chapter eleven

Ecological and community considerations in engineering arthropods to suppress vector-borne disease

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11.1 Introduction

Advances in molecular biology have encouraged major research efforts devoted to improving human health by reducing the ability of natural populations of vector arthropods to transmit certain pathogens. In 1986, a well-attended symposium on this subject was held at the national meeting of the American Society of Tropical Medicine and Hygiene. The speakers participating in this earliest of formal discussions on the subject agreed that the

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risk of vector-borne disease might be reduced if a genetic "construct" could be developed that would block development of certain pathogens in the vector arthropod and if that construct could be linked to a genetic "drive mechanism" that would cause a disproportionate portion of the descendants of the released arthropods to carry the construct. Malaria was the primary disease discussed at the symposium, and the main construct under consideration was a gene or combination of genes that destroyed one of the developmental stages of the malaria pathogen in the vector. At the time, the newly discovered global sweep by the P-element in natural populations of *Drosophila melanogaster* (Spradling and Rubin 1986) inspired the participants to identify transposable elements as the most feasible drive mechanism for the proposed public health intervention against *Anopheles gambiae*, the main African vector mosquito. The strategy proposed at that early symposium more recently has been extended to the *Aedes aegypti* mosquitoes that transmit dengue virus (Olson et al. 1996). The enduring spirit of optimism that began in the 1980s now causes a large share of the public health entomology research budget to be invested in the genetics of vector competence, transposable elements, and the structure of vector populations (Spielman 1994).

Although, clearly, the deliberate release of hematophagous arthropods into a site rife with vector-borne human infections would be designed to improve human health, such a release may also threaten well-being, both in the short and the long term. The released organisms or the first few generations of their descendants might themselves directly cause human annoyance or transmit agents of disease. More fundamentally, the disease burden might be exacerbated until the desired health effects were accomplished, but only temporarily. Another possible consequence is a resurgence of the disease should the manipulation not be sustained in the population. Accordingly, the discussion that follows examines the need for regulatory oversight that would govern the release of genetically modified vector arthropods. In particular, we identify potential unintended consequences of such releases and recommend a rationale for endorsing such releases.

11.2 *Ethics of releasing reared vector arthropods*

Unique ethical problems accompany any release of hematophagous arthropods because local residents who may be affected by the experiment must register their "informed consent." Presumably, each such "experimental subject" must be provided with relevant details of the research protocol, must agree to participate, must be monitored throughout the course of the experiment, must be able to withdraw from the experiment at will, and ultimately must be apprised of the results of the work. Conventional pharmaceutical or vaccine trials are levied against a closely defined group of people, every one of whom would be identified and interviewed. Phase 1 trials generally are conducted under laboratory conditions and involve a few tens of people; Phase 2 trials are conducted in endemic field sites and involve a few hundred subjects; and Phase 3 trials may include populations comprising many thousands of people. Entomological experiments, on the other hand, permit no such distinctions. Released vector arthropods may attack anyone in their vicinity, including transient visitors, and their attacks may continue for weeks. Once released, free-ranging arthropods cannot be recalled.

Debates over the implementation of public health interventions tend to pit conservative advocates of the "Precautionary Principle" against the more permissive advocates of the "principle of minimal risk." Few non-contained entomological releases could take place if a precautionary proof of safety were prerequisite. Certain kinds of entomological exposures may be so contained and apparently innocuous that little debate over their deployment is justified. A "vector competence" experiment using laboratory-reared mosquitoes, for example, would pose few problems. In those operations, pathogen-free insects

would be caged against an infected person's body upon which they would be permitted to feed. Eventually, the course of infection in these insects would be evaluated. Olfactometer experiments similarly would be subject to conventional rules of ethics. Institutional Review Boards (IRBs) could review such limited "releases" as though they were conventional Phase 1 drug or vaccine trials. Even experimental exposure of human subjects to ticks would present few ethical problems, despite the distastefulness of these organisms. Any possibility of inherited infection (= transovarial transmission, etc.) must be, of course, rigorously excluded. Although such "contained exposures" to vector arthropods would encounter few ethical obstacles, a "noncontained release" would be far more problematic.

11.2.1 Marked organisms

Mosquitoes have long been used in "mark–release–recapture" experiments that were designed to explore any of a variety of phenomena, but mainly dealing with dispersion behavior and longevity. Service (1993) listed hundreds of these experiments, including one that released 3 million hematophagous mosquitoes in 1951. Although no long-term damage has been reported, the regulatory climate has changed (Aultman et al. 2000). Any release of hematophagous female mosquitoes must now be reviewed and approved by IRBs or by other regulatory authorities. The heart of the difficulty lies in obtaining informed consent. Investigators who plan to release any hematophagous or potentially pestiferous insect might first inform and obtain the consent of all people who would find themselves in the vicinity of the release. Provision for withdrawing from such an experiment might be required, and such a provision could not be honored unless residents were willing to relocate. Even a single objection by an individual to the release of a potential pest might disqualify the entire experiment.

11.2.2 Infertile organisms

A large series of "genetic control" experiments were executed during the 1960s and 1970s, each aiming to suppress the density of one or another population of vector or pest insects. These experiments used strategies based on the release of laboratory-reared organisms exploiting sterile males, cytoplasmic incompatibility, sterile hybrid males, translocation heterozygotes, conditional lethals, meiotic drive, and compound chromosomes (Pal and LaChance 1974). The objectives of these experiments included: "1. eradication or suppression to an acceptable level, i.e., below the critical density for disease transmission; 2. long-term suppression without eradication (which might involve a commitment to continued releases); or 3. replacement of the target strain by an introduced strain that has low fertility or inability to transmit disease, conditional lethals, or some other desirable characteristics."

The late Edward Knipling's (1955) seminal demonstration that screwworm flies can be eliminated by "the use of sexually sterile males" stimulated diverse attempts designed to displace or modify various vector populations. The successful attack on those flies required the release of about 20 irradiated, factory-reared males for each female fly present at the site. Some 10 billion irradiated screwworm flies were released each year along the Texas–New Mexico border (Smith and von Borstel 1972). These insects are particularly vulnerable to such an approach because they tend to distribute themselves uniformly over the landscape and to mate randomly. Males can readily be distinguished from the much-larger female flies and, of course, are entirely innocuous — they lay no eggs. The ultimate objective of this highly successful operation was to limit the northern distribution of the "endemic population to the Isthmus of Panama, where continuous small releases would keep the North American continent free of the pest" (Smith and von Borstel 1972). Although screwworm flies originally were grown in whale meat, an environmentally

friendly vegetable diet now serves that purpose. Few ethical issues arise directly from such a release.

Laven's (1967) "Okpo experiment" was the first attempt to register similar gains against a vector mosquito. That sophisticated attack on the tropical house mosquito (*Culex pipiens quinquefasciatus*) in a village in Burma was based on the principle of "cytoplasmic incompatibility," i.e., failure of egg embryonation due to infection by a rickettsial symbiont. Every aquatic breeding site in that isolated community was identified, and the abundance of the larval and pupal stages of the mosquitoes in each was estimated. About as many male pupae deriving from an incompatible laboratory colony were placed in each water container each day as were present there naturally. Fertile eggs, thereafter, became increasingly scarce. Great care had to be taken to release no females of the incompatible strain; disastrous population replacement might then have occurred. Female pupae were excluded by examining each pupa microscopically. The apparently successful outcome of this experiment, however, remains in doubt because of the absence of comparison treatments; the experiment was uncontrolled. The Okpo experimental release presented few ethical issues because only non-hematophagous male mosquitoes were released.

Quite the opposite was the case, however, when World Health Organization (WHO) personnel attempted to apply sterile male technology in India against the tropical house mosquito. Intense controversy surrounded the fiasco, which is remembered as the "Delhi experiment." Politically motivated critics accused the WHO of practicing U.S. Central Intelligence Agency–inspired biological warfare against the Indian people (Curtis and von Borstel 1978). The Indian Parliamentary Public Accounts Committee concluded that these irradiated mosquitoes would sterilize male human residents at the release site. Alarm had been generated by the massive manner in which about half a million sterilized male mosquitoes were released daily from prominent, truck-mounted cages. The intense political response to the releases caused the program to be discontinued. The outcome was a lesson teaching that, even in cases with few objective problems to burden a release, the attitudes of people residing at the release site may be exceedingly costly.

A more objective problem accompanied the landmark "Lake Apastepeque experiment" in El Salvador. This trial, sponsored by the U.S. Department of Agriculture and the Centers for Disease Control, aimed to reduce the fertility of *Anopheles albimanus*, the dominant vector of malaria in the region (Lofgren et al. 1974). Chemosterilized laboratory-reared mosquitoes of both sexes were released into the study site. As pupae, the mosquitoes were sorted by sex on the basis of size. Unfortunately, because this distinction tends to be ambiguous, nearly 700,000 females accompanied the 4.4 million males that were released over a 10-month period into a 15-km^2 malaria-endemic agricultural community of about 1000 people (Breeland et al. 1974). To protect the released mosquitoes, no public health applications of insecticide were permitted in the vicinity of the release site throughout the 2-year duration of this experiment. Instead, additional supplies of antimalaria drugs were distributed there. The authors of the study noted that "the release of large numbers of females could be a [health] problem if their longevity is sufficient to permit them to become malaria vectors. This possibility must be investigated." No such investigation was recorded. The Lake Apastepeque experiment was a great success; the fertility of the target population was reduced sharply, and their abundance transiently declined. The immediate impact of this experiment on the well-being of the residents of the site, however, remains uncertain.

Releases such as that at Lake Apastepeque were conducted in a far more permissive environment than exists today. Problems deriving from the "release ratio," the proportion of released vector or pest insects compared with those in the ambient situation, encountered in the implementation of "genetic control" strategies, illustrate issues that ultimately may impede future release of genetically modified arthropods.

11.2.3 Exotic organisms

A novel attempt to modify the genetic composition of an established pest or vector population involved the release of tropical *Aedes albopictus* mosquitoes in a suburban site in the north-central United States (Hanson et al. 1993). The aim was to reduce the hibernal survival of a recently established infestation of a temperate zone variety of these mosquitoes by releasing males derived from a laboratory colony of similar but diapause-incapable mosquitoes that originated in tropical Malaysia. Approximately 40,000 adult male mosquitoes were released. Few female mosquitoes would have been released. An unusually detailed section dealing with "safety" in the project's report discusses the remote possibility that Japanese B encephalitis virus might somehow be introduced into North America as a result of this experiment (Hanson et al. 1993). We understand that the safety discussion was a reaction to severe criticism from the regulatory authorities. Eventually, the authorities were satisfied that this release of male mosquitoes posed no danger to the residents of the study site and that no ethical issues were evident in the situation. Although this first attempt at "genetic engineering" did proceed and apparently succeed, diverse obstacles to any release should be expected.

11.3 Diversity of transgenic strategies

Prospects for creating transgenic organisms for release against vector arthropods generally focus on attempts to modify the vector genome such that the organism cannot support the development of a pathogen. The most likely general target in the case of *Plasmodium falciparum* malaria is an oocyst melanization trait in Anopheles gambiae mosquitoes that is regulated at two major genetic loci (Vernik et al. 1989). Genes from alien kinds of organisms serve similarly as promising bases for devising intervention strategies. Promoter sequences that regulate gene expression generally must be present in the construct (James et al. 1991, Mueller et al. 1993). Alternatively, bacterial symbionts may serve as vehicles for expressing foreign genes in vector arthropods. Those arthropods that feed exclusively on blood generally depend on "nutritionally mutualistic" symbionts in the gut to digest their blood meal (Beard et al. 1998). Other kinds of microbial symbionts are present in some mosquitoes and in certain other flies. Mycobacteria genes, for example, have been introduced stably into the coryneform bacteria that are symbiotic in the guts of the kissing bugs that transmit Chagas' disease, and this promises to serve as a basis for public health interventions.

Yet another form of genetic manipulation, which only indirectly involves vector arthropods, focuses on the food that the candidate vector arthropod may ingest. Algae, for example, may be transformed to express *Bacillus thuringiensis israelensis* toxins to destroy larval mosquitoes that ingest them. This protein also may be expressed in the pollen of corn that has been transformed for similar purposes (Yihdego et al., in press). The primary anopheline vectors of malaria in Africa feed abundantly on pollen. The release of such "insecticidal" GEOs (genetically engineered organisms) also requires regulatory oversight because their use may select against conventional insecticides that could otherwise remain effective. Although diverse constructs, including arthropods, microbial symbionts, crop plants, and algae, are being developed for intervening against vector-borne infection, no practical system is yet in operation.

11.4 Drive mechanisms

Genetic drive mechanisms are required to disseminate a GEO rapidly and thoroughly through a wild population. Certain non-Mendelian genetic phenomena may transfer genes

most effectively between germ lines or favor the proliferation of particular reproductive combinations in a population. Favorable traits that could be linked to such entities therefore would be "driven" through a wild population. Patently unacceptable alternatives include sustained inundative releases of the modified vector or use of particular conditional lethals, such as a gene conferring insecticide insusceptibility. Conditional lethals might be more acceptable when used in a sexing scheme that ensures against the presence of females in the release population. In conjunction with direct drive mechanisms, such conditional lethals would serve as a precaution in the event that the diversity of drive mechanisms is limited.

The bulk of efforts seeking to design a transgenic vector with reduced competence has focused on methods for linking genetic constructs to a transposable element. Such transposons are parasite-like segments of DNA that reproduce within genomes. Retrotransposons function similarly but use an RNA intermediate. Certain types carry a gene for a transposase that facilitates copying and reinsertion of the transposon into a new site within the genome. Others must rely on their hosts for expression of transposition enzymes. A single kind of transposon may occupy multiple sites within a genome. A transposon named *hobo*, for example, occupies some 66 sites within the genome of *Drosophila simulans* populations throughout the world (Vieira et al. 1999). Replication frequency appears to be regulated by interactions between the host and the transposon (Hartl et al. 1997a, 1997b). A single host may carry numerous transposons within its genome. A global survey of *D. melanogaster* revealed that some populations contain more than 1000 copies of about 30 different transposons and retrotransposons. *Mariner*-like elements are unique in not requiring transmission through the germ line; they also can transpose within somatic cells. Such profligate transposition throughout evolutionary history has provided about 42% of the genetic material of the human genome, much of it residing in the less functional heterochromatin (Smit 1999). Fruit flies carry between a 5 and a 15% load of errors, and certain studies suggest that flies carrying few errors remain far from saturated. The great diversity of transposon varieties and the ability of genomes to function under heavy loads would appear to facilitate artificial introductions.

The stability of transposon-based constructs remains in question. Although copy number would be expected to increase after a release, it remains unclear how many copies would accumulate before reaching an equilibrium and how long they might persist intact. Degraded and inactive transposons predominate among those detected in genomes thus far. Although molecular geneticists have succeeded in transfecting mosquitoes with transposons in the laboratory with a 4% success rate (Coates et al. 1998), it is not clear how stable their constructs might be, how many generations a transgenic mosquito might remain incompetent, or how readily these elements would be incorporated into field populations. Considerable sequence diversity characterizes the 297 elements of *D. melanogaster*, including frequent internal rearrangement (Dominguez and Albornoz 1999). Frequent frame shifts, substitutions, insertions, and deletions, which can disrupt their open reading frame and render them nonfunctional, characterize other transposons in nature. A 2×10^{-5} mutation rate has been estimated for certain of these elements in *D. melanogaster*. Particular non-LTR retrotransposons often produce "dead-on-arrival" copies because of the frequent loss of fragments at their 5′ end (Petrov et al. 1996). Upon reaching a tenuous equilibrium, transposon copy numbers generally decline incrementally as a result of stochastic loss. Transposons have a limited life span and therefore are inherently unstable. Their instability must be considered when designing interventions based on these drive mechanisms.

Transgenic releases should have in place a series of different transposable element constructs before any proposed introduction commences. This permits a backup release in the event that poor saturation, impaired fitness, or instability compromises the effectiveness

of the previously released construct before the trait of interest becomes fixed in the wild population. Otherwise, a wild population inevitably would rebound after several generations because of its reproductive fitness advantage over transposon bearers. Fitness decreases as transposon copies increase in the genome. Cumulative fitness deficits may overwhelm the ability of an organism to cope with its environment. Each genetic construct should occupy a distinctly different set of transposition sites, and be demonstrated to be capable of transfecting its host in serial combination. Even with a backup, some minimum measure of confidence in the stability of each construct must be demonstrated. Releasing one unstable construct after another would be pointless.

Until recently, reliable methods for transforming mosquitoes have been elusive. A *Science Research News* report in 1993 commented that "most vector biologists [are] pinning their hopes on a genetic manipulation technique, [that] the race is on to find a transposable element that functions in *An[opheles] gambiae*," and *minos* appeared to be "one leading candidate" (Aldhous 1993). The pace was set when, nearly a decade later, the *minos* transposon was used to insert a stably inherited fluorescent marker into *A. stephensi* mosquitoes (Cattaruccia et al. 2000), and *hermes* was used to insert a defensin gene into *Aedes aegypti* (Kokoza et al. 2000). Neither accomplishment approached the ultimate requirement for a usable drive mechanism because separate promoter mechanisms were required. The power of these newly developed techniques, however, is likely to accelerate the pace of this perceived race to identify a suitable transposable element.

11.5 Theoretical considerations

Several other considerations arise in the context of designing successful interventions using GEOs. Although the following factors have not been documented as problematic, they nevertheless are areas of potential concern.

11.5.1 Competence

Most GEO interventions that have been proposed seek to reduce the suitability of the target vector population as a host for the pathogen. Although this strategy generally assumes that the pathogen develops and multiplies freely in natural vector populations, no estimates of the prevalence of malaria competence in nature appear to have been published, nor are estimates available that would lead us to anticipate the effect of a change in competence on human health. Indeed, competence is a weak element in the classical model of "vectorial capacity" (Garrett-Jones and Shidrawi 1969). Although vector longevity or narrowness of host range contribute exponentially or geometrically to the force of transmission, competence contributes only linearly. Thus, indoor applications of residual insecticide that reduce the likelihood that an adult mosquito would survive to become infectious would interrupt transmission far more powerfully than would any reduction in competence.

11.5.2 One-pathogen strategies

An arthropod vector of one infection is likely to transmit other infections, as well, and this presents special ethical problems. Anopheline vector mosquitoes, for example, may transmit at least six human pathogens: *P. falciparum, P. vivax, P. malariae, P. ovale,* O'nyong nyong virus, and *Wuchereria bancrofti.* In addition, numerous apomictic or partially panmictic vector populations may be present in the same site. A release directed against one pathogen in only one of these vector populations, therefore, may have little effect on the

overall force of transmission of these pathogens. Such an outcome may be complex. In the event that a given release results in an increase in vector abundance, human health may bear an added burden.

11.5.3 *Herd immunity*

Any temporary improvement in health tends to prejudice the future well-being of a population, a relationship that holds particularly true in the case of malaria. Where the force of transmission is intense, infection may be virtually universal. Episodes of patent disease, however, tend to be infrequent in adults. Although few indigenous adults express malaria-related symptoms, virtually all visitors from non-endemic sites would become incapacitated and suffer life-threatening episodes of illness. Such immune protection of perpetually infected residents dissipates when transmission ceases. This loss of herd immunity, as would follow transient elimination of infection in a site, may be mirrored by a devastating outbreak of disease in the event that transmission resumes. Reversal of a major public health gain also would be burdened by a series of social disruptions. An indigenous population adapts psychologically to frequent illness, an attitude that rapidly becomes reversed once the burden is relieved. Resumption of transmission in this "newly virgin" population, of course, would provoke outrage. In the event that an intervention were supported by outside funding, over time the donor community might become "fatigued" and make renewed funding difficult. Resumed transmission of malaria after a period of relief would produce damage that far exceeded the transient benefit that might have resulted from the period of relief.

11.5.4 *Maintaining the population density of GEOs*

Additional problems must be considered in the likely event that the density of the population of released organisms must be maintained at a specified level, relative to that of the population of native vectors. Toward this end, the density of the released population must be monitored and steps taken to ensure that its density remains at the specified level. Peridomestic insecticide use might have to be curtailed, as in the case of the Lake Apastepeque experiment. Similarly, the use of bednets or screening might be incompatible with the objectives of the release. Indeed, success might hinge on the availability of sites that are suitable for the breeding of the released arthropod, and peridomestic artificial breeding sites might be required. Even if the modified vector were completely incompetent as a vector of any human infection, presumably it would still be anthropophagous and such a pest cannot be nurtured.

11.6 *Temporal–spatial relationships*

Transposon-driven releases may be subject to strong environmental forces that create considerable uncertainty regarding the successful fixation of an accompanying construct, especially early in a release and when conditions for perpetuation of the released arthropods are marginal. Because vector populations are distributed patchily and are interrupted seasonally, a transgenic release should not be expected to progress smoothly, but rather in fits and starts. The instability of such progression toward fixation would tend to increase as conditions for the perpetuation of released transposon-bearing vectors become less optimal. The reproductive and ecological fitness of vectors bearing transgenic constructs is critical in determining the rate and stability of ultimate fixation.

The dissemination of transposon-driven constructs in a population may be limited by particular characteristics of the transformed organisms and by their interactions with wild

populations. Under ideal conditions with unrestricted, panmictic breeding and global dispersal, certain mathematical models indicate that a released construct may become fixed in about 30 generations as long as overall fitness is at least half that of wild types (Ribeiro and Kidwell 1994). Organisms that mate but once in their lifetime remain relatively close to discontinuous breeding sites (such as those of *An. gambiae* s.l.) and undergo seasonal constriction of their populations, and this may require greater fitness and much more time to become fixed. Certain models suggest that under such conditions, even constructs that preserve fitness at about 80% of that in wild-type mosquitoes would require many hundreds of generations to achieve fixation (Kiszewski and Spielman 1998).

The unstable progress toward fixation may endanger health in areas hyperendemic for malaria. The ebb and flow of transposon-bearing vector populations may create situations in which exposure to malaria disappears for a few years and then returns. This may compromise protective immunity in certain local populations, leading to epidemics with high rates of morbidity and mortality. Even where fixation progresses more stably, pockets of transmission may become interspersed with refugia, thereby exposing members of protected communities to infection when they travel relatively short distances from their homes.

Measures designed to enhance the ability of a transgenic organism to overtake a wild population may have limitations. Achieving highly favorable release ratios (1:1 or greater) will accelerate fixation but may require intensive interventions against wild populations prior to release. Stewardship strategies, including repeated release of transgenic organisms, may have a similar effect. Mathematical models suggest that, while the average time until fixation may be reduced considerably by such practices, considerable uncertainty and variability in outcomes remains, primarily as a result of stochastic instabilities associated with the early stages of a release (Kiszewski and Spielman 1998).

11.7 *Epidemiological objectives*

Although efforts to produce transgenic malaria-incompetent *Anopheles gambiae* mosquitoes have been pursued for more than a decade now, the health objective of such efforts has not precisely been defined. Simply put, the question remains: How would reduced vector competence translate into reduced public health burden? The question is most appropriately asked for malaria in sub-Saharan Africa because the burden imposed by vector-borne disease is greatest there and because the *A. gambiae* complex has been the most frequent subject of studies to reduce vector competence. Although these mosquitoes are exceptionally long-lived and human biting, their competence as malaria vectors is restrained because they tend to develop far fewer oocysts than do certain vector mosquitoes native to North America or India. The magnitude of the sporozoite inoculum, presumably, would reflect oocyst load, and pathogenesis would, presumably, relate to this quantum of infection. The proportion of field-derived mosquitoes that become infective after feeding on an infectious person, however, has not yet been determined. We lack a model that would help relate increments of reduced malaria burden with reduced vector competence.

Risk of malaria infection is estimated most readily by calculating an entomological inoculation rate (EIR) as the product of the mosquito biting rate times the proportion of mosquitoes with their salivary glands infected by sporozoites. The EIR measures the average number of infective bites per person per unit time. Annual EIRs throughout sub-Saharan Africa range from <1 to more than 1000, depending upon the environment and climate supporting vector populations (Beier et al. 1999). The frequency of symptomatic infection as well as the severity of symptoms in Africa relate directly to EIR (Mbogo et al. 1993). Interventions that reduce malaria competence in the vector population, therefore, would translate directly into a similar increment of improved health.

These considerations suggest that an effective anti-vector intervention would be likely to reduce malaria incidence but may improve human health only marginally because severe disease occurs even when the EIR exceeds 1 (Mbogo et al. 1993). Irrespective of natural immunity, severe malaria episodes frequently follow only a single infective bite. Malaria prevalence frequently exceeds 40 to 60% in African sites characterized by an EIR < 1. An EIR ≤ 1 would seem a likely minimal goal for antimalaria interventions. To be worthwhile, a GEO release against malaria must promise to reduce risk to at least one sporozoite inoculum per person per year.

Considerations relating to the structure of vector populations suggest difficulties that may obstruct proposed releases of transgenic malaria-refractory mosquitoes in real-world African communities. Many such sites are infested by three potent vector species, *A. gambiae* and *A. arabiensis* of the *A. gambiae* complex, and *A. funestus* (Powell et al. 1999). The *A. gambiae* s.l. mosquitoes populating individual African sites are likely to include such genetically isolated demes as those designated as Mopti, Bamako, and Savanna (Favia et al. 1997). If specimens of an *A. gambiae* GEO deme were to be released in such a site, prevalence would continue unabated if any of the resident non-GEO populations were to continue to transmit at EIRs that exceed one infective bite per year. In much of Africa, the proportion of EIRs due to non-*A. gambiae* mosquitoes ranges from about 10 to 90%. The species composition within projected release sites will, therefore, be a key determinant of the public health success of GEOs.

The requirement for sustainable gains introduces complexity into the planning of any intervention against a vector-borne disease. To be avoided, of course, is an intervention that increases the disease burden or produces an outcome that cannot be sustained or one that impedes subsequent interventions.

11.8 *Regulatory requirements*

The deployment of transgenic arthropods is regulated in the United States by the Biotechnology Permits Unit of the Animal and Plant Health Inspection Service (APHIS) of the Department of Agriculture operating under the provisions of the Federal Plant Pest Act and the Plant Quarantine Act. A description of these functions is displayed on the Internet at the following address:

www.aphis.usda.gov:80/bbep/bp/arthropod/

On that Web site, APHIS indicates that, "The fundamental risk assessment to be addressed is: Will the genetic alteration modify ecologically or environmentally relevant properties of the organism? Specific potential or perceived risks associated with the release of a transgenic arthropod could include the displacement of native populations, a change in host or prey utilization or ecological distribution, the transfer of exogenous DNA to other organisms, or, if one of the characteristics of the transgenic arthropod was increased resistance to herbicides or pesticides, subsequent usage of such chemicals." The description, however, includes no discussion of criteria specifically relating to human health. In commenting on this lacuna, Beard and colleagues (1998) suggest that the release of numerous genetically modified vectors may actually increase risk of human disease if numerous vectors are involved in transmission.

The complex interactions and unintended consequences that might derive from transgenic releases call for a careful approach to their planning and execution. Thorough field trials generally should precede large-scale interventions, except when the intervention methodology shows too little promise in laboratory experimentation to warrant such a test. Ease of containment must be considered in field trial design to limit any deleterious

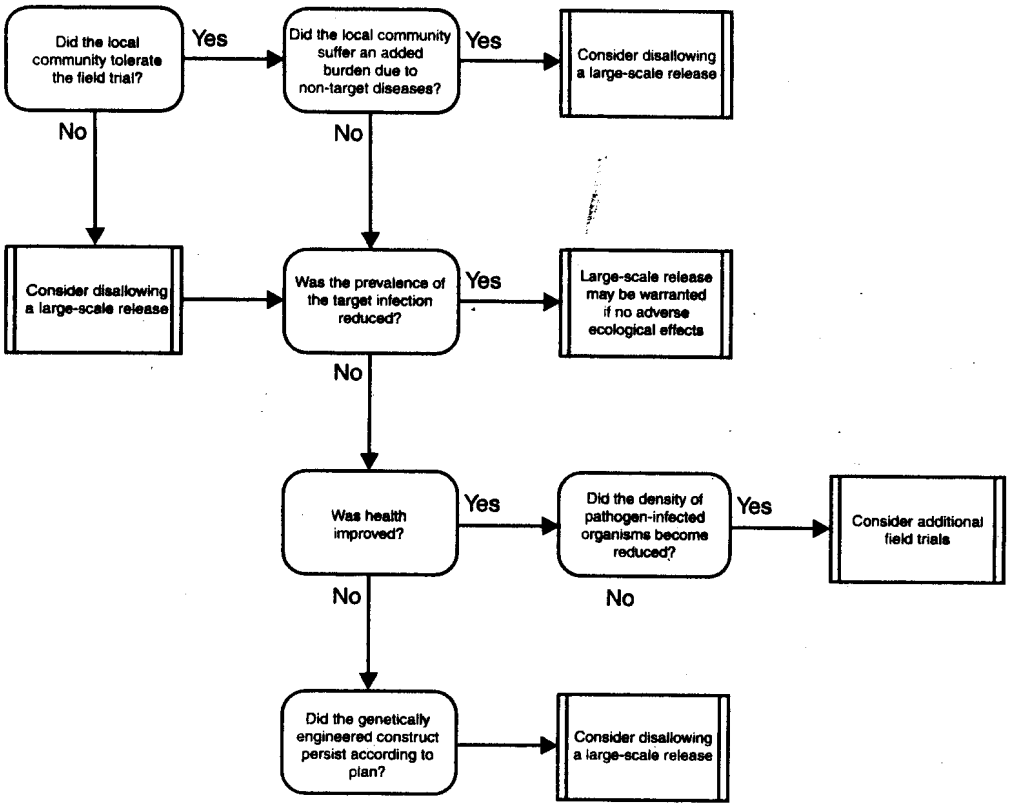

Figure 11.1 Decision tree for judging whether field trials of GEO releases are warranted based on the characteristics of the genetic construct, its drive mechanism, and the vector organism (Modified from Scientists' Working Group, *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms*, Part Two: *Flowcharts and Worksheets*, The Edmonds Institute, Edmonds, WA, 1998.)

effects, in case a release causes unanticipated complications. Isolated populations, such as occur on an island, would facilitate limiting the spread of a disadvantageous release. The likelihood of unintended consequences warrants a careful, stepped approach in designing release strategies.

To determine whether a preliminary field trial is warranted, one must first consider the pathogen-transmitting competence of a transgenic vector (see decision tree in Figure 11.1). There is little point in proceeding farther if tangible benefits cannot be realized. If drive mechanisms are required, some demonstration of their effectiveness must be provided experimentally. The stability of a drive mechanism is also critical in determining the feasibility of a release. Unstable construct–drive mechanism linkages can prove dangerous when they decay after a period of interrupted transmission. Immunological lapses in the population during this period of protection would render it susceptible to explosive outbreaks that would ensue when wild-type vectors returned. This threat proves less critical if alternative drive mechanisms are available that would allow protection to be restored through a new release. The detailed molecular characteristics of a genetic construct may, therefore, prove important in the field.

The scale required of a release also may provide an indication of its desirability. If transgenic vectors must outnumber wild types in order to drive replacement and if transgenic vectors are as pestiferous as the wild ones, it may not be ethical to impose such discomfort on the residents of a release site, and such residents may impose limits on the scale of further releases. Many vectors are capable of transmitting more

than one type of infection. Massive releases can decrease one infectious disease burden while promoting all others associated with a vector. Even if the disease whose incidence is reduced is associated with greater morbidity and mortality than all other local diseases combined, such an outcome may not necessarily be welcomed by the affected communities. If transgenic vectors provide little annoyance and if they do not transmit other infections that are unaffected by the genetic construct, then these points become moot.

To determine whether large-scale releases should follow after field trials, the response of local communities to the field trials must be closely monitored (Figure 11.2). Precedent indicates that certain communities may react violently even to relatively innocuous releases of sterile male insects. The biological success of a trial intervention is meaningless unless affected communities are able to perceive that a GEO release is desirable.

Trials should monitor the infectious disease burdens suffered by a community. Any unanticipated enhancement of burdens revealed by trials would provide further grounds for discontinuing a release program. Trials also must demonstrate that the prevalence of the target pathogen is reduced. Otherwise, a large-scale release would not confer sufficient benefits to the population to warrant the release. Reduction of prevalence, however, may not always provide a proportionate increase in level of health. In such cases, the criterion for continuation must be a decrease in overall morbidity or infection rates. Once evidence for a health benefit can be shown, its sustainability must be considered. Some indication of the stability of genetic constructs may be derived from field trials, although the time span for the work may be too limited to detect any potential for instability. As a result, investigators may be limited to theoretical considerations regarding sustainability.

If the design of a transgenic release meets each of the considerations discussed above, then a large-scale field trial may indeed be warranted, particularly if pilot field trials have already been performed and no evidence of adverse ecological effects has been identified. A limited set of field trials, however, may not provide sufficient confidence to persuade biologists, government officials, and the general public to form a consensus toward proceeding with an intervention. Although absolute confidence in outcomes may not be required, ambiguous or weak outcomes may indicate a need for additional testing. Furthermore, these decision trees consider only the biological and ethical rationality of GEO releases. Analyses of cost-effectiveness and operational feasibility also deserve serious consideration.

11.9 Conclusions

The release of genetically modified arthropods that would otherwise serve as vectors of human pathogens carries special burdens and requires regulation. In general, such a release should not be permitted unless:

1. The released organisms do not annoy local residents more than do any ambient vector organisms.
2. The release results in no increase in abundance of hematophagous arthropods.
3. The release requires no reduction in ongoing health-promoting activities.
4. The force of transmission of microbes other than the target pathogen would not increase.
5. The release does not compromise future interventions against the target disease.
6. Any improved state of health of people living in the release site is sustainable.

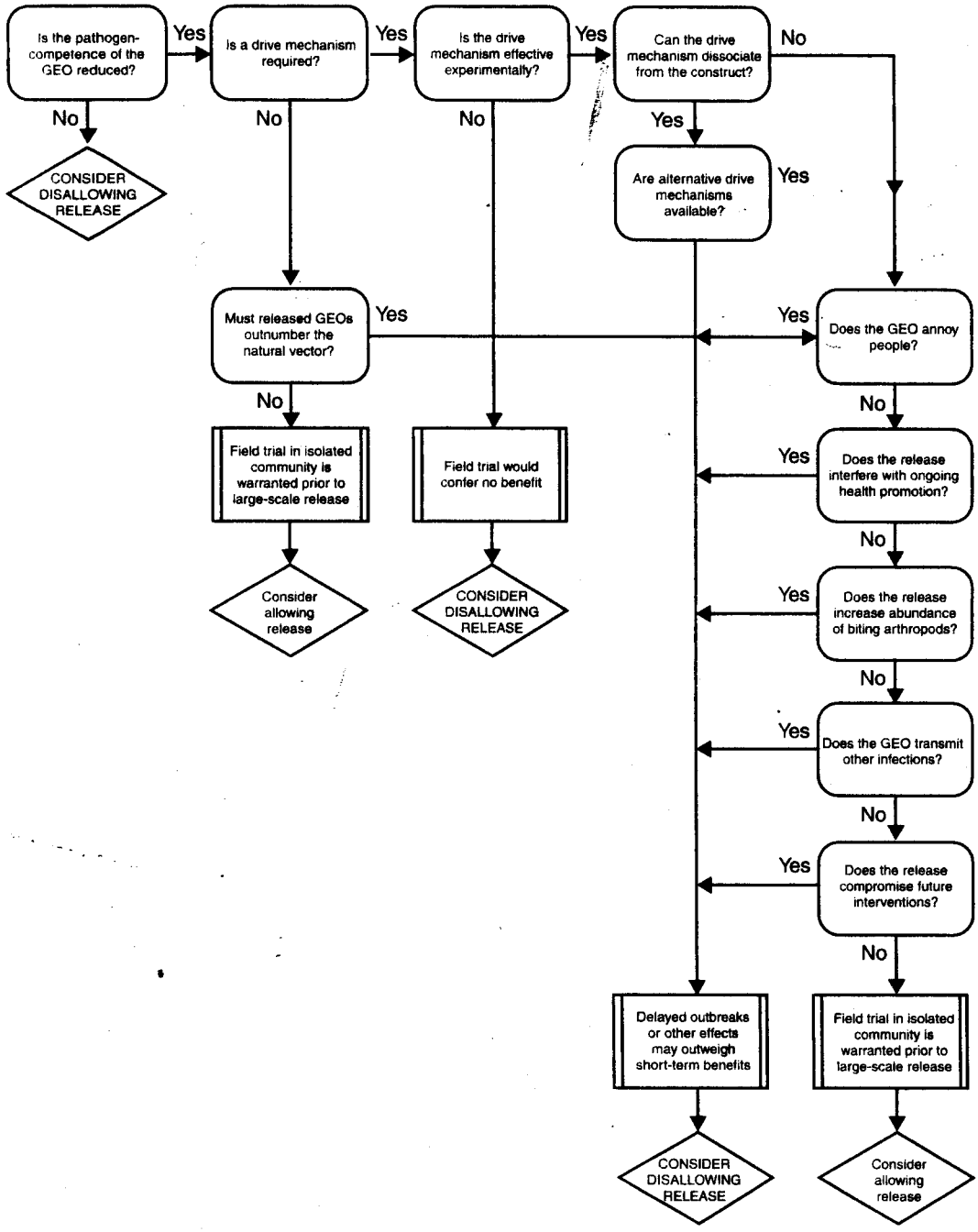

Figure 11.2 Decision tree for judging whether a large-scale GEO release should be allowed to proceed, based on the outcomes of isolated field trials. (Modified from Scientists' Working Group, *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms*, Part Two: *Flowcharts and Worksheets*, The Edmonds Institute, Edmonds, WA, 1998).

Literature cited

Aldhous, P., Malaria: focus on mosquito genes, *Science*, 261, 546, 1993.

Aultman, K. S., Walker, E. D., Gifford, F., Severson, D. W., Beard, C. B., and Scott, T. W., Managing risks of arthropod vector research, *Science*, 288, 2321, 2000.

Beard, C. H., Durvasula, R. V., and Richards, F. H., Bacterial symbiosis in arthropods and the control of disease transmission, *Emerg. Infect. Dis.*,4, 581, 1998.

Beier, J. C., Killeen, G. F., and Githure, J. J., Entomological inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa, *Am. J. Trop. Med. Hyg.*, 61, 109, 1999.

Breeland, S. G., Jeffery, G. M., Lofgren, C. S., and Weidhaas, D. E., Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador: I. Characteristics of the test site and the natural population, *Am. J. Trop. Med. Hyg.*, 23, 274, 1974.

Cattaruccia F., Nolan, T., Loukeris, T. G., Blass, C., Savakis, C., Kafotos, F. C., and Crisanti, A., Stable germline transformation of the malaria mosquito *Anopheles stephensi*, *Nature*, 405, 958, 2000.

Coates, C. J., Jasinskeine, N., Miyashiro, L., and James, A. A., Mariner transposition and transformation of the yellow fever mosquito, *Aedes aegypti.*, *Proc. Natl. Acad. Sci. U.S.A.*, 95, 3748, 1998.

Curtis, C. F. and von Borstel, R. C., Allegations against Indian research unit refuted, *Nature*, 273, 96, 1978.

Dominguez, A. and Albornoz, J., Structural instability of 297 element in *Drosophila melanogaster*, *Genetica*, 105, 239, 1999.

Favia, G., della Torre, A., Bagayoko, M., Lanfrancotti, A., Sagnon, N., Toure, Y. T., and Coluzzi, M., Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation, *Insect Mol. Biol.*, 6, 377, 1997.

Garrett-Jones, C. and Shidrawi, G. R., Malaria vectorial capacity of a population of *Anopheles gambiae*, *Bull. World Health. Org.*, 40, 531, 1969.

Hanson, S. M., Mutebi, J.-P., Craig, G. B., and Novak, R. J., Reducing the overwintering ability of *Aedes albopictus* by male release, *J. Am. Mosq. Control. Assoc.*, 9, 78, 1993.

Hartl, D. L., Lohe, A. R., and Lozovskaya, E. R., Regulation of the transposable element mariner, *Genetica*, 100, 177, 1997a.

Hartl, D. L., Lozovskaya, E. R., Nurminsky, D. I., and Lohe, A. R., What restricts the activity of mariner-like transposable elements? *Trends Gen.*, 13, 197, 1997b.

James, A. A., Blackmer, K., Marinotti, O., Ghosn, C. R., and Racciopi, J. V., Isolation and characterization of the gene expressing the major salivary gland protein of the female mosquito *Aedes aegypti*, *Mol. Biochem. Parisitol.*, 44, 245, 1991.

Kiszewski, A. E. and Spielman, A., Spatially explicit model of transposon-based genetic drive mechanisms for displacing fluctuating populations of anopheline vector mosquitoes, *J. Med. Entomol.*, 35, 584, 1998.

Knipling, E. F., Possibilities of insect control or eradication through the use of sexually sterile males, *J. Econ. Entomol.*, 48, 459, 1955.

Kokoza, V., Ahmed, A., Cho, W.-L., Jasinskeine, N., James, A. A., and Raikel, A., Engineering blood meal-activated systemic immunity in the yellow fever mosquito, *Aedes aegypti*, *Proc. Natl. Acad. Sci. U.S.A.*, 97, 9144, 2000.

Laven, H., Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility, *Nature*, 216, 383, 1967.

Lofgren, C. S., Dame, D. A., Breeland, S. G., Weidhaas, D. E., Jeffery, G., Kaiser, R., Ford, H. R., Boston, M. D., and Baldwin, K. F., Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador: III. Field methods and population control, *Am. J. Trop. Med. Hyg.*, 24, 288, 1974.

Mbogo, J., Snow, R. W., Kabiru, E. W., Ouma, J. H., Githure, J., Marsh, K., and Beier, J. C., Low level *Plasmodium falciparum* transmission and the incidence of severe malaria infections on the Kenyan coast, *Am. J. Trop. Med. Hyg.*, 49, 245, 1993.

Mueller, H. M., Crampton, J. M., della Torre, A., Sinden, R., and Chrisanti, A., Members of a trypsin gene family in *Anopheles gambiae* are induced in the gut by blood meal, *EMBO J.*, 12, 2891, 1993.

Olson, K. E., Higgs, S., Gaines, P. J., Powers, A. M., Davis, B. S., Ramrud, K. I., Carlson, J. O., Blair, C. D., and Beaty, B. J., Genetically engineered resistance to Dengue-2 virus transmission in mosquitoes, *Science*, 272, 884, 1996.

Pal, R. and LaChance, L. E., The operational feasibility of genetic methods for the control of insects of medical and veterinary importance, *Annu. Rev. Entomol.*, 19, 269, 1974.

Petrov, D. A., Lozovskaya, E. R., and Hartl, D. L., High intrinsic rate of DNA loss in *Drosophila*, *Nature*, 384, 346, 1996.

Powell, J. R., Petrarca, V., della Torre, A., Caccone, A., and Coluzzi, M., Population structure, speciation, and introgression in the *Anopheles gambiae* complex, *Parassitologia* (Rome), 41, 101, 1999.

Ribeiro, J. M. and Kidwell, M. G., Transposable elements as population drive mechanisms: specification of critical parameter values, *J. Med. Entomol.*, 31, 10, 1994.

Service, M.W., *Mosquito Ecology*, Elsevier Applied Science, London, 1993, 652.

Smit, A. F. A., Interspersed repeats and other mementos of transposable elements in mammalian genomes, *Curr. Opin. Genet.*, 9, 657, 1999.

Smith, R. H. and von Borstel, R. C., Genetic control of insect populations, *Science*, 178, 1164, 1972.

Spielman, A., Why entomological antimalaria research should not focus on transgenic mosquitoes, *Parasitol. Today*, 10, 374, 1994.

Spradling, A. C. and Rubin, G. M., Transposition of clonal P elements into *Drosophila* germ line chromosomes, *Science*, 218, 341, 1986.

Vernik, K. D., Collins, F. H., and Gwadz, R. W., A general system of resistance to *Plasmodium* infection in the vector *Anopheles gambiae* is controlled by two genetic loci, *Am. J. Trop. Med. Hyg.*, 40, 585, 1989.

Vieira, C., Lepetit, D., Dumont, S., and Biemont, C., Wake up of transposable elements following *Drosophila simulans* worldwide colonization, *Mol. Biol. Evol.*, 16, 1251, 1999.

Yihdego, Y., Pollack, R. J., and Spielman, A., Enhanced development in nature of larval *Anopheles arabiensis* mosquitoes feeding on maize pollen, *Am. J. Trop. Med. Hyg.*, in press.