Synthetic threads through the web of life

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CRISPR-Cas gene editing tools have brought us to an era of synthetic biology that will change the world. Excitement over the breakthroughs these tools have enabled in biology and medicine is balanced, justifiably, by concern over how their applications might go wrong in open environments. We do not know how genomic processes (including regulatory and epigenetic processes), evolutionary change, ecosystem interactions, and other higher order processes will affect traits, fitness, and impacts of edited organisms in nature. However, anticipating the spread, change, and impacts of edited traits or organisms in heterogeneous, changing environments is particularly important with "gene drives on the horizon." To anticipate how "synthetic threads" will affect the web of life on Earth, scientists must confront complex system interactions across many levels of biological organization. Currently, we lack plans, infrastructure, and funding for field science and scientists to track new synthetic organisms, with or without gene drives, as they move through open environments.

CRISPR | gene drives | ecological impacts | scale linkages | interaction strength

CRISPR-Cas gene editing tools have brought us into an era of synthetic biology that will change the world (1, 2). Having already enabled breakthroughs in basic research and medicine, CRISPR tools promise more in agriculture, public health, and conservation (1–3). For applications outside the laboratory or hospital, we need to anticipate how gene-edited organisms and their traits will spread, change, and affect other biota and ecosystems. As I write this in 2020, we in the United States find ourselves crippled in our efforts to contain and address a new human virus because we are blinded to its spread by a lack of field testing and monitoring. Will we do better as (or before) we unleash our own novel organisms into Earth's biosphere?

How will humans and nature be changed as threads of new, synthetic biology weave their way through our lives and our world? What must we do to anticipate, track, and steward these changes? Ecologists, particularly field ecologists, must engage with these issues before, during, and for prolonged periods after releases, if we are to realize CRISPR technology's benefits and reduce its potential for harm.

These questions require consideration: 1) If an edited organism is viable in the laboratory, what will the altered gene(s) do to the organism in an open environment, in the near term and over time? 2) If an organism with an edited genome can reproduce in an open environment, how will its progeny change and spread through populations? The development of gene drives in particular compels attention to this question (3–7). 3) How will these new organisms perform in different ecological contexts? 4) How will they affect their ecosystems? 5) How will we, or future humans, feel about these organisms? Will we like their direct and indirect effects?

Below, I review nested scales of interacting biological systems that determine fates and impacts of organisms modified by gene editing in open environments. Second, I address issues raised by applications of gene drives that can spread synthetic alleles through wild populations. Third, I explore the impact of nitrogen fixation by a river diatom under drought versus normal flow regimes. The questions become: "What could possibly go wrong?" and "How could we detect change, and early warnings of change going awry?" Promising methods once thought to be in the domain of either molecular biology or of field ecology are now increasingly used in both and will help us collaborate to meet these challenges. Next, I discuss values. To the credit of scientists and others at the forefront of the CRISPR revolution, broad societal values have been very much a part of the discussion from its onset (1–7). Last, I describe resources, efforts, and training that could help those who release edited creatures into open environments to meet their responsibility to track and steward their impacts.

Linking Genes, Traits, Organisms, and Ecosystems: Challenges to Prediction

> The systems we isolate mentally are not only included as part of larger ones, but they also overlap, interlock, and interact with each other.
>
> Arthur Tansley, 1935 (8)

Genes, including edited genes, may affect traits of organisms that in turn alter their performances and, more rarely, their impacts on ecosystems. Reciprocal feedbacks run countercurrent, from environment to ecological interactions to gene expression. Few if any pathways linking cause and effect across these scales of biological organization are straightforward. At every level, strongly nonlinear controls, variable time lags, and context dependence "change the ground rules for existence" (9) and the impacts of traits, sometimes abruptly (10). Add to this system-level complexity the rich diversity of components: genes, organisms, and landscapes with idiosyncratic evolutionary and natural histories, and it is clear why ecology, the culmination of all these nested interactions, can justifiably be called the most complex system science has ever tried to understand (11).

Predicting Traits from Genes

> It was long believed the sequence of genes in a genome was all that was needed to understand that organism's biology. Recently, scientists have realized there's another level of control: the epigenome.
>
> Joseph Ecker, 2020 (12)

Geneticists once hoped to predict phenotypes of organisms directly from their genetic codes, but this has not proven possible for most traits of ecological significance. As they probed gene regulation networks, molecular and developmental geneticists

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uncovered increasing complexity. Gene expression clearly changes during developmental ontogeny, but how it changes can be under strong environmental control, both at cellular and ecosystem scales. How given gene sequences, including edited sequences, will actually manifest as traits in living organisms depends on a variety of genomic features (transposable elements, copy number variations, chromosomal aneuploidy); epigenetic effects (methyl groups on cytosine [DNA] bases, chromatic modifications, small RNAs); as well as primary and secondary pleiotropy, homeobox regulation, phenotypic plasticity, and other phenomena (13–15). Epigenetic elements that alter gene expression interact with each other in complex, nonlinear ways, for example, during meiosis and mitosis in plants (16). Environmentally induced changes in gene transcription can have lifelong or even intergenerational phenotypic consequences (14, 17, 18).

Epigenetics are particularly exciting, and humbling, for the quest to predict phenotype from genotype. Empirical and experimental study of the phenotypic, ecological, and evolutionary consequences of epigenetics for nonmodel organisms in natural environments has just begun, and is particularly challenging for the many plants that have large, complex genomes or are polyploid (17). Ecological epigeneticists call for increased transfer of knowledge and methods from model species research to genomes of evolutionarily divergent species, and for more mechanistic, experimental studies in complex natural environments (17, 18). As Richards et al. (17) point out, to anticipate impacts of these genomic-level processes in diverse, nonmodel organisms in real (open) environments, we would need to know the extent and sources of epigenetic variation in natural populations, and whether such variation could alter ecological interactions and have evolutionary consequences.

Complex interactions of genomic elements, the general lack of detailed genomic information for most organisms, and feedbacks from complex ecological interactions make the consequences of gene editing hard to predict in whole organisms. However, if we could reliably map edited genes to traits of organisms released into natural environments, how well could we then anticipate their ecological impacts, particularly under different environmental conditions? Context dependence at all scales, but particularly at this largest scale, is a formidable challenge for predicting the fates and impacts of edited organisms in nature.

Predicting Performance from Genes and Traits

Selective environments shift, even in highly controlled agricultural, hospital, or laboratory ecosystems. Perhaps the most controlled environment in the history of life on Earth has still given rise to evolutionary surprises. Richard Lenski and his students and colleagues have tracked genetic changes in 12 initially identical populations of *Escherichia coli* through >70,000 generations as of April 2020 when the experiment was paused for COVID (19). Every day, 1% of each population was transferred to a flask of fresh identical medium. At 500-generation intervals, a subsample of each population was cryopreserved.

Despite this extraordinary control over their genotype and environment, the 12 *E. coli* clones delivered many evolutionary surprises. One is particularly cautionary for the use of gene drives to spread edited genes through wild populations. Two *E. coli* clones had mutations that would eventually take over the population, but they were initially were outcompeted by two other mutated clones that later went extinct. Clones that won over the longer term had greater potential for further adaptation due to a mutation that altered chromosomal supercoiling and affected gene transcription, enhancing mutation rates (20). Initially conferring a fitness disadvantage, over the longer term, the topoisomerase mutation that altered chromosomal coiling allowed these clones to generate descendants with new, beneficial mutations.

Another dramatic "reversal of fortune" was documented in *Drosophila* with an Sxl mutation that normally renders eggs of female flies completely infertile. Starr and Cline (21) found that parasitism by the bacterium *Wolbachia* allowed parasitized Sxl mutant female flies to lay viable eggs. The authors pointed out that such interactions (which would be impossible to anticipate) may shift the impact of species interactions from negative to positive, and even to obligate interactions of hosts with former parasites (21). Subsequent work showed that a bacterial endosymbiont of aphids made aphids resistant to parasitoid wasps if the bacteria were infected by phage with genes encoding Cytolethal Distending Toxins (22, 23). Whiteman, students, and colleagues (24) showed that one of these genes, *cdtB*, was encoded in the eukaryotic genomes of two host aphids (*Myzus* spp.), likely arriving via horizontal gene transfer. They also found that this "domestication" of the prokaryotic *cdtB* gene had occurred in several drosophilid lineages, possibly via horizontal gene transfer mediated by mites, brachoviruses, or direct integration of the phage into the aphid and fly genomes (24). For our discussion here, two points are critical. First, the change in gene sequencing and traits was triggered by species interactions of phage, bacteria, and insects. This vividly make Tansley's (8) point, that the ecosystems "we isolate mentally are not only included as part of larger ones, but they also overlap, interlock, and interact with each other." Second, these tortuous evolutionary steps and "worm holes" link genes to ecology via multilevel upscaling and downscaling processes. They are quite challenging to explain, let alone predict, as captured by the title of Jonathan Losos' (25) book, *Improbable Destinies: Fate, Chance, and the Future of Evolution*.

Gene Drives

In wild populations facing uncertain futures, maintaining grist for future evolution argues against tinkering with life in ways that diminish wild genetic diversity. However, gene drives that overwrite alternative "wild-type" alleles do exactly that as they spread through populations. At least drives using a genetic scalpel like CRISPR-Cas9 rather than the obsidian knives of earlier gene-altering technologies overwrite much shorter (typically <1 kb) stretches of the adjacent genomic sequence.

Gene drives are genetic elements that bias transmission of alleles in sexual species upward from the expected Mendelian probability of 50%. Drives have evolved in nature [e.g., *Wolbachia*, transposons, and other "selfish" genetic elements (3, 4)]. *Wolbachia* gene drives were proposed for biocontrol of pests (26) and have been deployed in open environments in Brazil, French Polynesia, southeastern Australia (27), and the Central Valley of California (28) for biocontrol of mosquito vectors of human disease. With CRISPR-Cas techniques, genetic cargos can be precisely edited, then driven with much higher efficiency (>99% copying fidelity and transmission rates) than achieved by *Wolbachia* drives (3, 29). CRISPR-Cas drives to render female *Anopholes gambiae* (which vectors malaria in Africa) sterile have been extensively studied in enclosures at Imperial College, London, and are being considered for release in Africa in the future (30). Gene drive applications to suppress mosquitos and insects in general, however, are currently challenged by rapid evolution of host resistance (31, 32). Various solutions are under investigation, including targeting a doublesex gene in which a mutation that blocked the drive would also render *Anopholes gambiae* females sterile. The chance of evolved resistance can be reduced if the genes at more than one locus were edited (30–32).

Another approach to counter evolved host resistance is to use drives that might enhance, rather than decrease, the fitness of the edited host. Anthony James and colleagues (33) have used CRISPR to edit *Anopholes stephensi*, which vectors malaria in India. They introduced two independent transgene alleles into the mosquito hosts. Each codes for antibodies that attack different life stages of *Plasmodium* parasite: one that infects the host's gut and the other, host salivary glands. Again, these two

gain-of-function alleles make the evolution of *Plasmodium* resistance less probable. In addition, resistance to *Plasmodium* may improve mosquito host fitness and increase the probability that gene-driven *Anopholes stephensi* could persist and spread after introduction to the Indian subcontinent. Such a release awaits considerable public and regulatory discussion, but meanwhile, the performance of edited *A. stephensi* is being carefully studied in increasingly realistic but contained environments—insectary cages in Irvine, California, where (as in the trials in London), the edited mosquitoes could not persist if they escaped (7).

Could we recall organisms with gene drives after they have been released? Creative ideas for controlling unwanted impacts or halt the spread of gene-driven organisms released into nature have been proposed (2, 29), and some tested in the laboratory (34). These include immunization drives (e.g., to protect species in their native range that are targeted as exotics elsewhere); recall drives that could be sent through a population to seek and destroy edited, dispersed organisms; chemical tethers already used successfully in laboratory (34) trials, to make edited organisms reliant on chemicals that they cannot obtain in the wild. We currently cannot assess how well immunization or recall drives might work over time in open natural environments. Nor do we have sufficient funding, organization, and personnel to track edited genes driven through open environments. It is unclear how we would learn whether genes in mosquitos that confer resistance to a particular parasite may leave the vector more vulnerable to hosting another pathogen (4). The low prevalence of infection of mosquitoes in most areas of malaria endemism (35) might reduce this concern (why are not uninfected mosquitoes hosting other pathogens?), unless gene editing itself improved the suitability of engineered mosquitoes as vectors for other pathogens, a change for which, at this time, there seems to be no obvious mechanism. If, however, genetic mutation or nongenetic mechanisms triggered such changes in gene-driven edited organisms over time, we would at this point be blind to them.

Other general concerns apply to gene drives, independent of our intended use of them to suppress, enhance, or simply change populations of wild plants and animals. In light of Lenski's long-term evolution experiment, it is concerning that CRISPR-Cas9 gene drives will efficiently overwrite homologous alternative alleles on paired chromosomes as they spread. Loss of allelic diversity might deprive descendant populations of their tickets through an unpredictable future (20, 25). Finally, context dependence thwarts prediction over larger scales. Novel traits that seem beneficial (to biota or society) in some settings may be harmful in others, and vice versa. These considerations underscore the need for careful evaluation, preparation, and consensus before organisms with gene drives are released (3–6), as well as chemical tethers, genetic time bombs, recall drives, or other methods to cope with unpleasant surprises after release.

Predicting Impacts from Traits

Ecology is classically defined as the study of factors affecting the distribution and abundance of organisms (36). Most observers, including many ecologists, assume that populations are primarily limited by environmental conditions and resources. Organisms should occur where they can tolerate physicochemical conditions and where resource supplies meet their requirements (37). To predict responses of species to greenhouse warming, for example, a popular approach is to analyze their climate envelopes (the ranges of temperature, moisture, sometimes other conditions where they are currently found) and assume that they will survive in or move to regions where such conditions occur in the future (38–40).

These views ignore species interactions, so are in a sense analogous to "bean bag" genetics (41). Resource supplies and conditions clearly matter, but organisms are often not where they could be, either because they have not arrived (dispersal limitation) or because of competitors or natural enemies. Impacts of consumers, particularly predators and pathogens, are difficult to observe in nature, so we tend to underestimate their importance. We often assume that if "the world is green," it is because conditions and resources support lush plant growth. If the world looks more barren, conditions for plants are not hospitable. More often than is generally appreciated without experiment or prolonged observation, consumers are exerting cryptic, strong direct and indirect effects on ecological communities: limiting some species, and indirectly releasing others: their prey's prey (42).

Paine (43–45) showed the explanatory, and potentially predictive power of identifying "trophic cascades"—strong chains of direct and indirect interactions that link plants through consumers to predators, and send ripples through ecosystems if the abundances or performances of "strong interactor" species change. Strong interactors are "foundational" or "dominant" species if they maintain sufficient biomass to physically structure their ecosystems, and "keystone species" if they are uncommon but nevertheless capable of strongly affecting ecosystem structure, sometimes by suppressing or releasing dominants (43, 46). If, as Paine and others have postulated, relatively few strong interactions trigger cascades of indirect effects that reverberate throughout communities, the dynamics of many co-occurring populations may be entrained to the fates of a few (43), making ecology more predictable.

Context dependence, however, remains a formidable challenge to ecological prediction (46–48). Species interaction strengths change across space and time as conditions and resources alter performance of web members. Adding or subtracting players can change everything. For example, the trophic cascade through which sea otters protect kelp forests from overgrazing throughout much of the northeastern Pacific (49) collapsed when killer whales began to prey on, and locally extirpate sea otters (50), possibly as a consequence of the depletion of great whales, once preferred prey of killer whales, followed by population collapses of other, fatter marine mammals (51). Although impacts of an organism cannot be predicted from traits, there should be rules that let us map changes in interactions and interaction strength over space and time. Developmental geneticists have discovered such rules as they painstakingly unraveled complex, idiosyncratic control paths underlying tissue-engendered effects on gene expression (14, 15). Despite the challenges of working over much larger scales, ecologists need to advance analogous efforts in landscapes, seascapes, and fresh waters, our domains of investigation.

Hidden Processes, Cryptic Players

In his remarkable book, *The Serengeti Rules*, Sean Carroll (52) pointed out the parallels between trophic cascades in ecosystems, and regulation networks governing gene expression in cells. Genetic circuits—molecular chains of command—regulate how, when, and if genes will be expressed, repressed, or induced. The Lac operon defied understanding until Monod and Jacob realized that another cryptic player was involved, a protein that repressed the beta-galactosidase gene until its substrate, lactose, repressed the repressor (52). Carroll pointed out that this control path functioned in the same way as a three-level trophic cascade, in which predators indirectly protect plants by suppressing herbivores (42). As we discover and unravel the often indirect, idiosyncratic control paths in gene regulation networks, metabolisms, or ecosystems, we improve our chances of anticipating, understanding, and managing the surprises. Another hopeful sign is that from genes to ecosystems, multiple manifestations first perceived as mutually contradictory can be conceptually unified when the underlying mechanism is revealed (14, 15, 53). This often involves the discovery of controls over phenomena that operate at higher levels of organization.

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Mutations in single genes can lead to multiple manifestation of brain disorders through pleiotropy, copy number variation, single-nucleotide substitutions, and epigenetic dysregulation, while different genes can lead to convergent phenotypes by disrupting common neurodevelopmental pathways (14, 15). Analogously, bird, lizard, and plant ecologists have argued for the primacy of competition as a force structuring ecological communities (e.g., ref. 54), while this view is more rare among insect ecologists (e.g., ref. 55). These views could be (somewhat simplistically) reconciled if three-level food chains are common, so that birds or lizards at the top and plants at bottom of the food chain are often resource limited, whereas insects (at the second trophic level) are predator limited, and therefore not in competition for limiting resources (53).

Context-Dependent Impacts of Traits: Nitrogen Fixation

Bioavailable nitrogen is broadly limiting to biota across terrestrial, marine, and many freshwater environments. Nitrogen fixation (reduction of N_2 gas to ammonia) appears to have evolved only once, before Earth's atmosphere was oxygenated, and is still restricted to only a few archaea and bacteria (56). A number of these have entered into symbiotic partnerships with eukaryotes, from diatoms (57) to vascular plants (56), in which microbial nitrogen fixers trade bioavailable nitrogen for reduced carbon and energy from their hosts. Since the 1970s, a major goal for gene editing in agriculture has been to extend the host range of these symbioses to nonlegume crops, particularly cereals (58). Another approach would be to transfer prokaryotic *nif* genes for nitrogenase biosynthesis and function directly into the plant genome (59). Broadly replacing synthetic nitrogen fertilizer with biological fixation in agriculture would have clear economic and environmental benefits, saving enormous energy costs from industrial Haber–Bosch nitrate–ammonia production, and reducing eutrophying runoff from agricultural fields to surface and coastal waters. What could possibly go wrong?

Environmental and social concerns echo those raised by environmentalists and the public since the original introduction of GMO (genetically modified organism) crops (60). These concerns include the spread of nitrogen-fixing genes into wild relatives, particularly via pollen for wind-pollinated cereals. Release from nitrogen limitation could make wild graminoids into aggressive weeds. On the other hand, with increased nitrogen content, crops and of nontarget recipients of edited genes could suffer more herbivory, due to their increased nutritional value (61). If root exudates or litter from edited crops or transformed wild plants enriched soil nitrogen, they could facilitate weed invasions and loss of native flora adapted to low nutrient conditions. When nonnative *Myrica* invaded and established as Hawaii's first nitrogen-fixing shrub, it changed the "ground rules for existence" for native plant species, and many went extinct (9). Native plant and arthropod diversity has been lost from patches where nitrogen fixers (vetches and lupine) enriched soils and facilitated weed invasion in coastal (62) and inland (63) California grasslands. In the Catalina Mountains of Arizona, invasive buffelgrass brought wildfire [still burning as of June 2020 (64)] that kills ancient saguaro forests.

Impacts of nitrogen fixation in river ecosystems can also change from beneficial to adverse (for humans), as hydrologic context shifts the fate of the fixed nitrogen. In clear, sunlit rivers, attached algae fuel food webs (65). Diatoms, often dominants in riverine algal biofilms, are among the most nutritious of all primary producers. With the exception of one toxic genus, diatoms are digestible, synthesize beneficial (to animals) secondary compounds like carotenoids, and are rich in lipids, including polyunsaturated fatty acids that animals require for membrane and nerve health, but cannot synthesize themselves (66). Freshwater attached diatoms in the family Epithemiaceae are particularly nutritious. *Epithemia* spp. contain what appears to be Earth's youngest endosymbiont: nitrogen-fixing "spheroid bodies" whose closest free-living relatives are cyanobacteria in the genus *Cyanothece* (67, 68). While other nitrogen-fixing cyanobacteria synthesize nitrogen-bearing toxins, *Epithemia*'s endosymbionts synthesize all 23 essential amino acids required by animals (68), despite genome reduction that eliminated both photosystems (68, 69). In unpolluted lakes and rivers where they often abound, *Epithemia*-rich algal assemblages are consumed voraciously and preferentially by algivorous insects (70) and tadpoles (71) and, when exported from rivers to estuaries, by amphipods and isopods that are important fish and shorebird prey (72). As diatom-cyanobacterial holobionts, *Epithemia* support much higher rates of growth and emergence in aquatic insects (70) and tadpoles (71) than do other detrital or algal diets.

Making a diatom into an even more complete "superfood" like *Epithemia* would seem a worthy goal for gene editing, had not evolution already done it. During drought, however, *Epithemia* blooms that fuel salmon-bearing river food chains during higher river flows can trigger indirect effects that threaten public health (73).

Under the Mediterranean seasonality of Northwestern California, rainy winters are followed by summer drought. Winter and summer flows govern algal phenology, and how river food webs assemble during the biologically active summer season (48, 73, 74). In spring, as river flow subsides, clears, and warms, filamentous green macroalgae (*Cladophora glomerata)* can grow several meters long, increasing surface habitat for attached diatoms and other epiphytes by five to six orders of magnitude (70). By midsummer, *Cladophora* streamers turn from green to rusty red as they are smothered by *Epithemia*. They nourish prey that support rearing salmonids, not only in the river, but also in the estuary. Diatom–*Cladophora* assemblages that drift or are experimentally introduced into the estuary are grazed away within minutes by swarms of amphipods and isopods, who greatly prefer them to the local soft green seaweeds (*Ulva* and *Enteromorpha*) generally assumed to be important food sources for coastal marine food webs (72). These observations suggest that drift algae from rivers can be important but cryptic energy and nutrient source to estuarine and coastal food webs, yet another example showing that strong top-down limitation can conceal strong links in food chains, until experiments or changing circumstances reveal them.

Under severe summer drought, the fate of *Epithemia*-rich assemblages and the nitrogen they fix changes. These proliferations stop fueling salmon-bearing food chains, and instead support blooms of potentially neurotoxic cyanobacteria (73, 75). If flows in sunlit river mainstems drop below critical levels [which can occur even after rainy winters due to human water extraction (76)], pools warm and stagnate, and nutritious *Cladophora–Epithemia* assemblages are overgrown by heat-tolerant, potentially neurotoxic cyanobacteria dominated by *Anabaena* spp. (75, 77). As fronts of *Anabaena* spread through *Cladophora–Epithemia* assemblages, they smother red-brown host assemblages under blue-green to black cloaks. *Anabaena* get access to sunlight and likely also nutrients or energy from their dying hosts. While *Anabaena* fixes nitrogen, it also takes up carbon heterotrophically, which extends its ability to fix nitrogen into hours of darkness (78). Over the last decade, neurotoxic cyanobacteria have killed more than a dozen dogs in the Eel, Russian, and other Northern California Rivers (73, 75). To the south, 20 sea otter deaths off Monterrey Bay were linked to microcystins (hepatotoxins) produced by other cyanobacteria in the agriculturally enriched rivers (79). Whether nitrogen-fixing diatoms or other nutrient sources deliver nourishment or toxins to food webs in rivers and linked upland and coastal ecosystems depends on winter and summer hydrology, climate, and increasingly, human choices (73, 76).

Tinkering with Genes, Organisms, and Ecosystems: Values

Charles Mann (80) contrasts two schools of 20th-century thinkers and scientists: "wizards"—engineers, inventors, or tinkerers

who use technology to fix problems—and "prophets" who revere nature: organisms and landscapes beyond human domination. The wizard "sees people as endlessly inventive ... wily managers and thinkers and doers that can expand endlessly. [Prophets] see us as fundamentally embedded in ... something larger, and we shouldn't wreck that larger thing" (81). Twenty-first century CRISPR scientists would at first seem to be in the techno-optimist wizard camp. A more nuanced view is raised by their hopes of applying CRISPR editing and gene drives for conservation of wild species and restoration of seminatural ecosystems (2). Gene editing, gene drives, and other modern genetic tools give conservationists new, and in some cases, perhaps our only hope of undoing some of the damage humans have done to wild species and seminatural ecosystems. Interspecies somatic cell nuclear transfer may enhance genetic diversity in species like black-footed ferrets or gray wolves that humans have reduced to low population sizes (82). Coral biologists are exploring gene editing to make corals, or *Symbiodinium*, their dinoflagellate endosymbionts, less stressed in the current and near-future oceans that our greenhouse gases have warmed and acidified (83). Gene drives are widely discussed as ways with fewer off-target impacts (than, for example poisoning) for eliminating pests, disease vectors, or predators we have introduced to islands, where they now threaten native species, including endemics (3, 4, 84). Their ethical use requires attempts to predict the full range of outcomes (my focus in this paper); analysis of risks, benefits, and opportunity costs; public engagement and acceptance; and oversight—what Sandler (85) has classified as criteria for "an instrumentalist ethical perspective," in which the technologies or tools are "neither good nor bad, but neutral."

Sandler argues that, while important, the instrumentalist ethical perspective is incomplete without another "form-of-life perspective" that considers how gene editing will restructure our activities and our relationships with life. Gene editing is not only an efficient, perhaps sometimes necessary means to certain ends—it will alter how we and future humans will feel about nature as it is increasingly populated by gene-edited creatures, even if these serve as our agents for species conservation or ecosystem restoration. For some of us, it is very appealing to "resurrect" species that could remake past landscapes and ecosystems that we mourn, from the American chestnut-dominated forests of central and eastern North America (86–88) to the mammoth steppes of Beringia (89, 90). If we restrict human activities and free enough space and time so that these species can evolve and reconfigure ecological systems on their own, we could possibly maintain aspects of wildness and wilderness important to the prophets (80) among us today. If, in an even more crowded, human-dominated world, we do not so restrict ourselves, we will have to endlessly tinker with Earth's biota and ecosystems to fix problems and maintain life-supporting parameters within acceptable ranges (91) (the Band-Aid on a Band-Aid problem).

How will current and future humans feel about gene-edited creatures, even as our agents of species conservation or ecosystem restoration? In his near-future science fiction novel about rampant synthetic biology and its potential, via competitive biowarfare among agrobusiness companies, to impoverish the human food supply, Paolo Bacigalupi (92) depicts the cultural revulsion some humans feel toward synthetic creatures as persisting. Human perceptions and values change, however. American conservationists of European descent once revered North America's "pristine wilderness" but have recently begun to appreciate the degree to which old growth forests and the diverse mosaics of plants and game that European colonists found in were, in fact, created and maintained by careful tending and fire management by Native Americans (93–95). Wildlands managers and conservation biologists increasingly realize that we need to return to such management to sustain western forests and their biota and life-supporting functions through changing regional fire, flood, and drought regimes (95). Indigenous people's views of using synthetic biology to restore species (e.g., American chestnuts or salmonids) or ecosystems in their lands appear, however, to be negative or mixed at present (93).

Constant Vigilance, Beyond the Laboratory: The Importance of Tracking Synthetic Threads through Real Environments

> [S]cientists want to transfer, enhance, or silence genes to make microbes work for us without having to hassle with natural selection. We will be the creators of microbial metabolism, and will design microbes to do our bidding. We have the power to do so, but that power does not appear to come with an understanding of the potential tremendous consequences for microbial evolution, let alone our role in altering the future trajectory of the planet.
>
> Paul Falkowski (56)

> Just by being vigilant—by being out there—one comes to recognize change. Why is the world we know so well changing?
>
> Robert T. Paine (96)

> Dr. Frankenstein's crime was not that he invented a creature through some combination of hubris and high technology, but rather that he abandoned the creature to itself. [LaTour then cites from Mary Shelley's *Frankenstein*: "Remember, I am thy creature," the monster protests, "I ought to be thy Adam; but I am rather the fallen angel, whom thou drivest from joy for no misdeed ... I was benevolent and good; misery made me a fiend. Make me happy, and I shall again be virtuous."]
>
> Bruno LaTour (97)

In these early days of gene editing of nonmodel systems, many synthetic biologists focus on whether their new creations will be viable when not coddled in the laboratory. However, it is not too early to ask "what happens if they survive, establish, and spread? What happens if they are 'driven' through wild populations?"

Forward prediction of their futures, and how they will affect ours, will be challenged and often thwarted by scale-spanning complexities and contingencies, including those I have reviewed here. The more we seek predictive mechanistic understanding of processes that drive change and link genes to ecosystems, however, the more nimble we will be in postdicting—explaining surprises. For either prediction or postdiction, 21st-century tools should help us realize some of the promise of synthetic biology and reduce its potential for harm. Methods once thought to be in the domain of either molecular biology or of field ecology are now are increasingly available in both (17, 98). Spillover of methods across fields should enhance the interchange among molecular geneticists, cell biologists, epigeneticists, ecologists, evolutionary biologists, and Earth scientists needed to meet the challenge and responsibility of CRISPR-Cas use in the world. LaGrangian observations of free-living individuals, which have greatly enlightened field biologists, are now enabling cell biologists to learn about the functioning and fates of single cells as they spread and change across space and time within their living microenvironments (99, 100). Quantitative stable isotope probing lets ecologists and ecosystem scientists identify key microbial taxa and track the elemental and molecular exchanges they mediate in nature (99, 101). Geneticists use "knockin" and "knockout" experiments to investigate how genes affect phenotypes; ecologists use enclosure/exclosures, and other manipulative experiments to reveal how particular species affect food webs. Ecologists (43–50, 102) searched for trophic cascades set off by strong interactors (foundation or keystone species), in hopes of explaining or predicting indirect "knockon" effects entrained to these. Similarly, molecular biologists search for critical "gene programs," those gene

regulatory pathways that entrain many others and determine higher order phenotypic characters, like sex (103). At all scales of biological organization, context controls interaction strengths and outcomes, with the effect of "pattern on process" in landscape ecology (104) scaling down to topologically associated domains of gene expression in spatial genomics (105). Remote sensing advances in Earth systems science enable us to track changes in the distributions, abundances, and physiological states of biota, as well as environmental conditions, from aircraft or space (106). Broad calls for "convergence" from funding agencies and organizations demand both intellectual humility and synthetic imagination from biologists and Earth scientists to breach silo walls and bridge deep historic divides among our subdisciplines.

Such teamwork will be crucial for building detection networks to track edited organisms through open environments. These should be designed to focus sampling effort on "hot spots or hot moments" where performances, selective forces, or impacts of edited organisms are most likely to change. We could map shifting environmental controls, as well as the spread or conspicuous change in edited organisms (e.g., trees or megafauna) using repeated photogrammetry from drones, aircraft, or space-based observation platforms (106). Organisms whose CRISPR-Cas altered DNA has no externally visible phenotypic expression would require genetic monitoring, either of trapped organisms or of environmental DNA (eDNA).

The last effort is growing more feasible due to scientific and technological innovations using CRISPR-Cas technology not only for editing cells or organisms, but also for tracking their eDNA. New CRISPR-Cas12a [e.g., DETECTR (107)] and CRISPR-Cas13 [SHERLOCK (108)] platforms, developed for clinical diagnoses, have detection sensitivity down to attomolar concentrations, and can discriminate nucleotide strands that differ in just a few base pairs. Following preamplification of target DNA with recombinase polymerase amplification, a guide RNA directs a CRISPR-Cas12a nuclease to the targeted site. Once the enzyme cleaves that site, CRISPR-Cas12a cleaves other single-stranded DNA indiscriminately ("transcleavage"). If a single-stranded DNA fluorophore-quencher molecule has been added, Cas12a transcleavage activity will release the fluorophore from its quencher, triggering fluorescence that can then be measured in the sample. These detection systems are potentially inexpensive and adaptable for field applications: lyophilized for cold-chain independence, then used with paper spotting (108) or smartphone-enabled detection devices (109); or a recently developed handheld fluorescent monitor developed for bacterial detection (110, 111).

Williams et al. (110, 112) appear to be the first to apply this technology for species detection in nature. Sampling eDNA in four Irish streams, they could detect and differentiate closely related Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Sensitive CRISPR-Cas12a detection that greatly enhances eDNA "early warning" detection of valued, rare, or potentially invasive exotic species (109, 110, 112) could also track edited organisms spreading through open environments via gene drives.

In one of the most exhaustive gene drive monitoring programs to date, Crawford et al. (28) have tracked *Aedes aegypti* mosquitos (vectors for dengue, chikungunya, Zika, and yellow fever) with *Wolbachia* gene drives over an area almost 400 ha (including 3,000 homes) in Central California. If *Wolbachia*-infected males mate with uninfected females, zygotes die. If infected females mate with infected or uninfected males, however, zygotes are viable. For this sterile insect biocontrol to work, it is imperative that no *Wolbachia*-infected females be released. In a robotic larval rearing system, Crawford and colleagues infected larvae of both sexes, but then separated female pupae with automated size-based sex sieving, then checked by an industrial image analysis and a machine learning classifier (with some human checks). Importantly for the argument here, the released *Wolbachia* males and the entire *Aedes aegypti* population were monitored over large areas. Carefully mapped release sites and subsequent trap monitoring let investigators check the success of their biocontrol program, and monitor for mishaps, like the accidental release of *Wolbachia*-infected females.

The strategic, extensive monitoring in this study shows that responsible tracking of released edited organisms is feasible, with sufficient support. The authors' technological innovations (28) could facilitate other efforts. These monitoring programs will need coordinated networks of field biologists—trained, funded, and organized on the ground. It would be ideal if teams were led by local scientists and employed local youth, like Dan Janzen's parataxonomists in Costa Rica (113). Ideally, teams would include members trained in genomics and epigenomics, ecophysiology, and the relevant "ologies," so they could anticipate behavior and fates of species with different evolutionary and natural histories. Team members educated in community and ecosystem ecology and remote sensing methods could design sampling across appropriate space–time intervals to determine whether traits and impacts of these organisms remain as intended.

Field monitoring programs to track our creatures, our "hopeful monsters," could not only provide meaningful work for people in impoverished regions of the world (including the United States) but should enlist people with crucial Traditional Ecological Knowledge to advise stewardship. Releases that can be spatially contained should occur only if all indigenous and other local people in the arena have consented. More uncontained releases that could obliterate a targeted species from the Earth should require more widespread agreement (6), although how to attain this from ~8 billion human individuals is an open question. Consent or consensus may or may not develop, even after prolonged engagement and intense listening, but the process would educate both scientists and stakeholders in the perils and promise of using edited organisms in nature. It also might help more of us think about where nature (including human nature) will find a place in our increasingly engineered future.

When could we relax our watchful stewardship of our "hopeful monsters"—our edited biotic agents, and the feedbacks they trigger? Perhaps never, although the more we seek predictive understanding of processes and linkages from functional genomics to species interactions and ecosystem responses, the better we will become at our two crucial responsibilities at the dawn of the CRISPR-altered Anthropocene: first, designing efficient schemes to track the spread, change, and impacts of edited genes across landscapes, and second, analyzing why things go awry, perhaps even soon enough to correct problems, and survive them.

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