Breeding Strategies for the Fruit Crop Japanese Quince (Chaenomeles japonica)

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#### SUMMARY

In this paper, an ideotype for the fruit crop Japanese quince (*Chaenomeles japonica*) is established and traits to be specifically considered for selection and breeding are discussed: adaptation and hardiness, disease resistance, thorns, suckering, growth, rooting, time of ripening, yield, amenability for mechanical harvesting and fruit quality. In addition, test guidelines and descriptors for *Chaenomeles* species are presented. Short-term and long-term breeding strategies are suggested, based on a study of general and specific combining ability for plant vegetative traits, fruit yield and morphology traits, and fruit biochemistry traits. An efficient breeding strategy for Japanese quince could be based on recurrent selection. However, extensive test crosses and progeny tests in well-designed field trials should also be considered since some important traits are controlled by additive as well as non-additive genes.

#### **IDEOTYPE FOR THE FRUIT CROP JAPANESE QUINCE**

In the beginning of a breeding programme, a preliminary ideotype (Donald 1968) should be explicitly defined, to determine the kind of plant material needed. The ideotype should be redefined continuously, as biological and genetic knowledge accumulates. The ideotype established here for Japanese quince (*Chaenomeles japonica*) as a fruit crop is based on: 1) available knowledge on variation in plant growth and development, 2) a target area for cultivation in the Baltic countries and southern Scandinavia, 3) organic production methods (no use of pesticides or herbicides), 4) mechanical harvest, and 5) industrial processing. Taking these factors into account, the ideal plant must be locally adapted, tolerant to diseases, erect, not too dense, easy to propagate, high yielding, have few branches and no thorns, produce few root suckers, ripen early and have fruits that drop easily at harvest. In addition, fruit internal quality must satisfy the specific demands of the food industry as regards juice, flavour and dietary fibre.

#### **Adaptation and hardiness**

Since the species *C. japonica* is not perfectly adapted to the areas in the Baltic region where fruit production is intended, efforts should be devoted towards increasing local adaptation. Selection of hardy plant material will ensure that a severe winter does not destroy plantations and will promote high annual yields. A lack of hardiness in the Baltic plant material of Japanese quince has previously been compensated for by selection of plants with a spreading rather than erect growth habit. Thus, a snow-cover could protect

the plants from low winter temperatures. Unfortunately, cold winters with insufficient snow cover are common, and a spreading or prostrate growth habit is not compatible with other demands, *e.g.* weed control, phytosanitation and mechanical harvest.

#### **Disease resistance**

As Japanese quince becomes more widely cultivated, the crop will probably become more attacked by fungi, which are already a problem in other crops within the Rosaceae. Furthermore, since Japanese quince is a long-lived woody perennial, special attention must be paid to pest and diseases, and the plant material available should be screened for field resistant genotypes. Fungal populations and disease pressure can vary according to environmental conditions at the local site, which must be taken into account when field trials are designed. The most serious damage to fruits and shrubs seems to be caused by *Phlyctema vagabunda* (fruit spots and fruit rot) and *Botrytis cinerea* (fruit rot and die-back of twigs) (Rumpunen 2002). There is variation among populations in susceptibility to *P. vagabunda*, allowing resistance breeding (unpublished results). The unspecific behaviour of *B. cinerea* instead suggests the use of an avoidance strategy. Since the fungus seems to enter the twigs through remaining fruits in late fall, selection for fruit drop at maturity should reduce growth of grey mould from fruits into shoots and thus reduce die-back damage.

#### Thorns

Thorns are undesirable since they make handling of plants difficult and can also damage fruits during management and harvest. In contrast to many other fruit crops, the development of thorns is an adult character in Japanese quince. Selection cannot be successfully undertaken until the third year since juve-nile plants generally lack thorns altogether (Kviklys 1998, Rumpunen 2001).

#### Suckering

The production of root suckers is abundant in many genotypes of Japanese quince and is correlated to shrub density. However, suckering seems to be independent of yield parameters (Kviklys 1998). Selection for a lower number of suckers should therefore not have any negative impact on yield.

#### Growth

Growth can be separated into plant height, plant habit and branching. A high and erect plant with few branches would facilitate mechanical harvest. On a high plant, fruits would also become less contaminated by soil and therefore possibly less infected by fungal diseases. As the shrubs become less branched, they also become less dense. Thus the leaf foliage would dry more quickly, which might decrease attacks from leaf fungi, and more sunlight could reach the fruits, promoting a high internal fruit quality.

#### Rooting

The capacity for softwood cuttings to form roots is an important character, which seems to be predominantly governed by additive genes (Kviklys 1998). Shoots from erect plants are slightly more difficult to root than shoots from spreading plants. The capacity for root formation should be evaluated when promising genotypes are propagated and finally selected for evaluation in comparative trials.

#### **Time of ripening**

In the Baltic countries and southern Scandinavia, fruit ripening starts in mid-August with a peak in mid-September. Early ripening is especially important in marginal areas of cultivation, whereas varieties with a different ripening period may be valuable for harvesting and processing. Non-additive gene action (dominance and epistasis) seems to be pronounced for start of ripening (Kviklys 1998), and screenings should therefore be conducted in progenies derived from crosses among many selected parents.

# Yield

Yield is the best measure of integrated performance (Austin 1993) and at the same time, potential yield sets the limit for profitability. Selection for annual and high yields will integrate a range of physiological characters and plant response to a changing environment, and yield is thus a measure of overall adaptation provided that it is estimated over several years. A yield of 3 kg per plant in the fourth season and 4 kg or more in the fifth season (Rumpunen & Kviklys 2001) is considered relatively high, and should constitute the lower boundary for selection.

# Amenability for mechanical harvesting

To enable mechanical harvest, the plant should be erect, and fruits should drop easily when the shrub is shaken or when combed off the twigs. Fortunately, considerable variability exists in this trait (unpublished results), which besides being dependent on morphology of fruit and petiole, may also be associated with adaptation. At maturity, a natural fruit drop occurs, but late ripening plants may not develop a functional abscission layer.

# Fruit quality

Fruit quality can be separated into external and internal characters. Since the Japanese quince fruits are primarily intended for industrial use, appearance is of minor importance. However, fruits should be smooth to facilitate washing. The skin should have a sticky cuticle since this appears to be associated with strong fragrance. Skin bruises due to mechanical harvesting are a problem only when fruits are stored for a longer period. High content of fruit flesh and low content of seeds is desirable since the primary products are juice, aroma and dietary fibre.

# Test guidelines and descriptors

At present there are no specific UPOV test guidelines or official descriptors available for species in the genus *Chaenomeles*, either as fruit crops or as ornamental plants. An official list of descriptors would be highly useful, not only when new varieties are submitted for test of distinctness, uniformity and stability, but also when plants are described and core collections set up. Therefore, test guidelines and descriptors were developed and compiled for *Chaenomeles* species (see Appendix). The suggested test guidelines are based on UPOV test guidelines for apple, pear and quince.

# GENETIC CONTROL OF TRAITS IN JAPANESE QUINCE

A prerequisite for development of efficient breeding strategies is knowledge about the mating system and the mode of inheritance of specific traits (Moreno-González & Cubero 1993). Simple selection of parents based on their phenotypes is efficient only for highly heritable traits (Falconer & Mackay 1996). Therefore, different methods based on prediction of breeding values (Tancred *et al.* 1995, Durel *et al.* 1998, de Souza & Byrne 2000) and based on progeny testing (Nyquist 1991, Simmonds 1996, Oraguzie *et al.* 2001) have been developed. For this purpose, estimates of combining ability are particularly useful (Griffing 1956, Falconer & Mackay 1996). The presence of significant general combining ability (GCA) and lack of significant specific combining ability (SCA) indicates that the trait studied is mainly governed by additive gene action. The presence of significant differences in specific combining ability indicates that the trait is also in part attributable to non-additive gene action (dominance and epistasis). These estimates can thus provide information on patterns of inheritance of various traits and make it possible to predict the performance of progenies in controlled crossings. When additive effects predominate, parents for a breeding programme based on controlled crosses can be selected on the basis of their phenotypic (but preferably on their genotypic) performance. When non-additive effects are also important, selection of parents for creation of breeding populations should be based on progeny tests.

Despite the above-mentioned benefits, few quantitative genetic studies have been conducted in fruit crops (for references, see Durel *et al.* 1998). This is partly because many fruit crops have a long juvenile period and in applied breeding, crosses are not designed for genetic studies, plants are not randomised, and records of complete progenies are seldom kept. However, the short juvenile stage and the moderate plant size facilitate genetic studies of Japanese quince.

Phenotypic variation and patterns of inheritance of several plant and fruit traits have been studied in Japanese quince (Rumpunen & Kviklys 2002). Plants with contrasting characters were selected in seed-propagated commercial orchards in Lithuania, and crossed. Fourteen hybrid families were obtained with a total of 684 seedlings, for which five plant vegetative traits, eight fruit yield and morphology traits, and five fruit biochemistry traits were evaluated over seven years. General and specific combining ability were estimated and correlation coefficients were calculated among traits.

#### Inheritance of thorns

When thornless plants were inter-crossed, very few thorny plants were obtained in the progeny (4.8%). When thorny plants were inter-crossed, 65.7 % of the progeny became thorny, whereas crossing thorny plants with thornless plants produced 54.4% thorny offspring (Table 1). This suggests that thorniness is controlled by a single dominantly inherited gene, and by some modifiers.

Crosses	Thorns	(+/-)	Frequer	ncy(n) in	classes ((	)-3)	Total ( <i>n</i> )
Dam x Sire	Dam	Sire	0	1	2	3	
9219 x 9241	+	+	16	5	6	23	50
9260 x 9241	+	+	22	1	4	21	48
9222 x 9241	+	+	13	4	5	28	50
9217 x 9241	+	+	17	3	6	24	50
Total			68	13	21	96	198
Percentage in classes (%)			34.3	6.6	10.6	48.5	
9261 x 9241	_	+	17	8	4	20	49
9221 x 9241	_	+	27	1	6	15	49
9218 x 9241	_	+	34	2	4	9	49
Total			78	11	14	44	147
Percentage in classes (%)			53.1	7.5	9.5	29.9	
9217 x 9226	+	_	34	3	9	4	50
9219 x 9226	+	_	11	3	3	26	43
9260 x 9226	+	_	34	3	1	10	48
9222 x 9226	+	_	31	10	6	3	50
Total			110	19	19	43	191
Percentage in classes (%)			57.6	9.9	9.9	22.5	
9261 x 9226	_	_	44	3	1	0	48
9221 x 9226	_	_	48	1	1	0	50
9218 x 9226	_	_	49	1	0	0	50
Total			141	5	2	0	148
Percentage in classes (%)			95.3	3.4	1.4	0.0	
Total number of plants							684

**Table 1.** Segregation for thorns in 14 offspring families of Japanese quince (*C. japonica*) divided into four groups according to cross combination. The plants were scored in four classes: no thorns (0), very few thorns (1), few thorns (2) and many thorns (3) (Rumpunen 2001).

Trait	ACY96–00 (plant) r, (n)	ACY96–00 (family mean) r, (n)	
Annual yield (A	NY)		
1996	0.249*** (430)	0.350 ns (14)	
1997	0.599*** (627)	<b>0.940***</b> (14)	
1998	<b>0.806</b> *** (663)	0.933*** (14)	
1999	<b>0.729</b> *** (651)	0.958*** (14)	
2000	0.811*** (666)	<b>0.952</b> *** (14)	
Accumulated yi	ield (ACY)		
1996–97	<b>0.629***</b> (671)	<b>0.910***</b> (14)	
1996–98	<b>0.909</b> *** (671)	0.985*** (14)	
1996–99	<b>0.956***</b> (671)	<b>0.994***</b> (14)	

**Table 2.** Pearson correlation coefficients, r, for yield traits and accumulated yield 1996–2000 (ACY96–00), based on single plant estimates and on family means, respectively. Number of counts is given within parenthesis and correlation coefficients higher than 0.5 are printed in bold (Rumpunen 2001).

ns = non-significant, \*\*\* p < 0.001

#### Yield

A short juvenile period and annual yields are two valuable features of Japanese quince. Previously it was shown that non-additive effects were pronounced, *e.g.* for onset of bearing, onset of ripening and yield (Kviklys 1998). Reliable estimates of potential yield were available from three years of harvesting data when based on single plants, and from only two years of observations when based on family means (Table 2). A significant SCA detected in some years for annual yield and accumulated yield, respectively, indicates that not only additive genes but also non-additive genes are important for this trait (Table 3).

#### Plant and fruit morphology

Plant vegetative traits (branching, density, plant habit and height) seem to be primarily controlled by additive genes in Japanese quince as inferred from significant GCA estimates. However, for plant habit and height, non-additive genes are also important (Table 3).

Fruit number, fruit weight, fruit flesh, seed weight and number of seed chambers seem to be primarily governed by additive genes. A significant SCA obtained for fruit weight in one year shows that variation in the environment may also influence the perceived pattern of gene action (Table 3).

#### Fruit biochemistry traits

GCA was significant for the seed parents for all fruit biochemistry traits analysed. A significant SCA was detected for total antioxidant activity and for content of soluble solids (Table 3), which indicates that both additive and non-additive gene actions are important for the expression of these traits. However, the relative contribution of SCA to the overall genetic variance was low.

A significant SCA was also obtained for galacturonic acid, when estimates were based on fresh weight, and for malic acid, when estimates were based on dry weight (Table 3). Thus both additive and non-additive genes are important for the expression of these traits. The lack of SCA for succinic acid indicates that primarily additive genes govern this trait.

**Table 3.** Analysis of variance (probability shown) for yield related traits, plant vegetative traits, fruit traits, and fruit biochemistry traits, as well as the relative contribution of SCA to the overall genetic variance ( $RV_{SCA}$ ) for each trait, respectively (Rumpunen & Kviklys 2001).

Trait	GCA <sub>D</sub>	GCAs	SCA <sub>DxS</sub>	RV <sub>sca</sub>	Trait	$\operatorname{GCA}_{D}$	GCA <sub>s</sub>	SCA <sub>D x S</sub>	RV <sub>SCA</sub>
Annual yie	eld (ANY	<u>/)</u>			Plant vegetative traits				
1996	***	ns	ns	20.1	Branches (PBR)	***	***	ns	2.0
1997	***	***	ns	2.2	Density (PDE)	***	*	ns	2.1
1998	***	***	*	7.4	Habit (PHA)	***	***	ns	1.7
1999	***	***	*	2.9	Height (PHÉ)	***	***	*	2.2
2000	*	***	ns	6.0	8				
					Fruit traits				
Accumulat	ted vield	(ACY	)		Average weight (AFW)	***	***	*	1.8
1996–97	2	***	ns	0.7	Flesh (FFL)	***	***	ns	3.6
1996–98	***	***	ns	2.6	Seed chambers (SCH)	***	***	ns	4.0
1996–99	***	***	*	2.5	Seed weight (SWE)	***	**	ns	2.4
1996-200	)0 ***	***	*	2.9	<b>3</b> ( <b>1 1 )</b>				
					Fruit biochemistry traits				
Fruit weigl	ht (FWE	=ANY	/FNU)		Antioxidant activity (AA)	***	***	**	4.6
1996	***	***	ns	7.4	Soluble solids (SS)	**	ns	*	16.0
1997	***	ns	ns	5.7	Galacturonic acid fw (GalA fw)	***	**	**	13.3
1998	***	***	ns	2.2	Galacturonic acid dw (GalA dw)	***	ns	ns	13.7
1999	***	***	ns	1.2	Malic acid fw (MalA fw)	***	***	ns	4.2
2000	***	***	**	3.8	Malic acid dw (MalA dw)	***	***	*	2.5
					Succinic acid fw (SucA fw)	***	ns	ns	2.0
Fruit numb	ber (FNU	Ŋ			Succinic acid dw (SucA dw)	***	***	ns	2.1
1996	**	ns	ns	36.2					
1997	**	***	ns	3.6					
1998	***	ns	ns	18.9					
1999	***	***	ns	3.8					
2000	**	ns	ns	12.1					

ns = non-significant, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

#### **BREEDING STRATEGIES**

Whereas growers and the industry demand rapid progress and immediate access to new varieties, sufficient diversity should be maintained in breeding populations to ensure breeding progress also in the long-term perspective. It is therefore necessary to develop strategies for both short-term (< 30 years) and long-term breeding (Bosemark 1993). For this purpose, a population-based breeding approach has previously been advocated not only for seed propagated crops but also for vegetatively propagated woody species such as apples (Noiton & Shelbourne 1992) and sea buckthorn (Yao 1994). This approach also seems to be appropriate for Japanese quince, with some modifications (Figure 1). The suggested breeding strategy is also based on previous experience of *Chaenomeles* breeding and selection in Finland (Tigerstedt 1996), in Latvia (Ruisa 1996, Tiits 1989), in Lithuania (Kviklys 1998, Ratomskyte 1996), in Moldavia (Ponomarenko 1996), in Poland (Lesinska & Kraus 1996), in Sweden (Rumpunen & Kviklys 1996) and in Ukraine (Mezhenskij 1996).

Rapid and cost-effective screening and selection methods are needed for examination of numerous traits in large numbers of genotypes. Morphological traits are often easy to score and therefore useful in initial screening of populations, whereas biochemical analyses and molecular methods are expensive. Since many characters need to be simultaneously improved in the long-term perspective, an index-based selection could be useful (Bos & Caligari 1995, Yao 1994).

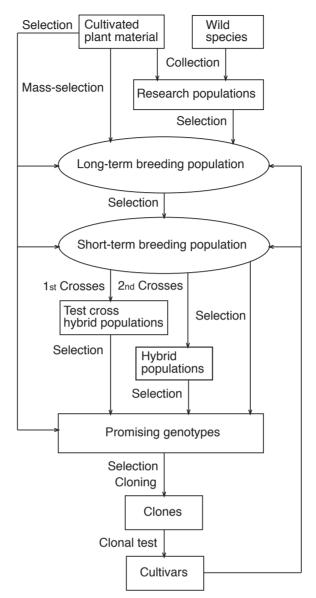


Figure 1. A short- and long-term breeding strategy for the fruit crop Japanese quince (C. japonica).

#### Short-term breeding strategy (for the perspective 5–10 years)

Short-term breeding strategies should be aimed at rapid development of locally adapted and vegetatively propagated cultivars. It would be possible to improve Japanese quince rapidly just by selection, taking advantage of the wide phenotypic variation among plants (genotypes) in orchards and germplasm collections. Screening of older orchards with seed propagated plants would ensure the selection of material that is already adapted to the local climate, at least to some extent. Promising selections (20–40) should then be vegetatively propagated and evaluated in comparative clone trials for at least 3–4 years of fruiting before their release as cultivars (Figure 1). Commercial plantations must rely on more than one genotype to ensure proper pollination since Japanese quince is self-incompatible. The use of at least 5–10 cultivars would reduce the potential spread of epiphytic diseases.

At the same time, populations derived through mass selection of plants in orchards (open pollination of promising selections based on their phenotypic performance) should be established. These will serve as a basis for the initial long-term breeding population (1000 genotypes) from which promising seedlings (50–100) will be selected to constitute the short-term breeding population (Figure 1).

#### Short-term breeding strategy (for the perspective 10–30 years)

Since some important traits appear to be governed by both additive and non-additive genes, a breeding strategy for Japanese quince could be based on extensive test crosses and progeny tests in well-designed field trials. Standards should be included in the field trials to enable comparison with superior clones (Simmonds 1996). It should be remembered that the GCA effects are useful for precise prediction only within the parental set studied in a particular trial, and that the SCA effects have no use outside the actual crosses on which they were measured (Tancred *et al.* 1995). Useful estimates of combining ability would already be available from an initial set of crosses conducted within an applied breeding programme. Valuable cross combinations should then be repeated with a larger progeny, and new combinations with predictable progeny performance should be made. Screening efforts should then be focused on the most promising families.

The best performing of the new cultivars should thus be subject to test crosses (1st crosses) with preliminary selections and other selected genotypes in the short-term breeding population. It would probably be realistic to make 25–50 crosses with a family size of 25–50 plants, resulting in a total of 625–2500 hybrids, depending on resources. To achieve this in Japanese quince, one or two successful crosses per combination would be sufficient due to the high number of seeds in each fruit and their high germinability. The five best test combinations should then be repeated (2nd crosses) with a family size of 500 to 1000 plants, depending on available resources, from which new promising selections could be made (Figure 1).

In a low-budget breeding programme, a breeding strategy based instead on recurrent selection could be efficient. Screening should be focused on morphological characters, yield and diseases (primarily fruit spots) in the years 3–5. Chemical analyses could then be restricted to promising genotypes that have passed the first stage of evaluation.

#### Long-term breeding strategy (for the perspective >30 years)

A long-term breeding strategy should aim at producing widely adapted populations with large genetic diversity from which selected genotypes could be incorporated into the short-term breeding population when appropriate (Yao 1994). In a long-term breeding population, genetic diversity is maintained through mild selection primarily aimed at increasing adaptability. Selection should take place after extreme winters, which occur in approximately one of ten years.

A long-term breeding strategy should also include research aimed at inducing variation in useful traits and developing efficient screening and selection methods. Increased variability can be obtained in several ways, *e.g.* by interspecific and intergeneric hybridisation, polyploidisation and development of double haploids. Methods for marker-assisted selection should be developed to enable fast and efficient screening for valuable traits.

#### Interspecific hybridisation

Since there are no barriers against interspecific hybridisation in the genus *Chaenomeles* (*C. thibetica* not yet tested) this is the obvious method for transferring useful characters between species. Whereas the three Chinese species are possible donors of genes for larger fruits and higher plants, they would probably also contribute inferior hardiness. Repeated backcrosses to *C. japonica* might restore cold hardiness and improve adaptability. However, plants of *C. thibetica* seem to be better adapted to the Baltic climate than either *C. speciosa* or *C. cathayensis* plants (unpublished results). Interspecific crosses between *C. japonica* and *C. thibetica* should therefore be given priority.

#### Intergeneric hybridisation

A few intergeneric hybrids have been produced between *Chaenomeles* and *Cydonia*, *Malus* and *Pyrus*, respectively (Mezhenskij 1989, 1996, Rjabov 1983 cited by Friedrich 1985, Ponomarenko 1990). How-

ever more efforts should be devoted to intergeneric hybridisation within the Maloideae, since this could be a way to create new interesting combinations. Besides, *Chaenomeles* plants seem not to be susceptible to mildew and scab, and the species in the genus *Chaenomeles* may thus serve as possible new sources of resistance that could be transferred to apple and pear.

### Polyploidisation

Polyploidisation is another approach to induce variation in Japanese quince. Through polyploidisation gigas effects may be obtained, *e.g.* in plant growth habit and fruit size. Tetraploids could be used to develop triploids, which are likely to have reduced seed set and an increased proportion of fruit flesh. Procedures for successful polyploidisation of Japanese quince are now available (Stanys *et al.* 2002).

#### Development of homozygous doubled haploids

Selection on the haploid level could increase the potential for efficient breeding since negative recessive alleles are revealed, and phenotypic selection for both qualitatively and quantitatively inherited characters can thus be simplified. Furthermore, since Japanese quince is self-incompatible and produces large amounts of seeds with high germinability, the possibility of producing hybrid seeds (F1) from selected double haploids should be studied. Production of homogeneous plants from seeds would considerably reduce the cost of propagation and establishment of plantations, thereby increasing the competitive ability of the crop. The development of double haploids would benefit from selection using molecular markers. Plants of haploid or double haploid origin regenerated from microspores of a heterozygous individual would all be homozygous, whereas individuals arising from diploid anther wall tissue would be heterozygous (Arús & Moreno-Gonzáles 1993). Haploid induction *in vitro* has, however, not been very successful in other pome fruits such as apple (Höfer & Grafe 2000) and may therefore also be difficult to achieve in *Chaenomeles*.

#### **DNA-markers**

The possibility of developing DNA-markers for characters that are difficult to score in young plants should be investigated. For instance, the use of molecular markers for self-incompatibility alleles would make it possible to select highly compatible varieties early in the breeding process (Kaufmane & Rumpunen 2002). A molecular marker linked to the presence of thorns would enable a reduction in population size at the seedling stage, thereby saving management costs. Molecular markers for quantitative characters expressed in the adult stage, such as content of pectin and resistance towards various diseases, would also be most useful. Molecular markers would be especially useful when several generations of back crosses are needed after interspecific hybridisation to restore valuable gene combinations.

#### **Breeding achievements**

In the ongoing European breeding programme for Japanese quince, selection has taken place in orchards, the selected plants have been micropropagated and clone trials have been established in Finland, Italy, Latvia, Lithuania and Sweden. In addition, breeding populations have been created and hybridisation programmes have been initiated. Polyploids have been developed, but have not yet set fruit. The first varieties should be available for marketing within a few years.

# CONCLUSION

The large phenotypic and genetic diversity in the genus *Chaenomeles*, as inferred from morphological and biochemical traits and from molecular markers, is advantageous for crop improvement through breeding and selection.

A breeding strategy for Japanese quince could be based on extensive test crosses and progeny tests in well-designed field trials, since many important traits are controlled by additive as well as non-addi-

tive genes. An efficient strategy for a low-budget breeding programme could instead be based on recurrent selection.

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# APPENDIX

Provisional test guidelines, and descriptors, for *Chaenomeles* species and hybrids. The guidelines are based on UPOV guidelines for the conduct of tests for distinctness, uniformity and stability of varieties of fruits.

#### I. Subject of the Guidelines

These test guidelines apply to all vegetatively propagated fruit and ornamental varieties of *Chaenomeles* Lindley species.

#### II. Material required

1. The competent authorities decide when, where and in what quantity and quality the plant material required for testing the variety is to be delivered. Applicants submitting plant material from a state other than that in which the testing takes place must make sure that all customs formalities are complied with. As a minimum, the following quantity of plant material is recommended: 10 two-year-old plants.

The plant material supplied should be healthy, not lacking in vigor or affected by any important pests or diseases.
The plant material must not have undergone any treatment, which may affect the subsequent growth of the plants, unless the competent authorities allow or request such treatment. If the plant material has been treated, full details of the treatment must be given.

#### III. Conduct of Tests

1. To assess distinctness, it is essential that the plants under test should produce at least two satisfactory crops of fruit.

The tests should normally be conducted at one place, representing a central hardiness zone for the variety. If any important characteristics of the variety cannot be seen at that place, the variety may be tested at an additional place.
Additional tests for special purposes may be established.

#### IV. Methods and Observations

1. Experience in testing uniformity and stability has shown that, in the case of vegetatively propagated *Chaenomeles* genotypes, the standards are met if the plant material supplied is uniform in the states of expression of the characteristics observed and that no mutations or mixtures are present.

2. Unless otherwise stated, all observations determined by measurement, weighing or counting should be made from a minimum sample of 10 typical organs or plant parts per genotype from a minimum of three ortets.

3. The dormant one-year-old shoot for testing should be taken in winter from plants that are at least two years old and have completed at least one growing season at the testing centre.

4. All flower characteristics should be measured at the start of anther dehiscense.

5. Observations on shoots of the current season should be made on a well growing and representative twig while the plant is still in active growth.

6. For observations on the fruit, 10 typical fruits should be selected out of a minimum of 30 fruits from three ortets. All fruit characteristics should be measured at peak maturity when the seeds are brown.

7. Flower colour should be determined in the middle of the day in a room facing north. Colour of the flowers should be determined by placing a flower on white paper. A standard colour chart, e.g. Royal Horticultural Society Colour Chart, should be used, when determining flower colour.

#### V. Grouping of Varieties

1. The collection of varieties to be grown should be divided into groups representing defined hardiness zones to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience to be stable within a variety. Their various states of expression should be fairly evenly distributed throughout the collection.

2. It is recommended that the competent authorities use the following characteristics for grouping varieties:

- (I) Plant: habit
- (ii) Flower: colour
- (iii) Fruit: average fresh weight

## VI. Characteristics and Symbols

1. To assess distinctness, uniformity and stability, the characteristics and their states as given in the Table of Plant Characteristics (Table VII) should be used.

2. Notes (1 to 9), for the purposes of electronic data processing, are given opposite the states of expression for each characteristic.

3. Legend:

\*: Characteristics that should be used on all varieties in every growing period over which examinations are made and always be included in the variety descriptions, except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible.

+: See Explanations on the chapter IX.

VII. Accession and Collecting Descriptors

#### 1 Accession

(A unique number for each accession in this database)

#### 2 Location

(The country where the accession is kept)

#### 3 Institute

(The institute where the accession is kept)

#### 4 Taxon

- 1 = unknown
- 2 = C. japonica
- 3 = C. speciosa
- 4 = C. cathayensis
- 5 = C. thibetica
- 6 = C. x suberba
- 7 = C. x clarkiana
- 8 = C. x vilmoriniana
- 9 = C. x californica

#### 5 Curator ID

(An ID number of the curator of the collection)

#### 6 Accession ID

(Number assigned to the accession by the curator)

#### 7 Synonym of the accession ID

#### 8 Parentage

#### 9 Fruit: use

- 1 = no use
- 2 = processing
- 3 = ornamental

#### 10 Plant: use

- 1 = no use
- 2 =fruit crop
- 3 = ornamental
- 4 = other

#### 11 Country

(Country of origin of the accession)

#### 12 Origin

(Site of origin of the accession)

#### 13 Genotype status

- 1 = unknown
- 2 = genotype (seedling) of wild plant material
- 3 = genotype (seedling) of cultivated plant material
- 4 = genotype (clone) of wild plant material
- 5 = genotype (clone) of cultivated plant material
- 6 = breeder's advanced selection
- 7 =variety

#### 14 Virus indexing

- 1 = not tested
- 9 = virus tested

#### VIII. Plant Characteristics

#### 1\*+ Plant habit

- 1 = prostrate shrub
- 2 =decumbent shrub
- 3 =semi-erect shrub
- 4 = erect shrub
- 5 = shrub (higher than wide) with a few main trunks
- 6 = shrub (higher than wide) with one main trunk
- 7 = tree

#### **2\*** Shoot: branching

(Number of branches on upright standing shoot, average distance between branches in cm. The length of the whole shoot or primary branch to be measured and the secondary branches to be counted in the spring or after leaf fall.)

- 1 = absent or very few (> 39 cm)
- 3 = few (30-39 cm)
- 5 = medium (20-29 cm)
- 7 = many (10–19 cm)
- 9 = very many (< 10 cm)

#### **3\*** Shoot: thorns on dormant one-year-old shoot

(Thorns on 10 cm of the shoot from the tip, the uppermost 5 cm excluded, to be counted after leaf fall.)

- 1 = absent
- 3 = few(0.1-2)
- 5 = medium (2.1-4)
- 7 = many (4.1-6)
- 9 = very many (> 6)

#### 4 Shoot: thorns on two-year-old shoot

(Number of thorns on 10 cm of the shoot from the upper part, the uppermost 5 cm excluded, to be counted after leaf fall.)

- 1 = absent3 = few (0.1–1)
- 5 = medium (1.1-2)
- 7 = many (2.1-3)
- 9 = very many (> 3)

#### 5\* Thorns

(Evaluated in the middle of a one-year-old shoot after leaf fall.)

1 = absent

2 = short slender thorns (< 10 mm)

- 3 =long slender thorns (> = 10 mm)
- 4 = short thick thorns (< 10 mm)

#### 6 Shoot: epidermis on a one-year-old shoot

- (To be observed after leaf fall.)
  - 1 =glabrous
  - 2 = covered with scabrous tomentum
  - 3 = pubescent

#### 7 Shoot: epidermis on a two-year-old shoot

(To be observed after leaf fall.)

- 1 = glabrous
- 2 = verruculose

#### 8 Leaf: blade length

(Length of a leaf in mm, the leaf sampled from the middle of a one-year-old shoot)

- 3 =short
- 5 = medium
- 7 = long

#### 9 Leaf: blade width

(Maximum width of a leaf in mm, the leaf sampled from the middle of a one-year-old shoot)

3=narrow

5=medium

7=wide

#### 10\* Leaf: blade ratio

(Ratio of length / width. Length measured from the tip of the leaf to the base including petiole. Width measured from the widest point of the cross section.)

- 3 = small
- 5 = medium
- 7 = large

#### 11\*+ Leaf: shape

- 1 = lanceolate C. cathayensis, C. thibetica
- 2 =narrowly elliptic *C. speciosa*
- 3 = elliptic C. japonica
- 4 = obovate C. japonica

#### 12\*+ Leaf: apical angle

 $1 = \text{obtuse} (> 90^\circ)$ 

- $2 = \text{acute (between 45^{\circ}-90^{\circ})}$
- $3 = \text{acuminate} (< 45^{\circ})$

#### 13\*+ Leaf: margin shape

1 = coarsely crenate	C. japonica
2 = doubly serrate	C. speciosa
3 = serrate with irregular spacing	C. cathayensis
4 = doubly serrate with irregular spacing	C. thibetica

#### 14+ Leaf: tooth apex shape

1 = cuspidate	C. japonica, C. speciosa
2 = obtuse	C. japonica, C. speciosa
3 = acute	C. cathayensis, C. thibetica
4 = acuminate	C. cathayensis, C. thibetica

#### 15\* Flower: cluster

(Number of buds to be counted just before the beginning of flowering.)

- 1 = single (1 bud)
- 3 = few (2-3 buds)
- 5 = medium (4-5 buds)
- 7 = many (6-7 buds)
- 9 =very many (more than 7 buds)

#### 16\* Flower: petals

(Relative position of margins when the detached flower is gently pressed into horizontal position.)

- 1 = free
- 2 =touching
- 3 = overlapping

#### 17\*+ Flower: petal shape

- 1 = circular
- 2 = transversely oblong
- 3 = elliptic
- 4 = obovate
- 5 = widely obovate
- 6 = other

#### 18+ Flower: petal's degree of concavity

(Petal is pressed and the degree of concavity is determined by the length of the tear, from the tip of the petal towards the middle.)

- 1 =flat (no tear)
- 5 = shallowly concave (tear shorter than half of the petal)
- 9 = deeply concave (tear longer than half of the petal)

#### 19\* Flower: diameter

(Flower diameter is measured when the flower is fully opened and the detached flower is gently pressed into horizontal position.)

- 1 = very small (< 4.0 cm)
- 3 = small (4.0 4.4 cm)
- 5 = medium (4.5-4.9 cm)
- 7 = large (5.0 5.5 cm)
- 9 = very large (> 5.5 cm)

#### 20\* Flower: form

- 1 = single
- 2 = double
- 3 = filled

#### 21\* Flower: colour

- 1 = white
- 3 = pink
- 5 = orange
- 7 = red
- 9 = dark red

#### 22\* Flower: colour code, according to *e.g.* the colour-chart of the Royal Horticultural Society

#### 23 Flower: percentage of imperfect flowers

- 1 = < 5%
- 2 = 5 9%
- 3 = 10 19%
- 4 = 20–29%
- 5 = 30–39%
- 6 = 40–49%
- 7 = 50-59%
- 8 = 60 69%
- 9=>70%

#### 24 Fruit: ratio length / width

(The fruit ratio is measured in mm for the longest and widest part of the fruit.)

- 1 = very small
- 3 = small
- 5 = medium
- 7 = large
- 9 = very large

#### 25\* Fruit: average fresh weight measured in g

- 1 = very low (0-9 g)
- 2 = very low to low (10-19 g)
- 3 = low (20-29 g)
- 4 =low to medium (30–39 g)
- 5 = medium (40-49 g)
- 6 = medium to heavy (50–59 g)
- 7 = heavy (60-69 g)
- 8 = heavy to very heavy (70–79 g)
- 9 = very heavy (> 80 g)

#### 26\* Fruit: ratio of area of locules / area of fruit flesh

(Measured in  $cm^2$  in a sample, which is sectioned transversely, the cut passing through the mid-position of the locules.)

- 1 = very small
- 3 = small
- 5 = medium
- 7 = large
- 9 = very large

#### 27\* Fruit: number of locules

- 3 = few (< 5)
- 5 = medium(5)
- 7 = many (> 5)

#### 28\*+ Fruit: shape, vertical cross-section

- 1 = circular
- 2 = oblate
- 3 = oblong
- 4 = ovate
- 5 = pyriform
- 6 = irregular

#### 29 Fruit: ribbing, horizontal cross-section

- 1 = absent
- 2 = weak
- 3 = strong

#### **30\*** Fruit: ground colour at maturity

- 1 = green
- 2 = yellowish green
- 3 = yellow

#### 31 Fruit: amount of over colour

- 1 = absent
- 3 = low
- 5 = medium
- 7 = high

#### 32 Fruit: over colour

- 1 = absent
- 2 = orange
- 3 = pink
- 4 = red
- 5 = greenish red
- 6 = green

#### 33 Fruit: greasiness of skin

- 1 = absent
- 2 = weak
- 3 = strong

#### 34\* Fruit: easiness of picking at the time of maturity

- 1 = very loose so that the fruit drops by itself when ripe
- 3 = quite loose
- 5 = medium
- 7 = hard
- 9 = very hard, almost impossible to pick without injuring the branch

#### 35\* Seed: number

(Number of developed seeds at maturity, to be counted in a minimum of 10 fruits of each clone from three different ortets.)

3 = small (< 60)

5 = medium (60 - 80)

7 = large (> 80)

#### 36 Seed: length

(To be measured by 10 seeds of each ortet from a minimum of three fruits on three ortets.)

3 =short (< 6 mm)

- 5 = medium (6-7 mm)
- 7 = long (> 7 mm)

#### 37 Seed: dry weight

(To be measured by 100 seeds of each clone, from a minimum of 3 ortets.)

#### 38 Seed: shape

(Biological data missing, to be established later)

#### 39\* Seed: share

(Seed fresh weight percentage of fruit total fresh weight to be measured from minimum 10 fruits of each clone from a minimum of 3 ortets.)

3 = small (< 6%) 5 = medium (6–10%) 7 = large (> 10%)

#### 40 Seed: colour

(Colour of the seeds, to be measured by 5 typical fruits from each clone.)

1= white

- 2= very light brown
- 3= light brown
- 4= brown
- 5= dark brown

#### 41\* Time of leaf bud burst

(Effective temperature sum when 5% of the buds are open.)

#### 42\* Time of beginning of flowering

(Effective temperature sum when 5% of flower buds are open.)

#### 43\* Duration of flowering

(Effective temperature sum, and number of days from beginning of flowering, when 5 % of the buds are open, to the end of flowering, when 95 % of the flowers have faded.)

#### 44\* Time of fruit maturity

(Effective temperature sum at which the colour of the seed coat has turned brown.)

#### 45\* Time of leaf fall

(Effective temperature sum and date of complete defoliation.)

#### 46\* Disease resistance

- 3 = weak
- 5 = medium
- 7 = strong

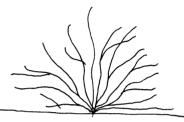
#### IX. Explanations

#### 1 Plant habitus

1= prostrate shrub



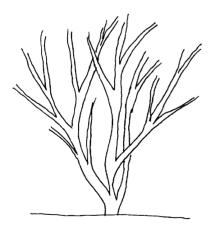
2= decumbent shrub



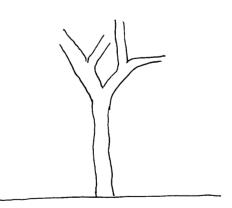
3= semi-erect shrub

4= erect shrub

- 5= shrub (higher than wide) with a few main trunks

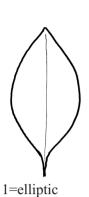


6= shrub (higher than wide) with one main trunk



7= tree

#### 11 Leaf: shape



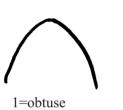






4=lanceolate

12 Leaf: apical angle

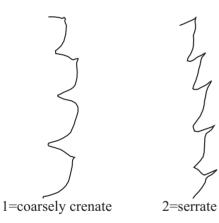




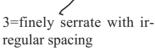


3=acuminate

#### 13 Leaf: margin shape









4=doubly serrate with irregular spacing

14 Leaf: tooth apex shape





2=obtuse



3=acute



# 17 Flower: petal shape





1=circular

2=transversely oblong



3=elliptic



4=obovate



5=widely obovate

### 18 Flower: petal's degree of concavity





5=shallowly concave



9=deeply concave

#### 28 Fruit: shape, vertical cross-section

