

CHAPTER 22

WHEN CAN INTELLIGENT DESIGN OF CROPS BY HUMANS OUTPERFORM NATURAL SELECTION?

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Abstract. Natural selection operated on the wild ancestors of crop plants for millions of years. Many seemingly intelligent design changes that we could make to enzyme structure or gene expression would duplicate (at least in phenotypic effect) variants already rejected by past natural selection. These variants died out because they decreased individual plant survival or reproduction under preagricultural conditions. Many of the variants rejected by past natural selection would also reduce crop yield or quality today, so it would be a waste of time to duplicate them using molecular methods. For example, most changes to rubisco will decrease photosynthesis (and crop yield) under current conditions, just as they would have decreased photosynthesis (and individual plant fitness) under preagricultural conditions.

A few of natural selection's 'rejects', however, would be genuine improvements by human criteria. Can we identify these promising rejects? Opportunities for crop genetic improvement that were missed by past natural selection are likely to fall into three major categories. First, and most important, conflicts of interest among competing plants, or between plants and their microbial symbionts, can cause trade-offs between individual plant fitness (favoured by past natural selection) and the collective performance of the crop community. Therefore, we can sometimes increase yield by reversing the effects of past natural selection for individual competitiveness. Second, changes in climate, soil fertility and pest populations mean that some variants that were less fit in the past will be more fit today. In this case, crop genetic improvement may accelerate changes that are already favoured by ongoing natural selection in an agricultural context. Third, eventually molecular methods may produce genotypes so different from anything that existed in the past that we cannot assume they were tested and rejected by natural selection. C₄ photosynthesis has evolved repeatedly, however, so a proposed innovation would have to be more radical than C₄ photosynthesis before we can assume it was missed by past natural selection.

The relative importance of these three kinds of opportunity is likely to change over the next few decades. Some trade-offs between individual competitiveness and the yield of the crop community have already been exploited, as in dwarf wheat and rice, but other opportunities may remain. Our ability to design radical new enzymes from scratch, or to predict the consequences of major changes in gene expression patterns, may improve over coming decades. Even after most significant opportunities to improve yield potential (yield in the absence of pests and diseases) have been fully exploited, ongoing evolution of pests and pathogens will create a continual need for 'Red Queen Breeding', generating a stream of new cultivars to keep up with the latest biotic threats.

“Natural Selection ... is as immeasurably superior to man’s feeble efforts, as the works of Nature are to those of Art.” – Darwin (1859).

“There is nothing in the process of evolution that has any aspect of community behaviour as a goal” – C.T. De Wit (1978).

INTRODUCTION

For 30 years, some biotechnology enthusiasts have been predicting that major increases in crop yields will come from improving fundamental physiological processes, such as photosynthesis or N₂ fixation (Shanmugam and Valentine 1975). For example, Zelitch (1975), claiming to have mutant plants with lower photorespiration and 40% greater net photosynthesis, suggested that “large increases in yields should be obtainable”. Yet there is no crop grown commercially today whose higher yield results from genetic engineering of photosynthesis, N₂ fixation or similar processes. In fact, there has been little or no improvement in yield potential (i.e., yield without abiotic stress, diseases or pests) of major crops over the last 20 years (Cassman 1999). This lack of progress, together with evolutionary arguments presented here and previously (Denison et al. 2003a; 2003b) suggest that ongoing emphasis on improving physiological efficiency (e.g., Long et al. 2006) is misplaced. We need a new theoretical framework to guide future crop improvement.

We should begin by recognizing that natural selection has already optimized much of the genome of our crops, mostly prior to domestication. Attempts to improve most genes are therefore likely to be futile. Fortunately for crop geneticists, however, the genotypes that were favoured by natural selection are not always best by human criteria. Conflicts between past natural selection and present human goals represent the best opportunities for significant genetic improvement of crops.

How can we identify these opportunities, ‘missed’ by natural selection? First, do not assume that the application of human intelligence over a few years is intrinsically superior to natural selection over millennia. Engineers are increasingly recognizing that processes analogous to natural selection, such as genetic algorithms (Cogan 2001) and simulated annealing (Kirkpatrick et al. 1983) can often solve baffling optimization problems. The development of an enzyme made from DNA (Breaker and Joyce 1995) is a biochemical example of using a process analogous to natural selection to solve an otherwise intractable design problem. Human ingenuity was required to create the conditions that selected for successive improvements in enzyme activity, but the actual process of selection was independent of human judgement. What these methods have in common with natural selection is non-random selection of each new generation from a randomly varying population. Given a large population of random variants from which to select each new generation, and given enough generations – each generation typically represents only a small improvement – natural selection generates solutions so effective that they can give the superficial appearance of having been designed by an individual (Behe 1996), or perhaps a team, of superhuman intelligence.

So, for most genes, improving on natural selection will be difficult. This is

especially true for many of the genes that are most important to crop yield. Enzymes involved in photosynthesis, synthesis of seed storage proteins, etc., are critical to crop yield. But, because they were also critical to the reproductive success of the wild ancestors of crops, they will already have been subject to strong selection for efficiency over millions of generations. Similarly, over-expression of a 'key enzyme' will almost always represent an option that was previously rejected by natural selection, so it is unlikely to increase crop productivity today. Crop yields may increase when photosynthesis increases with N fertilization or CO₂ enrichment (Long et al. 2006), but that is hardly evidence for the existence of trade-off-free opportunities for genetic improvement of photosynthesis that will increase yield. Similar conclusions have been drawn previously from more detailed analyses based on evolutionary theory (Denison et al. 2003b) and whole-plant physiology (Sinclair et al. 2004).

Nonetheless, it is clear that domestication and subsequent breeding of crops have resulted in enormous improvement in crop productivity under agricultural conditions, in addition to improvements in their suitability for our uses. Many opportunities for further improvements doubtless remain. The purpose of this chapter is to help those involved in crop improvement to identify areas where the chances for further progress are greatest, i.e., to identify opportunities for improvement (by human criteria) missed by past natural selection.

These opportunities can be grouped into three broad categories:

- Opportunities linked to conflicts of interest among organisms;
- New human goals or new crop environments; and
- Radical innovations not previously tested by natural selection.

The first two categories, which collectively account for most crop genetic improvement to date, will each be divided into two subcategories.

OPPORTUNITIES LINKED TO CONFLICTS OF INTEREST

Most past increases in crop yield potential have involved reversing the effects of past natural selection, in cases where conflicts of interest among individual plants have limited the collective performance of plant communities. Molecular methods have created new ways of implementing this approach.

Natural selection favours the spread of genes whose phenotypic effect favours their own spread (Dawkins 1976), whatever the consequences for a species as a whole, or for the plant communities and ecosystems where a species lives. Conflicts of interest even within an individual genome can sometimes be detected, as in cytoplasmic genes for male sterility, which favour their own spread by redirecting plant resources to seed production at the expense of pollen production (Dominguez 1995). The focus here, however, will be on conflicts of interest between individual plants and the plant community, and on conflicts between plants and their microbial symbionts. Both types of conflicts have limited the power of natural selection to optimize the collective performance of plant communities. Therefore, ameliorating the effects of such conflicts can provide opportunities for crop genetic improvement.

Trading individual competitiveness for community performance

It might seem that natural selection, operating on the wild ancestors of plants that reproduce mainly by producing seed, would already have maximized 'seed yield', at least in environments that resemble those where the ancestral plant evolved. But 'seed yield' can have at least three different meanings, even if expressed on a dry-weight basis: g plant^{-1} , g m^{-2} or g seed^{-1} . Natural selection will tend to increase some combination of g plant^{-1} and g seed^{-1} , subject to physiological and environmental constraints. But increases in g m^{-2} – the collective seed production of a group of plants – will be favoured by natural selection only as a side-effect of selection for individual seed production. Such side-effect benefits are probably common, as any increase in the inherent efficiency of an enzyme, for example, would tend to increase the productivity of individual plants. It would therefore be favoured by individual-based natural selection, while also increasing the collective productivity of groups of plants. This would leave little room for further genetic improvement by humans, except possibly through innovations so radical they have never arisen in the past, as discussed below.

There are, however, many cases in which past natural selection has favoured traits that reduce the productivity or efficiency of plant communities, despite their beneficial effects on individual productivity in a competitive environment. Taller rice plants out-compete their neighbours, but the collective seed production of the whole community is reduced by excessive investment in stems. Shorter, 'Green Revolution' varieties are much more productive (g m^{-2}) when grown together, but less competitive against taller genotypes (Jennings and De Jesus 1968). Dwarf fruit trees are less competitive than neighbours that keep growing (vertically or horizontally), but limited growth reduces pruning costs and allocates more resources to fruit production. Similarly, traits such as erect leaves and reduced branching reduce competitiveness while increasing yield potential (Donald 1968).

In all of these cases, changes in crop management may be required to achieve this potential. Kokubun (1988) showed that a population of single-stem soybean plants had higher seed yield (g m^{-2}) than branched soybeans, but only at high seeding rates (seeds m^{-2}). Similarly, a short wheat cultivar with erect leaves had the highest yield of all cultivars tested when weeds were controlled, but the lowest yield rank when forced to compete with weeds (Tanner et al. 1966).

Trade-offs between individual competitiveness and community-level traits will not always result in a negative relationship between weed-free yield and competitiveness with weeds (Gibson et al. 2003). A given genotype may have some defect, such as poor local adaptation, which affects both competitiveness and productivity. Major increases in yield potential may often require sacrificing some competitiveness, but that does not mean that all non-competitive genotypes have higher yield potential.

Community-level traits other than yield may also have been undermined by past natural selection for individual competitiveness. Consider water use efficiency. Natural selection will tend to favour high water use efficiency at the leaf level, increasing the ratio of CO_2 uptake per $\text{g H}_2\text{O}$ transpired. But past natural selection may have rejected some strategies that could have increased community-level water

use efficiency (Zhang et al. 1999). For example, conserving water in the soil for later use will not be favoured by natural selection if the conserved water is then used by a competing plant.

General strategies by which plant breeders might exploit individual versus community trade-offs have been discussed previously (Denison et al. 2003b), but a more recent example illustrates how molecular tools can facilitate this approach. Many plants respond to the presence of neighbours (using light cues detected by phytochrome) with an increase in stem elongation. Transplant experiments have shown that elongated plants are more fit under crowded conditions and less fit under less-crowded conditions (Dudley and Schmitt 1996).

Boccalandro et al. (2003) reasoned that, although elongation increases the fraction of total available light intercepted by taller individuals, it does not increase total light interception (and hence photosynthesis) of the plant community. The elongation response, therefore, represents a waste of resources in excessive allocation to stems. So they used genetic modification of phytochrome to make potato plants less responsive to crowding. The transgenic plants had higher tuber yield (both g plant^{-1} and g m^{-2} , as neighbouring plants all had the same genotype), although a decrease in g tuber^{-1} could affect the commercial value of the transgenic genotype. There were also effects on flowering time and stomatal conductance, so yield increases may not be due to effects on elongation alone, but this approach seems promising enough to merit further research in various crops.

Manipulation of genes related to crowding responses below-ground could also be worthwhile. Zhang et al. (1999) modelled root allocation in a water-limited environment and suggested that natural selection would favour excessive allocation to roots because of competitive interactions. Experiments by Gersani et al. (2001) confirmed this prediction, showing that interactions between roots of different soybean plants lead to excessive root proliferation at the expense of seed production.

Increasing benefits from symbiosis

Natural selection may also have failed to optimize below-ground interactions between plants and their microbial symbionts. This discussion will emphasize rhizobium symbiosis, so it is directly relevant only to legume crops and forages. Similar considerations may apply to mycorrhizas (Kiers et al. 2002), however, and perhaps also to disease-suppressing rhizosphere bacteria (Denison et al. 2003a).

Genes for nodulating plants persist in rhizobium populations because rhizobia reproduce inside nodules and are released into the soil in greater numbers than if they stayed in the soil. A legume species or cultivar that allows a given rhizobium strain to nodulate increases the soil abundance of that strain after nodule senescence, relative to rhizobia limited to soil and rhizosphere. This has been shown in the field both for soybean nodules (Kuykendall 1989), in which the differentiated, N_2 -fixing bacteroid form of rhizobia retains the ability to reproduce (citations in Denison 2000) and for pea nodules (Kucey and Hynes 1989), from which only undifferentiated rhizobia escape and reproduce in soil.

After reproducing manyfold inside a nodule, why bother to fix N_2 ? Fixing N_2 consumes resources that rhizobia might otherwise use for their own current or future reproduction. For example, rhizobia can fix more N_2 if they use all available C as an energy source than if they accumulate some C as polyhydroxybutyrate (PHB, Cevallos et al. 1996). Rhizobium cells that hoarded PHB should have greater survival and reproduction inside senescing nodules or subsequently in the soil, however. (Experiments underway in my laboratory support this hypothesis.)

In species where N_2 -fixing bacteroids will have no direct descendants in the soil, hoarding of PHB by bacteroids has no fitness benefit for rhizobia. It is therefore not surprising that only undifferentiated rhizobia, and not bacteroids, accumulate PHB in those species (Denison and Kiers 2004). Terminally differentiated bacteroids could still enhance their inclusive fitness at the expense of N_2 fixation, however, by diverting C to the production of rhizopines for consumption by undifferentiated rhizobia, presumably clonemates inside the same nodule (Denison 2000).

It might seem that rhizobia and plant have a shared interest in increasing the photosynthate supply they share. If rhizobia fix more N_2 , they can increase host-plant photosynthesis (Bethlenfalvay et al. 1978) and growth, potentially increasing total photosynthate supply to nodules. The problem is that each individual plant is typically infected by several different rhizobium strains (Hagen and Hamrick 1996). These rhizobia compete for host resources, at least in the sense that a given sugar molecule cannot be shared by two different nodules. After rhizobia escape into the soil during nodule senescence, strains from the same plant are each others' most likely competitors, for the next host plant and possibly for soil resources as well. By investing in N_2 fixation, therefore, rhizobia in a nodule may benefit their worst competitors, with negative consequences for their own fitness (survival and reproduction) and that of their descendants in the soil. Thus, multiple strains per plant create a 'tragedy of the commons'. Genes that enhance rhizobium reproduction at the expense of N_2 fixation should therefore completely displace more mutualistic genes that enhance N_2 fixation, over the course of evolution. Why has this not happened?

I have suggested that the most likely explanation for the persistence of more-mutualistic genes in rhizobium populations is that legumes monitor the actual symbiotic behaviour of rhizobia in nodules – N_2 fixation, not easily-mimicked 'recognition signals' – and then impose 'sanctions' that reduce the fitness of less-mutualistic strains (Denison 2000). A mathematical model confirmed that less-mutualistic strains would spread in the absence of host sanctions (West et al. 2002). The existence of host sanctions has since been confirmed experimentally, by manipulating the N_2 concentration around individual soybean nodules. Rhizobia allowed to fix only trace amounts of N_2 reproduced at only half the rate of genetically identical rhizobia fixing N_2 normally (Kiers et al. 2003).

Sanctions reverse our earlier theoretical prediction and raise a new question. If, because of host sanctions, rhizobia that fix N_2 have twice the fitness of rhizobia that do not, then why are strains that fix little or no N_2 with local crops common in some soils worldwide? In some cases, rhizobium strains that are poor N_2 fixers on the locally dominant crop species might do better (fix more N_2 and avoid sanctions) on another species (Bala and Giller 2001), which may once have been common locally

and which may persist as a weed. Unless fields are very weedy, however, release of rhizobia from nodules of this alternative host should be swamped by release from crop legume nodules. Therefore, if a sanctions-imposing crop is the dominant legume species, then the subset of locally adapted rhizobia that is most mutualistic on that crop should come to dominate the soil. Instead, less-mutualistic strains often dominate (Erdman 1950; Labandera and Vincent 1975; Denton et al. 2000).

Why are less-mutualistic strains sometimes common, if the dominant host species imposes sanctions on poorly performing nodules? If mixed nodules are common in the field, as may be the case for soybean (Moawad and Schmidt 1987), then less-mutualistic rhizobia sharing a nodule with a more-mutualistic strain might escape nodule-level sanctions. If so, then developing crops that are better at limiting the number of founding rhizobia to one per nodule could solve this problem. Alternatively, sanctions imposed by existing cultivars may not be stringent enough to prevent the spread of marginally effective rhizobia. If even mediocre rhizobia still provide a net benefit to an individual legume plant, then natural selection among legumes would have limited the imposition of sanctions, except against rhizobia that fix essentially no N_2 (Denison 2000). Improvements in host sanctions may therefore represent an opportunity for genetic improvement of legume crops and forages. A crop that killed all rhizobia inside nodules with mediocre fixation rate, while directing abundant resources to the best nodules, would tend to enrich the soil with the best local strains of rhizobia, released from its best-performing nodules.

Plants with this positive effect on rhizobium communities in the soil might be identified using a relatively simple screen. First, grow a genetically diverse population of plants, with each plant in a pot with soil containing rhizobia differing in mutualism, and save seed. Then grow a genetically uniform test cultivar in the same pots. Select seed from the first generation based on growth of the test cultivar. Plant genotypes with a beneficial effect on other soil microbes could be identified similarly, if such genotypes exist. For example, genotypes that enrich the soil with mycorrhizae more beneficial to the next crop, which may be the reverse of the current situation (Johnson et al. 1992), might be identified.

Another interesting approach was developed by Rosas et al. (1998), who designed an innovative method for identifying plant genotypes that nodulate preferentially with a specific inoculum strain. If local rhizobia are all ineffective, it would be easy to screen for green plants nodulated by the inoculum strain. But local rhizobia are often mediocre, making it difficult to identify plants nodulated mainly by the more mutualistic inoculum strain. So they made a non-fixing mutant of the inoculum strain, and screened for yellow plants. The genotypes selected were subsequently shown to admit selectively the N_2 -fixing version of the inoculum strain, despite the abundance of mediocre rhizobia in the soil.

This approach, improved control of the initial infection process, has potential advantages, relative to attempting to improve post-infection sanctions. Rhizobia reproduce many-fold inside the nodule before starting to fix N_2 , so unless sanctions were very effective at killing rhizobia inside nodules, there might still be many bad rhizobia released into the soil. Total exclusion from nodulation would be better. On the other hand, mediocre rhizobia might acquire the recognition signals of the inoculum strain fairly quickly, especially given the possibility of horizontal gene

transfer among rhizobia (Sullivan et al. 1995). It is not clear whether an exclusion system based on recognition signals would last long enough in the field to justify the effort to develop such selective cultivars.

NEW OBJECTIVES AND NEW ENVIRONMENTS

'Collective performance' is itself a new objective, as indicated by the quotation from Professor C.T. de Wit (of Wageningen) at the beginning of this essay. Although we can sometimes predict how the traits of individual plants will affect community-level performance (Donald 1968), actual success can only be measured at the community level, that is, using field plots rather than individual plants. This section, in contrast, discusses traits that can be effectively evaluated in individual plants.

Many new objectives will still require reversal of past natural selection, although the trade-offs are between the individual competitiveness of plants and a variety of human goals, rather than collective performance of the plant community. On the other hand, improved adaptation to new environments, including new pests and pathogens, will not always require significant reversal of past evolutionary trends. For these traits, human ingenuity is used to accelerate genetic changes that are also favoured by natural selection (to the extent that it is allowed to operate) in the new environment.

New goals

Trade-offs between seed production in g plant^{-1} versus g m^{-2} were discussed in the previous section. At the level of the individual plant, there is also a trade-off between seeds plant^{-1} and g seed^{-1} . Larger seeds may be able to use seed energy reserves to out-compete neighbouring seedlings during the critical days after germination (Darwin 1859). On the other hand, smaller seeds may be dispersed farther by wind, and a plant can produce more of them per g of C and N available. Natural selection has often favoured seed sizes that are less than optimal by human criteria, although there are exceptions (e.g., coconut). Breeding for larger seed size is often an appropriate and readily achievable goal.

Major changes in seed composition have also been achieved through selection (Dudley and Lambert 1969). There are some significant biochemical constraints on this process. A higher-protein seed requires more nitrogen, of course, but also more photosynthate (g C per g seed), because of the greater energetic requirements of protein relative to starch (Sinclair and De Wit 1975). Similar constraints limit the yield of crops whose seeds have a high lipid content (Penning de Vries et al. 1974).

Plant breeders have a long history of success in improving the flavour of fruits and vegetables, sometimes by reducing levels of plant secondary compounds involved in defence against herbivores or pathogens (Ames 1983). More radical changes to plant biochemical composition are becoming easier to achieve using molecular methods, although the impact of these developments may be less than has been claimed (Schnapp and Schiermeier 2001). Production of pharmaceuticals

should be limited to non-food crops, such as tobacco or guayule, to reduce the risk of contaminating food supplies (Daniell and Gepts 2004).

It is easy to imagine novel breeding objectives that would be useful in particular situations. For example, in compacted soils, roots sometimes reach deeper into the soil by following channels left by roots that grew and decayed in previous years. In one study, 41% of corn roots grew in channels left by a previous alfalfa crop (Rasse and Smucker 1998). The ideal root, from the standpoint of a subsequent crop, might consist of a coarse-weave 'basket' made of materials resistant to microbial degradation (e.g., lignin), surrounding a core of readily degradable materials (e.g., cellulose and protein). Once the core degraded, the basket could hold the channel open for smaller roots. Natural selection would favour such a root design only to the extent that it preferentially benefits the offspring of the plant leaving the channel, relative to competitors of the same species. Someday, humans might understand root developmental anatomy well enough to design rotation crops with such roots, although such a design might conflict with other objectives related to water and nutrient transport in roots. Or could we develop a practical screening method to select mutants that approach this root design by successive approximation? If each plant were grown in a long vertical tube containing compacted soil, differences in residual root channels after growth of each genotype might be detected simply by moistening the soil to field capacity and then looking for differences in the rate at which water added to the top of the tube drains out of the bottom.

New physical environments

A new environment may be a new location (e.g., the introduction of potatoes to Europe) or a change in biotic or abiotic conditions over time. For example, changes in the photoperiod response of flowering in soybean have been essential to the success of this crop over a wide range of latitudes in North America.

Some widespread trends in abiotic conditions include increases in atmospheric CO₂ or soil nitrogen and (with irrigation) increased predictability of soil water status (Denison et al. 2003b). The density of stomata per cm² leaf and the nitrogen content of leaves both appear to have decreased over time, based on comparisons of herbarium specimens collected over the past 200 years (Woodward 1987). This may represent a combination of long-term evolutionary changes of species and short-term acclimation by individual plants. Higher atmospheric CO₂ means that fewer stomata are needed to achieve a given CO₂ content in the leaf interior. A decrease in stomatal density may decrease the risk of dehydration due to excessive transpiration. The lower leaf N content presumably reflects a decrease in the concentration of the photosynthetic enzyme, rubisco. With higher CO₂ concentration, fewer rubisco molecules are needed to fix a given amount of CO₂.

To varying extents, natural selection can still occur in plant populations that are also subject to selective breeding, so stomatal density and leaf nitrogen of crop plants will continue to evolve even without deliberate selection by humans. However, evolutionary responses to changes in atmospheric CO₂ will always lag behind current conditions. Furthermore, we can predict the future, including further

increases in CO₂, in ways that the 'blind watchmaker' (Dawkins 1985) of natural selection cannot. Therefore, breeding for lower stomatal density and leaf N may increase crop yield and/or water use efficiency under some conditions. However, under hot conditions, the higher stomatal conductance of modern Pima cotton cultivars is associated with higher yields, apparently because of lower leaf temperature (Lu and Zeiger 1994). Similarly, rubisco serves as an important store of nitrogen in leaves, in addition to its photosynthetic function (Stitt and Schulze 1994). Therefore, whether a decrease in stomatal density or leaf nitrogen is an improvement may depend on climate, irrigation frequency, soil fertility and the ability of crops to take up soil nitrogen late in the growing season (Denison et al. 2003b). These complications are in addition to the technical difficulty of achieving the proposed change.

The need to consider, and perhaps modify, how the crop is grown may seem burdensome, but future improvements in crop production may be just as dependent on interactions between genetics, environment and management as past improvements have been. For example, dwarf wheat and rice have higher yield potential than taller traditional cultivars, but they require better weed control, as they are less able to compete with tall weeds for light.

New pest and pathogen genotypes

'New environments' may also include newly arrived or newly evolved pests and pathogens. Conventional breeders have a long history of success in developing crops resistant to or tolerant of biotic threats. More recently, molecular methods have been used with some success. Given the theme of this essay, it is useful to divide molecular approaches to disease and pest resistance into two categories: those that probably duplicate phenotypes previously rejected by natural selection, and those that are sufficiently novel that they may not have been tested by past natural selection. This is analogous to the difference between tinkering with an existing design and engineering a new design.

Disease-related traits that we can assume were previously rejected by natural selection include increased (or more constitutive) expression of genes already present in a crop. Before using this approach to crop genetic improvement, we should at least ask why mutants with higher expression of the target gene failed to out-compete those with 'normal' levels. Assuming that the increased expression did indeed increase disease resistance, did it also impose some cost that, on average, reduced seed production?

There are many reasons why constitutive expression might reduce yield, relative to inducible expression of the same defence. For example, inducible chemical defences against insect herbivores reduce synthesis costs, avoid autotoxicity, create spatial patterns that make herbivores more evident to birds (by increasing movement from one leaf to another), limit chemical deterrence of pollinators, and provide various other benefits, relative to constitutive defences (Agrawal and Karban 1999). The ubiquity of inducible defences against pathogens suggests that inducible pathogen defence was also beneficial, at least under pre-agricultural conditions. Has

this changed? In some crop fields, plant species diversity is less today than it was when these defences evolved, but this may not be true for some important crops, whose wild ancestors also grew in monospecific stands (Wood and Lenné 2001). Either way, are constitutive defences now the best solution, or should we consider increasing crop diversity in space or time (Denison et al. 2003b)?

Expression of the *NPR1* gene increases twofold in response to infection by a pathogen, and Cao et al. (1998) found that overexpression of *NPR1* in *Arabidopsis thaliana* increased resistance to two different pathogens. However, overexpressing the same gene in rice increases susceptibility to leaf lesions, especially under low light (Fitzgerald et al. 2004). This problem may explain why genotypes with higher constitutive expression of *NPR1* were rejected by past natural selection.

More innovative approaches to disease- and pest-resistance are also being developed. It might seem that an evolutionary perspective, which predicts that increased expression of existing genes will usually fail to increase yield, has little predictive power when it comes to truly novel genotypes. But how novel is novel?

Because the *Bt* toxin is not closely related to any plant toxin, insect herbivores may not evolve resistance to it as quickly as they would to new variants of toxins to which they are already resistant. This did not prevent evolution of *Bt* resistance in field populations of diamondback moth, however. An analysis of this case led Tabashnik et al. (1997) to predict that evolution of resistance in some insect pests may be 'faster than previously expected'. Subsequent evolution of *Bt* resistance in pink bollworm in the US has been slower than expected, however, apparently because of resistance management regulations requiring *Bt*-free insect refuges (Tabashnik et al. 2005). Reliable comparisons of evolution of *Bt* resistance in countries differing in resistance management would be of interest.

Using a vertebrate antibody to detect a plant pathogen and trigger chemical defences is certainly a clever idea (Bohlmann 2004). But it is probably safe to predict that ongoing pathogen evolution will overcome this new defence, sooner or later, either through mutations that prevent the antibody from recognizing the pathogen, or through mutations that reduce susceptibility to the plant's induced chemical defences. What makes the vertebrate immune system a major innovation is, among other things, its ability to generate millions of different antibodies. We will not be able to endow plants with that level of sophistication anytime soon. Meanwhile, developing crops resistant to evolving pathogens and pests "takes all the running you can do, to keep in the same place" (Carroll 1872), which led us to coin the term 'Red Queen Breeding' (Denison et al. 2003b).

RADICAL ALTERNATIVES NOT TESTED BY NATURAL SELECTION

The distinction between tinkering and engineering, applied to pest resistance in the previous section, can also be applied to physiological improvement of crop yield potential, i.e., yield per unit area, under non-limiting abiotic and biotic conditions (Evans and Fischer 1999). Simply increasing the expression of existing genes related to photosynthesis, nitrogen assimilation, drought tolerance, etc., is unlikely to increase yields reliably, except perhaps to the extent that crops are grown in (man-

made) environments very different from where their ancestors evolved. Similarly, tinkering with the active site of key enzymes is unlikely to result in further increases in efficiency, beyond what millions of years of natural selection have already achieved. On the other hand, innovations so novel that they never arose over the evolutionary history of the crop and its wild ancestors might, in theory, result in substantial increases in yield potential or in their utility for humans.

Again, how novel is novel? C_4 photosynthesis, which can enhance both photosynthesis and water use efficiency, has evolved repeatedly (Kellogg 1999), so evidently natural selection has no difficulty with changes as 'minor' as the conversion of C_3 plants to C_4 (Berry 1975; Denison et al. 2003b). Therefore, if there is a superior photosynthetic system not yet found by natural selection, it is presumably even more different from C_3 . A red algal rubisco with superior specificity for CO_2 , relative to O_2 (Uemura et al. 1997) was hailed as a major step towards higher photosynthesis in crops (Mann 1999), but that may have been overoptimistic. The maximum turnover rate for CO_2 , which is considered slow in terrestrial-plant rubisco, was even slower in the algal enzyme (Table 1 in Uemura et al. 1997).

Other novel genotypes that may someday be developed include N_2 -fixing wheat or rice, a goal that is perhaps more remote today than it was thought to be more than 30 years ago (Shanmugam and Valentine 1975). One problem is the simultaneous requirement for high O_2 flux for respiration, to meet the energy requirements of N_2 fixation, together with low O_2 concentration, to protect nitrogenase from inactivation. Legumes solve this problem with adaptations including a variable gas diffusion barrier and leghemoglobin (Jacobsen et al. 1998), which would probably require tens or hundreds of genes in a new N_2 -fixing crop. The alternative approach of modifying cereals to host N_2 -fixing bacteria in nodule-like structures (Christiansen-Weniger 1998) has this same problem as well as the conflict of interest between host and rhizobia, discussed above.

Crops that leave more persistent root channels in soil might someday be achieved through intelligent design of root-related genes, rather than by the selection procedure outlined above. Similarly, perhaps a cover crop could be designed to shade out weeds until an overseeded crop has germinated and emerged, but then drop its leaves and die, maybe in response to a photoperiod achievable only with supplemental light. Perhaps crops could be designed to interfere with pollination in nearby weeds, by producing pollen that mimics the early steps of fertilization.

Like all new technologies, these suggestions could have unanticipated side-effects, but they illustrate the sorts of things we may eventually be able to do, once we can design a better crop 'from scratch'. However, we are unlikely to achieve this capability soon enough to help alleviate the competing demands of feeding a growing world population sustainably, while preserving enough natural ecosystems to prevent unacceptable losses of biodiversity.

THE FUTURE OF CROP GENETIC IMPROVEMENT

Any physiological improvements in yield potential are likely to be more durable than comparable improvements in resistance to pests and pathogens. The effects of climate change are essentially random with respect to their interactions with physiological adaptations. Change in the physical environment may decrease the benefit from a new genotype – increasing CO₂ will eventually decrease the comparative advantage of C₄ rice (Sage 2000) – but they may also increase that benefit. Evolution of pests and pathogens, however, is not random with respect to their ability to infect or feed on crops. This is good news for crop geneticists, in terms of job security.

The most promising route to increasing yield potential over the next two decades is to continue exploiting trade-offs between the collective performance of communities of plants (and their symbiotic partners) and the competitiveness of individual plants, sacrificing the latter to improve the former. Accelerating adaptation to the changing physical environment will probably also contribute to higher yield potential. The contributions from radical physiological innovations, not previously tested by natural selection, are unlikely to be significant over the next decade or two, but could be very important in the longer term. Meanwhile, Red Queen Breeding will be an important activity for as long as the human food chain relies on plants.

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