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MORPHOLOGICAL AND CHEMOTAXONOMIC STUDIES OF GENUS *MALUS*

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DOCTOR IN PHILOSOPHY

BY

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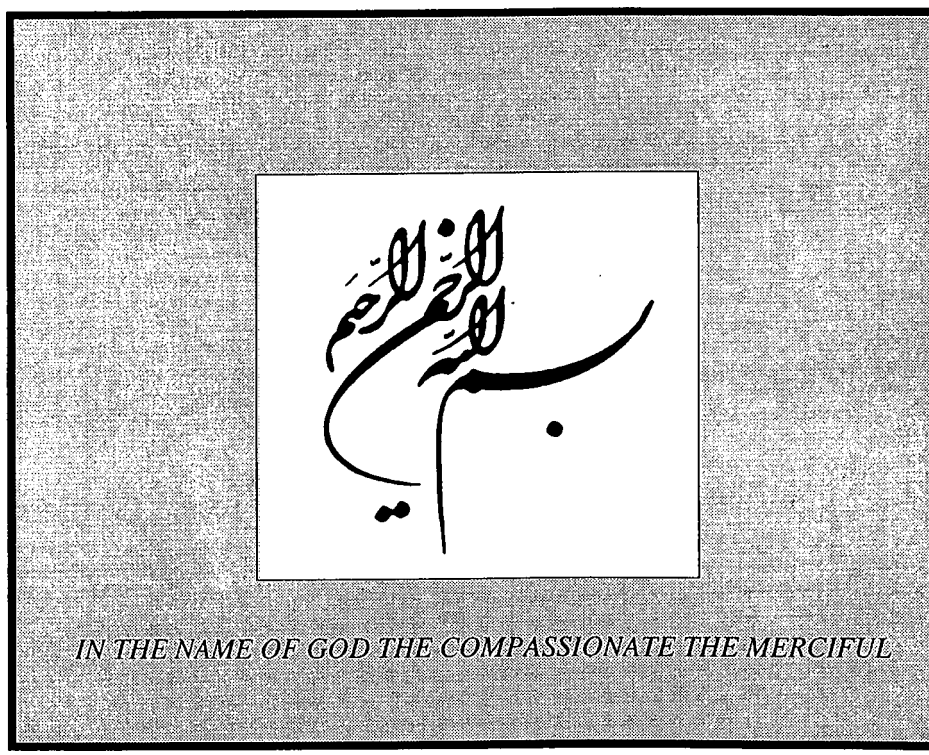

IN THE NAME OF GOD THE COMPASSIONATE THE MERCIFUL

ABSTRACT

A detailed, many faceted study of all available species of *Malus* (apple) largely confirms the sub-generic classification proposed by Rehder (1940). The revision of the subfamily Maloideae of the Rosaceae by Phipps *et al.* (1991), Robertson *et al.* (1991, 1992) and Rohrer *et al.* (1991, 1994) formed a basis for an examination of many more species of *Malus* than have previously been included in a single study. Fresh living material was used for almost all the work but herbarium specimens were also examined to check the identity of the living material and confirm the conclusions reached from the small number of living clones available.

The morphology of all parts of the trees was studied with special attention paid to the fruits which are known to yield many characters of value in determining evolutionary relationships and degree of advancement. The application of ideas from cladistics has allowed the postulation of evolutionary pathways for individual characteristics and taxa. Many new observations have been made and these have allowed the construction of an improved key to all the species studied. The absence of glands (colleters) on the leaves of *M. tschonoskii* is yet another character cofirming the isolation of this species in the genus.

Anatomical examination of wood structure has revealed much more variation than has been previously reported in the genus. Section *Chloromeles* is unique in having exclusively uniseriate rays and *M. fusca* has a unique arrangement of patches of apotracheal xylem parenchyma.

As with wood anatomy, pollen is much more variable than previously reported, and it has been possible to construct a key differentiating among almost all species. This key should now be tested on a wider range of material. Similar exine patterns in *M. trilobata* and *M. florentina* and dissimilarity from others is in line with other similarities between these species and their isolated position in the genus. Similarities between these two species and *M. tschonoskii* may be the persistence of an ancestral condition.

Analysis of flavonoid composition was more detailed than any previous study. On the whole RehderUs (1940) classification is supported. *M. trilobata* and *M. florentina* are again shown to be similar and the suggestion of a close relationship between *M. florentina* and *Sorbus torminalis* is refuted. A close relationship between *M. tschonoskii* and the *Chloromeles* is suggested, *M. toringoides* is shown to have an affinity with the *Baccatae*, and the placing of *M. fusca* in the *Kansuenses* is supported.

Isozyme studies also place *M. florentina* and *M. trilobata* close together and confirm the distinctness of that species pair and section *Chloromeles* from the rest of the genus.

Little evidence was found of a close relationship with the most similar species of some genera said to be most closely related to *Malus*, namely *Chaenomeles*, *Pseudocydonia* and *Aria* (*Sorbus* sect. *Aria*). No material of *Docynia* was available for study.

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CHAPTER 1

CHAPTER 1

INTRODUCTION

The Rosaceae exhibit considerable diversity in chromosome numbers and fruit types. Fruit characters are usually preferred over chromosome numbers for dividing Rosaceae into subfamilial groups. However, the four subfamilies of Rosaceae defined by fruit types conflicts with the distribution of chromosome numbers (Morgan *et al.*, 1994):

1. Spiraeoideae with follicles, x = 8, 9 (*Lindleya* and *Kageneckia* x = 17, *Vauquelinia* x = 15).
2. Rosoideae with achenes or drupelets, x = 7, 9 (occasionally 8 in tribe Ulmariae).
3. Amygdaloideae (Prunoideae) with drupes, x = 8.
4. Maloideae (Pomoideae) with pomes, x = 17.

1.1. Maloideae

The first formal recognation of Maloideae was by de Jussieu in 1791. The Maloideae has been treated at several different ranks as discussed by Huckins (1972): tribe (de Jussieu, 1785; Hutchinson, 1964); subfamily (Koehne, 1893); and family (Bartling, 1830; Spach, 1834; Endlicher, 1836-1840). Rehder (1940) described the subfamily Maloideae as follows:

> "Trees or shrubs; leaves simple or pinnate stipulate; flowers solitary or in umbels, racemes, panicles or corymbs; carpels 2-5, usually 2-ovuled, more or less united and adnate to the cup-shaped calyx-tube, forming an inferior ovary; fruit a fleshy pome."

The Maloideae contain about 28 genera and 940 species of mostly north temperate trees and shrubs, including economically and ecologically important groups, such as apples, pears, cotoneasters, moutain ashes, hawthorns, and shadbushes (Robertson *et al.*, 1991). This subfamily is remarkable for its hypothesized allopolyploid origin, extensive intergeneric hybridization, intergeneric grafting compatibility, and high incidence of polyploidy and apomixis.

Some characteristic features which differentiate the Maloideae from other subfamilies are:

1. Chromosome number of x = 17.
2. Syncarpic pome fruit.
3. Occurrence of the rust parasite genus *Gymnosporangium* exclusively in Maloideae (also found in *Vauquelinia*, subfamily Spiraeoideae, Salvile, 1979).
4. The widespread occurence of apomixis in Maloideae.

Apomixis has otherwise only been reported in the Rosoideae, being unknown in the Spireaoideae and Amygdaloideae. Sax (1931) suggested that all of the present genera of the Maloideae might be classified under one genus.

1.2. Tribal classification

According to Koehne (1891 cited by Huckins 1972), the Maloideae can be divided into two tribes based on the fruit core.

1. The Crataegeae with a hard pyrene for each carpel, almost all parts of the carpel becoming bony in the mature fruit and the fruit becoming drupe-like.
2. The Sorbae (Maleae) with a membranous to cartilaginous multilocular core. He further divides the Sorbae into four subtribes, the Sorbineae, the Arineae, the Pirineae and the Malineae.

Robertson (1974) suggests that no flower characters are correlated with this difference which develops only as ovaries mature into fruits. The consistency of the

endocarp and degree of lateral connation of carpels seems to be more variable than originally thought, and the division of the subfamily into two tribes as mentioned above may not best reflect the genetic relationships. In at least one case, two genera (*Sorbus* and *Aronia*) should be combined into one genus. Rohrer *et al.* (1991) also mentioned that the division of the Maloideae into two tribes based on type of the fruit core, is not supported by other fruit characters, e.g. although *Crataegus* and *Cotoneaster* are similar in having a pyrenous core, other fruit characters such as calyx lobe morphology or ovary connation and adnation would more closely link *Crataegus* with *Amelanchier* or *M. trilobata* and *Cotoneaster* with *Heteromeles* or *Sorbus.*

The traditional division of the Maloideae into two tribes can therfore no longer be considered to reflect evolutionary relationships.

1.3. Origin of Maloideae

One of the most extensively discussed evolutionary problems in the Rosaceae is the origin of Maloideae. Most authors accept that the Maloideae are polyploid, but how the base number x = 17 arose is much debated. Dickson *et al.* (1992) support the polyploid origin of Maloideae and found that, among Rosaceae, Maloideae have a relatively large amount of DNA per nucleus (C- value) and that the Spiraeoideae C-values are the smallest in the Rosaceae.

Nebel (1933) suggested that the Maloideae are aneuploids derived from a pentaploid ancestor with x = 7 and 35 somatic chromosomes. Darlington and Moffett (1930) found that in the diploid species of the Maloideae (Pomoideae) studied by them the most frequent pattern of arrangement of the bivalents was in three groups of 3 bivalents and four groups of 2. They interpreted this to mean that the tribe Maloideae originated from diploid species with x = 7 by doubling of the entire set plus the addition of a third partial set consisting of 3 of the original 7 bivalents (7+7+3 = 17). The presence of x = 7 as the basic haploid number of many genera in the family, (tribe Rosoideae) was used as supporting evidence for this hypothesis.

Sax (1931, 1932, cited by Stebbins, 1950) proposed that the Pomoideae (Maloideae) are more nearly related to the Prunoideae, and suggested that the Maloideae are hyper tetraploids derived from primitive Prunoideae with x = 8, (8+8+1 = 17).

On morphological grounds, Stebbins (1950) concluded that the Maloideae arose as amphiploids between primitive or ancestral members of the tribes Spiraeoideae (x = 9) and Prunoideae (x = 8). Stebbins (1950) mentioned some morphological evidence to support his hypothesis. The Rosoideae contain a number of characteristics and tendencies not found at all or relatively uncommonly in the Maloideae: the tendency toward the herbaceous or scandent habit; the tendency toward yellow flowers and numerous carpels containing only one ovule. On the other hand, the Prunoideae more nearly resemble the Maloideae in habit, leaf shape, inflorescence and character of sepals and petals than does any other tribe of the family Rosaceae. Also, these two tribes possess the cyanogenic glucoside, amygdalin in common, which is not found in the Rosoideae or in any other tribe of the family. There are, however, certain morphological and anatomical characteristics in which most genera of the Maloideae resemble the Spiraeoideae more than they resemble the Prunoideae. These include the presence of five carpels with several or numerous ovules.

Comparision of the flavonoid chemistry of the Maloideae, Spiraeoideae and Prunoideae (Challice, 1973, 1974, 1981) clearly shows that the Maloideae has strongest affinity with the Prunoideae and to a lesser extent with Spiraeoideae.

Phipps *et al.* (1991) deduce an allotetraploid origin of Maloideae from morphological character analysis. Their character analysis suggests a broadly equal similarity of maloids to spiraeoids and amygdaloids and supported the Sax - Stebbins allopolyploid theory.

They compared the morphological characters of three subfamilies, Maloideae, Amygdaloideae and Spiraeoideae:

1. In habit the Maloideae are most like amygdaloids. The tree habit frequent in Maloids (e.g. *Sorbus*, *Aria*, *Cormus*, *Torminalis*, *Malus*, *Pyrus*, ... etc.) is also found

commonly in amgdaloids (e.g. *Prunus*, *Padus*, *Cerasus*, ... etc.). Several of the Maloid genera are multistemmed shrubs which are also quite common in amygdaloids (e.g. *Prunus*, *Padus*, *Laurocerasus*).

2. The dimorphic shoots (long shoots and short shoots) found in most Maloideae (except in *Mespilus canescens*, *Photinia* and *Cotoneaster*) are similar to those of Amygdaloideae and are not known in spiraeoids. The development of thorns from the short shoots as is found in *Crataegus* and *Pyracantha* is paralleled in *Prunus*.

3. The diverse foliage types of maloids find counterparts in other Rosaceae. The *Sorbus* pinnate leaf, for instance, is extremely similar to spiraeoid foliage such as that of *Sorbaria* species even down to the adaxial glands at the base of the pinnae. Pinnate foliage is unknown in Amygdaloideae. The simple-leaved, camptodromus (CA) type of venation found in some Maloideae is however the universal type for the Amygdaloideae and it is often gland mariginal. No very similar CA type occurs in the spiraeoids, the foliage of Spiraeoideae being basically lobed and small, in many species being craspedodromous (CR) and in others CA type .

4. In both inflorescence and flower, at least in presumed plesiomorphic (primitive) maloid forms, there is a great similarity to spiraeoids, but amygdaloids are not totally dissimilar.

The Maloideae inflorescence shows considerable variation. The most common kind of inflorescence is a convex panicle of small white flowers forming a discrete platform. These are indistinguishable from inflorescences that can be found in the spiraeoid genera (*Spiraea, Sorbaria* and *Vauquelinia*). In the Rosaceae this type cannot be found outside Maloideae and Spiraeoideae. Transition series to fewer flowers per inflorescence, sometimes only one, occur, and are frequently correlated with much larger and coloured flowers. Umbelliform inflorescences are intermediate and found in *Crataegus* series *Aestivales*, many *Pyrus* and some *Malus* especially section *Pumilae*. *Cydonia oblonga* and several other taxa have uniflorus inflorescences. The racemose inflorescence of *Amelanchier* with its often narrow petals is very similar to that of *Padus* and *Osmaronia* (Amygdaloideae).

5. Fruits show very clear relationship to the Spiraeoideae. The fruiting carpel may be homologized with the spiraeoid follicle. The only difference between Spiraeoideae and Maloideae is the more or less adnate fleshy hypanthium of the Maloideae.

Dickson *et al.* (1991) support the suggestion of ancient polyploidy in the evolution of *Malus* through their finding of a high number of isozymes compared to diploid plant species.

Phipps *et al.* (1991) hypothesized that several phylogenetic lines originated very early from an original maloid melting pot because of great variation in habit and inflorescence in Maloid genera. Therfore, some major divergence in these characters took place initially, due to segregation. There was no trace of such variability in floral characters. The population of the original maloid quickly grew and became subject to differential selection by the environment. At first, hybridization easily took place with few barriers but gradually barriers developed due to spatial isolation and phenological factors. The main genera fairly rapidly stablized and spread around the northern hemisphere. The scheme for polychotomous early evolution of Maloideae given by Phipps *et al.* (1991) is presented in Figure 1.1.

Phipps *et al.* (1991) also suggested that late Eocene might be the time of origin of Maloideae as Hickey (1984 cited by Phipps *et al.*, 1990) identified *Crataegus*, *Malus*, *Sorbus*, *Amelanchier*, and *Photinia* in the Oligocene in North America.

Vauquelinia, Lindleya and *Kageneckia* are three controversial genera usually placed in Spiraeoideae. It has been suggested that phylogenetically they are related to Maloideae (Goldblatt, 1976). Phipps *et al.* (1991) rejected any direct relarionship between these three genera and subfamily Maloideae due to the woody capsule of *Vauquelinia* and *Lindleya* and the follicle in *Kageneckia* .

Recently, the results of rbcL (chloroplast genome) sequence variation studied by Morgan *et al.* (1994) suggested the Spiraeoids as sole ancestors of the Maloideae. Their results also suggest the inclusion of *Vauquelinia, Lindleya* and *Kageneckia* in Maloideae. Their ideas are supported by following evidence:

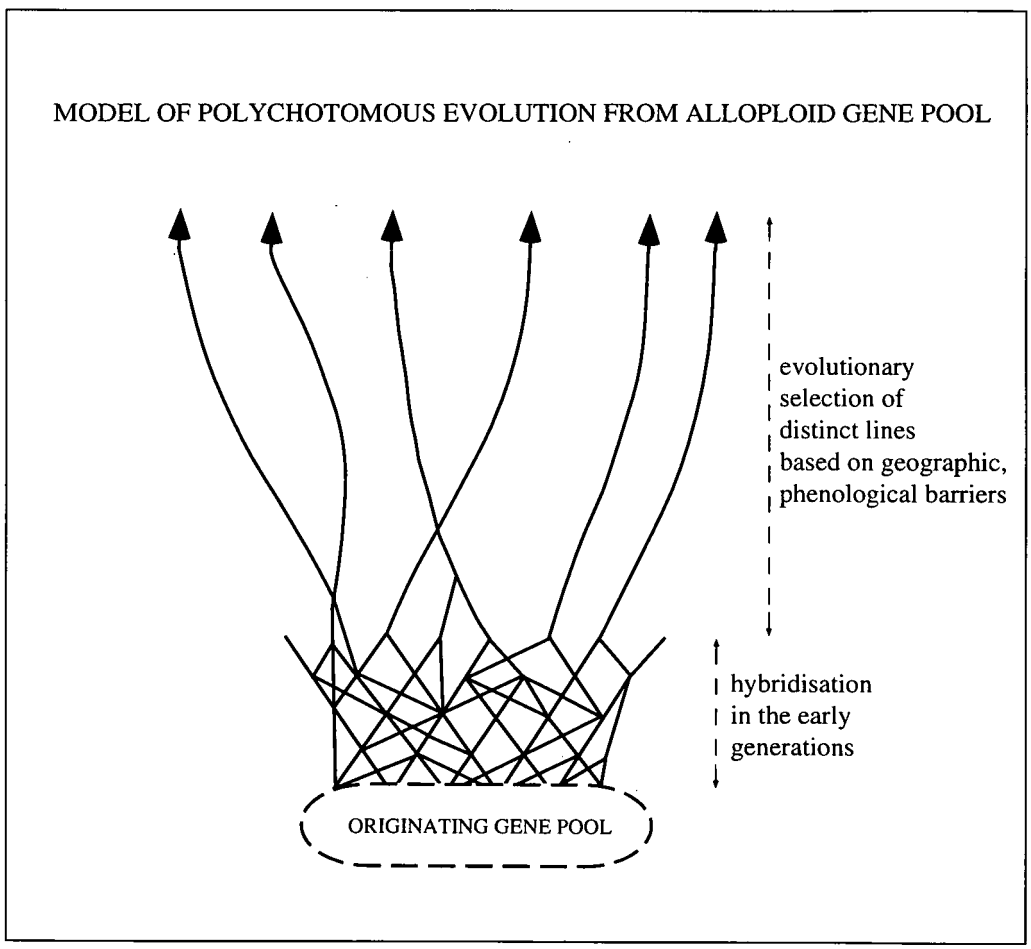


Figure 1.1. Scheme for polychotomous early evolution of Maloideae. After Phipps *et al.*, 1991.

1. Cytologically *Vauquelinia* (x = 15), *Lindleya* and *Kageneckia* (x = 17) are closely related to Maloideae. *Lindleya* and *Kageneckia* have a chromosome number of 17, which is the characteristic base number of Maloideae (Goldblatt, 1976) and occurs nowhere else in Rosaceae. *Vauquelinia* has n = 15 (Goldblatt, 1976), a number that could have arisen from x = 17 through aneuploid reduction (Robertson, 1974; Goldblatt, 1976; Challice, 1981; Kalkman, 1988).

2. Both *Vauquelinia* and *Lindleya* have connation among the carpels as well as adnation between the carpels and hypanthium. This is similar to the carpel fusion and adnation of many members of Maloideae (Phipps *et al.*, 1991).

3. Carpels are connate at both dorsal and ventral edges in *Vauquelinia* and *Lindleya*, a feature that is also characteristic of Maloids that have connate carpels (Sterling, 1966).

4. *Lindleya* synthesizes isochlorogenic acid which in Rosaceae is otherwise known only from Maloideae (Challice, 1973, 1974, 1981).

5. The rust parasite genus *Gymnosporangium*, which in flowering plants occurs almost exclusively on Maloideae is also found on *Vauquelinia* (Savile, 1979).

6. Campbell *et al.* (1993) found that analysis of sequenses from the internal transcribed spacer of nuclear r RNA also suggest that *Vauquelinia*, *Lindleya* and *Kageneckia* are closely releated to Maloideae.

Therefore they concluded that the ancestory of Maloideae s.l. can be traced to x = 9 spiraeoid ancestors. They also suggested that the nearest spiraeoid relatives outside Maloideae s.l. are members of Sorbarieae, a relationship that is also suggested by carpel anatomy and the prescence of dihydrochalcones and arbutin in Maloideae and *Sorbaria.* The dry-fruited taxa with n = 17 and n = 15 they placed in Maloideae s.l.; *Vauquelinia* and *Lindleya* with capsules and *Kageneckia* with follicles are usually treated as spiraeoids and provide links with proposed spiraeoid progenitors. They deduced that the Maloideae arose entirely from spiraeoid progenitors (by autopolyploidy or allopolyploidy) or that hybridization between spiraeoids and another lineage produced the x = 17 maloid ancestors. If the Maloideae originated in this way

the maternal ancestor(s) were spiraeoids, because the chloroplast DNA inheritance in Rosaceae is maternal. He also suggested the Sorbarieae as the nearest spireaoid relatives.

Phytoalexin production, studied by Kokubun and Harborne (1995) did not confirm or refute the allopolyploid origin of the Maloideae. In contrast to the Maloideae, the proposed parental Prunoideae and Spireaoideae show no sign of phytoalexin production, though no species of *Sorbaria* was tested.

The work of Morgan *et al.* (1994) showed clearly that the Spriaeoideae are not a monophyletic group and the very different lines within it therefore, need to be considered separately. The lack of information on the Sorbariae is therefore highly significant.

1.4. Geographic origin of Maloideae

The Maloideae are nearly restricted to the temperate northern hemisphere. Fifteen of 18 genera enumerated by Rehder (1940) are represented solely by Asiatic species. According to Sax (1931) and Phipps *et al.* (1991), the Maloideae must have originated in Asia before starting their migration toward America (1 genus, *Osteomeles* in Hawaii), (1 genus, *Chamaemeles* endemic to Madeira), and north Africa. Only *Hesperomeles* reached south America, following the Andes as far as Chile.

1.5. *Malus*

The genus *Malus* belongs to family Rosaceae, subfamily Maloideae. It is a north temperate genus of 25-35 species which are difficult to circumscribe due to lack of distinguishing characters, widespread crossability, transportation by people, escapes from cultivation, and introgression. These factors may blur taxonomic boundaries. Nomenculatural confusion also results from the naming as species of variants known only in cultivation. Phipps *et al.* (1990) reported 50 species in *Malus*.

In Prodromus Systematis Naturalis, De Candolle (1825) placed *Malus* in the genus *Pyrus* and recognized *Malus* as a section. De Candolle recognised: *Pyrus*

acerba, P. malus, P. proica, P. astracanica, P. spectabilis, P. prunifolia, P. baccata, P. coronaria, and *P. angustifolia.*

Rehder (1940) placed *Malus* in subfamily Maloideae with other genera including: *Cotoneaster, Mespilus, Pyracantha, Crataegus, Osteomeles, Sorbus, Aronia, Photonia, Stranvaesia, Eribotrya, Docynia, Chaenomeles, Cydonia, Rhaphiolepis, Pyrus, Amelanchier* and *Peraphyllum.* He defined *Malus* as follow:

> "*Malus* Mill . Apple. Deciduous, rarely half-evergreen trees or shrubs, rarely with spinescent branches; buds ovoid, with several imbricate scales; leaves serrate or lobed, folded or convolute in bud, stipulate: flowers white to pink or carmine, in umbel-like racemes; petals usually suborbicular or obovate; stamens 15-50, with usually yellow anthers, ovary inferior, 3-5-celled; styles 2-5, connate at base; fruit a pome without or with some grit-cells. with persistent or deciduous calyx. About 25 species in the temperate region of North America, Europe, and Asia; in North America south to Florida and Texas, in Asia to the Himalaya. Some species are important fruit-trees, others belong to our most valuable ornamental trees and shrubs with showy flowers in spring and attractive fruit in summer."

Classifications of *Malus* to species by different authors are presented in Table 1.1. Spach (1834, cited by Huckins, 1972) used the inflorescence type to subdivide *Malus.* He recognised two species groups differentiated by type of inflorescence:

1. Species with subssessile corymbs including *M. spectabilis, M. sempervirens* (= *M. angustifolia*), *M. coronaria* and *M. heterophyla.*

2. Species with sessile umbels including *M. prunifolia* and *M. paradisiaca* (= *M. pumila* var.).

Carrire (1883, cited by Huckins, 1972) divided the genus into two sections based on anther colour, the first with yellow, the second with red anthers. Koehne (1893, cited by Huckins, 1972) divided *Malus* into two section, *Calycomeles* including species possessing fruit with persistent calyces and *Gymnomeles* including

Table 1.1. Comparision of different treatments of *Malus* at the subgeneric level.

Rehder (1920)	Van Esteline (1933)	Koidzumi (1934)	Rehder (1940)	Vavilov (1968)	Huckins (1972)	Robertson *et al.*, (1991)
section 1: ***Eumalus***	**section 1:** ***Eumalus*** subsection: *Pumilae* subsection: *Baccatae*	***Gymnomeles*: *Baccata*** ***Sorbomalus*** including *M. florentina* and *M. fusca*	**Section 1:** ***Eumalus*** ser. 1 *Pumilae* ser. 2: *Baccatae*	**section 1:** ***Calycomeles*** subsection : *Eumalus* subsection 2: *Prunifoliae* subsection 3: *Chloromeles* subsection 4: *Eriolobus*	**section 1: *Malus*** subsection 1: *Malus* series 1: *Pumilae* series 2: *Baccatae* subsection 2: *Seiboldianae* subsection 3: *Kansuenses* series1: *Kansuenses* series 2: *Yunnanenses*	**subgenus 1:** ***Malus***
section 2: ***Sorbumalus*** subsection 1: *Florentinae* subsection 2: *Sieboldianeae* subsection 3: *Kansuenses* subsection4: *Yunnanenses* (*M. prattii, M. yunnanensis*)	**section 2:** ***Sorbomalus*** subsection 1: *Sieboldianae* subsection 2: *Florentinae* subsection 3: *Fuscae* subsecion 4: *Kansuenses* (including *Yunnanenses* Rehd.)	***Calycomeles:*** ***Chloromeles*** ***Eumalus***	**section 2:** ***Sorbomalus*** ser. 3: *Sieboldianeae* ser. 4: *Florentinae* ser. 5: *Kansuenses* ser 7: *Yunnanenses*	**section 2:** ***Gymnomeles*** subsection 1: *Baccatae* subsection 2: *Sorbomalus*	**section 2:** ***Sorbomalus*** *M. florentina*	**subgenus 2:** ***Sorbomalus***
section 3: ***Chloromeles***	**section 3:** ***Chloromeles***		**section 3:** ***Chloromeles***		**section 3:** ***Eriolobus***	**subgenus 3:** ***Chloromeles***
section 4: ***Eriolobus***	**section 4:** ***Eriolobus***		**section 4:** ***Eriolobus***		**section 4:** ***Chloromeles***	
section 5: ***Docyniopsis***	**section 5:** ***Docyniopsis***		**section 5:** ***Docyniopsis***		**section 5:** ***Docyniopsis***	

species with deciduous calyces. Zable (1903, cited by Williams, 1982) divided *Malus* species into two sections based on leaf characteristics: Section *Malus* with leaves convolute in the bud and unlobed at maturity and section *Sorbomalus* with leaves conduplicate in the bud and more or less lobed at maturity. According to this classification *M. florentina* and section *Chloromeles* were separated from *Calycomeles* and united with *M. sieboldii* and *M. fusca* to yield *Sorbomalus*. The remainder of the *Calycomeles* was united with *Malus baccata* and *M. halliana*, resulting in *Malus*. The use of these two characters, leaf characteristics and calyx persistence which were used by Koehne and Zabel, is founded on the morphological studies of many authors and followed by Rehder (1920, 1940).

Schneider (1906, cited by Huckins, 1972) added two sections *Eriolobus* (for *M. trilobata*) and *Docyniopsis* (for *M. tschonoskii*) by incorporating the genus *Eriolobus* within *Malus*. He also transferred to *Malus* from *Pyrus*, *M. pravingttii, M. fusca*, *M. florentina* and *M. transitoria*. He placed *M. florentina* in *Sorbomalus* with ecalyculate fruit in contrast to Koehne's assignment to *Calycomeles,* and also placed *M. prattii* with calyculate taxa of *Malus*.

Rehder (1920) divided the first two sections into subsections and created a fifth section. The European, Asian and western American species of *Sorbomalus* Zabel were grouped into three subsections: *Florentinae*, *Sieboldianae* and *Kansuenses*, while the eastern and central North American species were expelled to form a fifth section, *Chloromeles*. In addition, a fourth subsection, *Yunnanenses,* with persistent calyces, was added to section *Sorbomalus*. Rehder (1920) also transferred *Malus prattii* from *Malus* to *Yunnanenses* and *M. fusca* from *Sorbomalus* to *Kansuenses*.

Van Eseltine (1933b) modified Rehder's classification by removing *M. fusca* from *Kansuenses*, and placing it to a new subsection, *Fuscae*, and by submerging the subsection *Yunnanenses* in *Kansuenses*. Henning (1947, cited by Huckins, 1972) accepted these modifications and also returned *M. prattii* to series *Pumilae*, section *Malus*, where it originally had been placed by Schneider (1906). Koidzumi (1934) used the basic dichotomy of Koehne's subdivision to classify *Malus*. Yuzepchuk

(1939, cited by Huckins, 1972) subdivided the *Pumilae* into two series, *Silvestres* and *Prunifoliae*, and seperated *M. prunifolia* from the remainder of the subsection. Rehder (1940) retained his arrangement, only reducing his six subsections in *Malus* and *Sorbomalus* to the rank of series.

Rehder (1940) recognized 25 species in the genus divided into two groups and 5 sections:

I. The species with undivided leaves convolute in the bud, in one section.

Section 1. *Malus*, comprising series *Pumilae* with persistent calyx (*M. pumila*, *M. prunifolia*, *M. spectabilis* and *M. micromalus*) and *Baccatae* with deciduous calyx (*M. baccata*, *M. hupehensis*, *M. halliana*).

II. The species with leaves folded in the bud, sharply serrate and at least those of the short shoots more or less lobed including section 2. *Sorbomalus*, section 3. *Chloromeles*, section 4. *Eriolobus* and section 5. *Docyniopsis*.

Section 2. *Sorbomalus* with deciduous calyx, comprising series *Sieboldianae* (*M. sieblodii*, *M. floribunda*, *M. sargentii* and *M. zumi*), *Kansuenses* (*M. fusca*, *M. kansuensis*, *M. toringoides* and *M transitoria*), *Florentinae* (*M. florentina*) and series *Yunnanenses* (*M. yunnanensis* and *M. prattii* and more recently also *M. honanesis* and *M. ombrophila*, Phipps *et al.* 1990).

Section 3. *Chloromeles* including species with persistent calyx, fruit without sclerids, with impressed calyx.

Section 4. *Eriolobus* including only one species (*M. trilobata*) with fruits with sclerids, the calyx not impressed and leaves deeply lobed.

Section 5. *Docyniopsis* included only one species (*M. tschonoskii*) with fruit with sclerids, the calyx not impressed and leaves not or sligthly lobed. It now also includes several other species, *M. doumeri*, *M. formosana*, *M. melliana* and *M. tschonoskii* (Phipps *et al.*, 1990).

Terpo (1968) included *Malus florentina* in section *Eriolobus* with *M. trilobata.* Vavillov (1968) followed Koidzumi's classification and recognized the two groups of Koidzumi as sections and distinguished four subsections *Malus, Prunifoliae* and *Cholormeles* and *Eriolobus* in section *Calycomeles* and two subsections, *Baccatae* and *Sorbomalus* in *Gymnomeles.* Vavilov's classification is shown in Table 1.1.

It is noteworthy that there is no agreement on the rank of the infrageneric taxa and the placement of some species, especially the species of the sections of *Sorbomalus*, *Eriolobus* and *Docyniopsis.*

1.5.1. Section *Malus*

Series *Pumilae*

Huckins (1972) agrees with Yuzepchuk's (1936. cited by Huckins) division of *Pumilae* to two groups but recognises them as subseries: *Prunifoliae* and *Silvestres* and suggested the following key to the subseries of series *Pumilae*:

> "1- Inflorescence with 9-12 (means 9.8-11.4) nodes; flowers with 17-28 (means 19.4-22.6) stamens, without fusion of ventral carpellary bundles of adjacent carpels, locules with a false septum; fruit without or with basin at apex 1 mm or less deep and stone cells sparsely to moderately distributed in ovarian parenchyma .... subseries *Prunifoliae*
>
> 2- Inflorescence with 5-11 (means 6.2-9.8) nodes: flowers with 10-22 (means16.1-20.1) stamens, with or without fusion of ventral carpellary bundles of adjacent carpels, locules without a false septum; fruit with basin at apex 1-5 mm deep and stone cells absent from ovarian parenchyma .....Subseries *Silvestres*"

His morphological and anatomical results show that the *Prunifoliae* is more primitive than the *Silvestres.* He also suggested that the *Pumilae* are one of the more derived taxa of the genus and supported his ideas with other characters:

1. *Pumilae* have the most widespread geographical distribution of the genus, extending throughout Europe and Asia, including Japan, and have fewer geographical discountinuities.

2. It is also relatively homogeneous based on morphological, anatomical and biochemical evidence which indicate a relatively recent differentiation and distribution.

3. The species have a great diversity of reproductive systems, which range from completely sexual in *M. prunifolia* to apomictic cultivars of *M. pumila* .

4. They have a relatively high incidence of polyploidy.

He also proposed that the *Pumilae* and the *Baccatae* are closely related and recently evolved because of similarity in rolled leaves in bud and unlobed leaves on both long and short shoots.

The large fruited *Pumilae* and the small fruited *Kansuenses*, *Baccatae* and *Sieboldianae* are considered to be derived from a generalized small fruited ancestral type as exhibited by the *Yunnanenses*. The results of successful crosses of the cultivar *Malus* 'Tolman' and members of *Sieboldianae* (Grandal, 1928 cited by Huckins 1972) and budding of *Malus* 'McIntosh' on to *M. hupehensis, M. sikkimensis* and *M. toringoides* suggests that the *Pumilae*, although morphologically distinct in many characters, are similar genetically to the *Baccatae, Kansuenses* and *Sieboldianae.*

Series *Baccatae*

Huckins (1972) divided the *Baccatae* into four subseries based on morphological characters:

1. The *Baccatae* including *M. baccata* and its variants.

2. The *Hupehenses* including *M. hupehensis* and *M. halliana* .

3. The *Robustae* including *M. robusta* .

4. The *Sikkimenses* including *M. sikkimensis* and *M. rockii.*

His results suggest the *Sikkimenses* as most primitive and the *Baccatae* as the most derived subseries within the series. The larger, broader, more veined leaves of the *Sikkimenses* are intermediate between the pattern exhibited by the *Yunnanenses* and that of the series *Baccatae*. Huckins (1972) supported Simpson's idea about the natural position of subseries *Robustae* and reported that the *Robustae* have no particular features which distinguish them from any other taxa of series *Baccatae* and this contradicts the general belief in the hybrid nature of *M. robusta* resulting from crosses between *M. prunifolia* and *M. baccata*. On the basis of comparative morphology and anatomy, Huckins (1972) concluded that the *Sikkimenses* are most closely aligned with *Robustae* and the *Hupehenses* most closely aligned with subseries *Baccatae*. Subseries *Baccatae* have only the diploid number 34, while their presumably more primitive counterparts, the *Hupehenses*, are known primarily as triploids and tetraploids. Similarly the *Robustae* have diploids, and their presumably primitive counterparts, the *Sikkimenses*, are known only as triploids, tetraploids, and pentaploids. Thus, in series *Baccatae*, it seems that the more primitive the taxon the higher its level of the ploidy. All the polyploid species of series *Baccatae* are apomictic (i.e. *M. hupehensis*, *M. rockii*, *M. sikkimensis*) and all diploid species are completely sexual (i.e. *M. baccata*, *M. robusta*). It is possible that the more primitive, polyploid species, spun off from the sexual mainline of the series relatively early in its phylogeny and have persisted because of their ability to reproduce, at least in part, asexually. He also concluded that in series *Baccatae* facultative apomixis appears to be responsible for the maintenance of polyploid species with less advanced structural characteristics. Sexuality has enabled diploid species with more advanced structural characteristics to continue along the mainline of progressive evolution.

1.5.2. Section *Sorbomalus*

Series *Sieboldianae*

The *Sieboldianeae* is probably the most derived taxon of the genus. Though Huckins (1972) suggested that *Sieboldianae* are more primitive than *Baccatae* only

because of leaf vernation (conduplicate in *Sieboldianae*, involute or convolute in *Baccatae*). However, with reference to some characters, the *Sieboildianae* are more derived than the *Baccatae*, for example the degree of carpellary connation and of adnation of the floral cup to the gynoecium in the *Sieboldianae* is greater than in the *Baccatae*. There are other characteristics which support the derived status of *Sieboldianae*: 1. two of five species of this series are or have polyploid populations (*M. sargentii* and *M. sieboldii*) 2. presence of a diversity of reproductive systems, ranging from completely asxual systems in *M. atrosanguinea* and *M. zumi* to facultative apoomictic systems in *M. sargentii* and *M. sieboldii.* The *Sieboldianae* are a group of closely related and homogeneous species, with a combination of conduplicate vernation and lobed leaves, umbellate inflorescences, and ecalyculate fruit without or with only few stone cells. This conclusion is also supported by biochemical evidence, the series being distinguished from others by the presence of the sieboldin, a variant of phloridzin (Williams, 1966).

There is some evidence which indicates the *Sieboldianae* derives from *Eriolobus* including presence of trilobatin in *Eriolobus* and one seedling of *M. sieboldii* var. *arborescens* (Williams, 1966). Thus it may be possible that the *Sieboldianeae* are derived from *Eriolobus.*

Huckins (1972) believed that the *Sieboldianae* can be derived from the *Kansuenses* or from an ancestral pattern common to both. While there are differences in the stone cell distribution in the fruits of *Kansuenses* and *Sieboldianae*, they are differentially expressed in members of the *Kansuenses*. While the *Transitoriae* exhibit stone cells neither in the parenchyma of the floral cup nor along the core line, the *Sieboldianae* simply exhibit a more derived expression of this character in the absence of stone cells in the ovarian tissue. Presence of both cymose and umbellate inflorescences in *M. fusca* and *M. toringoides* is a transition to the completely umbellate inflorescences in the *Sieboldianae*. Huckins (1972) placed *Sieboldianae* in section *Malus sensu novo* with the *Pumilae* and *Baccatae* and suggested a subsectional rank.

Series *Kansuenses*

According to Huckins (1972) the *Kansuenses* are a transitional taxon, intermediate in overall pattern between the presumably primitive *Yunnanenses* and the presumably derivative *Sieboldianae*. On the basis of petal morphology and connation of the carpels and their vasculature, and meristematic characteristics of styles and stamens, *M. fusca* and its variants appear to be the most derived members of the *Kansuenses*. *M.kansuensis* has a number of characters which are intermediate between the *Yunnanenses* and *Kansuenses*. In some characteristics it looks primitive within the *Kansuenses* (eg. larger number of flowers per inflorescence; smaller, broader petals and degree of adnation of floral cup to the carpels; and, in fruit, greater abundance of stone cells than other members of the *Kansuenses*.

Van Eseltine (1933b) merged the two series *Yunnanenses* and *Kansuenses* into one subsection, *Kansuenses*. The affinity of the two series is also supported by the analysis of the flavonoid constitution of leaf tissue by Henkes (1963, cited by Huckins, 1972). On the basis of morphological and biochemical data, Huckins (1972) considered the two series, *Kansuenses* and *Yunnanenses*, a separate subsection of section *Malus*.

Vavilov (1968) believed that *M. fusca* in North West USA, a brown apple with small fruits with caducous sepals, is closer to *M. baccata* than to other north American species which have large, green fruits, non-caducous sepals and characteristic strong odour. Rehder (1940) placed it in series *Kansuenses*. *Malus fusca* is a diploid species and has never been reported to be apomictic. According to morphological and anatomical characters, *M. fusca* has derived status and this makes unlikely any possibility of affinity with the North American section *Chloromeles* (Huckins, 1972).

Series *Florentinae*

M. florentina

M. florentina has been the subject of much debate since it was described by Zuccagni in 1809 (cited by Cullen *et al.*, 1955), as *Crataegus florentina,* from the vicinity of Florence. This species has been included in different genera by different authors as discussed by Browicz (1970). Tavhioni-Tozzeti (1811, cited by Browicz, 1970) included it in *Pyrus*; Bertolini (1817) in *Mespilus*; Roemer (1847) in *Torminaria.*; Nyman (1855) in *Sorbus* (*S. florentina*); Decaisne (1874) in *Cormus*. In the same year Wenzig (1874) suggested that Zuccagni's species arose from a cross between *Sorbus torminalis* and *Pyrus malus*. However, in 1883 he changed his mind and placed this species within the genus *Sorbus* as *S. crataegifolia*. Koehne (1893) placed this species in *Malus* and used the specific name *crataegifolia* in section *Calycomeles* Koehne, and in 1903 Zabel placed it in a new section *Sorbomalus* Zabel. In 1903, Schneider suggested placing *Crataegus florentina* in the genus *Malus* under the name *M. florentina*. Rehder (1920) placed *M. florentina* in the monotypic subsection *Florentinae* Rehder. In 1940 Rehder lowered the systematic rank of subsection *Florentinae* to a series and in 1968 Terpo placed *Malus florentina* in section *Eriolobus* along with *M. trilobata.*

Browicz (1970) rejected the placing of the species in *Mespilus* and *Crataegus* because of the bony nature of the core. He also rejected inclusion of the species in *Eriolobus* because of the presence of numerous sclerids and the non deciduous calyx in *Eriolobus*. He supported his idea by the chemical evidence reported by Williams (1966), the leaves of *M. trilobata* containing a dihydrochalcone trilobatin which is not found in other *Malus* species containing phloridzin. Browicz (1970) pointed out that in this species leaves, as well as flowers, inflorescences and fruits are very variable. He considered the species as a hybrid between *Malus* and *Sorbus* and suggested that *Crataegus florentina* is a hybrid between *Malus* and *Sorbus* and that the species had similarities with both genera. According to Browicz the parental forms are most likely:

1. *Sorbus torminalis* from which species the leaf form is inherited as well as the presence of grit cells in the fruits and some anatomical characters of wood.

2. *Malus sylvestris*, the influence of which is manifest in the few flowers in the inflorescence, in the presence of 5 styles in the flowers, in the pubescence of leaves and in the presence of phloridzin in the leaves.

Huckins (1972) suggests a relict status rather than a hybrid origin for this taxon. He proposed that "*M. florentina* may represent a line of divergence from basic pomoid stock early in radiation of the *Maloideae*, and at the present time its affinity with *Malus* appears to be stronger than with *Sorbus* or any other genus". The chromosome number of 2n = 34 is reported for this species by Huckins (1972).

Series *Yunnanenses*

Geographically, the *Yunnanenses* are more or less confined to a few provinces of central China, primarily Yunnan and Szechuan (Yu and Yu, 1956). This distribution corresponds closely to the region of greatest concentration of *Malus* species in China, according to Yu and Yu the provinces of Shensi and Szechuan.

Leppik (1970) suggested that eastern Asia might be the original area of *Malus,* on the basis of his study of the genetic sources of disease resistance within the genus. Huckins (1972) showed that, based on morphological and anatomical characters, *Yunnanenses* is the most primitive taxon in the genus and supported Leppik's idea. He also proposed that the section *Malus* may have evolved from the *Yunnanenses.*

No hybridization, natural or artificial has been reported involving species of series *Yunnanenses* and any other.

Only diploidy is known to occur in members of this series. Rybin (1927) reported *Malus prattii* to be a diploid and Huckins (1972), Liang and Li (1993) and Schuster and Buttner (1995) reported that *M. yunnanensis* is also diploid. There are also no reports of apomixis in the literature.

1.5.3. Section *Chloromeles*

Henning (1947, cited by Vavilov, 1968) suggested considering the apple species of north America, described by Rehder (1940) and later by Van Eseltine (1933a), as subspecies, retaining two with the status of species, *M. coronaria* and *M. ioensis.* Robertson (1974) considers that although this section is quite distinct from other sections of *Malus*, it is very plastic, its members being particularly variable in leaf shape and degree of lobing, indumentum density, and fruit size. It is on the basis of these characters that nine species and numerous varieties have been recognized. The situation evidently has been complicated by hybridization and perhaps introgression among the native crabs and between them and cultivated apple. Polyploidy has been reported in *Chloromeles* (see Table 1.3). The only known wholy diploid species is *M. soulardii.* Species with high levels of diploidy are: *M. angustifolia* and *M. ioensis* both with diploids and tetraploids. *M. bracteata* is known as triploid while *M. coronaria, M. lancifolia* and *M. platycarpa* are known only as tetraploids. There is no report of apomixis in any of the *Chloromeles*. Huckins (1972) suggested that "*Chloromeles* has been native of the westerm hemisphere for a considerable time. The general homogeneity of the group, together with considerable differences in level of ploidy, indicate that it has probably differentiated in its present location". He also considered the *Chloromeles* as a relict for the following reasons:

"1. High incidence of polyploids and sexual species.

2. Widespread distribution and diversity of habitat.

3. Considerable degree of minor morphological differentiation indicated by the relatively large number of species and varieties described.

4. Development of adaptive complexes related to pollination and dissemination which are as advanced as any in the genus".

Decaisne (1874, cited by Robertson *et al.*, 1991) for the first time and then Robertson *et al.* (1991) considered the *Chloromeles* as a subgenus in *Malus*.

1.5.4. Section *Docyniopsis*

This section has been considered close to *Yunnanenses* by some authurs. Koidzumi (1934) united members of both taxa in the genus *Docyniopsis*. Likhonos (1964 cited by Huckins, 1972) considered *Malus prattii* (*Yunnanenses*) and *M. formosana* (*Docyniopsis*) as members of one section *Eriomeles*.

Docyniopsis is considered one of the most primitive taxa in the genus by Huckins (1972). He proposed that it is likely that *Docyniopsis* diverged from the main line of the genus before the *Yunnanenses* and their subsequent radiation. Robertson *et al.* (1991) excluded the *Docyniopsis* from *Malus* and gave generic rank to it.

1.5.5. Section *Eriolobus*

Section *Eriolobus* contain only one species, *M. trilobata*. It has been treated as a distinct genus by botanists. It is a very distinct species. The distinguishing characters are the combination of lobed leaves; large flowers in a simple umbel, the petals concave, woolly-ciliate at base, and notably clawed; sepals longer than the densely tomentose receptacle; and densely hairy cone from which arise five styles united at the base into a densely tomentose column, and mature fruit with stone cells (Bean, 1923). Huckins (1972), based on morphological and anatomical characters, considers this species the most primitive in the genus. Its percentages of primitive characters is similar to *Yunnanenses*. This species is considered one of the more ancient of the genus. This is supported by its relatively restricted and disjunct geographic distribution in Western Asia.

Huckins (1972) stated, "the particular combination of characters which is pecular to *M. trilobata* and which distinguishes it from the remainder of the genus supports its maintenance as a monotypic section of the genus". Robertson *et al.* (1991) raise the rank of the *Eriolobus* to genus.

1.6. Affinity of *Malus* to other genera

Chaenomeles, *Pseudocydonia* and *Docynia* are recognized by Robertson *et al.* (1991) as most closely related to *Malus*. Weber (1964) points out that *Chaenomeles* is similar to most species of *Malus* in having few or no stone cells in most of the pulp. However, *Chaenomeles* has many stone cells around the dorsal bundles of the carpels, and in this respect it differs from *Malus*. *Docynia* and *Malus* are similar in having a sclerenchymatous inner epidermis in the fruit and underlying cells of the inner epidermis. In particular *M. tschonoskii* is very similar to *Docynia* in having clusters of stone cells in the pulp (Iketani and Ohashi, 1991).

The occurrence of phloridzin in *Docynia* leaves suggests a close relationship with *Malus* as it is only found in these two genera (Challice, 1973b, Williams, 1982). *Malus* was for a long time treated as a section within *Pyrus* (De Candolle, 1825). However, the degree of density of stone cells in the pulp is a useful character for distinguishing *Pyrus* and *Malus*. There are many stone cells in the pulp of *Pyrus* but only a few or no stone cells in that of *Malus* with the exception of sections *Docyniopsis* (*M. tschonoskii*), *Eriolobus* (*M. trilobata*) and series *Yunnanenses*. In pulp structure *Pyrus* is similar to *M. tschonoskii*, but *Pyrus* doesn't have the sclerenchymatous inner epidermis found in all *Malus* species. This evidence reinforces the slender morphological distinction between *Malus* and *Pyrus*. It is also significant that *Malus* and *Pyrus* do not readily intergraft and are apparently unable to interbreed (Taylor, 1983), although Huckins (1972) cites successful crosses involving *Malus* and *Pyrus* reported by Grane and Marks (1952, cited by Huckins 1972).

Hybridization with other genera has not been reported in the literature, only Browicz (1970) proposed that *M. florentina* is the product of hybridization between *Malus* and *Torminaria*. Robertson *et al.* (1991) included this cross in their scheme for intergeneric hybrids in subfamily Maloideae (Figure 1.2).

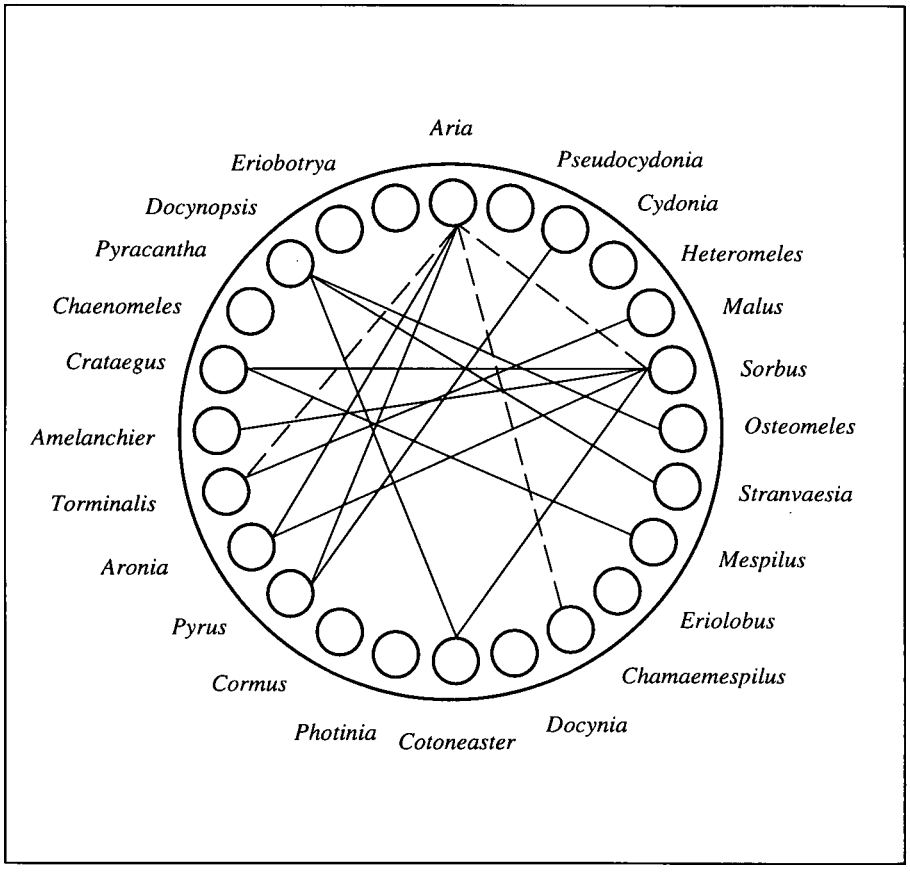


Figure 1.2. Published intergeneric hybrids in subfamily Maloideae. Dashed lines represent frequently occurring hybrids within *Sorbus* s.l. After Robertson *et al.* (1991).

1.7. Geographical origin of *Malus*

According to Vavilov's theory (1968) of the centre of origin of cultivated plants the origin is the region with greatest diversity. For apple species this is eastern Asia (particularly the Far East, Japan and China) so he suggests the centre of specific variation of the apple as consequently also its centre of origin. Liang and Li (1993) support Vavilov's theory by karyotype study of the genus and describe the section *Docyniopsis* and the series *Yunnanenses* as the relict primitive forms. The results of an investigation of the Karyotypes of the genus by Liang, *et al.* (1994) indicate an alloploid origin of *Malus*, with a base number of 17. According to studies by Leppik (1970) on the genetic sources of disease resistance within the genus, the original area of *Malus* might be eastern Asia.

1.8. Morphological characters in *Malus*.

1.8.1. Bark

Huckins (1972) categorized the rhytidome or outer bark, of *Malus* into three groups: firm, platey, and scaly, and noted that the type of bark at the basal portion of the main trunk is characteristic of particular taxa. In *Sieboldianae* and *Chloromeles* it is entirely scaly. *Pumilae*, *Baccatae*, and *Kansuenses* also have scaly bark at maturity with the exception of *Malus prunifolia* var. *rinki* (*Pumilae*), *M. sikkimensis* (*Baccatae*) and *M. kansuensis* f. *calva* (*Kansuenses*), which have platey bark. The *Florentinae*, *Yunnanenses* and *Docyniopsis* are characterized by entirely firm or only basally platey bark at maturity.

1.8.2. Leaves

A few species of *Malus* are evergreen or semi-evergreen, although most are deciduous (Robertson *et al.*, 1992). Some of their leaf characters are useful in distinguishing the taxa in *Malus*.

Leaf vernation

In *Malus* three types of vernation have been recognized and this character has served to provide the primary taxonomic division of Zabel's classification. Huckins (1972) demonstrated that the genus can not be seperated into clear cut infrageneric taxa only on the basis of leaf vernation. Sections *Sorbomalus, Docyniopsis* and *Eriolobus* have folded (conduplicate) vernation while the members of section *Malus* have rolled vernation. Series *Baccatae, M. toringoides* and *M. transitoria* have regularly involute vernation. Leaves in bud are arranged independently or associated in groups of two or more which are folded or rolled within one another. Vernation in *Yunnanenses, Florentinae*, *Chloromeles*, and *Docyniopsis* is independent, conduplicate. The *Sieboldianae* and *Kansuenses* are basically conduplicate but exhibit some tendency toward the convolute and associated conditions. Many *Baccatae* are characterized by independently involute leaves, but exceptions occur in some individual trees. The *Pumilae* are generally convolute, although independent-convolute leaves are also exhibited.

Leaf lobing

Taxa of *Malus* have always been characterized by some degree of leaf lobing or at least having the potential for such expression. Leaf lobing is correlated with leaf vernation. In section *Malus* Rehder with rolled vernation, all the leaves, regardless of position on the tree, are generally unlobed. Huckins (1976) noted that both Asami and Passecker noticed taxa of this section with sublobed or lobed leaves. In other sections of the genus characterized by folded vernation the leaves, at least on long shoots, are lobed. Robertson *et al.* (1992) pointed out that the only genera with consistently lobed leaves in Maloideae are *Torminalis* (=*Sorbus torminalis*) and *Eriolobus*. The leaves of *Eriolobus trilobatus* (Poiret) M. Roemer are deeply trilobed and can appear almost palmate. However, the termminal lobe is usually further divided into three lobes, and the overall lobing pattern and the secondary venation are pinnate. Within *Malus*, slightly to prominently lobed leaves occur at least on the long shoots and sometimes

also on the short shoots of subgenus *Malus* sect. *Sieboldianae* (the section as designated in Rehder, 1940); subsection *Sieboldianae* and *Kansueneses* of Huckins (1972), subgenus *Choloromeles*, and subgenus *Sorbomalus* (Robertson *et al.*, 1991). In *M. florentina*, all leaves, regardless of type of shoot, are lobed (Browicz,1970, Huckins, 1972).

Deeply lobed leaves are extremely common in the Asiatic and North American apples (*Malus*) and are found in most western Eurasian *Crataegus* (Sections, *Oxyacanthae*, *Azaroli*, and *Pentagynae*), in some North American *Crataegus* (e.g. Series *Cordatae*), in *Docynia* and in some pears (*Pyrus*) (Phipps *et al.*, 1991). Nearly palmately lobed foliage occurs in *Eriolobus (Malus) trilobata*, and there is a fully trilobed leaf in *Crataegus trilobata* (Phipps *et al.* 1991).

Huckins (1972) noticed that differential expression in lobing varies from individual to individual with age and vigor appearing to be important factors. Leaves on juvenile plants and vigorous, especially sucker and epicormic, shoots exhibit more pronounced lobing than do leaves on plants in advanced maturity or on less vigourous portions of a plant. This phenomenon is most evident in *M. sargentii, M. sieboldii, M. zumi, M. fusca, M. toringoides, M. coronaria, M. glaucescens* and *M. platycarpa.* Huckins (1972) expressed the lobing of the leaves in *Yunnanenses* and *Docyniopsis* as lobulate. The tendency to lobing in the spiraeoid genera *Neillia* and *Physocarpus* produces leaves resembling the *Malus fusca* lobed type in shape but not in sinus venation.

Leaf toothing

Within *Maloideae* it is common for teeth to be more conspicuous toward the apex of the leaves, decreasing in size toward the bases. It is rare for the teeth to be uniformly distributed from apex to base. Toothing is predominantly serrate, with a tendency to become crenate in some genera, most notably *Aria, Chaenomeles, Malus, Photinia* and *Pyrus.*

The toothing can be markedly serrate or dentate in species of *Malus*. There is a strong correlation between lobing, toothing and type of secondary venation. Lobed leaves always have craspedodromous venation. In some species of *Malus* the secondary veins go to the tips of the lobes and also to the base of the sinuses. The doubly toothed leaves found in some species of *Aria, Crataegus, Docyniopsis* and *Malus* are also craspedodromus. Camptodromous venation is characteristic of leaves with simply serrate to entire margins (Robertson *et al.*, 1992).

In *Crataegus* and *Malus* some groups of species have consistently both craspedodromous and camptodromous leaves on the same plant: craspedodromus on long shoot; camptodromus on short shoots.

Leaves with cordate bases are found in *M. trilobata* and truncate to slightly cordate bases are found in some species of *Malus*. Unlobed toothed leaves with camptodromous venation found in *Malus* and many Maloideae are characteristic of Amygdaloideae such as *Padus virginica* and *Prunus domestica.*

Maloid vegetative characteristics manifest a considerable range with nearly exact matches occuring in both *Spiraeoideae* and Amygdaloideae and fewer and poorer matches with some Rosoideae.

1.8.3. Inflorescence

The inflorescence of *Malus* is simple umbellate. However, in the inflorescence of a few taxa (*M. baccata* var. *gracillis*, *M. rockii*, *M. sikkimensis*, *M. sargentii*) the most basal fertile node may give rise to a secondary axis bearing two or three flowers (Huckins, 1972).

1.8.4. Flower

The perianth in *Malus* is always pentamerous. However, some species show variation in number of both sepals and petals. Huckins (1972) noticed that series *Baccatae* showed the greatest amount of variation; he observed tetramerous perianths in *M. baccata* f. *gracilis* and *M. halliana* var. *spontanaea* and hexamerous perianths in

M. hupehensis. He showed that *M. sikkimensis* exhibited the greatest degree of variation in the *Baccatae* as well as in the genus. *M. kansuensis* var. *calva* shows additional petals (9.8%) 6 to 12, *M. fusca* shows tetramerous perianths and *M. toringoides* shows (3.1%) flowers with four sepals but six petals. In Sieboldianeae, *M. sieboldii* (3.6%) and *M. sargentii* (9.3%) show tetramerous perianths. In *Chloromeles*, *M. platycarpa* (5.4%) shows a tetramerous corolla. In *Pumilae, M. prunifloia* (0.7%) shows staminoid petals and (7.1%) shows four petals and 21 stamens. *Malus florentina* shows 5.0% variation in perianth and 4.0% of *M. tschonoskii* show tetramerous perianths.

Rohrer *et al.* (1994) remarked on some flower characters common to most species of *Malus;* spreading orientation of the calyx lobes, except in *M. trilobata*; the presence of pink petals; 20 stamens, except more than 25 stamens in *Malus*, Section *Docyniopsis*; deeply inferior ovary which is overtopped by tissue of the hypanthium; glabrous base of cavity of hypanthium where the connate styles emerge, styles fused above the base and becoming densely hairy distally to about where they split into several free styles. However, some species of *Malus* have completely glabrous styles.

Huckins (1972) reported that carpel number in *Malus* usually is 5, but it differs in some species. He has also shown that in one individual of *M. baccata* an increase in vigor is accompained by an increase in carpel number and vice versa.

Fusion of styles in species of *Malus* has been used for a long time to separate this genus from *Pyrus*. However, within *Malus* there are some species which show separate styles. Series *Chloromeles* and *Docyniopsis* are characterized by seperate styles. Styles with canals (i.e. central cavities which result from the direct continuation of the locule into the style) are found in *M. tschonoskii, M. trilobata,* some species of *Chloromeles* and *Yunnanenses*, and late flowers of *M. florentina* (Huckins, 1972). Such canals are completely absent from the *Pumilae, Baccatae, Sieboldianae* and *Kansuenses.* The greatest degree of carpel connation and carpel and floral tube adnation has been reported in *Sieblodianae* and *Baccatae* while *M. tschonoskii* expresses the least degree of both types of fusion. The *Kansuenses*, express relatively

high degrees and the *Florentinae, Chloromeles* and *Eriolobus* express relatvely low degrees of both types of fusion (Huckins, 1972).

Based on floral characters, Robertson *et al.* (1991) raise *M. trilobata* and *M. tschonoskii* to generic rank, in *Eriolobus* and *Docyniopsis,* respectively. In *M. trilobata* the calyx lobes are strongly recurved, the hypanthium floor is pubescent and the ovary and hypanthium are only partially adnate. *M. tschonoskii* has flowers similar to those of *Docynia* and unlike those of *Malus*, both having large flowers with 40-55 stamens and a five-carpelate ovary that is fully connate but only partially adnate to the hypanthium. The nonadnate portion of their ovaries is a cone with long woolly pubescence.

1.8.5. Fruit

The morphological interpretation of the apple fruit has for long generated theoretical controversy. There are two divergent of lines of thought:

1. Kraus and Ralston (1916 cited by Tukey and Young 1942) reported that the apple consists of five drupelike carpels contained within the fleshy torus or receptacle and is therefore in large part a fleshy development of the stem or axis.

2. MacDaniels (1940) reported that the fleshy portion of the apple, exclusive of the included carpels, is composed of the fused and enlarged bases of the floral appendages and represents the floral tube.

Other studies have suggested that the hypanthium is derived both from tissues of the receptacle and floral appendages (Gracza,1970 cited by Rohrer *et al.*, 1991). The friuts of Maloideae vary considerably in degree of carpel connation, in adnation between hypanthium and ovary, and in core texture (Rohrer *et al.*, 1991).

Rohrer *et al.* (1991) described the internal structure of all pomes by recognising three regions;

1. Skin, as epidermal plus any subepidermal layers differentiated by cell size or colour from the bulk of the flesh.

2. The flesh as a paranchymatous region between the skin and the core.

3. The core, which occurs in the centre of fruit and contains the seeds.

He recognized two type of core: a pyrenous core as in tribe Crataegeae and a nonpyrenous core as in the tribe Sorbae. Iketani and Ohashi (1991) provide a description of the structure of fruit in *Malus*.

> "Inner epidermis and neighbouring cells are sclerified and usually the shape of these sclereids is radially elongated. Stone cells are absent or only a few in the flesh except for *Malus tschonoskii*, in which small clusters of stone cells appear sparsely or rather densely in the flesh. Pigment cells are absent or appear rarely in the flesh except for *M. tschonoskii* in which there are a few pigment cells in the flesh. A portion of free hypanthium and the sepals fall off after anthesis or they are persistent in the mature fruit."

Fruit formation in apple

Grane and Lawrence (1929) found that often a single seed is sufficient for the development of a fruit and even this seed may be imperfect. This approaches parthenocarpy and renders fruit production still less dependent on the formation of seeds. Fruitfulness in apple may therefore be maintained in spite of a high degree of sterility.

Origin of the pome

Rohrer *et al.* (1991) hypothesised that the ancestral pome fruit had five carpels, minimal connation of carpels, minimal adnation of ovaries to the hypanthium, two ovules per carpel and a leathery core. Iketani and Ohashi (1991) and Rohrer *et al.* (1991) reported that the fruit of Crataegeae is more primitive than that of Sorbae. Two types of evolutionary trend in the fruit in Maloideae may exist (Iketani and Ohashi, 1991). One proposal (MacDaniels, 1940) for the ancestoral type may be an achene derived from a follicle through the indehiscence of the ventral suture, all parts of the carpels becoming sclerified at the maturity of the fruit, subsequently the hypanthium

became fleshy and enlarged, enclosing the carpels.The other proposal is derivation from a 5-carpellary amgdaloid drupe together with hypanthial adnation in which only the innermost part of the carpels becomes sclernchymatous in the drupes (Kraus and Ralston, 1916). Phipps *et al.* (1991) mentioned that if the pome is derived from an amygdaloid drupe, then the soft carpellary tissue derived from the drupe must grade into the soft hypanthial tissue seen in the maloid pome. Therefore, if maloids are related to the amygdaloid evolutionary line, then it must have been at quite an early, or proto-drapaceous, stage of amygdaloid evolution. They concluded that the nature of the pome is an autapomorphy certainly related to the spiraeoid follicle and only possibly to an amygdaloid drupe. The first hypothesis is favoured by Wiley (1981, cited by Iketani and Ohashi, 1991), and Iketani and Ohashi, 1991). Morgan *et al.* (1994) included three genera with dry fruits, *Vauquelinia, Lindleya* and *Kageneckia* in Maloideae and suggest that the pome is derived from follicular or capsular fruits by the development of an expanded, fleshy hypanthium enclosing the ancesteral follicules or capsules. They also concluded that *Vauquelinia,* which has partially connate carpels and *Lindleya* with completely connate carpels, represent intermediate stages between the apocarpus follicles of *Kageneckia* and the pomes of more advanced taxa.

1.8.6. Seeds

According to Pelc (1984) in *M. sylvestris* seeds the site of attachment is wide and frequently surrounded by a fold of the seed coat. In this species, the hilum is least pronounced, sometimes visible in the form of a slight tuberosity. The site of attachment of the free part of the funiculus to the seed usually appears as a fold formed by the seed coat and surrounding a pit-like cavity. On the surface of the seed there are greatly elongated fusiform papillae arranged along the long axis of the seed. The colour of the seeds is solid light brown to brown with a slight grey tinge.

Seed germination

Investigation of seed germination of *M. niedzetzkyana* and a variety of *M. malus* by Lewis and Grane (1938) showed that by removing the endosperm and nucellar tissue germination was stimulated and the embryo germinated before the seed was mature. However, even after a few months, the seedlings remained stunted. Whole seeds develop normally. He describes the germination of the embryo as follows. In twelve hours the cotyledons separate appreciably, traces of anthocyanin and chlorophyll appear by the second or third day. The cotyledons green fully in 6- 8 days, at which time also the radicle begins to grow. After 14 days the seedlings which had developed normally could be potted up into a standard compost. Staito, *et al.* (1993) produced normal plantlets of *M. hupehensis* by embryo culture at low temperature (4° C) in the dark for 80 days. They also reported that treatment of embryos at 26° C in light gave rise to abnormal plantlets with short hypocotyls.

Seedlings *in Malus*

Thomas (1914) found that the radicles of the members of Spiraeoideae and Rosoideae, examined by him are diarch while those of the Prunoideae are tetrarch and in the Pomoideae both tetrarch and diarch forms are present as well as hexarch and even octarch arrangements. Seigler and Bowman (1939) reported that the primary root of the apple is usually characterised by a tetrarch radicle protostele.

1.9. Geographical distribution of *Malus*

The distribution of the species of *Malus* is presented in Table 1.2. There is no record of any apple growing wild in the southern hemisphere. All of the species reported are native to the northern hemisphere. The largest number of species are in eastern Asia, chiefly Japan and China. In North America there are several species belonging to section *Chloromeles* that are quite distinct from European and Asiatic species, the only North American species not belonging to *Chloromeles* is *Malus fusca* of section *Kansuenses*, from West North America. This differs from the other

Table 1.2. Geographical distribution of genus *Malus*. After Phipps *et al.* (1990).

Taxon	Distribution
Section *Malus* [section Eumalus Zabel]	
Series *Malus* [series *Pumilae*]	
1. *M. asiatica* Nakai	Xinjiang and Liaoning to Yunnan
2. *M. chitralensis* Vassilcz.	W Pakistan
3. *M. dasyphylla* Borkh.	Danubia, Balkans
4. *M. domestica* Borkh.	Europe, Russia (incl. naturalized range)
5. *M. kirghisorum* Al. and An Theod.	Iran
6. *M. micromalus* Makino	Yunnan, NE China, Japan
7. *M. montana* Uglitzk.	C Asia
8. *M. orientalis* Uglitzk.	Caucasus
9. *M. praecox* (Pall.) Borkh.	Russia
10. *M. punifolia* (wild.) Borkh.	NE China
11. *M. pumila* Mill.	Europe
12. *M. sieversii* (Ledeb.) Roem.	W Xinjiang, C Asia
13. *M. niedzwetzkyana* (Diek ex Koehne	C Asia
14. *M. spectabilis* (Ait.) Borkh.	Yunnan, E China
15. *M. sylvestris* Mill	Europe
16. *M. turkmenorum* Juz. and M. Pop.	C Asia
Series *Baccatae* (Rehder) Rehder	
17. *M. baccata* (L.) Borkh.	NE China
18. *M. halliana* Koehne	C and E China, Japan
19. *M. hupehensis* (Pamp.) rehder	C and E China
20. *M. manshurica* (Maxim.) Kom.	Far east USSR, NE China, Japan
21. *M. pallasiana* Juz.	Mongolia, Far east USSR, NE China, Japan
22. *M. robusta* Rehder	China
23. *M. rockii* rehder	Xizang, NW Yunnan, SW Sichuan
24. *M. sachalinensis* Juz.	Sakhalin
25. *M. sikkimensis* Koehne ex Schneider	E Himalayas
26. *M. spontanea* (Makino) Maniko	Japan
Sectiom *Sorbomalus* Zabel ex Schneider	
Series *Sieboldianae* (Rehder) Rehder	
27. *M. floribunda* Sieb.	Japan
28. *M. sargentii* Rehder	Japan
29. *M. sieboldii* Rehder	Japan, C and E China

Table 1.2. Geographical distribution of genus *Malus*. After Phipps *et al.* (1990).

Taxon	Distribution
30. *M. toringo* Sieb.	Japan, China
31. *M. zumi* Rehder	Japan
Series *Florentinae* (Rehder) Rehder	
32. *M. florentina* (Zucc.) Schneider	Italy, Yugoslavia, Greece, Turkey
Series *Kansuenses*	
33. *M. fusca* (Raf.) Schneider	Alaska
34. *M. kansuensis* (Batal.) Schneider	C China
35. *M. komarovii* (Sarg.) Rehder	Jilin
36. *M. toringoides* Hughes	Gansu, S Sichuan
37. *M. transitoria* (Batal.) Shneider	NC China
Series *Yunnanenses* (Rehder) Rehder	
38. *M. honanensis* Rehder	NC China
39. *M. ombrophila* Hand.-Mazz.	NW Yunnan
40. *M. prattii* (Hemsl.) Schneider	NW Yunnan, W Sichuan
41. *M. yunnanensis* (Franch.) Schneider	SC China
Section *Chloromeles* (Decne.)Rehder	
42. *M. angustifolia* (Ait.) Michx.	S North America
43. *M. bracteata* Rehder	S. U S.
44. *M. coronaria* (L.) Mill.	E North America
45. *M. glabrata* Rehder	E North America
46. *M. glaucescens* Rehder	E North America
47. *M. ioensis* (Wood) Britt.	C North America
48. *M. lancifolia* Rehder	E North America
49. *M. platycarpa* Rehder	E North America
50. *M. soulardii* (Biley) Britt.	C North America
Section *Docyniopsis* Schneider	
51. *M. doumeri* (Boiss.) Chev.	Taiwan, SE Asia
52. *M. formosana* (Kaw. & Koidz.)Kaw & Koidz.	Taiwan
53. *M. melliana* (Hand.-Mazz.) Schneider	SE China
54. *M. tschonoskii* (Maxim.) Chev.	Japan
Section *Eriolobus* (DC.) Schneider	
55. *M. trilobata* (Labill.) Schneider	E Mediterranean

American crab apples and resembles some of the Asiatic crab apples in several characteristics.

The northernmost species is the Siberian crab (*M. baccata*) and the nearest species to the equator is *M. formosana* at altitudes of 2100 to 2400 meters above sea level in Taiwan (Boer, 1959). In the eastern hemisphere, the genus is distributed in Asia and Europe.

Series *Pumilae* contains species which are distributed in Europe and Asia. Members of the series *Baccatae* are distributed in E. Siberia, China, Korea and Japan. In section *Sorbomalus*, the series *Sieboldianeae* is restricted to Japan with the exception of *M. sieboldii* which also occurs in China. The only species of series *Florentinae*, of section *Sorbomalus, M. florentina*, is restricted to Italy, Yugoslavia, Greece and Turkey (Browicz,1970 and Phipps *et al.*,1990). In series *Kansuenses,* one species, *M. fusca*, is distributed from Alaska to California. Other species occur in China. *M. kansuensis* and *M. transitoria*, in N. W. China and *M. toringoides* in W. China. Two species of series *Yunnanenses,* occur in China, *M. yunnanensis* in W. China and *M. prattii* in central and west China. Section *Docyniopsis* occurs in Japan, China and Taiwan, S.E. Asia. *M. tschonoskii* is confined to Japan (Phipps *et al.*, 1991). Section *Chloromeles* is found only in United State and Canada in the wild.

1.10. Chromosome number

Malus does not have the uniformity of chromosome number of genus *Pyrus*. Diploid, triploid and tetraploid species occur in the genus, pentaploidy, hexaploidy and aneuploidy apparently exist only in cultivars and interspecific hybrids. The base chromosome number similar to subfamily is 17 (Robertson, 1976).

The first report of chromosome numbers in *Malus* was by Nebel (1929). Table 1.3 shows the chromosome numbers reported by previous authors .

Liang and Li (1993) reported that the karyotype evidence shows that the most primitive groups in *Malus* are section *Docyniopsis* and series *Yunnanenses.* Section

Table 1.3. Chromosome numbers of some species of *Malus*.

species	Nebel (1929)	Sax (1959)	Rui-Yang *et al.* (1986)	Liang and Li (1993)	Schuster and Buttner (1995)
M. asiatica	-	-	34	68	-
M. baccata	34	-	34	34	34
M. baccata var. *mandshurica*	-	-	34	34	34
M. coronaria	68 ± 2	-	-	-	34
M. floribunda	-	-	-	-	34
M. florentina	-	-	-	-	34
M. fusca	-	-	-	-	34
M. formosana	-	-	-	34	-
M. glaucescens	68	-	-	-	68
M. halliana	34	-	51	34	-
M. hunnanensis	-	-	34	34	-
M. hupehensis	-	51	51	51	34, 51
M. kansuenesis	-	-	34	-	-
M. melliana	-	-	-	34	-
M. micromalus	-	-	34	34	34
M. ombrophila	-	-	-	34	-
M. prunifolia	34	-	34	34	34
M. prunifolia var. *macrocarpa*	34	-	-	-	34
M. adstringenes	-	-	-	-	51
M. niedzwetzkyana	-	-	-	-	34
M. pumila	-	-	34	34	34
M. rivularia	34	-	-	-	-
M. rockii	-	68	51	34,51	34
M. sargentii	34	68	-	-	68
M. sieboldii	-	-	51	51	34
M. sieversii	-	-	34	34	34
M. sikkimensis	-	51	51,68	34	34
M. soulardii	34	-	-	-	34

Cont'd. Table 1.3. Chromosome numbers of some species of *Malus*.

M. spectabilis	51 ± 1	-	34	51	34
M. sylvestris	-	-	-	-	34
M. baccata Jackii	-	-	-	-	34
M. toringoidea macrocarpa	-	-	-	-	68
M. toringoides	-	51	34, 68	34,51,68	51
M. transitoria	-	-	34, 51	34 ,51	34
M. xiaojiinensis	-	-	-	68	-
M. baccata columnaris	-	-	-	-	34
M. baccata var. *himalaica*	-	-	-	-	34
M. sieboldii arborescens	-	-	-	-	34
M. robusta	-	-	-	-	34
M. zumi	-	-	-	-	34
M. halliana 'parkmanni'	-	-	-	-	34
M. halliana spontanea	-	-	-	-	34
M. sargentii 'osea'	-	-	-	-	51
M. dawsoniana	-	-	-	-	34
M. platycarpa	-	-	-	-	68
M. ioensis	-	-	-	-	34
M. ioensis var. *palmeri*	-	-	-	-	51
M. tschonoskii	-	-	-	-	34
M. trilobata	-	-	-	-	34
M.yunnanensis	-	-	-	34	34

Pumila and Series *Baccatae* are the most advanced or derived and it is supposed that series *Sieboldianae* and *Kansuenses* are intermediate in evolution. They also showed that *M. sieversii* may be relatively primitive in series *Pumilae* and might be an ancestor of the cultivated apple (*M. pumila*). Liang *et al.* (1994) studied the karyotype of *Malus* species having 2n = 34 and reported that in the karyotype most chromosomes are metacentric and submetacentric and 1-2 pairs of subtelocentric homologues chromosomes are always present.

Schuster and Buttner (1995) reported 2n = 34 for all the species of series *Pumilae* examined by them, they also reported diploid, triploid, and tetraploids species in series *Pumilae* and proposed that the diploid and tetraploid genotypes of *M. hupehensis* are probably the result of hybridization of tetraploid with diploid species. The reason for different ploidy levels within species is the possibility of autoploidy.

1.11. Polyploidy

Polyploidy in the *Maloideae* is generally limited to triploids and tetraploids, although pentaploid and hexaploid progeny have been reported in *Malus* and other genera of this subfamily. The wild polyploids of *Malus* are usually considered as allopolyploid. Liang and Li (1993) reported allotriploidy in *M. hupehensis* and segmental allotriploidy in *M. spectabilis*, *M. sieboldii, M. toringoides*, *M. transitoria* and autotetraploidy in *M. xiaojinensis.*

Some characters correlated with ploidy level in *Malus*:

1. Pollen germination decreases (Grane and Lawrence, 1931) and pollen diameter increases with ploidy (Olden, 1953).

2. Polyploids have larger stomata (Heilborn, 1932. Bishop, 1953).

3. Leaf thickness, roundness, and area, number of mariginal serrations increase with higher level of polyploidy (Bishop, 1953).

4. Size of flowers and fruit increases with higher level of polyploidy (Bishop, 1953).

1.12. Sterility and incompatibility in *Malus*

The triploid cultivars of apples are characterised by a high degree of generational sterility but it is expressed in the formation of imperfect seeds and weak offspring rather than by failure to form fruits. The expression of incompatibility is more complex and variable in polyploids than in diploids. This is attributable to the polysomic condition of the factors which determine incompatibility in polyploids and consequent interactions favourable to greater variation in pollen-tube growth (Grane and Lawrence, 1931). They also reported that the proportion of good pollen in triploids varies from 4 to 27 percent, whereas that of known diploids ranges from 50 to 97 percent.

1.13. Propagation

In general, tree fruit varieties are propagated either by budding or by grafting. By means of root cuttings, however, the practicability of building up populations of clones from apple (*Malus*) seedlings has been demonstrated by several authors. Sielgler and Bowman (1939) reported that in general cuttings from roots that exhibit macroscopically a large number of primordia cushions are propagated most successfully. They concluded that adventitious roots and shoots have their origin in tissues that have made considerable secondary growth. Adventitious root primordia originate in the region of the vascular cambium or in near derivatives of cambial cells not associated with rays. Shoot primordia are organised as a result of meristematic activity of ray parenchyma cells.

1.14. Apomixis

Apomixis is used here to denote as asexual reproduction, strictly agamospermy. In Maloideae apomixis occures through apospory. In *Malus* the megaspore mother cell degenerates, an adjacent somatic cell of the ovule develops into a megagametophyte with an unreduced chromosome number, and the embryo then develops without fertilization (Robertson, 1974). Apomixis occurs only in polyploid

species of the Maloideae. As in all other Maloid apomicts, *Malus* apomicts are pseudogamous requiring pollination and fertilization of the central fusion nucleus to stimulate endosperm formation before an apomictic embryo can develop.

Apomixis in all known species and cultivars of *Malus* is facultative, with a balance between apomictic and sexual formation of seed. The percentage of apomictic seed produced varies with genotype. Apomixis can be nearly absolute in some species, such as *Malus hupehensis*. The degree of apomixis also is affected by environmental conditions during embryo sac development; apomixis is favoured by short day length and low temperature. Sax (1959) reported *M. hupehensis, M toringoides* and *M. sikkimensis* as apomictic species in *Malus*, all asiatic and triploid with 51 chromosomes. Apomictic *Malus* spp. vary in the percentage of apomictic seed produced from 20% to more than 90%. The degree of apomixis in *M. sargentii, M. sieboldii* and *M. sikkimensis* ranged from 21% to 63%, while *M. hupehensis* produced nearly 100% apomictic seeds (Olien, 1987). Luckwill and Campbell (1953) used *M. toringoides* and *M. sikkimensis* as rootstocks for apples. Robertson (1974) also reported the occurrence of apomixis in several species of section *Malus* and *Sorbomalus*. In most cases the plants are facultative apomicts, with the embryo sacs developing from unreduced megaspores.

Hjelmqvist (1957) showed that partial apomixis, combined with pseudogamy occurs in tetraploid forms of *Malus sieboldii*. Aposporic embryo sacs are formed which compete with the normal one, formed through meiosis, and often oust the latter. Hjelmquist (1959) investigated the occurrence of apomixis in hybrids between *M. sieboldii* and *M. baccata* var. *mandshurica* (Max.) Schneid. [generally known as *M. zumi* (Mats) Rehd.]. He found that while the tetraploid form of *Malus sieboldii* is partially apomictic, *M. zumi* is amphimictic. He believed that the chromosome number of hybrids may explain the amphimixis of hybrids. While the form of *M. sieboldii* that showed apomixis had the tetraploid number, *M. zumi* is diploid and apomixis has proved to be favoured by polyploidy. Related forms with a diploid chromosome number often are amphimictic. Therefore, he thought that the difference in

chromosome number is connected with the amphimictic development. More investigation by Hjelmquist showed that apomixis depends apparently on genetical factors which, through hybridisation and polyploidy, have greater possibilities to come into dominance and assert themselves.

The triploid *M. hupehensis* is almost an obligate apomictic with the embryo sacs produced aposporously. The aposporous egg cells are occasionally fertilized by pollen from other species giving rise to hybrids of a higher polyploid level.

1.15. Interspecific hybridizatiion in *Malus*

Interspecific hybridization is commom in *Malus*. Species of section *Chloromeles* cross with members of section *Malus* but not with those of section *Sorbomalus* or other sections, hybrids between section *Malus* and *Sorbomalus* also occur (Robertson, 1974).

No hybridization has been reported between sections *Eriolobus*, *Docyniopsis*, *Florentinae* and other sections. Figure 1.3 shows interspecific hybridization reported in *Malus.*

1.16. Genetic relationships in *Malus*

Savolainen *et al.* (1995) found 5 mutations in the chloroplast atpB-rbcL spacer of 55 apples of which three were autapomorphic transversions. *M. trilobata* has a guanine at position 30 instead of thymine, *M. ioensis* a thymine at position 31 instead of adenine, and *M. kansuensis* a thymine at position 463 instead of guanine. One mutation is a thymine at position 326 instead of cytosine, a synapomorphic transition for seven taxa, *M. pumila, M. baccata, M.x floribunda, M. sieboldii, M. sargentii* and *M. toringoides.*

The origin of *Malus x domestica* is hybrid and it has been suggested to be derived from *M. sylvestris, M. dasyphylla, M. pumila*, and some Asiatic species (Terpo, 1968). Savolainen *et al.* (1995) excluded ten wild species from being the female parent of *Malus x domestica. M. kansuensis, M. trilobata and M. ioensis,*

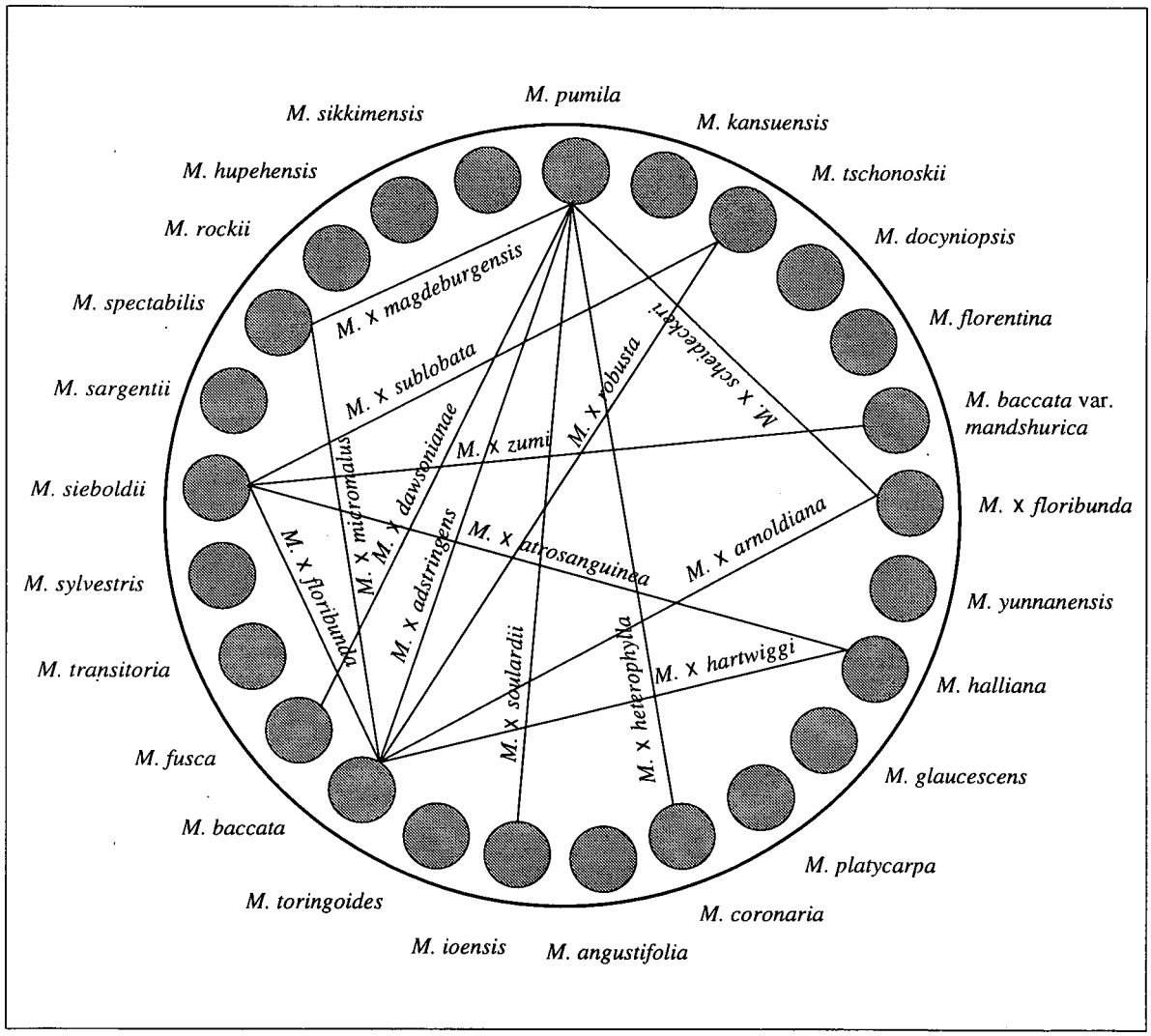


Figure 1.3. Reported interspecific hybrids in genus *Malus*.

which originated respectively from China, the Middle East, and North America, were excluded because of their characteristic autapomorphies. *M. pumila, M. baccata, M. halliana, M. x floribunda, M sieboldii, M. sargentii* and *M. toringoides*, which originated from Eastern Asia, were also excluded because of the shared mutation at position 326. Among the species analysed by them only three European species, *M. sylvestris, M. florentina and M. dasyphylla* or two North American species, *M. platycarpa* and *M. fusca* could have transmitted their plastid genome and thus be the potential female parent of *Malus x domestica*. Their results indicate that several European and American wild species might be candidates for the female parent. Moreover, if the Asiatic wild species are involved, then they would be the male parent.

1.17. Aim

Dispite the large amount of research done on the taxonomic relationship within the Maloideae, and the economic value of the apple (*Malus*) crop worldwide , there are still doubts about evolutionary relationships and taxonomy.

To help us unravel these problems, new material is becoming available for study from wild collections from China and elswhere in Far East.

Indeed, with the Phipps *et al.* (1990, 1991), Rohrer *et al.* (1991,1994) Robertson *et al.* (1992), Campbell *et al.* (1991, 1995) and Morgan (1994) re-assessments of the Maloideae, an overall study of the genus *Malus* could increase our understanding of the relationships of species within this important genus.

The aim of this study is to reassess the genus *Malus*, to help improve our general understanding of the relationships among the species. To this end, I have studied using living material a wide range of morphological characters, wood anatomy, pollen morphology, flavonoid compounds and isozymes.

CHAPTER 2

MORPHOLOGY

2.1. Introduction

Morphological and anatomical characters of forty one species and varieties of the genus *Malus* were studied. Material was kindly provided by Ness Botanic Gardens, Royal Botanic Gardens Kew, Royal Botanic Gardens Edinburgh and the National fruit collection, Brogdale. Identification of all the material has been checked against published descriptions and herbarium specimens.

2.2. Material and methods

Material was collected by the author or sent by the botanic gardens and studied fresh. Herbarium specimens of all the material studied were made and deposited in the herbarium of the National Museums and Galleries on Merseyside (LIV).

2.3. Results

2.3.1. Leaf

Petiole

The leaves of all the *Malus* species are petiolate. Length of petiole is a characteristic feature in species identification. Analysis of variance on petiole length is presented in Appendix Table 1. Species differ significantly ($P<0.05$) in petiole length. The longest petiole is found in *M. trilobata* (mean = 65 mm), while the shortest petiole belongs to *M. halliana* (mean = 13.2 mm) (Table 2.1).

Leaf size

Lamina length and lamina width are presented in Tables 2.1 and 2.2.

Table 2.1. Leaf caracteristics on long shoots of individuals of *Malus* examined.

Taxon	Leaf length (mm) X ± SD	Leaf width (mm) X ± SD	L/W ratio X ± SD	Petiole length (mm) X ± SD	No. leaves examined
Pumilae					
M. kirghisorum					
766237 RBGK	57.38 ± 6.28	36.75 ± 4.33	1.57 ±0 .15	13.88 ± 4.22	8
M. prunifolia					
156.80.01585 RBGK	85 ± 19.49	53.83 ± 19.49	1.60 ± 0.44	29.67 ± 4.93	6
M. pumila					
1973.1922 RBGK	40.43 ± 18.55	24.66 ± 5.47	1.37 ± 0.65	22.43 ± 2.15	7
M. sylvestris					
10083 NBG	67 ± 14.09	36 ± 4.08	1.89 ± 0.53	19.25 ± 1.5	4
Baccatae					
M. baccata					
RBGE	51.1	21.1	2.00	20	4
M.baccata var. *macrocarpa*					
1973-1182 RBGK	73.3 ± 7.51	52.2 ± 6.36	1.41 ± 0.14	39.8 ± 7.99	10
M. baccata var. *lutea*					
1947.19402 RBGK	78.75 ± 12.42	47.62 ± 5.53	1.65 ± 0.17	42.5 ± 6.19	8
M. baccata var. *jackii*					
1982-8354 RBGK	86.58 ± 9.99	45.36 ± 10.04	1.919 ± 0.41	22.83 ± 4.13	12
M. hupehensis					
8354 RBGK	71.57 ± 3.69	33 ± 3.74	2.20 ± 0.32	23.5 ± 4.23	10
1777 NBG	73.6 ± 6.80	37.2 ± 2.49	1.98 ± 0.17	15.2 ± 4.59	10
M. halliana					
1912003 RBGK	75.8 ±1 2.25	38.2 ± 7.21	2 ± 0.25	13.2 ± 7.32	10
39281.08313RBGK	64.71 ± 6.78	35.71 ± 3.15	1.82 ± 0.16	25.86 ± 4.53	7
M. robusta					
1986.8360 RBGK	88.7 ± 10.87	43.8 ± 18.83	2.35 ± 0.91	32 ± 8.06	10
Sieboldianeae					
M. sieboldii					
1993-385 RBGK	71.8 ± 6.07	75.2 ± 7.97	1.16 ± 0.10	16.7 ± 4.08	10
M. sargentiti					
1981.1591 RBGK	63.3 ± 5.06	65 ± 10.63	0.99 ± 0.11	14 ± 1.41	10
1973.19539 RBGK	61.56 ± 4.72	46.22 ± 15.72	1.49 ± 0.55	18.11 ± 6.66	9

Cont'd Table 2.1. Leaf caracteristics on long shoots of individuals of *Malus* examined.

Taxon	Leaf length (mm) X ± SD	Leaf width (mm) X ± SD	L/W ratio X ± SD	Petiole length (mm) X ± SD	No. leaves examined
M. zumi					
851.3085123 RBGK	71.1 ± 6.10	32.1 ± 6.33	2.27 ± 0.37	20.7 ± 5.31	10
Florentinae					
M. florentina					
19665032 RBGE	57.67 ± 6.92	49.2 ± 5.85	1.20 ± 0.12	36.3 ± 5.64	10
Kansuenses					
M. kansuensis					
1908.103 RBGE	69 ± 11.92	49.75 ± 4.27	1.24 ± 0.29	21 ± 6.27	4
1931. 1119 RBGE	47.7 ± 5.21	38 ± 3.59	1.30 ± 0.51	24.13 ± 2.95	8
1156.86.01573 RBGK	72.6 ± 9.13	46.4 ± 7.29	1.62 ± 0.44	25.6 ± 6.80	10
M. transitoria					
1986.1601 RBGK	52.54 ± 11.31	36.25 ± 7.65	1.55 ± 0.36	25.13 ± 6.49	8
M. toringoides					
19451001RBGE	65	28.7	2.26	-	4
Yunnanenses					
M. yunnanensis					
156.866.8230 RBGK	94.43 ± 14.71	57.85 ± 6.15	1.64 ± 0.25	26 ± 3.46	7
220113 RBGE	111 ± 25.02	70.8 ± 18.87	1.56 ± 0.32	47.8 ± 4 08	10
M. prattii					
19091013 RBGE	94.6 ± 21.15	60.8 ±1 1.43	1.55 ± 0.18	32 ± 6.60	4
Chloromeles					
M. angustifolia					
1986.8314 RBGK	79.71 ± 18.95	62.86 ± 15.23	1.30 ± 0.30	23.86 ± 6.38	7
1986.1546 RBGK	75.57 ± 7.85	47.14 ± 5.76	1.61 ± 0.12	32.86 ± 4.95	7
1980.1556RBGK	76.82 ± 12.41	63.64 ± 1 7.88	1.25 ± 0.27	22.82 ± 9.53	11
M. coronaria					
1973.939 RBGK	94.43 ± 10.78	63.71 ± 18.3	1.64 ± 0.67	28.57 ± 2.70	7
1968.47713 RBGK	82.4 ± 7.62	79 ± 13.68	1.06 ± 0.93	23.8 ± 7.51	10
M. ioensis var. *palmeri*					
1986.8032 RBGK	82.4 ± 7.62	79 ± 13.68	1.34 ± 0.17	23.8 ± 7.51	10
M. platycarpa					
1960.1592 RBGK	88.9 ± 17.09	56 ± 12.22	1.60 ± 0.09	28.1 ± 5.17	10

Cont'd Table 2.1. Leaf caracteristics on long shoots of individuals of *Malus* examined.

Taxon	Leaf length (mm) X ± SD	Leaf width (mm) X ± SD	L/W ratio X ± SD	Petiole length (mm) X ± SD	No. leaves examined
Dosyniopsis					
M. tschonoskii					
1989.5030 RBGE	64.8±5.71	49.9±4.25	1.30±0.08	25.9 ± 1.91	10
Eriolobus					
M. trilobata					
156.86.08210 RBGK	79±13.63	97.38±21.33	0.83±0.15	65 ± 17.56	8
1986.0351 RBGE	58±5.77	62.3±12.88	0.96±0.20	39 ± 9.35	10

Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.
X mean; SD standard deviation; L Length; W Width

Table 2.2. Leaf characteristics of sections of *Malus*.

Taxon	Lamina length		Lamina width	
	w(mm)	x	w(mm)	x
Section *Malus*				
Pumilae	50-100	57.3-85	30-60	36.5-53.8
Baccatae	30-120	51.7-86.5	15-62	21.1-52.2
Section *Sorbomalus*				
Sieboldianae	56-82	63.3-71.8	21-85	31.1-74.2
Florentinae	49-67	58.6	43-60	49
Kansuenses	35-80	47.7-72.6	25-75	28.7-62.5
Yunnanenses	67-130	94.2-111.2	45-99	57.8-70.8
Section *Chloromeles*	59-110	75.5-95.7	25-111	47.1-79
Section *Docyniopsis*	55-74	61.6-62.8	36-58	47.3-49.9
Section *Eriolobus*	45-96	56-79	49-120	62.3-97.3

Abbreviations; w range; x means

Analysis of variance on lamina length and lamina width are presented in Appendix Tables 2. and 3. Species differ significantly ($P<0.05$) in lamina length and lamina width.

The lamina length varies from 50 to 130 mm. The longest leaves belong to series *Yunnanenses* and the shortest to the series *Kansuenses*. The narrowest leaves occur in *Kansuenses* (*M. toringoides*) and *Sieboldianae* (*M. zumi*) with the length to width ratio of 2.2 and 2.27, respectively and the widest leaves occur in *Eriolobus* with the length to width ratio of 0.83 and 0.96.

Leaf lobing

Leaves of all the species of *Malus* are simple. However, leaves have different degrees of lobing. Heteroblastic leaves also are common in some series.

Leaves with 3 or more lobes were observed in some series of the genus. Leaves of none of the species of series *Pumilae* and *Baccatae* are lobed, neither on short shoots nor on long shoots. An exception occurs in *M. spectabilis* (*Pumilae*) with both kinds of leaves. Only in one individual of series *Baccatae*, *M. baccata* 19081034 RBGE, were lobed leaves observed on short shoots. It has also reported that lobed leaves occur in some varieties of *M. prunifolia* (Asami, 1927).

In series *Sieboldianae* lobed leaves were observed on both kinds of shoots, while entire leaves were mostly restricted to the short shoots. In *M. sieboldii* var. *arborescens* both lobed and unlobed leaves were observed on short shoots. The only individual of *M. zumi* examined had unlobed leaves on short shoots.

In series *Kansuenses*, only *M. kansuensis* has lobed leaves on both kind of shoots, while the other members of the series have both kind of leaves. In *Eriolobus* and *Florentinae*, only lobed leaves were observed on both kind of shoots. In series *Yunnaneses*, *M. yunnanensis* possesses lobed leaves on both kinds of shoots but in *M. prattii* none of the shoots had lobed leaves.

The only species studied of series *Docyniopsis, M. tschonoskii*, has unlobed leaves with a very slightly lobed margin (lobulate). Series *Chloromeles* have lobed leaves, at least on long shoots (Table 2.3).

Venation of leaves

All the individuals of *Malus* species examined in this study had pinnate venation with two distinct types of secondary venation. Curving lateral veins dissolving at a distance from the leaf margin (camptodromous venation) are found throughout the subfamily Maloideae and in most species of *Malus*. The other type, straight lateral veins ending in the teeth or leaf lobes (craspedodromous venation), is characteristic of the species with lobed or lobulate leaves. In all the species of series *Pumilae* and *Baccatae* the venation is camptodromous. In series *Kansuenses, M. fusca* has camptodromous, while *M. kansuensis, M. transitoria and M. toringoides* have craspedodromus venation.

In *M. tschonoskii, M. florentina* and *M. trilobata* venation is craspedodromous. In series *Yunnanenses* both kinds of venation was observed. In *M. coronaria, M. ioensis* var. *palmeri* and *M. lancifolia* venation of lobed leaves is craspedodromous. In series *Sieboldianae*, unlobed leaves on short shoots have camptodromous and lobed leaves on long shoots have craspedodromous venation.

Adaxial glands (colleters)

These glands consist of a multicellular and cylindrical base and an elliptic head. They are reddish to reddish black and may be straight or bend over the surface of the leaf. They are mostly on the midrib of the adaxial surface of leaves and sometimes they occur along secondary veins as well.

All the species examined, except *M. tschonoskii*, have these glands on the adaxial surface. These glands also occur at the base of petiole where it joins the stem. This characteristic occurs in all the species examined except *M. coronaria. M. tschonoskii*, which lacks glands on the surface of the leaf, has these petiolar glands.

Table 2.3. Leaf lobing in individuals of *Malus* examined.

Taxon	Lobing on long shoot leaves	Lobing on short shoot leaves
Pumilae		
M. domestica		
1973.19912 RBGK	-	-
M. niedzwetzkyana		
1973.11840 RBGK	-	-
M. prunifolia var. *rinki*		
19071032 RBGE	-	-
Bacatae		
M. baccata		
19744220 RBGE	-	-
19081034 RBGE	-	+ and -
M. baccata var. *mandshurica*		
19665030 RBGE	-	-
M. halliana		
19121003 RBGE	-	-
M. hupehensis		
19081017 EBGE		
M. rockii		
19642282 RBGE	-	-
M. sikkimensis		
19665029 RBGE	-	-
M. robusta		
197319959 RBGE	-	-
Sieboldianae		
M. sieboldii		
19872240 RBGE	+	+
19801986 RBGE	+	+ and -
M. sieboldii var. *arborescens*		
19051019 RBGE	+	+ and -
M. x floribunda		
19665033 RBGE	-	-
M. zumi		
19171011 RBGE	+	–
Florentinae		
M. florentina		
19665032 RBGE	+	+
Kansuenses		
M. kansuensis		
19381119 RBGE	+	+
M. toringoides		
19451001 RBGE	+	+ and -
M. transitoria		
1986.1601 RBGK	+	+
M. fusca		
NBG	+	+ and -
Yunnanenses		
M. yunnanensis		
19220113 RBGE	+	+
M. prattii		
19091013 RBGE	-	-
Chloromeles		
M. angustifolia		
1986.1556 RBGK	+	-
M. coronaria		
1968.47713 RBGK	+	+
M. glaucescens		
1986.8280 RBGK	+	-
M. lancifolia		
19868281 RBGK	+	-

Cont'd. Table 2.3. Leaf lobing in individuals of *Malus* examined.

Taxon	Lobing on long shoot leaves	Lobing on short shoot leaves
Eriolobus		
M. trilobata		
196917535RBGK	+	+
Docyniopsis		
M. tschonoskii		
4434 NBG	+*	+*

Symbols; * Leaves lobulate; + present; - absent
Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.

This new observation that *M. tschonoskii* lacks glands on the surface of the leaves is further evidence for the isolation of this species within *Malus*. However, before too much is made of this, observations should be made on other individuals of the species and other species of section *Docyniopsis*.

2.3.2. Inflorescence

Inflorescence characteristics

There are two different kinds of inflorescence in the genus; umbellate and corymbose. Members of the series *Pumilae, Baccatae* and *Sieboldianae* have umbellate and members of the series *Kansuenses, Florentinae, Yunnanenses, Chloromeles* and *Eriolobus* have corymbose inflorescences. An exception to this is the occurrence of corymbose inflorescences in *M. sikkimensis* and *M. rockii* (*Baccatae*).

Analysis of variance on number of flowers per inflorescence are presented in Appendix Table 4. Species differ significantly ($P<0.05$) in number of flowers per inflorescence.

In most species the number of flowers per inflorescence is about 5. Series *Yunnanenses* and *Kansuenses* have the highest number of flowers per inflorescence (means 9.28 and 6.79, respectively). Series *Docyniopsis* and *Eriolobus* have the lowest number of flower per inflorescence (means 4.8 and 4.99, respectively), Table 2.4. Number of flowers is correlated with the size of the flower. The biggest flowers occur in series *Pumilae, Chloromeles, Eriolobus* and *Docyniopsis* which have the lowest number of flowers, whereas *Kansuenses, Florentinae* and *Yunnanenses* have more numerous small flowers.

Variation in number of flowers per inflorescence within series

Species of series *Pumilae* show a relatively similar number of flowers per inflorescence. The greatest number of flowers occurs in *M. prunifolia* and *M. pumila* (means 6.1 and 6, respectively), while the lowest number of flowers occurs in *M.*

Table 2.4. Number of flowers per inflorescence in individuals of *Malus* examined.

Taxon	X ± SD	No. of inflorescences.
Pumilae		
M. pumila		
1973.1922 RBGK	6.10 ± 0.64	13
M. prunifolia		
1986.1585 RBGK	6.00 ± 1.07	8
M. prunifolia var. *rinki*		
1986.8411 RBGK	3.44 ± 1.13	9
M. niedzwetzkyana		
197311840 RBGK	4.38 ± 1.19	8
NFC	5.80 ± 0.63	10
M. spectabilis		
07029.7004 RBGK	3.57 ± 1.13	7
M. sylvestris		
10083 NBG	5.29 ± 0.75	7
M. domestica		
1973.19912 RBGK	5.22 ± 0.67	9
Baccatae		
M. baccata		
1776 NBG	6.8 ± 1.23	23
M. baccata var. *mandshurica*		
1923.33803 RBGK	5.10 ± 0.74	10
M. baccata var. *gracilis*		
1986.1559. RBGK	3.12 ± 1.27	17
M. halliana		
1986.8345 RBGK	6.17 ± 0.51	18
M. hupehensis		
1777 NBG	5.25 ± 0.95	8
M. sikkimensis		
3406 NBG	7.2 ± 1.03	14
M. rockii		
NBG	6.00	12
M. robusta		
NFC	6.29 ± 0.49	7
Sieboldianae		
M. sieboldii		
1781 NBG	4.89 ± 1.12	36
M. sargentii		
4433 NBG	6.80 ± 0.45	5
NFC	7.33 ±0.87	9
M. zumi		
1930.85117 RBGK	4.58 ± 1.38	12
M. x floribunda		
NFC 1	4.822 ± 1.33	11
NFC 2	6.75 ± 0.71	8
M. x floribunda 'Hillieri'		
NFC	7.87 ±1.21	7
Florentinae		
M. florentina		
1986.8398 RBGK	6.22 ± 1.35	18
Kansuenses		
M. kansuensis		
1986.1573 RBGK	7.71 ± 2.29	7
M. toringoides		
1986.8287 RBGK	5.38 ± 0.89	16
M. transitoria		
1986.1601 RBGK	5.56 ± 0.81	7
M. fusca		
NBG	8.50	4

Cont'd Table 2.4. Number of flowers per inflorescence in individuals of *Malus* examined.

Taxon	X ± SD	No. of inflorescences.
Yunnanenses		
M. yunnanensis		
3407 NBG	9.28 ± 2.81	7
M. prattii		
NFC	14.17 ± 1.47	6
Chloromeles		
M. angustifolia		
1986.1554RBGK	5.00 ± 1.07	8
M. coronaria		
477.68421 RBGK	5.07 ± 1.14	14
M. glaucescens		
119868280 RBGK	5.00 ± 0.71	13
M. ioensis var. *palmeri*		
1986.8032 RBGK	5.63 ± 0.52	8
M. lancifolia		
1986.8281 RBGK	6.35 ± 1.06	17
Docyniopsis		
M. tschonoskii		
4437 NBG	4.4 ± 0.84	10
NFC2	4.86± 0.38	7
NFC1	5.14 ± 0.38	7
Eriolobus		
M. trilobata		
1969.17535 RBGK	4.99	11

Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool. X mean; SD standard deviation.

spectabilis and *M. prunifolia* var. *rinki* (means 3.6 and 4.1). In series *Baccatae*, *M. baccata* and *M. sikkimensis* have the greatest number of flowers (means 6.8 and 7.2, respectively), while *M. hupehensis* and *M. baccata* var. *gracilis* have the lowest number of flowers per inflorescence (means 5.25 and 3.12, respectively). In series *Sieboldianae* the highest numbers were observed in *M. floribunda* and *M. sargentii*, while the lowest occur in *M. sieboldii* and *M. zumi*. In series *Kansuenses*, *M. fusca* and *M. kansuensis* have the highest number (means 8.5 and 7.7, respectively), while *M. toringoides* and *M. transitoria* have the lowest number (means 5.56 and 5.38). Series *Chloromeles* shows a great homogenity in the number of flowers among the species and ranges between 5 and 6.35.

2.3.3. Petals

Colour

In *Malus* the petals may be white, pink, pinkish white or yellowish white. Petal colour may change during development from bud to flower. Most of the species show red or pink colour in bud and change to white in flower. In series *Pumilae*, *Sieboldianae* and *Baccatae* petal colour in bud is pink or red and changes to white or pinkish white in flower. In series *Pumilae*, *M. niedzwetzkyana* is distinct from the others in its pink-red petals. In series *Kansuenses* petals are white or pink to red in bud and change to white or yellowish white in flower. In *Yunnanenses* and *Florentinae* they are white in both cases. In *Chloromeles* petals are pink or red in bud and flower and gradually change to pinkish white or white. In *M. tschonoskii* the colour in bud is pinkish red and changes to pinkish white in flower (Table 2.5).

Petal apex

The petal apices of most species of *Malus* are rounded. Exceptions are *M. pumila*, *M. niedzwetzkyana*, *M. sikkimensis*, *M. tschonoskii*, *M. trilobata* and *M. kansuensis* with emarginate or rounded, and *M. toringoides* and *M. transitoria* with dentate petal apices (Table 2.5).

Table 2.5. Petal characteristics of individuals of *Malus* examined.

Taxon	Colour (Bud)	Colour (Flower)	Base	Marigin	Apex
Pumilae					
M. pumila					
1973.1922 RBGK	pink	white	cuneate	entire	rounded (emarginate)
M. niedzwetzkyana					
1973.11840 RBGK	red	pink	cuneate	entire	rounded (emarginate)
NFC	red	red-pink	cuneate	entire	rounded
M. spectabilis					
1970.29.07004 RBGK	pink	pinkish white	-	-	-
M. prunifolia					
1986.1585 RBGK	pink	white	acuminate	entire	rounded
M. sylvestris					
10083 NBG	red	pinkish white	cunnate	entire	rounded
Baccatae					
M. baccata					
1776 NBG	pink	pinkish white	cordate	entire	rounded
M. baccata var. *mandshurica*					
388.23.38803 RBGK	pink	white	truncate	entire	rounded
M. hupehensis					
1777 NBG	pink	white	truncate(cuneate)	crenulate	rounded
M. sikkimensis					
3406 NBG	pink	white	subcordate(truncate)	entire	rounded (emarginate)
M. halliana					
1986.8345 RBGK	pink	white	truncate	crenate	rounded
M. robusta					
NFC	pink	white	truncate (cuneate)	entire	rounded
Sieboldianeae					
M. sieboldii					
1781 NBG	pink	white	truncate	entire	rounded
M. x floribunda 'Excellenz Theil'					
NFC	pink- red	pinkish white	truncate	entire	rounded
M. x floribunda					
NBG	red	pinkish white	cuneate	entire	rounded

Cont'd. Table 2.5 Petal characteristics of individuals of *Malus* examined.

Taxon	Colour (Bud)	Colour (Flower)	Base	Marigin	Apex
Florentinae					
M. florentina					
1986.1595 RBGK	white	white	cuneate-truncate	entire	rounded
Kansuenses					
M. kansuensis					
1986.1573 RBGK	white	yellowish white	cordate	crenulate	emerginate (rounded)
NBG	white (pink)	white	cordare	crenulate	emarginate (rounded)
M. toringoides					
1986.8287 RBGK	white	white	truncate	entire	dentate
M. transitoria					
1986.1601 RBGK	white	yellowish white	truncate	entire	dentate
M. fusca					
NBG	pink	white	semicordate	entire (wavy)	rounded
Yunannenses					
M. prattii					
NFC	white	white	cordat+ truncate	entire	rounded
Chloromeles					
M. angustifolia					
156.860154 RBGK	pink	white	acuminate	crenate	rounded
M. ioensis var. *palmeri*					
1986.8032 RBGK	pink	pinkish white	acuminate	entire	rounded
M. lancifolia					
1986.8281RBGK	pink	pinkish white	acuminate	entire	rounded
M. glaucescens					
1986.8280 RBGK	pink	white	acuminate	crenate	rounded
M. platycarpa					
NFC	white	pink	acuminate	crenate	rounded
Docyniosis					
M. tschonoskii					
4437 NBG	pink red	white pink	truncate	entire	emarginate
Eriolobus					
M. trilobata					
1969-17535 RBGK	white	white	truncate	waved	emarginate

Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.

Petal margin

The condition of the petal margin is of some value in characterizing certain taxa of *Malus* (Table 2.5), some being entire, others not. In *Pumilae*, *Sieboldianae*, *Baccatae*, *Kansuenses* and *Docyniopsis*, the margins of the petals are usually entire. Exceptions occur in the *Baccatae*, in which *M. halliana* and *M. hupehensis* have crenulate margins. *M. kansuensis* is distinct from other members of series *Kansuenses* in its crenulate petal margins. In series *Chloromeles* the margin is crenulate except in *M. ioensis* and *M. lancifolia*, in which it is entire. In *M. trilobata* the margin of the petals is wavy (Table 2.5).

Petale base

Petals with acuminate bases characterize the series *Chloromeles* and *M. prunifolia* of series *Pumilae*. In series *Baccatae* and *Kansuenses* petals with truncate bases are commoner than other shapes. Exceptions occur in *M. baccata* and *M. sikkimensis* of series *Baccatae* with cordate to subcordate bases and *M. kansuensis* of series *Kansuenses* with cordate bases. In *Yunnunenses* both truncate and cordate petal bases occur. The petals of *Florentinae* are distinct from the other series in the lack of claws (Table 2.5).

Size of petals

Analysis of variance on petal length and petal width are presented in Appendix Tables 5 and 6. Species differ significalntly ($P<0.05$) in petal length and petal width.

Mean values for petal lengths in the genus ranges from 7.1 mm in *M. transitoria* to 26 mm in *M. niedzwetzkyana.* Means of this character in the series range from 14.09-26 mm in the *Pumilae*, 15-20.5 mm in *Chloromeles*, 12.76-19.89 mm in *Baccatae*, 19.8 mm in *Eriolobus,* 7.1-10.64 mm in *Kansuenses* and 12.57 mm in *Docyniopsis*. The narrowest petals occur in *Kansuenses* (5-12.82 mm), *Sieboldianae* (5.92-10.8 mm) and *Docyniopsis* (7.64 mm). The wider petals belong to series

Pumilae (9-15.06 mm), *Eriolobus* (14.4 mm), *Baccatae* (9.2-13.5 mm) and *Chloromeles* (8.56-15.30 mm), Table 2.6.

Abnormal petaloid structures have been observed in some species. In *M. sylvestris* a petaloid segment was observed in the calyx of a flower. An increase in the number of petals from 5 to 7 was observed in one flower of *M. ioensis* var. *palmeri*, and in *M. spectabilis* the number of petals tended to increase to 10 or 15, the petals being arranged in five groups of two or three petals. Such double flowers are common in genera which have been subject to much artificial breeding but, unlike cherries, (*Prunus* spp.), there are few 'double' flowered apples (e.g. *M. halliana*).

Variation of petal characters within series

Overall, in considering petal characters in series *Pumilae*, *M. pumila* and *M. niedzwetzkyana* are distinct from the others in having the widest petals with emarginate apices. In series *Baccatae*, there is a homogenity in the size of petals, the narrowest petals occur in *M. baccata* var. *gracilis*. *M. baccata* and *M. sikkimensis* are distinct from the others by cordate and subcordate base, while other members have truncate bases. *M. hupehensis* and *M. halliana* are distinguished by their crenate and crenulate margin. In series *Sieboldianae*, *M. sargentii* has the widest petals (mean 10.8 mm), while the mean of the other members ranges between 5.9 and 7.4 mm. All the members examined show similar bases and apices. In series *Kansuenses*, *M. fusca* is very distinct in having the largest petals (16.09 x 12.82 mm) and *M. transitoria* has the smallest petals (7.1 x 5 mm). *M. kansuensis* and *M. fusca* are distinct in having cordate and semicordate petal base respectively, while others have truncate bases. *M. kansuensis* also has crenulate margins, while others have entire margins. *M. toringoides* and *M. transitoria* are distinct in having dentate petal apices, while others have emarginate or rounded apices. Therefore it is possible to distinguish two subseries in *Kansuenses* as follow:

1. Subseries I, including *M. kansuensis* and *M. fusca* with cordate or semicordate petal base.

Table 2.6. Petal characteristic of individuals of *Malus* examined.

Taxon	Length (mm) X ± SD	Width (mm) X ± SD	L/W ratio X ± SD	No. petals examined
Pumilae				
M. domestica				
1973.19912 RBGK	16.9 ± 1.45	10.9 ± 1.10	1.56 ± 0.12	10
M. niedzwetzkyana				
197311840 RBGE	23.1 ± 2.51	13.2 ± 2.30	1.78 ± 0.27	10
NFC	26.0 ± 1.76	15 ± 1.86	1.75 ± 0.15	11
M. spectabilis				
070.2907004 RBGK	20.83 ± 3.79	12.30 ± 2.64	1.74 ± 0.40	11
M. pumila				
1973.1922 RBGK	24.06 ± 1.60	15.06 ± 1.75	1.61 ± 0..20	18
M. prunifolia				
1986.1585 RBGK	14.09 ± 3.11	9.72 ± 0.65	1.44 ± 0.30	11
M. sylvestris				
10083 NBG	16.66 ± 2.18	9 ± 0.71	1.85 ± 0.25	10
Baccatae				
M. baccata				
1776 NBG	15.9 ± 1.73	13.5 ± 1.43	1.17 ± 0.06	10
M. baccata var. *gracilis*				
1986.1559 RBGK	16.8 ± 2.04	9.2 ± 1.08	1.85 ± 0.28	16
M. baccata var. *mandshurica*				
1923.38803 RBGK	17.1 ± 1.29	12.5 ± 0.97	1.37 ± 0.10	10
M. hupehensis				
1777 NBG	19.8 ± 1.62	13 ± 0.94	1.53 ± 0.94	10
M. sikkimensis				
3406 NBG	12.76 ± 1.59	11.64 ± 1.57	1.09 ± 0.21	14
M. halliana				
1986.8345 RBGK	16.2 ± 1.73	12.14 ± 1.29	1.35 ± 0.12	14
M. rockii				
NBG	17.3 ± 0.82	8.5 ± 0.71	2.04 ± 0.14	10
M. robusta				
NFC	19.89 ± 1.91	12.56 ± 1.01	1.59 ± 0.16	10

Cont'd. Table 2.6. Petal characteristic of individuals of *Malus* examined.

Taxon	Length (mm) X ± SD	Width (mm) X ± SD	L/W ratio X ± SD	No. petals examined
Sieboldianae				
M. sargentii				
NFC	13 ± 1.05	10.8 ± 0.92	1.21 ± 0.08	11
M. sieboldii				
1781 NBG	10.3 ± 0.48	5.9 ± 0.74	1.77 ± 0.19	
NFC	11.53 ± 0.77	6.74 ± 0.54	1.71 ± 0.11	19
M. x *floribunda*				
4558 NBG	14.88 ± 0.86	7.41 ± 0.79	2.02 ± 0.21	17
Kansuenses				
M. kansuensis				
1986.1573 RBGK	8.11 ± 1.45	8.22 ± 1.56	0.99 ± 0.14	10
M. toringoides				
1986.8287 RBGK	10.64 ± 0.74	8.306 ± 0.50	1.28 ± 0.09	14
M. transitoria				
1986.1601 RBGK	7.1 ± 90	5.00 ± 0.43	1.42 ± 0.16	12
M. fusca				
NBG	16.09 ± 1.04	12.82 ± 1.46	1.26 ± 0.11	11
Yunnanenses				
M. prattii				
NFC	7.9 ± 0.88	7.4 ± 1.02	1.08 ± 0.11	10
Chloromeles				
M. coronaria				
1968.4213 RBGK	17.23 ± 2.24	11.92 ± 1.89	1.45 ± 0.11	13
M. glaucescens				
1986.8280 RBGK	20.5 ±0.71	15.30 ± 0.82	1.34 ± 0.09	10
M. lancifolia				
1986.8281 RBGK	15 ± 0.87	8.56 ± 0.88	1.77 ± 0.23	9
M. ioensis				
1986.8032 RBGK	16.58 ± 1.24	10.91 ± 1.08	1.52 ± 0.13	13
M. angustifolia				
1986.0154 RBGK	17.4 ± 0 .84	13.10 ± 1.79	1.35 ± 0.16	10
M. platycarpa				
NFC	18.27 ± 2.05	13 ± 1.91	1.42 ± 0.12	11

Cont'd. Table 2.6. Petal characteristic of individuals of *Malus* examined.

Taxon	Length (mm) X ± SD	Width (mm) X ± SD	L/W ratio X ± SD	No. petals examined
Docyniopsis				
M. tschonoskii				
4337 NBG	12.57 ± 0.76	7.64 ± 0.50	1.65 ± 0.11	14
Eriolobus				
M. trilobata				
1969.17535 RBGK	19.8	14.4	1.38	11

Abreviations; L Length; W Width; X Mean; SD Standard deviation
RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.

2. Subseries II, including *M. toringoides* and *M. transitoria* with truncate petal base.

In series *Chloromeles*, *M. glaucescens* is distinct from others in having the largest petals (20.5 x 15.3 mm), other species having petals of much the same sizes (15-17.4 x 8.56-13.1 mm). This may be due to ploidy level. *M. glaucescens* is always reported to be tetraploid while, the other species examined may be diploid.

2.3.4. Stamens

Stamen characteristics

Analysis of variance on number of stamens per flower are presented in Appendix Table 7. Species differ significantly ($P<0.05$) in the number of stamens.

The number of stamens per flower in individuals of *Malus* is presented in Table 2.7. Stamen number in most *Malus* species examined is usually 20 per flower. It varies from 11 in a flower of *M. toringoides* to 61 in a flower of *M. tschonoskii.*

The stamen number of 20 is particularly constant in series *Chloromeles*. In *M. coronaria, M. lancifolia* and *M.glaucescens* 10 out of 10 specimens examined had 20 stamens. In *M. pumila* of series *Pumilae* all the specimens examined showed the constant number of 20 per each flower although Huckins (1972) reported stamen number of 10-20 for *M. pumila.*

M. trilobata has stamen numbers ranging from 20-23 and a mean value of 21.1. Van Esteline (1933) reported 20-30 stamens for *Eriolobus* and 30-50 for *M. tschonoskii* and Huckins (1972) reported a mean of 50.1 for *M. tschonoskii.* My results show 39-61 stamens per flower of *M. tschonoskii* and 20-23 for *M. trilobata.*

In *M. ioensis* var. *palmeri* some irregularity was observed in stamen form. The changing of an anther sac to a petaloid segment was observed in two flowers. The fusion of two filaments together also was observed in one flower. The fusion of anthers also was observed in *M. prunifolia, M. angustifolia* and *M. ioensis* var. *palmeri.* Transformation of an anther to a petaloid segment also was observed in *M.*

Table 2.7. Staminal characteristics of individuals of *Malus* examined.

Taxon	Anther colour	Stamen number X ± SD	No. flowers examined
Pumilae			
M. domestica			
197319912 RBGK	Yellow	19.9 ± 0.31	11
M. niedzwetzkyana			
197311840 RBGK	Pink	19.9 ± 0.32	10
NFC	Pink	19.55 ± 1.04	11
M. spectabilis			
07027907004 RBGK	Yellow	34.8 ± 5.36	5
M. pumila			
1973.1922 RBGK	Yellow	20 ± 0	13
M. prunifolia			
1986.1585 RBGK	Yellow	19 ± 1.26	11
M. sylvestris			
10083 NBG	Yellow	20.03 ± 0.67	30
Baccatae			
M. baccata			
1776 NBG	Yellow	21.00 ±1 .51	31
M. baccata var. *gracilis*			
1986.1559 RBGK	Yellow	22.18 ± 3.19	13
M. hupehensis			
1777 NBG	Yellow	25.57 ± 0.98	7
M. sikkimensis			
3406 NBG	White or pink	24.33 ± 2.90	12
M. halliana			
1986.834 RBGK	Yellow	18.21 ± 1.12	14
M. rockii			
NBG	Yellow	15.71 ± 1.77	27
Sieboldianae			
M. sieboldii			
1781 NBG	Yellow	20.8 ± 1.46	32
M. xfloribunda 'Excellenz Theil'			
NFC	Yellow	18.73 ± 1.68	15
M. xfloribunda			
NBG	Yellow	18.89 ± 0.95	19
M. sargentii			
4433 NBG	Yellow	18.95 ± 1.23	
NFC	Yellow	19.45 ± 0.80	27
Florentinae			
M. florentina			
1986.1595 RBGK	White	19.9 ± 0.32	14
Kansuenses			
M. kansuensis			
1986.01573 RBGK	Yellow	20.25 ± 0.62	12
NBG	Yellow brown	15.23 ± 1.37	40
M. toringoides			
1986.8287 RBGK	White cream	20 ± 0	18
NFC	Yellow	20.73 ± 1.53	15
M. transitoria			
1986.1601 RBGK	Yellow	21.38 ± 1.76	13
M. fusca			
NBG	Yellow brown	19.88 ± 0.5	16
Yunnanenses			
M. prattii			
NFC	Yellow	20.1 ± 0.55	20

Cont'd Table 2.7. Staminal characteristics of individuals of *Malus* examined.

Taxon	Anther colour	Stamen number X ± SD	No. flowers examined
Chloromeles			
M. angustifolia			
1986.1556 RBGK	Pink	19.21 ± 1.19	14
M. coronaria			
1968.4213 RBGK	Red	20 ± 0	9
M. glaucescens			
1986.8280 RBGK	Red	20 ± 0	11
M. ioensis var. *palmeri*			
1986.8032 RBGK	Red	19.9 ± 0.99	10
M. lancifolia			
1986.8032 RBGK	Red	20 ± 0	10
M. platycarpa			
NFC	Red	19.71 ± 0.55	24
Docyniopsis			
M. tschonoskii			
4437 NBG	Yellow	47.33 ± 3.80	18
NFC (2)	Yellow	53.73 ± 3.65	15
Eriolobus			
M. trilobata			
1969.17535 RBGK	Yellow	21.1	10

Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool. X mean; SD standard deviation.

spectabilis. In this species also transformation of a stigma to an anther was observed. Another irregularity in this species was the extra thickening and hairiness of 3 out of 27 stamens in one flower.

Colour of the anther varies from white or yellow to pink or red and is of some value in distinguishing taxa in *Malus*. Anther colour is yellow in *Pumilae*, *Baccatae* (except *M. sikkimensis* and *M. niedzwetzkyana*), *Sieboldianae* and *Docyniopsis* though *M. sikkiminsis*, *M. florentina* and *M. toringoides* have white anthers. All the members of series *Chloromeles* and *M. niedzwetzkyana* of series *Pumilae* have red or pink anthers. *M. sikkimensis* also may have pink or white anthers. Anther colour is correlated with petal colour. Red or pink anthers are correlated with pink petals (e.g. *Chloromeles* and *M. niedzwetzkyanea*) and yellow or white anthers are correlated with white or pinkish white petals (other series), although exceptions occur (e.g. *M. sikkimensis*).

Variation in stamen characters within series

Series *Pumilae* shows homogeneity in yellow anthers with the exception of *M. niedzwetzkyana* which has pink anthers. The number of stamens in series *Pumilae* is about 20, deviation from this number occurs in *M. spectabilis* with mean of 34.8 ± 5.36. Huckins (1972) reported a dicotomy in number of stamens of series *Pumilae* and divided this series to two subseries: *Prunifoliae* tend toward an increase from 20 and *Pumilae* with a trend toward a decrease from 20. I did not find such a dicotomy in the *Pumilae*. In series *Pumilae*, *M. spectabilis* shows a range of 28-40 with mean of 34.8, and therefore shows a trend to an increase in the number of stamens. *M. domestica*, *M. niedzwetzkyana*, *M. pumila*, *M. prunifolia* and *M. sylvestris* showed a stamen number of about 20 (Table 2.7). In series *Baccatae*, *M. hupehensis* and *M. sikkimensis* there is a trend toward an increase from 20, while *M. halliana*, *M. rockii* and *M. baccata* var. *gracilis* the trend is toward a decrease in the number of stamens from 20 and *M. baccata* shows trends in both directions.

In series *Sieboldianae* also there is a tendency towards a decrease in number of stamens, only *M. sieboldii* shows an increase from 20. In series *Kansuenses*, although species usually have about 20 stamens, 40 specimens of *M. kansuensis* collected from Ness Botanic Gardens showed a large decrease from 20 toward 15.23. Series *Chloromeles* are very distinct in having pink anthers and a constant number of 20 stamens per flowers, slight variation only being observed in *M. angustifolia* and *M. lancifolia.*

2.3.5. Carpel

Carpel characteristics

Analysis of variance on number of carpels per flower are presented in Appendix Table 7. Species differ significantly ($P<0.05$) in number of carpels.

The fusion of the styles in species of *Malus* has been used for a long time to separate this genus from *Pyrus*. However, within *Malus* there are some species which show free styles. Series *Chloromeles* and *Docyniopsis* are characterized by free styles.

The colour of the styles is a useful character to distinguish series *Chloromeles* from other series. In this series, the styles are red while in other series they are green. In most species of *Malus* styles are pubescent. Exceptions are *M. kansuensis* and *M. transitoria* which have glabrous styles. *M. florentina* and series *Yunnanenses* usually have slightly pubescent or glabrous styles (Table 2.8).

The number of carpels per flower has a diagnostic value in distinguishing series of *Malus*, the data are presented in Table 2.9. It is exclusively 5 in series *Chloromeles* and very close to 5 in series *Eriolobus* (4.9), *Yunnanenses* (4.67-5), *Docyniopsis* (5.10-5.53) and *Florentinae* (4.94-5). The lowest number of carpels occurs in series *Sieboldianae* (3.4-4.1), series *Kansuenses* (2.23-4.63), and series *Baccatae* (3-5).

Table 2.8. Style characteristics in individuals of *Malus*.

Taxon	Stigma colour	Style hairness	Style connation
Pumilae			
M. domestica			
1973.19912 RBGK	green	pubescent	connate
M. niedzwetzkyana			
1973.11840 RBGK	red	pubescent	s.connate
M. pumila			
1973.1922 RBGK	green	pubescent	connate
M. prunifolia			
1986.1585 RBGK	green	pubescent	connate
M. prunifolia var. *rinki*			
19071032 RBGE	green	pubescent	connate
M. sylvestris			
10083 NBG	green	s.pubescent	connate
Bacatae			
M. baccata			
1776 NBG	green	pubescent	connate
M. baccata var. *mandshurica*			
388.23.38803 RBGK	green	pubescent	connate
M. halliana			
1986.8345 RBGK	green	pubescent	connate
M. hupehensis			
1777 NBG	green	pubescent	connate
M. sikkimensis			
3406 NBG	green	s. pubescent	connate
1986.1594 RBGK	green	pubescent	connate
M. robusta			
NFC	green	pubescent	connate
Sieboldianae			
M. sieboldii			
1781 NBG	green	Pubescent	connate
M. x floribunda			
NBG	green	pubescent	connate
Florentinae			
M. florentina			
1986.1595 RBGK	green	glabrous	connate
Kansuenses			
M. kansuensis			
19381573 RBGK	green	glabrous	connate
NBG	green	glabrous	connate
M. toringoides			
1986.8287 RBGE	green	s. pubescent	connate
M. transitoria			
1986.1601 RBGK	green	glabrous	connate
M. fusca			
NBG	green	glabrous	connate
Yunnanenses			
M. yunnanensis			
NBG	green	glabous	connate
M. prattii			
NFC	green	glabous	connate
Chloromeles			
M. angustifolia			
1986.1556 RBGK	pink	pubescent	free
M. ioensis var. *palmeri*			
1986.8032 RBGK	pink	pubescent	free
M. coronaria			
1968.47713 RBGK	pink	pubescent	free
M. glaucescens			
1986.8280 RBGK	pink	pubescent	free

cont'd. Table 2.8. Style characteristics in individuals of *Malus*.

Taxon	Stigma colour	Style hairness	Style connation
M. lancifolia			
19868281 RBGK	pink	pubescent	free
M. platycarpa			
NFC	pink	pubescent	free
Eriolobus			
M. trilobata			
196917535RBGK	green	pubescent	–
Docyniopsis			
M. tschonoskii			
4434 NBG	green	pubescent	free

abbreviations; s. slightly
RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.

Table 2. 9. Number of carpels per flower in *Malus* species examined.

Taxon	Carpel numbers X ± SD	No. of flowers examined
Pumilae		
M. domestica		
1973.19912 RBGK	5 ± 0	12
M. niedzwetzkyana		
1973.11840 RBGK	5 ± 0	11
NFC	4.86 ± 0.35	15
M. prunifolia		
1986.1585 RBGK	4.92 ± 0.30	10
M. prunifolia var. *rinki*		
1986.842 RBGK	3.76 ± 0.66	8
197311836 RBGK	3.4 ± 0.89	5
M. pumila		
1973.1922 RBGK	5 ± 0	21
M. sylvestris		
1980.8440 RBGK	4.33 ± 0.57	3
10083 NBG	4.98 ± 0.15	44
M. spectabilis		
1929.7004 RBGK	8.86 ± 1.77	7
Baccatae		
M. baccata		
805047 RBGE	4.14 ± 0.38	7
594.82084 RBGK	5 ± 0	4
1982.8352 RBGK	5 ± 0	4
1776 NBG	4.98 ± 0.15	43
M. baccata var. *jackii*		
1982.6316 RBGK	5 ± 0	5
M. baccata var. *himalaica*		
1986.1560 RBGK	3.5 ± 0.53	10
M. baccata var. *mandshurica*		
388.2338803 RBGK	3.91 ± 0.54	21
NBG	5 ± 0.33	20
M. baccata var. *gracilis*		
1986.1559.RBGK	3.64 ± 0.81	11
M. halliana		
19121003 RBGE	3 ± 0	5
3928108313 RBGK	4.6 ± 0.70	10
1986.8345 RBGK	4.23 ± 0.60	13
M. hupehensis		
19081017 RBGE	3 ± 0	5
1988.8233 RBGK	4.6 ± 0.55	5
1777 NBG	4.2 ± 0.62	30
M. sikkimensis		
1933.61408 RBGK	4.6 ± 0.52	10
19665029 RBGE	3.8 ± 0.45	5
3406 NBG	4.25 ± 0.62	12
M. robusta		
NFC	4.93 ± 0.37	30
156.86.08360 RBGK	3.8 ± 0.42	10
M. rockii		
NBG	4.03 ± 0.71	31
Sieboldianeae		
M. x *floribunda*		
1933.3517 RBGK	4 ± 0	2
NFC	4.30 ± 0.57	30
4558 NBG	4.58 ± 0.50	33

Cont'd. Table 2. 9. Number of carpels per flower in *Malus* species examined.

Taxon	Carpel numbers X ± SD	No. of flowers examined
M. x *floribunda* 'Exellenz Theil'		
NFC	4.60 ± 0.54	41
M. x *floribunda* 'Hillieri'		
NFC	5.61 ± 0.60	34
M. sargentii		
1986.1591 RBGK	3.9 ± 0.74	10
1973.19539 RBGK	4.57 ± 0.79	
4433 NBG	4.27 ± 0 .51	37
M. sieboldii		
1781 NBG	4.04 ± 0.68	49
19801986 RBGE	3.4 ± 0.55	6
1988.655 RBGK	3.4 ± 0.52	7
M. sieboldii var. *arborescens*		
156.8601533 RBGK	3.66 ± 0.5	9
M. zumi var. *calocarpa*		
851.3085123 RBGK	3.75 ± 0.75	12
Florentianae		
M. florentina		
1595 RBGK	4.94 ± 0.24	17
19665032 RBGE	5 ± 0	3
Kansuenses		
M. fusca		
19340528 RBGE	3.80 ± 0.45	5
NBG	4.63 ± 0.50	20
M. kansuensis		
19381119 RBGE	3 ± 0	10
NBG	2.2 3± 0.45	41
M. kansuensis var. *calva*		
156.8601573 RBGK	3.57 ± 0.68	30
M. toringoides		
101.131014 RBGK	4.18 ± 0.83	10
19451001 RBGE	3.67 ± 0.51	6
1981.8440 RBGK	3.37 ± 0.50	19
1981.8524 RBGK	3.44 ± 0.53	9
NFC	3.94 ± 0.77	16
M. transitoria		
1986.1601. RBGK	4.2 ± 0.83	4
Yunnanenses		
M. yunnanensis		
19220113 RBGE	5 ± 0	5
1981.4267 RBGK	5 ± 0	4
156.8608236 RBGK	5 ± 0	7
M. prattii		
19091013 RBGE	4.67 ± 0.52	6
NFC	5 ± 0	30
Chloromeles		
M. angustifolia		
1986.1556 RBGK	5.07 ± 0.27	16
M. coronaria		
1968.47713 RBGK	5 ± 0	3
1973.939 RBGK	5 ± 0	4
477.684213 RBGK	5 ± 0	9
M. glaucescens		
1986.8280 RBGK	5 ± 0	18
M. ioensis var. *palmeri*		
1986.8032 RBGK	5 ± 0	14

Cont'd. Table 2. 9. Number of carpels per flower in *Malus* species examined.

Taxon	Carpel numbers X ± SD	No. of flowers examined
M. lancifolia		
1986.8281 RBGK	5 ± 0	10
NFC	5 ± 0	22
Eriolobus		
M. trilobata		
1969.17535 RBGK	4.9	10
Docyniopsis		
M. tschonoskii		
4437 NBG	5.19 ± 0.51	21
NFC (1)	5.4 ± 0.5	20
NFC (2)	5.53 ± 0.72	17

Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool. X mean; SD standard deviation.

Connation of the carpels

The degree of connation of the carpels, investigated in available species, is shown in Table 2.10 and defined as the ratio of the distance between the centre of the carpels and the dorsal primary vascular bundles of the carpels minus the length of the suture to the distance between the centre of the carpels and the dorsal primary vascular bundles of the carpels (Figure 2.1 shows this ratio).

All individuals of the *Chloromeles* and *M. tschonoskii* had connation of less than 16 %. Connation in series *Kansuenses* ranged from 68.57% in *M. fusca* to 100% in *M. toringoides* and *M. transitoria*. In *M. baccata* var. *mandshurica*, *M. rockii* and *M. halliana* connation was 100%.

In series *Chloromeles* and *Docyniopsis* carpels tend to be apocarpic, while in series *Baccatae*, *Kansuenses* and *Folrentinae* they tend to be syncarpic (Table 2.10).

Variation in number of carpels within series.

A dichotomy is observed in series *Pumilae* in number of carpels per flower. In *M. prunifolia* var. *rinki* there is a tendency to decrease the number of carpels to a range of 3-5 with a mean of 3.4-3.76, while the other members tend to have 5 carpels, except *M. spectabilis* with a mean of 8.86. Deviation from 5 in *Chloromeles* is only found in *M. angustifolia*, which shows a tendency for carpel number to increase to 6, at least in the one specimen seen. In the *Yunnanenses*, *M. prattii* shows a slight reduction in the basic value of 5 to 4.67. In *M. tschonoskii* deviation is toward an increase to 7 carpels per flower. The major reductions in number are observed in series *Baccatae*, *Sieboldianae* and *Kansuenses*, which tend to show a decrease in the number of carpels toward 4 and 3. Huckins (1972) reported deviation from 5 in *M. glaucescens* but none of the instances in this study showed deviation from 5. He reported a mean value of 5 for *M. prunifolia* var. *rinki,* while this study showed mean values of 3.4 and 3.5 for this species. He also reported a mean value of 3 for carpel number in *M. hupehensis*. One specimen in the present study has a carpel number of 3 while for two other specimens mean value are 4.2 and 4.6.

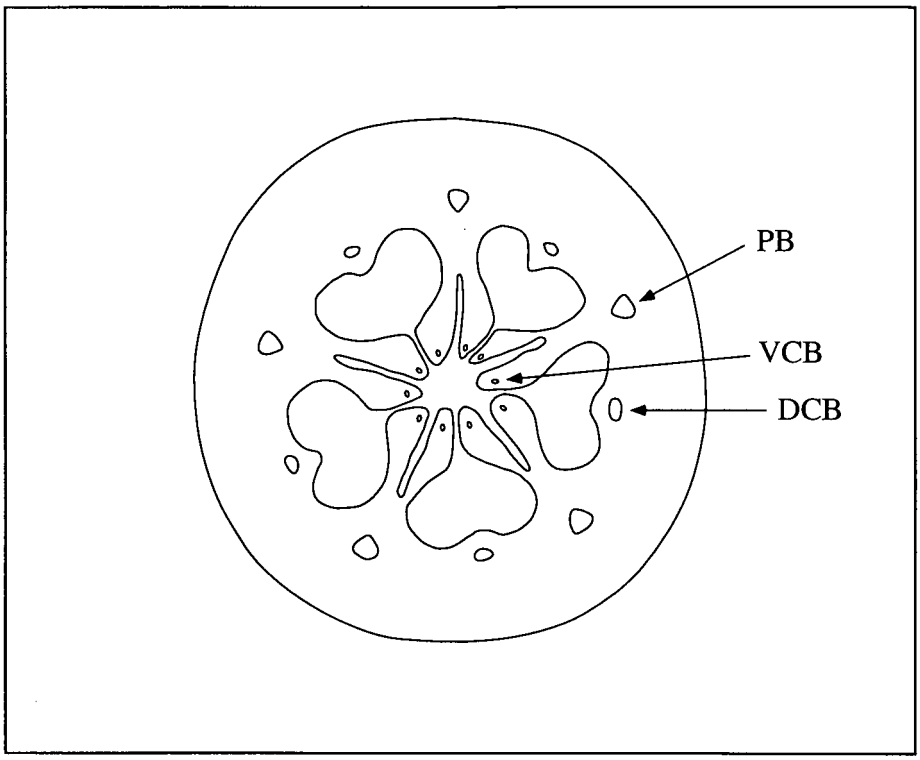


Figure 2.1. Cross section of *Malus* hypanthium showing connation of carpel.

PB - Petal bundle

VCB - Ventral carpelary bundle

DCB - Dorsal carpelary bundle

Table 2.10. Carpel connation in *Malus* species.

Taxon	% Connation*
Pumilae	
M. domestica	36.6
NFC	
M. niedzwetzkyana	
1973.11840 RBGK	56.7
NFC	45
M. prunifolia	
1986.1585 RBGK	65
M. pumila	
19731922 RBGK	53.8
M. sylvestris	
10083 NBG	100
Baccatae	
M. baccata	
1776 NBG	91
M. baccata var. *mandshurica*	
388.2338803 RBGK	100
M. halliana	
1986.8345 RBGK	100
M. rockii	
NBG	100
Sieboldianeae	
M. sieboldii	
1781 NBG	100
M. sargentii	
4433 NBG	100
M. x floribunda	
NFC	75
M. x floribunda 'Excellenz Theil'	
NFC	73
Florentinae	
M. florentina	
1595 RBGK	73.3
Kansuenses	
M. fusca	
NBG	68.57
M. kansuensis	
NBG	77.6
M. kansuensis var. *calva*	
19861573 RBGK	84.4
M. transitoria	
1986.1601 RBGK	100
M. toringoides	
1981.8440 RBGK	100
NFC	93
Yunnanenses	
M. prattii	
NFC	68
Chloromeles	
M. angustifolia	
156.8601556 RBGK	15.3
M. glaucescens	
156.8608280 RBGK	2.70
M. lancifolia	
156.8608281 RBGK	11.50
M. ioensis var. *palmeri*	
156.8608032 RBGK	10.41

Cont'd Table 2.10. Carpel connation in *Malus* species.

Taxon	% Connation*
M. coronaria	
447.684213 RBGK	8.5
Docyniopsis	
M. tschonoskii	
NFC 2	12.3

* Connation described as the ratio of the distance between the centre of the carpels and the dorsal vascular bundles of the carpels minus the length of the suture to the distance between the centre of the carpels and dorsal vascular bundles of the carpels (see Figure 2.1).
Abreviations; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.

The number of carpels is correlated with the size of the fruit. In series *Pumilae* and *Chloromeles* with large fruits, number of carpels is 5, it is particularly constantly 5 in series *Chloromeles*. In series *Sieboldianae*, *Baccatae* and *Kansuensis* with small fruits, the number of carpels is 3-4. It is significant that the relatively small fruits of series *Yunnanenses* and *Florentinae* possess 5 carpels, almost certainly a persistence of the ancestral state.

2.3.6. Fruit

Shape

Generally the shape of the fruit is globose but some are oblong or obovate and some are depressed or flattened at the poles of the fruits. Globose fruits are common in *Baccatae*, *Sieboldianae* and *Yunnanenses,* except in *M. sikkimensis* and *M. rockii,* which have obovate fruits. Fruits of *Pumilae*, *Chloromeles* and *Docyniopsis* are generally flattened and depressed at both apex and base (i. e. 'apple-shaped'). An exception is *M. prunifolia* var. *rinki* which shows no depression at apex. Fruit of *Kansuenses* are usually oblong, but sometimes obovate or globose. *M. kansuensis* has oblong fruit and *M. transitoria* and *M. fusca* obovate ones. *M. florentina* also shows oblong fruits.

Colour

The fruits of *Malus* may be green, orange, orange brown or red depending on the species. *Chloromeles* mature fruits are always green. In series *Kansuenses*, *M. toringoides* and *M. transitoria* have red fruits, while *M. kansuensis* and *M. fusca* have orange brown fruits. Series *Baccatae*, *Sieboldianeae* and some species of series *Pumilae* and *Kansuenses* have fruits which are red at maturity.

Size

Analysis of variance on fruit length and fruit diameter are presented in Appendix Tables 8 and 9. Species differ significantly ($P<0.05$) in fruit length and fruit diameter.

Dimensions of fruits of *Malus* species are presented in Table 2.11. Species of the series *Pumilae*, *Chloromeles*, and *Docyniopsis* have the largest fruits. *Sieboldianae* are characterized by small fruit. In *Baccatae, Kansuenses, Yunnanenses, Florentianae* and *Eriolobus* fruits are of medium size. These data show that size of the fruit usually correlates with carpel number as was described in section 2.3.5. The largest fruits belong to *M. niedzwetzkyana* and the smallest ones belong to *M. sieboldii* and *M. zumi.*

Condition of calyx

The persistence of the calyx is a useful character in differentiating between series in *Malus,* the series having been largely defined using this character. In series *Pumilae*, *Yunnanenses*, *Docyniopsis*, *Chloromeles* and *Eriolobus* the calyx is persistent on the fruit, but in series *Baccatae*, *Sieboldianeae*, *Kansuenses* and *Florentinae* the calyx is deciduous (Table 2.11). It seems that the presence or absence of the calyx is correlated with the size of fruit. In *M. florentina* the condition of the calyx varies from one fruit to another, some being deciduous, others persistent. This variability could be the result of a hybrid origin in this species. The theory of hybrid origin for this species considers *M. sylvestris* and *Sorbus torminalis* as parents (Browicz 1970). However, both of these have fruits with persistent calyces.

I have also found some calyculate (with calyx) fruits in *M. sikkimensis* with erect calyces presisting on fruits. It has been reported that in series *Yunnanenses* ecalyculate fruits are also sometimes present (Huckins 1972).

Table 2.11. Fruit characteristics of individuals of *Malus* examined.

Taxon	Length (mm)	Diameter (mm)	L/Diam.	Calyx	Ratio. Flesh/Diam.
Pumilae					
M. niedzwetzkyana					
1973.11840RBGK	32	30	1.06	Present	0.67
M. prunifolia var. *rinki*					
1986.842RBGK	17.9 ± 2.29	17.20 ± 1.62	1.03 ± 0.08	Present	0.49 ± 0.05
197311836RBGK	17.4 ± 1.82	16.8 ± 2.48	1.06 ± 0.11	Present	0.52 ± 0.04
M. pumila					
1973.1922 RBGK	32.2 ± 2.17	36 ± 2.55	0.90 ± 0.06	Present	0.56 ± 0.01
M. sylvestris					
1980.8440 RBGK	25 ± 2.83	29 ± 1.41	0.86 ± 0.06	Present	0.57 ± 0.02
Baccatae					
M. baccata					
594.820841 RBGK	16.75 ± 2.22	19.25 ± 2.06	0.87 ± 0.11	Absent	0.44 ± 0.04
1982.8352 RBGK	18.50 ± 1.05	22.67 ± 1.75	0.82 ± 0.07	Absent	0.46 ± 0.01
M. baccata var. *jackii*	10.11 ± 0.92	9.78 ± 0.83	1.03 ± 0.05	Absent	0.43 ± 0.07
594.8206316 RBGK	8 ± 1	8 ± 1	1 ± 0	Absent	0.48 ± 0.06
M. baccata var. *himalaica*					
156.8601560RBGK	8 ± 0.93	7 ± 0.53	1.14 ± 0.10	Absent	0.45 ± 0.08
M. baccata var. *mandshurica*					
388.2338803 RBGK	8.33 ± 0.5	8.33 ± 0.5	1.02 ± 0.08	Absent	0.48 ± 0.06
M. halliana					
121003 RBGE	8 ± 0	9.2 ± 0.45	0.87 ± 0.04	Absent	-
3928108313 RBGK	10.1 ± 1.29	9.4 ± 0.84	1.07 ± 0.09	Absent	0.40 ± 0.06
156.86.01560 RBGK				Absent	
M. hupehensis					
19081017 RBGE	9 ± 0.58	9.75 ± 0.46	0.93 ± 0.10	Absent	-
1988.8233 RBGK	13.5 ± 1.22	15 ± 2.09	0.91 ± 0.15	Absent	0.49 ± 0.03
M. sikkimensis					
1933.61408RBGK	14.2 ± 1.35	14.2 ± 1.48	1 ± 0.05	Absent	0.49 ± 0.03
19665029RBGE	13.8 ± 2.28	14.6 ± 1.52	0.94 ± 0.06	Absent	
M. robusta					
1986.8360	13.1 ± 1.8	14.8 ± 1.32	0.88 ± 0.06	Absent	0.45 ± 0.10

Cont'd. Table 2.11. Fruit characteristics of individuals of *Malus* examined.

Taxon	Length (mm)	Diameter (mm)	L/Diam.	Calyx	Ratio. Flesh/Diam.
Sieboldianae					
M. x *floribunda*					
1933.3517 RBGK	11.23 ± 1.54	9.33 ± 1.53	1.23 ± 0.23	Absent	0.50 ± 0.05
M. sargentii					
1986.1591 RBGK	7.56 ± 0.53	8.33 ± 0.71	0.91 ± 0.07	Absent	0.46 ± 0.09
1973.19539 RBGK	8.11 ± 1.05	8.2 ± 1.14	0.97 ± 0.06	Absent	0.54 ± 0.05
M. sieboldii					
19801986 RBGE	6.85 ± 0.69	6.5 ± 0.84	1.07 ± 0.21	Absent	-
1988.655 RBGK	6.67 ± 1	7.11 ± 0.93	0.94 ± 0.07	Absent	0.45 ± 0.09
M. sieboldii var. *arborescens*					
156.86.01533 RBGK	6.86 ± 0.69	7 ± 0.58	0.98 ± 0.05	Absent	0.47 ± 0.13
M. zumi 'calocarpa'					
851.3085123 RBGK	6.67 ± 1.03	6.17 ± 1.03	1.1 ± 0.11	Absent	0.42 ± 0.07
Florentinae					
M. florentina					
1595 RBGK	16.43±2.06	12.07±1.83	1.40 ± 0.32	Absent	0.32 ± 0.05
19665032 RBGE	11.67±0.58	11.16±1.17	1.13 ± 0.06	Absent*	-
Kansuenses					
M. fusca					
19340528 RBGE	11.8 ± 0.84	10.6 ± 1.14	1.13 ± 0.17	Absent	-
M. kansuensis					
1986.1573 RBGK	9.25 ± 1.5	7.25 ± 0.5	1.27 ± 0.15	Absent	0.52 ± 0.07
19051031 RBGE	10.4 ± 0.55	9 ± 0	1.16 ± 0.06	Absent	-
M. kansuensis var. *calva*					
156.86.01573	10 ± 1.15	10 ± 1.41	1 ± 0.08	Absent	0.48 ± 0.12
M. toringoides					
19451001 RBGE	13.2 ± 2.05	12.4 ± 0.89	1.06 ± 0.14	Absent	-
101.131014 RBGK	12.27 ± 2.57	12.09 ± 1.5	1.02 ± 0.21	Absent	0.52 ± 0.07
1981.8440 RBGK	13.8 ± 2.17	11.4 ± 1.52	1.21 ± 0.10	Absent	0.52 ± 0.06
1981.8524 RBGK	12.22 ± 1.56	11.89 ± 1.27	1.02 ± 0.08	Absent	-
M. transitoria					
1986.1601.RBGK	-	-	-	Absent	-

Cont'd. Table 2.11. Fruit characteristics of individuals of *Malus* examined.

Taxon	Length(mm)	Diameter (mm)	L/Diam.	Calyx	Ratio. Flesh/Diam.
Yunnanenses					
M. yunnanensis					
19220113RBGE	13 ± 0.82	17.75 ± 1.26	0.73 ± 0.06	Present*	-
1981.4267 RBGK	8.75 ± 0.96	8.75 ± 0.96	1 ± 0	Present	0.12 ± 0.01
156.8608236 RBGK	11.5 ± 1.07	12.13 ± 0.99	0.95 ± 0.06	Present	0.21 ± 0.06
M. prattii					
19091013 RBGE	11.67 ± 1.03	13.67 ± 1.96	0.87 ± 0.17	Present	-
Chloromeles					
M. angustifolia					
1986.1556 RBGK	24.33 ± 2.06	25 ± 3	0.47 ± 0.04	Present	0.58 ± 0.04
M. coronaria					
1968.47713 RBGK	20 ± 1.60	23.75 ± 2.12	0.85 ± 0.11	Present	0.67 ± 0.03
1973.939 RBGK	25.25 ± 3.30	27.75 ± 2.36	0.90 ± 0.07	Present	0.76 ± 0.03
M. glaucescens					
1986.8280 RBGK	18.27 ± 1.90	23.36 ± 1.21	0.78 ± 0.07	Present	0.66 ± 0.06
M. ioensis var. *palmeri*					
1986.8032 RBGK	21 ± 2.83	18 ± 8.49	1.27 ± 0 .44	Present	0.75 ± 1.83
M. platycarpa					
1980.1592 RBGK **	28	37	0.76	Present	0.65
Eriolobus					
M. trilobata					
1969.17535 RBGK	11.75 ± 2.22	11.75 ± 1.5	0.99 ± 0.07	Presenet	0.63 ± 0.06
Docyniopsis					
M. tschonoskii					
4437 NBG**	18	15	1.2	Present	0.67

* Calyx is present in some fruits

** Only one specimen was examined

Abbreviations; L Length; Diam Diameter; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool

X mean; SD standard deviation

Proportion of flesh to diameter

The amount of flesh varied from a mean of 58-76% of the diameter in *Chloromeles*, 49-67% in *Pumilae,* 48-52% in *Kansuensis*, 40-48% in *Baccatae*, 42-54% in *Sieboldianeae*, 32% in *Florentineae* to 12-21% in *Yunnanenses*. A large amount of flesh is correlated with large size of fruit. An exception occurs in series *Kansuenses* with relatively small fruits. Therefore, proportionately the largest cores occur in *Yunnanenses* and the smallest in *Chloromeles* (Table 2.11).

Sclerids

The fruits of most *Malus* species contain sclerids. The four species with the greatest abundance of sclerids in the fruits are *M. yunnanensis*, *M. prattii*, *M. tschonoskii* and *M. trilobata*. In these species sclerids occur in the flesh (hypanthial tissue), ovarian tissue, as well as at the core line. Other species possessing sclerids in the flesh are: *M. kansuensis*, *M. ioensis*, *M. fusca*, *M. rockii* and a small amount in *M. sikkimensis*. In all the species of series *Sieboldianeae* and *Chloromeles*, as well as *M. tschonoskii* and *M. trilobata* sclerids build a line which seperates ovarian tissue and hypanthial tissue (core line). A core line occurs in *M. halliana,* but with few sclerids. In all the species examined, except *M. sylvestris* and *M. florentina*, sclerids occur around the primary vascular bundles of ovarian tissues. In *M. sikkimensis, M. zumi*, *M. glaucescens, M. ioensis*, *M. tschonoskii*, *M. trilobata*. and *M. yunnanensis* sclerids occur between endocarp and vascular bundles as well (Table 2.12).

Hypanthium

Hypanthium tissues in all the species examined are homogenous parenchyma, but in *M. tschonoskii* the presence of sclerids in clusters and radial arrangement of parenchyma around them make a kind of heterogenous flesh (Plate 1).

Table.2.12. Distribution of sclerids in fruits of *Malus* examined.

Taxon	Flesh	Core line	Vas. bundles	Ovarian
Pumilae				
M. prunifolia				
1968.1585 RBGK	-	-	*	-
M. sylvestris				
10083 NBG	-	-	-	-
Baccatae				
M. baccata				
1776 NBG	-	-	+	-
M. sikkimensis				
1781 NBG	*	-	+	*
M. halliana				
19121003 RBGE	-	*	+	-
M. hupehensis				
1777 NBG				
M. rockii				
NBG	+	-	+	-
Sieboldianeae				
M. sieboldii				
1781 NBG	*	**	+	-
M. zumi				
19171011 RBGE	-	+	+	*
M. sargentii				
4433 NBG	-	+	+	-
M. x *floribunda*				
19400229 RBGE	-	+	+	-
Florentineae				
M. florentina				
19665032 RBGE	-	-	-	-
Kansuenses				
M. kansuensis				
RBGE	+	+	+	-
M. toringoides				
NBG	-	-	+	-
M. fusca				
NBG	*	-	*	-
Yunnanensis				
M. yunnanensis				
1981.4267 RBGK	++	-	+	++
M. prattii				
19091013RBGE	++	+	+	-
Chloromeles				
M. glaucescens				
19868280 RBGK	-	+	+	+
M. ioensis var. *palmeri*				
19868032 RBGK	+	+	+	+
Docyniopsis				
M. tschonoskii				
4437 NBG	++	+	+	++
Eriolobus				
M. trilobata				
1969.17535 RBGK	++	+	+	++

Symbols: + sparse; ++ abundant; - absent; * small amounts; ** discontinuous core line.
Abreviations; Vas Vascular; RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool.

2.3.7. Seed

Seeds of the genus *Malus* are similar in shape and they are almost pyriform. Their colour changes from yellow brown to brown black on ripening.

Seed length varies from 2mm in series *Sieboldianae* to 9 mm in series *Chloromeles* (Table 2.13).

The anatomy of the seed coat in different species is similar. The surface of the epidermis is covered with a layer of wax, which varies in thickness among the species but follows the contours of the epidermal cells. Only in *M. ioensis* var. *palmeri* is the wax layer piliform because of piliform cells of the epidermis layer under it. *M. pumila* has a dome like layer of wax. Other species show a smooth layer. Cross patterning of the epidermis varies from round, domed, oblong to piliform. Under the epidermis there are several layers of sclerenchyma cells, which may be 4-5 layers or 9-12 layers deep. *M. halliana, M. yunnanensis, M. fusca, M. prunifolia, M. toringoides, M. florentina, M. baccata,* and *M. sargentii* show 4-5 layers. In *M. sylvestris, M. pumila, M. kansuensis, M. coronaria, M. ioensis* var. *palmeri, M. glaucescens, M. tschonoskii, M. niedzwetzkyana, M. sieversii* and *M. angustifolia* the sclerenchyma is 9-12 cells thick. In species of series *Chloromeles* and *M. pumila, M. sylvestris* and *M. fusca* the sclerenchyma cells have very thick cell walls, while others have relatively thin cell walls. Under the sclerenchyma layers there are three to four layers of paranchyma cells which are horizontally elongated.

2.3.8. Genera related to *Malus*

Introduction

Genera *Docynia*, *Pesudocydonia*, *Chaenomeles* and *Sorbus* (*Aria* group) have been suggested to be the most related genera to *Malus* (Robertson *et al.*, 1991).

Based on morphological characteristics, Phipps *et al.* (1991) showed that *Malus* is closer to *Docynia* and *Chaenomeles* than other genera in Maloideae. To investigate more about these relationships some species of the *Aria* group of *Sorbus*

Table. 2.13. Seed characteristics of *Malus* examined.

Taxon	Seed Length X ± SD (mm)	Seed Width X ± SD (mm)	L/W ratio X ± SD	No. seeds examined
Pumilae				
M. kirghisorum				
5374.01 NFR	6.18 ± 0.70	3.17 ± 0.31	1.95 ± 0.24	15
3545.01NFR	6.43 ± 0.63	3.84 ± 0.51	1.71 ± 0.33	16
M. micromalus				
3458.01 NFR	4.87 ± 0.35	2.57 ± 0.50	1.97 ± 0.45	15
M. prunifolia				
1994 RBGK	4.80 ± 0.44	2.87 ± 0.23	1.71 ± 0.23	8
M. prunifolia var. *rinki*				
197311836RBGK	4.67 ± 0.43	3.06 ± 0.39	1.55 ± 0.25	9
198608412 RBGK	4.75 ± 0.42	3.1 ± 0.32	1.54 ± 0.17	10
M. pumila				
1973.1922 RBGK	6.80 ± 0.42	3.65 ± 0.67	1.95 ± 0.58	10
M. sieversii				
3249.01 NFR	7.37 ± 0.48	3.77 ± 0.48	1.98 ± 0.24	15
5326 NFR	6.34 ± 0.47	2.88 ± 0.47	2.26 ± 0.40	
M. sylvestris				
RBGK	6.69 ± 0.46	3.63 ± 0.52	1.88 ± 0.31	8
10083 NBG	5.80 ± 0.59	4.00 ± 0.47	1.47 ± 0.24	10
Baccatae				
M. baccata				
1776 NBG	4.94 ± 0.39	2.44 ± 0.46	2.08 ± 0.41	9
198208481 RBGK	5.44 ± 0.53	3.33 ± 0.50	1.68 ± 0.35	9
1982.8352RBGK	4.20 ± 0.42	2.80 ± 0.42	1.53 ± 0.28	8
M. baccata var. *himalica*				
1198601560 RBGK	4.15 ± 0.34	2.30 ± 0.35	1.84 ± 0.33	10
M. baccata var. *jackii*				
594.8206316 RBGK	4.25 ± 0.35	2.00 ± 0.00	2.13 ± 0.18	2
M. halliana				
RBGK	4.90 ± 0.32	2.25 ± 0.35	2.22 ± 0.31	10

cont'd. Table 2.13. Seed characteristics of *Malus* examined.

Taxon	Seed Length X ± SD (mm)	Seed Width X ± SD (mm)	L/W ratio X ± SD	No. seeds examined
M. hupehensis				
RBGK	6.13 ± 0.35	3.00 ± 0.00	2.04 ± 0.12	8
3453.01 NFR	3.23 ± 0.56	1.96 ± 0.43	1.71 ± 0.47	13
3234 NFR	4.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	4
3454.01 NFR	4.38 ± 0.62	2.38 ± 0.47	1.90 ± 0.43	16
3456.01	4.23 ± 0.60	2.08 ± 0.49	2.12 ± 0.47	1
1777 NBG	4.35 ± 0.41	2.06 ± 0.16	2.13 ± 0.18	10
M. rockii				
642282 RBGE	5.33 ± 0.5	2.72 ± 0.44	2.04 ± 0.56	9
NBG	2.88 ± 0.63	1.75 ± 0.29	1.65 ± 0.29	4
M. robusta				
156.8608360 RBGK	5.78 ± 0.44	3.00 ± 0.00	1.93 ± 0.15	9
M. sikkimensis				
3406 NBG	4.5 ± 0.45	2.00 ± 0.00	2.25 ± 0.22	6
1933.61408 RBGK	5.78 ± 0.44	3.33 ± 0.50	1.76 ± 0.24	9
Sieboldianeae				
M. sieboldii				
1988.655 RBGK	3.22 ± 0.36	2.00 ± 0.25	1.62 ± 0.18	9
M. sieboldii var. *arborescens*				
156.8601533	3.00 ± 0.00	2.00 ± 0.00	1.50 ± 0.00	8
M. sargentii				
4433 NBG	2.88 ± 0.35	1.50 ± 0.46	2.10 ± 0.77	8
1986.1591 RBGK	3.08 ± 0.29	2.00 ± 0.00	1.54 ± 0.14	12
M. floribunda				
1993.3517 RBGK	3.00 ± 0.00	2.00 ± 0.00	1.50 ± 0.00	2
M. zumi				
RBGK	3.00 ± 0.00	2.00 ± 0.00	1.50 ± 0.00	2
Kansuenses				
M. fusca				
340528 RBGE	4.85 ± 0.34	2.35 ± 0.41	2.13 ± 0.44	10
2511.01 NFR	4.90 ± 0.94	1.53 ± 0.43	3.00 ± 0.43	15
2510.01 NFR	4.76 ± 0.53	1.94 ± 0.17	2.48 ± 0.37	16

cont'd Table. 2.13. Seed characteristics of *Malus* examined.

Taxon	Seed Length X ± SD (mm)	Seed Width X ± SD (mm)	L/W ratio X ± SD	No. seeds examined
M. kansuensis				
156.8601573 RBGK	5.05 ± 0.47	2.45 ± 0.52	2.16 ± 0.56	11
M. toringoides				
451001RBGE	3.83 ± 0.26	1.83 ± 0..26	2.14 ± 0.42	6
101.131014 RBGK	3.91 ± 0.54	2.00 ± 0.00	1.95 ± 0.27	11
1981.8440 RBGK	3.80 ± 0.42	2.00 ± 0.00	1.90 ± 0.21	10
1981.8524 RBGK	3.70 ± 0.48	2.10 ± 0.00	1.78 ± 0.28	10
M. transitoria				
1601 RBGK	3.75 ± 0.50	2.75 ± 0.50	1.38 ± 0.08	4
Yunnanenses				
M. prattii				
19091013 RBGE	5.69 ± 0.46	3.81 ± 0.65	1.52 ± 0.17	8
M. yunnanensis				
19220113 RBGE	5.28 ± 0.36	3.83 ± 0.56	1.41 ± 0.24	9
1981.4267 RBGK	4.57± 0.53	2.86 ± 0.38	1.62 ± 0.23	7
156.8608236 RBGK	4.38 ± 0.52	3.38 ± 0.52	1.30 ± 0.04	8
5201 NBG	4.33 ± 0.58	3.00 ± 0.00	1.44 ± 0.19	
Florentinae				
M. florentina				
19665032 RBGE	3.95 ± 0.83	2.95 ± 0.37	2.05 ± 0.44*	10
Chloromeles				
M. angustifiolia				
1986.1556 RBGK	8.66 ± 0.51	4.85 ± 0.32	1.79 ± 0.16	12
2577.01 NFR	5.75 ± 0.55	3.56 ± 0.48	1.65 ± 0.26	18
2546.01 NFR	6.97 ± 0.29	3.75 ± 0.45	1.89 ± 026	16
M. coronaria				
1968.47713 RBGK	5.40 ± 0.52	3.90 ± 0.52	1.40 ± 0.15	10
2589.01 NFR	6.08 ± 0.63	3.34 ± 0.47	1.85 ± 0.32	19
2588.01 NFR	5.91 ± 0.49	3.53 ± 0.46	1.69 ± 0.22	16
1973.939 RBGK	7.20 ± 0.45	3.40 ± 0.55	2.17 ± 0.40	10

Cont'd. Table 2.13. Seed characteristics of *Malus* examined.

Taxon	Seed Length X ± SD (mm)	Seed Width X ± SD (mm)	L/W ratio X ± SD	No.seeds examined
M. ioensis				
2973.01 NFR	6.78 ± 0.41	3.88 ± 0.29	1.76 ± 0.13	16
2972.01 NFR	5.56 ± 0.56	3.21 ± 0.47	1.77 ± 0.27	17
M. glaucescens				
08280 RBGK	5.88 ± 0.35	3.63 ± 0.35	1.63 ± 0.19	20
M. platycarpa				
80.01592RBGK	8.00 ± 0.00	5.33 ± 0.58	1.51 ± 0.15	3
Docyniopsis				
M. tschonoskii				
4434 NBG	5.00 ± 0.00	3.88 ± 0.25	1.29 ± .09	4

Abbreviations; L Length; W Width; X mean; SD standard deviation
RBGK Royal Botanic Gardens Kew, London; RBGE Royal Botanic Gardens Edinburgh; NFC Brogdale, National fruit collection; NBG Ness Botanic Gardens, Neston, Liverpool. NFR National Germplasm Repository, Geneva, NY.

and the genera *Pseudocydonia* and *Chaenomeles* available in Ness Botanic Gardens were selected and studied. The species investigated were:

Sorbus caloneura, Sorbus chamaemespilus, Sorbus meliosmifolia, Sorbus vestita, Sorbus wardii, Sorbus zahlbruckneri and *Chaenomeles* sp. Unfortunately no material of *Docynia* was available.

Leaves

Leaves of all the specimens examined were simple with craspedodromous or camptodromous venation. In *Sorbus caloneura* leaves are elliptic, glabrous, with craspedodromous venation, crenate margin, acuminate apex, rounded base. In *Sorbus chamaemespilus* leaves are ovate or oblong, slightly pubescent in bud very soon glabrous, lobulate with camptodromous venation which veins eventually running to the tips of teeth, margin double serrate, entire toward the base. In *Sorbus lanata* leaves are elliptic, lobulate, glabrous above and pubescent below, with craspedodromous venation. This species is distinct from the others in having secondary veins parallel to each others. Leaf margin is serrate-crenulate, acute at apex and cuneate at base. In *Sorbus vestita* leaves are broadly elliptic, pubescent, white below and glabrous above with craspedodromous venation, serrate margin, acuminate at apex and cuneate at base. In *Sorbus meliosmifolia* leaves are elliptic, pubescent in bud and very soon become glabrous, venation craspedodromous, margin bi- to triserrate, acuminate at apex and cuneate at base. In *S. wardii* leaves are eliptic, slightly pubescent below, craspedodromous venation, acute at apex, rounded at base, margin serrate and entire at base of the blade. In *Sorbus zahlbruckneri* also leaves are elliptic with craspedodromous venation, margin double serrate or dentate, acuminate at apex and cuneate to rounded at base and slightly hairy above. *Sorbus chamaemespilus* is distinct from others in having oblong or ovate leaves with camptodromous venation. In *Chaenomeles* leaves are spathulate, margin serrate, obtuse at apex and cuneate at base.

Size of the leaves is presented in Table 2.14. Largest leaves belong to *Sorbus vestita* and shortest to *Chaenomeles.*

From this comparision very little can be deduced about the primitive (plesiomorphic) state in *Malus*. Lack of leaf lobing might suggest that lobed leaves are an apomorphy in *Malus*.

Petals

Among the species examined *Chaenomeles* has the largest petal length, while *Sorbus meliosmifolia* has the shortest petals.

The size of petals in *Chaenomeles* is very close to that of *Malus*, but the small petals of the *Sorbus* species examined in this study find a parallel only in sections *Kansuenses* and *Yunnanenses*. Creamy white, white or yellowish white petals as found in *Sorbus* are only found in *Kansuenses*, *Yunnanenses* and *Florentinae*. Orange petals as observed in *Chaenomeles* were not observed in any of *Malus* species (Table 2.15).

This might indicate that the pinkish colouration in the petals of some *Malus* is apomorphic.

Stamens

Number of stamens is about 20 in nearly most species examined. However, reduction from 20 to 15 (ranges from 11-18) is observed in *S. chameamespilus*. In *Sorbus vestita* and *Chaenomeles* increases in the number of stamens from 20 to 26 and 52, respectively are observed.

In *Malus* the increase in the number of stamens observed in *M. tschonoskii* and *M. spectabilis* is paralleled by a similar increase in *Chaenomeles,* mean of 52.29, very close to the mean of 53.73 in *M. tschonoskii*. Reduction from 20 to 15 was observed in series *Baccatae* of *Malus* as in *Sorbus chamaemespilus*. (Tables 2.8 and 2.16).

Table 2.14. Leaf characteristics of some species in genera related to *Malus*.

Taxon	Leaf length (mm) X ± SD	Leaf width (mm) X ± SD
Chaenomeles sp.		
NBG	28 ± 6.2	19.4 ± 2.9
Sorbus caloneura		
NBG	86.1 ± 7.1	38.9 ± 3.3
S. chamaemespilus		
NBG	104.2 ±7.2	54.4 ± 4.5
S. meliosmifolia		
NBG	103.7 ± 9.2	47 ± 5.7
S. vestita		
NBG	162.2 ± 13.6	63.8 ± 8.9
S. wardii		
NBG	132 ± 14.5	54.2 ± 7.1
S. zahlbruckneri		
NBG	78.1 ± 11.6	38.9 ± 6.1

Abreviations; NBG Ness Botanic Gardens, Neston, Liverpool.
X mean; SD standard deviation.

Table 2.15. Petal characteristics of individuals of some species in genera related to *Malus*.

Taxon	Length (mm) X ± SD	Width (mm) X ± SD	L/W ratio X	No. petals examined
Chaenomeles japanica				
NBG	14.4 ± 1.7	12.4 ± 1.6	1.16	10
Sorbus caloneura				
NBG	5.2 ± 0.5	4 ± 0	1.3	5
S. chamaemespilus				
NBG	6.8 ± 0.6	3.3 ± 0.5	1.3	10
S. lanata				
NBG	6.4 ± 0.5	3.6 ± 0.5	1.77	7
S. meliosmifolia				
NBG	3.6 ± 0.9	4 ± 0.7	0.9	7
S. vestita				
NBG	8.5 ± 0.5	6.3 ± 0.7	1.3	10

Abreviations; NBG Ness Botanic Gardens, Neston, Liverpool.
X mean; SD standard deviation; L Length; W Width.

Table 2.16. Staminal characteristics of individuals of some species in genera related to *Malus*.

Taxon	Anther colour	Stamen number X ± SD	No. flowers examined
Chaenomeles sp.			
NBG	Yellow	52.3 ± 3	14
Pseudocydonia			
NBG	Yellow	19.7 ± 2.7	4
Sorbus caloneura			
NBG	Yellow	20 0 ±.32	20
Sorbus chamaemespilus			
NBG	Pink	15 ± 1.8	28
S. lanata			
NBG	Yellow	19.3 ± 1.4	14
S. meliosmifolia			
NBG	Yellow	20 ± 0.2	30
S. vestita			
NBG	Red	20.8 ± 2.7	30
S. wardii			
NBG	Yellow	22.6 2.8	19
S. zahlbruckneri			
NBG	Yellow	19 ± 1.3	20

Abreviations; NBG Ness Botanic Gardens, Neston, Liverpool; X mean; SD standard deviation.

Table 2.17. Number of carpels per flower of some species in genera related to *Malus* .

Taxon	Carpel numbers X ± SD	No. of flowers examined
Chaenomeles sp.		
NBG	5.1 ± 0.3	8
Pseudocydonia		
NBG	4.3 ± 0.9	4
Sorbus caloneura		
NBG	4.7 ± 0.5	40
S. chamaemespilus		
NBG	2± 0	30
S. lanata		
NBG	3.3 ± 0.5	15
S. meliosmifolia		
NBG	3.6 ± 0.6	30
S. vestita		
NBG	3.9 ± 0.6	30
S. wardii		
NBG	2.6 ± 0.5	19
S. zahlbruckneri		
NBG	2 ± 0	20

Abreviations NBG Ness Botanic Gardens, Neston, Liverpool. X mean; SD standard deviation.

Styles

Fusion and hairness of styles of related genera to *Malus* are presented in Table 2. 17. All the species examined have fused styles, at least in lower 1/3. However, the only species with free styles is *S. chamaemespilus.* In *S. caloneura, S. meliosmifolia, S. zahlbruckneri, Chaenomeles* and *Pesudocydonia* styles are glabrous, while *S. chamemespilus, Sorbus lanata, S. wardii* and *S. zahlbruckneri* have pubesent styles.

Free styles also were observed in section *Docyniopsis*, *Chloromeles* and *Eriolobus* of genus *Malus* and glabrous styles were observed in series *Kansuenses, Yunnanenses* and *M. florentina..*

Among the species examined *Sorbus chamemespilus* and *Sorbus zahlbruckneri,* are very distinct from others by the constant number of 2 carpels per flowers. This reduction in number of carpels was also found in series *Kansueneses* and *Sieboldianae*. Other species have 3-5 carpels. Reduction from 5 was observed also in *S. vestita, S. lanata, S. meliosmifolia* and *S. wardii,*

It is usually presumed that the plesiomorphic state is 5 free carpels. Fusion of styles and reduction in carpel number in *Malus* is presumed to be paralleled by a similar event in *Sorbus* and *Chaenomeles.*

Carpel connation and adnation

None of the species examined showed the suture in lateral joining as occurs in some *Malus* species, but a hole is seen in the center of the carpels in most species examined except *S. vestita* and *S. meliosmifolia.*

In all the species examined the ovary was fused with the surrounding fleshy tissue. In other words, the ovary is adnate to the hypanthium.

As with carpel number and style fusion, there is a presumed polarity with increasing connation and adnation being apomorphies, but perhaps not synapomorphies, so much as parallelisms (Cronquist, 1987).

Hypanthium

The fruit flesh of all species of *Sorbus* examined, including *S. chamaemespilus,* is heterogeneous. There are groups of large cells with brown contents surrounded by the small cells of ground tissue. However, in *Chaenomeles* and *Pseudocydonia* the flesh is homogeneous as occurs in all the species of *Malus*. The only exeption in *Malus* with heterogenous flesh is *M. tschonoskii*, in which clusters of sclerids make a distinctly heterogeneous flesh but this is clearly a different kind of heterogenity from that found in the *Sorbus* species.

2.4. Discussion

2.4.1 Introduction

With *Malus* the difficulty in defining a sister group makes the polarity of some characters difficult to define, but in most cases the polarities proposed for the Maloideae (Table 2.18) seem reasonable to apply within *Malus* as comparision with *Chaenomeles*, *Pseudocydonia* and *Aria* show. Carpel adnation and carpel connation are clearly apomorphies and reversals in degree of fusion are presumed to be unlikely. However, the occurrence of wholly adnate and connate carpels in different genera or species may not be synapomorphies but the result of parallel evolution.

2.4.2. Leaves

The variation in leaves within the genus can be used for the classification of *Malus* for delimiting subgeneric taxa. In brief the characteristics of leaves in different series of *Malus* are as follows:

Series *Baccatae* and *Pumilae* have unlobed leaves with camptodromous venation on both long and short shoots. Variation in this character in these series is very rare, although trees labelled *M. spectabilis* and *M. prunifolia* may rarely have lobed leaves because of hybridization. Series *Kansuenses* and *Florentinae* have lobed leaves with craspedodromous venation on both long and short shoots, although in *M. toringoides* and *M. fusca* unlobed leaves may occur on short shoots. Series *Sieboldianae* and *Chloromeles* have lobed leaves with craspedodromous venation, at least on long shoots, and lobed or unlobed leaves on short shoots. Section *Eriolobus* has lobed leaves and section *Docyniopsis* has lobulate leaves on both kind of shoots. Robertson *et al.* (1992) postulated an evolutionary sequence in *Maloideae* from ancestral to derived state as follows:

a. Pinnately lobed with sharp teeth and craspedodromous venation.

b. Coarsely doubly serrate or dentate with craspedodromous venation with the secondary veins going to the tips of the largest teeth.

c. Unlobed but toothed and craspedodromous with the secondary veins going to the tips of some or all of teeth.

d. Unlobed and toothed with camptodromous venation.

e. Unlobed with entire margins and camptodromous venation. The most derived foliage type would be group e without heteroblastic foliage.

Therefore, the pinnately lobed leaves with craspedodromous venation of subgenus *Eriolobus* and the lobulate leaves of *Docyniopsis* and *Florentinae* without heteroblastic foliage are the most primitive (plesiomorphic) foliage type, while the unlobed and toothed leaves with camptodromous venation of the *Pumilae* and *Baccatae* are the most derivative (apomorphic) foliage types. *Sieboldianae*, *Kansuenses*, *Yunnanenses* and *Chloromeles* with heteroblastic foliage are intermediate.

This evolutionary sequence for leaf characters is in agreement with the order of series proposed on the basis of other characters, especially those such as degree of carpel fusion, with a generally accepted direction of polarity. Robertson *et al.*'s (1992) proposals for Maloidedae can therefore be applied to *Malus*.

M. trilobata, the sole species of the section *Eriolobus* has the widest leaf blade. This characteristic causes *M. trilobata* to be a distinctive taxon within the genus *Malus*.

2.4.3. Flower

The usual flower form in the *Malus* is large, pentamerous, with small triangular calyx lobes, pinkish-white petals, 20 stamens, 5 carpels with various degrees of carpel fusion and an inferior ovary.

Phipps *et al.* (1991) considered there to be three main trends in flower evolution of Maloideae as follows:

1. The carpels sunk ever deeper into the hypanthium, generating the fully inferior ovary of, for instance, many *Malus* species.

2. Increased connation of carpels generally accompanies their increased adnation to the hypanthium.

3. The inflorescence evolution leading to fewer flowers per inflorescence, the flowers tending to increase in size and stamen number as well as develop visual levels of pigmentation (pink, red orange).

The polarities of characters according to Phipps *et al.* (1991) for the Maloideae in general is given in Table 2.18.

Therefore, the small flowers of series *Yunnanenses*, *Kansuenses*, and *Florentinae* are plesiomorphic and the large flowers of section *Docyniopsis*, *Eriolobus*, *Pumilae* and, *Chloromeles* are apomorphic. *Baccatae* and *Sieboldianae* are intermediate.

White flowers of *Yunnanenses*, *Florentinae*, *Kansuenses* and nearly white petals of *Docyniopsis* and *Eriolobus* are plesiomorphic, while pink or red petals of series *Pumilae*, *Sieboldianae*, *Chloromeles* and *Baccatae* are apomorphic.

On this interpretation the stamen number (mean 47.33-53.73) in *M. tschonoskii* is an apomorphic character while in series *Florentinae* (19.9), *Yunnanenses* (20.1), *Chloromeles* (19.2-20) *Eriolobus* (21.1), and *Pumilae* (19.9-20.03) [except *M. spectabilis* mean 34.8] it is plesiomorphic while, in series *Kansuenses* (15.23-21.38) and *Baccatae* (15.71- 25.57) *Sieboldianae* (18.73-20.8) it is a transitional character. Large number of stamens (39-61) in *M. tschonoskii* is a distinctive floral character of this species and has been a subject of debate for classification of this species (Robertson *et al.*, 1991). Based on number of stamens, *M. tschonoskii* is close to the genus *Docynia* with 30-50 stamens per flower (Rehder, 1967).

Carpel number of 5 in *Chloromeles*, *Yunnanenses*, *Eriolobus* and *Florentinae* is plesiomorphic, a number lower than 5 in series *Sieboldianae*, *Kansuenses* and *Baccatae* can be considered as apomorphic and *Pumilae* with both reduction and

Table 2.18. Evolutionary polarity of flower and fruit characters in Maloideae (summarised after Phipps *et al.*, 1991).

	Plesiomorphic	Apomorphic
Flower no.	40 ± (20)	ca. 50, or 1
size	smallish (10 mm)	larger (>20 mm)
stamen no.	about 20	5-10 or 30-50
petal shape	circular	narrow
petal colour	white	pink, orange or red
Carpel no.	5	1
styles	free	fused
carpel connation	free	fused

increase from 5 is intermediate. Free styles in *Chloromeles* and *Docyniopsis* is a plesiomorphic state. Low degree of carpel connation in *Chloromeles*, *Docyniopsis*, and *Eriolobus* is plesiomorphic.

Small and white flowers with glabrous styles are characteristics of series *Kansuenses* and *Yunnanenses.* The large, pink to red flowers with red stamens, low degree of carpel connation and low degree of carpel adnation to the hypanthium are some characteristics to distinguish section *Chloromeles* from the other sections of the *Malus*. Although, *Malus niedzwetzkyana* of series *Pumilae* also has large and red flowers with red stamens, it differs from *Chloromeles* in having unlobed leaves rolled in bud and red fruit.

The results obtained from flower and vegetative parts of *Malus* led to the making of a key to species of *Malus* in the flowering state (Figure 2.2).

Figure 2.2. Key to the species of *Malus* using flowers and vegetative parts

1. Anthers red 2
   2. Leaves unlobed; carpel connation >20 % *M. niedzwetzkyana*
   2. At least leaves of long shoots lobed; carpel connation <20% 3
      3. Petal margin entire 4
         4. Calyx lobes, tube and pedicel pubescent, leaves tomentose below and strongly veined *M. ioensis*
         4. Calyx lobes, tube and pedicel glabrous, leaves glabrous or slightly pubescent 5
            5. Leaves glabrous, unlobed or slightly lobed on short shoots, ovate to oblong-lanceolate, light green on both sides; 5-8 flowers per inflorescence, carpel connation slightly >10% *M. lancifolia*
            5. Leaves pubescent, lobed, broad-ovate to oblong, light green above and lighter below; 4-6 flowers per inflorescence; carpel connation <10% *M. coronaria*
      3. Petal margin crenate 6
         6. Leaves ovate or oblong lanceolate, calyx lobes shorter than or equal to the tube, glabrous, carpel connation> 10%, styles slightly connate *M. angustifolia*
         6. leaves broad ovate to triangular ovate, calyx longer than tube, slightly pubescent; carpel connation <5% percent, styles free, glabrous *M. glaucescens*
1. Anthers white or yellow (except *M. niedzwetzkyana*) 7
   7. Leaves unlobed 8
      8. Leaf about as broad as long *M. prattii*
      8. Leaf longer than broad 9
         9. Ratio of petal length to width >1.5; degree of carpel connation lower than 50% (except *M.sylvestris*) 10
            10. Anthers pink; petals red *M. niedzwetzkyana*
            10. Anthers yellow; petals pinkish white 11
               11. Anthers (17) 20; petals 5 12
                  12. Young leaves purple or bronze; petals 21-26 mm; calyx pubescent *M.pumila*
                  12. Young leaves green; petals 12-18 mm; calyx glabrous 13
                     13. Leaves broad elliptic to obovate; sharpely serrate, acuminate at apex, pubescent below *M. prunifolia*
                     13. Leaves orbicular, crenate; acuminate to mucronate at apex, glabrous below *M. sylvestris*
               11. Anthers 28-40, petals 5 or more *M. spectabilis*
         9. Ratio of petal length to width ≤1.5; degree of carpel connation high, more than 50 % 14
            14. Leaves sharply serrate *M. x floribunda*
            14. Leaves fine serrate or crenate 15
               15. Sepals green; petal base cordate or subcordate, margin entire 16
                  16. Leaves pubescent below, leathery, apex acuminate to attenuate, margin finely serrate; petals <15mm, white, styles 4(5) *M. sikkimensis*

16. Leaves glabrous below, not leathery, apex acute or acuminate, margin crenate, entire at base, petals>15 mm, pinkish white, styles (4)5 *M. baccata*

15. Sepals red; petal base truncate to tapering, margin entire to crenate or crenulate 17

17. Petal margin entire, style (3)4(5) pedicel <25mm, petals <19 mm *M. halliana*

17. Petal margin crenulate, style 4 (5); pedicel >30 mm,; petals mostly > 19 mm *M. hupehensis*

7. Leaves lobed or lobulate 18

18. Degree of carpel connation high more than 60 % 19

19. Styles glabrous: leaf base rounded to cuneate 20

20. Short shoot leaves unlobed or slightly lobed; petal base semicordate *M. fusca*

20. Short shoot leaves lobed; petal base cordate or truncate 21

21. Calyx tube and pedicel glabrous, petal base cordate, margin crenulate; leaves rounded at base *M. kansuensis*

21. Calyx tube and pedicel tomentose, petal base truncate, margin dentate; leaves tapering to cuneate at base 22

22. Leaves lobed >3/4 way to midrib *M. transitoria*

22. Leaves lobed <3/4 way to midrib *M. toringoides*

19. Styles pubescent; leaf base cuneate 23

23. Carpels 3 (5) 24

24. Leaves unlobed, narrowly elliptic to obovate, acute to rounded at apex, cuneate at base, margin sharply serrate, glabrous; calyx lobes and tube glabrous, styles 4-5, degree of lateral carpel connation 73-75 % *M. x floribunda*

24. Leaves lobed at least on long shoots, ovate to elliptic, degree of lateral carpel connation 100% 25

25. Petals large >11× 9 mm; leaves pubescent beneath; carpels (3)-5 *M. sargentii*

25. Petals smaller, <11× 9 mm slightly pubescent on both side, carpels 3(-5) *M. sieboldii*

23. Carpels 5 *M. florentina*

18. Degree of carpel connation low, less than 60% 26

26. Styles glabrous; leaves slightly lobed or unlobed; tree with spreading branches 27

27. Leaves lobed,venation craspedodromous, pubescent below *M.yunnanensis*

27. Leaves unlobed, venation camptodromous, glabrous above *M. prattii*

26. Styles pubescent; leaves lobed or lobulate; tree with erect branches 28

28. Leaves lobulate; stamens 40-60; tree with erect branches; calyx<5mm *M. tschonoskii*

28. Leaves trilobed; stamens 20; tree with spreading branches; calyx about 1 mm *M. trilobata*

2.4.4. Fruit

The typical pome of *Malus* is a false fruit with an enlarged and fleshy hypanthium, generally 5 carpels, persistent or deciduous calyx, a thin endocarp which makes a cartilaginous core, carpels usually connate or adnate, and two ovules per locule.

According to Rohrer *et al.* (1991) major trends in the evolution of the pome include reduction in the number of carpels, increase in the number of ovules per carpel, to many or their reduction to one, greater fusion among carpels and greater adnation of ovary to hypanthium. They deduced that the primitive pome had 5 carpels, minimal carpel connation, minimal adnation of ovaries to the hypanthium and 2 ovules per carpel.

As all these states exist within *Malus*, the trends proposed for the Maloideae as a whole can be identified within *Malus*.

In *Malus* the number of carpels varies between 2 and 5. Therefore it can be concluded that the reduction from 5 in the number of carpels in series *Baccatae*, *Kansuenses* and *Sieboldianae* indicates an apomorphy while, the number of 5 in *Chloromeles*, *Docyniopsis*, *Yunnanenses* and *Eriolobus* is a plesiomorphy. Carpels of the *Pumilae* show both reduction and an increase from 5 and therefore are considered intermediate.

Low connation of carpels in *Chloromeles*, *Docyniopsis,* and *Eriolobus* is a plesiomorphic character, while high degree of carpel connation in *Baccatae*, *Sieboldianae* and *Kansuenses* is an apomorphic character and *Yunnanenses* and *Florentinae* and *Pumilae* are in a transition state.

Based on fruit characters, *Malus* can be divided into two major groups as follows:

1. A group with persistent calyx and different degrees of carpel adnation and connation including series *Pumilae* of section *Malus*, *Yunnanenses* of section *Sorbomalus* and sections *Eriolobus*, *Chloromeles*, and *Docyniopsis*.

2. A group with deciduous calyx and fully adnate and connate carpels. Including series *Baccatae*, *Sieboldianae*, *Kansuenses* and *Florentinae*.

In the second group section *Chloromeles* is distinct from others in having fruit green at maturity and sclerids absent or sparse. Section *Eriolobus* is distinct from the others in having recurved calyx lobes and many sclerids.

Section *Docyniopsis* with erect and fleshy calyx, flattened fruit, and numerous cluster of sclerides throughout the flesh is distinct from all others.

Based on fruit morphology and anatomy section *Chloromeles* is strongly differentiated from other species of *Malus* as follows:

1. Large and green fruits.
2. Absence of sclerids in flesh.
3. Low degree of carpel connation.
4. Incomplete carpel adnation with free carpel apices protruding at fruit apex.

Robertson *et al.* (1991) retained *Chloromeles* as subgenus *Chloromeles* based on fully adnate carpels, whereas this study reveals the adnation of carpels to the hypanthium to be incomplete. This perhaps reveals the problems Robertson *et al.* (1991) encountered through using only representative species of each section.

The results obtained from fruit and vegetative parts of *Malus* led to the making of a key which is shown in Figure 2.3. This key identify the species in the fruiting state.

Figure 2.3. Key to the species of *Malus* using fruit and vegetative parts

1. Calyx persistent 2
   2. All leaves unlobed 3
      3. Stone cells in flesh aboundant, fruit lenticellate *M. prattii*
      3. Stone cells rare or absent, fruit not conspicuously lenticellate 4
         4. Fruit diameter more than 28 cm, fruit stalk short (1-2 cm) 5
            5. Young branches and young leaves purple; fruit conic and red, flesh with red or pink pigment *M. niedzwetzkyana*
            5. Young branches and leaves green or bronze; fruit globose green to greenish yellow flushed with red 6
               6. Young leaves on long shoots bronze or reddish, ovate elliptic, acute at apex, obtuse to rounded at base, slightly pubescent; fruit impressed at both ends; fruit stalk shorter than 1 cm *M. pumila*
               6. Young leaves on long shoots green, obovate to short and broad elliptic to orbiculate, long to short acuminate at apex, rounded to cuneate at base, glabrous; fruit not impressed at the ends, fruit stalk 1-1.5 cm *M. sylvestris*
         4. Fruit diameter less than 28 mm, fruit stalk long (2.5-4 cm) 7
            7. Calyx persistent or deciduous on fruit of the same plant *M. micromalus*
            7. Calyx always persistent on the fruit 8
               8. Fruit yellow at maturity, not impressed at apex; leaves sometimes lobed *M. spectabilis*
               8. Fruit yellowish red or yellow green, impressed at apex, leaves unlobed9
                  9. fruit stalk 3.5-4 cm; fruit yellow green *M. sieversii*
                  9. Fruit stalk 2-3.5 cm; fruit yellowish red *M. prunifolia*
   2. At least long shoot leaves lobed or lobulate 10
      10. Carpel connation complete, fruits yellow; leaves sometimes unlobed *M. spectabilis*
      10. Carpel connation incomplete, fruit green, yellow, yellow green or red, leaf lobing various 11
         11. Stone cells rare, fruit green or yellow-green to yellow 12
            12. Fruit green 13
               13. Fruit large, about 37 mm or more; long shoot leaves sligthly lobed to unlobed *M. platycarpa*
               13. Fruit smaller than 37 mm; leaves at least on long shoots lobed 14
                  14. Leaves strongly pubescent, veins prominent below, short shoot leaves unlobed or shallowly lobed *M. ioensis*
                  14. Leaves glabrous or slightly pubescent, veins not prominent, short shoots leaves more or less lobed 15
                     15. Leaves glabrous, short shoots leaves unlobed, finely or coarsely serrate, ovate or oblong lanceolate, light green on both sides, chartaceous, margin serrate, base cuneate or rounded *M. lancifolia*

15. Leaves slightly pubescent below, usually lobed, broad ovate or oblong, light green above and lighter below, base cordate, cuneate or rounded *M. coronaria*

12. Fruit yellow or yellow-green 16

16. Fruit yellow green; leaves dark green above, ovate to oblong lanceolate, short shoot leaves unlobed, margin often entire, sometimes serrate *M. angustifolia*

16. Fruit yellow, leaves light green above, broad ovate or triangular ovate, long and short shoot leaves lobed, margin serrate *M. glaucescens*

11. Stone cells abundant, fruit red 17

17. Leaves 3-lobed, glabrous on both sides; calyx recurved and long; fruit not lenticellate *M. trilobata*

17. Leaves lobulate, tomentose on both sides; fruit lenticels conspicuous 18

18. Calyx erect and short, lobes succulent and contiguous; carpel apices conical; fruit not ribbed, broader than long *M. tschonoskii*

18. Calyx recurved and long, lobes not touching, not succulent; carpel apices rounded; fruit ribbed, at least as long as broad 19

19. Leaves lobed, venation craspedodromous pubescent below *M. yunnanensis*

19. Leaves unlobed, venation camptodromous, glabrous below *M. prattii*

1. Calyx deciduous

20. Leaves unlobed 21

21. Leaf margin sharply serrate, fruit ≤1 cm *M. x floribunda*

21. Leaf margin finely serrate ; fruit > 1cm 22

22. Inflorescence corymbose; fruit with conspicuous white lenticells on the surface 23

23. Leaves tomentose and grey beneath, slightly pubescent above; fruit elliptic, stalk 16-35 mm, *M. sikkimensis*

23. Leaves pubescent or glabrous beneath except on veins; fruit pyriform, stalk 10-24 mm *M. rockii*

22. Inflorescence umbellate; fruit not conspicuously lenticellate 24

24. Locules 5 rarely 4 *M. baccata* and *M. robusta*

24. Locules (3) 4- 5 25

25. Calyx scar on fruit 2-6 mm diameter, fruit red, globose, slightly pyriform, core about 1/2 fruit diameter, locules (3)4(5) *M. hupehensis*

25. Calyx scar on fruit 1-2 mm diameter, fruit brown red, oblong, core 1/4-1/2 fruit diameter, locules (3) 4-5 *M. halliana*

20. Leaves lobed 26

26. Lobes less than 1/2 distance to midrib; locules 5 *M. florentina*

26. Lobes more than 1/2 distannce to midrib; locules 3-5 27

27. Inflorescence umbellate 28

28. Fruit > 8 mm; globose to conic; some leaves often unlobed *M. x floribunda*

28. Fruit ≤ 8 mm; leaves deeply or shallowly lobed 29

29. Long shoot leaves shallowly lobed, sharply serrate, light green, thin, style bases usually persistent on fruit *M. zumi*

29. Long shoot leaves deeply lobed, serrate, green; style bases not persistent on the fruit 30

30 Leaves slightly pubescent beneath; the side of fruit exposed to the sun red and the shade side yellow *M. sieboldii*

30. Leaves scabrous beneath, fruit red *M. sargentii*

27. Inflorescence corymbose 31

31. Leaves 3-lobed in upper half 32

32. Leaves 3-lobed on long and short shoots, rounded at base; fruit brown, 9-11 mm in diameter, locules 2-3 (5), stone cells present on core line *M. kansuensis*

32. Leaves often unlobed, especially on short shoots, cuneate at base, fruit red, 11-13 mm in diameter, locules 3(5), no stone cells on core line *M. fusca*

31. Leaves 3 or more lobed, lobes not confined to upper half of leaf 33

33. Core less than1/3 fruit diameter *M. transitoria*

33. Core 1/2-2/3 fruit diameter *M. toringoides*

2.4.5. Study of *Malus* sections

Section *Malus*

Series *Pumilae*

Simple leaves without lobes but with serrate margins, folded leaves in the bud, large pinkish white flowers, and in one case red petals, umbellate inflorescence and sclerids sparse to absent are considered apomorphic, stamen number of about 20 and persistant calyx considered plesiomorphic, number of flowers per inflorescence, carpel number (3-5) and connation of the carpels are considered intermediate.

In considering polarity of morphological characters, of 11 characters 2 are plesiomorphic, 3 intermediate and 6 apomorphic (Table 2.19). Therefore, *Pumilae* is considered as one of the most advanced taxa in the genus.

There is some evidence to support the advanced position of *Pumilae* including:

1. Widespread distribution in Europe and Asia.
2. Relatively little differentiation among species.

The *Pumilae* have some characters shared with plesiomorphic taxa. With *Chloromeles*, *Docyniopsis* and *Eriolobus* it shares persistent calyx and large flowers. With *Chloromeles* only it also has in common pinkish white to pink flowers, large fruits, and sclerids sparse to absent. *Pumilae* is distinguished from *Chloromeles* by white anthers and green stigma, umbellate inflorescence and yellow to red fruits. The affinity of *Pumilae* and *Chloromeles* is supported by the occurrence of hybridization between *M. pumila* and members of *Chloromeles* e.g. *M. pumila* x *M. ioensis* = *M.* x *soulardii* (Beans, 1923) and *M. pumila* x *M. coronaria* = *M.* x *heterophylla* (Rehder, 1967).

With *Florentinae* it only has in common an intermediate number of flowers per inflorescence, stamen number of about 20 and few to sparse sclerids in the fruit.

With apomorphic taxa it shares an intermediate number of flowers, umbellate inflorescences, pinkish petals, and sclerids sparse or absent.

Table.2.19. Polarity of morphological and anatomical characters in *Malus*.

characters / series	leaf shape	vernation	size of flowers	no. of flowers per Inf.	inf. types	petal colour	stamens no.	carpels no.	carpel connation	calyx condition	sclerids occurrence
Pumilae	A	A	A	I	A	A	P	I	I	P	A
Baccatae	A	A	I	I	A	A	I	A	A	A	A
Sieboldianae	I	P	I	I	A	A	I	A	A	A	A
Florentinae	P	P	P	I	P	P	P	P	I	I	I
Kansuenses	I	P	P	I	P	P	I	A	A	A	I
Yunnanenses	I	P	P	P	P	P	P	P	I	I	P
Chloromeles	I	P	A	I	P	A	P	P	P	P	A
Eriolobus	P	P	A	A	P	P	P	P	P	P	P
Docyniopsis	P	P	A	A	P	P	A	P	P	P	P

With *Baccatae* it shares rolled leaf vernation, intermediate number of flowers per inflorescence, sclerids sparse to absent, unlobed leaves on both short and long shoots, and pinkish white petals. *Pumilae* is much closer to the *Baccatae* than to any other taxon in the genus.They therefore probably have a recent common ancestor. The shared derived characters (rolled leaves in the bud and unlobed leaves) are evidence of exclusive common ancestry.

Pumilae is much closer to *Docyniopsis* than other primitive taxa. They share:

1. Unlobed leaves or tendency toward unlobed leaves.
2. Five carpels.
3. Presence of sclerids in *M. prunifolia* around the vascular bundle.
4. Relatively low degree of carpel connation.

With *Sieboldianae* the *Pumilae* have in common an intermediate number of flowers, umbellate inflorescence, and petal colour. With *Kansuenses,* it shares an intermediate number of flowers and stamens. With *Yunnanenses* it shares stamen number of about 20 per flowers.

Therefore it has more affinity with *Baccatae* than other taxa. Rehder (1940) placed two taxa in one section *Malus*. It is also supported by the occurrence of many hybrids between series *Baccatae* and *Pumilae* (e.g. *M. pumila* x *M. baccata* = *M.* x *adstringens*, *M. prunifolia* x *M. baccata* = *M.* x *robusta*). The occurrence of some hybrids between *Pumilae* and *Sieboldianae* also support the close relationship between these two taxa (e.g. *M. prunifolia* x *M. sieboldii* = *M.* x *sublobata*), Figure 1.3.

The occurrence of *M.* x *dawsonianae* = *M. fusca* x *M. pumila* also supports the relationship between *Pumilae* and *Kansuenses*.

Series *Baccatae*

Unlobed leaves without heteroblastic forms, petal colour, carpel number, connation of carpel, rolled vernation, umbellate inflorescence, persistent calyx and

absence of sclerids are considerd apomorphic. Intermediate number of flowers, stamen number and number of flower per inflorescence are considered intermediate.

Of 11 characters, 8 are apomorphic and 3 intermediate and there are no plesiomorphic characters (Table 2.19). The *Baccatae* are considered the most advanced taxon in the genus.

The close relationship between *Baccatae* and *Pumilae* was discussed in under series *Pumilae*.

Baccatae has some characters in common with *Sieboldianae*, intermediate number of flowers per inflorescence, high degree of carpel connation, umbellate inflorescence, pinkish white flowers, deciduous calyx, small fruits and abscence of sclerids. The *Baccatae* are distinguished from *Sieboldianae* by unlobed leaves and rolled vernation.

Broad leaves, which are pubescent beneath, white petals, corymbose inflorescence, and the presence of some calyculate fruits in *M. sikkimensis* shows the affinity of series *Baccatae* with *Yunnanenses*.

Section *Sorbomalus*

Sieboldianae

Pinkish white petals, carpel number (4-5), high degree of carpel connation, type of inflorescence, deciduous calyx and absence of sclerids are considered apomorphic. Heteroblastic leaves, size of flowers and number of flowers per inflorescence and the stamen number (15-24) are considered intermediate. Conduplicate vernation of leaf in the bud, is considered plesiomorphic.

The presumed polarity of morphological characters is as follows. Of 11 characters 6 are apomorphic, 4 intermediate, and 1 plesiomorphic (Table 2.19). The *Sieboldianae* are considered as a derivative taxon in the genus.

Sieboldianae are much closer to *Kansuenses* than to other taxa. They share in leaves conduplicate in bud, heteroblastic leaves, intermediate stamen number, high

degree of carpel connation, deciduous calyx and fruit with few sclerids or sclerids absent.

Sieboldianae are different from *Kansueneses* in having pubescent styles in contrast to the glabrous styles of *Kansuenses*. *Kansuenses* are considered more primitive than *Sieboldianae* because of their smaller flowers, white petals and corymbose inflorescence.

With other advanced taxa *Sieboldianae* have in common pinkish white petals, umbellate inflorescences, absence of sclerids and intermediate number of flowers per inflorescence.

Sieboldianae are also close to *Baccatae* in having an intermediate number of flowers, pinkish white petals, intermediate stamen number, carpel number, high degree of carpel connation, type of inflorescence, deciduous calyx and few or no sclerids.

The *Sieboldianae* and *Baccatae* have the same apomorphic characters (synapomorphies). *Sieboldianae* is more primitive than *Baccatae* in having heteroblastic leaves, folded vernation of leaves in the bud and number of stamens about 20. The occurrence of polyploidy in *M. sieboldii* and *M. sargentii* and the occurrence of both sexual and apomictic reproduction in the species of the series indicate the advanced nature of series *Sieboldianae*. The *Sieboldianae* is a very distinctive series in the genus because of the homogeneous characters in the species including conduplicate vernation, deciduous calyx, umbellate inflorescences and few sclerids in the fruits.

Kansuenses

Presence of both lobed and unlobed leaves, number of flowers, stamens number and sparse to moderate number of sclerids are considered intermediate. Conduplicate vernation, size of flowers, type of inflorescence, petal colour are considered plesiomorphic, while number of carpels per flower, carpel connation and condition of calyx are considered apomorphic.

The presumed polarity of morphological characters in *Kansuenses* shows the series to occupy an intermediate position. Of 11 characters 4 are plesiomorphic, 4 intermediate and 3 apomorphic (Table 2.19).

With *Eriolobus*, *Docyniopsis*, *Yunnanenses*, and *Florentinae* it has in common white petals, conduplicate vernation and corymbose inflorescence.

With *Yunnanenses* and *Florentinae* it has in common white petals, small flowers, conduplicate vernation and corymbose inflorescence.

Kansuenses has in common with *Yunnanenses,* white flowers, lobed and unlobed leaves (heteroblastic leaves), glabrous style, increased number of flowers per inflorescence and is distinguished from it by narrower leaves, smaller number of flowers per inflorescence, smaller number of sclerids in fruits, deciduous calyx and greater degree of carpel connation. These similarities between the two taxa are the reasons for Van Eseltine (1933) including the two series in one subsection, *Kansuenses*, and for Huckins (1972) to include them in one section, *Kansuenses*.

With *Chloromeles*, it has in common sparse numbers of sclerids and lobed to entire leaves. From *Chloromeles* it is distinguished by small flowers, white petals, white anthers, green stigmas, greater degree of carpel connation, deciduous calyx, glabrous and fused styles, variation in number of stamen from 20 to (11-25), variation of carpels from 5 to 2-5 and relatively small, red to brown fruits.

With *Docyniopsis* they have in common white flowers, corymbose inflorescence and white anthers. It is clearly distinguished from *Docyniopsis* by the greater number of flowers per inflorescence, small number of stamen (11-25) against 39-61, carpel number of 2-5 in *Kansuenses* against 5 in *Docyniopsis*, sparse sclerids in *Kansuenses* versus abundant sclerids in fruit of *Docyniopsis*, relatively small fruit in *Kansuenses* versus large fruits of *Docyniopsis*.

With *Sieboldianae* and *Baccatae* it has in common carpel number about 5, high degree of carpel connation, deciduous calyx. With *Pumilae* and *Baccatae* it shares intermediate number of flowers per inflorescence.

With *Sieboldianeae* it shares conduplicate vernation, deciduous calyx, high degree of carpel connation (synapomorphy) which indicate that they may have evolved from a common ancestor.

Yunnanenses

In a consideration of the polarity of morphological characters, *Yunnanenses* is the most primitive taxon in the genus. Of 11 characters, 8 are plesiomorphic, 3 intermediate and none apomorphic. Although *Eriolobus* has 1 plesiomorphic character more than *Yunnanenses*, it also has 2 characters which are considered apomorphic. *Docyniopsis* has also the same number of plesiomorphic character but it has also 3 apomorphic characters.

Shape of the leaves, connation of carpels, and condition of calyx are considered intermediate in *Yunnanenses* .

In comparison with other series and sections, *Yunnanenses* have more plesiomorphic characters than series *Pumilae* and *Baccatae*. It has also a number of characters in common with the other sections of *Malus* including *Florentinae*, *Chloromeles, Docyniopsis* and *Eriolobus*. Common plesiomorphic characters to these taxa are carpel number of 5, conduplicate vernation of leaves and corymbose inflorescences.

The *Yunnanenses* differ from these other primitive taxa in some characters. For example from *Docyniopsis* in its relatively greater number of flowers per inflorescence, smaller petals, smaller number of stamens, glabrous styles, the smaller fruit size, cartilaginous and recurved calyx against erect and fleshy calyx and greater degree of carpel connation and adnation. They are also close to each other in having conduplicate vernation, lobulate to entire leaves with serrate margins, white petals in flowers and abundant number of sclerids in the flesh and core line.

With *Eriolobus* it has in common white petals, relatively small fruits, abundunt sclerids and recurved calyx while it differs in having lobulate or entire leaves against

trilobed leaves, short calyx against long calyx and a greater number of flowers per inflorescence.

The characters which *Yunnanenses* have in common with *Florentinae* are white petals in bud and flowers, stamen number about 20, carpel number of 5, nearly the same degree of carpel connation, glabrous styles and persistent or sometimes deciduous calyx. The *Yunnanenses* differ from *Florentinae* in large leaves, short clawed petal, and fruit with abundunt sclerids.

The characters in common with *Chloromeles* are stamen number of about 20 and 5 carpels. The characters which distinguish *Yunnanenses* from *Chloromeles* are white petals versus pink petals, white anthers versus pink or red anthers, green stigma versus red stigma, small fruits versus large fruits, yellow to red fruits versus yellow to green fruits, nearly high degree of carpel connation vesus very low degree of carpel connation and fruits with abundunt sclerids versus sclerids few or absent.

The distribution of this series in China and its primitive position, support the idea of Leppik (1970) about the origination of *Malus* in the western part of China.

Florentinae

In considering the polarity of morphological characters, of 11 characters, 7 are plesiomorphic, 4 intermediate and none apomorphic (Table 2.19).

Similarities between *Florentinae* and other presumably primitive taxa will be discussed later. With each taxon it has some characters in common; with *Chloromeles,* sparse or absence of sclerids in the flesh of the fruit; with *Docyniopsis* carpel number of 5; with *Eriolobus* lobed leaves, white petals, stamen number about 20 and, small fruit; with *Yunnanenses* small flowers, small and white petals, stamen number of 20, glabrous styles and relatively small fruit.

It is distinguished from all other species in the genus by petals without claws and clavate styles.

Section *Chloromeles*

In a consideration of the polarity of morphological characters; of 11 characters, 6 are plesiomorphic, 2 intermediate and 3 apomorphic (Table 2.19). Their large, pink petals and absence of or few sclerids are apomorphic characters. The lobed or entire leaves and small number of flowers per inflorescence (mean 5.4) are considered intermediate.

The *Chloromeles* have some characters in common with *Pumilae* and *Docyniopsis,* including high carpel number, persistent calyx, large amount of hypanthial tissue in fruit and large fruits. *Chloromeles* is similar to *Florentinae* in having similar stamen and style numbers per flower, free styles and few sclerids distributed in fruit.

Chloromeles are distinguished from the *Florentinae* and the rest of the genus by having pink or red anthers and stigma contrary to the yellow or white anthers and green stigmas of the reminder of the genus, large size of petals, green fruits versus red, orange and brown fruits of the rest of the genus.

Chloromeles is a very distinct taxon in the genus in its red or pink anthers and stigmas, green fruits, large fruits, large amount of flesh and sclerids sparse or absent.

Section *Docyniopsis*

In a consideration of the polarity of morphological characters *Docyniopsis* is one of the most primitive taxa of the genus. Of 11 characters examined 8 are plesiomorphic, none intermediate and 3 apomorphic (Table 2.19). Although *Docyniopsis* has the same number of plesiomorphic characters as *Yunnanenses* it has 2 more characters which are apomorphic. *Docyniopsis* has in common with other primitive taxa, conduplicate vernation, corymbose inflorescence and carpel number of 5.

Docyniopsis is closer to *Yunnanenses* than other sections. They have in common lobulate or entire leaves, conduplicate leaves, white petals, sclerids abundunt

in fruit. It is distinguished from *Yunnanenses* by larger petals, greater number of stamens, pubescent styles, small number of fruit per inflorescences, and larger fruits. In comparison to *Yunnanenses*, *Docyniopsis* have 2 apomorphic characters and no intermediate ones. It is therefore unlikely that *Docyniopsis* is derived from *Yunnanenses,* especially with having more plesiomorphic characters like low degree of carpel connation and adnation.

M. tschonoskii the only representative of section *Docyniopsis* available for this study, is very similar to *Docynia* in having lobulate leaves, 5 carpels and large number of stamens (30-50) (Rehder,1940) but is distinguished by 3-10 ovules per carpel (Rehder, 1940).

According to Phipps *et al.* (1991) evolutionary trends in flower characters of Maloideae are in the direction of fewer flowers per inflorescence and increase in size and stamen number. There is also a trend from the 2- ovulate carpel to a multiovulate condition. In *M. tschonoskii* increase in stamen number and decrease in number of flowers per inflorescence is not in favour with increase the number of ovule per carpel.

Docyniopsis and *Chloromeles* share pubescent and free styles, large fruit, large percentage ratio of flesh to diameter, 5 carpels, low degree of carpel connation and adnation and persistent calyx. *Docyniopsis* is distinguished from *Chloromeles* by white versus pink petals, white versus pink anthers, stamen number of 39-61 versus 20, red fruits versus green fruits, abundunt sclerids in fruits versus rare or absence of sclerids in fruits.

Docyniopsis also has some characters common with *Eriolobus* inluding white petals, corymbose inflorescence, carpel number of 5, presence of great number of sclerids and persistent calyx. They differ from each other in lobulate leaves in *M. tschonoskii* and deeply trilobed leaves in *Eriolobus*, and having recurved calyx versus erect calyx.

With *Florentinae*, *M. tschonoskii* shares, white petals and prescene of 5 carpels. *M. tschonoskii* differs from *Florentinae* in leaf lobing, lobulate or unlobed

versus lobed; clawed versus unclawed petals, stamen number per flower (39- 61 versus 19-20), pubescent styles versus glabrous to sligthly pubescent, sclerids abundant versus sparse or absent, degree of carpel connation less than 15% versus about 70% .

Although there is some affinity with all other primitive sections, this section is closest to *Yunnanenses* as discussed before.

Section *Eriolobus*

In a consideration of the polarity of morphological characters, *Eriolobus* is one of the primitive taxa in the genus. Of 11 characters 9 are considered plesiomorphic, none intermediate and 2 apomorphic.

Comparision of Section *Eriolobus* with *Chloromeles* and *Docyniopsis* have been discussed in their sections.

With *Florentinae*, *Eriolobus* has in common lobed leaves,white petals, intermediate number of flower per inflorescence, intermediate carpel connation, five carpels and small fruit. They both also occur in the mediterranean area. Therefore they may have been originated at the same time during the early radiation of *Maloideae.* Although these two taxa have very similar characters in common, *Eriolobus* is distinguished from *Florentinae* by pubescent styles and presence of sclerids.

2.5. Descriptions and synonomy

Descriptions of sections, series and species are based on results obtained from available cultivated living material in this study, the habit of the plants was derived from the literature.

The arrangement of the species is based on Rehder's classification (1940), and references given in { }.

Section *Malus*

Series *Pumilae* (Rehder) Rehder

Subsection *Pumilae* Rehder; series *Malus* (Phipps *et al.*, 1990)

Trees without thorns. Leaves rolled in the bud, unlobed on both kind of shoots. Inflorescence 2-8 flowered. Petals red or white in bud and white, pinkish white or red in flower, margin entire, apex rounded, base acute, acuminate or cuneate. Stamens 16-40 per flower; anthers yellow or pink. Styles (3-) 5, green and connate and pubescent at base. Fruit large, yellow to red, globose, depressed at both ends; calyx persistent; stone cells absent or sometimes few; degree of carpel connation ranges between 36.6 and 100%.

1. *M. pumila* Mill.

Described (1753) (Ponomarenko 1986)

M. pumila Miller {Cullen *et al.*, 1955}
Pyrus pumila (Miller) Tausch {Cullen *et al.*, 1955}
M. communis Poiret {Cullen *et al.*, 1955}
M. domestica Borkhausen {Cullen *et al.*, 1955}
M. pumila var. *paradisiaca* (Linnaeus) Schneider {Cullen *et al.*, 1955}
M sylvesteris var. *paradisiaca* (Linnaeus) Bailey {Cullen *et al.*, 1955}
M. dasyphylla Borkh. {Cullen *et al.*, 1955}
P. malus var. *pumila* (Miller) Henry {Cullen *et al.*, 1955}

Hybrids
M. adsteingens Rehder = *M. baccata* x *M. pumila* {Cullen *et al.*, 1955}
M. x *astracanica* dumont de Courset = *M. prunifolia* x *M. pumila* {Cullen *et al.*, 1955}
M. x *heterophylla* Spach {Cullen *et al.*, 1955}
P. malus Durand and Jackson {Cullen *et al.*, 1955}
M. x *purpurea* (Barbier) Rehder = *M. floribunda* var. *purpurea* Barbier = *Pyrus purpurea* of gardens = *M.* x *moerlandsii* Doorenbos = *M. purpurea* ' Lemoinei' x *M. sieboldii* {Cullen *et al.*, 1955}

Tree 5 to 15 m tall; young shoots tomentose, purple. Leaves broad elliptic or ovate, 4.5-10 x 3-5.5 cm, pubescent on both sides when young, then glabrous, slightly pubescent on veins of adaxial surface, acute at apex, obtuse at base, margin serrate. Flowers 5-7, in umbel; calyx pubescent sometimes glabrous. Petals 21-26 x 11-18 mm, white in flower, obovate, base acute, apex rounded sometimes emarginate, margin entire. Stamens (19)-20. Styles 5, pubescent at base. Fruits subglobose, impressed at base, 29-35 x 34-40 mm; core 1/2 of the fruit diameter; calyx persistent. Seed 6-7 x 2-4 mm.

2. *M. prunifolia* (Willd.) Borkh.

Described 1794 (Ponomarenko, 1986)

M. prunifolia (Willdenow) Borkhauden {Rehder, 1967}, {Cullen *et al.*, 1955}, {Bean, 1923}
Pyrus prunifolia Willdenow {Cullen *et al.*, 1955}, {Bean, 1923}, {Hooker, 1875}
M. prunifollia (Wild.) Spach. {Hooker, 1875}
M. hybrida Desf. {Hooker, 1875}

Varieties
M. prunifolia var. *rinki* Rehder {Rehder, 1967}, {Rehder, 1916}
M. prunifolia var. *rinki* (Koidzumi) Rehder{Cullen *et al.*, 1955}, {Bean, 1923}
M . pumila var. *rinki* Koidzumi{Cullen *et al.*, 1955}, {Bean, 1923}
M. ringo Siebold.{Cullen *et al.*, 1955}, {Bean, 1923}, {Rehder, 1916}
M. asiatica Nakai {Cullen *et al.*, 1955}, {Bean, 1923}, {Rehder, 1967}
Pyrus. ringo Wenzig{Cullen *et al.*, 1955}, {Bean, 1923}
Pyrus malus Siebold {Rehder, 1916}
P. praecox Miquel {Rehder, 1916}
P. Malus 'tomentosa' Maxim. {Rehder, 1916}
M. sinensis Wenzig {Rehder, 1916}
P. prunifolia Debeaux{Rehder, 1916}
M. microcarpa 'Ringo' Carriere{Rehder, 1916}
M. matsumurae Koidzumi{Rehder, 1916}
M. communis Lamk. var. *typica* Matsumura{Rehder, 1916}
M. yezoensis Koidzumi{Rehder, 1916}

Hybrids
M. p. x *pumila* {Rehder, 1967}
M. scheideckeri (spaeth) Zab. = *M. p.* x *floribunda* {Rehder, 1967}
M. robusta (Carrier) Rehder = *M. p.* x *M. baccata* {Rehder, 1967}
Pyrus microcarpa C. Koch. var. *robusta* Carriere
M. sublobata (Zab.) Rehd. = *M. p.* x *M. sieboldii* {Rehder, 1967}

A small tree to 3-8 m tall. Young shoots pubescent later glabrous. Petioles slender 1-5 cm long, pubescent at first then glabrous; leaf blade obovate or elliptic, 5-9 x 4-5 cm, apex short acuminate, base obtuse or rounded, margin sharply serrate, young leaves pubescent, finally glabrous; stipules filiform. Flowers 4-7, in umbels;

hypanthium, calyx and pedicels villous. Sepals slightly longer than hypanthium. Petals, 14-16 x 9-11 mm, white in flower, obovate or elliptic, apex rounded, base acuminate, margin entire, short clawed at base. Stamens (16-)20, anthers yellow. Styles (4)5, tomentose at base. Fruits yellow to red, subglobose, 2-3.5 cm in diameter, with a cavity at base; calyx persistent; the sepals connate at base into a short tube. Seed 4-5 x 2-3 mm.

M. prunifolia* var. *rinki (Koidz) Rehd.

Leaves pubescent beneath; flowers pinkish, calyx villose, pedicel usually shorter.

3. *M. spectabilis* (Ait.) Borkh.

Described 1789 (Ponomarenko, 1986)

***M. spectabilis* (Aiton) Borkhausen**
Pyrus spectabilis Aiton {Korban and Skirvin, 1984},{Rehder, 1916}, {Rehder, 1967)
M. sinensis (Dumont de Courset ex Jackson) Dumont de Courset{Rehder, 1916}
Pyrus sinensis Dumont de Courset ex Jackson {Rehder, 1916}
M. microcarpa var.*spectabilis* Carrier {Rehder, 1916}

Varieties
M. spectabilis riversii (Booth) Nash {Rehder, 1967}
Pyrus spectabilis var. *riversii* Booth
M. s. albi- plena Schelle {Rehder, 1967}

Hybrids
1. *M. s.* x *M. pumila* = *M. magdeburgensis* Schoch {Rehder, 1967}
2. *M. s.* x *M. baccata* = *M. micromalus* Mak. {Rehder, 1967}

Trees to 8 m tall. Young shoots pubescent, later glabrous, red-brown. Petiole 1-3 cm; leaf blade elliptic, 5-8 x 2-3 cm, apex acuminate, base cuneate, pubescent beneath, glabrous above, margin serrate. Flowers 2-5 in umbel; calyx, hypanthium and pedicel glabrous. Pedicels 2-3 cm. Calyx shorter than hypanthium. Petals 17-25 x 7-8 mm, pink or red in bud, pinkish white in flower, ovate, apex rounded, base cuneate, margin entire, short clawed at base. Stamens 28-40; anthers yellow. Styles pubescent at base, (4)5. Fruits yellow, subglobose, not impressed at apex, about 2 cm in diameter; calyx present; fruiting pedicel 3-3.5 cm, thickened at apex, subglabrous.

M. spectabilis has long been cultivated in North China but is not recorded there or elsewhere in the wild state.

The increase of the number of petals in this crab apple (double flowers) indicates that it is probably the product of long cultivation and selection.

4. *M. micromalus* Makino.

Described 1908 (Ponomarenko, 1986)

***M. micromalus* Makino**
M. spectabilis var. *kaido* Siebold {Rehder, 1916}
M. spectabilis, kaido Kirchner {Rehder, 1916}
M. microcarpa 'Kaido' Carriére {Rehder, 1916}
Pyrus kaido Mouillefert non *M. kaido* Dippel {Rehder, 1916}
M. spectabilis var. *micromalus* Koidzumi {Rehder, 1916}

Small tree, to 2.5- 5 m tall. Leaves long elliptic, 5-10 x 2.5-5 cm, pubescent at first, glabrous later, apex acute or acuminate, margin serrate, base cuneate or subrounded. Flowers 4-7 in umbel. Pedicel 2-3 cm, pubescent when young, glabrescent; hypanthium tomentose outside. Sepals as long as or slightly longer than hypanthium. Petals pink, suborbicular or long-elliptic, ca, 1.5 cm, short clawed at base. Stamens about 20. Styles 5, pubescent at base. Fruit red, subglobose, 1-1.5 cm in diameter, with cavity at base; calyx deciduous or a few persistent; fruiting pedicel 2-3 cm, subglobose. Seed 4-5 x 2-3 mm.

It has been suggested that it is a hybrid between *M. spectabilis* and *M. baccata.* This could be supported by occurrence of both kinds of calyx condition in a plant.

5. *M. sylvestris* (L) Mill.

Described 1753 (Ponomarenko 1986)

***M. sylvestris* (Linnaeus) Miller**
{Cullen *et al.*, 1955}, {Bean, 1923}, {Hillier, 1981}
Pyrus malus Linnaeus var. *sylvestris* Linnaeus {Cullen *et al.*, 1955}, {Bean, 1923}
M. acerba Merat {Bean, 1923}, {Hillier, 1981}, {De Candolle, 1825}
M. communis ssp. *sylvestris* (L.) Gams
M. sylvestris ssp. *mitis* (Wallroth) Mansfeld {Cullen *et al.*, 1955}
M. domestica Borkh. {Cullen *et al.*, 1955}

Tree to 8 m. Leaves broad ovate, elliptic to rounded, glabrous, slightly pubescent on veins, apex acuminate to mucronate, base obtuse or cuneate, margin serrate, convulate in bud. Flowers 4-6, in umbels. Calyx longer than hypanthium; calyx, hypanthium and pedicel almost glabrous, sometimes pubescent. Petals 12-18 x 8-10 mm, red in bud pinkish white in flower, apex rounded, base cuneate, margin entire. Stamens 20-23; anthers yellow. Styles 4-5, slightly pubescent at base. Fruits globose, greenish yellow, lenticilate, 23-27 x 28-30 mm; core diameter 1/2 of the fruit diameter, cavity at both ends; calyx present and recurved on the fruit. Seed 5-7 x 3-4.5 mm.

6 *M. niedzwetzkyana* (Hemsl.) Dieck.

Described 1891 (Ponomarenko, 1986)

***Malus niedzwetzkyana* (Hemsl.) Dieck.** {Hemsley, 1904}
Pyrus niedzwetzkyana Hemsl. {Hemsley, 1904}
Malus medwietzkyana Dieck. {Hemsley, 1904}
M. heterophylla Spach. {Cullen *et al.*, 1955}
M. pumila 'Niedzwetzkyana' (Dieck) Schneid. {Rehder, 1967}

Hybrids
M. x prupurea Barbier) Rehd.= M. niedzwetzkyana x M. x atrosanguinea
M. floribunda 'purpurea' Barbier

Young leaves and shoots slightly red or purple, pubescent, later slightly pubescent only on veins. Leaves elliptic-oblong, obovate or oblanceolate, apex acuminate, base obtuse, margin serrate. Flowers 3-7 in umbels; calyx, hypanthium and pedicels slightly pubescent and red. Petals 17-25 x 8-15 mm, red in bud, pink or red in flower, apex rounded sometimes emarginate, base acute, margin entire. Stamens (17)-21; anthers and filaments pink or red. Styles (4)5, pubescent at base. Fruits globose, red, about 32 x 30 mm; core less than 1/2 of the fruit diameter.

Series *Baccatae*

Leaves involute (rolled) in the bud, blades unlobed on both short and long shoots. Inflorescence umbellate, of 4-9 flowers. Petals pink to pinkish white in the bud and pinkish white or white in flower, truncate, subcordate or cordate at base,

margin crenate or entire, apex rounded. Stamens 14-31 per flower, anthers yellow. Syles (3)4-5 per flower, connate at the base, green. Fruit small, globose sometimes obovate, red, without depression at the ends; calyx deciduous. Fruit without or with few stone cells, degree of carpel connation high (91-100%).

7. *M. baccata* (L.) Borkh.

Described 1769 (Ponomarenko, 1986)

M. *baccata* (Linnaeus) Borkhausen {Cullen *et al.*, 1955},{Rehder, 1967}
P. baccata L. {Rehder, 1916}
M. baccata Linnaeus {Rehder, 1916}, {Korban *et al.*, 1984}
M. sibirica Borkhausen {Cullen *et al.*, 1955}
M. baccata (L.) Desf. {Hooker, 1874}
P. baccata var. *leiostyla* Ruprecht and Maximowicz {Rehder, 1916}
P. baccata a *sibirica* Maximowicz {Rehder, 1916}
M. baccata var. *sibirica* (Maxim) Schneider {Rehder, 1916}
M. microcapa baccata Carriere {Rehder, 1916}
M. baccata a sibirica Schneider {Rehder, 1916}

Varieties
1. *M. baccata* var. *mandshurica* (Maximowicz) Schneider {Cullen *et al.*, 1955}, {Rehder, 1967}
Pyrus baccata Linnaeus var. *mandshurica* Maximowicz {Cullen *et al.*, 1955}
M. baccata Desf. var. *mandshurica* Schneider, 1906. Matsumura, 1912. Koidzumi, 1913. {Rehder, 1916}
M . cerasifera Spach {Rehder, 1916}, {Rehder, 1967}
Pyrus spectabilis A. Gray {Rehder, 1916}
P. prunifolia Maximowicz {Rehder, 1916}
M. baccata var. *mandshurica*, f. *latifolia* Matsumura {Rehder, 1916}, {Rehder, 1967}
Pyrus cerasifera Tausch {Rehder, 1967}
2. *M. baccata* var. *himalaica* (Maxim.) Schneider
Pyrus baccata var. *himalaica* Maxim.{Rehder, 1916}, {Rehder, 1967}
M. baccata Hemsley {Rehder, 1916}
Pyrus spectabilis Hemsley {Rehder, 1916}
3. *M. baccata* f. *jackii* Rehder {Rehder, 1916}
4. *M. baccata gracilis* Rehd. {Rehder, 1967}
5. *M. baccata* var. *columnaris* Rehd. {Rehder, 1967}

Hybrids
1. *M.* x *hartwigii* Koehne = *M. baccata* x *M. halliana* {Cullen *et* al., 1955}, {Hillier, 1981}
2. *M. arnoldiana* Sarg. = *M. baccata* x *M. floribunda* {Hillier, 1981}
3. *M. robusta* (Carr.) Rehd. = *M. baccata* x *M. prunifolia* {Cullen *et al.*, 1955}
Pyrus cerasifera Wenzig not Tausch {Rehder, 1967}
M. robusta erecta Rehd. f. {Rehder, 1967}
4. *M. r.* var. *persicifolia* Rehd.{Rehder, 1967}
5. *M. adstringens* Zabel. = *M. baccata* x *M. pumila* {Rehder, 1967}

Tree to 10-14 m tall. Shoots red-brown, slender, slightly curved or pendulous, glabrous. Leaves elliptic, ovate or ovate-oblong, apex acuminate, base acute or obtuse, margin finely serrate, especially at the apex, and toward the base coarsely

serrate, young leaves tomentose, very soon glabrous except on veins, Flowers 5-9 in umbels, calyx lobes, hypanthium and pedicels glabrous; pedicels slender. Petals 13-18 x 11-16 mm, pink in bud, pinkish white in flower, obovate, apex rounded, base cordate, margin entire, short clawed at base. Stamens 18-25; anthers yellow. Styles 3-5, pubescent. Fruits 8-20 x 8-26 mm, red or yellow; calyx deciduous; core 1/2 of the fruit diameter or more. Seed 4-6 x 2-4 mm.

8. *M. hupehensis* (Pamp.) Rehd.
Described 1910 (Ponomarenko, 1986)

***M. hupehensis* (Pampanini) Rehder** {Rehder, 1967}, {Cullen *et al.*, 1955}, {Bean, 1923}
Pyrus hupehensis Pampan {Rehder, 1967}, {Bean, 1923}
M. hupehensis Rehder {Rehder, 1967}
Pyrus theifera Bailey {Rehder, 1967}
M. theifera (Bailley) Rehd. {Rehder, 1967}
P. hupehensis f. *rosea* Redh. {Sealy, 1875}
Pyrus baccata L. sec. Hemsel. {Sealy, 1875}
P. spectabilis Ait. sec. Hemsel. {Sealy, 1875}
P. theifera (Rehder) Bailey {Sealy, 1875}

Tree to 8 m tall. Young shoots slightly pubescent, very soon glabrous. Leaves pubescent on both sides when young, later glabrous, slightly pubescent above, ovate or ovate-elliptic, apex acuminate, margin fine serrate, cuneate or obtuse at base, unlobed. Stipules on young leaves bract like, later filiform. Flowers 4-7 in umbel; calyx lobes hypanthium and pedicel slightly pubescent or glabrous. Calyx purple. Petals, 12-22 x 12-14 mm, pink in bud, white in flower, rounded at apex, truncate or acute at base, margin crenulate, short clawed. Stamens 24-27; anthers yellow. Styles 3(-5), pubescent. Fruits globose, yellow green tinged with red, elliptic or subglobose, 8-18 x 9-18 mm; calyx deciduous; fruiting pedicels 3-6 cm, glabrous. Seeds 2-7 x 1-3 mm.

9. *M. halliana* (Voss)Koehne
Described 1890 (Ponomarenko, 1986)

***M. halliana* (Voss) Koehne** {Hillier, 1981}, {Rehder, 1916}
Pyrus halliana Voss. {Rehder, 1916}
Pyrus malus parkmanii Temple {Rehder, 1916}

Pyrus parkmanii Hort. ex Sargent {Rehder, 1916}
Pyrus halliana Hort. ex Sargent {Rehder, 1916}
M. halliana var. *spontanea* (Makino) Koidzumi {Cullen *et al.*, 1955}, {Bean, 1923}
M. floribunda var. *spontanea* Makino {Cullen *et al.*, 1955}, {Bean, 1923}
M. halliana Parkmanii Rehd. {Bean, 1923}
M. parkmanii Rehd.

Hybrids
M. x*atrosanguinea* (Spath) Schneider = *M. halliana* x *M. sieboldii* {Cullen *et al.*, 1955}, {Bean, 1923}
P. atrosanguinea Spath {Cullen *et al.*, 1955}

Tree to 5 m. Young shoots soon glabrous and purple. Leaves leathery, ovate, elliptic, apex acuminate to acute, base cuneate or obtuse, margin finely crenate-serrate, glabrous. Pedicels purple. Flowers in corymbs; calyx lobes, hypanthium and pedicels glabrous. Petals white in flower, apex rounded, base truncate, margin crenate. Stamens 16-20; anthers yellow. Styles 3-4(5); pubescent at base. Fruits 7-11 x 6-11 mm, obovoid, purplish; calyx deciduous; core 1/2-3/4 fruit diameter. Seed 4-5 x 2-3 mm.

10. *M. sikkimensis* (Wenzig) Schneid.

Described 1890 (Ponomarenko, 1986)

***M. sikkimensis* (Wenzig) Schneider** {Cullen *et al.*, 1955}
M. pashia Wenzig var. *sikkimensis* Wenzig {Cullen *et al.*, 1955}, {Bean, 1923}
Pyrus baccata indica Hort.{Hooker, 1995a}

Trees small, 6-8 m tall. Young shoots tomentose, later slightly pubescent. Leaves ovate to elliptic ovate, apex acuminate to acute, base rounded or broad cuneate, margin serrate, pubescent on both side. Pedicels tomentose, later subglabrous, 1.5-5 cm. Flowers 5-8 in corymb; calyx lobes longer than hypanthium; calyx lobes, hypanthium and pedicel tomentose, later subglabrous. Petals 10-15 x 9-14 mm, pink in bud, white in flower, apex rounded sometimes emarginate, base subcordate sometimes truncate, margin entire, short clawed at base. Stamens 20-31; anthers yellow sometimes pink. Styles 4-5, pubescent or slightly pubescent. Fruits obovate, red, lenticillate, 11-16 x 11-16 mm; calyx deciduous, rarely persistent. Seed 4-6 x 2-4 mm.

11. *M. rockii* Rehd.

Described 1933 (Ponomarenko, 1986)

Trees 8-10 m tall. Leaves elliptic ovate or oblanceolate, acuminate at apex, cuneate to rounded at base, slightly pubescent on both sides. Pedicels 2-4 cm, pubescent. Flowers 5-7 in corymbs or umbels; calyx, hypanthium and pedicels pubescent. Sepals slightly longer than or as long as hypanthium. Petals white, obovate, short clawed at base. Stamens 14-20; anthers yellow. Styles 4, pubescent at base. Fruits subglobose or ovoid, red, 10-15 mm in diameter, red, lenticillate; calyx deciduous; fruiting pedicel 2-4 cm, pubescent. Seed 4.5-6 x 2-3 mm.

Section *Sorbomalus*
Series *Sieboldianae*

Leaves conduplicate (folded), lobed on long shoots and lobed or unlobed on short shoots. Inflorescence umbellate, with 2-9 flowers. Petals red to pink in bud and pinkish white to white in flower, margin entire, base truncate, apex rounded. Stamens 15-24 per flower; anthers yellow. Styles (3)4-5 per flower, green, connate, pubescent at base. Fruit small, red, without depressions at the ends; calyx deciduous; stone cells very few; degree of carpel connation high, 73-100%.

12. *M. floribunda* Van Houtte

Described 1865 (Ponomarenko, 1986)

***M. floribunda* Van Houtte** {Cullen *et al.*, 1955}, {Hillier, 1981}
Pyrus floribunda Kirchner not Lindley {Cullen *et al.*, 1955}
M. pulcherrima (Ascherson and Graebner) K. R. Boynton {Cullen *et al.*, 1955}

Tree or shrub up to 10 m. Young leaves and branches tomentose very soon glabrous or slightly pubescent. Leaves elliptic ovate or obovate, apex acute or acuminate, serrate, cuneate at base. Flowers 2-9 in umbels; calyx lobes, hypanthium and pedicels glabrous. Sepals as long as hypanthium. Petals pink or red in bud white or pinkish white in flower. Stamens 16-20, anthers yellow. Styles (3)4(5), pubescent

at base. Fruits globose, red, 10-12 x 8-11 mm; core 1/2-3/4 of the fruit diameter. Seed 3 x 2 mm.

This large shrub which has been known in cultivation for nearly a century has not been found wild. It is probably a hybrid rather than a valid species. It is considered to be a hybrid from *M. sieboldii* and perhaps *M. baccata* or *M. prunifolia.* Unlobed leaves of long and short shoots indicate the affinity with the *M. baccata* or *M. prunifoila* and the occurrence of occasionally lobed leaves on short shoots indicate affinity with *M. sieboldii.*

13. *M. zumi* (Mats.) Rehd.
Described 1899 (Ponomarenko, 1986)

***M. zumi* (Mats.) Rehder**
Pyrus zumi Mats.
Pyrus toringo 'γ' *integrifolia* Franchet and Savatier{Rehder, 1916}
M. toringo, α *integrifolia* Zabel apud Dippel{Rehder, 1916}

Young leaves pubescent on both sides, soon glabrous. Leaves elliptic oblong, acuminate to acute at apex, cuneate at base, margin serrate, long shoot leaves lobed, leaves on short shoots unlobed or rarley lobed. Flowers 2-7 in umbel; calyx lobes, hypanthium and pedicels glabrous or slightly villous. Petals pink in bud, becoming white in flower, elliptic. Styles 3-4(5), pubescent at base. Fruits globose red, 5-7 mm in diameter; Calyx deciduous .Seed 2 x 3 mm.

14. *M. sieboldii* (Regel) Rehder
Described 1856 (Ponomarenko, 1986)

***M. sieboldii* (Regel) Rehder.**{Rehder, 1916}, {Bean, 1923}, {Rehder, 1923}, {Cullen *et al.*, 1955}
Pyrus sieboldii Regel. {Rehder, 1916}, {Cullen *et al.*, 1955}, {Bean, 1923}, {Rehder, 1923}
P. toringo Siebold {Rehder, 1916}
M. toringo (Sieb.) Nakai {Rehder, 1967}
M. microcarpa toringo (Nakai) Carriere. {Rehder, 1916}
M. baccata ssp. *toringo* (Nakai) Koidzumi. {Rehder, 1916}
Pyrus. rivularia Gray {Rehder, 1916}
P. mengo Siebold ex Koch. {Rehder, 1916}
Sorbus toringo (Sieb) Koch. {Rehder, 1916}

Varieties
1. *M. sieboldii* var. *sargentii* Rehder {Cullen *et al.*, 1955}
2. *M. sieboldii* var. *arborescens* Rehder
Pyrus baccata Thunberg (1784) {Rehder, 1916}
Crataegus alnifolia Regel. {Rehder, 1916}
Pyrus toringo γ typica Franchet and Savatier. {Rehder, 1916}
M. toringo, f. typica Matsumura. {Rehder, 1916}
M. sieboldii var. *calocarpa* {Rehder, 1916}

Hybrids
M. sieboldii x *M. prunifolia* = *M. sublobata* (Dippel) Rehder = *M. ringo* forma *sublobata* Kipple {Cullen *et al.*, 1955}, {Bean, 1923}
M. sieboldii x *M. baccata* var. *mandshurica* = *M. zumi* (Matsumura) Rehder
Pyrus zumi Matsumura
M. sieboldii x *M. halliana* = *M. atrosanguinea* (Spath) Schneid. {Rehder, 1916}

Shrubs 2-6 m tall. Young shoots pubescent, later glabrous. Leaves lobed on long shoots, 3-lobed, the middle lobe long with one small lobe on each side, the two lower lobes never further lobed, elliptic, young leaves pubescent later glabrous, apex acuminate, rounded or broadly cuneate at base, margin serrate; leaves on short shoots unlobed or lobed, glabrous above and slightly pubescent below on veins. Flowers 3-7 in umbel. Sepals and hypanthium, glabrous. Petals pink in bud, white in flower, apex rounded, base truncate, margin entire, short clawed at base. Stamens 18-24; anthers yellow. Styles 3-4(5), pubescent at base. Fruits globose, red or yellow brown, 5-8 x 5-8 mm; calyx deciduous; core 1/2-3/4. Seed 3-4 x 1.5-2.5 mm.

15. *M. sargentii* Rehd.

Described 1903 (Ponomarenko, 1986)

***M. sargentii* Rehder {Rehder, 1967}**
M. sieboldii var. *sargentii* (Rehd.) Asami {Rehder, 1967}
Pyrus sargentii (Rehd.) Bean {Rehder, 1967}
M. sargentii (Rehd.) Bean {Rehder, 1967}
variety: *M. sieboldii* var. *toringo* f. *sargentii* Koidz. {Rehder, 1967}, {Hillier, 1981}

Low shrub to 2 m. Young shoots tomentose. Leaves on short shoots small and ovate, long shoot leaves ellipic to broad ovate, apex acute or acuminate, base subcordate to cuneate, margin serrulate and entire at base, mostly 3-lobed, pubescent on both sides, 6.6-7.5 x 3.5-5 cm. Petioles 2-3 cm, pubescent. Pedicels glabrous or sparsely hairy, up to 4 cm. Flowers 6-7 in umbel, hypanthium slightly pubescent.

Calyx glabrous. Petals pink in bud, white in flower, ovate, short clawed at base. Styles (3)4(5), pubescent. Stamens 18-24; anthers yellow. Fruit obovate to globose, 7-10 x 7-10 mm; fruiting pedicel glabrous; calyx deciduous; core 1/2-3/4 of fruit diameter. Seed 2-4 x 1-2 mm.

Series *Florentinae*

16. *M. florentina* (Zucc.) Scneid.

Described 1809 (Ponomarenko, 1986)

***M. florentina* (Zuccagni) Schneider** {Bean, 1923}, {Rehder, 1967}
Crataegus florentina Zuccagni{Cullen *et al.*, 1955}, {Bean, 1923}
Pyrus crataegifolia Savi. {Cullen *et al.*, 1955}, {Bean, 1923}, {Hooker,1895b}
Eriolobus florentina (Zuccagni) Stapf
Pyrus florentina (Zucc.) Targ. {Rehder, 1967}, {Browicz, 1970}
M. florentina = *M. sylvestris* x *Sorbus torminalis* = *Malus pumila* x *Sorbus torminalis* {Browicz, 1970}

Small tree, with rounded habit; without thorns. Leaves conduplicate (folded) in bud, hawthorn-like, lobed on both kinds of shoots, broad-ovate, apex acute, base truncate, obtuse or cordate, margin serrate, lobed, tomentose beneath, 4.9-6.7 x 4.3-6 cm, folded in bud. Petioles 3-4.6 cm. Flowers 4-7 in corymb; calyx lobes, hypanthium and pedicels tomentose. Pedicels slender and long, 2-3 cm. Petals white in bud and flower, clawless. Stamens (19)20; anthers yellow. Styles (4)5. Fruit 13-19 x 8-15 mm, ovate to rounded, yellowish orange changing to red; calyx usually persistent, sometimes deciduous; core 1/2-3/4 fruit diameter; stone cells absent or few; degree of carpel connation high, about 73.% . Seed 3-4 x 2-3 mm (Plate 25).

The idea of the hybrid origin of this crab apple from *M. sylvestris* and *Sorbus torminalis* has been proposed by Browicz (1970). Although its affinity with *Sorbus torminalis* is supported by similarity in leaf shape (Plate 27), comparision of flavonoids in the two species (chapter 5 this thesis) do not support this affinity. Clawless petals are observed only in this species within the genus, a distinctive pattern of peroxidase isoenzyme and flavonoids reveal this crab apple as a species.

Series *Kansuenses*

Leaves conduplicate (folded) in the bud, lobed on both shoot types but sometimes unlobed on short shoots. Inflorescence 3-10 flowered. Petals pinkish white or white in bud and white to yellowish white in flower, margin entire or crenulate, apex emarginate, dentate or rounded, base cordate or truncate. Stamens 11-25; anthers yellow or creamy white. Styles (2) 3-4 (5), green. Fruit brown to red, obovate or oblong,with slight depressions at both ends; calyx deciduous; degree of carpel connation high (68%- 100%). Stone cells few and not present in ovarian tissues.

Based on morphological characteristics it is possible to distinguish two subseries in the series *Kansuenses:*

a. Subseries *Kansuenses*; number of flowers per inflorescence more than 7, petal base cordate or semicordate, petal apex emarginate or rounded, including *M. kansuensis* and *M. fusca.*

b. Subseries *Transitoriae*; number of flowers per inflorescence less than 6, petal base truncate, petal apex dentate, including *M. toringoides* and *M. transitoria.*

17. *M. fusca* (Rafin.) Schneid.

Described 1830 (Ponomarenko, 1986)

***M. fusca* (Rafinesque) Schneider**
Pyrus fusca Rafinesque {Hillier, 1981}
Pyrus rivularis Dougl. {Rehder, 1967}
Pyrus. diversifolia Bong.{Hillier, 1981}, {Rehder, 1967}

Variety
M. fusca Levipes (Nutt.) Schneid.{Rehder, 1967}

Hybrid
M. fusca x *pumila* = *M. Dawsoniana* Rehd. {Rehder, 1967}

Shrub or tree up to 12 m tall. Young branches pubescent. Young leaves pubescent on both side, very soon glabrous above, ovate to lanceolate, apex acuminate, margin doubly serrate, base cuneate, obtuse to slightly cordate, sometimes lobed. Stipules bract like. Flowers 7-10 in corymbs. Petals pink in bud, white in

flower, apex rounded, base semicordate, margin entire and wavy. Stamens 18-20; anthers yellow. Styles (3)4-5, glabrous. Fruits elliptic to ovate, slightly pubescent, red or yellow, depressed at base, 11-13 x 9-12 mm; fruiting pedicel pubescent. Seed 4-6 x 1-2 mm.

18. *M. toringoides* (Rehd.) Hughes
Described 1919 (Ponomarenko, 1986)

***M. toringoides* (Rehder) Hughes**
M. transitoria var. *toringoides* Rehder {Stapf, 1922}
Pyrus transitoria var. *toringoides* (Rehder) Bailey {Stapf, 1922}
P. toringoides (Rehder) Osborne {Koidzumi, 1934}
Sinomalus toringoides (Rehder) Koidzumi {Koidzumi, 1934}

Tree up to 3-6 m. Shoots pubescent when young, later glabrous. Leaves 5-8 x 2.5-3 cm, lobed or sometimes unlobed, lobed leaves usually with 2 lobes on each side, elliptic-oblong, apex acute or attenuate, base cuneate, margin doubly serrate sometimes crenate, young leaves pubescent, very soon glabrous, except on veins. Flowers 4-7 in corymb; calyx lobes, hypanthium and pedicel pubescent. Petals 10-12 x 8-9 mm, pinkish white in bud white in flower, apex emarginate, base cordate, margin crenulate. Stamens (19)20(25), anthers yellow,or creamy white. Styles 3-4 (5), usually glabrous sometimes pubescent. Fruits elliptic to obovate yellow tinged with red, 10-19 x 9-14 mm, lenticillate; calyx deciduous; core 1/3-1/2 fruit diameter. Seed 3-5 x 2-3 mm.

19. *M. kansuensis* (Batal.) Schneid.
Described 1893 (Ponomarenko, 1986)

***M. kansuensis* (Batalin) Schneider** {Hillier, 1981}
Pyrus kansuensis Batalin {Rehder, 1967}
Eriolobus kansuensis (Batalin) Schneid. {Bean, 1923}, {Rehder, 1967}, {Rehder, 1916}
Variety
M. kansuensis var. *calva* Rehder {Bean, 1923}, {Rehder, 1967}

Shrub or small tree to 8m tall. Leaves ovate, 4-8.6 x 3.2-7.5 cm, apex acuminate, base truncate or obtuse, 3 or 5-lobed in upper half, margin doubly serrate,

pubescent beneath at least on the veins. Petioles 1.3- 3.7 cm. Flowers 3-10 in corymb; calyx, hypanthium and pedicels glabrous. Petals 6-10 x 6-10 mm, white or pink in bud white in flower, apex emarginate or rounded, base cordate, margin crenulate. Stamens 20(22). Styles 2-4(5), glabrous. Fruit 9-11 x 9-12 mm, yellowish red, elliptic oblong, or obovoid; calyx deciduous; core 1/2 fruit diameter. Seeds 4-6 x 2-3 mm (Plate 26).

20. *M. transitoria* (Batal.) Schneid.
Described 1893 (Ponomarenko, 1986)

***M. transitoria* (Batalin) Schneider** {Cullen *et al.*, 1955}, {Rehder, 1916}
Pyrus transitoria Batalin
Sinomalus transitoria (Batalin) Koidzumi {Cullen *et al.*, 1955}

Variety
M. transitoria var. *toringoides* Rehder {Rehder, 1916}

Young branches and leaves tomentose, very soon glabrous, leaves deeply lobed small, acuminate, serrate, with two lobes on each side, 3.5-7.3 x 2.8-5.2 cm. Stipules bracte like. Petioles 1.9-3.5 cm. Flowers 4-7 in corymb; calyx lobes, hypanthium and pedicle tomentose, calyx lobes shorter than hypanthium. Pedicel 1.5-2.5 cm. Petals 6-8 x 4-6 mm, yellowish white or white in flower, apex dentate, base truncate, margin entire. Stamens (17)20(24). Styles 3-4(5), glabrous;. Fruits 9-14 x 10-13 mm; calyx deciduous; core less than 3/4 fruit diameter. Seed 3-4 x 2-3 mm.

Series *Yunnanenses*

Leaves conduplicate or involute, slightly lobed or unlobed. 6-14 flowered. Petals white in bud and in flower, margin entire, base cordate or truncate, base rounded. Stamen about 20. Style 5(4), green. Fruit globose, green or yellow green; stone cells present and abundant; degree of carpel connation around 68%.

21. *M. prattii* (Hemsel) Schneid.

Described 1895 (Ponomarenko, 1986)

M. prattii (Hemsley) Schneider
{Rehder, 1967}, {Cullen *et al.*, 1955}, {Hillier, 1981}, {Rehder, 1916}, {Bean, 1923}
Pyrus prattii Hemsley {Rehder, 1967}, {Cullen *et al.*, 1955}, {Bean, 1923}
Docyniopsis prattii (Hemsley) Koidzumi {Cullen *et al.*, 1955}

Tree to 10 m tall. Leaves 6.7-12 x 4.7-7.5 cm, broad elliptic to ovate, unlobed, apex short acuminate, base cordate or obtuse, sometimes cuneate, serrate or double serrate, young leaves tomentose, soon glabrous or slightly pubescent, petioles 2.5-4 cm. Flowers 12-16 in corymb; Petals white in bud and flower, apex rounded, base cordate or truncate, margin entire. Stamens (19)20(21). Styles (4)5, glabrous. Fruits subglobose, ribbed, lenticillate, pubescent, yellow or red; calyx persistent. Seeds 5-6 x 3-5 mm.

22. *M. yunnanensis* (Franch.) Schneid.

Described 1890 (Ponomarenko, 1986)

***M. yunannensis* (Franchet) Schneider** {Cullen *et al.*, 1955}, {Hillier, 1981}, {Rehder, 1916}
Pyrus yunannensis Franchet {Cullen *et al.*, 1955}, {Rehder, 1916}
Eriolobus yunnanensis (Franchet) Schneider {Rehder, 1916}
Cormus yunnanensis (Franchet) Koidzumi {Rehder, 1916}
Pyrus veitchii Hort.{Rehder, 1967}, {Rehder, 1916}

Variety
M. y. var. *veitchii* Rehder {Cullen *et al.*, 1955}, {Bean, 1923}

Tree to 10 m tall. Young shoots tomentose. Leaves ovate to oblong-ovate, apex short acuminate, base rounded or subcordate, sharply and doubly serrate, usually with 3-5 pairs of broad short lobes, 7.2-13 x 4.6-9.9 cm, tomentose beneath. Petioles 2.2-8.5 cm, tomentose. Pedicels 1-2 cm long. Flowers 6-14 in corymbs; calyx lobes, hypanthium and pedicels tomentose. Calyx lobes as long as hypanthium. Petals white, suborbicular, about 8 mm long, short clawed at base. Stamens 20-25. Syles 5, glabrous at base. Fruits subglobose, red, 8-14 x 8-19 mm, white lenticillate; calyx

reflexed and persistent sometimes deciduous (Huckins, 1972); core more than 3/4 fruit diameter. Seeds 4-5 x 2-4 mm.

Section *Chloromeles*

Trees with thorns. Bark scaly on main trunk. Leaves conduplicate in the bud, lobed on the long shoots, lobed or unlobed on short shoots. Inflorescence corymbose, 4-8 flowered. Petals pink in bud, gradually tending to white or pinkish white in flower, large and broad, with entire or crenulate margins and truncate, acuminate or cordate bases and rounded apex. Stamens 18-22 per flower: anthers red. Styles (4) 5 (6) per flower, red, pubescent, free. Fruits medium to large, globose, depressed at both ends, green, calyx persistent. fruits without or with many stone cells along the core line; degree of carpel connation low 2-15%.

Characters which distinguish this section from others are; pink flowers, red anthers and stigmas, very low degree of carpel connation and carpel -hypanthium adnation, free styles, large green fruit and absence of sclerids in flesh.

23. *M. platycarpa* Rehd.

Described 1913 (Ponomarenko, 1986)

Tree to 6 m tall; young branches tomentose, becoming glabrous. Leaves ovate to elliptic 6.6-11.5 x 4.4-8 cm, apex rounded with short acute point, base rounded, sharply and doubly serrate, long shoot leaves lobed, pubescent beneath; petioles 2-3.3 cm. Flowers in corymbs, calyx lobes, hypanthium and pedicel glabrous. Petals white in bud, pink in flower, apex rounded, base cuneate, margin crenate. Stamens 20. Styles 5, pubescent at base. Fruits yellowish green, depressed at both ends, calyx persistent, 28-37 mm, core less than 1/2 fruit diameter. Seeds 8 x 5-6 mm.

24. *M. glaucescens* Rehd.

Described 1911 (Ponomarenko, 1986)

***M. glaucescens* Rehder**
P. glaucescens (Rehder) L. H. Bailey {Cullen *et al.*, 1955}, {Hillier, 1981}

Shrub or small tree. Branches sometimes spiny; Leaves broadly ovate or triangular-ovate, apex acute or short acuminate, base truncate, obtuse or rounded, young leaves lobed, leaves on flowering shoots lobed or unlobed, glabrous, margin serrate. Flowers 4-6 in corymbs; calyx, pedicel and hypanthium glabrous. Calyx lobes longer than hypanthium. Petals 20-21 x 9-16 mm, pink in bud and flower, gradually becoming white, apex rounded, base cuneate, margin crenate. Stamens 20; anthers red. Styles 5, pubescent or slightly pubescent, red. Fruits subglobose, slightly depressed above, yellowish green, 19-24 x 12-24 mm; calyx persistent; core between 1/3 to 1/2 fruit diameter. Seed 5-6 x 3-4 mm.

25. *M. coronaria* (L.) Mill.

Described 1753 (Ponomarenko, 1986)

***M. coronaria* (Linnaeus) Miller** {Cullen *et al.*, 1955}, {Hillier, 1981}, {Bean, 1923}
P. coronaria Linnaeus {Cullen *et al.*, 1955}
M. bracteata Rehder {Cullen *et al.*, 1955}
M. fragrans Rehder {Rehder, 1967}

Varieties
M. coronaria var. *dasycalyx* Rehder {Cullen *et al.*, 1955}, {Bean, 1923}
M. coronaria var. *lancifolia* (Rehder) Fernald {Cullen *et al.*, 1955}
Pyrus lancifolia (Rehder) Bailey {Cullen *et al.*, 1955}
M. coronaria 'Charlottae' Rehder f. {Cullen *et al.*, 1955}, {Bean, 1923} {Hillier, 1981}
M. coronaria var. *elongata* Rehder {Rehder, 1967}

Hybrid
M. coronaria x *pumila* = *M. hetrophylla* Spach {Rehder, 1967}

Tree to 10 m. Young shoots tomentose later glabrous. Leaves ovate to ovate-oblong, 7-11 x 4-11.1 cm, apex, acuminate, cordate at base, margin serrate or doubly serrate, slightly lobed, pubescent on both sides when young, later glabrous, dark green above, ligth green beneath. Petioles 1-3.2 cm. Flowers 4-6 in corymbs. Petals 13-21 x 9-16 mm, pink in bud and flower gradually becoming white, apex rounded,

base cuneate, margin entire. Stamens 20; anthers red. Styles 5, pubescent; at base. Fruits green, globose, 18-30 x 21-31 mm, white lenticillate, depressed at both ends; calyx persistent; core less than 1/2 fruit diameter. Seeds 5-7 x 3-5 mm.

26. *M. lancifolia* Rehd.

Described 1911 (Ponomarenko, 1986)

***M. lancifolia* Rehder**
Pyrus lancifolia (Rehd.) Bailey {Rehder, 1967}, {Van Eseltine, 1933a}

Tree to 8 m, branches often spiny , young shoots slightly pubescent or nearly glabrous. Young leaves on long shoots pubescent later glabrous, ovate, serrate and slightly lobed, short shoot leaves elliptic-ovate to lanceolate, margin serrate. Flowers 5-8 in corymbs; calyx lobes, hypanthium and pedicel glabrous. Calyx lobes longer than hypanthium. Petals 13-16 x 7-10 mm, apex rounded, base cuneate, margin entire. Stamens 20; anthers red. Styles 5, red, pubescent at base. Fruit subglobose, about 2.5 cm diameter, green.

27. *M. angustifolia* (Ait.) Michx.

Described 1789 (Ponomarenko, 1986)

***M. angustifolia* (Aiton) Michaux**
Pyrus angustifolia Aiton {Cullen *et al.*, 1955}, {Bean, 1923}, {Rehder, 1967}
M. coronaria Brit. not Mill. {Rehder, 1967}
M. sempervirens Desf. {Rehder, 1967}

Variety
M. angustifolia pendula Rehder, f.

Shrub or tree to 10 m. Shoots glabrous or slightly pubescent when young. Leaves ovate-oblong, oblong or lanceolate, glabrous, 5.9-11.5 x 4.4-8 cm acuminate or sometimes obtuse at apex, rounded or subcordate at base, margin serrate or sometimes entire, long shoot leaves broader, coarsely serrate and often lobed, slightly tomentose below. Petioles 1.5-4 cm. Flowers 4-7 in corymb; calyx, hypanthium and pedicels glabrous. Calyx shorter or equal to hypanthium. Petals 16-19 x 11-16 mm, pink in bud and flower gradually becoming white, apex rounded, base cuneate,

margin crenate. Stamens (18-)20(-22); anthers red. Styles 5(6), red. Fruits, green, depressed at apex. 22-28 x 22-28 mm, white lenticillate; core about 1/2 fruit diameter. Seeds 5-9 x 3-5 mm.

28. *M. ioensis* (Wood) Brii.

Described 1860 (Ponomarenko, 1986)

***M. ioensis* (Wood) Critton**
Pyrus coronaria var. *ioensis* Wood {Cullen *et al.*, 1955}, {Bean, 1923}
P. ioensis (Wood) Bailey {Cullen *et al.*, 1955}, {Bean, 1923}
M. coronaria var. *ioensis* Schneid. {Cullen *et al.*, 1955}, {Bean, 1923}
Varieties
M. i. plena (Schneid) Rehd. {Cullen *et al.*, 1955}
Pyrus angustifolia f. plena Hort. {Cullen *et al.*, 1955}
P. angusifolia 'Bechtellii' Rehd. Hort. {Cullen *et al.*, 1955}
M. i. fimbriata Salvin, f. {Cullen *et al.*, 1955}
M. ioensis palmeri Rehder var. {Cullen *et al.*, 1955}
M. i. var. *texana* Rehder {Cullen *et al.*, 1955}
M. i. var. *bushii* Rehder {Cullen *et al.*, 1955}

Hybrids
M. i. x *pumila* {Bean, 1923}
M. soulardii (Bailey) Britton {Bean, 1923}
Pyrus soulardii Bailey = *M. i.* x *M. niedzwetzkyana* {Bean, 1923}

A small tree up to 10 m, some branches with spine like spurs. Leaves oblong ovate to elliptic-obovate, apex acute or short acuminate, base rounded or broad cuneate, coarsely serrate or short lobed, young leaves tomenetose below then becoming glabrous, except on the veins. Petioles tomentose. Flowers 5-6 in umbels; calyx, hypanthium and pedicels tomentose. Calyx lobes longer than hypanthium. Petals oblong to narrowly ovate, 15-19 x 10-13 mm, pink in bud, pinkish white in flower, gradually becoming white, apex rounded, base cuneate, margin entire, short clawed. Stamens about 20. Styles 5, pubescent at base. Fruits, green; subglobose, 19-23 x 12-24 mm. Seeds 5-7 x 2-4 mm.

Section *Eriolobus*

29. *M. trilobata* (Poir.) Schneid.

Described 1809 (Ponomarenko, 1986)

***M. trilobata* (Poir.) Schneid.** {Rehder, 1967}
Crataegus trilobata Poiret {Rehder, 1967}
Sorbus trilobata (Poiret) Heynold {Rehder, 1967}
Eriolobus trilobata (Poirt) Roem. {Rehder, 1967}

A small tree to 6 m or shrub of upright habit. Young shoots and leaves tomentose becoming glabrous. Petioles 2.3-8 cm long and slender. Leaves 4.5-9.6 x 4.9-12 cm, deeply 3-lobed and serrulate, the middle lobe with one or two smaller lobes on each side, the lateral lobes usually with a basal lobe. Flowers 3-6 in corymb. Sepals longer than hypanthium, pedicel pubescent. Petals 15-23 x 11-17 mm, white in bud and flower, apex emarginate, base truncate with a clawed, margin entire and wavy. Stamens 20-23; anthers yellow. Styles (4)5, pubescent on both side. fruits obovate, red or yellow, 18 x 25 mm; core more than 1/2 fruit diameter; calyx presistent; stone cells abundant.

Section *Docyniopsis*

30. *M. tschonoskii* (Maxim) Schnied.

Described 1874 (Ponomarenko, 1986)

***M. tschonoskii* (Maximowicz) Schneider** {Hillier, 1981}
Pyrus tschonoskii Maximowicz {Cullen *et al.*, 1955}, {Rehder, 1967}
Eriolobus tschonoskii Rehder {Cullen *et al.*, 1955}
Cormus tschonoskii Koidzumi Cullen *et al.*, 1955}

Variety

M. tschonoskii var. *hoggii* Franchet and Savatier

Tree to 12m.Young shoots tomentose; Leaves 5.5-7.4 x 3.6-5.8 cm, elliptic to ovate, apex acuminate, base cordate or rounded, margin serrate, doubly serrate or sometimes lobulate, tomentose when young and finaly glabrous or slightly tomentose on veins on adaxial surface and tomentose below, veins pink, leaves in bud folded. Petioles 1.7-3 cm, pubescent. Flowers 3-6 in corymb. Petals 15-23 x 11-17 pinkish

red in bud, pinkish white in flower, apex emarginate, base truncate, margin entire. Stamens 39-61; anthers yellow. Styles (4)5(7). Fruit subglobose, flattened, with depression at the calyx end, red; fruiting pedicel pubescent, short and thick; calyx presistent, erect; sclerids abundant; degree of carpel connation low (12.3%). Seeds about 5 x 3.5-4 mm.

This species is distinguished from other species by erect habit, craspedodromous venation with conspicuous veins, lobulate leaves, lack of adaxial glands, small number of flower per inflorescence, numerous number of stamens, very low degree of carpel connation and carpels and hypanthium adnation.

CHAPTER 3

CHAPTER 3

WOOD ANATOMY

3.1. Introduction

Members of subfamily Maloideae have very similar wood anatomy (Burgerstein, 1895 cited by Huckins, 1972). Burgerstein examined the woods of subfamily Prunoideae and did not find many absolute differences from subfamily Maloideae. The most important differences detected were:

1. All taxa of Prunoideae examined by him had strong development of tertiary thickenings in the vessels but in the genera of Maloideae these thickenings were absent. However, in 1898 he reported that tertiary thickenings of the vessel walls were completely absent in *Malus*, *Pyrus*, *Crataegus* and *Pyracantha*, while they clearly occur in all species of *Sorbus* (including *Aria, Torminaria*, *Cormus*) *Micromeles*, *Amelanchier* and *Aronia.*

2. Vascular rays are uniseriate to triseriate (mostly uniseriate or biseriate, or in *Mespilus* uniseriate to quadriseriate) in the Maloideae. In contrast, vascular rays are uniseriate to 10-seriate (mostly uniseriate to 4-seriate) in the Prunoideae.

Zhang *et al.* (1992) reported that the origin of the Maloideae, presumably through alloploidy, was associated with reduction in ray size, an increase in parenchyma abundance and ray homogeneity, and attaining tree stature. They also noted that the predominance of helical thickenings in the Maloideae may point to (a) diploid ancestor(s) which had already acquired this feature.

Zhang and Bass (1992) reported that Maloideae are quite homogeneous in wood anatomy and characterised by a high percentage of solitary vessels. The ground tissue consists exclusively of fibres tracheid with bordered pits, relatively abundant axial parenchyma, 1-3 (-5) seriate rays, and large prismatic crystals in enlarged, sclerified and/or chambered axial parenchyma cells. Based on ray composition they divided the Chinese genera of Maloideae to two groups:

1. *Sorbus, Pyrus, Malus, Crataegus, Amelanchier, Cydonia,* and *Docynia,* with multiseriate rays composed of procumbent cells only, or having at most one row of square or upright marginal cells.

2. *Dichotomanthes, Cotoneaster, Stranvaesia, Photinia, Eriobotrya, Raphiolepis,* and *Chaenomeles* with multiseriate rays composed of procumbent body cells and 1-6 rows of square to upright marginal cells.

3.2. Material and methods

Transverse, tangential and radial longitudinal sections of the wood of 22 available species (Table 3.1) were made by hand. Sections were treated with sodium hypochlorite (2.5 % for 15 minutes) then rinsed with distilled water three times and stained in 1% safranin (Dickison *et al.*, 1994) for two minutes. Specimens were then rinsed with distilled water and mounted on slides and observed by light microscopy. Cell diameters were measured from transverse sections and do not included walls. Terminology used to describe wood is that advocated by the IAWA Committee on Nomenclature (1989).

3.3. Results

The anatomy of the woods of species of *Malus* are very similar. Growth rings and late wood consist of rows of radially flattened fibres. Growth rings are distinct through differences in vessel frequency between late wood and subsequent early wood. Vessels are usually angular and in solitary and diffuse patterns (204- 647 per mm^2) (Table 3.1), rarely in the clusters of two or three vessels. Ground tissue is composed exclusively of fibres tracheids with distinctly bordered pits.

The largest vessel diameter occurs in series *Chloromeles* and the smallest in series *Docyniopsis* (Table 3.2). Mean of tangential diameter varies from 15.5 μm in *M. tschonoskii* to 27 μm in *M. lancifolia.* Mean value for radial diameter varies from 19.5 μm in *M. tschonoskii* to 30.1 μm in *M. coronaria.* Radial to tangential diameter ratio ranges from 0.9 in *M. baccata* to 1.5 in *M. kirghisorum.*

Crystals are present in some species. I could not find any crystals in species of series *Pumilae*, *Baccatae* and *Sieboldianae*, but they were found in series *Yunnanenses*, *Docyniopsis*, *Chloromeles*, *Florentinae* and *Eriolobus* (Plate 2.A and Table 3.2). Crystals are usually present in parenchyma cells or ray cells. They are usually cubic, but in *M. trilobata* they are oblong.

The lowest number of vessels per square millimeter are found in *M. fusca,* mean value 204, while the highest values are found in *M. yunnanensis* var. *veitchii*, mean value 647 and *M. angustifolia* mean value 623. Analysis of variance on vessel number per square millimeter is presented in Appendix Table 11 and shows that species differ significantly in density of vessels.

Rays may be uniseriate or multiseriate. Multiseriate rays are usually of two (biseriate) or rarely three cells wide. In most species of *Malus,* except in series *Chloromeles*, rays are biseriate.

In *Chloromeles* uniseriate rays are the dominant kind (more than 90%). I could not find any biseriate rays in *M. angustifolia*, *M. lancifolia*, *M. glaucescens* and *M. ioensis* var. *palmeri* (Plate 3.A) but a few were found in *M. coronaria* and *M. platycarpa.*

The only species of series *Pumilae* with almost equal amounts of both kinds of rays is *M. niedzwetzkyana.* In all other series biseriate rays are dominant (Plate 3.B).

The number of cells in uniseriate rays varies between 3 and 29 with mean values ranging between 6 in *M. pumila* and 13 in *M. angustifolia.* In biseriate rays the number of the cells varies from 4 to 55 with mean value ranging from 11.5 in *M. hupehensis* to 32.18 in *M. toringoides* (Table 3.2).

The general shape of rays in longitudinal tangential sections is long elliptic, usually with a long cell at each end but in some cases biseriate rays contain more than one long terminal cell. Two to five cells make a long tail at one end or both ends of the rays e.g. in *M. toringoides* the tail is composed of 2 or 3 cells.

In all species examined rays are heterogeneous, 3-6 rows of procumbent cells in the middle and 1-6 rows of square or upright cells on both margins of the rays

Table 3.1. Wood characteristics of *Malus* examined.

Taxon	No. of vessels/mm^2	Ray size* (μm)		No. of cells per ray	
		Uniseriate X ± SD	Biseriate X ± SD	Uniseriate X ± SD	Biseriate X ± SD
Pumilae					
M. spectabilis	377 ± 69	-	139.9 ± 52.2	-	20.54 ± 7.67
M. kirghisorum	492 ±35	-	257.4 ± 110.4	8.67 ± 1.15	21.21 ± 9.01
M. sylvestris ssp. *orientalis*	372 ± 103	-	222.6 ± 77.3	-	22.14 ± 11.29
M. niedzwetzkyana	519 ± 58	107.0 ± 33.2	191.4 ± 38.4	6.82 ± 3.57	15.14 ± 5.19
M. pumila	311 ± 36		224.11 ± 117	6 ± 1.73	19.22 ± 5.38
Baccatae					
M. baccata	293 ± 39	-	-	11.15	17.17
M. sikkimensis	383 ± 35	166.2.± 93.8	312 ± 112.7	6.44 ± 2.13	15.5 ± 7.64
M. hupehensis	280 ± 31	219.0 ± 104	243.1 ± 111.9	6.73 ± 3.71	11.05 ± 4.40
Sieboldianae					
M. sieboldii var. *arborescence*	432 ± 28	139.2 ± 86.6	246.4 ± 1108.1	9.87 ± 6.55	20.8 ± 8.13
M. sargentii	406 ± 92	-	297.5 ± 187.4	7.75 ± 4.6	13.75 ± 5.18
M. zumi	571 ± 50	117.1 ± 21.5	180 ± 57.5	6.58 ± 2.47	13.6
Kansuenses					
M. kansuensis	566 ± 111	273.1 ± 142	289.7 ± 66.2	8.43 ± 4.72	21.38 ± 12.24
M. toringoides	456 ± 152	166.4 ± 45.8	492.9 ± 187.8	10 ± 4.31	32.18 ± 14.38
M. fusca	204 ± 53	-	189.7 ± 67.5	7.86 ± 3.18	17.62 ± 7.83
Yunnanenses					
M. yunnanensis var. *veitchii*	647 ± 96	-	197.3 ± 99.2	-	-
Florentinae					
M. florentina	372 ± 38	177.75 ± 75.9	325.4 ± 147.6	8.5 ± 3.30	20.57 ± 5.97
Chloromeles					
M. coronaria	357 ± 41	348.8 ± 120.2	348.8 ± 120.2	10.47 ± 5.08	21.31 ± 15.64
M. angustifolia	623 ± 43	360.1 ± 198.4	-	12.67 ± 8.83	-
M. lancifolia	370 ± 77	249.7 ± 158.9	-	8.94 ± 3.32	-

Cont'd. Table 3.1. Wood characteristics of *Malus* examined.

Taxon	No. of vessels/mm^2	Ray size* (μm)		No. of cells per ray	
		Uniseriate X ± SD	Biseriate X ± SD	Uniseriate X ± SD	Biseriate X ± SD
M. platycarpa	530 ± 52	258.3 ± 137.7	226.7 ± 70.8	10 ± 4	17.4 ± 3.78
M. glaucescens	465 ± 49	245.2 ± 81	-	13 ± 6.78	-
Docyniopsis					
M. tschonoskii	568 ± 132	-	239.1 ± 77.5	-	-

Abreviations; M mean; SD standard deviation.
* Length of the vessels in tangential longitudinal section.

Table. 3.2 Wood characteristics of *Malus* species examined.

Taxon	Vessel diameter			
	Tangential diam. X ± SD	Radial diam. X ± SD	Radial / Tangential ratio X ± SD	Crystals
Pumilae				
M. spectabilis	20.00 ± 3.41	23.54 ± 4.88	1.17 ± 0.40	-
M. kirghisorum	15.59 ± 3.62	22.20 ± 3.65	1.50 ± 0.44	-
M. sylvestris ssp. *orientalis*	20.83 ± 3.05	25.45 ± 4.76	1.25 ± 0.34	-
M. niedzwetzkyana	22.56 ± 4.92	26.25 ± 5.24	1.24 ± 0.39	-
M. pumila	23.07 ± 4.05	26.06 ± 5.34	1.17 ± 0.33	-
Baccatae				
M. baccata	24.56 ± 5.81	25.15 ± 5.94	0.91 ± 0.55	-
M. sikkimemsis	24.36 ± 4.14	27.96 ± 5.41	1.17 ± 0.31	-
M. hupehensis	24.18 ± 5.52	26.01 ± 5.24	1.17 ± 0.42	-
Sieboldianae				
M. sieboldii var. *arboresence*	25.45 ± 4.78	25.67 ± 6.91	1.08 ± 0.48	-
M. sargentii	20.09 ± 5.35	23.39 ± 6.73	1.25 ± 0.51	-
M. zumi	22.33 ± 3.44	25.06 ± 5.34	1.11 ± 0.27	-
Kansuenses				
M. kansuensis	18.15 ± 3.54	21.75 ± 4.44	1.27 ± 0.46	-
M. toringoides	19.81 ± 4.25	24.90 ± 6.28	1.27 ± 0.39	-
M. fusca	20.85 ± 4.63	27.70 ± 8.27	1.37 ± 0.49	-
Florentinae				
M. florentina	21.64 ± 3.75	22.88 ± 5.07	1.03 ± 0.29	+
Chloromeles				
M. coronaria	25.98 ± 5.39	30.05 ± 6.34	1.24 ± 0.39	-
M. angustifolia	18.32 ± 3.64	24.90 ± 5.60	1.42 ± 0.42	+
M. lancifolia	26.99 ± 5.05	28.07 ± 7.85	1.06 ± 0.32	+
M. ioensis var. *palmeri*	25.13 ± 5.96	22.63 ± 4.32	0.98 ± 0.35	-
M. platycarpa	20.42 ± 3.21	27.55 ± 4.82	1.40 ± 0.38	-
M. glaucescens	25.09 ± 5.73	29.38 ± 8.02	1.22 ± 0.51	+

Cont'd. Table. 3.2 Wood characteristics of *Malus* species examined.

Taxon	Vessel diameter			
	Tangential diam. X ± SD	Radial diam. X ± SD	Radial / Tangential ratio X ± SD	Crystals
Docyniopsis				
M. tschonoskii	15.53 ± 3.10	19.45 ± 3.90	1.29 ± 0.35	+
Eriolobus				
M. trilobata	-	-	-	+

Abreviatons; X mean; SD standard deviation; diam diameter.

(Plate 2.B). In some species rays may have the above arrangement or each ray may have long uniseriate tails (e.g. *M. pumila*). Uniseriate ray height varies from 107 μm in *M. niedzwetzkyana* to 360.1 μm in *M. angustifolia*. In biseriate rays vertical extent varies from 139.9 μm in *M. spectabilis* to 492.9 μm in *M. toringoides* (Table 3.1).

Intervessel pits in all the species are bordered pits with alternate arrangement, and slit like apertures 5-10 μm in diameter, while vessel-rays pits are much reduced bordered pits 2-4 μm in diameter (Plate 4.A).

Perforations in the vessels are in most cases simple and exclusively in oblique end walls, but in some species sporadic scalariform perforations with 1-8 bars were also found; e.g. in *M. ioensis* var. *palmeri, M. baccata, M. sargentii M. angustifolia, M. toringoides* and *M. glaucescens* (Plate 4.B).

Helical thickening was found in *M. tschonoskii, M. florentina* and *M. ioensis* var. *palmeri.*

Fibres are exclusively fibres tracheids with bordered pits. Fibre lumina are less than 3 times the double wall thickness, therefore they fall into the category of thin to thick walled of the IAWA list of microscopic features for hardwood identification. In all the species examined axial parenchyma is apotracheal and diffuse (not associated with the vessels), and parenchyma cells are distributed irregularly among the fibrous elements of the wood). An exception occurs in *M. fusca* which has axial parenchyma diffuse in aggregates (parenchyma strands grouped into short discontinuous tangential or oblique lines).

3.4. Discussion

Malus species are very homogenous in wood anatomy and it is difficult to separate species by wood characteristics. However, two groups based only on the kind of rays can be recognised as follows:

1. Species with more than 90% uniseriate rays: species of series *Chloromeles.*

2. Species with both uniseriate and biseriate rays in which biseriate rays are predominate (80-90%): all other series of *Malus.*

Although Zhang *et al.* (1992a) placed *Malus* in the group which have only one row of square or upright cells bounding the procumbent cells, this study shows that in most species of *Malus* there are more than one (1-4) rows of square and upright cells bounding the procumbent cells, and *Malus* was misplaced in the second group of Zhang (1992).

The only species with very distinct wood anatomical characters is *M. fusca* with the largest ratio of radial to tangential vessel diameter, lowest number of vessels per square millimeter, and diffuse in aggregate apotracheal axial parenchyma (grouped patches). It is also geographically very distinct by distribution in north west America.

The occurrence of helical thickening (plesiomorphic character) in the wood of *M. tschonoskii* and *M florentina* show the similarity between these species, this indicates the retaining of this primitive character. It can be concluded that these two species evolved from the separate line than other *Malus* species.

CHAPTER 4

CHAPTER 4

POLLEN MORPHOLOGY

4.1. Introduction

Differences in size and surface ultrastructure of pollen grains are useful for distinguishing species of fruit trees. Thakur and Thakur (1970) stated that "pollen exine pattern is so genetically stable for the different species that it can be used for species identification". Hebda *et al.* (1988) showed that exine sculpturing, aperture and aperture zone structure, grain shape, and grain size are all useful characters to distinguish genera and even species of rosaceous pollen. Fogle (1977a,b) used the length and width of the exine, depth of exine ridges and prominence of pores in the exine to distinguish peach, nectarine, plum, cherry, apricot, apple and pear. He reported that peach and nectarine have the largest pollen grains and their ridges tend to be shallow and longitudinally oriented, with some curved patterns. Pollen morphology of the Rosaceae of western Canada was investigated by Hebeda and Chinnappa (1990). They concluded that *Amelanchier alnifolia* pollen grains exhibit systematic geographic variability.

Until this work, no one had investigated the pollen of the species of *Malus*. Although pollen of some varieties of *M. domestica* was occasionally identified (Fogle, 1977b). Xiang and Sheng (1991) reported the pollen morphology of sections and series of *Malus* but only included *M. coronaria* of the American species. Also, they did not investigate the pollen of *M. trilobata, M. fusca* and *M. florentina*, which are included in this study.

4.2. Material and methods

Nineteen *Malus* species were examined in ultrastructural studies of pollen grains. Flowers with dehiscent anthers were collected from herbarium specimens. Small quantities of pollen of each species were sifted onto separate polished

aluminium disk stubs covered with double-sided transparent tape (Fogle, 1977a,b), sputter-coated with 60% gold-palladium in a polaron E 5100 coater and viewed in a Philips 501B scanning electron microscope at accelerating voltages of 7.2 and 15.KV (Veltkamp *et al.*, 1994). The middle part of the exine of pollen grains was photographed at x10,000 and ten pollen grains was measured.

4.3. Results

4.3.1. General information

The shape of the all pollen grains examined was elliptical, tricolpate with three germinal furrows with each furrow extending almost the full length of the pollen grain (Plate 5).

The dimensions of the pollen grains of the species examined are shown in Table 4.1. Mean size of the pollen grains ranges from 38.75 μm to 57.69 μm in length and from 19.15 μm to 28.74 μm in width. The length/width ratios were from 1.78 to 2.35. All the American species except *M. lancifolia* have very large pollen. The largest mean pollen length occurs in *M. glaucescens, M. angustifolia* and *M. coronaria* and the smallest average pollen length occurs in *M. prattii*. The largest average pollen width occur in *M. glaucescens, M. coronaria, M. angustifolia* and *M. sargentii* and the smallest width occurs in *M. kansuensis*. Species could be classified into 2 general groups based on the length of the pollen grains:

1. Those with the length less than 45 μm.
2. Those with the length larger than 45 μm.

4.3.2. Exine sculpture

The general pattern of the exine differs from species to species. I recognised 5 different patterns based on the ridge patterns on the surface of the exine.

1. Smooth
2. Parallel

Table 4.1. Dimensions of pollen grains of *Malus* species.

Taxon	Length (μm)		Width (μm)		Length/Width ratio	
	Range	X ± SD	Range	X ± SD	Range	X ± SD
M. niedzwetzkyana	49.6-52.2	50.81 ± 1.01	21.4-24.4	23.19 ± 1.14	2.02-2.36	2.20 ± 0.13
M. domestica	46.0-48.3	47.1 ± 0.86	18.8-21.2	20.18 ± 0.78	2.23-2.45	2.33 ± 0.07
M. baccata	45.4-49.8	47.88 ± 1.45	22.9-25.8	24.40 ± 1.01	1.78-2.12	1.96 ± 0.09
M. sargentii	50.6-53.3	52.35+0.75	23.2-25.5	24.72+0.94	2.04-2.25	2.12 ± 0.07
M. sieboldii	46.4-49.7	47.85+1.22	20.2-22.2	21.55+0.69	2.15-2.33	2.22 ± 0.06
M. xfloribunda	46.0-51.6	48.54 ± 1.95	21.9-23.5	22.63 ± 0.61	1.98-2.37	2.15 ± 0.01
M. fusca	41.5-47.2	44.24 ± 2.20	19.4-21.6	20.32 ± 0.71	2.02-2.80	2.18 ± 0.10
M. kansuensis	41.4-46.8	44.19 ± 1.63	18.1-20.4	19.15 ± 0.76	2.03-2.45	2.31 ± 0.12
M. transitoria	38.1-47.4	43.35+2.59	18.6-21.7	20.20+0.98	1.76-2.35	2.15 ± 2.15
M. prattii	38.2-39.7	38.75 ± 0.07	19.6-22.0	20.56 ± 0.75	1.81-1.97	1.89 ± 0.06
M. florentina	39.9-43.8	42.06 ± 1.43	22.0-24.8	23.60 ± 0.86	1.65-1.90	1.78 ± 0.07
M. angustifolia	52.9-58.4	55.96 ± 1.84	23.8-25.8	24.74 ± 0.70	2.06-2.04	2.26 ± 0.11
M. coronaria	54.2-59.0	56.03 ± 1.24	23.3-27.6	25.34 ± 1.15	2.13-2.38	2.21 ± 0.07
M. glaucescens	54.2-61.2	57.69 ± 2.27	23.3-28.5	26.64 ± 1.69	1.97-2.34	2.17 ± 2.34
M. ioensis var. *palmeri*	50.0-56.7	52.64 ± 1.94	19.9-24.8	22.43 ± 1.30	2.20-2.54	2.35 ± 0.09
M. lancifolia	40.5-46.7	42.89 ± 2.09	19.2-22.3	20.64 ± 0.95	1.94-2.18	2.07 ± 0.07
M. platycarpa	48.9-54.3	51.12 ± 1.72	26.6-31.7	28.74 ± 1.61	1.69-1.90	1.78 ± 0.08
M. tschonoskii	44.2-49.1	46.10 ± 1.51	20.6-22.9	21.94 ± 0.88	1.99-2.21	2.10 ± 0.07
M. trilobata	44.7-48.5	46.78 ± 1.34	20.6-23.7	22.26 ± 1.02	1.93-2.22	2.10 ± 0.10

Abreviations; X mean; SD standard deviation.

3. Curved

4. Reticulate

5. Tangled thread

Among the species examined, some are recognised by relatively smooth surfaces, others by rough surfaces. Those with smooth surfaces have very shallow ridges with 3 or more of them fused together to make a smooth compound ridge. In other words, the ridges are not easily distinguished. Examples are *M. sieboldii* and *M. prattii*. Pollen of the above species are distinguished by more numerous and larger pores on the exine of *M. sieboldii* and fewer and smaller pores and the presence of a lot of pits on the exine of *M. prattii*.

Pollen grains with rough surfaces have deep and distinguishable ridges. Among the species with rough exine the most conspicuous species is *M. lancifolia* with tangled thread decoration on the surface of the exine with ridges which are very short, thick and confluent. Pollen of the other species tends to have patterning which is longitudinally oriented, branched and sometimes with some of the ridges curved.

The spaces between the longitudinal ridges are wide in some species, (e.g. *M. tschonoskii, M. floribunda, M. sargentii, M. kansuensis, M. baccata, M. niedzwetzkyana, M. platycarpa* and *M. domestica*) while in other species they are very narrow and close. Examples are *M. ioensis* var. *palmeri, M. trilobata* and *M. coronaria.* However, in *M. coronaria, M. transitoria* and *M. fusca* both types could be seen in a single pollen grain.

4.4. Discussion

Examination of more samples of each species might reveal more intra-specific variation and some of the distinctions found here might no longer hold. This is a preliminary study but the significant variation found suggests the potential taxonomic usefulness of pollen characteristics. The correlation of much of this variation with existing taxonomic divisions reinforces the existing classification.

In all the species examined, tricolpate pollen is characteristic without any exceptions. Exine sculpturing is not the same in different parts of the grain. Variation in exine sculpture was observed in the pollen of all taxa. However, the most constant part is the middle of each pollen grain. Exine sculpturing is extremely variable but is mostly striate. However, species may differ in degree of density of ridges and their orientaition. The ridges may be densely packed with obscured perforations, or they may be widely spaced resulting in reticuloid patterns. Ridges also vary from long and parallel to short and irrigular.

The mean size of pollen grains shows considerable variation among species (Table 4.1). Species of series *Chloromeles*, *Pumilae,* and *M. sargentii* of series *Sieboldianae* have the largest pollen. The significance of pollen grain size is uncertain as diploids and tetraploids and sometimes also triploids have been recorded within some species and pollen size is often closely linked with ploidy level (Böcher, 1940).

4.4.1. Characteristics of sections and series of *Malus* and descriptions of pollen by species

The characters of pollen morphology of sections and series are as follows:

Section *Malus*

1. Series *Pumilae*

Exine sculpture rough, striae parallel to the colpi, long, conjugate or curved at the poles; pores present.

M. domestica

Pollen 46.0-48.3 μm in length and 18.8-21.2 μm in width; surface rough, exine sculpture striate, ridges long, straight and parallel to the furrows, slightly curved at the ends; pores sparsely distributed (Plate 6).

M. niedzwetzchyana

Pollen 49.6-52.2 μm in length and 21.4-24.4 μm in width; surface rough; exine sculpture parallel, straight and sometimes curved at the end of the grain; ridges far from each other, pores abundant (Plate 7).

2. Series Baccatae

Exine sculpture smooth, striae parallel, curved at the poles, pores present.

M. baccata

Pollen 45.4-49.8 μm in length and 22.9-25.8 μm in width; pollen surface almost smooth; exine sculpture striate, short and occasionally branched, straight at the middle and curved at the ends; pores small (less than 0.12 μm), (Plate 8).

Section *Sorbomalus*

3. Series *Sieboldianae*

Strongly perforate, irregular, nearly reticulate (except *M. sargentii*) with parallel striae.

M. floribunda

Pollen 46.0-51.6 μm in length and 21.0-23.5 μm in width; surface rough; exine sculpture striate, ridges far from each other, very branched; pores abundunt variable in sizes (Plate 9).

M. sargentii

Pollen grains 50.6-53.3 μm in length and 23.2-25.5 μm in width; surface rough; exine sculpture striate; ridges long, straight sometimes curved at the ends or near the edge of furrows; pores sparsely distributed, elliptic or sometimes rounded (Plate 10).

M. sieboldii

Pollen 46.4-49.7 μm in length and 20.2-22.2 μm in width; pollen surface smooth, exine sculpture reticulate; pores abundant and very large (0.24-0.29 μm), pore shape irregular (Plate 11).

4. Series *Florentinae*

M. florentina

Pollen 39.9-43.8 μm in length and 22.0-24.8 in length, surface rough, exine sculpture striate, not very straight and slightly curved at the ends. Ridges short and very branched, pores small (0.12-0.24. μm) (Plate 12).

5. Series *Kansuenses*

Striae regular, parallel to colpi, not conjugate, but curved at the poles (except *M. fusca*).

M. fusca

Pollen 41.5-47.2 μm in length and 19.4- 21.6 μm in width; surface rough, exine sculpture striate, straight and curved, both types occurring on each pollen grain; pores present especially abundant in area of curved ridges (Plate 13).

M. kansuensis

Pollen 41.4-46.8 μm in length and 18.1-20.4 μm in width; surface rough, striae straight and nearly parallel to the colpi at the middle of the grain and curved at the ends, far from each other, very porous (Plate 14).

M. transitoria

Pollen 38.1-47.4 μm in length and 18.6-21.7 μm in width; surface rough, exine sculpture striate, mostly straight and parallel to the colpi, sometimes curved at the ends; ridges long and branched, close to each other; pores very rare and small at the middle and more numerous at the poles (Plate 15).

6. Series *Yunnanenses*

Exine sculpture smooth, striae irregular, perforation present.

M. prattii

Pollen 38.2-39.7 μm in length and 19.6-22.0 μm in width; surface smooth, exine sculpture striate, irregular, ridges very close to each other usually with two or three fused to make a compound wide ridge; pores abundant, large and round although Xiang and Sheng (1991) reported that pores are absent in *M. prattii.*

Distinct from other species by very smooth and fused ridges (Plate 16).

Section *Chloromeles*

Exine sculpture rough, striae very variable in the species, *M. ioensis*, *M. platycarpa* and *M. lancifolia* have irregular striae; *M. coronaria*, *M. glaucescens* and *M. angustifolia* have regular and parallel striae.

M. angustifolia

Pollen grains 52.9-58.4 μm in length and 23.8-25.8 μm in width; pollen surface rough, exine sculpture striate; ridges long, parallel with furrows and curved at the ends; pores mostly at the ends sparse at the middle of the grain (Plate 17).

M. coronaria

Pollen grains 54.2-59.0 μm in length and 23.3-27.6 μm in width; surface rough, exine sculpture slightly striate and mostly parallel, rarely branched sometimes

curved and may be different in each lobe of a pollen grain; pores rare, especially when the ridges are curved (Plate 18).

M. glaucescens

Pollen grains 54.2- 61.2 μm in length and 23.3-28.5 μm in width; surface rough; exine sculpture striae mostly unbranched and close to each other; ridges parallel to the furrows and curved at the ends, far from each other; pores sparsely distributed and round (Plate 19).

M. ioensis

Pollen 50.0-56.7 μm in length and 19.9-24.8 μm in width; ridges close in the middle and far apart at the poles and edge of the furrows. Very similar to *M. trilobata* in exine sculpture but can be distinguished by the absence of pores and presence of pits on the middle of the pollen grain (Plate 20).

M. lancifolia

Pollen grains 40.5-46.7 μm in length and 19.2-22.3 μm in width; surface rough, exine sculpture striate and tangled thread; ridges very short; pores very few.

This species can easily be distinguished by the tangled thread sculpture (Plate 21).

M. platycarpa

Pollen grains 48.9-54.3 μm in length and 26.6-31.7 μm in width; surface rough; exine sculpture striate, striae mostly unbranched and close and merging into each other; pores absent in the middle and very rare at the ends.

The pollen of this species is very distinct in its greater width than measured in any other species (Plate 22).

Section *Docyniopsis*

Exine sculpture rough, striae irregular; ridges very thick.

M. tschonoskii

Pollen grains 44.2-49.1 μm in length and 20.6-22.9 μm in width; surface very rough, exine sculpture striate; ridges very thick, far from each other, both kinds of striate (straight and curved) could be seen. This species is easily distinguished by the very thick ridges (Plate 23).

Section *Eriolobus*

Striae very dense and smooth in the middle, well separated at the poles and near colpi.

M. trilobata

Pollen grains 44.7-48.5 μm in length and 20.6 -23.7 μm in width; surface rough, exine sculpture striate with short and branched ridges; ridges far from each other except in the middle of the pollen grains where the ridges are very close to each other and look smooth; pores are elliptic and scattered all over the surface. However, they are more distinct at the ends and edges of furrows. All the grains show the same patterns in each lobe (Plate 24).

4.5. Discussion

Variation within the species of the series is observed in series *Sieboldianae*, *Kansuenses* and *Chloromeles*. In series *Sieboldianae*, *M. sargentii* shows variation from other species with regular striae and reduction in porosity. *M. fusca* of series *Kansuenses* is rather different from other *Kansuenses* with ridges parallel to the colpi. *M. fusca* has irregular striae which curve at the margins of the colpi.

M. trilobata and *M. florentina* are very similar to each other and to *Chloromeles* in having few pores and dense ridges which distinguish these taxa from other species in the genus.

The evolution of exine sculpture may be from rough and dense ridges with small and few pores in *Chloromeles* to smooth or sparse ridges and large and more numerous pores in *Sieboldianae*. In this case *Yunnanenses* with smooth and sparse ridges with pores may be considered derivative but this is not in accordance with the postulated evolutionary sequence of morphological characteristics in which *Yunnanenses* are considered a primitive taxon.

Based on the differences in pollen size and exine pattern a key to the species was developed to use the observed differences for identifying *Malus* species (Figure 4.1).

Figure 4.1. Key to the species of *Malus*

1. Pollen length less than 45 μm 2
2. Exine sculpture smooth *M. prattii*
2. Exine sculprure rough 3
3. Ridges tangled thread *M. lancifolia*
3. Ridges not tangled thread and longitudinally oriented 4
4. Ridges long, mostly parrallel at mid - grain, sometimes curved at the poles 5
5. Ridges close to each other, prominant; pores rare *M. transitoria*
5. Ridges far from each other, less prominant; pores aboundant *M. kansuensis*
4. Ridges short, not parallel; mostly branched, curved at the edge of colpi and poles 6
6. Ridges thin, longitudinaly oriented in the middle of the grains; pores small (0.12-0.24 μm) *M. florentina*
6. Ridges thick, curved in some parts; pores large (0.24-0.29 μm) *M. fusca*

1. Pollen length larger than 45 μm 7
7. Exine sculpture smooth 8
8. Ridges irrigularly reticulate, pores large (0.24-0.29 μm) *M. sieboldii*
8. Ridges parallel at mid - grain, pores small (0.12 μm or less) *M. baccata*
7. Exine sculpture rough 9
9. Pollen ridges close to each other (dense) and smooth at mid - grain, branched 10
10. Ridges short, branched, pores absent or very rare in the middle and also at edges of the colpi *M. ioensis*
10. Ridges long, branched, pores abundant at the poles and edges of colpi *M. trilobata*
9. Pollen ridges far from each other 11
11. Ridges very branched, pores large *M. x floribunda*
11. Ridges rarely branched 12
12. Ridges straight throughout the pollen's length, only at the ends slightly curved 13
13. Pores numerous *M. niedzwetzkyana*
13. Pores few 14

14. Pollen length less than 49 µm — *M. domestica*

14. Pollen length larger than 49 µm — *M. glaucescens*

12. Ridges curved in different directions — 15

15. Ridges very thick, never straight — *M. tschonoskii*

15. Ridges not very thick, straight or curved — 16

16. Pollen width more than 26 µm — *M. platycarpa*

16. Pollen width less than 26 µm — *M. coronaria, M. angustifolia, M. sargentii*

CHAPTER 5

CHAPTER 5

STUDIES ON FLAVONOID PATTERN IN *MALUS*

5.1. Introduction

It is not always easy to decide whether apparently equivalent morphological features in different taxa indicate a close relationship or have arisen as a result of parallel or convergent evolution (Davis and Heywood, 1963). There is also no way of knowing what magnitude of genetic change, and hence what importance, should be ascribed to it in the evolutionary sense. It is useful, therefore, to consider whether other information, for example biochemical data, might be used to overcome some of these difficulties.

Flavonoids are probably the most useful class of secondary plant constituents for systematic purposes, because of their widespread distribution, stability and ease of detection (Harborne, 1973). Furthermore, they can be detected in small fragments of leaf from herbarium specimens. Flavonoids which are useful in systematic studies must obviously show correlation in their distribution patterns with morphological or other biological characters. For example, in *Pinus* the heartwood flavonoids vary according to whether the species have a single or a double vascular bundle in the needle leaves (Davis and Heywood, 1963). A number of interesting correlations with phytogeography have been observed in *Brazia*, for example, the diploid Eurasian species have a different set of flavonoids in the leaves from the tetraploid south American species (Harborne 1973).

5.1.1. Chemistry of Flavonoids

The flavonoid structures are derived from the aromatic nucleus of flavon or 2-phenylbenzopyran. The simplest member is flavone, which occurs naturally as the farina on leaves and stems of many *Primula* species. The majority of flavonoids have phenolic hydroxyls attached at the 5-,7-,3' and 4' positions.

Flavonoids are usually divided into classes depending on the degree of oxidation of the central pyran ring, the two most important classes being the flavonols or 3- hydroxyflavones (e.g. quercetin) and the anthocyanidins (e.g. cyanidin).

While about 150 different flavonoid aglycones have been isolated from plants, only eleven of these occur at all commonly. The structures of these eleven compounds are similar in their basic hydroxylation pattern, and they differ only in the number of hydroxyl groups attached to the B ring.

In the case of anthocyanidins, the number of B- ring hydroxyls present is correlated with colour properties, thus, pelargonidin with one hydroxyl is scarlet, cyanidin with two is crimson, and delphinidin with three is mauve.

The flavonols corresponding to the three main anthocyanidin types are kaempferol, quercetin and myricetin. They are colourless at the pH of cell sap. They occur in flowers and leaves. Flavones lack the hydroxyl group present in flavonols and anthoxyanidins and only two structures are common: apigenin and luteolin (Harborne, 1973).

5.1.2. The function of flavonoids in plants

The anthocyanins are the source of orange to blue colours in petals, fruits, leaves and roots. Flavonoids contribute to yellow flower colour, either by co-occurring with yellow carotenoids or by replacing them in about 15% of plant species. The so-called colourless flavones and flavonols make an important contribution to plant colour either by acting as copigments to the anthocyanins or by providing body to cream and ivory flowers. Both yellow and colourless flavonoids can also provide ultraviolet (UV) -absorbing honey guides in yellow petals; these guides are perceived by bees, which are then able to detect the flowers more effectively among green leaves. UV- absorbing flavonoids are universally present in leaves and petals, either on the surface or in the epidermal cells and here they appear to provide protection from the potentially damaging effect of UV radiation (Harborne, 1988).

Flavonoids such as quercetin are physiologically active, they may play an indirect role in germination and plant growth. Claims have also been made for a protective function for certain classes of flavonoids in plant desease resistance e.g. (pisatin in *Pisum sativum*).

Topical interest in flavonoids has also centered on their taxonomic distribution, as chemical markers in the biosystematics of higher plants (Harborne, 1973).

5.1.3. Flavonoids and evolution

Bate-Smith (1962) has discussed the possibility that irreversible changes in phenolic biosynthesis have occured during the evolution of plants. In particular he suggests that the ability to form leuco-anthocyanins and trihydroxy-substituted derivatives, once lost, cannot be regained. Families or other taxa lacking one or both of these classes of compound would be regarded as evolutionarily more advanced than taxa possessing both. Bate-Smith also notes that in plants where the common flavonols and hydroxy-acids are not found, they seem to be replaced either by flavones or by sinapic or ferulic acids, respectively. He believes that the replacements arise from a gradual loss of specific oxidative enzymes during evolution, so preventing development of some or all of the commoner phenolics in more highly evolved plants. Such losses of the synthetic power might occur in several unrelated lines of descent, so that it is not suggested, for example, that all plants with flavones are related, merely that they are all to some degree advanced in terms of phenolic metabolism. However, more complex metabolic changes, involving additional stages of hydroxylation or modification of the flavonoid nucleus, might well be rarer, and the plants possessing them thereby more likely to be related phylogenetically.

A consequence of Bate-Smith's ideas include the general point that woody plants (usually containing leuco-anthocyanins) are, on the whole, more primitive than herbs. The Rosales show the greatest consistency in production of leuco-anthocyanins and trihydroxy-acids, irrespective of habit, and so may occupy a central position among orders of dicotyledons. Another corollary would be that taxa characterized by

high frequency of unusual phenolics, and deficiencies of commoner ones, are evolutionarily advanced.

Harborne (1966) has further developed the suggestions made by Bate-Smith and categorized flavonoid characters as primitive, advanced or isolated; isolated characters appear to be not especially primitive or advanced. The recognition of such characters as primitive or advanced rests largely on their distribution in plants believed to be primitive or advanced on other criteria.

Flavone C-glycosides are a class of flavonoid in which the glycoside moiety is attached directly to the flavonoid skeleton by a carbon-carbon bond, rather than by the more usual carbon-oxygen-carbon linkage, as in the more common flavone and flavonol O-glycosides. These O-glycosides are readily hydrolysed to flavonoid + sugar by hot acid or by enzymic action, whilst C-glycosides under these conditions remain intact. It is generally considered that these C-glycosides are biosynthetically and phylogenetically more primitive than O-glycosides; Challice (1981) states that this is a chemotaxonomic character of considerable potential usefulness in the Rosaceae. He reported the occurrence of flavone C-glycosides in the subfamily Maloideae. They are absent in *Malus* and related genera like *Chaenomeles*, *Docynia*, and *Pyrus* but present in *Sorbus* sections *Aria*, *Torminaria* and *Chamaemespilus*.

5.1.4. Flavonoids in Rosaceae

Bate-Smith (1961) showed that because of the lack of trihydroxy flavonoid constituents in Rosoideae, Pomoideae, Spiraeoideae and Prunoideae and their presence in *Chrysobalanaceae*, this latter group is distinct from the remainder of the *Rosaceae*. He also examined the phenolic constituents of the leaves of *Rosaceae* and observed that the woody plants in this family have the common phenolic compounds of woody plants (leuco - anthocyanins and flavonols). All the members of subfamily *Rosoideae* except those in the tribe *Kerrieae* contain the trihydroxy acid, ellagic acid. Challice (1973a) found flavone C-glycosides in *Pyracantha, Dichotomanthes, Osteomeles, Chamaemeles, Malacomeles, Aronia, Hesperomeles* and *Crataegus,*

flavone O-glycosides were found to co-occur in the latter genera and flavone O-glycosides occur alone in *Sorbus* (Section *Aria*), *Chaenomeles* (section *Pseudocydonia*), *Malus* (rarely) and in *Pyrus*. The occurrence of flavone C-glycosides was considered to indicate primitive status (see section 5.1.3). Challice (1973) supported the hypothesis that the south American *Hesperomeles* evolved from primitive North American *Crataegus* and that the two endemic genera *Aronia* (N. America) and *Malacomeles* (Mexico and Guatemala) may represent surviving relicts of this evolutionary line as it moved southwards. He also suggested *Dichotomanthes* as a relict of primitive *Pomoideae*.

5.1.5. Affinity of *Malus* with other genera of Maloideae

The occurrence of phloridzin in leaves of *Docynia* suggests a close relationship with *Malus* (Challice, 1973 b).

Chaenomeles, Pseudocydonia and *Docynia* are recognized by Robertson *et al.* (1991) as the genera most closely related to *Malus*. In this study I investigated flavonoids of some *Sorbus* species of section *Aria*, *Chaenomeles* sp. and *Pseudocydonia sinensis* to study relationship between these genera and the genus *Malus*.

5.1.6. Flavonoids in *Malus*

Malus is distinct from all but one other genus in the subfamily by having dihydrochalcones in all the species. The only other genus in the subfamily with dihydrochalcone is *Docynia*. *Malus* is distinguished from *Pyrus* by existence of the dihydrochalcone phloridzin in *Malus*. Harborne (1967) has pointed out that in Maloideae, *Malus* and *Pyrus*, both have cyanidin 3- galactoside, which is absent from the other sub-families. He noted that there is a relationship between the chemistry of flavonoids and geographic distribution in *Pyrus* and *Malus*. In *Malus*, Western Asian species have a dihydrochalcon, phloridzin, while Eastern Asian species have a glucoside of 3-hydroxyphloretin. Chemical data would indicate that both *Malus* and

Pyrus have evolved in an eastward direction, the Chinese and Japanese species having the more advanced characteristics. The presence of distinctive phenolics such as arbutin in all species of *Pyrus* and dihydrochalcones in all species of *Malus* can be regarded as good evidence for the monophyletic nature of these genera (Challice and Kovanda, 1978a).

Williams (1970) reported that some species of *Malus,* for example the very distinctive *M. florentina, M. trilobata* and *M. tschonoskii*, appear to be consistent in composition, whatever their source. He also reported that native wild material of *M. fusca*, obtained from two different sites gave the same phenolic pattern as material from Kew. He also reported that the distinctive ultraviolet blue fluorescent material found in the bark of North American *Malus* species of the *Chloromeles* group is a 3-glucoside of azeleatin.

The flavonoid pattern of many species have not been investigated so this study was planned to investigate all available species.

5.1.7. Paper chromatography techniques for flavonoid isolation

The technique of paper chromatography occupies a dominant position in the field of flavonoid analysis and separation.

Paper chromatography is suitable for the separation of complex mixture of all types of flavonoids and their glycosides. Its continuing appeal is also due to the ease of obtaining acceptable chromatographic separations by this method, its convenience for isolating both small and large amounts of flavonoids and the low cost of the necessary equipment and materials (Markham, 1975).

5.2. Materials and methods

5.2.1. Investigation of leaf and bark flavonoids

Extraction methods and Chromatography:

The procedures for the extraction and subsequent acid hydrolysis of the flavonoids are basically as described in Challice and Kovanda (1978a). 0.5 g of dried

leaf of each specimen was weighed out, ground, and 4 ml of 80% methanol was added and the mixture boiled in a water bath for 5 min and the supernatant decanted. This was repeated three times to get efficient extraction. The extracts were then dried under nitrogen and kept in a freezer until required. For bark extraction, 0.2 g of bark was used. Dried extracts were dissolved in a few drops of 90% ethanol and then applied to the paper at a point about 8 cm in from the side edge and on the last fold of the paper. Whatman no.1 (46 x 57cm) chromatograph paper was used for separation of flavonoids and the chromatograms run in glass Shandon chromatotanks. The spotted papers were run first in an alcoholic solvent, n-butanol-acetic acid-water (BAW), (4:1:5), in the longer dimension for 17-18 hrs. The chromatograms were then removed from the chromatotank and dried in a fume cupboard. 15% acetic acid was used for second dimension and papers run for 4-5 hrs. in this solvent. The chromatograms were then dried and viewed under ultra violet light, wavelength 366 nm, with and without NH_3 vapour for spot detection.

Most flavonoids appear as coloured spots on paper chromatograms when viewed in UV light. Fuming with ammonia often produces significant changes in these colours (Maberry *et al.*, 1970).

Acid hydrolysis

One millilitre of 2 N hydrochloric acid was added to 1 ml of methanolic extraction and boiled in a boiling water bath for 40 min. The tubes were then cooled and extracted with 1ml ethyl acetate and dried by leaving on the bench overnight. These extractions were examined separately by thin layer chromatography on cellulose plates of 0.1 mm thickness and run in 3 different solvents; BAW, Forestal (acetic acid : water : hydrochloric acid; 30 : 10 : 30) and CAW (chloroform : acetic acid : water; 2 : 1 : 0.13) against markers of Quercetin, Kaempferol, and Myrecetin (Table 5.3).

Spray reagents

Three paper chromatograms of each specimen were prepared and 3 different reagents were applied for detection of flavonoids (Table 5.1).

Ortho-dihydroxy phenols were detected by spraying with a ferric chloride-potassium ferricyanide solution (0.2% w/v solution; 1:1), whereupon a strong blue colouration was developed after washing with dilute hydrochloric acid. Flavonol glycosides were detected by their yellow colour on the use of a diphenyl boinic acid ethanol amine spray. 5-hydroxylated flavonoids appeared as yellow green fluorescent spots on spraying with aluminium chloride ($AlCl_3$) and examined under UV light (Dass and Weaver, 1972 and Gupta, 1968).

5.2.2. Cluster analysis

A total of 41 species and varieties of *Malus* have been analysed. Presence or absence of flavonoids of selected compounds of the leaf and bark were then subjected to a cluster analysis using the software package " Data desk" in an Apple Macintosh computer.

5.3. Results

5.3.1. Leaf flavonoids

Flavonoid constitutents of the leaf are very simple compared to those of the bark and very similar among the species.

In series *Sieboldianae* and *Eriolobus* phloridzin has been substituted by sieboldin and trilobatin, respectively.

Compound Fc is found in all the series except in *Docyniopsis*, *Eriolobus* and *Yunnanenses*. Compound Fd is found only in section *Malus* and *M.* x *floribunda* of series *Sieboldianae*.

5.3.2. Bark flavonoids

Bark chromatograms of all the species examined are presented in Figures 5.1 and 5.2. Rf. value and colour reactions of flavonoids in the bark are presented in Table 5.1. Presence or absence of the compounds is also shown in Table 5.2. One of the compounds which is taxonomically valuable is compound Fa. In all the series recognized by Rehder Fa is present. In series *Pumilae* all contain Fa except *M. prunifolia*, *M. pumila* and *M. domestica*. In series *Baccatae*, *M. baccata* and *M. baccata* var. *columnaris* lack this compound. It is noteworthy that in series *Kansuenses* all except *M. toringoides* do not possess this compound. In all the *Chloromeles*, *M. florentina*, *M. tschonoskii* and *M. trilobata*, this compound was found. In series *Yunnanenses*, *M. yunnanensis* did not show Fa.

Compound E, which is made visible only by ammonium fumigation, is characteristic of series *Yunnanenses*. This compound also occured in *M. micromalus* of series *Pumilae* and *M. halliana* of series *Baccatae*.

Compound G occurs in *Chloromeles* (except *M. platycarpa*), *Docyniopsis*, *Eriolobae*, *Florentinae* and all the *Kansuenses* except *M. toringoides*, another character suggesting that *M. toringoides* may be misplaced in this series in Rehder's classification.

Another characteristic compound of series *Eriolobus, Docyniopsis, Florentinae* and *Chloromeles* (except *M. coronaria* and *M. angustifolia*) is compound Flavonol glycoside C (Fc). This compound is not present in series *Pumilae* (except *M. micromeles*), *Baccatae, Sieboldianeae* or *Yunnanenses*. In series *Kansuenses* only *M. toringoides* containes flavonol glucoside C.

None of the series *Pumilae*, *Sieboldianae*, and *Baccatae* and *Yunnanenses* showed compound H, this compound is characteristic of series *Chloromeles*, *Kansuenses, Florentinae*, *Docyniopsis,* and *Eriolobus*. Compound H is usually accompained by compound G in all species which contain these compounds except in *M. coronaria, M. platycarpa, M. ioensis* and *M. angustifolia* of series *Chloromeles*.

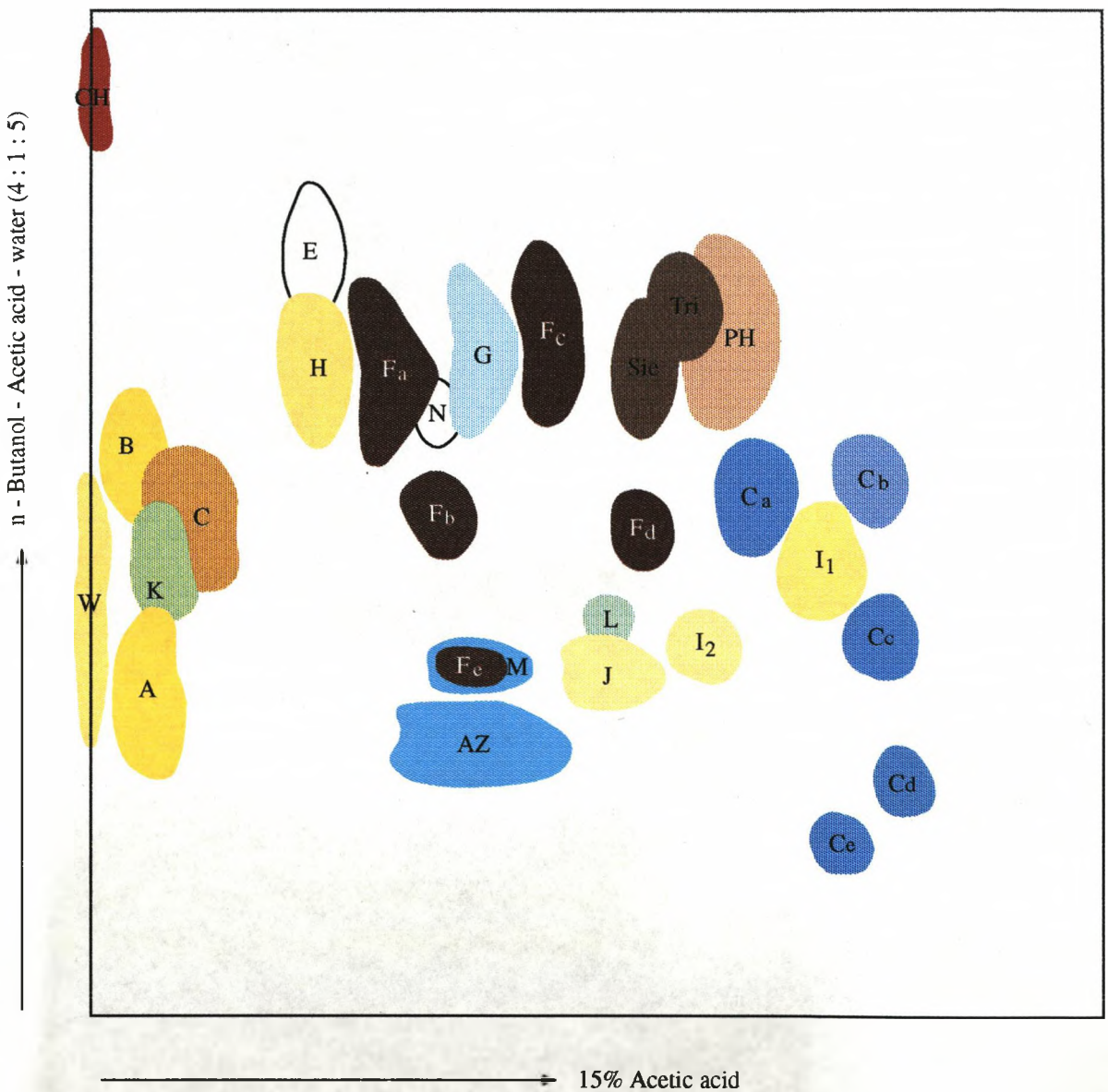


Figure 5.1. Paper chromatographic location of *Malus* flavonoids. See table 5.1 for key to letters.

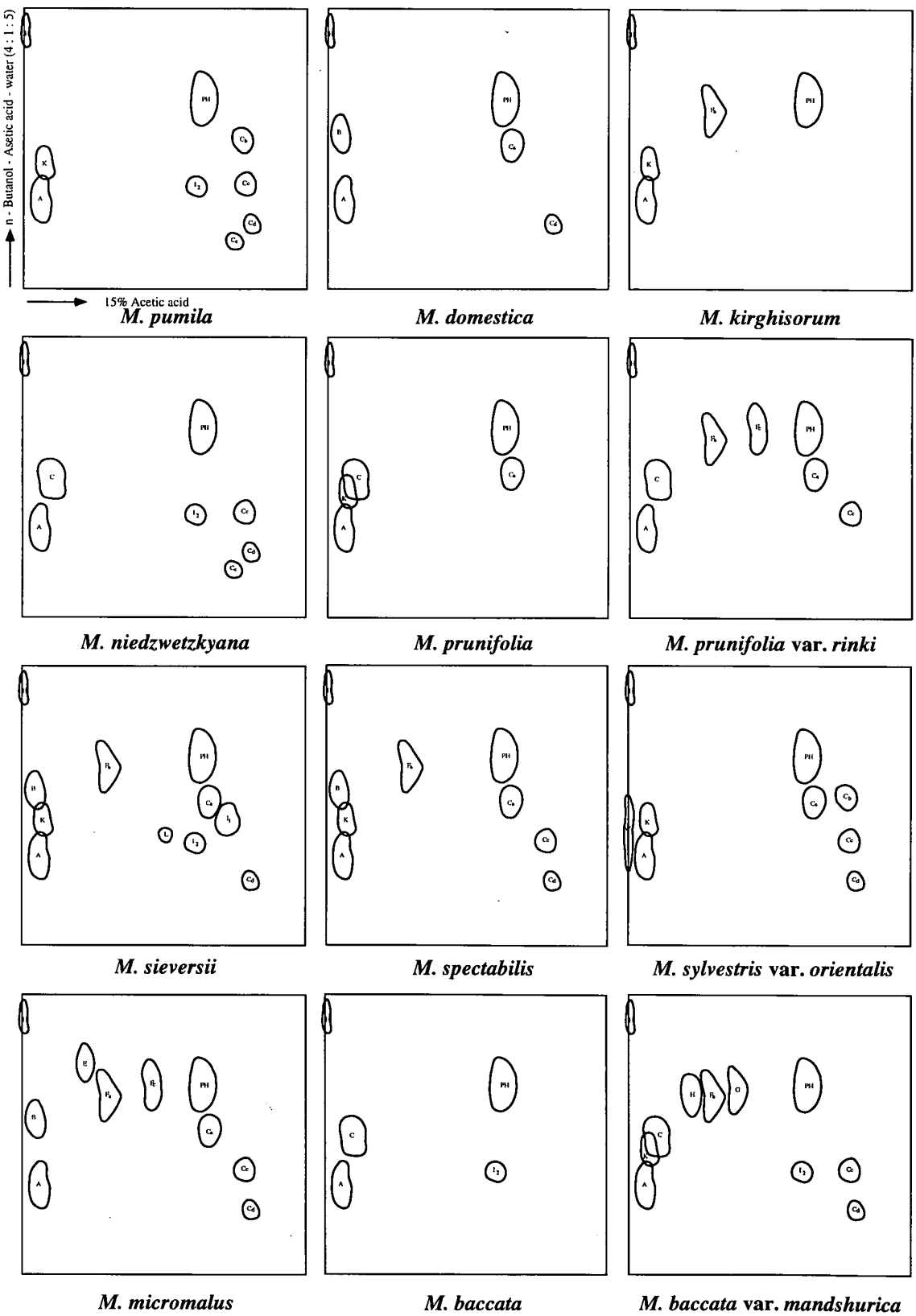
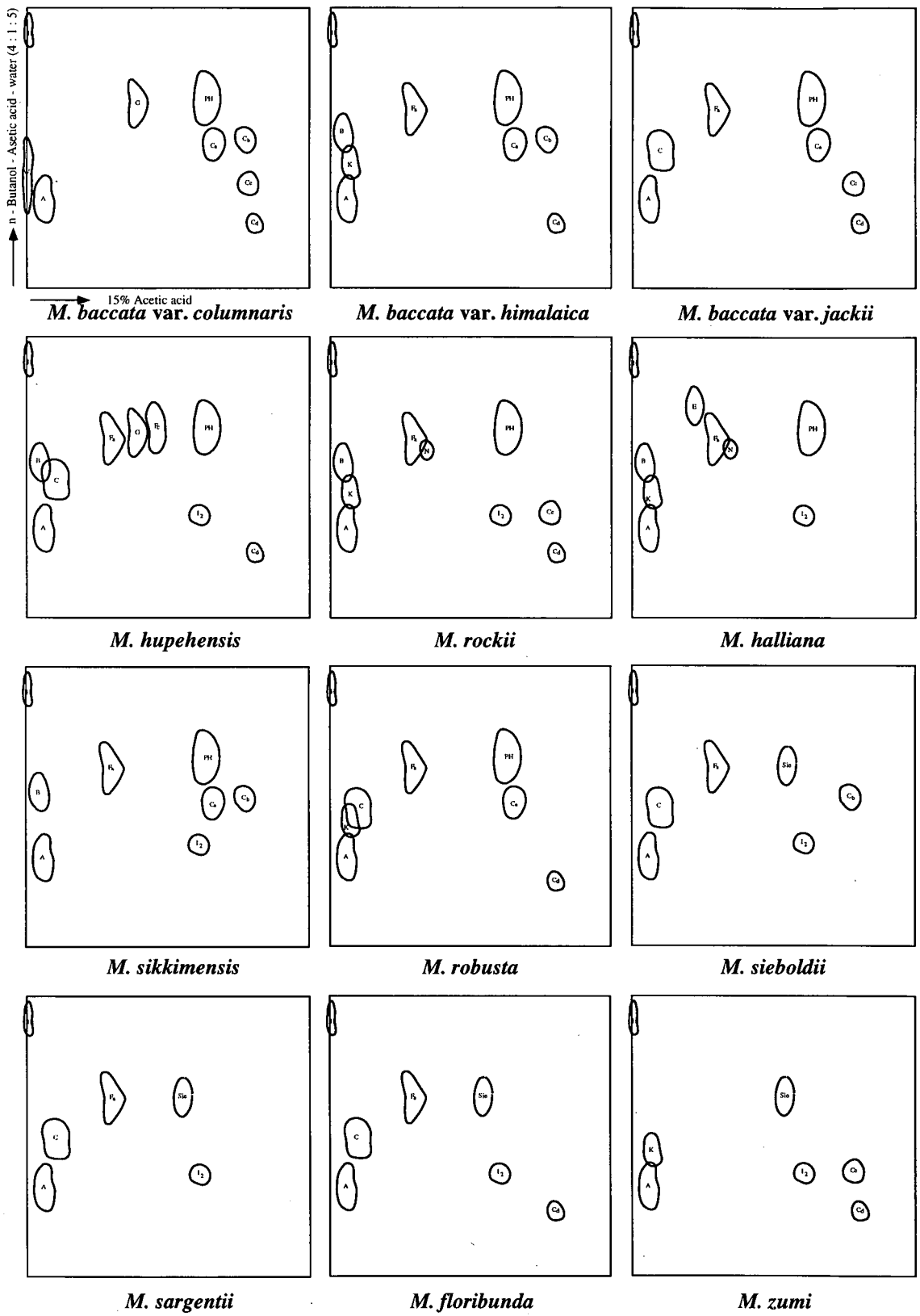
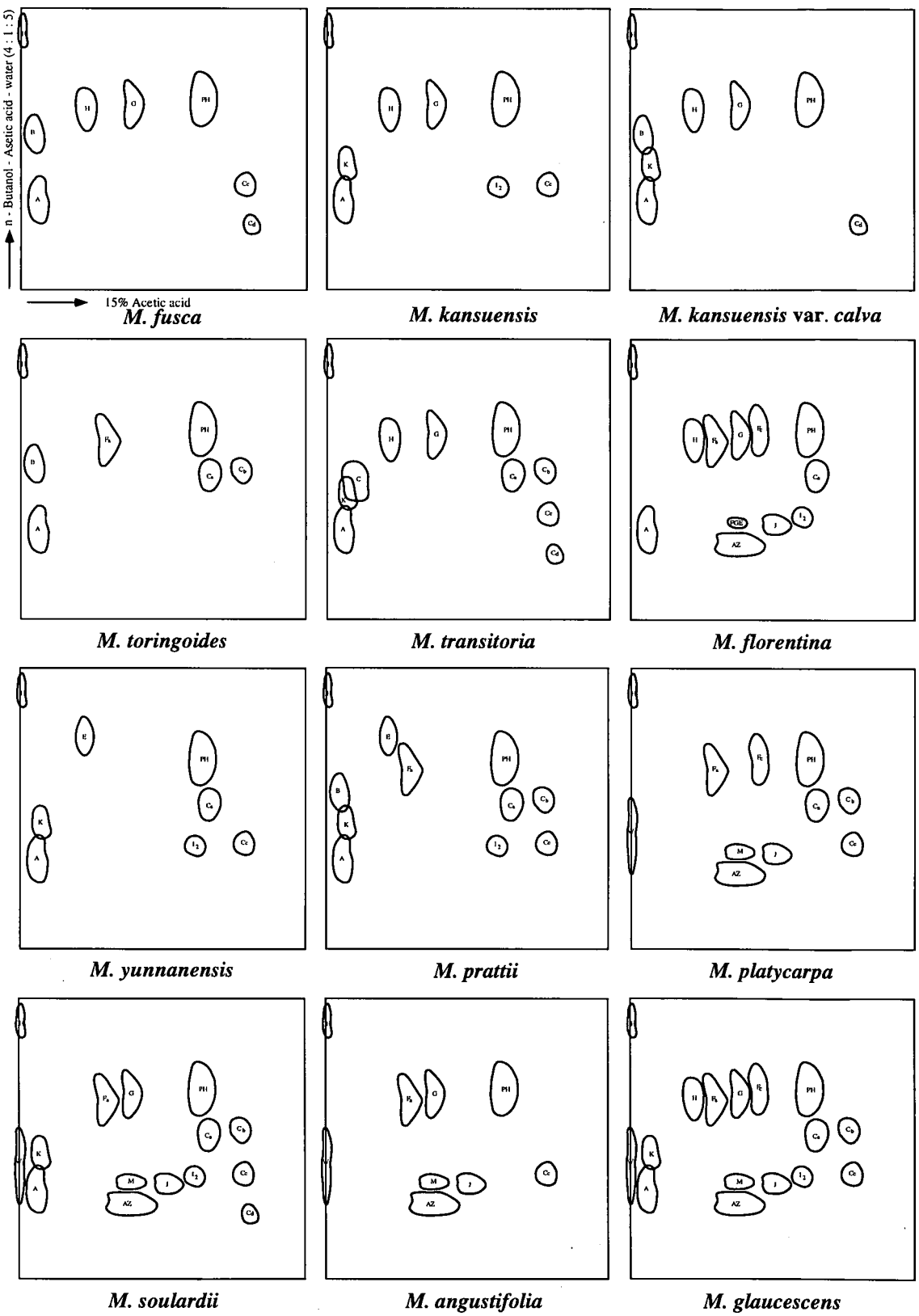


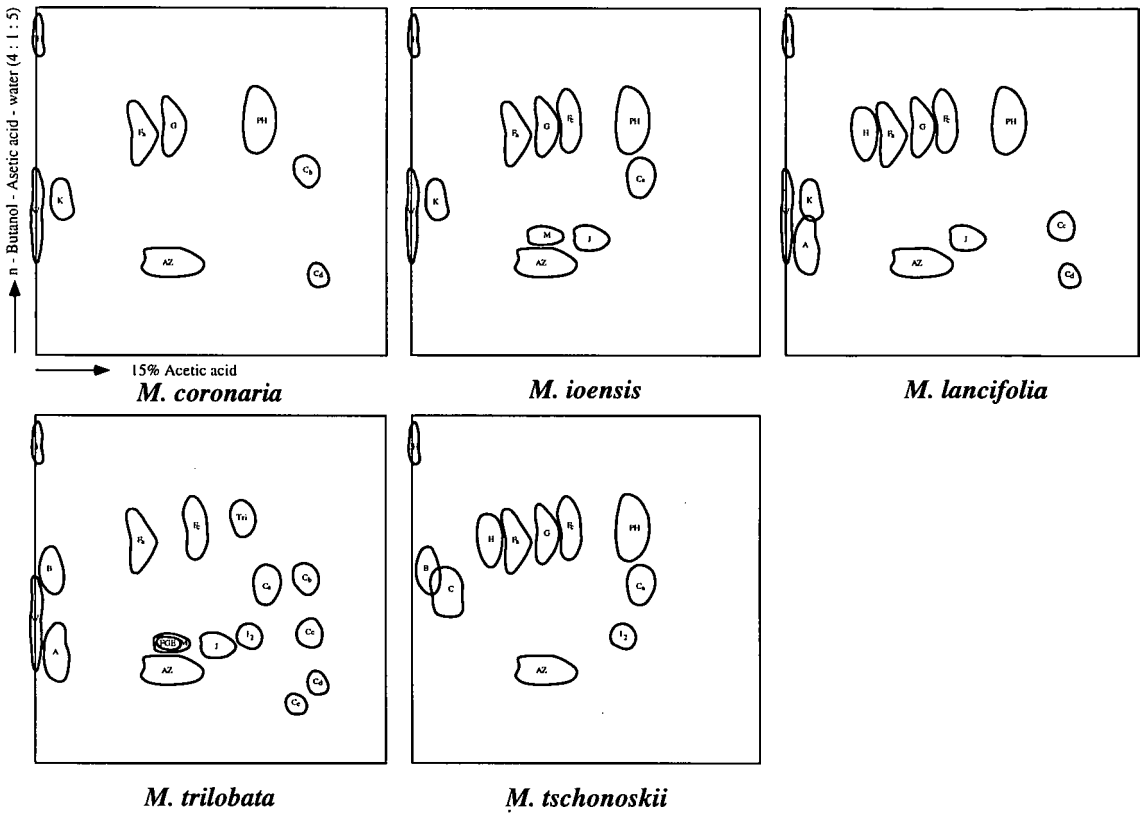
Figure 5.2. Paper chromatographic location of bark flavonoids of individual species of *Malus*.



Cont'd. Figure 5.2. Paper chromatographic location of bark flavonoids of individual species of *Malus*.



Cont'd. Figure 5.2. Paper chromatographic location of bark flavonoids of individual species of *Malus*.



Cont'd. Figure 5.2. Paper chromatographic location of bark flavonoids of individual species of *Malus*.

Table 5. 1. Rf. value and colour reactions of flavonoids in *Malus* bark

Spot	Rf. values x100		Colour reaction by different reageants					
	BAW	HoAc 15%	UV light	UV+NH3	Reagent A	Reagent B	Reagent C	Identification*
A	32	6	Fl. yellow	u.c	Strong blue	u.c	u.c	-
Az**	28	39	Blue	Yellow	Blue	Blue green	Fl. yellow green	Azeleatin 3-glucoside
B	56	4	Fl yellow	u.c	Strong blue	u.c	u.c	-
C	49	10	Dull yellow	u.c	Strong blue	Blue green	Green	-
E	70	21	Invisible	Blue	Pale yellow	-	-	-
Fa	65	29	Dark brown	Yellow	Strong blue	Yellow orange	Green	Flavonol glucoside
Fc	68	44	Dark brown	Yellow	Strong blue	Yollow orange	Yellow	Flavonol glucoside
G	60	37	Light blue	u.c	u.c	u.c	u.c	-
H	60	21	Pale Yellow	u.c	u.c	u.c	u.c	-
I_1	46	72	Yellow	Yellow	Pale blue	Green	u.c	-
I2	36	60	Pale yellow	u.c	Strong blue	-	Yellow	-
J	35	52	Bluish yellow	u.c	u.c	u.c	Yellow	-
K	46	7	Green	Yellow	u.c	Yellow orange	u.c	-
L	38	51	Blue	u.c	-	-	-	-
M.	35	38	Light blue	Yellow	-	-	-	-
N	63	39	Invisible	Yellow	-	-	-	-
Ca	51	64	Blue	Fl. Blue	Strong Blue	-	u.c	Cinamic ester

Cont'd. Table 5. 1 Rf. value and colour reactions of flavonoids in *Malus* bark

Spot	Rf values x100		Colour reaction by different reageants					
	BAW	HoAc 15%	UV light	UV+NH3	Reagent A	Reagent B	Reagent C	Identification*
Cb	53	76	Blue	u.c	Pale Blue	u.c	u.c	Cinamic ester
Cc	39	77	Blue	u.c	Strong Blue	u.c	u.c	Cinamic ester
W	42	0	Yellow	Y. Orange	-	-	-	-
Ph.	68	62	Faint yellow	Y. Brown	Strong Blue	Bluish green	Blue green	Phloridzin
Sieb**	65	54	Dark	u.c	Strong blue	Y. green	Y. green	Sieboldin
Tri**	75	57	Dark	u,c	Strong yellow	Y. green	Y. green	Trilobatin

Key: * Identification by Rf value and colour changing; ** Williams, 1961; .Fl. Florescent; Y Yellowish; u.c, unchanged.
Reagent A: ferric chloride- potassium ferricyanide.
Reagent B: diphenyl boinic acid ethanol amine.
Reagent C: aluminium chloride.

Table. 5.2. Occurrence of flavonoids in *Malus* species.

Taxon	Ph.	Sie.	Tri	A	B	C	K	G	E	Fa	Fc	Fe	H	I_1	I_2	Ca	Cb	Cc	Cd	Ce	Cf	Az	J	L	M	N	W	X
Pumilae																												
M. domestica	+	-	-	*	*	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
M. kirghisorum	+	-	-	*	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. niedzwetzkyan	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-
M. prunifoilia	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
M. prunifolia var. *rinki*	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
M. pumila	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-
M. sieversii	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-
M. spectabilis	+	-	-	*	*	-	+	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
M. sylvestris var. *orientalis*	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-
M. micromalus	+	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-
Baccatae																												
M. baccata	+	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
M. baccata var. *mandshurica*	+	-	-	+	+	-	*	+	-	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-
M. baccata var. *columnaris*	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-
M. baccata var. *himalaica*	+	-	-	+	*	-	+	-	-	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-
M. baccata var. *jackii*	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
M. hupehensis	+	-	-	+	+	+	-	+	-	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
M. rockii	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-
M. halliana	+	-	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
M. sikkimensis	+	-	-	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
M. robusta	+	-	-	+	-	+	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
Sieboldianae																												
M. sieboldii	-	+	-	+	-	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
M. sargentii	-	+	-	+	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
M. floribunda	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
M. zumi	-	+	-	*	-	-	*	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-
Kansuenses																												
M. fusca	+	-	-	+	*	-	-	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
M. kansuensis	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-

Cont'd. Table. 5.2. Occurrence of flavonoids in *Malus* species.

Taxon	Ph.	Sie.	Tri	A	B	C	K	G	E	Fa	Fc	Fe	H	I_1	I_2	Ca	Cb	Cc	Cd	Ce	Cf	Az	J	L	M	N	W	X
M. kansuensis var. *calva*	+	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
M. toringoides	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
M. transitoria	+	-	-	+	-	+	+	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+
Yunnanenses																												
M. prattii	+	-	-	+	*	-	+	-	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
M. yunnanensis	+	-	-	+	-	-	+	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Florentinae																												
M. florentina	+	-	-	+	-	-	-	+	-	+	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
Chloromeles																												
M. angustifolia	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	-	+	-
M. coronaria	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+	-
M. glaucescens	+	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	+	+	-	-	-	+	+	-	+	-	+	-
M. ioensis	+	-	-	-	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	+	-
M. lancifolia	+	-	-	+	-	-	+	+	-	+	+	-	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+	-
M. platycarpa	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	-	+	-	+	-
M. soulardii	+	-	-	+	-	-	+	+	-	+	-	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	+	-
Docyniopsis																												
M. tschonoskii	+	-	-	-	+	+	-	+	-	+	+	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-
Eriolobus																												
M. trilobata	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	+	+	-	+	-	+	-

Symbols: + presence of compound; - absence of compound; * trace amount of compound.
Si.e. and Tri. only in leaves.

Compound Az is only found in all the species of series *Chloromeles*, *Florentinae*, *Docyniopsis* and *Eriolobus*. Compounds W and M are only found in *Chloromeles*. Series *Florentinae* contains the flavonol glycoside E (Fe) which is only found in this series.

Series *Baccatae* and *Sieboldianeae* are characterized by the prescence of both flavonol glycoside A and compound I_2. But series *Sieboldianae* is distinct from *Baccatae* in having sieboldin in leaves. In series *Sieboldianae* all the members except *M. zumi* contain flavonol glycoside A.

The results of acid hydrolized analysis are presented in Table 5.3. None of the species examined contain myricitin. All the species shows quercetin, while kaempferol is found only in series *Chloromeles* and *Docyniopsis*. Azeleatin is present in series *Florentinae*, *Chloromeles* and *Docyniopsis*. These data shows a close relationship between these three series.

5.4. Discussion

Based on flavonoid compounds of bark, 6 species clusters (lettered A to F) have been recognized by cluster analysis in genus *Malus* (Figures 5.3 and 5.4) as follows:

Cluster A:

There is a basic similarity in flavonoid constituents of bark tissue of series *Baccatae* and *Pumilae* which made cluster A. Rehder (1940) placed series *Pumilae* and *Baccatae* in one section based on rolled leaves in the bud and unlobed leaves. This is confirmed by the flavonoid pattern of these two groups. Therefore cluster A is compatible with Rehder´ s classification with the addition of *M. toringoides*.

Cluster B:

Cluster B comprises all the members of series *Kansuenses* Rehder, except *M. toringoides*, which occurs in cluster A, along with members of series *Baccatae*. Based on glabrous styles and leaves with 4-5 lobed, Rehder (1940) placed *M. toringoides* in

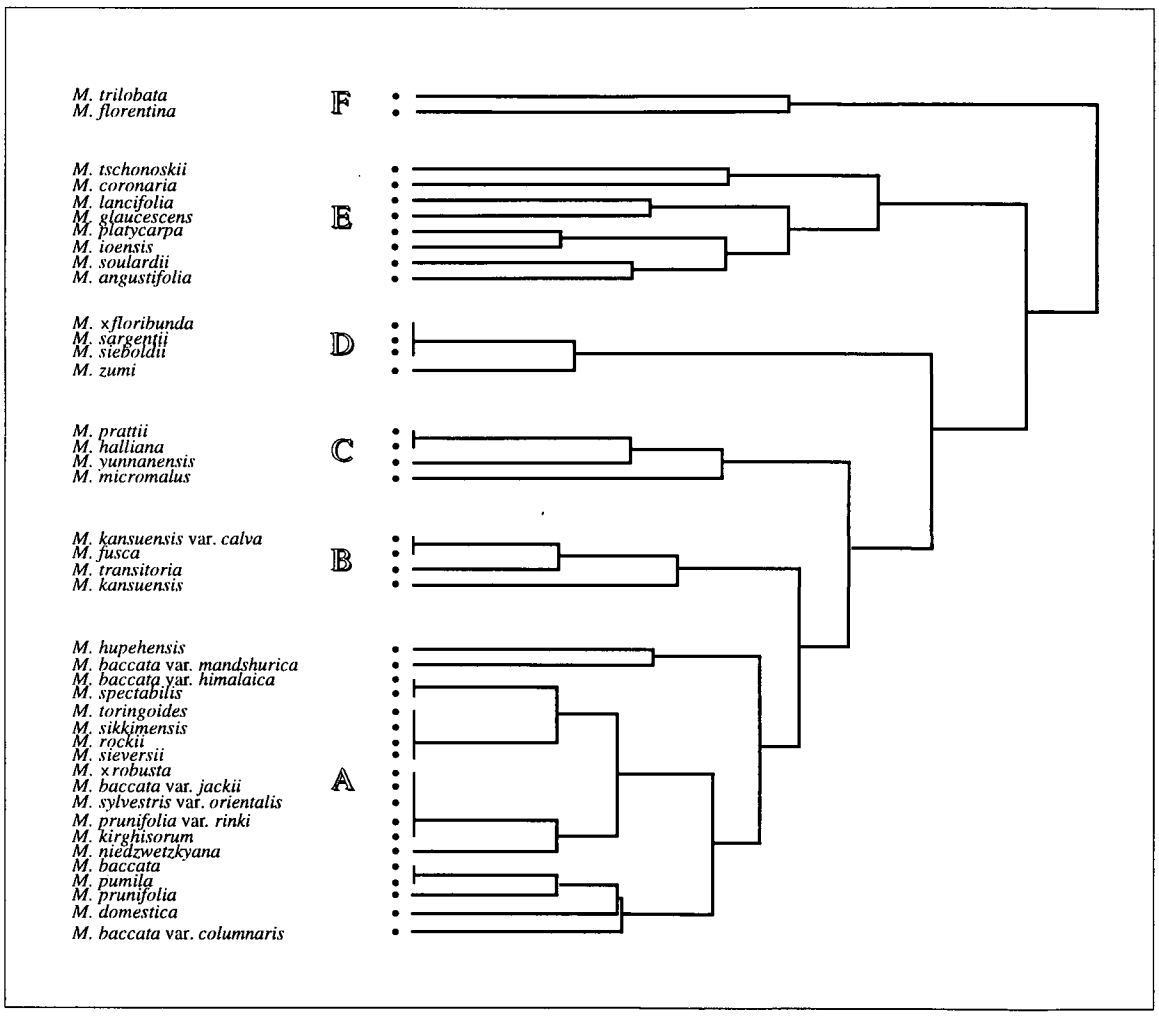


Figure 5.3. Cluster analysis of flavonoid compounds of the bark and leaf in *Malus* spp. Six different clusters are lettered A - F.

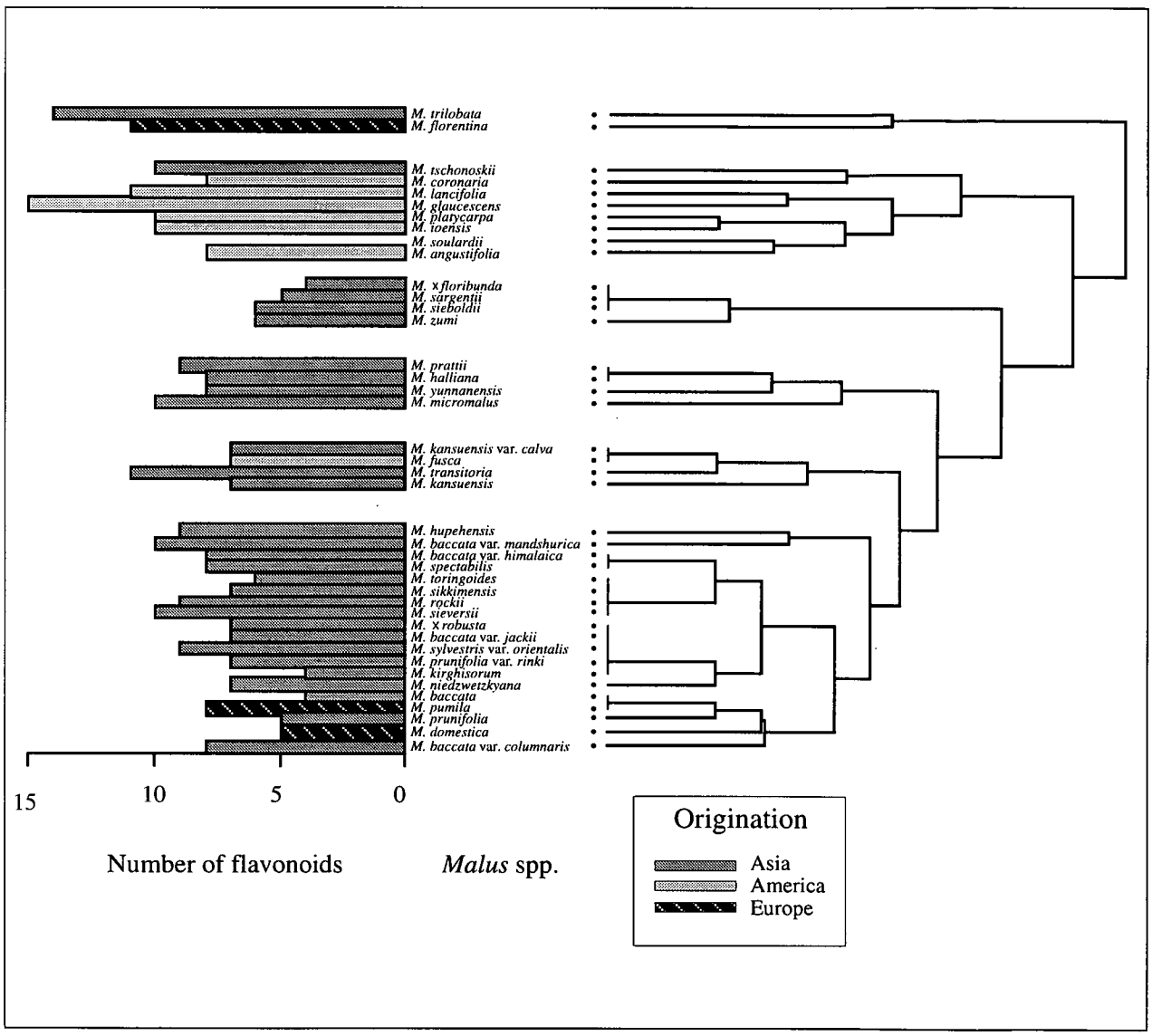


Figure 5.4. Cluster analysis of flavonoid compounds and number of flavonoids in *Malus* spp.

Table 5.3. Acid hydrolised compounds of crude extract of *Malus* bark.

Taxon	Flavonoids aglycones			
	Quercetin	Kaempferol	Azeleatin	Isorhamnitin
M. niedzwetzkyana	+	-	-	?
M. kirghisorum	+	-	-	-
M. prunifolia	+	-	-	?
M. domestica	+	-	-	?
M. sylvestris ssp. *orientalis*	+	-	-	-
M. spectabilis	+	-	-	-
M. baccata	+	-	-	-
M. baccata var. *mandshurica*	+	-	-	?
M. halliana	+	-	-	?
M. sikkimensis	+	-	-	?
M. rockii	+	-	-	-
M. floribunda	+	-	-	-
M. zumi	+	-	-	-
M. sieboldii	+	-	-	-
M. sargentii	+	-	-	-
M. florentina	+	-	+	-
M. toringoides	+	-	-	-
M. kansuensis var. *calva*	+	-	-	-
M. kansuensis	+	-	-	-
M. prattii	+	-	-	-
M. yunnanensis	+	-	-	-
M. glaucescens	+	+	+	-
M. coronaria	+	+	+	-
M. lancifolia	+	+	+	+
M. ioensis	+	+	+	+
M. tschonoskii	+	+	+	-

Symbols; + present; - absent; ? doubtful identification

series *Kansuenses*. However, this is not compatible with a classification based on flavonoid analysis.

Cluster C:

M. yunnanensis and *M. prattii* from series *Yunnanenses*, *M. halliana* of series *Baccatae* and *M. micromalus* of series *Pumilae* have been grouped in Cluster C. Therefore this cluster is not completely compatible with Rehder's classification.

Cluster D:

Cluster D consists of members of series *Sieboldianae*, which is completely compatible with Rehder's classification.

Cluster E:

Cluster E includes section *Chloromeles* along with *M. tschonoskii*. Presence of componds Az and G make these two taxa cluster close together. However, in Rehder's classification *M. tschonoskii* is a member of section *Docyniopsis*.

Cluster F:

Cluster F, which shows the least similarity with the other clusters, includes *M. trilobata* and *M florentina.* In Rehder's classification *M. florentina* is a member of section *Sorbomalus*, while *M. trilobata* constitues the unique species of section *Eriolobus*. Therefore cluster F is not compatible with Rehder's Classification.

Williams (1961, 1966) found that all the species of series *Sieboldianae* contain a dihydrochalcone, sieboldin, which does not occur in other species of *Malus*. He also found another dihydrochalcone, trilobatin, confined to series *Erioiobus*. In 1966 Williams found that phloridzin occurs in all other species of genus *Malus* except the two mentioned series. In the present study also I found the compound phloridzin in all the series except *Sieboldianae* and *Eriolobus*. In the two mentioned series two other compounds with slightly different Rf. values were found which according to Williams should be sieboldin and trilobatin, respectively.

The presence of dihydrochalcones (phloridzin and its derivatives, sieboldin and trilobatin) in all the species of *Malus* shows the monophyletic origin of this genus.

M. sieversii and *M. micromalus* are distinct from other members of series *Pumilae* in having compound B. *M. sieversii* also contain compounds L and I_1 which are not found in any other members of series *Pumilae.*

It has been suggested that, in the angiosperms, advancement is accompanied by reduction in the complexity of the phenolic pattern and also the production of a few new compounds (Gornall and Bohm, 1978, cited by Williams, 1982, see also section 5.4). Having two more compounds in *M. sieversii* than other species of series *Pumilae* could confirm that *M. sieversii* is more primitive than other species of series *Pumilae.* This result supports Ponomarenko (1991) who suggested that *Malus sieversii,* a wild central - Asian species, is the ancestor of *Malus domestica.*

In series *Kansuenses* Rehder all the members except *M. toringoides* possess compounds G, and H, while *M. toringoides* possess compound flavonol glycoside A (which was not observed in other members of series *Kansuenses*) and also I_2, characteristic of the *Baccatae.* It seems that *M. toringoides* from the point of view of its chemical constitution is different from other members of *Kansuenses* and closer to the *Baccatae.* Morphologicaly, *M. toringoides* has involute vernation in common with the *Baccatae.* There are two possibilities to explain the lack of compounds G and H, one possibility is that *M. toringoides* during its evolution has lost the ability to produce these two compounds or that primitively never acquired the ability to make them.

In series *Sieboldianae* all the members contain flavonol glycoside A except *M. zumi.*

Rehder (1940) placed *M. florentina* in Section *Sorbomalus. M. florentina* contains flavonol glycoside E, and C and compounds H, Az and J which do not occur in any other members of section *Sorbomalus,* therefore chemical evidence confirms the Huckins modification (1976) of placing *M. florentina* in a seperate section *Florentinae.*

M. fusca, the north American species of the series *Kansuenses* has similar chemical constitution to *M. kansuensis* and *M. transitoria* from north west China, This suggests that it is correctly placed with other members of series *Kansuenses.* Although

M. fusca and series *Chloromeles* occur in North America, *M. fusca* is chemically distinct from section *Chloromeles* in lacking Az , M, and W and it is also quite morphologically distinct from them.

Huckins (1972) placed *Yunnanenses* and *Kansuenses* close together in section *Sorbomalus*. Chemically compounds G and H which exist in all *Kansuenses* except *M. toringoides* are not present in *Yunnanenses*. Therefore chemical data do not support this relationship, although peroxidase phenotypes are very similar.

The flavonoid patterns in *M. trilobata* and *M. florentina* are very similar which shows affinity between two species but they differ in the presence of compounds G and H in *M. florentina* which are absent from *M. tribobata*.

Although morphologically *Yunnanenses* is the most primitive taxon in the genus. Considering the idea of Bate-Smith that reduction in the complexity of the phenolic pattern is an advancement. Chemotaxonomic evidence is not compatible with morphological results. *M. trilobata* has the most complex pattern of distinctive compounds, G, H, Az, M, W, J, Fe which are absent in series *Pumilae, Sieboldianae, Yunnanenses,* and most species of the series *Baccatae*.

M. tschonoskii and section *Chloromeles* have G, H, Az, M, W and J. *M. florentina* has G, H, Az and J. Series *Kansuenses* has G and H. This succession of reduction of compounds shows that chemically *M. trilobata* is the most primitive taxon in the genus. The other primitive taxa are *M. tschonoskii*, *M. florentina* and series *Chloromeles.* Series *Pumilae*, *Sieboldianae*, *Baccatae* and *Yunnanenses* are considered advanced. Series *Kansuenses* is considered an intermediate taxon in the genus. This evidence is compatible with morphological results except for series *Yunnanenses* that morphologically is considered a primitive taxon.

5.5. Genera related to *Malus*

5.5.1. Introduction

There is no reported naturally occurring intergeneric hybrid between *Malus* and any other genera of Maloideae, although there are hybrids produced during plant

breeding programs, such as *Pyrus* x *Malus*, *Crataegus* x *Malus* and *Chaenomeles* x *Malus*. The only reported natural intergeneric hybrid in *Malus* is *Malus* x *Torminaria* postulated by Browicz (1970) as the origin of *M. florentina.*

The aim of this section is to investigate chemical similarity between *Malus* and the related genera, *Pseudocydonia*, *Chaenomeles* and *Sorbus* section *Aria.*

5.5.2. Result and discussion

The flavonoid constitution of the species examined is presented in Table 5.4. Their chromatograms are in Figure 5.5 and 5.6. Rf value and colour reaction are presented in Table 5.5.

Phloridzin, a compound which is characteristics of *Malus* species is not found in any other related genus examined.

Only compounds Fb, K, Ca, Cb, Cd, Fd and G are shared by *Malus* and the other genera examined. Among these compound Fb, Fd and G are taxonomically important in *Malus*. Although it has been suggested that *Chaenomeles*, *Pseudocydonia* and *Sorbus* section *Aria* have affinity with *Malus*, this is not supported by the chemotaxonomic data.

Among the *Sorbus* species examined, *S. vestita* is distinct from others by having compounds m, n, o and p, which are not found in others specimens examined.

Leaves of all the species examined contain Ca and Cb except one specimen of *S. aria* and *S. takhtajanii* which lack compound Cb.

S. alnifolia, *S. caloneura*, *S. keisseleri* and *S. zahlbruckneri* make a group containing compound c which is not found in other species.

S. chamaemepilus and *S. torminalis* are very distinct from other species of the genus *Sorbus*. *S. chamemespilus* is the only species to contain compound r. Compound s is only found in *S. chamaemespilus* and *S. torminalis*, which represent separate monotypic subgenera of *Sorbus*. Compound s also is found in *Pseudocydonia*. Compound q is only found in *S. torminalis* and *Pseudocydonia.*

Table 5.4. Occurrence of flavonoids in leaf and bark in some species in genera related to *Malus*.

Taxon	Fa	b	c	Ca	Cb	Fd	g	G	i	j	B	l	m	n	o	p	q	r	s	Cc	v	w	x	y
Sorbus wardii (l)	+	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aria (l)	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
E3																								
S. aria (l)	+	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Strasburg																								
S. lanata (l)	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. takhtajanii (l)	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. chamaemespilus (l)	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
S. alnifolia (l)	+	-	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. caloneura (l)	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. keissleri (l)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. meliosmifolia (l)	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. torminalis (l)	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Hungary																								
S. torminalis (l)	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Kent																								
S. zahlbruckneri (l)	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. vestita (l)	+	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Pseudocydonia sinensis (l)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Chaenomeles cathayensis (l)	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-

Cont'd. Table 5.4. Occurrence of flavonoids in leaf and bark in some species in genera related to *Malus*.

Taxon	Fa	b	c	Ca	Cb	Fd	g	G	i	j	B	l	m	n	o	p	q	r	s	Cc	v	w	x	y
Chaenomeles x hybrida (l)	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaenomeles lagenaria (l)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
Sorbus wardii (b)	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E3																								
S. aria (b)	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srasburg																								
S. lanata (b)	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
S. takhtajanii (b)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. chamaemespilus (b)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. alnifolia (b)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. caloneura (b)	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
S. keissleri (b)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. meliosmifolia (b)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. torminalis (b)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hungary																								
S. torminalis (b)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kent																								
S. zahlbruckneri (b)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudocydonia sinensis (b)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyrus pashia (b)	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Cont'd. Table 5.4. Occurrence of flavonoids in leaf and bark in some species in genera related to *Malus*.

Taxon	Fa	b	c	Ca	Cb	Fd	g	G	i	j	B	l	m	n	o	p	q	r	s	Cc	v	w	x	y
Pyrus cordata (b)	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

All the specimens in this study were provided from Ness Botanic Gardens, Neston, Liverpool.

Abbreviation; l: leaf, b: bark

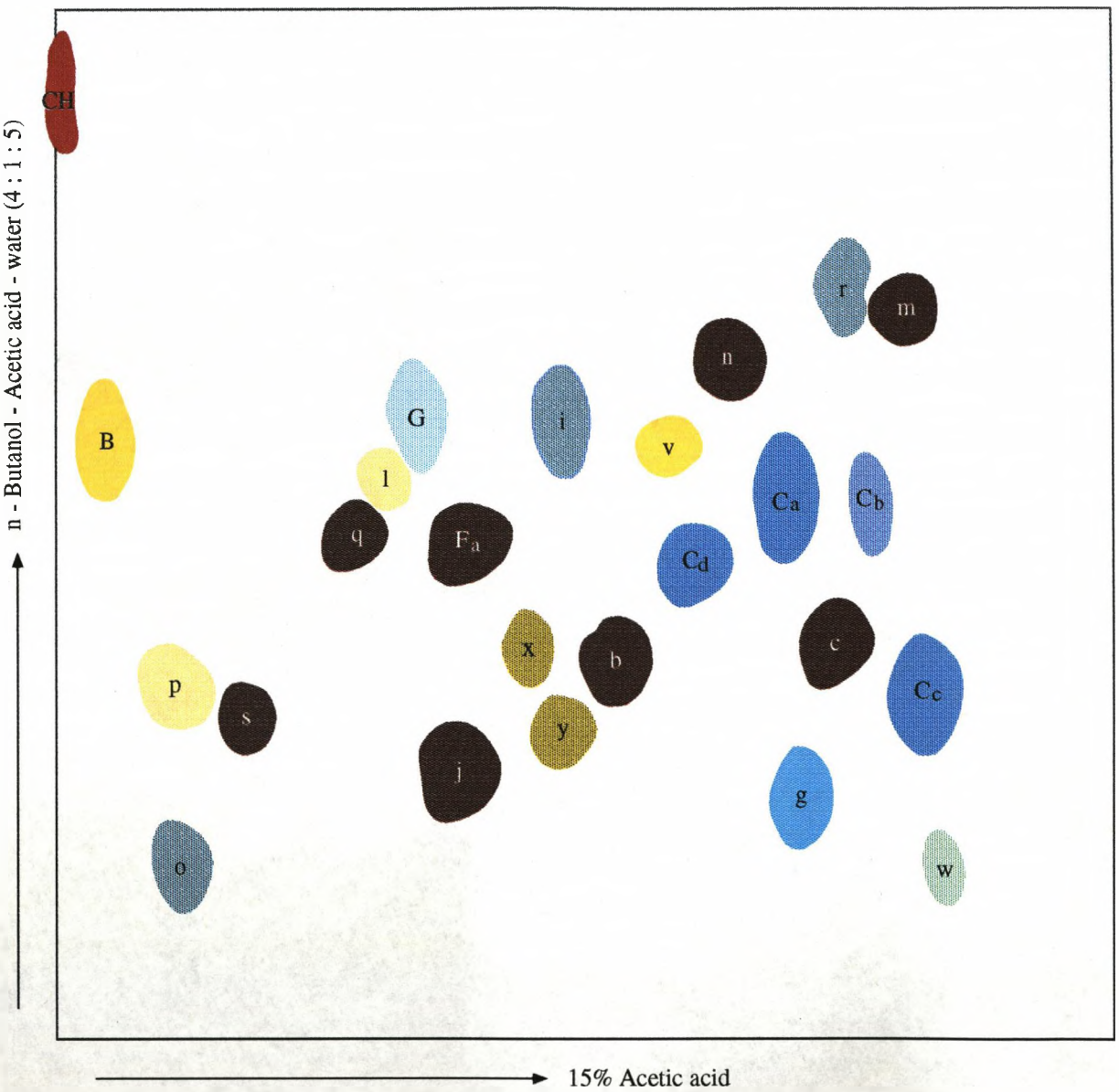


Figure 5.5. Paper chromatographic location of flavonoids of some *Sorbus* section *Aria*, *Sorbus chamaemespilus*, *Chaenomeles* and *Pseudocydonia*.

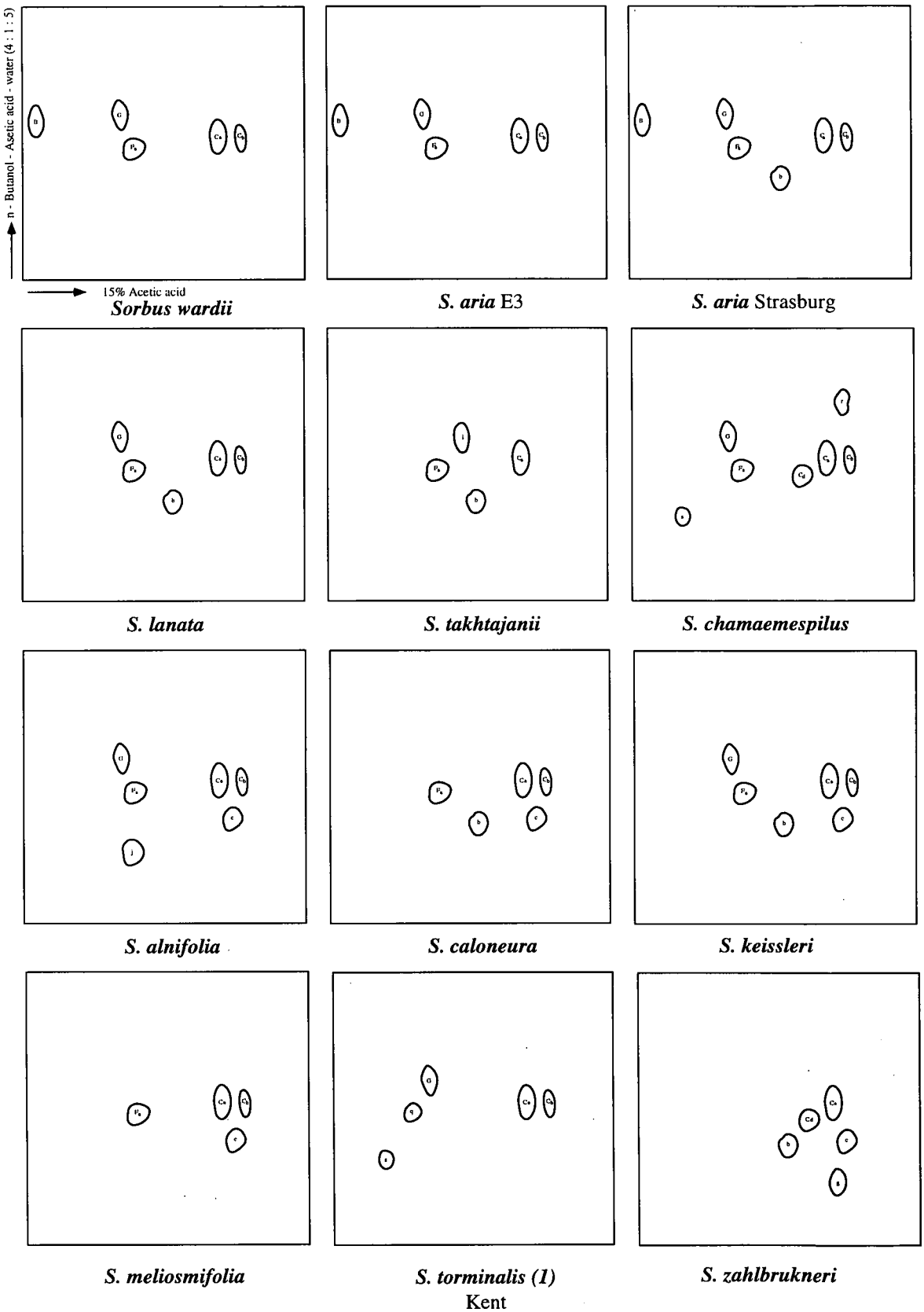
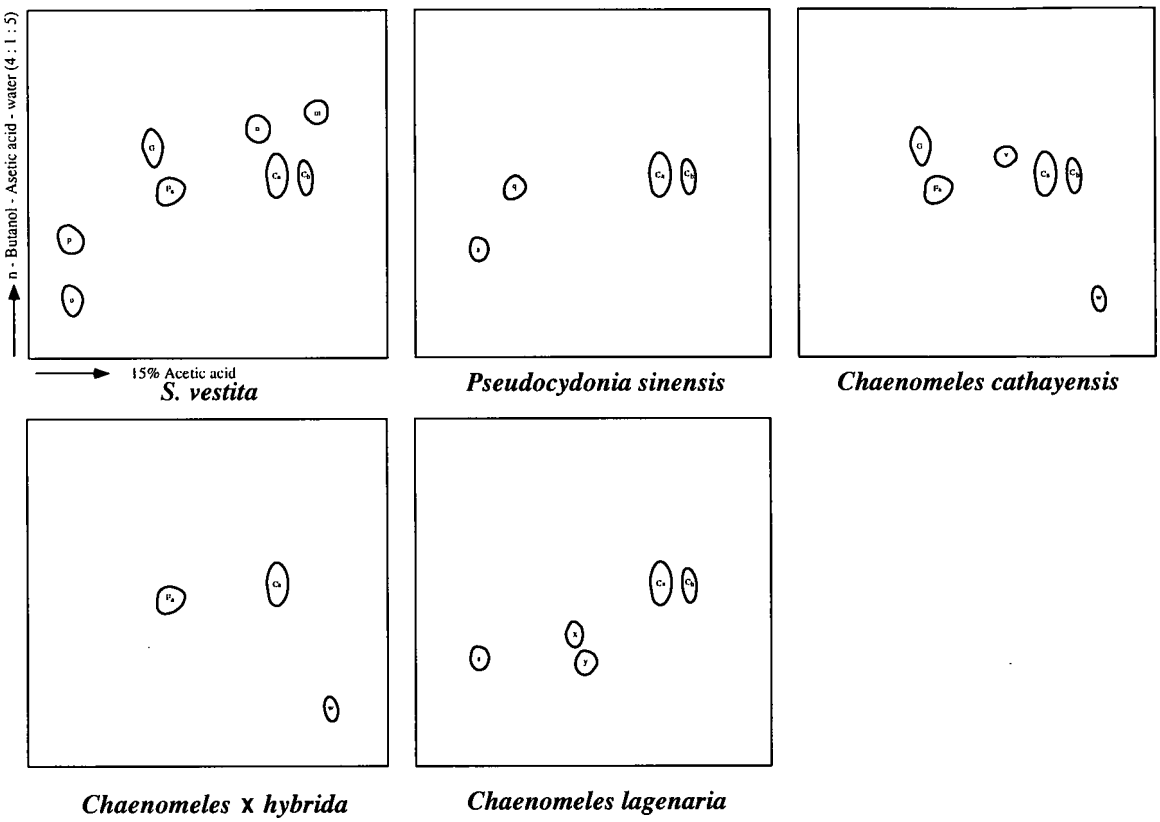


Figure 5.6. Paper chromatographic location of bark flavonoids in some species related to *Malus*.



Cont'd. Figure 5.6. Paper chromatographic location of bark flavonoids in some species related to *Malus*.

Table 5.5. Flavonoid spots, Rf. values and colour reaction of some species related to *Malus*.

Spots code	UV. colour	UV+ NH_3	Rf. values	
			BAW	HoAc
Fa	dark	yellow	49	39
b	dark	yellow	37	52
c	dark	yellow	39	74
Ca	blue	green- blue	53	68
Cb	blue	u.c	53	77
Fd	dark	yellow	46	60
g	blue	u.c	27	70
G	blue	u.c	61	33
i	blue	u.c.	61	47
j	dark	yellow	26	36
B	yellow	yellow	59	3
l	yellow	orange	54	30
m	dark	yellow	71	79
n	dark	yellow	66	63
o	blue	u.c	18	11
p	yellow	u.c	35	9
q*	dark	yellow	50	26
r	blue	u.c	73	74
s**	dark	yellow	32	16
Cc	blue	blue	34	81
v	dark pink	pink	58	58
w	greenish yellow	u.c	17	84
x	dull yellow	u.c.	38	45
y	dull yellow	u.c.	31	48

abbreviation; BAW butanol: acetic acid: water; HoAc acetic acid : water; u.c unchanged

* probably vitexin (Challice and Kovanda, 1978a,b)

**probably luteolin 7-O- diglucoside (Challice and Kovanda, 1978a,b)

Therefore *Pseudocydonia* is closer in flavonoid constitution to *S. chamemespilus* and *S. torminalis* than to *Malus*.

It has been suggested that *Pseudocydonia* is intermediate between *Cydonia* and *Chaenomeles* (Robertson *et al.*, 1991). Chemically *Pseudocydonia* contains compound s which is found elsewhere only in *Chaenomeles*.

It has been suggested that *M. florentina* has hybrid origin and its parents are *M. sylvestris* (European wild crab apple) and *Sorbus torminalis*.

Study of the flavonoids pattern in all three species shows that;

1. *M. sylvestris* contains 8 compounds: k, Fb, Fd, Ph, Ca, Cb,Cc and B.
2. *M. florentina* contains 11 compounds: K, Fa, Fb, Fc, Ph, Ca, Cb, Cc, Cd and B.
3. *S. torminalis* contains 5 compounds: Ca, Cb, Cd, G, q and s.
4. *M. florentina* and *M. sylvestris* have compounds K, Fb, Ph, Ca, Cb, Cc and B in common, most of these also being found in many other *Malus* species.
5. *M. florentina* and *Sorbus torminalis* only have compounds, Ca, Cb, and Cd in common. Compound Cd is not found in any other *Sorbus* spp. examined in this study. However, it is found in *Pseudocydonia* and *Pyrus cordata* bark.

Meanwhile compound s and q which occur in *Sorbus torminalis* are not observed in any other *Malus* species.

Sorbus torminalis bark is very poor in flavonoid compounds, the only compound found in a specimen from Hungary is compound Ca. In contrast *M. florentina* bark is very rich in flavonoids and has compounds which neither *M. sylvestris* nor *M. torminalis* contain: h, Fc, Az, Fe, J and I_2. The only taxonomically valuable compound which is found in both *M. florentina* and *Sorbus torminalis* is compound G in bark of *M. florentina* and leaf of *Sorbus torminalis*. This compound is

also found in other *Sorbus* and *Malus* species leaves as well. Therefore, it can be concluded that *M. florentina* is likely to be a distinct species with no relationship with *S. torminalis*. It is one of the species which contain many flavonoids (12) compared to other *Malus species*. It has been suggested that species with more flavonoids are more primitive than the species with fewer flavonoids. On this basis, *M. florentina* can be considered as a primitive species with its special characteristics and close to *M. trilobata* in flavonoids, as shown in Figures 5.3 and 5.4.

This study showed that chemically the genus *Malus* is very distinct from the proposed related genera.

CHAPTER 6

CHAPTER 6

ISOZYME VARIATION IN *MALUS*

6.1. Introduction

6.1.1. Definition

Enzymes may exist in the same species in more than one molecular form. Such multiple molecular forms of an enzyme in a single species have been designated isozymes (Markert and Moller, 1959 cited by Scandalios, 1969). Isozymes may differ in primary structure because they are encoded in different genes, either allelic or non allelic. The primary structure may be further modified by conjugation of molecules with reactive groups, such as amino, carboxyl, or hydroxyl groups of the amino acid residues of polypeptide chain.

Electrophoretic analysis is used to produce zymograms which show discrete bands representing isozymes, the products of distinct alleles. Isozyme analysis has offered a possible alternative method for cultivar identification of several crops including apple (Weeden and Lamb, 1985).

6.1.2. Starch gel electrophoresis

Starch gel is one of a wide variety of supporting media that can be used for horizontal zone electrophoresis. Such gels are prepared by disolving starch in an appropriate buffer solution. The choice of buffer is somewhat empirical and a wide variety of compositions have been used successfully. A characteristic feature of starch gels is that they exhibit molecular sieving effects. Separation of proteins is achieved, therefore, not only on the basis of differences in charge, but also of differences in molecular size and shape. The contribution of these factors, however, is difficult to control because a starch gel of any particular concentration will contain a range of pore sizes and there is no way of knowing what these are (Shaw and Prasad, 1970).

6.1.3. Previous studies on isozyme systems in apple

Previous studies on several isozyme systems have demonstrated that isozyme polymorphism does exist in apple. Weeden and Lamb (1985) pointed out that 2 isozyme systems, 6-phosphogluconate dehydrogenase and asparate aminotransferase are useful for differentiating among the cultivars because of high levels of genetic polymorphism in these systems. The reason for such considerable variability may be that the loci specifying the isozymes are in regions of the genome that have particularly diverse origins within *Malus*. The study of polymorphic enzyme systems in apple can generate information on linkage groups present in the genome, phylogenetic relationships among species, and biochemical evolution of a polyploid (Weeden and Lamb, 1987). Published isozyme analyses at the species level in *Malus* are few.

Peroxidases are one of the most widely investigated enzyme systems, being highly variable within higher plants and highly polymorphic in many genera. Peroxidases are usually characterized as monomeric enzymes and by the occurrence of null alleles. Phenotypic comparison of banding patterns observed in zymograms of peroxidases have been used for the identification of apple cultivars and rootstocks. (Misic *et al.* 1980; Mendendez,1986; Vinterhalter and James 1983; Quarta and Arone 1987, cited by Manganaris and Alston, 1992, 1993).

Cheng (1986) examined isoperoxidases in 13 species in the genus *Malus* by polyacrylamide gel plate electrophoresis. He recognised 3 regions with slow, medium and fast mobility, and a total of 20 isoperoxidases were distinguished. Manganaris and Alston (1993) suggested that four loci, PRX2, PRX3, PRX4 and PRX7 are involved in peroxidase phenotypes including 15 alleles, the first two recognised in species. They classified the species and derivatives examined by them into 20 phenotypic classes. They reported that two apples *M. trilobata* and *M.* x *robusta* 'Erecta' did not show any activity in the PRX2 and PRX3 zone.

Comparison of *M. fusca* and section *Chloromeles* by Dickson *et al.* (1991) showed that although *M. fusca* displays isozyme mobility patterns quite distinct from those of section *Chloromeles*, the number of loci expressed is the same for at least

four of the six enzymes. Genetic identity estimates between *M. fusca* and section *Chloromeles* species are relatively low when compared to those of other congeneric plant species. They concluded that there has been no recent gene flow between eastern and western North American *Malus* species.

6.2. Material and methods

6.2.1. Samples' sources

Materials for this study were kindly provided by Royal Botanic Gardens Kew; Royal Botanic Gardens Edinburgh; Ness Botanic Gardens, Liverpool; OBST Gene Bank, Germany; and National fruit Collection, Brogdale.

6.2.3. Starch gel electrophoresis

Starch was selected as the preferred medium for electrophoresis because of its non-toxic nature, the relatively simple and inexpensive apparatus required for such gels, and the capability of obtained multiple slices from a single gel.

Preparation of starch gel

A technique for horizontal starch gel electrophoresis was developed from the procedure of Weeden and Lamb (1987). A former made of four perspex strips of the following dimensions , two being 215 mm and two being 140 mm was laid out on a glass plate using 6 clips as holders to give a muld of internal dimensions 140 x 180 x 6 mm.

A 12% starch gel was prepared by suspending 36 g of Sigma potato starch in 300 cm^3 of 9 parts Tris - Citrate pH 8.1, 0.2 M and 1 part lithium borate buffer (pH 8.1, 0.2 M). This suspension was heated with vigorous stirring until a low viscosity starch solution was obtained which was then rapidly degassed by applying a vacuum to the flask for approximately 5s. A glass lid was pressed into place, the gel allowed to cool to room temperature during the night and then placed in cold room at 4° C prior to use. Gels were used a day after preparation.

Sample preparation and application

Extracts were prepared by macerating 4 cm^2 leaves with 1 cm^3 of 50 mM Tris malaete pH 8.5 containing: 20% glycerol, 10% soluble polyvinyl prolidin (PVP 100), 0.5% of Triton X-100 and one drop of 2- mercaptoethanol. The last two constituents being added immediately before use. Sample preparation was carried out on ice to prevent the denaturation of enzymes. Wicks were made of 2 cm long and 4 mm wide chromatography paper Whatman no. 3 soaked in the sample solution.

A cut was made 6 cm from the cathode end of the gel and samples lined up evenly along one surface of the origin. The cut gel was then pressed tightly against the samples.

Electrophoresis

The gel plate was placed in an electrophoresis tank and sponge sheets soaked in tank buffer (lithium - borate, pH 8.1, 0.2 M) applied to both ends of the gel, overlapping the gel surface by 3-4 cm. The surface of the gel was covered by cling film to prevent evaporation from the gel. The whole tank was kept in a cold room at 4°C to keep the gel cold during electrophoresis. The electrophoresis was carried out with a constant 35 mA current for 20 min. after which the power supply was switched off, the wicks were removed, the gel sections pushed firmly together, and electrophoresis continued with a 50 mA current until the dye front had moved 8 cm anodal to the sample origin. Electrophoresis took approximately 4 hours.

The gel was then sliced using nylon line with double 2 mm thick perspex strips used as guides. Three slices were obtained, the top being discarded and the others being transferred to staining boxes.

Staining techniques and enzyme assays

Assay for Peroxidase

Assay for peroxodase followed the method of Weeden (1984) as presented below:

0.04g 3- amino- 9 ethylcarbazol dissolved in 4 ml N, N dimethylformamide and added to 50 ml sodium acetate buffer 0.1 M, pH 5 and 2 drops of hydrogen peroxide added just before use. The gel was kept in the dark at room temperature and after 10 min the appeared bands were read.

Assay for Glucose Phosphate Isomerase

Assay for Glucose Phosphate Isomerase following Chyi and Weeden (1984) and Weeden and Lamb (1987) as presented below:

Fructose 6- Phosphate	0.012 g
NAD	0.016 g
Leuconostoc glucose 6- phosphate dehydrogenase	24 units
MTT	0.006 g
Meldula Blue	0.0001 g

The above material added to 50 ml of 0.1 M. Tris HCl pH 8. The gel kept in dark at 37°C for 30 min until the bands appeared.

Assay for Glutamate Oxaloacetate Transaminase (GOT)

Tris -HCL . 0.1 M. , pH. 7.5	50 ml
L Aspartic acid	0.05 g
α - Ketoglutaric acid	0.05 g
Pyredoxal	0.0025 g
Fast green	0.005 g

6.3. Results

6.3.1. Peroxidase phenotypes

I recognised a total of 11 bands of peroxidase isoenzymes in my gels, shown diagramatically in a composite zymogram in Figure 6.1. Peroxidase phenotypes of individuals examined are presented in Figure 6.2.

The zone of Rf. values is from 0.45 to 0.76. The Rf. value of each bands is shown in Table 6.1.

Table 6.1. Rf. value of peroxidase bands. x100

Bands	Rf.
A	46
B	48
C	50
D	54
E	56
F	58
G	60
H	67
I	70
J	73
K	76

Three different zones of peroxidase activity were recognized based on Rf. value of the bands including PRX-I, PRX-II and PRX-III (Figure 6.1). PRX-I including bands A, B, C, peroxidase II including D, E, F, G and peroxidase III including bands H, I, J, K. Isozyme phenotypes for peroxidase phenotype of individuals of species (based on Manganaris and Alston, 1993) is given in Table 6.2.

Table 6.2. Isoenezyme phenotypes for Peroxidase in *Malus* species.

Malus sp.	PRX I	PRX II	PRX III
Pumilae			
M. pumila	ab	ad	-
M. pumila var. *nervosa*	aa	ad	-
M. prunifolia 156.86.01585 RBGK	bb	ab	-
M. prunifolia 73.11832 RBGK	aa	ad	-
M. prunifolia 3.10 OBST	bc	aa	-
M. prunifolia 4.31 OBST	ac	ad	-
M. prunifolia var. *rinki* 0007311.836 RBGK	bb	ad	-
M. p. var.*rinki* 156.86.08412 RBGK	-	ad	-
M. p. var. *rinki* RBGE	bc	dd	-
*M. p.*var.*rinki* 071032 RBGE	bc	dd	-
M. niedzwetzkyana 86.8282 RBGK	aa	ad	-
M. niedzwetzkyana 77.772RBGK	bb	acd	-
M. niedzwetzkyana 7311840 RBGK	bb	acd	-
M. niedzwetzkyana NFC	bc	ad	-
M. kirghisorum 19800633 RBGE	bc	ad	-
M. kirghisorum 76.6237 RBGK	ab	ad	-
M. sylvestris NBG	bb	aa	-
M. sylvestris var. *orientalis* 1988.332 RBGK	bb	aa	-
M. spectabilis 1929.7004 RBGK	-	ad	-
M. spectabilis 158.86.08235 RBGK	bb	ad	-
M. domestica 19912. RBGK	abc	aa	-
M. micromalus 86.8282 RBGK	aa	aa	-
M. micromalus 6.02 OBST	bb	ad	-
Baccatae			
M. baccata 5000416 RBGQ	-	aa	-
M. baccata NBG 1	-	ad	-
M. baccata NBG 2	-	ad	-
M. baccata 13.6 OBST	-	aa	-
M. baccata 5.31 OBST	aa	ab	-
M. baccata NFC.	ab	aa	-
M. baccata H1 RBGE	-	aa	-
M. baccata H2 RBGE	bb	aa	-
M. baccata H3 9103 RBGE	cc	aa	-
M. baccata 19744220 RBGE	-	aa	-
M. baccata 3 NBG	bb	ad	aa
M. baccata 5000416 RBGK	-	aa	-
M. b. var. *mandshurica* 66530 RBGE	-	ad	aa
M. b. var. *mandshurica* 156.86.01560	-	ad	-
M. b. var. *himalaica* 156.86.01560 RBGK	bb	ad	-
M. b. var. *jackii* 1982.8354RBGK.	bb	ad	-
M. b. var. *jackii* RBGK	-	ad	-
M. b. var. *columnaris* RBGK	aa	aa	-
M. halliana 6.25 OBST	-	aa	-
M. halliana 5.67 OBST	-	ad	-
M. halliana 1912003 RBGE	-	ab	-
M. halliana 1986.8395 RBGK	aa	ad	-
M. halliana 1982.8313 RBGK	-	aa	-
M. halliana 1912003 RBGE	-	ab	-
M. hupehensis 1981017 RBGE	-	ad	-
M. hupehensis 8354 RBGK	bc	aa	-
M. hupehensis	bc	aa	-
M. hupehensis 5.64 OBST	aa	aa	-
M. hupehensis 5.58 OBST	bc	ac	-
M. hupehensis NFC	bc	ad	-
M. sikkimensis 1 NBG	-	ad	-
M. sikkimensis 2 NBG	-	ad	-
M. sikkimensis 156.86.01594 RBGK	-	ad	-
M. sikkimensis 1966.7029 RBGE	aa	ad	-

Cont'd. Table 6.2. Isoenezyme phenotypes for Peroxidase in *Malus* species.

Malus sp.	PRX I	PRX II	PRX III
M. rockii NBG	cc	ad	-
M. rockii 156.86.015090 RBGK	aa	ad	-
Sieboldianae			
M. sieboldii 872249 RBGE	-	ab	-
M. sieboldii 872249 (2) RBGE	-	ad	-
M. sieboldii 872249 (3) RBGE	-	ad	-
M. sieboldii 872249 (4)RBGE	-	ad	-
M. sieboldii 1993.385 RBGK	-	ad	-
M. sieboldii 1 NBG	-	ad	-
M. sieboldii 2 NBG	-	ad	-
M. sieboldii 1985.655 RBGK	-	ad	-
M. sieboldii 4437906357 RBGK	-	ad	-
M. sieboldii 1979.6351 RBGK	-	ad	-
M. sieboldii 872249 (5) RBGE	-	abc	-
M. sieboldii (6) RBGE	-	abc	-
M. sieboldii 6.76 OBST	-	aa	-
M. sieboldii 6.7 OBST	-	ad	-
M. sieboldii 13.22.1 OBST	-	ad	-
M. sieboldii 6.73 OBST	aa	aa	-
M. sieboldii 6.79 OBST	-	ad	-
M. s. var. arborescens RBGE	-	ad	-
M. s. var. *arborescens* RBGK	-	ad	-
M. sargentii 1986.1591 RBGK	aa	ad	-
M. sargentii 1973.19539 RBGK	-	ab	-
M. sargentii NBG	-	ad	-
M. sargentii 5.85 OBST	cc	ad	-
M. sargentii 6.82 OBST	-	aa	-
M. sargentii NFC	-	ad	-
M. x floribunda 1993.3517 RBGK	ab	ad	-
M. x floribunda 1986.1566 RBGK	-	abd	-
M. x floribunda	-	ad	-
M. x floribunda 6.67 OBST	bb	abd	-
M. x floribunda 'Exzellens Thiel' NFC	-	ad	-
M. x floribundas'Hillieri' NGC	-	ad	-
M. x floribunda NFC	-	ad	-
M. x floribunda 0229 RBGE	-	ad	ab
M. x zumi 19171011 RBGE	-	ab	-
M. x zumi 7.01 OBST	aa	abd	-
M. x zumi 6.85 OBST	-	ad	-
M. x zumi	cc	ad	-
M. x zumi 8.07 OBST	bb	ad	-
M. x zumi 13.25 OBST	bb	ad	-
Florentinae			
M. florentina 8.13 OBST	bb	-	-
M. florentina 1966.5032 RBGE	bb	-	-
M. florentina 86.083398 RBGK	bb	-	-
M. florentina 156.86.08361 RBGK	bb	-	-
Kansuenses			
M. fusca 156.86.08237 RBGK	aa	ad	-
M. fusca 1981.1060 RBGE	bc	-	-
M. fusca 1980.554 RBGE	ab	-	-
M. toringoides 1981.8440 RBGK	bb	aa	-
M. toringoides 101.13.1014 RBGK	bb	ac	ab
M. toringoides 7.25 OBST	cc	aa	-
M. toringoides 1986.8287 RBGK	bb	aa	-
M. kansuensis 156.86.01573 RBGK	bb	aa	-
M. kansuensis 1908. 1034 RBGE	-	aa	ab
M. kansuensis 10341 RBGE	-	bb	bcd
M. kansuensis 1931. 1119 RBGE	bb	aa	bc

Cont'd. Table 6.2. Isoenezyme phenotypes for Peroxidase in *Malus* species.

Malus sp.	PRX I	PRX II	PRX III
M. kansuensis 8.31 OBST	-	abd	aa
M. transitoria 1986.160 RBGK	-	abd	bb
M. transitoria 7.28 OBST	ab	ab	-
Yunnanenses			
M. yunnanensis 1988.4303 RBGK	bb	ad	-
M. yunnanensis 156.860.8236 RBGK	ab	ad	-
M. yunnanensis 868397 RBGK	bc	dd	-
M. yunnanensis 1981.4267 RBGK	-	ad	-
M. yunnanensis 1992.3418 RBGK	cc	aa	-
M. yunnanensis 1 NBG	bb	ad	-
M. yunnanensis 2 NBG	bb	ee	abcd
M. yunnanensis 8.34 OBST	-	aa	-
M. yunnanensis 220113 RBGE	bb	aa	ab
M. y. var. *veitchii* 19250024 RBGE	-	acd	abc
M. y. var. *veitchii* 9.36a OBST	ac	ac	aa
M. y. var. *veitchii* RBGE	-	ad	abcd
Chloromeles			
M. coronaria 1973. 939 RBGK	aa	ad	-
M. coronaria 1968.47713 RBGK	cc	aa	-
M. coronaria Charlottae NFC	cc	aa	-
M. coronaria 9.37 OBST	-	aa	-
M. coronaria NFC	cc	-	-
M. platycarpa 7.37 OBST	bb	aa	-
M. platycarpa 8.37 OBST	-	aa	-
M. platycarpa NFC	bc	aa	-
M. platycarpa 601592 RBGK	bc	aa	-
M. ioensis 8.64 OBST	cc	aa	-
M. ioensis 1933.35418 RBGK	cc	aa	-
M. ioensis 8357 RBGK	cc	aa	-
M. ioensis var. *palmeri* 1986.8032 RBGK	bb	aa	-
M. angustifolia 1986.8314 RBGK	bc	ab	-
M. glaucescens 08280 RBGK	aa	ad	-
Eriolobus			
M. trilobata RBGK	bb	-	-
M. trilobata 7.73 OBST	ab	-	-
M. trilobata 912969 RBGE	bc	-	-
M. trilobata 1980.0351 RBGE	bb	-	-
Docyniopsis			
M. tschonoskii RBGE	-	aa	-
M. tschonoskii NFC	-	aa	-
M. tschonoskii 73.1185.1 RBGK	-	aa	-
M. tschonoskii 86.8211 RBGK	-	ab	-
M. tschonoskii NBG	aa	ad	-
M. tschonoskii 8.73 OBST	-	aa	-

The frequency of peroxidase bands in 9 Series of the genus *Malus* (according to Rehder's classification, 1940) is presented in Table 6.3.

Band D is dominant (highest frequency) in all groups except in series *Florentinae* and *Eriolobus* (frequency = 0). Bands J and K were found only in series *Yunnanenses* and *Kansuenses*. The highest frequency of band A occurred in series *Pumilae* (43%), but this band was absent in series *Florentinae*. Only two series, *Florentinae* and *Eriolobus,* had 100% occurrence of band B, while it is absent in series *Docyniopsis.*

Band C showed the highest frequency in series *Yunnanenses* and was not found in Series *Docyniopsis* and *Florentinae.* This band was fairly evenly distributed amongst the other series. Band E is absent in *Docyniopsis*, *Florentinae*, *Eriolobus* and *Yunnanenses*. Band F is only found in series *Pumilae*, *Baccatae*, and *Yunnanenses* with frequency of 4%, 6%, and 56%, respectively. The highest frequencies of band G were observed in series *Sieboldineae* and *Yunnanensis* (75%). Bands H, I, J, K appeared only in some individuals of series *Pumila*, *Yunnanenses*, and *Kansuenses*. The highest frequencies of bands H, I, J are in series *Yunnanenses* (38%), *Kansuenses* (39%) and *Yunnanenses* (22%), respectively.

The peroxidase zymogram of each individual examined in this study is presented in Figure 6.1 and a composite pattern of all individuals of each species shown in Figures 6.2.

In series *Pumilae* three bands were observed at PRX I, two at PRX II, while PRX III was monomorphic in the samples of series *Pumilae*. Only one individual, *M. prunifolia* shows one band in the PRX III zone. Similarly 3 bands were observed at PRX I and II in series *Baccatae*, while two bands were scored at PRX III.

In *Sieboldianae* there were 3 bands at PRX I and II and two bands at PRX III. In series *Kansuenses* PRX I and II have 3 bands and PRX III has 4 bands.

Table. 6.3. Frequency of peroxidase bands in series of *Malus*.

Series ↓ / Peroxidase bands →	A	B	C	D	E	F	G	H	I	J	K	Total of specimens
Pumilae	0.43	0.73	0.34	0.96	0.02	0.04	0.64	0.02	0	0	0	24
Baccatae	0.13	0.43	0.09	0.99	0.05	0.05	0.58	0	0	0	0	33
Sieboldineae	0.11	0.16	0.33	1	0.19	0	0.75	0	0	0	0	38
Kansuenses	0.29	0.58	0.21	0.78	0.29	0	0.13	0.24	0.39	0.10	0.10	14
Yunnanenses	0.16	0.19	0.53	0.95	0	0.55	0.75	0.38	0.27	0.22	0.11	10
Chloromeles	0.20	0.46	0.18	0.98	0.37	0	0.52	0	0	0	0	15
Docyniopsis	0.14	0	0	1	0	0	0.14	0	0	0	0	7
Florentinae	0	1	0	0	0	0	0	0	0	0	0	5
Eriolobus	0.25	1	0.25	0	0	0	0	0	0	0	0	4

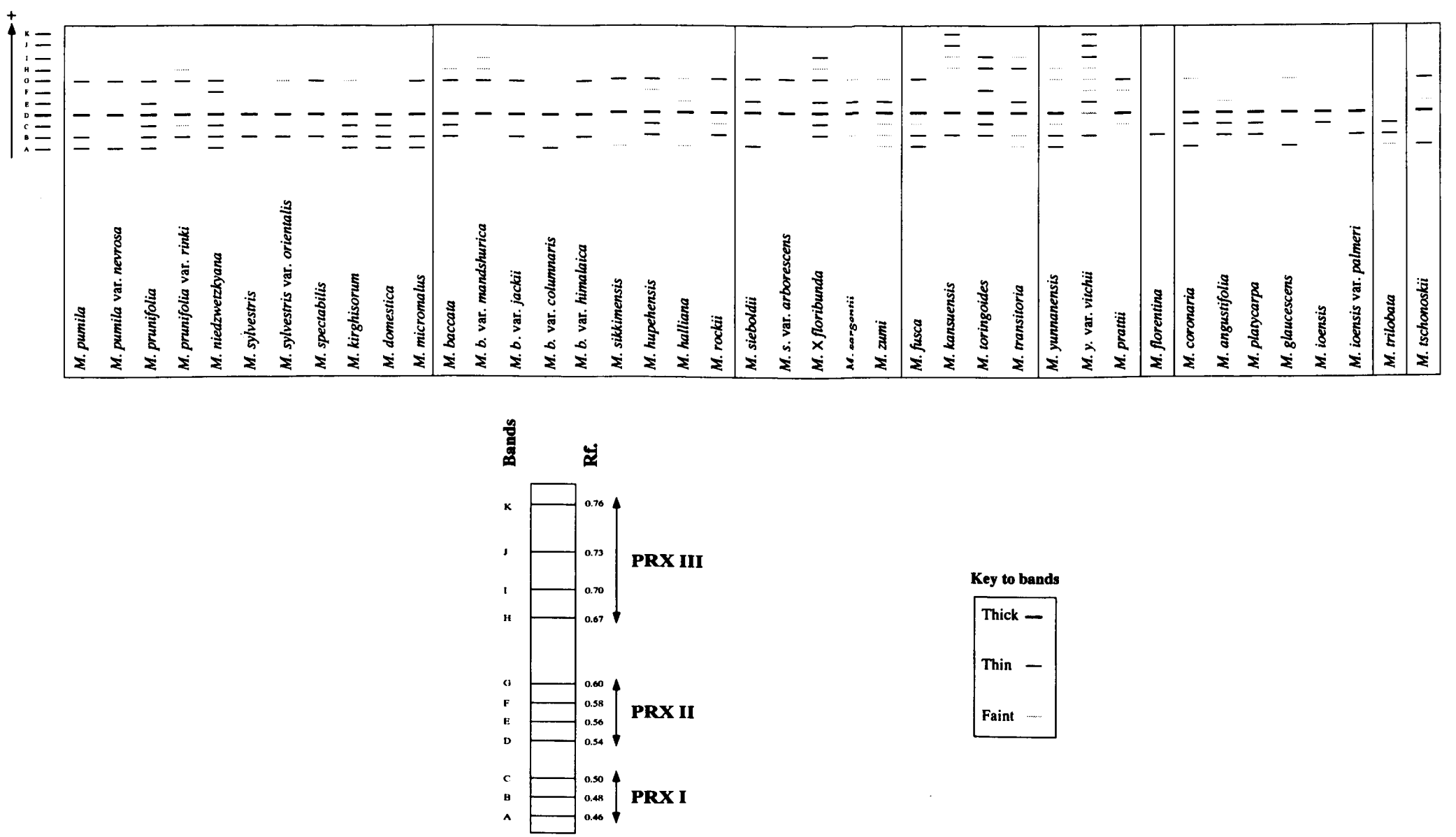


Figure 6.1. The composite peroxidase phenotype pattern of *Malus* species and composite zymogram of peroxidase phenotype.

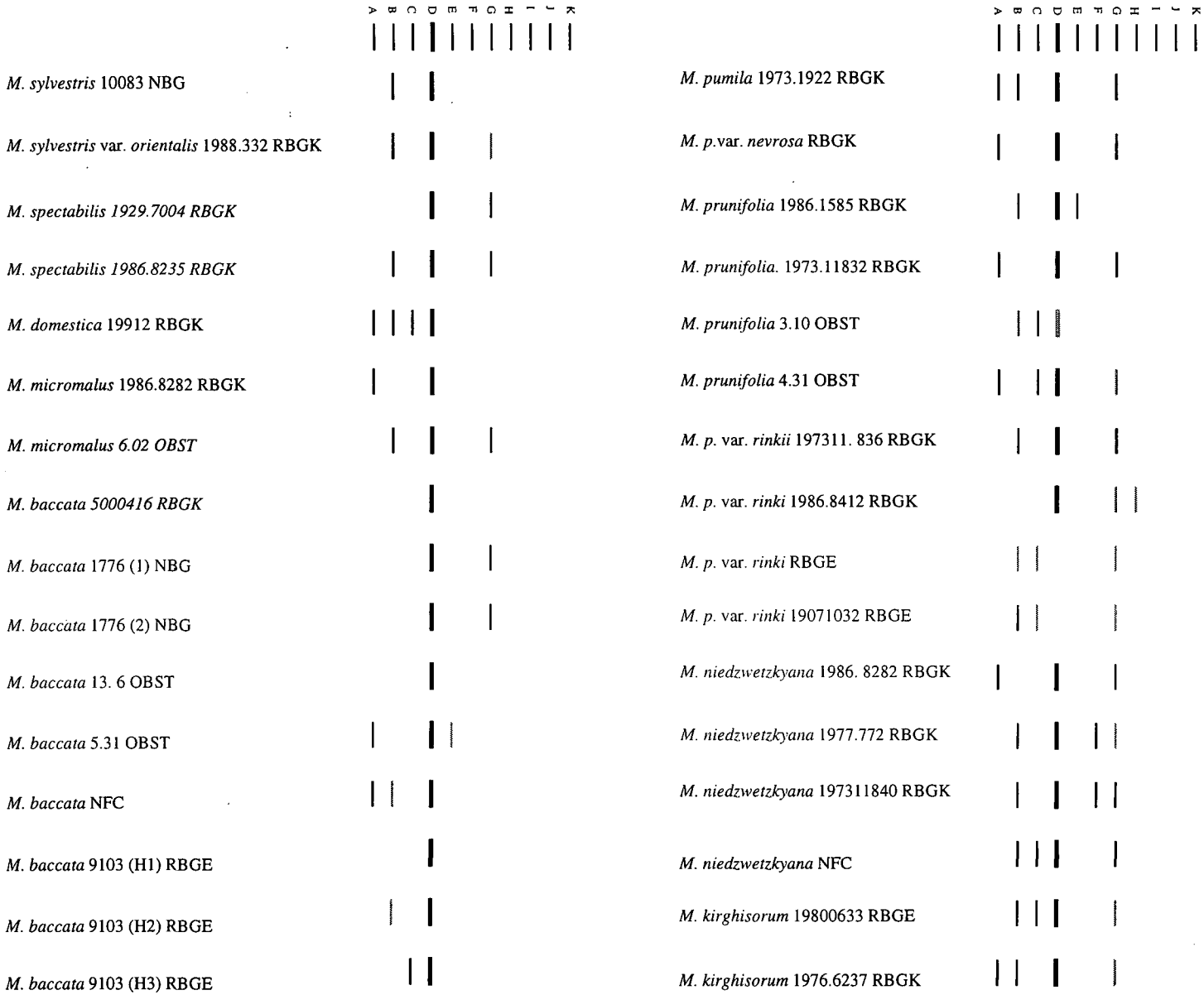
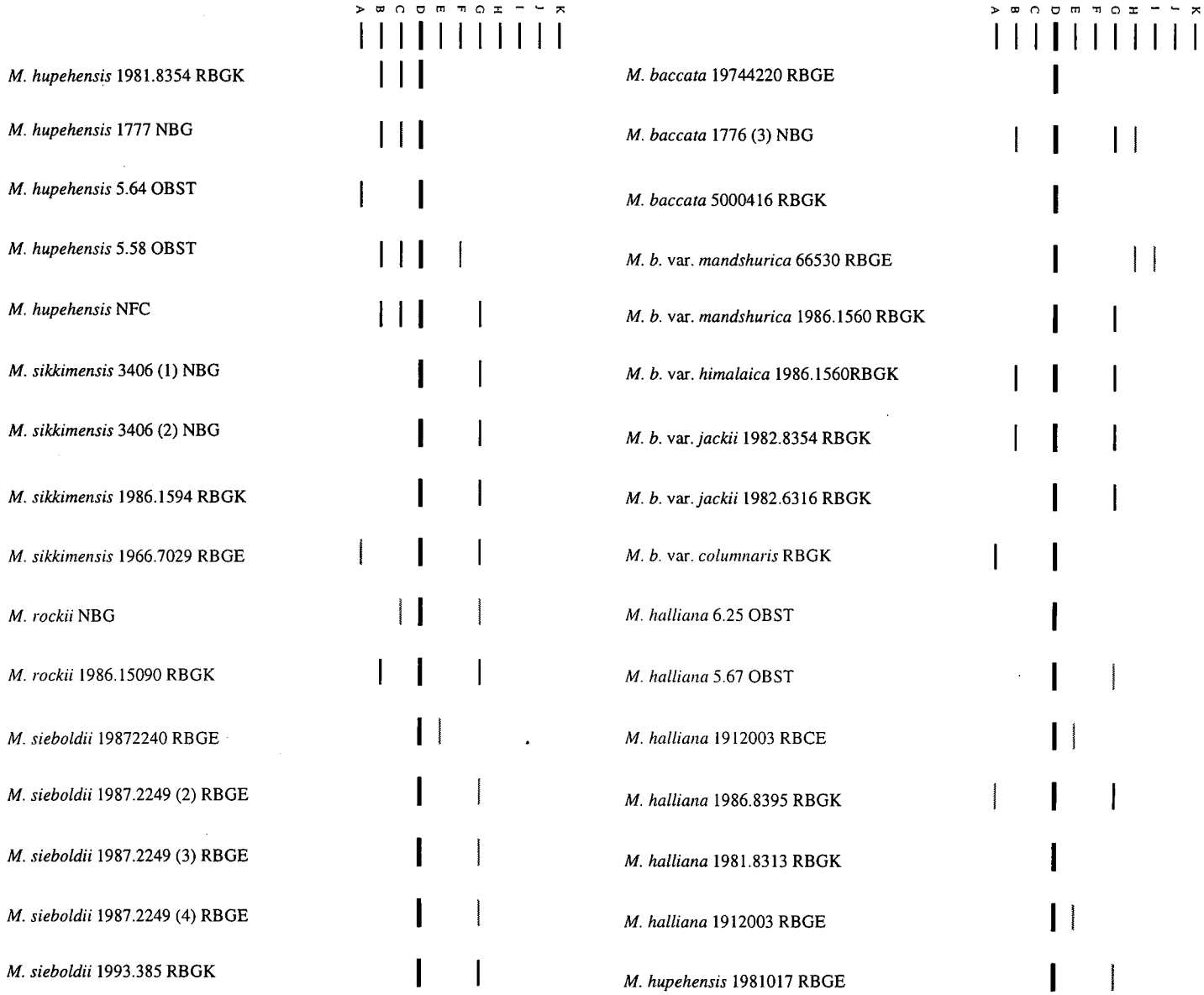


Figure 6.2. Peroxidase phenotype patterns of *Malus* species.



Cont'd. Figure 6.2. Peroxidase phenotype patterns of *Malus* species.

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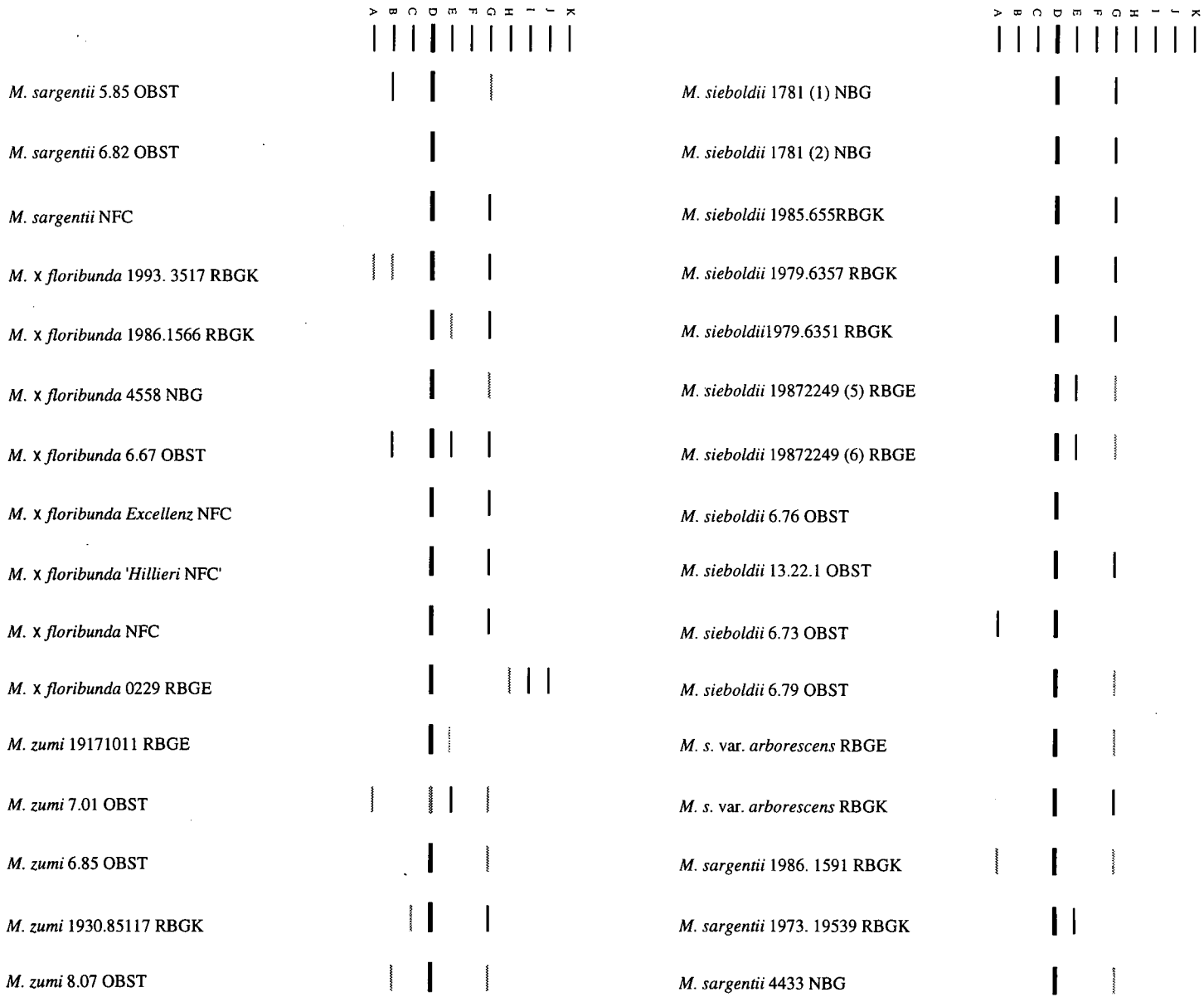
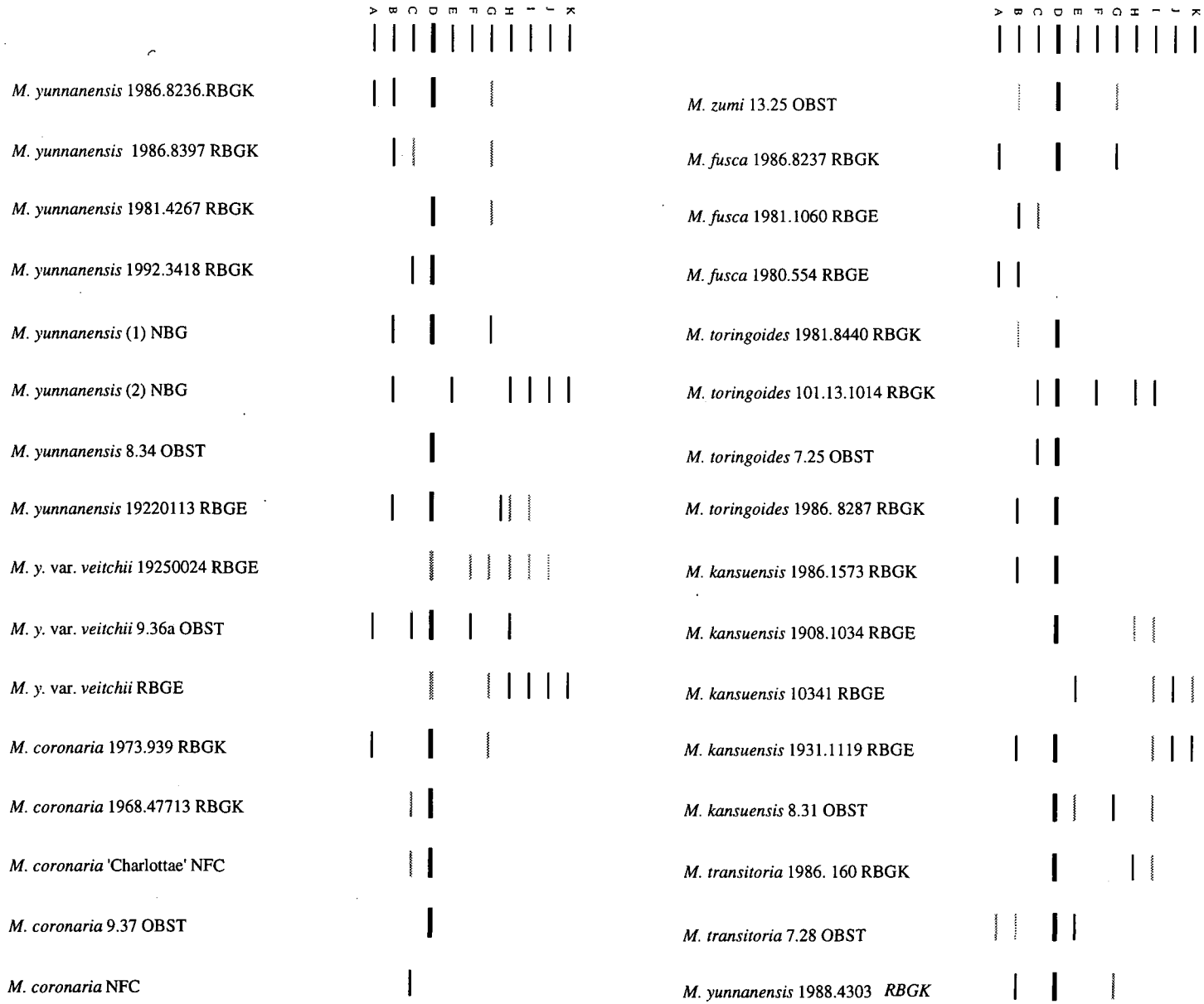
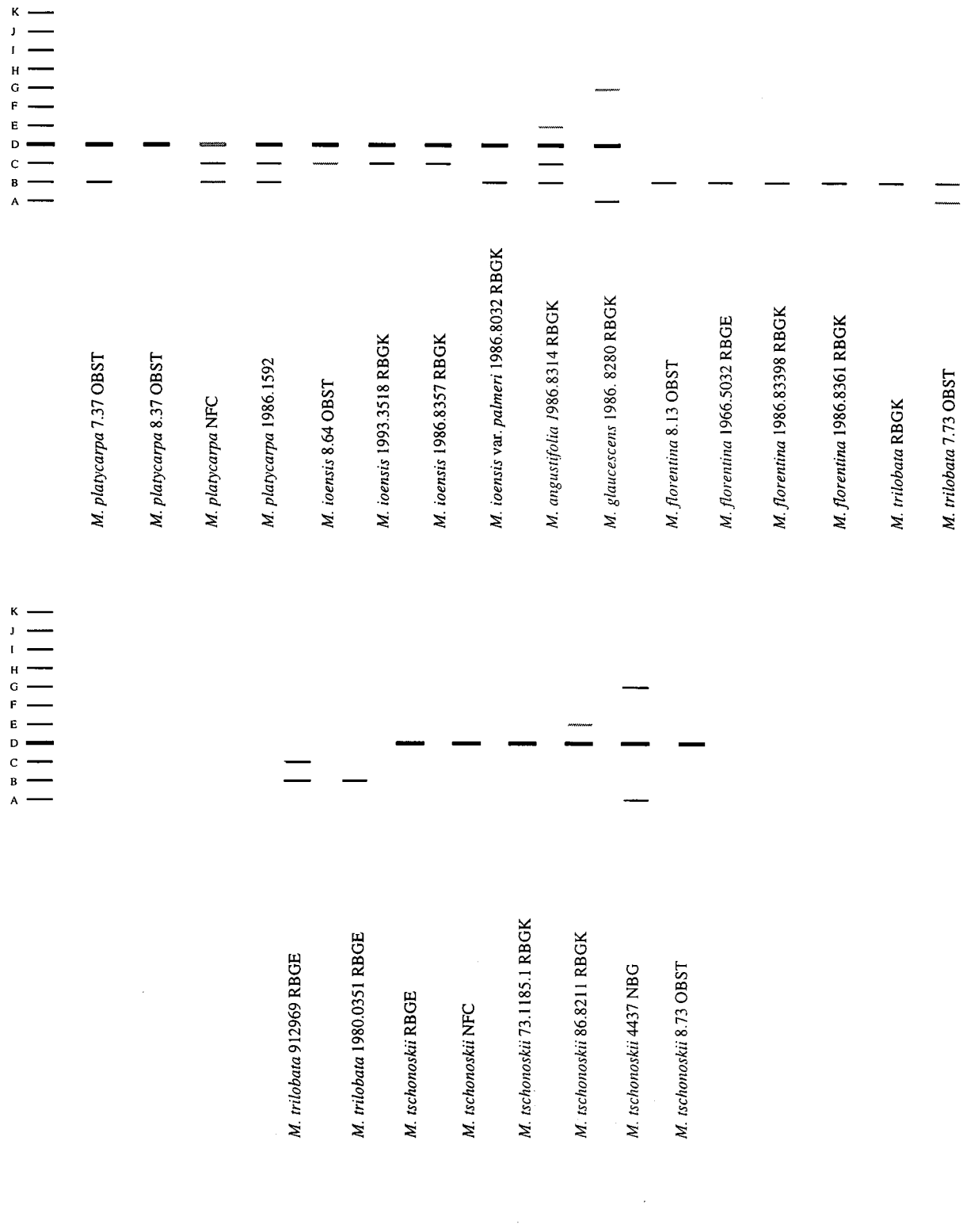




Cont'd. Figure 6.2. Peroxidase phenotype patterns of *Malus* species.



Cont'd. Figure 6.2. Peroxidase phenotype patterns of *Malus* species.

In series *Chloromeles* there are 3 bands were scored for PRX I and PRX II and no activity was observed at PRX III.

In series *Yunnanenses* 3 bands were scored at PRX I, 3 at PRX II and 4 at PRX III. In series *Florentinae* only one band observed at PRX I and no activity observed in location of PRX II and III. *M. trilobata* also shows 3 bands at PRX I and no activity at PRX II and III. In section *Docyniopsis* one band at PRX I and 3 bands at PRX II while no activity was scored at PRX III.

6.3.2 Glucose phosphate isomerase phenotype

Twenty six different species were examined. Due to difficulty in reading the bands of this enzyme only species showing clear bands are discussed.

Two zones of activity were recognised for GPI (GPI I and GPI II) in species of *Malus* examined. No variation among the taxa was detected in the more anodal zone designated GPI I. A total of 5 bands were scored for GPI II in my gel and the Rf. value varied from 0.15 to 0.24. A zymogram of Glucose phosphate isomerase II is presented in Figure 6.3.

The numbers of bands in series *Pumilae* varies from 2 in *M. niedzwetzkyana* to 4 in *M. domestica, M. pumila* and *M. spectabilis.* All the species examined showed bands C and D. The only species showing band B in this group was *M. spectabilis.* Bande A appeared in all the species except *M. niedzwetzkyana.* Band D appeared as a faint band in *M. prunifolia* and *M. domestica.*

In series *Baccatae*, all the species showed band D, while band A appeared in *M. hupehensis* and *M. robusta.* In *M. baccata* and *M. halliana* only band D was observed. *M. rockii* and *M. baccata* var. *himalaica* showed similar patterns (bands D and E). In this group band B appeared only in *M. hupehensis. M. sikkimensis* did not show band A.

Figure 6.3. Glucose phosphate isomerase II phenotypes in *Malus* species.

In series *Chloromeles* only 2 bands are recognised (C and D). *M. coronaria*, *M. angustifolia* and *M. glaucescens* showed band C only, whereas in *M. lancifolia* both bands C and D were observed.

All the bands found in section *Chloromeles* appeared in *M. fusca.*

In *M. tschonoskii* of section *Docyniopsis* three bands B, C, and D were found. *M. florentinae* showed 3 bands C, D, and E.

6.3.3. Glutamate Oxaloacetate Transaminase

Three zones of activity were noted in *Malus* species (GOT I, GOT II and GOT III). Variation in these regions was poorly resolved and was not included in the results. The only noticeable point is that in *M. trilobata* GOT I was slightly lower than GOT I in the other species examined.

6.4. Discussion

6.4.1. Distinguishing among species of sections and series by peroxidase phenotype

Section *Malus*

Series *Pumilae*

Peroxidase enzyme systems showed no fixed allelic differences between the morphological species of series *Pumilae.*

Bands G, D, and B are common in series *Pumilae*. The peroxidase pattern of *M. domestica, M. kirghisorum, M. niedzwetzkyana* and *M. prunifolia* are very similar to each other, while one individual of *M. prunifolia* has also band E which is not found in the others. *M. niedzwetzkyana* was recognisable by the existence of band F.

Series *Baccatae*

Band E is absent in *M. hupehensis* and *M. rockii* but appeared as a faint band in *M. halliana* and *M. sikkimensis.* Band H is restricted to one individual each of *M.*

baccata and *M. baccata* var. *mandshurica*, while band I is only found in *M. baccata* var. *mandshurica.*

Band F only occurs in series *Pumilae* and *Baccatae* confirming the close relationship of the two series and supporting the definition of section *Malus* comprising series *Pumilae* and *Baccatae* based on morpholigical similarities. It is also supported by the similarity of their flavonoid pattern presented in this study (see chapter 5, Figure 5.3).

Section *Sorbomalus*

Series *Sieboldianae*

In series *Siebodianae* bands D and G are the common bands. *M.* x *floribunda* showed band E which is found in *M. sieboldii* as well. Bands H and I were only found in *M.* x *floribunda*. In none of the species of series *Sieboldianae* is band F observed. Bands B and C were never observed in the individuals of *M. sieboldii* examined. Individuals of *M. sargentii* did not show bands A and C as well as band E. The only species in *Sieboldianae* which showed bands in H and I was *M. xfloribunda* which is probably of hybrid origin.

Series *Kansuenses*

M. transitoria, M. kansuensis and *M. toringoides* show bands H and I but these two bands are not found in *M. fusca* of the same Series. Thus the *M. fusca* peroxidase phenotype could be consistently distinguished from the other *Kansuenses* by the lack of H and I activity bands. *M. fusca* does not show a pattern distinct from *Chloromeles*, all the bands found in *Chloromeles* being observed in *M. fusca* except band E.

Series *Yunnanenses*

In 8 samples of series *Yunnanenses* bands E and F were not found. *M. yunnanensis* var. *veitchii* showed 9 bands. Band H is common in *M. yunnanensis* and

bands I, J, and K are found exclusively in *M. yunnanensis* var. *veitchii.* The only individual of *M. prattii* examined did not show band H but contained bands C, D, G in common with *M. yunnanensis.*

M. yunnanesis var. *veitchii* of series *Yunnanenses* and *M. kansuensis* of series *Kansuenses* are very distinct from all other species of *Malus* by presence of bands J and K.

Series *Florentinae* and section *Eriolobus*

All the samples of *M. florentina* and *M.trilobata* lack the prominent band D common to all the other species. They also have no activity in PRX I and PRX II. Thus the *M. trilobata* and *M. florentina* peroxidase phenotype could be consistenly distinguished from that of other species, (Figures 6.1 and 6.2). Samples of *M. trilobata* had the same character as *M. florentina* but they have bands A and C as well as band B.

Lack of band D, which occurs in all other species of *Malus*, in *M. florentina* and *M. trilobata* suggests less similarity between these two species and the rest of the genus. Besides, these two species exhibit a low number of bands (only one in *M. trilobata* and three in the *M. florentina*, Figure 6.1 and 6.2).

Terpo (1968) included *M. florentina* in section *Eriolobus* with *M. trilobata.* Similarity in the patterns of isoenzymes and similarity in flavonoid patterns in these two species (this study) support this suggested relationship.

Series *Chloromeles*

All the species of section *Chloromeles* have band D, the common band in all other series of *Malus* except series *Florentinae* and section *Eriolobus*. This supports the placing of this section in the genus *Malus.*

M. coronaria, M. angustifolia and *M. ioensis* of series *Chloromeles* showed bands G, E and G respectively as faint bands. Therefore in series *Chloromeles*, the main bands are present in the region between bands A to D, not above that. *M.*

angustifolia and *M. platycarpa* showed the same pattern (B, C, D). Amongst the species of this group, *M. glaucescens* is distinct from the other in the absence of bands B and C.

Section *Docyniopsis*

Malus tschonoskii the only species examined of this series did not show a distinctive peroxidase phenotype.

CHAPTER 7

CHAPTER 7

GENERAL DISCUSSION

7.1. Introduction

This work builds on the studies of Phipps, *et al.* (1990, 1991), Robertson *et al.* (1991, 1992) and Roher *et al.* (1991, 1994) and applies many of their ideas to a detailed study of all available species of *Malus* and investigates further characteristics.

A traditional taxonomic approach has been adopted though using some of the ideas and terms developed in Hennigian cladistics. Zoological taxonomic studies have been much advanced by cladistic studies but many problems have been encountered when applying cladistic methods to plant taxonomy, chiefly those caused by parallel evolution in related groups, allopolyploidy and the consequent net - like relationships among species (Cronquist, 1987). However, the establishment of polarity in direction of evolution, though often much more difficult in plants than animals because of reversals, has been an advance in botanical evolutionary thinking. The sharing of ancestral characteristics (plesiomorphies) is much less significant than the sharing of derived (apomorphic) characteristics, especially where the apomorphy is likely to have evolved only once and is therefore a synapomorphy. However, we must be aware of how many presumptions are involved as emphasised by Cronquist (1987).

With *Malus* the difficulty in defining a sister group makes the polarity of some characters difficult to define, but in most cases the polarities proposed for the Maloideae seem reasonable to apply within *Malus*. Carpel adnation and connation are clearly apomorphies and reversal in degree of fusion are presumed to be unlikely. However, the occurrence of wholly adnate and connate carpels in different species may not be synapomorphies but the result of parallel evolution.

7.2. Summary of discussions

7.2.1. Relationship between sections of *Malus*

By considering the polarity of morphological characteristics studied for this thesis series *Yunnanenses* and *Florentinae* of section *Sorbomalus* with having the most primitive characters and no apomorphic ones, represent the most primitive taxa in the genus. Primitive status of *M. florentina* is supported by its complex pattern of flavonoids (see chapter 5) but primitive status of *Yunnanenses* is not supported because of few flavonoids detected.

Section *Chloromeles*, *Docyniopsis* and *Eriolobus* share large flowers (synapomorphy) and they probably have a common ancestor, and evolved earlier than other taxa in the genus because of low degree of carpel connation. The primitive status of these three sections is supported by morphological characters and also their greater number of flavonoids. Similarity between section *Chloromeles* and *Docyniopsis* is supported by flavonoid constituents.

In series *Chloromeles* the very low degree of carpel connation is considered plesiomorphic. This character, besides the other plesiomorphic characters, indicates that this taxon has probably evolved in isolation for a considerable time.

The number of plesiomorphic characters and geographic distribution of the one species which is restricted to West Asia, *M. trilobata*, suggest that this is one of the primitive and relict species in the genus.

Both *Yunnanenses* and *M. trilobata* have the most plesiomorphic characters. There are two very primitive characters in *M. trilobata* not present in series *Yunnanenses,* trilobed leaves and low degree of carpel connation. *Yunnanenses* could not be derived from *M. trilobata* because of the presence of apomorphic characters like large petals, large flowers and small number of flowers per infloresence. Therefore they may be derived from two close ancestors.

Series *Pumilae*, *Baccatae*, and *Sieboldianae* share umbellate inflorescence, pinkish white petals, and a few sclereids in the flesh (synapomorphy). Thus, they

probably have a common ancestor. They are also considered advanced taxa because of the large number of apomorphic characters and also relatively lower number of flavonoids than primitive taxa.

Pumilae is much closer to the *Baccatae* than to any other taxon in the genus. They probably have a common ancestor. Some characters retained the ancestral state while others have evolved in divergent ways. Leaves rolled in bud is common to all species of *Pumilae* and *Baccatae* while size of the fruit, condition of the calyx and number of carpels per flower indicate the divergent evolution of these characters in both series.

Similarity in flavonoid constituents in *Baccatae* and *Pumilae* supports the close relationships between the two taxa.

Persistence of calyx and stamen number of about 20 shared by *Yunnanenses* and *Pumilae* show the relationship between these two taxa. Due to low degree of carpel connation in most species of *Pumilae,* derivation of *Pumilae*, directly from *Yunnanenses* is unlikely. Although due to similarity between the *Pumilae* and *Baccatae*, the *Pumilae* could be derived from the line from which *Baccatae* has been derived.

Sieboldianae are close to *Baccatae* in having flowers in umbels, pinkish white petals, carpel number, high degree of carpel connation, deciduous calyx and few or no stone cells (synapomorphies). Thus they probably have a common ancestor. If these characters evolved in parallel direction from a common ancestor, leaf characters might have evolved in divergent ways.

Affinity of *Sieboldianae* with *Eriolobus* has been demonstrated by Williams (1960) who reported the presence of the dihydrochalcone trilobatin in one seedling of *M. sieboldii* var. *arborescens* and *M. trilobata.* Morphologically, there are some plesiomorphic characters shared between the two taxa which indicate the retention of

some ancestral characters but there is no synapomorphy to indicate a direct common ancestor, except perhaps the presence of trilobatin.

Series *Kansuenses* is close to *Sieboldianae* and *Baccatae* in having same apomorphic characters such as carpels number, carpel connation and calyx condition which suggests they may have a common ancestor.

Morphologically *Yunnanenses* and *Kansuenses* have no apomorphic character in common but they share some retained primitive characters (plesiomorphies). Although Henke (1963, cited by Huckins, 1972) reported similarity between flavonoid constituents of both taxa, the work carried out in this study (chapter 5) does not show this similarity in taxonomically valuable flavonoid compounds.

7.2.2. Characteristics of sections of *Malus*

Section *Malus*

Section *Malus* comprises two series *Pumilae* and *Baccatae*. Similarity between these two series is confirmed by morphological, anatomical and flavonoid constituents. Series *Pumilae* and *Baccatae* are very similar in flavonoid pattern. This similarity coincides with the morphological similarity in rolled leaves and unlobed leaves in both series. The large number of known and suspected inter serial crosses involving members of the *Pumilae* and *Baccatae* also suggests a close relationship between *Pumilae* and *Baccatae* (Figure 1.3) though there may have been parallelism in the evolution of the flavonoids in these two groups.

The exine structure of pollen grains in the two series are similar in striae parallel to the colpi and curved at the poles and presence of pores. Anatomy of wood of both series is also similar.

Section *Sorbomalus*

This section is comprised four series including *Sieboldianae*, *Kansuenses*, *Florentinae* and *Yunnanenses*. Morphologically they share folded leaves in the bud and presence of lobed or lobulate leaves.

The homogeneity of series *Sieboldianae* is emphasised by morphological (see chapter 2) characters and the occurrence of sieboldin in the leaves of all species. It is also a distinctive character for the series.

Morphologically, *Sieboldianae* is considered one of the most advanced taxa in the genus. This is also supported by the smooth exine and large and numerous pores of its pollen grain.

Series *Kansuenses* is considered a transition taxon in the genus because of its intermediate morphological character. Morphologically it is distinguished from other series by having white petals and fused and glabrous style bases.

Peroxidase isozymes analysis indicates a similarity between sections *Yunnanensis* and *Kansuenses*. This similarity has also been supported by morphological analysis as shown by Van Eseltine (1933) and this study. Chemically *Kansuenses* has not a distinctive flavonoid pattern but there is a homogeneity in its flavonoids constituents. However, *M. toringoides* shows different flavonoids.

On morphological grounds Rehder (1940) placed *M. toringoides* in series *Kansuenses,* due to its leaves having 4-5 lobes and the glabrous styles. However, as discussed in section 5.3.2 *M. toringoides* differs in two major characteristics from other species of the series *Kansuenses* as below:

1. In having compound Fa, which is absent in all the other species of the series examined.
2. In absent of compound G, which occurs in all the other species of the series examined.

The above characteristics suggest that chemical evidence (flavonoid compounds) does not support the position of *M. toringoides* within series *Kansuenses*.

Cluster analysis of the *Malus* species based on flavonoid constituents shows that *M. toringoides* clusters with members of series *Baccatae* and *Pumilae*, because of presence of compounds Fa in *M. toringoides* which do not occur in other members of

series *Kansuenses* but is present in series *Pumilae* and *Baccatae*. This species can be classified close to the *Baccatae* due to its fruits with deciduous calyces and a low percentage of stone cells in the flesh and the involute leaves, although none of the members of series *Baccatae* as currently defined shows lobed leaves.

M. fusca, another species of series *Kansuenses*, shows differences in wood anatomy but similar morphology and flavonoid pattern to other members of the series. In this species axial parenchyma is apotracheal diffuse in aggregate type, a feature which is absent in all the other species of *Malus* examined. The few similarities between the pollen of *M. fusca* and other species of series *Kansuenses* makes the position of this taxon within this series somewhat anomalous. These characters define *M. fusca* as an isolated species.

Morphologically series *Yunnanenses* is considered the most primitive taxon in the genus. However, chemically the series is considered one of the advanced taxa in the genus because of few taxonomically valuable flavonoids.

Series *Florentinae* is also very distinct in the lobulate to lobed leaves and clawless petals. It is also very distinct in flavonoid pattern (cluster F). Peroxidase isozymes also reveal that *M. florentina* is distinct from others by lacking the common band present in all other species of *Malus* except *M. trilobata*.

A suggested relationship between *M. florentina* and *Sorbus torminalis* investigated through flavonoid constituents was not confirmed. The above evidence is in agreement with changing the rank of *M. florentina* to the separate section *Florentinae* as suggested by Huckins (1972).

Terpo (1968) suggested that *M. florentina* and *M. trilobata* be placed in section *Eriolobus*. This proposal is supported here by flavonoid analysis (cluster F,

Figures 5.3, 5.4). However, there are some significant differences between these two species;

a. Presence of abundant sclerids in fruits of *M. trilobata* versus low number or absence in *M. florentina* .

b. Persistence of calyx in *M. trilobata* versus deciduous calyx in *M. florentina.*

c. Presence of trilobatin in *M. trilobata* versus phloridzin in *M. florentina.*

The presence of compounds Fc and Az in *Chloromeles, Eriolobus, Docyniopsis* and *Florentinae* and their absence in all other species shows a close relationship among these taxa. The cladogram of Phipps *et al.* (1991) and Robertson *et al.* (1991) also suggest a close relationship between these three taxa.

Section *Chloromeles*

Morphologically it is considered a primitive taxon in the genus (chapter 2). This was confirmed by the large number of taxonomically valuable flavonoids. It is a very distinct section, recognisable by (morphologically) pink flowers, red anthers, low degree of carpel connation, green fruits and few stone cells in the flesh. The wood anatomy of the section is quite distinct, the parenchyma rays being almost exclusively uniseriate. This section comprising the largest pollen length except *M. lancifolia.* Flavonoid compounds are complex including taxonomically important compounds Az, Fa, Fc, G and H.

As mentioned in section 5.3. the flavonoid compounds of section *Chloromeles* and *M. tschonoskii* show similarity (cluster E, Figure 5.3). Morphologically this relationship can be supported by the common characteristics of low degree of carpel connation and low adnation of carpels to the floral tube, persistence of the calyx in fruits and the presence of 5 carpels. However, there are some differences. These differences are as follows:

a. Green fruits of series *Chloromeles* versus orange-red fruits of *M. tschonoskii.*

b. Spreading shape of the tree in the *Chloromeles* versus erect shape in *M. tschonoskii.*

c. Red anthers in *Chloromeles* versus yellow anthers in *M. tschonoskii.*

d. Constant stamen number of 20 in *Chloromeles* versus 39-62 stamens in *M. tschonoskii.*

e. Pink flowers of *Chloromeles* versus pinkish white in *M. tschonoskii.*

f. Low percentage or absence of stone cells in fruit flesh in *Chloromeles* versus high percentage of stone cells in *M. tschonoskii.*

g. Presence of adaxial glands on leaves of *Chloromeles* and absence of them on leaves of *M. tschonoskii.*

h. Uniseriate parenchyma rays of *Chloromeles* versus biseriate rays in *M. tschonoskii.*

i. Geographic distribution of section *Chloromeles* exclusively in North East America versus *M. tschonoskii* restricted to Japan, with other species of *Docyniopsis* in China. However, this disjunction between closely related species is common. Although characters d, f, g and h are the most significant it would be most interesting to know more of Chinese species of *Docyniopsis*. There is probably a close relationship between *Docyniopsis* and *Chloromeles.*

Section *Docyniopsis*

Morphologically this is considered one of the most primitive taxa in the genus. This is confirmed by the presence of large number of taxonomically valuable flavonoids. *M. tschonoskii* the only representative species of this section studied shows many differences from other species of the genus as follows:

1. Morphologically, high number of stamens per flower, low degree of carpel connation, fleshy and erect calyx, lack of adaxial glands on the leaves and heterogeneous parenchyma of flesh.
2. Chemically distinctive flavonoid pattern.
3. Anatomically small vessel diameter.

4. Distinctive pollen grain with thick and prominent ridges.

5. Distribution restricted to Japan.

Cluster analysis of flavonoids compounds show similarity between *Chloromeles* and *Docyniopsis* discussed under section *Chloromeles*.

Section *Eriolobus*

The primitive status of section *Eriolobus* was confirmed by morphological and chemical characters. *M. trilobata*, the only species of this section, is distinguished by :

1. Having widest leaves and leaves always lobed.

2. Low degree of carpel connataion and adnation.

3. Distinctive flavonoid pattern and presence of the dihydrochalcon trilobatin.

4. Lack of band D, the band common in almost all other species of *Malus*.

Cluster analysis on flavonoid constitutes shows a close relationship between *M. trilobata* and *M. florentina*, this relationship is supported by similarity in peroxidase phenotypes (lack of band D) and also similarity in exine sculpture of pollen grains.

Morphologically, *Pseudocydonia*, *Chaenomeles* and section *Aria* of *Sorbus* show some similarities with *Malus*. However, flavonoid compounds do not support a close relationship. Also, there is no report of intergeneric hybrids between *Malus* and these related taxa.

CHAPTER 8

CHAPTER 8

CONCLUSION

This work largely supports the subgeneric classification of *Malus* as proposed by Rehder (1940). The investigation of previously little studied characteristics, flavonoid constituents, pollen morphology, wood anatomy, isozymes, largely reinforcing the current sub-generic classification.

1. The distinctness of section *Chloromeles* is emphasised by further distinctions not previously described.

a. High percentage or exclusive occurrence of uniseriate rays in wood.

b. The presence of some flavonoid compounds not found in any other species of *Malus*.

c. Absence of peroxidase III in all the members.

d. Large length of pollen grains.

2. The distinctness of *M. tschonoskii* is emphasised by the following previously unrecognised characteristics.

a. Morphologically, number of stamens of 30-61 versus mean of 20 in other species, the only case in the genus of significant increase in stamen number.

b. Heterogeneous parenchyma of flesh versus homogeneous flesh of others species.

c. Low degree of carpel adnation and connation.

d. Lack of adaxial glands on the leaves.

e. A different flavonoid compound pattern from most species of *Malus*, the presence in cluster E, with section *Chloromeles* .

Besides previously described characters including:

a. Numerous sclereids in flesh.

b. High density of lenticels (white dots) on the surface of fruits.

A close relationship with the *Chloromeles* is suggested by the following character common to both groups:

a. Low degree of carpel connation and carpel to hypanthium adnation.

b. Similar flavonoid compounds.

Although *Chloromeles* (N. E. American) and *M. tschonoskii* (Japan) are geographically separate this common geographical disjunction is seen in many close genera.

3. A close relationship between *M. trilobata* and *M. florentina* was shown by cluster analysis based on flavonoid compounds. This relationship is supported by :

a. Similarity in lack of band D which occurs in other sections of the genus.

b. Pollen structure of both species also show similarity in the thickness and patterns of ridges.

c. Morphologically they share some plesiomorphic characters (lobed leaves, folded vernation, corymbose inflorescence, white petals, stamen number about 20, and 5 carpels).

d. Chemically, they also have a large number of taxonomically valuable flavonoids which indicate the primitive status.

Shared pleisiomorphies indicate persistence of ancestral features and often denote relict taxa.

4. Although *M. florentina* has some characteristics which make its position uncertain within the genus *Malus* (lack of band D in peroxidase zymogram and different flavonoid compounds from other species of *Malus*), it seems reasonable to retain it in genus *Malus*. This proposal relies on:

a. Presence of phloridzin, which occurs in all the species of *Malus* (except *M. trilobata* and series *Sieboldianae*).

b. Fusion of carpels, and inferior ovary.

c. Similarities in wood anatomy with the other species of *Malus*.

Results obtained in this study show more similarity between *Eriolobus* and *Florentinae* than other series or sections.

5. The retention of *M. fusca* in series *Kansuenses* is emphasised by morphological and chemical evidence (flavonoid compounds). There are two characters which make the position of *M. fusca* in series *Kansuenses* uncertain and distinct it from other *Kansuenses*.

a. The apotracheal diffuse grouped patches of axial parenchyma in woods.

b. The presence of parallel and curved ridges in pollen grain.

6. Series *Kansuenses* and *Yunnanenses* show much similarity in morphological characters, peroxidase phenotypes and pollen morphology, these results are in support of placing the two series in one section *Sorbomalus* as proposed by Rehder (1940).

7. Morphologically series *Kansuenses* can be divided to two subseries *Kansuenses* and *Transitoria*.

8. Comparison between flavonoid compounds in genus *Malus* and related genera *Chaenomoles, Aria, Pseudocydonia* indicates that there is not any significant similarity among these genera. Future work should perhaps concentrate on *Docynia* which may be as closely related to some species of *Malus* such as *M. trilobata, M. florentina* or section *Chloromeles*.

9. Comparision of flavonoid compound of *M. florentina* with *Sorbus torminalis* and *M. sylvestris* does not support the theory of a hybrid origin for *M. florentina.*

Future studies

This work has revealed gaps in our knowledge in certain areas where a very limited amount of material was available for study. Investigation of intraspecific variation in such species as *Malus tschonoskii*, *M. florentina*, and *M. trilobata* and of other species of section *Docyniopsis* might confirm or refute the distinctness of these taxa of which perhaps only a single clone is in cultivation.

In many groups wild source material is gradually becoming available from recent expeditions and should be included in any future study. Suggested relationships e.g. *Docyniopsis* and *Chloromeles*, could be investigated using more species and wild source material. Similarly, the relationships among species of *Malus* and *Docynia* could be investigated, material of the latter genus unfortunately not being available for this study.

DNA studies on both chloroplast and nuclear genomes might yield useful results. Unfortunately financial support was not available for such work in this study but suggestions are made as to which species could most variably examined.

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Plate 1

Transverse section, 400x. Clusters of sclerids and radially arranged parenchyma in the flesh in *Malus tschonoskii*.

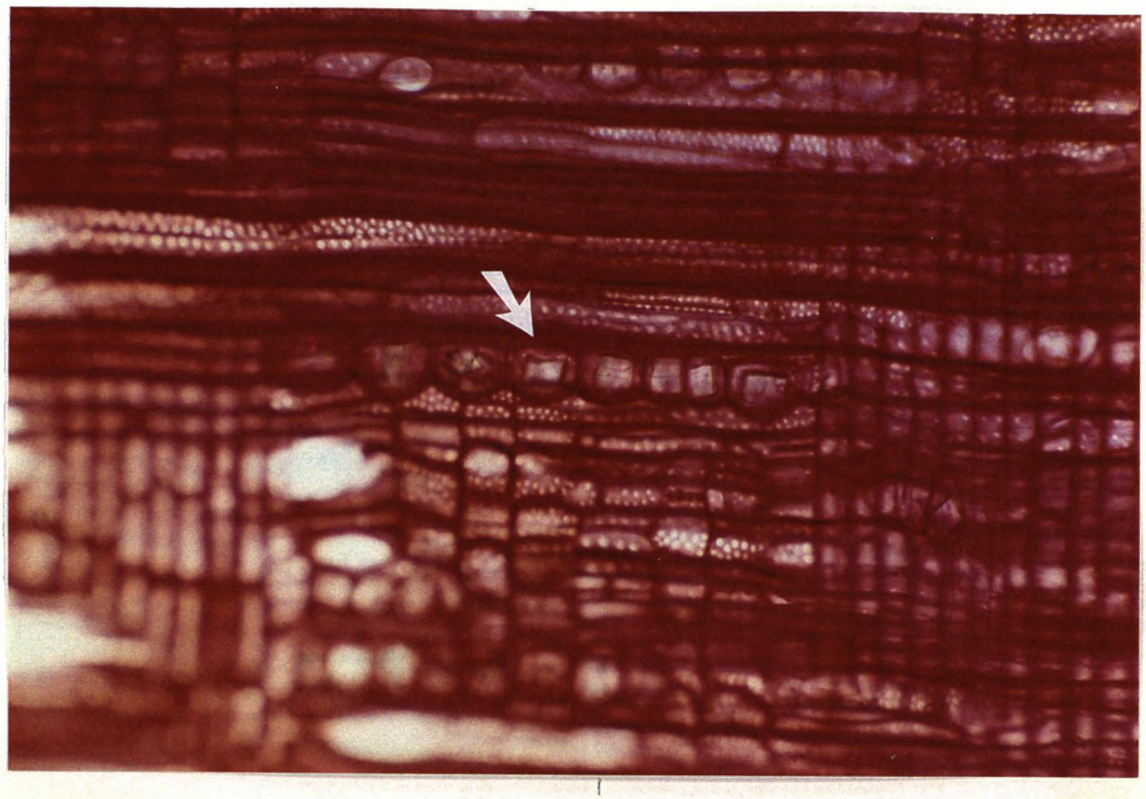
PLATE 1

Plate 2

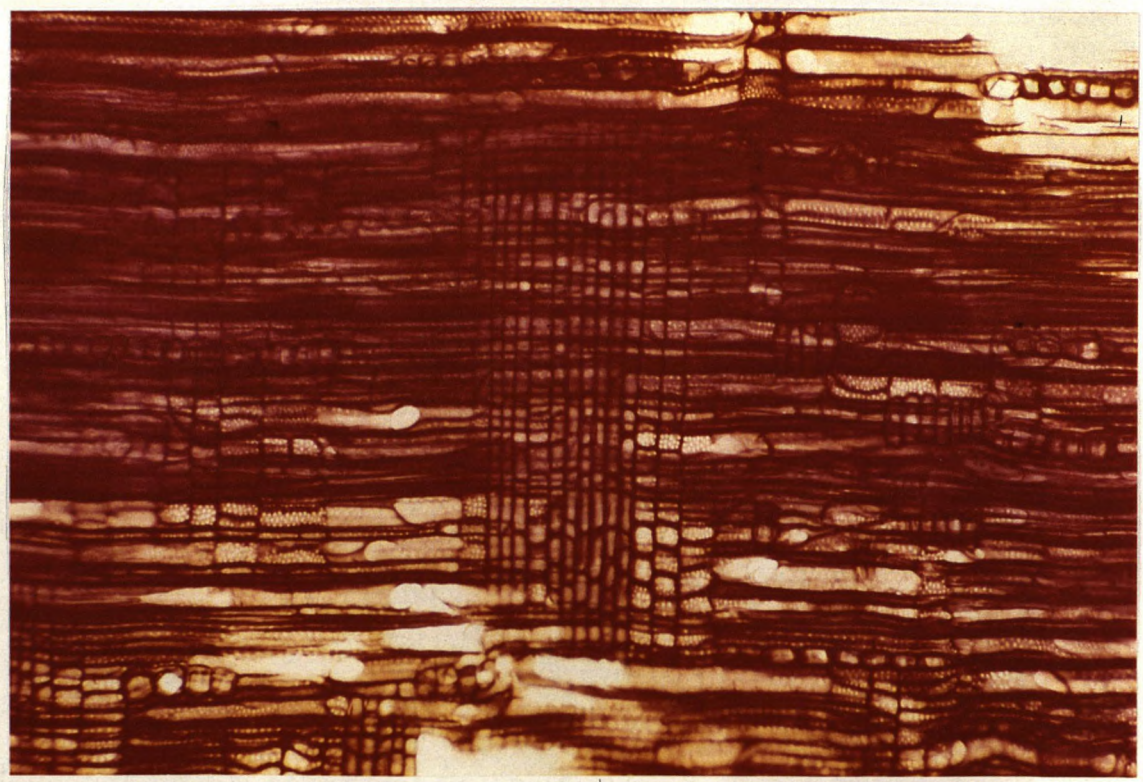
Wood structure of *M. tschonoskii.*

Plate 2. A. 100x. Radial longitudianal section. Prismatic crystals in enlarged parenchyma cells in *M. tschonoskii.*

Plate 2. B. 100x. Radial longitudianal section. Heterogeneous rays composed of procumbent body cells and 1-3 rows of spuare to upright cells in *M. tschonoskii.*



A



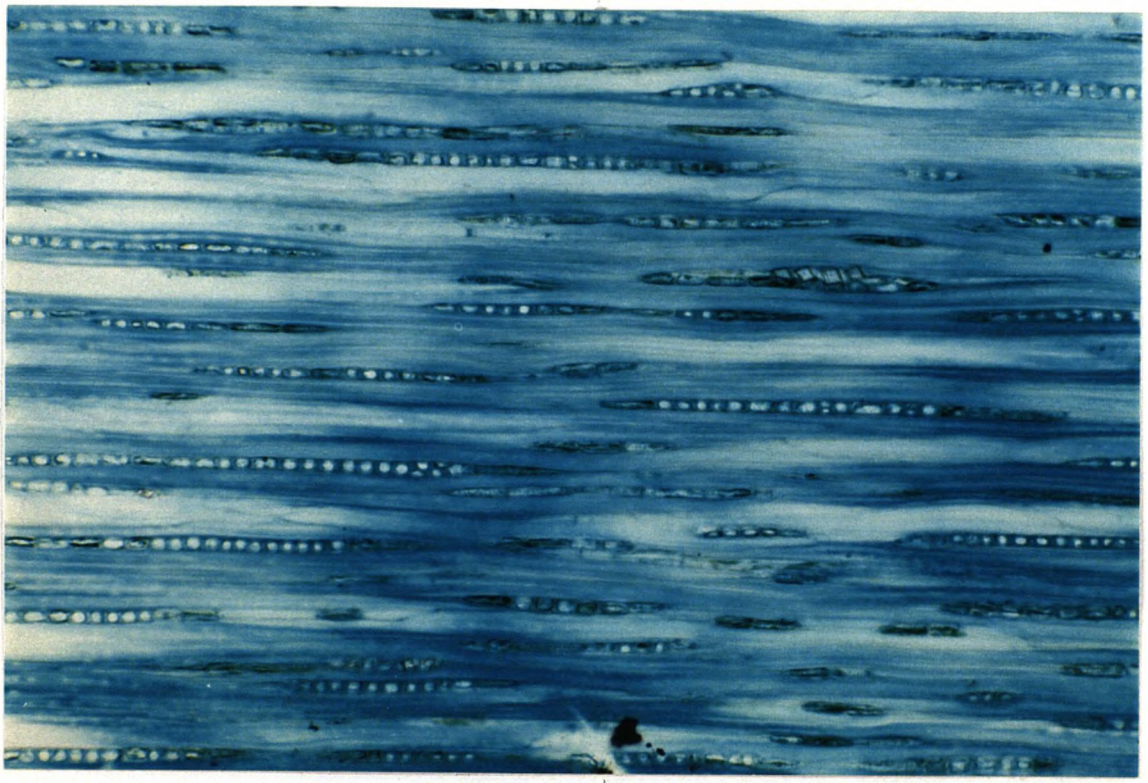
B

Plate 3

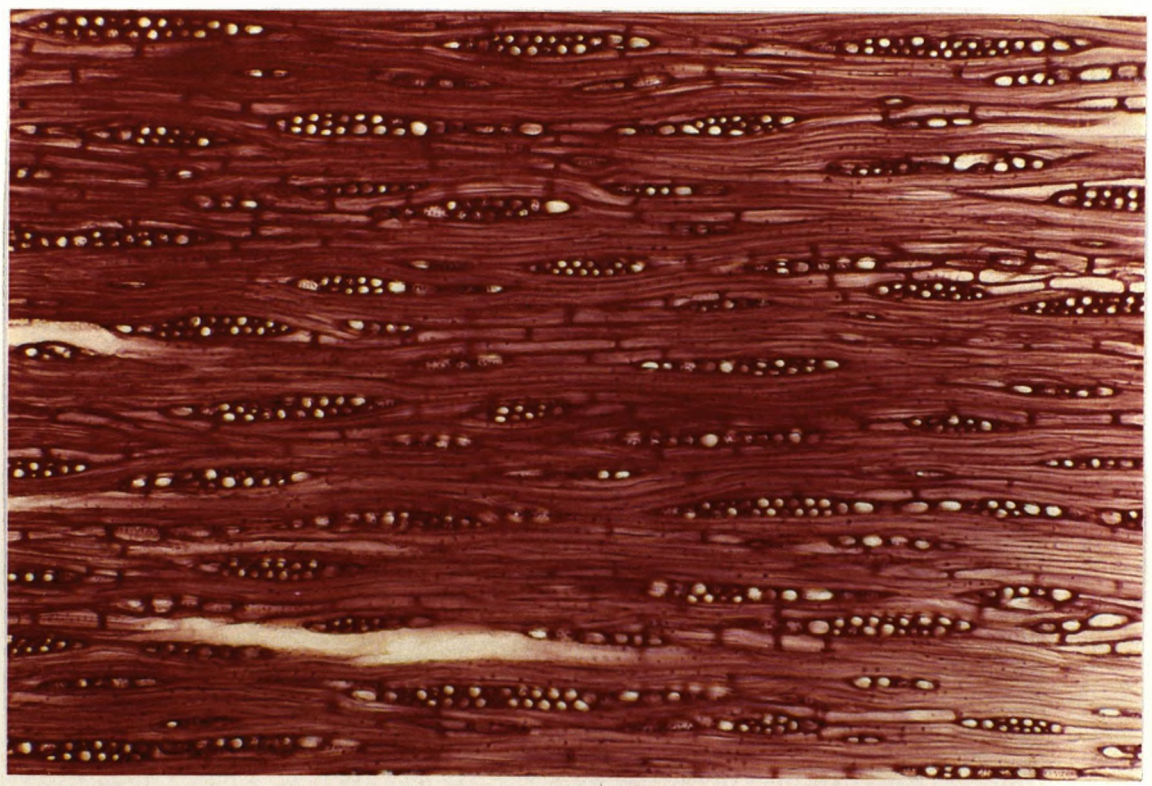
Uniseriate and multiseriate ray in *M. angustifolia* and *M. fusca.*

Plate 3. A. 100x. Tangential longitudianal section. Heterogeneous and uniseriate rays in *M. angustifolia.*

Plate 3. B. 100x. Tangential longitudianal section. Heterogenous and 1-2(-3) seriate rays in *M. fusca.*



A



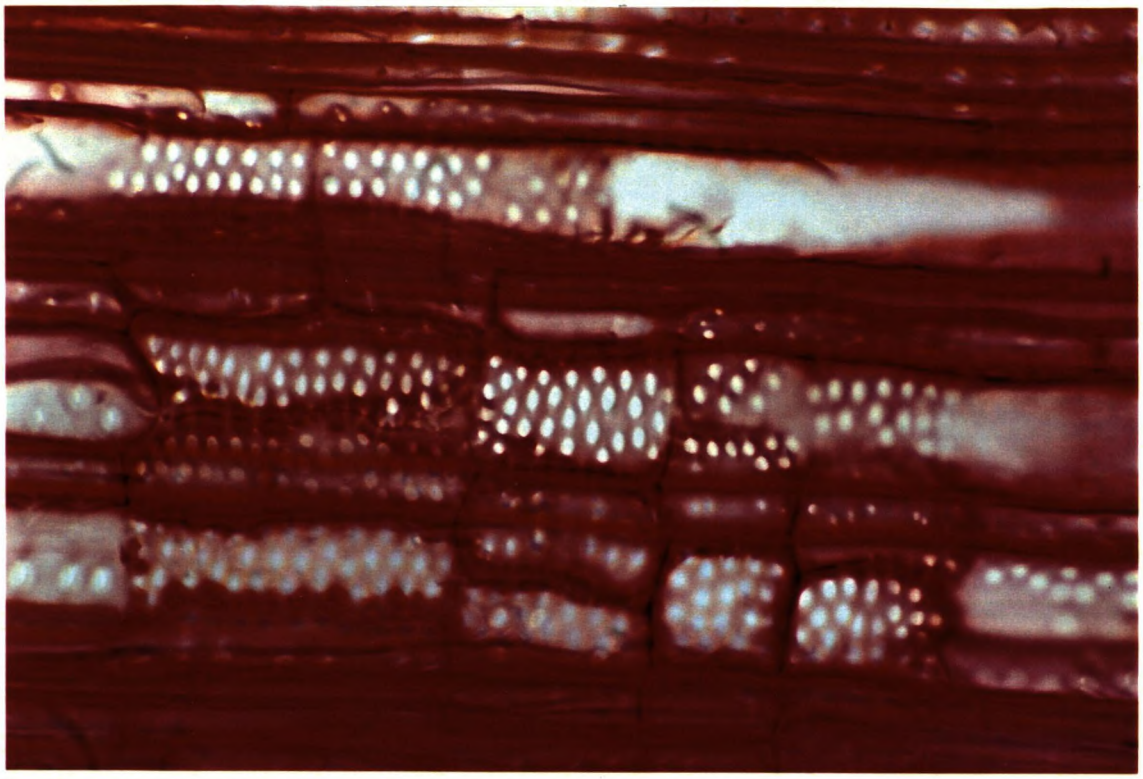
B

Plate 4

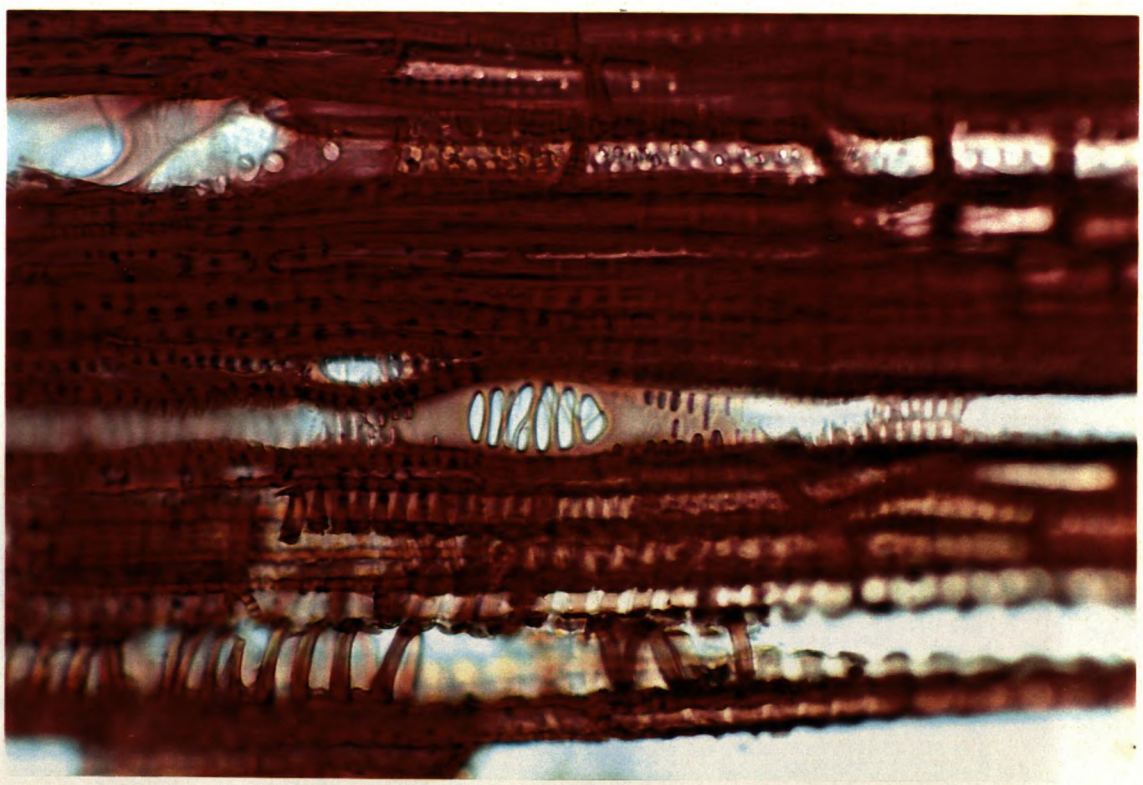
Vessel ray pits and scalariform perforation plates in *M. tschonoskii.*

Plate 4. A. 400x. Radial longitudianal section. Vessel ray pits (much reduced borderd pits) in *M. tschonoskii.*

Plate 4. B. 400x. Radial longitudianal section. Scalariform perforation plate with 5 bars in *M. tschonoskii.*



A



B

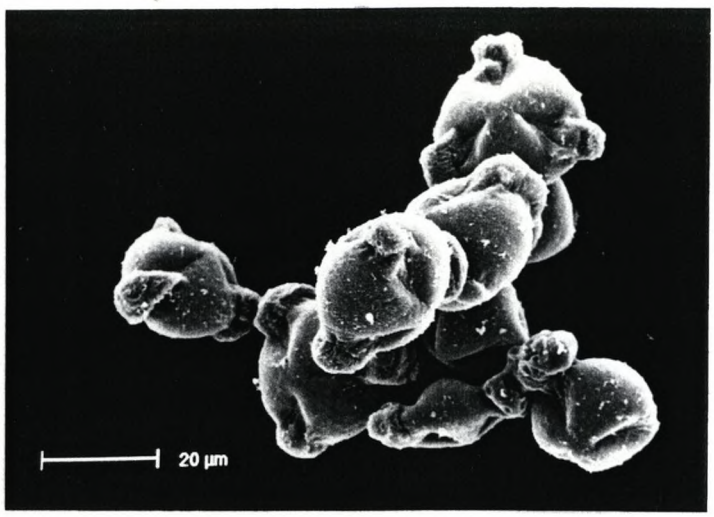
Plate 5

Scanning electron micrographs of *M. trilobata* pollen prepared by acetolysis.

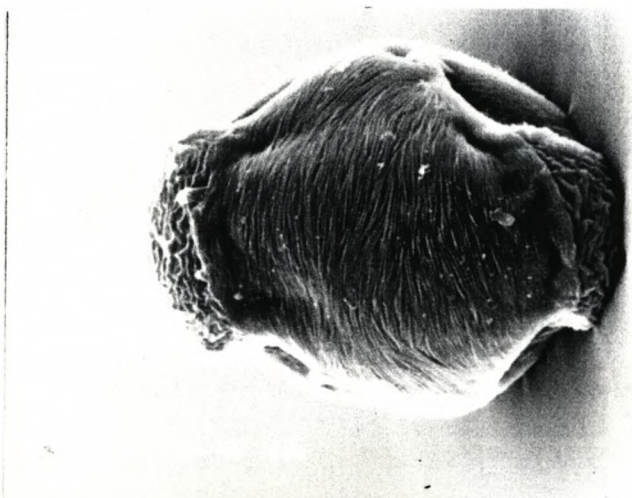
Plate 5. A. Pollen grains.

Plate 5. B. Equatorial view showing striate sculpturing.

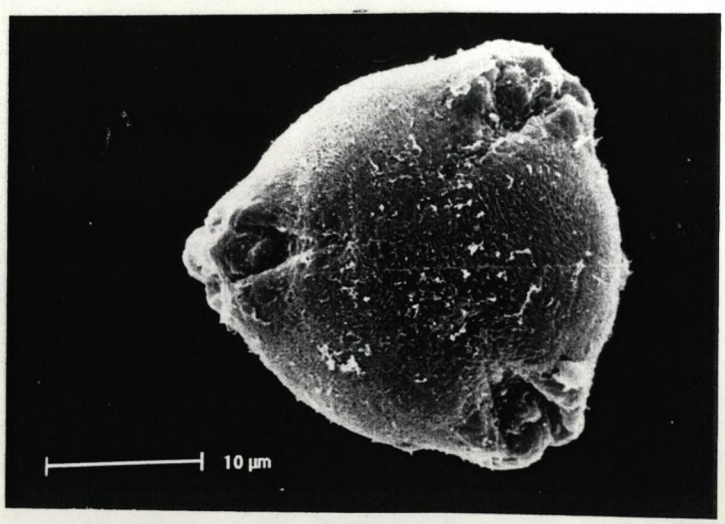
Plate 5. C. Polar view showing tricolpate pollen.



A



B



C

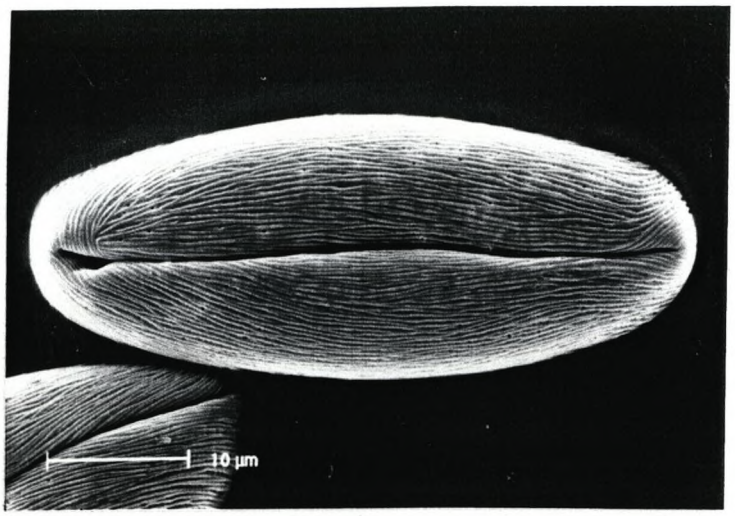
Plate 6

Scanning electron micrographs of *M. domestica* pollen.

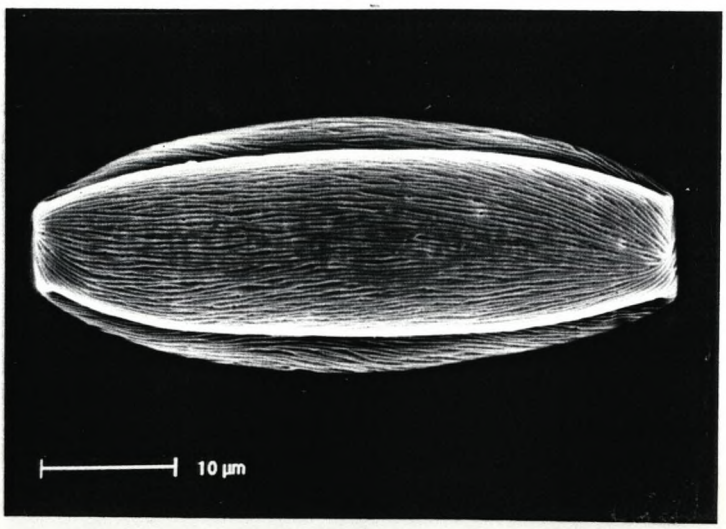
Plate 6. A. Side view.

Plate 6. B. Equatorial view.

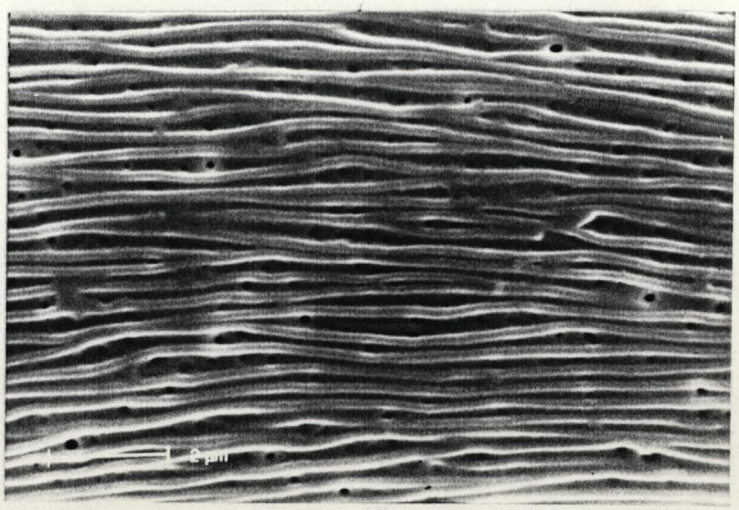
Plate 6. C. 10000x. Exine sculpture showing the ridges .



A



B



C

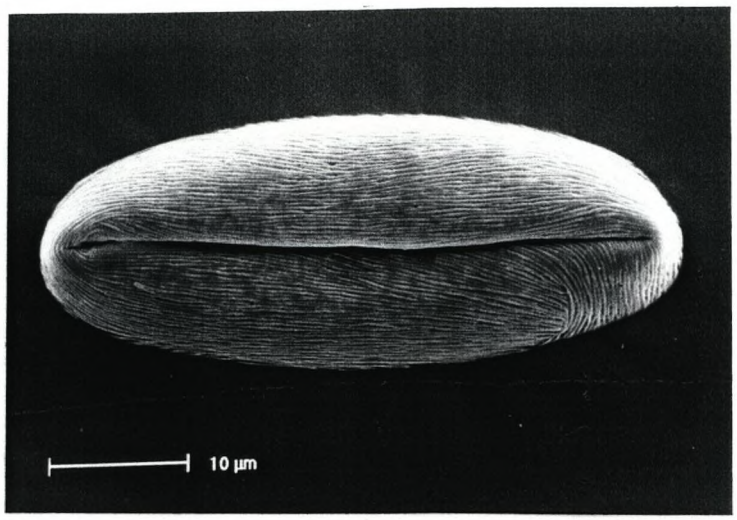
Plate 7

Scanning electron micrographs of *M. niedzwetzkyana* pollen.

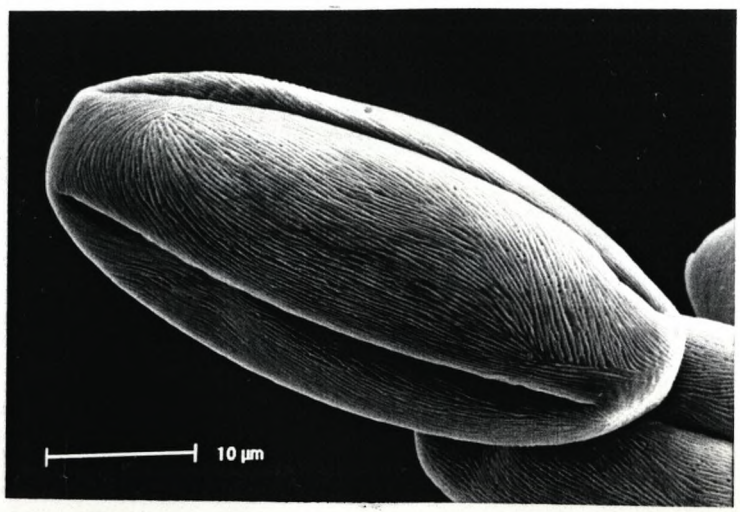
Plate 7. A. Side view.

Plate 7. B. Equatorial view.

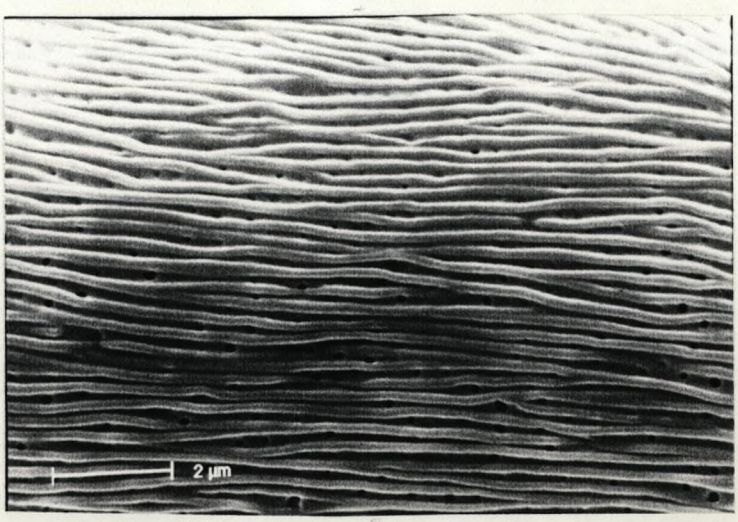
Plate 7. C. 10000x. Exine sculpture showing the ridges .



A



B



C

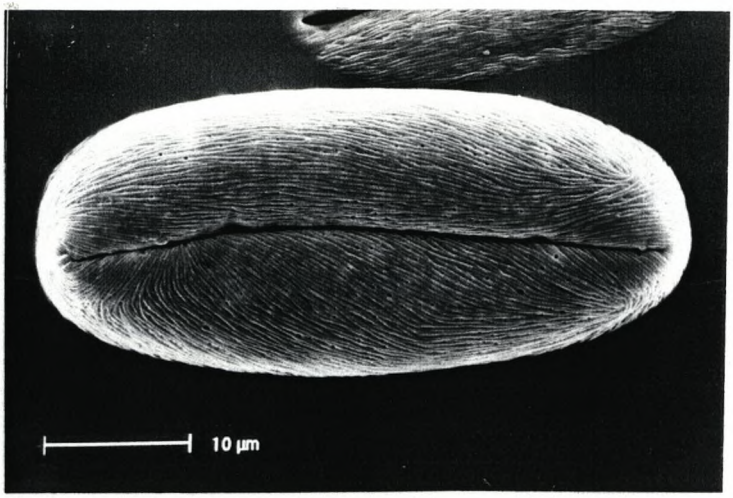
Plate 8

Scanning electron micrographs of *M. baccata* pollen.

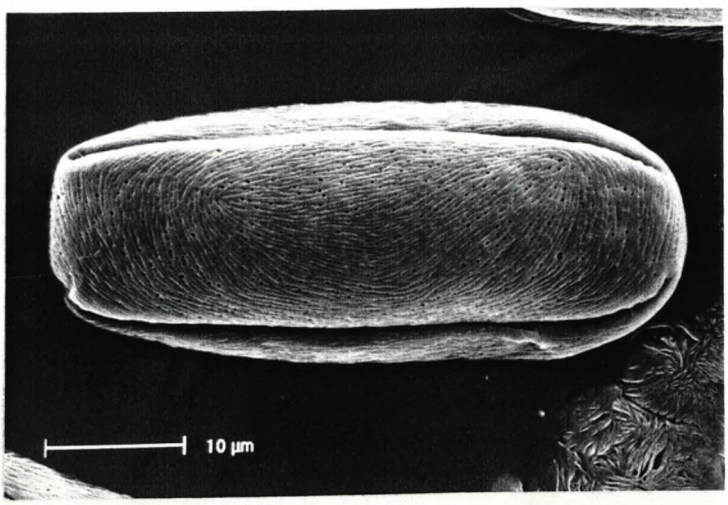
Plate 8..A. Side view.

Plate 8. B. Equatorial view.

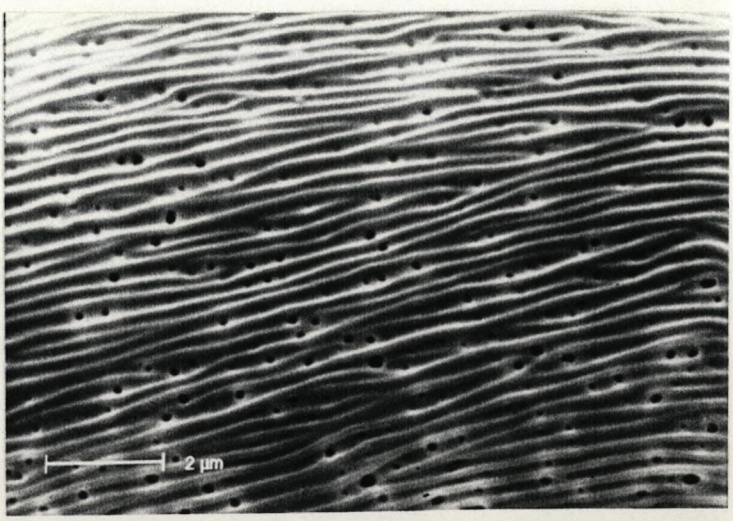
Plate 8. C. 10000x. Exine sculpture showing the ridges .



A



B



C

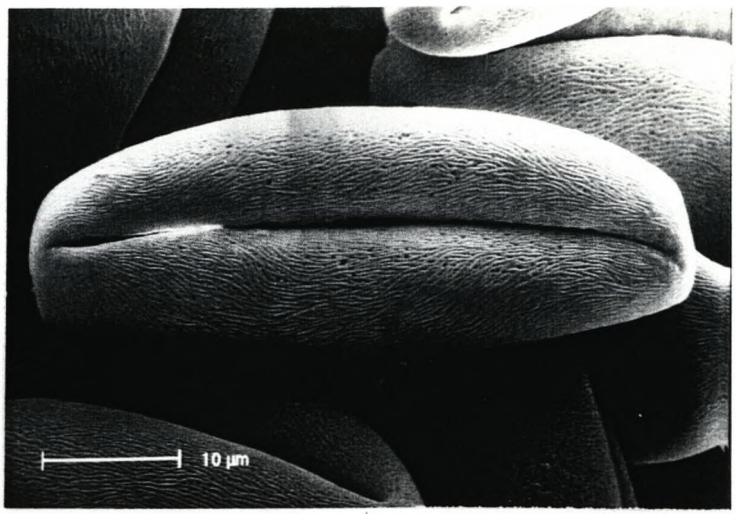
Plate 9

Scanning electron micrographs of *M. x floribunda* pollen.

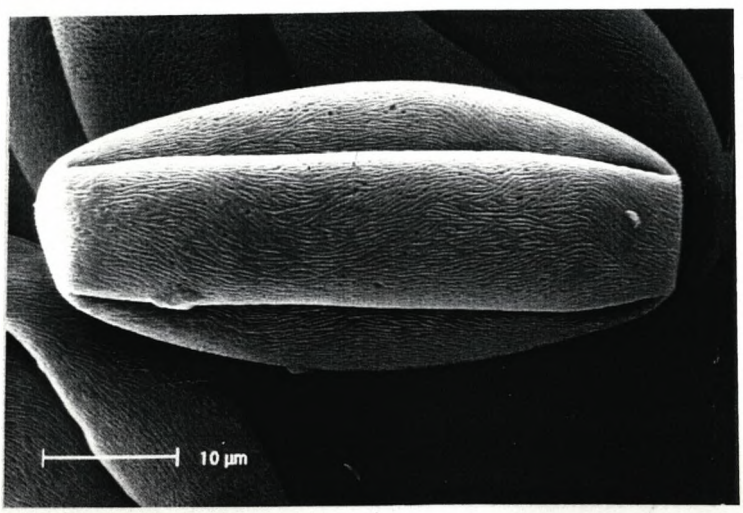
Plate 9. A. Side view.

Plate 9. B. Equatorial view.

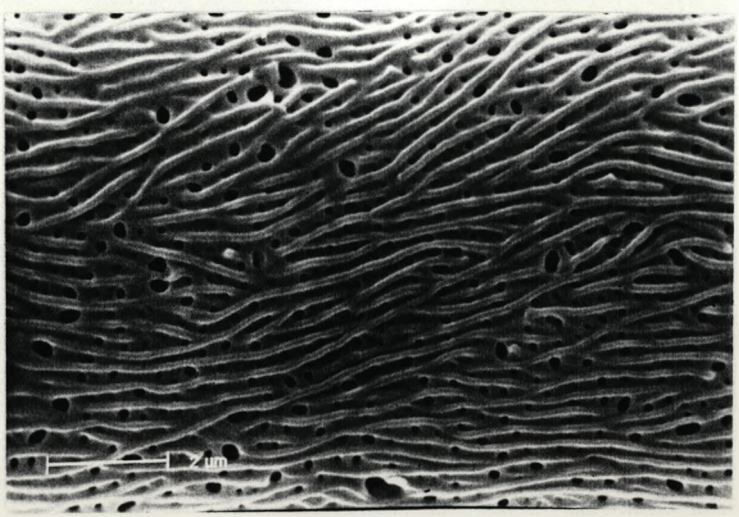
Plate 9. C. 10000x. Exine sculpture showing the ridges .



A



B



C

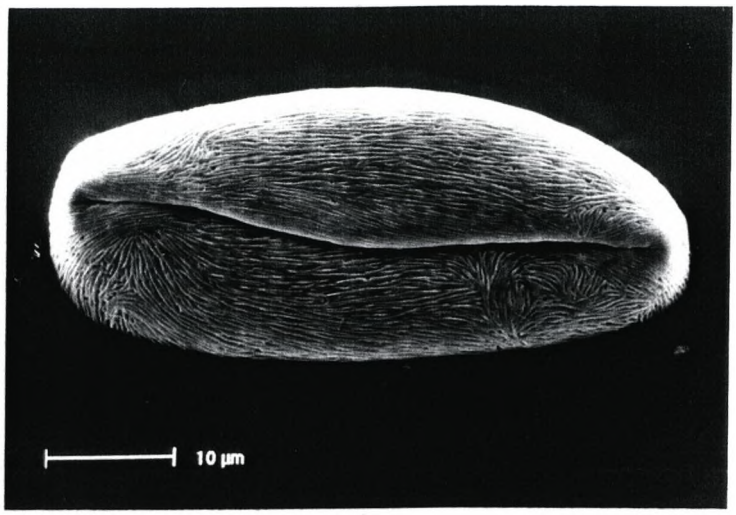
Plate 10

Scanning electron micrographs of *M. sargentii* pollen.

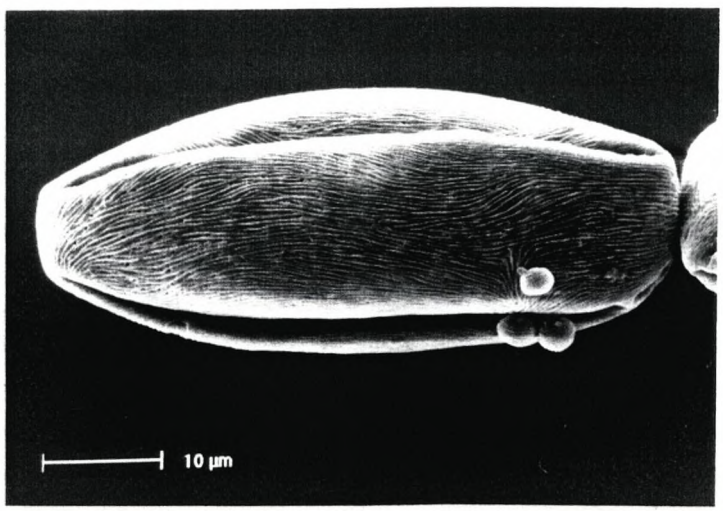
Plate 10. A. Side view.

Plate 10. B. Equatorial view.

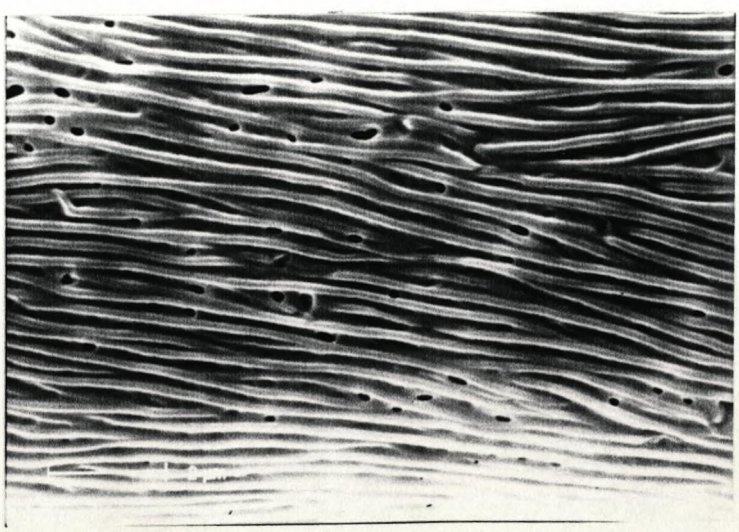
Plate 10. C. 10000x. Exine sculpture showing the ridges .



A



B



C

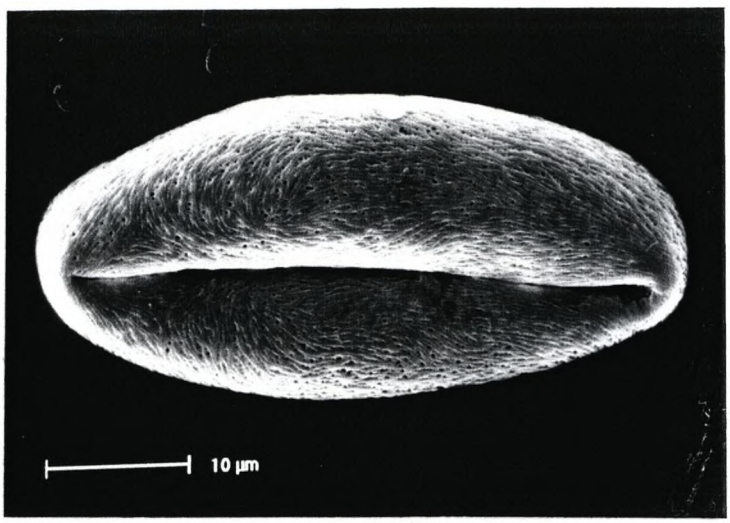
Plate 11

Scanning electron micrographs of *M. sieboldii* pollen.

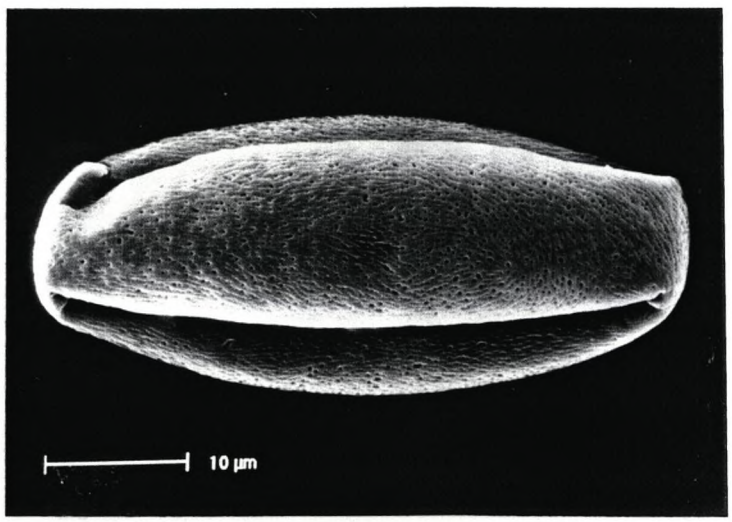
Plate 11. A. Side view.

Plate 11. B. Equatorial view.

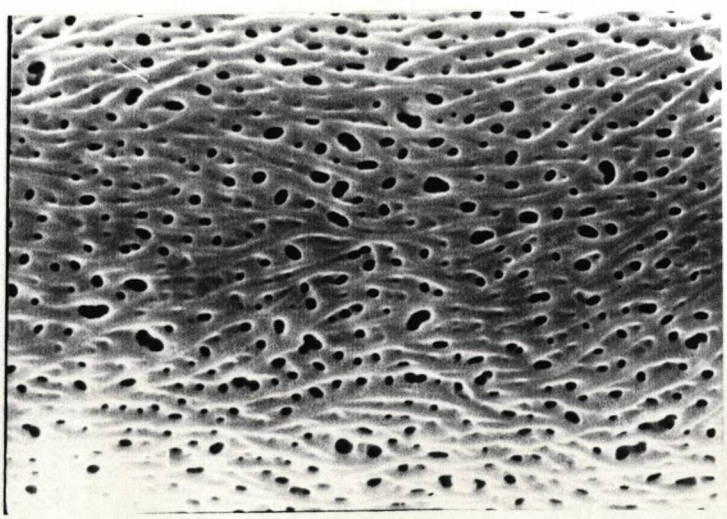
Plate 11. C. 10000x. Exine sculpture showing the ridges .



A



B



C

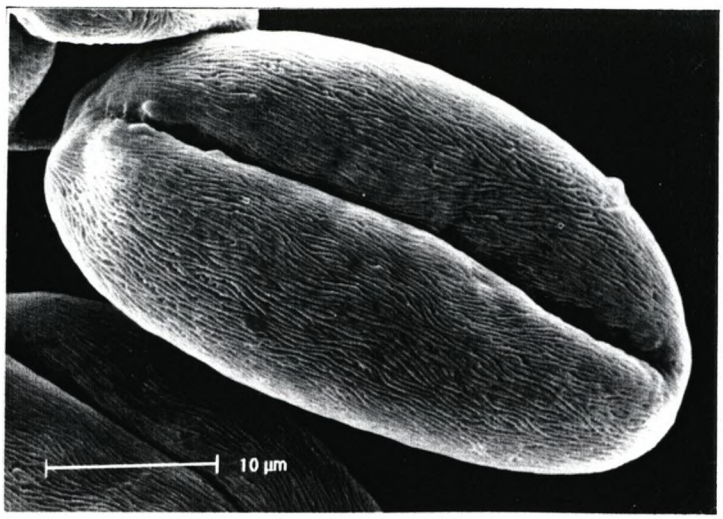
Plate 12

Scanning electron micrographs of *M. florentina* pollen.

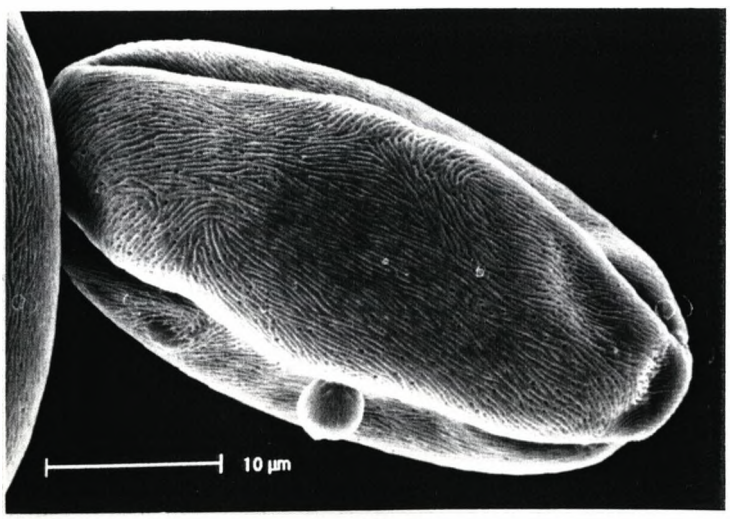
Plate 12. A. Side view.

Plate 12. B. Equatorial view.

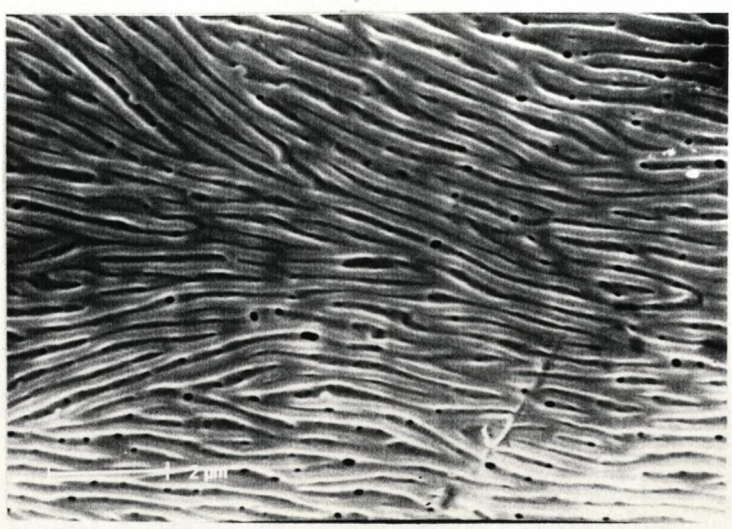
Plate 12. C. 10000x. Exine sculpture showing the ridges .



A



B



C

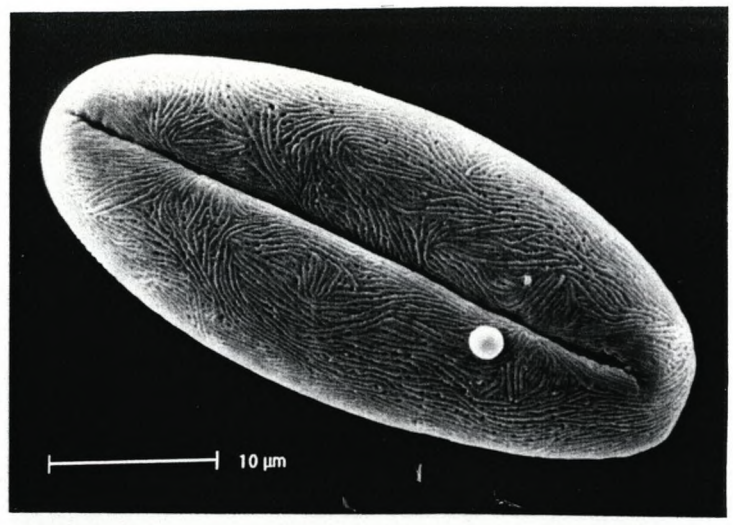
Plate 13

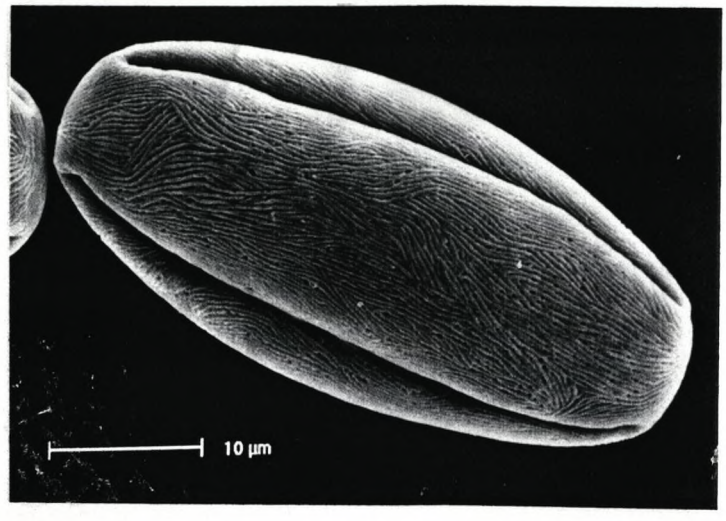
Scanning electron micrographs of *M. fusca* pollen.

Plate 13. A. Side view.

Plate 13. B. Equatorial view.

Plate 13. C-D. 10000x. Exine sculpture showing the ridges .


A
10 µm


B
10 µm

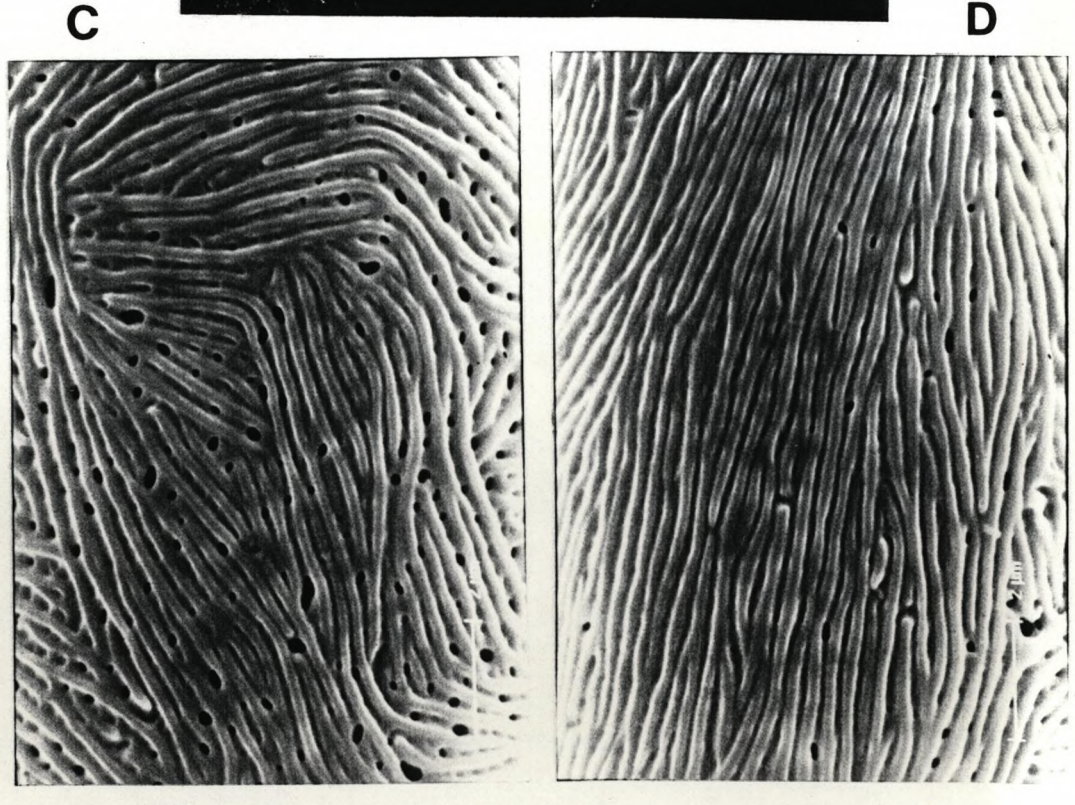
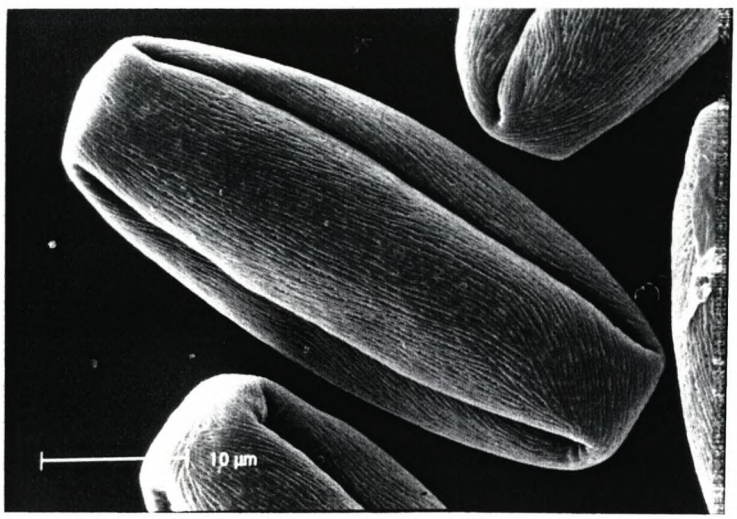

C
D

Plate 14

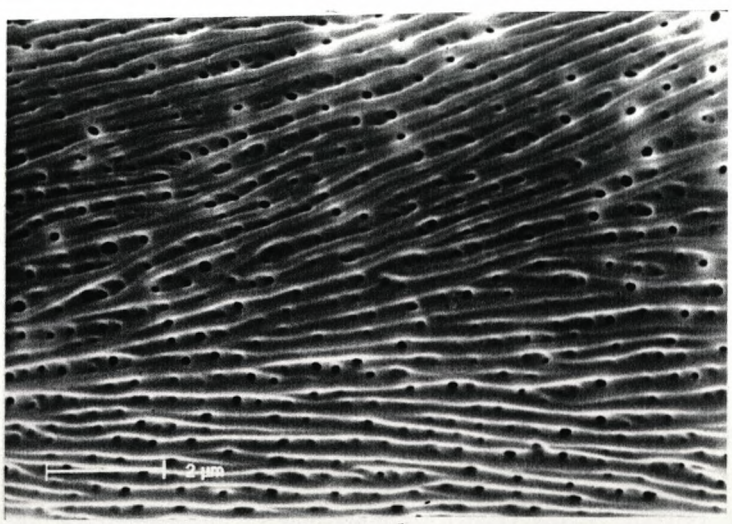
Scanning electron micrographs of *M. kansuensis* pollen.

Plate 14. A. Equatorial view.

Plate 14. B. 10000x. Exine sculpture showing the ridges .



A



B

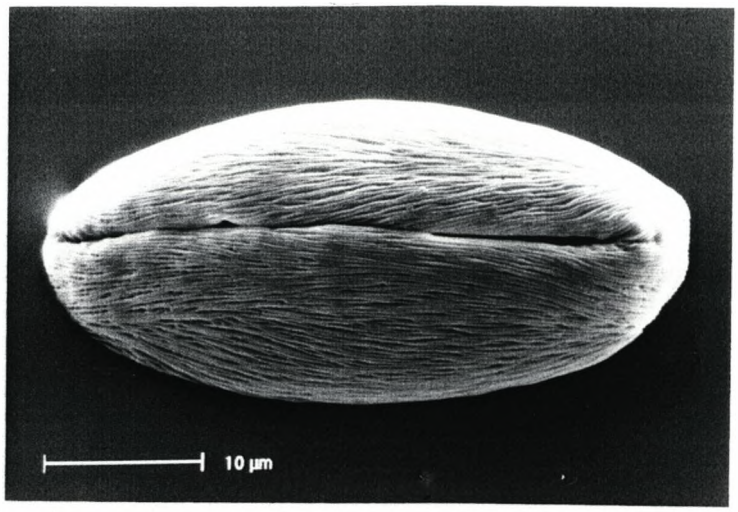
Plate 15

Scanning electron micrographs of *M. transitoria* pollen.

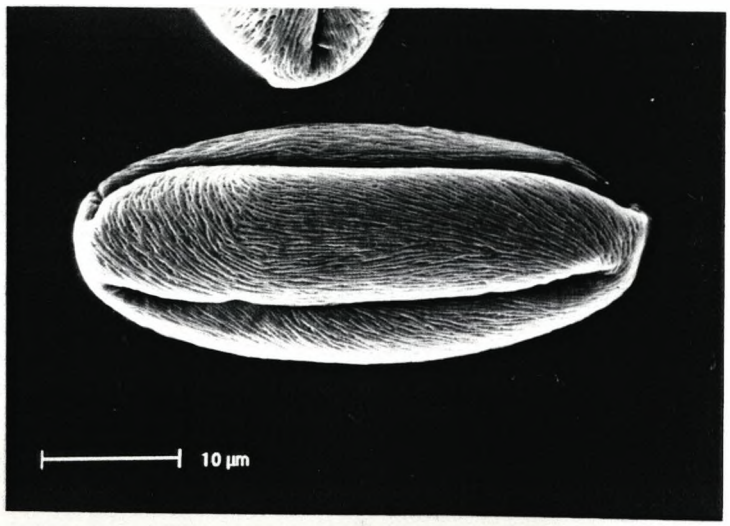
Plate 15. A. Side view.

Plate 15. B. Equatorial view.

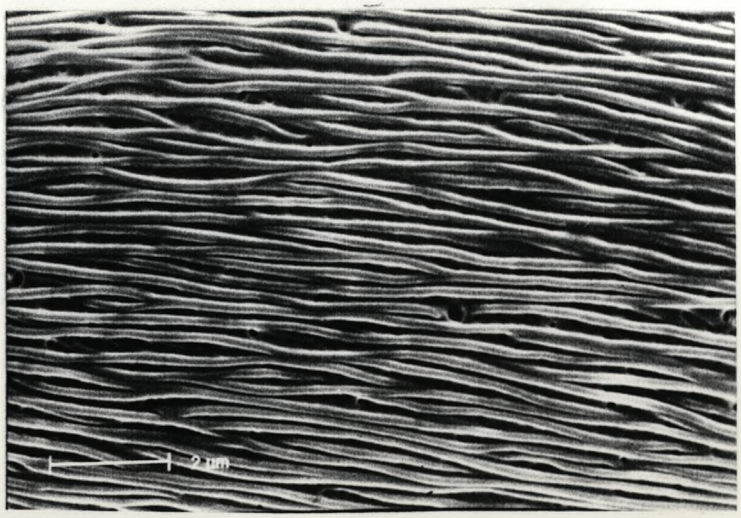
Plate 15. C. 10000x. Exine sculpture showing the ridges .



A



B



C

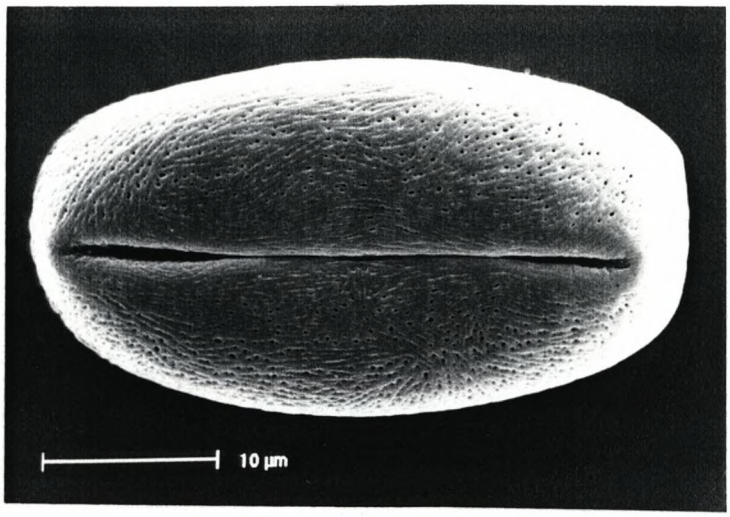
Plate 16

Scanning electron micrographs of *M. prattii* pollen.

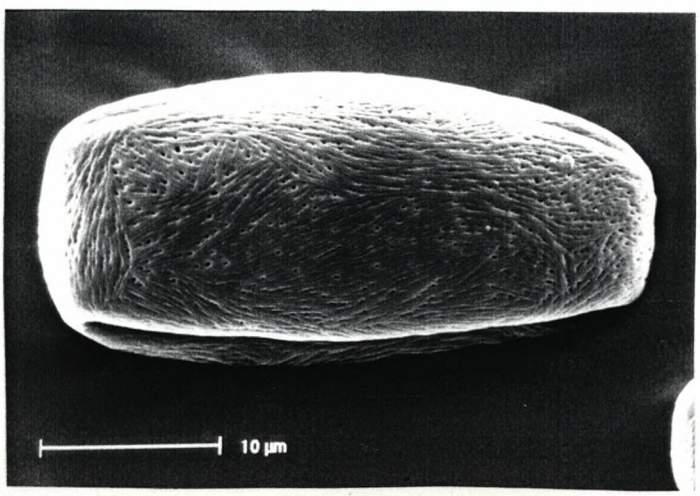
Plate 16. A. Side view.

Plate 16. B. Equatorial view.

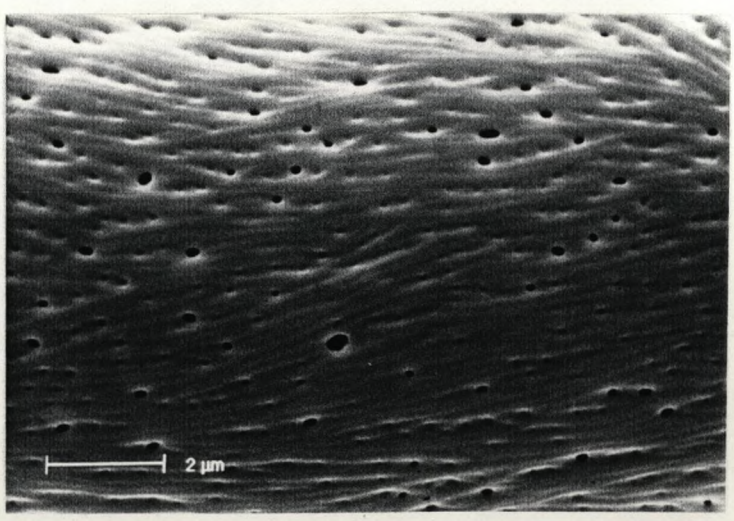
Plate 16. C. 10000x. Exine sculpture showing the ridges .



A



B



C

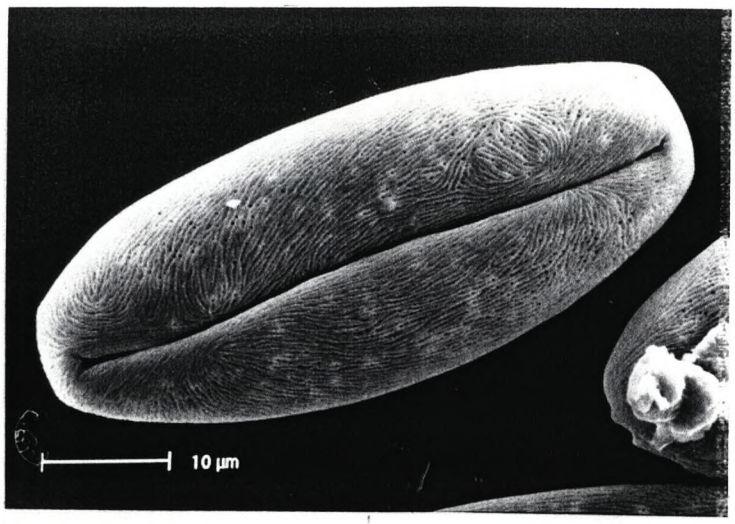
Plate 17

Scanning electron micrographs of *M. angustifolia* pollen.

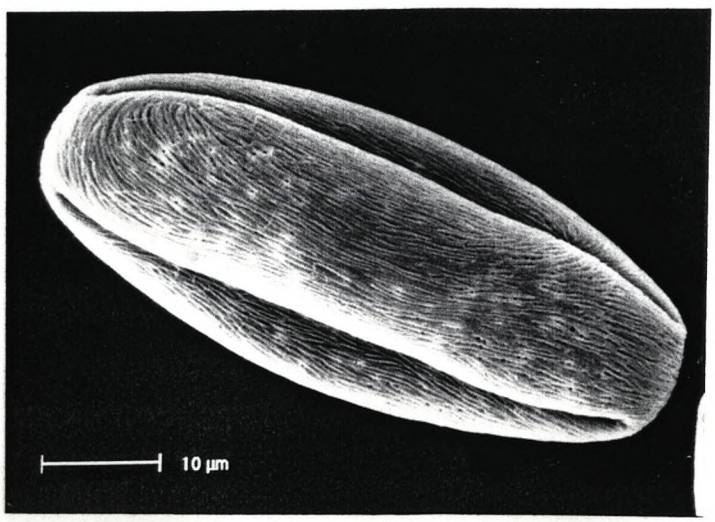
Plate 17. A. Side view.

Plate 17. B. Equatorial view.

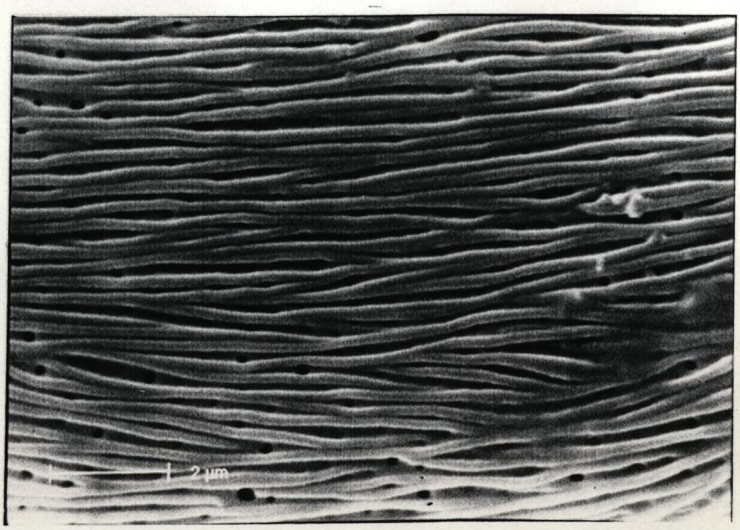
Plate 17. C. 10000x. Exine sculpture showing the ridges .



A



B



C

Plate 18

Scanning electron micrographs of *M. coronaria* pollen.

Plate 18. A. Side view.

Plate 18. B. Equatorial view.

Plate 18. C-D. 10000x. Exine sculpture showing the ridges .

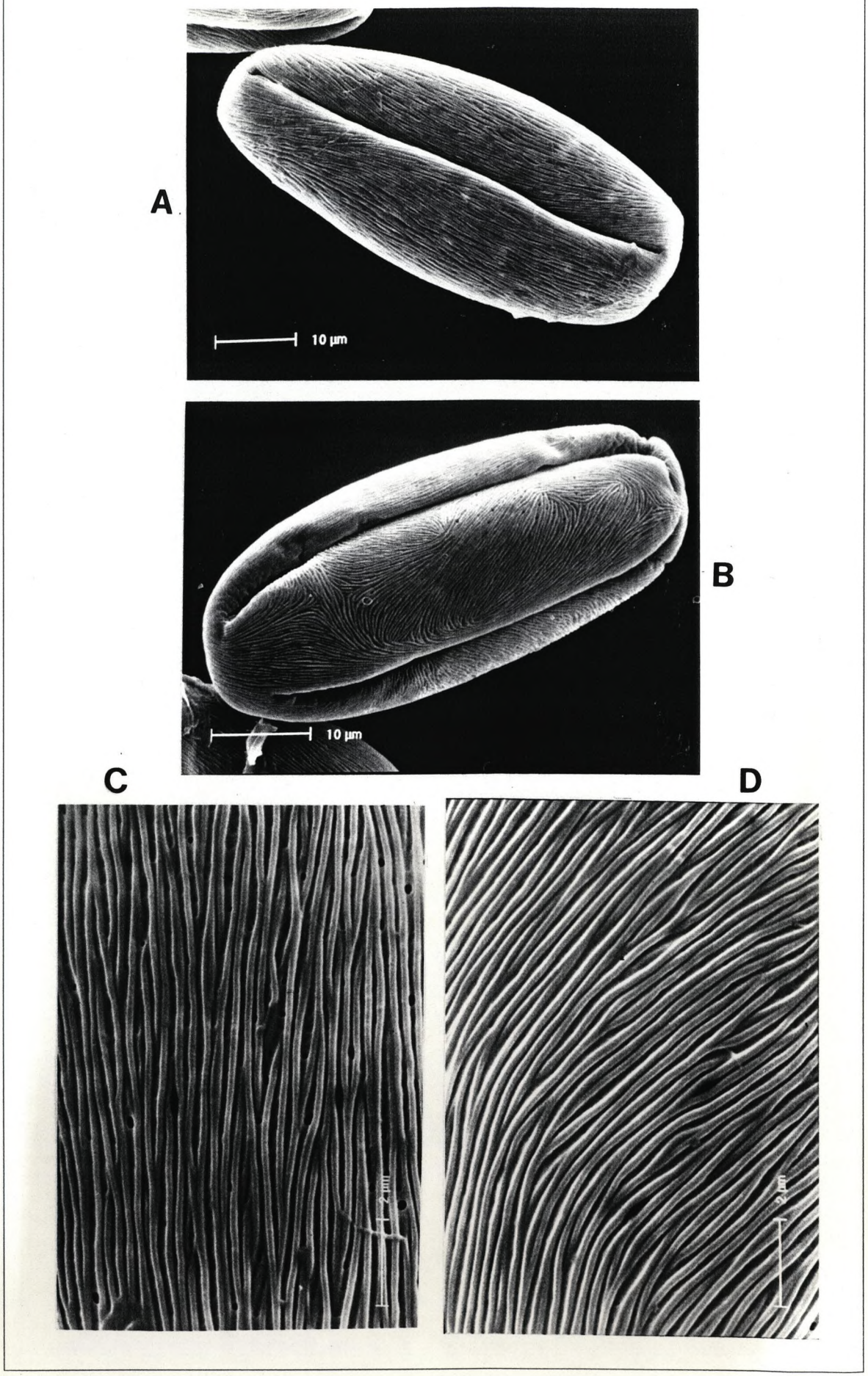

A
10 µm
B
10 µm
C
D

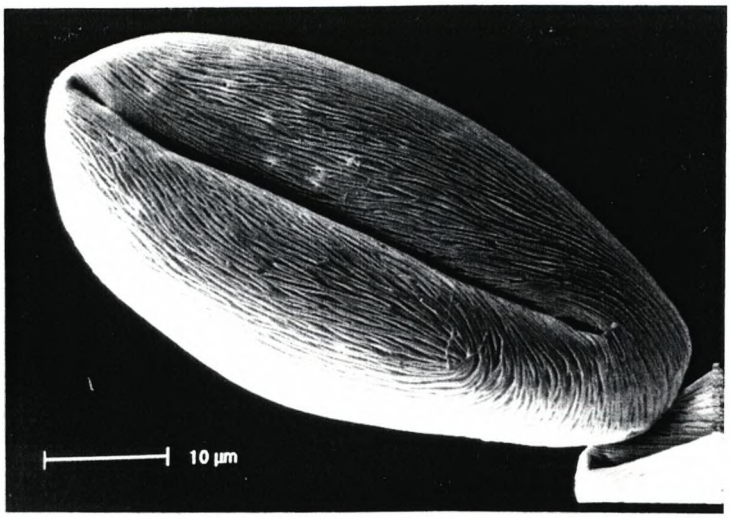
Plate 19

Scanning electron micrographs of *M. glaucescens* pollen.

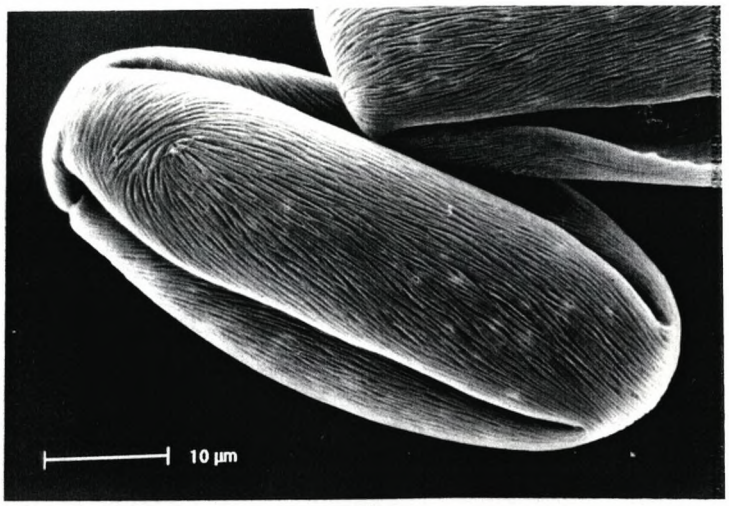
Plate 19. A. Side view.

Plate 19. B. Equatorial view.

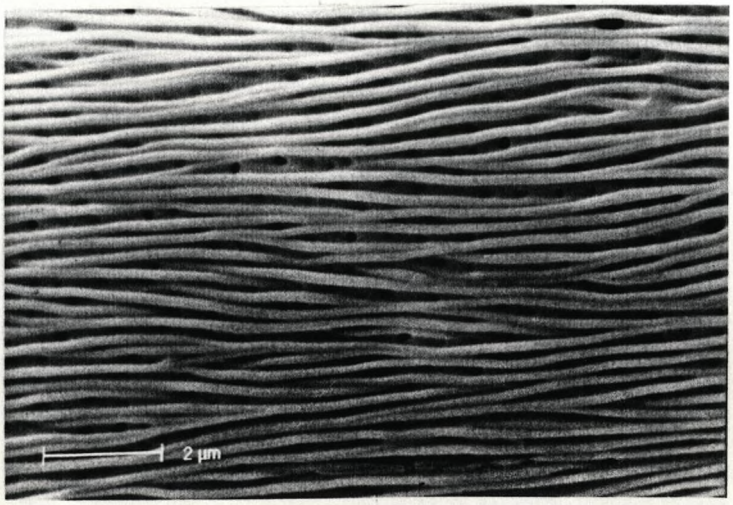
Plate 19. C. 10000x. Exine sculpture showing the ridges .



A



B



C

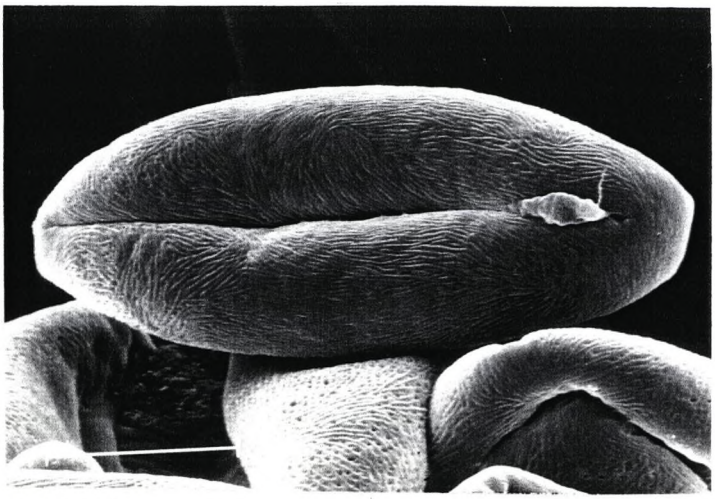
Plate 20

Scanning electron micrographs of *M. ioensis* pollen.

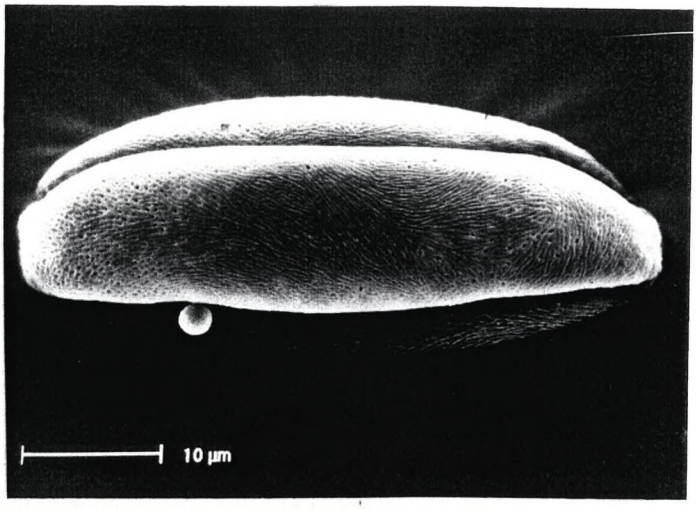
Plate 20. A. Side view.

Plate 20. B. Equatorial view.

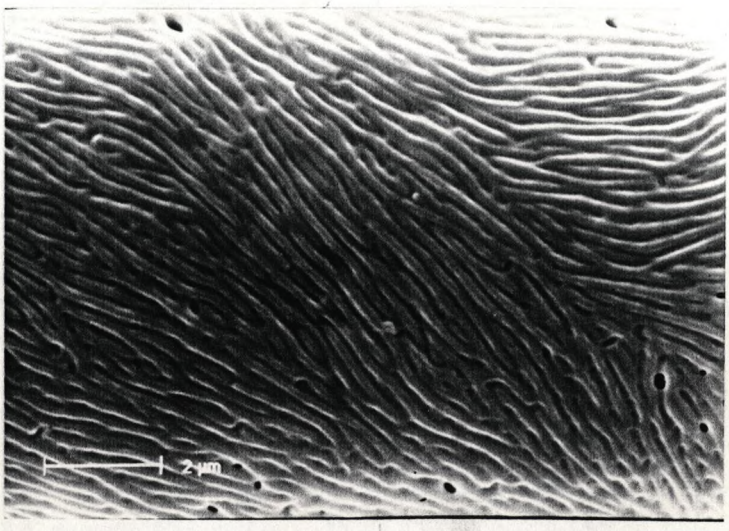
Plate 20. C. 10000x. Exine sculpture showing the ridges .



A



B



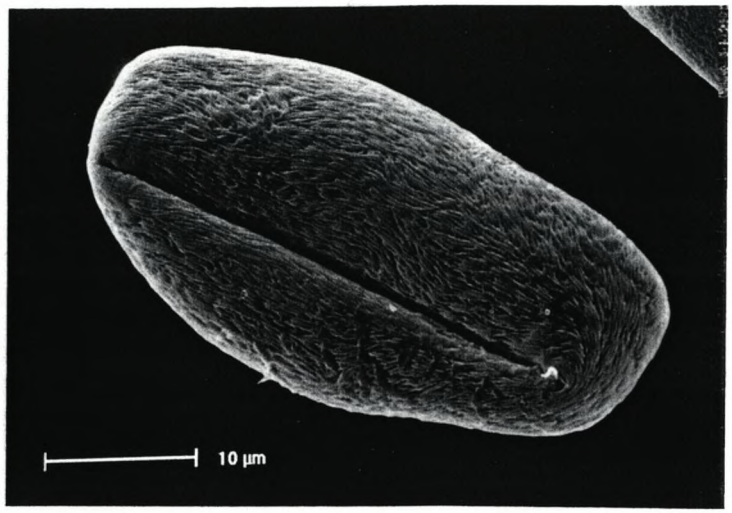
C

Plate 21

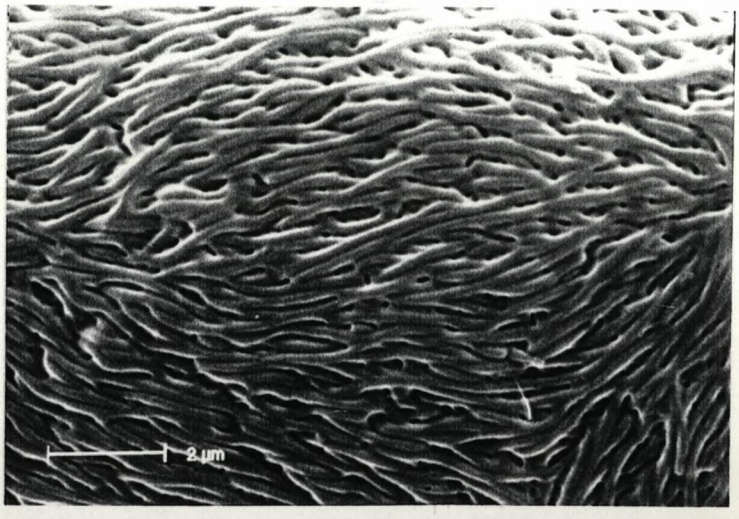
Scanning electron micrographs of *M. lancifolia* pollen.

Plate 21. A. Side view.

Plate 21. B. 10000x. Exine sculpture showing the ridges .



A



B

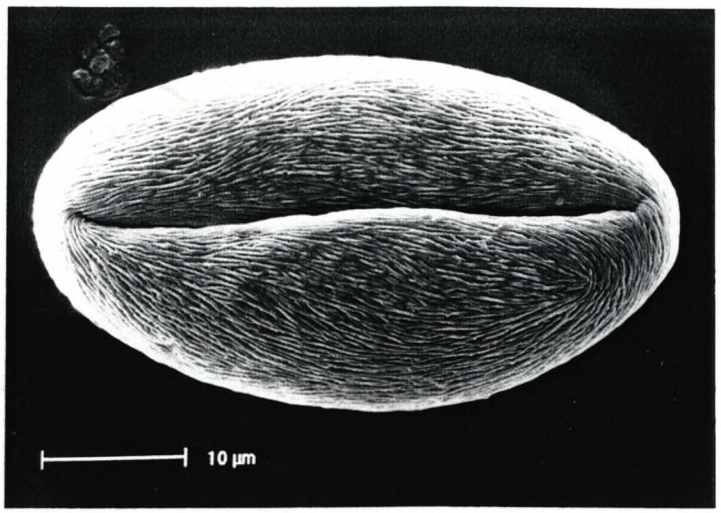
Plate 22

Scanning electron micrographs of *M. platycarpa* pollen.

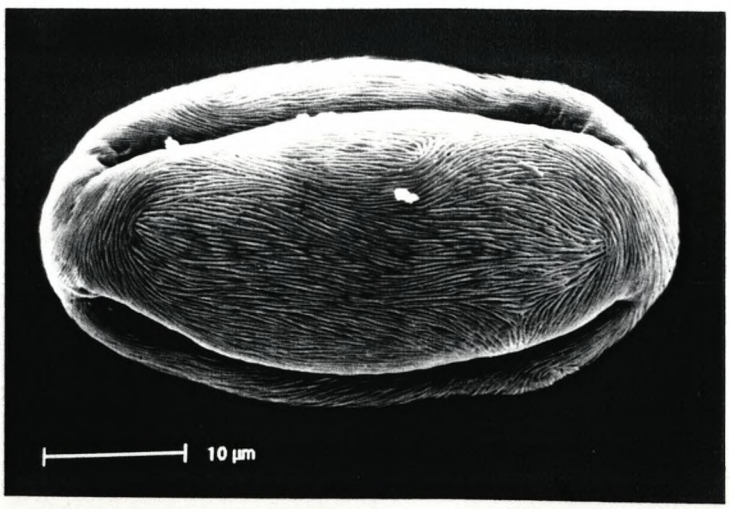
Plate 22. A. Side view.

Plate 22. B. Equatorial view.

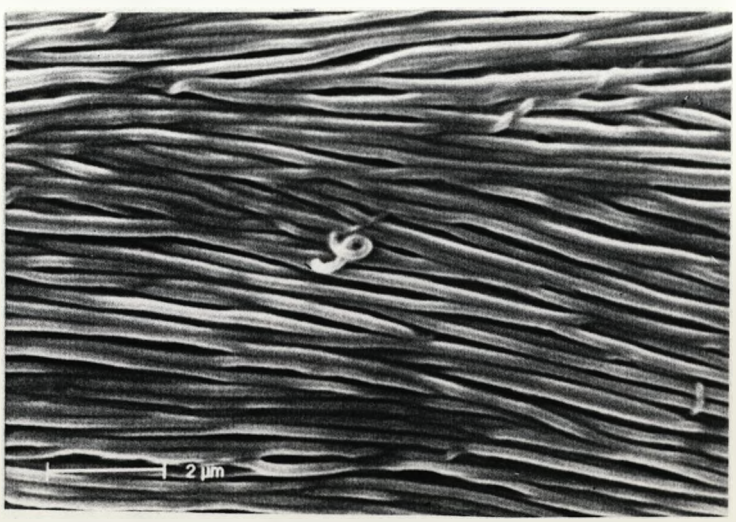
Plate 22. C. 10000x Exine sculpture showing the ridges .



A



B



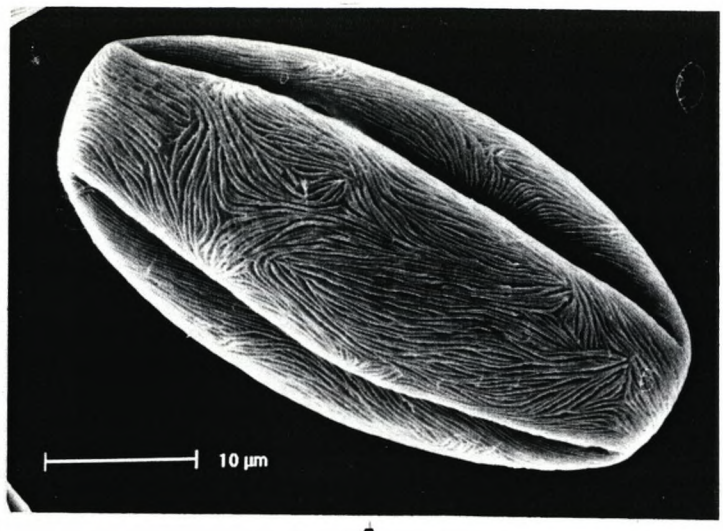
C

Plate 23

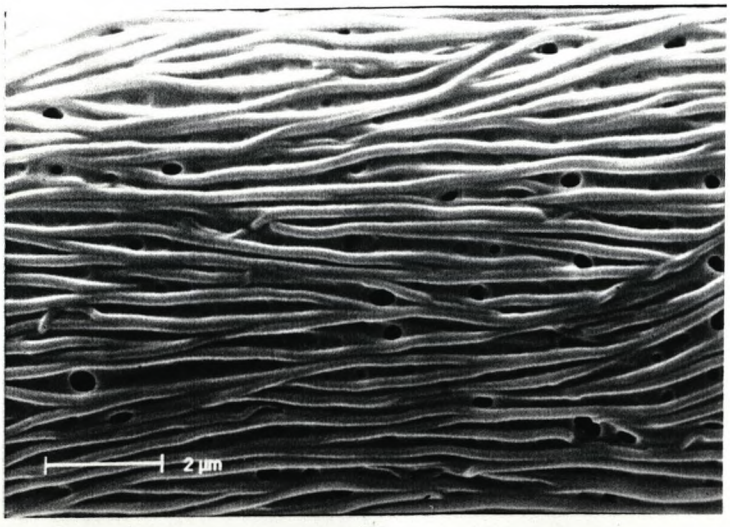
Scanning electron micrographs of *M. tschonoskii* pollen.

Plate 23. A. Equatorial view.

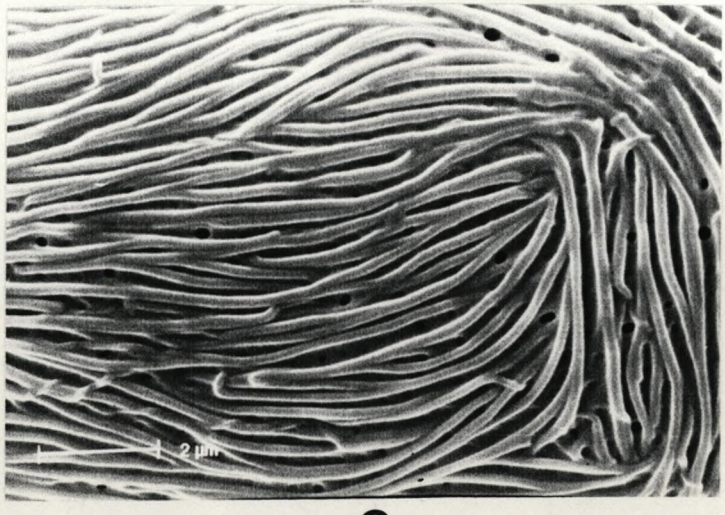
Plate 23. B-C. 10000x. Exine sculpture showing the ridges .



A



B



C

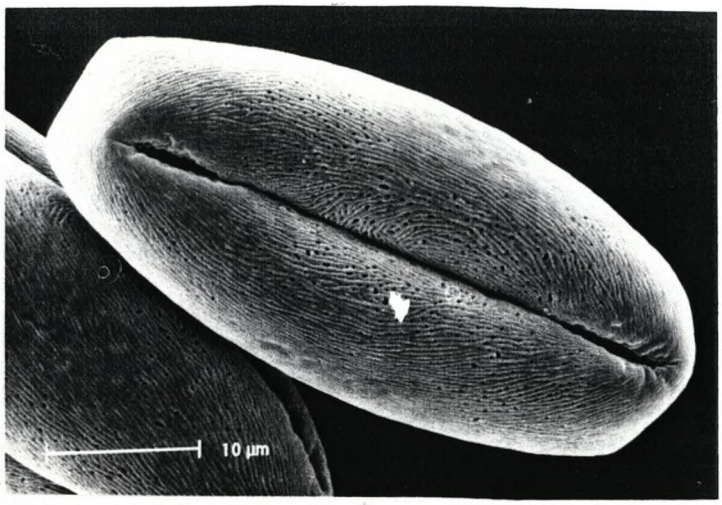
Plate 24

Scanning electron micrographs of *M. trilobata* pollen.

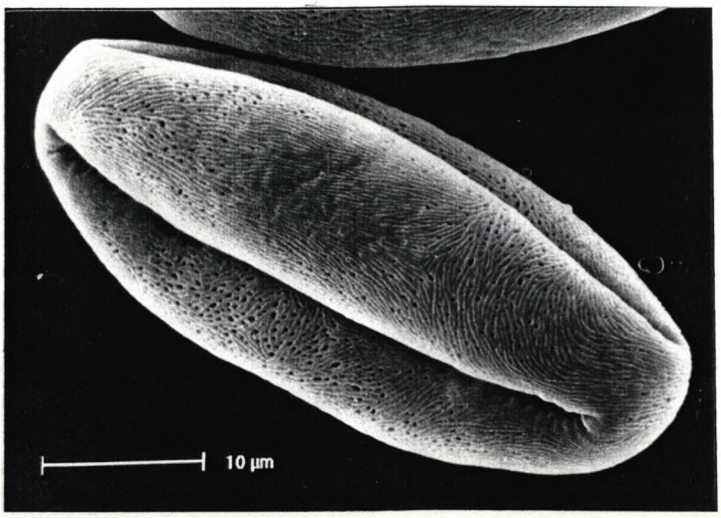
Plate 24. A. Side view.

Plate 24. B. Equatorial view.

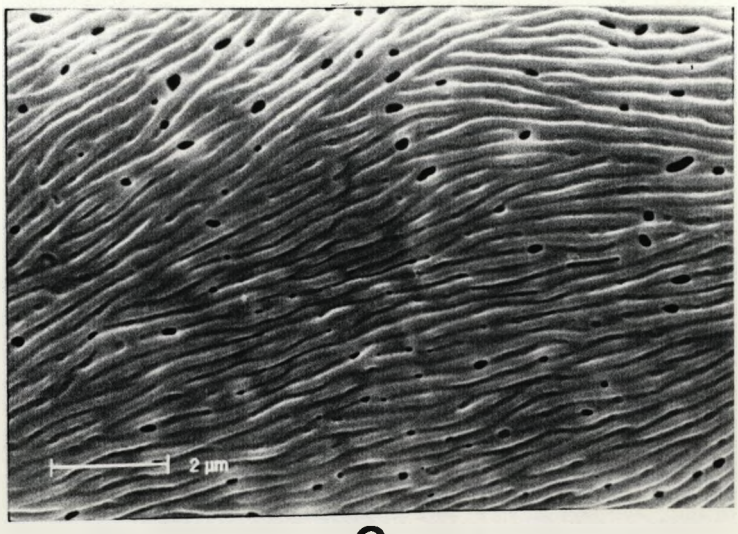
Plate 24. C. 10000x. Exine sculpture showing the ridges .



A



B



C

Plate 25

Tree of *M. florentina.*

Plate 25. A. Tree with spreading head.

Plate 25. B. Showing lobed leaves and red fruits.

A

B

Plate 26

Tree of *M. Kansuensis.*

Plate 26. A. Tree with spreading head.

Plate 26. B. Showing lobed leaves and red fruits.

A

B

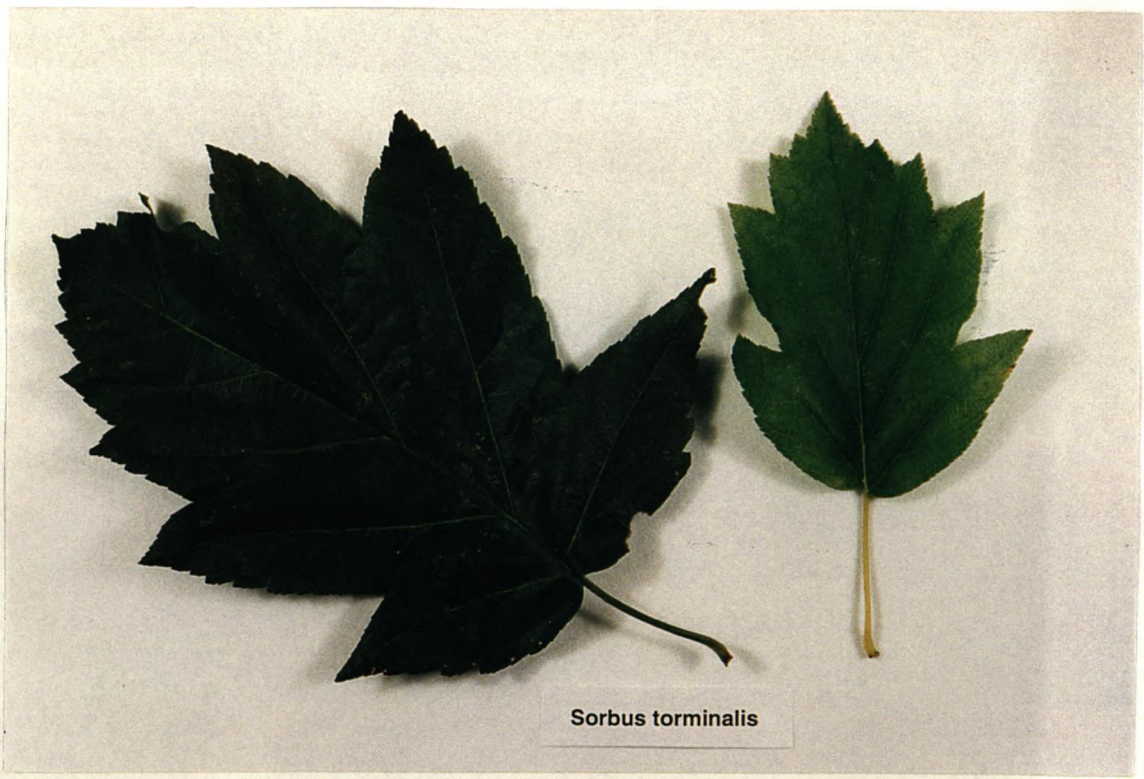
Plate 27

Comparison of *M. florentina* and *Sorbus torminalis* leaves.

Plate 27. A. *M. florentina* leaf.

Plate 27. B. *Sorbus torminalis* leaves.

A



B

Appendix

TABLE 1. ANALYSIS OF VARIANCE ON PETIOLE LENGTH

SOURCE	DF	SS	MS	F	p
S*	39	38480.3	986.7	21.35	0.000
ERROR	298	13773.2	46.2		
TOTAL	337	52253.5			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	9	19.778	3.866	(-*-)
2	10	25.900	1.912	(-*-)
3	10	47.800	14.078	(-*-)
4	10	13.200	7.315	(--*-)
5	7	25.857	4.525	(--*-)
6	8	53.125	14.126	(--*-)
7	7	22.429	2.149	(-*--)
8	6	41.167	3.764	(--*-)
9	10	14.000	1.414	(-*-)
10	8	25.125	6.490	(--*-)
11	10	25.600	6.802	(-*-)
12	7	26.000	3.464	(--*--)
13	7	32.857	4.947	(-*--)
14	7	65.000	17.559	(--*-)
15	11	22.818	9.527	(-*-)
16	5	32.000	6.595	(--*--)
17	4	19.250	1.500	(---*--)
18	10	25.900	3.635	(-*-)
19	11	32.000	8.062	(-*-)
20	4	21.000	6.272	(---*--)
21	10	20.700	5.012	(-*-)
22	6	25.167	4.070	(--*-)
23	10	39.800	7.700	(-*-)
24	10	15.200	4.590	(--*-)
25	10	23.900	6.641	(-*-)
26	8	13.875	4.224	(-*-)
27	10	39.000	9.345	(--*-)
28	10	20.600	2.797	(-*-)
29	10	36.300	5.638	(-*-)
30	8	24.125	2.949	(-*-)
31	7	23.857	6.362	(--*-)
32	8	42.500	6.188	(-*--)
33	6	23.500	4.231	(--*-)
34	9	18.111	6.660	(-*-)
35	12	22.833	4.130	(-*-)
36	10	28.100	5.174	(-*-)
37	10	16.700	4.084	(-*-)
38	7	28.571	2.699	(-*--)
39	10	23.800	7.510	(-*-)
40	6	29.667	4.926	(--*--)

POOLED STDEV = 6.798

20 40 60 80

KEY TO LEVELS:

1. *M. ioensis* var. *palmeri*, 2. *M. tschonoskii*, 3. *M. yunnanensis*, 4. *M. halliana*, 5. *M. halliana*, 6. *M. dasyphylla*, 7. *M. pumila*, 8. *M. fusca*, 9. *M. sargentii*, 10. *M. transitoria*, 11. *M. kansuensis*, 12. *M. yunnanensis*, 13. *M. angustifolia*, 14. *M. trilobata*, 15. *M. angustifolia*, 16. *M. prattii*, 17. *M. sylvestris*, 18. *M. sieboldii*, 19. *M.* x *robusta*, 20. *M. kansuensis*, 21. *M. zumi*, 22. *M. kansuensis*, 23. *M. baccata* var. *mandshurica*, 24. *M. hupehensis*, 25. *M. solardii*, 26, *M. kirghisorum*, 27. *M. trilobata*, 28. *M. tschonoskii*, 29. *M. florentina*, 30. *M. kansuensis*, 31. *M. angustifolia*, 32. *M. baccata* var. *lutea*, 33. *M. hupehensis*, 34. *M. sargentii*, 35. *M. baccata* var. *jackii*, 36. *M. platycarpa*, 37. *M. sieboldii*, 38. *M. coronaria*, 39. *M. coronaria*, 40. *M. prunifolia*.

* S = Number of specimens

TABLE 2. ANALYSIS OF VARIANCE ON LEAF LENGTH

SOURCE	DF	SS	MS	F	p
S*	38	62533	1646	12.77	0.000
ERROR	297	38275	129		
TOTAL	335	100808			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	9	83.33	12.65	(--*--)
2	10	64.80	5.71	(--*--)
3	10	111.00	25.02	(-*--)
4	10	75.80	12.25	(--*--)
5	7	64.71	6.78	(--*--)
6	7	80.86	12.97	(--*---)
7	7	40.43	18.55	(--*---)
8	10	63.30	5.06	(--*--)
9	11	52.55	11.31	(--*--)
10	10	72.60	9.13	(--*--)
11	7	94.43	14.71	(---*--)
12	7	75.57	7.85	(--*---)
13	8	79.00	13.66	(---*--)
14	11	76.82	12.41	(--*-)
15	5	94.60	21.15	(---*---)
16	4	67.00	14.09	(----*---)
17	10	69.20	4.96	(--*--)
18	10	88.70	10.87	(-*--)
19	4	69.00	11.92	(----*---)
20	10	71.10	6.10	(-*--)
21	6	72.00	10.49	(---*--)
22	10	73.30	7.51	(--*--)
23	10	73.60	6.80	(-*--)
24	11	72.73	12.95	(--*--)
25	8	57.37	6.28	(--*--)
26	10	58.00	5.77	(--*--)
27	10	61.60	5.30	(--*-)
28	10	58.60	6.92	(-*--)
29	8	49.00	5.21	(---*--)
30	7	79.71	18.45	(--*--)
31	8	78.75	12.42	(---*--)
32	7	71.57	3.69	(---*--)
33	9	61.56	4.72	(--*--)
34	12	86.58	10.00	(--*-)
35	10	88.90	17.10	(--*-)
36	10	71.80	6.07	(--*--)
37	7	94.43	10.78	(---*--)
38	10	82.40	7.62	(--*--)
39	6	85.00	19.49	(---*---)

POOLED STDEV = 11.35

50 75 100

KEY TO LEVELS:

1. *M. ioensis* var. *palmeri*, 2. *M. tschonoskii*, 3. *M. yunnanensis*, 4. *M. halliana*, 5. *M. halliana*, 6. *M. dasyphilla*, 7. *M. pumila*, 8. *M. sargentii*, 9. *M. transitoria*, 10. *M. kansuensis*, 11. *M. yunnanensis*, 12, *M. angustifolia*, 13. *M. trilobata*, 14. *M. angustifolia*, 15. *M. prattii*, 16. *M. sylvestris*, 17. *M. sieboldii*, 18. *M.* x *robusta*, 19. *M. kansuensis*, 20. *M. zumi*, 21. *M. kansuensis*, 22. *M. baccata*, 23. *M. hupehensis*, 24. *M. solardii*, 25. *M. kirghisorum*, 26. *M. trilobata*, 27. *M. tschonoskii*, 28. *M.* x *floribunda*, 29. *M. kansuensis*, 30. *M. angustifolia*, 31. *M. baccata* var. *lutea*, 32. *M. hupehensis*, 33. *M. sargentii*, 34. *M. baccata* var. *jackii*, 35. *M. platycarpa*, 36. *M. sieboldii*, 37. *M. coronaria*, 38. *M. coronaria*, 39. *M. prunifolia*.

* S = Number of specimens

TABLE 3. ANALYSIS OF VARIANCE ON LEAF WIDTH

SOURCE	DF	SS	MS	F	p
S*	38	65638	1727	12.60	0.000
ERROR	291	39892	137		
TOTAL	329	105530			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	9	63.22	12.69	(--*--)
2	10	49.90	4.25	(--*--)
3	10	70.80	18.87	(--*--)
4	10	38.20	7.21	(--*--)
5	7	35.71	3.15	(--*---)
6	6	38.67	4.55	(--*---)
7	7	36.71	32.26	(---*--)
8	10	65.00	10.03	(--*--)
9	8	36.25	7.65	(---*--)
10	10	46.40	7.29	(--*-)
11	7	57.86	6.15	(--*---)
12	7	47.14	5.76	(---*--)
13	8	97.37	21.33	(--*--)
14	11	63.64	17.88	(-*--)
15	5	60.80	11.43	(---*---)
16	4	36.00	4.08	(---*----)
17	10	60.10	5.47	(--*--)
18	10	43.80	18.83	(--*-)
19	4	49.75	4.27	(----*----)
20	10	32.10	6.33	(--*--)
21	6	62.50	8.22	(---*---)
22	10	52.20	6.36	(--*--)
23	10	37.20	2.49	(--*--)
24	11	48.09	12.21	(--*--)
25	8	36.75	4.33	(---*--)
26	10	62.30	12.88	(--*--)
27	10	48.30	7.76	(--*--)
28	10	49.20	5.85	(--*--)
29	8	38.00	3.51	(--*--)
30	7	62.86	15.23	(--*---)
31	8	47.62	5.53	(--*--)
32	6	33.00	3.74	(---*---)
33	9	46.22	15.72	(--*---)
34	11	45.36	10.04	(--*--)
35	10	56.00	12.22	(--*--)
36	10	75.20	7.97	(--*--)
37	7	63.71	18.30	(--*---)
38	10	79.00	13.68	(--*--)
39	6	53.83	6.79	(---*--)

POOLED STDEV = 11.71

25 50 75 100

KEY TO LEVELS:

1. *M. ioensis* var. *palmeri*, 2. *M. tschonoskii*, 3. *M. yunnanensis*, 4. *M. halliana*, 5. *M. halliana*, 6. *M.dasyphylla*, 7. *M. pumila*, 8. *M. sargentii*, 9. *M. transitoria*, 10. *M. kansuensis*, 11. *M.yunnanensis*, 12, *M. angustifolia*, 13. *M. trilobata*, 14. *M. angustifolia*, 15. *M. prattii*, 16. *M. sylvestris*, 17. *M. sieboldii*, 18. *M. robusta*, 19. *M. kansuensis*, 20. *M. zumi*, 21. *M. kansuensis*, 22. *M. baccata*, 23. *M. hupehensis*, 24. *M. solardii*, 25. *M. kirghisorum*, 26, *M. trilobata*, 27. *M. tschonoskii*, 28. *M. florentina*, 29. *M. kansuensis*, 30. *M. angustifolia*, 31. *M. baccata* var. *lutea*, 32. *M. hupehensis*, 33. *M. sargentii*, 34. *M. baccata* var. *jackii*, 35. *M. platycarpa*, 36. *M. sieboldii*, 37. *M. coronaria*, 38. *M. coronaria*, 39. *M. prunifolia*.

* S = Number of specimens

TABLE 4. ANALYSIS OF VARIANCE ON NO. OF FLOWERS PER INFLORESCENS

SOURCE	DF	SS	MS	F	p
S*	34	450.64	13.25	12.77	0.000
ERROR	353	366.35	1.04		
TOTAL	387	816.99			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	10	4.400	0.843	(--*--)
2	7	5.286	0.756	(--*---)
3	10	6.800	1.229	(--*--)
4	37	4.892	1.125	(*-)
5	7	5.286	0.951	(--*---)
6	5	6.800	0.447	(---*---)
7	10	7.200	1.033	(--*--)
8	14	5.071	1.141	(-*--)
9	8	5.625	0.518	(--*---)
10	17	6.353	1.057	(--*-)
11	13	5.000	0.707	(--*--)
12	8	5.000	1.069	(---*---)
13	16	5.375	0.885	(--*-)
14	16	5.563	0.814	(--*-)
15	7	7.714	2.289	(---*--)
16	18	6.222	1.353	(-*-)
17	13	6.077	0.641	(-*--)
18	8	6.000	1.069	(---*---)
19	18	6.167	0.514	(--*-)
20	17	3.118	1.269	(--*-)
21	7	3.571	1.134	(---*---)
22	9	5.222	0.667	(--*--)
23	8	4.375	1.188	(---*--)
24	12	4.583	1.379	(--*--)
25	10	5.100	0.738	(--*---)
26	9	3.444	1.130	(--*---)
27	11	4.818	1.328	(--*--)
28	10	5.800	0.632	(--*--)
29	7	4.857	0.378	(---*---)
30	7	5.143	0.378	(---*---)
31	8	6.750	0.707	(---*--)
32	7	7.857	1.215	(---*---)
33	7	6.286	0.488	(--*---)
34	8	6.750	0.707	(---*--)
35	9	7.333	0.866	(---*--)

POOLED STDEV = 1.019

4.0 6.0 8.0

KEY TO LEVELS:

1. *M. tschonoskii*, 2. *M. sylvestris*, 3. *M. baccata*, 4. *M. sieboldii*, 5. *M. hupehensis*, 6. *M.sargentii*, 7. *M. sikkimensis*, 8. *M. coronaria*, 9. *M. ioensis* var. *palmeri*, 10. *M. lancifolia*, 11. *M. glaucesens*, 12, *M. angustifolia*, 13. *M. toringoides*, 14. *M. transitoria*, 15. *M. kansuensis*, 16. *M. florentina*, 17. *M. pumila*, 18. *M. prunifolia*, 19. *M. halliana*, 20. *M. baccata* var. *gracilis*, 21. *M. spectabilis*, 22. *M. domestica*, 23. *M. niedzwetzkyana*, 24. *M. zumi*, 25. *M. baccata* var. *mandshurica*, 26. *M. prunifolia* var. *rinki*, 27. *M.* x *floribunda*, 28. *M. niedzwetzkyana*, 29. *M. tschonoskii*, 30. *M. tschonoskii*, 31. *M.* x *floribunda*, 32. *M. floribunda* var. *hillieri*, 33. *M.* x *robusta*, 34. *M.* x *floribunda*, 35. *M. sargentii*.

* S = Number of specimens

TABLE 5. ANALYSIS OF VARIANCE ON PETAL LENGTH

SOURCE	DF	SS	MS	F	p
S*	32	8826.08	275.82	104.07	0.000
ERROR	357	946.19	2.65		
TOTAL	389	9772.27			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	9	16.667	2.179	(-*-)
2	10	15.900	1.729	(*-)
3	10	10.300	0.483	(-*-)
4	10	19.800	1.619	(-*-)
5	13	12.769	1.589	(*-)
6	13	17.231	2.242	(-*)
7	12	16.583	1.240	(-*)
8	9	15.000	0.866	(-*-)
9	10	20.600	0.699	(*-)
10	10	17.400	0.843	(-*-)
11	14	10.643	0.745	(-*)
12	12	7.083	0.900	(-*)
13	9	8.111	1.453	(-*)
14	17	24.059	1.600	(*)
15	11	14.091	3.113	(*-)
16	14	16.286	1.729	(*-)
17	15	16.800	2.042	(*)
18	12	20.833	3.786	(-*)
19	10	16.900	1.449	(-*-)
20	10	23.100	2.514	(*-)
21	10	17.100	1.287	(*-)
22	10	19.100	1.287	(-*-)
23	10	17.300	0.823	(-*-)
24	9	19.889	1.900	(-*-)
25	19	11.526	0.772	(*)
26	14	12.571	0.756	(*)
27	11	16.091	1.044	(-*)
28	10	13.000	1.054	(-*)
29	20	26.050	1.761	(*-)
30	17	14.882	0.857	(*)
31	10	7.900	0.876	(-*-)
32	11	18.273	2.054	(*-)
33	9	9.000	0.866	(-*-)
				12.0 18.0 24.0
POOLED STDEV =		1.628		

KEY TO LEVELS:

1. *M. sylvestris*, 2. *M. baccata*, 3. *M. sieboldii*, 4. *M. hupehensis*, 5. *M. sikkimensis,* 6. *M. coronaria*, 7. *M. ioensis* var. *palmeri*, 8. *M. lancifolia, 9. M. glaucescens*, 10. *M. angustifolia*, 11. *M. toringoides*, 12, *M. transitoria*, 13. *M. kansuensis*, 14. *M. pumila*, 15. *M. prunifolia*, 16. *M. halliana*, 17. *M. baccata* var. *gracilis*, 18. *M. spectabilis*, 19. *M. domestica*, 20. *M. niedzwetzkyana*, 21. *M. baccata* var. *mandshurica*, 22. *M. prunifolia*, 23. *M. rockii*, 24. *M. robusta*, 25. *M. sieboldii*, 26. *M. tschonoskii*, 27. *M. fusca,* 28. *M. sargentii*, 29. *M. niedzwetzkyana*, 30. *M.* x *floribunda*, 31. *M. prattii*, 32. *M. platycarpa,* 33. *M. kansuensis.*

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* S = Number of specimens

TABLE 6. ANALYSIS OF VARIANCE ON PETAL WIDTH

SOURCE	DF	SS	MS	F	p
S*	32	3201.36	100.04	57.97	0.000
ERROR	357	616.10	1.73		
TOTAL	389	3817.46			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	9	9.000	0.707	(--*-)
2	10	13.500	1.434	(--*-)
3	10	5.900	0.738	(-*-)
4	10	13.000	0.943	(-*-)
5	13	11.846	1.573	(-*-)
6	13	11.923	1.891	(-*-)
7	12	10.917	1.084	(-*-)
8	9	8.556	0.882	(-*--)
9	10	15.300	0.823	(--*-)
10	10	13.100	1.792	(-*--)
11	14	8.357	0.497	(-*-)
12	12	5.000	0.426	(-*-)
13	9	8.222	1.563	(-*--)
14	17	15.059	1.784	(-*-)
15	11	9.727	0.647	(-*-)
16	14	12.143	1.292	(-*-)
17	15	9.200	1.082	(-*-)
18	12	12.333	2.640	(-*-)
19	10	10.900	1.101	(-*-)
20	10	13.200	2.300	(--*-)
21	10	12.500	0.972	(--*-)
22	10	12.100	0.876	(--*-)
23	10	8.500	0.707	(-*--)
24	9	12.556	1.014	(--*-)
25	19	6.737	0.452	(*-)
26	14	7.643	0.497	(-*-)
27	11	12.818	1.401	(--*-)
28	10	10.800	0.919	(-*-)
29	20	15.000	1.864	(-*-)
30	17	7.412	0.795	(-*-)
31	10	7.400	1.075	(-*-)
32	11	13.000	2.000	(-*-)
33	9	7.000	1.225	(-*-)
POOLED STDEV =		1.314		7.0 10.5 14.0

KEY TO LEVELS:

1. *M. sylvestris*, 2. *M. baccata*, 3. *M. sieboldii*, 4. *M. hupehensis*, 5. *M. sikkimensis*, 6. *M. coronaria*, 7. *M. ioensis* var. *palmeri*, 8. *M. lancifolia*, 9. *M.* glaucescens, 10. *M. angustifolia*, 11. *M. toringoides*, 12, *M. transitoria*, 13. *M. kansuensis*, 14. *M. pumila*, 15. *M. prunifolia*, 16. *M. halliana*, 17. *M. baccata* var. *gracilis*, 18. *M. spectabilis*, 19. *M. domestica*, 20. *M. niedzwetzkyana*, 21. *M. baccata* var. *mandshurica*, 22. *M. prunifolia*, 23. *M. rockii*, 24. *M. robusta*, 25. *M. sieboldii*, 26. *M. tschonoskii*, 27. *M. fusca*, 28. *M. sargentii*, 29. *M. niedzwetzkyana*, 30. *M.* x *floribunda*, 31. *M. prattii*, 32. *M. platycarpa*, 33. *M. kansuensis*.

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* S = Number of specimens

TABLE 7. ANALYSIS OF VARIANCE ON STAMEN NUMBER

SOURCE	DF	SS	MS	F	p
S*	32	31961.48	998.80	381.42	0.000
ERROR	461	1207.20	2.62		
TOTAL	493	33168.68			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	18	47.333	3.804	
2	29	20.034	0.680	
3	29	20.897	1.496	
4	7	25.571	0.976	
5	12	24.333	2.902	
6	9	20.000	0.000	
7	10	19.900	0.994	
8	10	20.000	0.000	
9	11	20.000	0.000	
10	14	19.214	1.188	
11	18	20.000	0.000	
12	13	21.385	1.758	
13	12	20.250	0.622	
14	10	19.900	0.316	
15	12	20.000	0.000	
16	11	19.000	1.265	
17	14	18.214	1.122	
18	11	22.182	3.188	
19	5	34.800	5.357	
20	11	19.909	0.302	
21	10	19.900	0.316	
22	11	18.818	1.401	
23	9	14.333	1.225	
24	10	18.300	1.059	
25	40	15.225	1.368	
26	20	20.100	0.553	
27	11	19.545	1.036	
28	15	53.733	3.654	
29	15	18.867	1.685	
30	22	19.455	0.800	
31	19	18.895	1.243	
32	15	19.867	0.516	
33	31	20.839	1.463	

POOLED STDEV = 1.618

24 36 48

KEY TO LEVELS:

1. *M. tschonoskii* Ness, 2. *M. sylvestris* Ness, 3. *M. baccata* Ness, 4. *M. hupehensis.* Ness, 5. *M. sikkimensis*, Ness. 6. *M. coronaria*, 7. *M. ioensis* var. *palmeri*, 8. *M. lancifolia*, 9. *M. glaucesens*, 10. *M. angustifolia*, 11. *M. toringoides*, 12, *M. transitoria*, 13. *M. kansuensis*, 14. *M. florentina*, 15. *M. pumila*, 16. *M. prunifolia*, 17. *M. halliana*, 18. *M. baccata* var. *gracilis*, 19. *M. spectabilis*, 20. *M. domestica*, 21. *M. niedzwetzkyana*, 22. *M. baccata* var *mandshurica*, 23. *M. prunifolia* var. *rinkii*, 24. *M. baccata*, 25. *M. kansuensis*, 26, *M. prattii*, 27. *M. niedzwetzkyana*, 28. *M. tschonoskii*, 29. *M. floribunda* 'Excellenz theil', 30. *M. sargentii*, 31. *M. sargentii*, 32. *M. fusca*, 33. *M. sieboldii.*

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* S = Number of specimens

TABLE 8. ANALYSIS OF VARIANCE ON CARPEL NUMBER

SOURCE	DF	SS	MS	F	p
S*	73	539.473	7.390	27.34	0.000
ERROR	1024	276.794	0.270		
TOTAL	1097	816.267			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	17	4.9412	0.2425	(-*)
2	19	5.0000	0.0000	(*)
3	12	5.0000	0.0000	(*)
4	14	5.0714	0.2673	(*-)
5	1	5.0000	0.0000	(----*----)
6	16	4.1875	0.8342	(*)
7	1	5.0000	0.0000	(----*----)
8	4	5.0000	0.0000	(--*--)
9	4	5.0000	0.0000	(--*--)
10	11	4.0909	0.7006	(*-)
11	19	3.3684	0.4956	(*)
12	9	3.4444	0.5270	(*-)
13	10	3.9000	0.7379	(-*)
14	7	4.5714	0.7868	(-*-)
15	17	3.7647	0.5623	(*)
16	21	3.9048	0.5390	(-*)
17	10	3.5000	0.5270	(-*)
18	10	4.6000	0.5164	(-*-)
19	30	3.5667	0.6789	(*)
20	5	5.0000	0.0000	(-*-)
21	2	4.0000	0.0000	(---*---)
22	3	4.3333	0.5774	(--*--)
23	21	5.0000	0.0000	(*)
24	4	5.0000	0.0000	(--*--)
25	7	5.0000	0.0000	(-*-)
26	10	3.8000	0.4216	(-*-)
27	5	4.6000	0.5477	(-*-)
28	9	3.6667	0.5000	(*-)
29	11	5.0000	0.0000	(-*-)
30	5	3.4000	0.8944	(-*-)
31	12	3.7500	0.7538	(-*)
32	4	3.0000	0.8165	(--*--)
33	10	3.4000	0.5164	(-*-)
34	9	4.8889	0.3333	(*-)
35	10	4.6000	0.6992	(-*-)
36	5	5.0000	0.0000	(-*-)
37	6	4.6667	0.5164	(-*-)
38	10	3.0000	0.0000	(-*-)
39	3	5.0000	0.0000	(--*--)
40	4	4.5000	0.5774	(--*-)
41	6	3.6667	0.5164	(-*-)
42	7	4.1429	0.3780	(-*-)
43	5	3.0000	0.0000	(-*-)
44	5	3.8000	0.4472	(-*-)
45	5	3.0000	0.0000	(-*-)
46	5	3.4000	0.5477	(-*-)
47	21	5.1905	0.5118	(*)
48	44	4.9773	0.1508	(*)
49	43	4.9767	0.1525	(*)
50	49	4.0612	0.6585	*)
51	29	4.2069	0.6199	(*)
52	12	4.2500	0.6216	(*-)
53	10	5.0000	0.0000	(-*-)
54	10	5.0000	0.0000	(-*-)
55	11	4.9091	0.3015	(-*)
56	13	4.2308	0.5991	(*-)

57	11	3.6364	0.8090	(*-)
58	7	8.8571	1.7728	(-*-)
59	12	5.0000	0.0000	(*)
60	20	5.4000	0.5026	(*)
61	17	5.5294	0.7174	(-*)
62	20	4.3000	0.5712	(-*)
63	19	4.6316	0.4956	(*)
64	15	4.8667	0.3519	(*-)
65	22	5.0000	0.0000	(*)
66	30	5.0000	0.0000	(*)
67	16	3.9375	0.7719	(-*)
68	34	5.6176	0.6038	(*)
69	29	4.9310	0.3714	(*)
70	41	4.6098	0.5421	(*)
71	42	4.4048	0.6270	(*)
72	33	4.5758	0.5019	(*)
73	37	4.2703	0.5082	*)
74	31	4.0323	0.7063	(*)
POOLED STDEV =		0.5199		4.0 6.0 8.0

KEY TO LEVELS:

1. *M. florentina*, 2. *M. glaucescens*, 3. *M. coronaria*, 4. *M. angustifolia*, 5. *M. trilobat*, 6. *M. tansitoria*, 7. *M. tschonoskii*, 8. *M. baccata*, 9. *M. baccata*, 10. *M. toringoides*, 11. *M. toringoides*, 12, *M. toringoides*, 13. *M. sargentii*, 14. *M. sargentii*, 15. *M. prunifolia* var. *rinkii*, 16. *M. baccata* var. *mandshurica*, 17. *M. baccata* var. *himalaica*, 18. *M. sikkimensis*, 19. *M. kansuensis*, 20. *M. baccata* var. *jackii*, 21. *M. floribunda*, 22. *M. sylvestris*, 23. *M. pumila*, 24. *M. yunnanensis*, 25. *M. yunnanensis*, 26, *M. robusta*, 27. *M. hupehensis*, 28. *M. sieboldii*, 29. *M. niedzwetzkyana*, 30. *M. prunifolia* var. *rinkii*, 31. *M. zumi* var. *calocarpa*, 32. *M. kansuensis*, 33. *M. sieboldii*, 34. *M. baccata* var. *jackii*, 35. *M. halliana*, 36. *M. yunnanensis*, 37. *M. prattii*, 38. *M. kansuensis*, 39. *M. florentina*, 40. *M. sikkimensis*, 41. *M. toringoides*, 42. *M. baccata*, 43. *M. halliana*, 44. *M. fusca*, 45. *M. hupehensis*, 46. *M. siebolidii*, 47. *M. tschonoskii*, 48. *M. sylvestris*, 49. *M. baccata*, 50. *M. sieboldii*, 51. *M. hupehensis*, 52. *M. sikkimensis*, 53. *M. ioensis* var. *palmeri*, 54. *M. lancifolia*, 55. *M. prunifolia*, 56. *M. halliana*, 57. *M. baccata* var. *gracilis*, 58. *M. spectabilis*, 59. *M. domestica*, 60. *M. tschonoskii*, 61. *M. tschonoskii*, 62. *M. floribunda*. 63. *M. fusca*, 64. *M. niedzwetzkyana*, 65. *M. platycarpa*, 66. *M. prattii*, 67. *M. toringoides*, 68. *M. floribunda* var *hillieri*, 69. *M. robusta*, 70. *M. floribunda* 'Excellenz Theil', 71. *M. sargentii*, 72. *M.* x *floribunda*, 73. *M. sargentii*, 74. *M. rockii*.

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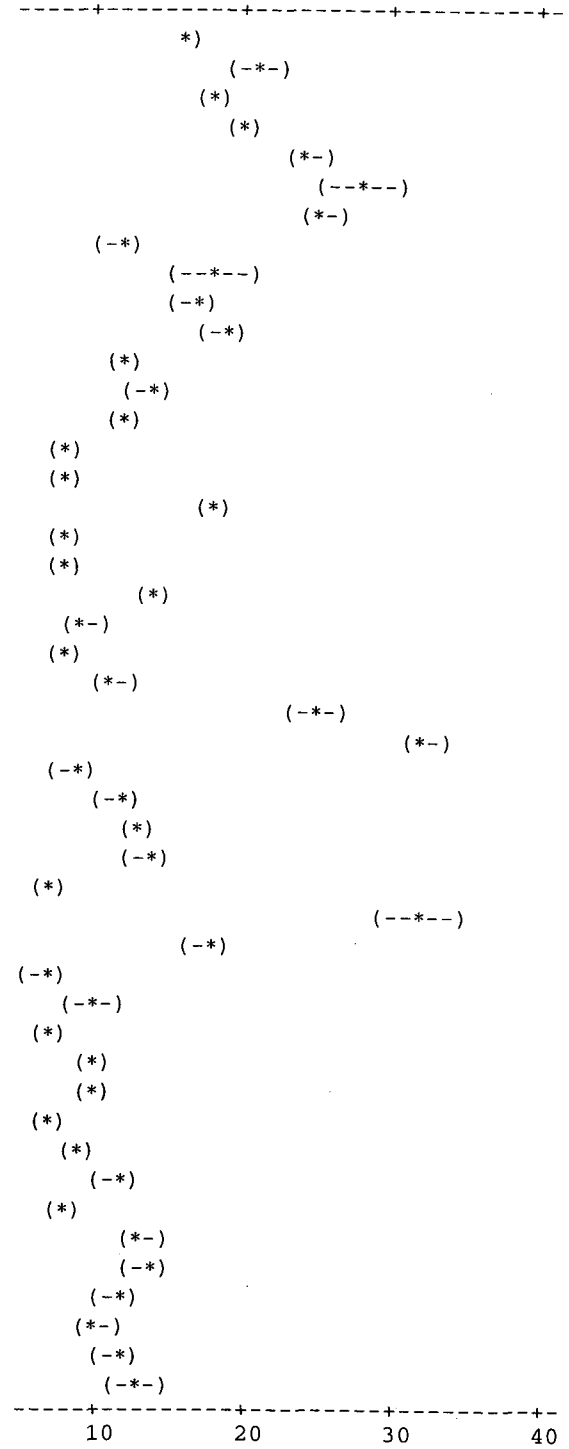
* S = Number of specimens

TABLE 9. ANALYSIS OF VARIANCE ON FRUIT LENGTH

SOURCE	DF	SS	MS	F	p
S*	46	8147.61	177.12	73.87	0.000
ERROR	242	580.29	2.40		
TOTAL	288	8727.90			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	14	16.429	2.065	
2	2	21.000	2.828	
3	11	18.273	1.902	
4	8	20.000	1.604	
5	3	24.333	3.215	
6	1	28.000	0.000	
7	4	25.250	3.304	
8	4	11.750	2.217	
9	1	18.000	0.000	
10	4	16.750	2.217	
11	6	18.500	1.049	
12	11	12.273	2.573	
13	5	13.800	2.168	
14	9	12.222	1.563	
15	9	7.556	0.527	
16	10	8.000	1.054	
17	10	17.900	2.283	
18	9	8.333	0.500	
19	8	8.000	0.926	
20	10	14.200	1.135	
21	4	9.250	1.500	
22	5	8.000	1.000	
23	3	11.333	1.155	
24	2	25.000	2.828	
25	5	32.200	2.168	
26	4	8.750	0.957	
27	8	11.500	1.069	
28	10	13.100	1.595	
29	6	13.500	1.225	
30	7	6.857	0.690	
31	1	32.000	0.000	
32	5	17.600	1.817	
33	6	6.667	1.033	
34	4	10.000	1.155	
35	9	6.667	1.000	
36	9	10.111	0.928	
37	10	10.100	1.287	
38	7	6.857	0.690	
39	7	9.000	0.577	
40	5	11.800	0.837	
41	5	8.000	0.000	
42	5	13.200	2.049	
43	5	13.800	2.280	
44	3	11.667	0.577	
45	5	10.400	0.548	
46	6	11.667	1.033	
47	4	13.000	0.816	



POOLED STDEV = 1.549

KEY TO LEVELS:

1. *M. florentina*, 2. *M. ioensis* var. *palmeri*, 3. *M. glaucescens*, 4. *M. coronaria*, 5. *M. angustifolia*, 6. *M. platycarpa*, 7. *M. coronaria*, 8. *M. trilobata*, 9. *M. tschonoskii*, 10. *M. baccata*, 11. *M. baccata*, 12, *M. toringoides*, 13. *M. toringoides*, 14. *M. toringoides*, 15. *M. sargentii*, 16. *M. sargentii*, 17. *M. prunifolia* var. *rinki*, 18. *M. baccata* var. *mandshurica*, 19. *M. baccata* var. *himalaica*, 20. *M. sikkimensis*, 21. *M. kansuensis*, 22. *M. baccata* var. *jackii*, 23. *M.* x *floribunda*, 24. *M. sylvestris*, 25. *M. pumila*, 26. *M. yunnanensis*, 27. *M. yunnanensis*, 28. M. x *robusta*, 29. *M. hupehensis*, 30. *M. sieboldii* var. *arborescens*, 31. *M. niedzwetzkyana*, 32. *M. prunifolia* var. *rinki*, 33. *M. zumi* var. *calocarpa*, 34. *M. kansuensis*, 35. *M. sieboldii*, 36. *M. baccata* var. *jackii*, 37. *M. halliana*, 38. *M. sieboldii*, 39. *M. hupehensis*, 40. *M. fusca*, 41. *M. halliana*, 42. *M. toringoides*. 43. *M. sikkimensis*, 44. *M. florentina*, 45. *M. kansuensis*, 46. *M. prattii*, 47. *M. yunnanensis*.

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* S = Number of specimens

Table 10. ANALYSIS OF VARIANCE ON FRUIT DIAMETER

SOURCE	DF	SS	MS	F	p
S*	46	11597.32	252.12	114.05	0.000
ERROR	246	543.80	2.21		
TOTAL	292	12141.12			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	15	12.067	1.831	(*)
2	2	18.000	8.485	(-*-)
3	11	23.364	1.206	(*)
4	8	23.750	2.121	(*)
5	3	25.000	3.000	(-*-)
6	1	37.000	0.000	(--*--)
7	4	27.750	2.363	(-*)
8	4	11.750	1.500	(-*)
9	1	15.000	0.000	(--*--)
10	4	19.250	2.062	(*-)
11	6	22.667	1.751	(-*)
12	11	12.091	1.514	(*)
13	5	11.400	1.517	(*-)
14	9	11.889	1.269	(*)
15	9	8.333	0.707	(*)
16	10	8.200	1.135	(*)
17	10	17.200	1.619	(*)
18	9	8.333	0.500	(*)
19	8	7.000	0.535	(*)
20	10	14.200	1.476	(*)
21	4	7.250	0.500	(*-)
22	5	8.000	1.000	(*)
23	3	9.333	1.528	(*-)
24	2	29.000	1.414	(-*-)
25	5	36.000	2.550	(*)
26	4	8.750	0.957	(-*)
27	8	12.125	0.991	(*)
28	10	14.800	1.317	(*)
29	6	15.000	2.098	(*)
30	7	7.000	0.577	(*)
31	1	30.000	0.000	(--*--)
32	5	16.800	2.490	(-*)
33	6	6.167	1.472	(*)
34	4	10.000	1.414	(*)
35	9	7.111	0.928	(*)
36	9	9.778	0.833	(*)
37	10	9.400	0.843	(*)
38	6	6.500	0.837	(-*)
39	8	9.750	0.463	(*)
40	5	10.600	1.140	(-*)
41	5	9.200	0.447	(*-)
42	5	12.400	0.894	(*-)
43	5	14.600	1.517	(-*)
44	6	11.167	1.169	(*)
45	5	9.000	0.000	(*)
46	6	13.667	1.966	(-*)
47	4	17.750	1.258	(-*)

POOLED STDEV = 1.487

10 20 30 40

KEY TO LEVELS:

1. *M. florentina*, 2. *M. ioensis* var. *palmeri*, 3. *M. glaucescens*, 4. *M. coronaria*, 5. *M. angustifolia*, 6. *M. platycarpa*, 7. *M. coronaria*, 8. *M. trilobata*, 9. *M. tschonoskii*, 10. *M. baccata*, 11. *M. baccata*, 12, *M. toringoides*, 13. *M. toringoides*, 14. *M. toringoides*, 15. *M. sargentii*, 16. *M. sargentii*, 17. *M. prunifolia* var. *rinkii*, 18. *M. baccata* var. *mandshurica*, 19. *M. baccata* var. *himalaica*, 20. *M. sikkimensis*, 21. *M. kansuensis*, 22. *M. baccata* var. *jackii*, 23. *M.* x *floribunda*, 24. *M. sylvestris*, 25. *M. pumila*, 26. *M. yunnanensis*, 27. *M. yunnanensis*, 28. *M.* x *robusta*, 29. *M. hupehensis*, 30. *M. sieboldii* var. *arborescens*, 31. *M. niedzwetzkyana*, 32. *M. prunifolia* var. *rinki*, 33. *M. zumi* var. *calocarpa*, 34. *M. kansuensis*, 35. *M. sieboldii*, 36. *M. baccata* var. *jackii*, 37. *M. halliana*, 38. *M. sieboldii*, 39. *M. hupehensis*, 40. *M. fusca*, 41. *M. halliana*, 42. *M. toringoides*. 43. *M. sikkimensis*, 44. *M. florentina*, 45. *M. kansuensis*, 46. *M. prattii*, 47. *M. yunnanensis*.

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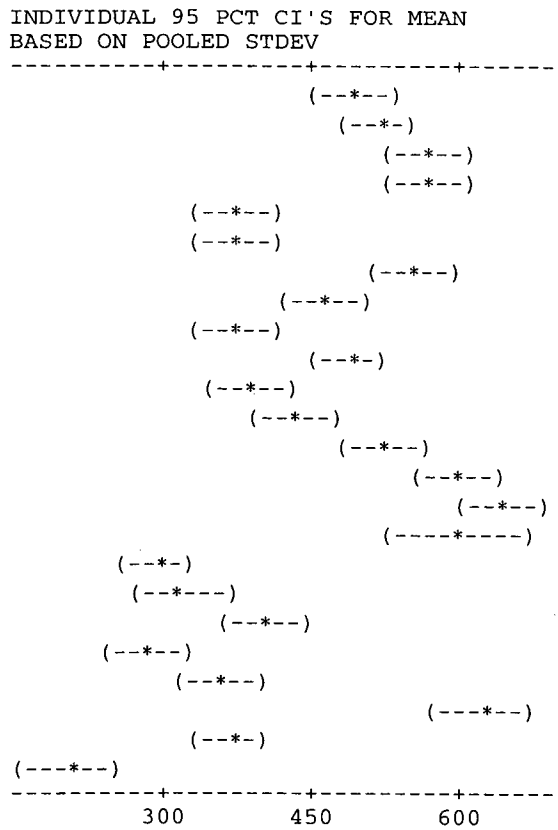
* S = Number of specimens

TABLE 11. ANALYSIS OF VARIANCE ON NO. OF VESSELS PER mm2

SOURCE	DF	SS	MS	F	p
S*	23	2973403	129278	27.24	0.000
ERROR	199	944328	4745		
TOTAL	222	3917730			

LEVEL	N	MEAN	STDEV
1	9	501.29	61.85
2	10	519.24	58.03
3	10	568.01	132.42
4	10	571.04	50.39
5	10	377.06	69.45
6	10	372.26	103.48
7	9	556.87	111.28
8	9	465.80	49.60
9	10	372.25	38.81
10	10	489.43	33.45
11	9	383.47	35.40
12	10	432.48	28.09
13	10	530.07	52.90
14	9	604.36	94.04
15	11	647.27	96.58
16	3	598.37	12.55
17	10	293.31	39.27
18	9	322.31	36.17
19	10	406.58	92.03
20	10	280.68	31.54
21	9	357.37	41.73
22	8	623.45	43.32
23	11	370.72	77.68
24	7	204.77	53.19

POOLED STDEV = 68.89



KEY TO LEVELS:

1. *M. toringoides*, 2. *M. niedzwetzkyana*, 3. *M. tschonoskii*, 4. *M, zumi*, 5. *M. spectabilis*, 6. *M. sylvestris*, 7. *M. kansuensis*, 8. *M. glaucescens*, 9. *M. florentina*, 10. *M. kirghisorum*, 11. *M. sikkimensis*, 12, *M. sieboldii* var. *arborescens*, 13. *M. platycarpa*, 14. *M. kansuensis*, 15. *M. yunnanensis* var. *veitchii*, 16. *M. ioensis* var. *palmeri*, 17. *M. baccata*, 18. *M. pumila*, 19. *M. sargentii*, 20. *M. hupehensis*, 21. *M. coronaria*, 22. *M. angustifolia*, 23. *M. lancifolia*, 24. *M. fusca*.

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* S = Number of specimens

TABLE 12. ANALYSIS OF VARIANCE ON POLLEN LENGTH

SOURCE	DF	SS	MS	F	p
S*	18	4744.61	263.59	98.38	0.000
ERROR	170	455.47	2.68		
TOTAL	188	5200.08			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
1	10	55.960	1.843	(*-)
2	9	57.689	2.272	(-*-)
3	10	42.780	2.093	(*-)
4	10	44.190	1.634	(-*)
5	10	46.780	1.336	(-*-)
6	10	42.060	1.426	(-*-)
7	10	56.030	1.237	(*-)
8	10	38.750	0.584	(-*)
9	10	51.120	1.719	(-*-)
10	10	47.880	1.453	(-*-)
11	10	44.240	2.104	(-*)
12	10	43.350	2.593	(*-)
13	10	46.100	1.508	(-*-)
14	10	50.810	1.007	(-*)
15	10	48.540	1.953	(-*-)
16	10	47.100	0.860	(*-)
17	10	52.640	1.940	(-*)
18	10	47.850	1.220	(-*)
19	10	52.350	0.753	(*-)

POOLED STDEV = 1.637

42.0 48.0 54.0

KEY TO LEVELS:

1. *M. angustifolia*, 2. *M. gaucescens*, 3. *M. lancifolia*, 4. *M. kansuensis*, 5. *M. trilobata*, 6. *M. florentina*, 7. *M. coronaria*, 8. *M. prattii*, 9. *M. platycarpa*, 10. *M. baccata*, 11. *M. fusca*, 12, *M. transitoria*, 13. *M. tschonoskii*, 14. *M. niedzwetzkyana*, 15. *M. floribunda*, 16. *M. domestica*, 17. *M. ioenesis* var. *palmeri*, 18. *M. sieboldii*, 19. *M. sargentii*.

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* S = Number of specimens