BROADENING THE APPLICATION OF EVOLUTIONARILY BASED GENETIC PEST MANAGEMENT

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Insect- and tick-vectored diseases such as malaria, dengue fever, and Lyme disease cause human suffering, and current approaches for prevention are not adequate. Invasive plants and animals such as Scotch broom, zebra mussels, and gypsy moths continue to cause environmental damage and economic losses in agriculture and forestry. Rodents transmit diseases and cause major pre- and postharvest losses, especially in less affluent countries. Each of these problems might benefit from the developing field of Genetic Pest Management that is conceptually based on principles of evolutionary biology. This article briefly describes the history of this field, new molecular tools in this field, and potential applications of those tools. There will be a need for evolutionary biologists to interact with researchers and practitioners in a variety of other fields to determine the most appropriate targets for genetic pest management, the most appropriate methods for specific targets, and the potential of natural selection to diminish the effectiveness of genetic pest management. In addition to producing environmentally sustainable pest management solutions, research efforts in this area could lead to new insights about the evolution of selfish genetic elements in natural systems and will provide students with the opportunity to develop a more sophisticated understanding of the role of evolutionary biology in solving societal problems.

Since the 1940s, researchers have been experimenting with approaches for manipulating the genetic systems of pests to control their populations, or for replacing pathogen-vectoring pest strains with genotypes incapable of pathogen transmission. Past efforts with insects have met with some notable successes including the regional eradication of major pests. However, the number of these successes has been limited, by the lack of sophisticated genetic tools (for reviews see Gould and Schliekelman 2004; Dyck et al. 2005), and no successful programs have been conducted with taxa other than insects.

A major effort is now underway to use the ever-increasing array of molecular genetic methods to more efficiently manipulate mosquitoes that vector human diseases (http://www.gcgh.org/Projects/ControlInsectVectors/GeneticStrategy/ default.htm). A few smaller programs are aimed at insects of agricultural importance (Gong et al. 2005). Although insect pests are certainly worthy targets for management, the major objective of this article is to demonstrate the potential for broadening the scope of species targeted for genetic manipulation beyond the Insecta to encompass an array of sexually reproducing plant and animal pests. Although I argue for broader use of genetic control methods, I also discuss economic, ecological, evolutionary, and ethical reasons for restricting their use.

Evolutionary biologists have much to contribute to this applied research area because it is heavily based on evolutionary concepts such as frequency-dependent selection, gene by environment interactions, the evolution of selfish DNA, and the impacts of population and age structure on evolutionary dynamics.

History

One of the early triumphs of genetic control was the eradication of the cattle-killing screwworm fly from the United States around 1966 (Klassen and Curtis 2005). This program, which was later expanded to encompass Mexico and Central America, is estimated to currently save the cattle industry in North and Central America $1.3 billion per year (Vargas-Teran et al. 2005). The screwworm eradication program entailed rearing up to 500 million sterile screwworms per week on artificial foods (Klassen and Curtis 2005). The males were irradiated at levels that caused chromosomal breakage in germ line cells. After being released from airplanes over wide areas, these males far outnumbered native males and mated with the local females. The embryos that resulted from these matings died during development. This genetic control method was dubbed the sterile insect technique (SIT) (see Dyck et al 2005 for a review). A similar approach continues to be used for combating repeated foreign introductions of the Medfly in California and Florida before this crop pest can establish large population in the United States and cause widespread damage to agricultural commodities. It is estimated that an established population of Medfly would cause a loss of $1.3–1.9 billion per year, just in California (Enkerlin 2005).

In the early years of work on the SIT approach, researchers and administrators dreamed of using SIT for control or eradication of a large number of insect pest species (Knipling 1960). This dream was never realized because of economic, ecological, and genetic limitations. Rearing enough screwworm flies and Medflies to overwhelm their natural male populations was feasible because the targeted populations of these insects were small (e.g., 40–80 screwworms per km^2—Lindquist 1955) and factories could produce them at low costs (e.g., One million Medflies cost about $300 to produce—Parker 2005). The cotton bollworm that was at one time targeted for eradication, reaches high densities, can move hundreds of miles, and lives in many habitats; a formidable challenge for any genetic control program. A trial project for eradicating the Gypsy moth yielded disappointing results and was dropped.

Although the SIT approach has not been broadly applied to pest problems, enough technical advances have been made in insect rearing, sexing, and radiation treatments (Parker 2005) to insure a useful place for classical SIT in specific pest systems (Krafsur 1998). Klassen and Curtis (2005) discuss seven insect pest species that have been or are being controlled on a large scale by use of the SIT.

The SIT approach is very straightforward; break chromosomes, kill embryos. But, even before the first experimental tests of the SIT approach, a few researchers were theoretically and empirically testing genetic control methods based on more sophisticated evolutionary principles. In the early 1940s,

F. L. Vanderplank was working on a number of tsetse fly species that vector trypanosomes, the cause of sleeping sickness. Some of the tsetse species could freely interbreed but their hybrid offspring were less capable of surviving than the parent species (Vanderplank 1944, 1947). Evolutionary theory predicts that with such underdominance, one of the two genetic variants will become extinct. Which variant goes extinct depends on how fit each parental type is in the local environment, and the initial frequency of the two variants. Vanderplank understood that because of frequency-dependent selection, even if one variant is more fit than a second variant, the first variant could go extinct if the second variant was initially more common. Vanderplank used this evolutionary principle in developing and conducting a genetic control experiment in an isolated 26-km^2 area of Tanzania, where *Glossinia swynnertoni* was the indigenous, well-adapted species. He released a large number of a second species, *G. moristans*, which was less adapted to the area. Over time the introduced *G. moristans* genome outcompeted the indigenous species but later it became nearly extinct, presumably because it was not adapted to the dry environment. This enabled local people to reintroduce cattle that are highly susceptible to tsetse vectored diseases (Klassen and Curtis 2005).

Around the time that Vanderplank was conducting his empirical experiments, Aleksandr Serebrovskii was using his understanding of the population genetics of chromosomal translocations to develop related concepts for genetic control of pests. He examined expected evolutionary outcomes of mating one normal insect with a second insect that was homozygous for a chromosomal translocation (Serebrovskii 1940). The first generation offspring from such a cross are often expected to survive because they have all of the critical DNA sequences in the species genome, but in the second generation the aneuploid offspring that are produced are typically inviable or have very low fitness. From a population genetic perspective this system would have similar properties to the underdominance system investigated by Vanderplank with the exception that the underdominance would mostly be expressed in the grandchildren. Serebrovskii was especially interested in the fact that during the period when one chromosomal type was replacing the alternate type, there would be a substantial genetic load on the polymorphic population that could decrease its pest status.

Serebrovskii did his work in the Soviet Union during the Lysenko era (Roll-Hansen 2005). His work was not viewed favorably by the political establishment and was unknown to scientists in Western Europe until the 1960s when Chris Curtis independently rediscovered this approach for genetic control. Interestingly, Curtis took this idea one step further than Serebrovskii by exploring the consequences of releasing a strain with a chromosomal translocation that also harbored alleles that made mosquitoes refractory (i.e., unable to transmit a pathogen—Curtis 1968). Curtis and his colleagues moved this idea to the laboratory bench and to the

field where they had limited successes with prototype mosquitoes (Asman et al. 1981; Whitten 1985).

Because only females produce offspring, a genetic manipulation that resulted in female death or sterility but had no effect on males could be very effective. Indeed computer models predicted that under some conditions a manipulation that only killed females would be more debilitating than one like the SIT that caused male and female mortality (Foster et al. 1988; also see Schliekelman and Gould 2000). From a practical perspective this became feasible through a translocation between the Y chromosome and an autosome (Whitten et al. 1977). A related tool was developed based on discovery of a meiotic drive mechanism in *Aedes aegypti* that continuously altered the sex ratio in favor of males (Craig et al. 1960) and would theoretically reduce this mosquitoes density. A twist in the use of meiotic drive was to find a gene that was resistant to the meiotic drive and couple to it a gene that made the mosquito refractory to dengue virus. Selection would then be expected to increase the frequency of both genes (Wood et al. 1977).

Curtis who had been a major proponent of these more advanced genetic control techniques later wrote an article in which he bemoaned the fact that these more sophisticated genetic control concepts would never come to practical use because the available methods were too crude (Curtis 1985). By the late 1990s I could find no research articles documenting practical use of classical genetic methods other than the SIT.

Adding Molecular Biology

Just as most researchers in the area of genetic control were contenting themselves with a limited role for their methods (Krafsur 1998) the first genetically engineered strains of *Drosophila* were being created (Rubin and Spradling 1982). It did not take long for applied research scientists to recognize that there might be potential for these new developments in molecular biology to help broaden the potential for genetic control. Initial efforts were focused on genetic transformation of *A. aegypti* and *Anopheles gambiae*, the main vectors of dengue fever and malaria, respectively. Although transformation has still not become routine, it is very feasible in both species (e.g., Nimmo et al. 2006).

Initially, there was excitement about using the same transposable elements (transposons) that enabled insertion of foreign DNA into the mosquitoes as a mechanism for driving genes for refractoriness into mosquito populations (Ribeiro and Kidwell 1994). This excitement was in part based on population survey data demonstrating that based on its ability to move to a new location while maintaining itself in the old location, the *P* element transposon had spread itself throughout most of the range of *Drosophila melanogaster* within only a few decades. This seemed like a perfect way to get pathogen-resistance genes into a mosquito population. If molecular biologists could insert a novel transpo-

son sequence with an embedded refractory gene sequence into a disease-vectoring mosquito, the refractory gene would be expected to hitchhike along with the transposon as it spread through native mosquito populations (Ribeiro and Kidwell 1994).

Much effort has gone into this approach but a number of problems have revealed themselves. Perhaps the most troubling is that the process of replication in transposons is not perfect, so over time the sequence of the pathogen refractory gene could accumulate mutations, or even be entirely lost from the transposon. This latter case was seen in a *Drosophila* experiment in which a novel transposon "loaded" with another gene was released into a laboratory population. The transposon spread successfully into the population, but when the sequence of the transposon was examined months after the experiment was initiated it was clear that it had unloaded its extra DNA cargo (Carareto et al. 1997). If this happened after release into a wild mosquito population of a transposon loaded with a refractory gene, the refractory gene would be lost and the mosquito population would simply contain a novel transposon.

Aside from the problem with genetic stability of the transposons, it was also found that currently available transposons did not move frequently enough in mosquitoes to enable rapid spread of the transposon and its cargo (O'Brochta et al. 2003). A simple population genetics model showed that even if transposition rates could be increased, genetic drive would be inefficient if there were linkage between the insertion sites (Rasgon and Gould 2005). Molecular biologists could benefit from more detailed evolutionary analyses of transposon drive because attempts are still being made to find more effective, naturally occurring transposons, and to engineer transposons with better replication and movement characteristics. In the meantime, molecular biologists and population geneticists have turned their attention to other approaches for spreading useful genes. Theoretical and empirical research on these alternative approaches has just begun, but they hold promise of being more stable and controllable than transposons. A recent breakthrough in developing a prototype for one of these approaches will be discussed after a review of the basic evolutionary concepts and molecular mechanisms behind these alternative approaches.

One of the alternate approaches is conceptually similar to the underdominance methods proposed by the classical geneticists, with the exception of being more precise and powerful. Instead of relying on naturally occurring translocations or incompatibility between two species, Davis et al. (2001) examined the theoretical possibility of genetically engineering underdominance into a pest strain. As with the classical approaches to underdominance, release of the engineered strain in high numbers would result in the engineered constructs becoming fixed in the population. By using Curtis' idea of linking a refractory gene to the construct, the resulting population would no longer vector disease pathogens.

Davis et al. (2001) and Magori and Gould (2006) worked out specific properties of the engineered constructs that would decrease the number of insects that need to be released to about one-third the numbers required for the classical approach.

In 2003, Austin Burt proposed another approach; the possibility of reengineering an existing selfish genetic element called a homing endonuclease gene (HEG) in a manner that could either spread refractory genes or decrease density of pest populations (Burt 2003). The basic evolutionary history and population genetic principles that impact HEGs are discussed in Burt and Trivers (2006). HEGs do not move around the genome like transposons, but when they are found on one of two homologous chromosomes (hemizygous) in a diploid species, they have the capacity to insert themselves into the second homologue in the identical location during gametogenesis. If even one individual that is hemizygous for an HEG is released into a population, the HEG element is expected to reach a high frequency in the population if it does not decrease the fitness of individuals that carry it. Burt (2003) demonstrated theoretically that HEGs could be used for debilitating a pest population if the HEG disrupted certain gene functions only when it was homozygous. Burt and his colleagues, as well as researchers working in the area of human gene therapy, are making progress in manipulating these genetic elements, but a working system in a pest species is not on the immediate horizon.

The breakthrough alluded to earlier involves yet another curious selfish genetic element. This one, which is found in beetles, fungi, and plants, is sometimes called a Medea element (Beeman et al. 1992; Burt and Trivers 2006; Raju et al. 2007), referring the Greek goddess who killed her children. When a female that is heterozygous for a rare genetic allele mates with a male that lacks the allele, Mendelian rules indicate that one half of the offspring will be heterozygous for the allele, and that the overall frequency of the allele in the offspring will be 0.25. However, when that allele is the Medea element, almost all of the surviving offspring are heterozygous, so the frequency of the Medea element increases to nearly 0.50. Investigations of *Tribolium* beetles have shown that initially only one-half of the embryos have the Medea element, but as development of the embryos proceeds, almost every embryo without the Medea element dies. Wade and Beeman (1994) have developed population genetic models to predict the spread of Medea in structured and unstructured populations. The expected result is that the Medea element reaches a very high frequency even from an initial frequency of 2%, as long as it does not substantially decrease the fitness of individuals that carry it. And, if individuals carrying the element have lower fitness, the release threshold for spread to high frequency can still be much lower than for engineered underdominance. This would seem like an ideal driver for a linked gene that conferred refractoriness.

Richard Beeman's group who first discovered this element in *Tribolium* noted its potential for driving useful genes into popula-

tions, but the molecular mechanism by which the Medea element operates is not understood. It is generally thought that the Medea element must contain at least two distinct genes, one that lays down an embryo-specific toxin in the egg and another that removes or provides resistance to the toxin in the developing embryo. Without being able to identify the actual genes involved, it is impossible to know if these genes would only function in *Tribolium* beetles or could be used to drive refractory genes into mosquitoes and other disease vectors.

Bruce Hay and his research team at Cal Tech were intrigued by the Medea element's properties. As *Drosophila* molecular biologists they took the risk that they could build a synthetic genetic element that would function just like Medea. In building their synthetic Medea construct, they relied on developmentally regulated expression of a specific miRNA that shuts down expression of a gene critical for embryogenesis (*myd88*), and on the replacement of the endogenous *myd88* with a synthetic DNA sequence unaffected by the miRNA (Chen et al. 2007). When a female *D. melanogaster* that was heterozygous for the synthetic Medea element mated with a normal male, all of her progeny that lacked the Medea element died. When flies with the synthetic Medea element were introduced to a normal fly population in the laboratory at a frequency of 0.25, the element rapidly increased in frequency as was expected by population genetic theory. After 10–12 generations none of the flies were wild type. Chen et al. (2007) used some interesting molecular genetic tricks to produce a construct that was less prone to loss of function over time. This is a very exciting result because it proves that engineered genetic drive can be achieved. Because the molecular mechanisms are well understood they should be transferable to other species. These results beg the question of whether it is best to continue work on modifying naturally occurring gene-drive elements or to invest in genetically less-complex synthetic drive elements. Even if the answer is to only develop synthetic drive elements it is likely that a better understanding of natural drive systems will contribute to building better synthetic systems.

Next Steps

Adapting the Medea system to other species will take time, and as discussed in the case studies accompanying this article, it may not be the optimal drive mechanism for all target species. Progress is expected with the other drive systems described, and more novel approaches may be invented (Sinkins and Gould 2006). This too will take time, but there is a realistic expectation that robust, engineered drive systems will become available.

To be useful, these driver mechanisms will need to be tightly linked to genes that make disease vectors refractory, or to genes that decrease the fitness of pest species that have direct negative environmental or economic impacts. Engineered genes that confer

at least partial refractoriness to *A. aegypti* and *Anopheles stephensi* have been developed (Ito et al. 2002; Franz et al. 2006). They are not effective enough for field deployment in their current forms, but improvements are being made. Other genes have been engineered into *Drosophila* and Medflies that kill almost all female progeny (Heinrich and Scott 2000; Thomas et al. 2000; Gong et al. 2005) and similar genes are being developed for other species. The catch-all name for these type of genes is "effector genes." It is not news to evolutionary biologists that if effector genes lower the fitness of pest, vector, or pathogen populations, there will be selection to adapt to these effector genes. Evolutionary theory could certainly be used to design more temporally sustainable effectors.

In 5–10 years, fully functioning gene drive systems with effector genes could be available in target species. It is now a good time to assess the potential of these gene drive systems as well as associated risks. So far, funding and research has focused on insects, and especially on mosquito vectors of human diseases. There has been little discussion or research on the utility of these systems for controlling vertebrates, plants, and other sexually reproducing organisms. Choosing the most appropriate targets for the first applications of engineered genetic pest management will not be simple. Evolutionary biologists will have much to contribute in making these choices, but they will need to work within a broader context to be most helpful.

Below, is a brief discussion of the general genetic, evolutionary, ecological, economic, and ethical criteria that must be considered in determining whether a population of a pestiferous species is an appropriate target for one or more genetic control approaches. In the boxes accompanying this article, these criteria are applied to two potential target taxa (Scotch broom and rodents).

GENETIC FACTORS

At the molecular genetic level many of the factors relate to technical feasibility of engineering a species and can be listed as a set of questions about a target species

(1) How difficult is it to incorporate a foreign gene into the target genome?
(2) How difficult is the establishment of stable transgene expression?
(3) How much functional and structural genomic information is currently available about the species and especially about genes involved in gametogenesis and embryogenesis?
(4) Does information on the functional genomics of the species or related species suggest that important fitness traits such as sex ratio could be genetically manipulated?
(5) Are there taxonomically closely related genomic model species (e.g., *Arabidopsis*, *Drosophila*, zebra fish) that provide guidance about questions 1–4?

For a species that has already been the subject of considerable genomic research it may be easy to answer these questions. If a species and its close relatives have never been subjected to any genomic analysis, a research plan would need to be devised to make general assessments about the likely answers to these questions. In many cases this will not be straightforward. For example, past efforts indicate that developing a transformation system for a given species could take years, and that initial progress in this endeavor may be a poor indicator of the final transformation efficiency that is achieved. If the answers to the above questions for a target species suggest major hurdles, it would probably be best to put that species on a "waiting list" instead of dismissing it. Given the pace of discovery in molecular genetics, the answers may change dramatically in 1–5 years.

EVOLUTIONARY ISSUES

In contrast to molecular genetic attributes, the population genetic characteristics of a species are not likely to change substantially prior to release of the transgenic strain. A partial list of questions about the evolutionary characteristics of a species follows:

(1) Is the species obligately sexual?
(2) If the species is hermaphroditic, as with many plant species, is it self-incompatible?
(3) Is mating typically among individuals from a very local area (highly structured populations) or are individuals highly mobile, resulting in random mating over a large area?
(4) Are high rates of recombination expected among multiple inserted genes (e.g., many chromosomes, low levels of chromosomal inversions)?
(5) Are barriers to mating between native and engineered strains likely to evolve quickly?
(6) Are there simple mutations or existing genetic variation that could enable adaptation to the effector gene(s).
(7) What will be the selection pressure for adaptation to the effector gene(s).

As can be imagined from the discussion of gene drive mechanisms, if some of the individuals composing the target population do not reproduce sexually, or if there is genetic variance in the population for becoming asexual, gene drive will be less efficient or it could potentially stall. If there is genetic variance in the natural population for characteristics that decrease mating between natural and engineered individuals, this too could cause a problem. However, evolved mating barriers could be countered by continually backcrossing the transgenic release strain with the current natural population. Perhaps the most daunting evolutionary issue will be how to design an effector gene or a set of effector genes that would suppress the vector or pathogen population without resulting in rapid adaptation. Although some concepts are available from past attempts to develop evolutionarily

sustainable pest control strategies (Roush and Tabashnik 1990; Gould 1991, 1995), there is a need for consideration of novel approaches.

ECOLOGICAL FACTORS

An understanding of the target pest species' population dynamics is critical for determining what type of genetic intervention would lessen its pest status. Understanding the pest's impact on species in the community and on ecosystem functions will help in judging how eradication or suppression of its population could affect the environment (Scott et al. 2002). For example, before the screwworm eradication program was started it was known that in addition to attacking cattle, the screwworm was attacking a number of wild mammals species and causing substantial mortality (Lindquist 1937). In a posteradication analysis, Reichard (2002) found survey information indicating that white-tailed deer and feral pig populations increased substantially after screwworm eradication. When the original screwworm eradication program was being developed in the 1960s these nontarget effects were not assessed as potential risks. Any similar program proposed in the 21st century would certainly need to conduct such an assessment. (Related to this, the emergency international effort for eradication of an invasive screwworm population in Libya was in part motivated by concern over potential screwworm impacts on African large mammal populations [Reichard 2002]).

Some broad ecological questions that should be asked before starting any genetic control program are:

POPULATION DYNAMICS:

(1) How hr# much of a decrease in the pest's population density would substantially decrease direct damage to the environment, agriculture, or forestry?
(2) How much of a decrease in a pest's ability to transmit a single pathogen (or what percent of the pest population with reduced transmission) would be needed to prevent epidemics of a disease?
(3) Are there density-dependent factors regulating the pest population that will make decreasing pest density especially difficult?
(4) Will the size of the pest population make it difficult for certain gene drive mechanisms to reach threshold frequencies from which they can spread?
(5) Is the pest population spatial structure localized in a way that will prevent interpopulation spread of transgenes that have high threshold frequencies?
(6) Does the life history of the organism involve high levels of mortality of immature stages? (This could make transformation efforts more difficult).

Community and ecosystem ecology:

(1) If one pest species is removed from an ecological community will it be replaced by a competing species that will cause similar direct or indirect damage?
(2) If the targeted pest is removed will the community structure be destabilized or become substantially less diverse?
(3) Does the targeted species have a role in nutrient dynamics that will not be assumed by other species?
(4) If the transgene moves across species boundaries, could it have negative effects on beneficial species?

ECONOMIC FACTORS

The balance sheet between costs and benefits, defined broadly, will determine which if any genetic pest management approach is worth pursuing instead of a nongenetic approach. Such an analysis will include development and implementation costs as well as assessment of the economic gains (i.e., money, human lives, environmental quality).

Here, the major questions framed in a very general way are:
(1) What is the current damage caused by the pest?
(2) How expensive will the development and implementation be, and how long will it take?
(3) How much of a decrease in the damage will be achieved and for how long?

ETHICAL FACTORS

Ethical factors defined broadly encompass all societal issues including those that are spiritual, political, or involve social justice.

(1) Is the target organism a pest to some people but beneficial to others?
(2) Is the transgenic technology considered spiritually damaging to the local community?
(3) Will the use of the transgenic technology present a personal risk to some groups that do not benefit from the technology?
(4) Will those who benefit from the implementation be the ones who pay for the implementation?
(5) Is there a means to obtain truly informed consent from the affected community?

The Need for a Real Interdisciplinary Approach

The list of questions above is very long and it is clearly not a complete list. In the accompanying boxes, two examples are given of how these questions can be applied to specific, target species. These examples should make the point abundantly clear that for these pest and disease-control strategies to work there is a need for nontrivial, interdisciplinary interaction. Not only will this demand interaction among all of the disciplines listed here, it will also require understanding and respect between basic and applied workers within each of these disciplines. Although the concrete

products of such interactions would make the effort worthwhile, there will also be important intellectual growth that will emerge from these interactions.

Evolutionary biologists have been studying the dynamics of selfish genetic elements since the 1920s, but we still have only a rudimentary understanding of their origins and dynamics (Burt and Trivers 2006). Although one prototype of a synthetic selfish genetic element has been developed, knowing more about the molecular and population genetics of naturally occurring selfish genetic elements would most likely provide new ideas about how to design and use synthetic elements. If evolutionary biologists had a better understanding about why some selfish elements have flourished in specific taxa, and in populations with specific structures, we would be in a better position to offer advice to applied researchers who are trying to use the tools described in this article. On the other hand it is likely that the need of applied researchers for more precise predictions about evolutionary trajectories will push evolutionary biologists toward development of new and more sophisticated theoretical models and experimental designs.

One important spin-off from faculty involvement in this research area will be the intellectual development of their students. These students will spend their careers in a world that is reliant on application of biological knowledge in areas well beyond medicine. It is easy to say that we need to train our students in interdisciplinary approaches to prepare them for this future, but often such training involves lip-service to any fields beyond biology. Participation in the research endeavor outlined here will require students to develop a more sophisticated understanding of how evolutionary biology, coupled with knowledge from other fields beyond biology can be used to affect the future of our society.

CASE STUDY: MICE AND RATS

At an economic level, the worldwide losses caused by mice and rats are enormous. It has been conservatively estimated that in Asia, each year, rats consume over 30 million tons of rice. That is enough to feed 180 million people per year (Singleton 2003; Stenseth et al. 2003). In outbreak years, *Mus domesticus* damage to Australian wheat reduces that country's total agricultural production by 3–4% (Singleton 1997). In addition to agricultural impacts, a number of mice and rats serve as reservoirs for diseases of humans and livestock. For example, the white-footed mouse (*Peromyscus leucopus*) is a major reservoir for Lyme disease, and it has been shown that when wild populations of this mouse are made incapable of maintaining the Lyme disease pathogen, *Borrelia burgdorferi*, transmission is substantially curtailed (Tsao et al. 2004).

It is hard to find citizens with sympathy for rats and mice, but these rodents are integral parts of many ecosystems. Although the economic and ecological impacts of decreased density or elimination of one mouse or rat species from a local area might be beneficial, its elimination from a broad geographic area could have negative impacts. Because interspecific competition is common among rodents, it is also feasible that in some habitats, elimination of one species would simply result in increased numbers of another native or introduced species. The spread of an engineered construct into a rodent species that only decreased its ability to serve as a reservoir for an emergent human disease would be less likely to have off-target ecological impacts.

From a molecular genetic perspective, we have highly developed tools for manipulating the genomes of laboratory mice and rats, and these tools should be transferable to wild strains and related species. Effective methods for transgenesis, heterologous gene replacement, and age/tissue specific gene silencing are all available (e.g., Waterston et al. 2002; Gibbs et al. 2004; Yu and McMahon 2006; Dann et al. 2006). Furthermore, we have a reasonable understanding of genes involved in gametogenesis, embryo development, and sex determination (e.g., Naz and Rajesh 2005, Surani et al. 2007) that will make the development of useful gene drive constructs much easier than for most other target species.

At the population, genetic, and ecological level we also have more information on mice and rat species than for most other potential targets of genetic pest management. *Mus domesticus* and its wilder relative *M. musculus* have been subjects of detailed population dynamic and evolutionary studies (Pocock et al. 2005; Berry and Scriven 2005). Capture–Mark–Recapture (CMR) studies in many environments have demonstrated the extent of short and long distance movement and the variation in such movement. These studies as well as population differentiation studies based on allozymes, Y chromosomes, and mitochondrial genes have provided a good description of population structure in multiple habitats (Pocock et al. 2005). There are a number of *M. domesticus* populations with single and multiple translocations (Riginos and Nachman 1999; Berry and Scriven 2005), and there is a naturally occurring selfish genetic element, the T-locus, that increases based on super-Mendelian inheritance ratios in the offspring of heterozygotes (Carroll et al. 2004). Studies of the dynamics of hybrid zones between populations with and without translocations provide some initial predictions

about how transgenic gene drive mechanisms with high release thresholds might behave after release (Chatti et al. 2005). Although the T-locus is only partially understood at the molecular level, the pattern of its frequencies in wild populations has reinforced other data suggesting that *M. domesticus* populations are locally structured. This type of structure could slow the spread of certain gene drive elements. Because of their pest status, a number of conventional approaches for controlling *M. domesticus* are available and can reduce densities by over 90% (Brown 2006, Brown et al. 2007). If a transgenic strain were introduced following use of conventional control, the threshold frequency could be achieved based on release of a much smaller number of transgenic individuals.

Mus domesticus lives in some habitats in which it competes with other species, but it also inhabits niches such as houses in which interspecific competition is lacking or is inconsistent. In some habitats removal of *M. domesticus* is likely to lower overall rodent density, but in some areas *M. musculus* or other similar species are likely to replace it. Removal of *M. domesticus* over large areas could have a substantial effect on ecosystem functioning or diversity, but data are lacking. A bigger issue is the potential for transgenes to move from *M. domesticus* to *M. musculus* (Payseur and Nachman 2005). If a Medea type of element were transferred from an engineered strain of *M. domesticus* to even a few offspring of *M. musculus*, it could spread through most of that species populations because of the potential low percentage threshold for increase in Medea frequency (but see Chen et al. 2007 for potential molecular safeguards). If the Medea element were carrying a recessive lethal transgene, the populations of *M. musculus* could be severely decreased and its geographic range could shrink. It is difficult to estimate the ecological impacts of such an occurrence. Although it might be possible to engineer a specific Medea construct with fitness costs that raised its threshold frequency for increase, a detailed understanding of genotype by environment impacts on the fitness cost would be needed before any release was considered. It would seem safer to drive a gene into a single *M. domesticus* population using an underdominance drive where the frequency threshold for spread was relatively high. With such a system it would be unlikely for a deleterious transgene to spread between species. Even in this case, careful ecological studies would be needed to assess the risk of spread.

Fortunately, past research involving the release on isolated islands of strains with T-locus and with chromosomal translocations provides protocols for testing the limits of invasiveness of specific strains with gene drive mechanisms (Pocock et al. 2005). Before any experimental releases were done with strains carrying an effector gene, a number of release exper-

iments into natural populations would need to be conducted on isolated islands to follow the population genetic behavior of the gene drive mechanism alone. These experiments would not only provide information for risk analysis, they would also offer more detailed information on the population structure of *M. domesticus* than has been available. In the end, it could be that for *Mus* it may be best to use genetic manipulations like insertion of multiple copies of sex ratio distortion genes without a drive gene (e.g., Schliekelman et al. 2005). Release of strains with these constructs would only limit or eradicate a local population, and would not persist in any population.

At a practical level, it is important to understand how much of a reduction in a population's fitness would be sufficient to have an economic impact (Brown et al. 2007). Jacob and Matulessy (2004), and Jacob et al. (2004, 2006) conducted a series of experiments on ricefield rats (*Rattus argentiventer*) in Indonesia that offer predictions about the level of female sterility or female killing that would be needed to reduce populations. Experimental females were sterilized by tubal ligation and released into field enclosures as 25%, 50%, and 75% of all females. After three generations, the population density in the 50% and 75% treatments was less than half that in the control and crop damage was decreased from about 85% to about 30% (Jacob et al. 2004a). In another experiment in which there was potential for immigration, no differences were found (Jacob et al. 2006). It was concluded that releases (or chemical treatments that sterilized females) would need to be conducted over a large area to have an effect. If a female killing allele could be driven to a frequency of over 0.90, then a substantial decrease in density would be expected based on these data. However, longer duration experiments of this kind would be needed to insure that the long-term dynamics would be similarly affected.

Given the defined problems with mice and rats, the molecular tools available, and the preliminary data on population structure and dynamics, it would be feasible to develop a detailed, multidisciplinary research project with the goal of either locally or regionally manipulating populations of these pests. It is too early to determine which genetic strategies would best fit specific populations or which would be most feasible at the molecular level. The first molecular experiments would need to focus on laboratory strains, and during that time period more detailed field studies on wild populations would provide insight about the most promising strategies and practical targets.

CASE STUDY: SCOTCH BROOM

Scotch broom, *Cytisus scoparius* (L.) Link (Fabaceae), is a European shrub that has become an invasive weed in New Zealand, Australia, and the East and West coasts of the USA

and Canada (Parker 1997, 2000; Simpson et al. 2005; Zouhar 2005; Jarvis et al. 2006). It causes losses to biodiversity, tourism, and the forestry industry. Just in Oregon, it is estimated to cause losses of \$47 million per year, mostly to the forestry industry (Radtke and Davis 2000). In New Zealand it has the status of "Weed of National Significance" and is now encroaching on an area in Barrington Tops National Park that is inhabited by 35 rare or threatened plant species (Odom et al. 2005). In recent years Scotch broom has also become a pest in certain areas of Europe (Prévosto et al. 2006). In New Zealand it has one redeeming quality as a source of pollen for honey bees, but this benefit is far outweighed by the damage caused (Jarvis et al. 2006).

Our knowledge of the molecular biology of this species is extremely limited (Kang et al. 2007). However, Scotch broom is a member of the plant family, Fabacae. This family includes a number of important agricultural crops such as soybean, alfalfa, peanut, and chickpea. For this reason, a great deal of effort has gone into studying the molecular biology and genetics of this family. Transgenesis is not routine (Somers et al. 2003) but has resulted in commercially marketed soybean and alfalfa varieties, with transgenic varieties of other legumes in the pipeline (Popelka et al. 2004). As long as transgenesis in Scotch broom is not especially problematic, it should be feasible in coming years. A few studies have been conducted on female sterile mutants (e.g., Kato and Palmer 2004) and reproductive anatomy (Rodriguez-Riano et al. 2006).

At the population genetic level, a number of studies have demonstrated that Scotch broom is self-incompatible (Parker 1997; Simpson et al. 2005). Rodriguez-Riano et al. (1999) demonstrated that self-incompatibility extends beyond the genus *Cytisus* to another species in the Papilionoideae. Therefore, self-incompatibility may be an evolutionarily constrained trait in this taxon.

Because of its pest status, the population dynamics of Scotch broom has been carefully studied and simulation models have been developed to predict its spread and the impact of control measures. Invasiveness of this species is generally pollinator limited, and exotic honey bees or bumblebees have been essential for its spread (Parker 1997; Simpson et al. 2005). Results of a simulation model developed by Stokes et al. (2006) indicate that spatial spread in parts of Australia that have honey bees could be greater if bumblebees were introduced. In general, control of a well-established population is difficult because the plants produce huge numbers of seeds that can remain in the soil for over five years. The plants live approximately eight years and have no vegetative reproduction, so if seed production is halted the population will go extinct. Simulation models indicate that reduction of seed production by 70% and

99% will, respectively, curtail spread in slow and rapidly invaded areas (Parker 2000). Lesser impact on seed production would substantially slow spread (Parker 2000, Stokes 2006), and in combination with biological control could stop invasion.

These biological characteristics suggest a number of genetic control measures. Planting of a barrier of female sterile plants could interfere with spread. More powerful would be a female sterility gene that was linked to an HEG or a male-biased, meiotic drive mechanism. If plants of this type were seeded or transplanted into a population, male-only plants would compete with, and replace the native, monoecious plants.

On continents where Scotch broom is not native, its limitation or elimination are not expected to have negative ecological consequences. Care would need to be taken to insure that Scotch broom was not hybridizing with any native species. If a dominant-acting, female sterility gene were used, the threshold for spread would be high, even with an efficient drive mechanism. This would make spread into any sexually compatible species much less likely. If the female sterility trait were absolute, no seeds would be produced that contained the transgene, but pollen with the transgene would continue to be produced. To the extent that the trait were not completely penetrant, transgenic seed would be produced. It would clearly be important to expand on existing population dynamics models in testing for potential ecological risks. Findings from such models and from empirical ecological experiments could increase the accuracy of risk estimation.

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