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

Between the two *R. agrestis* populations, intraspecific variation was observed (Figures 4.72 – 4.74). In the Maasvallei population, the rachides and hips were significantly shorter and the pedicels tended to be shorter compared to the congeners from Brabants District Oost. In addition, the *R. agrestis* population sampled in Maasvallei showed more variation concerning the glands on pedicels on hips, and the pubescence on upper side of the leaflets compared to the congeners from Brabants District Oost. The individuals sampled at Brabants District Oost were characterised by mostly glabrous or sparsely pubescent leaflet upper sides, multiserrated leaflet margins, ovoid to elliptical hips, and eglandular hips and pedicels. This was in contrast to the Maasvallei population, where the glandular state of hips and pedicels varied from eglandular to moderately glandular, the pubescence of the upper side of the leaflets could be glabrous or moderately pubescent and the leaflet margins could vary from bi- to multiserrated. In addition, the hips varied from ovoid to elliptical, or even to bottle-shape or reversed ovoid.

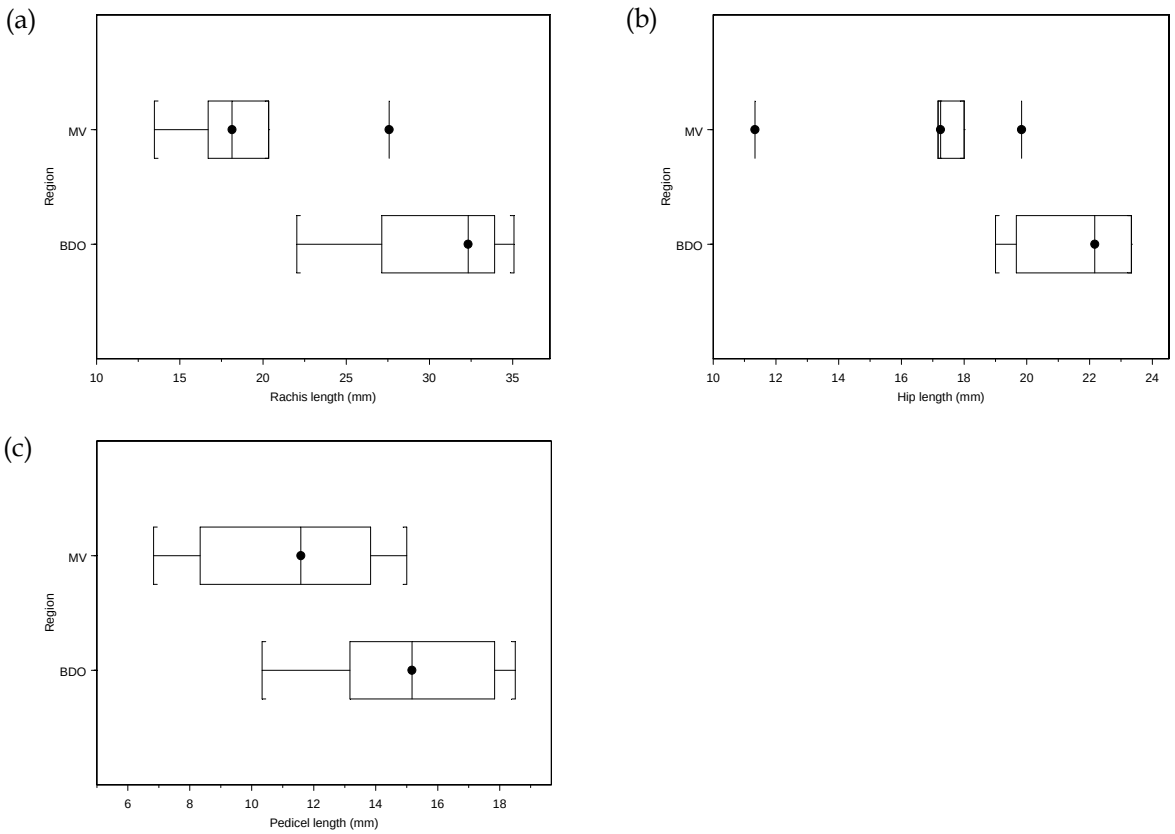


Figure 4.73: Box-and-Whisker plot of intraspecific variation in *R. agrestis* based on (a) rachis length; (b) hip length; (c) pedicel length. With Maasvallei (MV); Brabants District Oost (BDO).

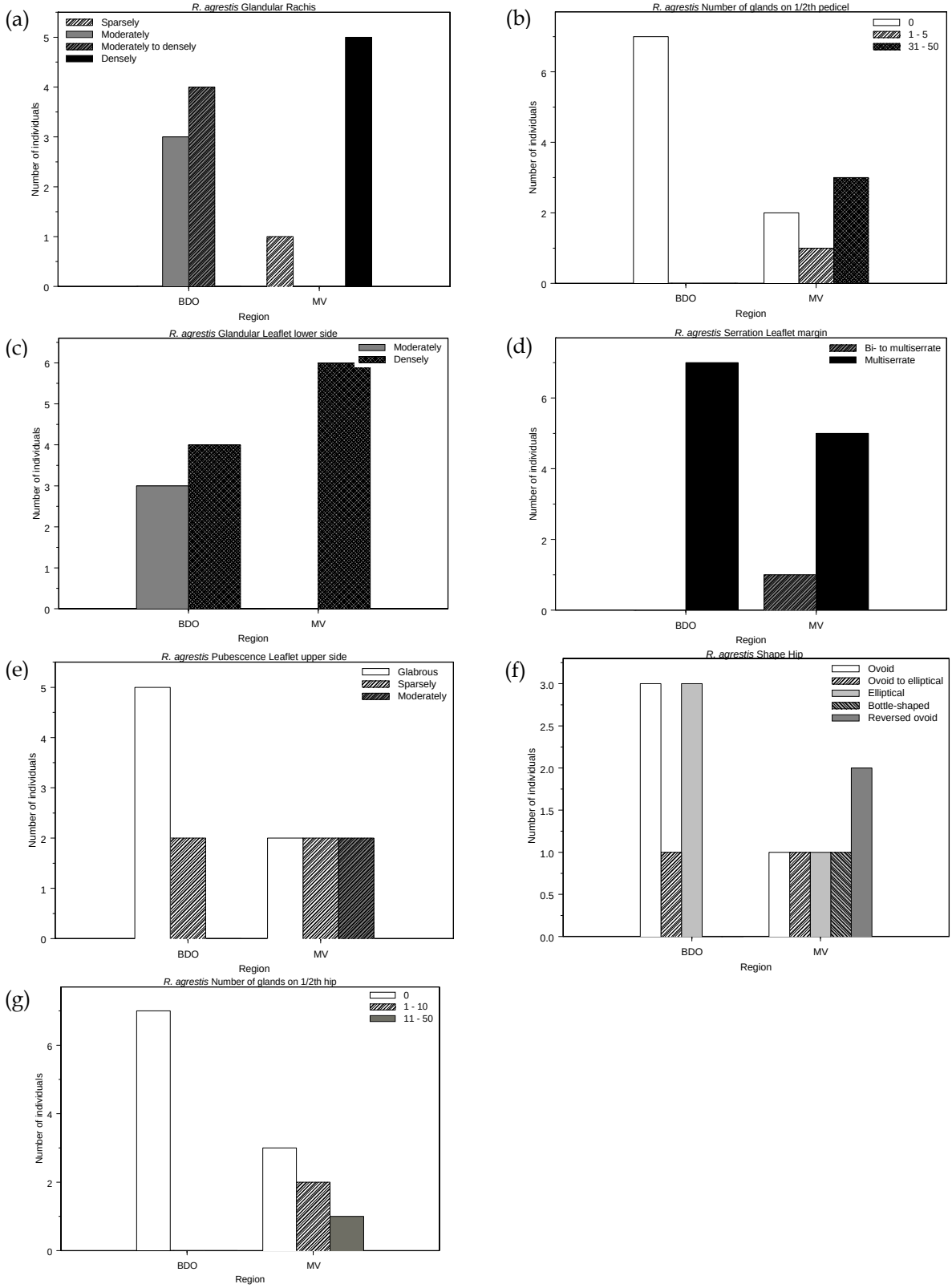


Figure 4.74: Histograms of intraspecific variation in *R. agrestis* based on (a) glandular rachis; (b) number of glands on 1/2th pedicel; (c) glandular leaflet lower side; (d) serration leaflet margin; (e) pubescence leaflet upper side; (f) shape of hip; (g) number of glands on 1/2th hip. With Brabants District Oost (BDO); Maasvallei (MV).

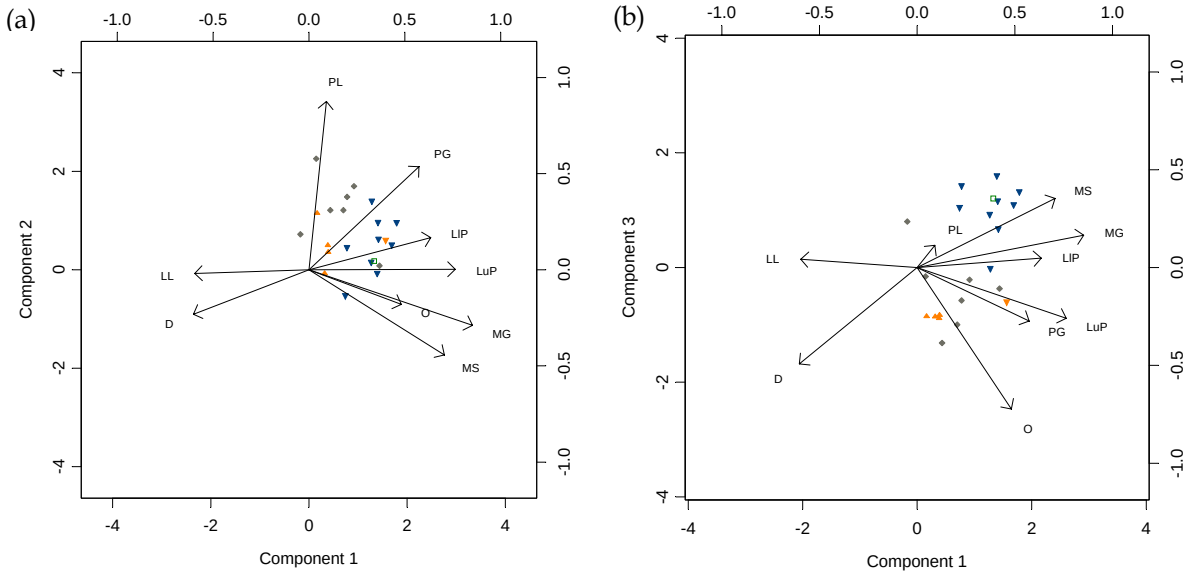


Figure 4.75: Biplot of the Principal components of *R. tomentosa* based on the nine selected morphological characters. (a) The first two components; (b) the first and the third component. Individuals are labelled with region of provenance: Westkust (▼); West-Vlaams Heuvelland (▼); Vlaamse Ardennen (▲); Brabants District Oost (◆); Maasvallei (□). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

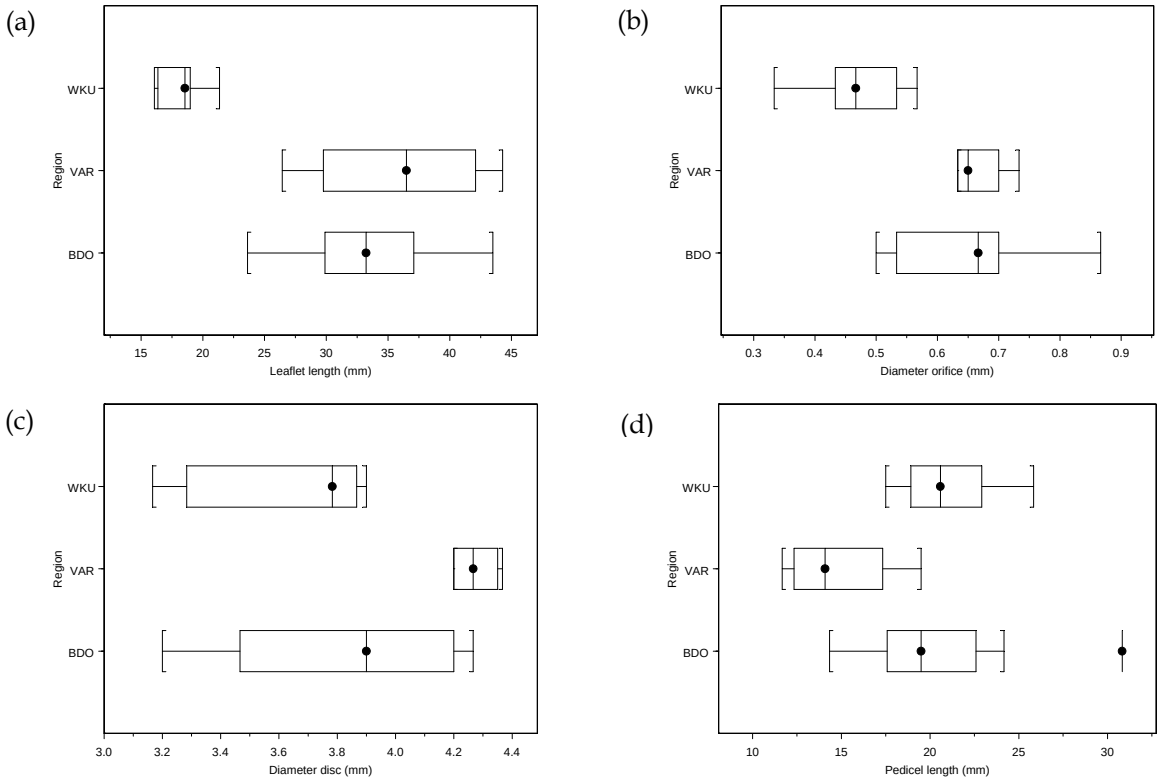


Figure 4.76: Box-and-Whisker plot of intraspecific variation in *R. tomentosa* based on (a) leaflet length; (b) diameter of orifice; (c) diameter of disc; (d) pedicel length. With Westkust (WKU); Vlaamse Ardennen (VAR); Brabants District Oost (BDO).

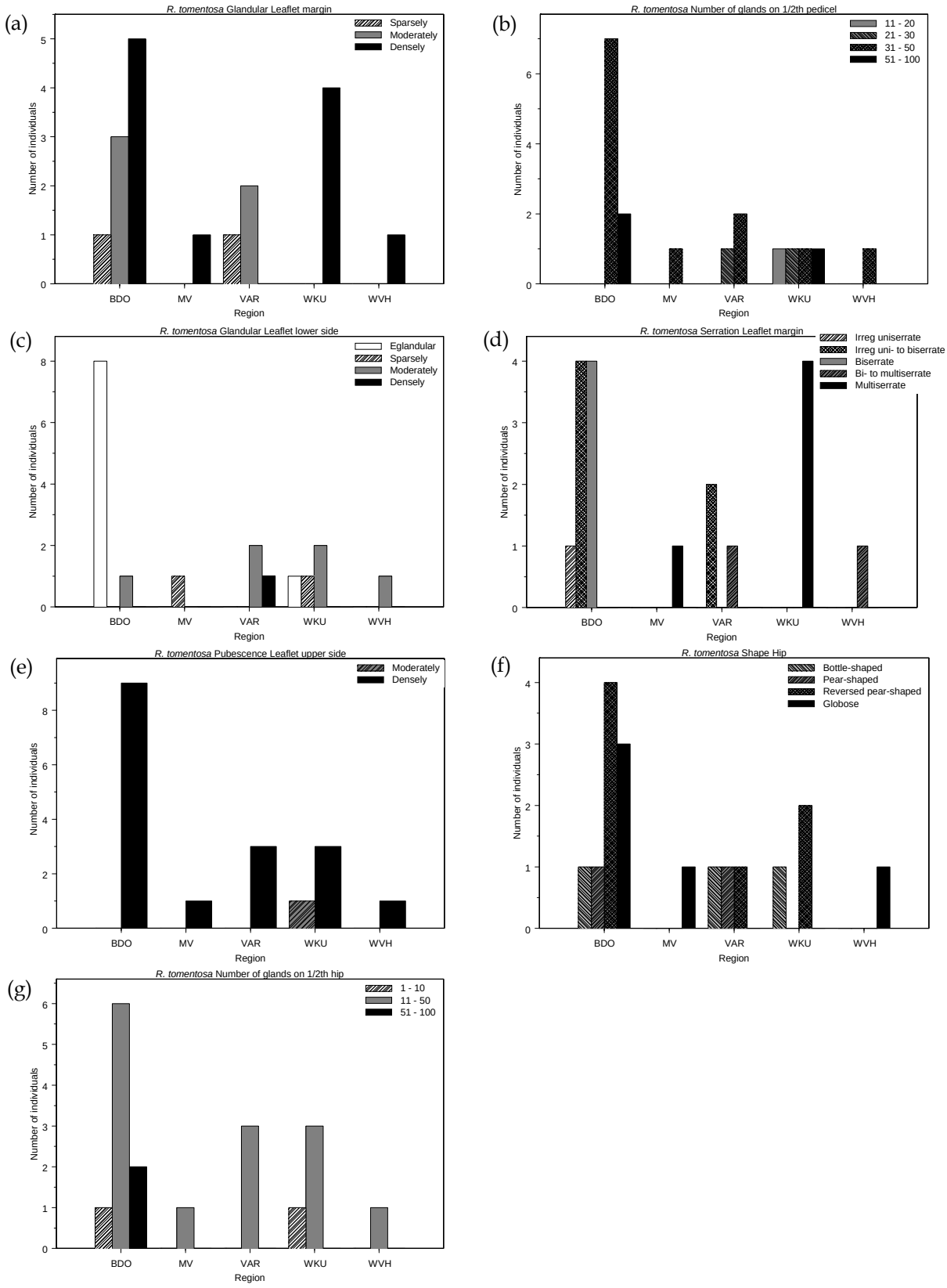


Figure 4.77: Histogram of intraspecific variation in *R. tomentosa* based on (a) glandular leaflet margin; (b) number of glands on the 1/2th pedicel; (c) glandular leaflet lower side; (d) serration of the leaflet margin; (e) pubescence leaflet upper side; (f) shape of hip; (g) number of glands on the 1/2th hip. With Brabants District Oost (BDO); Maasvallei (MV); Vlaamse Ardennen (VAR); Westkust (WKU); West-Vlaams Heuvelland (WVH).

Based on the species descriptions of Henker (2000) and Wissemann (2003), it was not possible to determine the Flemish *Vestitae*. According to Henker (2000), *R. tomentosa* displays smaller diameters of the orifice (<0.8 mm) and longer pedicels compared to *R. pseudoscabriuscula*. In addition, the leaflet margins are uniserrated and eglandular, the sepals reflexed and deciduous, and the receptacle is bouquet-shaped. In contrast, *R. pseudoscabriuscula* has multiserrated and densely glandular leaflet margins, spreading but erect sepals, and head- to bouquet-shaped receptacles. In the Flemish *Vestitae*, both uni- and multiserrated leaflet margins were observed with orifice diameters varying between (0.3 mm-) 0.5 mm - 0.7 mm (-1.3 mm). We could not find an association between the serration of the leaflet margins and the diameter of the orifice. As the majority of the Flemish *Vestitae* displayed a diameter of the orifice smaller than 0.8 mm, all Flemish *Vestitae* were identified as *R. tomentosa*.

Only the *R. tomentosa* populations sampled in Brabants District Oost, Vlaamse Ardennen, and Westkust were sufficiently represented in the data set to show relevant intraspecific variation (Figures 4.75 – 4.77). The shrubs sampled at the Westkust were characterised by significant shorter leaflets and a strong tendency towards more narrow orifice compared to their congeners from Brabants District Oost and Vlaamse Ardennen. However, the differentiation in diameter of the orifice was not confirmed in the PCA. In addition to the significant shorter leaflets, all the studied leaflet dimensions differed significantly. Moreover, the individuals originating from Vlaamse Ardennen tended towards larger diameters of the disc and shorter pedicels compared to the congeners from Brabant District Oost and Westkust.

The population from Westkust was characterised by densely glandular and multiserrated leaflet margins, while the congeners sampled at Brabants District Oost and Vlaamse Ardennen varied from sparsely to densely glandular leaflet margins with serrations varying from irregular uniserration, or uni- to biserration, to bi- and even bi- to multiserration. Moreover, the hips of the Westkust population were sparsely to moderately glandular and mainly reversed pear-shaped, while those of Brabants District Oost could also be densely glandular and globose.

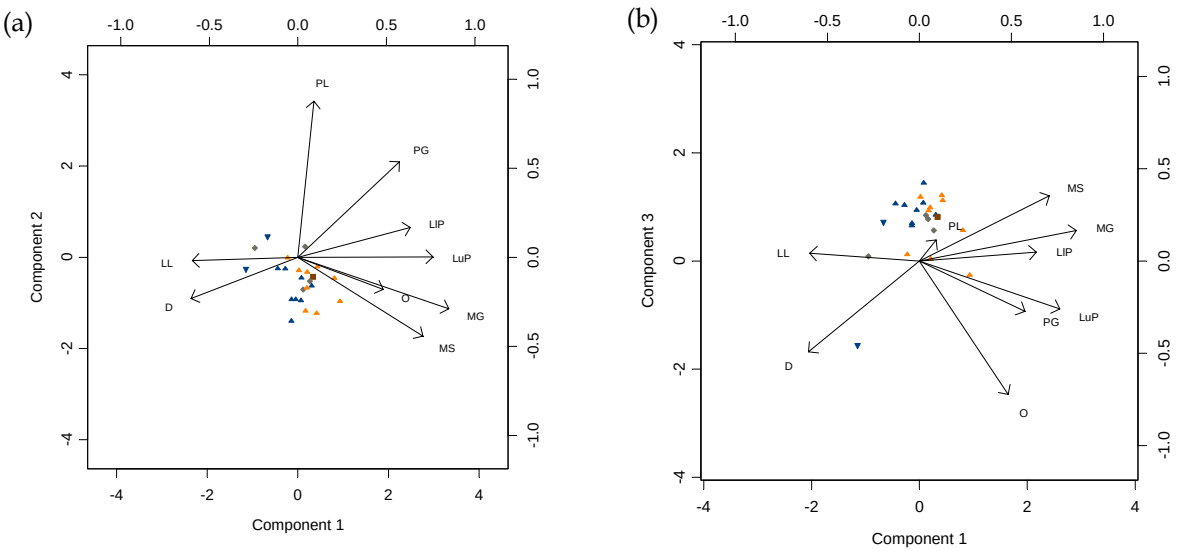


Figure 4.78: Biplot of the Principal Components of *R. balsamica* based on the diagnostic morphometric characters. (a) The first two components; (b) the first and third component. Individuals are labelled with regions of origin: Westkust (▼); Oostkust (▲); Vlaamse Ardennen (▲); Kempen (■); Brabants District Oost (◆); Maasvallei (□). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

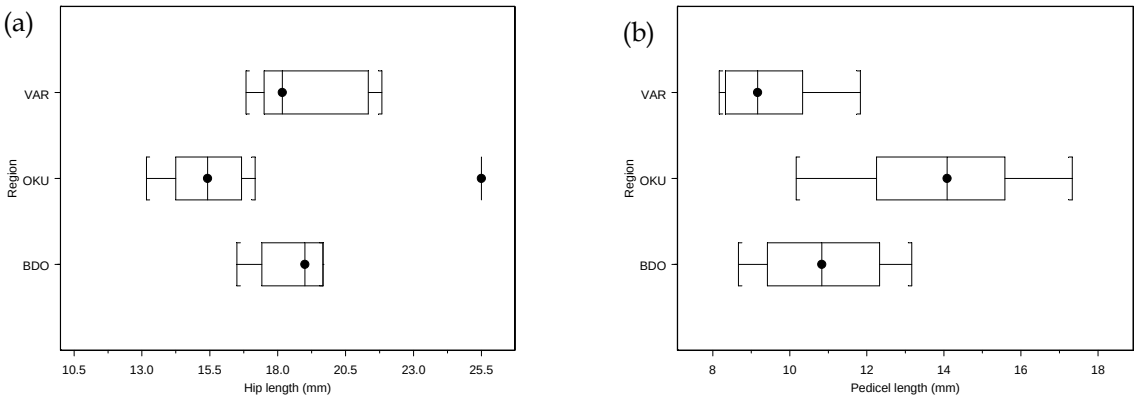


Figure 4.79: Box-and-Whisker plot of intraspecific variation in *R. balsamica* based on (a) hip length; (b) pedicel length. With Vlaamse Ardennen (VAR); Oostkust (OKU); Brabants District Oost (BDO).

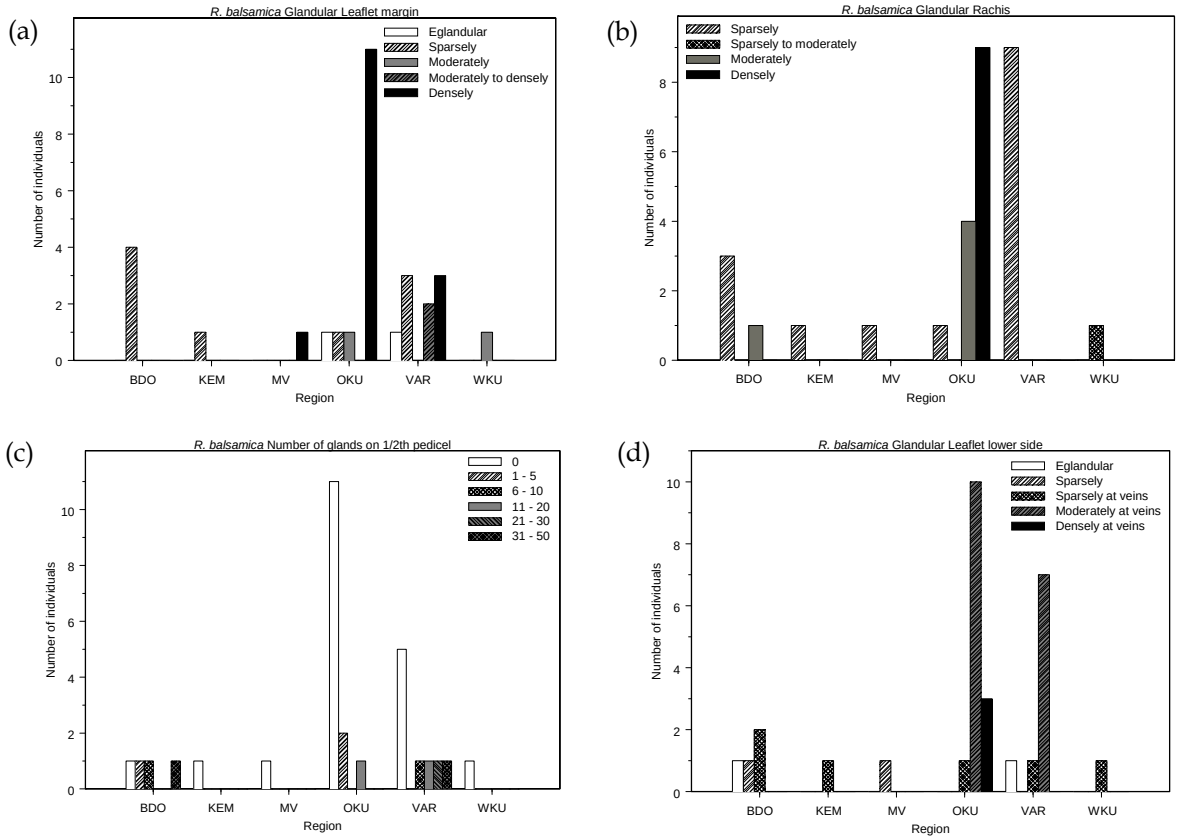


Figure 4.80: Histogram of intraspecific variation in *R. balsamica* based on (a) glandular leaflet margin; (b) glandular rachis; (c) number of glands on 1/2th pedicel; (d) glandular leaflet lower side. With Brabants District Oost (BDO); Kempen (KEM); Maasvallei (MV); Oostkust (OKU); Vlaamse Ardennen (VAR); Westkust (WKU).

Only the *R. balsamica* populations from Oostkust, Brabants District Oost, and Vlaamse Ardennen could be compared due to restricted number of sampled individuals in the other populations (Figures 4.79 – 4.81). The PCA of *R. balsamica* suggested little intraspecific variation for the population of Oostkust. In the Box-and-Whisker plots, the individuals from Oostkust had shorter hips and longer pedicels compared to the populations sampled at Vlaamse Ardennen and Brabants District Oost. Moreover, the majority of the Oostkust individuals had glabrous leaflet upper sides. This is in contrast with the congeners from Brabants District Oost and Vlaamse Ardennen of which the leaflet upper sides were mainly sparsely pubescent. In addition, the individuals of Oostkust had moderately glandular veins at the lower side of the leaflets and the rachides were even densely glandular. The congeners from Brabants District Oost were described by eglandular, to sparsely glandular, and the individuals from Vlaamse Ardennen by moderately glandular veins on the lower leaflet sides. The majority of the individuals had sparsely glandular rachides.

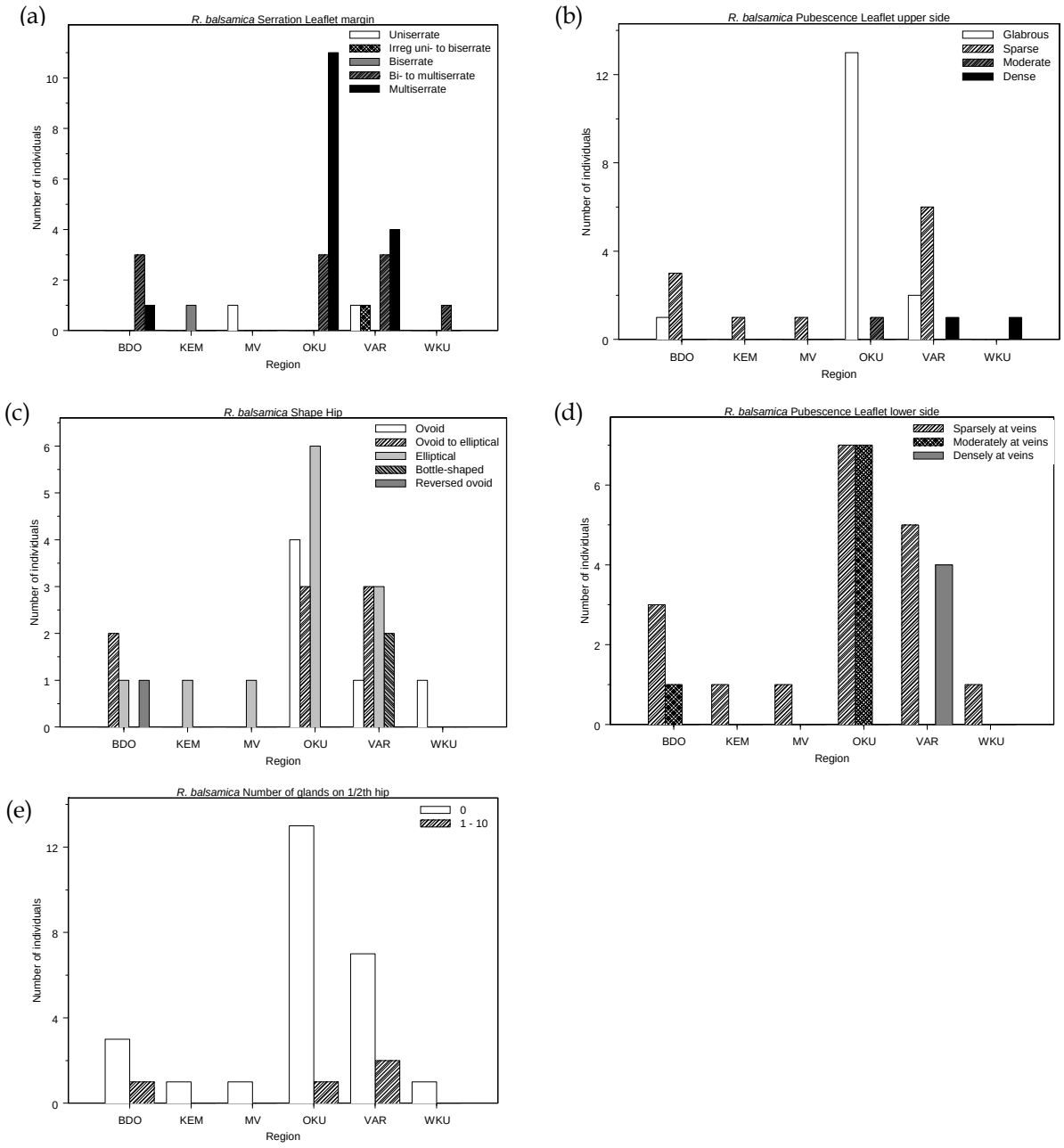


Figure 4.81: Histogram of intraspecific variation in *R. balsamica* based on (a) serration leaflet margin; (b) pubescence leaflet upper side; (c) shape of hip; (d) pubescence leaflet lower side; (e) number of glands on 1/2th hip. With Brabants District Oost (BDO); Kempen (KEM); Maasvallei (MV); Oostkust (OKU); Vlaamse Ardennen (VAR); Westkust (WKU).

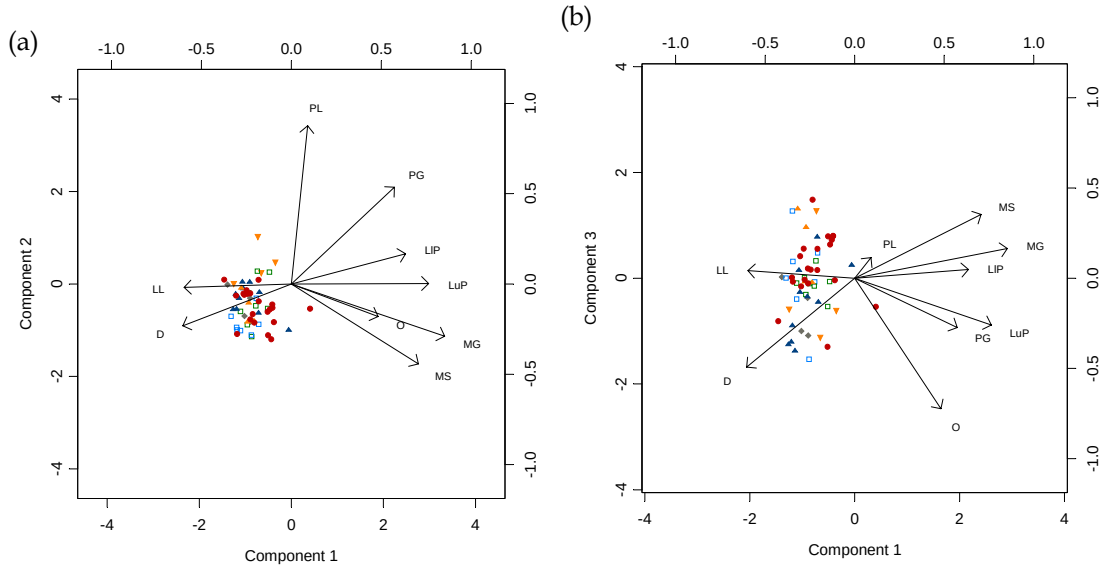


Figure 4.82: Biplot of the Principal components of *R. canina* based on the nine selected morphological characters. (a) The first two components; (b) the first and third component. Individuals are labelled with region of provenance: Westkust (▼); Oostkust (▲); West-Vlaams Heuvelland (▼); Vlaamse Ardennen (▲); Vlaamse Zandstreek (□); Brabants District Oost (◆); Maasvallei (□); Viroin (●). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

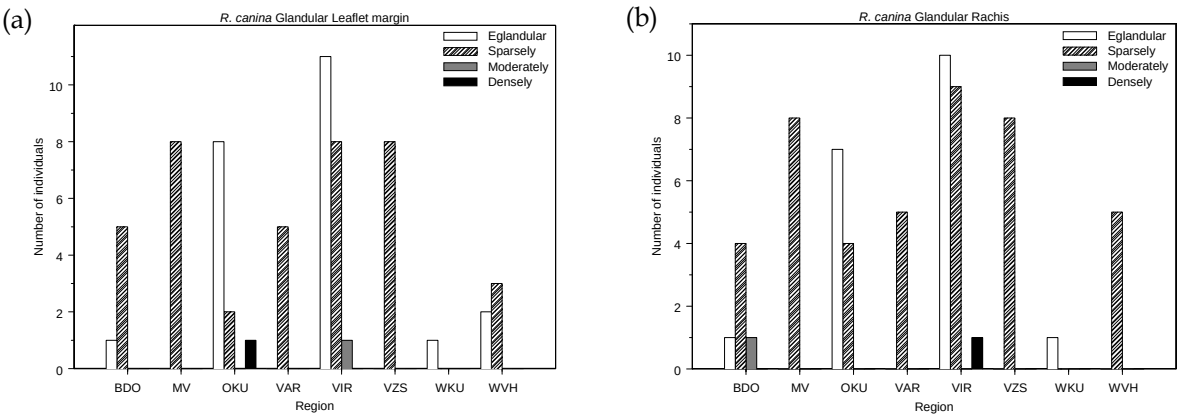


Figure 4.83: Histogram of intraspecific variation in *R. canina* based on (a) glandular leaflet margin; (b) glandular rachis. With Brabants District Oost (BDO); Maasvallei (MV); Oostkust (OKU); Vlaamse Ardennen (VAR); Viroin (VIR); Vlaamse Zandstreek (VZS); Westkust (WKU); West-Vlaams Heuvelland (WVH).

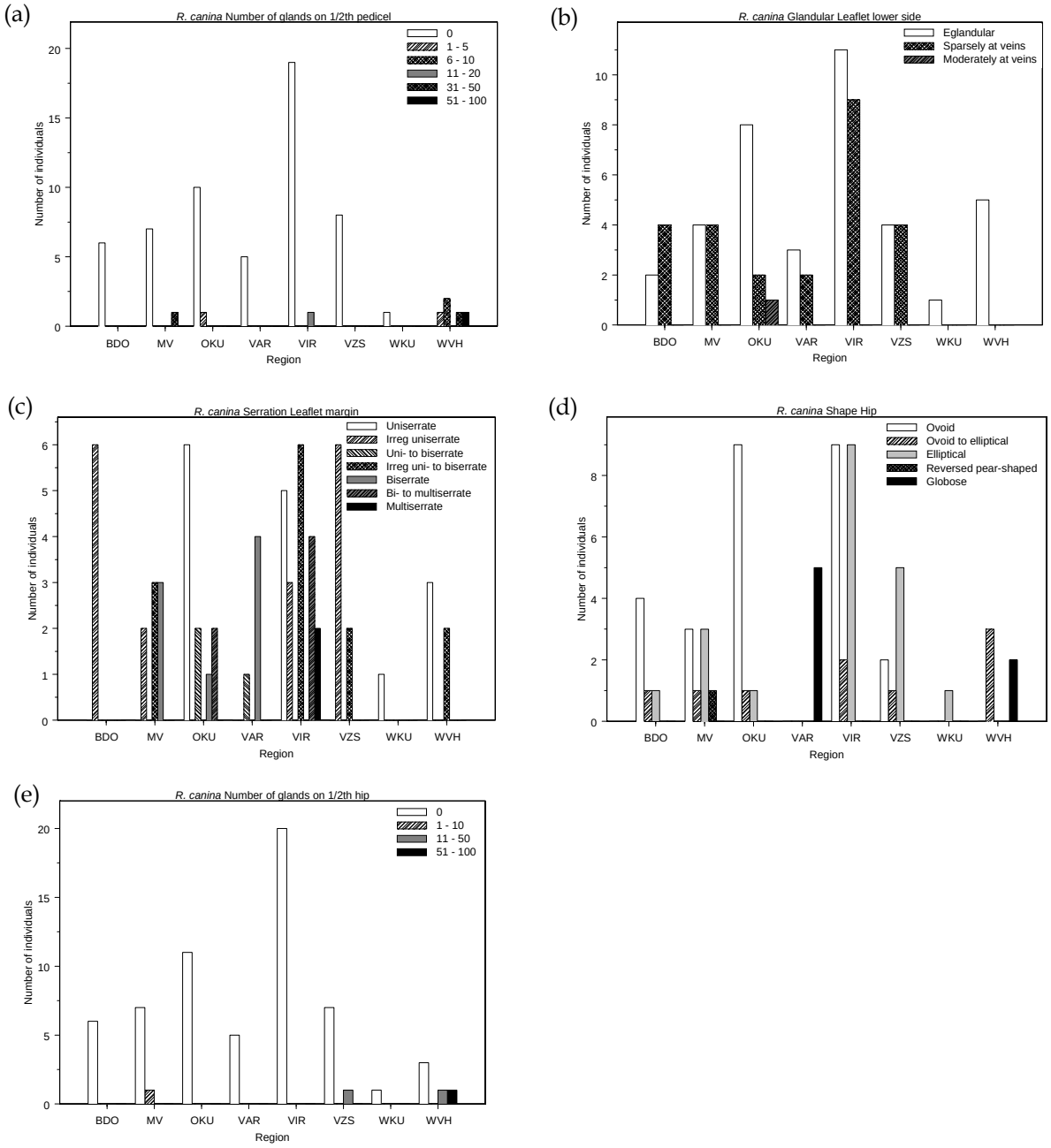


Figure 4.84: Histogram of intraspecific variation in *R. canina* based on (a) number of glands on 1/2th pedicel; (b) glandular leaflet lower side; (c) serration leaflet margin; (d) shape of hip; (e) number of glands on 1/2th hip. With Brabants District Oost (BDO); Maasvallei (MV); Oostkust (OKU); Vlaamse Ardennen (VAR); Viroin (VIR); Vlaamse Zandstreek (VZS); Westkust (WKU); West-Vlaams Heuvelland (WVH).

R. canina (Figures 4.82 – 4.84) is the most common taxon in Flanders and therefore sampled in seven Flemish regions and in the Walloon region, Viroin. In general, no intraspecific variation was observed in the diagnostic characters. However, some remarkable differences were observed in the distribution of the descriptive characters.

All the individuals sampled in West-Vlaams Heuvelland had glandular pedicels and hips, typical for *R. canina* var. *andegavensis*. In contrast, the pedicels and

hips were mostly eglandular or occasionally sparsely glandular in the other populations. This unequal distribution was caused by the specific search and sampling of *R. canina* var. *andegavensis* in West-Vlaams Heuvelland as this variety is rare in the other regions.

Other striking variations were the moderately and densely glandular leaflet margins and veins on the lower side of the leaflets sampled at the Oostkust. Also in the Viroin one individual with glandular leaflets was sampled. These individuals were determined as *R. canina* var. *dumalis*. Although some very specific differentiations were found, they did not influence the global position of *R. canina* individuals towards each other.

R. corymbifera

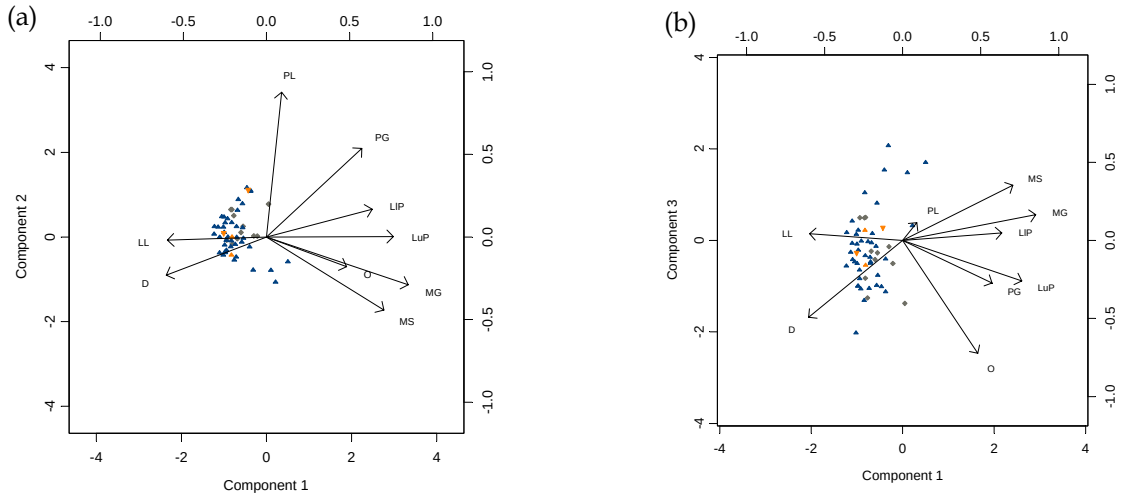


Figure 4.85: Biplot of the Principal components of *R. corymbifera* based on the nine selected morphological characters. (a) The first two components; (b) the first and third component. Individuals are labelled with region of provenance: Oostkust (▲); West-Vlaams Heuvelland (▼); Vlaamse Ardennen (▲); Brabants District Oost (◆). With: pubescence leaflet upper side (LuP); pubescence leaflet lower side (LlP); serration leaflet margin (MS); glandular leaflet margin (MG); glandular pedicel (PG); pedicel length (PL); diameter of disc (D); diameter of orifice (O); leaflet length (LL).

The outcomes of the common taxon *R. corymbifera* (Figures 4.85 – 4.87) were very similar to those of *R. canina*. *R. corymbifera* was also sampled in different regions and lacked the presence of intraspecific variation in the majority of the diagnostic characters. However, few individuals sampled at West-Vlaams Heuvelland, Oostkust, Viroin, and Brabants District Oost had glandular leaflet margins, hips or pedicels. Similarly to the *R. canina*, the observed differentiations did not cause intraspecific variation, but characterise the variety *R. corymbifera* var. *deseglisei*.

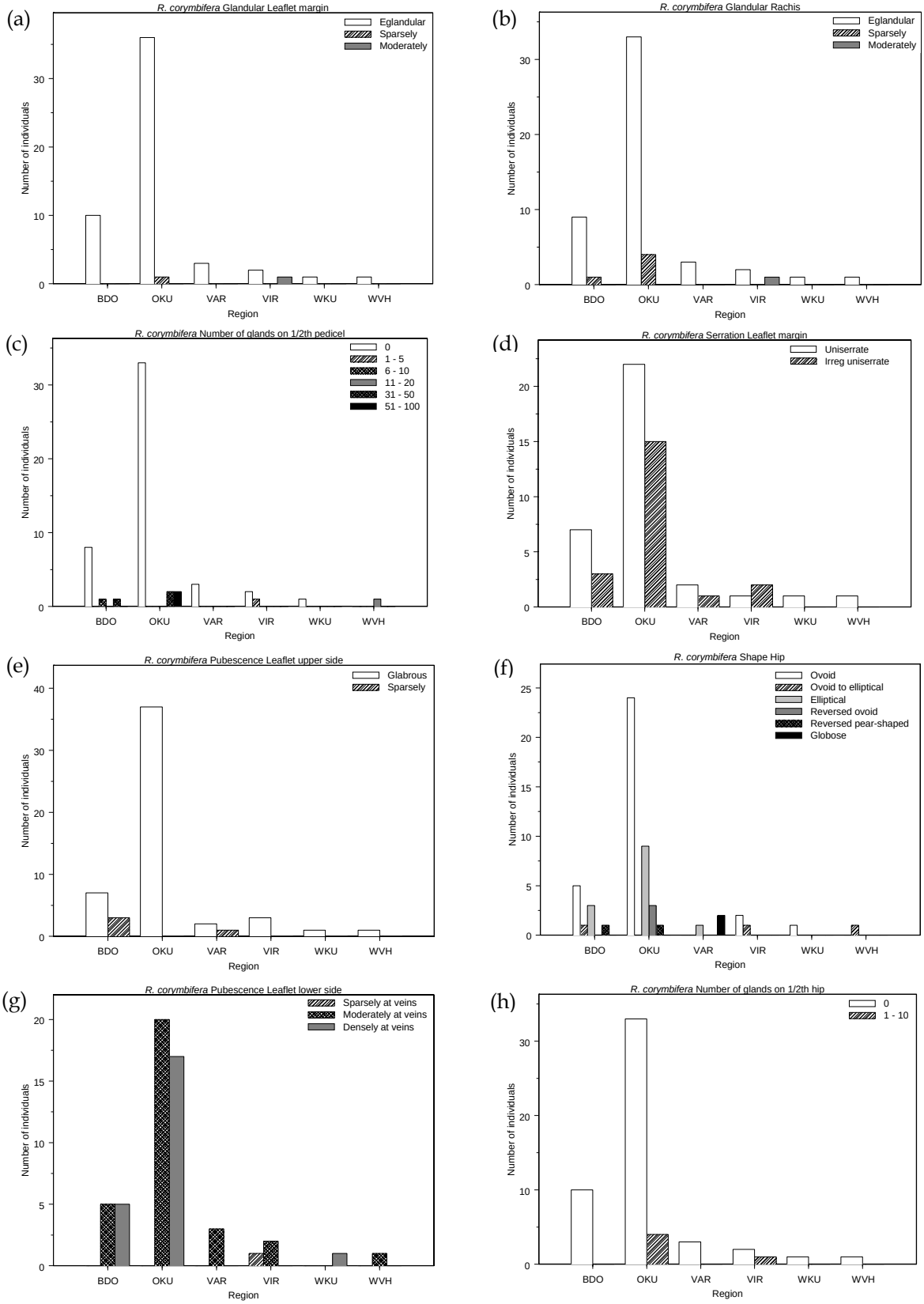


Figure 4.86: Histogram of intraspecific variation in *R. corymbifera* based on (a) glandular leaflet margin; (b) glandular rachis; (c) number of glands on 1/2th pedicel; (d) serration leaflet margin; (e) pubescence leaflet upper side; (f) shape of hip; (g) pubescence leaflet lower side; (h) number of glands on 1/2th hip. With Brabants District Oost (BDO); Oostkust (OKU); Vlaamse Ardennen (VAR); Viroin (VIR); Westkust (WKU); West-Vlaams Heuvelland (WVH).

4.3.2.4. Comparing observations with descriptions in literature

The set of analysed characters (Table 3.6) was based on previously published studies. Here, the observations on the Flemish wild individuals are compared with different publications, in chronological order: "Drawings of Scandinavian Plants" (Nilsson 1967, 1999), "Roses of Great-Britain and Ireland" (Graham and Primavesi 1993), "Hegi Illustrierte Flora van Mitteleuropa" (Henker 2000), and "Classification: conventional taxonomy (wild roses)" (Wissemann 2003). A more detailed description for each publication is given in §2.1.2 "The section *Caninae*". For our observations, the minima and maxima of the morphometric characters and the most frequently observed states of the descriptive characters were mentioned. Only the differences between the publications and our observations were highlighted below. The complete overview is summarised in §Appendix.

All the Flemish *R. arvensis* (Table A.1) individuals showed glabrous leaflet lower sides and glabrous to sparsely pubescent leaflet upper sides, while in literature (Graham and Primavesi 1993, Henker 2000) the lower sides were described as glabrous to pubescent at the veins and the upper sides were always glabrous. In addition to the eglandular leaflet margins and hips described by Henker (2000), also sparsely glandular forms were observed on the Flemish individuals.

The leaflets of the Flemish *R. rubiginosa* (Table A.2) were longer compared to the ones measured by Graham and Primavesi (1993). According to Henker (2000), the rachides were glabrous or slightly pubescent, while the Flemish rachides were densely pubescent. In addition, the pubescence of the Flemish leaflet upper sides was observed to be moderate or dense, while in literature (Nilsson 1967, Graham and Primavesi 1993, Henker 2000) only glabrous to slightly pubescent leaflet upper sides were described. Both Nilsson (1967) and Graham and Primavesi (1993) mentioned biserrated leaflet margins, while those described by Henker (2000) were multiserrated. In the Flemish *R. rubiginosa*, mainly multiserrated margins were observed, with intermediate forms to biserration. The diameters of the orifice were clearly smaller on the Flemish hips (0.7 mm – 1.3 mm) compared to those of Henker (2000) (1.2 mm and 2 mm) and slightly smaller compared to Nilsson (1967) (1 mm - 1.2 mm).

Only Graham and Primavesi (1993) stated the absence of acicles in *R. micrantha* (Table A.3). According to the other authors and our observations, acicles were (occasionally) mixed with hooked and curved prickles. The presence of heteracanthy indicates the close relationship with *R. rubiginosa* in which the presence of acicles mixed with large hooked prickles is frequently observed. The upper side of the leaflets were always glabrous according to Nilsson (1967) and Graham and Primavesi (1993), but could vary towards slightly pubescent (Henker 2000). In our samples even moderately pubescent leaflets were observed. Only Nilsson (1967) described biserrated leaflet margins, while the other authors observed multiserrated leaflet margins like we did.

The Flemish hips were longer compared to the ones described in literature, 1.4 - 2 cm and 1 – 1.7 cm, respectively. In addition, the diameters of the disc were smaller

in the Flemish individuals (2.6 - 4.5 mm) compared to the ones measured by Nilsson (1967) (4 - 4.5 mm). Moreover, the presence of glands on the pedicels varied from eglandular to densely glandular according to all sources. However, in the literature the eglandular state was observed rarely while the Flemish pedicels were equally divided over the different stages. There is also disagreement on the shape of the receptacle, Henker (2000) described it as bouquet-shaped, while Graham and Primavesi (1993) mentioned a subglobose head and Nilsson (1967) a conoidal somewhat flattened head. In the Flemish individuals, the head-shaped was most frequently observed.

According to Nilsson (1967), *R. agrestis* (Table A.4) displayed glabrous or pubescent lower leaflet sides, whereas the other authors did not mention glabrous individuals. In the Flemish individuals the veins were sparsely pubescent. In addition, only the *R. agrestis* studied by Nilsson (1967) had uni- or biserrated leaflet margins, while the other descriptions mentioned multiserration. The Flemish individuals had also multiserrated leaflet margins. Finally, only on the Flemish individuals sparsely pubescent pedicels were observed, while they were mostly described as being glabrous by the other authors.

In contrast to the glandular lower sides of the leaflet as described in literature (Nilsson 1967, Graham and Primavesi 1993, Henker 2000, and Wissemann 2003), also eglandular lower leaflet sides were observed in the Flemish *R. tomentosa* (Table A.5). As the observations were performed on dried leaflets, this eglandular state might be described as subfoliar glands being hidden in the strongly tomentose surface on the lower leaflet side. Additionally, the glands are known to lose their translucence and dry out leaving behind a few specks of grey cellular tissue (Graham and Primavesi 1993).

The literature only mentioned uni- or biserrated leaflet margins, while in the Flemish individuals also transitional forms to multiserration were observed. Henker (2000) observed mostly eglandular and rarely sparsely glandular leaflet margins, while Nilsson (1967) only mentioned glandular margins. In the Flemish individuals mostly densely glandular, but also intermediate forms towards sparsely glandular margins were observed. In addition, the Flemish hips (1.5 - 2 cm) were larger than those of Nilsson (1967) and Graham and Primavesi (1993), both 1 - 1.5 cm, while the pedicels were shorter (Flemish: 1.2 - 2.6 cm versus values in literature: 2 - 4 cm). These data indicate that Flanders lacks pure *R. tomentosa* and *R. pseudoscabriuscula* individuals. Instead, the Flemish *Vestitae* can be described by combining the species specific characters of both taxa as they are mentioned in literature. This might urge for a new taxon description or indicate the lack of difference between both taxa.

The nomenclature of *R. balsamica* is an example of the complexity within the subgenus *Rosa*. Comparing the four different determination keys, three different botanical names refer to this taxon (Table A.6). Recently, the name *R. balsamica* was accepted (Kurtto *et al.* 2004) and therefore used in this study. Both Nilsson (1967) and Graham and Primavesi (1993) referred to this taxon as "*R. obtusifolia*".

The different publications did agree on the variation of glands on the lower side of the leaflets, but differed in their frequency and/or position. According to Nilsson (1967), the main veins were usually glandular, but completely eglandular leaflets were also described. The serration was biserrated according to Nilsson (1967) and Graham and Primavesi (1993), multiserrated according to Wissemann (2003), and Henker (2000) observed intermediate states of bi- and multiserration. These intermediate states were also observed on the Flemish samples. In contrast to the eglandular hips and glabrous pedicels, the Flemish individuals occasionally showed the presence of glands or hairs.

Only Nilsson (1967) described a possible pubescent leaflet lower side of *R. canina* (Table A.7), while these were mostly glabrous. Similarly, Henker (2000) noted that the majority of the leaflet margins and hips were eglandular although rarely glandular margins were observed. In the Flemish data set also glandular margins were observed. In literature, the leaflet serration was described as uni- or occasionally biserrated, however in our observations also multiserration was observed. Henker (2000) also mentioned the presence of multiserration in *R. canina* var. *andegavensis*. However, due to his species-concept, these individuals are classified as hybrids. The pedicels measured in the Flemish *R. canina* were shorter (0.5 - 1.7 cm) and could be glabrous or sparsely pubescent compared to the ones described in literature. According to Nilsson (1967) and Graham and Primavesi (2003), the pedicels are 1 - 2.5 cm long and glabrous.

Within *R. canina*, the differences in glandular states on leaflets or pedicels are the basis of classification in varieties. In addition, the variation in serration of the leaflet margins and glands on leaflets are correlated. The most frequently observed *R. canina* in Flanders is var. *canina*, which is characterised by eglandular leaflets, hips, and uniserrated leaflet margins. When the leaflet margins vary from bi- to multiserrated and the main veins and rachides are glandular, the individuals were described as var. *dumalis*. However, if at least a small part of the pedicels are glandular; these var. *canina* or var. *dumalis* shrubs are defined as var. *andegavensis*. When var. *dumalis* has additionally glandular veins or mesophyll with eglandular pedicels, they belong to the var. *scabrata*. With glands on the pedicel, they are var. *blondaeana*. The two latter are very rare in Flanders.

R. corymbifera (Table A.8) was characterised by uniserrated and eglandular leaflet margins, pubescent and eglandular lower sides of the leaflets and rachides. However, according to Henker (2000), *R. corymbifera* could be occasionally multiserrated with glandular leaflet margins. According to Graham and Primavesi (1993), the rachides were eglandular, whereas the individuals of Henker (2000) and those of Flanders seldomly were sparsely glandular. The Flemish individuals showed a varying pubescence at the veins, moderately to densely; whereas the literature mentioned sparsely to mild pubescent lower sides of the leaflets. The length, pubescence and presence of glands on the pedicels varied enormously between the different sources. According to Graham and Primavesi (1993) they were smooth and 1.5 – 2.5 cm long, whereas Henker (2000) and the Flemish observations mentioned mostly glabrous and eglandular pedicels, that were rarely pubescent or

glandular. According to the latter, the pedicel length varied between 0.3 - 2 (-2.7) cm and 0.5 – 2 cm, respectively. Although Henker (2000) and the Flemish observations displayed similar lengths of the pedicel, the relative length differed enormously: 1.5 – 2 (Henker 2000) and 0.4 - 1.3 in the Flemish individuals.

5. DISCUSSION

5.1. The challenge of analysing wild roses

5.1.1. Tackling polyploid genomes

Due to the polyploid and heterogamous chromosome constitution of the section *Caninae*, two major restrictions had to be considered while interpreting the AFLP and STMS polymorphisms.

First, the section *Caninae* is characterised by two types of genomes, each being subject to different evolutionary forces. They are suggested to reflect the interspecific relationships among the section *Caninae* taxa differently (Nybom *et al.* 2006). The univalent-forming chromosomes are presumed to reflect the taxonomical affinities, whereas the bivalent-forming chromosomes are likely to be exchanged within and between taxa (Nybom *et al.* 2006). Consequently, as neutral genetic markers are considered to be spread randomly throughout the whole genome, only three fifth of the scored AFLP markers will be positioned on the univalent-forming chromosomes and thus be able to differentiate among the taxonomical groups. Since the two sets of bivalent-forming chromosomes are suggested to be exchangeable across taxa, they are presumed to add noise to the taxonomical subdivision of the section *Caninae*. Unfortunately, it was not possible to differentiate between the AFLP markers situated on the bi- or univalent-forming chromosome sets.

Moreover, polyploidy and the highly homologous state of the bivalent-forming chromosomes did not allow the quantification of the allelic frequencies of the co-dominant STMS markers. Therefore the presence of alleles was interpreted as phenotypes, the so-called "allelic phenotype" (after Becher *et al.* 2000), which enabled the observation of tendencies. In combination with the other results and additional information on locality, some specific topics regarding clonality and origin of hybrid taxa could be addressed.

Second, the wild and mostly pentaploid *Caninae* individuals do not fulfil the Hardy-Weinberg assumptions, such as the diploid chromosomal structure and Mendelian meiosis, that are required in the general accepted population genetic statistics. Similar deviating chromosomal constitutions are observed in other species (e.g. *Andropogon ternatus*, Norrmann and Quarin 1987). To our knowledge, none was analysed with molecular-genetic methods. Therefore, alternative and more descriptive strategies were used to analyse the AFLP polymorphisms.

Several distance-based analyses (Jaccard similarity coeffients, PCO, and cluster analysis) were combined with a model-based approach (Bayesian statistics) allowing us to formulate well-supported conclusions. The weaknesses and restrictions of one method can be detected by a non-confirming result obtained by another method. For instance, the output of a PCO biplot is known to be highly dependent on the set of individuals included in the analyses. Consequently, emphasising different taxonomical levels and population sizes might reveal structures in the data that are

not present or (biological) relevant in a global picture. In order to confirm or reject the presence of these substructures, both descriptive analyses and Bayesian statistics were performed on the same groupings. Moreover, as the PCO biplot only visualises two components at once, cluster analysis considers all the relevant components. In contrast, Bayesian statistics will attempt to assign individuals to populations based on their genotypes, while simultaneously estimating population allele frequencies. In conclusion, in the distance-based methods, the pair wise distances were calculated between every pair of individuals.

The Bayesian statistics approach, developed by Pritchard *et al.* (2000), is proven to be useful for two to four highly differentiated populations, that are evenly distributed in number or in space (Evanno *et al.* 2005). However, in a complex and large taxon such as *Rosa* much more populations may be present displaying complex hierarchical migration schemes. By adapting the calculation of Pritchard *et al.* (2000), Evanno *et al.* (2005) were able to reveal the real number of populations in such complex structures. Applying this strategy, the population structure could be revealed in only a few of our analysed taxa (e.g. section *Rosa*). In other taxa (e.g. section *Cinnamomeae*, section *Caninae*), the outcome suggested the presence of two populations. Unfortunately, the approach of Evanno *et al.* (2005) is restricted to reveal the population structure when less than three different groups are present. Consequently, in the cases that two gene pools were suggested no decisive population structure could be assessed, and feedback from the morphology and/or other genetic techniques was required to establish whether one or two gene pools would be the most likely outcome.

The above-mentioned approaches are basic statistical methods to assess the genetic diversity between- and within-taxa or -populations. Moreover, each individual method is not able to make decisive conclusions about biological relevant groupings or subdivisions. We assume that the combination of the different approaches is the best strategy to handle complex and polyploid taxa. The set of different strategies (e.g. dominant versus co-dominant markers, distance-based versus model-based methods, different calculation methods to assess pair wise distances) resulted mostly in complementary patterns. Supplementary information and contradictive results were taken into account and interpreted carefully. The congruent outcomes of the European and Flemish data sets confirmed that the sampling of the populations and individuals occurred randomly, and that the analysed samples are a representative subset for the different taxa of the subgenus *Rosa*.

5.1.2. Tackling hybridogenic species-complexes

In the past, the taxonomical structure of species, sections, and subgenera was mainly based on the observable morphological variations. As the environment may influence morphological characters, the phenotypic variation cannot be assumed to be a direct consequence of genetic mutations (Nybom *et al.* 1997). Moreover, DNA markers may differentiate slower than morphological characters. The integration of different sets of characters, such as morphological and different DNA markers, is important in getting the whole picture. This is especially true in taxa with a problematic morphological differentiation, such as hybridogenic species-complexes. Usually, hybrids display a continuous variation in morphological, ecological, and genetic traits, due to shared ancient polymorphism and/or hybridisation, causing a difficult determination. In addition, they have a complex character expression pattern, displaying a mosaic of parental, intermediate, and transgressive characters (Lihová *et al.* 2007). Although some morphological characters could be used as reliable criteria for their discrimination, the detection of hybrids is not always possible given the considerable within-species variation and only a small or non-existent between-species gap. For instance, the analysis of AFLP polymorphisms did allow the recognition of three genetically different groups in the *Vicia sativa* aggregate. In this complex, a stable classification based on the morphology was hampered by the large and almost continuous morphological variation within the six taxa (Van de Wouw *et al.* 2001). In contrast, AFLP data appeared to be less informative for the identification of hybrids between *Quercus crispula* and *Q. dentate*. Although these taxa displayed differences in leaf traits, no species-specific AFLP markers were obtained (Ishida *et al.* 2003).

Combining the outcome of our morphological and genetic approaches, we had to conclude that none of the applied techniques was able to discriminate the subsections or lower taxonomical groups in clearly well-defined clusters. Several tendencies towards intersubsectional differentiation were observed. Within the subsections *Rubigineae* and *Vestitae,* two largely overlapping clusters could be identified. The subdivision within these subsections is supported by morphological dissimilarities. This differentiation was more pronounced in the morphological analyses, as very little to no genetic differences were found using AFLP and STMS markers.

The results of the morphological and genetic analyses of the subsections *Caninae* and *Tomentellae* appeared to be incongruent as subtle but consistent morphological differentiations were not reflected in the genetic analyses. In literature, similar incongruity was reported for the taxonomically confused genus *Gentianella* section *Gentianella* (Winfield *et al.* 2003), and in hybrids of *Cardamine pratensis* and *C. raphanifolia* (Lihová *et al.* 2007). In both species-complexes, hybridisation, backcrossing, and introgression are presumed to occur frequently, and generate hybrid swarms.

5.2. Population differentiation, taxonomical aspects, and implications for conservation

5.2.1. General remarks

Population differentiation and conservation

The assessment of locally adapted populations is of major importance in the framework of conservation and use of autochthonous genetic resources. A first step is to assess whether populations sampled at different localities, regions, or countries display intraspecific differentiation. This differentiation can be expressed in a variation in morphology, and/or genetic markers. In addition, it should alter the fitness of the populations (Krauss and Koch 2004). Our results suggested the occurrence of intraspecific differentiation in some wild roses, such as *R. spinosissima*, *R. arvensis*. The main consideration is whether introgression of non-adapted genes will reduce the fitness of a (presumed) locally adapted population. Or, in contrast, will introgression increase the genetic diversity of the local population and augment the response to a changing environment. Next, the general impact of introgression on the fitness of the individuals or populations should be tested in provenance trails.

Both AFLP and SSR markers are known to provide information for randomly distributed and neutral loci. Previous studies have shown that large differences in morphology can be governed by small changes at a limited number of genes (Bradshaw *et al.* 1995, Andersson 2001, Doebley *et al.* 1997), or by epigenetic influences. Consequently, one cannot expect neutral markers to distinguish between highly related taxa (Winfield *et al.* 2003).

In the framework of the conservation of autochthonous genetic resources, it is important to maintain a sufficiently high level of genetic variation, and to conserve the diversity and purity of the presumed autochthonous plants. Roses display a range of reproduction strategies. The seeds could develop in an apomictic way, which means that they are identical to the mother plant and to each other, and contain no additional genetic variation. Alternatively, in the case of outcrossing, one can never be sure about the origin of the pollen grains that fertilise the presumed autochthonous mother plant. The purity of the descendants has to be questioned as both intraspecific hybridisation with allochthonous pollen grains and interspecific hybridisation may occur. In a small-scale experiment, we have confirmed the occurrence of spontaneous interspecific hybridisation in the field as one of the seedlings of an isolated *R. micrantha* plant displayed an STMS allele that was absent in the mother plant. Until now, isolated mother plants were assumed to reproduce through apomixis (Werlemark *et al.* 1999, Wissemann and Hellwig 1997), or through selfing (Nilsson 1999). Our small-scale experiment did not reject the occurrence of these reproduction strategies, but confirmed the statement of Nilsson (1999) that within mixed populations (the occurrence of multiple taxa on one locality), intra- and interspecific hybridisation may occur, and may cause genetic pollution in

descendants of presumed wild mother plants. In addition, Nilsson (1999) states that it might account for the variation that is observed within species of such mixed populations. For the conservation of rare rose species in the long run, it is recommended to construct living gene banks or seed orchards. Planting stock can be used for (re)introduction as it is important to collect the basic material for the orchards as cuttings of wild plants instead of using seeds collected in the field. Although cuttings are more labour-intensive and it is harder to end up with sufficient genetic variation, they have a quicker seed set and a more reliable genetic constitution regarding the authenticity of the species.

Conservation guidelines should be formulated to maintain the observed morphological and genetic variation, within or among taxa, subsections, or among populations. Regarding the conservation of wild roses, different strategies should be taken into account. Each taxon is characterised by special life history features (e.g. different reproduction and dispersal strategies, requiring different habitat conditions), and each population is affected by different influences (e.g. the occurrence of different taxa at one locality). The suggestions and guidelines concerning the conservation strategies of the different taxa are discussed separately.

Conservation of section Caninae *taxa*

Although the occurrence of interspecific hybridisation and introgression in the section *Caninae* is commonly accepted, few additional considerations should be taken into account. Apart from their impact on the taxonomy, the polyploid and atypical chromosomal constitution with the related canina meiosis have a unusual influence on hybridisation and introgression events.

First, polyploids display a higher genetic diversity and heterozygosity compared to diploids. They are also presumed to have an increased ability to colonise unoccupied niches (Vamosi and Dickinson 2006).

Secondly, the two types of genomes that are present in all the *Caninae* individuals display different inheritance patterns. The bivalent-forming chromosome sets act as a diploid genome and are inherited through both parents by Mendelian meiosis. The same chromosome sets will act as bivalents and are presumed to be highly homologous in order to recombine during meiosis (Nybom *et al.* 2006). In contrast, the uniparental inheritance of non-recombinant univalent-forming sets strongly resembles apomixis. This means that three fifth of the entire genome in the descendents is identical to that of the seed parent. Moreover, these chromosomes will only change by mutation instead of recombination (Lim *et al.* 2005, Nybom *et al.* 2006). The evolutionary fate for such "asexual" chromosomes is genetic degradation through the accumulation of mutations (Lim *et al.* 2005).

The introgression of non-adapted genes in a locally adapted section *Caninae* population will only occur when the non-adapted genes are located on the recombining bivalent-forming chromosomes. In addition, the homology of the bivalent-forming chromosomes has to be sufficiently high to allow recombination in the introgressed F1 hybrids which is essential as it is presumed to influence the

success of the canina meiosis in the F1 hybrids (Nybom *et al.* 2006). A fertile F1 generation is required before backcrossing and consequently introgression can occur.

Small mutations are known to cause mal-adaptations influencing the fitness of the individual (Doebley *et al.* 1997). If small mutations have occurred on the bivalent-forming chromosomes, the locally adapted univalent-forming chromosomes inherited through the seed parent can act as a buffer to hamper the reduction of the fitness. Alternatively, the non-local genes might influence the local population in a positive way, e.g. by enlarging the genetic variation of an endangered population, and increasing the fitness of these F1 hybrids. Still, the homology between the two bivalent-forming chromosome sets should be sufficiently high.

Alternatively, if non-adapted genes are situated on the non-recombinant univalent-forming chromosomes through either mutations or seed transfer, the fitness of the F1 hybrid can be influenced displaying an alternative reproductive potential. This can lead to an indirect negative impact, or a positive influence of more suitable genes.

5.2.2. Classification of the subgenus *Rosa*

In the past, several classifications of the subgenus *Rosa* have been suggested. However only in 2000, a taxonomical structure was proposed by Henker and was accepted by the majority of the taxonomists on the European continent. In this classification, the European wild roses are divided into five sections. The sections *Pimpinellifoliae, Rosa, Cinnamomeae,* and *Synstylae* all have only few representatives in Europe. In contrast, the section *Caninae* contains over 20 taxa and forms the largest and most complicated group. The clustering of this polymorphic group is based on the common presence of the unique chromosomal constitution, the heterogamous canina meiosis, and the autapomorphic bivalent-forming chromosomes (both of the nrITS-*Caninae*-type) (Wissemann 2002a). According to Koopman *et al.* (2008), polymorphisms in related species groups are the result of the adaptation to different selection pressures; whereas character similarity in evolutionary divergent species is an adaptation to similar conditions.

The analysis of the AFLP polymorphisms confirmed the subdivision of the subgenus *Rosa* into different groups. Depending on the followed approach, the number of groups differed: the European subgenus *Rosa* was divided in five (using cluster analysis and Jaccard similarity coefficients), three (based on PCO analyses), or in two gene pools (using the model-based approach). The results of each approach are congruent and confirm the general subdivision of the subgenus *Rosa* in the five sections: *Pimpinellifoliae*, *Cinnamomeae*, *Synstylae*, *Rosa,* and *Caninae*. A higher similarity among the sections *Pimpinellifoliae* and *Cinnamomeae* on the one hand and among the sections *Synstylae* and *Rosa* on the other hand was observed. The close relatedness of the latter two was already suggested in the phylogenetic analyses within the subgenus *Rosa* (Koopman *et al.* 2008). The fifth cluster was formed by the section *Caninae* that appeared to be a very dense and well-defined genetic unit in contrast to its polymorphic character. The unique and peculiar position of the section

Caninae in the subgenus *Rosa* was confirmed by the model-based approach that indicated that the section *Caninae* was the most distinct group within the subgenus.

The intersectional hybridisation between *R. arvensis* and section *Caninae* taxa is possible as the bivalent-forming chromosomes are closely related to chromosomes of the section *Synstylae* (Wissemann and Ritz 2005). This higher similarity was not confirmed by our results as no distinction could be made between the genetic markers of the bivalent- and univalent-forming chromosome sets, and the distinct origin of the univalent-forming chromosomes sets.

Although interspecific hybridisation within the section *Caninae* is known to occur, a hierarchical subdivision was observed. The subsection *Rubigineae* appeared to be the most distinct group, followed by the subsection *Vestitae*. Nevertheless, the section *Caninae* subsections overlapped largely and lacked clear and well-defined boundaries. The differentiation of the subsections might be explained by the very strict conditions under which hybrids will be fertile. According to Nybom *et al.* (2006), interspecific hybrids will only be fertile and able to contribute their genetic material to the next generation if the bivalent-forming chromosomes are sufficiently homologous to recombine, thus they are able to follow the canina meiosis. This means that even within the section *Caninae*, interspecific hybridisation may fail and some interspecific crossings may be more successful than others. This will depend on the differentiation of the bivalent-forming chromosome sets originating from different parental taxa.

The differentiation in sections and subsections was more pronounced in the Flemish section *Caninae* compared to the European data set. This might be due to the smaller number of sections and/or taxa included in the Flemish analyses. For instance, the section *Rosa* which was absent in the Flemish data set appeared to take in a more intermediate position between the European sections *Caninae* and *Synstylae*. In addition, in the Flemish data set a smaller number of individuals and less geographical differentiation were included. The allelic phenotypes of the Flemish subgenus *Rosa*, being assessed with STMS markers, supported the differentiation between the sections, but also confirmed the lack of clear subsection-boundaries.

5.2.3. Polymorphisms within the section *Caninae*

In the section *Caninae,* a huge, continuous, and consistent variation in pubescence and glands on leaflets, pedicels, and hips is present. This variation forms the basis of the taxonomical subdivision in different subsections or groups (Henker 2000, Nilsson 1999). In the combined morphological analysis, the Flemish section *Caninae* formed a sphere consisting of different portions. Each portion was characterised by a combination of a few striking morphological characters typifying the three groups of Nilsson, and three out of the six subsections of Henker. The overlap between the portions indicates that transition states and combinations of species-specific characters were observed. This stresses the hybridogenic character of the section *Caninae* even more. Similar tendencies were observed in the molecular-genetic analyses (AFLP). Our results are in contrast with the conclusions of Atienza

et al. (2005), who studied the variability within the subgenus *Rosa* with RAPD polymorphisms, and tried to assess the consistency of the subdivision within the section *Caninae*. They could not observe a consistent subdivision within the section *Caninae*. In their opinion, this was due to the many intermediate forms that were considered in the analyses. Alternatively, we assume that this lack of consistency might be explained by the used set of markers, since the differentiation at the subgenus level was addressed with the same set of markers.

In both the morphological and the genetic analyses, the subsection *Rubigineae* was identified to be the most differentiated group within the section *Caninae*. These taxa were characterised by strongly glandular leaflets spreading a typical apple-like odour. This outcome supports the conclusion of Koopman *et al.* (2008) who stated that the subsection *Rubigineae* is a derived and genetically defined group within the section *Caninae*. Similarly, taking *R. rubiginosa* as a representative of the *R. rubiginosa*-group, it was described as a clearly delimit unit based on morphometrical analyses (Nybom *et al.* 1996), RAPD (Olsson *et al.* 2000) and STMS analyses (Nybom *et al.* 2006). The differentiation of the subsection *Rubigineae* within the section *Caninae* taxa might be due to a distinct non-*Caninae* parent in the historical hybridisation events providing one or more dissimilar univalent-forming chromosome set(s). Alternatively, the somewhat higher differentiation of the bivalent-forming chromosomes compared to the other *Caninae* subsections might impose a barrier in the interspecific hybridisation. Werlemark (2000a) stated that differences in flowering phenology or perhaps interspecific pollen competition may have caused, or is still causing, the differentiation of the subsection *Rubigineae* as the taxa have different inclinations to hybridise in nature. In contrast, the presence of wild interspecific progeny (Graham and Primavesi 1993, Feuerhahn and Spethmann 1995) and the success of controlled interspecific crossings with subsection *Rubigineae* as parental taxa (e.g. Werlemark *et al.* 1999) was reported multiple times. These successful interspecific hybridisations suggest the presence of a sufficiently high homologous state of the bivalent-forming chromosomes of the subsection *Rubigineae* with the other *Caninae* subsections. Therefore, it is more likely that one or more univalent-forming chromosome set(s) of the subsection *Rubigineae* is (are) strongly differentiated. In addition, they might be responsible for the observed morphological differentiation.

Subsequently, the subsection *Vestitae* displayed typical tomentose pubescent leaflets and persistent stipitate glands on pedicels and hips and was differentiated from the subsections *Tomentellae* and *Caninae* in both the morphological and genetic analyses. Although intersubsectional differentiation was observed, the boundaries were more faint compared to the differentiation of the subsection *Rubigineae*. In contrast, the phylogenetic analyses of Koopman *et al.* (2008) did not subdivide the subsection *Vestitae* from the remaining subsections. This discrepancy with our results may be due to the restricted number of representatives included in the phylogenetic analyses. Moreover, they attempted to clarify the global picture of the subgenus *Rosa* and the section *Caninae*, instead of gaining insight in the taxon structure within the subsections. Alternatively, cpDNA analyses performed by Wissemann and Ritz

(2005) split the whole section *Caninae* into two major clades: the taxa that are characterised by eglandular and non-odorant glands (cfr. subsections *Caninae* and *Tomentellae*), and the taxa with odorant, both apple- and turpentine-scented, glands (cfr. *Rubigineae* and *Vestitae*). This outcome supports our result as it indicates a difference in the historical maternal line of the groups *Rubigineae*-*Vestitae* and *Caninae*-*Tomentellae*. Regarding the presumed common maternal ancestor of the subsections *Rubigineae* and *Vestitae* as was suggested by Wissemann and Ritz (2005), we could not drawn any conclusions based on our outcomes.

Although both Henker (2000) and Wissemann (2003) made a distinction between the subsections *Tomentellae* and *Caninae*, the systematic position of *R. balsamica* and *R. abietina,* the two taxa of the subsection *Tomentellae*, is known to be uncertain (Wissemann 2000b). The combined morphological analysis showed tendencies to intersubsectional differentiation between the *Caninae* taxa (*R. canina* and *R. corymbifera*) and *R. balsamica,* the only analysed representative of the subsection *Tomentellae*. This differentiation was not supported by the molecular-genetic analyses.

Depending on the analysed sections or subsections, the AFLP analyses suggested different subdivisions of the section *Caninae*. Several arguments favour the assignment of all the section *Caninae* taxa into one gene pool. First, the so-called species-specific morphological characters displayed a continuous variation. Second, these taxa share the unique polyploid chromosomal constitution and the heterogamous canina meiosis. Third, the autapomorphic bivalent-forming chromosome sets are exchangeable among the different subsections, and finally, these taxa have the theoretical ability to hybridise intersubsectional. However, within the section *Caninae*, some restrictions to the hybridisation processes are known that might favour the assignment of the taxa in two gene pools. For instance, the lack of compatible bivalent-forming chromosome sets hampering the sexual reproduction of the viable F1 hybrids (Nybom *et al.* 2006), the presence and consistency of few well-observable and clearly defined morphological characters of the pure individuals, and the presence of the derived and genetically defined subsection *Rubigineae* within the section *Caninae*. Alternatively, a third hypothesis might suggest that both the bivalent and the univalent genomes could be assigned to one or more gene pools. This hypothesis is supported by the allopolyploid origin of the section *Caninae* in which the integrity of the original genomes is still maintained (Nybom *et al.* 2006). The bivalent genomes, probably originating from the Protocanina, are observed in all the section *Caninae* taxa and are proven to be two highly homologous and exchangeable chromosome sets (Nybom *et al.* 2006). This "diploid" genome allows interspecific hybridisation and can be assumed to act as one gene pool within the section *Caninae*, providing that homology remains sufficiently high. In contrast, the univalent-forming chromosome sets originated from different non-*Caninae* species through multiple ancient hybridisations (Nybom *et al.* 2006). Consequently, these (mostly) three haploid and non-recombining genomes could be assigned to one, two, or three distinct gene pools (Nybom *et al.* 2006). Taking this into account, the subsection *Rubigineae* appeared to contain the most distinct univalent genome(s) compared to

the other subsections, indicating the presence of, or the evolution to a separate gene pool. The high similarity of the subsections *Caninae* and *Tomentellae* genomes was confirmed in all outcomes. Consequently, it is most likely that the univalent-forming chromosome sets of these two subsections belong to the same or highly related gene pool(s). Explaining the origin of the intermediate position of the subsection *Vestitae* is less straightforward. Our morphological and genetic polymorphisms indicated a higher similarity with the subsections *Caninae*-*Tomentellae* compared to the subsection *Rubigineae*. Alternatively, cpDNA analyses formed the clades *Rubigineae*-*Vestitae* and *Caninae*-*Tomentellae* suggesting both clades to have a different maternal influence (Wissemann and Ritz 2005). One explanation might be that several Protocanina individuals have hybridised multiple times with the non-*Caninae* species. If so, the present-day subsections *Rubigineae* and *Vestitae* are presumed to share a similar Protocanina, whereas subsections *Caninae* and *Tomentellae* share the ancient influence of another Protocanina. This might explain the results of cpDNA sequence analysis. In addition, the higher similarity of the subsection *Vestitae* with the *Caninae*-*Tomentellae* might be caused by the more similar origin of the univalent-forming chromosome sets. Unfortunately, we are not able to distinguish among the polymorphisms observed on the bivalent- or the univalent-forming chromosome sets. Therefore, it could not be proven that the assignment of the taxa to the different subsections was based on the univalent-forming chromosome sets. Until now no conclusive evidence is found to validate the hypothesis on the existence of the Protocanina (Wissemann and Ritz 2007).

From an evolutionary point of view, several Rosaceae members have probably developed fairly recently. In addition, the section *Caninae* is assumed the most recently formed section (Atienza *et al.* 2005). This means that the intersubsectional boundaries are still being created, or are disappearing. Our data supported the idea of a young section *Caninae* in an evolutionary time-scale as the morphology showed clear distinctions and many intermediate forms. Presumably, the partly apomictic character of the canina genome hampers quick evolutionary species formations and prevents large-scale species differentiation.

5.2.4. Hybridisation processes

Hybrid swarms within the subsection Rubigineae

Taxonomical issues

The shape of the leaflets divided the subsection *Rubigineae* in two groups. One group contained taxa with slender leaflets and wedge-shaped bases, such as *R. elliptica* and *R. agrestis*. The second group consisted of the taxa with broad leaflets and well-rounded bases: *R. rubiginosa*, *R. micrantha*, and *R. henkeri-schulzei*. This subdivision based on the morphology was also observed in the results of the genetic analyses. However, distinction between the two clusters was vague. The overlap was quantified by the species assignment of the European *R. rubiginosa* and *R. micrantha*,

65% was assigned to group 2, and the remaining 35% were clustered in group 1 together with the slender leaflet-taxa. In contrast, the Flemish *R. agrestis* appeared to display a higher genetic similarity with *R. rubiginosa* compared to the similarity among *R. rubiginosa* and *R. micrantha*. This is in contrast with both the morphological analyses and the outcome of the European genetic analyses. This incongruence should be interpreted carefully as the rare presence of *R. micrantha* in Flanders allowed the sampling of only few individuals, and may distort the global output. Moreover, the almost equal partitioning of the *R. rubiginosa* individuals originating from the St-Pietersberg (Maasvallei) to the two assumed gene pools (45/55 ratio) might support the idea that the subsection consists of only one gene pool.

Within each group based on the shape of the leaflets, different taxa were characterised by the theoretically well-defined L and D type differences (habit, orifice, sepals, etc.). These differences can be very subtle in the field. The lack of clearly observable morphological differentiation among *R. rubiginosa, R. henkeri-schulzei*, and *R. micrantha,* on the one hand, and *R. agrestis, R. inodora*, and *R. elliptica* on the other was confirmed by an almost complete overlap of the taxa within each group. As a consequence, all the taxa of one group are presumed to belong to the same gene pool. This was supported by the paternal inheritance pattern of the L and D type characters, being the diameter of the orifice and the persistence of the sepals. These should not be regarded diagnostic to the determination of the parental taxa, as they are located on the bivalent-forming chromosomes and are transferred among the taxa. In addition, we assume that the variation in diameter of the orifice and the persistence of the sepals is caused by small mutations as hybrids between L and D type taxa might still be fertile.

The individuals of the supposable "species-pure" populations, consisting of only one taxon and lacking any indication of past hybridisations or presence of other taxa, display morphological characters that were consistent to the taxon description. For instance, the species-specific characters of *R. rubiginosa*, such as diameter of orifice, persistence of the sepals, heteracanthy of the prickles, etc., were clearly observable in the coastal populations. These individuals can be assigned to a certain species or taxon. This was in contrast with the populations with a mixed presence of *R. rubiginosa* and *R. micrantha*, e.g. St-Pietersberg (Maasvallei). In this mixed population, the *R. rubiginosa* individuals clearly displayed the influence of *R. micrantha*. For instance, *R. rubiginosa* had a clearly more narrow orifice, longer leaflets, and larger disc index compared to the congeners from the "species-pure" *R. rubiginosa* populations at the Westkust, and to the descriptions in literature (Henker 2000). In addition, *R. micrantha* individuals of this hybrid population showed more narrow leaflets, larger hips, larger diameters of orifice and disc, a head-shaped stigma, and shorter pedicels compared to those described in literature (Henker 2000). Unfortunately, no "pure" Flemish *R. micrantha* populations could be included as a reference. Apart from the lack of consistent species-specific morphological characters, these individuals also lacked a genetic base required to be considered as a specific unit. Accepting the loss of the species level in mixed populations, we suggest to describe these interspecific hybrids as intermediate forms and assign them to a

species-complex instead of trying to reconstruct the history of the ancestral crossings. In our opinion, each species-complex consists of two presumably pure parental taxa and a range of intermediate individuals or hybrids, more specifically, the *R. micrantha – R. henkeri-schulzei - R. rubiginosa*-complex, and the *R. agrestis – R. inodora - R. elliptica*-complex. The spontaneous hybrids are characterised by a range of variable transitional forms between the parental taxa. The parental taxa that display well-defined species-specific characters are mostly absent in the mixed population.

Geographical differentiation

Although a large geographical area was sampled in Europe, little to no geographical (AFLP-based) genetic differentiation was observed among the populations of the subsection *Rubigineae*. This confirmed the conclusions based on morphometrical characters and RAPD analysis that *R. rubiginosa* is a highly homologous taxon (Nybom *et al.* 1996, Olsson *et al.* 2000). The lack of geographical genetic differentiation within the subsection might be explained by the densely covered distribution area in Europe, or by the chromosomal constitution of which the non-recombining univalent-forming chromosome sets were inherited apomictical, whereas the recombining bivalent-forming chromosomes were exchangeable and highly homologous in order to be fertile. Alternatively, the heterogamous meiosis restricts the introgression of tetraploid cultivars and interspecific gene flow will only be successful if the introgressed bivalent-forming chromosome set is sufficiently homologous with the maternally inherited bivalent-forming chromosome set. Also the extended use of *R. rubiginosa* as rootstock, the past human distribution and the dispersal of seeds by birds or small mammals might have contributed to the genetic uniformity of the European *Rubigineae*.

The observed deviations in morphology between our Flemish *R. rubiginosa* and the few *R. micrantha* individuals, and the descriptions in literature might be caused by a different origin of provenance, a different constitution of the population (species-pure versus mixed populations), etc. The main deviation is the higher frequency in pubescence and glands on the *R. rubiginosa*, *R. micrantha,* and *R. agrestis* populations sampled at the Maasvallei compared to the description in literature. This variation is known to indicate hybridisation or introgression events (Graham and Primavesi 1993).

The few analysed *R. agrestis* individuals showed a tendency to morphological differentiation between the Flemish populations of Maasvallei and Brabants District Oost. The pedicels and hips from the population of Brabants District Oost tended to be larger compared to the Maasvallei population and to the descriptions in literature. This difference might be the expression of local adaptation, and can only be confirmed in provenance trails. Supposed that local adaptation caused this differentiation, the urge to prevent hybridisation or introgression among the two populations might not be as crucial as with diploid species such as *R. arvensis*. The univalent-forming chromosomes in the section *Caninae* taxa buffer the influence of non-adapted genes in the F1 generation, whereas the bivalent-forming chromosomes

prevent the introgression if the non-adapted genes disrupt the required homology. Anyhow, many questions concerning the mechanism of the canina meiosis remain open. For instance, which level of homology among the bivalent-forming chromosomes is required to allow sexual reproduction? Therefore, the principle of precaution should be taken into account and the population *R. agrestis* of Brabants District Oost and Maasvallei should be kept apart. At least until the presence of local adaptation was confirmed or rejected.

The status of *R. henkeri-schulzei*

It can be assumed that hybridisations have occurred in the mixed populations of *R. micrantha* and *R. rubiginosa* since a long time. Mixed populations are characterised by the fading of the species-specific characters of the two parental species, indicating the presence of little to no pure individuals. The rather difficult morphological distinction of the spontaneous individuals in such a mixed population is reflected by the lack of genetic differentiation. It increases the ability of the F1 hybrids to backcross with one of the parental taxa, or with other hybrids. These unverifiable hybridisation and backcrossing paths in combination with the unique chromosomal constitution, the unequal meiosis, and the different patterns of inheritance lead to a huge variability in the descendents. This is observed through the presence of both intermediate and species-specific parental characters (e.g. diameters of orifice, leaflet dimensions), and signs of introgression (variable occurrence of glands and pubescence).

In literature, individuals combining characters of both *R. rubiginosa* and *R. micrantha,* and displaying transitional forms are described as the intermediate species *R. henkeri-schulzei* (syn. *R. columnifera* Henker 2000) or as the subspecies *R. rubiginosa* subsp. *columnifera* (Wissemann 2003). Although they are assumed to be (sub-)species, no consistent or detailed description was found in literature. Alternatively, Graham and Primavesi (1993) described the descendant of *R. micrantha* x *R. rubiginosa* as *R.* x *bigeneris*. Unfortunately, they did not mention the reciprocal hybrid as they followed Melville who stated that reciprocal hybrids have not been recorded. The occurrence of reciprocal hybrids among *R. rubiginosa* and *R. micrantha* has been confirmed by successful controlled crossings (unpublished results).

Apart from the morphological differences between *R. rubiginosa* and *R. micrantha*, we were not able to distinguish these taxa in the molecular-genetic analyses. In addition, the intermediate species *R. henkeri-schulzei* overlapped completely with both parental species clouds. The high similarity between parental and intermediate species can only encourage further backcrossings among these taxa, creating a complex of intermediate forms. The combination of the different patterns of inheritance of species-specific characters expands the complexity of determining spontaneous wild hybrids.

In conclusion, since this intermediate taxon lacks a clear morphological description in literature, one might question the species-position "*R. henkeri-schulzei*"

and instead assume it to be a fertile hybrid "*R.* x *henkeri-schulzei*". Alternatively, as both the parental and the intermediate "species" lack any genetic differentiation, one might argue about the use of defining different species in a mixed population. It might be more convenient to accept the presence of hybridisations and the young evolutionary state of the whole section *Caninae*. Therefore, we suggest assigning all the presumed *R. rubiginosa*, *R. henkeri-schulzei,* and *R. micrantha* individuals in a mixed population to the same hybrid swarm or species complex: *R. rubiginosa-R. henkeri-schulzei-R. micrantha*. Elaborating this is the work of taxonomists.

Conservation

Regarding the conservation of the subsection *Rubigineae*, two items should be considered. On the one hand, the present-day division of regions of provenance in Flanders might be sufficient for the subsection *Rubigineae* given the lack of genetic geographical differentiation. In addition, the possible impact of the tendencies of morphological differentiation observed among the *R. agrestis* populations of the region Brabants District Oost and Maasvallei should be investigated in provenance trails. For now, these populations should be handled separately. On the other hand, are the observed differences between the presumed pure and mixed populations worth conserving? Should we focus on the *ex situ* conservation of the remaining pure individuals, or do we accept evolution and selection to act on these populations with the possible consequence of losing the typical wild parental species. Are the present-day intermediate individuals in the mixed populations worth to conserve, and how should this be handled? *In situ* conservation will allow further influence of both evolution and selection creating a diversity of individuals with new character-combinations, and perhaps a new species-form. The main dilemma is do we want to conserve the present-day status of the wild individuals, or conserve the processes and the species-complexes. These questions have to be evaluated for each taxon or population separately by a group of experts.

Hybrid swarms within the subsection **Vestitae**

Taxonomical issues

The AFLP analyses divided the five European *Vestitae* taxa into two partly overlapping clusters that were supported by species-related morphological characters. The first gene pool mainly consisted of *R. pseudoscabriuscula* and *R. tomentosa*, both taxa are characterised by uni- to multiserrated leaflets, and narrow diameters of the orifice (smaller or equalling 1 mm). The second gene pool contained the taxa *R. sherardii*, *R. villosa*, and the majority of *R. mollis* all characterised by a broader orifice (larger than 1 mm), erect and persistent sepals, and (irregular) multiserrated leaflet margins. However, as was suggested previously, the L and D type characters should not be regarded diagnostic to the determination of taxa, as they are located on the bivalent-forming chromosomes and are transferred among the taxa. Consequently, the interspecific differentiation might be the result of

different univalent-forming chromosome sets, originating from different non-*caninae* parental taxa.

The two taxa *R. pseudoscabriuscula* and *R. tomentosa* are assumed to be autochthonous in Flanders (Maes 2006). According to the literature (Henker 2000), few well-defined and remarkable differences distinguish these two taxa. In practice, it was rather difficult to identify the wild Flemish *Vestitae* as *R. pseudoscabriuscula* or *R. tomentosa*. Our morphological study could not distinguish the correlations between the presumed species-specific characters, such as diameters of orifice, the serration, and presence of glands on leaflet margins. The decision to determine all the Flemish *Vestitae* as *R. tomentosa* was based on the diameter of the orifice that was smaller than 1 mm. Moreover, a high genetic similarity was observed between the European *R. pseudoscabriuscula* and *R. tomentosa*. The Flemish *R. tomentosa* displayed a larger variability in serration, in presence of glands on the leaflet margin, and in hip and pedicel length compared to the descriptions in literature. This dissimilarity can be explained by the difference in species description.

Similar to the *R. tomentosa* - *R. pseudoscabriuscula* issue is the lack of a genetic base to differentiate between *R. mollis* and *R. villosa*. The kinship between these two morphologically very similar taxa is stressed in the taxonomy of Nilsson (1967). He classified these taxa as subspecies: *R. villosa* ssp. *mollis* and *R. villosa* ssp. *villosa*.

The high genetic similarity among *R. sherardii* and *R. villosa* is explained by the presumed hybridogenic origin of *R. sherardii*. This was suggested by RAPD- and STMS-based investigations and *R. villosa* ssp. *mollis* or a closely related taxon acted as seed parent (Olsson *et al.* 2000, Nybom *et al.* 2004). We suggest that both *R. tomentosa* - *R. pseudoscabriuscula*, and *R. mollis* - *R. villosa* are considered to be handled as one species-complex. Although *R. sherardii* and *R. mollis* – *R. villosa* have a common ancestor, we tend to divide the subsection *Vestitae* in three species(-complexes): *R. tomentosa* - *R. pseudoscabriuscula*, *R. sherardii,* and *R. mollis* - *R. villosa*. Similar to the subsection *Rubigineae*, the individuals characterised by pure species characters can be identified as species, whereas individuals displaying a whole range of intermediate forms will be described as the intermediate forms.

Geographical Differentiation

Although the five taxa of the subsection *Vestitae* were well sampled in Europe (51 populations originating from five countries), the AFLP polymorphisms did not show a geographical differentiation pattern. This is in contrast to the AFLP analyses of the Flemish *R. tomentosa*, where the genetic differentiation in two clearly separated gene pools appeared to be a reflection of the variation in population structure (density and distribution of the individuals). The sampled regions of provenance are characterised by a typical population structure. In the regions, West-Vlaams Heuvelland and Vlaamse Ardennen, the *Vestitae* were very scarce and only two or three individuals could be sampled. This is in contrast to the two large populations of the Westkust, each sampled at a well-defined locality. Within the population Oostvoornduinen, little to no other *Caninae* taxa were observed; whereas in the

Doornpanne, the *Vestitae* individuals were mingled with *R. rubiginosa*. Finally, the population at Brabants District Oost consisted of a compilation of solitary growing individuals sampled along edges of forests and sunken roads on different localities spread out in the region. The observed small-scale differentiation lies within the observed species variation at the European scale. This might be explained by the presence of local adaptation within the species range. It also stresses the fact that none of the applied methods can give an indication about the biological relevance of the observed subdivision.

The small populations from Vlaamse Ardennen and West-Vlaams Heuvelland showed genetic similarity towards each other, while they differed from their congeners from the larger populations Westkust and Brabants District Oost. Therefore, these two small populations might be valuable relict populations. Apart from the individuals of West-Vlaams Heuvelland, also interpopulational morphological differentiation was observed. The Westkust population displayed more narrow diameters of the orifice and shorter leaflets, a higher frequency in densely glandular and multiserrated leaflet margins, and less glandular hips compared to the congeners from Vlaamse Ardennen and Brabants District Oost. This distinction might be caused by a different occurrence of taxa in the sampled populations. The individuals of the Doornpanne (Westkust) are mingled with a dense *R. rubiginosa* population that might explain the shorter leaflets and more dense glandular leaflets margins, but contradicts the more sparsely glandular hips and the narrow orifice. The deviating morphology could also be the result of local adaptation, or a rare ancient and untraceable hybridisation event.

The solitary living shrubs scattered throughout the Brabants District Oost showed a higher genetic variation compared to the dense populations at Westkust. This indicates that the gene flow among the individuals of the dense population is higher than among solitary and presumed isolated shrubs. In addition, an enhanced level of clonality was observed in the Westkust population, which reduces the total intrapopulational variation. As this taxon is known to be very difficult to reproduce vegetatively, these clones might be the result of apomictic reproduction. Alternatively, the lower variation might be explained by founder or bottleneck effects. The solitary shrubs in Brabants District Oost are assumed relict individuals from larger populations, in which reduction and fragmentation of habitats (edges of forest, along sunken roads, etc.) may limit the exchange of pollen and increase the differentiation from each other.

Conservation

For *R. tomentosa,* the subdivision of the Flemish regions of provenance (Vander Mijnsbrugge *et al.* 2005) should be maintained as in each region of provenance different population structures were observed. Moreover, the genetic and/or morphological analyses displayed similar differentiation within this taxon. For Brabants District Oost, there is no control on the maintenance of the sunken roads in which the strongly isolated shrubs grow. In addition, most shrubs are situated along

fields of maize or wheat being sprayed or fertilised. These treatments have a negative influence on the growth and fitness of the shrubs, therefore they are under severe pressure and very vulnerable. Revisiting the sampling localities with only a few months in-between, several shrubs were already disappeared or died. The genetic analyses did not indicate the presence of differentiation within the region Brabants District Oost, so compiling these solitary shrubs will probably enhance the genetic constitution of this taxon. The collection of genotypes through cuttings and the centralisations in supervised living gene banks that can serve as seed orchards might be an important contribution towards the maintenance of this taxon in Flanders in the long run. Concerning the few relict individuals in Vlaamse Ardennen and West-Vlaams Heuvelland, similar conservation strategy should be followed in order to maintain these relict genotypes. In contrast, in the larger and denser Westkust populations more than 50 shrubs grouped together. These populations are more sheltered towards external threats, as the localities are protected areas and responsible agencies are aware of these hot spots of biodiversity for wild roses, such as Oostvoornduinen and Doornpanne.

Hybridisations among the taxa of the subsections Caninae *and* Tomentellae

In Europe, eight taxa of the subsection *Caninae* were sampled intensively, with *R. canina* and *R. corymbifera* as the most common taxa. Of the subsection *Tomentellae* only *R. balsamica* was sampled since the other taxon, *R. abietina*, was too rare in the sampled countries. The morphology of these three taxa was studied intensively.

Taxonomical issues

Although both Henker (2000) and Wissemann (2003) made a distinction between the subsections *Tomentellae* and *Caninae*, the systematic position of *R. balsamica* and *R. abietina,* both taxa of the subsection *Tomentellae*, is known to be uncertain (Wissemann 2000b). In addition, the nomenclature of *R. balsamica*, *R. obtusifolia,* or *R. tomentella* has been a subject of discussion. Only recently, a consensus was reached on *R. balsamica* being the most correct name and *R. tomentella* is suggested to be a synonym (Kurtto *et al.* 2004). In the same publication, the name *R. obtusifolia*, which both Graham and Primavesi (1993) and Nilsson (1999) used as a synonym of *R. tomentella*, is mentioned as a synonym of *R. corymbifera* (Kurtto *et al.* 2004).

In contrast to the subsections *Rubigineae* and *Vestitae*, the morphological differences among the taxa of the subsections *Caninae* and *Tomentellae* were more subtle. The three analysed taxa displayed few clear morphological subsection- and species-related characters (e.g. presence and frequency of pubescence and glands on leaflets, hips, and pedicels). Nevertheless, the combined analysis of morphometric and descriptive characters showed little differentiation between the taxa of the subsection *Caninae*, *R. canina* and *R. corymbifera*. A similar degree of differentiation was present between the taxa of the subsection *Caninae* and *R. balsamica*, and no clear

subsection or species-boundaries could be defined. Apart from the little morphological differentiations, the AFLP and STMS polymorphisms were unable to detect a consistent taxonomical differentiation, neither among the subsections *Caninae* and *Tomentellae*, nor among the three taxa. This was confirmed by the similar Jaccard similarity coeffients calculated among and within *R. canina*, *R. corymbifera,* and *R. balsamica*. Even more striking was the result of the additional analyses comparing the partitioning of the diversity within and among taxa and localities. This suggested that the genetic similarity among individuals of a mixed population (e.g. *R. canina* Zwin versus *R. corymbifera* Zwin) is higher, irrespective of the taxonomical position of the individuals (based on the morphological characters), compared to the genetic similarity with congeners from other localities (e.g. *R. canina* Zwin versus *R. canina* Heers).

The combined morphological analyses suggested a hybridogenic origin of the subsection *Tomentellae*. *R. balsamica* tended towards an intermediate position between the subsections *Caninae* and *Rubigineae*. The subsection *Caninae* was characterised by long, mostly glabrous and eglandular uniserrated leaflets with a correlated variation in presence of glands and serration of the leaflet margins. The rachides or veins could be pubescent. The morphometric characters of *R. balsamica* (subsection *Tomentellae*) showed little to no difference with the subsection *Caninae* taxa. In addition, *R. balsamica* was characterised by glandular leaflet margins, rachides, and veins on the lower leaflet sides, and a varying serration of pubescence on the leaflets. The pedicels were glabrous or sparsely pubescent and mostly eglandular. Moreover, analysing the epicuticular wax structure of the section *Caninae*, the taxa of the subsection *Tomentellae* and the majority of the subsection *Caninae* share the triangular rodlet type that differs from the type observed in the subsection *Rubigineae* (Wissemann 2000b). In contrast and based on the analysed AFLP or STMS polymorphisms, the subsections *Caninae* and *Tomentellae* did not show any differentiation, whereas the *Rubigineae* taxa formed the most distinct cluster in the section *Caninae*. Moreover, calculating the Jaccard similarity coefficient within and among the subsections *Caninae* and *Tomentellae*, the similarity among the subsections equalled the within-similarity.

Both AFLP and SSR markers are known to provide information for neutral loci, and previous studies have shown that large differences in morphology can be governed by small changes at a limited number of genes (Bradshaw *et al.* 1995, Andersson 2001, Doebley *et al.* 1997). Consequently, if only a small number of genes, or even a single gene, separate the taxa *R. canina*, *R. corymbifera,* and *R. balsamica*, one cannot expect neutral markers to distinguish these taxa (Winfield *et al.* 2003). In any case, it is less presumable to accept that the morphological differences between *R. canina*, *R. corymbifera,* and *R. balsamica* are caused by environmental plasticity instead of having a genetic basis.

The Flemish *R. canina*, *R. corymbifera,* and *R. balsamica* individuals occasionally displayed a variation in presence and frequency of glands or pubescence on leaflets, hips, or pedicels compared to the descriptions in literature. This variation in glands

and pubescence was already mentioned by Henker (2000) and partly by Nilsson (1967). Within the taxa *R. canina* and *R. corymbifera*, a correlation with the serration of the leaflet margins was observed. Based on these differences, Henker (2000) divided the species into varieties. In Flanders, these varieties were not equally distributed over the different regions in Flanders. In some populations a high frequency of varieties was observed, while they were completely absent in other populations. Remarkable was the higher frequency of *R. canina* var. *andegavensis* (with glandular pedicels and hips) and *R. corymbifera* var. *deseglisei* both in West-Vlaams Heuvelland and at the coastal area. We can only hypothesise why these localities have a higher frequency of varieties. As these varieties only differ in the presence of glands and serration on the leaflets, it can be assumed that these formerly pure subsection *Caninae* taxa were influenced by subsection *Rubigineae* taxa through (ancient) hybridisations events. The variation in glands is, in addition to the variation in pubescence, accepted as an indicator of past hybridisation and introgression (Graham and Primavesi 1993). We were not able to detect any correlation between the presence of glands (~ variety) and the genetic structure of these individuals within the subsections *Caninae* and *Tomentellae*.

So far, little to no arguments were found to support the subdivision of the subsections *Caninae* and *Tomentellae*, or more specifically the distinction of *R. balsamica* from the subsection *Caninae* taxa. The morphological variation expressed in *R. balsamica* falls into the variation present among the two subsection *Caninae* taxa. In addition, the genetic similarity among the individuals of the subsections *Caninae* and *Tomentellae* in the mixed population at Het Zwin (Oostkust) appeared to be higher than among congeners sampled at other localities. In this case, the factor locality is more important than similar morphological characters on subsection or taxon level. In the past, independent investigations have also tackled the taxonomical position of *R. balsamica* and/or *R. abietina*. The epicuticular wax type characterising the majority of the section *Caninae* taxa is also observed in the taxa *R. balsamica* and *R. abietina* (Wissemann 2000b). Secondly, the analyses of cpDNA sequences showed the clustering of *R. abietina* within the subsection *Caninae* clades. Unfortunately, *R. balsamica* was not included in this analysis (Wissemann and Ritz 2005).

All together, we suggest both *R. balsamica* and *R. abietina* being included in the subsection *Caninae*, as the morphological and genetic similarity of the taxa of both subsections appeared to be very high and clear-cut, and consistent interspecific boundaries were absent. Moreover, the locality of origin appeared to be more important regarding the genetic similarity than the common presence of morphological characters.

Geographical differentiation

The European taxa *R. canina*, *R. corymbifera,* and *R. balsamica* displayed no consistent wide-scale geographical differentiation. Within Flanders, the comparison of the genetic similarity of mixed versus pure populations suggested that the genetic similarity of an individual is largely influenced by the surrounding of other section

Caninae taxa. This means that the individuals sampled at one locality, even displaying morphological dissimilarities, had a higher genetic similarity with the other individuals of this locality than with congeners sampled at other localities. All the individuals sampled at Het Zwin (Oostkust) were, irrespective of their morphological taxon determination (*R. canina*, *R. corymbifera* or *R. balsamica*), more similar to each other than to their congeners sampled at other localities, e.g. the pure *R. canina* population sampled at Deinze (Vlaamse Zandstreek). This rather unique and unexpected observation could indicate that the evolutionary differentiation among these subsections and taxa is a relative young phenomenon and is still in progress. A plausible explanation can be the occurrence of historical hybridisation processes after which the more species-specific phenotypes could recover through several generations of backcrossing. But genetic structures are still the testimony of the historical hybridisation resulting in the observed similarity on locality instead of on taxon basis.

Conservation

In comparison with the subsections *Rubigineae* and *Vestitae*, the taxa *R. canina* and *R. corymbifera* are more common species, and therefore less threatened at the Flemish scale. However, they might be threatened locally. Emphasising on the intraspecific variation, the guidelines are to conserve both pure species and species complexes at mixed populations. For these taxa, no arguments have been found to maintain the subdivision in regions of provenance. The conservation guidelines should be focussed on the character of the locality or population and should be evaluated for each population separately. For instance, the unique genetic diversity of the mixed population at Het Zwin (Oostkust) is the most striking example that favours the isolated conservation of this population. In addition to the conservation of this present-day genetic variation, it is important to allow the different evolutionary processes to act on the mixed populations, as evolution and formation of new and rather unusual hybrids form the basis of the subgenus *Rosa* and section *Caninae* in general.

Hybridisation among* R. arvensis *and section* Caninae *taxa

Interspecific or even intersectional hybridisation between parental taxa with different ploidy levels that are able to produce fertile descendants is a unexpected phenomenon. Both Henker (2000) and Graham and Primavesi (1993) described the occurrence of interspecific hybridisation among polyploid taxa of the section *Caninae* and diploid section *Synstylae* species. The heterogamous canina meiosis (producing haploid pollen grains) allows interspecific hybridisation with diploid species resulting in F1 progeny that differs in morphology, genetic constitution (ploidy), and fertility. Although little is known about the viability and fertility of interspecific descendants, it has been shown that the viability of interspecific pollen is clearly lower compared to those of the pure parental taxa (Wissemann and Hellwig 1997, Werlemark 2000a). When the pollen grains are viable, the fertility of the F1

generation with a section *Caninae* seed parent will be influenced by the homology of the two (bivalent-forming) chromosome sets. The homology has to be sufficiently high to allow recombination and canina meiosis in these F1 hybrids. NrITS sequence analyses have indicated that the section *Synstylae* forms a direct sister group to the section *Caninae* (Wissemann and Ritz 2005). One might thus assume that the similarity of the bivalent-forming chromosomes of the section *Caninae* is sufficiently homologous to the chromosomes of *R. arvensis*. Moreover, it can be assumed that the degree of homology among the bivalent-forming chromosomes is variable, and will determine whether the F1 hybrids will be fertile or sterile.

If *R. arvensis* acts as the pollen donor, the descendants will show large morphological similarities with the *Caninae* seed parent, as 4/5th of the genome was contributed by the *Caninae* mother parent. The hybrids will only show a slight influence of the *R. arvensis* pollen parent. Theoretically, when *R. arvensis* is the seed parent, the descendents will be diploid, receiving one chromosome set of *R. arvensis*, and one of bivalent-chromosome sets of the *Caninae* pollen parent. All the analysed section *Caninae* individuals, including *R. stylosa,* and the hybrid *R.* x *irregularis* were pentaploid, whereas the *R. arvensis* individuals were diploid, as stated in literature (Henker 2000, Darlington and Wylie 1961). The presence of the pentaploid chromosomal constitution of *R. stylosa* and the derived putative hybrids *R. stylosa* x *R. canina* confirmed the maternal influence of the section *Caninae*. At this moment, we have found no proof that the reciprocal crossings are able to deliver viable seeds or progeny.

The morphology of both *R. stylosa* and *R.* x *irregularis* indicated the influence of *R. arvensis* (section *Synstylae*) and of possibly three section *Caninae* taxa: *R. canina*, *R. corymbifera,* and/or *R. balsamica*. The elliptic-lanceolate leaflets with uniserrated eglandular margins of *R. stylosa* showed high similarity with *R. canina*, *R. corymbifera,* and *R. balsamica*. However, the pubescence on the lower sides and rachides would rather indicate the influence of *R. corymbifera* or *R. balsamica,* as *R. canina* has glabrous and rarely glandular leaflets, hips, and pedicels. In addition, the rachides were seldom glandular in *R. corymbifera*, but more frequently glandular in *R. balsamica*. These three *Caninae* taxa had mainly smooth hips and pedicels, while the pedicels of *R. stylosa* were longer and more comparable to those of *R. arvensis*. In addition, the styles of *R. arvensis* were extremely long exserting the disc, and the pedicels were densely glandular with stipitate of subsessile glands as was observed in some *R. stylosa*. The orifice diameter of *R. stylosa* was situated within the range of *R. arvensis* and the *Caninae* taxa. In addition, *R. stylosa* had some newly formed and typical characters: the lower leaflets were back-folded, the prickles were delta-shaped, and the disc was strongly conical shaped. Henker accepted *R. stylosa* to be a fixed crossing that once found its origin as a fertile hybrid that was able to cross with one of the parental taxa or with other *R. stylosa* individuals. This is in contrast with *R.* x *irregularis*, a very rare and sterile taxon. The habit, inflorescence, and colour of flowers are described to be similar to *R. arvensis*, however the shoots are strong and erect, and the hooked prickles are similar to those of the *Caninae*-*Tomentellae* taxa. Occasionally, hips are produced; nevertheless, they are infertile or mal-formed. Remarkably, in our AFLP-based species assignment *R. stylosa* and the hybrid *R.*

stylosa x *R. canina* displayed a higher similarity with the subsection *Rubigineae* compared to the subsections *Caninae*-*Tomentellae*. On the other hand, in the analysed *R. stylosa* individuals, species-related STMS alleles of each of the three *Caninae* taxa and of *R. arvensis* were observed, confirming the parental influence of these four taxa. Unfortunately, as these genetic markers were not able to discriminate between the three *Caninae* taxa, it was not possible to elect or eliminate one of them to be the most possible seed parent.

These apparently contradictory results reflect the incongruence in literature concerning the taxonomical position of *R. stylosa* in the whole section *Caninae*. Henker (2000) and Wissemann (2003) placed *R. stylosa* within the subsection *Caninae*, whereas Graham and Primavesi (1993) created a separate subsection *Stylosae*. In addition, describing the epicuticular wax structures, Wissemann (2000b) suggested that the subsection *Rubigineae* had influenced *R. stylosa* as a seed parent through an ancient hybridisation event. Both taxa carried the granule type and the matroclinal inheritance pattern was observed. However, Wissemann (2000b) did not include any *R. balsamica* individuals in the analyses. Moreover, he did not take into account the morphological and wax type similarity of *R. stylosa* with *R. corymbifera*. In contrast, cpDNA analyses of the subgenus *Rosa* showed a high similarity of *R. stylosa* with taxa of the subsection *Caninae*-*Tomentellae* (Wissemann and Ritz 2005). Finally, the phylogenetic analyses based on AFLP polymorphisms stressed the controversial relationship of *R. stylosa* with the subsection *Rubigineae* even more (Koopman *et al.* 2008). Looking at the whole subgenus *Rosa* including both wild and cultivated accessions, the similarity with the subsection *Caninae* was high. However, when the analysis was restricted to the wild taxa, *R. stylosa* appeared to be associated with the subsection *Rubigineae* (Koopman *et al.* 2008).

The difference in fertility among *R. stylosa* and *R.* x *irregularis* has been the reason why *R. stylosa* is now accepted as a species, instead of a hybrid. In addition, the genetically analysed *R.* x *irregularis* individuals were completely mingled with the *Caninae*-*Tomentellae* taxa, whereas the *R. stylosa* individuals displayed a high similarity with the *Caninae*-*Tomentellae* taxa was positioned in-between the *Caninae*-*Tomentellae* and *R. arvensis* clusters.

In conclusion, the historical hybrid origin of *R. stylosa* and *R.* x *irregularis* with *R. arvensis* as the pollen parent was supported by the STMS polymorphisms. However, the indications towards a possible mother taxon for *R. stylosa* were not straightforward. Based on the morphology of the leaflets (shape, serration, pubescence, and glands), on the presence of species-related or -specific STMS alleles, and on the sequence of cpDNA, we might conclude that *R. corymbifera* or *R. balsamica* could be the most likely maternal taxon. If this hypothesis is true, the glands on pedicels and rachides would be inherited through the pollen parent. Alternatively, the influence of the subsection *Rubigineae* through ancient hybridisation was suggested by the phylogenetic analyses, and could explain the glandular pedicels and rachides. However, descendents of the *Rubigineae* are expected to have densely glandular leaflets spreading a typical apple-scented fragrance that was not observed

in *R. stylosa*. One of the possible explanations is that the odour-spreading and densely glandular leaflets of the *Rubigineae* only developed after the interspecific crossing of a pre-*Rubigineae* with *R. arvensis* to form *R. stylosa*. The observed epicuticular wax types (Wissemann 2000b) did not favour or undermine either of these hypotheses.

Conservation

The most numerous *R. stylosa* population in Flanders is situated in Ter Yde (Westkust). Apart from this valuable taxon, a larger population of *R. spinosissima*, and some hybrids of *R. canina* and *R. stylosa* are present. Although more than ten well-developped *R. stylosa* shrubs were analysed, they appeared to belong to the same genotype. The value of this locality is already acknowledged, as it is a protected area by the Flemish decree of dunes.

5.2.5. The influence of locality on the genetic constitution of a section *Caninae* population

We have found several indications that confirm the occurrence of (ancient) interspecific hybridisation events. They also stress the far-reaching influence of the presence of multiple section *Caninae* taxa on the morphological and/or genetic variation of the taxa in particular, and the populations in general.

The influence of hybridisation events on the morphology of the individuals in mixed populations was confirmed by comparing them with individuals of presumed "species-pure" populations or the descriptions in literature. *R. rubiginosa* individuals sampled at the mixed Maasvallei population were characterised by more narrow diameters of orifice and longer leaflets, compared to the literature and to congeners sampled in populations that lack the (historical) presence of *R. micrantha*. Similarly, the *R. micrantha* individuals sampled at the same mixed population tended towards smaller leaflets, larger hips, and larger diameters of disc and orifice compared to the literature. Unfortunately, no representative pure (and large) *R. micrantha* populations are present in Flanders. Nevertheless, this outcome indicates the fading of the species-specific characters (e.g. small versus large leaflets, or diameters of orifice) in mixed populations. In contrast, no genetic differentiation was observed between the *R. rubiginosa* and *R. micrantha* individuals, disregarding the mixed or presumed pure state of the populations.

The influence of mixed populations is not restricted to the fading of morphological species-specific characters of the original taxa. At the locality Het Zwin (Oostkust), *R. canina*, *R. corymbifera,* and *R. balsamica* are all present in large frequencies. Although the morphological differences among these taxa were subtle, they were consistent and allowed the identification of the individuals in this mixed population. Most striking was the observation of the high genetic similarity among these morphological different individuals. Moreover, comparing the genetic

similarity of these three taxa sampled at Het Zwin (Oostkust) with the congeners sampled at other localities, the genetic similarity was the highest among the different taxa of the mixed locality, disregarding their morphological subtle but consistent differences.

Similarly, the similarity within the mixed *R. rubiginosa*, *R. micrantha*, *R. henkeri-schulzei,* and *R. canina* population sampled at the slope of St-Pietersberg (Maasvallei) was studied. In contrast to the more subtle morphological differences observed at the populations of Het Zwin, the morphological differences between *R. rubiginosa* and *R. canina* were well observable and consistent. Yet, the genetic similarity between *R. rubiginosa* and *R. canina* both sampled at this slope was higher compared to the similarity between these *R. canina* individuals and the congeners sampled at other localities, e.g. Heers. The more striking morphological differences were not reflected in the genetic constitution, as the genetic similarity was higher among different taxa of the same locality compared to congeners of different localities.

We assume that a representative sample of the populations was analysed, as Het Zwin (Oostkust) populations were sampled randomly and all individual shrubs were collected at the slope of St-Pietersberg (Maasvallei). Based on these outcomes, we suggest that in the absence of other section *Caninae* taxa, the genetic identity of the taxon will be more pronounced in comparison to situations where several taxa are present in a sufficiently high frequency. In these mixed populations, the taxa appeared to be influenced by each other through probably rare hybridisations that result in genetic similarity and the fading of morphological species-specific characters.

The fact that the input of other species was not always clearly detected in the morphological or genetic study might be a consequence of the heterogamous canina meiosis. This means that the mother donates $4/5^{th}$ of the chromosomes and the pollen donor only $1/5^{th}$ to the descendents. Additionally, the univalent-forming chromosomes are presumed to determine the species-specific characters and the morphological influences of historical hybridisation will fade more quickly through backcrossing with a parental species than the genetic constitution. This is due to the faster mutation rates of noncoding DNA compared to coding DNA.

5.2.6. Differentiation of *Rosa* species at different geographical scales

At the European scale, certain taxa displayed intraspecific geographical differentiation by comparing AFLP markers. The in-depth molecular-genetic and morphological investigation at the small geographical scale (Belgium, or even Flanders) suggested the presence of genetic and/or morphological intraspecific differentiation.

Population differentiation at European scale

In Western Europe, *R. spinosissima* is mainly distributed along the coasts, although inland populations, such as in the Alpine area in France or on calcareous open areas in Belgium, are known. AFLP polymorphisms indicated intraspecific population differentiation. At the European level, the Danish populations, which are situated on the Northern boundary of the distribution area, appeared to be the most distinct compared to their Belgian, Dutch, German, and French congeners.

The absence of *R. spinosissima* in some well-suited (e.g. area of the Delta in the Netherlands), or rather young areas [e.g. in areas younger than 75 years such as the Westhoek (De Panne, Westkust, Belgium)] (pers. com. M. Leten) indicates that, even if the species is locally abundant, the generative dispersal is not as common as one might expect based on the fruit dispersal strategy. The fleshy and nutrient-rich hips display all characters to be digested and dispersed by birds (ornithochory). In addition, a higher genetic variation was observed in the centre of one widespread patch compared to the edges. This might indicate that the distribution of the species is restricted by the demanding and specific habitat requirements instead of the production and dispersal of fertile seeds. Thus, we might state that vegetative reproduction is important in a patch, but *R. spinosissima* has found a way to maintain the genetic diversity within a dense thicket.

The distribution area of *R. gallica* is mainly situated in the Southern and Eastern part of Europe, with in addition a highly fragmented area in Central Europe. The Alps form a natural boundary preventing the gene flow between the Central and Southern populations. The occurrence of intraspecific genetic differentiation was observed as the German population clearly differed from the French populations.

In addition, the two French populations originating from Alpes Maritimes displayed a higher interpopulational similarity compared to the other French populations. These two populations were located only ten km apart, whereas the other populations were situated more distantly. As the interest in cultivating *R. gallica* was and is still very high, the human-influenced distribution might have influenced the genetic patterns.

The distribution area of both *R. majalis* and *R. pendulina* appears to be discontinuous and barriers for gene flow are observed among the analysed populations (Kurtto *et al.* 2004). The French *R. pendulina* populations showed genetic differentiation with the German congeners. Although *R. pendulina* did not show a large discontinuum in its distribution area, the Alps might hamper gene flow which can result in the differentiation of the French and German populations.

Within *R. majalis*, the Swedish populations differed from the German ones. This genetic differentiation might be caused by the large geographical distance among the analysed populations, by the hampering of gene flow by both the North Sea and the Baltic Sea, and apparently the isolation of the German populations.

Small-scale population differentiation (Belgium)

R. spinosissima

In addition to the differentiation assessed on the European scale, the Belgian inland population, Viroin, displayed genetic differentiation compared to the Belgian coastal populations. This observed variation stresses the marginal character and high value of the inland population. Although *R. spinosissima* was once frequently observed in the calcareous grasslands of the Viroin, today only a few branches were found, probably suffering from bottleneck effects and isolation. The remaining individuals can assumed to be relicts. This idea is stressed by both an increased genetic differentiation compared to the coastal populations and the presence of a rare allele (257 bp) and a unique allelic phenotype (presence of both 257 and 258 bp) for the allele RhO517. In addition, none of the analysed samples displayed the presence of glands, enlarging the value of this population even more as no signs of introgression were observed (Maes 2006). Finally, these impoverished populations are under severe threat as the past and/or current mowing management emphasises the maintenance and conservation of the herbaceous flora of the calcareous grasslands (especially the orchids). This causes a negative impact on the valuable relict and inland populations of woody shrubs such as *R. spinosissima*. An alternative management is suggested to enhance the growth and survival chances of the few young and vulnerable sprouts that are still present on the open and sunny spaces in the forests. Little to no harm will be done to the orchids if few well chosen spots will be skipped during mowing. Thus, the shoots of *R. spinosissima* will get the opportunity to grow, develop, and reproduce, both through seed setting and rootstocks. The endeavour to combine the management in favouring herbaceous and woody shrubs will enhance the ecological value of the area in general. The woody shrubs will serve as an additional harbour for fauna in open grassland; whereas the fleshy hips serve as an additional food source for birds and even for smaller animals (Bouman *et al.* 2000).

Along the Belgian coast, *R. spinosissima* populations were sampled at different localities. In each population the species has a typical habit. The individuals of the Oostvoornduinen (Westkust) population are all part of one large and dense thicket, whereas the populations of the Middenkust consisted of few small and single branches, or occasionally a well-developed shrub. The population of Ter Yde (Westkust) displayed an intermediate habit, with both small continuous thickets and isolated sprouts.

The difference in population characters (size, habit, etc.) should be taken into account, when evaluating the genetic differentiation. In contrast to the rare presence at Middenkust, the two populations of the Westkust were larger and therefore sampled more intensively. This different sampling strategy might explain the larger genetic variation that was observed in the Westkust populations. Alternatively, a different genesis of these coastal regions may also have influenced the genetic constitution of the populations. In the late Middle Ages, the Westkust was assumed to be one big unit of formerly old cores of dunes. Presumably, the newly formed

dunes were colonised with relict populations and species of the old dunes. Most of these species were restricted to this region, or had their main core here (*Rosa spinossissima*, *Potentilla neumanniana*, *Primula veris*, *Helianthemum nummularium*, *Thesium humifusum*, *Asperula cynanchica*, *Viola hirta*, etc.). In contrast, the environment of the Middenkust was too dynamic, too small, and too recent for the fauna and flora of the calcareous grasslands, including *R. spinosissima*. Although it is hard to argue whether a species was present or absent at a certain region, it was only observed to be present at Middenkust in the last decades. So, assuming that these "individuals" are relicts of old populations, these were probably very small and well-isolated. Alternatively, assuming that the localities were newly colonised, they would be very likely to be allochthonous populations. The latter hypothesis was supported by the atypical morphology of the Middelkerke population compared to the description of wild individuals in literature. The fewer prickles on the branches and densely glandular pedicels both could indicate the influence of cultivated *R. spinosissima* (Maes 2006, Graham and Primavesi 1993). However, this presumed introgressed, or cultivated population did not display a deviating genetic constitution compared to the wild populations which indicates the importance of morphological studies in addition to genetic diversity analyses. Moreover, the occurrence of cultivated genes in presumed natural populations stresses the vulnerability of small and wild populations and the threat of introgression of cultivated genes (Maes 2006). This is especially true for the *R. spinosissima* populations in the coastal regions as lots of cultivated *R. spinosissima* are available on the market. These presumed introgressed populations might be worth monitoring to study the evolution and the distribution of the introgressed individuals. As the colonisation of new areas seems rather hard for *R. spinosissima*, conservation guidelines should emphasise on the maintenance of the valuable present-day populations, both the highly endangered and the well established coastal populations.

R. arvensis

In Belgium, *R. arvensis* populations were intensively sampled in two geographic regions: West-Vlaams Heuvelland and Vlaamse Ardennen. Both regions belong to Brabants District West, one of the Flemish regions of provenance. Although both populations are only situated less than 70 km apart, they displayed both intraspecific morphological and genetic differentiation.

The populations of the Vlaamse Ardennen had significantly narrow diameters of the orifice and tended towards more narrow diameters of the disc and longer pedicels compared to their congeners at West-Vlaams Heuvelland. Moreover, the leaflet margins and rachides were always sparsely glandular at the Vlaamse Ardennen. The leaflet margins varied from eglandular to sparsely glandular and the rachides varied from eglandular to moderate glandular for the individuals from West-Vlaams Heuvelland. In contrast, the hips were eglandular to moderately glandular at Vlaamse Ardennen, while being eglandular to sparsely glandular at West-Vlaams Heuvelland. Based on the AFLP and STMS polymorphisms, genetic differentiation was observed among the two regions.

The striking morphological and genetic differentiation among the populations of *R. arvensis* could be caused by effects influencing marginal populations. Both populations are situated along the Northern boundary of the distribution area of *R. arvensis*. In general, marginal populations can show unique alleles as they adapt to the threats and higher pressures of the environment. The combination of both genetic and morphological intraspecific variation might suggest local adaptation. However, until now we have no idea about ecological significance of differences in diameters of orifice or disc, or on longer pedicels.

In a small-scale provenance test, about 20 different genotypes from both regions were sampled, and planted at the same locality. After one growing season for the origin Vlaamse Ardennen and two for West-Vlaams Heuvelland, the morphological characters were reanalysed. The preliminary results indicated that the grown up cuttings of the wild plants did not display any difference in presence or frequency of the glands on leaflet margins, rachides and hips. In addition, the observed tendencies in larger diameters of the orifice and disc, and longer pedicels of the West-Vlaams Heuvelland origin were still present but less pronounced compared to the original locality (pers. com. K. Vander Mijnsbrugge). If these differentiations were caused by phenotypic plasticity, thus being influenced by the environment, they would be or absent in the provenance trail, or present in both origins in the provenance trail. We might reject that this variation was caused by phenotypic plasticity. As we don't know whether this relatively small-scale population differentiation is a result of local adaptation or of local genetic drift (caused by e.g. habitat fragmentation), the precautionary principle urges the split of Brabants District West, the region of provenance where both regions belong to, specifically for this species. In this way, the populations will not be mingled. Previously, the occurrence of small-scale differentiation was also observed even at scales of 500 m or less (Waser and Price 1985). For instance, within populations of both *Hydrocotyle bonariensis* and *Ranunculus reptans* a different flood frequency required local adaptation (Knight and Miller 2004, Lenssen *et al.* 2004).

5.2.7. Intrapopulational clonality of *R. spinosissima* and *R. arvensis*

R. spinosissima is known to cover the dunes, forming dense thickets through rootstocks. Within such a thicket, the identification of different genotypes on the site is hardly possible. Therefore, the genetic diversity and clonality within a dense thicket was studied. However, the tetraploid chromosomal constitution of *R. spinosissima* did not allow the assessment of the STMS allele frequency, and therefore the clonality could not be validated. By combining identical allelic phenotypes and the sampling localities of the branches, we were able to assume the clonality of the samples. On the other hand, the observation of different allelic phenotypes in a dense and well-spread *R. spinosissima* thicket, confirmed the presence of genotypic variation within one continuous population. We conclude that vegetative reproduction by rootstocks occurs within a densely grown thicket. However, the

clonality of *R. spinosissima* should not be overestimated as different allelic phenotypes were observed in one thicket.

The hanging and weak branches of *R. arvensis* that form a large sprawl are able to root when contacting soil. As most *R. arvensis* shrubs are situated along edges of forests or on open and mostly well-shaded places in the woods, the hip production is rather restricted. The rooting of the branches forms an important alternative reproduction strategy. The sampling of all the well-developed *R. arvensis* shrubs along the edge of the forest at Hayeweg (Brakel, Vlaamse Ardennen) confirmed the presence of vegetative propagation in the field as these ten shrubs could be assigned to only four different genotypes (STMS polymorphisms). Nevertheless, the lack of genetic differentiation in one population should not be overestimated as different genotypes were observed in all sampled populations.

5.3. General conclusions

The taxonomical subdivision of the subgenus *Rosa* into the sections *Pimpinellifoliae, Rosa, Cinnamomeae*, *Synstylae,* and *Caninae* was confirmed by our AFLP-based analyses. In addition, the very dense and well-defined genetic unit supported the unique position of the polymorphic section *Caninae* within this subgenus. Although interspecific hybridisation within the section *Caninae* is known to occur, the combination of the morphological and molecular-genetic approaches allowed the observation of a hierarchical subdivision. Three major, and partly overlapping, groups could be identified within the section *Caninae*: the *Rubigineae*, the *Vestitae*, and the *Caninae-Tomentellae.* The lack of clear and well-defined boundaries will be the result of interspecific hybridisation among these groups. However, the impact and frequency of the interspecific hybridisation in the field is disguised by the predominant maternal inheritance of the morphological characters. This stresses the importance of combining different approaches to be able to reconstruct the phylogeny of the taxa and to delimit the species boundaries. The subdivision of the subsections *Caninae* and *Tomentellae*, as suggested by Henker (2000) and Wissemann (2003) was not reflected in our analyses. We did observe subtle but consistent morphological and little to no molecular-genetic differentiation among *R. canina*, *R. corymbifera* (subsection *Caninae*), and *R. balsamica* (subsection *Tomentellae*).

Within each group, some parallel morphological characters, such as diameter of the orifice and persistence of the sepals, were observed and described to be diagnostic. This distinction was not reflected in the genetic (AFLP-based) structure. In addition, the paternal inheritance of these characters suggests an interspecific exchangeability of the responsible genes, therefore they are located on the bivalent-forming chromosome sets.

In species-pure populations, the identification of species was possible. This is in contrast to the mixed populations where the individuals displayed a morphological and genetic intermediate position in-between the parental species. In addition, certain morphological well-defined parental taxa were hardly distinguishable based on their genetic structure. This outcome suggests that the

identification of species-complexes would be more appropriate in mixed populations.

The population and life history traits should be evaluated for every species or taxon separately. This is especially true for the section *Caninae* taxa, on which the impact of the polyploid and heterogamous chromosomal constitution has to be considered and evaluated while

- describing the morphology of wild rose shrubs as predominant maternal inheritance disguises the spontaneous hybrids
- interpreting the molecular-genetic polymorphisms as the Hardy-Weinberg assumptions required for the generally applied population genetic analyses are not met
- attempting to subdivide the section *Caninae* into different subsections and taxa as the recombining bivalent-forming chromosome sets are exchangeable among taxa and subsections, and a huge, continuous, and consistent variation in pubescence and glands on leaflets, pedicels and hips is observed within this section
- interpreting the observed intraspecific differentiation and defining conservation measurements as the chromosomal constitution is assumed to prevent or buffer introgression of non-local genes
- delineating the conservation units, the character of the population would be relevant compared to the delineation of the regions of provenance

The analyses of the morphological and molecular-genetic (AFLP and STMS) characters confirmed the hybridogenic origin of *R. stylosa*. In the historical hybridisation process, *R. arvensis* was suggested to have acted as the pollen donor and the seed parent should be a *Caninae-Tomentellae* taxon. Unfortunately, we were not able to elect or eliminate one of the three possible taxa *R. canina*, *R. corymbifera,* and *R. balsamica* as the most possible seed parent. In addition, we were not able to clarify the historical relationship between *R. stylosa* and the subsection *Rubigineae*.

The occurrence of (ancient) interspecific hybridisation events and the far-reaching influence of the presence of multiple section *Caninae* taxa on the morphological and/or genetic variation of the populations were confirmed. Therefore, the character of the population (species-pure versus mixed) should be considered while delimitating the conservational units. For some taxa, the regions of provenance will be a suitable unit, for others several units should be defined within one region of provenance. Remarkable was that the genetic character of the population was not always expressed in the morphology of the individuals. This rather unique and unexpected observation can indicate that the evolutionary differentiation among these subsections and taxa is a relative young phenomenon that is still in progress. A plausible explanation can be the occurrence of historical hybridisation processes after which the more species-specific phenotypes could recover through several generations of backcrossing. But genetic structures are still the testimony of the historical hybridisation resulting in the observed similarity on locality rather than taxon basis. Consequently, the conservation guidelines should be

focussed on the character of the locality and population and should be evaluated for each population separately.

Intraspecific geographical differentiation was observed at the large European scale, but also at the smaller scale, within Belgium and Flanders. Within the species *R. spinosissima*, *R. gallica, R. majalis*, and *R. pendulina* intraspecific genetic differentiation was observed using AFLP polymorphism. In addition, at the small geographical scale within Belgium genetic differentiation was assessed e.g. among the inland and coastal populations of *R. spinosissima*. The inland *R. spinosissima* population is considered a highly vulnerable and relict population. In addition, at an even smaller scale, only 70 km apart, both genetic and morphological intraspecific differentiation was assessed among the *R. arvensis* populations of West-Vlaams Heuvelland and Vlaamse Ardennen.

For both *R. spinosissima* and *R. arvensis* one should not overestimate the presence of clonality within a population.

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Curriculum vitae

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1 jan 2002 – 30 nov 2005
Wetenschappelijk medewerker aan het Centrum voor Landbouwkundig Onderzoek, Departement Plantengenetica en –veredeling (CLO-DvP, Vlaamse Gemeenschap) te Melle. Onderzoek van de morfologische en genetische diversiteit van de autochtone rozen (*Rosa* spp.) in Vlaanderen. Project werd gefinancierd door Afd. Bos & Groen, Ministerie van de Vlaamse Gemeenschap. Standplaats: IBW (Geraardsbergen).

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1 dec 2007 – 13 jan 2008

Onbezoldigd werk in het kader van het behalen van mijn doctoraat: "Diversity of European wild roses (*Rosa* spp.), with an emphasis on Flanders".

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PUBLICATIES

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Deelname met actieve bijdrage

VAN HUYLENBROECK J., DE COCK K., LEUS L. AND DE RIEK J. Genetic relationships of wild and cultivated roses and their potential for breeding (poster). SHE - First Symposium on Horticulture in Europe. Vienna. 17 - 20 February 2008.

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Symposium on Rose Research and Cultivation, Santa Barbara, California. September 18-22, 2005.

DE COCK K. Vlaamse rozen onder de loep. (voordracht) Starters in het bosonderzoek: jonge wetenschappers stellen hun resultaten voor. Brussel. 22 maart 2005.

DE COCK K., VERSCHAEVE L., VANDER MIJNSBRUGGE K., BREYNE P., VAN SLYCKEN J. AND VAN BOCKSTAELE E. Characterisation of autochthonous roses (*Rosa* spp.) in Flanders using morphology and molecular markers. (poster) Ecological Genetics. Antwerp, Belgium. February 2-4, 2005.

DE COCK K., VERSCHAEVE L., BREYNE P., VAN SLYCKEN J. AND VAN BOCKSTAELE E. Morphological and genetic research of autochthonous roses (*Rosa* spp.) in Flanders. (poster) Biodiversity: state, stakes and future. Louvain-la-Neuve, Belgium. April 7-9, 2004.

DE COCK K., VERSCHAEVE L., BREYNE P., VAN SLYCKEN J. AND VAN BOCKSTAELE E. Populatiebiologie van autochtone rozen (*Rosa* spp.) in Vlaanderen. (poster) Studiedag: Toepassingen van DNA-onderzoek in Bos- en Faunabeheer, Brussel, Belgium. September 19, 2003.

DE COCK K., LYBEER B., ZWAENEPOEL A., VANDER MIJNSBRUGGE K., BREYNE P., GOETGHEBEUR P., AND VAN SLYCKEN J. Phenotypic and genotypic variation among *Salix alba – S.* x *rubens – S. fragilis* in Flanders. (poster) Dynamics and conservation of genetic diversity in forest ecosystems. Strasbourg, France. December 2-5, 2002.

VANDENBROECK A., DE COCK K., VANDER MIJNSBRUGGE K., VAN SLYCKEN J. AND BREYNE P. Characterization and conservation of the genetic diversity of some autochthonous woody species in Flanders. (abstract) 3rd International symposium on the Molecular and cellular biology of bananas, Leuven, Belgium. September 9-11, 2002.

DE COCK K., LYBEER B., ZWAENEPOEL A., VANDER MIJNSBRUGGE K., BREYNE P., GOETGHEBEUR P., AND VAN SLYCKEN J. Morphological and molecular diversity of the willow complex *Salix alba – S.* x *rubens – S. fragilis* in Flanders. (poster) IUFRO Symposium: Population and Evolutionary Genetics of Forest trees, Stara Lesna, Slovakije. August 25-29, 2002.

DE COCK K., LYBEER B., ZWAENEPOEL A., VANDER MIJNSBRUGGE K., BREYNE P., GOETGHEBEUR P. AND VAN SLYCKEN J. Morphological and molecular diversity of the willow complex *Salix alba – S.* x *rubens – S. fragilis.* (poster) International Poplar Symposium III, Uppsala, Sweden. August 26-29, 2002.

DE COCK K., HENDRICKX F. AND MAELFAIT J.-P. Population genetic study of the sister species *Pardosa saltans* and *P. lugubris* (*Aranea*, *Lycosidea*). (poster) Sixth Benelux Congress of Zoology, Utrecht, Nederland, 19-20 november 1999.

Passieve deelname aan studiedagen en congressen

Informatienamiddag: 'Autochtone bomen en Struiken.' 10/12/2001, Oudenaarde

Workshop: Vlaams Impulsprogramma natuurontwikkeling. 26/04/2002, Brussel

History and Forest Biodiversity. 13-15/1/2003, Leuven

Studiedag: 'Bruggen tussen wetenschap en samenleving' 17/11/2003, Brussel

Workshop: 'Studie van appeldiversiteit: Kansen voor het behoud en duurzaam gebruik van genetische bronnen.' 23/11/2004, Brussel

LiCor User Meeting: 14/12/2004, Westburg, Leusden Nederland

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Deelname aan internationale en nationale onderzoeksprojecten

GENEROSE (Genetic evaluation of European rose resources for conservation and horticultural use), Europees project onder de sleutelactie 5.1.1 "Sustainable agriculture" in het Vijfde framework "Quality of Life" programma.

Populatiebiologie van autochtone rozen (*Rosa* spp.) en meidoornen (*Crataegus* spp.) in Vlaanderen. Afd. Bos&Groen, Ministerie van de Vlaamse Gemeenschap. 01/01/2002-19/12/2005.

Populatiebiologie van het wilgencomplex *S. alba* – S. x *rubens* - *S. fragilis* in Vlaanderen. Vlaams Impulsprogramma Natuurontwikkeling (VLINA). 01/01/2001-14/12/2001.

Begeleiding van eindewerken

"Inventarisatie van autochtone rozen (*Rosa*, Rosaceae) in Vlaanderen: een morfologisch-taxonomisch onderzoek", scriptie voorgelegd door Marijn Vanloosveldt tot het behalen van het diploma: licentiaat in de biologie. 2003-2004, Universiteit Gent, Promotor: Prof. P. Goetghebeur.

"Taxonomie van het *Crataegus*-complex (*Rosaceae*-Maloideae) in Vlaanderen, een ergelijkend morfologisch en genetisch onderzoek", scriptie voorgelegd door Leander Depypere tot het behalen van het diploma: licentiaat in de biologie. 2003-2004, Universiteit Gent, Promotor: Prof. P. Goetghebeur.

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ArcGIS: Basiscursus voor nieuwe GIS gebruikers, 2002-2003, ESRI Benelux

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Introductory course on Bioinformatics, 2003-2004, Instituut voor Permanente Vorming in de Wetenschappen (ICES)

Access 2002: Eenvoudige databanken maken en gebruiken, 2004-2005 Xylos

Academic English: Writing skills, Universitair Centrum voor Talenonderwijs, 2004-2005, Universiteit Gent

Appendix

Distribution maps of subgenus Rosa *taxa in Europe, and the Netherlands and Flanders*

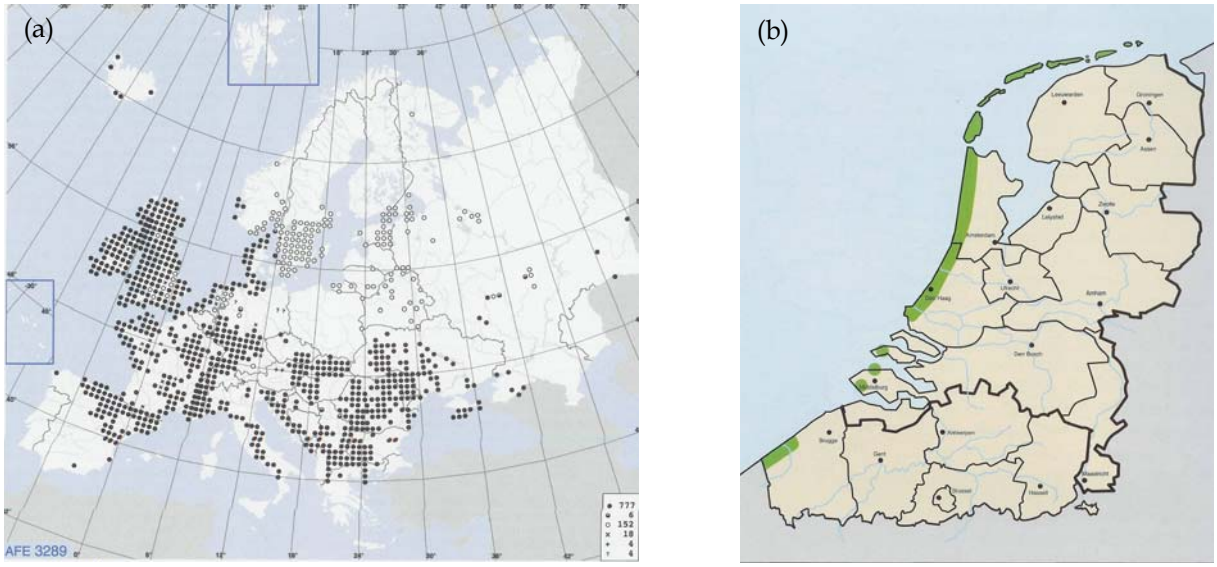

Figure A.1 : Distribution of *R. spinosissma* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

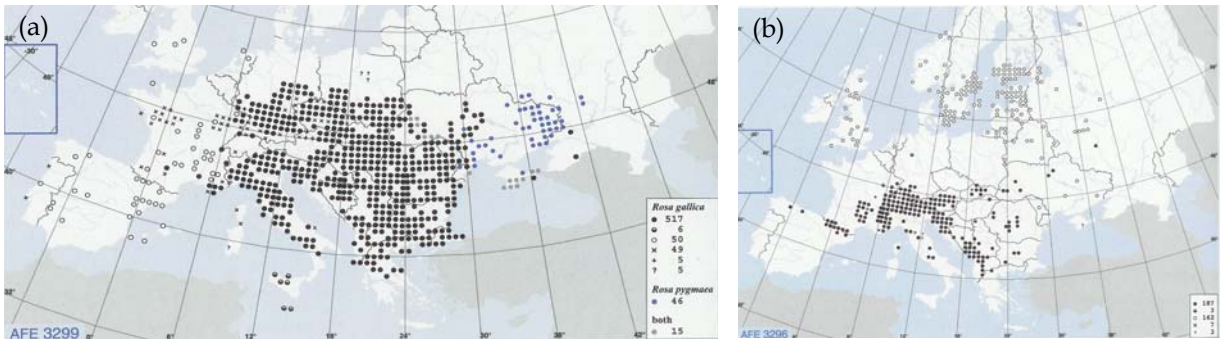

Figure A.2: Distribution of (a) *R. gallica* and *R. pygmaea* in Europe; (b) *R. glauca* in Europe (Kurtto *et al.* 2004).

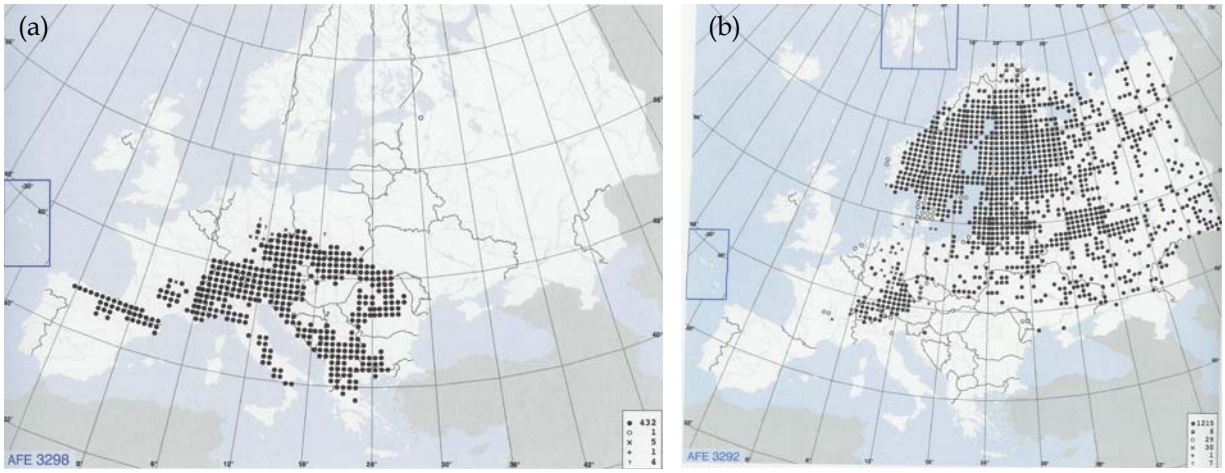

Figure A.3: Distribution of (a) *R. pendulina;* (b) *R. majalis* in Europe (Kurtto *et al.* 2004).

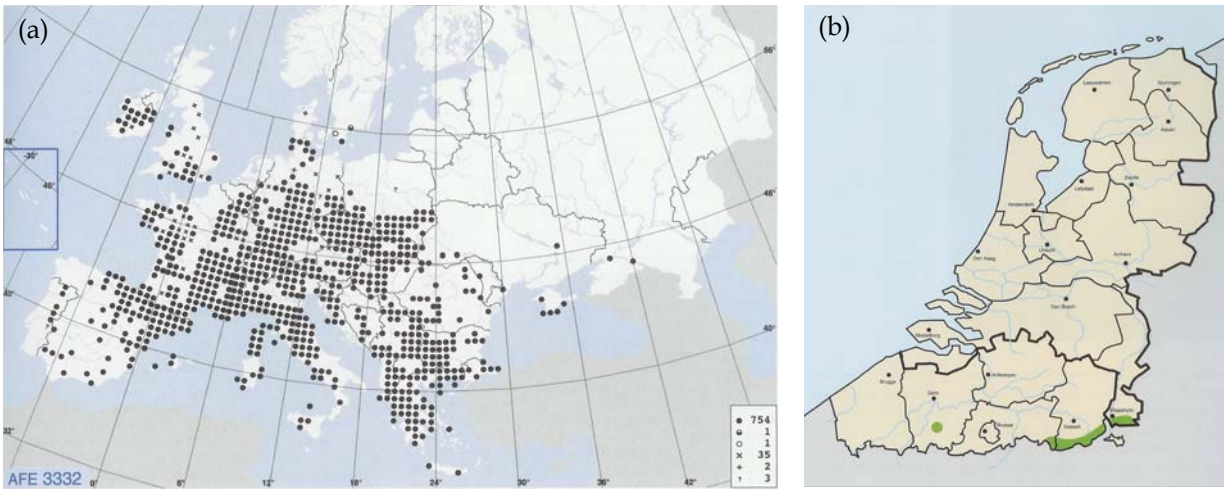

Figure A.4: Distribution of *R. agrestis* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

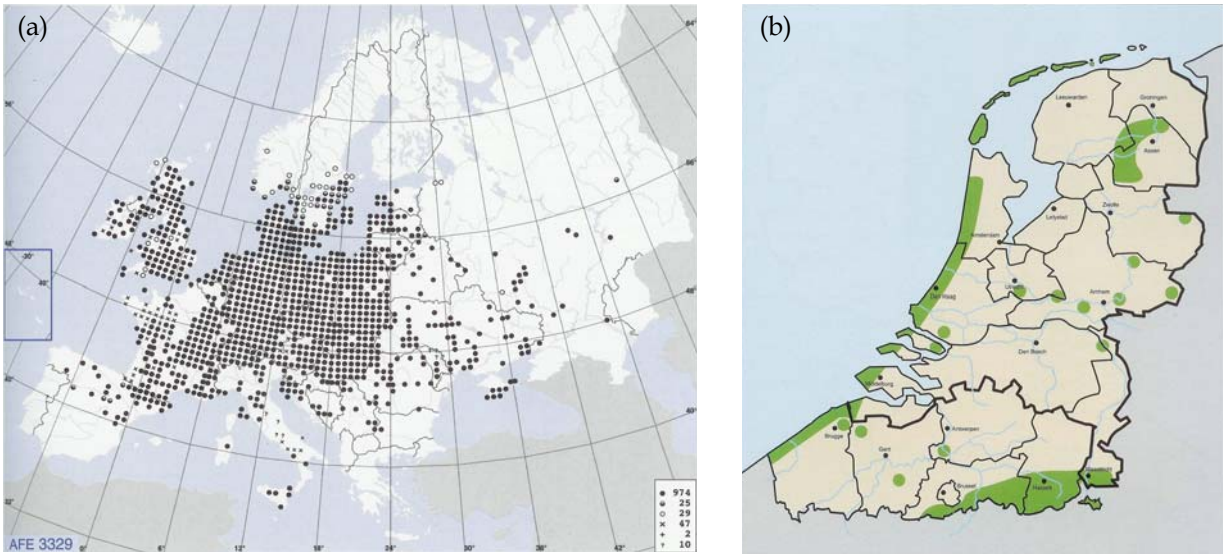

Figure A.5: Distribution of *R. rubiginosa* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

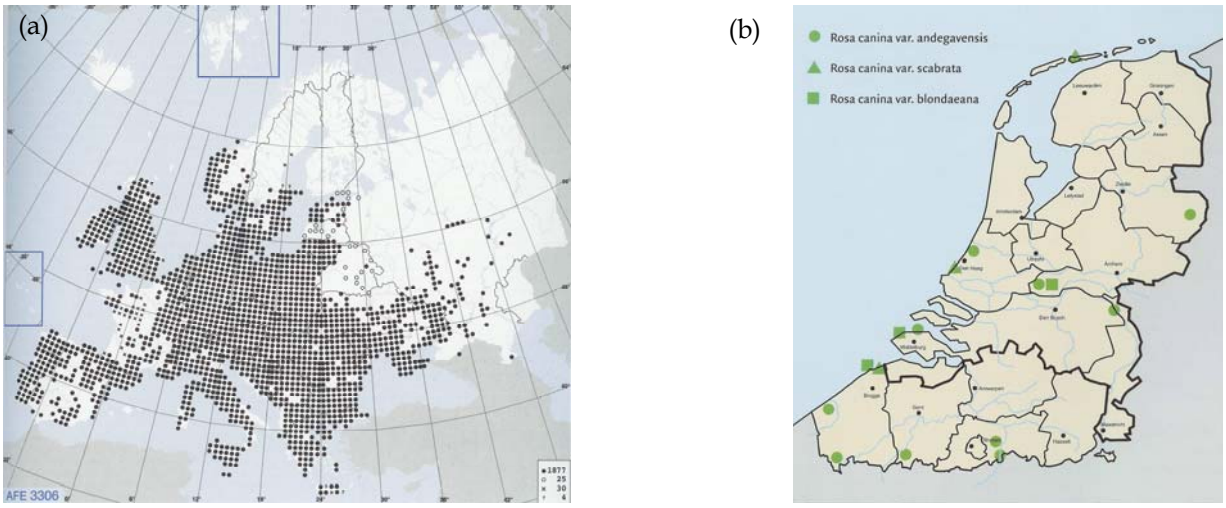

Figure A.6: Distribution of *R. canina* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

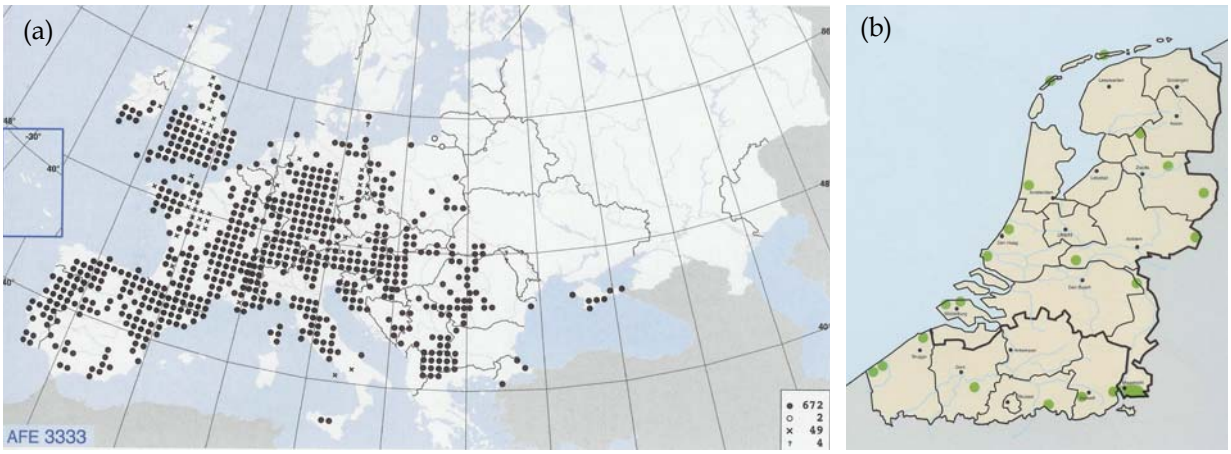

Figure A.7: Distribution of *R. micrantha* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

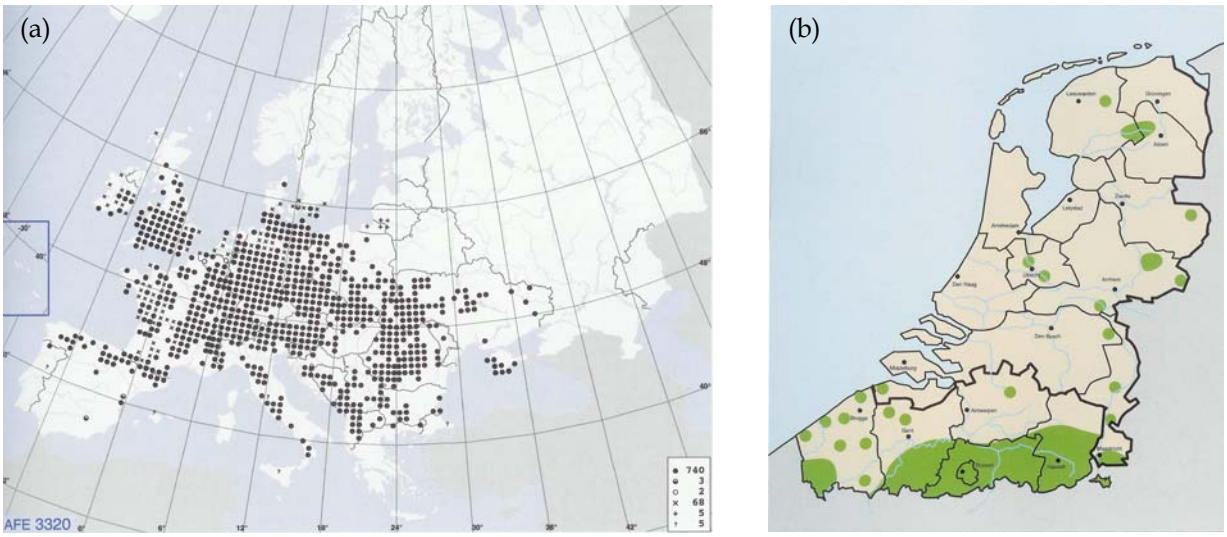

Figure A.8: Distribution of *R. tomentosa* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

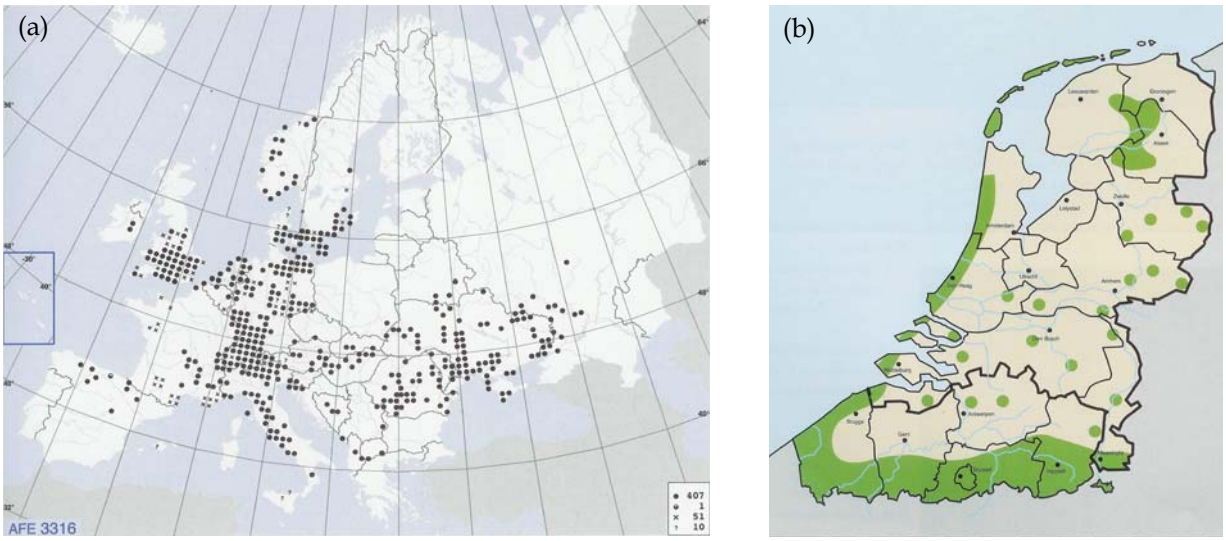

Figure A.9: Distribution of *R. balsamica* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

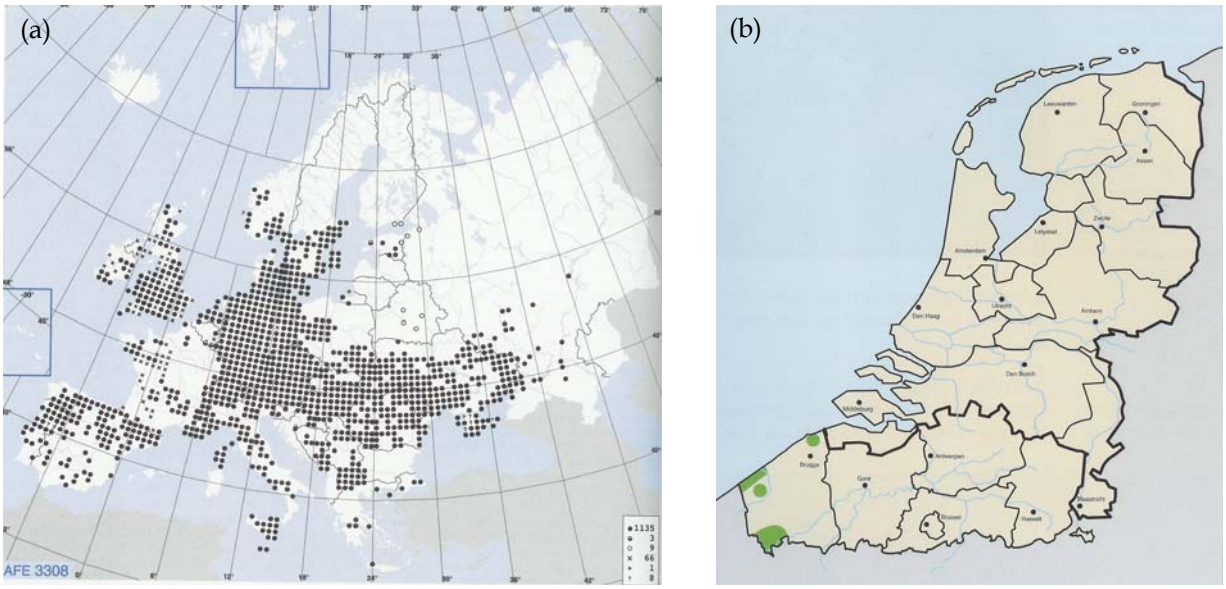

Figure A.10: Distribution of (a) *R. corymbifera s. lato* in (a) Europe (Kurtto *et al.* 2004); (b) *R. corymbifera* in the Netherlands and Flanders (Maes *et al.* 2006).

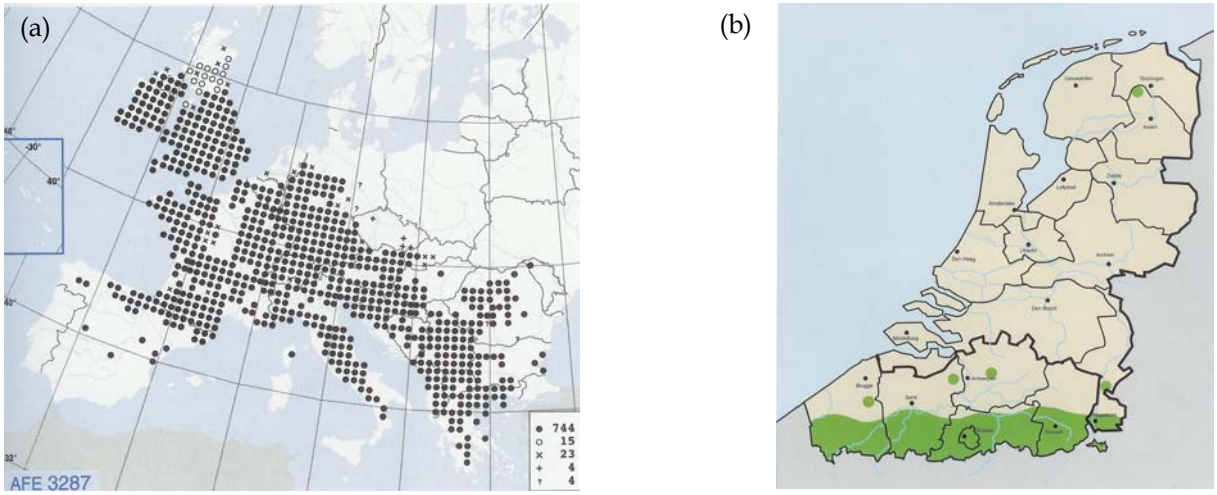

Figure A.11: Distribution of *R. arvensis* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

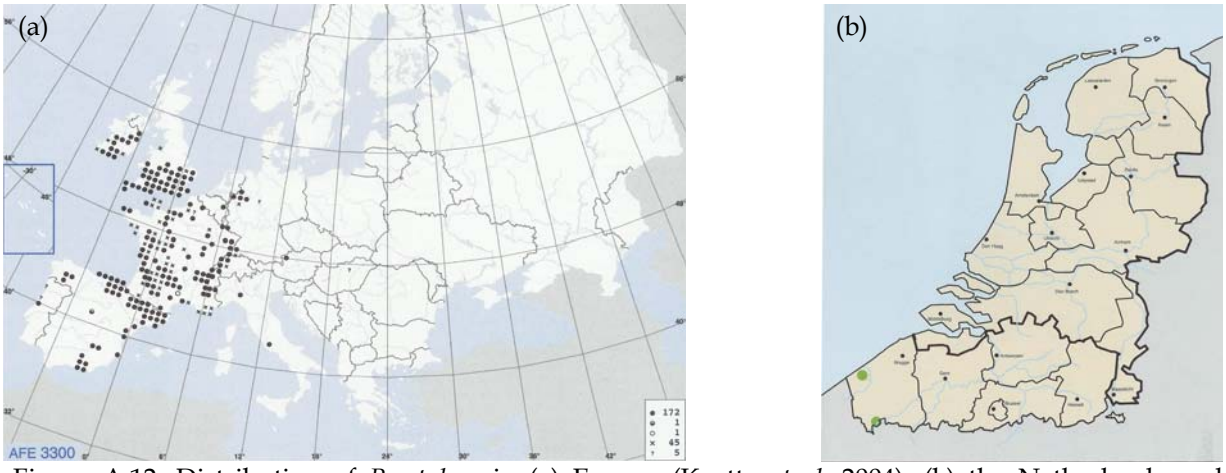

Figure A.12: Distribution of *R. stylosa* in (a) Europe (Kurtto *et al.* 2004); (b) the Netherlands and Flanders (Maes *et al.* 2006).

Figure A.13: Distribution of *R. henkeri-schulzei* in the Netherlands and Flanders (Maes *et al.* 2006).

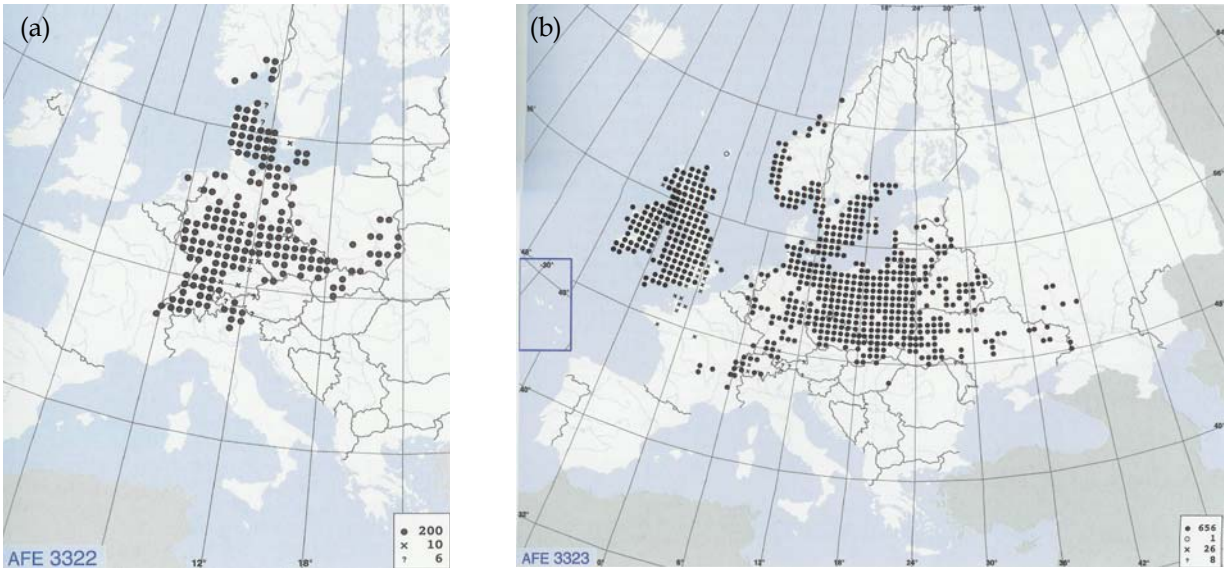

Figure A.14: Distribution of (a) *R. pseudoscabriuscula*; (b) *R. sherardii* in Europe (Kurtto *et al.* 2004).

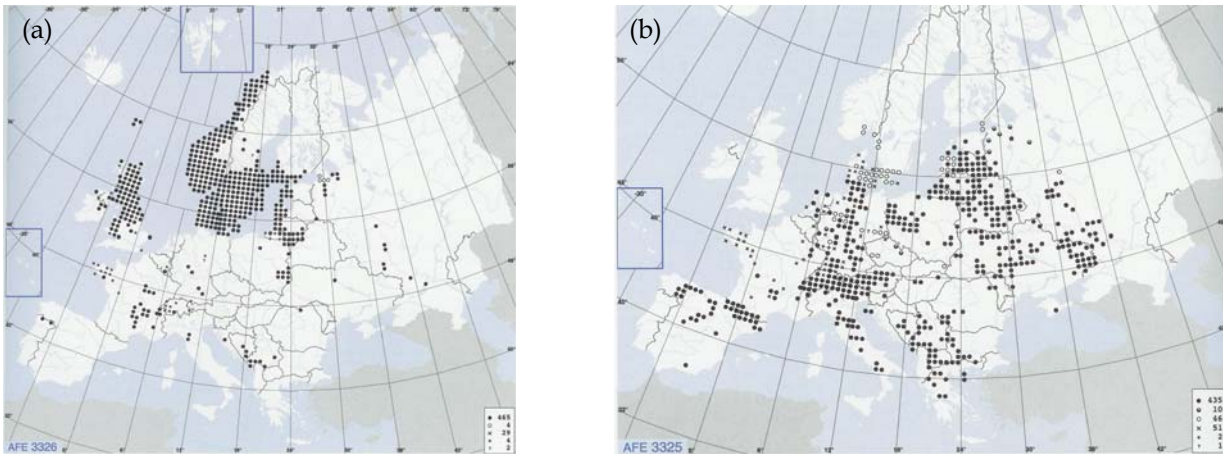

Figure A.15: Distribution of (a) *R. mollis* ; (b) *R. villosa* in Europe (Kurtto *et al.* 2004).

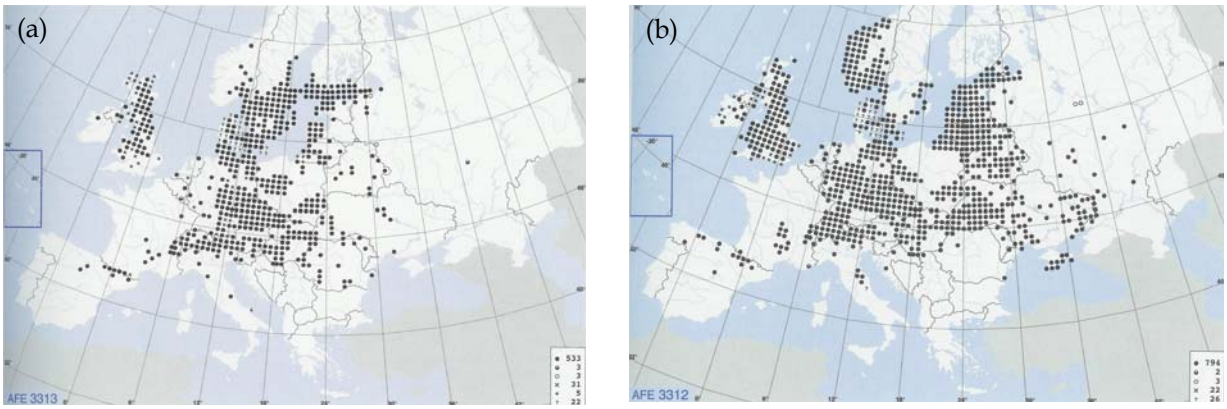

Figure A.16: Distribution of (a) *R. caesia*; (b) *R. subcanina* in Europe (Kurtto *et al.* 2004).

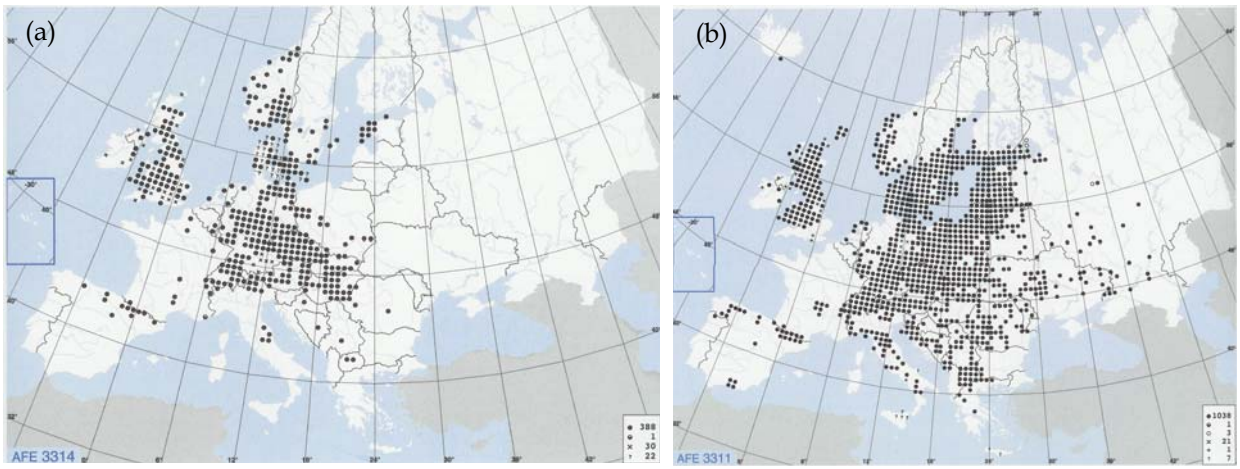

Figure A.17: Distribution of (a) *R. subcollina*; (b) *R. dumalis* in Europe (Kurtto *et al.* 2004).

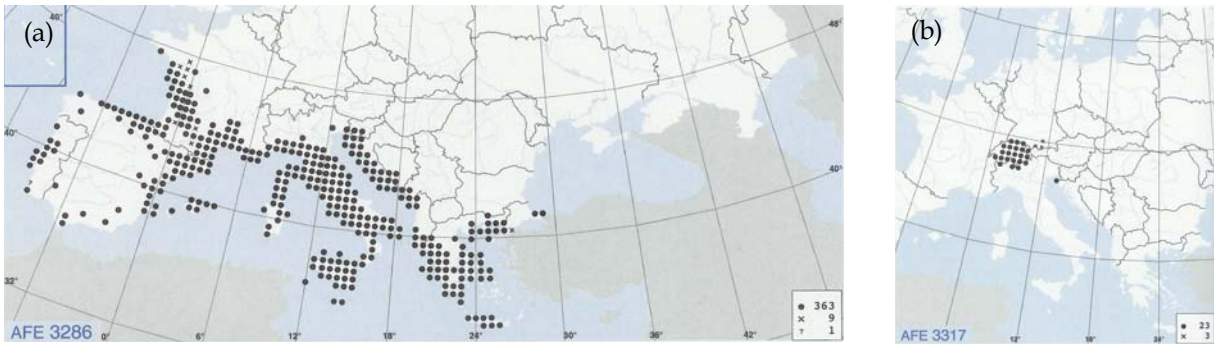

Figure A.18: Distribution of (a) *R. sempervirens*; (b) *R. abietina* in Europe (Kurtto *et al.* 2004).

Situation of the sampling sites of subgenus Rosa *taxa in Europe and Belgium*

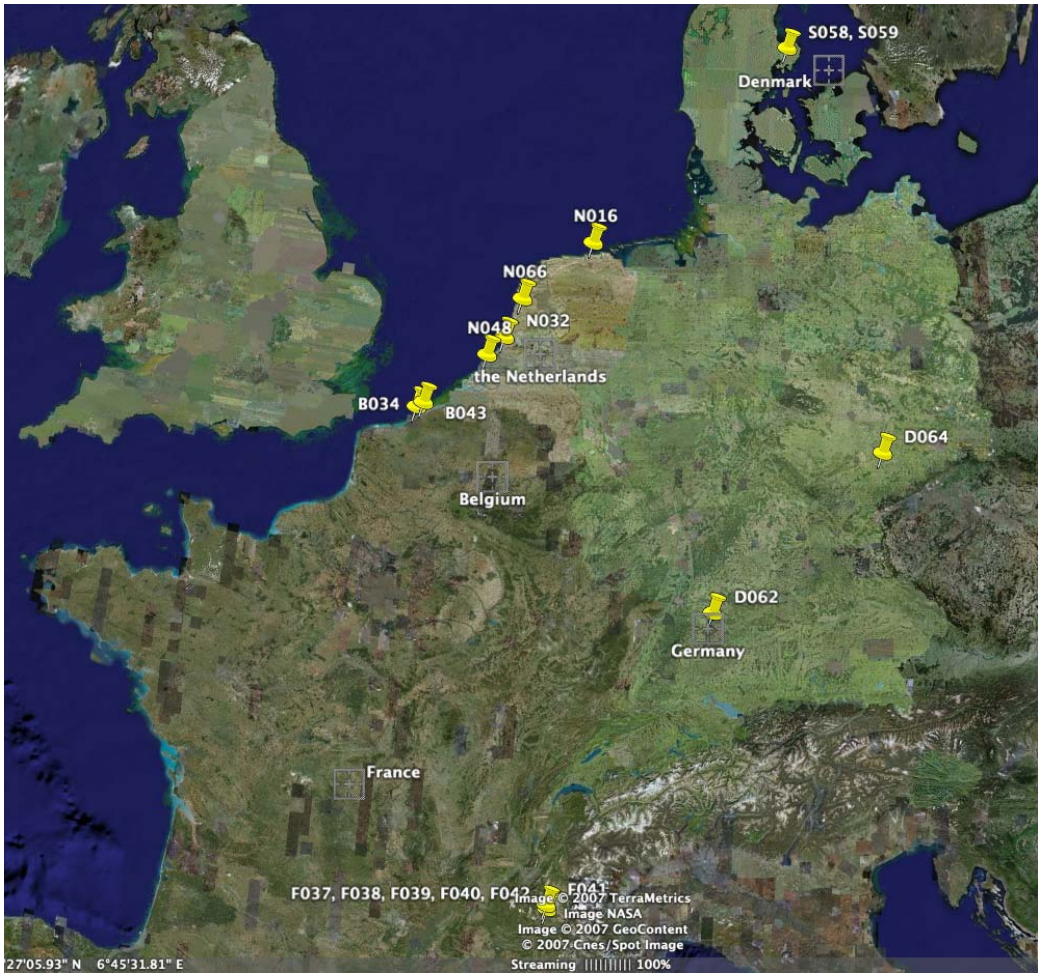

Figure A.19: Map of Western Europe. Sampling sites of *R. spinosissma* (section *Pimpinellifoliae*) are indicated. Used population codes see table 4.7.

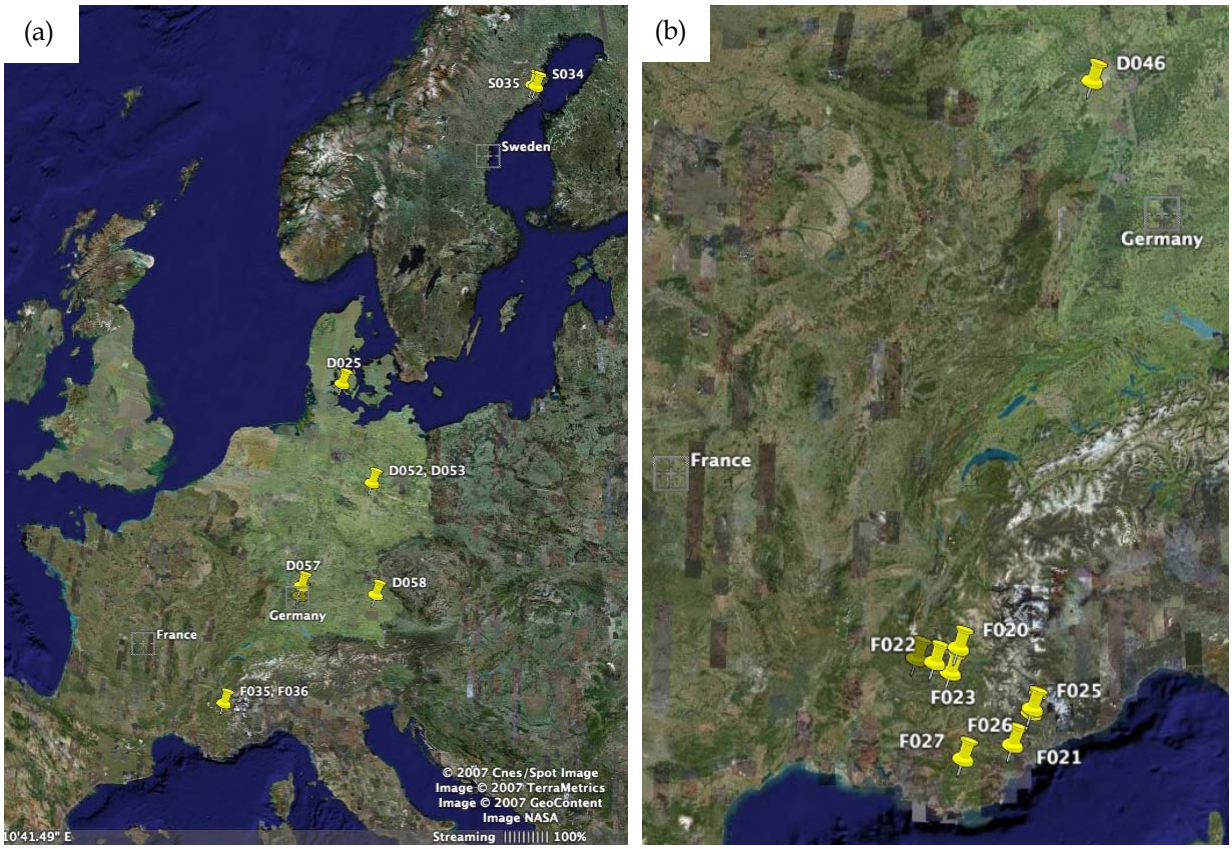

Table A.20: Map of Western Europe. Sampling sites of (a) the species *R. majalis*, and *R. pendulina* (section *Cinnamomeae*); (b) *R. gallica* (section *Rosa*) are indicated. Used population codes see tables 4.11 and 4. 9, respectively.

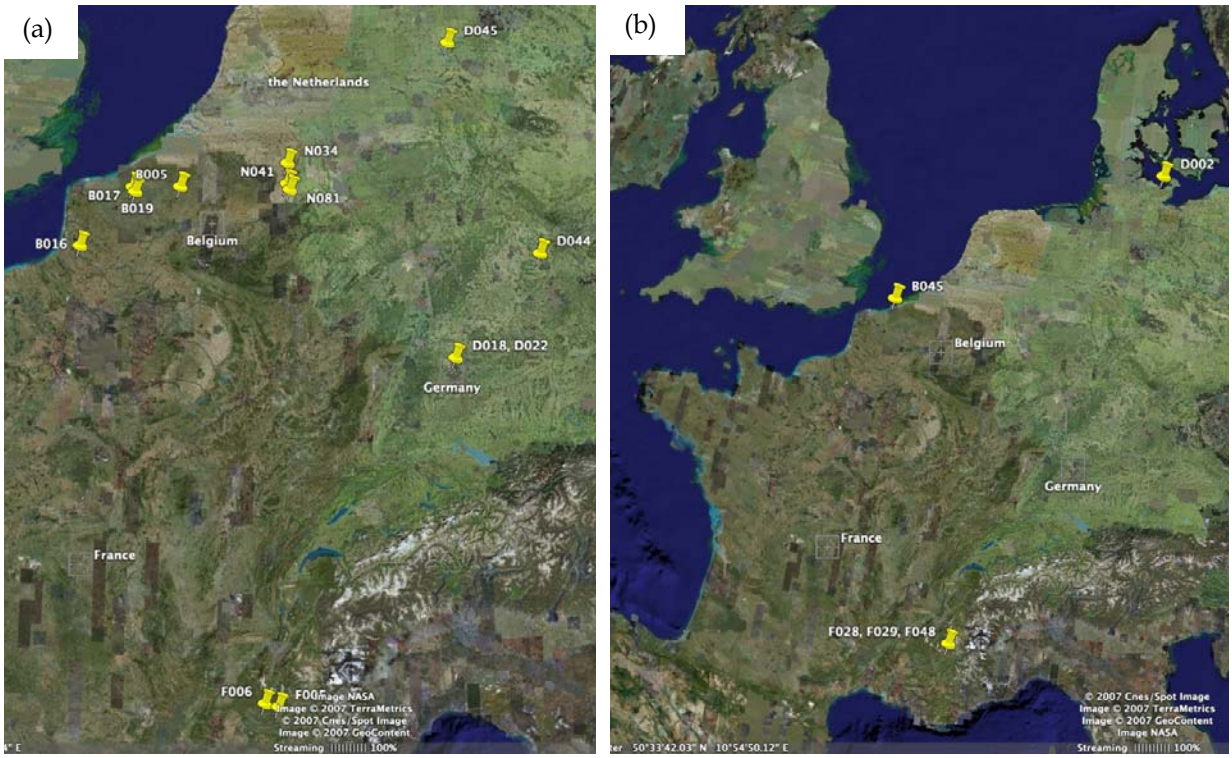

Figure A.21: Map of Western Europe. Sampling sites of (a) *R. arvensis* (section *Synstylae*); (b) *R. glauca* (section *Caninae*, subsection *Rubrifoliae*) are indicated. Used population codes see tables 4.14 and 4.19, respectively.

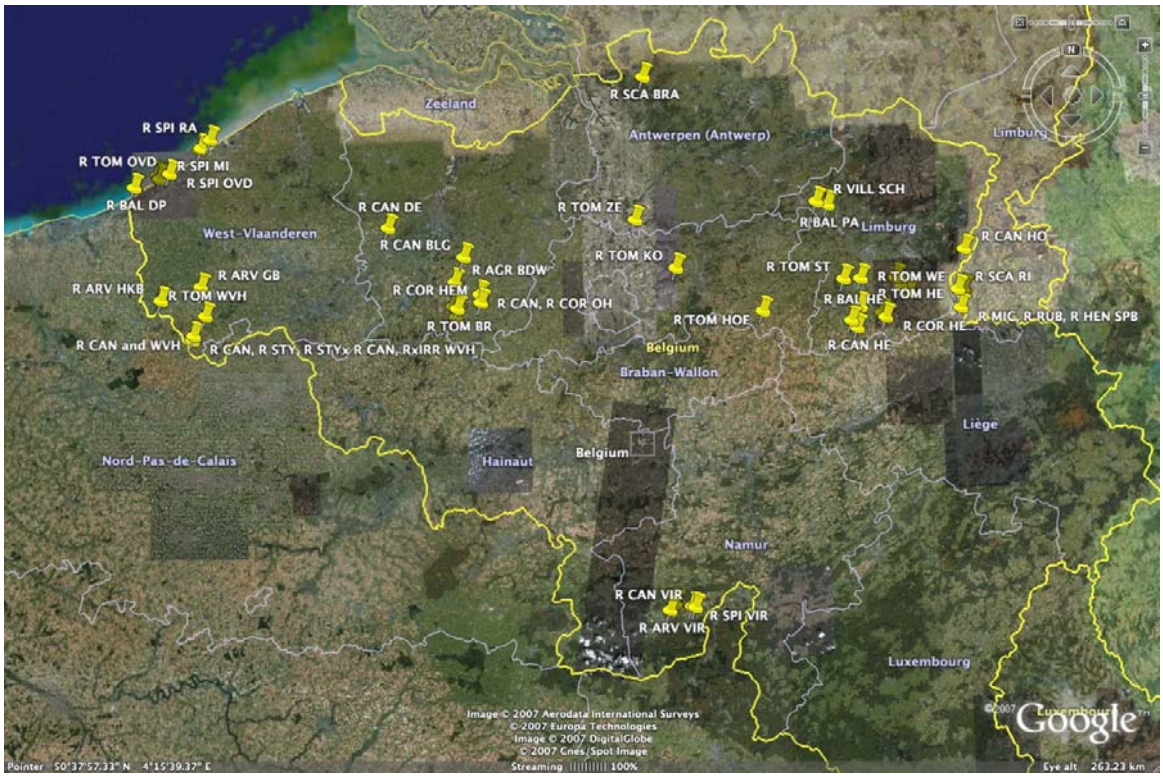

Figure A.22: Map of Belgium. Overview of all the sampled populations in Belgium. Used abbreviations for taxon see table 4.2; for region or locality see table 4.34.

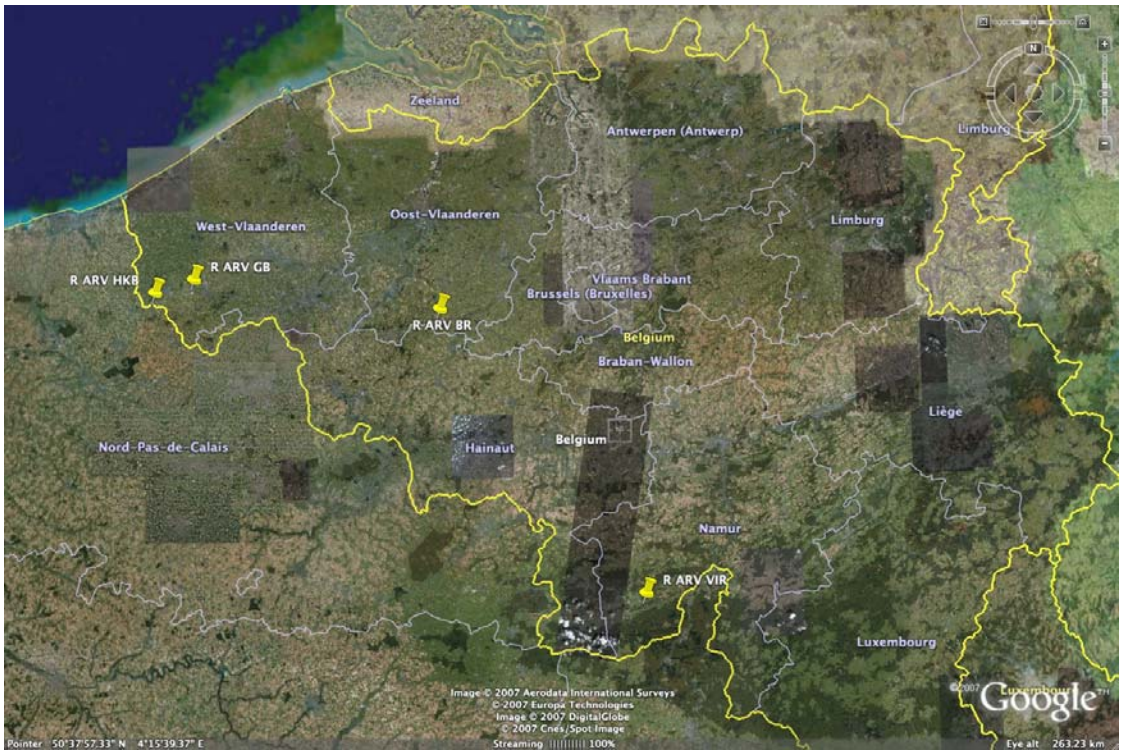

Figure A.23: Map of Belgium. Sampling sites of *R. arvensis* (section *Synstylae*) are indicated. Used abbreviations for taxon see table 4.2; for region or locality see table 4.34.

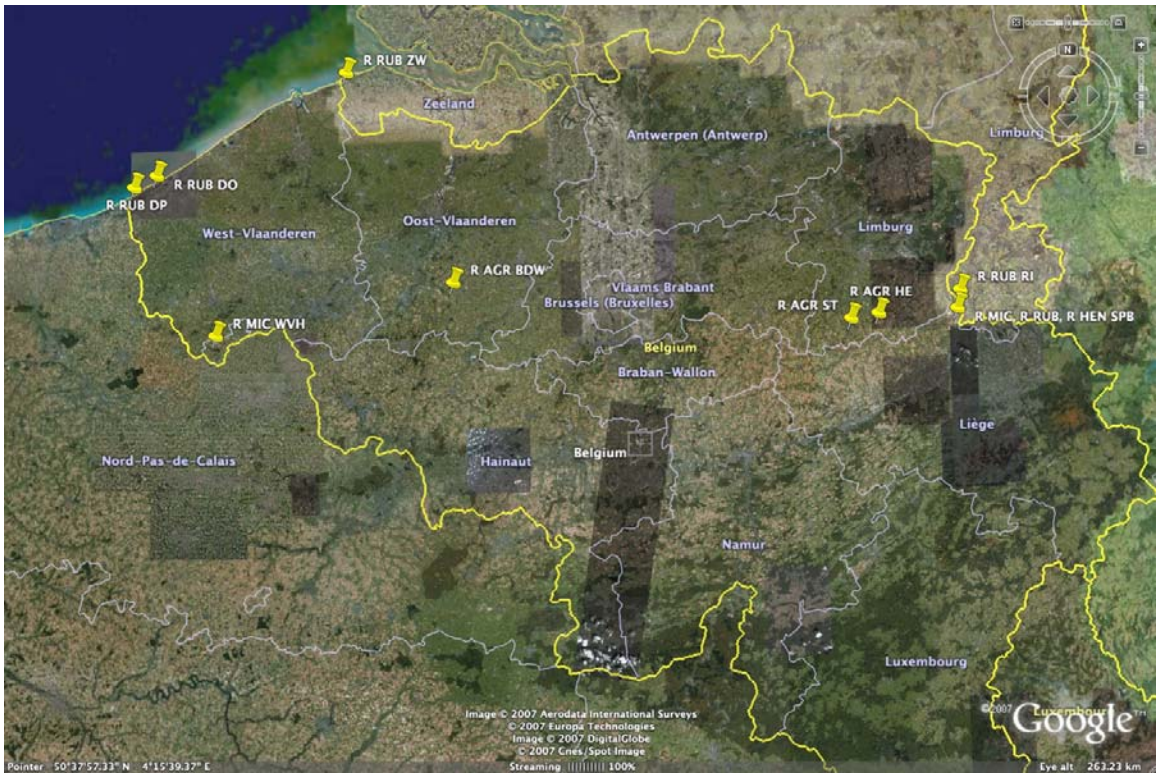

Figure A.24: Map of Belgium. Sampling sites of the taxa of the subsection *Rubigineae* (section *Caninae*) are indicated. Used abbreviations for taxon see table 4.2; for region or locality see table 4.34.

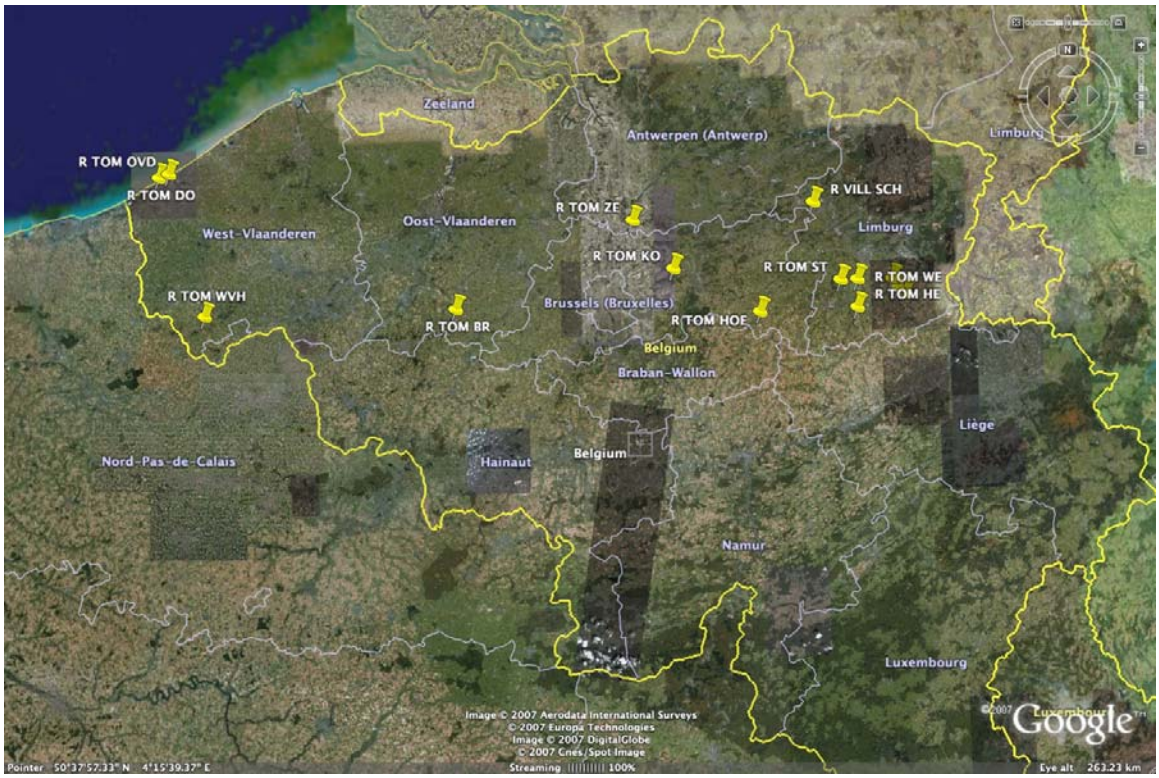

Figure A.25: Map of Belgium. Sampling sites of taxa of the subsection *Vestitae* (section *Caninae*) are indicated. Used abbreviations for taxon see table 4.2; for region or locality see table 4.34.

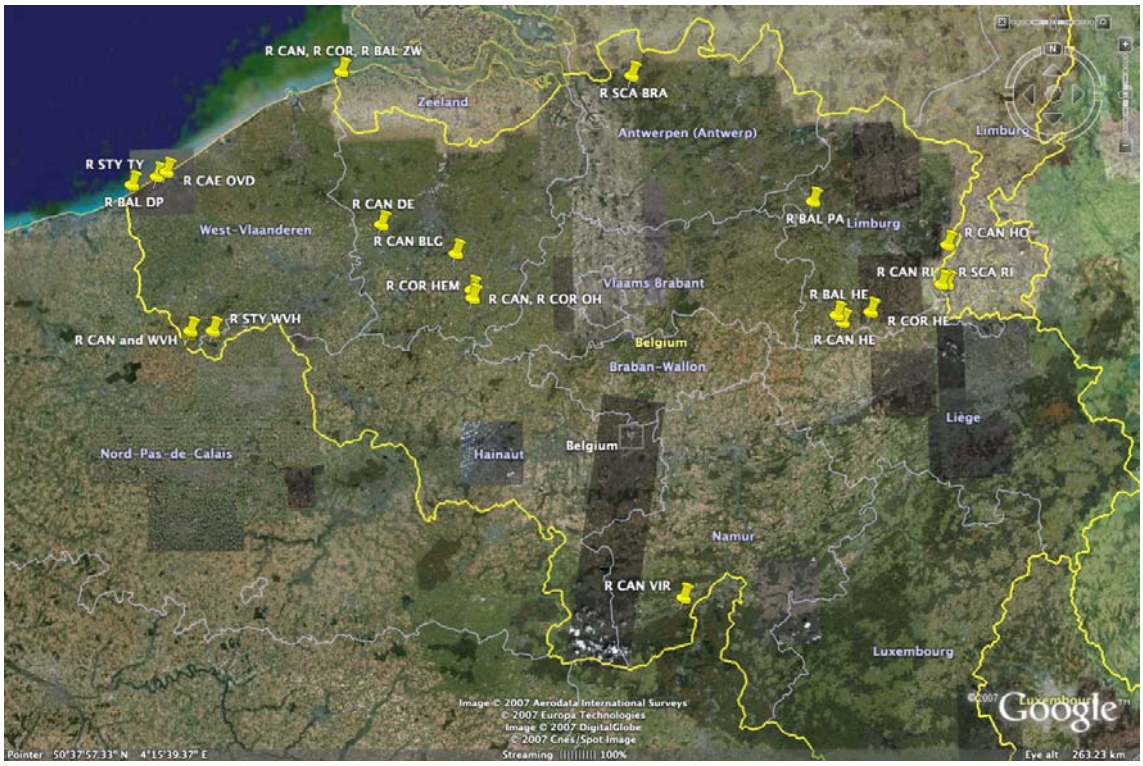

Figure A.26: Map of Belgium. Sampling sites of the taxa of the subsections *Tomentellae* and *Caninae* (section *Caninae*) are indicated. Used abbreviations for taxon see table 4.2; for region or locality see table 4.34.

Comparing the morphological descriptions in literature with the observations on the Belgian species

R. arvensis

STRUCTURE	CHARACTER	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Curved and slender	Sickle-shaped to slightly curved, sometimes hooked, or straigth		Curved to sickle-shaped, or straight
Leaflet	Shape	Ovate-elliptical	Ovoid to broad-elliptical, seldom broad-elliptical or spherical		Ovoid to elliptical, or reversed ovoid
	Base (cm)				0,6-1,6
	Length (cm) x	1-3 x			1.4-3.5-(-3.8) x
	Width (cm)	0.6-1.3			0.7-1.6 (-2)
	Pubescence leaflet lower side	Glabrous or pubescent on veins	Glabrous or slightly pubescent on veins		Glabrous
	Pubescence leaflet upper side	Glabrous	Glabrous		Glabrous or sparsely pubescent
	Glandular leaflet lower side	Eglandular	Eglandular	Eglandular	Eglandular
Leaflet margin	Serration	Crenate-serrate	Uniserrate, sometimes biserrate		(Irregular) uniserrated, teeth with a typical accolade-shape
	Glands?		Eglandular		Eglandular or sparsely glandular
Rachis	Length (cm)				1.6-3.7 (-4.3)
	Pubescence		Downy hairs		Glabrous to densely pubescent
	Glands?	Usually with stalked glands	Eglandular or with few glands		Eglandular to sparsely glandular

Table A.1: Description of leaflet and hip characters of *R. arvensis* (66/19 individuals/ including hip data) according to Graham and Primavesi (1993), Henker (2000), Wissemann (2003), and our observations.

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)
Hip	Shape	Narrowly ovoid to globose	Spherical to elongated elliptical		Mostly elliptical, bottle-shaped or reversed pear-shaped, sometimes ovoid and reversed ovoid hips
	Length (cm)	0.8-1.5	1-1.5		1-1.8
	Glands	Smooth	Eglandular		Eglandular or sparsely to moderately glandular
	# glands on half a hip				0-50
	Disc index	6	(5-) 6-10 (-11)		4.2-8.6
Sepals	State and persistence at anthesis	Falling early	Reflexed and deciduous (L type)		
Orifice	Diameter (mm)		0.3-0.6		0.4-0.8
Disc	Shape	Almost flat	Flat to slightly convex. seldom explicitly convex		Flat or convex
	Diameter (mm)		(2.5-) 3-3.5 (-4.5)		2.8-4.6 (-5)
Receptacle	Shape	Small globose head			Head type (D type)
Styles	Type	Fused into a slender, exserted column	Agglutinated and elongated like a column	Agglutinated after anthesis like a column	Agglutinated and elongated like a column
Pedicel	Length (cm)	2-5	(1.5-) 2-4 (-5)		1.7-4.8
	Relative length		2-5		1.2-3.2
	Pubescence				Glabrous
	Glands?	Short-stalked glands; rarely smooth	Strongly glanded with stipitate or subsessile glands, seldom completely eglandular		Densely glandular
	# glands on half a pedicel				(11-) 51-100

Table A.1 continu: Description of leaflet and hip characters of *R. arvensis* (66/19 individuals/ including hip data) according to Graham and Primavesi (1993), Henker (2000), Wissemann (2003), and our observations.

R. rubiginosa

STRUCTURE	CHARACTER	NILLSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Curved or falcate, often interspersed with small, straight prickles or acicles	Very curved, acuminate, unequal and often mixed with scattered stout acicles	Hooked to sickle-shaped mingled with slightly curved or straigth prickles (heteracanth)	Hooked and straight prickles mixed (heteracanth)	Curved to sickle-sha hooked, mixed with straight prickles. Heteracanth
Leaflet	Shape	Broadly ovate, or obovate	Suborbicular to ovate-elliptical	Circular-elliptical to broad-elliptical, seldom oval or circular		Ovoid, or elliptical v transitions
	Base (cm)					0.5-1.3 (-2.2)
	Length (cm) x		1-2 (-2.5) x			1.4-3 (-4.6) x
	Width (cm)		1-2			0.8–1.8 (-2.3)
	Pubescence leaflet lower side	Pubescent	Pubescent on veins	Felty, especially on the veins		Densely pubescent c veins
	Pubescence leaflet upper side	Glabrous or shortly pubescent	Sligthly pubescent	Glabrous to sligthly pubescent		Moderately to dens pubescent
	Glandular leaflet lower side	Densely spaced, sessile glands, with strong scent of apples	Numerous viscid, brownish or translucent, sweet-smelling glands	Numerous sticky glands smelling like apples or vine	Numerous sticky glands smelling like apples or vine	Densely glandular, smelling like apples
Leaflet margin	Serration	Biserrate	Biserrate	Multiserrate		Multiserrated, with intermediate forms biserrated
	Glands?	Glandular	Glandular	Glandular		Densely glandular
Rachis	Length (cm)					1 - 4.1 (-5.5)
	Pubescence			Slightly pubescent or glabrous		Densely
	Glands?		Glandular	Densely glandular		Densely glandular

Table A.2: Description of leaflet and hip characters of *R. rubiginosa* (49/31 individuals/ including hip data) according to Nilsson (1967), Graham a Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Hip	Shape	Long, subglobose, ovoid or ellipsoid	Ovoid, obovoid or globose	Elliptical to oval, seldom spherical		Variable: reversed ovoid, and pear-shaped, or bottle-shaped, ovoid to elliptical
	Length (cm)	1.0-1.8	1.2-2.5	> pedicel		1.4-1.9 (-2.1)
	Glands	Eglandular or with acicles near the base	Smooth or glandular-setose at base	Eglandular or glandular		Eglandular or sparsely glandular
	# glands on half a hip					0-10 (-11-100)
	Disc index		3	1.8-3.2		2.7-5 (-5.7)
Sepals	State and persistence at anthesis	Erect or ascending after flowering, falling somewhat before fruit ripens	Spreading-erect, usually persistent	Erect and persistent, seldom spread out and deciduous (D type)	Erect and persistent	
Orifice	Diameter (mm)	1- 1.2		(1-) 1.2-2 (-2.4)		0.7-1.3 (-1.4)
Disc	Shape	Flat or somewhat concave	Shallowly concave	Flat, also slighlty concave or slightly convex		Flat, also slighlty concave or slightly convex
	Diameter (mm)	4.5				(2.6-) 3.3-4.4 (-5.1)
Receptacle	Shape	Dense flattened or slightly convex head covering most of the disc	Wedge-shaped head	Head type (D type), seldom bouquet-shaped (L type)		Head type (D type)
Pedicel	Length (cm)	1-1.5	1	(0.3-) 1-1.5 (-2.5)		0.7-1.4 (-1.7)
	Relative length	1				0.4-1.1
	Pubescence		Hispid			Glabrous, seldom sparsely or moderately pubescent
	Glands?	Densely stipitate- or setose-glandular	Glandular, mixed with small acicles	Stipitate-glandular, seldom eglandular		Eglandular or densely glandular
	# glands on half a pedicel					0 (-1-30-) 31-100

Table A.2 continu: Description of leaflet and hip characters of *R. rubiginosa* (49/31 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

R. micrantha

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Curved or falcate, occasionally interspersed with smaller prickles or acicles	Very curved with long bases, more or less equal, acicles absent	Hooked, seldom sickle-shaped, rarely mixed with needle prickles	Hooked and straight prickles mixed (heteracanth)	Hooked, curved to sickle-shaped, sometimes mixed with straigth acicles (Heteracanth)
Leaflet	Shape	Ovate or elliptical	Ovate, obovate or elliptical, rounded at base	Elliptical, broad-elliptical to ovoid		Ovoid, or elliptical with transitional forms, seldom reversed ovoid
	Base (cm)					0.6-1.6
	Length (cm) x		1.5-3 (-3.5) x			1.4-3.2 x
	Width (cm)		0.8-2.5			0.9-1.7 (-2)
	Pubescence leaflet lower side	Pubescent	Short pubescent	Hispid or downy hairs, seldom glabrous		Densely pubescent on the veins
	Pubescence leaflet upper side	Glabrous	Glabrous	Glabrous to slightly pubescent		Glabrous to moderately pubescent
	Glandular leaflet lower side	Glandular, strong scent of apples	Viscid, brownish, or translucent sweet-smelling glands	Numerous sticky glands, sometimes with few to no glands, smelling like apples	Numerous sticky glands smelling like apples or vine	Densely glandular
Leaflet margin	Serration	Biserrate	Multiserrate	Multiserrate		Multiserrated
	Glands?	Glandular	Glandular	Glandular		Densely glandular
Rachis	Length (cm)					1.5-3.9
	Pubescence			Pubescent to glabrous		Densely pubescent
	Glands?		Glandular	Numerous glands, rarely with little prickles		Mainly densely glandular

Table A.3: Description of leaflet and hip characters of *R. micrantha* (16/8 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Hip	Shape	Ovoid, globose, pyriform or fusiform	Urceolate or with a short neck	Slender, elliptical, ovate, bottle-shaped or fusiform		Ovoid- to elliptical, sometimes globose
	Length (cm)	1.2-1.7	0.9-1.7		Usually small	1.4-2
	Glands	Eglandular or often glandular		Mostly stipitate-glandular with acicles on the lower half, seldom on the whole hip		Eglandular or sparsely glandular
	# glands on half a hip					0–10 (-11-50)
	Disc index		5-6	(4.2-) 5-7 (-11.4)		3.4-7.2
Sepals	State and persistence at anthesis	Deflexed and falling soon after flowering	Reflexed, falling before ripening	Reflexed and deciduous early after anthesis (L type)	Reflexed and deciduous early after anthesis	
Orifice	Diameter (mm)	0.7		(0.4-) 0.6-0.8 (-1)		0.5-0.9
Disc	Shape	Flat, somewhat conoidal	Convex	Mostly convex, rarely slightly convex		Flat or convex
	Diameter (mm)	4-4.5				2.6-4.5
Receptacle	Shape	Conoidal, somewhat flattened head	Subglobose head	Bouquet-shaped (L type), rarely headtype (D type)		Head type (D type)
Pedicel	Length (cm)		1-1.7 (-2)	(0.5-) 1-2 (-3)		0.8-1.5
	Relative length	<1		2-3		0.5-1.1
	Pubescence					Glabrous
	Glands?	Stipitate- or setose-glandular, occasionally eglandular	Nearly always glandular, no acicles	Densely glandular, rarely eglandular		Eglandular or densely glandular with frequently intermediate forms
	# glands on half a pedicel					0-30 (-31-100)

Table A.3 continu: Description of leaflet and hip characters of *R. micrantha* (16/8 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

R. agrestis

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Curved, sometimes straight	Curved, stout-based	Hooked to sickle-shaped	Hooked and straight prickles mixed (heteracanth)	Sickle-shaped to curves, or curved or hooked
Leaflet	Shape	Elliptical to oblong-ovate	Narrowly elliptical, cuneate at base and acute at apex	Elongated elliptical, or reversed-ovate, with wedge-shaped or narrowed base		Elongated elliptical, with wedge-shaped or narrowed base
	Base (cm)					0.9-1.4
	Length (cm) x		2-2.5 x			1.9-2.7 (-3.6) x
	Width (cm)		1-1.3			1–1.3
	Pubescence leaflet lower side	Glabrous or pubescent		With downy or felty downy hairs		Sparsely pubescent on the veins
	Pubescence leaflet upper side	Glabrous or pubescent		Glabrous, or with downy or felty hairs		Glabrous or sparsely pubescent
	Glandular leaflet lower side	Densely glandular, scent of apples or resine	Scattered viscid or translucent, scarcely sweet-smelling glands	Numerous sticky glands smelling like apples, rarely eglandular	Numerous sticky glands smelling like apples or vine	Densely glandular
Leaflet margin	Serration	Mostly biserrate, sometimes uniserrate	Multiserrate	Multiserrate		Multiserrate
	Glands?	Glandular	Glandular	Glandular		Densely glandular
Rachis	Length (cm)					1.4-3.5
	Pubescence			Glabrous to felty pubescent		Sparsely to moderately pubescent
	Glands?		Glandular	Glandular		Varied from moderately to densely glandular

Table A.4: Description of leaflet and hip characters of *R. agrestis* (15/12 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Hip	Shape	Long, globose, ovoid or fusiform	Ovoid to globose or slightly urceolate	Elliptical to ovate, also spherical, elongated elliptical or ovate		Ovoid- to elliptical
	Length (cm)	1-1.5	1-2			1.1-2.3
	Glands	Eglandular	Smooth	Eglandular		Eglandular
	# glands on half a hip					0- (1-50)
	Disc index		5-6	4.5-7.0		(4.1-) 5-7.1 (-8.8)
Sepals	State and persistence at anthesis	Deflexed and falling soon after flowering	Reflexed, falling early	Reflexed and deciduous after anthesis (L type)	Reflexed and deciduous after anthesis	
Orifice	Diameter (mm)	0.5		0.5-0.8		0.4-0.7 (-0.9)
Disc	Shape	Conoidal	Slightly convex	Convex to slightly convex		Convex
	Diameter (mm)	3-4.5				2.6-3.7 (-4.3)
Receptacle	Shape	Small conoidal or ovoid head		Bouquet-shaped (L type), rarely also head type (D type)		Head (D type) or bouquet type (L type)
Pedicel	Length (cm)		1-1.5			0.7-1.9
	Relative length	1		1-2		0.5-0.8
	Pubescence	Glabrous	Smooth			Glabrous or sparsely pubescent
	Glands?	Eglandular, sometimes sparsely glandular		Eglandular or slightly covered with glands		Eglandular, sometimes moderately glandular
	# glands on half a pedicel					0 (1-10) and (31-50)

Table A.4 continu: Description of leaflet and hip characters of *R. agrestis* (15/12 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

R. tomentosa

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Slender and curved	Arcuate and slender	Slightly curved to completely straight	Slender and curved	Straigth to curved
Leaflet	Shape	Ovate-lanceolate	Ovate-lanceolate	Elliptic-lanceolate, to elliptical, seldom ovoid-lanceolate or ovoid- broad-elliptic		Ovoid or elliptical with transitional forms, sometimes lanceolate
	Base (cm)					0.6 – 2.1
	Length (cm) x	2-4 x	(1.5-) 2-4 x	Ratio: (1.2-) 1.8 (-3) / 1		1.6 – 4.4 x
	Width (cm)	1.2-2	1-2			0.9 – 2.5
	Pubescence leaflet lower side	Densely tomentose	Tomentose	Tomentose	Conspicuously hairy	Tomentose
	Pubescence leaflet upper side	Densely tomentose		Mostly dense, sometimes sparsely tomentose	Conspicuously hairy	Densely
	Glandular leaflet lower side	Glandular, smelling like turpentine	Faintly resin-scented or odourless glands (hidden in tomentum)	Subfoliar glands	Glandular. hidden in hairs. smelling like resin or turpintine	Eglandular or moderately glandular
Leaflet margin	Serration	Uni- or biserrated	Irregularly biserrate	Uniserrate to irregularly biserrate	Mostly uniserrate with conspicuous wide and short teeth. if biserrate than a slight occurrence of glands	Varied from irregularly uni- to biserrate. over biserrate. bi- to multiserrate. and multiserrate
	Glands?	Glandular		Mostly eglandular, rarely sparsely glandular		Densely glandular. also sparsely to moderately
Rachis	Length (cm)					0.6 – 4.1
	Pubescence		Tomentose	Densely and tomentose		Densely
	Glands?		Glandular	Sometimes with numerous short stipitated glands		Varying from moderately to densely glandular

Table A.5: Description of leaflet and hip characters of *R. tomentosa* (29/20 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Hip	Shape	Ovoid, sometimes globose	Ovoid or globose	Globose to oval, or elliptical, seldom pear-shaped		Reversed pear-shaped or globose, sometimes ovoid- to elliptical
	Length (cm)	1-1.5	1-1.5			1.5-2 (-2.2)
	Glands	Usually glandular, often smooth	Glandular-hispid	Densely glandular, rarely eglandular		Moderately glandular with persistent stipitate glands
	# glands on half a hip					(1 – 10-) 11-50 (51-100)
	Disc index		5	(3,5-) 4-6 (-8)		4,3-10,4
Sepals	State and persistence at anthesis	Patent, deflexed and soon falling after flowering	Spreading or spreading-erect after anthesis, but falling before hips have fully reddened	Reflexed and deciduous (L type)	Reflexed and deciduous	
Orifice	Diameter (mm)	0.5-1		0.5-1 (-1.2)		0.3– 0.9
Disc	Shape	Conoidal		Explicit convex, rarely slightly convex		Flat or convex
	Diameter (mm)	About 4				3 – 4.4
Receptacle	Shape	Diffuse globose or conoidal head	Small head	Bouquet-shaped (L type)		Head type (D type)
Styles	Type					
Pedicel	Length (cm)	2.5-3.5	2-3.5	(1.5-) 2-4		1.2 – 2.6 (-3.1)
	Relative length			(1-) 2-3 (-4)		0.6–1.5 (-2.1)
	Pubescence		Hispid	Glabrous or pubescent		Glabrous or sparsely pubescent
	Glands?	Densely glandular	Glandular	Stipitate-glandular		Densely glandular with stipitate glands
	# glands on half a pedicel					(11-30) 31-100

Table A.5 continu: Description of leaflet and hip characters of *R. tomentosa* (29/20 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations.

R. balsamica

STRUCTURE	CHARACTER	NILSSON (1967) †	GRAHAM & PRIMAVESI (1993) †	HENKER (2000) *	WISSEMANN (2003) *	OBSERVATIONS
Prickle	Shape	Strongly hooked	Broad-based, strongly curved	Hooked	Curved	Sickle-shaped, curved to hooked
Leaflet	Shape	Broadly ovate or oval	Broadly ovate with rounded base	Elliptical to broad-elliptical or ovoid, seldom elongated-elliptical, reversed ovoid or spherical-elliptic		Ovoid, elliptical with transitions
	Base (cm)					0.9-1.7
	Length (cm) x Width (cm)	1.5-3.5 x 1.5 – 2.5	2-3 (-3.5) x 1.5-2			2.1-3.8 x 1.1-2.1
	Pubescence leaflet lower side	Pubescent, sometimes restricted to veins	Pubescent, not tomentose	Veins are densely pubescent, leaflets sometimes glabrous, or slightly pubescent	Pubescent	Sparsely pubescent at veins, sometimes moderately or densely pubscent
	Pubescence leaflet upper side	Softly pubescent, sometimes glabrous	Sometimes pubescent	Glabrous or downy pubescent	Mostly pubescent	Glabrous or sparsely pubescent
	Glandular leaflet lower side	Usually glandular on main veins, sometimes eglandular, or glandular all over	Often glandular	Veins densely glandular	Veins densely glandular	Sparsely or moderately glandular at veins
Leaflet margin	Serration	Usually biserrated, occasionally uniserrate	Biserrate	Biserrate to multiserrate	Multiserrated	Bi- to multiserrated or multiserrated
	Glands?	Glandular	Numerous small, reddish-brown glands on teeth	Glandular	Glandular	Sparsely or intermediate forms to densely pubescent
Rachis	Length (cm)					1.6-4
	Pubescence	Densely pubescent	Pubescent	With downy hairs	Pubescent	Densely pubescent
	Glands?	Glandular	Often glandular	Numerous glands	Glandular	Sparsely to densely glandular

Table A.6: Description of leaflet and hip characters of *R. balsamica* (32/37 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations. With *: descriptions based on *R. tomentella*, and †: descriptions based on *R. obtusifolia*.

STRUCTURE	CHARACTER	NILSSON (1967) †	GRAHAM & PRIMAVESI (1993) †	HENKER (2000)*	WISSEMANN (2003)*	OBSERVATIONS
Hip	Shape	Ovoid or globose	Broadly ovoid or globose	Elliptical to spherical		Varied mainly from ovoid- to elliptical
	Length (cm)	1-2	1-1.5			1.3-2.2 (-2.6)
	Glands	Eglandular	Smooth	Eglandular		Mainly eglandular
	# glands on half a hip					0 (1 – 10)
	Disc index		5-6	(4-) 5-8 (-10)		(3.4-) 5.9-11.9
Sepals	State and persistence at anthesis	Deflexed and falling soon after flowering	Strongly reflexed after flowering, falling early	Reflexed and deciduous (L type)	Reflexed and deciduous	
Orifice	Diameter (mm)	0.5-1		0.5-0.9 (-1)		0.4-0.7 (-1.3)
Disc	Shape	Flat, somewhat conoidal	Convex	Clearly to sligth convex		Convex
	Diameter (mm)	About 5				3,6-4,8
Receptacle	Shape	A rather loose, more or less globose head	Small globose head	Head- (D type) or bouquet-shaped (L type)		Head- (D type) or bouquet-shaped (L type)
Pedicel	Length (cm)	0.5-1.5	0.5-1.5	(0.2-) 1-2 (-3)		0.8-1.7
	Relative length			1-2		0.4-1.2
	Pubescence	Glabrous			Smooth	Glabrous or sparsely pubescent
	Glands?		Eglandular	Eglandular, or seldom sligthly glandular	Smooth	Eglandular, seldom glandular
	# glands on half a pedicel					0 (-100)

Table A.6 continu: Description of leaflet and hip characters of *R. balsamica* (32/37 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations. With *: descriptions based on *R. tomentella*, and †: descriptions based on *R. obtusifolia*.

R. canina

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)°	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Curved or hooked	Broad-based and strongly curved	Hooked, seldom sickle-shaped	Hooked	Sickle-shaped or hooked
Leaflet	Shape	Ovate, elliptic or broadly lanceolate	Ovate or ovate-lanceolate	Variable: elliptical to ovate, rarely elliptic-lanceolate to spherical-elliptic		Ovoid, elliptical with transitionals forms, or lanceolate, seldom reversed ovoid
	Base (cm)					0.8-1.8 (-2.2)
	Length (cm) x Width (cm)		1.5-4 x 1-1.8			1.8-4.3 (-4.7) x 1.1-2.1
	Pubescence leaflet lower side	Glabrous or less frequently pubescent	Glabrous	Glabrous	Glabrous	Glabrous
	Pubescence leaflet upper side	Glabrous		Glabrous	Glabrous	Glabrous
	Glandular leaflet lower side	Eglandular, or occasionally with few glands on main veins		Eglandular, or glandular		Eglandular or sparsely glandular at veins
Leaflet margin	Serration	Uniserrated, or occasionally biserrate	Uniserrated	Regular or irregular uni- to biserrate		Varied from (irregular) uniserrate, to bi-, and occasionally to multiserrate margins, including intermediate forms
	Glands?		Eglandular	Rarely glandular		Eglandular or sparsely glandular
Rachis	Length (cm)					1.4-4.9 (-5.7)
	Pubescence			Glabrous, rarely on the ridge	Glabrous	Glabrous
	Glands?	Eglandular, or rarely with few glands	Eglandular	Mostly eglandular, rarely sparsely glandular		Eglandular to sparsely glandular

Table A.7: Descriptions of leaflet and hip characters *R. canina* (82/65 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations. With °: description based on *R. canina* with adaptations mentioned for the Group *Lutetianae* (Corresponds to the lectotype of *R. canina* in LINN).

STRUCTURE	CHARACTER	NILSSON (1967)	GRAHAM & PRIMAVESI (1993)°	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Hip	Shape	Long, ovoid, or ellipsoid	Very variable, globose, ovoid, obovoid, ellipsoid or urceolate	(Elongated- or spherical) elliptical, (reversed) ovate, spherical, pear-, or bottle-shaped		Ovoid- to elliptical, or globose
	Length (cm)	1-2	1-2.5			1.3-2.5 (-2.6)
	Glands	Eglandular	Smooth	Eglandular, rarely glandular		Eglandular
	# glands on half a hip					0 (1-100)
	Disc index		5-6	(4.5-) 5-6 (-10)		4.5-11.1 (-19.8)
Sepals	State and persistence at anthesis	Reflexed and falling soon after flowering	Mostly reflexed, sometimes rising to the horizontal, falling early	Reflexed and deciduous (L type)	Reflexed and deciduous	
Orifice	Diameter (mm)	0.5-1		0.4-0.8 (-1)		(0.2-) 0.3-0.8
Disc	Shape	Conoidal	Flat or convex, occasionally conoidal	Clearly to sligthly convex, rarely extremely convex		Flat or convex
	Diameter (mm)	4.5-5.5		3.2-5 (-6)		(2.9-) 3.5-5.4
Receptacle	Shape	Loose, diffuse, globose or conoidal head	Small globose or conical head	Headtype (D type), sometimes bouquet-shaped (L type)		Headtype (D type) or bouquet-shaped (L type)
Pedicel	Length (cm)	1-2.5	1.5-2.5	1-2 (-3)		0.5-1.7 (-2)
	Relative length	1		(0.1-) 0.5-1 (-1.4)		0.2-1
	Pubescence	Glabrous	Smooth	Glabrous or pubescent		Glabrous or sparsely pubescent
	Glands?		Smooth	Eglandular, rarely glandular, occasionally with numerous glands		Eglandular, seldom glandular
	# glands on half a pedicel					0 (-100)

Table A.7 continu: Descriptions of leaflet and hip characters *R. canina* (82/65 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations. With °: description based on *R. canina* with adaptations mentioned for the Group *Lutetianae* (Corresponds to the lectotype of *R. canina* in LINN).

R. corymbifera

STRUCTURE	CHARACTER	GRAHAM & PRIMAVESI (1993) °	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Prickle	Shape	Broad-based and strongly curved	Hooked	Hooked	Sickle-shaped to curved or hooked
Leaflet	Shape	Ovate or ovate-lanceolate	Broad-elliptical, spherical-elliptical to elongated-elliptical		Ovoid, elliptical with transitional forms
	Base (cm)				0.9-1.7 (-2.3)
	Length (cm) x	1.5-4 x			2-3.8 (-5) x
	Width (cm)	1-1.8			1-2.2 (-2.4)
	Pubescence leaflet lower side	Sparsely pubescent, or only on the midrib	Slightly to mild pubescent	Sometimes pubescent	Moderately to densely pubescent at the veins
	Pubescence leaflet upper side		Glabrous, rarely slightly pubescent	Sometimes pubescent	Glabrous or sparsely pubescent
	Glandular leaflet lower side		Eglandular, rarely glandular on main vein		Eglandular
Leaflet margin	Serration	Usually uniserrated	Uniserrate, seldom multiserrate		(Irregularly) uniserrated
	Glands?		Eglandular, if multiserrated: glandular		Eglandular
Rachis	Length (cm)				1.7-4.5 (-5.4)
	Pubescence	Pubescent, sometimes densely	Feltig to slightly pubescent	Pubescent	Densely pubescent
	Glands?	Eglandular	Eglandular, rarely with few glands		Mostly eglandular to sparsely glandular

Table A.8: Descriptions of the leaflet and hip characters of *R. corymbifera* (58/54 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations. With °: description based on *R. canina* with the adaptations mentioned for the Group *Pubescentes* (*R. corymbifera* Borkh.)

STRUCTURE	CHARACTER	GRAHAM & PRIMAVESI (1993) °	HENKER (2000)	WISSEMANN (2003)	OBSERVATIONS
Hip	Shape	Very variable, globose, ovoid; obovoid, ellipsoid or urceolate	Elliptical, seldom elliptical-spherical, to spherical		Ovoid- to elliptical
	Length (cm)	1-2.5			1.3-2.2
	Glands	Smooth	Eglandular, rarely glandular		Mainly eglandular
	# glands on half a hip				0 (1 – 10)
	Disc index	5-6			4.2-10.7
Sepals	State and persistence at anthesis	Mostly reflexed, sometimes rising to the horizontal, falling early	Reflexed and deciduous (L type)	Reflexed and deciduous	
Orifice	Diameter (mm)		0.5-0.9 (-10)		0.4-0.7 (-1)
Disc	Shape	Flat or convex, occasionally conoidal	Flat to clearly convex		Convex
	Diameter (mm)				3.7-5.3
Receptacle	Shape	Small globose or conical head	Head- (D type) or bouquet type (L type)		Head- (D type) or bouquet type (L type)
Styles	Type				
Pedicel	Length (cm)	1.5-2.5	0.3-2 (-2.7)		0.5-2
	Relative length		(1-) 1.5-2		0.4-1.3
	Pubescence	Smooth	Glabrous or pubescent		Glabrous or moderately pubescent
	Glands?	Smooth	Eglandular, rarely glandular		Eglandular, seldom glandular
	# glands on half a pedicel				0 (-100)

Table A.8 continu: Descriptions of the leaflet and hip characters of *R. corymbifera* (58/54 individuals/ including hip data) according to Nilsson (1967), Graham and Primavesi (1993), Henker (2000), Wissemann (2003) , and our observations. With °: description based on *R. canina* with the adaptations mentioned for the Group *Pubescentes* (*R. corymbifera* Borkh.)