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Evaluating the fate of genetically modified microorganisms in the environment: Are they inherently less fit?

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Abstract. Genetically modified microorganisms hold great promise for environmental applications. Nonetheless, some may have unintended adverse effects. Of particular concern for risk assessment is the simple fact that microorganisms are self-replicating entities, so that it may be impossible to control an adverse effect simply by discontinuing further releases of the organism. It has been suggested, however, that genetically modified microorganisms will be poor competitors and therefore unable to persist in the wild due to energetic inefficiency, disruption of genomic coadaptation, or domestication. Many studies support the hypothesis that genetically modified microorganisms are less fit than their progenitors, but there are a few noteworthy counter-examples in which genetic modifications unexpectedly enhance competitive fitness. Furthermore, subsequent evolution may eliminate the maladaptive effects of some genes, increasing the likelihood that a modified organism or its engineered genes will persist. Evaluating the likelihood that a genetically modified microorganism or its engineered genes will persist is a complex ecological and evolutionary problem. Therefore, an efficient regulatory framework would require such evaluations only when there are plausible scenarios for significant adverse environmental effects.
Key words. Genetically modified microorganisms; competitive fitness; environmental effects; genomic coadaptation; energetic inefficiency; domestication.

For thousands of years, humans have manipulated their environment in order to increase its productivity and suitability for the human population. The intended benefits of such manipulations have often been accompanied by unintended deterioration of the environment, as manifest by the loss of sustainable production due to over-exploitation of resources and by fouling of the environment with wastes. For most of that time, environmental degradation due to human activities was presumably a minor problem in comparison with the challenges for human existence imposed by the unmanipulated environment. But as the technological capacity and the size of the human population have increased, environmental degradation has become more important. Consequently, human societies have begun to weigh the possibility of environmental degradation in evaluating the merits of proposed activities.

Unfortunately, assessing the environmental impact of human activities is difficult in several respects. There is the socio-political issue of whose costs and benefits are to be weighed, with the possibility of conflicting determinations between, for example, local and global communities. There is also the socio-economic problem of assigning values to such disparate entities as, for example, marketable products and biological diversity. Finally, there is the scientific challenge of predicting the ecological consequences of a proposed activity. Such predictions are difficult because of the intrinsic complexity of most ecological systems and our ignorance of many of the components and interactions comprising these systems. Among the complications that may arise in ecological systems are scale-dependence, time-lags and other nonlinear phenomena, which may cause cascading and even irreversible changes in ecological systems[51]. A well-known example of a cascading effect is the increasing concentration of certain chemical pesticides at higher trophic levels[53], while the extinction of any species clearly represents an irreversible outcome.

One of the most important ways in which humans have manipulated their environment is through the genetic modification of plants, animals, and microorganisms. Historically, genetic modification has been accomplished by selective breeding, in which variants are selected for their useful properties and allowed to breed disproportionately relative to their less desirable counterparts. Recent advances in molecular biology have enhanced the opportunities for genetic modification of organisms. In particular, new technologies enable the directed production of specific genotypes, rather than relying on selective breeding among the haphazard genetic variants produced by spontaneous mutation and sexual recombination. These molecular approaches will sometimes be used to produce more quickly and with greater precision varieties that could also have been produced by selection among spontaneous genetic variants. In other cases, these molecular approaches will be used to produce specific combinations of genes from widely divergent taxa, which cannot recombine sexually, thus allowing the production of varieties that cannot be achieved by traditional selective breeding.

The new molecular technologies for genetically modifying organisms may be applied to such diverse goals as

improving human health, increasing agricultural productivity, processing raw materials for industry, and degrading waste products. Some proposed applications are especially promising because they may alleviate environmental problems that have been caused by the use of chemicals as pesticides, fertilizers, and so on. For example, plant varieties are being engineered to better resist damage by herbivores and pathogens, which might otherwise require chemical control; bacteria and viruses are also being engineered to serve as new biological control agents of these pests. New symbioses between plants and nitrogen-fixing bacteria may be engineered to reduce the need for fertilizers. And bacteria may be engineered to degrade otherwise recalcitrant chemical pollutants that foul the environment.

While these objectives are certainly desirable, it is appropriate to ask whether specific applications may also have potentially adverse environmental effects. For example, might some virus that has been genetically modified to control a particular insect pest inadvertently also infect some non-target species, including perhaps some that are beneficial? In the past, applications of chemical pesticides have sometimes exacerbated damage to crops by releasing the target species from control by a predatory insect, which is itself susceptible to the pesticide[11,15]. Or might a bacterium that has been modified to degrade some pollutant under certain conditions actually convert that pollutant into an even more toxic compound? For example, under methanogenic conditions the biodegradation of tetrachloroethylene produces the more toxic vinyl chloride[52].

Such scenarios do not imply environmental catastrophe, but they do suggest that some applications of genetically modified organisms, if not properly evaluated, may exacerbate a problem rather than alleviate it. Of course, the mere possibility of adverse effects should not preclude any particular application; rather, one must weigh the likelihood and magnitude of the potential adverse effects relative to the likelihood and magnitude of the proposed benefits. A proper evaluation should also consider the cost of measures to control or mitigate an adverse effect, in the event that it actually occurs.

Of particular concern for risk assessment is the fact that organisms, unlike chemicals, are self-replicating entities. In particular, a population of genetically modified organisms, once released into the environment, may be self-sustaining. In that case, it may not be possible to control or mitigate some adverse effect simply by discontinuing further releases of the organism. Instead, more active and costly measures would be required to control the inadvertently engineered pest or to mitigate its unintended effect. If, on the other hand, a population of genetically modified organisms cannot sustain itself, then the organism may be effectively controlled simply by discontinuing further releases. Whether an introduced organism can become established and persist will depend primarily on its Darwinian fitness in the environment where it is introduced. Thus, an organism's fitness in an environment determines persistence, and persistence amplifies the organism's effects on the environment.

[However, not all classes of environmental risk associated with genetically modified organisms require that a population be self-sustaining after release. For example, excessive use of an agent for biological pest control may lead to the rapid evolution of resistance by the target species, which can be regarded as the squandering of a valuable natural resource[27]. And the deployment of herbicide-resistant crop varieties might be detrimental if, instead of encouraging the use of chemicals that are less toxic, such varieties encouraged the use of greater quantities of toxic chemicals.]

A general hypothesis and alternative underlying models

It has frequently been suggested that genetically modified microorganisms, including those produced by modern molecular methods as well as by traditional selective breeding, are unfit for survival and reproduction in nature[8,13,14]. For example, Davies (ref. 13, p. S11) says that 'Recombinant microorganisms are essentially non-competitive and are unlikely to have much chance of establishing themselves in highly competitive natural environments'. If so, this would provide a substantial measure of environmental safety.

Several explanations have been put forward as to why genetically modified microorganisms should be systematically unfit. According to one explanation, offered by Brill (ref. 8, p. 383), 'The extra burden to the organism carrying new genes should decrease its ability to compete and persist'. In other words, a genetically modified microorganism may be disadvantaged in the wild because of the energetic costs associated with carriage and expression of additional genes. This view is particularly attractive because it suggests a universal effect and implies that the loss of fitness might even be quantified directly from the bioenergetic burden imposed by the syntheses of extra nucleic acids and proteins. Moreover, according to this explanation, a genetically modified microorganism cannot recover its progenitor's fitness in the wild except by eliminating the engineered genes or suppressing their expression.

A second explanation was advanced by Davis (ref. 14, p. 1334), who argued that 'a recombinant cannot be dangerous unless it can survive, and such survival, like earlier successes in evolution, depends on a harmonious balance (coadaptation) of the total genome. Hence to be effective any new gene must interact well with the others. But foreign genes from a distant source will not fit well in the recipient genome, so they can be expected to produce noncompetitive (or even nonviable) monstrosities, rather than dangerous monsters'. Here, the

reduction in fitness of a recombinant genotype is not due simply to the energetic burden of additional DNA and protein synthesis. Instead, the loss of fitness results from the perturbation of biochemical, physiological or developmental processes due to pleiotropic effects of the genetic modification. Because the cost of the genetic modification is explicitly contextual, its magnitude would depend on the genetic background in which it is found, precluding the possibility of calculating the cost from bioenergetic considerations. Moreover, the maladaptive effect of an engineered gene might be reduced or even eliminated during subsequent evolution – without necessarily diminishing the gene's expression – if it is transferred by recombination onto another genetic background or if a compensatory mutation occurs in the original genetic background that restores genomic coadaptation. Regal[49] refers to these first two explanations together as the 'pregnant pole-vaulter' model. In order to distinguish them, I will refer to the first and second explanations as the 'excess baggage' and 'untuned engine' models, respectively.

According to a third explanation, also put forward by Davis (ref. 14, p. 1329), 'the use of modified microorganisms is not entirely novel but is an extension of the old process of domestication of wild organisms including the selection of microbial variants to make bread, wine, antibiotics, and vaccines'. Regal[49] refers to this explanation for the reduced competitive fitness of genetically modified organisms as the 'domesticated species' model. However, as Regal[50] has emphasized, genetic engineers using modern molecular methods will not be restricted to further modifying those organisms that have already been domesticated. During selective breeding, organisms tend to become increasingly dependent on human activities and concomitantly less dependent on those traits that facilitate their survival and reproduction in the wild. This process thus allows those traits that adapt the organism to the wild to be diminished, while the organism becomes better adapted to the purpose for which it is selectively bred by humans. The degree of dependency on humans (i.e., domestication) varies widely among genetically modified organisms obtained by selective breeding; certain varieties are unable to propagate themselves without deliberate human intervention, whereas other varieties may interbreed with their natural relatives and some can establish feral populations[49,50]. This explanation, unlike the preceding explanations, does not imply that the same genetic changes that enhance the traits primarily useful to humans diminish the 'wild-type' traits that allow survival and reproduction in the wild. Rather, the loss of fitness in the wild may also be due to a long history of relaxed selection, which allows these wild-type traits to be lost by random genetic drift or secondary selection for other traits (e.g., docile behavior in animals bred primarily for milk production) during domestication.

The table summarizes the similarities and differences among these three models. I recognize that the distinctions between these models are not always sharp. And proponents of the arguments cited here might disagree with some of the inferred characterizations of the models. Nonetheless, I believe that these distinctions serve to emphasize some of the subtle issues that bear on the general hypothesis that genetically modified microorganisms pose few environmental risks because they are unfit for survival in nature.

Fitness: Definition and estimation

A textbook definition for fitness is 'The average contribution of one allele or genotype to the next generation or to succeeding generations, compared with that of other alleles or genotypes' (ref. 26, p. 552). The Darwinian fitness of an organism therefore refers to its capacity for survival and reproduction, which depends on its environmental circumstances as well as on its genotype. As a consequence of any difference in average contribution, the relative abundance of alleles or genotypes that are competing in a particular environment will change systematically over a period of generations.

Comparisons among three alternative models concerning the competitive fitness of genetically modified microorganisms

	Model		
	Excess baggage	Untuned engine	Domesticated species
A genetically modified microbe is less fit than its unmodified counterpart	Yes	Yes	Yes
The same genetic modification that produces the engineered trait reduces fitness	Yes	Yes	No
A genetically modified microbe cannot recover its progenitor's fitness except by losing the engineered trait	Yes	No	No

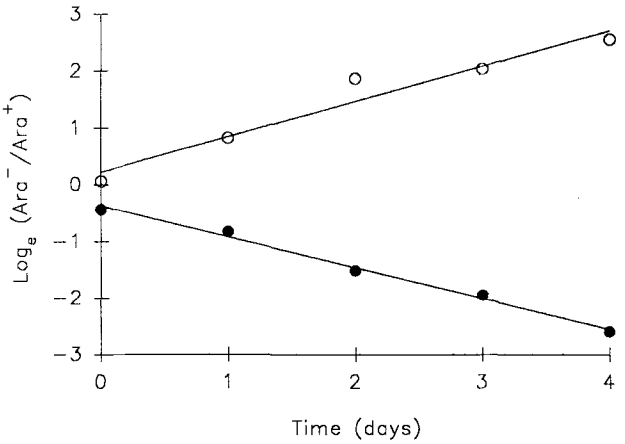

Figure 1. Illustration of the estimation of relative fitness from competition experiments. An arabinose-utilization marker was used to distinguish an *E. coli* B strain that carried plasmid pBT1071 from its plasmid-free progenitor. Strains competed in a glucose-limited minimal salts medium without antibiotic, with an overall growth rate, c, of 4.605 day^{-1}. *Filled symbols*: Ara$^-$/pBT1071 versus Ara$^+$; selection rate constant, $r = -0.543$ day^{-1}; fitness of modified genotype relative to its progenitor, $W = 1 + r/c = 0.882$. *Open symbols*: Ara$^-$ versus Ara$^+$/pBT1071; $r = -0.619$; $W = 0.866$. Thus, the plasmid-bearing cells were competitively inferior to their unmodified progenitors, irrespective of the marker states used to score the genotypes (R. E. Lenski, unpublished data).

For plant and animal species, it is usually quite hard to observe directly any change in the relative abundance of alleles or genotypes, owing to their long generations and complex life cycles as well as difficulties in determining genotype and parentage easily and unambiguously. But these limitations do not apply to many bacteria and other microorganisms, whose generations may be rapid (on the order of hours) and life cycles simple (binary fission). Moreover, because many microbial species are predominantly or exclusively asexual, the determination of genotype and parentage is facilitated by employing genetic markers that can be easily scored and do not become dissociated from the traits of interest by sexual recombination.

Therefore, the fitness of a genetically modified microorganism relative to its unmodified progenitor can be directly inferred by allowing the two genotypes to compete in a defined environment and monitoring the rate of change in their relative abundance over a number of generations. Figure 1 illustrates the outcome of competition between two bacterial genotypes, one carrying a plasmid and the other its plasmid-free progenitor, using a reciprocal design to control for any effect of the genetic marker used to distinguish the competitors. From such data, a selection rate constant, r, may be estimated by linear regression using the following equation:

$$\log_e(M_t/N_t) = \log_e(M_0/N_0) + rt,$$

where M and N are the abundances of the modified genotype and its progenitor, respectively, and subscripts denote time[22]. If the overall rate of population growth (or the rate of turnover at steady state) is also known, then the selection rate constant can be converted to a relative fitness, W, by:

$$W = 1 + r/c,$$

where c is the overall growth rate. A relative fitness greater than 1 indicates that the modified genotype is more fit, while a value less than 1 implies that its progenitor is more fit. Discussions of some factors that may complicate interpretation of fitness assays, along with more detailed descriptions of experimental design and statistical inference, are presented elsewhere[22,35,36].

A review of the evidence

Are genetically modified microorganisms always less fit than their progenitors? According to all three models, genetically modified microorganisms should be less fit than their unmodified counterparts. Data bearing on this general hypothesis are available for at least four rather different types of genetic modification. The most familiar type includes mutations that cause defects in metabolic functions. Of course, most such mutations are deleterious and many are lethal. For example, spontaneous mutants of *E. coli* that have defects in the lipopolysaccharide core of their cell envelope are markedly inferior competitors to their progenitors in a glucose-limited minimal salts medium[33]. However, mutants that are defective in the *tonA*-encoded outer membrane protein do not show any loss of fitness under similar conditions[22,39], perhaps because the protein is only useful in certain environments. These sorts of metabolic defects may be similar to the losses of functions important for survival in the wild that occur during prolonged domestication, and these defects are like those that might be used to deliberately disable genetically modified microorganisms in order to prevent their persistence. But such metabolic defects are not typical of the primary genetic modifications that would characterize microorganisms engineered for environmental applications, in which an existing metabolic activity would be enhanced or some new metabolic function added.

In a second type of experiment, genotypes that express some metabolic function compete against genotypes that are repressed or deficient for that function, in an environment where the function serves no purpose. For example, several studies have shown that *E. coli* mutants expressing the lactose operon constitutively are less fit than their progenitors, whose *lac* operons are repressed, when the two genotypes compete in an environment containing some other sugar as the limiting resource[2,20,31]. Similarly, when the amino acid tryptophan is supplied exogenously, bacteria that synthesize tryptophan, prototrophs, are out-competed by other-

wise identical genotypes that do not produce tryptophan, auxotrophs[16,54]. These results support the supposition that expression of unnecessary metabolic functions impairs fitness. However, unregulated expression of an unnecessary metabolic function may be much more costly than mere possession of that function. For example, constitutive expression by *E. coli* of a protein that pumps tetracycline out of the cell reduces fitness in the absence of antibiotic by 3% or more, depending on the strength of the promoter, whereas the corresponding repressed operon reduces fitness by less than 0.3%[48]. Evidently, tight regulation of gene expression may virtually eliminate the fitness cost of possessing a metabolic function when it is not being used, while still allowing the corresponding function to be expressed when it is useful to the microorganism.

In the third type of experiment, bacteria transformed with an accessory genetic element (a transposon, plasmid or prophage) are allowed to compete against their untransformed progenitors, in an environment that contains no antibiotic or other agent known to favor carriage of the element. Most of these studies have shown significant reductions in fitness associated with the possession of superfluous elements (e.g., ref. 38, which also cites ten earlier studies reaching the same conclusion). However, a few studies have compellingly demonstrated unexpected competitive advantages associated with the possession of these seemingly superfluous elements: the transposon Tn*5* enhances the fitness of certain genotypes of *E. coli*[6,28], as does the Lambda viral prophage[19,24,25,43].

These exceptions may be over-represented in the scientific literature because they are so unexpected. Even so, they indicate that one cannot automatically assume that the addition of seemingly superfluous genetic material invariably reduces competitive fitness, as supposed by the 'excess baggage' model. One might counter by arguing that the added genes cannot be superfluous if they enhance competitive fitness, but this only begs the question of how to define 'superfluous' in a non-circular fashion. These exceptions also seem to contradict the 'untuned engine' model, since foreign genes have been added that do not disrupt genomic coadaptation but actually increase competitive fitness. One might counter here that these accessory genetic elements have a long evolutionary history of interaction with their hosts, which has resulted in their coadaptation[40,42]. It would be interesting to compare the fitness effects associated with the addition of accessory element from the same versus distantly related host species.

The final type of genetic modification occurs during prolonged propagation of a microbial population in a defined laboratory environment. During the course of such experiments, one almost invariably detects some mutant that is more fit than its progenitor in that environment, ususally within a few hundred generations (see refs 17, 22, 37 for reviews; see refs 5, 41 for two recent studies). This common finding does not imply that most or even many spontaneous mutations are beneficial, but rather it illustrates the amplifying effect of natural selection. These mutants may be similar to those genetic modifications that occur during domestication by artificial selection, although in these experimental studies the investigator is not usually deliberately selecting for a specific phenotypic property. As far as I am aware, none of these studies has examined how quickly microorganisms lose their competitive fitness in the wild during evolutionary adaptation to a laboratory environment. This interesting issue is also worthy of investigation. In any case, laboratory studies of evolution remind us that even rare genetic events may have important consequences for fitness.

It is necessary to reiterate that the effects of genetic modifications on fitness are often highly dependent on environmental circumstances. For example, mutations that cause defects in the cell envelope, which are often severely deleterious in environments where nutrients are limiting, can be advantageous when viruses are present[39]. Similarly, constitutive expression of the *lac* operon is costly when cells are competing for glucose, but this phenotype is beneficial when lactose is the sole source of energy[20]. Many plasmids that are burdensome to their hosts in most ecological circumstances confer resistance to antibiotics[42]. And mutants selected during long-term propagation in a defined laboratory environment often have no advantage when they are competed against their progenitors under even slightly different conditions[4,18]. By the same token, a bacterium that has been engineered to catabolize some pollutant, or a virus modified to infect a particular insect pest, may be disadvantaged relative to their unmodified counterparts in most environments. But the engineered bacterium may be more fit in environments where the pollutant is found at a high concentration, as may the modified virus in environments where the target species is abundant.

What are the energetic costs of possessing unnecessary genetic functions, and how do fitness effects compare with these costs? According to the 'excess baggage' model, the carriage and expression of unnecessary gene functions will impose energetic burdens on the microorganism that should reduce its competitive fitness. In principle, one can calculate the bioenergetic costs of synthesizing known quantities of additional nucleic acids and proteins as a fraction of the cell's total energy budget. By contrast, the adverse fitness effects of genetic modifications are explicitly contextual in the 'untuned engine' model, and these effects may be much greater than expected from strictly energetic costs.

An *E. coli* cell carrying 50 copies of a 4.4 kb plasmid and expressing a cloned gene product as 20% of its total protein, for example, has been estimated to require

about 0.1% and 13% more ATP for DNA and protein syntheses, respectively, than its plasmid-free counterpart[12]. One study found no relationship between the physical size or copy number of plasmids and their effect on bacterial growth[55], indicating that the energetic cost of additional DNA synthesis must indeed be of minor importance for fitness relative to the effects (energetic or otherwise) of gene expression.

In a benchmark study, Dykhuizen[16] found that the fitness advantage for tryptophan auxotrophs relative to prototrophs, when the amino acid is supplied in the medium, is about three orders of magnitude greater than was predicted on the basis of energetic efficiency. The physiological explanation for this discrepancy is not known, but the effect is manifest whether the auxotrophic mutants express defective tryptophan-synthesizing enzymes or none at all; perhaps the prototrophs accumulate some metabolite that disrupts another physiological process. Other studies have also indicated fitness effects due to protein expression that are not commensurate with strictly energetic costs. *E. coli* that produce MalE-LacZ hybrid proteins grow very poorly, probably because these molecules become physically stuck in the cytoplasmic membrane, thereby interfering with the transport of periplasmic and outer membrane proteins to their respective sites[3,29]. Low-level expression of the tetracycline-resistance protein by *E. coli* substantially reduces competitive fitness and high-level expression is lethal[46,48]; these effects are apparently due to loss of membrane potential caused by the protein's efflux activity[23].

Can genetically modified microorganisms recover their fitness during subsequent evolution, and does recovery necessarily require loss of the engineered trait? Like all other organisms, genetically modified microorganisms may continue to evolve after their release into the environment. Such further evolution will tend to increase the competitive fitness of the introduced microorganisms, and thereby enhance the likelihood of their persistence, whether or not this outcome is in the best interest of the humans that seek to use them[30,40]. A microorganism that has been handicapped ecologically by genetic modifications might recover its competitiveness either by reversing these modifications or via secondary changes elsewhere in the genome that ameliorate the maladaptive side-effects of the primary modifications. The 'excess baggage' model implies that a genetically modified microorganism can recover only by the first mechanism, whereas the 'untuned engine' and 'domesticated species' models also allow recovery by the second mechanism.

At least three studies with bacteria in the laboratory have quantitatively addressed these issues, as has one elegant study of an insect population in the field. In the first study, *E. coli* mutants resistant to infection by the virus T4 were, on average, about 35% less fit than their T4-sensitive progenitors in the absence of that virus[33,34]. Populations of mutants and their progenitors were allowed to evolve in the laboratory for 400 generations (60 days), again in the absence of the virus. During this time, none of the T4-resistant populations reverted to sensitivity, although their mean fitness increased to a level that was indistinguishable from their sensitive progenitors (fig. 2). (The failure of the resistant mutants to revert to sensitivity, despite the high cost of resistance, reflects the fact that resistance is due to the loss of receptors on the cell surface; deletions and other mutations that cause losses of function are often difficult to revert.) During this same period, the sensitive control populations also adapted to the laboratory environment, but their improvement averaged only about 10%. Thus, the marginal fitness cost of the resistance phenotype was reduced by more than two-thirds in only 60 days.

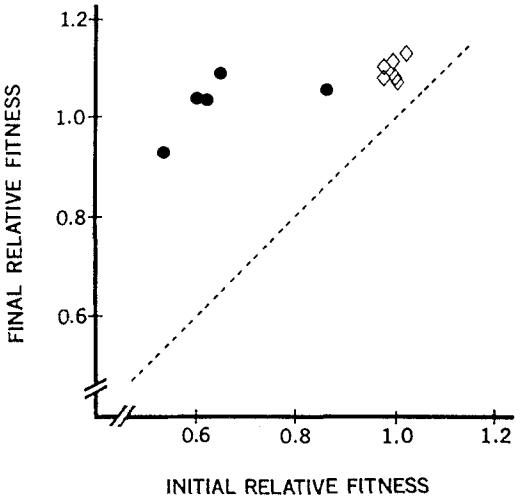

Figure 2. Compensation for deleterious genetic modifications during subsequent evolution. The abscissa shows that T4-resistant mutants (filled symbols) had initial fitnesses well below their sensitive progenitors (open symbols). The ordinate shows that, after the resistant and sensitive populations had evolved for 400 generations, their final fitnesses were very similar. None of the resistant populations reverted to their progenitor's sensitive state. (Reprinted from Lenski[34], with permission of Evolution.)

In another study, *E. coli* carrying the plasmid pACYC184, which encodes resistance to two antibiotics, were shown to be less fit than their isogenic plasmid-free counterparts in the absence of antibiotic[7,38]. And because plasmid-free segregants arise spontaneously at a fairly high rate, the plasmid tends to be lost from the population when plasmid-bearing cells are propagated in the absence of antibiotic. But when plasmid-bearing cells are propagated in the presence of antibiotic, plasmid-free segregants cannot take over and the plasmid-bearing population evolves. After 500 generations (75 days) in the presence of antibiotic, plasmid-free segregants still arise spontaneously in the evolved cell population. However, quite remarkably, the evolved

plasmid-bearing cells out-compete their plasmid-free derivatives *even in the absence of antibiotic*. In other words, a genetic modification (i.e., plasmid carriage) that was formerly costly to the bacteria had become beneficial in only 75 days. Further experiments showed that the amelioration of the cost of plasmid carriage was due entirely to mutations in the bacterial chromosome, rather than in the plasmid itself.

The third study was similar in design to the second one, but it differed in the particular bacterium, plasmid, and culture conditions that were employed[45]. In this evolutionary experiment, the cost of plasmid carriage was substantially reduced but not entirely eliminated over 800 generations. Secondary genetic changes in both the bacterium and the plasmid were responsible for the reduced cost of plasmid carriage.

In addition to these direct demonstrations that secondary mutations can ameliorate the maladaptive side-effects of certain genetic modifications, other studies have shown that the fitness effects due to a particular genetic modification may depend on the genetic background in which it is made[6,10,21]. If an engineered gene can be transferred by conjugation or other mechanisms of genetic exchange, then that gene will tend to be concentrated by natural selection in genetic backgrounds where it has less of a disadvantage; and if there are any backgrounds where that gene actually confers an advantage, then it may presist[30,32,50]. Therefore, even though a genetically modified microorganism may itself be unable to persist and have no adverse effect, a descendant genotype might persist and cause some environmental problem. For example, when different viruses infect the same host, they may recombine genetically. A narrow host-range virus that has been engineered for greater virulence against an insect pest (e.g., by the addition of a toxin-encoding gene) might transfer the engineered trait to another virus with a broader host-range that includes some beneficial species[1,32].

Perhaps the most striking example of the amelioration of maladaptive side-effects of a genetic modification comes from the work of McKenzie and co-investigators[9,44]. After diazinon was used to control the Australian sheep blowfly for ten years, resistant genotypes became prevalent in some areas. These resistant flies showed abnormal development and were less fit than their sensitive progenitors in the absence of diazinon. Had diazinon treatments been curtailed at that point in time, the resistant genotypes presumably would have been out-competed and disappeared. However, diazinon continued to be used in the field, and after several more years the resistant flies showed normal development and were as fit as the sensitive flies even in the absence of diazinon. In fact, a single secondary mutation, on a separate chromosome from the primary mutation conferring resistance, was responsible for this striking recovery of competitive fitness.

Scientific summary

1) Any environmental effects due to the release of a genetically modified microorganism are likely to be more persistent if the microorganism establishes a self-sustaining population. Whether an introduced genotype persists depends primarily on its competitive fitness relative to its indigenous counterparts.

2) It has often been suggested that genetically modified microorganisms will be poor competitors and therefore unable to persist in the wild. Possible explanations for this loss of competitive fitness include energetic inefficiency, disruption of genomic coadaptation, and domestication.

3) Most experimental studies support the general hypothesis that genetically modified microorganisms are less fit than their unmodified progenitors. But there are a few noteworthy exceptions in which genetic modifications unexpectedly enhance competitive fitness. Also, many genetic modifications that are disadvantageous under certain environmental conditions are favorable under others.

4) The loss of fitness due to the disruption of physiological processes is often much greater than the strictly energetic cost of possessing an unnecessary genetic function. Tightly regulated expression of engineered functions may minimize deleterious fitness effects.

5) The deleterious fitness effects associated with genetic modifications may be reduced or even eliminated during subsequent evolution, in some cases without loss of the primary modification. Natural selection favors new genetic combinations, arising through mutation or recombination, that are more likely to persist.

Implications for environmental risk assessment

1) Genetically modified microorganisms have many promising applications in the environment, including some that may alleviate problems caused by the excessive use of chemical treatments. However, microorganisms are self-replicating entities, and so it cannot be assumed that any unintended adverse effects they might cause can be rectified simply by halting their further application.

2) Many genetically modified microorganisms are competitively inferior to their unmodified counterparts, and so these modified genotypes will often be unable to persist following their release. However, some genetically modified microorganisms are competitively fit, at least in certain environments, and subsequent evolution may give rise to more persistent genotypes.

3) Evaluating the likelihood that an introduced population or its genes will persist is a complex ecological and evolutionary problem, even in a simple laboratory setting. Consequently, an evaluation of this likelihood for every genetically modified microorganism would be costly, and such evaluations should be required only when justified on other grounds.

4) An efficient framework for evaluating possible environmental risks associated with the release of genetically modified microorganisms would take into consideration the familiarity of the proposed application, in terms of its similarity to previous applications that have a safe environmental record[47]. Implementation of such a framework would allow environmental risk assessment research to focus on those proposed applications that pose the greatest risks.

5) An evaluation of the likelihood that a genetically modified microorganism or its engineered genes will persist following release into the environment should be required when the intended application is not similar to previous applications with a safe record of use and there exist plausible scenarios for significant adverse environmental effects.

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