15

Release ratios employed for genetically modifying populations of mosquitoes

Andrew Spielman[#]

Abstract

To begin to define the mass of transgenic vector-incompetent mosquitoes that might be required for modifying a natural vector population, the release ratios that have been employed in genetic-control experiments are reviewed. Proposed releases incorporating genetic-drive mechanisms may require somewhat smaller masses of released mosquitoes. Because pathogen-incompetent mosquitoes tend to be less fit than are those in natural target populations, at least as many construct-bearing mosquitoes must be released as are present in the target site at the beginning of the intervention. A series of well documented attempts to reduce the fertility of natural populations of mosquitoes were reported during 1967 through 1982. Those that succeeded generally released more than ten modified mosquitoes for each mosquito present at the time of the release. In the event that the entire vector population of the region is not immediately rendered incapable of supporting the development of the pathogen, some specified prevalence of construct-bearing must indefinitely be sustained there. Existing anti-malaria measures may be incompatible with such an intervention. A successful genetic intervention may require the sustained release of more human-biting mosquitoes than would otherwise be present in the target site.

Keywords: *Diptera*; *Culicidae*; genetically modified mosquito; fitness; release ratio; transgene

Introduction

A rising level of excitement has followed the recent simultaneous descriptions in Nature and in Science (Gardner et al. 2002; Holt et al. 2002) of the genomes of the most important malaria parasite, *Plasmodium falciparum*, and of its principle African vector, *Anopheles gambiae*. These revelations stimulated intense speculation over the possibility that anopheline populations may one day be rendered incompetent as hosts for this pathogen. Toward that end, mosquitoes carrying an engineered gene might be released in nature such that the transgene will sweep through the natural vector population. Such a moiety is not likely, by itself, to increase in frequency. One solution, then, is to conduct a straightforward inundative release, involving vastly more release insects than would then be present in the site. Such a release might be preceded by extensive applications of conventional insecticide, followed by the withdrawal of these materials. Alternatively, the transgene might be linked to a drive

[#] School of Public Health and Center for International Development, Harvard University, Boston MA 02115, USA. E-mail: aspielma@hsph.harvard.edu

mechanism, such as a transposable element or a cytoplasmic incompatibility factor in the form of a durable construct.

Several obstacles confront the concept of a driven release. One lies in the pattern of Mendelian inheritance that characterizes those transposable element systems in mosquitoes that recently have been investigated (Catteruccia et al. 2000). Because the transposons used for those transformations appear not to be mobile, offspring of a pair that carry such an element would be as likely as not to carry that sequence themselves. The sequence, therefore, would not drive. Another lies in the requirement that the transgene must never disassociate from its drive mechanism. The likelihood that this requirement will be met, however, seems remote (Spielman 1994, see also Curtis elsewhere in this volume). Yet another consideration focuses on the idea that a small inoculum involving only a few transposon-carrying individuals probably would not take hold in a natural population of mosquitoes. When released into caged populations of *Drosophila melanogaster*, P-elements frequently fail to become established at a 1% release ratio (Carareto et al. 1997). This transposon increases in frequency solely when the release ratio exceeds 10%. Even under ideal cage conditions and using naturally receptive insects, this classical transposable element requires a non-trivial initial inoculum. Another drive mechanism in a less well-adapted mosquito genome would require an even larger inoculum before it can become established. Even in the case of a transposon-driven release, therefore, consideration must be devoted to the number of mosquitoes that are to be released.

To modify a population of vector mosquitoes usefully, a defined mass of construct-carrying insects must initially be released into its midst, and the desired genetic trait must thereafter be maintained at some specified frequency. It may be that the local density of such a target population can transiently be reduced by conventional insecticidal means and replaced by a mass of released mosquitoes. Insecticide resistance might be used as a conditional lethal that would promote such a replacement effort. The work of the first quarter century of "genetic-control" research provides insight into the release ratios that might be required for the anticipated efforts (see Reisen and Lounibos elsewhere in this volume).

Fitness

Vector mosquitoes must pay some price for the microbes that develop and multiply within their bodies. Such an extraneous biomass requires nourishment, and those host tissues that are destroyed must be repaired. But, any antimicrobial response that is mounted may very well cost the host mosquito more than would be saved if the pathogen were to mature. An antibacterial immune response as well as an encapsulation response is associated with decreased fecundity. The pathology produced by plasmodial or arboviral pathogens is mainly evident in the insect's salivary glands (Rossignol, Ribeiro and Spielman 1986), a burden that is imposed solely on the miniscule portion of the vector population that lives long enough to become infective. Salivary production is diminished. Such an infectious mosquito subsequently becomes a "super-spreader" of pathogens because it tends to probe numerous hosts without imbibing blood and is functionally sterile. The vector population, however, reaps a "group selection" benefit from arboviral or plasmodial pathogens because they induce a prominent thrombocytopenia in vertebrate hosts, a condition that facilitates blood-feeding by vector insects. Even the human reservoir is spared in the ancestral African malaria cycle. This exquisite set of reciprocal adaptations preserves the fitness of vector populations in the face of their parasitic

relationships. Pathogen-incompetence does not necessarily convey a fitness advantage to a vector population (Catteruccia, Godfray and Crisanti 2003).

Vector-competent mosquitoes appear to be more fit than are those that fail to support the development of pathogens (Boëte and Koella 2002). Indeed, field-derived *An. gambiae* only rarely melanize malaria parasites, a finding that presumably reflects relative fitness. Paradoxically, the "quantum of infection" component of vector competence in nature appears to correlate inversely with vectorial capacity; fewer oocysts develop in those mosquitoes that contribute more powerfully to the force of transmission than in those that contribute less. No more than a few oocysts mature on the midgut of each African *An. gambiae s.s.*, while a hundred or more develop in a South-Asian *An. stephensi* mosquito, and these anthroponotic parasites arose in Africa. The mechanism of this apparent adaptation remains entirely unexplored. What price does competence exact from a mosquito population, and wherein lies any profit?

If construct-bearing mosquitoes are no more fit than is the natural target population, the release ratio must somewhat exceed 1:1. Pal and LaChance formalized this concept in 1974, relating release ratio to R_0 and fitness in a model that includes a density-dependent factor (Pal and LaChance 1974). The resulting relationship served as a critical basis for a major sterile-male release effort against Indian *Culex pipiens* that was partially implemented. The program simulates random pairings by wild females where fertile wild males and sterile released males coexist, and evaluates incremental values of R0. Threshold values for release ratios determine the boundary between perpetuation and elimination of the natural population.

Experience relating to release ratios

The first attempt to reduce the density of vector mosquitoes by genetic means was conducted in Okpo, a village in Burma where the *Culex pipiens quinquefasciatus* vector of lymphatic filariasis constituted the target population (Laven 1967). This village is surrounded by paddy fields in which these mosquitoes cannot breed, and every breeding site was identified. The goal was to eliminate *C. pipiens* from this isolated site by releasing male pupae derived from a population that was "cytoplasmically incompatible" with those in Okpo. Sperm of the released males could not fertilize the *Wohlbachia*-exposed eggs of Okpo females. During the breeding season, some 4,000-10,000 native male pupae appeared to be present in those breeding sites that were present in the village, and 5,000 incompatible male pupae were placed in these bodies of water each day. An appropriate number of pupae were placed in each breeding site. Egg viability declined steadily during the 12 weeks of the breeding season. Although no comparison treatments or follow-up observations were described, it seems evident that a 1:1 release ratio resulted in the intended effect in this limited site.

Another release against *C. pipiens* mosquitoes in an even more limited site was conducted in a cesspit in a farmyard located near Montpellier in France. The objective of this experiment was to reduce vector density by means of translocation heterozygotes (Laven 1972). Some 300-20,000 adult mosquitoes appeared to be originating in the site each day. The release ratio reached 5:1 per day, and the number of egg rafts deposited in the cesspit declined from 20,000 to 100 over 6 weeks.

A much larger genetic-control translocation-heterozygote experiment was conducted against *Culex pipiens* in a village complex located near Delhi in India (Brooks et al. 1976). The 228 homes in the village contained as many as 743 breeding sites, from which 525-2,000 adults emerged each day. Some 5,000-40,000 mosquitoes

were released each day. The release ratio was about 17-225:1, with a mean of 60:1 during the seasonal peak, resulting in a two-third reduction of fertility in wild females. The experiment was discontinued before it could be brought to fruition.

Another translocation-heterozygote experiment was directed against *Aedes aegypti* during the late 1970s in an isolated Kenyan village (McDonald, Häusermann and Lorimer 1977). Mosquito abundance in the village was estimated at about 1,000 during the dry season, and some 814 male mosquitoes were released each day for nine weeks. The released mosquitoes appeared to be about 13% as fit as were those in the natural population. This estimated release ratio of 10:1 resulted in a 37% reduction in fertility.

A population of chromosomally aberrant *Culex tritaeniorhynchus* was released in a village near Lahore, Pakistan during the late 1970s (Baker et al. 1979). The village contained 143 homes and several ponds that produced numerous mosquitoes, including about 147,000 males. A total of 167,000 males were released at a rate of about 12,000 per day. The actual release ratio was estimated at 1:4. Fitness, unfortunately, proved to be nil. All anticipated matings failed. Although mosquitoes may appear to be fully fit in population cages, genetically altered mosquitoes may not be competitive when released in nature.

Radiation-sterilized *Culex pipiens* were released on an uninhabited Florida island in the United States in a classical attempt to adapt Knipling's (Knipling 1955) original methodology against mosquitoes (Patterson et al. 1970). An estimated 40,000 adults were present there, and some 8,000-15,000 sterilized adults were released each week for a release ratio of about 9:1. This overwhelming inundative release resulted in an apparent 40-fold reduction in abundance, to about 1,000 mosquitoes.

A well-documented release of chemosterilized mosquitoes was conducted against *Anopheles albimanus* in a virtually isolated 150 km2 mainland site near Lake Apastepeque in El Salvador (Dame, Lowe and Williamson 1981). Some 1,500 mosquitoes were present there when these insects were most abundant, and extensive larvicidal applications preceded each release. Their Ro was estimated at between 7 and 21. In an initial series of observations, in 1972, as many as 30,000 sterile males were released each day. Sterility became evident in more than half of the target population when the release ratio exceeded 20:1. Another such experiment, performed in 1978, attained sterility in only about a quarter of the population after the mass of the release was increased to 1 million sterile males, including 5,000 females, per day.

Sustainability

It seems likely that the sustainability of any transgenic release will require that the released mosquitoes must permanently be nurtured in each release site (Spielman 1994). In the event that too few of these mosquitoes are present in the village environment, their abundance must be enhanced. Severe ethical problems would, thereby, arise because such transgenic mosquitoes presumably would require human blood to reproduce. After a vector population has been rendered incompetent, the released mosquitoes must be nurtured such that a particular proportion of construct-bearing insects will be present in the site, and that proportion may exceed unity. In the event that other anti-malaria measures must be discontinued, such as source reduction and the use of insecticide-treated bed-nets, insuperable ethical problems would arise.

In the event that a transgenic release immediately renders the entire mosquito population of a malaria-endemic region incapable of supporting the development of these pathogens, this health gain must be sustained. An intervention against endemic

malaria that results in a temporary reduction in transmission would be disastrous because it would be followed by a loss of herd immunity and an unrealistically elevated expectation of health. The malaria outbreak that follows would be particularly burdensome. Sustainability, then, requires a system for monitoring the relative proportion of malaria-competent vs. incompetent mosquitoes and maintaining the density of these non-vector mosquitoes at some beneficial level. Although steps must be taken to preserve their abundance, little consideration has yet been devoted to such a sustained release ratio.

Considerations affecting release ratio

Various considerations seem relevant to efforts designed to estimate the magnitude of the release ratio. Questions relating to these issues include:

1. Structure of the target population. How panmictic is the target vector population within each village, between villages and across the region?
2. Critical degree of competence. How prevalent is pathogen-competence in the indigenous vector population? What level of competence is required to attain the desired health result?
3. Fitness of the release population. Will the fitness of the released mosquitoes be sufficient for them to exchange genetic material with those mosquitoes already present in the site? Will the fitness of the resulting modified mosquitoes be sufficient for them to persist?
4. Release ratio. Must the density of the released population exceed that of existing wild-type mosquitoes? Must the human residents of the release site be exposed long-term to increased biting density?
5. Need for nurturing the modified population. Will it be necessary to create artificial breeding sites for the released mosquitoes and for their modified progeny? Must bed-net and insecticide use in the release site be discouraged?
6. Schedule of dissemination. How rapidly and how extensively will a transgenic release modify the regional population?
7. Ethics of a release. Will the ethical requirements for informed consent be satisfied? Will residents of the release site be able to withdraw from the trial?
8. Multiplicity of sympatric vector populations. If more than one vector population is present in the release site, what will the health impact be if only the target population is rendered incompetent?
9. Multiplicity of pathogens in the local vector population. What is the health relevance of any other pathogens that might be transmitted by the target population?
10. Sustainability of health gains. How likely is it that the transgene will become disassociated from the drive mechanism or that the transgene will become inactivated by mutation? In the event that the transgenic release must be repeated, are alternative drive mechanisms available?

Together, answers to these ten sets of questions should shape the strategies that govern any projected release of transgenic mosquitoes. Biological considerations will be balanced against ethical issues. One would seek to maximize the magnitude of the release and the other would minimize this parameter.

Interpretation

The magnitude of such an initial inundative release of transgenic mosquitoes directed against an anopheline vector of malaria might be estimated from published material. To determine how many *An. gambiae s.l.* were present in a Malian village, marked mosquitoes were released and subsequently recaptured while resting indoors (Touré et al. 1998). A total of 938 marked mosquitoes were released in the first year and 1,900 in the second. The recapture rate was estimated at 4-11%, which indicated that 5,000-17,000 of these mosquitoes were present in the village during the first year of observation and 15,000-43,000 during the second. If an effective release of one million mosquitoes per day were required in Mali, as suggested in the Salvadorian "Lake Apastepeque experiment," an operational effort might require the release of as many as 10 million reared mosquitoes per African village per day, a non-trivial number of hematophagous mosquitoes.

Genetic mechanisms that may drive transgenes through vector populations are burdened because they are likely not to drive, because they may dissociate from the accompanying transgene, because they spread excessively slowly and because they reduce the fitness of the target insects. A genetic intervention may require the sustained release of many more mosquitoes each day than would otherwise be present in the village environment.

References

Baker, R.H., Reisen, W.K., Sakai, R.H., et al., 1979. Field assessment of mating competitiveness of male *Culex tritaeniorhynchus* carrying a complex chromosomal aberration. *Annals of the Entomological Society of America,* 72, 751-758.

Boëte, C. and Koella, J.C., 2002. Evolutionary thoughts about the use of genetically manipulated mosquitos for malaria control. *Trends in Parasitology,* 19, 32-38.

Brooks, G.D., Curtis, C.F., Grover, K.K., et al., 1976. *A field trial on control of Culex pipens fatigans Wied. by release of males of a strain integrating cytoplasmic incompatibility and a translocation*, WHO/VBC/76.635.

Carareto, C.M.A., Kim, W., Wojciechowski, M.F., et al., 1997. Testing transposable elements as genetic drive mechanisms using *Drosophila* P element constructs as a model system. *Genetica,* 101 (1), 13-33.

Catteruccia, F., Godfray, H.C.J. and Crisanti, A., 2003. Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. *Science,* 299 (5610), 1225-1227.

Catteruccia, F., Nolan, T., Loukeris, T.G., et al., 2000. Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature,* 405 (6789), 959-962.

Dame, D.A., Lowe, R.E. and Williamson, D.L., 1981. Assessment of released sterile *Anopheles albimanus* and *Glossina morsitans morsitans*. *In:* Pal, R., Kitzmiller, J. B. and Kanda, T. eds. *Cytogenetics and genetics of vectors: proceedings of a symposium of the 16th international congress of entomology, Kyoto, Japan, 1980*. Elsevier Biomedical Press, Amsterdam, 231-248.

Gardner, M.J., Shallom, S.J., Carlton, J.M., et al., 2002. Sequence of *Plasmodium falciparum* chromosomes 2, 10, 11 and 14. *Nature,* 419 (6906), 531-534.

Holt, R.A., Subramanian, G.M., Halpern, A., et al., 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science,* 298 (5591), 129-130,141-149.

Knipling, E.F., 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology,* 48, 459-467.

Laven, H., 1967. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature,* 216 (113), 383-384.

Laven, H., 1972. Eradicating mosquitos using translocations: a first field experiment. *Nature,* 236, 456-457.

McDonald, P.T., Häusermann, W. and Lorimer, N., 1977. Sterility introduced by release of genetically altered males to a domestic population of *Aedes aegypti* at the Kenya coast. *American Journal of Tropical Medicine and Hygiene,* 26 (3), 553-561.

Pal, R. and LaChance, L.E., 1974. The operational feasability of genetic methods for control of insects of medical and veterinary importance. *Annual Review of Entomology,* 19, 269-291.

Patterson, R.S., Weidhaas, D.E., Ford, H.R., et al., 1970. Suppression and elimination of an island population of *Culex pipiens* quinquefasciatus with sterile males. *Science,* 163, 1368-1370.

Rossignol, P.A., Ribeiro, J.M.C. and Spielman, A., 1986. Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene,* 35, 277-279.

Spielman, A., 1994. Why entomological antimalaria research should not focus on transgenic mosquitoes. *Parasitology Today,* 10, 374-376.

Touré, Y.T., Dolo, G., Petrarca, V., et al., 1998. Mark-release-recapture experiments with *Anopheles gambiae* s.l. in Banambani Village, Mali, to determine population size and structure. *Medical and Veterinary Entomology,* 12 (1), 74-83.