Genetic Diversity and Disease Resistance of Wild *Malus orientalis* from Turkey and Southern Russia

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ABSTRACT. Genetic diversity and disease resistance are described for 496 seedlings from wild populations of *Malus orientalis* Uglitzh. collected in southern Russia and Turkey in 1998 and 1999. Eighty-five half-sib families were genotyped using seven microsatellite markers, and disease resistance was determined for apple scab (*Venturia inaequalis* Cooke), cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein), and fire blight (*Erwinia amylovora* Burrill). Individuals from the two Russian Caucasus collection locations were homogeneous compared with populations from the four Turkish collection locations. Within three of the Turkish collection locations, some half-sib families were highly diverse and several of these families had unusually high levels of disease resistance. In all, twenty individuals exhibited resistance to all three diseases. Bayesian analyses of the population structure revealed six distinct clusters. Most of the individuals segregated into two clusters, one containing individuals primarily from southern Russia and the other containing individuals from both Russia and northern Turkey. Individuals in the four small clusters were specific to Turkish collection locations. These data suggest wild populations of *M. orientalis* from regions around the Black Sea are genetically distinguishable and show high levels of diversity.

The domestication of *Malus ×domestica* Borkh. has not been fully documented. *Malus sieversii* (Ledeb.) M. Roem. from central Asia is thought to be a major species contributor, while *Malus orientalis* and *Malus sylvestris* (L.) Mill. are potential minor species contributors (Buttner, 2001; Watkins, 1995). Human domestication and incorporation of *M. orientalis* into other genetic backgrounds may have occurred during its westward movement from Armenia and the Transcaucasus into the area of the ancient Greek civilization (Buttner, 2001; Ercisli, 2004). Within Turkey, introgression may have also occurred along regions of the silk trade route which extended from China through habitats of *M. sieversii* in central Asia and across Turkey into Europe.

Fruit of wild *M. orientalis* trees growing in the mountainous regions of Turkey, Iran, and the Caucasus region of Russia are a source of local foods, beverages, and medicines (Buttner, 2001; Khoshbakht and Hammer, 2005). These wild trees were once widespread in forests, mixed scrubs, and in rocky slopes by streams and field edges (Browicz, 1972); but in some areas

these wild trees are being depleted by human encroachment (P. Forsline, personal communication). Across the *M. orientalis* natural habitat, trees exhibit a range of vegetative and fruit characters (Güleriyüz, 1988). Fruit are often small (2–3.5 cm), sour/sweet, astringent, and bitter (Khoshbakht and Hammer, 2005).

Evaluations of genetic diversity of wild *Malus* L. populations have provided useful assessments of germplasm diversity in the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS) National Plant Germplasm System (NPGS) collection (Hokanson et al., 1998, 2001; Lamboy et al., 1996; Volk et al., 2005). For example, *M. sieversii* populations in Kazakhstan were shown to have regional structure with most of the diversity represented within half-sib families. *Malus sieversii* wild apples are generally larger and sweeter than the apples of *M. orientalis* and Chinese species of wild apples. This may be a result of widespread movement and selection during the domestication of apples by humans over thousands of years.

Apple scab and fire blight are two diseases that are particularly damaging to apple crops. Some resistant cultivars are available to breeding programs, but additional sources of resistance are desirable. Phenotypic screening is the primary source of disease resistance data for *Malus* collections, but specific molecular markers for disease resistance genes are being developed (Bus et al., 2005; Cheng et al., 1998; Gianfranceschi et al., 1996; Hemmat et al., 1998). As transformation technologies become more established for apple breeding, incorporation of alleles from wild apple types would potentially bring new sources of resistance into highly desirable cultivars (Norelli et al., 1998). In addition, efforts to breed disease-resistant rootstocks using both traditional and

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transgenic approaches could use wild germplasm as sources of disease resistance (Borejsza-Wysocka et al., 1999; Norelli et al., 2003).

USDA-sponsored germplasm expeditions to southern Russia and Turkey were conducted to systematically collect *M. orientalis* from 32 sites. Elevation of the sites ranged from 100 to 1950 m. In total, seeds were collected from 85 maternal tree sources (families) to provide wild germplasm for inclusion in the NPGS. The genetic diversity of *M. orientalis* from Turkey and southern Russia has not been previously described. An understanding of disease resistance and genetic diversity in this germplasm collection may increase use of *M. orientalis* genetic resources in breeding programs.

Materials and Methods

PLANT MATERIALS. *Malus orientalis* plant materials were collected from five sites in the Caucasus, Russia, in 1998 and

from 27 sites in Turkey in 1999. Materials from these sites were classified to comprise six locations which were separated by at least 100 km (Table 1). Generally, the two Russian locations (RA, RB) were densely forested and the four Turkish locations (TA, TB, TC, TD) represented dry, rural regions that were sparsely populated with *M. orientalis* trees.

Seeds were brought through quarantine to the USDA-ARS Plant Genetic Resources Unit in Geneva, NY. Between 1 and 30 seeds from one to nine maternal trees were planted to represent selected seed lots from the collection sites. Leaf samples were collected from each *M. orientalis* tree and sent to the USDA-ARS National Center for Genetic Resources Preservation in Ft. Collins, CO, and kept at -80°C until DNA extractions were performed.

MOLECULAR ANALYSIS. Genomic DNA from leaf tissue from two replicate samples of 496 individual *M. orientalis* trees was extracted using DNeasy 96 plant kits (Qiagen, Valencia, CA). *Malus* SSR (SSR) were amplified using unlinked primers

Table 1. Description of *Malus orientalis* collection sites from Russia in 1998 and Turkey in 1999.^z

Location	Collection site no.	Latitude (degrees)	Longitude (degrees)	Elevation (m)	Country	State	Total no. of families	Total no. of individuals	Families with ≥ 5 half-siblings	
									No. of families	No. of individuals
RA	2	44.92	38.00	100	Russia	Caucasus	6	27	4	20
RB	3	44.43	40.15	400	Russia	Caucasus	8	35	3	18
RB	4	44.41	40.12	500	Russia	Caucasus	6	59	6	59
RB	5	44.45	40.21	300	Russia	Caucasus	6	36	5	34
RB	7	44.23	40.18	700	Russia	Caucasus	2	41	2	41
TA	2	41.29	41.51	750	Turkey	Artvin	2	13	1	9
TA	7	41.25	42.37	1,125	Turkey	Artvin	1	17	1	17
TA	8	41.22	42.38	1,470	Turkey	Artvin	2	14	1	10
TB	10	40.39	40.54	1,380	Turkey	Bayburt	2	7	0	0
TB	11	40.42	40.46	1,580	Turkey	Bayburt	4	11	1	5
TB	12	40.45	40.46	1,700	Turkey	Bayburt	1	6	1	6
TB	13	40.34	40.40	1,730	Turkey	Bayburt	6	36	5	32
TB	14	40.47	40.05	1,770	Turkey	Gumushane	1	3	0	0
TB	15	40.47	40.03	1,950	Turkey	Gumushane	4	11	1	5
TB	16	40.37	39.70	1,550	Turkey	Gumushane	1	12	1	12
TB	17	40.22	39.28	1,610	Turkey	Gumushane	1	3	0	0
TB	18	40.21	39.17	1,470	Turkey	Gumushane	1	7	1	7
TB	19	40.21	39.16	1,450	Turkey	Gumushane	1	6	1	6
TC	1	41.06	35.98	590	Turkey	Samsun	1	5	1	5
TC	20	40.55	36.63	1,030	Turkey	Tokat	1	16	1	16
TC	23	40.24	36.42	1,320	Turkey	Tokat	2	11	1	11
TC	24	40.26	36.41	1,320	Turkey	Tokat	1	1	0	0
TC	25	40.93	35.39	1,320	Turkey	Amasya	9	30	2	11
TC	26	40.92	35.36	1,300	Turkey	Amasya	3	30	3	30
TD	27	41.66	33.59	1,120	Turkey	Kastamonu	1	12	1	12
TD	28	41.66	33.35	1,180	Turkey	Kastamonu	1	8	1	8
TD	29	41.71	33.45	1,080	Turkey	Kastamonu	1	1	0	0
TD	30	41.70	33.53	1,140	Turkey	Kastamonu	1	2	0	0
TD	32	41.65	33.12	750	Turkey	Kastamonu	5	12	0	0
TD	33	41.75	33.69	1,080	Turkey	Kastamonu	2	8	0	0
TD	34	41.79	33.66	1,040	Turkey	Kastamonu	1	8	1	8
TD	35	41.82	33.68	990	Turkey	Kastamonu	1	8	1	8
Total							85	496	46	390

^zThe first set of columns displays the total number of seed families and the total number of individual genotypes represented by those families. The second set of columns displays the subset of families with five or more half-siblings and the number of individuals in those families. Families represented by five or more half-siblings were used in differentiation estimates.

(GD12, GD15, GD96, GD100, GD142, GD147, GD162) (Hemmat et al., 2003; Hokanson et al., 1998). Standard cultivar controls were Golden Delicious, Rome Beauty Law, and Cox Orange Pippin. Forward primers, labeled with either IRD 700 or IRD 800, were obtained from MWG-Biotech (High Point, NC), and unlabeled reverse primers were purchased from IDT (Coralville, IA). All polymerase chain reaction amplifications were performed as described previously (Volk et al., 2005). Amplified products were separated on denatured acrylamide gels using a DNA sequencer (model 4200; LI-COR Inc., Lincoln, NE) (Volk et al., 2005).

Digital images were collected from the sequencer using LI-COR Saga™ Generation 2 software and were manually interpreted and scored using the Saga™ software. Alleles from replicate samples were examined at each locus, and when alleles for replicates were not identical, data for that locus were entered as “missing” in subsequent analysis. Individuals were included in the analyses when they had missing data for no more than one marker.

MOLECULAR DATA ANALYSIS. Of the 496 individuals in the data set, 390 were from families that contained five or more half-sib seedlings (Table 1). Descriptive statistics, including average differentiation between groups (F_{st}) and diversity within groups as measured by the number of polymorphic alleles and allelic richness, were estimated from genotypic data using the software packages GDA (Lewis and Zaykin, 2001) and FSTAT (Goudet, 1995).

Measurements of allelic richness were normalized using the method of El Mousadik and Petit (1996). This approach uses a rarefaction method which weights comparisons among groups by the smallest sample of genotyped individuals.

Population graph-theoretical methods were used to display the genetic variation within and among the six locations. Graphed node sizes were proportional to the within-population genetic variability, and edge lengths represented the among population component of genetic variation (Dyer and Nason, 2004).

Nonhierarchical genotypic clustering was performed using the genotypes obtained for all 496 samples in a manner independent of location or family structure. Clusters of individuals were identified using Bayesian methods that minimize genetic linkage disequilibrium (LD) among alleles at each of the marker loci within putative subpopulations (Pritchard et al., 2000). The number of clusters (denoted k) in a data set was identified using a combination of three methods (C.M. Richards, G.M. Volk, A.A. Reilley, A.D. Henk, D. Lockwood, P.A. Reeves, and P.L. Forsline, unpublished). STRUCTURAMA (Huelsenbeck and Andolfatto, 2007) software was employed for Markov chain Monte Carlo (MCMC) calculations. The mode cluster assignment across these independent runs was used to assign individual genotypes to discrete clusters in each of 10 MCMC runs in STRUCTURAMA. Successive changes in posterior probabilities and variances among independent runs were determined using methods described previously (C.M. Richards, G.M. Volk, A.A. Reilley, A.D. Henk, D. Lockwood, P.A. Reeves, and P.L. Forsline, unpublished; Evanno et al., 2005). Pie charts were constructed to represent the proportion of individuals from each cluster for each of the six collection locations.

DISEASE RESISTANCE. Potted seedling plants were inoculated with conidia of mixed races (1–5) of *Venturia inaequalis* for two consecutive years. Seedlings scored as resistant had the

Table 2. Descriptive statistics for each of seven microsatellite loci based on genotypic data from 496 *Malus orientalis* individuals that originated from seeds collected in Russia and Turkey.

Marker	Total no. of alleles	Avg gene diversity	Minimum allele size (bp)	Maximum allele size (bp)
GD12	27	0.83	138	198
GD15	2	0.34	142	145
GD96	20	0.85	150	190
GD100	11	0.74	217	237
GD142	20	0.72	128	172
GD147	20	0.79	117	173
GD162	26	0.77	212	268
Total	126			
Avg/locus	18	0.72		

following response: no symptoms, pin-point lesions, chlorotic lesions, necrotic lesions, nonsporulating, cupped or convoluted leaves. Seedlings that produced conidia and exhibited signs of sporulation were considered susceptible.

Cedar apple rust susceptibility was determined based on the presence of pycnidia on seedling tissues after inoculation with basidiospores of *Gymnosporangium juniperi-virginianae*.

Fire blight resistance was determined by resistance to *Erwinia amylovora* strain Ea273 inoculation greenhouse plants. Shoots were inoculated by transversely bisecting the two youngest actively growing leaves with scissors dipped in a suspension of *E. amylovora* (1×10^9 cfu/mL). Necrotic lesion lengths were expressed as a percentage of the current season's shoot length, and plants with <20% of shoot length blighted were characterized as resistant.

Results

Malus orientalis accessions from Turkey and southern Russia represent rich sources of genetic diversity. The 496 seedlings resulting from two collection trips represent 85 maternal sources of seeds (families) (Table 1). All 496 individuals were analyzed using the seven microsatellite markers and a total of 126 alleles were amplified with an average gene diversity of 0.72 (Table 2). Markers GD12, GD96, GD142, GD147, and GD162 amplified 20 or more alleles, and markers GD15 and GD100 amplified two and 11 alleles, respectively. Allelic data for all individuals are publicly accessible using the Germplasm Resources Information Network database (USDA, 2008).

Table 3. Levels of *Malus orientalis* genetic differentiation as estimated by F_{st} (with confidence intervals) calculated for within family, collection location, and cluster sources.^z

Source	N	F_{st}	F_{st} confidence interval	No. of genotypes
Family (within location)	46	0.208	0.18–0.25	390
Location	6	0.046	0.03–0.06	390
Cluster	6	0.076	0.06–0.09	496

^zOnly individuals in half-sib families with five or more individuals were included in family and location calculations. All 496 individuals were assigned to clusters and were included in cluster genetic diversity calculations.

Genetic structure estimates were calculated using only the 46 families with more than five half-sibs per family. These families were chosen to adequately estimate within- and among-family genetic variance. These results demonstrate a high F_{st} , 0.208, among families within locations (Table 3). The F_{st} value of 0.046 among locations was relatively low (Table 3). These results suggest high levels of diversity within the outcrossing families.

Mean allelic richness (as calculated using the 46 families with more than five half-sibs per family) was higher in the Turkish locations compared with the Russian locations (Table 4A). The Russian locations had only slightly fewer alleles per location than the Turkish locations, and the number of uniquely found, or so-called private alleles, per location ranged from 1 to 5. A visualization of the genetic variation within and among collection locations reveals the relatively low levels of diversity in the Russian locations compared with the highly diverse and disparate Turkish locations (Fig. 1).

Clustering methods inferred the number of clusters as well as the assignment of all 496 individuals to the clusters. Clustering algorithms converged on $k = 6$, and a graphical representation of this data shows the relationships among the six clusters (Fig. 2). Clusters 1 and 2, while represented by 228 and 178 individuals, respectively, had lower intracluster genetic variation than the other clusters which were represented by 13–24 individuals each (Table 4B, Fig. 2).

High levels of diversity were present among the individuals within some locations (Fig. 3). Most of the individuals in the cluster analyses were assigned to Clusters 1 and 2, which contained individuals from all locations. Clusters 3, 4, 5, and 6 were more localized. Individuals collected from the Russian locations were assigned primarily to Clusters 1 and 2. The individuals collected from Turkey showed much higher levels of diversity. Clusters 5 and 6 contained individuals exclusively from location TA, Cluster 3 was primarily found in location TC, and Cluster 4 was almost entirely specific to location TD (Fig. 3).

Genetic structure estimates revealed a small but significant F_{st} value of 0.076 for variation among clusters (Table 3). The allelic richness per locus and cluster have means of 5.4–7.5 alleles per cluster for all but Cluster 5, which shows a reduced number of 3.6 alleles per cluster across the seven loci (Table 4B). A high number of private alleles (15) was found in Cluster 1 compared with the other five clusters (0–4). The many private alleles are not surprising given the large number of individuals from many locations included in this cluster.

DISEASE RESISTANCE. Novel sources of disease-resistance alleles are valuable to apple breeding and research programs. *Malus orientalis* populations in southern Russia and Turkey have moderate levels of disease resistance. On average, more than half of the individuals per family in Russian locations RB and Turkish location TA were resistant to scab (Fig. 4A). In

Table 4. Allelic richness (see text) was calculated for all *Malus orientalis* individuals collected from Russia (RA, RB) and Turkey (TA, TB, TC, TD) and grouped according to collection location (section A) or cluster (section B).^z

Section	Locus	Location					
		RA	RB	TA	TB	TC	TD
A	GD12	8.68	11.47	15.02	11.78	14.05	16.1
	GD15	2	2	2	2	1.997	2
	GD96	10	11.37	11.15	12.61	11.44	14.45
	GD100	3	6	7.5	4.98	6.77	5.47
	GD142	12.66	10.59	9.75	10.22	11.55	10.82
	GD147	10.63	8.63	9.32	10.58	12.33	10.68
	GD162	8.51	9.13	9.6	11.61	12.62	14.28
	Mean/location	7.8	7.95	8.22	8.67	9.45	9.62
	Private alleles	1	4	2	5	2	4
	<i>N</i>	27	171	44	102	93	59
B	Locus	Cluster					
		1	2	3	4	5	6
B	GD12	9.53	7.62	5.48	9.1	5.17	8.55
	GD15	2	1.98	1.98	1.75	2	2
	GD96	9.31	8.14	7.99	9.39	4.29	6.87
	GD100	5.05	4.73	5.36	4.35	5	5
	GD142	8.26	7.91	6.9	5.5	2	5.19
	GD147	9.1	6.96	6.26	5.75	4	4.44
	GD162	3.36	6.26	8.76	6.57	3	5.47
	Mean/cluster	7.52	6.23	6.1	6.06	3.63	5.36
	Private alleles	15	4	2	1	0	2
	<i>N</i>	228	178	17	24	13	13
	He	0.8	0.74	0.7	0.7	0.64	0.74

^zAllelic richness per locus was calculated based on genotypic data collected from seven microsatellite loci. Section A: Comparison of allelic richness and private alleles across collection locations (with *N* representing the number of individuals in each location). Section B: Comparison of allelic richness, private alleles, and heterozygosity (He) across clusters (with *N* representing the number of individuals in each cluster). Comparisons were independent of sample size.

contrast, nearly all the individuals collected from location TB were susceptible to scab. More than half of the individuals in the families at location TA were resistant to cedar apple rust (Fig. 4B). In addition, more than 50% of the individuals in the families of the RA, RB, TB, and TC locations were resistant to fire blight (Fig. 4C).

An analysis of disease resistance across the six clusters revealed that some clusters were composed of individuals or families that are particularly resistant to one or more disease (Table 5). For example, 74% of the individuals in Cluster 2 and 71% of the individuals in Cluster 5 were resistant to scab compared with 51% in the total data set (Table 5). Within Cluster 2, 67% of the individuals that are resistant to scab are from location RB (data not shown). The Cluster 5 scab-resistant individuals are from family GMAL4511, collected from site 7 in location TA. Cedar apple rust resistance was 15% overall, but Clusters 5 (represented by family GMAL4511) and 6 had resistance levels of 36% and 67%, respectively (Table 5). In Cluster 6, all four individuals in family GMAL4512 and six of the nine individuals in family GMAL4513 were resistant to cedar apple rust (data not shown).

Families GMAL4511, GMAL4512, and GMAL4513 were collected from sites 7, 8, and 8, respectively, in location TA. In family GMAL4511, individuals GMAL4511.f, GMAL4511.g, GMAL4511.l, and GMAL4511.m were resistant to both scab and cedar apple rust. Individual GMAL4511.j was resistant to

Fig. 1. Population network of *Malus orientalis* individuals from collection locations in Russia (RA, RB) and Turkey (TA, TB, TC, TD). Node diameter is proportional to intralocation genetic variation. Lengths of edges connecting nodes are proportional to genetic differentiation among the connected locations. Both node sizes and edge lengths are rendered in three-dimensional space.

Fig. 2. Population network of the six clusters as determined by nonhierarchical genotypic clustering of 496 individuals of *Malus orientalis* collected from Russia and Turkey. Node diameter is proportional to intracluster genetic variation. Lengths of edges connecting nodes are proportional to genetic differentiation among the connected clusters. Both node sizes and edge lengths are rendered in three-dimensional space.

Fig. 3. Genetic diversity of *Malus orientalis* accessions from six Russian and Turkish collection locations. Pie charts located at each of the six collection locations reflect the proportion of individuals assigned to each of the six genetic clusters identified using Bayesian population-structure inference.

Fig. 4. Fractional level of disease resistance of *Malus orientalis* trees among collection locations. The fraction of individuals resistant to (A) apple scab, (B) cedar apple rust, and (C) fire blight was calculated for each family within each location. Mean values $\pm$ SE were determined among families for each location. Significant differences were calculated by ANOVA, and significantly different means were identified by Tukey–Kramer HSD multiple-range tests, and lowercase letters denote significant differences among means.

both scab and fire blight, and three individuals, GMAL4511.q, GMAL4511.x, and GMAL4511.y, were resistant to scab, cedar apple rust, and fire blight. The four members of family GMAL4512 had 100% resistance to cedar apple rust but were not resistant to scab or fire blight. The GMAL4513 family also had remarkable levels of disease resistance. Individuals GMAL4513.a, GMAL4513.c, GMAL4513.f, GMAL4513.g, and GMAL4513.m were all resistant to both scab and cedar apple rust, and GMAL4513.o was resistant to all three diseases.

Resistance to fire blight was more uniformly spread across the clusters, although Clusters 3 and 6 had considerably lower

Table 5. The 496 *Malus orientalis* individuals from seed collected in Russia and Turkey were organized into six clusters based on linkage disequilibrium estimates for seven microsatellite loci.^z

	Total no. of individuals	No. of families	Scab resistance		CAR resistance		Fire blight resistance	
			No.	(%)	No.	(%)	No.	(%)
Cluster 1	237	62	80	34	32	14	153	65
Cluster 2	187	39	139	74	18	10	104	56
Cluster 3	18	3	6	33	3	17	6	33
Cluster 4	25	6	8	32	5	20	11	44
Cluster 5	14	1	10	71	5	36	7	50
Cluster 6	15	3	9	60	10	67	2	13
Overall	496	114	252	51	73	15	283	57

^zThe number of individuals (*N*) and the percent of those individuals resistant to apple scab, cedar apple rust (CAR), and fire blight (*Erwinia amylovora*) were calculated for the individuals included in each of the six clusters.

levels of resistance (33% and 13%, respectively) compared with the overall resistance levels between 44% to 65% among the clusters). Across all 496 *M. orientalis* individuals, 76 were resistant to scab and fire blight, 16 were resistant to scab and cedar apple rust, 11 were resistant to fire blight and cedar apple rust, and 20 were resistant to all three diseases (accessions GMAL4487.i, GMAL4552.e, GMAL4554.f, GMAL4556.b, GMAL4556.c., GMAL4556.f, GMAL4556.g., GMAL4556.p, GMAL4557.a, GMAL4483.m, GMAL4483.n, GMAL4485.k, GMAL4485.o, GMAL4486.o, GMAL4490.f, and GMAL4494.o in addition to the GMAL4511 and GMAL4513 accessions listed above). Family GMAL4556 has a total of 12 members and was collected from site 27 in location TD. All of the individuals in family GMAL4556 were assigned to Cluster 1.

Discussion

Genetic and disease resistance analyses of the *M. orientalis* populations collected from southern Russia and Turkey in the NPGS reveal localized regions of diversity and high overall levels of disease resistance. These disease-resistant individuals may provide valuable new alleles to breeding programs. The data presented provide much greater detail than that previously published for *M. orientalis* collected from Russia and Turkey (Aldwinckle et al., 2002).

The use of clustering to identify groups of genetic lineages of individuals was valuable for identifying diverse sets of individuals as well as pockets of high-level disease resistance. Clusters 5 and 6 exhibited high levels of cedar apple rust resistance that were specific to several families in location TA. The clustering of genotypes aided in the identification of families GMAL4511 and GMAL4513, which both have high levels of resistance but are genetically distinct from one another. Family GMAL4556 also emerged as a family in cluster 1 that has unusually high levels of resistance to scab, cedar apple rust, and fire blight.

It is interesting to compare the genetic structure between populations of *M. sieversii* in Kazakhstan and *M. orientalis* in southern Russia and Turkey. The allelic richness across the seven markers was similar for *M. sieversii* and *M. orientalis*. Markers GD15 and GD100 had the fewest number of alleles and GD162 and GD96 both had higher numbers of alleles per locus. *Malus orientalis* had more alleles at GD12, GD100, GD142, and GD147 than *M. sieversii* (C.M. Richards, G.M.

Volk, A.A. Reilley, A.D. Henk, D. Lockwood, P.A. Reeves, and P.L. Forsline, unpublished).

Malus sieversii populations formed four clusters across populations that were separated by thousands of kilometers. In Kazakhstan, locations containing individuals from several different clusters were predominantly found in the southwestern collection sites where individuals from many families contributed to each cluster (C.M. Richards, G.M. Volk, A.A. Reilley, A.D. Henk, D. Lockwood, P.A. Reeves, and P.L. Forsline, unpublished). Several specific families

comprised each of the four much smaller clusters (3, 4, 5, and 6) identified for *M. orientalis*. These small clusters were mostly found in Turkish collection locations.

Distribution of *M. orientalis* individuals in Turkish collection locations was sparse compared with the collection locations in Russia. Many of the hillsides appeared barren and overgrazed, with only *M. orientalis* wild apple trees found widely spaced across the landscape (P. Forsline and H. Aldwinckle, personal communication). The source of disease resistance alleles in *M. orientalis* families may be different from that in *M. sieversii* and *M. ×domestica*.

Family structure in the *M. orientalis* and *M. sieversii* populations was comparable. Among-sampling location, among-cluster, and among-family F_{st} values were all very similar (<0.09 , ≈ 0.05 , and ≈ 0.2 , respectively). In both data sets, among-family F_{st} values were considerably larger than among-location and among-cluster values, suggesting high levels of outcrossing in these heterogeneous wild populations (C.M. Richards, G.M. Volk, A.A. Reilley, A.D. Henk, D. Lockwood, P.A. Reeves, and P.L. Forsline, unpublished).

In 2002, new populations of *M. orientalis* were sampled in Georgia and Armenia. These new collection sites in the Caucasus Mountains are nearest to collection location TA. Future genotyping and phenotyping efforts will reveal if additional novel alleles and sources of disease resistance are present in these populations.

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