

A multifaceted overview of apple tree domestication

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- 1 A multifaceted overview of apple tree domestication
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29 Abstract

Apple is an iconic tree and major fruit crop worldwide. It is also a model species for the study of the 30 evolutionary processes and the genomic basis underlying the domestication of clonally propagated 31 perennial crops. Multidisciplinary evidence from across Eurasia has documented the pace and 32 33 process of cultivation of this remarkable crop. While population genetics and genomics have revealed the overall domestication history of apple across Eurasia, untangling the evolutionary 34 processes involved, archeobotany has helped to document the transition from gathering and using 35 apples to the practice of cultivation. Further studies, integrating archeogenetics and -genomics 36 approaches, will definitively bring new insights about key traits involved in apple domestication. 37 Such knowledge has potential to boost innovation in present-day apple breeding. 38

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Keywords: Adaptive introgression, self-incompatibility, rootstock, pathogen, Silk Road, targeted
sequencing, plant remains, paleogenetics.

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48 Glossary

49 **Archeobotany:** The study of plant remains (seeds, fruits, wood, leaves, pollen, etc.) found in 50 archeological deposits with the aim of reconstructing the paleo-environment and the management of 51 plant resources (including the domestication of wild plants), their uses, and the waste disposal 52 practices of ancient societies.

Archeo/paleogenetics and –genomics: Research fields that use the genetic information gathered from archeological plant or animal remains (ancient DNA; aDNA) to address questions about the evolution, domestication and history of use and cultivation of plants and animals (e.g. [1–8]).

Balancing selection: Selection regimes that result in increased genetic diversity relative to neutral
 expectations in populations of living organisms.

Crabapple [9]: Wild apple species that usually blossom profusely and produce small, acidic fruits.
The word crab comes from the Old English 'crabbe' meaning bitter or sharp tasting. Many crabapples are cultivated as ornamental trees. In Western Europe the term crabapple is often used to refer to *Malus sylvestris* (the European crabapple), in the Caucasus to *M. orientalis* (the Caucasian crabapple) and, in Siberia, to *M. baccata* (the Siberian crabapple). The native North American

crabapples are *M. fusca*, *M. coronaria*, *M. angustifolia*, and *M. ioensis*. *Malus sieversii*, the main
 progenitor of the cultivated apple, is usually not referred to as a 'crabapple'.

65 **Grafting:** A technique of vegetative propagation whereby tissues of two plants are intentionally 66 joined and allowed to grow together. The upper part of the combined plant (which contributes the 67 aerial parts and produces fruit) is called the scion and the lower part (which provides the roots) is 68 called the rootstock.

Introgression: The transfer of genomic regions from one species into the gene pool of another
 species through an initial hybridization event followed by repeated backcrosses.

Taphonomy: A number of agents that determine the presence and preservation quality of items
 found in sedimentary deposits.

The cultivated apple: The only domesticated species in the genus *Malus*, here called *Malus domestica*. *Malus domestica* is often referred to as *Malus* × *domestica*, the "×" indicating a hybrid origin. However, from what we know so far, the cultivated apple did not originate from a hybrid speciation event. The cultivated apple originated from the Central Asian wild apple *M. sieversii* and was later introgressed by other wild species present along the ancient Silk Road trading routes. We therefore prefer *M. domestica*.

Water flotation: Laboratory technique used to process sediment samples from archeological deposits using the principle of density where a light fraction and a heavy fraction are separated inside the flotation tank. Usually both fractions are dried after the separation process is finished. When waterlogged sediments are processed, the fractions must be stored in water at a temperature at around 4°C.

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86 The history of apples: tangled, gnarly and otherwise hard to unravel

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Recent multi-proxy studies have unraveled the complex histories of seed-propagated crops (e.g. maize [10]), but equivalent research in long-lived, clonally propagating perennials is still lacking. One reason is the difficulty of retrieving suitable ancient remains; another is that genomic resources have only been deployed relatively recently in this type of crop, in comparison of seed-propagated crops. Attempts to combine the perspectives from studies in different disciplines, to make multipleproxy interpretations of the domestication history of apple are rare. As a consequence, answers to many questions are still left for future research.

Apple (Malus domestica Borkh.) is an iconic tree and a major fruit crop in Eurasia that 95 supports many local economies. Thousands of apple cultivars are grown worldwide to produce 96 high-quality fruit for the fresh market, a range of beverages and other food products [11] (Box 1). 97 The past decade has seen tremendous progress in the reconstruction of apple history due to the 98 99 increasing availability of genetic and phenotypic data and archeobotanical information for both wild and cultivated *Malus* species. It is now assumed that the cultivation origins of the apple tree are in 100 the Tian Shan mountains located in Central Asia [9] (Box 1). In classical times, apple cultivation 101 expanded along the Silk Road trade routes linking Asia and Europe and spread from there [9,12,13]. 102 The introduction of grafting techniques facilitated the fixation and propagation of superior 103 genotypes derived from open pollination (i.e. 'chance seedlings'), and caused a revolution in the 104 history of apple cultivation [13,14] (Box 2). For some apple species, including the closest wild 105 relatives to the cultivated apple, the combination of molecular markers and phenotypic traits that 106 were initially used to build the systematics of the genus Malus, has allowed to confirm that some 107 morphological groups are truly genetically differentiated [15-24]. However, the origin of this crop 108 109 and its domestication history - in particular the relationships between cultivated and wild apples - is not yet completely understood. 110

Botanical and archeobotanical evidence, together with genetic and genomic data, has laid 111 the groundwork for a multidisciplinary re-evaluation of the biogeography of wild apples and the 112 history of the cultivated species. Our previous review [9] highlighted the genetics and ecology of 113 apple domestication and its impact on wild apple evolution. In this review, results obtained using 114 botany, archeobotany, archeogenetics/genomics and genetics/genomics are merged to start 115 mapping the extensive history of the cultivated apple. We conclude this review with a discussion of 116 how multidisciplinary research, including recent technological advances in genomics, can be 117 applied to further study of the domestication process of the cultivated apple. 118

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Using archeobotany and the potential of archeogenetics and archeogenomics to reconstruct 120

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the history of cultivation of apples

By fingerprinting present-day apple samples using DNA markers (i.e. microsatellites), the pace and 123 124 process of apple domestication and diversification has been reconstructed. These data support the view that the cultivated apple was domesticated from the wild apple Malus sieversii (Ledeb.) M. 125 Roem. in Central Asia and was then transported westwards along trade routes [9]. Along the way, 126 the cultivated apple hybridized with other wild apple species including Malus sylvestris (L.) Mill, 127 the European crabapple [16,25]. Further data gathering using archeo/paleogenetics and -128 genomics approaches is however still relatively slow [26]. This is due to the shortage of suitable 129 ancient apple remains, slowing the data generation for comparative studies between past and 130 present-day apple samples [9]. Full documentation of this process of domestication, expansion and 131 introgressive hybridization (Box 1) requires suitable amounts and a wide diversity of archeological 132 apple remains from covering the range of the cultivated apple in Eurasia. But ancient human 133 populations did not store apple seeds on purpose at the same scale as this was done for seed-134 propagated crops. As a result, the number of apple seeds in archeological sites therefore remains 135 low in comparison. In addition, ancient apple seed remains from Central Asian archeological sites 136 dating to around 8-10,000 years Before the Common Era (BCE) (Box 3) are scarce due to 137 unfavorable preservation conditions and lack of excavations in certain regions. Additional data 138 could be gleaned by performing new campaigns to gather vegetal remains using water-based 139 sieving techniques in some relevant sites that are already being excavated, e.g. [27]. Another 140 possible source is archived ancient apple seed remains already present in collections (archaeological 141 or herbarium) that could be (re-)investigated using DNA-based techniques. 142

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Archeobotanical insights on apple cultivation 144

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Early apple use by humans started with the gathering of wild apples, then proceeded to increasingly 146 managed wild stands (including cultivation with protection) and ultimately the intentional 147 cultivation of apple trees. However, our knowledge of the evolution of these practices in Eurasia 148 remains patchy at best [28] (Box 3). The knowledge gaps can be explained by a lack of apple plant 149 remains (seeds, fruits, wood or pollen) in archeological sites in the region where apple 150 domestication and diffusion took place. The recovery of apple remains in archeological sites 151 depends heavily on preservation conditions and taphonomic agents. Apple seed testa (which have 152 the greatest chance of being identified to genus or species level) are abundant and frequent in sites 153 with waterlogged deposits (lakeshore settlements, wells, etc.) preserved under anoxic conditions 154

(Figure 1). In contrast, dry sites rarely contain apple seed testa remains, as they can only be 155 preserved in a charred or mineralized state. So far, waterlogged archeological deposits have 156 primarily been investigated in Europe. It is relatively common to find apple halves and some whole 157 apples (Figure 1) that were accidentally charred during drying in ovens prior to storage in Europe. 158 159 These remains would be ideal material for investigating changes in fruit size over time (Figure 1), but this has not yet been attempted. Such charred remains can also be found in dry sites, where their 160 detection depends primarily on the intensity of sampling and the use of water flotation to retrieve 161 small plant remains from archeological sediments. 162

This combination of factors has resulted in a higher number of apple seed remains from 163 Europe in comparison to those from Asia. We can now reconstruct apple use before and after the 164 arrival of cultivated apples in Europe (Box 3). Unfortunately, the few finds from Kazakhstan, 165 Mongolia, Nepal, Tajikistan and Turkmenistan only date to the last 3,000 years [27,29,30]. This 166 prevents us from tracing earlier cultivation or domestication in this area, which is supposed to be 167 the home of apple cultivation [9,31]. It is also difficult to distinguish between the remains from wild 168 and domestic forms of apple based on the morphological characteristics of seeds, fruits, pollen or 169 wood alone [32,33] (Figure 1 and Box 3). Often identification to the genus level is not possible, 170 leading to descriptions such as Malus/Pyrus or even Maloideae (also referred to as Pomoideae tribe 171 - we will use the term Maloideae hereafter) in case of wood or pollen. In only one medieval site in 172 the Nepalese Johng Valley, two seeds could be determined as *M. baccata* [34]. New digital image 173 analysis techniques such as those applied to the Rosaceae family, combined with genetic markers 174 and genome data (nuclear or chloroplastic, see below), might prove useful for the Maloideae and 175 could improve the available data at the genus level [35]. 176

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178 Ancient DNA investigations of apple remains

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Only two studies have used ancient DNA (aDNA) techniques to infer the history of apple gathering, 180 use and cultivation; both studies used data from European sites [28]. First, Schlumbaum et al. [28], 181 investigated waterlogged bulk samples of testa fragments from two Neolithic and two Roman sites 182 in the Alpine foreland using chloroplast and nuclear markers. Chloroplast aDNA, present in many 183 copies in one cell, has more chances to survive than single copy nuclear aDNA [36], but nuclear 184 aDNA bi-parental inheritance has the potential to reveal signatures of hybridization between 185 different species, sub-species and even cultivars. The use of chloroplast markers was unsuccessful 186 in that study, but a nuclear ITS1 region (internal transcribed spacer region 1 of the ribosomal DNA) 187 was successfully typed in two Roman samples, confirming that they belonged to the genus Malus. 188 The retrieved aDNA ITS1 sequences were identical to each other and were shared with wild M. 189

sylvestris and M. sieversii as well as with apple cultivars. Recent DNA studies also reported similar low power to discriminate among species [12,31,37]. This genome region was therefore not informative to discriminate among apple species, in particular between M. domestica and its close relatives M. sieversii and M. sylvestris. However, the study by Schlumbaum et al. [28] demonstrated that it is feasible to use waterlogged remains to at least identify the taxonomic status of seed remains as belonging to genus Malus vs. Pyrus.

More recently, Fietta et al. [38] used aDNA techniques to characterize 23 ancient apple 196 seeds from Europe, covering a large time scale and geographical range: ten archeological sites 197 spanning a period from the Bronze age (3300 years BCE) to the Middle Ages (1492 Anno Domini, 198 "AD" hereafter) collected in locations distributed from England to Estonia. A small target ITS1 199 region (about 80-150 bp) [39] was typed, but only four specimens out of the 23 (i.e. amplification 200 success of 17%) delivered useful data. Among these, two specimens had been retrieved at a Middle 201 Ages archeological site in Tartu (Estonia) and two at a late Roman (AD 300-400) archeological site 202 203 in London (UK). The two Estonian samples formed a monophyletic group with the available GenBank ITS1 sequences belonging to M. sylvestris, M. domestica, Malus pumila Mill., Malus 204 hupehensis (Pamp.) Rehder, and Malus caspiriensis Langenf., with a high similarity (98% blastn 205 sequence similarity) with a Russian specimen of *M. sylvestris*. One of the specimens from the 206 London site also showed a close similarity with the Russian M. sylvestris sample. Again, ITS 207 markers did not distinguish between cultivated and wild apples, but did allow the taxonomic 208 identification of the genus Malus of waterlogged seed remains. 209

These studies demonstrate that morphological and genetic identification results coincide and that morphological criteria for the distinction between *Malus* and *Pyrus* are reliable, at least in wellpreserved samples. However, these two studies also highlight that higher-resolution genetic markers are needed to be able to distinguish between wild and cultivated apple. Such markers are also needed to draw comparisons with present-day apple samples using population genomics and genetic approaches.

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217 Archeogenomics holds great promise

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Recently, advances in **archeogenomics** have opened new perspectives for aDNA studies in woody plant perennials and will likely offer new opportunities for the investigation of the origin and domestication of the cultivated apple. Plant aDNA extraction protocols, e.g. [40], and the use of next generation sequencing technologies allow the sequencing and reconstruction of plant genomes (e.g. [5,10]). This is especially true for DNA extracted from ancient apple remains, as it is currently accepted that either waterlogged or desiccated plant materials, including wood, are more suitable

for archeogenomics [41,42] than charred plant remains [43]. Many new techniques are introduced 225 in aDNA studies. Sequence capture or target enrichment is particularly elegant and effective [39] 226 because it reduces contamination risks and can target several genomic regions simultaneously. 227 DNA regions both in the nuclear and plastidial genomes, known to be informative when identifying 228 229 different Malus species and cultivars, can be targeted. Such archeogenomics data, combined with the increasing number of genomic resources available for the genus Malus (see [24] for a general 230 review), will enable tracing and follow-up of polymorphisms and alleles in functional genes that are 231 known to have played a role in domestication. Examples are genes involved in water and nutrient 232 use, dormancy, flowering time, fruit ripening, color, acidity, or disease resistance [24]. 233

In summary, current archeogenomics techniques open perspectives for significant progress in reconstructing the pace and process of apple domestication. The success of this endeavor will depend on strong collaboration between population genomicians, archeobotanists and archeogenomicians. First they will have to work together to retrieve biological apple remains from key archeological sites such as those located in Central Asia and along the Silk Road, and then to apply state-of-the-art sequencing and population genomics methods to the found remains.

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241 Genomic insights into apple history

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For decades already, population genetics and genomics studies have provided insights into the 243 history of the cultivated apple and its wild relatives, as well as some clues about the genomic basis 244 of domestication. However, genomic evidence has only been generated for a few traits relevant to 245 apple domestication and cultivation (e.g. fruit size and color). Little is known about the role of the 246 crop-wild hybridization that occurred between the cultivated apple and its wild relatives in the 247 248 adaptation of the cultivated apple to different environments in Eurasia and beyond. Knowledge of the untapped genetic diversity available in wild apple relatives is highly relevant to modern crop 249 improvement [44]; recent advances in genomics hold great promise in this area. 250

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252 Apple diversification and domestication: from population genetics to genomics

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Population genetics approaches have generated insights into the origins of the cultivated apple, its domestication history and its spread by human populations, and the biogeography of its wild relatives, i.e. *M. sieversii and M. sylvestris*. In a recent review we have summarized the available knowledge on these topics based on the use of classical genetic markers such as microsatellites [9]. Genetic analyses support a Central Asian origin for cultivated apple, together with a large secondary contribution from the European wild apple (*M. sylvestris*) to the genetic makeup of the crop. These genetic studies revealed that wild apple species display weak spatial population structure, reflecting high levels of gene flow and high levels of introgression from the domesticated apple [9,45]. Also within the cultivated gene pool, weak levels of structure have been detected [25,46–50]. Genetic research based on a few marker loci has thus revealed a major role of hybridization during the domestication of the apple tree.

Recently, next-generation DNA sequencing technologies have made it possible to generate 265 annotated genome assemblies of different genotypes and to produce vast amounts of DNA sequence 266 data for a relatively large sample of wild and cultivated apple genotypes, enabling (i) the 267 exploration of the genomic changes that have taken place during domestication and breeding, and 268 (ii) the analysis of wild apple species evolution and diversification. For example, a de novo 269 assembly of a doubled-haploid derived from the cultivar Golden Delicious (line GDDH13) using 270 third-generation sequencing technologies has provided a high quality reference genome [51] that 271 complements the previously published genome sequence of Golden Delicious [52]. Interestingly, 272 the exploration of the GDDH13 reference genome revealed a burst of transposable elements whose 273 timing coincided with the uplift of the Tian Shan Mountains. An additional high quality genome 274 assembly of the anther-derived homozygous HFTH1 revealed extensive genomic variation 275 compared to the GDDH13 reference genome, largely explained by the activity of transposable 276 elements [53]. In particular, the authors pinpointed a long terminal repeat retrotransposon insertion 277 ("redTE") upstream of a gene (MdMYB1) known to be a core transcriptional factor of anthocyanin 278 biosynthesis and associated with red skin color. Repetitive elements are therefore at the core of the 279 cultivated apple genomic architecture, and might have played a central role in the evolution of this 280 genus as demonstrated in other crops such as maize and sunflower [54,55]. Further, analysis of 281 patterns of DNA methylation showed that epigenetic factors may contribute to agronomically 282 283 relevant aspects such as fruit development [51]. Also recently, Duan et al. [12] used genome resequencing approaches to generate a detailed genome variation map of 117 apple accessions from 284 24 species, and comprising 35 samples of cultivated apple (scions and rootstocks), 39 samples from 285 recognized wild contributor species (10 M. sylvestris and 29 M. sieversii), and 21 from other species 286 neglected in previous studies. This genome-wide dataset has provided insights that complement 287 those generated by studies based on small sets of genetic markers regarding phylogenetic 288 relationships among the cultivated apple and its wild relatives (for an overview see [9]). Genomic 289 regions determining fruit firmness and flavor were detected using a genome-wide association study, 290 and the effect of domestication and selection on these regions was described [12]. All of the 291 gathered evidence supported a model of apple fruit size evolution comprising two major events: one 292 occurring prior to domestication and the other after/during domestication or recent breeding. 293 Similarly, but without the use of genome-wide data, Yao et al. [56] found that a microRNA whose 294

expression is associated with fruit size was fixed in cultivated apple and in its wild progenitors with 295 large fruits. This indicates that selection for fruit size was initiated before domestication. Genomic 296 analyses therefore suggest that apple differs from annual crop models, such as maize [57], where 297 selection for most key agronomic traits likely occurred after domestication [52]. To date, these 298 299 genomic studies of apple have not integrated an estimate of the timing of selection in the context of a pre-existing or post-domestication adaptation to a new environment (e.g. climate, pathogens, 300 humans). Nor have they tested whether such genomic regions are the result of crop-wild 301 introgressions following apple domestication. 302

Combined genomics and archeogenomics analyses of present-day and ancient samples (e.g. charred apples or apple halves found in archeological sites), respectively, could provide temporal multi-proxy data that would make it possible to decipher the origin and further selection of traits relevant for apple breeding such as dormancy, regulation of flowering, fruit size, ripening, color, acidity or disease resistance (see [24] for a general review).

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309 Introgression as an essential player in apple diversification

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While inference from quantitative genomics and phylogenomics contributes significantly to our 311 understanding of apple evolution, other issues may be better investigated at the population level. In 312 particular, as explained above, crop-wild hybridization is at the core of apple evolution. Such 313 hybridizations followed by successful establishment of wild beneficial alleles into domesticated 314 populations and vice versa can occasionally trigger adaptation. For example, adaptive introgression 315 from highland teosintes has contributed to maize highland adaptation [57]. However, to date no 316 studies have been published on the adaptive consequences of crop-wild introgressions in apple and 317 their genomic basis. The development of new population genomic inference methods makes it 318 possible to identify the dynamics of gene flow and positive selection between populations [24,58– 319 61]. In combination with a comprehensive sampling of both local wild populations and of landraces 320 cultivated along the Silk Road, this offers opportunities to explore the timing, genomic architecture, 321 and regimes of selection acting in the introgressed regions in wild and cultivated apple. 322

The role of adaptive introgression driven by **balancing selection** at self-incompatibility loci in favoring crop-wild pollination and introgression during domestication also deserves further investigation using next-generation sequencing [62]. It is widely assumed that natural selection should favor introgression for alleles at genes evolving under multi-allelic balancing selection including self-incompatibility in plants [63]. Apples are characterized by gametophytic selfincompatibility (GSI) which shows two distinct components: one that determines the pistil specificity (S-RNAse gene) and another that determines the pollen specificity (SFBB gene), called

S-genes. The locus that contains the genes determining GSI specificity is called the S-locus. The 330 studies that investigated the evolution at the GSI system in Malus (and other Rosaceae) have non-331 exhaustively described hundreds of alleles of the S-RNase and SFBB genes obtained by PCR-based 332 genotyping, Sanger sequencing, or DNA gel blotting for a limited set of accessions [64-72]. 333 334 However, the architecture of the S-locus, its variability and the respective selective regimes acting on it across wild and cultivated apple are still little explored. The extremely high nucleotide 335 diversity, structural rearrangements and repetitive sequences are the reason why traditional 336 sequence analysis is impractical [73–75]; however, see Veeckman et al. [76] for methods and tools 337 to reconstruct loci with a high level of complexity in highly heterozygous species using next-338 generation sequencing and advanced bioinformatics tools. To decipher the role of the S-locus in the 339 adaptive introgression during apple domestication, one must get a detailed view of polymorphism of 340 the whole region from geographically widely distributed wild and cultivated apple samples. 341 Recently, using RNA sequencing, Pratas et al. [77] identified 24 SFBB genes expressed in anthers 342 and determined their gene sequence in nine *M. domestica* cultivars. Whole-genome information has 343 also become publicly available for apples, including the high quality reference genome and the re-344 sequenced genomes for more than 100 wild and cultivated apple accessions. It is therefore timely to 345 take advantage of these data to explore species-wide polymorphism of the whole S-locus region and 346 its role in the evolution history of apples during domestication. Such investigations would 347 complement archeogenomics data that is difficult to use for the reconstruction of the S-locus, as that 348 would require high quality and long fragment data. 349

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351 Knowledge of apple domestication and diversification underpins future breeding

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Crop wild relatives (CWRs) are an invaluable resource for apple breeding [78]. However, the wild 353 genetic resources remain largely unexplored [44]. For instance, M. baccata (L.) Borkh., the Siberian 354 crab apple, is one of the main genetic resources for breeding programs, particularly in China, 355 because of its excellent resistance to cold stress and apple scab [79]. Likewise, Malus floribunda 356 Siebold ex Van Houtte, M. hupehensis, Malus zumi (Matsum.) Rehder, Malus × robusta (CarriŠre) 357 Rehder and *Malus sieboldii* (Regel) Rehder could provide valuable genetic variation for developing 358 elite cultivars and rootstocks that would be better adapted to specific biotic and abiotic stress 359 conditions [80]. The use of CWRs is also particularly relevant when considering the important role 360 played by hybridization and introgression in the domestication process of *M. domestica* [9]. Several 361 disease resistance genes implemented in commercial apple breeding were introgressed from a wild 362 Malus species. Well-known examples are the Rvi6 gene from M. floribunda, which confers 363 resistance to apple scab [81] or the FB_MR5 gene of $M. \times robusta$, which confers resistance to fire 364

blight [81,82]. In the M. sylvestris - M. domestica pair, the lack of strong reproductive barriers or 365 spatial distance between apple orchards and *M. sylvestris* populations [17] has resulted in the spread 366 of alleles from the cultivated gene pool to populations of the wild species [17,18]. This has 367 generated a number of unmanaged wild apple M. sylvestris populations in Europe with introgressed 368 369 crop alleles [17,18]. These trees may have become unintended repositories of alleles that are no longer available in breeding germplasm, or they may represent an untapped reservoir of adaptive 370 alleles. To date, the lack of cost-efficient methods to screen CWR genomes at high resolution to 371 identify genomic regions associated to traits of relevance in breeding has hindered the use of CWRs 372 in apple breeding. 373

Recent technological innovations have generated in-depth knowledge of the genes 374 underlying specific adaptations to relevant biotic and abiotic factors. These innovations hold great 375 promise to further accelerate this discovery process. For instance, next generation sequencing 376 technologies have been used to unravel the genes underlying resistance to pathogens, such as blue 377 mold (Penicillium expansum) infection in M. sieversii compared to M. domestica [83] and to 378 identify QTLs (Quantitative Trait Loci) controlling resistance to this pathogen in *M. sieversii* [84]. 379 These studies illustrate the efficient use of genomic tools to understand domestication and selection 380 in apple and to identify useful genetic variation in wild apple species [85,86] that might open new 381 perspectives for breeding. 382

A major bottleneck is the total lack of systematic, range-wide and genome-wide collections 383 for any of the wild apple relatives (M. sieversii, M. sylvestris, M. orientalis Uglitzk, M. 384 niedzwetzkyana Dieck) and more ancestral species (M. baccata, M. floribunda, M. florentina 385 (Zuccagni) C.K.Schneid.)). Sampling these taxa will offer clear opportunities to: (i) explore the 386 evolutionary history of apple; (ii) underpin the genomics of adaptation in vegetatively propagated 387 388 perennials; and (iii) tackle challenges of modern apple breeding by characterizing the genomic variation underlying fruit-related traits and apple adaptation to the biotic and abiotic environments it 389 encountered during its spread along the Silk Road. Archeobotany and archeogenomic studies of 390 apple seed remains from different periods of time will also perfectly complement present-day 391 genomics datasets to bring new insights regarding key traits involved in domestication and recent 392 diversification. 393

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395 Concluding remarks

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The botany, taxonomy and genetics of apples has been a tantalizing research subject for some time [13,31]. Interdisciplinary investigations have allowed (at least partial) elucidation of some of the processes underlying apple domestication and the history of wild apples in Europe, where apple

remains have been systematically recovered from archeological deposits since the beginning of the 400 Holocene. These studies have demonstrated that apple is a suitable study model to unravel a process 401 of divergence and adaptation with gene flow [9]. However, major issues remain to be resolved (see 402 Outstanding Questions). Current limitations include potential difficulties in species identification of 403 404 archeological remains and the lack of archeobotanical samples in and around the area of origin in Central Asia. However, all of the modern technologies can now be applied to address these issues: 405 easier genome retrieval from modern individuals, genomic approaches on ancient waterlogged and 406 desiccated plant remains including wood, and novel morphometric approaches in archeobotany. We 407 therefore anticipate that genomics and archeogenomics will assist in deciphering apple evolution. 408 Retrieval of representative samples from the centers of domestication in Central Asia and centers of 409 diversification in Europe and in the Caucasus remains an important concern. The risk of loss of wild 410 apple populations in their area of origin and beyond due to human encroachment and climate 411 change has become alarming, underscoring the urgency of investigating this iconic crop. These 412 findings should contribute to sustainable management of cultivated apple germplasm for future 413 breeding programs and the conservation of wild apple populations that are often located in 414 developing countries and subject to increasing anthropogenic threats. 415

416

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418

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Box 1. On the cultivation of apples, and its past and present use worldwide

The commercial apple (*Malus domestica* Borkh.), sometimes referred to as the "Grand King of Fruit Crops", is a deciduous species belonging to the Rosaceae family. It is cultivated mostly in temperate zones and consumed either fresh or in food products worldwide. Thanks to its (eco)physiological properties and grafting techniques, the species can survive in cold environments by reducing the risk of damage during autumn and spring frost events [87,88].

Known for its fruit qualities and characteristics, it has been cultivated since ancient times 633 [13]. The so-called Silk Road, a network of ancient trade routes from Asia to Europe in existence 634 from as 3500 BCE to the mid-1400s, promoted the spread of apples into the European continent. 635 The Greek philosopher and botanist Theophrastus (ca. 320 BCE) studied the apples brought to 636 Greece by Alexander the Great and described six apple cultivars and cultural practices such as 637 general tree care and grafting. It is believed that the Romans introduced some of today's apple 638 cultivars to the British Isles. Canadian settlers introduced this fruit to North America, with the first 639 commercial apple orchard in the U.S.A. established ca. 1600 [13]. 640

Apples are now consumed worldwide as either fresh fruit or as the basis for various food 641 products (e.g. dried, juice, cider, purees, etc.). Over 89 million tons of apples are produced annually 642 worldwide, making this fruit the third the most popular in world 643 (http://www.fao.org/faostat/en/#data/QC). The largest producer is China, representing around 40% 644 of the world's total production, followed by the United States, with over 4.5 million tons per year. 645 Italy, France, Germany and Poland are the biggest apple producers 646 In Europe. (www.yara.us/agriculture/crops/apple/key-facts/world-apple-production/). Product 647 quality is ensured by intensive cultural and pest management practices as well as harvesting at proper 648 maturity, storage, and shipping under highly managed conditions. 649

650

Box 2. The rootstock and the scion: Key components of large-scale apple cultivation

To obtain the uniformity required for profitable production, the commercial apple tree is a 652 composite biological unit: a combination of rootstock, scion, and sometimes an interstem. Such 653 rootstock/scion/interstem combination is created by either **grafting** or budding [89]. These asexual 654 propagation practices are essential for commercial production, because apple seedlings derived 655 from a single tree are genetically and phenotypically highly heterogeneous due to their forced cross-656 pollination due to self-incompatibility [77,90]. The cultivated apple tree is therefore a complex 657 biological entity that requires a vast array of knowledge from scientists, consultants and growers to 658 ensure a high quality product for the market. 659

660 Invented in East Asia about 4,000 years ago, the grafting of scion onto a rootstock created a 661 revolution for apple spread and cultivation [9,91,92] The use of dwarfing rootstocks has facilitated

the establishment of high-density orchards (2,000 to 3,500 trees/hectare). It has also been shown 662 that more dwarfing rootstocks induce the scion cultivar to flower and bear fruit earlier than less 663 dwarfing rootstocks [93-95]. Hypotheses of the biological mechanisms involved in scion size 664 control and precocity include hormone translocation and changes in fluxes of nutrients, water, and 665 assimilates between rootstock and scion [94]. Higher light-use efficiency in high-density orchards 666 can have a positive effect on fruit size and color as well as on fruit production. In general, the more 667 dwarfing the rootstocks, the better they perform in high-density planting, generating earlier 668 economic returns [96]. 669

The interaction between the rootstock and the scion is complex. For example, the most 670 commonly used M.9 rootstock (dwarfing) has the highest ratio of crop to scion when compared to 671 more vigorous rootstocks [97]. However, choice of rootstock can also influence productivity [93] 672 and other factors such as precocity, yield, environmental and edaphic adaptability, light 673 interception, and disease and pest resistance [97,98]. Scion selection is an existential question when 674 selecting for desired fruit qualities and tree characteristics. Important traits influenced by scion 675 include fruit quality (size, shape, color, sweetness, tartness, firmness, flesh color, etc.), vield, 676 disease resistance, time of harvest, and storage potential [93,97]. 677

Worldwide there are an estimated 6,000 to 10,000 scion cultivars [49]. Frequently, new cultivars arise as spontaneous mutations, often called 'bud sports' (i.e. 'Red Delicious'), but several private and public breeding programs also create new apple cultivars via crossing and selection.

681

682 **Box 3. Some archeobotanical evidence of apple cultivation**

A first glimpse into apple use and cultivation in Europe is coming into view. We have compiled archeobotanical data from about 600 sites located in 34 countries to give an overview of the use of apple in prehistoric and historic times (Figure I). The compilation is based on findings of apple in Eurasia, identified as *Malus* or *Malus/Pyrus* (or Maloideae). In these studies, the small plant remains from soil samples are retrieved using **water flotation** techniques [98].

Most remains of *M. domestica* identified are from the Roman period (ca. 2,100-1,500 BCE) 688 onwards (Figure I). The increase observed since the Roman period could be a result of the spread of 689 the cultivation of cultivated apple in central and northern Europe. The important representation of 690 wild apple remains during the Neolithic period can be interpreted as an evidence of the efforts made 691 to manage the landscapes around the sites in order to increase the productivity of certain trees such 692 as hazel, apple or oak, and to protect them from domestic animals (e.g. [99]). Malus sylvestris needs 693 a lot of light, thus it was unlikely to be common in prehistoric forests in Europe; instead it would 694 have been found on floodplains, hedges and thickets [100]. One good example for this is the Zürich-695 Parkhaus Opéra site (dated to ca. 5,100 BCE), where the excellent preservation conditions made it 696

possible to calculate that between 190 and 400 kg of apples were gathered yearly per domestic unit 697 in average over a period of 15 years [101]. These amounts clearly exceed the normal productivity of 698 a natural woodland environment in the Lake Zürich region. For some of the northernmost regions of 699 Europe it has been proposed that early farmers could have spread wild apples [100], which would 700 also indicate a high degree of management of M. sylvestris. The land use system changed 701 significantly afterwards, with larger areas of cultivated land and less access to woodland. The 702 consumption of wild apples also appears to have decreased (note, however, that this observation 703 may be biased due to the state of research and taphonomy issues). A significant increase of apple 704 705 consumption probably only started with domesticated apple cultivation (see also [100]).

- 707 Figure captions
- 708

Figure I. Presence of apple remains recorded from archeobotanical studies a. in different types of preservation conditions, b. for different regions, c. for different chronological periods.
Similar temporal trends are shown in the different regions, with a high number of records for the Neolithic period (ca. 8,000-4,500 BCE), a decrease in the Bronze and Iron ages (ca. 4,500-2,100 BCE), followed by an increase in Roman times and a generally good record of medieval and post-medieval finds.

715

Figure 1. Photography and sizes of seed remains of apples. From left to right and from top to 716 bottom: Seed of Malus sylvestris (Zürich-Parkhaus Opéra), pericarp of Malus/Pyrus (Zürich-717 Parkhaus Opéra, form (Photo credits: G. Haldimann) [102]; charred crabapple (Photo credits: W. 718 Kirleis); charred apple half (Photo credits: W. Kirleis). Fruit size is used as a criterion to distinguish 719 between wild and domesticated apple in Europe. Charring has an important effect on fruit size, as 720 fruits can become ca. 1/3 smaller after charring [33]. Before charring, wild apples were 20-36 mm 721 wide and 18-36 mm long (weight 5-16 g). After charring, wild apples measure 15-25 mm wide and 722 15-26 mm long. Most charred archeological remains of wild apples fit within these dimensions and 723 the results agreed with an average of 27 mm in diameter for charred wild apple [103]. Within this 724 genus, the differentiation of seed morphology between species has been little explored, but the 725 morphological differentiation of seeds between Malus sp. and Pyrus sp. is relatively well defined in 726 the archeobotanical literature. Seeds of apple are wider and rounded in the apex, in contrast to those 727 of pear. They also have fibrous lengthwise striations on their surface (e.g. [104]) that make them 728 well recognizable in both charred and uncharred states (Figure I). Pericarp fragments can be found 729 730 in archeological sites as well. In addition, the inner wall of the endocarp (also called pericarp or carpel in the literature) of apple seeds is shiny, and presents undulating fibers on the inner side 731 (Figure I). This allows identification of apple pericarp fragments found in archeological sites. 732

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