Submission for PLoS ONE

Phenotypic divergence between the cultivated apple (*Malus domestica*) and its primary wild progenitor (*Malus sieversii*)

Thomas Davies[1], Sophie Watts[1], Kendra McClure[1], Zoë Migicovsky[1] & Sean Myles[1]

Affiliations:

[1] Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University, Truro, NS, Canada

ABSTRACT

An understanding of the relationship between the cultivated apple (*Malus domestica*) and its primary wild progenitor species (*M. sieversii*) not only provides an understanding of how apples have been improved in the past, but may be useful for apple improvement in the future. We measured 10 phenotypes in over 1000 unique apple accessions belonging to *M. domestica* and *M. sieversii* from Canada's Apple Biodiversity Collection. Using principal components analysis (PCA), we determined that *M. domestica* and *M. sieversii* differ significantly in phenotypic space and are nearly completely distinguishable as two separate groups. We found that *M. domestica* had a shorter juvenile phase than *M. sieversii* and that cultivated trees produced flowers and ripe fruit later than their wild progenitors. Cultivated apples were also 3.6 times heavier, 43% less acidic, and had 68% less phenolic content than wild apples. Using historical records, we found that apple breeding over the past 200 years has resulted in a trend towards apples that have higher soluble solids, are less bitter, and soften less during storage. Our

results quantify the significant changes in phenotype that have taken place since apple domestication, and provide evidence that apple breeding has led to continued phenotypic divergence of the cultivated apple from its wild progenitor species.

INTRODUCTION

The domesticated apple (*Malus domestica*) belongs to the genus *Malus*, which consists of 30-55 interfertile species that grow primarily in temperate climates. Archaeological evidence suggests that apples have been cultivated for at least 3,000 years [1] and that they have had immense cultural, religious, culinary and economic importance for centuries [2–4]. Genomic evidence suggests that as apples were transported west into Europe along the Silk Road from Central Asia, hybridization and introgression from multiple *Malus* species created the modern cultivated apple (*M. domestica*) [2,5]. While there has been introgression from multiple species, including *Malus sylvestris* and *Malus baccata*, to the *M. domestica* genome, *Malus sieversii* of Kazakhstan is widely recognized as the primary ancestor of the cultivated apple [5–7].

Today, the cultivated apple is the 3rd most produced fruit crop in the world [8]. Accordingly, apple fruit quality and phenology traits have been a major focus for breeding programs around the world [9–11], and both wild and domesticated germplasm are routinely evaluated for their potential use by apple breeders [12,13]. Traits such as precocity, harvest date and flowering date have practical implications for apple producers, as these traits influence investment timelines, crop quality and fruit damage risk. Weight, firmness, sugar content, acidity and phenolic content are important considerations for processors and consumers, who have specific preferences for these quality attributes when choosing to purchase apples [14]. Many of

these fruit quality traits have been targets for improvement in breeding programs around the world, and current genetic mapping efforts remain focused on these phenotypes [15–17].

Cost-effective trait improvement in apples is critical since the investment costs of growing apple trees are high. Apple trees are large plants with a long juvenile phase: new trees often only start bearing fruit 5 years into the life cycle, requiring growers to invest heavily before generating revenue. Thus, producers typically grow only thoroughly evaluated and historically successful apple varieties. As a result, a small number of well-established varieties dominate the cultivated population. For example, in 2019 over 50% of all commercially produced apples in the US consisted of only 4 apple cultivars [18]. The global population of apples is dominated by a small number of elite varieties, despite an immense source of genetic and phenotypic diversity available for apple improvement [19]. Decreased diversity in apples, and agricultural crops more broadly, has resulted in an increased interest in the use of crop wild relatives (CWRs) for agricultural improvement. CWRs offer genetic and phenotypic diversity that can be leveraged in the breeding of novel cultivars with desirable traits such as disease resistance or flesh colour [20]. By 1997 the world economy had gained approximately $115 billion in benefits from the use of CWRs as sources of resistance to environmental change and disease [21]. An understanding of how fruit quality and phenology vary within the cultivated apple's wild relatives is essential to future apple improvement.

Phenotyping large and diverse populations of plants is labour intensive and frequently results in a "phenotyping bottleneck" [22], leaving crop researchers without powerful fruit quality data for analysis. Recently, comprehensive phenotyping of Canada's Apple Biodiversity Collection (ABC) generated measurements for fruit phenotypes in a collection of more than 1000 wild and cultivated apple accessions [23]. In the present work, we explored ten phenotypes from

the ABC and determined the degree to which the cultivated apple differed from its primary wild progenitor, *M. sieversii,* and how cultivated apples have changed over the past 200 years of breeding and improvement.

MATERIALS AND METHODS

Phenotype data

The phenotype data analysed here were collected from Canada's Apple Biodiversity Collection (ABC) and were part of previously published work [23]. Briefly, the ABC is an apple germplasm collection located at the Agriculture and Agri-Food Canada (AAFC) Kentville Research Station in Nova Scotia, Canada (45.071767, -64.480466). The ABC contains 1119 unique accessions of apples planted in duplicate on M.9 rootstock in an incomplete randomized block design. The apple accessions in the ABC consist of accessions from the United States Department of Agriculture (USDA) Plant Genetic Resources Unit apple germplasm collection in Geneva, NY, USA; commercial cultivars from the Nova Scotia Fruit Growers' Association Cultivar Evaluation Trial; and diverse breeding material from AAFC Kentville. The orchard consists largely of *M. domestica* accessions, but also contains 78 *M. sieversii* accessions.

Phenotype data from the ABC were collected in 2016 and 2017 [23]. Here we focus on 10 phenotypes most relevant for assessing how apples have changed during domestication, breeding and improvement. Precocity was measured as a score of 1-4, indicating year of bloom; 1 (2014), 2 (2015), 3 (2016) and score 4 indicated that the tree had not yet bloomed as of 2016. Flowering date was measured in 2016 as the date in Julian days when the youngest wood displayed >80% of flowers at king bloom stage. Since it often took more than one day to harvest

the entire orchard, harvest date was recorded in Julian days as the Monday of the week of harvest. Firmness was measured as the average firmness in kg/cm^2 at harvest of five apples measured using a penetrometer. Weight was measured as the average weight in grams of five apples at harvest. Acidity was measured as the malic acid content in mg/mL of combined juice from five apples measured using titration. Soluble solids were measured as °Brix of the juice of five apples using a refractometer. Phenolic content was measured as µmol GAE/g of fresh weight. Percent acidity change was measured by subtracting the acidity at harvest from the acidity after 90 days storage and then dividing by the acidity at harvest. Percent firmness change was measured by subtracting the firmness at harvest from the firmness after 90 days storage and then dividing by the firmness at harvest. Sample sizes for each phenotype are listed in Table 1.

Table 1. Sample sizes by phenotype.

Phenotype	*M. domestica*	*M. sieversii*
Precocity	797	76
Flowering Date	768	74
Harvest Date	647	59
Firmness	644	59
Weight	644	58
Acidity	626	56
Soluble Solids	644	56
Phenolic Content	399	9
% Change in acidity during storage	449	19
% Change in firmness during storage	409	27

Data analysis

Principal components analysis (PCA) was conducted using a scaled and centered matrix of the 10 phenotypes listed in Table 1 using the prcomp() function in R 4.0.2 [24]. A Wilcoxon signed-rank test was used to determine whether the phenotypes and PC values differed significantly between wild and cultivated apples.

A Pearson correlation was used to assess relationships between phenotypes and the release year of cultivated apples. Where appropriate, the significance threshold was Bonferroni-corrected to account for 10 comparisons. Data visualization was performed using the ggplot2 R package [25].

RESULTS

PCA of the 10 phenotypes revealed modest overlap between cultivated and wild apples in phenotypic space (Fig. 1A, 1B). Wild and cultivated apples were significantly different along PC1 ($W = 53893$, $p = 3.56 \times 10^{-26}$), PC2 ($W = 13066$, $p = 2.07 \times 10^{-17}$) and PC3 ($W = 39203$, $p = 0.0002$; Fig. 1C).

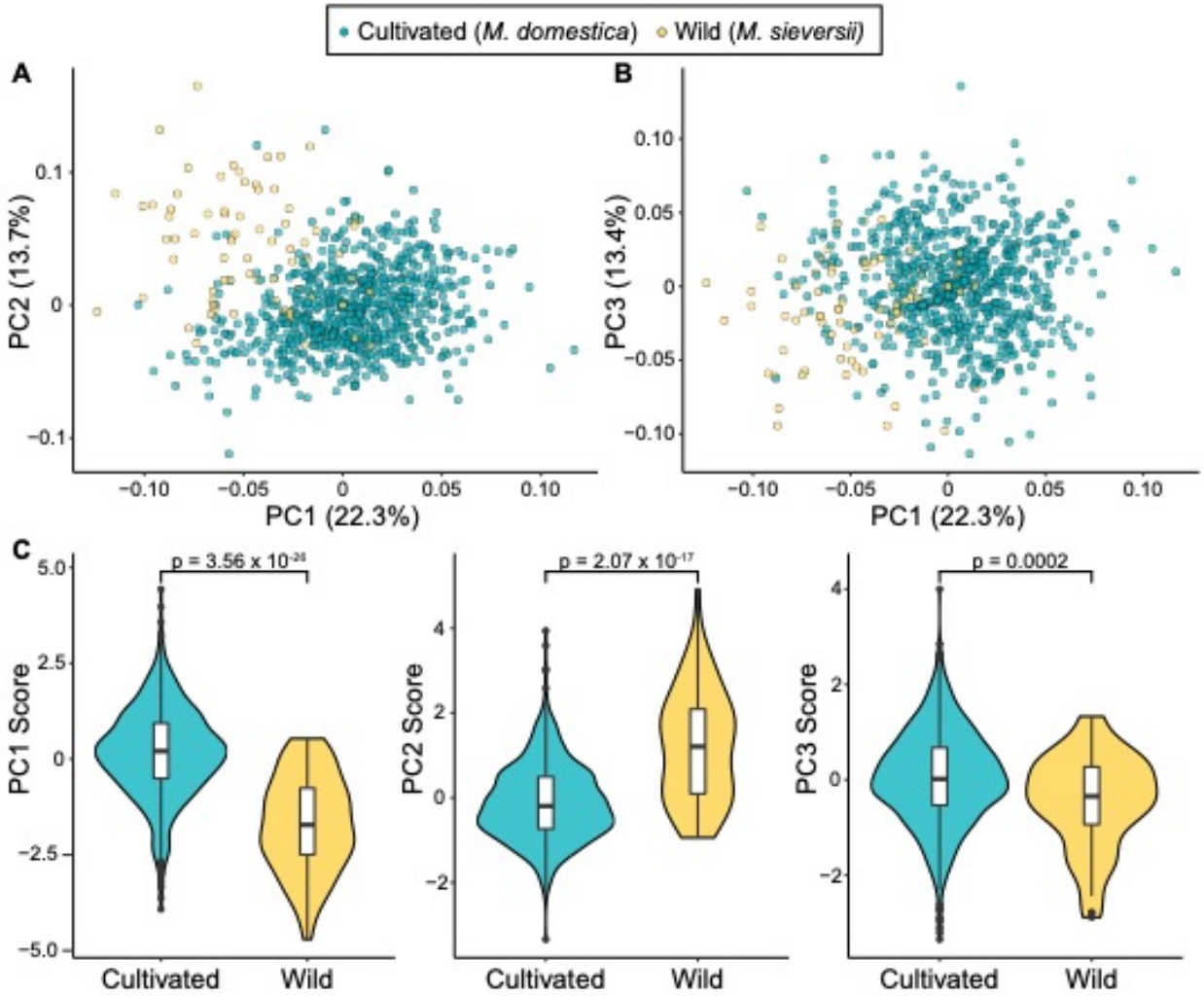
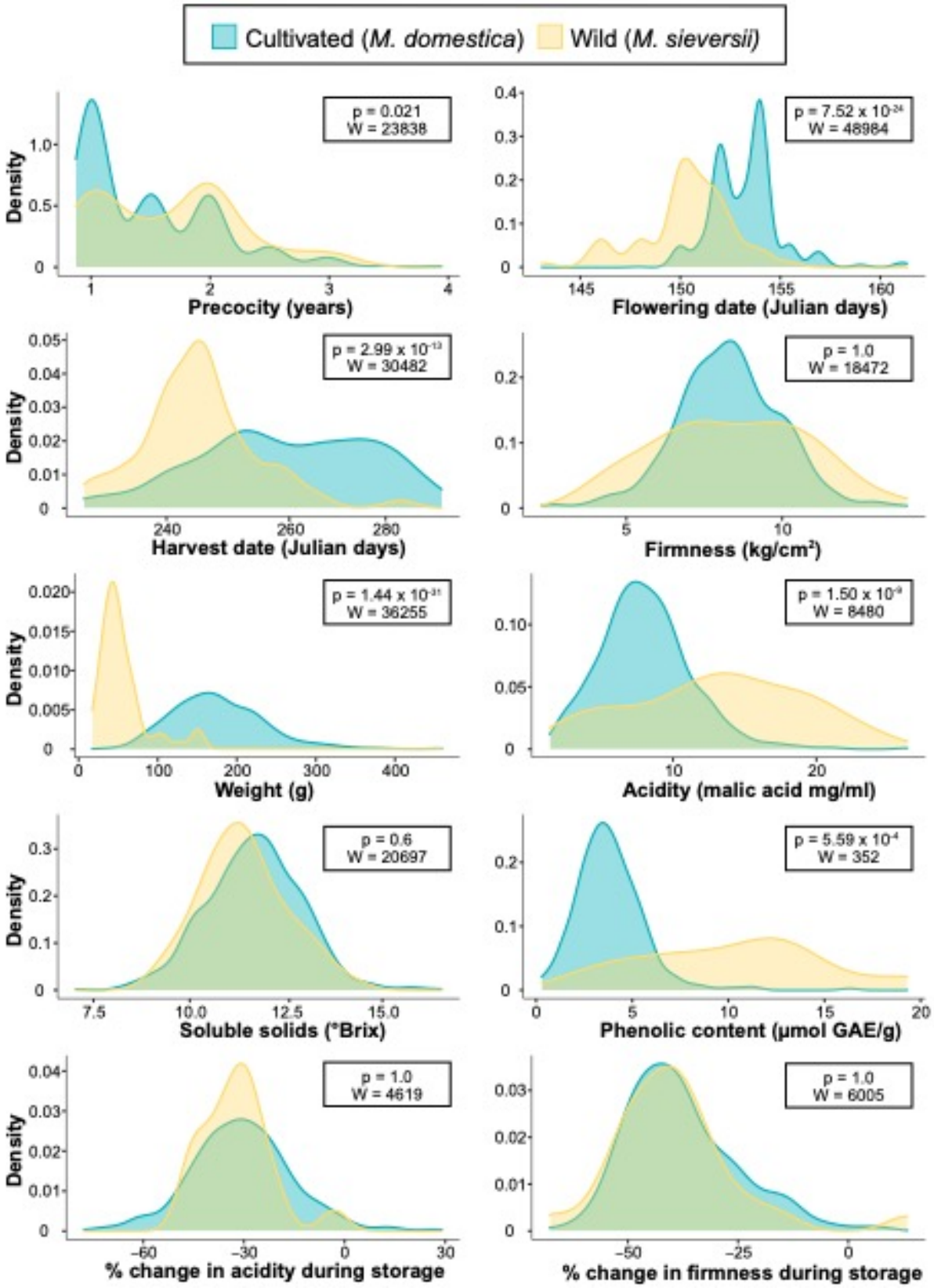


Fig 1. PCA of ten phenotypes in wild (N = 79) and cultivated apples (N = 801). A) PC1 vs PC2. B) PC1 vs PC3. The proportion of the variance explained by each PC is shown in parentheses on each axis. C) The difference between wild and cultivated apples for PCs 1, 2 and 3 are shown as violin plots. P values from a Wilcoxon test comparing PC values between cultivated and wild apples are shown for each of the first three PCs.

132 To visualize and assess the difference between cultivated and wild apples for each individual
133 phenotype, we produced density plots to visualize each species' distribution for each phenotype
134 and tested whether phenotypes differed between the two species (Fig. 2).



135

Fig 2. Overlapping density plots of 10 phenotypes comparing values from wild and cultivated apples. The phenotype associated with each plot is shown along the X axis. The W and Bonferroni-corrected p values report the results of performing a Wilcoxon rank sum test of the difference between the phenotypic distributions of wild and cultivated apples.

Wild and cultivated apples differed significantly for 6 of the 10 phenotypes tested, including precocity ($W = 23838$, $p = 0.021$), flowering date ($W = 48984$, $p = 7.52 \times 10^{-24}$), harvest date ($W = 30482$, $p = 2.99 \times 10^{-13}$), weight ($W = 36255$, $p = 1.44 \times 10^{-31}$), acidity ($W = 8480$, $p = 5.1 \times 10^{-9}$), and phenolic content ($W = 352$, $p = 5.59 \times 10^{-5}$). We found that, on average, cultivated apples produce flowers for the first time 21% (0.38 years) earlier than wild apples. Within a growing season, cultivated apples flower 3 days later, and are harvested 15 days later than wild apples. Cultivated apples are also 3.6 times heavier, 43% less acidic, and 68% lower in phenolic content than their wild progenitors. In comparison, wild and cultivated apples did not differ significantly for firmness, soluble solids, or changes in acidity or firmness during storage.

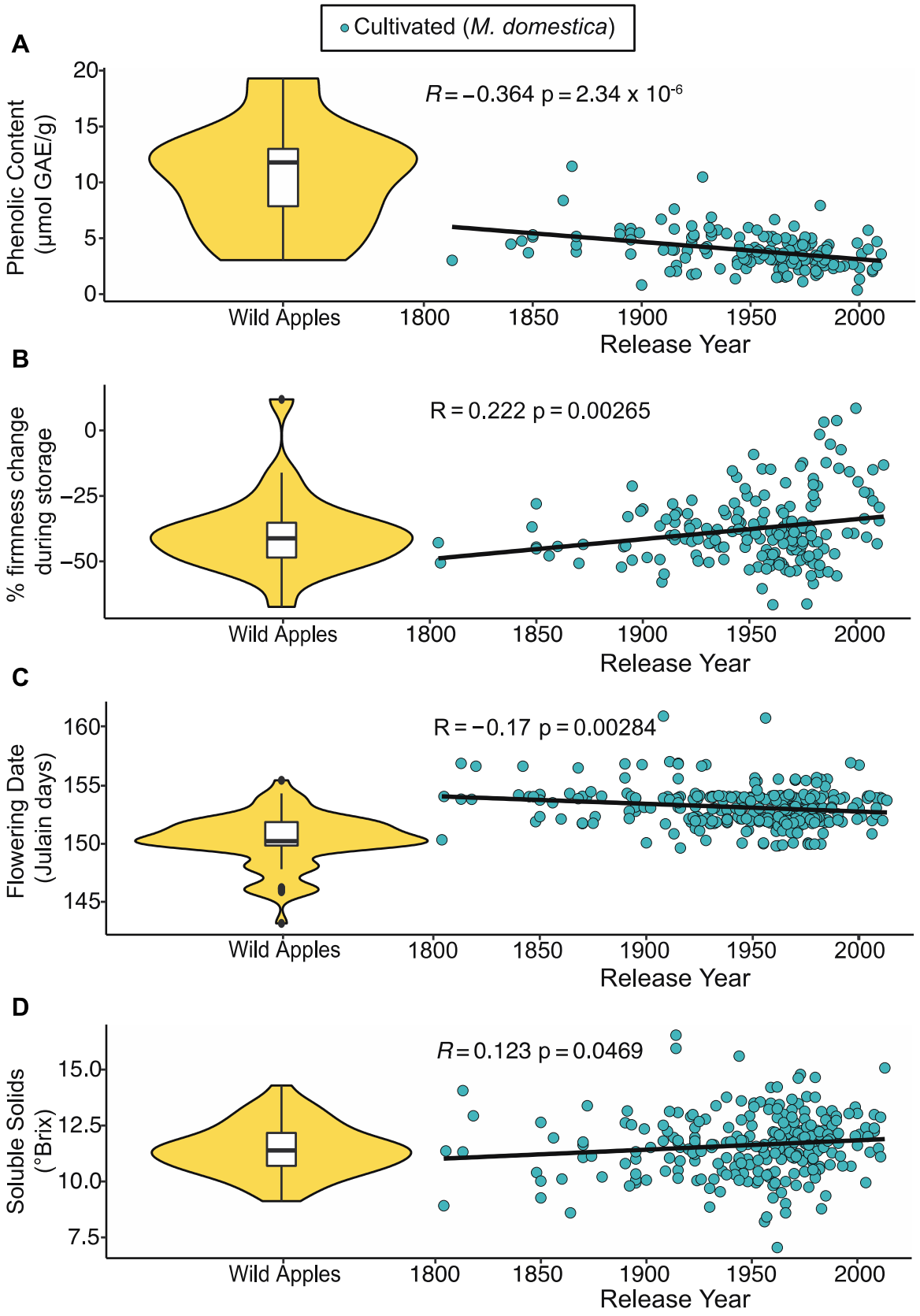


Fig 3. Phenotype values of cultivated apples as a function of their release year with a comparison to values in their wild ancestor, *M. sieversii*. Phenotypes include phenolic content (A), firmness change during storage (B), flowering date (C), and soluble solids (D). Values for cultivated apples are blue, and the values observed for *M. sieversii* are represented in yellow as a violin plot on the left side of each plot. The R and p values from a Pearson correlation between phenotypic values and release year are shown within each scatter plot.

To visualize phenotypic change within cultivated apples over time, apples' phenotypes are displayed as a function of their release year (Fig. 3 & Supplementary Fig. 1). We found significant correlations with release year for phenolic content ($R = -0.364$, $p = 2.34 \times 10^{-6}$), change in firmness during storage ($R = 0.222$, $p = 0.00265$), flowering date ($R = -0.172$, $p = 0.00247$), and soluble solids ($R = 0.123$, $p = 0.0469$) and determined that cultivated apples have shifted closer to the mean of wild apples for flowering date and firmness change, but further from the mean of wild apples for phenolic content and soluble solids.

DISCUSSION

Apples have been cultivated for over 3000 years, but because vegetative propagation has been practiced for 2000 years, it has been suggested that only about 100 generations have elapsed since apple domestication [26]. Despite this relatively short window for apple improvement, we found that cultivated apples are nearly entirely phenotypically distinct from their primary wild progenitor, *M. sieversii* (Fig. 1). Phenotypic differences are frequently used as an approximate measure of relatedness, and the separation in principal component space

174 observed here is in agreement with genomic studies that have shown significant differentiation
175 between the genomes of *M. domestica* and *M. sieversii* [5,19]. It is worth acknowledging that we
176 observed some overlap between wild and cultivated apples in phenotypic space. The PCA
177 performed here made use of only 10 phenotypes, and it is possible that more differentiation
178 would be observed with more measures of the apple phenome. Further, each variable in PCA
179 should ideally capture an independent biological feature of apples. However, some phenotypes
180 analysed here are correlated, such as harvest date and firmness [23], and their variation may be
181 driven by the same biological feature [27]. Therefore, interpreting our PCA as a quantification of
182 the degree of phenotypic differentiation between cultivated and wild apples should take these
183 caveats into consideration.

184 We found significant differences between wild and cultivated apples for several
185 phenology traits including precocity, flowering date, and harvest date (Fig. 2). Cultivated apple
186 trees flower and bear fruit at a younger age. Due to the long juvenile phase of apple trees, plants
187 with the ability to bear fruit earlier in their life cycle are desirable for growers because revenue is
188 generated earlier. It is therefore possible that precocity has been selected for during apple
189 improvement.

190 Flowering date was 17% (3 days) later in cultivated apples than wild apples. Frost during
191 blossoming can cause loss, damage or reduced marketability of fruits [28], making flowering
192 time an important consideration for growers when planting orchards. Additionally, apples with
193 later flowering dates tend to be firmer [23,29], and firmer apples are preferred by consumers
194 [30]. The later flowering date in cultivated apples could therefore be a by-product of selection for
195 firm apples. Similarly, selection for firm apples may explain why cultivated apples were
196 harvested 15 days later than wild apples, since harvest date and firmness are strongly correlated

197 [23,29]. It is well established that harvest date is a reliable predictor of fruit firmness, and these
198 two phenotypes may be regulated by a common molecular pathway [27]. Thus, preference for
199 firm fruit could be directly impacting the selection for apples with later harvest dates.
200 We found significant differences between cultivated and wild apples across multiple fruit
201 traits including weight, acidity, and phenolic content (Fig. 2). Cultivated apples are 3.6x heavier
202 than wild apples, in agreement with previous comparisons between these two species [31].
203 Consumers prefer large, visually appealing fruit [32,33], so selection for large fruit size may
204 explain our observation. We also found that cultivated apples are 43% less acidic than wild
205 counterparts. Acidity contributes to the sour taste of apples, and apple preference is heavily
206 influenced by acid/sugar ratios [34]. Given this relationship, it is not surprising that cultivated
207 apples, which are primarily consumed as fresh fruit [35], have lower acid than wild apples but do
208 not differ in soluble solid content. Finally, cultivated apples have, on average, 68% less phenolic
209 content than wild apples. Phenolic compounds, which offer nutritional benefits [36], are partially
210 responsible for the enzymatic browning that occurs when apple flesh is exposed to oxygen [37].
211 Browned flesh is visually unappealing and typically results in negative effects on flavour,
212 making apples that resist browning more appealing to producers and consumers [37]. In fact, the
213 only genetically modified apple variety on the market today, Arctic™ Apples, was designed to
214 silence genes related to enzymatic browning and was advertised as “the original nonbrowning
215 apple” [38]. The human aversion to apple browning has likely contributed to the decline in
216 phenolic content in cultivated apples, despite the nutritional benefits of such compounds. In
217 addition, some evidence suggests that fruit size impacts polyphenol accumulation in apples [39],
218 which could help explain why we observe lower phenolic content in cultivated apples.

219 According to the present analysis, many phenotypes of cultivated apples have
220 dramatically changed since divergence from the primary progenitor species, *M. sieversii*. These
221 differences represent phenotypic separation that could be leveraged in the improvement of
222 cultivated apples, and emphasizes the potentially functional diversity provided by CWRs. While
223 wild apples from this investigation may not offer improved fruit quality phenotypes that are
224 currently attractive to consumers, they hold phenotypic variation that could be important for
225 apple improvement in the future. For example, breeders could exploit the high phenolic content
226 of wild apples to improve the nutritional quality of cultivated apples. Further, traits from wild
227 apple varieties could potentially benefit the cider industry, which values high acidity and
228 phenolic content [40].
229 Analysis of cultivated apple phenotypes as a function of release year revealed changes
230 over the past 200 years in phenolic content, change in firmness during storage, flowering date,
231 and soluble solids (Fig. 3). In particular, as shown previously [23], phenolic content has
232 decreased over time. Phenolic content is associated with bitter taste [41], and modern varieties
233 therefore likely taste less bitter on average than older varieties. Although selection for decreased
234 bitterness could explain our observation, the relationship between low phenolic content and
235 decreased flesh browning could also explain why modern cultivated apples tend to have less
236 phenolics [42]. In comparison, wild apples tend to have higher phenolic content, indicating that
237 cultivated varieties are diverging from the ancestral state. Similarly, more recently released apple
238 cultivars soften less during storage than older cultivars, diverging from the ancestral state. The
239 extended storage and long-distance shipment of apples has become increasingly routine over the
240 past several decades, and selection for reduced softening during storage may explain why
241 firmness retention has improved over time. Storage and transport have also been key targets in

tomato breeding [43], and the demand for fruit that performs well during extended storage and transport is unlikely to subside.

Flowering date is an important trait for apple production, and varies widely across the genus *Malus* [13]. Later flowering apple trees are less likely to be impacted by frost damage [28] and more likely to be firm [23], which is preferred by consumers. Despite the understood benefits of growing apples with later flowering dates, we found that more recently released varieties had earlier flowering dates. The trend towards earlier flowering varieties could indicate that selection for other traits has indirectly impacted flowering date. Alternatively, growers could be preferring earlier flowering varieties in an attempt to manage fruit ripening times during the harvest season. Cultivated varieties are trending towards the ancestral state of earlier flowering dates, which suggests that wild apples could offer valuable genetic material for breeding earlier harvested varieties.

Finally, we found that more modern cultivated apples are only slightly higher in soluble solid content. Previous investigations have reported that firm apples tend to have higher sugar content [10,29,44], so our observation that modern apple varieties tend to have higher SSC may be at least partially be driven by recent selection for increased firmness. Further, a number of studies have suggested that the sugar content of apples is a key factor affecting consumer preference [14,30]. Although SSC is only a modest predictor of perceived sweetness [45], consumer's preference for sweet apples could underlie the upward trend in soluble solid content seen in modern cultivated apples.

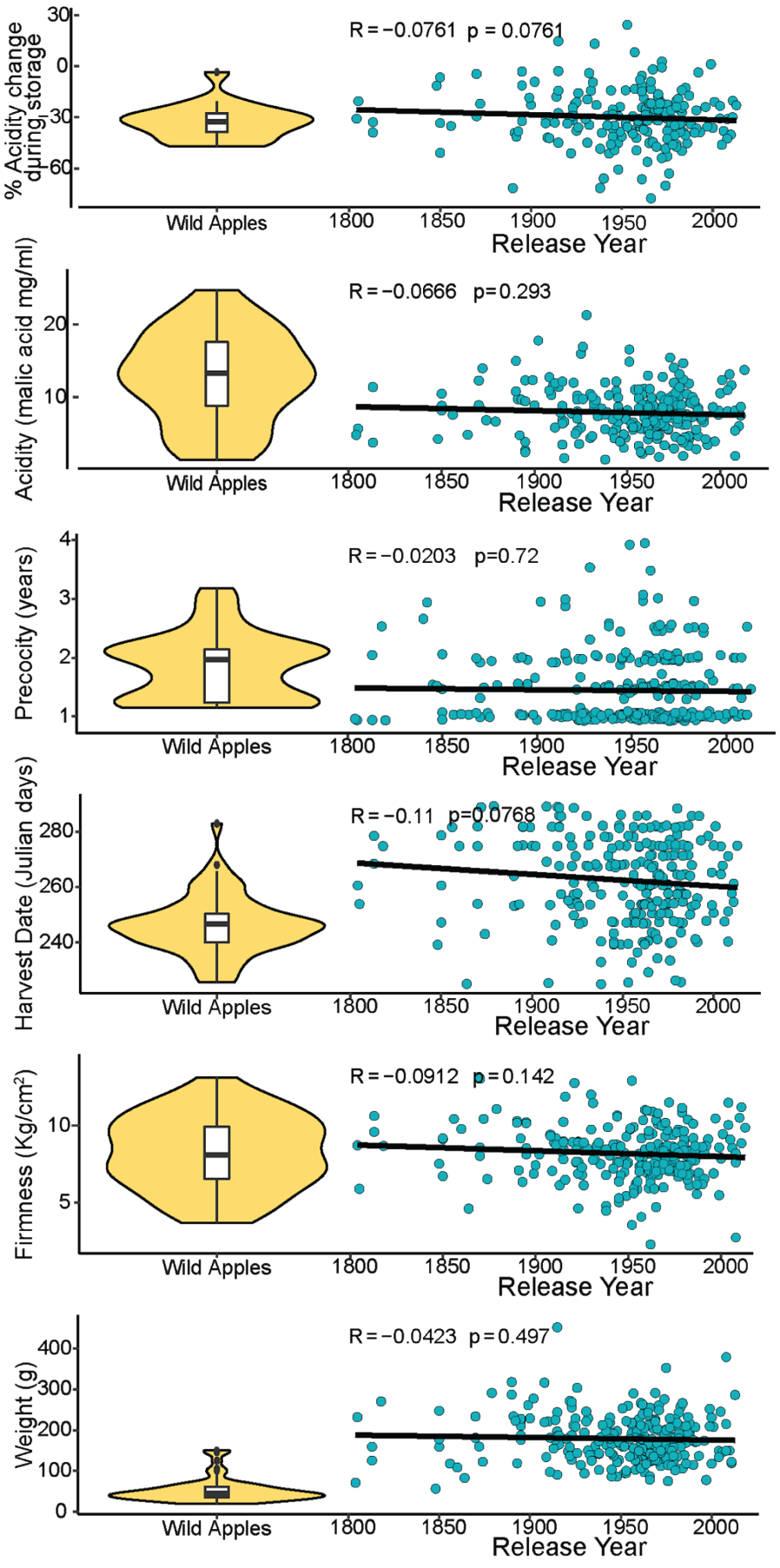
Several caveats of the present analysis are worth noting. First, we only considered one of the multiple progenitor species of *M. domestica* here [6]. Therefore, only a fraction of the ancestry of the cultivated apple is captured by *M. sieversii*, and a more inclusive pool of ancestral

265 species would yield a more comprehensive comparison of wild and cultivated apples. Second, it
266 is unknown how representative the current sample of wild apples is of the broader *M. sieversii*
267 population. It is possible that the wild apple varieties within the ABC represent only an
268 unrepresentative subset of *M. sieversii*, and thus do not accurately capture the diversity of the
269 species. Further, there has been evidence of gene flow between cultivated and wild apples [46],
270 which could mean that the wild species from the current investigation have experienced gene
271 introgression from cultivated trees, and thus do not accurately represent the wild progenitor.
272 Finally, the relatively small sample size in several comparisons limited the power of some of our
273 analyses (Supplementary Fig. 2).

274 Our work demonstrates that cultivated and wild apples have diverged phenotypically, and
275 that hundreds of years of apple improvement have shaped the variation in fruit and phenology we
276 observe among cultivated apples today. Wild apples offer potentially valuable pools of genetic
277 material that may be helpful for apple improvement. Future comprehensive phenomic
278 evaluations, including metabolomic and transcriptomic analyses, across diverse wild apple
279 species will help further assess the degree to which the apple's wild relatives may contribute to
280 improving apple cultivar development.

281

282 SUPPLEMENTARY FIGURES



Supplementary Figure 1. Phenotypes of cultivated apples as a function of their release year with a comparison to the ancestral state. Phenotypes include acidity change during storage, acidity, precocity, harvest date, firmness, and weight. Cultivated apple scores for each phenotype are shown in blue, and the ancestral state of each phenotype is represented in yellow as a density distribution of values from *M. sieversii*. The R and p values from a Pearson correlation between phenotypic values and release year are shown within each scatter plot.

ACKNOWLEDGMENTS

General: The authors thank the Nova Scotia Fruit Growers' Association and the Farm Services team at AAFC-Kentville for their work in establishing and maintaining the trees studied here. We thank Tayab Soomro for useful discussion.

Funding: This work was supported by the National Science and Engineering Research Council of Canada. ZM was supported by the National Science Foundation Plant Genome Research Program 1546869.

Competing interests: The authors declare no competing interests.

Data availability: All data presented are freely available to the public via Watts et al. [23]. Statistical analyses are on GitHub at https://github.com/MylesLab/Wild_vs_cultivated.

REFERENCES

1. Zohary D, Hopf M. Domestication of plants in the Old World: The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Oxford University Press; 2000.

2. Cornille A, Giraud T, Smulders MJM, Roldán-Ruiz I, Gladieux P. The domestication and evolutionary ecology of apples. Trends Genet. 2014;30: 57–65.

3. Juniper BE, Mabberley DJ. The story of the apple. Timber Press (OR); 2006.

4. Ferree DC, Warrington IJ. Apples: Botany, Production, and Uses. CABI; 2003.

5. Duan N, Bai Y, Sun H, Wang N, Ma Y, Li M, et al. Genome re-sequencing reveals the history of apple and supports a two-stage model for fruit enlargement. Nat Commun. 2017;8: 249.

6. Sun X, Jiao C, Schwaninger H, Chao CT, Ma Y, Duan N, et al. Phased diploid genome assemblies and pan-genomes provide insights into the genetic history of apple domestication. Nat Genet. 2020;52: 1423–1432.

7. Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, et al. The genome of the domesticated apple (Malus × domestica Borkh.). Nat Genet. 2010;42: 833–839.

8. FAOSTAT. FAOSTAT. In: Food and Agriculture Association of the United States [Internet]. 22 Dec 2020 [cited 7 Mar 2021]. Available: http://www.fao.org/faostat/en/

9. Jung M, Roth M, Aranzana MJ, Auwerkerken A, Bink M, Denancé C, et al. The apple REFPOP-a reference population for genomics-assisted breeding in apple. Hortic Res. 2020;7: 189.

10. McClure KA, Gardner KM, Douglas GM, Song J, Forney CF, DeLong J, et al. A genome-wide association study of apple quality and scab resistance. Plant Genome. 2018;11: 1–14.

11. Urrestarazu J, Muranty H, Denancé C, Leforestier D, Ravon E, Guyader A, et al. Genome-Wide Association Mapping of Flowering and Ripening Periods in Apple. Front Plant Sci. 2017;8: 1923.

12. Khan MA, Olsen KM, Sovero V, Kushad MM, Korban SS. Fruit quality traits have played critical roles in domestication of the apple. Plant Genome. 2014;7: 1.

13. Gottschalk C, van Nocker S. Diversity in seasonal bloom time and floral development among apple species and hybrids. J Am Soc Hortic Sci. 2013;138: 367–374.

14. Cliff MA, Stanich K, Lu R, Hampson CR. Use of descriptive analysis and preference mapping for early-stage assessment of new and established apples. J Sci Food Agric. 2016;96: 2170–2183.

15. McClure KA, Gong Y, Song J, Vinqvist-Tymchuk M, Campbell Palmer L, Fan L, et al. Genome-wide association studies in apple reveal loci of large effect controlling apple polyphenols. Hortic Res. 2019;6: 107.

16. Wu B, Shen F, Wang X, Zheng WY, Xiao C, Deng Y, et al. Role of MdERF3 and

MdERF118 natural variations in apple flesh firmness/crispness retainability and development of QTL-based genomics-assisted prediction. Plant Biotechnol J. 2020. doi:10.1111/pbi.13527

17. Iezzoni AF, McFerson J, Luby J, Gasic K, Whitaker V, Bassil N, et al. RosBREED: bridging the chasm between discovery and application to enable DNA-informed breeding in rosaceous crops. Horticulture Research. 2020;7: 177.

18. WAPA. U.S. Apple and Pear Forecast. In: WAPA - The World Apple and Pear Association [Internet]. Dec 2018 [cited 26 Oct 2020]. Available: http://www.wapa-association.org/asp/page_1.asp?doc_id=447

19. Migicovsky Z, Gardner KM, Richards C, Thomas Chao C, Schwaninger HR, Fazio G, et al. Genomic consequences of apple improvement. Hortic Res. 2021;8: 9.

20. McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, et al. Agriculture: Feeding the future. Nature. 2013;499: 23–24.

21. Pimentel D, Wilson C, McCullum C, Huang R, Dwen P, Flack J, et al. Economic and Environmental Benefits of Biodiversity. Bioscience. 1997;47: 747–757.

22. Furbank RT, Tester M. Phenomics--technologies to relieve the phenotyping bottleneck. Trends Plant Sci. 2011;16: 635–644.

23. Watts S, Migicovsky Z, Yu C, McClure K, Butler L, Sawler J, et al. Quantifying apple diversity: a phenomic characterization of Canada's Apple Biodiversity Collection. In Review. 2021.

24. R Core Team. R: A language and environment for statistical computing. 2020.

25. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2016.

26. Spengler RN. Origins of the Apple: The Role of Megafaunal Mutualism in the Domestication of Malus and Rosaceous Trees. Front Plant Sci. 2019;10: 617.

27. Migicovsky Z, Yeats TH, Watts S, Song J, Forney CF, Burgher-MacLellan K, et al. Apple ripening is controlled by a NAC transcription factor. Cold Spring Harbor Laboratory. 2021. p. 708040. doi:10.1101/708040

28. Eccel E, Rea R, Caffarra A, Crisci A. Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. Int J Biometeorol. 2009;53: 273–286.

29. Nybom H, Ahmadi-Afzadi M, Sehic J, Hertog M. DNA marker-assisted evaluation of fruit firmness at harvest and post-harvest fruit softening in a diverse apple germplasm. Tree Genet Genomes. 2013;9: 279–290.

30. Harker FR, Kupferman EM, Marin AB, Gunson FA, Triggs CM. Eating quality standards

for apples based on consumer preferences. Postharvest Biol Technol. 2008;50: 70–78.

31. Kumar S, Raulier P, Chagné D, Whitworth C. Molecular-level and trait-level differentiation between the cultivated apple (Malus× domestica Borkh.) and its main progenitor Malus sieversii. Plant Genetic Resources; Cambridge. 2014;12: 330–340.

32. Carew R, Smith EG. The value of apple characteristics to wholesalers in western Canada: A hedonic approach. Can J Plant Sci. 2004;84: 829–835.

33. Skreli E, Imami D. Analyzing consumers' preferences for apple attributes in Tirana, Albania. International Food and Agribusiness Management Review. 2012;15: 137–157.

34. Hampson CR, Quamme HA, Hall JW, MacDonald RA, King MC, Cliff MA. Sensory evaluation as a selection tool in apple breeding. Euphytica. 2000;111: 79–90.

35. Lutes H. The Facts on Conventional and Non-Browning Apples - CropLife.ca. CropLife Canada; 23 Jul 2019 [cited 26 Oct 2020]. Available: https://croplife.ca/facts-figures/apples/

36. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, et al. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Molecules. 2016;21: 1374.

37. Holderbaum DF, Kon T, Kudo T, Guerra MP. Enzymatic Browning, Polyphenol Oxidase Activity, and Polyphenols in Four Apple Cultivars: Dynamics during Fruit Development. HortScience. 2010;45: 1150–1154.

38. Stowe E, Dhingra A. Development of the Arctic® Apple. Plant Breed Rev. 2020;44: 273–296.

39. Busatto N, Matsumoto D, Tadiello A, Vrhovsek U, Costa F. Multifaceted analyses disclose the role of fruit size and skin-russeting in the accumulation pattern of phenolic compounds in apple. PLoS One. 2019;14: e0219354.

40. Mattila P, Hellström J, Törrönen R. Phenolic acids in berries, fruits, and beverages. J Agric Food Chem. 2006;54: 7193–7199.

41. Soares S, Kohl S, Thalmann S, Mateus N, Meyerhof W, De Freitas V. Different phenolic compounds activate distinct human bitter taste receptors. J Agric Food Chem. 2013;61: 1525–1533.

42. Toivonen PMA. Fresh-cut apples: Challenges and opportunities for multi-disciplinary research. Can J Plant Sci. 2006;86: 1361–1368.

43. Kramer MG, Redenbaugh K. Commercialization of a tomato with an antisense polygalacturonase gene: The FLAVR SAVR^{TM} tomato story. Euphytica. 1994;79: 293–297.

44. Migicovsky Z, Gardner KM, Money D, Sawler J, Bloom JS, Moffett P, et al. Genome to Phenome Mapping in Apple Using Historical Data. Plant Genome. 2016;9.

doi:10.3835/plantgenome2015.11.0113

45. Aprea E, Charles M, Endrizzi I, Laura Corollaro M, Betta E, Biasioli F, et al. Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds. Sci Rep. 2017;7: 44950.

46. Cornille A, Gladieux P, Giraud T. Crop-to-wild gene flow and spatial genetic structure in the closest wild relatives of the cultivated apple. Evol Appl. 2013;6: 737–748.