Diversity in Seasonal Bloom Time and Floral Development among Apple Species and Hybrids

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ABSTRACT. Timing of flowering is a fundamental developmental transition that has great ecological and agricultural importance. For perennial plants, seasonal timing of bloom and anthesis, which is the ultimate stage of flowering, can be determined by the net effect of several preceding developmental steps: seasonal timing of floral initiation, rate and extent of floral development before winter dormancy, duration of dormancy, and rate of floral development after release from dormancy. In the domestic apple (*Malus ×domestica*), fruit production has generally favored cultivars that bloom relatively early in the season. However, floral tissues are easily damaged by freezing temperatures, and freeze injury is especially problematic in years when abnormally warm temperatures in early spring lead to rapid floral development. To facilitate identification of genes/alleles that govern bloom time, and that could add versatility to production systems for apple, we evaluated seasonal bloom time for accessions of *M. ×domestica*, wild apple species (*Malus* sp.), and *Malus* hybrids maintained in a large germplasm diversity collection.

One of the most important plant traits, in terms of both ecology and agricultural production, is seasonal timing of flowering. Seasonal flowering is driven by the interaction of endogenous genetic pathways with environmental cues such as photoperiod, light intensity and wavelength, and temperature (Amasino, 2009; Andrés and Coupland, 2012; Lang, 1965). In this way, flowers are produced at a time of the season that maximizes the potential of a given plant to produce abundant fruit and seed. For self-incompatible plants, precise control of flowering in a population is especially important to ensure cross-pollination, and for plants that rely on biotic pollination, seasonal control is important to ensure flowering at a time when specific pollinators are present and active.

For many woody perennials growing in temperate climates, including several major horticultural fruit species, flowering spans two growing seasons interrupted by an extended period of winter dormancy. Thus, flowers are differentiated on the shoot meristem during the first season and develop incompletely before the onset of dormancy. During the subsequent spring, flowers complete development, culminating in bloom and anthesis. When plants have perceived an appropriate period of winter temperatures (chilling requirement or endodormancy), this further development is dependent only on environment including warmer temperatures (ecodormancy) (Lang et al., 1987). In many seasons, extensive development associated with abnormally warm temperatures after completion of endodormancy leads to susceptibility to frost damage. A striking example was seen in 2012 throughout the midwestern United States, where prolonged high temperatures in early spring led to bloom of many horticultural crops more than 1 month before normal (Fig. 1). This period was followed by a series of hard freezes, which led to widespread and extensive losses for many of these including peach (*Prunus persica*), cherry (*Prunus cerasus* and *Prunus avium*), and apple [U.S. Department of Agriculture (USDA), 2012]. For apple, which can be strongly biennial for flowering and fruit production,

this degree of loss may lead to overcropping the subsequent year, a problem that must be countered by expensive chemical or manual thinning of flowers or fruit.

If climate change leads to more variability in early-season temperatures, then crop losses such as that seen in 2012 will become increasingly common. An important strategy to sustain current production, therefore, will be to develop cultivars that are less prone to early bloom. This will in turn require the integration of phenological and genetic studies to identify genes/alleles that control aspects of flowering. Timing of spring bloom is expected to be under complicated genetic control, because it can be influenced by several factors including seasonal timing of floral initiation, rate and extent of floral development before winter dormancy, duration of endodormancy, and rate of floral development after release from dormancy (Bubán, 1996; Hänninen and Tanino, 2011; Soltész, 1996). However, the genetic control of such individual traits is mostly unknown, and general diversity in these traits among species and domesticated cultivars has generally not been explored.

To hasten the identification of the potential range of genes/alleles that govern timing of bloom, and that could add versatility to production systems for apple, we sought to establish the extent of natural variation seen in bloom time throughout the genus *Malus*. An extensive collection of *Malus* species, hybrids, and domestic apple cultivars is maintained at the USDA-Agricultural Research Service (ARS) Plant Genetic Resources Unit in Geneva, NY. A core subset considered to represent the diversity of the entire collection is also maintained at the Geneva site, allowing for efficient evaluation of important traits relevant to industrial production (Hokanson et al., 2001; Kresovich et al., 1995). The objectives of this study were to 1) document relative flower development within the core germplasm collection at multiple points during the typical period of bloom; and 2) identify those accessions with the most extreme (early or late) bloom time within the core and extended collection.

Materials and Methods

This study used the extensive *Malus* germplasm collection maintained at the USDA-ARS Plant Genetic Resources Unit in

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Fig. 1. Average daily temperatures and winter chill unit accumulation for Geneva, NY, in 2009–10 and 2011–12. Chill units were determined by the method of Hauagge and Cummins (1991).

Geneva, NY. Plantings were managed in accordance with routine commercial practices for weeds, irrigation, nutrition, and microbial and insect pests. Trees were maintained as 16-year-old grafts of clonal material on rootstock ‘P.22’, 5-year-old grafts on ‘BUD 9’ (B.9), 8-year-old grafts on ‘Malling 7’ (M.7), and/or 10-year-old grafts on ‘EMLA 7’ (E.7). Evaluations were carried out on 21 Apr. 2010, 1 May 2010, 15 May 2010, and 2 May 2012. Floral development stage was evaluated for five representative inflorescences (flower clusters) for each individual using the numerical assessment scheme described in the text and an average value was calculated. Imprecision in evaluation was assessed by comparing ratings, taken on the same day by the same evaluator, for 171 accessions in the B9 planting. Most (61%) differences in measurements were 0.2 units or less, and 92% of differences in measurements were 1 unit or less. Those with measurement differences of 1 or greater were nearly all at a relatively early developmental stage for which developmental gradation was less obvious. Accumulated chilling hours as shown in Figure 1 were determined by the method of Hauagge and Cummins (1991), beginning on 1 Nov. Data from this study have been submitted to the USDA Germplasm Resources Information Network and are available by request from the corresponding author.

Results

DIVERSITY IN FLORAL DEVELOPMENT AMONG APPLE GENOTYPES. As an initial assessment of diversity in floral development, we catalogued developmental stage for 189 accessions of the core diversity collection on three dates in Spring 2010 (Table 1). An additional 50 individuals in this core collection either showed no flowering or exhibited only a few inflorescences and were not

Table 1. *Malus* specimens and accessions evaluated in this study in 2010.

USDA–Geneva planting	Available for study in 2010		Flowering in 2010 ^a	
	Specimens (no.)	Accessions (no.)	Specimens (no.)	Accessions (no.)
B9	239	238	189	189
P22	609	185	546	179
E7	759	658	591	533
M9	1872	1557	1356	1174
All	3473	2258	2685	1778

^aSpecimens with fewer than five inflorescences were not evaluated.

evaluated. Lack of flowering in apple can result from the natural phenomenon of biennial bearing. The evaluation dates were chosen in accordance with early (21 Apr.), intermediate (1 May), and late (15 May) bloom timing for the population in general in 2010. A numerical assessment scheme was developed (Fig. 2; Table 2) that is less sensitive to inflorescence and flower structural variation among species than a traditional apple bloom progression scale often used for *M. ×domestica* (Chapman and Catlin, 1976). For each of 68 accessions that were represented by one or more individuals, measurements varied only slightly with a median difference between replicates of 0.1 units and 90% of replicates within 0.3 units (data not shown).

The early evaluation date revealed a tremendous range in developmental stage among all accessions. The least developed among the 189 accession groups was a representative of *M. angustifolia* (southern crab apple), which had just broken dormancy and did not exhibit a visible inflorescence without dissection (Stage 0). Conversely, the most advanced was an accession of *M. sylvestris* (european crab apple) with all flowers open (Stage 8) (Fig. 3).

The early evaluation date also revealed an extensive developmental range within species groups. For example, for the species *M. hupehensis*, accessions ranged from just after Stage 1, with the inflorescence just visible within the new shoot, to after

Fig. 2. Stages of floral development in apple as designated in this study. (A) At the earliest stage (Stage 0), plants had just broken dormancy with vegetative or mixed vegetative/floral shoots apparent. Inflorescences were visible only upon macroscopic dissection of shoots. (B) At Stage 1, the inflorescence was macroscopically visible within the shoot without dissection (*Malus ×domestica*). (C–D) By Stage 2, individual flowers were easily apparent as a tight cluster [e.g., in (C) *M. ×domestica*] or loose cluster [e.g., in (D) *M. fusca*]. At this stage, in (C) *M. ×domestica*, petals were visible in the dominant “king” flower. (E–F) By Stage 3, most flowers within the inflorescence showed petals [(E) *M. ×domestica*, (F) *M. ioensis*]. (G–H) By Stage 4, petals were apparent in all flowers [(G) *M. ×domestica*, (H) *M. fusca*]. (I) Petals became dominant over sepals on the bud in Stage 5 (*M. ioensis*). (J) In Stage 6, petals of one or more flowers failed to totally enclose the floral bud. (K) One or more flowers was fully open at Stage 7. (L) At Stage 8, all flowers in an inflorescence were open.

Table 2. Stages of *Malus* floral development defined in this study.

Stage no.	Description of floral development
0	Plants have just broken dormancy; inflorescence visible only on dissection
1	Shoots have developed to the point where inflorescence is visible without dissection
2	Individual flowers are apparent within the inflorescence
3	Petals are visible on most flowers in the inflorescence
4	Petals are visible on all flowers in the inflorescence
5	Petals become dominant over sepals in flower buds
6	Petals of one flower in the inflorescence fail to enclose bud
6.5	Petals of one-half of flowers in the inflorescence fail to enclose bud
7	One flower in the inflorescence is fully open
7.3	One-half of flowers in the inflorescence are fully open
7.5	Approximately 70% of flowers in the inflorescence are fully open
7.8	Approximately 90% of flowers in the inflorescence are fully open
8	All flowers in the inflorescence are open
8.5	One-half of flowers in the inflorescence have shed petals
9	(Post-bloom) Nearly all flowers in the inflorescence have shed petals

Stage 6, when many flowers had begun to open (Fig. 3). A similar range was seen in *M. baccata*, where accessions ranged from just after Stage 2 (petals becoming visible in buds) to nearly Stage 8. Accessions that were included in the large *M. ×domestica* and *Malus* hybrid groups were similarly broadly distributed along developmental stage (Fig. 3). The *M. ×domestica* cultivar that had undergone the least development was ‘Cox’s Orange Pippin’, traditionally considered as late-blooming, whereas the named cultivar that had advanced furthest was ‘Novosibirski Sweet’, originally sourced from the former Soviet Union (USDA, 2013).

The intermediate evaluation date revealed the greatest range in floral development between species groups (Fig. 3). Some or all representatives of *M. prattii*, *M. angustifolia*, *M. florentina*, *M. ombrophila*, *M. rockii*, and *M. transitoria* exhibited extremely compact inflorescences with no petals visible (before Stage 2), whereas for some or all representatives of *M. sieversii*, *M. brevipes*, *M. orientalis*, and *M. sylvestris*, most flowers in an inflorescence had opened (Stage 7.5) (Fig. 3).

These data from the core collection showed that bloom development could be strongly variable both within a species group and between species groups. To focus analysis on bloom, we queried the entire *Malus* collection for specimens that represented the extreme range in bloom time. This effort considered nearly 2700 individuals representing nearly 1800 distinct accessions that were available for evaluation and were flowering in the year of this study (Table 1).

We first identified individuals that were most advanced at the time of the early assessment. We identified 121 individuals representing 76 accessions that had reached Stage 7 (one flower fully opened) and 46 individuals representing 31 accessions that had reached Stage 7.3 (50% open flowers). We defined these as “early blooming” and “extreme early blooming,” respectively (Tables 3 and 4). In addition to *M. sylvestris* (described previously), individual accessions of *M. baccata* and *M. orthocarpa* had reached Stage 8. The *M. ×domestica* cultivar

that was most advanced was ‘Shinibalt 1’, originally sourced from Pakistan, which had reached Stage 7.8 (90% of flowers open) (Tables 3 and 4). When considering those accessions that had reached Stage 7, *M. ×domestica* was underrepresented, whereas *M. kirghisorum*, *M. orientalis*, *M. sieversii*, and *M. sylvestris* were overrepresented ($P \leq 0.0015$, Fisher’s exact test) relative to the collection population in general (Table 5).

We then identified individuals that had not yet reached Stage 8 by the time of the late assessment. Here, we found 68 specimens representing 49 accessions that were at or less advanced than Stage 8 and 14 specimens representing eight accessions that were at or less advanced than Stage 7. We defined these as “late blooming” and “extreme late blooming,” respectively (Table 6). The developmentally least advanced accessions, which were at or before Stage 6, were of *M. ombrophila*, *M. florentina*, and *M. angustifolia* (Table 6). The developmentally least advanced accession of *M. ×domestica* was ‘Koningszuur’, a cultivar developed in The Netherlands, possibly incorporating *M. sylvestris* (USDA, 2013). Among late-blooming accessions, *M. ×domestica* and *Malus* hybrids were underrepresented, whereas *M. angustifolia*, *M. coronaria*, *M. florentina*, *M. ioensis*, and *M. yunnanensis* were overrepresented [$P \leq 0.001$ Fisher’s exact test (Table 7 and data not shown)].

YEAR-TO-YEAR VARIATION IN BLOOM STAGE. To assess year-to-year variability in floral development in relative terms among genotypes, we recorded developmental stage for 172 accessions in the B.9 planting in 2012 on a date (2 May) similar to that for the intermediate evaluation in 2010 (1 May). As expected as a result of warmer than typical early spring conditions in 2012 (Fig. 1), most accessions (118) were more advanced in 2012 relative to 2010 on this date (Table 8 and data not shown). Interestingly, a small subset of accessions (38) was less advanced on this date in 2012. Analysis of the species composition of this subset (Table 8) revealed that *M. ×domestica* accessions were significantly overrepresented, representing 55% of this subset but only 28% of the analyzed population [χ^2 P value 0.007 (data not shown)]. Conversely, *M. ×domestica* accessions were underrepresented among those that showed the most advancement of development relative to 2010 [$P = 0.022$ (data not shown)]. Accordingly, relative bloom stage between 2010 and 2012 was well correlated for non-*domestica* accessions (Pearson’s correlation 0.8826, $n = 123$) but only poorly correlated for *domestica* accessions [0.4478, $n = 49$ (data not shown)]. This may suggest that many *domestica* accessions may be recalcitrant to excessive promotion of bloom by early spring warmth. Alternatively or in addition, this effect could result from variation in chill requirement among accessions and the dissimilar winter conditions experienced in 2009–10 vs. 2011–12. We analyzed our data with respect to a study of variation in chill requirement among selected representatives of the same germplasm collection carried out by Hauagge and Cummins (1991). For the 43 accessions that were analyzed in both studies, we found a negative correlation between chill requirement and developmental stage, which was stronger in 2012 (Pearson’s correlation -0.5696) than in 2010 (-0.3185) (data not shown). The exceptional low chill-requiring cultivar Anna (Hauagge and Cummins, 1991) was among those accessions showing the greatest advancement in 2012 relative to 2010. Whereas these observations would not be inconsistent with delayed development response for higher chill-requiring

Fig. 3. Stage of floral development for representatives of *Malus* species groups. Chart depicts individual observations for 189 accessions of the core *Malus* diversity collection on three dates in 2010 representing early [21 Apr. (open)], mid [1 May (gray)], and late [15 May (black)] development for the population in general. The number of accessions evaluated within each species group is indicated by size of the circle; here the smallest circle represents one accession. Medians of observations are shown as a short horizontal bar. Data represent ratings of floral development where 0 = buds that have just broken dormancy and 9 = nearly all flowers have shed petals, as described in Table 2 and Figure 2.

accessions in 2012, calculated chill unit accumulation in the relatively warm winter of 2011–12 surpassed that of the more typical winter of 2009–10 as a result of extended periods of temperatures just above freezing in 2011–12 (Fig. 1). Further speculation on genetic response to variation in seasonal temperature regimes will be facilitated by a morphological or genetic marker for the completion of endodormancy (Soltész, 1996).

Discussion

Timing of spring bloom and its response to seasonal temperatures is a critical trait for production, and development

of new cultivars that bloom later in the season should be an increasing priority. To facilitate identification of genetic allele(s) that control this trait, we thus initiated assessment of bloom stage in an extensive diversity collection. Our results reveal substantial range of bloom stage both between and within species. Whereas a wide range was not unexpected among the many species of apple, the range seen within some species [e.g., *M. hupehensis* (Fig. 3)] was unanticipated, and the expression of such diversity within a species suggests this trait may be controlled by one or a small number of alleles. Simple genetic control of flowering has been seen in *Arabidopsis thaliana* in nature, in which a single gene controls the striking

Table 3. Extreme early blooming accessions^z identified in the USDA–Geneva *Malus* collection.

Accession no.	Species	Name	Stage on 21 Apr. 2010 ^y
589392	<i>M. orthocarpa</i>		8
633819	<i>M. orientalis</i>	RUS 98 02-01	7.8
644163	<i>M. ×domestica</i>	Shinibalt 1	7.8
633824	<i>M. sylvestris</i>	Oberwartha 5 × Klipphausen	7.5–8
286599	<i>M. baccata</i>		7.5–8
589170	<i>M. brevipes</i>		7.7
589255	<i>M. ×domestica</i>	Redspur Delicious	7.6
596283	<i>M. sieversii</i>		7.6
633797	<i>M. sieversii</i>	KAZ 93-24-01	7.6
633825	<i>M. sylvestris</i>	Barenhecke 3 × Klipphausen	7.6
644179	<i>Malus</i> sp.	Babarabkaturshi	7.6
437057	Hybrid	Roberts Crab	7.3–7.8
589252	Hybrid	Rosedale	7.5
594104	<i>M. sieversii</i>		7.3–7.7
619168	<i>M. sylvestris</i>		7.5
633800	<i>M. sieversii</i>	KAZ 95 18-10	7.5
633828	<i>M. orientalis</i>	99TU-08-02	7.5
588882	Hybrid	Ottawa 7	7.4–7.5
594101	<i>M. orientalis</i>		7.3–7.7
589024	<i>M. ×domestica</i>	Crimson Beauty	7.4
589312	<i>M. ×domestica</i>	Russian sdlg.	7.4
613959	<i>M. sieversii</i>	KAZ 95 17-14	7.4
633831	<i>M. orientalis</i>	99TU-26-02	7.4
588870	Hybrid	Dolgo	7.3–7.4
590043	<i>M. kirghisorum</i>		7.3–7.4
633831	<i>M. orientalis</i>	99TU-26-02	7.3–7.4
137581	<i>M. ×domestica</i>	Ottawa No. 322	7.3
280400	<i>M. ×domestica</i>	Anna	7.3
589478	<i>M. ×domestica</i>	Novosibirski Sweet	7.3
590079	Hybrid	PRI 1312-6	7.3
594100	<i>M. spectabilis</i>		7.3

^zAccessions were defined as “extreme early blooming” if they had reached Stage 7.3 (one-half of flowers within the inflorescence fully open) by 21 Apr. 2010.

^yStages are as defined in Table 2 and Figure 2 where Stage 7.3 represents one-half of flowers in the inflorescence fully open and where Stage 8 represents all flowers in the inflorescence fully open. Values represent the average for five inflorescences. A range of values is given where more than one specimen of an accession was evaluated.

difference in flowering habit between annual and winter-annual accessions (Gazzani et al., 2003).

The substantial variation in bloom stage within *M. ×domestica* cultivars was expected given the heterogeneous origins of domestic apple (Cornille et al., 2012). This variation did not seem to be conditioned by tree architecture, because the rootstocks, training systems, and management regimes were similar. Although flowers in lateral positions on long shoots can be delayed in opening relative to those arising on spurs (Soltész, 1996), in this study, we did not find obvious differences in developmental stage strictly associated with shoot-dominated vs. spur-dominated flowering architectures.

Malus ×domestica cultivars were, in general, earlier to bloom than most *Malus* species. Commercial apple production has

Table 4. Early blooming *Malus ×domestica* accessions identified in the USDA–Geneva *Malus* collection.^z

Accession no.	Name	Stage on 21 Apr. 2010 ^y
644163	Shinibalt 1	7.8
589255	Redspur Delicious	7.6
589312	Russian sdlg.	7.4
137581	Ottawa No. 322	7.3
292930	Kitaika Zolotaia Ranniaia	7.2
293887	Brunnichnoe	7.2
383526	Yellow Arkad	7.2
589451	CC-14-45	7.2
589024	Crimson Beauty	7–7.4
280400	Anna	7–7.3
104727	Irish Peach	7.1
437044	P.16	7.1
589155	Manito	7.1
589478	Novosibirski Sweet	7–7.3
589526	Zalesak #3	7.1
644162	Nurshah	7.1
199421	Safstaholm	7
267822	Vered	7
588837	Gravenstein Washington Red	7
588841	Idared	7
589056	Redford	7
589116	Pepinka Litowska	7
589483	Altaiski Sweet	7
589713	Early Bird Red	7
589956	Antonovka 172670-B	7
590152	Ottawa #524	7

^zAccessions were defined as “early blooming” if they had reached Stage 7 (one flower within the inflorescence open) by 21 Apr. 2010.

^yStages are as defined in Table 2 and Figure 2 where Stage 7 represents one flower within the inflorescence fully open and where Stage 8 represents all flowers in the inflorescence fully open. Values represent the average for five inflorescences. A range of values is given where more than one specimen of an accession was evaluated.

generally favored cultivars that bloom relatively early in the season, anecdotally to produce marketable fruit as early as possible in the season, and to ensure fruit maturity in short growing seasons (Soltész, 1996). In this study, we did not find a significant relationship between bloom stage and fruit maturity period for the 210 accessions of *M. ×domestica* for which seasonal period of fruit maturity was previously recorded (USDA, 2013) [Pearson’s correlation –0.2769 (data not shown)]. This lack of association is consistent with results of the majority of studies reviewed by Soltész (1996). Other factors that may be more critical for early maturity, including rapid rate of fruit development and early initiation of fruit ripening, are thus not incompatible with late bloom as breeding goals.

Timing of bloom may be influenced by the sum effect of several preceding developmental steps, including seasonal timing of floral initiation, rate and extent of floral development before winter dormancy, duration of dormancy, and rate of floral development after release from dormancy. Whereas timing of bloom is an easily accessible trait to evaluate, the remaining developmental steps are more cryptic. Initiation, for example,

Table 5. Early blooming species groups identified in the USDA–Geneva *Malus* collection.

Species	Early blooming accessions (no.) ^z	Extreme early blooming accessions (no.) ^y	Total representation (no.)
<i>M. baccata</i>	3	1	41
<i>M. brevipes</i>	1	1	2
<i>M. ×domestica</i>	26 ^x	7	986
<i>M. halliana</i>	1		11
<i>M. hupehensis</i>	1		15
Hybrid	11	5	268
<i>M. kirghisorum</i>	3 ^w	1	5
<i>M. orientalis</i>	6 ^w	4	11
<i>M. orthocarpa</i>	1	1	1
<i>M. prunifolia</i>	1		32
<i>M. sieversii</i>	13 ^w	5	97
<i>M. spectabilis</i>	2	1	6
<i>M. sylvestris</i>	4 ^w	3	13
<i>M. ×adstringens</i>	1		2
<i>M. ×robusta</i>	1		9
Total	75	29	1499

^zAccessions were defined as “early blooming” if they had reached Stage 7 (one flower within the inflorescence open) by 21 Apr. 2010.

^yAccessions were defined as “extreme early blooming” if they had reached Stage 7.3 (one-half of flowers within the inflorescence open) by 21 Apr. 2010.

^xUnderrepresented among early blooming accessions ($P \leq 0.0015$, Fisher’s exact test).

^wOverrepresented among early blooming accessions ($P \leq 0.0015$, Fisher’s exact test).

Table 6. Extreme late-blooming accessions and late-blooming *Malus* ×*domestica* accession identified in the USDA–Geneva *Malus* collection.^z

Accession no.	Species	Name	Stage on 15 May 2010 ^y
596278	<i>M. ombrophila</i>		5.3–5.4
588868	<i>M. florentina</i>		5.4–6.3
613880	<i>M. angustifolia</i>		6
589763	<i>M. angustifolia</i>		5.8–7
589426	<i>M. ioensis</i>	fimbriata	6.7
590063	<i>M. yunnanensis</i>		6.8
589385	<i>M. florentina</i>	Skopje P2	7
590116	<i>M. yunnanensis</i>	Veitchii	7
188517	<i>M. ×domestica</i>	Koningszuur	7.9

^zAccessions were defined as “extreme late blooming” or “late blooming” if they were at or less advanced than Stage 7 (one flower within the inflorescence open) or Stage 8 (all flowers open), respectively, on 15 May 2010.

^yStages are as defined in Table 2 and Figure 2: Stage 5, petals become dominant over sepals in flower buds; Stage 6, petals of one flower in the inflorescence fail to enclose bud; Stage 7, one flower in the inflorescence fully open; Stage 8, all flowers open. Values represent the average for five inflorescences. A range of values is given where more than one specimen of an accession was evaluated.

takes place at the shoot apex, which is both microscopic and encased in vegetative tissues within the bud. Extent of endodormancy can only be evaluated destructively through collection of shoots in the field and incubation under warm

Table 7. Late-blooming species groups identified in the USDA–Geneva *Malus* collection.

Species	Late-blooming accessions (no.) ^z	Extreme late-blooming accessions (no.) ^y	Total representation (no.)
<i>M. angustifolia</i>	13 ^x	2	14
<i>M. bhutanica</i>	2		8
<i>M. coronaria</i>	9 ^x		32
<i>M. ×domestica</i>	1 ^w		986
<i>M. florentina</i>	2 ^x	2	2
<i>M. fusca</i>	1		22
<i>M. honanensis</i>	1		1
Hybrid	1 ^w		268
<i>M. ioensis</i>	10 ^x	1	36
<i>M. ombrophila</i>	1	1	1
<i>M. prattii</i>	1		3
<i>Malus</i> sp.	2		22
<i>M. yunnanensis</i>	3 ^x	2	7
Total	47	8	1402

^zAccessions were defined as “late-blooming” if they were at or less advanced than Stage 8 (all flowers open) on 15 May 2010.

^yAccessions were defined as “extreme late-blooming” if they were at or less advanced than Stage 7 (one flower within the inflorescence open) on 15 May 2010.

^xOverrepresented among late-blooming accessions ($P \leq 0.0015$, Fisher’s exact test).

^wUnderrepresented among late-blooming accessions ($P \leq 0.0015$, Fisher’s exact test).

temperatures. The effect of environment on development is expected to be complex; for example, whereas heat accumulation after endodormancy release promotes floral development, lack of sufficient chilling hours over winter can delay bloom (Chandler, 1942; Hänninen and Tanino, 2011). The identification of species and *M. ×domestica* cultivars that exhibit extremes in bloom stage provides an entry point for more advanced and focused analyses of the genetic and environmental factors contributing to bloom time.

Our study population comprised much of the known cataloged germplasm for *Malus* species and *M. ×domestica* cultivars and provides a valuable resource for practical breeding and association mapping. That variation in development among species was wider than that within *M. ×domestica* suggests potential for genetic improvement of *M. ×domestica* through wide (interspecific) crosses. We noted that the three accessions of the *Malus* species native to the study area, *M. ioensis*, were markedly later blooming than nearly all *M. ×domestica* accessions; in 2010, none of the flowers of *M. ioensis* had opened at the time when most *M. ×domestica* accessions were nearly in full bloom (Fig. 3). Although most *M. ×domestica* accessions at the study site suffered complete loss of crop in 2012, *M. ioensis* escaped frost damage and produced abundant fruit. *M. ioensis* is easily intercrossed with *M. ×domestica* and thus is a potential source of wild germplasm for introduction of late-blooming genetic alleles. In contrast, *M. sieversii*, a large-fruited species from central Asia believed to contribute much of the *M. ×domestica* genome (Cornille et al., 2012), is currently being used in wide crosses to introduce disease resistance and novel production traits. However, nearly all accessions of *M. sieversii* studied here bloom early, even relative to *M. ×domestica* (Fig. 3),

Table 8. Accessions in the USDA–Geneva *Malus* collection that showed less advanced or more advanced floral development in 2012 relative to 2010.²

Species	Accessions that were less advanced in 2012 than in 2010 (no.)	Accessions that were more advanced in 2012 than in 2010 (no.)
<i>M. ×domestica</i>	21	21
<i>M. angustifolia</i>	0	1
<i>M. asiatica</i>	0	1
<i>M. baccata</i>	1	5
<i>M. bhutanica</i>	0	2
<i>M. brevipes</i>	0	1
<i>M. florentina</i>	0	0
<i>M. floribunda</i>	0	1
<i>M. fusca</i>	0	2
<i>M. halliana</i>	1	2
<i>M. hupehensis</i>	0	4
Hybrid	7	19
<i>M. ioensis</i>	1	1
<i>M. kansuensis</i>	0	2
<i>M. mandshurica</i>	0	1
<i>M. micromalus</i>	0	5
<i>M. ombrophila</i>	0	1
<i>M. orientalis</i>	0	9
<i>M. prattii</i>	0	2
<i>M. prunifolia</i>	2	3
<i>M. pumila</i>	0	1
<i>M. sargentii</i>	0	1
<i>M. sieversii</i>	1	13
<i>M. sikkimensis</i>	0	1
<i>M. spectabilis</i>	0	1
<i>M. sylvestris</i>	0	3
<i>M. toringo</i>	2	3
<i>M. transitoria</i>	0	2
<i>M. tschonoskii</i>	1	0
<i>M. yunnanensis</i>	0	1
<i>M. zhaojiaoensis</i>	1	0
Other	0	7
<i>Malus</i> sp.	0	2
Total	38	118

²Developmental stage was assessed on 1 May 2010 and 2 May 2012 for 172 accessions in the USDA–Geneva *Malus* collection.

and the responsible genetic alleles will need to be eliminated to produce an acceptable cultivar for frost-prone regions.

Literature Cited

- Amasino, R. 2009. Floral induction and monocarpic versus polycarpic life histories. *Genome Biol.* 10:228.
- Andrés, F. and G. Coupland. 2012. The genetic basis of flowering responses to seasonal cues. *Nat. Rev. Genet.* 13:627–639.
- Bubán, T. 1996. Flower development and formation of sexual organs, p. 3–54. In: Nyéki, J. and M. Soltész (eds.). *Floral biology of temperate zone fruit trees and small fruits*. Akadémiai Kiadó, Budapest, Hungary.
- Chandler, W.H. 1942. *Deciduous orchards*. Lea and Febiger, Philadelphia, PA.
- Chapman, P.J. and G.A. Catlin. 1976. Growth stages in fruit trees—From dormant to fruit set. *Plant Sci. Entomol. (Geneva, Switzerland)* 11:58.
- Cornille, A., P. Gladieux, M.J.M. Smulders, I. Roldán-Ruiz, F. Laurens, B. Le Cam, A. Nersesyan, J. Clavel, M. Olonova, L. Feugey, I. Gabrielyan, X. Zhang, M.I. Tenaillon, and T. Giraud. 2012. New insight into the history of domesticated apple: Secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genet.* 8:E1002703.
- Gazzani, S., A.R. Gendall, C. Lister, and C. Dean. 2003. Analysis of the molecular basis of flowering time variation in *Arabidopsis* accessions. *Plant Physiol.* 132:1107–1114.
- Hänninen, H. and K. Tanino. 2011. Tree seasonality in a warming climate. *Trends Plant Sci.* 16:412–416.
- Hauagge, R. and J.N. Cummins. 1991. Phenotypic variation and length of bud dormancy in apple cultivars and related *Malus* species. *J. Amer. Soc. Hort. Sci.* 116:100–106.
- Hokanson, S., W. Lamboy, A. Szewc-McFadden, and J. McFerson. 2001. Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. *Euphytica* 118:281–294.
- Kresovich, S., W. Lamboy, J. McFerson, and P. Forsline. 1995. Integrating different types of information to develop core collections, with particular reference to *Brassica oleracea* and *Malus × domestica*, p. 147–154. In: Hodgkin, T., A.H.D. Brown, T.J.L. Hintum, and E.A.V. Morales (eds.). *Core collections of plant genetic resources*. Wiley, Chichester, UK.
- Lang, A. 1965. Physiology of flower initiation, p. 1380–1536. In: Lang, A. (ed.). *Encyclopedia of plant physiology*. Vol. XV/1. Springer-Verlag, Berlin, Germany.
- Lang, G.A., J.D. Early, G.C. Martin, and R.L. Darnell. 1987. Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. *HortScience* 22:371–377.
- Soltész, M. 1996. Flowering, p. 80–131. In: Nyéki, J. and M. Soltész (eds.). *Floral biology of temperate zone fruit trees and small fruits*. Akadémiai Kiadó, Budapest, Hungary.
- U.S. Department of Agriculture. 2012. Crop production. 6 Mar. 2013. <http://www.nass.usda.gov/Publications/Todays_Reports/reports/crop0810.pdf>.
- U.S. Department of Agriculture. 2013. Apple. 6 Mar. 2013. <<http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?115>>.