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FROM FIELD TO TABLE: PERSPECTIVES AND POTENTIAL FOR FRUIT DOMESTICATION

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ABSTRACT

Domestication, the evolution of species in response to human selection, is the foundation upon which agriculture is built. Most contemporary crops are the products of evolutionary processes that began thousands of years ago, and that continue today as scientists harness emerging technologies to develop new crop varieties for a rapidly changing world. Current understanding of evolution under domestication is based primarily on annual plants, often self-compatible species that are propagated from seed each year. However, attention is refocusing on the development of perennial crops as concerns mount about a growing population, a changing climate, and sustainable agriculture. The domestication process in perennial fruit crops departs from that observed in annuals due primarily to differences in breeding systems (most perennial plants are outcrossing) and mode of reproduction (many perennial crops are clonally propagated). These differences have implications for two important aspects of perennial fruit crop domestication: 1) the extent and structure of population genetic variation in cultivated populations and their wild progenitors and 2) the genetic basis of agriculturally important traits. In order to better understand the hallmarks of perennial plant domestication, and to understand how perennial crop domestication might proceed in the future, we look to perennial fruit crops that have been evolving under domestication for thousands of years, including the apple (*Malus domestica*) and grape (*Vitis vinifera*) and their wild relatives. Using these examples, we explore the geographic and taxonomic mosaic of perennial crop domestication, the impact of genetic bottlenecks on variation in cultivated populations, crop-wild gene flow, and the genetic basis of phenotypic variation. We emphasize the importance of variation housed in wild-relatives for breeding fruits as well as rootstocks. These two iconic crops provide an important roadmap for exploring how best to conserve naturally occurring variation in perennial plant species and how to utilize it in plant breeding.

Keywords: perennial fruit crops, domestication, genetic variation, *Malus domestica*, *Vitis vinifera*, gene flow

INTRODUCTION

The global significance of plant domestication cannot be overstated, as all modern food plants are the products of domestication, and future improved or new crops will necessarily undergo this process as humans strive to meet the needs of a growing population and a changing climate. Crop populations originate with the transfer of seeds or cuttings from natural settings to agricultural landscapes. As farmers identify individuals with traits that enhance crop production, and remove individuals with undesirable traits, this selective cultivation over the course of many generations causes crop populations to diverge morphologically and genetically from their wild progenitors. Although the domestication process may have started thousands of years ago for many plant species, it is not merely a phenomenon of the past. Today, ongoing domestication efforts occur primarily through targeted plant breeding programmes guided by modern genetic and genomic approaches. Contemporary domestication includes both the continued improvement of crops that originated thousands of years ago (like wheat and beans), as well as attempts to domesticate species that have not previously experienced artificial selection (like some species being used for biofuels). Indeed, the evolution of crop plants under domestication is the primary pathway to improving nutrition, yield, and resistance to abiotic and biotic stress under current and future predicted climates in the world's food plants.

The origins of domestication trace back nearly 10 000 years to the transition of humans from hunter-gatherer populations to agricultural societies. Several lines of evidence support multiple, independent origins of agriculture in at least seven geographically distinct regions (Vavilov, 1992). These agricultural centres are also "centres of domestication", geographic regions where the majority of crops originated, and that today retain important genetically variable and agriculturally valuable wild progenitors of modern crops. Originally, it was thought that for a given crop species, wild plants from a single geographic region at a single time point were taken into cultivation, followed by subsequent rounds of selection on cultivated individuals to generate the domesticated lineage (Zohary, 2012). However, more recent analyses suggest domestication likely involved multiple origins of a crop from wild populations over the course of many years, and perhaps from different geographic regions within a domestication centre (Brown *et al.* 2009). Today, domestication is viewed as a dynamic evolutionary process that occurs across broad spatial and temporal scales. Founder effects associated with the establishment of crop populations, ongoing artificial selection, and other evolutionary processes such as crop-wild gene flow, continue to contribute to the pace of plant evolution under domestication.

Agricultural societies are based primarily on domesticated annual plants that are usually self-fertile, and are propagated from seeds (Glémin and Bataillon, 2009). Not surprisingly, much of our current understanding of plant evolution under domestication is based the effects of genetic drift and artificial selection on these annual plant species (Hancock, 2005). For example, scientists have described a suite of traits in members of the grass family that change in predictable ways under domestication, including loss of shattering, synchronous flowering, larger fruits/grains, and more numerous fruits/grain per inflorescence (Glémin and Bataillon, 2009). Surveys have also shown that annual plant domestication is often accompanied by a domestication bottleneck (i.e. a reduction in genetic variation in cultivated populations relative to their wild relatives) (Miller and Gross, 2011). Finally, a large body of work has identified the genetic basis of many domestication traits, and this work shows that some traits are the result of single or few loci of large effect, while other domestication traits result from myriad, interacting loci of small effect (Olsen and Wendel, 2013). The majority of the calories consumed by humans are derived from annual grains and legumes; without doubt, these plants will continue to form the foundation of agriculture. However, as concerns mount about a changing climate and the sustainability of modern agriculture, attention is focusing on the potential of perennial plants, which offer promising options for food production while decreasing environmental impacts, and will likely play an increasingly important role in food production in the future.

PERENNIAL PLANTS HAVE BEEN IMPORTANT COMPONENTS OF AGRICULTURAL SOCIETIES SINCE THEIR INCEPTION

Perennial species make up between 35 and 80 percent of the total number of plant species domesticated in each of the major centres of origin (Meyer *et al.* 2012; Zohary, 2012). Perennial crops include a variety of herbaceous and woody plants that live for more than two years. These crops represent a broad range of plant families, and generally fall into two categories: 1) perennial species that are grown for their roots or other below-ground vegetative components, and 2) perennial species that are grown for their fruits. Although perennial plants that produce edible roots, tubers, or fleshy fruits have been cultivated for thousands of years (e.g. apple, grape, horseradish, potato), to our knowledge perennial grains have been conspicuously absent from agriculture (Van Tassel *et al.* 2010). The wide diversity of geographic and phylogenetic origins of traditional perennial crops means that each domesticated perennial is likely to have some unique features. However, it is possible to identify a general domestication syndrome associated with the evolution of perennial plants in response to artificial selection, which will be informative as breeders look towards domesticating other perennial species, including grains.

Current understanding suggests that annual plants preceded perennials in domestication, with perennial plant domestication reaching its first peak of activity 4 000 years ago (Meyer *et al.* 2012; Miller and Gross, 2011). Evidence suggests that this first peak of perennial domestication coincides with the widespread adoption of vegetative propagation. Just as naturally self-compatible annual plants appeared to be predisposed to domestication, similarly, perennial species that could be easily vegetatively propagated were among the first perennials to enter the domestication process. Interestingly, it seems that perennial grasses and legumes may have been overlooked by early farmers because natural selection had not favoured high seed production and ability to self-pollinate in perennial species to the extent that it had in annuals. In other words, annual grains may have been favoured over perennial grains historically because of their wild ancestors' higher seed productivity and/or greater ease of propagation (Van Tassel *et al.* 2010).

How do perennial plant species evolve under domestication? This question lies at the core of contemporary research programmes aimed at developing perennial grains and legumes; however, compared with annual crops, relatively little is known about how perennial species change in response to human selection. For example, what are the main traits that are under selection during perennial crop domestication? What is the extent and impact of domestication bottlenecks and artificial selection on genetic variation in cultivated populations of perennial plants? What is the potential for crop-wild or wild-crop gene flow in long-lived species? What is the genomic basis of domestication traits in perennials? Understanding evolutionary processes associated with perennial crop domestication will inform conservation strategies aimed at preserving genetic variation in cultivated populations and their wild progenitors, and will facilitate breeding efforts that are based on targeted selection within existing domesticates as well as the development of new domesticates.

WHAT MAKES PERENNIAL PLANTS DIFFERENT FROM ANNUAL PLANTS?

Aside from living for more than one year, perennial plants have several attributes that differentiate them from annual plants and that play a significant role in their capacity for evolution (McKey *et al*. 2010; Zohary and Spiegel-Roy, 1975). Perennial plants often have longer juvenile phases and lengthy reproductive cycles; where it takes an annual one year to grow from a seed and produce another seed, it could take a perennial plant many years before a germinated seed is capable of producing viable offspring. This duration means that it can take several years or even decades for a single generation to be completed. In a one-hundred year time period, an annual plant will have 100 cycles of sexual reproduction on which selection can act; in contrast, a perennial plant could have half that, or much less. In practical terms, this means that evolution could take much longer in perennials than annuals because it takes many more years to achieve the same number of sexual cycles. All things being equal, under this scenario we might expect domesticated perennials to show less divergence from their wild progenitors over time relative to annual plants, because fewer cycles for selection have occurred. However, many perennial plants exhibit stark morphological contrasts compared with their wild progenitors, suggesting that evolution, although operating over fewer sexual cycles, results in clear morphological changes over relatively few generations. This suggests that there may be unique aspects to perennial plants that are not regularly observed in annuals, and that are contributing their evolutionary potential and trajectories.

There are two other features of perennial crops that stand in stark contrast to annual plants, and both are related to reproduction. The first distinguishing feature of perennial species is that they tend to be obligate outcrossers and exhibit a range of mechanisms that prevent self-pollination and/or self-fertilization, including dichogamy, dioecy, or self-incompatibility, among others (Zohary and Spiegel-Roy, 1975). This is in contrast to the self-compatible systems found in most annual crops, either the result of evolution of self-compatibility under domestication or because annual crops were domesticated primarily from wild populations with the capacity for self-fertilization (note that some annual crops are predominantly outcrossing; however, many of the outcrossing annuals, such as maize and pearl millet are self-compatible). Generally speaking, outcrossing functions to increase heterozygosity within individuals, increase variation within populations, and decrease differentiation among populations as individuals exchange genes with plants from nearby populations or wild relatives. Interspecific gene flow likely plays an important role in the origin and evolution of perennial crops (Hughes *et al.* 2007 and see below). In crop populations where reproduction is based solely on sexual reproduction by seed, obligate outcrossing may slow the breeding process because all individuals, including those with the most desirable combination of traits, must hybridize with other individuals to yield seed. This can lead to the dilution of favoured traits in the cultivated population and increase the rate of masking of recessive alleles. On the flipside, obligate outcrossing in crops produces a nearly limitless amount of variation on which natural and artificial selection can act.

The second distinguishing feature of perennial plant reproduction relative to annuals has to do with how the plants reproduce in nature relative to how they are propagated in cultivation. Some perennial plants in natural populations reproduce clonally. This is thought to be a mechanism to enhance the likelihood of long-term viability of a genotype by producing multiple ramets, increasing the probability that at least some part of a genotype could transcend negative stochastic events that occur over the course of an extended lifespan (Vallejo-Marín *et al.* 2010). In cultivation, the majority of perennial crops are vegetatively propagated through layering, cuttings, grafting, or some combination of these three. Vegetative propagation addresses breeding challenges associated with long juvenile phases by instantaneously replicating genotypes with favourable traits. Because perennial crops are outcrossing and individuals are usually highly heterozygous, clonal reproduction replicates those individuals, thus resulting in populations comprising largely heterozygous individuals. However, clonal reproduction can lead to a low level of population variation and a high degree of population structure as entire populations can consist of one or a handful of genotypes. Increasing clonality within populations may be associated with reduced sexual reproduction and/or reduced fertility due to trade-offs associated with increased allocation of resources to vegetative growth, inbreeding depression, or mate limitation (McKey *et al.* 2010). Indeed, mate limitation in clonally propagated perennial plants is consistent with observed shifts toward self-compatibility, or in dioecious species, to hermaphroditism or parthenocarpy (McKey *et al.* 2010; Zohary and Spiegel-Roy, 1975)

In short, evolutionary processes in perennial plants are unique due in part to the combination of long juvenile phases, obligate outcrossing, and clonal reproduction. Even though annual crops undergo yearly cycles of sexual reproduction, seed production in annuals results largely from self-compatible individuals that produce relatively homozygous offspring. In contrast, while perennial crops have to wait years or decades for each cycle of sexual reproduction, the heterozygous individuals produced via outcrossing may be immortalized through clonal propagation. These differences in reproduction have significant implications for the extent and structure of population genetic variation in cultivated populations and crop wild relatives, and also for the genetic basis of agriculturally important traits.

IMPLICATIONS FOR POPULATION STRUCTURE AND THE GENETIC RESPONSE TO SELECTION

Perennial wild species are the sources from which perennial crops are descended. Several properties of perennial plant populations, including an outcrossed breeding system, hybridization with sympatric congeners, and life history strategies, promote variation within natural populations and decrease differentiation among populations (Petit and Hampe, 2006). Evolutionary processes operating in nature establish the highly diverse genetic foundation on which the domestication process is based. Understanding natural genetic variation in crop wild relatives is important for characterizing resources for breeding. In addition, the same evolutionary processes that shape variation in natural populations (outcrossing, intra- and interspecific gene flow, clonal reproduction) have implications for the genetic basis of evolution under domestication.

The trend for wild perennial species, including many of the wild relatives of domesticated perennials, is low population structure and high genetic variation (Petit and Hampe, 2006). These patterns are products of the characteristic perennial life history traits and breeding systems discussed above. In terms of population structure, for example, tree species are predicted and observed to be resistant to founder effects during the colonization of new habitats. This is partially due to the long juvenile phase of most trees, during which time the population can only grow via the arrival of new migrants (Austerlitz *et al.* 2000), and partially due to the rapid restoration of genetic diversity via long-distance pollen dispersal (Hampe *et al.* 2013). Because the changes in allele frequency that occur during mild or severe genetic bottlenecks are an important source of population differentiation, and thus population structure, many tree species (especially wind-pollinated species) will not exhibit population structure. These processes continue in existing natural populations within a species range, and are combined with a pattern of loss of homozygous individuals in population cohorts (Jolivet *et al.* 2013) to contribute to the high levels of genetic diversity seen in many tree species, including crop progenitors. High levels of gene flow often extend to interspecific hybridization among closely related species. Gene flow among close relatives appears common in long-lived species, and has been well documented in systems such as the oaks and poplars (Petit *et al.* 2004; Stolting *et al.* 2013). Nonetheless, tree populations can be vulnerable to the effects of habitat fragmentation, and some studies show that trees in long-term fragmented habitats either show signs of inbreeding or increased genetic structure among younger cohorts (Vranckx *et al.* 2012). This may have important implications for the wild relatives of some crop species, especially those in areas with a long history of human habitation and high population densities.

Interestingly, the high levels of gene flow among populations of tree species do not prevent populations within those species from responding to geographically variable selective regimes. Studies in wild species consistently show that populations are locally adapted along biotic and abiotic gradients within a species range, some of which are quite extensive (González-Martínez *et al.* 2006). In cases where the genetic bases of these adaptive traits have been documented, the underlying loci appear to be numerous and of relatively small effect in terms of the percentage of variation explained (Eckert *et al.* 2010). The population structure, genetic variation, and currently documented genetic basis of adaptive traits in natural tree populations have important implications for the process and genetic basis of domestication in perennial plants. First, the lack of genetic structure means that genetic analysis of crop relatives might be less likely to result in false associations due to linked variation. Genetic structure has presented a major impediment to accurately identifying the genetic basis of selectively advantageous traits in many annual systems (wild and domesticated). Second, if wild perennial populations can adapt to different selective pressures via many changes of small effect, and in the face of gene flow, then it is possible perennial crops may respond to artificial selection in a similar manner. This would stand in contrast to the genetic basis of domestication documented in many (although not all) annual crops, in which domestication traits are controlled by loci or genes of major effect (Purugganan and Fuller, 2009). Progress and challenges in the effort to identify and characterize the genetic basis of adaptation in perennial crops are detailed below.

WHAT CAN BE LEARNED FROM PERENNIAL CROPS THAT HAVE BEEN UNDERGOING DOMESTICATION FOR SEVERAL CENTURIES?

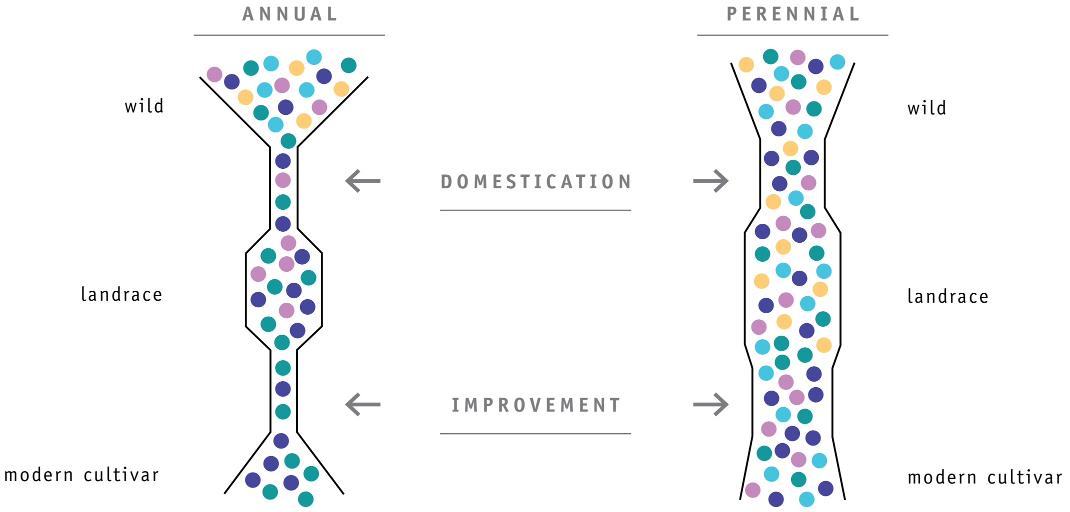
Research indicates that perennial crops originate and evolve in a fundamentally different way than annual crops, and these differences have important implications for crop breeding and improvement (McKey *et al.* 2010; Miller and Gross, 2011; Zohary and Spiegel-Roy, 1975). While more research on the topic is badly needed, current evidence indicates that multiple origins are the rule rather than the exception for perennials, with more than half of the perennial crops studied to date showing evidence of two or more origins (Miller and Gross, 2011). This is particularly interesting in light of the low levels of genetic structure detected in most of the wild relatives of perennial crops; the lack of structure should make estimates of multiple origins fairly conservative because there is not enough differentiation in most of the wild species' range to accurately detect distinct lineages. Moreover, the "more than half" estimate of multiple origins for perennial crops does not include the instances of multiple perennial crops of the same genus – at least six genera contain two or more domesticated species. Taken together, these trends may indicate that some species and genera of perennial crops should be considered good candidates for a new or re-domestication process, or simply for extensive improvement. It is certainly clear that desired traits in these polyphyletic crops can be assembled from a variety of starting points, so redeveloping these traits in a related species or from a new population within the same species should be feasible.

In addition, domesticated perennials appear to undergo crop-wild and wild-crop gene flow. Crop-wild gene flow is a broadly common phenomenon, and has been documented for annual and clonal crops (Ellstrand *et al.* 1999). In perennial crops, the best-documented cases are from some of the older domesticates (grape, olive, and apple), where gene flow has been demonstrated both from the crop into the wild species and vice versa. Gene flow from domesticated lineages into wild populations is an area of concern for conservation reasons; this scenario has been documented for apple and grape (Di Vecchi-Staraz *et al.* 2009; Gross *et al.* 2012). Hybridization between feral and domesticated olive may also be contributing to weed evolution in Australia (Besnard *et al.* 2007). However, there are also more positive outcomes in grapes and apples, were the wild species' contribution to the domesticated lineage may have been a key part of the domestication process, contributing to the development of unique varieties or facilitating the movement of a domesticated lineage into a new geographical region (Cornille *et al.* 2012; Lopes *et al.* 2009; Myles *et al.* 2011). Gene flow may well be more common than it appears to be based on existing studies, as it can be difficult to detect when there is insufficient genetic differentiation between the crop and its wild progenitor. Thus, it is likely that larger marker datasets (i.e. those resulting from next-generation sequencing approaches) will reveal more instances of gene flow in future studies. In light of the information in apple and grape, and in consideration of the propensity for multiple origins of several perennial crops, wild germplasm represents a powerful resource for perennial plant breeding. While the long juvenile phase of most perennial crops can justifiably make plant breeders cautious in the crosses they choose, the relative ease with which the domestication phenotype can be assembled (either from multiple starting points or in the face of gene flow from the wild species) suggests that the time investment may yield a high return.

Perennial crops appear to experience a relatively mild genetic bottleneck associated with domestication (Figure 1). While genetic bottlenecks vary in intensity from crop to crop, for annual species the average reduction in genetic variation during the domestication bottleneck is ~40 percent. This is in strong contrast to the average bottleneck in perennial crops, where the average reduction in diversity is only 5 percent (Miller and Gross, 2011). There are many factors that could contribute to the relatively mild genetic bottlenecks in perennial crops, including the aforementioned trends towards outcrossing (when sexual reproduction occurs), multiple origins of crop lineages, and crop-wild gene flow. Whatever the cause, the mild genetic bottlenecks mean that many domesticated perennials have high genetic variation, often close to what is seen in their wild relatives. The full extent of this variation has not yet been utilized in cultivation or breeding programmes (Myles, 2013; Myles *et al.* 2011). Ongoing efforts to preserve the range of variation under cultivation in living germplasm banks or repositories support an invaluable resource for crop improvement. It is also interesting to consider that the genetic bottleneck that accompanies annual plant domestication is one of the contributors to the phenotypic and genetic differentiation between the crop and the wild species. The loss of diversity during the bottleneck is random, and can lead to loss or fixation of alleles by chance alone; these changes in allele frequency allow the crop to be distinguished from the wild species based on genetic analysis. However, many domesticated perennials can also be effectively distinguished from their wild progenitors, *despite* a very mild bottleneck. Thus, the genetic and phenotypic differentiation must be attributed to other factors, including (both not limited to) artificial selection. Finally, from a plant breeding perspective, the mild genetic bottlenecks in perennial crops mean that genome scans designed to detect regions of low diversity (suggestive of genes under selection) will not be impeded by the confounding effects of a genetic bottleneck, as has been the case for some annual crops (e.g. Hamblin *et al.* 2006).

FIGURE 1. DOMESTICATION AND IMPROVEMENT BOTTLENECKS FOR ANNUAL VERSUS PERENNIAL CROPS

Different coloured circles represent variation in alleles or phenotypes present a given species undergoing domestication. The width of the funnel represents the relative population sizes during different stages of domestication. Variation is either lost during the domestication and improvement process in annual crops, or retained through these processes in perennial crops.



GENETIC BASIS OF DOMESTICATION TRAITS

Evolutionary biologists and plant breeders have pursued a variety of approaches to elucidate the genetic underpinnings of domestication traits in perennial crops. The primary tool used thus far has been QTL mapping, and it has been applied to a number of perennial crop systems (see Miller and Gross, 2011). When QTL maps are based on crosses between two cultivars, they are very useful for pinpointing the genetic basis of agronomically valuable traits that segregate within a domesticated lineage. However, they tell us little about the genetic basis of domestication traits – the traits that evolve during the domestication process and that differ between wild and domesticated plants. The general trend of the QTL mapping studies (either within the crop or in the few existing crop-wild crosses) indicates that domestication traits are underpinned by many QTL of small effect, although QTL of major effect have also been observed.

While QTL mapping has been very useful in the quest to identify loci associated with traits of agricultural importance with the goal of food improvement, it is also subject to several weaknesses. One of these is the high variability of QTL detection across populations and across years within populations (Crouzillat *et al.* 2000; Kenis *et al.* 2008). While this is expected to some extent (the expression of phenotypic traits in an orchard is dependent on environmental influences), the high variability makes it difficult to move forward to map-based cloning, a technique used to identify many domestication genes in annual plants. Efforts to clone the genes underlying these traits are also hindered by the fact that fine mapping requires analysis of hundreds of crossover events – the equivalent of a mapping population with at least 500 (and usually over 1 000) individuals. This is clearly not a realistic goal for every large-statured, perennial crop - the monetary and temporal investment required to maintain plants through their juvenile phase to maturity is beyond the reach of most individual researchers, and many institutions. Instead, investigations into domestication genetics and plant breeding are utilizing techniques that do not require mapping populations, but rely on existing variation and the power of massively parallel high-throughput sequencing techniques (i.e. “next generation sequencing” or NGS). Here we discuss the initial application of these techniques and their potential application to perennial crops in the future.

Genome-wide association studies (GWAS), also referred to as association mapping, is an excellent alternative to QTL mapping in perennial plants, because it takes advantage of the variation in existing populations (wild or domesticates), thus allowing breeders to skip the step of generating a new mapping population and simultaneously take full advantage of the large collections that exist for many perennial crops (Khan and Korban, 2012). However, there are two important considerations for the implementation of GWAS (Khan and Korban, 2012; Myles, 2013). One is that genetic structure or genetic differentiation within the surveyed population can lead to spurious associations. For example, the existing differentiation between wild and domesticated lineages means that a GWAS can be effectively carried out in *either* wild trees *or* domesticated trees – a GWAS conducted on a mixture of these two lineages would only reveal that all the wild phenotypes were in correlated with all the wild-specific markers. The second consideration is that linkage disequilibrium (LD) becomes a double-edged sword. Low levels of LD mean that significant correlations should be located in or (very close to) the gene controlling the trait of interest, but that it will require a very large number of markers to thoroughly cover the genome and actually capture these associations. High levels of LD can allow a large genome to be scanned using relatively few markers, but a significant association may still be very far from the functional gene or genomic feature. However, given the relatively low cost of generating SNP makers using NGS techniques, it seems unlikely that requiring a large number of markers will be a roadblock in the coming years. In light of this, it is fortunate that many perennial species have relatively low LD – this should make GWAS a powerful approach for elucidating the genetic underpinnings of phenotypic traits. In species with high LD, the targeted creation of QTL mapping populations that will generate recombinants in the genomic are of interest can complement the GWAS approach.

Association studies can also be carried out in a more targeted way if researchers have enough information to identify candidate genes that could contribute to the trait of interest. In this case, SNP markers can be genotyped in the region of interest, or the entire gene can be sequenced using targeted enrichment techniques (Cronn *et al.* 2012). This has been used to great effect in forest trees and fruit crops to confirm the association between candidate genes and traits of interest and to identify favourable alleles at these loci (Cevik *et al.* 2010; González-Martínez *et al.* 2007). This approach can be quite useful, but will ultimately be biased toward known genes. More 'agnostic' approaches like QTL mapping and GWAS still are necessary to identify genes that are not part of known families or have not been cloned in other species.

While both QTL mapping and GWAS are critical tools for dissecting the genetic basis of perennial crop domestication and improvement traits, they may not always be necessary to advance the crop domestication and improvement process from the perspective of breeders. Genomic selection (GS), the cousin of marker assisted selection (MAS), uses markers from across the genome to predict the phenotype of the plant in question (Heffner *et al.* 2009). This technique could be applied to the same types of populations used for GWAS studies, and utilizes the same type of markers, so the approaches are complimentary (Kumar *et al.* 2013; Myles, 2013). GS takes advantage of the fact that although many of the genes underlying traits are of small effect and difficult to map precisely, they still show detectable linkage with at least one marker. Once these patterns of linkage are established, it is possible to move forward to genotyping and phenotype prediction (based on a genotyped and phenotyped "training population"), allowing breeders to select seedlings for retention or removal long before they reach sexual maturity and set fruit for evaluation, thus speeding the process considerably.

All of these techniques have the potential to greatly advance the perennial crop improvement process, and can also increase our understanding of the genetic basis of perennial crop domestication. In particular, it is hoped that these techniques will allow crop breeders to take full advantage of the valuable genetic diversity present in most perennial domesticates. It should be noted, however, that while the high genetic diversity of most perennial crops will ultimately be an important resource to crop breeders, this same feature also presents a challenge for genomic approaches like GWAS and GS (discussed in Myles, 2013). Despite advances in sequencing technology and marker development, the option to start with inbred parents in crosses or in a population will greatly increase the power and accuracy of most NGS approaches. For example, SNP calls in heterozygous individuals are difficult because the heterozygous state has low support; more data is required to call these SNPs accurately. The overall diversity within perennials can also be difficult to accommodate, even if individuals are homozygous. This is because SNP data generated from NGS is too extensive to be checked manually, so its processing depends on the SNP and surrounding sequence matching a reference sequence almost exactly. This requirement is not always met in a species with high diversity, and results in many potentially variable sites being discarded. While analytical advances allowing for the imputation of missing data are being made, researchers and breeders working with these genetically diverse perennial crops must be prepared to generate an excess of data in order to have enough valid data to conduct their desired analyses.

WHAT CAN BE LEARNED FROM GRAPE AND APPLE?

In order to better understand the hallmarks of perennial plant domestication, and to predict how perennial crop domestication might proceed in the future, we look to two of the oldest and most economically important perennial fruit crops, apple (*Malus domestica*) and grape (*Vitis vinifera*), along with their wild relatives. This discussion follows on the heels of several recent papers that have drawn attention to these crops (Cornille *et al.* 2012; Meyer *et al.* 2012; Miller and Gross, 2011; Myles, 2013). Using these examples, we explore the geographic and taxonomic mosaic of perennial crop domestication, the impact of genetic bottlenecks on variation in cultivated populations, crop-wild gene flow, and the genetic basis of phenotypic variation. We emphasize the role of variation housed in wild-relatives for breeding fruits as well as rootstocks. These two iconic crops provide an important roadmap for exploring how best to conserve naturally occurring variation in perennial plant species and how to utilize it in plant breeding.

GRAPE

Cultivated grapevine (*Vitis vinifera* ssp. *vinifera*), the most economically important berry in the world, was domesticated from European grapevine (*Vitis vinifera* ssp. *sylvestris*) (This *et al.* 2006). The centre of diversity for *Vitis vinifera* appears to be in the Caucasus region, and multiple lines of evidence suggest that cultivated grapevines were domesticated from wild vines in this area (Grassi *et al.* 2006; Imazio *et al.* 2013; Myles *et al.* 2011; Pipia *et al.* 2012). Patterns of molecular genetic diversity point to a role for western European *V. vinifera* in the evolution of cultivated grapevine as well, either as a second source of cultivated materials (Arroyo-García *et al.* 2006), or as a participant in crop-wild gene flow in the area (DeAndres *et al.* 2012; Myles *et al.* 2011). The domestication process in grape is characterized by a shift from dioecious wild progenitors to hermaphroditic cultivars, the seedlessness resulting from parthenocarpy or stenospermocarpy (Cabezas *et al.* 2006), a broad domestication bottleneck with high levels of variation retained in cultivated populations, and rapid decay of LD (Myles *et al.* 2011). Extensive genetic variation in cultivated and wild grapevines have been confirmed in surveys of breeding collections (Aradhya *et al.* 2003; Myles *et al.* 2011), broad-scale analyses of natural grapevine diversity (Grassi *et al.* 2006) and regional analyses of wild populations in France, (Barnaud *et al.* 2009), Spain (DeAndres *et al.* 2012), and the Caucasus region (Pipia *et al.* 2012).

Although grapevine cultivation is based primarily on the European grapevine *V. vinifera*, other *Vitis* species play critical roles in grape production as well (Galet, 1979). Most *Vitis* species can be distinguished morphologically and genetically from one another (Aradhya *et al.* 2003; Miller *et al.* in revision; Péros *et al.* 2010; Zecca *et al.* 2012); however, the majority of subg. *Vitis* (the largest subgenus within *Vitis* and the one that includes the European grapevine) are interfertile. Interspecific hybridization has played an important role in the development of grapevine cultivars in some parts of North America (Ali *et al.* 2011): European grapevines do not grow well in the eastern and central United States due primarily to their susceptibility to native pests and pathogens. Early European colonists experimented with crossing *V. vinifera* and native North American *Vitis*, generating hardy hybrid grapevines that exist in vineyards in the eastern half of the United States today. In addition, North American grapevine species have contributed valuable rootstocks for the global grape industry (Galet, 1979; Peccoux, 2012). Widespread grafting of *V. vinifera* to North American species dates back to the mid-1800's when insects in the genus *Phylloxera* devastated the French grape industry (Sorensen et al. 2008). Starting with this crisis, North American grapevines have provided the foundation for rootstock development, and are the focus of research programmes working to elucidate molecular mechanisms and genetic underpinnings of abiotic and biotic stress resistance in rootstocks (Gong *et al.* 2011; Marguerit *et al.* 2012; Pavlousek, 2011; Polesani *et al.* 2012). Today, many vineyards consist of European *V. vinifera* grafted to North American *Vitis* species, including the river grape (*V. riparia*), the rock grape (*V. rupestris*), and Berlandieri's grape (*V. cinerea* ssp. *berlandieri*), and their hybrid derivatives (Galet, 1979).

Both grapevine scions and rootstocks are the focus of crop improvement efforts using molecular markers to facilitate selection, but both are the products of highly heterozygous, outcrossing populations that exhibit rapid LD decay. Given this, what is the genetic basis of phenotypic variation in grapevine? Traditional QTL analysis has been used to characterize genetic architecture of berry quality, yield, and pest/pathogen resistance. QTL studies identified a few loci of relatively large effect associated with variation in berry colour, berry weight, number of inflorescences per shoot, and seedlessness in table grapes (Cabezas *et al.* 2006; Costantini *et al.* 2008; Doligez *et al.* 2010), and phenological stages in wine grapes (Duchêne *et al.* 2012), also demonstrating that variation in a few regions of the genome is associated with traits of agricultural significance. In contrast, proanthocyanidin production has a more complex genetic basis with multiple loci of small effect contributing to phenotypic variation (Huang *et al.* 2012). An alternative to traditional QTL studies is GWAS, which makes use of existing germplasm collections, sidestepping the need to generate trait-specific mapping populations (Morrell *et al.* 2011; Myles *et al.* 2009). Given the extensive natural variation in *Vitis*, the outstanding germplasm collections that have been established for *Vitis* in Europe, North America, and elsewhere, and the developing genomic resources for this genus (Jaillon *et al.* 2007; Scalabrin *et al.* 2010), association mapping offers a promising approach for characterizing the genomic basis of phenotypic variation in grapes.

Another approach to exploring regions of the genome that are involved traits of agricultural importance involves transcriptomics, the analysis of the expressed portion of the genome. In grapevine, a growing body of work incorporates various methods of transcriptome analysis to identify genes that are active during key stages of fruit ripening, abiotic stress, or biotic stress. Pioneering work in this field analysed expressed sequence tags isolated from different plant organs, developmental stages, and cultivars to identify genes that were up- or down-regulated during various stages of grape growth (Goes Da Silva *et al.* 2005). Subsequent studies have characterized transcriptional activity during berry development (Ali *et al.* 2011), and have described genomic response to abiotic stress (Cramer *et al.* 2007; Liu *et al.* 2012; Tillett *et al.* 2011). Comparative transcriptomics studies across species have been particularly powerful in dissecting species or cultivar-level differences in pathogen susceptibility (Polesani *et al.* 2012) and flavonoid biosynthesis (Ali *et al.* 2011). Many of these studies used an Affymetrix gene chip that was developed for grapevine.

The transcriptome approach to identifying candidate genes associated with agriculturally important traits is only expanding with the advent of massively parallel sequencing of ribonucleic acid (RNA), (RNA-seq). RNA-seq offers an efficient, cost-effective way to access all species of transcript in a given tissue at a given time point, and can be used to describe both DNA sequence and transcript abundance (Wang *et al.* 2009). In grapevine, RNA-seq has been used to generate de novo transcriptomes for cultivars (Venturini *et al.* 2013), which can then be compared with reference genomes or transcriptomes of other cultivars facilitating the identification of cultivar-specific transcript. This promising approach is particularly powerful for clonally propagated perennial plants where individual genotypes live for multiple years and are replicated over diverse landscapes. For long-lived clones, RNA-seq offers the unparalleled opportunity to characterize temporal and spatial variation in a genotype's genomic response to whatever it may encounter.

APPLE

What is known about domesticated apple relative to the general features of perennial crop domestication? *Malus domestica* is one of the world's major fruit crops, is economically one of the most important, and is planted widely in the northern and southern hemispheres. As such, it has been the subject of intense study, and these studies show that apple demonstrates many of the major trends for perennial fruit crops detailed above. Genetic diversity in both wild and domesticated apple is very high, and individual plants are highly heterozygous (Cornille *et al.* 2012; Richards *et al.* 2009; Velasco *et al.* 2010). Wild populations of the progenitor, *Malus sieversii*, appear to have low population structure with high levels of outcrossing; this corresponds well with research on other wild tree species (Richards *et al.* 2009). Domesticated apple shows no sign of an improvement bottleneck, retaining the same high levels of diversity seen in its close wild relatives (Cornille *et al.* 2012). The results of one study indicate that this may be partially attributed to the influx of genetic variation from one of the close wild relatives, *Malus sylvestris* (Cornille *et al.* 2012). Gene flow from the domesticated species into *M. sieversii* has also been documented, which is an issue of possible conservation concern (Gross *et al.* 2012). As for many perennial crops, however, the broad genetic diversity of the crop and the wild species are not yet fully utilized or reflected in most modern breeding programme (other than introgression of disease-resistance genes) or in the active commercial orchards for this crop.

QTL mapping has been applied to several cultivar × cultivar crosses, and most domestication traits (mainly related to fruit quality) are controlled by loci of small effect (Kenis *et al.* 2008). Not surprisingly, these small-effect loci are inconsistent across populations and across years within a population, due to environmental effects and differences in genetic background. While these QTL studies have been enormously useful for MAS efforts dealing with disease resistance genes (which tend to have a larger effect) (King *et al.* 1999), a more targeted approach incorporating candidate genes has been necessary to identify and utilize alleles associated with fruit quality, and, more recently, growth habit (Baldi *et al.* 2012; Cevik *et al.* 2010). While these approaches are impressive, it is likely that important loci and genes remain undiscovered due to the lack of precision of QTL mapping and limited mapping population size used in most of these studies. GWAS approaches and genome scans for loci under selection offer promising avenues to identify these loci.

Both the breeding and genetics of apple were advanced with the resources building up to and culminating in the sequencing of the apple genome (‘Golden Delicious’ cultivar) followed by resequencing of 27 additional cultivars (Chagne *et al.* 2012; Velasco *et al.* 2010). While much work remains to curate the apple genome (a difficult task, due partially to the high heterozygosity), this has resulted in many advances. Analysis of SNPs developed based on these genomes indicate that LD in domesticated apples (outside of structured family populations) is low, which will make the link between significant markers in GWAS and the underlying gene more realistic than in a high LD species. Encouragingly, in the first GWAS approach for *Malus*, using a structured family population and 2 500 SNPs, several known candidate genes were recovered in the initial scans. Moreover, a GS approach to the same population indicate that fitting markers across the genome was effective in capturing phenotypic variation that is very difficult to track at the single-marker level (Kumar *et al.* 2013). This study also confirmed the difficulties inherent in working with a highly diverse species – the SNPs were based on an 8K SNP array, but only 2 500 were robust enough to be included in the final analysis. While even the number of SNPs used in this GS study may seem a daunting goal for non-model species, rapid advances in SNP generation technology and analysis are likely to level the playing field very rapidly. Instead, it is possible that the limiting resource for non-model crops might end up being the generation, maintenance, and phenotyping of large pedigreed populations such as nested association mapping (Kotoda *et al.* 2010) lines that are extremely useful for GWAS and GS studies. While these populations represent a substantial investment, they position researchers to immediately take advantage of developing technologies, and should be a priority for the research community.

REFERENCES

Ali, M.B., Howard, S., Chen, S., Wang, Y., Yu, O., Kovacs, L.G. & Qiu, W. 2011. Berry skin development in Norton grape: Distinct patterns of transcriptional regulation and flavonoid biosynthesis. *BMC Plant Biology*. 11: 7.

Aradhya, M., Dangl, G., Prins, B., Boursiquot, J., Walker, M., Meredith, C., Simon, C. 2003. Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. *Genetical Research*. 81: 179-192.

Arroyo-García, R., Ruiz-García, L., Bolling, L., Ocete, R., López, M., Arnold, C., Ergul, A., Söylemezo,˝lu.G., Uzun, H., Cabello, F., Ibáñez, J., Aradhya, M., Atanassov, A., Atanassov, I., Balint, S., Cenis, J., Costantini, L., Gorislavets, S., Grando, M., Klein, B., McGovern, P., Merdinoglu, D., Pejic, I., Pelsy, F., Primikirios, N., Risovannaya, V., Roubelakis-Angelakis, K., Snoussi, H., Sotiri, P., Tamhankar, S., This, P., Troshin, L., Malpica, J., Lefort, F. & Martinez-Zapater, J. 2006. Multiple origins of cultivated grapevine (*Vitis vinifera L. ssp. sativa*) based on chloroplast DNA polymorphisms. *Molecular Ecology*. 15: 3707-3714.

Austerlitz, F., Mariette, S., Machon, N., Gouyon, P-H. & Godelle, B. 2000. Effects of colonization processes on genetic diversity: Differences between annual plants and tree species. *Genetics*. 154: 1309-1321.

Baldi, P., Wolters, P.J., Komjanc, M., Viola, R., Velasco, R. & Salvi, S. 2012. Genetic and physical characterisation of the locus controlling columnar habit in apple (*Malus* × *domestica* Borkh.). *Molecular Breeding*. 31: 429-440.

Barnaud, A., Laucou, V., This, P., Lacombe, T. & Doligez, A. 2009. Linkage disequilibrium in wild French grapevine, *Vitis vinifera L*. subsp. *silvestris*. *Heredity*. 104: 431-437.

Besnard, G., Henry, P., Wille, L., Cooke, D. & Chapuis, E. 2007. On the origin of the invasive olives (*Olea europaea* L., Oleaceae). *Heredity*. 99: 608-619.

Brown, T.A., Jones, M.K., Powell, W., Allaby, R.G. 2009. The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology & Evolution*. 24: 103-109.

Cabezas, J.A., Cervera, M.T., Ruiz-García, L., Carreño, J., Martínez-Zapater, J.M. 2006. A genetic analysis of seed and berry weight in grapevine. *Genome*. 49: 1572-1585.

Cevik, V., Ryder, C., Popovich, A., Manning, K., King, G. & Seymour, G. 2010. A *FRUITFULL*-like gene is associated with genetic variation for fruit flesh firmness in apple (*Malus domestica* Borkh.). *Tree Genetics & Genomes*. 6: 271-279.

Chagne, D., Crowhurst, R.N., Troggio, M., Davey, M.W., Gilmore, B., Lawley, C., Vanderzande, S., Hellens, R.P., Kumar, S., Cestaro, A., Velasco, R., Main, D., Rees, J.D., Iezzoni, A., Mockler, T., Wilhelm, L., Van de Weg, E., Gardiner, S.E., Bassil, N. & Peace, C. 2012. Genome-wide SNP detection, validation, and development of an 8K SNP array for apple. *PLoS One*. 7: e31745.

Cornille, A., Gladieux, P., Smulders, M.J.M., Roldán-Ruiz, I., Laurens, F., Le Cam, B., Nersesyan, A., Clavel, J., Olonova, M., Feugey, L., Gabrielyan, I., Zhang, X-G., Tenaillon, M.I. & Giraud, T. 2012. New insight into the history of domesticated apple: Secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genetics*. 8: e1002703.

Costantini, L., Battilana, J., Lamaj, F., Fanizza, G. & Grando, M. 2008. Berry and phenology-related traits in grapevine (*Vitis vinifera L.*): From Quantitative Trait Loci to underlying genes. *BMC Plant Biology*. 8: 38.

Cramer, G., Ergül, A., Grimplet, J., Tillett, R.L., Tattersall, E., Bohlman, M., Vincent, D., Sonderegger, J., Evans, J., Osborne, C., Quilici, D., Schlauch, K., Schooley, D. & Cushman, J. 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Functional & Integrative Genomics*. 7: 111-134.

Cronn, R., Knaus, B.J., Liston, A., Maughan, P.J., Parks, M., Syring, J.V., Udall, J. 2012. Targeted enrichment strategies for next-generation plant biology. *American Journal of Botany*. 99: 291-311.

Crouzillat, D., Ménard, B., Mora, A., Phillips, W. & Pétiard, V. 2000. Quantitative trait analysis in *Theobroma cacao* using molecular markers: Yield QTL detection and stability over 15 years. *Euphytica*. 114: 13-23.

DeAndres, M.T., Benito, A., Perez-Rivera, G., Ocete, R., Lopez, M., Gaforio, L., Munoz, G., Cabello, F., Zapater, J.M. & Arrroyo-Garcia, R. 2012. Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Molecular Ecology*. 21: 800-816.

Di Vecchi-Staraz, M., Laucou, V., Bruno, G., Lacombe, T., Gerber, S., Bourse, T., Boselli, M. & This, P. 2009. Low level of pollen-mediated gene flow from cultivated to wild grapevine: Consequences for the evolution of the endangered subspecies *Vitis vinifera* L. subsp. *silvestris*. *Journal of Heredity*. 100: 66-75.

Doligez, A., Bertrand, Y., Dias, S., Grolier, M., Ballester, J., Bouquet, A. & This, P. 2010. QTLs for fertility in table grape (*Vitis vinifera L.*). *Tree Genetics & Genomes*. 6: 413-422.

Duchêne, E., Butterlin, G., Dumas, V. & Merdinoglu, D. 2012. Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theoretical and Applied Genetics*. 124: 623-635.

Eckert, A.J., van Heerwaarden, J., Wegrzyn, J.L., Nelson, C.D., Ross-Ibarra, J., Gonzalez-Martinez, S.C. & Neale, D.B. 2010. Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics*. 185: 969-982.

Ellstrand, N.C., Prentice, H.C. & Hancock, J.F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics*. 30: 539-563.

Galet, P. 1979. *A Practical Ampelography*. Translated and adapted by Lucie T. Morton. Cornell University Press, Ithaca, NY.

Glémin, S., Bataillon, T. 2009. A comparative view of the evolution of grasses under domestication. *New Phytologist*. 183: 273-290.

Goes Da Silva, F., Iandolino, A., Al-Kayal, F., Bohlmann, M., Cushman, M., Lim, H., Ergul, A., Figueroa, R., Kabuloglu, E., Osborne, C., Rowe, J., Tattersall, E., Leslie, A., Xu, J., Baek, J., Cramer, G., Cushman, J. & Cook, D. 2005. Characterizing the the grape transcriptome. Analysis of expressed sequence tags from multiple *Vitis* species and development of a compendium of gene expression during berry development. *Plant Physiology*. 139: 574-597.

Gong, H., Blackmore, D., Clingeleffer, P., Sykes, S., Jha, D., Tester, M. & Walker, R. 2011. Contrast in chloride exclusion between two grapevine genotypes and its variation in their hybrid progeny. *Journal of Experimental Botany*. 62: 989-999.

González-Martínez, S.C., Krutovsky, K.V. & Neale, D.B. 2006. Forest-tree population genomics and adaptive evolution. *New Phytologist*. 170: 227-238.

González-Martínez, S.C., Wheeler, N.C., Ersoz, E., Nelson, C.D. & Neale, D.B. 2007. Association genetics in *Pinus taeda* L. I. Wood property traits. *Genetics*. 175: 399-409.

Grassi, F., Labra, M., Imazio, S., Rubio, R.O., Failla, O., Scienza, A. & Sala, F. 2006. Phylogeographical structure and conservation genetics of wild grapevine. *Conservation Genetics*. 7: 837-845.

Gross, B.L., Henk, A.D., Forsline, P.L., Richards, C.M. & Volk, G.M. 2012. Identification of interspecific hybrids among domesticated apple and its wild relatives. *Tree Genetics & Genomes*. 8: 1223-1235.

Hamblin, M.T., Casa, A.M., Sun, H., Murray, S.C., Paterson, A.H., Aquadro, C.F. & Kresovich, S. 2006. Challenges of detecting directional selection after a bottleneck: Lessons from *Sorghum bicolor*. *Genetics*. 173: 953-964.

Hampe, A., Pemonge, M.H. & Petit, R.J. 2013. Efficient mitigation of founder effects during the establishment of a leading-edge oak population. *Proceedings of the Royal Society B: Biological Sciences*. 280: 1764.

Hancock, J.F. 2005. Contributions of domesticated plant studies to our understanding of plant evolution. *Annals of Botany*. 96: 953-963.

Heffner, E.L., Sorrells, M.E. & Jannink, J-L. 2009. Genomic selection for crop improvement. *Crop Science*. 49: 1.

Huang, Y., Doligez, A., Fournier-Level, A., LeCunff, L., Bertrand, Y., Canaguier, A., Morel, C., Miralles, V., Veran, F., Souquet, J., Cheynier, V., Terrier, N. & This, P. 2012. Dissecting genetic architecture of grape proanthocyanidin composition through quantitative trait locus mapping. *BMC Plant Biology*. 12: 30.

Hughes, C.E., Govindarajulu, R., Robertson, A., Filer, D.L., Harris, S.A. & Bailey, C.D. 2007. Serendipitous backyard hybridization and the origin of crops. *Proceedings of the National Academy of Sciences*. 104: 14389-14394.

Imazio, S., Maghradze, D., Lorenzis, G., Bacilieri, R., Laucou, V., This, P., Scienza, A. & Failla, O. 2013. From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera L.*) germplasm. *Tree Genetics & Genomes*. 9: 641-658.

Jaillon, O., Aury, J., Noel, B., Policriti, A, Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyère, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lecharny, A., Scarpelli, C., Artiguenave, F., Pè, M., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A., Weissenbach, J., Quétier, F. & Wincker, P. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*. 449: 463-467.

Jolivet, C., Rogge, M. & Degen, B. 2013. Molecular and quantitative signatures of biparental inbreeding depression in the self-incompatible tree species Prunus avium. *Heredity*. 110: 439-448.

Kenis, K., Keulemans, J. & Davey, M. 2008. Identification and stability of QTLs for fruit quality traits in apple. *Tree Genetics & Genomes*. 4: 647-661.

Khan, M.A. & Korban, S.S. 2012. Association mapping in forest trees and fruit crops. *Journal of Experimental Botany*. 63: 4045-4060.

King, G.J., Tartarini, S., Brown, L., Gennari, F. & Sansavini, S. 1999. Introgression of the *Vf* source of scab resistance and distribution of linked marker alleles within the *Malus* gene pool. *Theoretical and Applied Genetics*. 99: 1039-1046.

Kotoda, N., Hayashi, H., Suzuki, M., Igarashi, M., Hatsuyama, Y., Kidou, S-I., Igasaki, T., Nishiguchi, M., Yano, K., Shimizu, T., Takahashi, S., Iwanami, H., Moriya, S. & Abe, K. 2010. Molecular characterization of *FLOWERING LOCUS T*-like genes of apple (*Malus* x *domestica* Borkh.). *Plant and Cell Physiology*. 51: 561-575.

Kumar, S., Garrick, D., Bink, M., Whitworth, C., Chagne, D., Volz, R. 2013. Novel genomic approaches unravel genetic architecture of complex traits in apple. *BMC Genomics*. 14: 393.

Liu, G., Wang, J., Cramer, G., Dai, Z., Duan, W., Xu, H., Wu, B., Fan, P., Wang, L. & Li, S. 2012. Transcriptomic analysis of grape (*Vitis vinifera L.)* leaves during and after recovery from heat stress. *BMC Plant Biology*. 12: 174.

Lopes, M.S., Mendonça, D., Rodrigues dos Santos, M., Eiras-Dias, J.E. & da Câmara Machado, A. 2009. New insights on the genetic basis of Portuguese grapevine and on grapevine domestication. *Genome*. 52: 790-800.

Marguerit, E., Brendel, O., Lebon, E., Van Leeuwen, C. & Ollat, N. 2012. Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. *New Phytologist*. 194: 416-429.

McKey, D., Elias, M., Pujol, B. & Duputié, A. 2010. The evolutionary ecology of clonally propagated domesticated plants. *New Phytologist*. 186: 318-332.

Meyer, R.S., DuVal, A.E. & Jensen, H.R. 2012. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytologist*. 196: 29-48.

Miller, A.J. & Gross, B.L. 2011. From forest to field: Perennial fruit crop domestication. *American Journal of Botany*. 98: 1389-1414.

Miller, A.J., Matasci, N., Aradhya, M.K., Prins, B., Zhong, G.Y., Soimon, C., Buckler, E.S. & Myles, S. 2014. *Vitis* phylogenomics: hybridization intensities from a SNP array outperform genotype calls. *PLoS ONE* (in revision).

Morrell, P.L., Buckler, E. & Ross-Ibarra, J. 2011. Crop genomics: advances and applications. *Nature Publishing Group*. 13: 85-96.

Myles, S. 2013. Improving fruit and wine: what does genomics have to offer? *Trends in Genetics*. 29: 190-196.

Myles, S., Boyko, A.R., Owens, C.L., Brown, P.J., Grassi, F., Aradhya, M.K., Prins, B., Reynolds, A., Chia, J-M., Ware, D., Bustamante, C.D. & Buckler, E.S. 2011. Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences*. 108: 3530-3535.

Myles, S., Peiffer, J., Brown, P.J., Ersoz, E.S., Zhang, Z., Costich, D.E. & Buckler, E.S. 2009. Association mapping: Critical considerations shift from genotyping to experimental design. *Plant Cell*. 21: 2194-2202.

Olsen, K.M. & Wendel, J.F. 2013. Crop plants as models for understanding plant adaptation and diversification. *Frontiers in Plant Science*. 4: 290.

Pavlousek, P. 2011. Evalutaion of drought tolerance of new grapevine rootstock hybrids. *Journal of Environmental Biology*. 32: 543-549.

Peccoux, A. 2012. *Molecular and physiological characterization of grapevine rootstock adaptation to drought*. These pour le Doctorat de l'Université Bourdeuax 2. 186 pp.

Péros, J., Berger, G., Portemont, A., Boursiquot, J. & Lacombe, T. 2010. Genetic variation and biogeography of the disjunct *Vitis* subg. *Vitis* (Vitaceae). *Journal of Biogeography*. 38: 471-486.

Petit, R.J., Bodénès, C., Ducousso, A., Roussel, G. & Kremer, A. 2004. Hybridization as a mechanism of invasion in oaks. *New Phytologist*. 161: 151-164.

Petit, R.J. & Hampe, A. 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution, and Systematics*. 37: 187-214.

Pipia, I., Gogniashvili, M., Tabidze, V., Beridze, T., Mgamkrelidze Gotsidridze, V., Melyan, G., Musayev, M., Salimov, V., Beck, J. & Schaal, B. 2012. Plastid DNA sequence diversity in wild grapevine samples (*Vitis vinifera* subsp. *sylvestris*) from the Caucasus region. *Vitis*. 51: 119-124.

Polesani, M., Bortesi, L., Ferrarini, A., Zamboni, A., Fasoli, M., Zadra, C., Lovato, A., Pezzotti, M., Delledonne, M. & Poverari, A. 2012. General and species-specific transcriptional responses to downy mildw infection in a susceptible (*Vitis vinfera*) and a resistant (*V. riparia*) grapevine species. *BMC genomics*. 11: 117.

Purugganan, M.D. & Fuller, D.Q. 2009. The nature of selection during plant domestication. *Nature*. 457: 843-848.

Richards, C.M., Volk, G.M., Reilley, A.A., Henk, A.D., Lockwood, D., Reeves, P.A. & Forsline, P.L. 2009. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. *Tree Genetics & Genomes*. 5: 339-347.

Scalabrin, S., Troggio, M., Moroldo, M., Pindo, M., Felice, N., Coppola, G., Prete, G., Malacarne, G., Marconi, R., Faes, G., Jurman, I., Grando, S., Jesse, T., Segala, C., Valle, G., Policriti, A., Fontana, P., Morgante, M. & Velasco, R. 2010. Physical mapping in highly heterozygous genomes: a physical contig map of the Pinot Noir grapevine cultivar. *BMC Genomics*. 11: 204.

Sorensen, W.C., Smith, E.H., Smith, J. & Carton, Y. 2008. Charles V. Riley, France, and Phylloxera. *American Entomologist*. 54: 134-149.

Stolting, K.N., Nipper, R., Lindtke, D., Caseys, C., Waeber, S., Castiglione, S. & Lexer, C. 2013. Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. *Molecular Ecology*. 22: 842-855.

This, P., Lacombe, T. & Thomas, M. 2006. Historical origins and genetic diversity of wine grapes. *Trends in Genetics*. 22: 511-519.

Tillett, R., Ergul, A., Albion, R., Schlauch, K., Cramer, G. & Cushman, J. 2011. Identification of tissue-specific, abiotic stress-responsive gene expression patterns in wine grape (*Vitis vinifera L.*) based on curation and mining of large-scale EST data sets. *BMC Plant Biology*. 11: 86.

Vallejo-Marín, M., Dorken, M.E. & Barrett, S.C.H. 2010. The ecological and evolutionary consequences of clonality for plant mating. *Annual Review of Ecology, Evolution, and Systematics*. 41: 193-213.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications*. 3: 434-452.

Vavilov, N.I. 1992. *Origin and Geography of Cultivated Plants* (English translation). Cambridge University Press, New York.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S.K., Troggio, M., Pruss, D., Salvi, S., Pindo, M., Baldi, P., Castelletti, S., Cavaiuolo, M., Coppola, G., Costa, F., Cova, V., Dal Ri, A., Goremykin, V., Komjanc, M., Longhi, S., Magnago, P., Malacarne, G., Malnoy, M., Micheletti, D., Moretto, M., Perazzolli, M., Si-Ammour, A., Vezzulli, S., Zini, E., Eldredge, G., Fitzgerald, L.M., Gutin, N., Lanchbury, J., Macalma, T., Mitchell, J.T., Reid, J., Wardell, B., Kodira, C., Chen, Z., Desany, B., Niazi, F., Palmer, M., Koepke, T., Jiwan, D., Schaeffer, S., Krishnan, V., Wu, C., Chu, V.T., King, S.T., Vick, J., Tao, Q., Mraz, A., Stormo, A., Stormo, K., Bogden, R., Ederle, D., Stella, A., Vecchietti, A., Kater, M.M., Masiero, S., Lasserre, P., Lespinasse, Y., Allan, A.C., Bus, V., Chagne, D., Crowhurst, R.N., Gleave, A.P., Lavezzo, E., Fawcett, J.A., Proost, S., Rouze, P., Sterck, L., Toppo, S., Lazzari, B., Hellens, R.P., Durel, C-E., Gutin, A., Bumgarner, R.E., Gardiner, S.E., Skolnick, M., Egholm, M., Van de Peer, Y., Salamini, F. & Viola, R. 2010. The genome of the domesticated apple (*Malus* x *domestica* Borkh.). *Nature Genetics*. 42: 833-839.

Venturini, L., Ferrarini, A., Zenoni, S., Tornielli, G.B., Fasoli, M., Santo, S.D., Minio, A., Buson, G., Tononi, P., Zago, E.D., Zamperin, G., Bellin, D., Pezzotti, M. & Delledonne, M. 2013. De novo transcriptome characterization of *Vitis vinifera* cv. Corvina unveils varietal diversity. *BMC Genomics*. 14: 41.

Vranckx, G., Jacquemyn, H., Muys, B. & Honnay, O. 2012. Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. *Conservation Biology*. 26: 228-237.

Wang, Z., Gerstein, M. & Snyder, M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews: Genetics*. 10: 57-63.

Zecca, G., Abbott, J.R., Sun, W., Spada, A., Sala, F. & Grassi, F. 2012. The timing and the mode of evolution of wild grapes (*Vitis*). *Molecular Phylogenetics and Evolution*. 62: 736-747.

Zohary, D. & Spiegel-Roy, P. 1975. Beginnings of fruit growing in the Old World. *Science*. 187: 319-327.

Zohary, D., Maria, H. & Weiss, E. 2012. *Domestication of plants in the Old World*. Oxford University Press, New York, New York, USA.