Molecular and Morphological Diversity in the Plant Genus *Chaenomeles*

K. Rumpunen[a]*, I. Bartish[b], L. Garkava–Gustavsson[a], H. Nybom[a]

[a]Balsgård–Department of Horticultural Plant Breeding, Swedish University of Agricultural Sciences, Kristianstad, Sweden
[b]Institute of Botany, Academy of Science, Pruhonice, Czech Republic

*Correspondence to kimmo.rumpunen@vv.slu.se

SUMMARY

In this paper, five studies on diversity in the plant genus *Chaenomeles* are summarised. Genetic resources in the genus *Chaenomeles* were evaluated using molecular markers and morphological characters. Population differentiation and diversity were estimated in cultivated and wild plant material. In agreement with studies on cultivated *Chaenomeles* material, native material of *C. japonica* was strongly differentiated from *C. speciosa*, *C. cathayensis* and *C. thibetica*. Populations of *C. japonica* and *C. speciosa* were considerably more diverse than populations of *C. cathayensis* and *C. thibetica*. Furthermore, evidence of spontaneous hybridisation in native populations between *C. cathayensis* and *C. speciosa* was obtained. Random amplified polymorphic DNA markers and morphological characters revealed concordant patterns of genetic relatedness among the offspring families studied. To increase diversity in breeding populations and collections, different strategies for sampling of wild plant material are proposed for *C. japonica* and *C. speciosa* on the one hand, and *C. cathayensis* and *C. thibetica* on the other.

INTRODUCTION

The genus *Chaenomeles*

The East Asian plant genus *Chaenomeles* Lindley belongs to the subfamily Maloideae of the ecologically and economically important family Rosaceae (Phipps *et al*. 1990). Within the Maloideae, *Chaenomeles* is most closely related to the genera *Cydonia* (quince), *Docynia*, *Malus* (apple) and *Pyrus* (pear). Four species, *C. cathayensis* (Hemsl.) Schneider (Chinese quince), *C. japonica* (Thunb.) Lindl. (Japanese quince or dwarf Japanese quince), *C. speciosa* (Sweet) Nakai (flowering quince), and *C. thibetica* Yü (Tibetan quince) are now recognised in the genus *Chaenomeles* (Phipps *et al.* 1990). Throughout its history, however, taxonomic confusion has been extensive for *Chaenomeles*. Early mistakes were most likely made in the absence of complete herbarium specimens. Besides, there are few distinctive genus-specific and species-specific characters. The *Chaenomeles* plants are also phenotypically variable, which makes taxonomic work based on morphological characters difficult. However, Weber (1964) claims that: "by using the distinguishing characters of the adult leaves, the confusion between the three species (*C. thibetica* not studied by Weber) of *Chaenomeles* and the two of *Cydonia* could have been completely avoided". The separation of the two species in *Cydonia* is now supported by morphological studies of the fruits (Rataru & Ponomarenko 1993) and by molecular studies (Kaneko *et al*. 2000). Nevertheless, whereas separation of the genera *Cydonia* and *Chaenomeles* is possible, it is difficult to discriminate among some of the species within *Chaenomeles* using morphological characters only. This is especially valid for the

most variable species *C. speciosa* and its hybrids with *C. cathayensis* and *C. japonica*, as well as for the two similar species *C. cathayensis* and *C. thibetica* (Rumpunen 2002).

The taxonomic confusion and the fact that not all species had been studied previously were two reasons to investigate the genus using morphological traits and various molecular markers. We also wanted to obtain information on how genetic variation is partitioned in the genus, in order to develop strategies for collection and conservation of germplasm. Five investigations were performed and are reviewed in this paper, which to a large extent reproduces the summary of a thesis (Rumpunen 2001). The first and second investigations focused on diversity in cultivated plant material (Bartish *et al*. 1999, Garkava *et al*. 2000). The third and fourth investigations dealt with diversity in wild plant material (Bartish *et al*. 2000a, 2000b). In the fifth investigation, molecular and morphological estimates of diversity in wild plant material were compared (Rumpunen & Bartish 2002).

DIVERSITY IN CULTIVATED *CHAENOMELES* PLANT MATERIAL

Characterisation of genetic diversity using RAPDs

Various molecular marker systems have proved their value for characterisation of plant genetic resources both in collections and in wild populations. Thus the application of *e.g.* isozymes, RFLPs (restriction fragment length polymorphism), RAPDs (random amplified polymorphic DNA), AFLPs (amplified fragment length polymorphism) and STMS (sequence tagged microsatellite sites) has become an important feature in taxonomy and plant breeding. The application of these markers to plant collections and breeding material has been reported for a wide variety of crops (review in Weising *et al.* 1994, Goulão *et al*. 2001a, 2001b).

In spite of some well-documented concern about reproducibility and reliability (Thormann *et al*. 1994, Halldén *et al*. 1996) of RAPD analysis (Williams *et al*. 1990), this method has become a generally adopted technique for studying genetic relationships among and within groups of closely related plant taxa (Nybom & Bartish 2000). Single short random primers (usually 10 base pairs) are used in a polymerase chain reaction with extracted plant DNA to generate polymorphic markers. After separation by gel electrophoresis, the resulting band pattern of amplified fragments is visualised by ethidium bromide staining and scored.

The first study (Bartish *et al*. 1999) was undertaken to investigate the feasibility of using RAPD analysis for characterisation of genetic diversity among taxa, populations and individual plants, in a selected subset of a large collection of *Chaenomeles* genotypes at Balsgård–Department of Horticultural Plant Breeding, Swedish University of Agricultural Sciences. The collection was gathered from botanical gardens and from orchards, to provide a basis for a joint Latvian-Lithuanian-Swedish breeding project. Three species, *C. japonica*, *C. speciosa* and *C. cathayensis*, as well as the hybrid taxon *C*. x *superba* were represented in the collection. The fourth species, *C. thibetica*, was not available at that time.

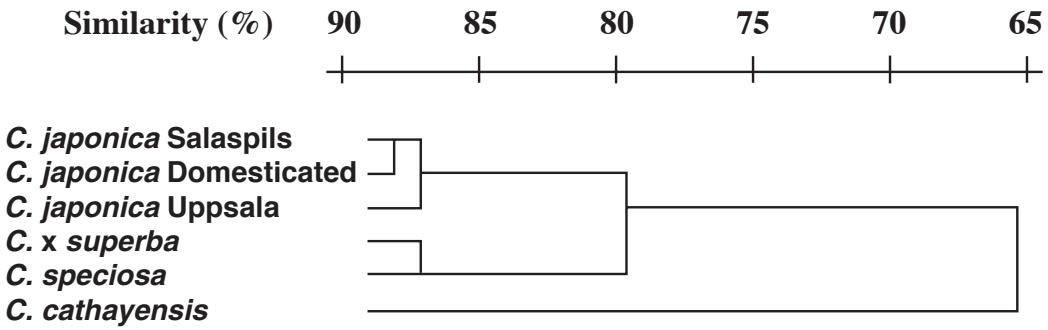

Figure 1. Dendrogram based on UPGMA analysis of genetic similarity estimates (cosine similarity matrix derived from mean RAPD band frequencies) among taxa and accessions of *Chaenomeles* (Bartish *et al.* 1999).

The results of the RAPD analysis (Bartish *et al*. 1999) were in general agreement with previous taxonomic investigations based on morphological characters. In this paper, interspecific variation was studied mainly in *C. japonica*; plants from different populations (sources), including one domesticated population, formed well separated groups in a cluster analysis (Figure 1).

Estimates of genetic relatedness through the application of coefficients of similarity, indices of uniformity, cluster analyses and multidimensional scaling (MDS), showed that *C. cathayensis* and *C. japonica* were the most distantly related species, and that *C. cathayensis* was comparatively homogeneous. *Chaenomeles speciosa* took a somewhat intermediate position between these two species as shown in a MDS plot (Figure 2), and by the identical similarity coefficients obtained from interspecific pairwise comparisons between this species and each of the other two species. Moreover, *C. speciosa* and the hybridogenous taxon *C.* x *superba* were considerably more heterogeneous than the other two species.

Characterisation of genetic diversity using isozymes

The second study (Garkava *et al*. 2000) investigated the feasibility of using isozyme analysis for characterisation of genetic diversity among taxa, accessions and individual plants of *Chaenomeles*. Only one minor isozyme study had previously been reported in *Chaenomeles* (Ponomarenko 1990). We also aimed to compare isozyme data with previously published RAPD data on the same plant material (Bartish *et al*. 1999), and to determine the relative efficiency of these two marker systems.

We noticed that a cluster analysis as well as a multidimensional scaling analysis (Figure 3), based on isozymes, grouped the taxa in agreement with previously published results obtained with RAPD analysis (Bartish *et al*. 1999). We concluded that *C. japonica* and *C. cathayensis* were the most distantly related species, whereas *C. speciosa* took an intermediate position together with the hybrid taxon *C*. x *superba*. Similarity matrices obtained with isozymes and RAPDs, respectively, were closely correlated, r=0.74. The previously noted low level of RAPD variability in *C*. *cathayensis* was also indicated with isozymes.

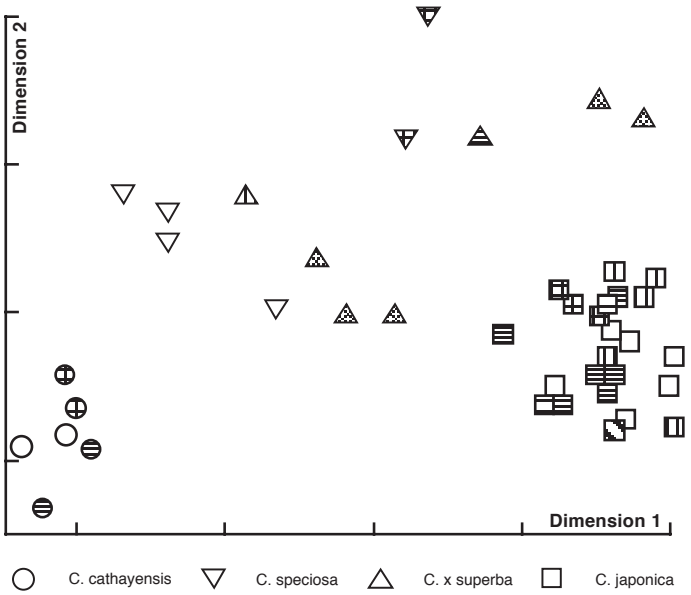

Figure 2. Two-dimensional plot from a multidimensional scaling analysis (MDS) of genetic dissimilarity (reversed and rescaled Jaccard's similarity coefficient based on RAPD data) among individual plants of *Chaenomeles* (Bartish *et al*. 1999).

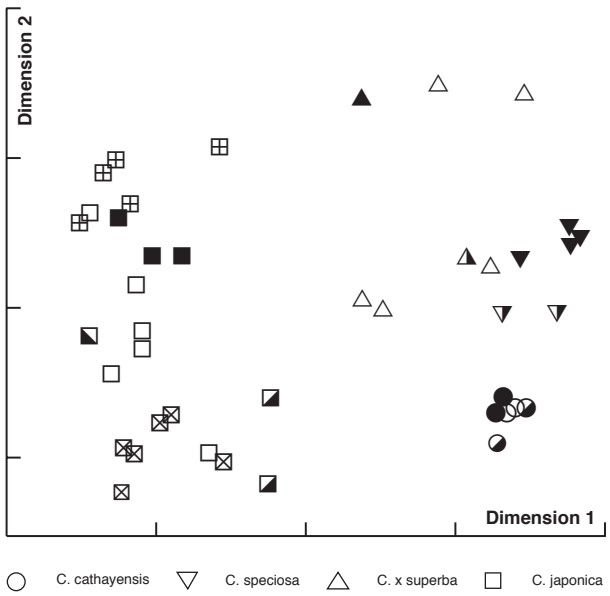

Figure 3. Plot of MDS analysis of genetic similarity estimates (Jaccard's coefficient) for isozyme data, showing groups among individual plants. *Chaenomeles cathayensis* and *C. japonica* form two well-separated groups, whereas *C. speciosa* and *C.* x *superba* together form a third intermediate group (Garkava *et al*. 2000).

However, the isozyme data were less efficient than the RAPD data for intraspecific grouping of the genotypes according to the origin of the plant material.

DIVERSITY IN WILD *CHAENOMELES* PLANT MATERIAL

Because of possible interspecific hybridisation among accessions from botanical gardens, it was not clear to what extent the results obtained (Bartish *et al*. 1999, Garkava *et al*. 2000) would be representative of indigenous populations of *Chaenomeles*. Furthermore, information on population structure of wild plant material would be useful for development of strategies for efficient sampling. A large collection of wild Chinese and Japanese *Chaenomeles* accessions was therefore assembled (Table 1). A population of *C. thibetica* was included which, for the first time, enabled a complete study of the genus *Chaenomeles*. It is our intention that this germplasm will be evaluated for adaptation and horticultural traits when adult plants of all species are available.

Diversity and phylogenetic relationships estimated by RAPDs and isozymes

The purpose of the third study (Bartish *et al.* 2000a) was to determine population structure and gene diversity for species and populations within *Chaenomeles,* and to investigate the correspondence between RAPD- and isozyme-based data sets in analyses of phylogenetic relationships, population structure and relative gene diversity estimates.

We found highly significant correlations between RAPD and isozyme analyses for both phenetic distances and gene diversity estimates (Bartish *et al.* 2000a). In agreement with previous studies on cultivated *Chaenomeles* material (Bartish *et al*. 1999, Garkava *et al*. 2000), *C. japonica* was clearly differentiated from *C. speciosa* and *C. cathayensis* (Figure 4). The recently recognised species *C. thibetica* appeared to be rather closely related to *C. cathayensis* (Figure 5). Populations of *C. japonica* and *C. speciosa* were considerably more diverse than populations of *C. cathayensis* and *C. thibetica* (Table 2). Correspondingly, most of the total variability could be attributed to within-population differentiation in the case of *C. japonica* and *C. speciosa,* and to between-population differentiation in the case of *C. cathayensis*. A difference in mating systems among the species was suggested as a possible explanation for these results. A discordant pattern was found between RAPDs and isozymes in the analyses of popu-

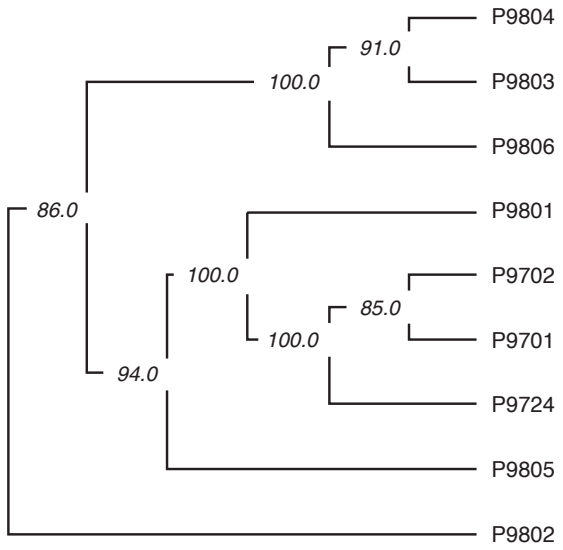

Figure 4. Dendrogram of phylogenetic relationships between populations of *Chaenomeles* (see Table 1 for ID) calculated on RAPD null allele frequencies by the neighbour joining method with bootstrap support (%) for each node of the tree (Bartish *et al.* 2000a).

Table 1. Accession identity (ID, for family and population), number of individuals (*n*) and origin of the nine *Chaenomeles* populations investigated (Bartish *et al.* 2000a).

ID	*n*	Species	Origin (co-ordinates[1,2] and altitude)
P9701–1, 2, 3	21	*C. japonica*	Shionomuro, Imaichi, Tochigi, Japan (36°45'N, 139°48'E[1]; 275 m)
P9702–1, 2, 3	19	*C. japonica*	Noguchi, Nikko, Tochigi, Japan (36°45'N, 139°43'E[1]; 450 m)
P9724–1	21	*C. japonica*	Ibuki, Shiga, Japan (35°15'N, 136°20'E[2]; 900 m)
P9801–8, 9, 14	19	*C. speciosa*	Zhenyuan, Yunnan, China (23°50'N, 101°10'E[2]; 1800–2300 m)
P9802–1, 2, 11	21	*C. speciosa*[3]	Hutiaoxia, Zhongdian, Yunnan, China (27°50'N, 99°30'E[2]; 1800–2100 m)
P9803–1, 2, 3	21	*C. cathayensis*	Hutiaoxia, Zhongdian, Yunnan, China (27°50'N, 99°30'E[2]; 1800–2100 m)
P9804–10, 11, 25	21	*C. cathayensis*	Caojian, Yunlong, Yunnan, China (25°35'N, 98°05'E[2], 2500–2600 m)
P9805–8, 9, 14	21	*C. speciosa*[3]	Dali, Yunnan, China (25°35'N, 100°10'E[2]; 1900–2400 m)
P9806–6, 9, 11	21	*C. thibetica*	Yi'ong, Bomi, Tibet (30°00'N, 95°00'E[2]; 2500 m)

[1]precise, [2]estimated, [3]hybrid (Bartish *et al*. 2000b)

Table 2. Gene diversity estimated by Shannon's index (*H*') and by the Lynch and Milligan index (*H*) with standard error, within populations and species of *Chaenomeles*, based on RAPD and isozyme data (Bartish *et al.* 2000a).

Group	H'_{pop} RAPD	H'_{pop} isozyme	H_{pop} RAPD
C. cathayensis			
P9803	0.140 (0.024)	0.069 (0.025)	0.066 (0.012)
P9804	0.282 (0.032)	0.081 (0.026)	0.139 (0.017)
C. japonica			
P9701	0.487 (0.032)	0.422 (0.047)	0.229 (0.017)
P9702	0.467 (0.032)	0.375 (0.050)	0.212 (0.016)
P9724	0.424 (0.031)	0.413 (0.050)	0.196 (0.016)
C. speciosa			
P9801	0.547 (0.030)	0.503 (0.049)	0.253 (0.015)
P9802	0.520 (0.030)	0.419 (0.044)	0.239 (0.014)
P9805	0.589 (0.030)	0.460 (0.044)	0.258 (0.014)
C. thibetica			
P9806	0.286 (0.029)	0.189 (0.052)	0.141 (0.015)
Group	H'_{sp} RAPD	H'_{sp} isozyme	H_{sp} RAPD
C. cathayensis	0.337 (0.032)	0.284 (0.056)	0.160 (0.015)
C. japonica	0.620 (0.032)	0.612 (0.050)	0.278 (0.016)
C. speciosa	0.706 (0.029)	0.655 (0.043)	0.306 (0.014)
Group	H'_{ge} RAPD	H'_{ge} isozyme	H_{ge} RAPD
Chaenomeles	0.746 (0.013)	0.665 (0.024)	0.282 (0.008)

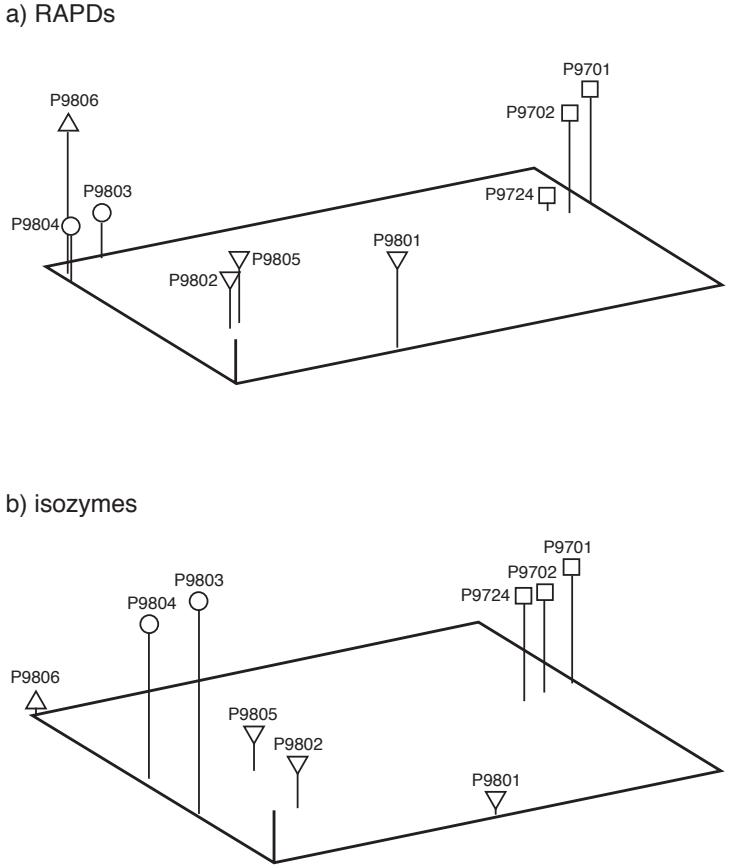

Figure 5. Three-dimensional representation of principal co-ordinate analysis of phenetic relationships between populations of *Chaenomeles* (see Table 1 for ID). Percentage explained variability was for the a) phenetic RAPD-based data set: PC1 62.4%, PC2 16.0% and PC3 5.9%; b) isozyme-based data set: PC1 44.2%, PC2 17.2% and PC3 12.9% (Bartish *et al*. 2000a).

lation structure within *C. japonica*. A higher proportion of non-neutral markers for isozymes compared to RAPDs may explain the result. This finding also demonstrates the importance of using multiple molecular marker systems in studies of population structure.

Interspecific hybridisation revealed

Both isozyme and RAPD data suggested that two of the populations analysed (P9802 and P9805) had been derived through interspecific hybridisation between *C. cathayensis* and *C. speciosa* (Bartish *et al*. 2000a). In a fourth study (Bartish *et al.* 2000b), the previously obtained RAPD data set was re-analysed to study within-family (family being siblings derived from one fruit as a result of open pollination) marker frequencies in five populations, including the parental species as well as the putative hybrids, to corroborate this hypothesis. In addition, chloroplast DNA polymorphism and numerous morphological characters were analysed in the same five populations.

The analysis of diagnostic RAPD markers and of chloroplast DNA haplotypes supported the notion of spontaneous hybridisation, and suggested that there had been symmetrical, rather than unidirectional, introgression between *C. cathayensis* and *C. speciosa*. Moreover, absolute concordance was found between the percentage of (inviable) albino seedlings and the cpDNA haplotype. Albinos thus always appeared in families with the *C. speciosa* haplotype but never in families with the *C. cathayensis* haplotype. The chloroplast genome in *Chaenomeles* is probably uniparental in origin and maternally inherited like in most other angiosperms. Consequently, an active selection/linkage mechanism of postzygotic isolation between *C. speciosa* and *C. cathayensis* was indicated. RAPDs and morphological characters revealed concordant patterns of genetic relatedness among the offspring families studied (Figures 6 and 7). Some putative hybrid families had mainly intermediate characters, whereas others appeared to be later generation hybrids since they were genetically and phenetically rather similar to families that appeared to

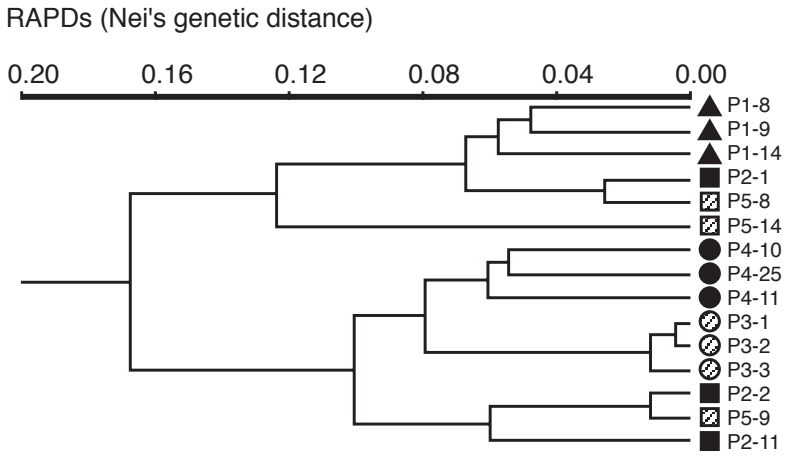

Figure 6. UPGMA dendrogram of a RAPD-based data set (Nei's genetic distances) for families of *Chaenomeles*. The different populations are represented by triangles (*C. speciosa*), circles (*C. cathayensis*) and squares (hybrids) (Bartish *et al*. 2000b).

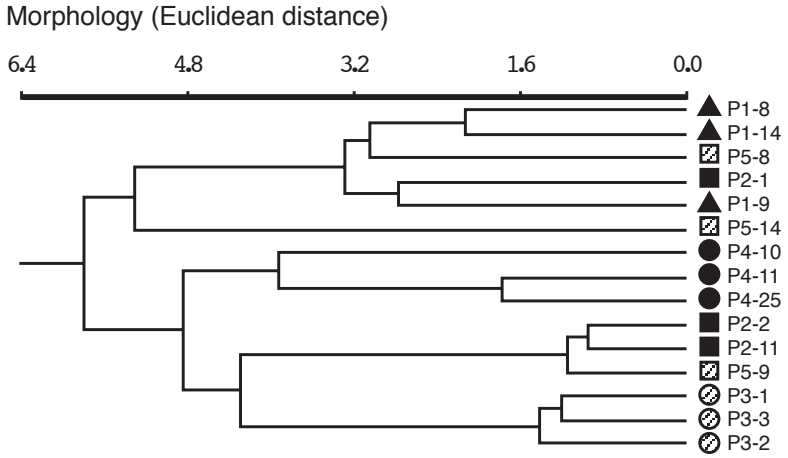

Figure 7. UPGMA dendrogram of a morphological data set (Euclidean distances) for families of *Chaenomeles*. The different populations are represented by triangles (*C. speciosa*), circles (*C. cathayensis*) and squares (hybrids) (Bartish *et al*. 2000b).

Table 3. Partitioning of genetic variability between families within populations, estimated for the RAPD data set as *G'*-statistics (derived from Shannon's Index) with standard error (SE) and *Φ*-statistics (AMOVA) with P-value. Values in bold refer to the largest estimates of variability (Bartish *et al*. 2000b).

Population	*G'*	(SE)	*Φ*	P-value
P9801 *C. speciosa*	0.520	(0.024)	0.193	(0.0001)
P9802 Hybrid	**0.740**	(0.032)	**0.530**	(0.0001)
P9803 *C. cathayensis*	0.182	(0.020)	0.025	(0.1965)
P9804 *C. cathayensis*	0.289	(0.022)	0.371	(0.0001)
P9805 Hybrid	**0.630**	(0.027)	**0.530**	(0.0001)

represent pure species. The RAPD-based proportion of between-family variability was considerably higher in the putatively hybridogenous populations than in populations of the pure species (Table 3). Within-family gene diversity estimates ranged from *C. speciosa* (max. *Hj* = 0.235) to *C. cathayensis* (min. *Hj* = 0.094) with the presumed hybrid families taking intermediate values.

COMPARING MORPHOLOGICAL AND MOLECULAR DIVERSITY

It is difficult or even impossible to predict the genetic structure of quantitative characters in populations from molecular marker genes alone (Podolsky & Holtsford 1995, Latta 1998, Waldmann & Andersson 1998, 1999). Empirical studies of variation in several quantitative characters are thus not only important for the development of strategies for long-term conservation of species, but also for the evaluation of potential use and gain in domestication and plant breeding. However, when molecular markers and quantitative characters are analysed in the same plant material, more conclusive information is obtained.

Statistical approaches

Evaluation of molecular and morphometric data usually involves different statistical methods, which make comparisons difficult. Furthermore, the evaluation of concordance is often qualitative rather than quantitative, thus precluding comparisons on an absolute scale. The array of commonly used statistical methods for data analysis comprises multivariate analyses (*e.g*. cluster analyses and ordination analyses), univariate analyses and different diversity indices which, in the case of molecular data, were most often developed for co-dominant markers.

Multilocus (CF_{ST}) and multitrait (CQ_{ST}) measures of differentiation have been developed (Kremer *et al*. 1997, Waldmann & Andersson 1999), which account for associations between alleles [G_{ST} (Nei 1973) and F_{ST} (Weir & Cockerham 1984, Weir 1990)] or traits [Q_{ST} (Spitze 1993, Podolsky & Holtsford 1995)]. These measures may yield estimates that are more appropriate for comparison, but the biological interpretation of the statistic parameters obtained is not clear (Latta 1998). Besides, hypotheses are tested by different parametric tests relying on the theory of normal distribution, which is not always appropriate. A permutational test, as implemented in the flexible AMOVA framework (Excoffier *et al*. 1992), which does not rely on the basic assumptions of the parametric tests, is therefore a useful complement, developed for analysis of molecular variance and Φ-statistics (F-statistic analogues).

Although AMOVA was intended for co-dominant RFLP-data, it has proved to be most useful also for dominant RAPD-data under certain assumptions (Huff *et al*. 1993, Stewart & Excoffier 1996). AMOVA has been used extensively in analysis of RAPD-data in studies of diploid population differentiation (for references see Bartish *et al*. 1999, Nybom & Bartish 2000). In the case of RAPDs, the statistical parameters are derived from a matrix of squared Euclidean distances between all phenotypes, instead of between haplotypes as in the original framework. This makes the AMOVA methodology most appropriate also for calculation of variance components for both qualitative and quantitative multi-trait data with the same statistics. Furthermore, it enables hierarchical partitioning of the variance and thereby should allow calculation of standardised differentiation estimates for *e.g.* RAPDs and morphological characters.

Image analysis of leaf shape

Morphological characters are often studied by automated image analysis. Objective information can be simultaneously captured for several descriptors and large amounts of samples can be analysed in a short time. In addition, a digitised image may be re-analysed, with new descriptors, without having to return to the original objects (White *et al.* 1988). Traditional measures, such as leaf area, different length and width ratios, dentation etc. can be obtained, but shape descriptors based on the digitised outline are also available. Most commonly, measures of landmarks and pseudo-landmarks (West & Noble 1984, Dickinson *et al.*1987) and the set of coefficients of Fourier transforms (Kuhl & Giardina 1982, Rohlf & Archie 1984) have been applied.

The elliptic Fourier method (Kuhl & Giardina 1982), a development of the classic Fourier transform, is particularly suitable for the quantitative description of a closed form, such as the outline of a leaf. Ellipses, defined by four coefficients for each harmonic, corresponding to Fourier series of sine and cosine curves of decreasing amplitude and period, are fitted to the object outline. The infinite Fourier series are truncated after a finite number of harmonics depending on the complexity of the object and the pre-determined error of fit. The number of harmonics needed to accurately describe the shape may also be empirically settled. By normalisation, it is possible to make the elliptic Fourier coefficients invariant to size, rotation and starting point of the trace (Kuhl & Giardina 1982). The first few Fourier coefficients are expected to define overall shape, whereas finer details may be described with an increasing number of harmonics. Since the elliptical leaves of all species within *Chaenomeles* lack clear landmarks, but are considered to be highly useful in taxonomy (Weber 1964), elliptic Fourier coefficients may be particularly appropriate to describe the shape of *Chaenomeles* leaves.

Comparing morphological and molecular data using AMOVA

The purposes of the fifth study (Rumpunen & Bartish 2002), were to evaluate and compare the discriminatory power of elliptic Fourier coefficients and metric descriptors for leaf shape data in the genus *Chaenomeles*. We also wanted to compare the partitioning of variance and estimates of differentiation for morphometric and molecular data, using the AMOVA approach as a tool for analysis of multi-trait data and molecular data in concert.

Variation in average leaf shape (based on standardised Elliptic Fourier coefficients), among species and families in the genus *Chaenomeles* is shown in Figure 8. From these plots the difference between the

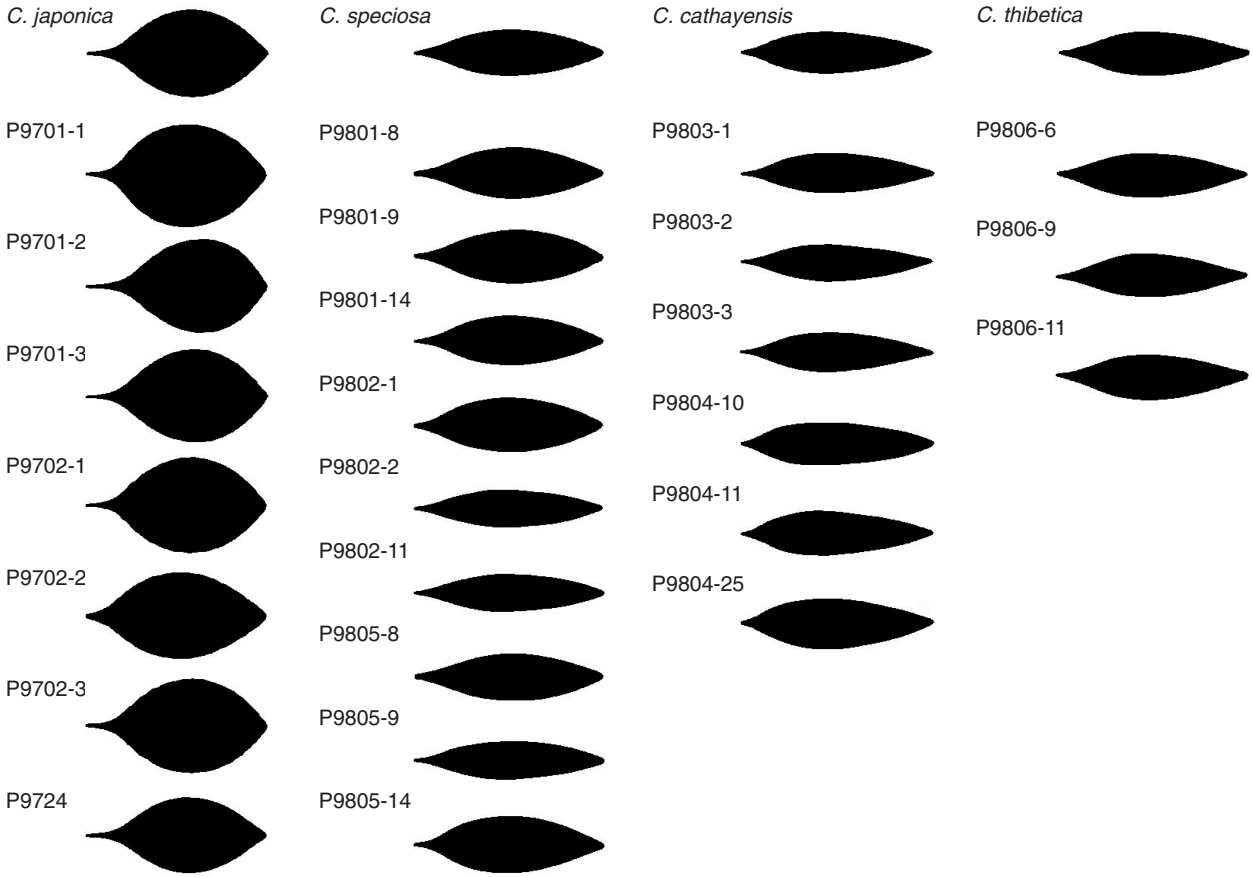

Figure 8. Variation in average leaf shape among species and families in the genus *Chaenomeles*. The illustration is based on average elliptic Fourier coefficients for species (uppermost in each column) and maternal families (below in the same column) (Rumpunen & Bartish 2002).

Japanese and the Chinese species is obvious. There is also a noticeable variation in leaf shape between families within all species.

We concluded that for normalised elliptic Fourier coefficients (EFC) 30 harmonics, and for power series of normalised elliptic Fourier coefficients (PEFC) 40 harmonics, were needed to achieve 100% correct reassignment of plants. By contrast, classical metric descriptors (MD) were considerably less efficient and only 87%, 77% and 66% of the plants were correctly reassigned to species, populations and families respectively. Furthermore, molecular RAPD data and data based on PEFC were the most concordant data sets as revealed by cluster analyses (Rumpunen & Bartish 2002).

All descriptor sets in the AMOVA partitioned the variance in an approximately similar way (Table 4). A surprisingly high correlation was found between the RAPD based data and the data based on elliptic Fourier transforms for the among-family estimates of variance components (RAPD vs. PEFC: 0.89, P=0.003, RAPD vs. EFC: 0.86, P=0.006). In contrast, only a moderate correlation was found between RAPD and MD data (0.71, P=0.049). This may indicate that shape *per se* (the size-component excluded) is a less biased estimator of genetic variation than metric leaf descriptors in the genus *Chaenomeles*. Estimates of population differentiation based on EFC and PEFC were always lower than differentiation estimates based on RAPDs, whereas the differentiation estimates based on MD were in general higher than estimates based on RAPDs, except for one species (*C. speciosa*) including hybrid populations (Table 5). The proposed and empirically tested AMOVA approach thus seemed most useful for evaluation of molecular and quantitative data and should be further tested on more data sets.

CONCLUSION

The large genetic diversity in the genus *Chaenomeles* as inferred from molecular markers is advantageous for crop improvement through breeding and selection. Isozyme markers were less efficient than RAPD (random amplified polymorphic DNA) markers for intraspecific grouping of the genotypes ac-

Table 4. Distribution of variation in three species of the genus *Chaenomeles* as revealed by variance components (AMOVA, nested analysis) for three morphometric data sets (elliptic Fourier coefficients, EFC; power elliptic Fourier coefficients, PEFC and metric descriptors, MD) and one molecular data set (RAPD). *Chaenomeles thibetica* was excluded from the analysis because it was represented by only one population. The result is presented as the percentage of the total variance (Rumpunen & Bartish 2002).

Group	Observed partition of variance (%)							
	MD	(SE)	EFC	(SE)	PEFC	(SE)	RAPD	(SE)
Among populations								
C. japonica	15.2	(3.5)	-0.4	(1.8)	4.2	(1.6)	9.5	(1.0)***
C. speciosa	5.2	(1.8)	2.5	(1.5)	3.4	(1.5)	8.2	(0.8)
C. cathayensis	57.6	(3.0)***	12.3	(2.8)***	22.0	(1.3)***	40.3	(1.1)***
Among families within populations								
C. japonica	27.7	(4.6)***	6.0	(1.8)**	8.6	(1.8)***	24.2	(0.6)***
C. speciosa	26.5	(3.1)***	8.5	(1.8)***	21.4	(0.5)***	38.1	(1.1)***
C. cathayensis	18.0	(3.9)***	-0.6	(1.3)	4.6	(1.2)**	13.0	(0.9)***
Within families								
C. japonica	57.0	(2.7)***	94.3	(1.6)***	87.2	(1.6)***	66.3	(1.1)***
C. speciosa	68.3	(1.5)***	89.0	(2.4)***	75.2	(1.2)***	53.7	(0.6)***
C. cathayensis	24.4	(1.7)***	88.3	(3.9)***	73.3	(2.0)***	46.8	(1.2)***

P-value estimated by permutational analysis of the null distribution of the variance components: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Standard error (SE) estimated by a jackknife procedure.

Table 5. Differentiation estimates (Φ-statistics) for species within the genus *Chaenomeles* based on hierarchical analysis of variance for different descriptor sets: metric descriptors (MD), elliptic Fourier coefficients (EFC), power elliptic Fourier coefficients (PEFC) and molecular phenotypes (RAPD). Φ_{POP} estimates differentiation of populations within species, $\Phi_{FAM(POP)}$ estimates differentiation of families within populations and Φ_{TOT} estimates differentiation for populations and families (Rumpunen & Bartish 2002).

Species	Φ_{POP}	(SE)	$\Phi_{FAM(POP)}$	(SE)	Φ_{TOT}	(SE)
C. japonica						
MD	0.152	(0.035)	0.327	(0.046)***	0.430	(0.027)***
EFC	-0.004	(0.018)	0.061	(0.018)**	0.057	(0.016)***
PEFC	0.042	(0.016)	0.090	(0.018)***	0.128	(0.016)***
RAPD	0.095	(0.010)***	0.268	(0.007)***	0.337	(0.011)***
C. speciosa						
MD	0.052	(0.018)	0.280	(0.028)***	0.317	(0.015)***
EFC	0.025	(0.015)	0.087	(0.018)***	0.110	(0.024)***
PEFC	0.034	(0.015)	0.221	(0.004)***	0.248	(0.013)***
RAPD	0.082	(0.008)	0.415	(0.009)***	0.463	(0.006)***
C. cathayensis						
MD	0.576	(0.030)***	0.425	(0.064)***	0.756	(0.017)***
EFC	0.123	(0.028)***	-0.007	(0.015)	0.117	(0.039)*
PEFC	0.221	(0.013)***	0.059	(0.016)**	0.267	(0.020)***
RAPD	0.403	(0.011)***	0.217	(0.014)***	0.532	(0.012)***

P-value estimated by permutational analysis of the null distributions. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Standard error (SE) estimated by a jackknife procedure.

cording to the origin of the plant material. However, highly significant correlations were found between the two different marker systems for gene diversity estimates. Populations of *C. japonica* and *C. speciosa* were considerably more diverse than populations of *C. cathayensis* and *C. thibetica*. Thus, different strategies should be employed when collecting and preserving plant material of *C. japonica* and *C. speciosa* on the one hand, and *C. cathayensis* and *C. thibetica* on the other.

LITERATURE

Bartish I.V., Jeppson N., Nybom H. 1999. Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by random amplified polymorphic DNA (RAPD) markers. Molecular Ecology 8: 791–802.

Bartish I.V., Garkava L. P., Rumpunen K., Nybom H. 2000a. Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes. Theoretical and Applied Genetics 101: 554–563.

Bartish I.V., Rumpunen K., Nybom H. 2000b. Combined analyses of RAPDs, cpDNA and morphology demonstrate spontaneous hybridization in the plant genus *Chaenomeles*. Heredity 85: 383–392.

Dickinson T.A., Parker W.H., Strauss R.E. 1987. Another approach to leaf shape comparison. Taxon 36: 1–20.

Excoffier L., Smouse P.E., Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Garkava L.P., Rumpunen K., Bartish I.V. 2000. Genetic relationships in *Chaenomeles* (Rosaceae) revealed by isozyme analysis. Scientia Horticulturae 85: 21–35.

Goulão L., Cabrita L., Oliveira C.M., Leitão J.M. 2001a. Comparing RAPD and AFLP analysis in discrimination and estimation of genetic similarities among apple (*Malus domestica* Borkh.) cultivars. Euphytica 119: 259–270.

Goulão L., Monte-Corvo L., Oliveira C.M. 2001b. Phenetic characterization of plum cultivars by high multiplex ratio markers: Amplified fragment length polymorphisms and inter-simple sequence repeats. Journal of the American Society for Horticultural Science 126: 72–77.

Halldén C., Hansen M., Nilsson N.-O., Hjerdin A., Säll T. 1996. Competition as a source of errors in RAPD analysis. Theoretical and Applied Genetics 93: 1185–1192.

Huff D.R., Peakall R., Smouse P.E. 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.). Theoretical and Applied Genetics 86: 927–934.

Kaneko Y., Nagaho I., Bang S.W., Matsuzawa Y. 2000. Classification of flowering quince cultivars (genus *Chaenomeles*) using random amplified polymorphic DNA markers. Breeding Science 50: 139–142.

Kremer A., Zanetto A., Ducousso A. 1997. Multilocus and multitrait measures of differentiation for gene markers and phenotypic traits. Genetics 145: 1229–1241.

Kuhl F.P., Giardina C.R. 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing 18: 236–258.

Latta R.G. 1998. Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. The American Naturalist 151: 283–292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America 12: 3321–3323.

Nybom H., Bartish, I. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in plant ecology, evolution and systematics 3: 93–114.

Phipps J.B., Robertson K.R., Smith P.G., Rohrer J.R. 1990. A checklist of the subfamily Maloideae (Rosaceae). Canadian Journal of Botany 68: 2209–2269.

Podolsky R.H., Holtsford T.P. 1995. Population structure of morphological traits in *Clarkia dudleyana* I. Comparison of F_{ST} between allozymes and morphological traits. Genetics 140: 733–744.

Ponomarenko N.S. 1990. Biological characteristic and intraspecific variability of *Chaenomeles japonica* (Thunb.) Lindl. ex. Spach in Moldova. Synopsis of thesis. Botanical garden, Kishinev 1–22. (in Russian)

Rataru G.I., Ponomarenko N.S. 1993. Anatomical features of pericarp structure in *Chaenomeles* Lindl. species. Buletinul Academiei de Stiinte a Republicii Moldova, Stiinte Biologice si Chimice 5: 9–15. (in Russian)

Rohlf F.J., Archie J.W. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). Systematic Zoology 33: 302–317.

Rumpunen K. 2001. Diversity in the plant genus *Chaenomeles*. Doctoral thesis. Acta Universitatis Agriculturae Sueciae, Agraria 293.

Rumpunen K. 2002. *Chaenomeles*: potential new fruit crop for northern Europe. In: Janick J. & Whipkey A. (Eds.) Trends in new crops and new uses. ASHA Press, Alexandria, VA, USA 385–392.

Rumpunen K., Bartish I.V. 2002. Comparison of differentiation estimates based on morphometric and molecular data, exemplified by various leaf shape descriptors and RAPDs in the genus *Chaenomeles*. Taxon 51: 69–82.

Spitze K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozyme variation. Genetics 135: 367–374.

Stewart C.N. Jr, Excoffier L. 1996. Assessing population genetic structure and variability with RAPD data: application to *Vaccinium macrocarpon* (American cranberry). Journal of Evolutionary Biology 9: 153–171.

Thormann C.E., Ferreira M.E., Camargo L.E.A., Tivang J.G., Osborn T.C. 1994. Comparison of RFLP and RAPD markers to estimating genetic-relationships within and among cruciferous species. Theoretical and Applied Genetics 88: 973–980.

Waldmann P., Andersson S. 1998. Comparison of quantitative genetic variation and allozyme diversity within and between populations of *Scabiosa canescens* and *S. columbaria*. Heredity 81: 79–86.

Waldmann P., Andersson S. 1999. Multilocus and multitrait differentiation of populations of the locally rare plant *Scabiosa canescens* and the more common *S. columbaria*. Hereditas 130: 341–343.

Weber C. 1964. The genus *Chaenomeles* (Rosaceae). Journal of the Arnold Arboretum 45: 161–205, 302–345.

Weir B.S., Cockerham C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Weir B.S. 1990. Genetic data analysis. Sunderland, MA.

Weising K., Nybom H., Wolff K., Meyer W. 1995. DNA fingerprinting in plants and fungi. CRC Press Inc., Florida, USA.

West J.G., Noble I.R. 1984. Analyses of digitised leaf images of the *Dodonaea viscosa* complex in Australia. Taxon 33: 595–613.

White R.J., Prentice H.C., Verwijst T. 1988. Automated image acquisition and morphometric description. Canadian Journal of Botany 66: 450–459.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6535.