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GUEST EDITORIAL

Genetic Transformation and its Potential in Insect Pest Control

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In a recent issue of *Science* (Holden, 1989) there was reported the laments of some of our more renowned insect scientists over the state of our discipline. For many reasons entomology as a whole, with the study of *Drosophila* being the most notable exception, has not kept up with some of the major scientific and technological advances in the last quarter of a century. The result, very generally speaking, has been that entomologists have not been shareholders in the new biology (actually not so new at this point), and they find themselves competing *against* molecular biology and molecular genetics both for money and positions. In 1990 it seems out of place to be selling the virtues of some of this technology and the molecular genetic analysis of biological systems. Many examples of the application of molecular genetics, both to practical and fundamental biological problems, greet us with every issue of *Science* and *Nature*. In this editorial I would like to discuss two points. First, I would like to reiterate the claim that recombinant DNA technology and its application to insect systems holds real potential as a tool to facilitate insect pest control practices currently employed, and may also lead to the development of novel strategies. Second, I would like to focus on one technical aspect of insect molecular genetics - gene transfer or genetic transformation—and discuss briefly not only why it is important but how it might be achieved.

Discussions of the benefits to be gained by entomologists through the study of insect molecular genetics and the use of recombinant DNA technologies have appeared over the years and not much new can be added (Cockburn *et al.*1984). It's potential application to pest problems is most easily illustrated with current examples of control practices and showing how the integration of molecular genetics might be useful.

Current biological control strategies in California for the control of spider mites in orchards involve the release of insecticide resistant phytoseiid mites of the species, *Metaseiulus occidentalis*, which has been developed using classical genetic methods. The ability to confer insecticide resistance to mite species using recombinant DNA methods might broaden the scope of this control strategy by permitting the transfer of the resistance phenotype to species with control potential but which are currently insecticide sensitive. Of course this will require considerable understanding of the biochemistry and genetics of resistance but this is already an area of research receiving considerable attention. In addition the success of such a scheme is contingent upon the ability to transfer a resistance gene into the desired host.

The Mediterranean fruit fly, *Ceratitis capitata* and the screwworm, *Cochliomyia hominivorax*, among other insects are controlled in part through the release of sterilized adults into the pest population. This method can be quite effective yet there are certain aspects of it which are open to improvement. For example, usually both males and females must be reared *en masse*, sterilized and released, although it is only the males which contribute

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positively to the efficacy of the method. In fact females are undesirable in this situation because they consume half the diet, which is not insignificant under mass-rearing conditions, and may actually be pests (by biting or causing oviposition damage, for example) even though strerile. Genetic methods for eliminating females from a mass-reared population can be developed using classical genetic methods, however a considerable knowledge of the genetics of the target insect needs to be in hand before such a genetic sexing strain can be produced. Using recombinant DNA technology such a genetic sexing strain might be developed more rapidly and without requiring extensive knowledge of the host's genetics. It is not unreasonable to think that a gene conferring a dominantly selectable phenotype could be introduced into the host species in such a way as to limit expression to males. These examples are fairly obvious and are intended to illustrate the potential of recombinant DNA technology in the field of insect pest control, given the right tools. I have no doubt that once the ability to manipulate insect genomes in this way is available more creative applications to pest control problems will emerge.

Currently it is difficult to foresee when in the future the release of recombinant insects (of any composition) will be socially acceptable. Even in cases where the recombinant insect has genetic qualities that could have been acquired by classical genetic manipulation, current attitudes would make such a release very difficult. Attitudes toward the release of recombinant organisms into the environment are changing and as more examples accumulate of the safe and ecologically sound release of such organisms, the release of recombinant insects will become accepted by the public. But this is certainly not a reason to abandon this approach. We are not discussing merits of a new insecticide whose efficacy is either demonstrable or not, and whose fate rests on the outcome of this test. We are discussing research tools which will make available the genetics of insects for our study and for our use. It can not be stated strongly enough that the greatest benefits from molecular genetics and recombinant DNA technology will be realized *not* as recombinant insects released into the environment but as increased understanding of the genetics, biochemistry, behavior, physiology, development and ecology of insects. It is this understanding which will have the greatest impact on insect pest control.

How will this potential be realized? By and large there is little preventing entomologists from capitalizing on this technology now. All insect systems will undoubtedly present their own set of unique problems which will have to be solved before a molecular genetic analysis can proceed, not the least of which will be the large genome sizes of some insects. However, most of the essential tools are available. One tool, which is not available and alluded to earlier, is some means by which genes manipulated *in vitro* can be introduced into the host of interest. While not essential for many types of molecular genetic studies, if this were available it would open the way to wonderfully powerful methods for isolating and analyzing genes and the biological processes with which they are involved. By way of example, since the development of a gene transfer method for *Drosophila* in 1982 (Rubin & Spradling, 1982), the advances made in understanding the genetics, development and neurobiology of these insects have been staggering. It is safe to say that the glut of knowledge acquired about this insect in the last five years is a direct result of the development of a gene transfer system. The value of the gene transfer system in *Drosophila* is based not only on the ability of this system to introduce DNA into the genome but also on its ability to be used as a mutagenesis system which, in turn, has permitted the identification and isolation of genes involved in particular processes. More recently methods have been developed, based on this gene transfer system, which have permitted the identification and isolation of genes using non-mutagenic methods, permitting the identification of genes with cryptic or lethal mutant phenotypes. It is safe to say that a similarly versatile system for non-drosophilids would encourage a similar flood of knowledge.

Conceptually the development of a gene transfer system is a simple problem, but unfortunately the reality of the biology has made this a challenging undertaking. Within the genomes of probably all organisms is a class of genetic elements which are inherently mobile, capable of literally transposing from one position in the genome to another. In some cases this movement can be rather frequent (Berg & Howe, 1989). One strategy for developing a gene transfer method is to harness the natural mobility of one of these elements and use it to literally carry DNA of interest into the genome of a host. This is how

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gene transfer is accomplished in *Drosophila*, the mobile genetic element in this case is called a *P*-element. By and large the majority of the effort spent to date on the development of a gene transfer system for non-drosophilids has focused on the *P* system. Attempts to use the *P*-element as a vector in three species of mosquitos, two species of tephritid fruit flies and a species of calliphorid and a grasshopper have all failed. There has been no evidence for the movement of the *Drosophila P*-element in any insect outside the family Drosophilidae. Many have concluded that the *P*-element will not function in non-drosophilids and alternative methods should be sought. This conclusion may prove, ultimately, to be correct but at this point it is premature.

The strategy for using the *P*-element as a gene vector is straightforward. The *P*-element consists of a unique sequence of DNA which are capable of transposing as a unit from one location in the genome to another. Significantly, when the *P*-element is attached to virtually any other piece of DNA these sequences also become mobile. But the *P*-element acts only as the vehicle which transports the DNA, a vehicle without an engine. The engine which drives this system is a protein called *P*-transposase. In the absence of *P*-transposase *P*-element DNA, like any other sequence of genomic DNA, is incapable of transposition. To use the *P*-element as a vector in *Drosophila*, or any other insect, requires that the gene vector be introduced into the germ cells along with the transposase gene. This is usually accomplished by direct injection using extremely fine needles. Following injection the transposase gene and the *P*-element vector exist as extrachromosomal DNA and at some frequency *P*-element DNA becomes integrated into chromosomes. Expression of the transposase gene is absolutely crucial for the success of this procedure and to facilitate the expression of the *P*-transposase gene it has been coupled to the regulatory region of another gene, the *Drosophila* heat shock gene. This results in abundant production of transposase, at least in *Drosophila* cells. While everyone has assumed that the *Drosophila* heat shock promoter used in this chimeric gene would function correctly in non-drosophilids and that expression results in the production of functional transposase protein, no one has demonstrated the latter and few the former. These assumptions seem reasonable but should be tested nonetheless. Preliminary results have indicated that functional *P* transposase mRNAs are not being produced in the embryos of the Caribbean fruit fly, *Anastrepha suspensa*, following injection of the transposase gene described above (David A. O'Brochta and Alfred M. Handler, unpublished observations). This is due to either improper or inefficient message processing, a problem which could be avoided with an alternative transposase gene which does not produce messages requiring extensive processing. In addition, the *Drosophila* heat shock promoter has been found to be virtually inactive in the sheep blow fly, *Lucilia cuprina*, even under conditions where expression is expected to be maximal (David A. O'Brochta and Peter W. Atkinson, unpublished observations). These results were unexpected but they lead us to the general conclusion that the existing gene transfer system, without modifications, is unlikely to be useful in non-drosophilid insects and quite importantly, each vector system must be tested and analyzed in the insects of interest. Given the inability to express transposase in *Lucilia cuprina* embryos and to process transposase messages in *Anastrepha suspensa P* mobility has not been adequately tested in these species. How can we be confident of the results of tests of *P*-element mobility in non-drosophilids when it is likely that they may have been conducted in the absence of transposase? Furthermore, we should anticipate that a 'universal' insect gene vector, useful in a phylogenetically broad range of insects, may never become a reality. A multiplicity of vectors may need to be developed and given the difficulties in dealing with the *P*-element system in non-drosophilids it is apparent that the scope of this gene transfer problem has grown.

While the results with the *P*-element still leave open the question of its utility as a non-drosophilid gene vector it would be prudent to consider other strategies. Two general strategies are worth pursuing. First, existing mobile DNA or gene transfer systems could be tested in non-drosophilids. This approach should not be confined to mobile DNA systems originating in insects. The *mariner* element from *Drosophila mauritiana*, the *Ac/Ds* elements from maize, the *Tc* element from the nematode, *Caenorhabditis elegans*, and the *FLP* recombinase system from yeast all are worth considering (Berg & Howe, 1989). Alternatively, mobile genetic elements with properties conducive to their development into gene vectors should be isolated from the insect species of interest. This

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is easier said than done. Space does not permit a discussion of this subject but efforts along these lines should focus on isolating elements on the basis of their mobility as opposed to structural criteria.

While considerable effort has been spent on either testing or looking for mobile DNA systems in non-drosophilids, even if it were available tomorrow, it is doubtful that such a system would be of great use immediately. The reason is that we would be almost completely unable at this time to recognize a transgenic non-drosophilid. That is, it is essential that one be able to recognize an animal which has acquired the gene vector and, as these are likely to be fairly rare, this is not a trivial problem. Generally, the strategy is to incorporate into the gene vector another gene which confers a recognizable phenotype. The transformed individuals can therefore be recognized by virtue of this new phenotype. The gene most often considered as a genetic marker is the prokaryotic gene neomycin phosphotransferase which, when expressed in some eukaryotes, can confer resistance to the drug neomycin. This selection system, however, is not very effective and unfortunately there are currently few alternatives. This problem is critical and deserves considerably more attention than it has received to date.

That molecular genetics and recombinant DNA technologies will become important tools in the study of non-drosophilid insects is a certainty. In fact, it is disappointing that in some circles the point is still open to serious debate. Technically there is little preventing this field from burgeoning. Progress now is largely dependent upon effort and support. The single major impediment to the advance of certain areas of this field is the development of non-drosophilid gene transformation technology. As discussed above the problem is not trivial and the potential to transfer *Drosophila* technology to non-drosophilids may be minimal. More overt support for this problem from agencies responsible for funding entomological research would be a prudent investment. It ultimately would go a long way to helping put entomology back on the map.

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