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Collection, Maintenance, Characterization, and Utilization of Wild Apples of Central Asia

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I. INTRODUCTION

Apple is the most ubiquitous and well-adapted species of temperate fruit crops. It is grown in high latitude regions of the world where temperatures may reach –40°C to high elevations in the tropics where two crops may be grown in a single year (Janick 1974). Apples are the fourth most important world fruit crop following all citrus types, grapes, and bananas.

The apple, along with many of the important temperate fruit crops, belongs to the Rosaceae or rose family. Apple, pear, quince, medlar and a few other species have been classified into the subfamily, Pomoideae, the pome fruits, having fruits with two to five carpels enclosed in a fleshy covering. The genus *Malus* consists of about 27 wild species (Table 1.1). Most of the species intercross and, since self-incompatibility is common, seed obtained from a botanic garden are mostly interspecific or intercultivar hybrids. It is therefore difficult to be certain of the authenticity of some species names. Some taxon formerly listed as species (Way et al. 1990) are now classified as cultivated species (Table 1.1) because there is no record of their having wild origins (Li 1989; Li 1996).

The cultivated apple is likely the result of interspecific hybridization and at present, the binomial *Malus* ×*domestica* Borkh. has generally been accepted as the appropriate scientific name, replacing the previously common usage of *M. pumila* (Korban and Skirvin 1984). *Malus sieversii* Lebed., a wild apple species native to Central Asia, has been recognized as a major progenitor of the domesticated apple, *M.* ×*domestica* (Way et al. 1990; Ponomarenko 1987 and 1992; Morgan and Richards 1993; Juniper et al. 1999). In ancient times, apple seeds and trees were

Table 1.1. *Malus* sections, series and primary species.

Sections	Series	Primary Species
Malus Langenf.	Sieversinae Langenf.	*M. sieversii* (Lodeb.) Roem.
		subsp. *kirghisorum* (Al.) Ponom.
		form. *niedzwetzkyana* (Dieck) Langenf.
		M. orientalis
		subsp. *montana* (Uglitz) Likh.
		subsp. *turkmenorum* (Juz.) Langenf.
		M. sylvestris (L.) Mill.
		var. *praecox* (Pall.) Ponom.
Baccatus Jiang	Baccatae (Rehd.) Rehd.	*M. baccata* (L.) Borkh.
		var. *mandshurica* (Komorov.) Likh
		var. *sachalinensis* (Juz.) Ponom.
		var. *himalaica* (M.) Vass.
	Hupehenses Langenf.	*M. hupehensis* (Pampan.) Rehd.
		M. halliana (Anon.) Koehne
	Sikkimenses Jing	*M. sikkimensis* (Wenzig) Koehne
Sorbomalus Zabel.	Sieboldiane (Rehd.)	*M. sieboldii* (Regel) Rehd.
		var. *sargenti* (Rehd.)
	Kansuenses (Rehd.) Rehd.	*M. kansuensis* (Batal.) Schneid.
		M. transitoria (Batal.) Schneid.
		M. toingoides (Rehd.) Hughes
		M. komarovii (Sarg.) Rehd.
		M. xiaojinensis Cheng et Jiang
		M. fusca (Raf.) Schneid.

(continued)

Table 1.1. *(continued)*

Sections	Series	Primary Species
	Yunnanenses Rehd.	*M. yunnanensis* (French) Schneid.
		M. prattii (Hemsl.) Schneid.
		M. honanensis Rehd.
		M. ombrophilla Hand.-Mazz.
	Florentinae Rehd.	*M. florentina* (Zuccagni) Schneid.
Chloromeles (Decne.) Rehd.		*M. ioensis* (Wood.) Brit.
		M. coronaria (L.) Mill.
		M. angustifolia (Ait.) Michx.
Docyniopsis Schneid.		*M. doumeri* (Bois.) Chev.
		M. melliana (Hand.-Mazz.) Rehd.
		M. tschonoskii (Maxim.) Schneid.
		M. laosensis Chev.
Eriolobus (D.C.) Schneid.		*M. trilobata* (Poiret) Schneid.

Note: Cultivated *Malus* species and *Malus* species hybrids (secondary species) include:

M. ×*arnoldiana* (Rehd.) Sarg. (*baccata* × *floribunda*)	*M. asiatica* Nakai
M. ×*atrosanguinea* ((Spaeth) Schneid. (*halliana* × *sieboldii*)	*M.* ×*dawsoniana* Rehd. (*fusca* × *domestica*)
M. ×*domestica* Borkh.	*M. floribunda* Siebold
M. ×*hartwigii* Koehne (*halliana* × *baccata*)	*M.* ×*magdeburgensis* Schoch. (*spectabilis* × *domestica*)
M. ×*micromalus* Mak. (*baccata* × *spectabilis*)	*M.* ×*platycarpa* Rehd. (*cornonaria* × *domestica*)
M. pumila Miller	*M. prunifolia* (Willd.) Borkh.
M. ×*purpurea* (Barbier) Rehd. (*neidzwetzkyana* × *atrosanguinea*)	*M.* ×*robusta* (Carr.) Rehd. (*baccata* × *prunifolia*)
M. ×*soulardii* (Bailey) Brit. (*ioensis* × *domestica*)	*M. spectabilis* (Ait.) Borkh.
M. ×*sublobata* (Dipp.) Rehd. (*prunifolia* × *sieboldii*)	*M.* ×*zumi* (Mats.) Rehd. (*mandshurica* × *sieboldii*)

Adapted from Way et al. (1990); Langenfelds (1991); Ponomarenko (1992); Li (1996); Li (pers. com.).

likely dispersed from Central Asia, east to China and west to Europe, via trade caravan routes popularly referred to as the "Silk Road" (Juniper et al. 1999). This flow of apple germplasm declined over the last few centuries as overland trade through the region decreased and ceased in the twentieth century as Central Asia was isolated for political reasons.

In the 1920s, Vavilov (1930) traveled through Central Asia and reported that large wild stands of *M. sieversii* existed in specific localities and suggested the region as a center of origin for the domesticated apple. Dzhangaliev (1977), while confirming the contemporary existence of the wild apple forests, also noted that they were under pressure in some areas due to urbanization, agriculture, grazing, and wood harvesting. In the 1980s, the U.S. Department of Agriculture (USDA) National Plant Germplasm System recognized that *M. sieversii* was a critical species that lacked representation in its *Malus* collection at the Plant Genetic Resources Unit (PGRU) in Geneva, New York. The material was critical because present cultivars of the commercial apple had a narrow genetic base and most commercial production was based on very few cultivars (Kresovich et al. 1988; Morgan and Richards 1993; Noiton and Alspach 1996; Hokanson et al. 1998). *Malus sieversii* could be a valuable genetic resource for the domesticated apple potentially containing more genetic diversity for important horticultural and environmentally adapted traits (Korban 1986; Way et al. 1990; Janick et al. 1996).

M. sieversii is diverse with the wild trees bearing a full range of forms, colors, and tastes. Recent collection trips to Central Asia (Section III) have verified that *M. sieversii* is very diverse and has all the qualities present in *M. ×domestica* (Forsline et al. 1994; Forsline 1995). The east/west trade routes that eventually became the "Silk Road" passed through this region on the way to China to the east and to the Middle East, past the Black Sea, to the west. Travelers on foot, camels, and horses likely began dispersing this germplasm as long ago as Neolithic times with routes being well established by the Bronze Age (Juniper et al. 1999). Ruminants such as deer native to the area and donkeys, mules, and horses used by humans along with humans themselves avidly ate these apples. No doubt the best were selected and this narrowed the genepool as it was dispersed. The seeds pass undamaged through alimentary canals. Thus seedlings would have been randomly established along the length of the trade routes and hybridization between previously isolated species then became possible.

A number of species likely contributed to the genetic makeup of the domestic apple. *Malus orientalis* found in western sections of the trade routes in the Russian Caucasus as well as in Turkey does not have the diversity of fruit quality, but may have contributed other valuable traits such as later blooming, adaptation to a wider array of habitats, and capacity for longer storage of the apples. Others that may have been contributing parents include, *Malus sylvestris* the European crabapple bearing small astringent, greenish-yellow fruits, native to an area from Britain across Europe to the Balkans, and *Malus baccata* and some of its subspecies or natural hybrids (*M. mandshurica*, *M. prunifolia*, and *M. asiatica*) on the eastern side of the trade routes. Recent evidence has shown *M. sylvestris* is an unlikely contributor to the genetic makeup of the commercial apple (Wagner and Weeden 2000). However, it may have been involved in the background of cider-type apples selected in Spain, France, and Britain.

Selected cultivars likely arose from random hybridizations and they were maintained by vegetative propagation, especially grafting which is a very ancient horticultural technology. B. E. Juniper (pers. comm.) reported that Oxford University scholars have found Babylonian cuneiform tablets dating to 2000 BCE depicting graftage. The Greeks knew about grafting and it was discussed in the writings of Theophrastus (487–287 BCE) as well as Roman agricultural writers such as Cato (234–149 BCE), Varro (116–27 BCE), Virgil (70–19 BCE), Pliny (12 BCE–70 CE), and Columella (first century CE). By the nineteenth century, England claimed over 2500 cultivars and many more were known at that time in Russian territories (Morgan and Richards 1993).

In North America, settlers relied on apple trees originating from seeds of apples collected from early plantings established in the Tidewater region of the East Coast (Calhoun 1995). Seedling orchard establishment continued in North America well into the nineteenth century. As a result, higher levels of genetic diversity accumulated in North America than in Europe at the time when selecting and grafting existing cultivars was the norm. The potential for hybridization was almost infinite and North America became a "vast experimental station," where selecting promising seedling apple cultivars was practiced on a large scale. From this vast grow-out, many of the world's important cultivars of unknown parentage arose such as 'Delicious' ('Hawkeye'), 'Golden Delicious', 'McIntosh', 'Jonathan', 'Rome Beauty', and 'Northern Spy'.

Many of these cultivars have been used to breed new apple cultivars such as 'Cortland', 'Empire', 'Jonagold', 'Fuji', 'Gala', and 'Pink Lady'. The introduction of resistance to apple scab (pathogen, *Venturia inaequalis* (Cke.) Wint.) with the incorporation of the V*f* gene from *M. floribunda* 821 indicated the potential of interspecific crosses to introduce new genes into commercial cultivars (Crosby et al. 1992). In addition, gene transformation is now able to insert genes from any source into existing high-quality cultivars. Some genetic transformants are now in the testing stage (Norelli and Aldwinckle 2000). It is from this history that we recognize the importance of establishing genebanks to preserve valuable germplasm that has been accumulated over many millennia by human activities.

The Plant Genetic Resources Unit (PGRU) in Geneva, New York is part of the National Plant Germplasm System (NPGS) administered by the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). The activities of the NPGS have been reviewed in a special volume of *Plant Breeding Reviews* (Janick 1989). Repositories for clonally propagated crops were not introduced to the system until the 1980s. An early assessment of the germplasm reserves of pome fruits (Lamb 1974) publicized the need for establishing clonal germplasm repositories. The PGRU is devoted to curating *Malus*, *Prunus*, and *Vitis* as well as many seed propagated vegetable crops. The *Malus* collection is the most extensive collection consisting of nearly 4000 accessions. Previous reports on the activities of PGRU have been published (Forsline 1987, 1988, 1992, 2000; Forsline and Way 1993) as well as the status of apple germplasm collections in the world (Way et al. 1990). The apple collection at PGRU was established in 1984 according to the clonal repository plan of the NPGS (Barton 1975; Brooks and Barton 1977). The majority of these accessions (2438) are clonally propagated and stored as duplicate orchard trees. Dormant buds of 2000 accessions are stored in a back-up collection in liquid nitrogen at the National Seed Storage Laboratory (NSSL) in Fort Collins, Colorado (Forsline et al. 1999). A core subset has been established including 206 clones (Forsline 1996) that is a test-array of the most genetically diverse accessions available for evaluation of specific genetic traits. Approximately 3000 accessions are distributed annually. Accession history, characterization, and evaluation are documented in the Germplasm Resources Information Network (GRIN) (USDA 2000). The core subset and 70 percent of the remainder of the collection has been characterized with 25 morphological descriptors.

In addition to the clonal collection, since 1988, approximately 1500 accessions of wild *Malus* spp. from centers of origin throughout the world are preserved at PGRU as seed lots. Accessions of *Malus sieversii* have been collected in Central Asia (mostly from Kazakhstan) from 12 distinct habitats and 894 tree sources (distinct accessions or seed lots). Collaborative evaluation for disease resistance and horticultural and molecular characterization is being conducted on 25,000 of these seedlings in 24 worldwide laboratories. Information on the *Malus* collection and other commodities in the NPGS can be accessed on the Web at: http://www.ars-grin.gov/npgs/. The procedures for collection, conservation, evaluation, and documentation of *Malus* germplasm have been described (Forsline 2000).

Until 1989, wild *Malus* germplasm from the Asian center of origin was unavailable (Dickson and Forsline 1994; Forsline 1995; Hokanson et al. 1997b). In 1989, policy changes in the former Soviet Union permitted U.S. scientists to establish collaborative efforts with Central Asian counterparts to conserve this germplasm. Subsequently, with funding through the USDA National Germplasm Resources Laboratory (NGRL) and effort from personnel at the NGRL, the PGRU, and cooperating scientists from other institutions, contacts with scientists and government officials in Central Asian countries were initiated and collaborative collection expeditions were undertaken in 1989, 1993, 1995, and 1996.

The four expeditions were conducted with equal funding from two programs in the USDA/ARS including the NPGS and the International Research Programs (IRP). Personnel participating in each expedition are listed in Table 1.2. The late Dr. Calvin Sperling led the initial trip with Herb Aldwinckle and Elizabeth Dickson in 1989 to develop contacts and determine the availability of *Malus* germplasm. Philip Forsline led the first follow-up trip in 1993 after detailed planning in 1992 with our main host, Professor Aimak Dzhangaliev. In 1994, a Specific Cooperative Agreement (SCA) was developed through USDA/ARS/IRP as a four-year project for research on Wild Apple Germplasm, funding the cooperative research activities of our main cooperator and expedition host in Kazakhstan, Professor Aimak Dzhangaliev. This agreement is one of many that IRP has funded for research in Central Asia. Following the development of the SCA, a third trip in 1995 included expeditions to sites that had not previously been accessed. In the fourth and final expedition in 1996, some of the objectives of the SCA were fulfilled with the cooperator, Professor Dzhangaliev. Dr. Stan Hokanson implemented expansion of our cooperative work in Kazakhstan and participated in this final expedition.

The expeditions focused mainly on Kazakhstan where the primary collaborator was Professor Aimak Dzhangaliev of the Academy of Sciences of the Republic of Kazakhstan whose laboratory had researched the variation in *M. sieversii* in Kazakhstan over several decades (Dzhangaliev 1977). These expeditions are documented in several publications (Dickson and Forsline 1994; Forsline et al. 1994; Forsline 1995, 2000; and Hokanson et al. 1997b) and in GRIN (USDA 2000) on the Web at: http://www.ars-grin.gov/ars/NoAtlantic/Geneva/kaz_trip.html.

Table 1.2. Participating personnel in four expeditions to Kazakhstan.

Expedition Personnel	Affiliation/Address	City/Country	Expedition Dates
Ahymbekov, S. S.	Minister for Kazakhstan Ministry of Agriculture	Almaty, Kazakhstan	1996
Aitkhozhina, Nagima	Director, M.A. Aitkhozhin Inst., Kazakhstan Natl. Acad. of Sci.	Almaty, Kazakhstan	1995, 1996
Aitkhozhina, Nazira	Professor, Dept. of Plant Pathology, Kazakhstan Natl. Acad. of Sci.	Almaty, Kazakhstan	1996
Aldwinckle, H. S.	Professor, Dept. of Plant Pathology, Cornell University	Geneva, NY, USA	1989
Amanbayev, A. K.	Deputy Minister, Ministry of Ecology and Bioresources, Chair, Committee of Forestry	Almaty, Kazakhstan	1993, 1995, 1996
Bekbaeva, B.	Host, Semipalatinsk Region of the Tarbagatai Area	Urdzhar, Kazakhstan	1995, 1996
Britz, G.	Pome Fruit Development, UNIFRUCO LTD	Bellville, South Africa	1995
Butenok, L.	Botanical assistant of Professor Dzhangaliev	Almaty, Kazakhstan	1993
Chekalin, S. V.	Vice Director, Main Botanical Garden, Kazakhstan Natl. Acad. of Sci.	Almaty, Kazakhstan	1995, 1996
Dickson, E.	L. H. Bailey Hortorium, Cornell University, presently U. of Calgary	Calgary, Al., Canada	1989, 1993, 1995, 1996
Dzhangaliev, A.	Main host for all collections, Professor, Head of Interbranch Lab for Protection of Germplasm, Main Botanical Garden, Kazakhstan Natl. Acad. of Sci.	Almaty, Kazakhstan	1989, 1993, 1995, 1996
Dusembina, S	Interpreter for expedition, student, Almaty Univ.	Almaty, Kazakhstan	1993
Forsline, P. L.	Leader of 3 expeditions from USA, Curator, PGRU, USDA-ARS, Cornell Univ	Geneva, NY, USA	1993, 1995, 1996
Gowhar, M.	Interpreter for expedition, junior scientist, Kazakhstan Natl. Acad. Sci.	Almaty, Kazakhstan	1995
Hokanson, S.	Post-Doc, PGRU, USDA-ARS, Cornell Univ.; presently, Professor, Univ. of Minnesota	St.Paul, MN, USA	1996
Human, T.	Fruit Breeder, INFRUTEC	Stellenbosch, South Africa	1995
Isetbaev, R.	Host in Karatau area, forestry officer of Boraldy forest	Chimkent, Kazakhstan	1993, 1995
Issayev, M. K.	Interpreter for expedition, Professor of Philology, Kazakh State Agrarian Univ.	Almaty, Kazakhstan	1996

(continued)

Table 1.2. (*continued*)

Expedition Personnel	Affiliation/Address	City/Country	Expedition Dates
Ivashenko, A. A.	Botanist for collection in the Karatau area, Main Botanical Garden	Almaty, Kazakhstan	1993
Luby, J. J.	Professor of Pomology and Plant Breeding, Univ. of Minnesota	St. Paul, MN	1995
Kashaganov, B.	Forestry officer in the Almaty region, host for collections in Ketmen area	Almaty, Kazakhstan	1996
Kunakbayev, Y.	Occasional interpreter, Science and Economic Assistant, Embassy, USA	Almaty, Kazakhstan	1996
Kolbintsev, V.	Assistant in Karatau area, Senior Science worker of Aksu-Jabagli Nature Preserve	Chimkent, Kazakhstan	1993
Kuzubaev, N. H.	Senior officer of Sarkand Forest, Host in Djungarsky area	Sarkand, Kazakhstan	1993, 1995, 1996
Mink, G.	Plant Pathologist, Washington State University	Prosser, WA, USA	1993
Noiton, D.	Apple Breeder, Hort. Res. Inst.	Havelock North, New Zealand	1993,1996
Ovchinnikov, I. D.	Senior officer of Sarkand Forest, main host in Djungarsky area	Sarkand, Kazakhstan	1993, 1995, 1996
Pellet, H.	Collector of ornamental species, Professor of Horticulture, Univ. of Minnesota	St. Paul, MN	1996
Rachimbaev, I.	Director, Main Botanical Garden, Kazakhstan Natl. Acad. of Sci.	Almaty, Kazakhstan	1993
Salova, T.	Wife of Professor Dzhangaliev, Pomologist (wild apricots), Main Botanical Garden	Almaty, Kazakhstan	1989, 1993, 1995, 1996
Serikbaev, S.	Senior forest officer of the Boraldy forest	Chimkent, Kazakhstan	1993, 1995, 1996

Smuryghin, A.	Cohost of 1993 expedition, Professor, Res. Inst. of Fruit and Viticulture	Almaty, Kazakhstan	1993
Sotnikov, V.	Botanical assistant of Professor Dzhangaliev, Almaty, Kazakhstan	Almaty, Kazakhstan	1995, 1996
Sperling, C.	Leader of 1989 expedition, Plant Exploration officer, USDA-ARS (deceased, May, 1995)	Beltsville, MD, USA	1989
Tulemisov, E.	Host for Karatau and Talasky areas, forestry officer for Chimkent region	Chimkent, Kazakhstan	1996
Turehanova, R.	Botanist, assistant for all expeditions, Main Botanical Garden	Almaty, Kazakhstan	1989, 1993, 1995, 1996
Turganov, G.	Host for all Karatau and Talasky areas, General Director, Chimkent region	Chimkent, Kazakhstan	1993, 1995, 1996
Unruh, T.	Research Entomologist, USDA, ARS, Yakima Agric. Res. Lab.	Yakima, WA, USA	1996
Urazaev, F. R.	Main guide in Tarbagatai area, forestry officer, Semipalatinsk region	Urdzhar, Kazakhstan	1995, 1996
Ustemirov, K. J.	Travel arrangements to Chimkent region, Foreign Relations, Com. of Forestry	Almaty, Kazakhstan	1993, 1995, 1996
Venglovsky, B.	Host for expedition in Kyrgyzstan, Professor, Inst. of Forestry and Walnut Breeding	Bishkek, Kyrgyzstan	1993

The expeditions successfully introduced large quantities of seeds as well as a limited number of clonal accessions to the *ex situ* collection at the PGRU. The collections aroused much interest among researchers in the United States, Canada, and several other countries. Upon request to the Germplasm Curator at the PGRU, scientists were provided with samples of the collections that they are currently evaluating for various traits at their respective home locales. Extensive evaluation is also being conducted at the PGRU in collaboration with Cornell University scientists. Thus, in less than a decade since the germplasm became available, a substantial international evaluation effort was mobilized rapidly and spontaneously. In this volume, we summarize progress in evaluation of the collections and prospects and plans for their utilization based on reports that were graciously provided to us by the cooperating researchers.

II. GERMPLASM ACQUISITION

Since 1984, 2438 *Malus* clones have been acquired at the PGRU. The majority were obtained early in the life of the apple repository from breeders' collections throughout the United States. Most were cultivars of *Malus* ×*domestica* such as those of the National U.K. Repository described by Smith (1971). The U.S. apple repository was established at the New York Agricultural Experiment Station at Geneva, in part because of the large collection of *Malus* (Way 1976) that Cornell apple breeders at the station had collected during the previous century. Nearly 50 percent of the 2438 clones were originally acquired by breeders at Cornell University (Lamb 1974; Way 1976), although other *Malus* species were collected from botanical gardens as well as breeders' collections. Significantly less than five representatives were available for each of 30 *Malus* species (Table 1.1). These species matched the published morphological descriptions (Yü 1979), but critical passport information including the country of origin, and information about the collectors and habitat of origin were generally lacking. The *Malus* in germplasm collections were considered genetically vulnerable (Way et al. 1990) because of the large gaps that existed in regard to wild apple germplasm. The first initiative to increase genetic diversity for wild germplasm was begun in 1987 with collection expeditions for four native North American *Malus* species (Dickson et al. 1991). Although North American germplasm has not thus far contributed to the cultivated genome of *M.* ×*domestica*, it will almost certainly have potential for future utilization.

Until 1989, wild *Malus* germplasm from the Asian center of origin was unavailable (Dickson and Forsline 1994; Forsline 1995; Hokanson et al.

1997b; Hokanson et al. 1999). The material was critical because present cultivars of the commercial apple have a very narrow genetic base (Morgan and Richards, 1993). This has subsequently been confirmed by genetic, biochemical, and molecular methods (Kresovich et al. 1988; Noiton and Alspach 1996; Hokanson et al. 1998) and assessment using biochemical and molecular tools confirmed this (Kresovich et al. 1988, 1990). The existence of wild stands of *M. sieversii*, the progenitor of cultivated apple, had been confirmed by Vavilov (1930) and Dzhangaliev (1977) in specific localities in Central Asia. These populations could be expected to contain genetic diversity for many important traits, such as resistance to biotic and abiotic stresses, fruit quality, tree attributes, and other genes that may solve as yet unforeseen problems (Korban 1986; Way et al. 1990; Janick et al. 1996).

The 1500 accessions of wild apple collected since 1987 include mostly seed populations from the following regions: (1) 200 accessions of four North American species across a broad geographic range; (2) 102 accessions of seven Chinese species from Sichuan; (3) 86 accessions of *M. orientalis* from the Russian Caucasus and the mountains of Turkey south of the Black Sea; (4) approximately 100 accessions of other *Malus* species obtained from direct exchange with collaborators around the world; and (5) 892 accessions of *M. sieversii* collected in four different years from 12 areas (Fig. 1.1) in Central Asia.

The Central Asian *M. sieversii* specimens are from Kyrgyzstan, Uzbekistan, Tajikistan, and Kazakhstan whose regions are characterized by mountain ranges (Zailisky, Djungarsky, Karatau, Ketmen, and Talasky) as depicted in Fig. 1.1 and described in Tables 1.4 and 1.5. Highlights of some of these collection activities (Dickson and Forsline 1994; Forsline 1995; Hokanson et al. 1997b) can be found on the Web at: http://www.ars-grin.gov/ars/NoAtlantic/Geneva/kaztrip.html. The Central Asian *M. sieversii* collections are primarily from Kazakhstan with additional samples from Kyrgyzstan, Uzbekistan, and Tajikistan. The species is a dominant overstory component in montane forests in these countries as depicted in Fig. 1.1 and described in Table 1.5.

Most collections were made as seeds, and altogether over 120,000 seeds were collected from 894 trees (Tables 1.6 and 1.7). Many of the seeds were selected at random from trees at the collection sites, but a concentrated effort was made in the 1995 and 1996 expeditions to identify and collect from trees that appeared to possess horticulturally desirable characters. These trees have been termed "elites" (Plate 1). Fruit size of *M. sieversii* from the different regions in Kazakhstan was diverse, but some accessions had fruit with an average diameter greater than 60 mm, approaching the size of many commercial cultivars (Table 1.8). Areas 5, 9, 11, and 12 (Fig. 1.1) had the largest fruit that also closely resembled

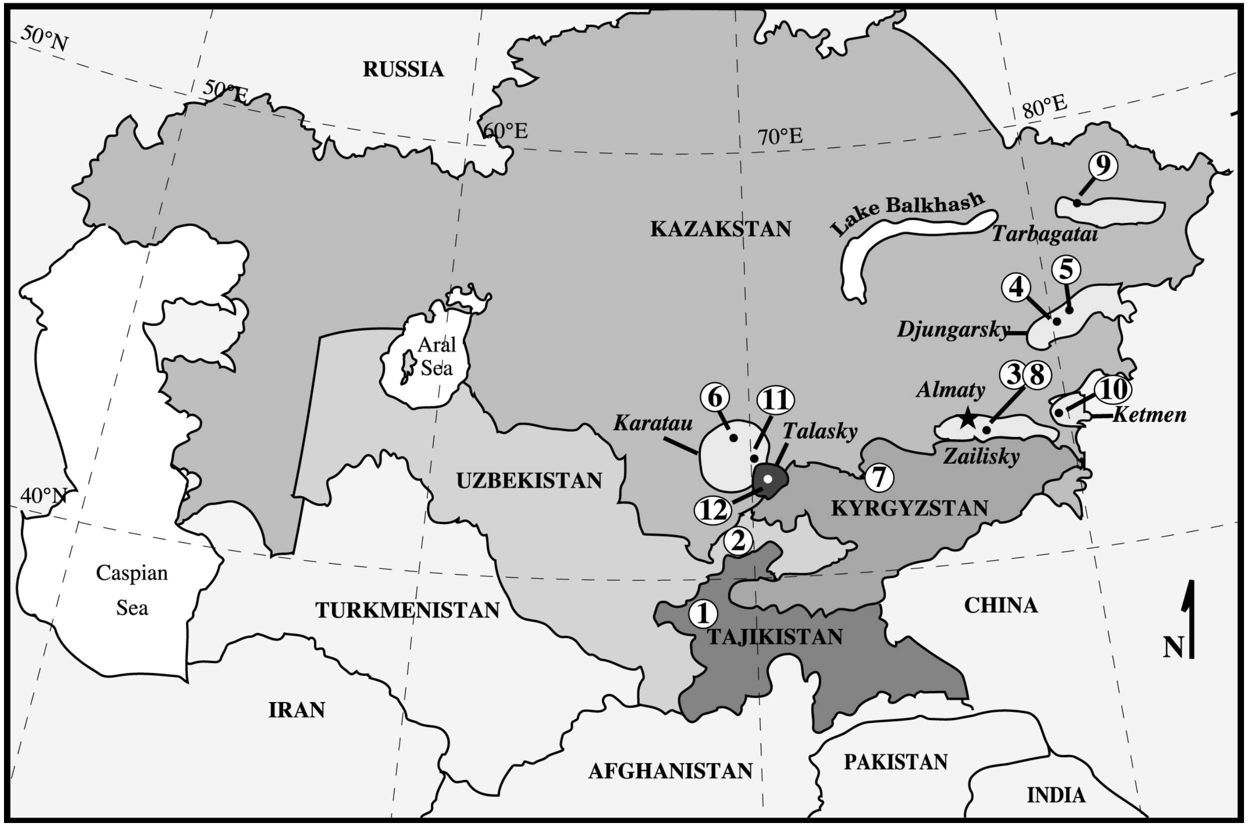

Fig. 1.1. Map of regions in Central Asia where *Malus sieversii* (Lebed.) was collected in 1989, 1993, 1995, and 1996.

Table 1.3. Descriptors used to characterize apple collections in Kazakhstan.

Category/ Descriptor No.[a]	Descriptor	Descriptor Defined	Code	Defined
Morphology/4	Calyx Basin (CALYXBASIN)	Appearance of Calyx Basin	1	none
			2	acute shallow
			3	acute medium
			4	acute deep
			5	obtuse shallow
			6	obtuse medium
			7	obtuse deep
Morphology/21	Fruit Bloom (FRUITBLOOM)	Rating of Natural Bloom (Wax) on Fruit at Maturity	1	absent
			2	slight
			3	moderate
			4	heavy
Morphology/22	Fruit Flesh Color (FRTFLSHCOL)	Fruit Flesh Color	1+1	white
			2+2	cream
			2+3	cream + green
			2+4	cream + yellow
			3+3	green
			3+4	green + yellow
			4+4	yellow
			4+5	yellow + orange
			4+7	yellow + red
			5+5	orange
			6+6	pink
			7+7	red
			8+8	rose red
Morphology/23	Fruit Flesh Firmness (FRTFLSHFRM)	Fruit Flesh Firm-ness at Maturity	1	soft
			2	semihard
			3	firm
			4	hard
Morphology/24	Fruit Flesh Flavor (FRTFLSHFLA)	Fruit Flesh Flavor	1	aromatic
			2	sweet
			3	subacid
			4	acid
			5	astringent
Morphology/26	Fruit Ground Color (FRTGRNDCOL)	Fruit Ground Color at Maturity	1	light green
			2	green
			2,4	green yellow
			3	light yellow
			4	yellow
			5	orange
			5,4	orange yellow
			6	brown
			7	pink
			8	red
			9	purple

(continued)

Table 1.3. (*continued*)

Category/ Descriptor No.[a]	Descriptor	Descriptor Defined	Code/Defined
Morphology/28	Fruit Length (FRTLENGTH)	Fruit Length (Ave of 10 Fruits from Vigorous Tree) in mm.	mm at maturity
Morphology/34	Fruit over Color (FRTOVERCOL)	Fruit Over Color at Maturity	1 none 2 green 2,5 green brown 3 yellow 3,2 yellow green 4 orange 5 brown 5,7 brown red 5,9 brown purple 6 pink 7 red 7,5 red brown 8 dark red 9 purple
Morphology/35	Fruit Russet Intensity (FRTRUSSET)	Percent of Fruit Surface with Russet (0–100%)	% of surface with russet (0–100%)
Morphology/36	Fruit Russet Loc. (FRTRUSSLOC)	Location of Russet on Fruit	1 pedicel end only 2 calyx end only 3 both pedicel and calyx end 4 entire fruit
Morphology/37	Fruit Russet Type (FRTRUSSTYP)	General Type of Russetting	1 extremely fine 2 medium heavy 3 surface cracks
Morphology/38	Fruit Shape (FRUITSHAPE)	Fruit Shape (Overall)	1.0 globose 1.1 globose-conical 1.2 short-globose-conical 2.0 flat 2.1 flat-globose (oblate) 3.0 conical 3.1 long-conical 3.2 intermediate-conical 4.0 ellipsoid 4.1 ellipsoid-conical 5.0 oblong 5.1 oblong-conical 5.2 oblong-waisted
Morphology/41	Fruit Size Uniformity (FRTSIZUNIF)	Consistency of Fruit Size	1 uniform 2 variable
Morphology/42	Fruit Stem Length (FRTSTEMLEN)	Stem Length (Average of 5) in mm	mm at maturity

Morphology/43	Fruit Stem Thickness (FRTSTEMTHK)	Rating of Stem Thickness	1 slender 2 medium 3 stout
Morphology/44	Fruit Texture (FRTTEXTURE)	A Rating of Fruit Flesh Texture at Maturity	1 fine 2 medium 3 coarse
Morphology/45	Fruit Width (FRUITWIDTH)	Fruit Width (Ave of 10 Fruits from Vigorous Tree) in mm	mm at maturity
Morphology/60	Overcolor Intensity (OVERCOLOR)	Percent of Overcolor on Fruit 0–100%	% overcolor on fruit at maturity
Morphology/82	Stem Cavity (STEMCAVITY)	Appearance of Calyx Basin	1 none 2 acute shallow 3 acute medium 4 acute deep 5 obtuse shallow 6 obtuse deep
Morphology/91	Tenacity of Fruit (FRUITTENAC)	Tendency of Fruit to Abscise or Hang	1 drops before mature 2 holds past maturity 3 persists into winter
Phenology/4	Harvest Season (HARVSEASON)	Harvest Season (days before or after Red Delicious—code no. 6)	1 >60 extremely early 2 50–60 very early 3 30–50 early 4 20–30 medium early 5 10 medium 6 RD season med. late 7 10 late 8 >30 extremely late
Production/21	Tree Bearing Habit (TREEBEAR)	Tree Bearing Habit	1 columnar 2 Type I spur 3 Type II semi-spur 4 Type III standard 5 Type IV tip-bearer 6 weeping
Growth/1	Tree Vigor (TREEVIGOR)	Tree Vigor from Small to Large When Grown on a Vigorous Rootsock	1 small 2 medium 3 vigorous 4 very vigorous

[a]As listed on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?115.

Table 1.4. Site descriptions for collection regions and years sites were accessed in Central Asia.

Country/Mt. Range/Area Site[a]	Site Name	Latitude/Longitude	Elevation (m)	Years collected
Tajikistan/—/1.00	Gazni	—	1490–1860	1989
Uzbekistan/—/2.00	Kondra	—	1100–1630	1989
Uzbekistan/—/2.01	Local Lake	—	1100–1630	1989
Uzbekistan/—/2.02	Tashkent	—	1100–1630	1989
Kazakhstan/Zailisky/3.00	Aksay River	43°07'N /77°47'E.	1260–1490	1995, 1996
Kazakhstan/Zailisky/3.01	Turgen, Kamenka	43°19'N /77°35'E.	1200–1360	1993, 1995
Kazakhstan/Zailisky/3.02	Almatinka River	43°06'N /76°54'E.	1440	1995
Kazakhstan/Zailisky/3.03	Talgar	43°17'N /77°23'E.	1490	1995
Kazakhstan/Zailisky/3.04	Kuznetzov	43°21'N /77°40'E.	1550	1989, 1995
Kazakhstan/Zailisky/3.05	Kotur Bulak	43°12'N /77°40'E.	1680	1993
Kazakhstan/Zailisky/8.00	Issyk Botanical Garden	—	—	1993
Kazakhstan/Djungarsky/4.00	Lepsinsk	45°31'N /80°43E.	1110–1360	1993, 1995, 1996
Kazakhstan/Djungarsky/4.01	Konstantinovka #1	45°38'N /80°55'E.	1260	1996
Kazakhstan/Djungarsky/4.02	Konstantinovka #2	45°41'N /80°52'E.	1185–1260	1996
Kazakhstan/Djungarsky/5.00	Topelevka, Low el.	45°24'N /80°24'E.	1180–1460	1993, 1995, 1996
Kazakhstan/Djungarsky/5.01	Topelevka, High el.	45°24'N /80°25'E.	1500–1760	1995, 1996
Kazakhstan/Karatau/6.00	Boraldy, Low el.	42°52'N /69°53'E.	600–620	1993, 1995
Kazakhstan/Karatau/6.01	Boraldy, High el.	42°52'N /69°53'E.	910	1995
Kazakhstan/Karatau/11.00	Kokbulak	42°40'N /70°16'E.	780–1230	1996
Kyrgyzstan/—/7.00	Fergansky #1	41°23'N /73°06'E.	1335	1993
Kyrgyzstan/—/7.01	Fergansky #2	41°16'N /72°53'E.	1550	1993
Kazakhstan/Tarbagatai/9.00	Alekseyevka site 05	47°14'N /81°34'E.	800–870	1995, 1996
Kazakhstan/Tarbagatai/9.01	Alekseyevka site 07	47°16'N /81°35'E.	820–960	1995, 1996
Kazakhstan/Tarbagatai/9.02	Alekseyevka site 09	47°16'N /81°34'E.	1030–1120	1995, 1996
Kazakhstan/Tarbagatai/9.03	Alekseyevka site 06	47°16'N /81°34'E.	860–990	1995, 1996
Kazakhstan/Tarbagatai/9.04	Alekseyevka site 08	47°15'N /81°34'E.	800–920	1995, 1996
Kazakhstan/Tarbagatai/9.05	Alekseyevka site 10	47°15'N /81°35'E.	860–1100	1995, 1996
Kazakhstan/Ketmen/10.00	Bolshoye Aksu	43°18'N /79°31'E.	1600–1660	1996
Kazakkstan/Talasky/12.00	Aksu Jabagli	42°19'N /70°22'E.	1010–1025	1996

[a]As depicted in Fig. 1.1.

Table 1.5. Site descriptions for collection regions in Central Asia.

Country/Mt. Range/ Area Site[a]	Forest Type	Elevation (m)	Annual Precipitation (mm)
Tajikistan/—/1.00	Xeric, mixed scrub forest	—	300
Uzbekistan/—/ 2.00-202	Xeric, mixed scrub forest	—	300
Kazakhstan/Zailisky/ 3.00–3.05	Humid-temperate, mixed forest	1170–1690	700
Kazakhstan/Djungarsky/ 4.00–4.02	Humid-temperate, mixed forest	1190–1360	800
Kazakhstan/Djungarsky/ 5.00	Humid-temperate, mixed forest	1170–1490	850
Kazakhstan/Djungarsky/ 5.01	Humid-temperate, mixed forest with diverse flora at high elevation	1500–1760	850
Kazakhstan/Karatau/ 6.00	Diverse stream habitat in xeric area	600–620	250
Kazakhstan/Karatau/ 6.01	Xeric, mixed scrub forest	880–910	250
Kazakhstan/Karatau/ 11.00	Xeric area and stream habitat	780–1230	250
Kazakhstan/Tarbagatai/ 9.00–9.05	Dry continental forest with –40°C common	870–1120	450
Kazakhstan/Ketmen/ 10.00	Semi-dry, temperate mixed forest	1600–1700	650
Kazakhstan/Talasky/ 12.00	Dry N. slope of canyon	1000–1025	320

[a]As depicted in Fig 1.1.

Table 1.6. Total *M. sieversii* seeds collected, distributed, and stored.

Year	No. Accessions	No. Seeds	No. Orders Processed	No. Distributed	No. at NSSL	No. at PGRU
1989	119	9,563	33	1,022	3,494	5,047
1993	60	23,406	147	8,486	7,290	7,630
1995	408	60,081	443	14,713	19,970	25,398
1996	307	28,039	279	6,454	8,344	13,241
Total	894	121,089	902	30,675	39,098	51,316

Table 1.7. Total collections, distribution, and storage of *M. sieversii* seeds from Central Asia.

Country/Mt. Range/Area Site[a]	No. Accession (trees harv).	No. Collected	No. Distributed	No. at NSSL	No. at PGRU	Years Collected
Tajikistan/—/1.00	19	1,267	108	449	710	1989
Uzbekistan/—/2.00	18	1,055	244	259	552	1989
Uzbekistan/—/2.01	8	385	24	130	231	1989
Uzbekistan/—/2.01	20	1,100	82	404	614	1989
Kazakhstan/Zailisky/3.00	53	4,449	681	1,525	2,243	1995, 1996
Kazakhstan/Zailisky/3.01	35	3,456	1,042	992	1,422	1993, 1995
Kazakhstan/Zailisky/3.02	1	374	50	160	164	1995
Kazakhstan/Zailisky/3.03	2	2,406	230	1,100	1076	1995
Kazakhstan/Zailisky/3.04	57	7,323	834	2,912	3,577	1989, 1995
Kazakhstan/Zailisky/3.05	3	949	643	125	181	1993
Kazakhstan/Zailisky/8.00	3	3,125	363	1,350	1,412	1993
Kazakhstan/Djungarsky/4.00	76	11,397	1,620	4,343	5,434	1993, 1995, 1996
Kazakhstan/Djungarsky/4.01	10	474	35	155	284	1996
Kazakhstan/Djungarsky/4.02	21	1,997	474	679	844	1996
Kazakhstan/Djungarsky/5.00	117	21,021	5,254	7,027	8,740	1993, 1995, 1996
Kazakhstan/Djungarsky/5.01	51	6,151	989	2,176	2,986	1993, 1995, 1996

Kazakhstan/Karatau/6.00	45	5,705	1,348	1,756	2,601	1993, 1995
Kazakhstan/Karatau/6.01	46	8,620	3,437	2,337	2,846	1993, 1995
Kazakhstan/Karatau/11.00	53	6,102	1,195	1,949	2,958	1996
Kyrgyzstan/—/7.00	6	3,862	997	1,450	1,415	1993
Kyrgyzstan/—/7.01	6	1,883	861	535	487	1993
Kazakhstan/Tarbagatai/9.00	45	4,656	1,506	1,339	1,811	1995, 1996
Kazakhstan/Tarbagatai/9.01	60	4,411	1,255	1,076	2,080	1995, 1996
Kazakhstan/Tarbagatai/9.02	49	4,482	1,448	1,177	1,857	1995, 1996
Kazakhstan/Tarbagatai/9.03	15	2,973	1,069	860	1,044	1995, 1996
Kazakhstan/Tarbagatai/9.04	7	1,878	1,025	400	433	1995, 1996
Kazakhstan/Tarbagatai/9.05	33	6,440	3,143	1,543	1,754	1995, 1996
Kazakhstan/Ketmen/10.00	22	2,118	513	613	992	1996
Kazakkstan/Talasky/12.00	13	1,050	205	277	568	1996
Total	894	121,809	30,675	39,098	51,316	1989, 1993, 1995, 1996

[a]As depicted in Fig. 1.1.

the cultivated apple in other quality traits. Likewise, some trees were nearly disease-free and had other desirable horticultural characteristics.

Accessions were described with 25 priority descriptors (Table 1.3) at collection time. These records can be accessed on the Germplasm Resources Information Network (GRIN) on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/search.pl (USDA 2000). For cataloging purposes, all collections received a sequential Geneva *Malus* (GMAL) number in order to distinguish our apple collection from others in the NPGS. Additionally, all permanent accessions receive a Plant Introduction (PI) number that can be used as well to search the GRIN database on the Web at http://www.ars-grin.gov/npgs/acc/acc_queries.html:

1989 collection	PI 600302–600409 (GMAL 3242–3360)
1993 collection	PI 600423–600482 (GMAL 3526–3585)
1995 collection	PI 600485–600584 (GMAL 3596–4003)
1996 collection	PI 600585–600624 (GMAL 4010–4317)

We also maintain inventory, accession, and passport records for each accession. For named cultivars, pedigree, developer, and general descriptive narrative are recorded. For collections of wild material, site records, including GPS data are recorded along with observations of each accession collected in situ.

The software used in the local data management is PARADOX by Borland International. Paradox Version 3.5 is a relational database management program that can be used either as a stand-alone system on a single computer or as a multi-user system on a network. This program is menu driven. Forms, reports, queries, and graphs are some of the capa-

Table 1.8. Mean range for fruit size in samples from random or elite *Malus sieversii* trees measured during collection in Kazakhstan.

		Fruit size mean (range) in mm	
Area[a]	Year(s)	Elite Samples	Random Samples
3	1995/1996	45 (32–56)	34 (25–49)
4	1995/1996	44 (32–55)	34 (28–44)
5	1995	46 (37–56)	36 (27–48)
5	1996	58 (54–65)	35 (25–49)
6	1995	42 (33–46)	41 (28–54)
9	1995/1996	56 (46–72)	43 (28–62)
10	1996	49 (47–51)	40 (29–51)
11	1996	55 (44–76)	42 (29–63)
12	1996	65 (60–74)	42 (32–50)

[a]See Fig. 1.1 and Table 1.2 for location and description of collection areas.

bilities of the program, as well as multi-table links using forms, reports, queries, or graphs. All data are loaded to the NPGS database management system, Germplasm Resources Information Network (GRIN), which uses Oracle and SQL programs. Information about GRIN can be found on the Web at: http://www.ars-grin.gov/npgs/.

The fruit size and quality varied each year in the sites that were visited. For example, in region 5, the size and quality of the fruit was much better in 1996 than in 1995 and fruiting was observed on a much higher percentage of the trees (Table 1.8). In addition to seed from 146 elite genotypes, vegetative material was collected from 44 of the elite accessions in the 1995 and 1996 expeditions and placed in quarantine at the USDA Plant Quarantine Facility in Beltsville, Maryland. Fruit color and fruit quality traits (apple flesh firmness and flavor) are highlighted in Tables 1.9 through 1.12 for collections made in 1995 and 1996.

In 1995 and 1996, material was also collected specifically for molecular studies of population genetics and biodiversity to follow preliminary work by Lamboy et al. (1996). These included randomly selected

Table 1.9. Red color of *M. sieversii* fruit from random populations collected from Kazakhstan.

		Red color		
Mt. Range/ Area Site[a]	Total Accessions	No. Accessions with Red Color[b]	Percent Accessions with Red Color[b]	Percent Red Intensity of Those with Red Color[c]
Zailisky/ 3.00–3.04	80	29	36	35
Djungarsky/ 4.00–4.02	87	42	48	27
Djungarsky/ 5.00–5.01	114	74	64	42
Karatau/ 6.00–6.01	64	34	53	37
Tarbagatai/ 9.00–9.05	149	101	68	46
Ketmen/10.00	20	13	65	38
Karatau/11.00	40	33	83	44
Talasky/12.00	10	8	80	51
Total	564	334	59	40

[a]As depicted in Fig. 1.1.

[b]Code 4–9 in Morphology descriptor 34; fruit over color (Table 1.3).

[c]Morphology descriptor 60; percent over color intensity (Table 1.3).

Table 1.10. Red color of *M. sieversii* fruit from elite selections collected from Kazakhstan.

		Red color		
Mt. Range/ Area Site[a]	Total Accessions	No. Accessions with Red Color[b]	Percent Accessions with Red Color[b]	Percent Red Intensity of Those with Red Color[c]
Zailisky/ 3.00–3.04	11	10	91	63
Djungarsky/ 4.00–4.02	14	10	71	41
Djungarsky/ 5.00–5.01	24	19	79	46
Karatau/ 6.00–6.01	21	15	68	45
Tarbagatai/ 9.00–9.05	58	55	91	60
Ketmen/10.00	2	1	50	50
Karatau/11.00	13	8	62	44
Talasky/12.00	3	3	100	44
Total	146	121	83	48

[a]As depicted in Fig. 1.1.

[b]Code 4–9 in Morphology descriptor 34; fruit over color (Table 1.3).

[c]Morphology descriptor 60; percent over color intensity (Table 1.3).

seeds from 304 trees across areas 3, 4, 5, 6, and 9 in 1995 (Fig. 1.1) and from 261 trees across areas 3, 4, 5, 9, 10, 11, and 12 (Fig. 1.1) in 1996. Leaves from the 261 maternal trees in 1996 also were collected and dried in silica gel, with DNA extracted later at PGRU.

III. CENTRAL ASIAN COLLECTIONS

Collections made in four expeditions (1989, 1993, 1995, and 1996) produced 121,089 seeds from 894 individual trees (Table 1.6). Localities of all collections are also listed in Table 1.4. A summary of total collections made in each year is listed in Table 1.6 and a summary of collections made at each site is listed in Table 1.7. A Global Positioning System device was available for collections made in 1993, 1995, and 1996 permitting latitude and longitude to be associated with each site (Table 1.4).

Table 1.11. Quality ratings of randomly collected fruit from Kazakhstan based on firmness and flavor.

Mt. Range/ Area Site[a]	Total Accessions	Distribution (%) Firmness[b] 1	2	3	4	Flavor[c] 1	2	3	4	5
Zailisky/ 3.00–3.04	80	39	49	10	3	10	3	48	14	25
Djungarsky/ 4.00–4.02	87	26	60	14	0	4	5	31	38	23
Djungarsky/ 5.00–5.01	114	21	45	29	5	7	11	32	23	28
Karatau/ 6.00–6.01	64	22	50	28	0	34	22	33	6	3
Tarbagatai/ 9.00–9.05	149	23	42	34	1	10	5	30	9	36
Ketmen/ 10.00	20	20	45	35	0	0	20	25	40	15
Karatau/ 11.00	40	20	35	40	5	35	20	15	15	15
Talasky/ 12.00	10	20	50	30	0	0	0	50	30	20
Total	564	25	47	26	2	15	9	33	18	25

[a]As depicted in Fig. 1.1.

[b]Morphology descriptor 23; fruit flesh firmness (Table 1.3).

[c]Morphology descriptor 24; fruit flesh flavor (Table 1.3).

A. 1989 Collection

The first trip to Central Asia included Calvin Sperling (leader) accompanied by Herb Aldwinckle and Elizabeth Dickson. Gratitude must be expressed to the opening of botanical exchange between the USSR and the United States (Tom Elias, formerly of Rancho Santa Ana Botanical Garden, Claremont, California, and Igor Smirnov, Moscow Botanical Garden). This trip took place between August 29 and September 14 in Tajikistan, Uzbekistan, and Kazakhstan identified as areas 1–3 (Fig. 1.1).

In 1989, 119 seedlots (9563 seeds) were collected (Table 1.6), including 19 seedlots (1267 seeds from Tajikistan and 46 seedlots (2540 seeds) from Uzbekistan (Table 1.7). While in Kazakhstan, the collectors met Professor Aimak Dzhangaliev who is the author and coauthor of two works of this present volume. He provided access to Kazakhstan/

Table 1.12. Quality ratings of elite fruit collections from Kazakhstan based on firmness and flavor.

Mt. Range/ Area Site[a]	Total Accessions	Distribution (%) Firmness[b] 1	2	3	4	Flavor[c] 1	2	3	4	5
Zailisky/ 3.00–3.04	11	27	46	9	18	36	0	55	9	0
Djungarsky/ 4.00–4.02	11	7	21	72	0	21	7	57	14	0
Djungarsky/ 5.00–5.01	24	8	25	58	8	38	8	46	4	0
Karatau/ 6.00–6.01	21	0	19	48	33	43	5	38	10	5
Tarbagatai/ 9.00–9.05	58	7	26	55	12	79	7	10	2	2
Ketmen/10.00	2	0	50	50	0	0	0	100	0	0
Karatau/11.00	13	8	23	54	15	69	0	31	0	0
Talasky/12.00	3	0	0	0	100	67	0	33	0	0
Total	146	8	25	51	16	56	5	32	5	2

[a]As depicted in Fig. 1.1.

[b]Morphology descriptor 23; fruit flesh firmness (Table 1.3).

[c]Morphology descriptor 24; fruit flesh flavor (Table 1.3).

Zailisky/3.04 where 54 of the 57 seedlots (5756 of 7323 seeds) were collected in 1989. The remainder of the seedlots/seeds were collected in that same site in 1995 (Table 1.7).

Fruit characterization records for collections in 1989 were not made, but the collectors observed that they ranged in size from small crabapples to commercial size, with color and quality being described as very diverse. Records and specific site data can be found on the Web. The data on the 1989 collection can be found by accessing catalog numbers PI 600302–600409 (GMAL 3242–3360).

In October 1992, Professor Dzhangaliev traveled to PGRU to meet members of the U.S. Apple Crop Germplasm Committee at their annual meeting. Cooperative agreements with Professor Dzhangaliev and the Kazakhstan Academy of Sciences were developed whereby three additional collection trips to diverse areas of Kazakhstan were planned.

B. 1993 Collection

A second expedition to Kazakhstan took place September 6–28, 1993. Philip Forsline led a team of collectors including Elizabeth Dickson, Gaylord Mink, and Dominique Noiton. Professor Dzhangaliev was again the main host and was assisted by others as listed in Table 1.2. Despite a severe spring frost in many of the forested areas of Kazakhstan and Kyrgyzstan, we were able to collect 60 seedlots (23,406) seeds of *M. sieversii* (Table 1.6) from the eight area/sites (Table 1.4 and Fig. 1.1).

Fruit characterization records for these collections were recorded using a narrative approach. These records, along with specific site data can be found on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/search.pl. The data on the 1993 collection can be found by accessing catalog numbers PI 600423–600482 (GMAL 3526–3585).

C. 1995 Collection

The material collected in 1989 and 1993 provided clues to enable future collection trips to be more effective (Hokanson et al. 1997b, 1999). A study using isozyme analysis (Lamboy et al. 1996) to determine levels of genetic variation present in the 1989 and 1993 collections of *M. sieversii* was conducted. Based on genetic analysis of sib families from four areas, the populations of *M. sieversii* surveyed appear to constitute a single panmictic population with more than 85 percent of the total genetic variation due to differences among families and only 15 percent due to differences among regions. Thus we determined that the most efficient strategy to acquire generalized genetic diversity and potentially useful alleles would be to explore as many unique ecological niches as possible.

This third expedition to Kazakhstan took place August 23 to September 16, 1995. Philip Forsline led a team of collectors including Elizabeth Dickson, James Luby, and two scientists from South Africa, Gary Britz representing that country's apple industry, and Taaibos Human, fruit breeder. Again, Professor Dzhangaliev was the main host assisted by others.

The main objectives of this expedition were to: (1) collect germplasm of *Malus sieversii* in its center of diversity, supplementing collections made in 1989 and 1993 by broadening the range of collections and returning to areas that had sparse fruiting in 1993; (2) collect other crop species as found in association with apple; and (3) expand contacts with Kazakh scientists to develop plans for further ex situ collections and develop strategies for in situ conservation.

This third collection trip for *M. sieversii* was the most successful expedition based on the diversity and quantity of material and characterization data collected. Previous collections made in 1993 were limited due to low fruiting from that season's spring frost. The 1995 collection was also timely since the social/political situation in Kazakhstan was deteriorating rapidly and future trips were jeopardized. Collections were made in four of the same areas as in 1993, but also included the northernmost site for *M. sieversii* (47°, 16′N) where wild apple trees were found with the largest size and highest quality fruit of any site explored thus far. Some elite selections were nearly 70 mm in diameter. Strategy for collection of apple in the wild forests was twofold: (1) random population sampling of 5 to 8 fruit/tree (average of 45 seed) from each of 30 trees in each site which appeared to be a unique ecosystem; and (2) selection of elite wild types (seed and occasionally scions) within these sites. Ten populations of 30 trees (5–8 fruit/tree) each were sampled which yielded 13,842 seeds. Additionally, 101 elite selections were made across all sites yielding 46,239 seeds and scions from 14 of the most elite types for a total of 60,081 seeds from 408 accessions (Table 1.6). In addition to the high-quality collections in the northern area, collections were made in a xerophytic area at 42°, 52′N (Karatau region) where trees were adapted to drought (Tables 1.4 and 1.5). Fruit at this site had excellent horticultural characteristics including the capacity for fruit to hang on the tree past maturity in an area with high heat units indicating potential for adaptation to areas with longer growing seasons. All collections were documented by 24 morphological descriptors (Table 1.3) along with associated site information. Those descriptors and codes for each can be found for the purpose of querying individual accessions on the Web at: http://www.ars-grin.gov/cgi-in/npgs/html/desclist.pl?115.

1. Geographic Sites in 1995

1. Zailisky, Sites 3.00, 3.01–3.04. Day trips were taken August 25–27 to the five sites (Table 1.4) near Almaty, an area with extensive human encroachment that has led to a decline in native habitats. Some of these were the same areas observed in 1989 and 1993. Two populations of 30 accessions each (GMAL 3700–3729 from site 3.00 and GMAL 3730–3759 from site 3.01) were collected in this area. From these 60 accessions, 2121 seeds were collected. The mean diameter of apples found in these random populations was 34 mm (Table 1.8). In addition, 11 wild elite samples selections from sites 3.00 (GMAL 3596–3598), 3.01 (GMAL 3602–3603), 3.02 (GMAL 3599), 3.03 (GMAL 3600–3601) and 3.04

(GMAL 3697–3699) averaged 46 mm (Table 1.8) from which 7878 seeds were collected. Complete data sets on all collections (populations and elites) with site and accession records can be accessed on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/search.pl. We were able to observe extensive diversity in each site throughout the expedition with 60 to 90 percent of the trees in the forests fruiting. Forests in the Almaty area, Topelevka, and Lepsinsk areas (areas 3, 4, 5) were very similar in habitat and fruit types. Fruit was not of high quality, and few elite samples were collected in these general areas. Fruit quality traits for all collections made in 1995–1996 are listed in Tables 1.9–1.12.

Tarbagatai, Sites 9.00–9.05. The second phase of our 1995 expeditions took us to the Tarbagatai mountain range (Fig. 1.1) which had not been visited in previous collection trips. Six sites (9.00–9.05) were examined from August 29 to September 1. An area of 3.5 × 3.0 km was covered, but different valleys and slopes were explored. Grazing has destroyed a large portion of this habitat. We discovered high-quality fruits (Plate 1A) in all sites within this area. Three populations of 30 accessions each: (1) GMAL 3760–3789 from site 9.00; GMAL 3790–3819 from site 9.01; and GMAL 3820–3849 from site 9.02 (Plate 2A) were collected in this area. The mean diameter of all fruits in the random populations (90 trees) was 43 mm (Table 1.8) of which 3630 seeds were collected. In addition, 14,414 seeds of 46 wild elites were collected including scions of the very best from sites: 9.00 (GMAL 3604–3607 with scions from GMAL 3607); 9.01 (GMAL 3616–3619 with scions from GMAL 3616 and GMAL 3619); 9.02 (GMAL 3626–3632); 9.03 (GMAL 3608–3615 with scions from GMALs 3608 and 3614); 9.04 (GMAL 3620–3625 with scions from GMALs 3623 and 3625); and 9.05 (GMAL 3633–3649 with scions from GMALs 3636, 3637, and 3643). The mean diameter of the wild elite selections was 56 mm (Table 1.8). The quality of some of these elite specimens was near to that of commercial apples. Therefore scions were collected for 10 of the 46 selections. The tree with the largest apples among these elites had fruits that averaged 72 mm in diameter. Most elite selections had aromatic qualities (Table 1.12), were extremely firm, and had little evidence of disease. Some red color was present on 68 percent of the random population (Table 1.9) and on 91 percent of the elites (Table 1.10). These figures are much higher than those in the forests at 43°N and 45°N latitude. Additionally, these apples have evolved in a harsh climate that often drops to –40°C. Spur-type trees, which have many horticultural advantages, were also prominent in this area and were seldom seen in the other forests. In this environment, we did not see trees that were greater than 50 years old as we had seen in other areas such as

Djungarsky and Zailisky; they were much smaller and more widely spaced here also. Fruit shapes from most areas were of the globose to globose conical type (Table 1.13).

Djungarsky, Sites 4.00, 5.00, 5.01. The third phase of our 1995 expedition took us to the Djungarsky mountain range where we had visited in 1993. Three sites (4.00, 5.00, and 5.01 were examined from September 2 to September 5. Djungarsky near Topelevka village (Fig. 1.1, area 5) was the same area that was visited in 1993, but fruiting was heavy this year. Two population samples of 30 accessions each were collected which included a lower elevation sample at 1170–1450 m (GMAL 3880–3909 from site 5.00) and a higher elevation sample from 1450–1690 m (GMAL 3850–3879 from site 5.01). The mean diameter of the fruit from the 60 random accessions was 36 mm. From these 60 random accessions, 3014 seeds were collected. In addition, seeds of 14 wild elites were collected from sites 5.00 (GMAL 3654–3663 with scions collected from GMAL 3654) and 5.01 (GMAL 3650–3653). The mean diameter of the wild-elite selections was 46 mm at site 5.00 and 38 mm at site 5.01. From these 14 trees, 7618 seeds were collected. Although the fruit was of similar size from the different elevations, both the 60 random accessions and 14 elites had variable horticultural characters and levels of disease susceptibility depending on elevation.

The Lepsinsk area (Fig. 1.1, Table 1.4, area/site 4.00) was visited in 1993 with minimal collections. One population of 30 accessions was collected along with eight elites in 1995. The random collection at site 4.00 included GMAL 3910–3939 with a mean fruit diameter of 34 mm with 1425 seed collected. A total of 6065 seeds from eight wild elites were collected from site 4.00 (GMAL 3664–3671), mean fruit diameter 37 mm. The diversity in this area was minimal with predominantly small, yellow, scab-infested fruit whose firmness and aromatics were low.

Karatau, Sites 6.00 and 6.01. Expeditions in the Karatau Mountains (Fig. 1.1, area 6) were made September 8 to 10. In site 6.00, a random population of 30 apples (GMAL 3940–3969) was collected along a stream tributary along with 12 elites (GMAL 3672–3681 and GMAL 3694–3696). The mean diameter of the apples from site 6.00 was 39–41 mm for both random and elites. We collected 1476 seed from the 30 random trees in site 6.00 and 4081 seed from the 12 elites. Site 6.01 was a xerophytic site 300 m above the stream area. There we collected a population of 34 apples (Plate 2B) and 12 wild elites (GMAL 3682–3693 with scions also collected from GMAL 3686 and GMAL 3693) that were completely without scab infection. We collected 2177 seed from the 34 random trees in site 6.01 and 6113 seed from the 12 elites. Because the area was so dry,

Table 1.13. Fruit shape of populations and elites from Kazakhstan.

Mt. Range/ Area Site[a]	No. Accessions	Percent of accessions rated for 13 shape[b] categories												
		1.0	1.1	1.2	2.0	2.1	3.0	3.1	3.2	4.0	4.1	5.0	5.1	5.2
Zailisky/ 3.00–3.04	91	39	35	1	1	16	3	1	0	0	0	1	1	0
Djungarsky/ 4.00–4.02	101	46	22	0	10	20	0	0	0	2	0	0	1	0
Djungarsky/ 5.00–5.01	138	39	20	0	7	28	2	1	0	0	2	1	2	0
Karatau/ 6.00–6.01	86	27	23	0	2	33	1	0	0	1	0	9	3	0
Tarbagatai/ 9.00–9.05	207	38	34	<1	1	17	3	0	<1	1	<1	1	2	0
Ketmen/ 10.00	22	5	55	0	0	32	0	0	0	0	10	0	0	0
Karatau/ 11.00	53	21	30	0	13	19	0	2	0	2	0	4	8	2
Talasky/ 12.00	13	0	8	0	15	38	0	8	0	0	8	0	23	0
Total	711	35	28	<1	5	22	2	1	<1	1	1	2	3	<1

[a]As depicted in Fig. 1.1

[b]Morphology descriptor 38; overall fruit shape (Table 1.3).

lack of scab on the fruit does not necessarily indicate scab resistance. However, the fruit was of good horticultural quality. Fruit from the random population at site 6.01 had the highest level of firmness and aromatics of any of the populations. Trees exhibited excellent fruit retention and the high level of fruit firmness was surprising given the very high level of heat units common in the area. These apples would likely be late harvest types in most apple-growing districts. They may also be of interest as a drought resistant source for rootstocks. The apples collected along the stream at site 6.00 were almost all drops and were difficult to characterize for quality since they were earlier maturing.

On September 13, we visited the ex situ collections of Professor Dzhangaliev and observed the collection of 600 apple accessions that he received over the last four years from PGRU in Geneva, New York, Corvallis, Oregon, and Davis, California, including 40 accessions that were recently grafted from our collection three weeks earlier. We observed other elite commercial types that his institute maintains as well as 70 wild 'selections' of *M. sieversii* from the Kazakhstan forests. In 1993, we imported five of these promising 'selections', which remain in quarantine in Beltsville, Maryland.

A total of 60,081 seeds were collected from 408 individual trees in 1995 (Table 1.6). Fruit characterization records for these collections were recorded using a descriptor approach (Table 1.3). These records along with specific site data can be found on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/search.pl. The data on the 1995 collection can be found by accessing catalog numbers: PI 600485–600584 (GMAL 3596–3699 elites) and GMAL 3700–4003 (random populations).

D. 1996 Collection

A fourth expedition to Kazakhstan took place August 24 to September 19, 1996. Collectors included Philip Forsline, Stan Hokanson, Thomas Unruh, and Harold Pellett. Professor Aimak Dzhangaliev was again the main host who was assisted by others.

Our objectives were threefold:

(1) collect seeds and leaf samples from trees at random throughout each site in order to characterize diverse populations using morphological and molecular methods;
(2) collect seeds and scions of superior types within each population based on horticultural and disease characteristics; and
(3) establish linkages with scientists in the Kazakh Academy of Sciences to continue cooperation on characterization of the Kazakh

apple forests, including studies to establish frameworks for in situ reserves, translation of previous works, and scientific exchange.

M. sieversii was collected in nine distinct ecosystems, five of which had never been accessed in previous years. In three of four sites previously visited, fruiting was heavier than in previous years allowing for more complete sampling. Only in the northernmost site, Tarbagatai, which was very productive in the previous year (1995), did we see sparse fruiting. In this expedition, collections included random samples of leaves and 14,000 seeds from 260 trees across the nine sites. Population sizes varied at each site from 10 to 60 trees depending on the size of the area and more significantly on the time allotted for collection at each site by our hosts. We collected an additional 14,500 seeds from 47 superior trees throughout all sites. Scions were collected for 30 of these to establish clones. All fruit and trees were characterized with up to 24 descriptors (Table 1.3) and associated site information can be found on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?115. The manuscript of an English translation of the book, *The Wild Apple Trees of Kazakhstan* was obtained from the author, our host, Professor Aimak Dzhangaliev and a revision of this book is published as part of this volume.

1. Geographic Sites in 1996

Ketmen, Site 10.00. On August 27, we left for our first expedition to the Ketmen mountains (Fig. 1.1, area 10) accompanied by Professor Dzhangaliev, Raisa Turehanova (botanist), and Valerie Sotnikov (horticulturist), Mukhamedjan Issayev (interpreter), Mr. Kashaganov (forestry director for the Ketmen area), and Dominique Noiton (apple breeder from New Zealand). We drove 350 km from Almaty, arriving in the village of Kolzhat in the Ketmen area near the Chinese border. We observed the Ketmen 'selections' in local gardens and then drove northwest 120 km to a Forestry Reserve that protects a rare species of ash (*Fraxinus sogdiana*).

The next morning, August 28, we drove 65 km to the Ketmen Mountains west of Kolzhat that we visited the previous day and collected *M. sieversii* near the village of Kirghiz Sai where time was limited. Three of the 20 random accessions collected were the red flesh form of *M. sieversii* that the Kazakhs classify as *Malus niedwetzkyana* (Table 1.1). In our three previous expeditions to Central Asia, we had never seen this in the wild, but Professor Dzhangaliev reported this species is found in most of the habitats in Kazakhstan. He maintains some forms at the Main Botanical Garden in Almaty. The size of the area covered was 5.0 km × 3.0 km. One population of 20 accessions (GMAL 4057–4076) was collected in this area. From these 20 accessions, we collected leaf samples for DNA extraction along with 1273 seeds. The mean fruit diameter in these 20 accessions was 40 mm. In addition, two wild elite samples from site 10.00 (GMAL 4010–4011) averaged 49 mm in diameter (Table 1.8) and 845 seeds were collected. The fruit was of poor quality (Tables 1.9–1.12).

Zailisky, Site 3.00. On August 30, collections were made in the Zailisky Mountains near Almaty, the same area as the collection in 1995 (site 3.00) along the Aksay River. This collection was valuable since 80 percent of the trees were fruiting in contrast to less than 50 percent in 1995. From a random population of 20 trees (GMAL 4077–4096) in a 0.5 km × 1.0 km area, we collected 1340 seeds and leaves; 35 percent of the trees were scab-free.

Tarbagatai, Sites 9.00–9.05. September 1 to 4 was spent in the same area (Fig. 1.1, area 9) in the Tarbagatai mountains, near the city of Urdzhar where we had collected in 1995 (sites 9.00–9.05). This is the area that produced some excellent superior selections in 1995 when nearly 90 percent of the trees were fruiting. However, as a result of cold weather during pollination in 1996, only 15 percent of the trees were fruiting, but we were able to make a random collection of 2440 seeds and leaves from 60 trees (GMAL 4097–4156) in a 3.0 × 5.5 km area; 11 trees in site 9.00 (GMAL 4136–4146); 23 trees in site 9.01 (GMAL 4097–4119); 10 trees in site 9.02 (GMAL 4147–4156); 7 trees in site 9.03 (GMAL 4120–4126); no trees in site 9.04; and 9 trees in site 9.05 (GMAL 4127–4135). The mean fruit diameter of 60 trees was 41 mm. We collected seeds and scions from 13 superior selections in sites 9.00–9.05 (GMAL 4012–4024) ranging in size from 45 mm to 62 mm with the mean diameter being 54 mm. We also collected scions of 11 of 13 elite selections and 4295 seeds. In this same area in 1995, having more diversity to select from with more precocious fruiting, 46 superior types were selected ranging in size from 46 mm to 72 mm. Very high levels of aromatic flavor, flesh firmness, red color, flesh firmness (Tables 1.9–1.12) and freedom from scab were observed. Although we did not record scab susceptibility on the 60 randomly selected fruit in 1995, the trees were very clean throughout the entire population. The grazing sheep and goats observed in this area in 1995 were not present in 1996.

Djungarsky, Sites 4.00, 4.01, 4.02, 5.00, and 5.01. On September 5 to 9, we made collections in four unique sites within the Djungarsky area (Fig. 1.1, areas 4 and 5). Sites 4.01 and 4.02, located near the city of Andreyevka were only 7 km apart but quite unique from each other and they had not been accessed in previous years. Collections were made very close to the village of Konstantinovka. Collections at site 4.01 were limited to seeds and leaves of 10 randomly selected trees (GMAL 4157–4166) from which we collected 474 seeds. These fruit had a mean diameter of 33 mm. We selected only those trees that were at least 50 years old since this is an area that Professor Dzhangaliev has reforested over the last 40 years with seedlings that may be native to other areas. This population area with a southwest aspect was 1.0 km × 1.0 km.

Site 4.02 (not part of the reforesting project) was located on a steep northwest-facing slope that was 1.5 km × 200 m. We collected leaves and seed of 17 randomly selected trees (GMAL 4167–4183) from which we collected 815 seeds and found four superior selected types (GMAL 4025–4028) from which 1172 seeds were collected. Scions were collected from GMALs 4027 and 4028). Mean fruit size of the random population was 33 mm and for the 4 elites was 48 mm. High levels of apple scab infection were observed in sites 4.01 and 4.02. We observed a full range of fruit diversity in these populations with 60 to 80 percent of the trees fruiting.

Site 4.00 in the Djungarsky range was located near the village of Lepsinsk 20 km southwest of sites 4.01 and 4.02. This was the same area accessed in 1993 and 1995. However, we were pleasantly surprised with the fruit diversity observed in 1996 since 80 percent of the trees were fruiting; in previous years, the fruiting intensity was less than 40 percent. The size of the population area was 1.5 km × 2.0 km with large variance in elevation, slope, and aspect. Thirty trees were sampled at random (GMAL 4184–4123) from which we collected 1535 seeds along with two superior selections (GMAL 4029–4030 with 641 seeds and scions were also collected for these two elite specimens). Mean fruit size of the random population was 34 mm and for the two elites was 44 mm. Apple scab was rather heavy in the area but 4 of 30 trees sampled along with the superior selections were impressively clean of scab.

We completed our collections in the Djungarsky range by returning to the site near the village of Topelevka (sites 5.00 and 5.01) about 35 km southwest of site 4.00. This same site was accessed in 1993 and 1995. We were impressed with the diversity available in 1996 with 95 percent of the trees fruiting. We sampled 54 randomly selected trees (GMAL 4214–4232 and 4249–4267 from site 5.00; GMAL 4233–4248 from site 5.01) from an area of 3.0 km × 5.0 km with a very large variance in elevation along with diverse slopes and aspects. The first ten trees sampled in the random populations were near an area of seedling reforestation. Trees GMAL 4224–4267 were collected on the last two days from localities of this area that had not been visited in 1993 or 1995. The mean diameter of the fruit from these 54 trees was 35 mm from which we collected 2928 seeds. We also collected 10 superior selections (GMAL 4031–4040 with scions collected from GMAL 4031–4036 and 4038) based on fruit quality, tree form, and disease resistance. The size range of these was 54 mm to 65 mm (mean fruit diameter was 58 mm) in comparison to the size range in 1995 of 37 mm to 56 mm (Table 1.8). From these 10 accessions 3190 seeds were collected. Quality of these fruits was also much more impressive than those collected during the sparse fruiting years of 1993 and 1995. All superior selections were without scab and 35 percent of the randomly selected materials were scab-free. We also collected seeds and scions of two genetic dwarfs (GMAL 4033 and 4034) found at an elevation of 1500 m to 1600 m. We initially suspected that they were dwarfed as a result of growing at high elevation, but vigorous, full sized trees were found in close association.

Karatau, Site 11.00. We arrived in the city of Chimkent 700 km west of Almaty on September 12 and drove 70 km northeast to the forestry camp named Kokbulak, meaning "blue spring." This site in the Karatau Mountains is 50 km southeast of the Boraldy forestry camp (sites 6.00/6.01) that we visited in 1993 and 1995 (also in the Karatau range). The habitat in Kokbulak, very similar to that in Boraldy, is a xerophytic area receiving only 250 mm of annual precipitation. However, there is a spring-fed stream running through the area. From September 12 to 14, we collected seed and leaf samples from 40 randomly selected *M. sieversii* (GMAL 4268–4307) over an area of 5.0 km × 7.0 km. The horticultural quality based on size, firmness, and flavor of these collections was good (Tables 1.9–1.12). Trees were 60 percent scab-free but this may be avoidance rather than resistance since the dry area may not be conducive to scab outbreaks. A population of 30 trees (GMAL 4268–4297) was collected near the active stream but may not be xerophytic types, and may be similar to those found along a stream in Boraldy in 1995 (site 6.00). The remainder of the random population (GMAL 4298–4307) was collected on a dry north-facing slope and may have drought tolerance similar to those we found in Boraldy in 1995 (site 6.01). The mean diameter of fruit from the 40 random collections (2295 seeds) was 42 mm. We collected 3990 seed from 13 superior types (GMAL 4041–4053) and scions from GMALs 4042–4043, 4049, and 4051–4053. Mean fruit size was 55 mm (Table 1.8), similar to the large fruit found in Tarbagatai in 1995. One accession had an average fruit size of 76 mm (Plate 1B). The quality of these fruits parallels those from the northern area of Tarbagatai.

Talasky, Site 12.00. We drove 60 km southeast of site 11.00 in Karatau (Fig. 1.1) to the Talasky Mountain area, an area that we had not previously visited. This was a restricted area listed as a National Park called Aksu Jabagli. The collection area is a deep narrow canyon 1270 m at the top and 1010 m at the river level. We hiked the steep winding trails on the southern slope down to the river level where we found *M. sieversii* growing up the face of the northern slope to the top of the canyon. We collected only along the river at 1010 m and up to 1025 m on the northern slope including leaves and 741 seeds from 10 randomly dispersed *M. sieversii* (Plate 2C) and 319 seeds and scions from three superior types (GMAL 4054–4056 with scions of GMAL 4054–4055). The quality of the fruit in this area was impressive (Tables 1.9–1.12) with mean fruit diameter of 42 mm. Mean fruit diameter of the three elites was 65 mm (Table 1.8). Two of the superior types (Plate 1C) had long ellipsoid/conical shapes that we had rarely seen before. This site had the highest levels of ellipsoid and oblong-shaped fruit (Table 1.13). The site was quite similar to that in Karatau with low rainfall and high summer temperatures. Although it would be difficult to collect along the entire steep north face, this area should be visited again in order to sample the trees on the upper slopes.

A total of 28,039 seeds were collected from 307 individual trees in 1996 (Table 1.6). Fruit characterization records for these collections were recorded using a descriptor approach (Table 1.3). These records, along with specific site data can be found on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/search.pl. The data on the 1996 collection can be found by accessing these catalog numbers: PI 600585–600624 (GMAL 4010–4056 elites) and GMAL 4057–4317 (random populations).

IV. MAINTENANCE

A system has been developed to back up the PGRU clonal collection by storing dormant buds in liquid nitrogen (LN). This system was first described by Sakai (1960), and refined for apple by Sakai and Nishiyama (1978) and Tyler et al. (1988). Stushnoff (1991) described how this method could be useful as a practical approach for storing germplasm in the NPGS. The protocol for storing germplasm as part of the base collection at the National Seed Storage Laboratory (NSSL) in Fort Collins, Colorado was developed as a pilot project on 64 diverse accessions (Forsline et al. 1998). This trial was also set up to determine longevity of storage over 40 years. No decline in viability was observed after four years of storage (Forsline et al. 1998) including some accessions now stored for 12 years (unpublished).

Nearly 85 percent of the clonal collection (2000 accessions) has been backed up in LN-storage at the base collection at NSSL. Of these, 1700 have been tested for recovery by grafting using the methods developed by Forsline et al. (1998) and refined for efficient storage and recovery (Forsline et al. 1995, 1996a, 1998). We have obtained good results for nearly all *Malus* accessions stored in the base collection. Grafted buds of 98 percent of *M. ×domestica* have shown adequate recovery. At least five, 10-bud containers of each accession are cryogenically stored. Adequate recovery following grafting onto seedling rootstock as the baseline test for the cryogenically stored material is 40 percent (Forsline et al. 1995, 1996a, 1998). Research is being conducted to determine a protocol to cryopreserve a small group of recalcitrant (nonhardy) apples. We have found that some of these such as 'Golden Delicious' vary in cryopreservability from year to year probably due to variable cold acclimation conditions. We concluded that most accessions can be successfully cryopreserved.

Storage of *M. sievesii* seed from Central Asia is done both at NSSL and PGRU (Table 1.7). Presently, 51,316 seeds are stored at PGRU at –20°C with 39,098 seed stored at NSSL in LN. In addition, over 5000 seedlings have been screened for disease resistance traits in a collaborative program with the Plant Pathology Department at Cornell University, Geneva. Many of these seedlings have been distributed to sites outside of PGRU. Seed germination for a subset of the stored seed was monitored (Table 1.14) with over 93 percent germination of *M. sieversii* seeds. In

Table 1.14. Germination of *M. sieversii* seeds from Kazakhstan.

Mt. Range/ Area Site[a]	No. Accessions Germinated	Percent Accessions with More Than 90 Percent Germ.	No. Seeds Stratified	Percent Seed Germinated
Zailisky/ 3.00–3.04	22	72	157	92
Djungarsky/ 4.00–4.02	43	58	319	92
Djungarsky/ 5.00–5.01	26	50	300	89
Karatau/ 6.00–6.01	44	88	663	97
Tarbagatai/ 9.00–9.05	98	64	1323	91
Ketmen/10.00	21	62	154	84
Karatau/11.00	24	83	255	91
Talasky/12.00	13	62	153	90
Total	291	68	3297	93

[a]As depicted in Fig. 1.1.

addition, chilling requirement was monitored for this subset (Table 1.15) with variable levels from 83 to 127 days. Longer chilling requirement in seeds may correlate with late bloom in those seedlings when they eventually begin to flower (Mehlenbacher and Voordeckers 1991). Areas 6 and 9 (Fig. 1.1) had the longest chilling requirements. Those seedlings are now being characterized for flowering times to determine if this correlation proves to be true. Over 1600 of these seedlings are being maintained at PGRU for horticultural characterization. When traits of interest are found in some of these seedlings, they too, will become potentially useful clones and propagated on rootstocks, established in orchards and backed up in cryogenic storage. In addition, when the 44 clonal accessions of *M. sieversii* are released from quarantine, they will also be maintained in orchards and backed up in cryogenic storage.

The size of the permanent field collection is being reduced utilizing cryogenic storage. Since all accessions in the permanent field collection will be backed up at NSSL, it is possible to consider reducing each accession to only one propagule in costly field plantings. Cryogenic storage is very cost-efficient. We have calculated that after an initial cost

Table 1.15. Chilling requirement for *M. sieversii* seed germination collected in Kazakhstan.

Mt. Range/ Area Site[a]	No. Accessions Germinated	Range of Chilling Requirement (days)	Accessions with More than 110 Days Chilling (%)	Ave. No. Days Chilling Needed
Zailisky/ 3.00–3.04	22	83–106	0	96
Djungarsky/ 4.00–4.02	43	88–116	2	101
Djungarsky/ 5.00–5.01	26	85–108	0	98
Karatau/ 6.00–6.01	44	106–127	52	114
Tarbagatai/ 9.00–9.05	98	83–127	22	108
Ketmen/10.00	21	80–108	0	94
Karatau/11.00	24	88–114	4	101
Talasky/12.00	13	83–106	0	97
Total	291	83–127	16	104

[a]As depicted in Fig. 1.1.

of $50 to process an accession and test for baseline recovery, the cost of LN for long-term storage is only one U.S. dollar per year, per accession, whereas the estimate to establish and maintain each accession in the field is $50 to $75 per year.

To facilitate protocols for cryogenic storage, buds can be collected from January to March at PGRU, and can be stored at –4°C for up to six months before processing and placing in LN storage (Forsline et al. 1996b). This greatly facilitates the implementation of the protocol. Cryogenic storage has given us a convenient way to ensure against losses from abiotic and biotic stresses. Fire blight (pathogen: *Erwinia amylovora* Burrill) has been our greatest challenge. We have had some severe epidemics in the PGRU orchards and have maintained records on levels of susceptibility for each accession. All accessions with high levels of susceptibility are stored in LN. In addition, we are repropagating our orchards on EMLA 7 rootstock, which is tolerant of fire blight and imparts less vigor than on seedling rootstock, which tends to exacerbate the problem of fire blight.

Seed accessions from collections of wild *Malus* are maintained in moisture-proof envelopes and stored at –20°C according to IBPGR guidelines (IBPGR 1985). Portions of original seed are stored at NSSL and seeds are distributed to collaborative evaluators from the active collection at PGRU.

V. DISTRIBUTION

Distributions include scions (dormant and summer bud wood), pollen, seeds, leaves for biochemical/molecular analysis, and recently, DNA samples from the core collection (Forsline 1996). The 206-member core collection has been distributed many times for characterization and evaluation. Distributions, begun in 1989, are tracked through GRIN (USDA 2000). The PGRU has distributed over 1300 seed populations (30,675 seeds, Table 1.6) of *M. sieversii* to 24 cooperators (Table 1.16) worldwide for evaluation and is summarized in Table 1.7. In addition, over 2000 seedlings that were resistant to apple scab in screening at Geneva, New York, were sent to five cooperators. Cooperators successfully germinated some 20,000 of the 30,765 distributed seeds. The stratification times reported for germination was highly variable, ranging from as few as 38 to over 200 days. Many cooperators noted that *M. sieversii* seeds required a longer stratification period than typically expected for domesticated apple. Following initial mortality and screening for disease resistance or vegetative traits, over 15,000 seedlings of *M. sieversii*

Table 1.16. Researchers evaluating *M. sieversii* collected in Central Asia.

Organization and Location	Name	Expertise of Evaluators	Traits of Special Interest
University of Alaska Fairbanks, USA	Jennifer McBeath	Horticulture	Cold hardiness
University of Arkansas Fayetteville, USA	Curt Rom	Breeding, pomology	High chilling requirement, fruit traits
Colorado State University Fort Collins, USA	Cecil Stushnoff	Plant breeding, physiology	Cold hardiness
University of Illinois Urbana, USA	Schuyler Korban	Plant breeding	Project terminated
Private Grower, Eaton Rapids, Michigan, USA	Joe Hecksel	Pomology	Horticultural traits
University of Minnesota, St. Paul, USA	David Bedford, James Luby	Plant breeding, pomology	Resistance to apple scab and fire blight, cold hardiness, fruit traits
Heartland Germplasm York, Nebraska, USA	John Kreutziger[a]	Plant breeding	Disease and pest resistance, late flowering and leafing dates, fruit traits
Rutgers Fruit Research and Ext. Center, New Brunswick, New Jersey, USA	Joseph Goffreda	Plant breeding	Resistance to apple scab, fire blight, and cedar apple rust, fruit traits and storage ability
USDA, ARS, Plant Genetic Resources Unit, Geneva, New York, USA	Philip Forsline, Stan Hokanson, Warren Lamboy, Jing Yu	Pomology, physiology, plant pathology, molecular genetics	Resistance to apple scab, fire blight, and cedar apple rust, fruit traits, molecular genetic studies of genetic diversity
Cornell University, Dept. of Plant Pathology, Geneva, New York, USA	Herb Aldwinckle, Ki-Sung Ko, Sang-Bum Lee, Tim Momol	Plant pathology fire blight, cedar apple rust,	Resistance to apple scab, *Rosellinia necatrix* and *Helicobasidium mompa*

(continued)

Table 1.16. *(continued)*

Organization and Location	Name	Expertise of Evaluators	Traits of Special Interest
Cornell University, Dept. of Hort Science, Geneva, New York, USA	Susan Brown, Norman Weeden	Plant breeding, molecular genetics	Dwarf growth habit
Cornell University, Dept. of Fruit & Vegetable Science, Ithaca, New York, USA	Ian Merwin	Pomology	Resistance to soil borne apple replant pathogens
Cornell University, Dept. of Entomology, Geneva, New York, USA	Harvey Reissig	Entomology	Resistance to apple maggot
Ohio Agricultural Research and Development Center, Wooster, USA	David Ferree, Diane Miller	Pomology	Resistance to apple scab, cedar apple rust, and fire blight, late flowering, fruit traits
The Dawes Arboretum Newark, Ohio, USA	Donald Hendricks	Botany	Resistance to apple scab, cedar apple rust, and fire blight, late flowering, fruit traits
Private Grower, Midwest Apple Improvement Association, Johnstown, Ohio, USA	Mitch Lynd	Pomology, botany	Resistance to apple scab, cedar apple rust, and fire blight, late flowering, fruit traits
Washington State University, Wenatchee, USA	Bruce Barritt	Breeding, pomology	Resistance to pests, fire blight and powdery mildew, juvenility, cold hardiness, sunburn tolerance, fruit traits
Private Grower, Madison, Wisconsin, USA	Kevin Bradley	Pomology	Horticultural traits
University of Wisconsin River Falls, USA	Brian Smith	Breeding, pomology	Disease resistance, yield, manageable tree stature and habit, winter hardiness, late bloom, short juvenility, early fruit maturity, fruit traits

Agriculture Canada Summerland, British Columbia, Canada	Cheryl Hampson, Harvey Quamme	Plant breeding, cold hardiness physiology	Disease resistance, cold hardiness, drought tolerance
Agriculture and Agri-Food Canada, Morden, Manitoba, Canada	Campbell Davidson	Plant breeding	Resistance to fire blight, cold hardiness, fruit traits
Kings Landing Historical Corporation, Fredericton, New Brunswick, Canada	Daryl Hunter	Pomology	Horticultural traits
Agriculture and Agri-Food Canada, Kentville, Nova Scotia, Canada	Christianne Deslauriers, Charles Embree	Plant breeding, physiology	Disease resistance, cold hardiness, growth habit, fruit traits
Genebank for Fruit, Dresden, Germany	Rolf Büttner, Martin Geibel	Pomology, plant breeding	Resistance to apple scab and powdery mildew (including molecular markers), plant stature, length of vegetative period, fire blight, fruit characters, genetic diversity for molecular and morphological traits
Ministry of Agriculture, Forestry & Fisheries, Yamanishi, Japan	Hideo Bessho, Junichi Soejima	Plant breeding	Horticultural traits
HortResearch Havelock North, New Zealand	Vincent Bus, Nadozie Oraguzie	Plant breeding, genetics, plant pathology, entomology, biometrics	Resistance to apple scab, powdery mildew, fire blight, woolly apple aphid, leafroller, apple leaf curling midge, leafing and flowering dates, juvenility, burr knots, stooling ability, tree habit and vigor, fruit traits
The Norwegian Crop Research Institute, Hermansverk, Norway	Dag Roen	Plant breeding	Resistance to apple scab and powdery mildew, fruit traits and storage ability, fruit rots and disorders

(continued)

Table 1.16. *(continued)*

Organization and Location	Name	Expertise of Evaluators	Traits of Special Interest
MULTIFRUIT EPPINDUST, South Africa	Gary Britz		
University of Reading, Reading, UK	Rita Farrel	Botany, molecular genetics	Genetic diversity
University of Oxford, Oxford, UK	Barrie Juniper	Botany, molecular genetics	Origin of domesticated apple
INFRUITEC, Stellenbosch, South Africa	Taiiboos Human	Plant breeding, pomology	Resistance to woolly apple aphid, Phytophthora, apple scab, powdery mildew and drought, chilling requirement, sunburn tolerance, fruit traits

[a]Deceased June 1999

remain in field plantings at PGRU and at sites of cooperators for further characterization.

The 44 clonal elite accessions remained in quarantine as of the end of 2001; however, permission for provisional release of 39 of the 44 accessions to the PGRU and Washington State University has been granted. They have been screened for apple scab by Cornell University researchers in the greenhouse at the PGRU and were planted on dwarfing rootstocks in the field in 1999 and 2000 according to guidelines of the provisional release. After three years in a field planting at PGRU, we have observed fruiting of 13 of these elites (Plate 1D) and have begun to characterize them for comparison with the characterization that was made when they were collected in Kazakhstan.

VI. CHARACTERIZATION AND EVALUATION

A. Core Collection and Main Collection

A core collection of apple has been established for the purpose of efficient characterization and evaluation. Brown (1989) first discussed the concept of core collections. The value for germplasm collections in the NPGS was further discussed (Kresovich et al. 1995) and developed for fruit crops including apple (Grauke et al. 1995). The core collection for apple includes 206 diverse accessions out of the total collection of 2438 clonal accessions. The initial core collection was designated in 1991 (Forsline 1996). It consists of: (1) 78 *M.* ×*domestica cultivars* with diverse pedigrees, a range of known traits, some with known disease resistance, and old and new types; (2) 27 PRI selections from the Purdue-Rutgers-Illinois cooperative breeding program (Crosby et al. 1992) with multiple disease/insect resistance; (3) 10 *Malus* species accessions with disease resistance traits; (4) 91 *Malus* species accessions with two to five representatives of each of 30 species (Way et al. 1990) having diverse geographic representation. This list can be found through GRIN (USDA 2000) on the Web at: http://www.ars-grin.gov/cgi-bin/npgs/html/desc_find.pl?crop=APPLE&115154=Y. Each accession in the core collection is replicated four times and planted in five climatically diverse sites in New York, North Carolina, Washington, Illinois, and Minnesota. The core collection is a dynamic entity whereby certain accessions may be removed or others added as we learn more about the genetic character of the entire collection including newly acquired accessions. The core collection has been completely characterized for 25 priority morphological descriptors as listed on the Web at: http://www.ars-grin.gov/

cgi-bin/npgs/html/crop.pl?115. In addition, we have made images which can be accessed on the Web at: http://www.ars-grin.gov/ars/NoAtlantic/Geneva/malus_proj.html for most of the core collection. The other members of the clonal collection (2208 accessions) have now been characterized using the same priority descriptors.

To better understand and utilize plant genetic resources, biochemical characterization (Weeden and Lamb 1985) has been used. More recently Kresovich et al. (1993), using molecular techniques, found efficient and informative DNA markers for germplasm characterization. Simple sequence repeats (SSRs) have a high level of polymorphism and a high-interspersion rate making them an abundant and effective genetic marker for maintenance of genebank diversity. Because of the potential of SSRs for automated analysis and their codominant nature, they are more useful than other PCR-based marker systems. SSRs have been identified in the entire *Malus* core subset (Hokanson et al. 1998, 2001). Automated fluorescence-based detection of polyacrylamide gels to improve analytical resolution, increase throughput and decrease unit cost, has been employed. In combination with passport and horticultural data, we intend to apply genetic information revealed by SSR primer pairs to better comprehend genetic identity and relatedness in the *Malus* germplasm collection, to help develop and refine the *Malus* core subset (Hokanson et al. 1997a, 1998, 2001; Szewc-McFadden et al. 1995 and 1996), and to advance our understanding of the extent and maintenance of genetic diversity of random *Malus* populations from Central Asia (Lamboy et al. 1996).

The collection is evaluated through collaborative research with cooperators from around the world. By using the core collection as a test array, Howell et al. (1996) found superior virus indicators for the apple stem grooving virus: (1) *M.* ×*micromalus*, Mak. (GMAL 273a, PI 594092); (2) *M. yunnanensis* (French.) Schneid. (GMAL 2342, PI 589758); and (3) *M. tschonoskii* (Maxim.) Schneid. (GMAL 1834, PI 589395).

The core collection has also been evaluated for: (1) disease resistance including: fire blight (*Erwinia amylovora* Burrill), apple scab (*Venturia inaequalis* (Cooke) Wint.), cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein.), sooty blotch (*Gloeodes pomigena* Schwein.), flyspeck (*Zygophiala jamaicensis* Mason), bitter rot (*Glomerella cingularia* Stonem.), black rot (*Botryosphaeria obtusa* Schwein.), and white rot (*Botryosphaeria dothidea*, Moug.); and (2) arthropod resistance including: European red mite (*Panonychus ulmi*, Koch), apple maggot (*Rhagoletis pomonella*, Walsh), and woolly apple aphid (*Eriosoma lanigerum* Hausm).

B. Central Asian Collections

Over 50 scientists (Luby et al. 2001) and technicians with diverse expertise have been evaluating *M. sieversii* accessions at locations in several countries (Table 1.16). Because most trees held by cooperators are not yet fruiting, evaluation has focused on disease resistance or vegetative traits. Currently HortResearch in New Zealand and the PGRU are the only sites with large numbers of fruiting trees. Most evaluators intend to evaluate for standard fruit traits such as size, color, texture, aroma, and flavor and vegetative traits such as growth habit and vigor. At PGRU, we have characterized many of the earliest fruiting seedlings (Plate 2D). Most evaluators also are targeting disease and pest resistances and other traits related to adaptation in their regions. These plans are summarized in Table 1.16. Progress for some traits is discussed in the following section.

1. Disease and Pest Resistance

Apple scab. Several cooperators are screening young seedlings for apple scab resistance. In New Jersey, nearly 2000 seedlings were screened for apple scab, and J. Goffreda (pers. comm. 1999) reported that a high proportion exhibited resistance similar to that conferred by the V_r gene.

In New York, nearly 5000 seedlings representing 12 regions (Fig. 1.1 and Table 1.17) have been evaluated for apple scab resistance with a total of 27 percent of those screened being resistant (Aldwinckle et al. 1997). Combining data from screenings of material collected in all the expeditions revealed major variation in the incidence of apple scab resistance related to region of origin (Table 1.17). This trend was also observed previously with a smaller subset. Area 4 had the highest proportion (49%) of the resistant seedlings while area 1 had the lowest proportion (5%).

In New Zealand, over 2500 seedlings from 60 seed lots from the 1995 and 1996 expeditions were evaluated in the greenhouse (Bus et al. 2002). Approximately 24 percent of the seedlings were resistant in these evaluations with a range among half-sib families from 0 to 70 percent. Areas 3, 10, and 11 had less than 10 percent resistant seedlings while areas 4, 5, 6, and 9 had greater than 25 percent resistant seedlings (Table 1.17). In addition, Vincent Bus (pers. comm.) also evaluated over 1400 trees from 52 seed lots from the 1993 expedition, following natural apple scab infection in the field. These trees form part of a large apple breeding population aimed at increasing genetic diversity for many traits (Noiton and Shelbourne 1992). Using estimated variance components, they found a low heritability (0.13 on family mean basis), due mainly to high levels of resistance and little variation among these families. They did

Table 1.17. Apple scab resistance of *Malus sieversii* seedlings from 12 regions in Central Asia screened in greenhouses in New York (NY, 1993, 1995, and 1996 collections), New Zealand (NZ, 1995 and 1996 collections), and Minnesota (MN, 1995 collection).

	No. of seedlings screened			Percent of seedlings resistant		
Area[a]	NY	NZ	MN	NY	NZ	MN
1	21	–	–	5	–	–
2	101	–	–	24	–	–
3	450	262	–	17	8	–
4	369	287	–	49	28	–
5	1175	277	–	27	45	–
6	705	133	362	37	25	74
7	383	–	–	25	–	–
8[b]	151	–	–	07	–	–
9	1125	684	1171	27	29	62
10	123	86	–	06	2	–
11	226	244	–	23	5	–
12	133	–	–	14	–	–
Total	4971	1973	1533	27	24	65

[a]Areas described in Figure 1.1 and Table 1.2.

[b]Open pollinated seedlings of Professor Dzhangaliev's forms at Almaty (Kazakhstan) Botanical Garden.

not detect any significant differences for scab incidence among the collection regions.

In Minnesota, seedlings from areas 6 and 9 were screened and 65 percent were resistant (Table 1.17). The results from the two areas were similar, but individual families ranged from 0 to 88 percent resistant seedlings.

Several seed lots have been screened at multiple sites (Table 1.18). Although some families consistently produced high proportions of resistant seedlings (e.g., GMAL 3607, 3631, 4024, 4089, and 4177) or high proportions of susceptible seedlings (GMAL 3609, 3643, 4011, 4068, 4071, 4086, 4171, 4309, and 4315) over multiple sites, others were quite variable. Inconsistencies in the seedling screenings may be due to different local inoculum sources, or to sampling effects from small sample sizes for some accessions at some locations. Alternatively, they may be due to a variation in age or physiological state of seedlings or to different test conditions that could influence infection success.

Of the 39 elite clonal accessions granted provisional release from quarantine, 30 have been evaluated for apple scab resistance in the

Table 1.18. Apple scab resistance of *Malus sieversii* seedlings in Central Asia from seed lots evaluated in greenhouses in New York (NY), New Zealand (NZ), and Minnesota (MN) after inoculation with local strains.

GMAL/PI Accession No.[a]	Seedlings screened			Seedlings resistant (%)		
	NY	NZ	MN	NY	NZ	MN
3604/600493	8	42	23	12	26	78
3605/600494	11	20	44	45	40	55
3607/600496	15	38	24	60	68	92
3608/600497	14	23	13	50	26	0
3609/600498	14	35	–	7	9	–
3618/600507	8	40	20	38	8	60
3625/600514	15	38	36	73	8	53
3627/600516	13	42	9	54	55	56
3631/600520	14	45	38	71	62	58
3634/600523	14	39	34	29	49	85
3636/600525	14	37	19	71	24	90
3643/600532	15	48	40	13	0	57
3683/600571	15	38	27	60	45	78
3688/600574	13	32	–	62	19	–
3691/600577	15	36	–	27	25	–
4011/600586	24	43	–	0	2	–
4024/600598	26	49	–	62	59	–
4032/600606	24	35	–	33	46	–
4038/600609	24	40	–	38	48	–
4068/—	7	19	–	14	0	–
4071/—	5	24	–	0	4	–
4086/—	7	22	–	0	0	–
4089/—	5	15	–	80	53	–
4171/—	6	20	–	0	5	–
4177/—	5	17	–	100	65	–
4190/—	7	23	–	29	70	–
4209/—	6	19	–	67	32	–
4302/—	5	13	–	0	23	–
4309/—	7	24	–	0	0	–
4315/—	7	17	–	0	0	–

[a]GMAL 4068–4315 are from the random collections with small seedlot quantities. No PI numbers have been assigned to those.

greenhouse using replicate, grafted plants. Four of these accessions show apple scab resistance on all ten replicate trees. Five others were resistant on some trees. In addition, Mehlenbacher and Weeden (pers. comm.) extracted DNA from five elite accessions that had 40 percent or more of their offspring resistant to apple scab and screened for the presence of markers that had been linked to apple scab resistance genes in other *Malus* accessions. All five *M. sieversii* had RAPD markers P415B and UBC562 markers for the V_r resistance gene. However, since these markers were also present in many other accessions, including susceptible ones, they may be of little value. Four of these five accessions also contained one or more markers that were quite rare in germplasm that did not exhibit apple scab resistance. GMAL 4326 (Q35771), GMAL 4331 (Q35777), GMAL 4334 (Q35780) had OPB12 for V_m. GMAL 4327 (Q35772) had OPB12 for V_m, the CS5 RAPD for V_f, and the UBC220 marker for V_b. Since the elite selections were initially identified when collected in Kazakhstan based on their superior fruit traits, these accessions represent potentially new sources of multiple resistance genes in a horticulturally desirable background.

Fire Blight. Extensive evaluations for fire blight (*Erwinia amylovora*) resistance have been conducted in New York and New Zealand. In a cooperative program of Cornell University and PGRU (Momol et al. 1999), 1125 seedlings from the 1989 and 1993 expeditions were inoculated with fire blight, and 29 percent were resistant. The status of the accessions from the 1989 collection is listed in Table 1.19. Work is in progress to screen subsequent collections. One fire blight resistant seedling, GMAL 3280.h (a single seedling designated as '.h' to distinguish from other half-sibs in the population), had desirable fruit quality and large fruit size (56 mm diameter). These results are significant because we have identified a resistant genotype with desirable horticultural traits.

Natural occurrence of fire blight has been monitored in the seedling grow-out at PGRU. After 3 to 4 years in the field, 25 percent of the seedlings are showing significant infection (Table 1.20). This method does not allow us to define resistance but it does indicate levels of susceptibility. There is a pattern that shows seedlings from some sites in Kazakhstan are much more susceptible than from other sites.

Table 1.19. Fire blight resistance of *M. sieversii* seedlings with artificial inoculation.

			Resistant seedlings	
Country/Area Site	No. Accessions	No. Inoculated	No.	Percent
Tajikistan/1.00	3	24	19	79
Uzbekistan/2.00–2.02	9	69	11	16
Kazakhstan/ 3.04[a]	34	266	80	30
Total	46	359	110	30

[a]Zailisky mountain range.

Table 1.20. Natural occurrence of fire blight in Kazakhstan seedlings at PGRU after three to four years in the orchard.

		Seedlings infected	
Mt. Range/Area Site[a]	No. Seedlings	No.	Percent
Zailisky/3.00–3.04	24	0	0
Djungarsky/4.00–4.02	100	7	7
Djungarsky/5.00–5.01	169	27	16
Karatau/6.00–6.01	195	36	19
Kyrgyzstan/7.00	5	2	40
Tarbagatai/9.00–9.05	372	162	44
Ketmen/10.00	54	4	8
Karatau/11.00	131	27	21
Talasky/12.00	101	18	18
Total	1151	283	25

[a]See Fig. 1.1.

In New Zealand, 936 trees from the 1993 expedition were evaluated in their fifth leaf for fire blight incidence following natural infection in the orchard. Approximately 87 percent of the trees remained disease-free in this field evaluation while Momol et al. (1999) found that 33 percent of 775 seedlings from the same 1993 collection expedition were resistant when inoculated as young seedlings. Seedlings from areas 3 and 6 were more susceptible than those were from areas 4, 5, and 7; whereas, no differences were observed in New Zealand following natural infection. Two of the most susceptible families (PI 600479 and PI 600480) were from a single site in Kyrgyzstan (area 7). Of the six most resistant families, three (PI 600428, PI 600429, and PI 600444) were from region 4 in east central Kazakhstan, and two (PI 600468 and PI 600476) were from region 6 in south central Kazakhstan. Inoculations of young seedlings by Momol et al. (1999) showed that PI 600468 was among the more resistant families. However, in contrast to the New Zealand field results, PI 600480 was among the more resistant families, but PI 600429 and PI 600444 were among the more susceptible ones. These apparent contradictions may result from a difference in natural versus artificial inoculation techniques, physiological differences in the trees due to age, genetic differences in the seed lots due to sampling, or different pathogen races.

Apple Replant Pathogens. In New York, Isutsa and Merwin (2000) evaluated some *M. sieversii* accessions, as well as other *Malus* species, for their resistance or tolerance to apple replant pathogens (ARP) based on their relative biomass accumulation when grown in orchard soils. The ARP included various species of *Pythium*, *Cylindrocarpon*, *Fusarium*, *Rhizoctonia*, and *Phytophthora*, as well as nematodes. Seedlings from two families (PI 600427 and PI 600563) were categorized as tolerant of ARP.

Powdery Mildew. In Germany and New Zealand, all seedlings are being evaluated for resistance to powdery mildew (*Podosphaera leucotricha* (Ell. & Ev.) E. S. Salmon). In Germany, the juvenile susceptibility to powdery mildew decreased significantly with the age of the plants (Geibel et al. 2000). Nearly all plants were susceptible when evaluated in the first season of growth, but mildew infections in 1998 were only 70 percent in the 2-year-old plants and 45 percent in the 3-year-old plants over all populations. Depending on the accession, 10 to 60 percent of the plants in a family were resistant.

Other Diseases. Seedling evaluations for cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein.) resistance are being conducted in New York and New Jersey. In New York, over 2200 seedlings were screened with approximately 50 percent of those resistant (Table 1.21). Resistance among the Kazakh sites ranged from 34 to 78 percent.

In New York, Lee, Ko, and Aldwinckle (2000) screened seedlings for resistance to western white root rot (*Rosellinia necatrix* Prill.) and violet root rot (*Helicobasidium mompa* Tanaka) and identified some populations with apparent resistance.

Table 1.21. Cedar apple rust resistance of *M. sieversii*.

		Resistant seedlings	
Mt. Range/Area Site[a]	No. Inoculated	No.	Percent
Zailisky/3.00–3.04	97	40	41
Djungarsky/4.00–4.02	194	107	55
Djungarsky/5.00–5.01	610	245	40
Karatau/6.00–6.01	207	115	56
Zailisky/8.00	90	31	34
Kyrgyzstan/7.00	164	78	48
Tarbagatai/9.00–9.05	520	404	78
Ketmen/10.00	66	25	38
Karatau/11.00	162	64	40
Talasky/12.00	104	36	35
Total	2214	1145	52

[a]See Fig. 1.1.

Insect Resistance. In New York, Reissig (pers. comm. 1999) conducted laboratory studies to compare the oviposition preference and survival of the apple maggot (*Rhagoletis pomonella* Walsh) in fruit from seedlings of *M. sieversii*. During the two years of the study, fruit from 43 different seedlings were evaluated from material collected in 1989. Oviposition preference was compared by picking the fruit in July and exposing the selections, along with fruit from 'McIntosh', to groups of apple maggot females from a laboratory colony in clear Plexiglas cages and counting subsequent oviposition punctures and eggs in the fruit. Apple maggot females oviposited in fruit from all of the M. sieversii seedlings tested, but fruit was generally less infested than the 'McIntosh' fruit by 3 to 94 percent.

Apple maggot females also infested fruit from all seedlings in a "no-choice" test. After infestation, fruit was incubated on racks over water-filled dishes and exiting larvae were collected to compare survival rates. Larvae survived in fruit from all of the selections tested, but numbers of larvae surviving in the *M. sieversii* fruit were generally lower than in 'McIntosh' standards. Although many of the *M. sieversii* selections were less favorable for apple maggot oviposition and survival than 'McIntosh', most of the fruit was somewhat smaller (avg. 2.1 to 3.3 cm in diameter) compared to the 'McIntosh' fruit (average 4.3 cm in diameter). Additional studies are needed to determine if the observed differences between these selections and the standard fruit are due to physical characteristics or chemical factors.

In New Zealand, seedlings from the 1993 collection were evaluated for resistance to woolly apple aphid (*Eriosoma lanigerum* Hausmann) in order to estimate heritabilities and combining abilities and identify resistant genotypes. In a preliminary study on trees grown from root cuttings from a subset of the 1993 seedlings, resistance to light brown apple moth (*Epiphyas postvittana* Walker) and apple leaf curling midge (*Dasyneura mali* Kieff.) were investigated. The findings were inconclusive, perhaps because the screening technique was based on leaf damage rather than development or survival rates (Wearing and Colhoun, 1999). However, the indication of a strong genetic component in the variation for incidence of apple leaf curling midge warrants further research.

Multiple Resistance. Several cooperators are screening seedlings for resistance to multiple diseases and pests. Some genotypes with resistance to multiple diseases have already been identified. In New York, for example, 775 seedlings from 33 seed lots collected in 1993 from regions 3, 4, 5, 6, 7, and 8 were inoculated with apple scab, cedar apple rust, and fire blight. Of these seedlings, 23 percent were resistant to apple scab (Aldwinckle et al. 1997), 38 percent were resistant to cedar apple rust and 33 percent were resistant to fire blight (Momol et al. 1997). As a result of these multiple disease resistance screens, 207 seedlings with putative multiple resistance were selected from the original 775 for further horticultural evaluation. We suspect that this group may be late blooming or have a mechanism to survive spring frosts since this 1993 collection was made after a severe spring frost in the wild apple forests of Central Asia that resulted in only 2 percent of the wild trees producing fruit. The majority of these selected seedlings were sent to the research group in Ohio (Table 1.16) where they are being observed at the Dawes Arboretum. Ohio was chosen since this is an area that is often challenged by early spring frosts.

Researchers in Germany noted that every population evaluated included some plants that were resistant to apple scab and powdery mildew. After two years of evaluation a total of 64 plants from nearly 1100 seedlings had neither scab nor powdery mildew symptoms (Geibel et al. 2000).

2. Environmental Stress Tolerance

Cold Hardiness. Several cooperators are interested in cold hardiness and will be evaluating material in the field at high latitude sites. Most material is too young to be evaluated, but all seedlings planted at Fairbanks, Alaska, were killed in their first winter.

In Colorado, Stushnoff (pers. comm. 1999) has screened young seedlings using the following acclimation protocol followed by a laboratory-freezing test (Stushnoff et al. 1983). When seedlings were 3 to 5 cm tall they were exposed to short days (12 h) at 10°C for one week, followed by 2 weeks at 4°C, and an overnight frost of –5°C, and then returned to 10°C day/4°C night with 12 h daylength for one week. The entire flat was then frozen with the temperature decreased at a rate of 2°C/h to –30°C and held for 1 h. The seedlings were then grown in a greenhouse and evaluated for injury by comparing them to 'Kerr' open pollinated seedlings. All seedlings with 75 percent or greater die back were considered not hardy. Of 720 seedlings evaluated, 86 were considered hardy in this test and have been retained for field evaluation.

Chilling Requirement and Late Bloom. In South Africa, Human (pers. comm. 1999) found that genotypes with shorter chilling requirements are sought for production areas with warm winters in contrast with the need in the central United States for material with a long chilling requirement to provide later bloom to avoid spring frosts. Researchers at these sites have been particularly interested in seedlings germinating after long periods in stratification, as this trait has been correlated with long chilling requirement and late bloom (Mehlenbacher and Voordeckers 1991). The chilling required for seed germination varied considerably as reported by the cooperators, ranging from 38 days in Germany to over 200 days in Nova Scotia.

Material collected in 1993 may be especially appropriate for this objective since only about 2 percent of the trees in Kazakhstan bore fruit that year by escaping late spring frost. A total of 173 seedlings with multiple disease resistance screened at Cornell University (Aldwinckle et al. 1997; Momol et al. 1997) are under evaluation at the Dawes Arboretum in Newark, Ohio, under the auspices of the Midwest Apple Improvement Association (MAIA). An additional 787 seedlings from the 1995 and 1996 collections with long seed chilling requirement (104 to 127 days, Table 1.15) were sent to the Dawes Arboretum to evaluate late blooming. These seedlings were sent to Dawes after being screened for apple scab and cedar apple rust (Aldwinckle et al. 1997).

Drought Tolerance and Sunburn Resistance. In warm, arid production regions such as Washington, British Columbia, and South Africa, cooperators indicated an interest in screening for drought tolerance. *Malus sieversii* is recognized as a drought tolerant rootstock in Kazakhstan and China. Regions 6, 11, and 12, in particular, have a hot, arid climate with high solar radiation (Table 1.5). Sunburn was surprisingly rare on fruit collected in these areas.

3. Plant Stature. Several cooperators indicated they were interested in obtaining manageable growth habits. Susan Brown in Geneva, New York, is interested specifically in obtaining genetic dwarfs and is evaluating seedlings from three accessions that Kazakh scientists described as genetic dwarfs. In New Zealand, the seedlings in the apple genetics population are rated for tree habit using the IBPGR descriptor and girth is assessed as a measure of vigor.

4. Molecular Genetic Diversity. Studies of molecular genetic diversity in the Central Asian apples at simple sequence repeat sites are continuing at the PGRU as a continuation of previous studies (Lamboy et al. 1996; Hokanson et al. 1998). In an initial study, Lamboy et al. (1996) reported that most allelic variation was among families within collection regions rather than among regions. The main objectives of the ongoing studies are to characterize (1) relative levels of diversity among and within populations, (2) variation between collecting years (1995 and 1996) at the same sites, and (3) diversity among maternal genotypes compared with the open-pollinated seedling populations derived from them using leaves and seeds collected in 1996. Several other cooperators plan studies of molecular genetic diversity (Table 1.16). The Oxford University, UK group is investigating the origin and migrations of the apple, including *M. sieversii* and its ancestors, in a comprehensive program using molecular markers as well as geological, historical, and anthropological approaches (Juniper et al. 1999, Robinson et al. 2001).

VII. UTILIZATION

The cooperators represent groups interested in cultivar development and genetic diversity. In addition to genebank programs in the United States, Germany, and New Zealand, there are several breeding programs at universities, government agencies, government-held corporations, and consortia of apple growers (Table 1.16). Since most seedlings held by cooperators were not yet fruiting in 2001, evaluation is ongoing and they have not yet been used in breeding. Many indicated their intentions for utilization, however. Nearly all cooperators viewed the *M. sieversii* germplasm as a means to broaden the genetic diversity in their breeding programs. Many cooperators indicated intentions to use *M. sieversii* selections in further breeding for rootstocks or scion cultivars. Most cooperators sought: (1) novel fruit quality characters such as color, texture, aroma, and flavor; (2) new sources for disease or pest resistance; and (3) stress tolerance for adaptation to their production regions. In addition, several were seeking easily managed growth habits for scion or rootstock cultivars.

The success of utilization of the *M. sieversii* germplasm in cooperators' breeding programs will not be known for many years. The ongoing evaluations will ensure that it is tested for critical traits in a large range of apple production regions. We anticipate that this germplasm will ultimately offer useful genetic diversity for several reasons. First, the ecological amplitude of the species in its native habitats is truly impressive. Samples were collected from diverse ecosystems ranging from lush, humid, temperate forests to sparse dry, cold northern forests to xeric, near-desert habitats (Table 1.5). Potential ecotypes from these regions should offer environmental adaptation as rootstock or scion for most apple production regions, except for subtropical areas. Second, *M. sieversii* in its native habitat has coevolved with several organisms that are pathogenic in orchards. Apple scab and codling moth (*Cydia pomonella* L.) were especially noted in collection sites. Other organisms such as ubiquitous apple replant pathogens are likely present in the montane apple forests, and natural selection for resistance may have resulted as a consequence of forest regeneration. Finally, some *M. sieversii* genotypes will be readily useful because they are already similar in phenotype to commercial cultivars for some critical horticultural traits. The elite clonal accessions (Plate 1D) and similar seedlings that will be discovered during evaluation may contribute to new cultivars without extensive back crossing.

VIII. CONCLUSION

Recent collections of wild apple in Central Asia follow the footsteps of early botanical explorers who first documented the geographic extent of the fruit forests of this region. Recognized as an important center of evolution by Vavilov and others, explorations, collections, and study of wild fruit species in the forests of Central Asia continue to this day. Scientists and workers from Russian and Central Asian botanical gardens (formerly within the USSR botanical system) shared their knowledge and experience of wild fruit relatives and led western explorers into their floristically rich forests. In particular, Professor Djangeliev and his associates in Almaty, Kazakhstan, guided the USDA-led teams to populations of apple adapted to various ecosystems in Kazakhstan. These areas are described in detail in the following sections of this volume.

In former times, wild species of scientific interest or ornamental/horticultural value were maintained in orchards and gardens. With human population growth increasing stress on natural environments, the need to protect the genetic diversity of wild species from degradation has mounting significance. Central Asian apple genetic diversity is now being maintained within orchards of Central Asia, North America, South Africa, Europe, and New Zealand. In addition, technological advances have allowed preservation by freezing scions and DNA samples. These efforts help insure against future genetic erosion. Characterization of apple selections begun in the orchards and laboratories of Kazakhstan by Professor Djangeliev continue there and internationally.

The applications for fruit breeding that result from recent wild fruit explorations in Central Asia demonstrate the synergistic effects of cooperative international endeavors. Distinct contributions by multiple disciplines from various societies and political systems enhance scientific dialogue by bringing together different views concerning genetics, breeding, and conservation. Apple cultivar development will benefit by this work, through the direct introduction of new selections and by introducing disease and insect resistant genes through breeding and gene transfer. The outcome has been cumulative, becoming larger than any single person's vision.

Through international agreements and collaboration, we envision apple cultivar improvement, model systems for collecting and preserving the genetic diversity of apple, and a need to develop plans for in situ conservation of these precious genetic resources. Conservation of apple as an "umbrella species" will conserve associated species and provide local economies with many benefits.

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