SPECIAL INVITED PAPER

Useful insights from evolutionary biology for developing perennial grain crops[1]

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Annual grain crops dominate agricultural landscapes and provide the majority of calories consumed by humanity. Perennial grain crops could potentially ameliorate the land degradation and off-site impacts associated with annual grain cropping. However, herbaceous perennial plants with constitutively high allocation to harvestable seeds are rare to absent in nature. Recent trade-off theory models suggest that rugged fitness landscapes may explain the absence of this form better than sink competition models. Artificial selection for both grain production and multiyear lifespan can lead to more rapid progress in the face of fitness and genetic trade-offs than natural selection but is likely to result in plant types that differ substantially from all current domestic crops. Perennial grain domestication is also likely to require the development of selection strategies that differ from published crop breeding methods, despite their success in improving long-domesticated crops; for this purpose, we have reviewed literature in the areas of population and evolutionary genetics, domestication, and molecular biology. Rapid domestication will likely require genes with large effect that are expected to exhibit strong pleiotropy and epistasis. Cryptic genetic variation will need to be deliberately exposed both to purge mildly deleterious alleles and to generate novel agronomic phenotypes. We predict that perennial grain domestication programs will benefit from population subdivision followed by selection for simple traits in each subpopulation, the evaluation of very large populations, high selection intensity, rapid cycling through generations, and heterosis. The latter may be particularly beneficial in the development of varieties with stable yield and tolerance to crowding.

Key words: bottleneck; domestication; epigenetics; epistasis; evolvability; genetic assimilation; genetic load; perennial grain; pleiotropy; trade-off.

Perennial grain crops have been proposed as an elegant solution to numerous problems confronted in modern agriculture. Perennial grains are expected to increase water quality, reduce soil erosion, increase soil carbon, and improve habitat for wildlife (Cox et al., 2006). Furthermore, perennials are predicted to be more resilient to abiotic stress resulting from climate change (Cox et al., 2006). Initiating and expanding perennial grain breeding programs has been proposed as a strategy to address the looming challenges of land degradation, food security, energy supply, and climate change (Glover et al., 2010).

Strategies proposed for developing a wide array of new perennial grain crops (Wagoner and Schaeffer, 1990; Cox et al., 2002) typically are separated into two groups: wide hybridization and de novo domestication. In de novo domestication, repeated cycles of selection are performed out of a wild perennial population to achieve a high-yielding domestic type. In wide hybridization, an existing grain crop is crossed with a wild perennial relative, and then either the hybrid population is improved, or repeated rounds of backcrossing are performed to introgress genes for perennial growth until the phenotype in other ways resembles the annual crop parent. Here, we will not address the array of genetic effects that are derived from wide hybridizations, but will rather focus our attention on the mechanisms specific to early stages of selection from wild populations.

The breeding strategies required for selecting out of wild material vs. improving yield of domesticated species are sure to differ. Whereas the first stages of domestication in cereal crops involve dramatic changes that facilitate uniform planting and efficient harvesting, crop improvement (of existing domestic species) involves improvements in yield and palatability or diversification based on regional adaptation or harvested product qualities (Olsen and Wendel, 2013). Although the early steps toward domestication will include yield gains, the primary objective is to transform the phenotype in ways that make a plant useful to meet human food needs. Subsequent crop improvement is associated with relatively modest incremental morphological changes.

Since few grain species have been recently domesticated (Meyer et al., 2012), most plant breeding theory and practice is based on improvement of previously domesticated species. With over a decade of experience domesticating new grain crops (described by Cox et al., 2010), we have been searching for genetic insights that are specific to taking wild species through the early stages of domestication. We have found strategies for the wholesale remodeling of plants (as is required to develop perennial grains) to be rare in the plant breeding literature. Therefore, we have turned to findings in other fields to develop more complete and efficient strategies for our own programs and, we hope, for the benefit of colleagues considering the domestication of other wild species.

Here we review literature in the fields of evolutionary biology and genetics that relates to the current effort to domesticate perennial grains. Although traditional plant breeding literature has abundant insights for all breeding programs, in this article we will primarily limit our consideration to topics from evolutionary biology and basic genetics. We will briefly review current literature

in the following areas and draw relevant conclusions for perennial grain domestication: ecological trade-offs, genetic load, purging of load, bottlenecks, pleiotropy, epistasis, heterosis, phenotypic plasticity, epigenetics, cryptic variation, robustness, evolvability, past domestication, and selection.

Trade-offs—A core component of evolutionary theory is the assumption of fitness trade-offs between life history traits (Roff and Fairbairn, 2007). Trade-offs can refer to both functional relationships between traits and to statistical correlations. No matter the statistical correlation between traits, functional relationships are generally regarded to be present, where resources allocated to one trait are unavailable for other traits.

It would be reasonable for anyone evaluating the feasibility of perennial grains to carefully consider a range of potential trade-offs. Even advocates of perennial grain crops have stated that a perennial grain cannot be expected to have yields comparable to annual grains due to trade-offs between seed and perennial organs (Wagoner and Schaeffer, 1990). Other authors have suggested that the trade-off is only necessary in the first year, because in later years the resources invested in overwintering can be used for growth in the following year (Cox et al., 2002). This analysis assumes that the respiration cost of overwintering organs will not exceed the energy savings obtained by not having to rebuild a root system annually.

Others have challenged the feasibility of domesticating perennial grains on the grounds that perennial plants must already be selected to produce as much seed as they can without sacrificing longevity (Denison, 2012). Under this model, selection for increased seed production must necessarily sacrifice odds of longevity. Although it may be possible to double seed production while still having a robust perennial plant, the argument goes, as yields approach those of annuals the inevitable result will be plants that seldom survive for multiple years. We in turn challenge this model: if seed yields can only be increased in perennials by sacrificing longevity, how have yield increases been achieved in annual crops over the past century, where perennating structures (overwintering crowns or rhizomes) could not be traded off for yield?

Elsewhere, we have challenged each of these trade-off predictions in light of both genetic and evolutionary theory and offered our own predictions (DeHaan et al., 2005; Van Tassel et al., 2010). Here we will briefly review a few relevant topics. To begin, reproduction can be generally understood as a substantial cost to a plant—reproductive allocation does indeed trade off with other potential sinks (Obeso, 2002). This negative statistical correlation may be observable, but negative genetic correlations can easily be selected against and do not represent effective constraints to evolutionary adaptation (Walsh and Blows, 2009). A statistical association can arise from many factors other than carbon allocation. For instance, long-lived plants can accumulate mutations that reduce fertility without impacting clonal growth (Ally et al., 2010). In a perennial grass, the high degree of seed abortion has been associated with genetic defects (which accumulate in outcrossing perennials) rather than a shortage of resources (Marshall and Ludlam, 1989). In essence, seed production in perennials may be sink, rather than source, limited.

The more important question is whether there is an ultimate functional trade-off between longevity and seed production, as suggested by Denison (2012). Will perennials selected for seed production similar to that of annual crops necessarily run out of resources for perennation? This will be the case only in a model where resource acquisition is constrained to not change, and selection is limited to shifting allocation among sinks. Pleiotropy, discussed in a later section, is an alternative hypothesis to resource allocation that may explain the often negative correlation between fecundity and lifespan.

Studies designed to identify trade-offs often find positive, rather than negative, correlations between sinks. To explain this paradox, the "Y" model of allocation has been developed (De Jong and van Noordwijk, 1992; Roff and Fairbairn, 2007). In this model, the two forks of the "Y" are regarded as competing sinks, and the base of the "Y" represents acquisition. When acquisition is allowed to vary and covary with allocation, situations where competing sinks can be positively rather than negatively correlated are easily understood.

For instance, longevity could be associated with greater acquisition. Therefore, a plant living longer may allocate less of its total resources to reproduction as a percentage, while the absolute quantity may increase. In agriculture, increased harvest index (units of grain per units of aboveground biomass) has been an important path to yield. But yield is the primary objective, not harvest index. A small amount of harvest index could be sacrificed if total productivity of the system were to increase.

So is it possible for total productivity to increase when perennials are grown in place of annuals? Let's consider a few examples. The perennial grass *Miscanthus* ×*giganteus* J.M. Greef & Deuter has produced 59% more aboveground biomass than grain maize (*Zea mays* L.) in Illinois. The difference was attributed to greater capture of solar radiation by developing a leaf canopy earlier and maintaining it later (Dohleman and Long, 2009). Similarly, the developing perennial grain kernza [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] averaged 37% greater aboveground biomass than annual wheat (*Triticum aestivum* L.) in the second year (Culman et al., 2013). In these cases, the perennials are achieving greater productivity by taking advantage of resources (primarily light and water) that are not captured by annuals. Note that in these examples, only aboveground biomass was included. The allocation to belowground perennial structures was not considered. So the perennials must be able to more than pay the respiration cost of their overwintering biomass. Additionally, comparisons of related perennials with annual crops indicate that the perennials can have a higher rate of photosynthesis (Jaikumar et al., 2013, 2014).

In accordance with the Y model, we would predict that in situations where annual crops can use a similar amount of water, light, and nutrients as the perennials, i.e., where the base of the Y is constrained, annuals will indeed have greater yield potential because overwintering via dormant seed is likely to incur at least slightly fewer costs than overwintering via dormant crowns, rhizomes, or tubers.

In perhaps more common situations where perennials are able to access more resources than annuals, as evidenced by higher aboveground biomass production, the primary question remaining is whether breeding can shift allocation from vegetative to reproductive structures. Modern breeding has successfully increased harvest index in many grains from about 0.3 to 0.5; many landraces may have had harvest indexes substantially below 0.3 (Sinclair, 1998). Because breeding has swiftly achieved this transformation, it is easy to imagine a gram-for-gram shift of carbon from vegetative structures to seed. However, this simplification ignores the fact that seeds are typically much higher in nitrogen content. Therefore, it has been hypothesized that a major limiting factor for seed production historically may have

been nitrogen (Sinclair, 1998). This hypothesis is consistent with the observation of low seed production in prairies, which often have low N availability. Thus we see that to achieve a high harvest index, the agricultural environment will need to provide abundant N supply to plants, in agreement with the model whereby high seed-producing herbaceous perennials, although rare in nature, can be created by first modifying the environment toward high resource availability and then selecting strictly for seed production on an area basis (DeHaan et al., 2005).

The final line of evidence we will present here supporting the possibility of artificially breeding perennials with high seed yield comes from yields of existing perennial plants. In an evaluation of native legumes for use as a grain, a majority of the species with the highest yield and reproductive allocation were perennials; two perennial species had a harvest index exceeding 0.5 (Bell et al., 2012). The annual net reproductive effort of modern apples can be 0.65, or similar to modern maize, even if the establishment years are included (Van Tassel et al., 2010). With these examples, we see that there is not likely to be an absolute barrier to high yields or harvest index in perennials. In the rest of this paper we will explore insights and likely strategies that will be helpful in creating herbaceous perennial crops with abundant seed production.

***Genetic load*—**We consider genetic load to be the presence of deleterious alleles in a population. In an evolutionary sense, these primarily recessive alleles cause a reduction in the average fitness of a population. The concept of genetic load stems from the understanding that plants lack a germ line; rather, sex cells are formed after a potentially large number of somatic cell divisions during which mutations are likely (Klekowski, 1988). Somatic mutations can be beneficial in the sense that if they are dominant and enhance growth in some manner, they can immediately benefit the sector of the plant in which they propagate. In plants with predominantly asexual reproduction, dominant beneficial mutations can be accumulated while dominant deleterious mutations can be eliminated in the individual where they occur through competition between chimeric plant parts (Klekowski, 2003; McKey et al., 2010). Thus, it is not surprising to find many clonally spreading species have strong vegetative vigor (Van Tassel et al., 2010).

However, a recessive mutation can occur somatically and have no impact on the vigor of the plant in which it appears. If the deleterious recessive allele is rare, it can begin to propagate in the population with little impact on fitness. But as the recessive deleterious allele becomes more frequent, it will occasionally appear in the fitness-reducing homozygous state. If the allele is lethal or strongly deleterious, it will be selected against and remain at a relatively low frequency. But if the allele is only mildly deleterious, it may gain moderate to high frequency. Additionally, dominant deleterious alleles can also increase in a population if they are only expressed at the juvenile, embryonic, or sexual reproduction growth stages (Klekowski, 1988). Although in annuals frequent sexual cycles will expose deleterious recessives to selection at the gamete stage and through recombination, in perennials highly recessive deleterious alleles can arise at a rate faster than they can be efficiently eliminated (Morgan, 2001).

Polyploids, upon formation, are expected to have an immediate advantage of reduced genetic load through the masking of somatic mutations (Otto, 2007). However, the larger mutational target size and lower efficiency of elimination of deleterious genes can result in higher-ploidy species carrying greater genetic load (Gerstein and Otto, 2009).

Abundant empirical evidence has accumulated showing high load in long-lived, larger, and clonal perennial plants. The percentage of ovules developing seeds is lower in perennials than in annuals, and even lower in woody plants (Wiens, 1984). Pollen abortion has been shown to increase with plant age (Bhattacharya, 2005). Clonal growth is correlated with self-incompatibility, and self-incompatibility is maintained due to the inbreeding depression associated with load (Morgan et al., 1997; Porcher and Lande, 2005; Vallejo-Marín and O'Brien, 2007). Plants that usually resprout rather than reseed after fire are believed to have higher genetic load and therefore lower seed set (Meney et al., 1997; Lamont and Wiens, 2003).

Genetic load has several implications for perennial grain breeding. First, if low seed set in perennials is due to genetic load, a reduction in the load could quickly improve yield. Second, large-statured, outcrossing and clonal plants are expected to carry more genetic load than small, cespitose, selfing species. And finally, strategies that account for and actively reduce genetic load will likely be important when breeding long-lived outcrossing species.

***Purging*—**If genetic load is substantial and limiting to seed production in perennials, purging the load could be an important strategy for perennial grain breeding. Since genetic load increases with population-average lifespan, plant size, and outcrossing rate, maintaining breeding populations in the reverse conditions through several generations is expected to purge deleterious alleles.

One of the primary roles of sex may be to eliminate deleterious genetic changes (Gorelick and Heng, 2011). Because load builds when the accumulation phase increases relative to the number of sexual cycles where purging can occur, shifting the balance in favor of more sexual cycles would be a logical step toward reducing load. This strategy to achieve purging is particularly applicable to genes harmful to embryonic development and seed set, which may only be selected against during sexual reproduction. Therefore, perennial grain breeding programs may benefit by using many rapid breeding cycles with strict selection for seed set. By avoiding periods of rhizomatous spread, opportunity for additional load accumulation will be reduced. Genomic selection may be a way to avoid long generation times in perennials, increasing the frequency of sexual reproduction (Harfouche et al., 2012).

Purging through inbreeding has been achieved in experimental populations. On average, purging through inbreeding in plants in one review was found to increase fitness-related traits by 24% (Crnokrak and Barrett, 2002). However, the authors caution that some of the fitness increase could have been due to laboratory conditions. When an outcrossed population of annual plants was selfed for several generations, fitness-related traits declined slightly. But when the inbred lines were then crossed, traits increased above those in the starting population, which is strong evidence for purging (Barrett and Charlesworth, 1991). This finding is also relevant to heterosis, which will be discussed in detail in a later section.

Evidence for purging has also been found in inbred populations of primarily outcrossing species. Despite decades of selection for forage yield, the outcrossing perennial alfalfa (*Medicago sativa* L.) still contains substantial genetic load. Inbreeding in alfalfa has improved forage yield, and inbred populations have been found to possess reduced numbers of deleterious alleles

(Kimbeng and Bingham, 1998, 1999). A substantial component of the breeding progress achieved in maize can be attributed to purging unfavorable alleles that were exposed through inbreeding (Walters et al., 1991; Brummer, 1999).

In one study of conifers, the average load was found to be about 10 lethal equivalents per zygote, but the range was about 9-fold (Sorensen, 1969). Given the wide range found, selection should be effective in identifying individuals with reduced load. Indeed, strategies for reducing load in conifer breeding programs have been proposed (Williams and Savolainen, 1996); we will summarize the main points here. If the average number of lethals per zygote is 10, roughly 1 in 20 000 trees would be free of lethals by chance. Rather than screening thousands of trees, selfing may be a more efficient strategy to purge. Selfing is expected to reduce the number of completely recessive lethals by one third per generation. Partially recessive alleles will be purged more quickly. When load is due to mildly deleterious rather than lethal alleles, it will be more difficult to purge. If deleterious alleles are fixed during inbreeding, populations can decline and lose fertility rapidly, so large starting populations may be required. Inbreeding depression may not be expressed in the most favorable growing conditions, so crowding can be used to expose some genetic load to selection. Selfing can often increase within-line variance when load is present, resulting in poor correlation between inbred line performance and outcrossed siblings. Therefore, selections based on inbred performance may be inaccurate. Potential solutions include inbreeding more slowly or removing the poorest-performing plants from each inbred family before analyzing the family's genetic value in a breeding program. Although selection based on selfed families has been performed in maize (Coors, 1988), we are not aware of breeding programs that have tried these approaches for increasing the accuracy of selection on inbreds.

Bottlenecks—The bottleneck concept refers to the loss of genetic diversity that occurs with reduction in population size. Past domestications have involved bottlenecks of varying size. In a study of domestic grain crops, cultivar-level diversity was found to range between 60 and 100% of progenitor diversity (Buckler et al., 2001). Strikingly, progenitor diversity itself varies widely, with the maize progenitor having six times the diversity of sorghum [*Sorghum bicolor* (L.) Monech]. The dominance of maize over sorghum in global acreage and yield potential may be partially attributable to this disparity in the genetic diversity available to their breeders. Reintroducing genetic diversity from prebottleneck populations into breeding gene pools could sustain breeding progress in soybean and other annual crops (Hyten et al., 2006).

As we begin to domesticate new species, we are then forced to ask what role bottlenecks will play in this process. Do substantial bottlenecks in domestic species represent a mistake that should be avoided at all cost? Or, are bottlenecks an unavoidable outcome of the rapid domestication that will be necessary to develop perennial grain crops in a period of decades?

First, we should contrast perennial grain crops with other currently domesticated outcrossing perennials, such as forage grasses. Forage grass breeding typically relies on maximum heterozygosity to cover genetic load and maximize biomass production (Ferdinandez and Coulman, 2004). Domesticating a forage requires the fixation of fewer traits than the domestication of a grain. Overall vigor is of primary importance in forages, while in grains, height, shatter resistance, free-threshing, locally adapted maturity, seed size, and grain quality are

more critical than sheer vigor. The strong selection required to fix these traits in a grain crop may cause bottlenecking. The other group of perennial crops to consider is the clonally propagated fruits and nuts. In these species, specific traits are required, but good genotypes are propagated indefinitely as clones. Each cloned variety can contain a vast amount of allelic diversity, meaning that progeny are not likely to perform as well as their parents. Seed propagated grains, however, are required to breed true.

To examine the impact that restricted population size can have on the rate of allelic fixation, we can consider the past domestication of wheat and barley. The critical genes for non-shattering could have been fixed in only a few centuries with self-pollinating species, but with outcrossers (and therefore large effective population size) fixation would have taken more than 8000 yr (Hillman and Davies, 1990). Another modeling study showed that domestication of a selfer could occur in 30 yr, while an obligate outcrosser would take about 3000 yr (Honne and Heun, 2009). If these models are correct, inbreeding and the associated bottlenecks may be useful in accelerating domestication.

Although bottlenecks reduce allelic diversity, they have often been found to increase additive genetic variation, that portion of diversity that is available for selection to act upon (Carson, 1990). One explanation for this increased variance is that when bottlenecks alter the genetic background of interacting alleles, epistatic effects can become additive. Additionally, bottlenecks may activate transposable elements, leading to increased mutation and crossing over (Carson, 1990; Hendry et al., 2011).

Others have argued that even if bottlenecks can increase genetic variance, the real question should be whether this is useful genetic variance. That is, do bottlenecks allow a population to reach a new stable state (Barton and Turelli, 2004)? In empirical tests, bottlenecked populations were unable to acquire new adaptations more quickly than nonbottlenecked populations (Frankham et al., 1999; van Heerwaarden et al., 2008). However, theory indicates that an extended bottleneck over several generations may be required to substantially increase epistatic genetic variance (Goodnight, 1987). Perhaps breeding programs would benefit most from extended bottlenecks of moderate constraint.

Increased genetic variance in bottlenecked populations may often be due to the accumulation of previously rare deleterious recessive genes (Bryant et al., 1986). If this is the case, these alleles can be regarded as nonadaptive, and little useful new variation will arise from a bottleneck. However, if the population enters a new environment simultaneous to the bottleneck, the newly exposed variation could now be adaptive. Such a possibility would be consistent with the observation that domestication events do not follow the usual pattern of reduced fitness following a bottleneck (Hendry et al., 2011) if movement from the wild to a field is understood as entering a new environment. If recessive alleles are beneficial, driving them to fixation will be achieved most rapidly with inbreeding and the associated bottleneck (Caballero et al., 1991).

Another important consideration is the size of the bottleneck. Many bottleneck experiments use an effective population size of two individuals. However, even effective population sizes of about 10 individuals can still allow dramatic evolution to occur (Hendry et al., 2011). Also, some models indicate that change in genetic variation with a bottleneck depends on the extent of inbreeding and is difficult to predict (Taft and Roff, 2011).

Current theory suggests that small populations should have reduced response to selection over the long term due to reduced initial variation and increased effect of drift (Willi et al., 2006). Indeed, small population size has been empirically demonstrated to substantially reduce response to selection (Weber, 1990). Most likely, the largest contributing factor to limited response in small populations is drift (Willi et al., 2006). Although effective population size (number of individuals mated) is important, the total number evaluated (and therefore selection intensity) may contribute more to selection response. In an experiment where population size, selection intensity, and total number evaluated were all varied, the total number scored was found to have greatest impact on response (Jones et al., 1968). The authors concluded that in general the real populations did not match theoretical predictions because much of the response was due to a few genes or gene combinations of large effect. Identifying such large-effect genes will of course be the objective of domestication programs.

In evolutionary studies of bottlenecks, the bottleneck is often treated as irreversible, but breeding programs will typically hold the full genetic diversity of a species in reserve while the breeding populations pass through bottlenecks. If additional genetic diversity is required, crossing with wild populations (possibly maintained in gene banks) will be a possibility. So long as wild populations are available, perennial grain breeding programs should be able to restore diversity as needed, as apparently occurred unintentionally during maize domestication and landrace evolution through natural gene flow from teosinte (Hufford et al., 2012).

Subdivided and structured populations—The effect of subdividing populations has been studied in natural populations and modeled extensively. In nature, new niches may develop, and species may diverge as the new niche is filled. There is evidence that species may diverge while gene flow continues between the subdivided populations (Osborne et al., 2013). Such a path to speciation may be similar to methods of domesticating new crops. Gene exchange will not necessarily prevent divergence, since strong selection can prevent much available genetic diversity from passing between the populations (Pinho and Hey, 2010). Although breeding populations are expected to be clearly separated from wild populations, intentional introgression from the wild to the domesticated population will likely be used in the breeding strategy. Nevertheless, as has been seen in natural speciation, rapid divergence is possible because artificial selection can be very intense.

Modeling of subdivided asexual populations showed that subdivision with migration allows accelerated crossing of fitness valleys when compared with a nonsubdivided population (Bitbol and Schwab, 2013). Another modeling study has shown that the chance of choosing the path of the largest initial fitness increase will increase with population size. Therefore, large populations may be "trapped" on peaks that are initially steep but are lower than other peaks that have gentle slopes at their bases (Jain et al., 2011). Furthermore, this study showed that the advantage of small populations will be greatest at intermediate time scales; the large population will quickly climb the steepest peaks, but may then be stranded until multiple mutants appear. Experiments with bacteria have shown that large populations are likely to be stranded in a rugged fitness landscape, but will have the advantage in a simple environment (Rozen et al., 2008). Rugged fitness landscapes are generated by antagonistic pleiotropic and epistatic effects; subdividing populations

by trait under selection (see below) could help the breeding program explore the landscape more effectively.

Small population size, as discussed in the previous section on bottlenecks, can reduce the number of deleterious recessive mutations. However, the fitness of individuals carrying the recessive alleles in the bottlenecked population will be reduced due to the increased number of homozygotes. Because selection can eliminate the deleterious homozygotes, the total number of deleterious alleles can further decrease. When a population has been subdivided and then the subdivided populations recombined, fitness can increase (Balick et al., 2013), a useful strategy to achieve purging (discussed above).

Particular population structures have been studied to determine their effect on evolution. One example is "star-structured" populations where nodes have only one connection to a central hub, while the hub connects to all nodes. Modeling has shown that various structures increase the fixation probability of beneficial mutations. However, time to fixation vs. probability of fixation have been shown to have opposite effects in determining the rate of evolution (Frean et al., 2013). In breeding programs, structured populations could be used to increase the probability that a new allele can sweep in, but then structure could be abolished by the breeder to allow rapid fixation.

Pleiotropy—Pleiotropy can be simply defined as a single gene affecting multiple traits. However, this important concept has grown in complexity beyond what we can describe here. Rather, we will point to a few theoretical and experimental topics that we find relevant to new domestication efforts.

Past domestication events have been able to take advantage of genes with profound pleiotropic effects on plant morphology. One example is the domestication gene *Q* of wheat, which confers free-threshing and pleiotropically influences rachis fragility, spike length, plant height, and spike emergence time (Simons et al., 2006). Another may be the *prog1* gene of rice, which likely influences height, tiller number, tiller angle, and panicle branch number (Sang and Li, 2013).

Genes with positive pleiotropic effects on multiple domestication traits may be pleasant surprises on the path to new domestication, but these few occurrences in past domestications give no assurance that positive pleiotropy will be common. Furthermore, positive pleiotropy in current varieties is no guarantee that the genes exhibited positive pleiotropy in the genetic context where they first arose. Indeed, for designing domestication programs, it would be useful to predict the prevalence of pleiotropy and whether to expect positive or negative pleiotropy to dominate.

As a generalization, *cis*-regulatory mutations are regarded as being less pleiotropic because they often affect only a subset of the expression domains of a gene, compared to protein-coding mutations, which alter the protein in every cell where it appears. Therefore *cis*-regulatory genes are hypothesized to be less constrained by the production of negative pleiotropic effects (Paaby and Rockman, 2013). Many domestication genes have in fact been found to be *cis*-regulatory mutations (Olsen and Wendel, 2013). A subtle *cis*-regulatory change dramatically altered the architecture of domestic maize (Wills et al., 2013), and similar changes are implicated in macroevolutionary shifts in part because plant genomes appear to "harbor rampant *cis*-regulatory flux" (Yant, 2012).

Literature reviews have shown *cis*-regulatory mutations to be more common among mutations affecting morphology than physiology, but an even stronger relationship exists for the

strength of selection and evolutionary timescales (Stern and Orgogozo, 2008, 2009). In domesticates and within species, polymorphisms between ecotypes or cultivars are commonly found within coding regions, whereas between species, where the divergence has taken place over longer evolutionary timescales, *cis*-regulatory polymorphisms dominate (Stern and Orgogozo, 2008; Seehausen et al., 2014). Strong selection, as is seen in domestication, can fix alleles with substantial pleiotropic effects (Otto, 2004). Therefore, we expect that in new domestications where time is severely limited we can expect to use many alleles with strong negative pleiotropy. Where *cis*-regulatory mutations can be identified, they would be preferred.

Pleiotropic effects can, in theory, create adaptive inertia. The inhibitory effect can be present even in the absence of genetic correlations, and the result can be lack of response to directional selection (Baatz and Wagner, 1997). Strong selection is expected to be able to overcome the inertia, but response may be slowed. To produce rapid response to selection, subdivided populations could individually be selected only for a single trait. Yield, which integrates many traits, could be reduced to a number of more fundamental traits for individual selection. For example, in wheat, seed length and width are under mostly separate genetic controls (Gegas et al., 2010). Therefore, progress may be more rapid by selecting for length and width separately, rather than mass.

Trade-offs between survival and reproduction are widespread, at least in animals (Paaby and Rockman, 2013). For instance, mutants and populations with increased lifespan typically have reduced fecundity, fertility, or later reproduction. However, examples are now being found where the expected trade-off does not exist. Flatt (2011, p. 374) stated, "Despite some evidence to the contrary, many of these observations are clearly at odds with the classical but simplistic notion of a competitive resource allocation trade-off between reproduction and survivorship. A more recent and now quite popular alternative view is that longevity–reproduction trade-offs might in fact be due to pleiotropic regulatory signaling processes that are independent of energy investment." If this statement is true, developing long-lived grain crops may be possible through selection, mutation, or alteration of environments to break pleiotropic relationships.

Epistasis—Epistasis is the interaction of alleles at different loci. In considering epistasis, we must distinguish between statistical and functional epistasis (Hansen, 2013). Statistical epistasis is defined by the presence of interaction effects in the regression analysis of phenotypes on gene content. Functional epistasis is observed when the effects of allelic substitutions depend on genetic background.

Strong evidence of statistical epistasis has been difficult to find and is often ignored in breeding and genetics. In maize, only a small role has been found for epistasis (Wallace et al., 2014). However, it is important to note that in statistical models interaction effects are inherently difficult to find and test without extremely large populations. Furthermore, statistical epistasis theory predicts that any effect of epistasis depends on linkage disequilibrium and will decay with recombination (Hansen, 2013). However, more recent theory has now shown that functional epistasis is likely to be a major contributor to additive genetic variance (Carter et al., 2005). The most important consideration is to distinguish the form of epistasis. While positive directional epistasis will contribute to additive genetic variance, negative directional epistasis will result in canalization, and nondirectional epistasis will not affect the response to selection.

Simulations and analytical arguments indicate that with directional selection the direction of epistasis will tend to become more negative; as positive interactions become weaker, the result will be an additive genetic architecture (Carter et al., 2005). The development of an additive genetic architecture in response to selection would explain observations with maize, where additive genetic architecture appears to dominate modern maize genetics (Wallace et al., 2014), while certain domestication genes are highly epistatic (Doebley et al., 1995; Lukens and Doebley, 1999; Durand et al., 2012).

If these theories are correct, we can expect epistasis to play a major role in new domestications. Large-effect domestication genes, as described in the section on pleiotropy, are expected to require a particular genetic background to function optimally. Hence, strong epistatic effects will be unavoidable. Furthermore in the early stages of domestication we might search for and find genes with strong positive directional epistasis. In fact, it has been our experience that when several genes conferring the domestication phenotype are "stacked", the result can be more than additive. These beneficial epistatic interactions can be seen in phenotypic distributions with a strong positive skew (Pooni et al., 1977; Cox et al., 2010). With large positive epistatic effects, higher selection intensities are expected to be optimal, since rare individuals with multiple positively interacting alleles can be selected.

One way to explain the abundance of epistasis is to place it in an evolutionary context. Because every change is built upon past changes, every solution arises in the context of an existing system. As solutions to one problem are fixed within an evolutionary lineage, new functional changes will generally be achieved by adding additional elements to those previously developed. Under this understanding, developmental pathways can become a "house of cards" where removing one critical element will severely impact the whole structure (Phillips, 2008). The "house of cards" may be a good way to conceptualize modern crop cultivars, where high yields are obtained through refined developmental pathways. Crossing with unimproved materials will often result in yield losses (Feuillet et al., 2008) since just a few exotic genes can easily disrupt the yield potential of elite cultivars.

If the "house of cards" model is common in nature, there are implications for developing perennial grains. Large-effect genes will be required to allow an annual grain to become a perennial or to allow for high seed yield in perennials. These genes of large effect will be almost certain to have profound epistatic interactions with the "background" in which they are placed. Thus, we would not expect that several genes conferring perenniality could be transferred to an annual grain crop without some impacts on traits such as yield or maturity. Under this model, transgenesis or wide hybridization cannot be seen as a clear short-cut. Although these approaches may help to provide a ready source of genetic variation, they will not eliminate the need for selection. Perhaps a lengthy period of selection should be anticipated to deal with the epistatic interactions resulting from most major-effect genes (except, perhaps, those caused by *cis*-regulatory mutations, as described in the previous section).

Mutagenesis followed by screening for changes in potentially important genes has been suggested as a pathway to rapid domestication of new species (Shapter et al., 2013). We strongly support the use of this technique, termed TILLING (targeting induced local lesions in genomes). A related approach, sometimes termed ecotilling, involves a directed search for useful mutations in wild populations (Harfouche et al., 2012; Henry, 2012). These strategies are worthy of expanded investment to

find major genes. However, we caution that many identified genes will produce epistatic and pleiotropic effects. Therefore, many generations of plant breeding may still be required to place the domestication genes within a genetic background where their benefits can be used without negative side effects.

Heterosis—Heterosis can be described as the difference between the performance of the progeny and the average of the parents (mid-parent heterosis) or the best parent (high-parent heterosis). Impressive yield gains have been made in crops like maize partially through the successful harnessing of heterosis (Brummer, 1999; Fischer and Edmeades, 2010).

The mechanisms behind the phenomenon of heterosis have been long debated, and consensus has yet to arise. However, heterosis is likely due to different mechanisms depending on species, individuals, and traits; overdominance, pseudo-overdominance, dominance, and epistasis may all be present (Hua et al., 2003; Zhou et al., 2012; Schnable and Springer, 2013). Another thought is that hybrids could involve genomic and epigenomic complementation (Schnable and Springer, 2013). A final theory is that organisms might be able to distinguish protein quality and only express the best allele. In this case, heterozygosity may save the cell energy by reducing protein production or different alleles may be used for particular environments (Goff and Zhang, 2013). No matter the single most important mechanism, we can at least conclude that heterosis is a unique effect that can be used through making F_1 hybrid varieties.

Stress tolerance is an important feature of heterotic grain crops, for which several mechanisms have been proposed (Blum, 2013). In the potential perennial biofuel, switchgrass (*Panicum virgatum* L.), heterosis was highest for biomass when the plants were grown in a dense competitive sward (Vogel and Mitchell, 2008). Stress and crowding tolerance may be particularly important for rhizomatous and tillering perennials that can become more dense over time. Therefore, heterosis should be explored as a means to deal with high-density perennial grain production environments.

Heterosis has potential for use in perennials, even though it has so far been infrequently used directly in breeding programs of perennials. While yield of hybrid maize has increased about 2% per year for decades, yield of perennial forages has been mostly stagnant; capturing heterosis is one proposal to obtaining increased yields in perennials (Brummer, 1999). Seed yield of perennial grasses has demonstrated particularly strong heterosis, exceeding 300% of the high parent in one case (Brummer, 1999).

Heterosis offers opportunity to increase yield without having to trade off other traits. Blum (2013, p. 4829) states that with heterosis "larger biomass and greater yield have been achieved without a change in growth duration, photosystem biochemistry, or harvest index." Indeed, lack of breeding progress in perennial forages has possibly been due to absence of tissue to trade-off, since yield consists of all aboveground biomass. Heterosis and/or purging may represent some of the few avenues available for increasing total aboveground biomass.

Brummer (1999) has provided practical strategies to developing hybrid and semihybrid varieties of outcrossing perennials. In general, the approach will rely upon keeping divergent and potentially heterotic populations separate in a breeding program and only combining them to produce the hybrid varieties. Such a method could be complementary with the strategies of subdivided populations, bottlenecks, and purging described above. Additionally, modern molecular genetic tools may be useful in identifying heterotic groups and particularly heterotic lines (Riedelsheimer et al., 2012).

Maternal and paternal effects can function similarly to heterosis and are worth a mention here (Roach and Wulff, 1987; Kirk et al., 2005). With new domestication, plant collection and hybridization may result in combining diverse cytoplasms with nuclear genes from distant geographic origins. In this case, strong nuclear–cytoplasmic interactions may arise, as have been observed in other species (Roach and Wulff, 1987).

Plasticity—Plant breeders have historically been leery of basing selection programs on traits in which a large part of the phenotypic variation is explained by environmental variance (and therefore less is explained by the genetic variance). Phenotypes are plastic if a single genotype can produce a range of phenotypes, canalized if multiple genotypes can produce "selectively equivalent phenotypes" (Schlichting and Wund, 2014). Breeders have historically considered plasticity "a nuisance" (Pigliucci, 2005) and might not predict that natural selection on highly plastic traits would produce examples of rapid evolution. However, that is what has been seen with invasive species that are characterized by high plasticity and evolvability (Maron et al., 2004).

The direction and magnitude of a plastic response is itself expected to vary from one genotype or population to another and thus be susceptible to alteration through adaptive selection; empirical evidence supports this prediction (Des Marais et al., 2013; Schlichting and Wund, 2014). One aspect of the domestication syndrome in grains appears to be general reduction in plasticity, including the reduction in opportunistic branching achieved in many crops through increased apical dominance and the fixation of dwarfing mutations and other genes that suppress the ability of densely spaced grain plants to manifest the shade-avoidance response (Ménard et al., 2013). The major maize domestication allele *tb1* is less environmentally plastic in its effects than the teosinte allele, and thus makes plants less responsive to changes in plant density (Doebley et al., 1995). Counterintuitively, while this finding reinforces the evidence that reduction in plasticity is a general domestication trait, the authors also suggest that teosinte's phenotypic plasticity may have predisposed it for domestication: "Maize evolution appears to fit the model that rapid morphological evolution can be achieved when selection favours one morph of a phenotypically plastic species" (Lukens and Doebley, 1999, p. 300).

Diggle and Miller (2013) found evidence to support the old but controversial idea of genetic assimilation—that constitutive phenotypes can be generated from plastic (environmentally produced) phenotypes by natural selection. Whereas most species are induced to produce more staminate flowers on branches where fruit was already setting (on earlier, hermaphroditic flowers), some species in the Solanaceae no longer produced staminate flowers while others produced the same proportion of staminate flowers after a certain number of nodes whether or not fruit was present on the branch. The authors make a useful distinction between the loss of plasticity in the first case, where altered floral forms are no longer produced, and true genetic assimilation of plasticity in the second case, where the previously environmentally induced phenotype (hermaphroditic flowers are considered the ancestral condition in the genus and the developmentally default pathway in most environments) is expressed constitutively.

Some authorities have recently suggested that genetic assimilation is an important evolutionary process that has been ignored because of its (superficial) association with Lamarckianism (Pigliucci and Murren, 2003). Crop domestication may

provide examples of genetic assimilation, in addition to the more obvious examples of loss of plasticity as demonstrated by the fixation of the maize *tb1* locus. The domestication syndrome for food plants is often described as including gigantism of fruits and seeds (Poncet et al., 2004). In many wild species, the size and number of fruits is highly plastic, affected by the quality of pollen, temperatures, proximity and number of competing fruits, stem position, and soil fertility (e.g., Matthies, 1990). For high value crops, especially asexually propagated ones, farmers intensively manipulate the fruit's environment to increase its size by pruning, thinning young fruit, manipulating branch angle, irrigation, etc. However, grain crops receive no such custom manipulation yet produce seed/fruit that is much larger than their ancestors', which may in part be the result of genetic assimilation of an environmentally inducible response.

It may be possible to replicate this genetic process during new domestication by selecting for yield and other domestication traits in a variety of environments early in the domestication process. Variant alleles capable of producing new phenotypes of great interest, such as increased seed size, may be present in the breeding population. However, if these phenotypes are seldom seen in individuals carrying these alleles because of the environment or presence of other genes, the alleles are described as having low penetrance. Selection in favorable environments in which normally low-penetrance alleles for traits such as seeds per head, seed size, and number of heads are expressed because of altered genetic correlations (Pigliucci, 2005) should "stack the deck" for the production of large changes in yield at a future point, even if in early generations this variation remains cryptic in the field. This strategy is most likely to succeed for traits that are known to be plastic but unlikely to be highly pleiotropic. *Thinopyrum intermedium*, for instance, produces much larger seeds in the greenhouse during the winter than ever seen in the field during the summer (L. DeHaan, unpublished observations). Because there is still variation between plants in the greenhouse, it might be possible to select for seed size potential, a trait that is normally hidden by some other genes that are epistatic to seed size genes in the field. Selection on spaced plants in well-fertilized or irrigated nurseries might accomplish something similar for this or other domestication traits. Selection in stressful environments could also be effective in revealing cryptic variation by inducing specific plastic responses and general epigenetic alterations. Whereas extremely large *Thinopyrum* seeds are seen more frequently in the greenhouse, an embryo-aborting phenotype is more frequent in (stressful) field conditions; highly sterile individuals become at least partially fertile (and more difficult to spot) in the greenhouse (L. DeHaan, unpublished observations). Stress has been associated with methylation changes, globally reduced RNA quantity, altered rates of RNA transcription and stability (Grossniklaus et al., 2013; Hodgins et al., 2013). Any of these mechanisms could affect the expression of an allele. To ensure that plants or individual tillers experiencing crowding or other stresses remain fertile, it may help to screen for fertility in such environments as a way of screening for alleles that are less susceptible to these expression-modifying processes (i.e., that are more penetrant).

For this strategy to accelerate the domestication program, cryptic variation preadapted in different experimental environments must be brought together at some point for selection for yield per unit area in a realistic agronomic environment and one of several events must happen to uncover it. A capacitor gene mutation could be found as a rare allele (e.g., by rescreening germplasm collections) or produced by mutagenesis. Such an al-

lele might be easier to find if its phenotypic effect has been magnified by preadaptation. Alternatively, it might be found that parallel selection in different environments (and/or for different traits) followed by recombination might produce altered genetic backgrounds in which the previously cryptic variation is more penetrant. Finally, we speculate that a single, apparently monomorphic epistatic (in a particular environment) locus may itself contain cryptic variation (and a multigene network almost certainly would). A transcription factor, for example, is likely to be robust to mutations, harboring numerous amino acid variations with very little alteration in specificity in normal conditions (Espinosa-Soto et al., 2011a). However, if this epistatic effect is plastic (e.g., reduced in a hypothetical favorable greenhouse environment), the protein itself may be plastic, occasionally assuming an alternative folding form. Selection in the environment favoring the plastic response followed by sexual recombination could incrementally stabilize the alternative form eventually making it the "native" form (Espinosa-Soto et al., 2011a). Mutations stabilizing alternate enzyme geometries have been directly observed in a directed evolution experiment (Tokuriki et al., 2012). Thus, selection in a nontarget environment could both preadapt normally cryptic variation for traits of interest, but could also result in the genetic assimilation of the trait.

Epigenetics—Several kinds of cellular changes can—without involving DNA sequence changes—induce mitotically stable changes in gene expression, enabling cell differentiation during development, whole organs (or organisms) to adjust gene expression in response to environmental signals, or genomic defense responses to new chromosomes (after wide hybridization) or newly inserted sequences from viruses, transposable elements, or transgenes (reviewed by Rapp and Wendel, 2005; Bond and Baulcombe, 2013; Diez et al., 2014). These cellular mechanisms include DNA methylation and histone modifications contributing to local chromatin remodeling that both affect the binding of the transcription apparatus to the template. Proteins and RNA can be transmitted mitotically even after the genes from which they were transcribed have ceased expression, and some of these are potent transcription factors or affect posttranscriptional steps in gene expression (Grossniklaus et al., 2013).

While most acquired epigenetic marks are erased during meiosis (and in the case of genomic imprinting replaced with pollen or egg-specific marks) there is good evidence that at least a few kinds of epigenetic states can be inherited transgenerationally. Until recently, evidence for the transgenerational inheritance of epigenetic suppression of transposable elements, duplicated genes or alien genomes (Freeling et al., 2012) was stronger than the evidence for the transgenerational inheritance of environmentally acquired epigenetic states. Several recent studies demonstrate transgenerational inheritance of induced plant defense states (reviewed by Holeski et al., 2012) or life-history states (Galloway and Etterson, 2007), although they are interpreted as examples of transgenerational plasticity rather than population-level evolutionary change in the Lamarckian sense (Grossniklaus et al., 2013). Other kinds of stresses are known to induce epigenetic changes, and some of these may eventually prove to be transgenerationally heritable as well (Bond and Baulcombe, 2013).

Other authorities caution that there are many kinds of parental and even grand-parental effects (seed provisioning, time of seed production/dispersal, etc.) that are difficult to distinguish from epigenetic effects (Klironomos et al., 2013). In part, our inability to clearly identify epigenetic effects is due to the inherently higher mutation rate for epialleles. Plant breeders have

prioritized additive genetic variance in calculating breeding values (e.g., Fehr, 1991). However Klironomos et al. (2013) pointed out that the distinction between genetic and epigenetic is somewhat artificial; many mechanisms can generate heritable variance in fitness, including maternal effects, epigenetic mutations, point mutations and (we would add) gene duplication, translocations, retroposition, and change in chromosome number. These range in the number of generations expected between successive mutations (fidelity) from 2 to tens of millions, but in principle natural selection can act on any form of heritable variation. Very strong selection is required to use low-fidelity variation (i.e., when the variant state is stable for few generations) for adaptation (Klironomos et al., 2013) but is exactly the kind of selection that domesticators can apply. These predictions from basic research are supported by recent investigations of the methylome of domesticated species, epimutagenesis, and artificial selection in genetically uniform populations. Examples of fixed epigenetic differences between the domesticated species and its wild ancestor have been found in soybean (Zhong et al., 2009), rice (Li et al., 2012), silkworm (Xiang et al., 2013), chicken (Nätt et al., 2012), fox (Trut et al., 2009), and finch (Wada et al., 2013). Although the potential for interaction between genetic changes and altered epigenetic states is great, making causality difficult to determine, several of these authors have hypothesized that "artificial selection might act on epigenomes to acquire human-preferred traits" (Xiang et al., 2013, p. 7).

Roux et al. (2011) experimentally perturbed the methylome in an inbred *Arabidopsis thaliana* (L.) Heynh. population and, after six rounds of selfing to create "eRILs", compared the phenotypic variation obtained with genetically diverse natural populations in a common garden. Similar types of heritable phenotypic variation were seen in both populations, suggesting that complex traits have polygenic architecture in which phenotypic variation can be produced by complementary epigenetic and genetic mechanisms. This is not surprising in light of the strong association of epigenetic polymorphisms with altered levels of gene expression (Bond and Baulcombe, 2013). More dramatically, Hauben et al. (2009) performed recurrent divergent selection on small populations of genetically identical (doubled haploid) canola (*Brassica napus* L.) plants and achieved stably inherited changes in respiration rate. Not only were the traits stably altered, but they contributed to significant increase (reduced respiration lines) or decrease in seed yield (elevated respiration lines). Specific methylation and histone modification patterns characterized each selected line. Altered respiration states were unstable in early rounds of selection, but stability increased with the cycles of selection. However, as the recurrent selection did not involve intermating, the gains seen with additional selection could not be attributed to fixation of alleles in a population as in classical plant breeding, but to some unknown "epigenetic stabilization" phenomenon, perhaps involving miRNA expression. This conclusion illustrates the uncertainty that remains about several transgenerational epigenetic phenomena but supports the argument that "evolutionarily significant phenotypic novelty can arise from environmental or epigenetic alterations of the genotype-phenotype map" (Schlichting and Wund, 2014, p. 662) even in the absence of genetic variation.

Noncoding RNA (ncRNA) can be transmitted in very different ways than DNA and histone modifications. It is beyond our scope to describe the somewhat bewildering array (and nomenclature) of ncRNA species, or to describe all of the cellular functions in which they participate (there are many reviews, including Amaral et al., 2013). Apparently important in all organisms— Mattick et al. (2010, p. 24) predicted that "the genomes of mammals are almost entirely transcribed"—they seem to have special importance in plant epigenetics because plants express RNA-dependent methyltransferases during gametogenesis and embryogenesis which, combined with the possibility of small RNA molecules diffusing from one cell to another, provides an explanation for why transgenerational epigenetic effects have been found more often in plants than other types of organisms (Bond and Baulcombe, 2013). They are of special interest here because of their emerging importance in understanding rapid evolutionary change. Because of the number of loci, their mutability, role in gene regulation, and plasticity of expression, they may "serve as central targets and substrates for regulatory change" (Amaral et al., 2013, p. 255).

Other previously puzzling effects that may be explained by ncRNA include paramutation and the ability of initially unstable epigenetic changes to be stabilized in subsequent generations (Bond and Baulcombe, 2013). This stabilization is not always a population allele-frequency effect as in classical plant breeding, but is thought to happen at the plant level or within selfed lineages and involves "feed forward" interaction between ncRNA and DNA methylation enzymes. Methylation appears to attract more methylation or chromatin modifications, and eventually the RNA-dependent pathway is not required.

RNA transcript concentrations can vary stochastically from cell to cell, potentially resulting in differential inheritance, and thus creating variation in methylation states between progeny. Environmental conditions, viruses and wide crosses could also induce ncRNA transcription or alter the stability of ncRNA species, and some of this new variation could be inherited transgenerationally as may be happening in the examples of inherited induced plant defenses (Grossniklaus et al., 2013).

In addition to deliberately accessing a new "reservoir of heritable variation" (Pigliucci, 2008, p. 894) using epimutagenesis, or screening genetically uniform populations for de novo epialleles, new information about epigenetic inheritance can help a domesticator avoid prematurely discarding phenotypic variants that do not appear to be stably inherited or not transmitted at Mendelian frequencies. Under a "mixed model" of inheritance, we would expect that novel phenotypes might be produced by a mixture of cellular and genetic systems that may be impossible to disentangle (on a timescale useful to a breeder). "Intrinsic stochastic fluctuations" (Masel and Siegal, 2009, p. 397) such as might be produced by cell division imprecisely partitioning maternally derived ncRNAs, transcription factors or hormones, interacting with a particular combination of large-effect alleles (epistasis) and numerous small-effect alleles ("genetic background") and signals transduced from environmental conditions could have created a new but ephemeral epigenetic state producing altered gene expression leading to the novel phenotype expressed in a chimeric and temporally unstable pattern. This variation could be stabilized by selection of feed-forward methylation events as described above and could also expose genetic variation normally hidden by epistatic interactions with epigenetically regulated loci. Selection could act on these low-penetrance genes, enriching the (normally) cryptic variation for variants with desirable effects, particularly if this state can be recreated repeatably by specific environmental triggers (Espinosa-Soto et al., 2011b).

Beyond simply using the hypothetical rare plant described above as a parent in a new subpopulation, this plant could be cloned (straightforward for most perennials) and used to identify environments (including meristem position on the plant) or major genes that might induce or suppress the unstable plastic phenotype.

Cryptic variation—Much of a population's genetic variation is cryptic, meaning the inheritance of a particular mutated allele has no effect on phenotype (in a given genetic and environmental background) and thus is hidden from natural and artificial selection (reviewed by Le Rouzic and Carlborg [2008], but see the section on genetic load for cumulative effects of many mildly deleterious alleles). In prehistoric and contemporary domestication efforts, novel phenotypes appeared in the populations under human-mediated selection. These phenotypes must have come from a combination of de novo mutations and previously cryptic variation. We consider cryptic variation here because outcrossing perennials may harbor a great deal of it (see earlier section *Genetic load*) and learning to use it could accelerate new domestication programs compared with relying solely on new mutations (induced or spontaneous).

In theory, cryptic variation can be revealed in a new environment, as when stress disrupts developmental canalization (Pigliucci and Murren, 2003; Badyaev, 2005) or by new mutations suddenly increasing phenotypic variance and thus facilitating rapid natural selection and, presumably, rapid artificial selection (see later section *Robustness and evolvability*). Experimental support for this prediction came from the in vitro evolution of novel ribozyme specificity: high cryptic variation populations were created experimentally using mutagenesis followed by stabilizing selection for the native function (to ensure that new mutations were cryptic in the normal environment). When the selection regime was subsequently altered, these high cryptic variation populations evolved an altered substrate specificity 6–8 times more rapidly than unmutagenized populations with the same native activity (Hayden et al., 2011).

DNA mutations can be noncryptic at one level of biological organization, yet cryptic at a higher level, so one must be clear about the phenotype being considered. For example, a base-pair mutation may produce a new phenotype in the form of a single amino acid substitution in an enzyme, but this enzyme may have unaltered activity due to the epistatic effects of the other amino acids, thus making the mutation ultimately cryptic (Tokuriki et al., 2012). At a higher level of organization, mutations producing altered protein abundance or activity may be cryptic if other genes with compensatory epistatic effects are present.

It is helpful to keep these cellular and genetic processes in mind when considering the circumstances in which cryptic variation is revealed or exposed and thus available for natural or artificial selection. We have discussed how environmental triggers can change gene expression states, (perhaps epigenetically) exposing low-penetrance alleles for at least one cycle of selection. Another scenario is the "co-option" (Rajon and Masel, 2013) of existing cryptic variation by a single new mutation at another locus. In the wild-type state, this epistatic allele has served as a "capacitor" allowing a genetic "charge" to build while preventing it from discharging (Le Rouzic and Carlborg, 2008). A mutation at this locus could produce extreme, novel phenotypes by instantly revealing many other mutations (Masel and Siegal, 2009). For example, mutations may freely accumulate in untranslated regions of a gene including introns and 3′ sequences. However, when a mutation-altering splice site or stop codon appears (and spreads through the population), these divergent regions may now be expressed, creating longer proteins with potentially altered function. *Cis*-regulatory regions of long-silenced genes (e.g., duplicated genes that have accumulated mutations) may suddenly mutate and become active (Rajon and Masel, 2013).

In these examples, the new mutation must spread through the population and expose variation likely to have additive effects on phenotypes. However, co-option mutations may produce larger phenotypic effects epistatically where a history of compensatory evolution resulted in "a diversity of cryptic effect sizes across sites rather than across individuals" (Rajon and Masel, 2013, p. 1209). This scenario is consistent with the results of mapping studies finding that alleles fixed at various loci within a line often have opposing phenotypic effects (reviewed by Rieseberg et al., 1999).

Simulations by Masel and Siegal (2009) suggested that the quality and quantity of previously cryptic variation was correlated with the strength of previous selection against lethals leading to increased chances of the appearance of "hopeful monster genotypes that appear in individuals lacking hopeless monster alleles" (Masel, 2006, p. 1985). "Hopeless monster" mutations, those that are strongly deleterious in all environments, are distinguished from "hopeful monster" mutations that are more mildly deleterious and, occasionally, adaptive (Masel, 2006).

This enrichment effect was much larger when numerous cryptic loci were involved in producing the phenotype, consistent with the capacitor gene models described above involving encryption by epistatic interactions between many loci in a gene network. An even stronger preadaptation (or exaptation) effect could be produced by a history of positive selection operating on cryptic variation. While recently shielded from selection, this variation could have been less shielded in previous generations. Microarray studies often show that environmental changes can alter the expression of hundreds of genes, temporarily exposing them to selection (Schlichting and Wund, 2014). If previous environments shared features with a new environment, the standing variation might be considerably more adaptive than might be predicted (Pigliucci and Murren, 2003).

We see an application of this theory to the process of ranking candidate species for their likely domesticability. While "[h]opeful monster mutations … are the raw material of evolutionary innovation in response to environmental change" (Masel, 2006, p. 172), they are exceedingly rare by chance alone, and anything we can do to favor their appearance will accelerate domestication. By prioritizing species or ecotypes likely to have experienced useful selection in the past (preadaption), it may be possible to increase the odds of finding new mutations or rare gene combinations that produce novel domestic phenotypes. For example, because the agricultural environment is likely to have more available nutrients and less competition than almost any natural plant community, the best candidates for domestication may have a native distribution that includes favorable landscape positions and fertile soils and may have experienced episodic or mild selection for the ability to rapidly respond to favorable conditions with high growth rate and fecundity. This variation could accelerate a domestication program even if this variation is cryptic in plants growing in the wild or in early generations of selection. In wild/semidomesticated populations, this useful variation may remain invisible due to epistatic loci with compensatory effects on this variation that may have been fixed by thousands of years of drift, stabilizing selection (Rajon and Masel, 2013), or selection for positive pleiotropic effects on traits currently under strong selection in the wild such as root competition, pollen production, or stress tolerance. In later generations of artificial selection, new phenotypes may appear: previously hidden alleles with small additive effects may increase in frequency; new "capacitor" alleles with weaker epistatic effects may arise.

Species or ecotypes found mostly in resource-limited environments may have never experienced this selective enrichment of

(normally) cryptic variation; these alleles may have actually been selected against if slow growth and conservative investment in reproduction sometimes increase longevity in this environment. Such species may prove to respond slowly to domestication efforts.

Robustness and evolvability—Developmental and cell biology seek to understand the processes that mediate the genetic information and the phenotype—the genotype–phenotype map. Broadly, trait robustness is the degree to which many genetic or environmental states map to a single phenotypic trait, thereby buffering the phenotype from disruption (Espinosa-Soto et al., 2011a).

Environmental robustness measures the insensitivity of a phenotype to multiple environments and may require particular homeostatic adaptations for achieving it, since the default state for most chemical reactions is strong sensitivity to changes in abiotic conditions such as temperature, pH, light, etc. (Pigliucci and Murren, 2003). Phenotypic plasticity then can either be the ancestral character or derived from a previously evolved robust state.

Genetic robustness measures the insensitivity of a phenotype to genetic perturbations (Espinosa-Soto et al., 2011b). Many molecular markers are associated with robustness in *Arabidopsis*, even for traits like flowering, implying that robustness in many traits could be selected for or against but has not recently been under strong stabilizing selection (Fraser and Schadt, 2010). Since genetic robustness QTLs by definition suppress changes in the expression of other genes, cryptic variation will be exposed in individuals with reduced robustness. Genetic and environmental robustness phenotypes map to different, non-overlapping QTLs in *Arabidopsis* (Fraser and Schadt, 2010), but genes associated with environmental robustness also "buffer the effects of genetic change" in yeast (Lehner, 2010, p. 1).

Genetic robustness is created when phenotypes are produced by redundant alleles through gene duplication and polyploidy, "fantastically complex" gene regulatory networks (Yant, 2012) with epistatic genetic architecture (Le Rouzic and Carlborg, 2008), complex developmental pathways (Badyaev, 2005), convergent biological pathways, and compartmentalization, which reduces the potential of mutations to be lethal when expressed (Kopp and Matuszewski, 2014). Cellular mechanisms may provide buffering, including types of negative feedback and nonlinear relationships between gene transcription and phenotype, such that moderate changes in gene expression do not affect the phenotype (Hartman et al., 2001; Springer and Stupar, 2007).

Counterintuitively, robustness is thought to increase evolvability even though robustness reduces the range of phenotypes currently available for natural selection (Wagner, 2008; Masel and Trotter, 2010; Espinosa-Soto et al., 2011b; Kopp and Matuszewski, 2014). This apparent paradox reveals a trade-off between the processes of short-term adaptation versus long-term evolvability as illustrated by the following scenario: an increase in robustness relaxes the effects of stabilizing selection on mutant alleles, allowing a population to accumulate cryptic genetic variation. In the short term, this variation may reduce average fitness (see section *Genetic load*). In the long term, change in the genetic or environmental background may alter the genetic buffering, allowing the "discharge … of potentially adaptive genetic diversity" (Le Rouzic and Carlborg, 2008, p. 35). Preadaptation of this cryptic variation also increases evolvability. Population size, structure, and mating system can affect evolvability through their effects on standing genetic variation. Traits with both heritable genetic and epigenetic regulation—a type of multiheritability-system epistasis—could evolve orders of magnitude faster than when a single system is responsible, according to recent simulations (Klironomos et al., 2013), perhaps because phenotypic and genotypic change can be decoupled in this way.

Although genetic robustness increases evolvability, canalization of development that creates environmental robustness may constrain evolvability. "The relaxation of constraint also increases the intrinsic capacity of organisms within the population to vary phenotypically and increases the potential of that population to generate novel mutations useful for future adaptation" (Brown, 2014, p. 5). Attempting to explain the extremely rapid and dramatic de novo domestication of the fox, Belyaev (1979) suggested that destabilizing selection had overcome the robustness and canalization mechanisms built up by a long history of stabilizing selection in a particular environment. Early allele-frequency or environmental changes leading to altered neurohormones may have resulted in epigenetic-mediated changes in gene expression in key brain genes that "sharply destabilized the correlated developmental systems and led to an increase in phenotypic variation" (Trut et al., 2009, p. 356).

Organismal complexity may reduce the rate of adaptation, as discussed in the pleiotropy section. Gene modularity, where genes' pleiotropic effects are restructured through development- or organ-specific expression, can therefore increase the evolvability of complex organisms (Pavlicev and Wagner, 2012). Gene duplication/polyploidy can have a general cryptic-variation accumulating effect but also enable modularity to evolve, allowing the evolution of gene families with different temporal, spatial, or environmental expression between paralogs, as has been shown in maize and wheat (Buckler et al., 2001). Subfunctionalization after gene duplication resulted in four paralogs of the flowering time (*FT*) locus in sunflower (*Helianthus annuus* L.); these paralogs have played distinct roles during sunflower speciation, domestication, and recent genetic improvement through breeding (Blackman, 2013).

Traits requiring epistatic interaction of mutations will have to cross a rugged fitness landscape with features that impede and promote adaptation. Simulations show that an intermediate level of epistasis and pleiotropy maximizes adaptation by increasing the average effect of a single mutation (compared with a smoother landscape) but reducing the chances of the population becoming "stuck on a suboptimal fitness peak" (Ostman et al., 2012). It may be necessary for the population to accumulate mutations in a particular order to avoid being stranded in this way. Historical contingency in the rate of evolution may be common. The evolution of most yeast life-history and metabolic traits in a recent experiment depended on the identity of the initial genotype used to found the population; phenotypic evolution was better explained by the initial genotype than by selection regime treatment (Spor et al., 2014). True evolutionary innovation was observed when after 30 000 generations, a single population among replicate *Escherichia coli* lineages (derived from a single ancestor) evolved the ability to use citrate as a carbon source under oxic conditions, something never seen in this species before (Blount et al., 2008). A "potentiating" but invisible mutation at some point in this one population appears to have made the citrate phenotype mutationally accessible to a second mutation. The authors conclude this trait must have been difficult to evolve because prior to the novel phenotype appearing, the populations experienced far more mutations than the number of possible point mutations in the bacterial genome and could have tried "every typical one-step mutation many times" (p. 7900).

Experimental evolution using microbes has shown the dependence of evolvability on genetic background and historical contingency perhaps in part because it is possible to control these factors. These factors are not controllable in the case study (Maron et al., 2004) provided by invasive plants, where attention has focused on the role of phenotypic plasticity in evolvability.

In their new environments, successful invaders often grow larger and more rapidly than they did in their native environment, suggesting, either (or both) that these species were capable of plastic responses, allowing initial colonization of new environments, or that plasticity itself may have been selected for in the new environment (Jakobs et al., 2004). Consistent with the selection model, invasive genotypes from several species have been shown to have greater phenotypic plasticity than native genotypes (Leger and Rice, 2003; Lavergne and Molofsky, 2007). More surprisingly, exotic populations of *Solidago gigantea* Aiton in Europe were shown to have both higher phenotypic and genetic variation than native North American populations.

From "natural experiments" like these, it is impossible to know whether phenotypic plasticity is the cause or the result of invasiveness; at the minimum, we can infer that plasticity has not been an impediment to rapid adaptation to new, generally more favorable, environments. In evolutionary simulations, populations evolved novel, adaptive traits faster when traits were allowed to be plastic, despite a trade-off with the rate of fixation of plastic phenotypes due to their lower penetrance (Espinosa-Soto et al., 2011a). One explanation is that in new environments, multiple individuals in populations with plastic traits may discover new phenotypes simultaneously, reducing the chances of the loss of rare adaptive changes by drift (Pigliucci and Murren, 2003). With rapid artificial selection, perennial grains could also "invade" nutrient-rich and competition-poor cropping systems. As with invasive weeds, plasticity may be less of an impediment than classical plant breeding theory would suggest.

Domestication progress will depend in part on the evolvability of the breeding population. It would therefore be useful to test hypotheses about evolvability of plants under artificial selection: (1) The level of environmentally inducible plasticity for a trait in a wild plant can be used as a predictor of the response to selection for that trait in a domestication program. If this hypothesis finds experimental support in the future, it would be useful to develop a standard set of controlled environments in which to observe candidate species' plasticity. Likewise, it will be useful to prioritize plant traits for early observation. In our experience, wild traits such as seed shattering create so many difficulties in breeding programs that they should be ranked very high. We would also place seed dormancy and low seedling vigor in this category.

(2) A population based on an individual with even a developmentally transient, environment-specific, or non-Mendelian new phenotype is likely to be more evolvable (with respect to the particular phenotype) than a plant selected at random from the base population. This hypothesis is based on the following logic. We have seen that genotypes capable of producing multiple phenotypes are of special interest because of the opportunity for assimilating the alternative phenotype or "morph" (Lukens and Doebley, 1999). We have also seen that some novel phenotypes may have to be assembled by a particular sequence of mutations (e.g., the evolution of citrate-metabolizing *E. coli*; Blount et al., 2008) or gene complexes (as in the case of maize architecture; Lukens and Doebley, 1999). Thus, the progeny of an unusual plant may not be expected to rapidly ascend an adaptive peak (as defined by the breeder) due to insufficient additive genetic

variation, but may have explored that part of phenotypic space better than other subpopulations and may be better positioned for rapid change if new additive genetic variation became available in this population through controlled crosses, mutagenesis, fixation of epistatic variation, etc.

(3) A related hypothesis is that subdividing the breeding population and allowing independent evolution (for several generation) may be a more efficient use of breeding resources than maintaining a single large population. This idea was presented in its own section above. Here we emphasize the bet-hedging benefits of this approach, given the unpredictable effect of particular mutations or genetic background on subsequent evolvability. Chance events (including founder effects and drift) may allow at least one subpopulation to evolve rapidly.

(4) It has been suggested that fewer genetic changes are required for phenotypic and taxonomic divergence between lineages of plants than for animals, due to plants' simple embryogenesis, iterative/modular development, and low number of cell and tissue types (Gottlieb, 1984), leading, along with a history of polyploidy and a higher mutation rate (Freeling et al., 2012), and more heritable epialleles (Bond and Baulcombe, 2013) to higher evolvability. Extending this logic, we hypothesize that plant forms with simpler, smaller, shorter-lived, less physiologically integrated modules are likely to be more evolvable than more complex forms. Thus, annuals may be more evolvable than perennials, and large tree-like perennials less evolvable than herbaceous perennials, everything else being equal. Whether this hypothesized gradient in evolvability due to growth form is steep or not—and therefore a major factor in selection of new candidates for domestication in comparison with other contributors to evolvability such as standing genetic variation, or preadaptations (e.g., seeds already large)—remains to be seen.

Past domestication—Domestication can be understood as the occurrence of evolutionary changes that may decrease the fitness of a species in the wild but increase it under human management (Meyer and Purugganan, 2013). Historical domestication events were likely driven by unconscious selection pressures, so many of the mechanisms may be similar to those occurring in natural selection (Purugganan and Fuller, 2011). Current domestication efforts will be driven by conscious selection, so the paths traversed in past domestication events need not be retraced. Specifically, modern domestication events are hoped to proceed over decades rather than centuries to millennia. Nonetheless, we examine a number of findings made in the study of past domestications to find direction for our current efforts.

Proto-agriculturalists created new, highly disturbed environments as they built villages, burned forests and cleared competitors from favorite stands of wild food plants (reviewed by Cox, 2009); annual (or weakly perennial) plants invaded these resource-rich habitats (McKey and Elias, 2012), evolving to take advantage of services now provided by humans. These services included agricultural practices that reduce plant competition, herbivory and pathogen load. Natural selection subsequently favored a shift in development and allocation from resource conservation and defenses to rapid growth, resource acquisition, and seed production.

Long-lived perennials, being even more conservative in use of resources and allocation to defenses than wild annuals, could not invade the original agricultural environments. However, through artificial selection informed by the domestication of wild annuals, they may be able to adapt to human-created, resource-rich, low-risk habitats. Clearly, the domestication of

perennial grains requires increased allocation to seed production, but attention to more fundamental traits may make this possible. Their history of natural selection under resource-limiting conditions may explain why perennials often have lower relative growth rates than annuals (Garnier, 1992). Direct selection for increased relative growth rate in a resource-rich environment could cause domestic perennials to establish and become sexual earlier, reduce their allocation to defenses, purge mildly deleterious alleles, flower earlier in the season, and produce larger organs, including reproductive structures.

Past domestication events have been found to move through several somewhat predictable stages (Meyer and Purugganan, 2013). In the first stage, grain size increased, growth habit became more determinate, and inflorescence architecture changed. Later stages involved increased seed number, dwarfism, and improved stress tolerance. Another author suggested that in small grains, increased seed size preceded shatter resistance (Fuller, 2007). However, in other species the order of traits arising was different, implying different selection pressures or underlying genetic architecture (Fuller, 2007). Current domestication efforts might not follow historical precedent, but the order in which traits are subjected to strong selection may be important. Some traits should be primary (such as nonshattering) because they allow breeding work on other traits to progress more quickly. If progress cannot be readily made on these traits, then other species may be better candidates for domestication.

A major domestication gene of rice, *qSW5*, has been identified (Shomura et al., 2008). This gene increases sink size by increasing the cell number in the outer glume of the rice floret. Genes such as *qSW5* point to the importance of increasing sink strength in grain domestication. In this case, the rice flower needed to be enlarged to allow wider seeds to develop. Similar constraints may exist in many future domesticates.

Studies of genes involved in domestication phenotypes have revealed a wide array of underlying mutations (Gross and Olsen, 2010; Olsen and Wendel, 2013). Of nine domestication loci studied across several species, eight were found to encode transcriptional factors (Purugganan and Fuller, 2009). About five genomic regions have been found to be responsible for the basic maize domestication phenotype (Tian et al., 2009). Genetic analysis of one maize domestication gene showed that a subtle *cis*-regulatory change in gene expression caused a profound change in the plant form (Wills et al., 2013). So dramatic changes can be achieved in plant morphology with fixation of a small number of major-effect genes.

In addition to major-effect genes that might lead to the domesticated form, a large number of genes have been selected for what might be termed adaptation or improvement. In maize, about 1200 genes, primarily of small effect, have been targets of selection (Tian et al., 2009). Maize, being an outcrossing species, had a high level of recombination during domestication. The result was that domestication proceeded slowly, but substantial diversity was retained (Wang et al., 1999; Flint-Garcia, 2013). The largest studies have found that in maize, traits are controlled by many genes, their effect sizes are small, and there is little to no pleiotropy or epistasis (Wallace et al., 2014).

Dramatic changes can be achieved in plant morphology with fixation of a small number of major-effect genes. Studies of genes involved in domestication phenotypes have revealed a wide array of underlying mutations, all with substantial impact (Gross and Olsen, 2010; Olsen and Wendel, 2013). About five genomic regions have been found to be responsible for the basic maize domestication phenotype (Tian et al., 2009), although

individual traits such as height have been found to be highly polygenic (Peiffer et al., 2014). Genetic analysis of the *prol1.1* maize domestication gene showed that a subtle *cis*-regulatory change in gene expression caused a profound change in the plant form (Wills et al., 2013).

These findings contrast with the view that in adaptation "the single most important genetic mechanism … was the assembly of favorable epistatic combinations of alleles of different loci by means of recurring cycles of selection …" (Allard, 1997, p. 1). The strong epistatic combination viewpoint can be reconciled with the finding of little epistasis in modern corn cultivars with the knowledge that about 1000 genes have been essentially fixed in modern corn cultivars, so that these genes will be invisible in genetic studies involving maize × maize crosses (Flint-Garcia, 2013). Although genes in strong epistatic networks may have been very important in domestication and early selection, the diversity remaining is mostly additive.

If domestication proceeds in a manner where genes of large effect that are generally epistatic are fixed first, then new domestication projects could differ substantially from breeding programs designed to fine-tune existing crops. Breeding methods that actively seek out and use genes (as in TILLING) with large epistatic effects may be useful in the early stages of domestication (Shapter et al., 2013).

Sunflower (*Helianthus annus*) is worthy of mention because its domestication appears to have been distinctly different than other species. In sunflowers, major domestication genes have not been identified; rather, many genes of small effect have been found (Burke et al., 2002; Wills and Burke, 2007). Presumably, other future domesticates may follow the sunflower model. Whether domestication can proceed more rapidly with or without major domestication genes is not known. Although large-impact genes can quickly produce a domesticated phenotype, they may be difficult to find and they may have undesirable pleiotropic effects. Probably, domestication efforts would do well to first search for useful major genes and evaluate their effects before moving on to strategies designed to accumulate genes of small effect.

Because we are considering the domestication of perennial plants with high reproductive allocation, some consideration should also be given to fruit and nut trees. In general, trees were domesticated more slowly, show fewer domestication traits than grains, have few common domestication traits, and were domesticated in association with shifts to vegetative propagation (Meyer et al., 2012). Trees are generally cross-pollinated and have high heterozygosity (Zohary and Spiegel-Roy, 1975). In these ways, they are similar to herbaceous perennials. However, herbaceous perennials have small stature and are therefore unlikely to be easily propagated vegetatively—although it is being investigated (New Energy Farms, 2014). Creating F_1 hybrids would likely be a more cost-effective way of distributing highly heterozygous, but uniform, genotypes. At any rate, apple breeding may be a good model to follow, where there is very low diversity between individuals of a cultivar, high heterozygosity, and a high level of diversity between cultivars through constant introgression of genes from wild germplasm (Cornille et al., 2014).

Tree domestication is hypothesized to have taken an interesting course, described by Goldschmidt (2013). First, selection was for release from genetic constraint on reproductive allocation. Essentially, sink strength had to be increased genetically to enable the production of many large fruits. The result was alternate year bearing, where high productivity years exhaust available resources and cause little production the following

year. Subsequent breeding and agronomic practices have refined allocation patterns and increased resource supply, allowing high productivity every year. Perennial grain breeding may follow a similar course. In the early stages of domestication, most selection will have to focus on increasing sink strength. Only when sink strength has been dramatically increased will traits affecting photosynthate supply be "visible" to selection.

Selection—Kenneth Mather, an eminent geneticist of the last century, has been quoted as writing "given time, genes and gene combinations can arise to do anything. The nature of the selection itself then becomes decisive in determining the response" (Futuyma, 2010). Of course in the short term, there are many good examples of genetic variation limiting response to selection, but in general, rates of evolution have been found to be slower than would be expected given mutation and drift (Futuyma, 2010). Therefore, to provoke rapid domestication of new crops, we must carefully consider the nature of selection applied to obtain the desired response.

Futuyma (2010) listed four major limits to selection. We summarize each, followed by implications for domestication programs.

First, the character may only exist in our imagination. An example may be longevity in a perennial grain. When there is no programmed death, selecting for increased lifespan by the breeder may be relatively meaningless. Instead, the useful traits to consider may be disease resistance, cold tolerance, or heat tolerance. Another example may be high grain yield under low nitrogen availability. Selection for high grain yield under nitrogen limitation may be impossible unless the species is capable of symbiotic nitrogen fixation.

Second, the necessary genetic variation may not exist in the population, and so response may be limited by the rate at which mutations arise. If the mutational target size is small, the required mutations may seldom arise. In such a situation, mutagenesis would be a practical solution.

Third, negative genetic correlations between characters being selected will slow response to selection. To accelerate progress, a breeder can subdivide populations and focus selection on each of the individual traits for a time. Furthermore, genetic structure and environmental change are known to disrupt genetic correlations, so the populations may be evaluated in a new environment or crossed with other populations to increase genetic diversity or change allele frequencies.

Fourth, insufficient genetic correlation between traits may reduce the response to selection. Along this line, if response to selection requires simultaneous changes in genetically distinct but functionally interacting characters, finding superior genotypes will be difficult. An example could be yield measured across many years and environments. Yield in any given year may be influenced by a wide array of biotic and abiotic stresses. Therefore, finding a single genotype that yields well in all situations will be unlikely. A better approach would be to breed independently for yield potential under a uniform environment, and then accumulate genes for tolerance to specific stresses in separate populations. Selection for very simple metric traits are likely to produce the most rapid response (Walsh and Blows, 2009). Once genes have been identified for multiple traits, they can be pyramided in varieties, provided negative epistasis is not severe.

Although the above considerations point to the value of selecting on fairly simple traits independently to achieve rapid progress, in some situations, preconceived notions of what is needed may be restrictive. If a very particular phenotype is required, populations are likely to get stuck on suboptimal adaptive peaks, but if fitness can be determined by a functional property rather than the exact form of the phenotype, there are many pathways to the adaptive peak, and the optimum can generally be reached (Salazar-Ciudad and Marín-Riera, 2013). It follows that breeders working on perennial grains should not place excessive phenotypic restrictions on the developing crop. For instance, we can look at existing grain crops and mistakenly conclude that perennial grains must have short stature, fewer but larger heads, and thicker stalks. Actually, specific functions—high yield and lodging resistance—are needed, not morphologies. The path to a perennial grain may be traveled more easily if morphological phenotypes are not preconceived. In other words, the perennial grains of the future will be more readily obtained if they are not required to look like modern annual grains in all ways other than lifespan.

If selection to produce domesticated phenotypes is performed on relatively simple traits to insure good progress, the breeder should be aware of the potential to select for increased resource use. Longer growing seasons can be beneficial to capture more resources, but may not translate into grain yield in a sward (Egli, 2011). Increased resource use may enable increased yield, as it has in maize (Fischer and Edmeades, 2010), but in situations where water is limiting, resource conservation may be more important (Black and Reitz, 1969).

Selection experiments often show diminishing-returns epistasis (Barrick and Lenski, 2013). In this situation, each additional mutation will increase fitness to a lesser extent based on the presence of other mutations already in place. This scenario explains the rapid progress obtained in the early generations of perennial grain breeding programs and cautions that we should anticipate that gains will be slower over time. The result is that phenotypic selection can be done in a rather crude manner in early generations and make rapid progress. More refined progeny testing, multiple environments, and molecular tools will not be necessary until the first round of variation is exhausted.

The strength of selection is an important dial that can be adjusted (Barrick and Lenski, 2013). At the extreme, selection can become so strong that only rare mutants with potentially innovative phenotypes will be selected. To make very rapid progress with a new species, the breeder can evaluate very large numbers of individuals to find a few with outstanding and novel traits.

In breeding programs, transgressive segregation is desired to make rapid progress. Somewhat surprisingly, the more similar the phenotype of the parents, the more likely that transgressive segregation will occur. Genetic divergence should increase transgressive segregation, but phenotypic divergence can begin to override it (Rieseberg et al., 1999). To reduce the confounding effect of phenotypic divergence, we suggest that when the elite gene pool needs to be widened, the value of particular wild or non-elite parents should be assessed only after at least one backcross to elite parents.

In contrast to diminishing-returns epistasis, increasing returns are also possible in some cases. Genetic networks with multiple synergistic epistatic interactions may be possible and produce "selection-induced genetic variation" (Carlborg et al., 2006). As beneficial alleles accumulate in this model, portions of the genetic network previously hidden by epistasis can be activated, releasing standing variation. Since many domestication genes of current crops are highly epistatic, this effect is likely to be common during new domestication projects, and

breeding progress in the first cycles of domestication may underestimate the potential for future progress.

Outcrossing perennials, as discussed earlier, can have substantial genetic load and strong competitive ability. Selection strategies should account for these traits. For identifying outstanding segregants in the face of high genetic load, large populations must be used, and to avoid the effects of competition, wide plant spacings are needed (Fasoula and Fasoula, 2002). The result is that very large nurseries of space-planted individuals are ideal for domesticating perennial grains. The large nurseries can result in large impacts of environmental variation, but this variability can be efficiently controlled using the AR1XAR1 spatial model (Qiao et al., 2000; Funda et al., 2007).

Conclusions—Plant breeding for new domestication is best regarded as distinctly different from breeding for adaptation or yield increase in previously domesticated species. Modern cultivars can be seen nearing the top of an adaptive peak. At this point, small refinements are necessary, as any major change to the genetic architecture is likely to be detrimental because, near the top of a peak, there are many more ways to go down than climb farther up. In new domestication, breeders are searching for the best adaptive peak to climb, and since the wild populations are in valleys, many opportunities are available for climbing rapidly.

The choice of adaptive peak to climb will be important. However, from the bottom of any given peak, the breeder cannot know for sure whether it will lead far or if it is only a small bump. In fact, some peaks may even be surrounded by moats—only by passing through a phase of maladaption can the peak be reached. This situation may be particularly true when pleiotropy and epistasis are important. Populations with abundant interplant allelic variation, intraplant allelic variation (polyploidy, gene duplication), plastic phenotypes, and an evolutionary history that includes adaptation to high resource environments are more likely to be able to navigate a complex adaptive landscape. Thus, candidate species for domestication should be prioritized by predicted evolvability in addition to morphological and life history traits. Breeding should focus more on the required functions than on morphological traits, and the first traits to focus on in breeding should be those that are required by the plant breeder to make selections accurately (such as nonshattering) and to make it feasible to grow and evaluate very large experimental populations (such as seed fertility and seedling vigor).

Given these considerations, we conclude that population subdivision is the most efficient way to explore many possible avenues to achieving a domesticated perennial grain crop. Populations may be divided based on selection strategies (traits measured in different ways or single trait vs. multiple trait indices), environment (including environments known to produce specific plastic phenotypes), presence of major genes (perhaps identified by ecotilling), mode of inheritance (e.g., selection using breeding values predicted only from additive genetic variance vs. approaches designed to use low fidelity inheritance).

Very large numbers of individuals should be evaluated, but selection should be strong to fix critical domestication alleles. Bottlenecks do not need to be necessarily avoided and can even be used beneficially. Inbreeding depression as a result of bottlenecks can be addressed with purging, intermating diverse populations, introgression of diverse germplasm, and development of F_1 hybrid varieties.

Selection is likely to be effective when performed on simple metric traits and on younger plants. A useful strategy for early steps in domestication, though not for later yield improvement, is likely to be evaluation of widely spaced plants to avoid competition. Selecting in the first growing season may be beneficial because the important domestication traits can be scored while avoiding confounding effects caused by aging plants. Short generation times, critical to achieving rapid progress, may be aided by genomic selection.

Because perennial grains will need major-effect genes for perenniality, we expect that pleiotropic and epistatic effects will prevent the creation of perennial grains that are "just like" their annual counterparts in all aspects but lifespan. Therefore, breeding perennial grain crops will require openness to new plant forms and the breeding strategies to create them.

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