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POPULATION GENETICS OF AUTOCIDAL CONTROL AND STRAIN REPLACEMENT

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■ **Abstract** The concept that an insect species' genome could be altered in a manner that would result in the control of that species (i.e., autocidal control) or in the replacement of a pestiferous strain of the species with a more benign genotype was first proposed in the mid-twentieth century. A major research effort in population genetics and ecology followed and led to the development of a set of classical genetic control approaches that included use of sterile males, conditional lethal genes, translocations, compound chromosomes, and microbe-mediated infertility. Although there have been a number of major successes in application of classical genetic control, research in this area has declined in the past 20 years for technical and societal reasons. Recent advances in molecular biology and transgenesis research have renewed interest in genetically based control methods because these advances may remove some major technical problems that have constrained effective genetic manipulation of pest species. Population genetic analyses suggest that transgenic manipulations may enable development of strains that would be 10 to over 100 times more efficient than strains developed by classical methods. Some of the proposed molecular approaches to genetic control involve modifications of classical approaches such as conditional lethality, whereas others are novel. Experience from the classical era of genetic control research indicates that the population structure and population dynamics of the target population will determine which, if any, genetic control approaches would be appropriate for addressing a specific problem. As such, there continues to be a need for ongoing communication between scientists who are developing strains and those who study the native pest populations.

INTRODUCTION

The concept that an insect's genetic system could be redirected for the destruction of insect populations or for transforming an insect pest into a less harmful life form appears to have been independently proposed at least twice in the 1940s (81, 89), once in the 1950s (44), and once in the 1960s (14, 96). Serebrovskii (81) provided a theoretical description of how the release of insect strains with chromosomal translocations could lower the overall fitness of pest populations. Serebrovskii intended to carry out experiments with house flies and grain weevils to prove his thesis, but his work on this topic was discontinued owing to World War II (C. Curtis, personal communication) and Lysenko's rise to power in the Soviet Union's scientific bureaucracy (57a, 96). His work was rediscovered in the late 1960s (96). Vanderplank (89) predicted that release of an insect species with postmating but not premating isolation from a local pest species would result in sterile F_1 hybrids. This would lower the overall fitness of the pest population and could even lead to species replacement. By the late 1940s, Vanderplank had demonstrated this method through a field test with tsetse flies (42a, 90).

Large-scale empirical efforts in the area of genetic control began in earnest when Knipling and his colleagues convinced the USDA to undertake the eradication of the screwworm fly in the 1950s through massive releases of insects with chromosomal abnormalities induced by radiation (44). The ultimate area-wide success of this program (47) was instrumental in ushering in a golden era of research on autocidal control and strain replacement (16). In addition to a great deal of pragmatic work on such issues as mass rearing, sterilization techniques, and release methods, theoretical population genetic studies were conducted to determine how best to design genetic control programs for a target insect in a specific environment. Between 1974 and 1981, four review articles that focused on population genetics aspects of these methods were published in the *Annual Review of Entomology* alone (3, 58, 60, 97). A number of authoritative books on the subject were also published at that time (23, 38, 61).

Research projects were initiated to develop genetic methods for control or eradication of at least 31 species in designated regions (48). High on the priority list were agricultural pests (e.g., boll weevils, bollworms, fruit flies) and insects of medical importance (e.g., tsetse fly and *Culex*, *Anopheles*, and *Aedes* mosquitoes). In 1968, Curtis (14) published an influential population genetics paper demonstrating that Serebrovskii's method of introducing strains with translocations for pest control could also be used as a means of replacing a pathogen-transmitting mosquito population with a strain that was not a disease vector. A number of projects were designed to test this general idea under laboratory and field conditions (96).

Although research on and application of genetic control methods are ongoing (46), the golden era seems to have passed. It is noteworthy that from 1981 to 2003 there has not been a single review in the *Annual Review of Entomology* focused specifically on this topic. This decreased emphasis was likely due to the lack of further successes as dramatic as that of the screwworm eradication program, to the

large governmental infrastructure needed to carry out genetic control projects, and to successes of alternative control tools (e.g., chemical pesticides) and strategies (e.g., IPM) (16, 46, 98).

The question today is whether the scientific revolution in molecular biology and insect transgenic technologies will usher in a new golden era of genetic control methods and strategies, and if so, will it be long-lived (1, 30, 33). Considerable financial resources have been invested in insect molecular genetics and transgenic techniques, and one major justification has been the potential to use the accrued knowledge in developing genetic control methods (41). Unfortunately, the investment in molecular techniques has not been paralleled by an equal investment in population dynamics and population genetics research that is needed to assess, and potentially improve, the utility of the molecular techniques (78).

In this article, we first review the classical research on genetic manipulation of pests, which was mostly conducted from the 1940s through the 1980s, in order to provide the reader with an overview of the relevant general concepts and techniques. We then describe and assess recent research that suggests that transgenic methods could improve the efficiency of genetic control. Some of these potential improvements involve modification of existing classical approaches, whereas others introduce truly new approaches. We have placed a set of figures as supplementary material to aid the reader with concepts discussed in the review and to provide teaching resources (follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org).

CLASSICAL APPROACHES FOR GENETIC CONTROL

Mutagen-Induced Sterility

The production and release of millions of insects, sterilized by exposure to mutagenic chemicals or radiation (31, 83), is generally considered the simplest and most crude form of genetic manipulation. By adjusting the dose of these mutagenic agents, and the application procedures, researchers aim to achieve a level of chromosome breakage in male gametes that will disrupt embryo development or result in offspring sterility. A dose leading to the above result without loss of male mating ability and sperm viability has been the Holy Grail for work in this area. For any project with the goal of population eradication, 100% F_1 mortality or sterility was considered a requirement [but see (98)]. Sterile release theory focuses on the importance of the number of males released, whereas the number of sterile females released is considered a neutral factor on the basis of an assumption that there is an excess of male mating potential in a population [but see (52)]. However, if females are released along with males, it is important that they be sterile or they will add extra offspring to the population. Achieving female sterility is a major problem in species where the male is more susceptible to the effects of the mutagen than the female [e.g., boll weevils (57)].

The simple algebraic models of sterile insect release dynamics developed by Knipling (44, 45) were used to guide development of the screwworm eradication program. On the basis of his "familiarity" with agricultural pests, Knipling assumed that a technique that could chronically reduce reproduction by even 50% was likely to cause a general decline in a pest population. His first discrete generation model (44) assumed a default net replacement rate of 1.0 (i.e., stable population size) and no density-dependent population regulation. He predicted that, under these conditions, if one started with a native population of 1 million female insects and released 2 million sterile males per generation, total eradication would be achieved in five generations. An interesting property of this simple model was that if the same number of insects were released in each generation, the percent decline in the pest population per generation would increase as time went on. This follows from the fact that as the population density of native insects declines in each successive generation, the fixed number of sterile insects from the rearing facility would make up a greater fraction of the total pest population.

Two basic but unrealistic assumptions of this model may have helped to sell the sterile insect approach to USDA decision makers. First is the assumption of density-independent population regulation. If there is nonlinear density-dependent regulation, compensation in the population growth rate is expected when population density is lowered and could result in population numbers rebounding after a release. Under these conditions the "increasing effect over time" property of Knipling's model may not occur. Second, if the target species is composed of many subpopulations, some of which are not accessible to released insects, eradication might be unattainable in any subpopulation. For example, if a subpopulation beyond the release area was exporting 1000 individuals per generation to the release area, the impact of these 1000 individuals would not be evident in early stages of a release aimed at an initial 1 million insects in the release area. However, the closer a project came to the goal of eradication, the more obvious the effect of 1000 immigrants would become. Prout (66) examined a general situation where there was both immigration and density dependence and concluded that density dependence could sometimes lessen the impact of migration, but that eradication would still be impossible. A number of other models examined more specific pest systems (25, 32). If immigrating females were already fertilized, their impact was predicted to be stronger.

Because the impact of a sterile release is typically expected to be inversely density dependent (increased impact as population declines), it is generally agreed that there should be benefits from combining sterile releases with other tactics that have density-independent effects or have greater impacts at high densities. For example, conventionally applied insecticides are generally expected to kill the same percentage of an insect population when its density is high or low. Many specialist biological control agents are expected to be positively density dependent: As pest density declines and fewer pests are available to be captured/parasitized, the biological control agent population declines and becomes less effective. It is

exactly at this time of low pest density when the release of sterile males would be most effective assuming no substantial rebound effect. A set of models was developed to examine the simultaneous and sequential use of the above tactics in concert with a sterile release program (4, 5, 34).

Hybrid Sterility

Releasing individuals of a species that can mate with the target species but produce unfit hybrid offspring can be considered a subset of the sterile release approach to population suppression, but this approach has some special properties if males and females are released. Depending on the species pair involved, hybrid offspring may die as embryos, die at a more advanced stage, or be sterile. Releases into a native population of species A could involve males of species B or could involve laboratory-bred F_1 hybrid males from a mating of species A and species B. Here the dynamics are the same as sterile release, but any premating isolation factors between the species would limit the utility of the method.

If males and females of species B are released, the population dynamics can be qualitatively different from release of only males because there is potential for species B to persist in the habitat. In a dual sex release, under the simplest conditions, the species with the highest density is expected to sexually outcompete the less abundant species. Assume that there are 100 males and 100 females of species A and 300 of each sex in species B. If mating is random, a female of species A will have a 75% chance of inappropriately mating with a male of species B. A female of species B would only have a 25% chance of mating with a male of the wrong species. This results in an asymmetric fitness reduction for the two species, which leads to a further increase in the density disparity between the species. The greater the difference between densities becomes, the greater the negative fitness impact on the less abundant species. Finally, species A will be replaced by species B. This is exactly what happened in the experiments of Vanderplank with tsetse flies (42a). In that case, species B (*Glossina morsitans centralis*) replaced species A (*G. swynnertoni*), but it could not survive in the habitat of species A during the subsequent dry seasons. This resulted in local near extinction of both species.

Conditional Lethals

A conditional lethal gene is one that causes mortality only when an organism is exposed to specific environmental conditions. Release of insects with dominant conditional lethal genes is not expected to have an immediate effect on the target population. Instead, released insects carrying the conditional lethal alleles in homozygous form would mate with native insects that would continue to pass on the conditional lethal alleles to their offspring. The spread of the conditional lethal would go on until environmental conditions triggered its expression (or inappropriate lack of expression in the case of a trait such as inability to diapause).

Translocations

Chromosomal translocation is defined as the mutual exchange of chromosome sections between terminal segments of nonhomologous chromosomes (29, 70); it can occur naturally or be induced by mutagens (96). The potential use of insects with translocations, initially proposed by Serebrovskii (81), and enlarged upon by Curtis (14), received a great deal of attention in the 1970s and 1980s as a means of suppressing population growth and/or replacing harmful native insect strains with more benign strains. In contrast to sterile insect releases, translocation releases are theoretically expected to have a weaker first generation effect on the population fitness but the effect of any single translocation release will continue for multiple generations (70). Over time the total number of genetically based deaths from the two approaches could be equal, but in a population with density-dependent regulation the small losses per generation from translocations may have little impact on population size.

The mechanism by which translocations affect native populations derives from the fact that when a translocation heterozygote undergoes meiosis, about one half of its gametes have a deficiency of one chromosomal segment and a duplication of another. These gametes are functional, but when they fuse with native insect gametes, they result in offspring that are inviable. When translocation heterozygotes mate with each other, the offspring viability is somewhat higher than the product of the frequency of genetically balanced gametes from each individual because unbalanced gametes compensate for each other in some embryos. In a randomly mating population with a 0.5 frequency of a single translocation, the theoretical expectation for inviable offspring becomes 43.75% instead of 50% because of this complementation. Robinson (70) summarized 12 empirical studies of translocation heterozygotes and found variation from 41% to 70% fertility. The reasons for the variation are mostly not understood, but choice of a translocation with the lowest heterozygous viability would be best in control programs as long as the homozygous translocation strain itself is reasonably viable. Releases can be of individuals that are homozygous or heterozygous for the translocation. The maximal debilitating effect of the release should theoretically occur when the frequency of the translocation after the release is 0.5. At frequencies of 0.5 or less, selection is expected to eliminate the translocations because of the low heterozygote fitness, but this could take over 15 generations (14). Theoretically, translocation releases can, at most, reduce population fitness by 50% in a single generation.

Theory indicates that a strain with multiple translocations would have lower heterozygous viability than a single translocation strain (70, 81) and should therefore be more effective at suppressing a target population. A number of empirical studies with agricultural pests and mosquitoes demonstrated that double-translocation heterozygotes could be produced and typically did have substantially lower offspring viability than single-translocation heterozygotes (70).

Serebrovskii (81) and Curtis (14) pointed out that if a large release of a single translocation strain raised the frequency of translocation chromosomes in a

population above 0.5, instead of the selection eliminating the translocation, it would eliminate individuals with the normal chromosome arrangement if fitness of the translocation homozygote was high. Curtis (14) proposed that if a translocation strain was developed that had a genetic trait tightly linked to the translocation that was beneficial to humans, then that beneficial trait could be fixed in a population as the native chromosomal arrangement was replaced by the translocation. It might even be feasible to use translocations to drive genes for conditional lethality into populations before the onset of the triggering environmental conditions such as winter or high temperatures (54). Theory predicted that a double-translocation strain would again be more effective than a single-translocation strain because natural selection could result in fixation of the translocations, even if the initial frequency was around 0.3, if the translocation homozygotes had high fitness. Unfortunately, most laboratory-generated translocations caused substantially reduced fitness when in homozygous form (70).

Computer simulation models were developed to examine the dynamics of releasing single- and multiple-translocation-bearing strains into specific native populations (21, 22, 26, 55, 79, 91, 93, 94, 99). Curtis & Robinson (22) produced a model that compared the effectiveness of release of a double-translocation strain with the effectiveness of sterile male releases (in a population with density-dependent regulation). Under specific conditions, release of the double-translocation strain caused a longer-lasting and more substantial decline in population numbers than sterile releases.

Detailed ecological studies were conducted to determine population parameter values that could affect model predictions (e.g., adult movement and density dependence in different environments). A study of *Culex pipiens quinquefasciatus* (67) demonstrated fluctuations in the degree of density dependence over time. Calculations from this work indicated that a 50% reduction in egg fertility would cause pupal production to be 64.5%, 70.2%, 53.1%, 104.2%, and 111.7% of normal production in early summer, late summer, monsoon, post monsoon, and winter periods, respectively. The prediction that at some times releases could increase population size (presumably owing to a decrease in scramble-type competition) emphasizes the need to understand local pest population dynamics before any attempt at population suppression.

A genetic method conceptually related to the use of translocations is the release of strains with compound chromosomes. Whereas translocations are produced by exchange of segments between nonhomologous chromosomes, compound chromosomes are produced by exchange of chromosome arms between homologous chromosomes. This results in one homologous chromosome having two right arms attached at the centromere, and the second homologous chromosome having two left arms attached at the centromere. Strains with compound chromosomes tend to have somewhat reduced fertility when the compound chromosome is maintained in homozygous form, and they are expected to have zero fertility when they are mated to an insect with a normal chromosomal type. These characteristics are expected to result in replacement of a native strain with the less fit compound

chromosome strain if the compound chromosome strain is released at high densities. If the compound chromosome carries a desirable gene, it will be driven into the population. Use of compound chromosomes is expected to be more efficient than use of translocations because of the zero fertility from matings to native strains. Compound chromosome strains have been developed in *Drosophila melanogaster* (36) and in *Lucilia cuprina* (28). Laboratory experiments with *Drosophila* demonstrated strain replacement (9, 36).

Female-Killing Systems

The inclusion of sterile females in a release program is generally thought to have a neutral effect on the growth of the target population because they are expected to attract both released and native males. The number of times that native females are mated will be lower with release of sterile males and females than with a release of only sterile males, but as long as males mate repeatedly, most native females are expected to mate [but see (52)]. When release of sterile females has a neutral effect, then production and release of sterile females is considered problematic because (*a*) the cost of production is up to twice as great as the cost of producing only males, (*b*) sterile adult females of some species can cause damage or transmit disease, and (*c*) if less than 100% of females are actually sterile, their release can interfere with some programs aimed at eradication.

A great deal of effort has gone into development of strains in which the females will die under specific environmental conditions, or can at least be sorted from the males before a release. Some of the traits bred into potential release strains include sex-specific temperature lethality (53, 72, 73), pesticide resistance (80), and altered coloration (73). All these traits have utility, but those in which females can be eliminated early in development are best for cost reduction in an insectary that is producing millions of insects per week. A translocation-based, temperature-triggered sexing strain has been developed for the Mediterranean fruit fly, *Ceratitis capitata*. However, rearing costs for this strain are similar to those for typical Mediterranean fruit fly strains because the translocation reduces the number of progeny per female in the rearing (7, 73).

In the approach above, females are never released. Another use of a female-killing gene involves release of males and females where females but not males of the F_1 (or later) generation die, or females cannot mate in the field. In such systems the F_1 males are expected to transmit the female-killing (or debilitating) genetic factor to their offspring. Two computer simulation models have examined the efficacy of releasing strains with recessive female-killing genes. In the first case the females are blind (27). In the second case, high temperatures trigger the female-killing trait (39). In both cases the difference between males and females is established by a translocation between the Y chromosome and an autosome carrying a dominant mutant allele, so that in addition to the impact of the female-killing gene, the native population is suppressed by the low fertility of translocation-bearing males. Both models predict that, under conditions where high ratios of released to native insects cannot be achieved, the female-killing system can sometimes be more efficient than a traditional sterile male release.

Cytoplasmic Incompatibility

In 1971, Yen & Barr (100) demonstrated that a *Rickettsia*-like organism, *Wolbachia*, caused the well-known cytoplasmic incompatibility between certain subspecies of *Culex* mosquitoes. When males carrying a *Wolbachia* strain mate with females that are not infected by *Wolbachia* or with females that are infected by a different strain, most or all of the embryos are not viable. Therefore, if *Culex* males carrying a novel, incompatible *Wolbachia* strain are released into a population, they will have dynamics similar to release of a sterile male. Laven (50) conducted releases of an incompatible *C. pipiens quinquefasciatus* strain in a village near Rangoon, and 100% inviability of naturally deposited eggs was achieved after 12 weeks of daily releases of incompatible males.

If males and females of a cytoplasmically incompatible strain are released, a more complex dynamic is expected. Outcomes can range from population suppression to strain replacement if both the native and released strain carry distinct *Wolbachia* strains. If only the released strain carries *Wolbachia*, and males and females are released, strain replacement is expected under many conditions, even with low initial release rates (<10%) [(88); JL Rasgon & TW Scott, manuscript submitted].

Assessment

LaChance (48) indicates that by 1979 the sterile release approach had been tested with at least partial success on 31 insect species. Asman et al. (3) provide a detailed review of the tests conducted on eight species of mosquitoes on four continents prior to 1981. The outcome of most of these tests was less than hoped for. Analysis of the factors impeding success clearly brought home the lessons that it was critical to understand the ecology of the target insect within each specific release area and that it was critical to study the biology and behavior of the genetically manipulated insects in a natural habitat.

It is worth reviewing some of the general statistics from the tests included in Asman et al. (3). The genetic manipulation methods examined were sterilization (4 cases), genetic sexing (2 cases), translocations (5 cases), sex ratio distortion (1 case), and cytoplasmic incompatibility (1 case). Some of the problems encountered in the tests were excess immigration of mated females, unsynchronized photoperiods between released and native insects, lower than expected mating competitiveness of released insects, recombination in translocation heterozygotes, and poor dispersal of males. Excess immigration of native insects was a major problem even when barrier zones were developed by use of pesticides. The fitness of release males in field cage releases and in true field releases was often much lower than that predicted from laboratory studies.

Outcome

A number of retrospective commentaries on the classical era of genetic manipulation have offered explanations for its limited success. Few authors dispute the success and importance of the screwworm sterile release programs (16, 46), and

most find some of the sterile release programs for fruit fly eradication and interdiction to be biological and economic successes. But given that over 31 genetic manipulation projects were initiated, the percent of success is low. Krafsur (46) suggests a number of reasons for the decline in work on genetic manipulation. Among them are (*a*) the need for long-term start-up research, (*b*) expense of scaled-up production units, (*c*) university emphasis on quick and fashionable research, and (*d*) lack of political power among stakeholders affected by human disease vectors.

Two highly regarded genetic manipulation efforts were terminated when they were well into the long methods development and testing process. A six-year multinational effort in India (15) that had done rigorous assessments of sterile release and translocation methods for a few mosquito species was abruptly halted after accusation by the Indian press that the project was related to a covert operation by the United States aimed at biological warfare (2). The productive sheep blow fly program in Australia ended in 1992, apparently owing to loss of interest by the sheep industry, as it experienced other production and marketing problems (71).

Curtis (16) concluded that the simplest genetic manipulation, adult sterilization, may be the way to move forward. He states, "It would perhaps be premature to write off all the excitement about inherited factors for genetic control as a 'lead balloon,' but we have not seen lift off yet and, meanwhile, it is the conventional approach to (sterile insect releases) which has matured into a cost-effective and growing industry." Robinson (71) seems to share this view in questioning the Australian decision to move away from male sterility methods (92) and embrace more sophisticated translocation and compound chromosome approaches (95).

Because they directly affect only the target species, classical genetic manipulation methods have a major environmental advantage over other pest control methods, but this advantage comes with the financial and intellectual costs involved in designing a system with precise genetic and ecological requirements. The question remains of whether recent advances in molecular genetics and in ecology will substantially lower these costs and barriers to success (1, 33, 30). The remainder of this article reviews and assesses these recent advances.

TRANSGENESIS: A NEW OPPORTUNITY?

Throughout the era of classical genetic pest manipulation, one of the greatest obstacles was the lack of appropriate genetic materials. Insects could be sterilized by radiation or chemical mutagens, but these processes, as well as laboratory rearing, often caused greatly reduced male fitness (37, 56). In other cases males were more sensitive to the mutagenic agent than females were, posing a dilemma (57). Although theoretically exciting, strains with translocations or compound chromosomes often had low or no viability in homozygous condition. All the best-laid plans to link a conditional lethal gene to a translocation were compromised by the rarity of single-locus conditional lethals.

To a *Drosophila* biologist today, all the methods described above would at least seem crude. Although genomic techniques and transgenesis methods are less advanced in nondrosophilid species, much progress has been made with economically important species, and a number of new methods for genetic manipulation of pests have been proposed. Some of the new methods are modifications to classical approaches, whereas others are completely novel.

Transposable Elements

The discovery and characterization of transposable elements in *Drosophila* and other insects led to the novel proposal that these elements could be used to drive desired genes into targeted populations (17, 40). A DNA sequence of interest could be inserted within a transposon, and the now "loaded" transposon could be inserted into a chromosome of the target organism along with the appropriate transposase gene. Because under proper conditions transposons can replicate and move to other chromosomal locations in germline cells, such transposons could be inherited in a larger proportion of an individual's offspring than is predicted by the simple Mendelian inheritance paradigm. Therefore, if a loaded transposon was released into a native population lacking a similar transposon (and therefore did not repress transposon movement), the introduced transposon should increase in frequency within that population. Ribeiro & Kidwell (69) developed a simple population model to describe the expected change in frequency of transposons with specific intergeneration transmission rates, i, and with fitness of transposon-bearing individuals, r_t, being equal to or less than fitness of uninfected individuals, r_w. If $i > (0.5)^* r_w/r_t$, then the transposable element spreads in the population. So a transposon with up to a 50% fitness cost could spread in a population.

Kiszewski & Spielman (42) reexamined the expected dynamics of transposon spread using a spatially explicit model of about 300 villages that each contained about 100 mosquito breeding sites. Even though all their simulations assumed 100% transmission ($i = 1.0$), they found that a transposon would need to have less than a 30% cost in order to spread and become fixed in each of the mosquito subpopulations. By using a flexible spatial model, Kiszewski and Spielman assessed how a number of environmental and operational factors influenced transposon spread. When they assumed a short dry season with a continuous low level of breeding, the transposon fitness cost could not be more than 20% or spread would not occur. When a long dry season was modeled, there was interrupted breeding and a decrease in the number of breeding sites to 1% of their prevalence during the wet season. These conditions slowed transposon spread in many cases, but transposons could sometimes spread when fitness cost was 30%. Another finding was that when transposon-bearing mosquitoes were randomly released throughout the modeled region, fixation was achieved more rapidly than when an equal number of engineered mosquitoes were released in an aggregated pattern or in a band (as if along a road through the region). The Kiszewski and Spielman model always assumed that $i = 1.0$. It would be interesting to know how spatial factors affect regionwide spread when i is less than 1.0 and there is a fitness cost to bearing

the transposon. Because the impact on malaria control might be different depending on transposon fixation patterns within and among villages, it is unfortunate that results of the Kiszewski and Spielman model were reported only in terms of generations to regionwide fixation.

Both the Ribeiro and Kidwell model and the Kiszewski and Spielman model classify individuals simply as transposon-bearing or not transposon-bearing. They assume a constant rate of transmission of transposons from all infected individuals and a constant fitness effect of infection. These assumptions are unlikely to hold for all transposons, and violation of these assumptions could have important effects on transposon spread within the targeted populations. The effects of transposons on fitness are at least in part due to disruption of gene function at or near the site of insertion. Therefore, the more a transposon replicates within a genome, the greater the expected initial fitness decline. This fitness impact would decrease spread through the target population. This negative effect is balanced by the fact that high rates of intragenomic replication can increase the transmission rate. For example, if on the basis of replication characteristics of a specific transposon an insect that receives one inserted transposon (in hemizygous condition) from one of its parents has, on average, two hemizygous insertions on different chromosomes of its germ cells, then the expected transmission to its offspring would be $1 - (0.5^*0.5) = 0.75$. If another rate of replication led to transposons on three chromosomes, then the transmission rate would be expected to be $1 - (0.5^*0.5^*0.5) = 0.875$. If the rate of replication in germ cells was similar to that in somatic tissue, and each insertion had on average a fitness cost of 10%, then the higher transmission rate from the three chromosome insertions would be somewhat balanced by the approximately 30% decrease in fitness caused by the three insertions. The above example assumes that replication is on different chromosomes. Replication can also be at sites close to those of the initial insertion. If there is little recombination between replicated transposons, the replication adds little to transmission rates but still is expected to decrease fitness. If there is an excess of such local replication, spread could be inhibited or halted.

Another factor not considered in current models is the potential of loaded transposons to "lose" the loaded desirable gene. Work by Carareto et al. (8) demonstrates this possibility. Because unloaded transposons appear to replicate faster than the larger, loaded transposons, it is possible for the final population to be fixed for the transposon without the desirable gene (8). Another concern related to transposon drive systems is that once a specific transposon establishes itself in a population, that transposon cannot be used to drive another gene into the population (63). Therefore, it is critical that the first use of a transposon have the desired impact.

Engineered *Wolbachia* Strains

A number of researchers have suggested that *Wolbachia* could be used to drive genes into native populations because of the reduced fitness of uninfected females that mate with *Wolbachia*-infected males (11, 18, 82). The desirable gene could

either be engineered into the *Wolbachia* strain itself, engineered into the DNA of an obligate endosymbiont of the insect strain to be released, or engineered into the mitochondrial DNA of the insect to be released. Turelli & Hoffmann (88) analytically examined the feasibility of these approaches. For engineered *Wolbachia* the ideal situation would be a *Wolbachia* strain that causes complete inviability in incompatible matings, has 100% transmission in compatible matings, causes no fitness decrease in the infected insect, and has no potential for loss of the transgene. As the fitness cost associated with *Wolbachia* infection increases, a higher initial release rate is needed. If some of the incompatible matings result in viable offspring, again, the initial release size will need to be larger and fixation will take longer. If transmission is less than 100%, then fixation is never achieved (although in cases where the desirable gene eliminates pathogen transmission by the insect vector, such fixation may not be needed for success).

If the desirable gene is carried on the target species' mitochondrial DNA or on an endosymbiont's DNA, then when the cost of carrying the desired gene is low, fixation of the desired gene can occur even if there is not 100% transmission of the *Wolbachia*. If the *Wolbachia* strain has some paternal transmission or horizontal transmission, this could decouple the *Wolbachia* from the desired transgene, resulting in the native population fixed for the *Wolbachia* but not the transgene (88). Of course, for the *Wolbachia* approach to work, it is critical that the desired gene is not silenced or mutated over time.

Although simple population genetics models have been successful in predicting the overall dynamics of *Wolbachia* in natural populations of *Drosophila* spp. (88) and *C. pipiens* (J. Rasgon & T. Scott, manuscript submitted), they make simplifying assumptions that may hamper their predictive value for transgenic insect releases. Using a spatially explicit modeling framework, Schofield (77a) demonstrated that both the average insect dispersal rate and the spatial pattern of dispersal affected *Wolbachia* dynamics. J. Rasgon & T. Scott (manuscript in preparation) demonstrated that in an age-structured mosquito population with overlapping generations, introduction thresholds can be up to 10 times higher than those predicted by simpler Turelli-Hoffmann-type models and that releases of gravid female mosquitoes would be approximately 5 times more efficient than releases of teneral adults.

Engineered Underdominance

The potential of translocations (described above) to cause strain replacement is based on heterozygotes for a translocation having lower relative fitness compared with the fitness of the native strain and the translocation homozygote. This condition of the heterozygote having the lowest fitness is called underdominance. When fitness is underdominant, selection will tend to fix one of the homozygote allelic/chromosomal genotypes. The more common an allele is, the higher its fitness, because it will occur more frequently in homozygotes. Thus, the initial frequency of the chromosomes/alleles determines which is fixed. As described in the section on translocations, it was hard to achieve useful underdominance

because most translocation chromosomes had low fitness in homozygous condition. Davis et al. (24) examined the potential for more efficiently developing underdominance by use of transgenic methods. They envisioned three potential approaches for engineering a release strain that could cause decreased population fitness and/or drive a desirable allele into a population on the basis of underdominance. The most efficient method based on their population genetics model involved insertion of two distinct constructs on different chromosomes. Each construct included the following DNA sequences: a structural lethal gene, a promotor for the lethal gene, a *trans*-acting suppressor sequence that acted on a specific promotor, and an independently regulated desirable gene. The construct on the first chromosome would have a promotor of the lethal gene that can only be suppressed by the suppressor sequence on the second chromosome. The promotor on the second chromosome can only be suppressed by the suppressor sequence on the first chromosome. The same structural lethal genes can be inserted on the first and second chromosomes. An individual that is carrying both constructs in at least hemizygous condition does not have expression of the lethal gene of either construct because both suppressor sequences are present. The desirable gene is expressed in these individuals because it is under regulation of an independent promotor.

When a strain containing both constructs in homozygous condition is released into a native population, the F_1 offspring that are hemizygous for both constructs are viable, but any F_1 that mates with a native insect will produce 25% offspring with neither construct, 25% offspring with both constructs, and 50% offspring with only one of the constructs. This last type of offspring is inviable because they lack the appropriate suppressor. When the average frequency of the constructs is somewhat below 0.30, there will be a decrease in population fitness and selection for elimination of the two constructs. If the average combined frequency of the constructs is above approximately 0.30 [for the exact proportions see (24)], and the insects bearing both constructs are about equal in fitness to the native insects, then selection should lead to fixation of both constructs, including the desirable gene. Davis et al. (24) demonstrate that under certain ecological conditions it is possible to achieve the 0.30 frequency through a series of releases in which the number of insects in each release is only 3% of the native population numbers. One positive characteristic of this system would be very low expectation of recombination between the desirable gene and the rest of the introduced construct. The model used by Davis et al. (24) assumes nonoverlapping generations and random mating. There are general population genetics calculations indicating that if these assumptions were relaxed, the constructs, including the desirable gene, could still become fixed in the population if barriers to gene flow were mild (65). However, a detailed spatial model would be useful in elucidating the limiting conditions for spread.

Development of constructs with the exact properties described by Davis et al. (24) may be difficult because of molecular genetic factors such as leakiness of promoters and incomplete action of suppressors. Nevertheless, engineered underdominance has such great potential that this and other approaches to achieve it deserve to be fully explored at the empirical level.

Sinkins et al. (82) proposed another approach that is conceptually similar to an underdominance drive mechanism [as described in (88)]. If the *Wolbachia* genes that cause cytoplasmic incompatibility could be engineered into the nuclear genome of a release strain, then matings between males with these genes and females lacking them would result in inviable offspring. When the frequencies of these genes were low, the male carrier's fitness would on average be more affected than the fitness of females not carrying the genes because most females would be mating with native males. This results in a decline of the incompatibility genes. If the frequency of the incompatibility genes was high, then it would be the noncarrying females that would be most debilitated, so the incompatibility genes would have an overall fitness advantage and would become fixed in the population. As with translocations and the engineered underdominance of Davis et al. (24), there is an unstable inflection point; above this point the endpoint is fixation, and below it the endpoint is extinction. If the incompatibility genes were engineered into the release strain on the same construct as a desired gene, both would become fixed in the population if the release frequency was above the inflection point. In the analysis by Turelli & Hoffman (88), the inflection point would be about 0.36 if the incompatibility trait was dominant and had no fitness costs, and 0.71 if the trait was recessive. Turelli & Hoffman (88) express concern that use of this type of underdominance would be problematic because of the high release ratios needed and the potential that any system with an inflection point above 0.50 could not spread among subpopulations (6). If multiple copies of incompatibility alleles could be engineered into the release strain, this problem is predicted to be diminished (76, 95).

Improving Conditional Lethality

The use of single genes for conditional lethality has some merit for population suppression, as described earlier in this article. In genetic models of the classical approach, a single gene with 100% conditional lethality (49) or multiple genes with partial lethality were examined (43). This focus was reasonable in the pretransformation era, because at that time a major factor limiting the use of the conditional lethal approach was the restricted availability of any appropriate genes in the target species. The ability to transform insects with foreign genes has removed this constraint, so it is now important to assess whether these molecular genetic advances will improve the utility of the conditional lethal approach.

Schliekelman & Gould (76) developed a model to determine if the efficiency of the conditional lethal approach could be increased substantially by developing an engineered pest strain that had multiple copies of a single transgenic construct, where even a single copy caused 100% mortality of insects exposed to the appropriate environmental conditions. This model was motivated by general equations in population genetics for the decay of linkage disequilibrium among physically unlinked genes (i.e., decay of aggregation of groups of genetic alleles in individuals within a population). The model output predicted the optimal number of physically

unlinked copies of a single transgene that should be added to a release strain in a defined situation. Parameters explored were as follows: (*a*) number of insertions of the conditional lethal in the release strain (number of alleles), (*b*) number of generations between the time of release and the time at which the conditional lethals were triggered, (*c*) ratio of released to native pest individuals, (*d*) release of only males versus release of both sexes, (*e*) fitness cost to pest individuals that carried 1 to 20 insertions during the time before environmental conditions triggered the lethal effects, (*f*) general form of population regulation for the pest species, and (*g*) extent of inbreeding depression in the released strain.

Each of these parameters had an impact on effectiveness of the release, and many of the factors interacted with each other in influencing efficacy. When nonconditional fitness costs (i.e., fitness reduction before the trait becomes lethal due solely to gene disruption at the transgene insertion site, or by leaky expression of the transgene) are not caused by the conditional alleles, the more copies inserted the greater the effectiveness at any given release size, and this approach is always expected to be far superior to sterile releases.

The effectiveness of conditional lethal releases decreases as nonconditional fitness cost associated with the conditional lethal alleles increases. When the average fitness cost to each inserted copy is 5%, this approach is still much more efficient than a sterile male release. However, when there is any nonconditional, per-allele reduction in fitness, there is a tradeoff between the fitness of the released insects (which carry all of the inserted copies) and the final fraction of offspring carrying at least one conditional lethal allele when environmental conditions trigger the gene. Therefore, in many cases the optimal number of insertions is only 4 to 8.

At a more specific level, the model addressed a question relevant to molecular biologists. If 3 insertion events were found that had no fitness costs associated with them, and an additional 10 insertion events each had a 5% cost, would it be best to only use the 3 insertions with no cost? The predictions from the model indicated that if the release ratio were 2:1, then it would be optimal to use the 3 no-cost insertions plus 6 insertions with the 5% cost, as long as the triggering time were past generation 3 (see 76 for details).

This model only examined cases with random mating, nonoverlapping generations, no physical linkage among inserted alleles, and specific multiplicative fitness costs. Models with more biological detail are needed to determine the applicability of these results to specific pest populations. For example, although Lepidoptera pests have about 30 chromosomes, other pest species have few chromosomes, so the assumption of no linkage between 20 insertions is problematic.

Engineered Female-Specific Lethality

As with the use of conditional lethals, most of the pretransformation era modeling and empirical work with female lethality traits focused on single genes. Using the same basic model structure as that described in Reference 76, Schliekelman & Gould (77) developed a second model to examine the efficiency of releasing a

strain with multiple insertions of female lethality alleles. This model predicts that a single release of insects with multiple copies of a female-killing gene results in the female-killing allele becoming widely spread by the carrier males while reducing the population for a number of generations by killing females. In the most ideal circumstances (i.e., no density dependence and no fitness cost to males), single releases of strains with multiple, physically unlinked copies of female-killing genes are several orders of magnitude more effective than equal-sized single sterile male releases or releases of insects with a single copy of the female-killing allele (see Reference 77 for details). As in the analysis of the conditional lethal approach, fitness cost of the female-killing alleles to males impacts the efficacy of the technique and the optimal number of insertions.

If instead of a single release the same number of insects is released each generation, population extinction can be achieved at release ratios of only 0.76:1 if the replacement rate of the native population is 4.0 and the cost of each allele is 0.05. From an operational perspective, an unexpected but important result of this work is that when there is a fitness cost to males from each insertion, it is best for the first releases in a series of releases to use strains with only a few insertions, but as the releases proceed it is best to increase the number of insertions in the released strain (77).

One major practical problem with a female lethality strategy is maintenance of such a strain prior to release. Heinrich & Scott (35) and Thomas et al. (87) developed a method that offers an ingenious, practical solution. They used *Drosophila* transcriptional control elements to drive expression of the tetracycline-repressible transactivator fusion protein (tTa). In the absence of tetracycline, the tTa element turns on expression of any gene controlled by the tetracycline-responsive element (tRe), but in the presence of tetracycline there is no expression. If a female-killing gene was engineered to be controlled by the tRe in a pest species, then the transgenic strain would produce highly fit females as long as tetracycline was added to the diet. The engineered strain could be maintained on a tetracycline-containing diet in the rearing facility until the generation before release so only males would be produced for release. The absence of tetracycline in the field would maintain the female-specific gene in an activated state. Multiple copies of such genes could be fixed in the release strain, and these transgenes should have dynamics similar to those predicted by the model unless the presence of multiple copies resulted in gene silencing (62). Even if there was within-generation gene silencing, the method could work because individuals bearing single female-killing alleles would continue to be produced as linkage disequilibrium decreases with time.

The Promise of Sex Ratio Distortion

The general concept of using sex ratio distortion genes for population suppression/replacement was explored by a number of researchers in the 1970s (12, 51, 85, 86), but little empirical progress toward field release was made (but see

Reference 15). It now seems feasible to engineer genes into insect strains that would cause a bias in favor of Y-bearing sperm of a target pest population or would directly convert XX insect embryos into phenotypic males (74). Alleles that interfere with the ability of X sperm to fertilize eggs have been studied as a subset of the general phenomenon of meiotic drive (38a). The potential to genetically alter sexual developmental pathways via transgenesis or transient gene expression is a new area of research, but successful production of fertile XX males has already been attained in house flies (24a, 34a) and Mediterranean fruit flies (59a). Pane et al. (59a) produced fertile XX Mediterranean fruit flies by injecting embryos with an RNAi construct that interfered with expression of the single gene *Cctra*, which is the structural and functional homolog of the *Drosophila* sex-determination gene transformer (*tra*). Current work is aimed at stable genetic transformation with a similar RNAi construct (A. Pane, personal communication).

Use of sex ratio distortion genes could have an advantage over female-killing genes, because instead of females being killed they become males that add to the spread of two types of sex ratio distortion alleles. Schliekelman et al. (75) compared the population suppression expected from release of strains with multiple insertions of sex ratio distortion alleles (that produce an excess of males) to release of strains with multiple female-killing alleles. When conditions of the release were identical to that for a female-killing approach, the sex ratio distortion approach due to interference with Y-bearing sperm was far more efficient. For example, in a multiple release program where the native population replacement rate (R_o) was 4.0, the cost of each insertion was 5%, and the strain had 6 insertions; the release ratio needed to cause extinction was only 1:48 for the sex ratio distortion alleles. This contrasts with the need for a 0.76:1 release for a female-killing allele, and a 2.7:1 ratio for a sterile release where the radiation causes no loss in adult fitness. The effects due to sex change are generally less pronounced. When compared with female-killing alleles, the success of sex distortion alleles due to interference with Y-bearing sperm is predicted to be less affected by an equal level of fitness cost. However, genes for altering sexual development may have larger negative effects on fitness than female-killing alleles do. Although the XX male Mediterranean fruit flies appeared to act normally in the laboratory (59a), their fitness has not been quantitatively assessed. Alleles that interfere with Y-bearing sperm may have fewer pleiotropic effects, but the overall lower sperm production may lead to lower sexual competitiveness in some cases (38a). Developing an effective sex ratio distortion technology may be difficult, but the payoff could be substantial.

Potential Early Targets and Methods

There has been much discussion and debate about using strain replacement approaches to establish populations of *Anopheles* and *Aedes* mosquitoes that cannot transmit malaria parasites and viruses, respectively (10, 13, 64, 84). Some of the debate focuses on whether genetic control could be as economically efficient as some proven approaches that use low-level technologies (19), and whether a mosquito

population engineered to be refractory to a human pathogen will remain so in face of pathogen evolution (20). In the agricultural arena, transgenic technologies have been considered as ways to improve current genetic control programs for Mediterranean fruit fly, codling moth, and pink bollworm.

As methods for insect transgenesis improve, and more pest species become amenable to manipulation, it will be important to reexamine target species priorities. In the past the major targets were widespread pest species. Perhaps the initial use of transgenic techniques should be targeted at control of pests in limited areas. Greenhouse pests could be a good target for first tests of a genetic control method because their confinement within controlled conditions would decrease confounding environmental factors and would be amenable to experimental analysis. Classical sterile male release programs had problems gaining stakeholder participation because the gains from the program were for an entire community. With less-mobile pests, an individual grower would have a more direct connection to the outcome of a local program. This is not to say that area-wide programs should not be attempted.

Most of the theoretical and empirical work on genetic control has focused on diploid, obligately sexual species. One classical program worked on haplodiploid mites (59), but little has been done with cyclically sexual aphids or other species with unusual genetic systems (e.g., whiteflies). One group of insects for which genetic control methods seem to have no relevance are parthenogenetic insects. Indeed, one fear of researchers in the 1970s was that genetic control programs would select for parthenogenetic insect strains within typically sexual species.

Selection of the first targets for transgene-based control will need to balance economic, biological, and social factors. The impact of the media on the demise of the classical genetic control program in India (2) attests to the need for a first transgenic release with clear and well-communicated societal benefits, rigorous environmental and health risk analysis with public involvement, and a high probability of project success. It may be best for the first programs to be conducted in geographically or physically contained areas such as islands or temperate zone greenhouses in winter months. As argued by Benedict & Robinson (6a), if the first transgenic releases involved stable insertions that produced sterile males, public concern would be much less than if the first releases involved transposons.

LESSONS FROM THE CLASSICAL ERA

One of the major constraints to success of classical genetic control approaches was the lack of tools for developing appropriate release strains. Breakthroughs in molecular genetics appear to be on the verge of removing at least some of these constraints. The fields of ecology and population genetics have also moved forward since the 1980s, but unfortunately, estimating the pattern of density dependence in a population and testing male mating fitness in the field are still extremely labor intensive. Researchers working with classical genetic manipulations learned over and over again that there is no substitute for examining behavior of a

genetically manipulated strain under local field conditions. This will not change in the future.

During the era of classical genetic control research there was incredibly good communication and cooperation between theoretical and empirical researchers. Indeed much of the empirical work was inspired by results of population genetics studies. There has been a tendency for the sophistication of modern science to isolate researchers involved in molecular work from those doing ecological and population genetics studies. We think that more interaction between these scientists at the early stages of genetic control projects could increase the chances of producing useful strains and ushering in a new and long-lived golden era of genetic control.

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