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A CASE STUDY

Scope of genetically engineered predator and parasitoid

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ABSTRACT

In order to reduce environmental contamination from chemical pesticides renewed emphasis has been placed on the development of effective bio-control agent for management of insect and mite pests. Genetic engineering can provide increased understanding of the biology and pathogenicity of the organism. Genetic engineering, also called genetic modification, is the direct human manipulation of an organism's genome using modern DNA technology. It involves the introduction of foreign DNA or synthetic genes into the organism of interest. Genetic engineering of arthropod natural enemies to improve their effectiveness as biological control agents requires the identification of beneficial traits, the cloning of genes that influence such traits, and the development of techniques for introducing these genes into the natural enemy species in such a way that they are appropriately expressed and stably transmitted to progeny. The genetic improvement of beneficial organisms can be approached from two different aspects *viz.*, increasing genetic diversity and artificial selective breeding. Different workers have carried out selective breeding procedures on beneficial organisms with some degree of success.

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INTRODUCTION

The biological environment of any living thing consists of two components: external and internal. Most aspects of insect pest suppression by biological or other means concern themselves with the external environment and how it can be managed or modified to the detriment of the pest (e.g. increased mortality). However, the internal biological environment also lends itself to manipulations which can work against the pest insect. In this regard, particular attention has been accorded to the pest's genetic constitution and its potential for useful modification (e.g. decreased fertility). It is surprising that more attention has not been given to the application of genetic principles in insect pest suppression. This is especially true when we consider that so much of modern genetics has its foundation in work done on insects, particularly a vinegar fly, *Drosophila melanogaster* Meigen, the honeybee, *Apis mellifera* L. and the silkworm *Bombyx mori* L.

In order to reduce environmental contamination from chemical pesticides renewed emphasis has been placed on the development of effective bio-control agent for management of insect and mite pests. Among the advantages of bio-insecticides are their safety to non-target organisms and the environment, lack of mammalian toxicity, and absence of toxic residues. Some of the limitations of bio-insecticides, such as slow action, restricted host range, and limited persistence in the field, can be addressed by various strategies involving genetic manipulation. In addition to providing a means for improving a given insecticidal product, genetic engineering can also provide increased understanding of the biology and pathogenicity of the organism.

Genetics has found wide practical application in many phases of modern agriculture exclusive of insect control. However, the very characteristics which made insects ideal basic research subjects are not fully recognized for their equivalent value in applied areas of pest suppression. High reproductive potential, short life cycles, and relative ease in mass rearing are all qualities that can be used against insect

pests. Also important are both the great genetic plasticity of insects (witness the widespread development of insecticide resistance) and their genetic diversity, either in naturally occurring races, or induced with various mutagenic agents to which they are vulnerable.

Finally, as suggested by Whitten (1970), the successful use of genetic manipulation for insect pest suppression sometimes requires far less basic biological and ecological information about the target organism (aside from numerical data) than other forms of biological control, at least when overwhelming numbers are available for release. The most critical factor is that random mating can be demonstrated between the liberated insects and the wild population. Hindsight indicates, however, that extensive basic ecological research might avoid many of the problems inherent in genetic manipulation programs on a limited budget (Whitten and Foster, 1975).

Genetic manipulation is easily included under the broad definition we have adopted for biological insect pest suppression because it involves the use of a living organism (the pest itself) for the population suppression of a pest insect considered detrimental to man. The great specificity and environmental safety of the technique are also unsurpassed by other methods. Genetic engineering, also called genetic modification, is the direct human manipulation of an organism's genome using modern DNA technology. It involves the introduction of foreign DNA or synthetic genes into the organism of interest.

Genetically modified organism (GMO) or genetically engineered organism (GEO) is an organism whose genetic material has been altered using genetic engineering techniques.

These techniques, generally known as recombinant DNA technology, use DNA molecules from different sources, which are combined into one molecule to create a new set of genes. This DNA is then transferred into an organism, giving it modified or novel genes.

Recombinant DNA (rDNA) molecules are DNA sequences that result from the use of laboratory methods (molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms.

Genetic engineering of predators and parasitoids :

Genetic improvement of arthropod natural enemies to enhance their capacity to control pests has been achieved previously by artificial selection (Beckendorf and Hoy, 1985; Johnson and Tabashnik, 1994). An integrated pest management program featuring a predatory mite strain selected for insecticide resistance has been successfully implemented (Headley and Hoy, 1987). However, the development of recombinant DNA techniques has made it possible or at least conceivable to transfer genes specifying beneficial traits directly to arthropods (Ashburner *et al.*, 1998; Heilmann *et al.*, 1994; Hoy, 1994). The use of genetic engineering methods for the improvement of beneficial arthropods has two advantages over artificial selection:

The goal of genetic improvement can be achieved rapidly, without the generations of rearing required for classical selection protocols;

Rather than selecting solely from the available gene pool of the arthropod natural enemy, any gene from any species can be used, in principle, for genetic improvement.

Genetic engineering of arthropod natural enemies to improve their effectiveness as biological control agents requires the identification of beneficial traits, the cloning of genes that influence such traits, and the development of techniques for introducing these genes into the natural enemy species in such a way that they are appropriately expressed and stably transmitted to progeny.

Heilmann *et al.* (1994) identified three beneficial traits that can be conferred to natural enemies: (1) pesticide and disease resistance; (2) cold hardiness; (3) sex ratio alteration for species where only one sex attacks the pest. These traits are monogenic (controlled by one gene) or are likely to be influenced strongly by a single gene. Complex, polygenic traits such as host finding ability and host preference are not well-understood at the molecular level and hence are less amenable to modification.

Improving natural enemies by genetic engineering requires vectors for the stable and heritable introduction and expression of foreign genes in arthropods. Progress toward the routine transformation of arthropods other than drosophilid flies has been reported with transposable elements, and "paratransformation" has been achieved by engineering symbiotic bacteria. Pantropic retrovirus vectors also show much potential as vectors for the transformation of a wide variety of arthropods.

The genetic improvement of beneficial organisms can be approached from two different aspects,

– Increasing genetic diversity and,
– Artificial selective breeding.

The importance of the Increasing Genetic Diversity was first recognized by Clausen (1936), who pointed out that beneficial organisms are not necessarily constant in their abilities and adaptations throughout their natural range of occurrence. Therefore, he suggested, it is best to make use of these available differences by importing the chosen beneficial organisms from many different regions, assuring the best chance of obtaining an effective strain well-adapted to the new environment. This may be even more important if the spatial distribution of the target pest in the new environment covers a diverse range of conditions.

The second method of improving beneficial organisms

for use in biological insect pest suppression involves the process of artificial selective breeding for increased fitness to the conditions of the new environment. The entire subject still remains largely conjectural even though the idea was suggested at least 60 years ago. The premise behind the suggestion is that, in light of the great successes achieved by selective breeding in improving varieties of domestic plants and animals, it should be possible to prove parasitoids, predators, and insect disease organisms in the same manner. Whereas, in the method of genetic improvement suggested above, a diverse gene pool is presented to the environment in hopes of a post-colonization natural selection of a well-adapted strain, in the second method, discerned weaknesses in adaptation are improved upon in the laboratory by artificial election before colonization.

Simmonds (1963) and Messenger and Van den Bosch (1971) have pointed out several disadvantages and difficulties in carrying out the laboratory selections. For example, because of the inherent complexity of the natural environment, the exact factors to which a beneficial organism may be maladapted are frequently impossible to determine ex situ, and therefore cannot be dealt with in the laboratory. Lack of knowledge concerning the genetic basis for inheritance of desirable characteristics in beneficial organisms makes intelligent selective breeding a difficult and lengthy proposition. It is necessary to obtain a sufficiently broad genetic diversity to assure the availability of suitable characters on which to base selections.

Finally, after a suitable strain has been developed and released, will it breed true under natural conditions, or will reversion to the wild type occur, or natural selection act to produce the best adapted strain despite any amount of effort expended in the laboratory before colonization.

Case studies :

A number of workers have carried out selective breeding procedures on beneficial organisms with some degree of success.

In the classical study of this kind, Wilkes (1942) was able to influence the temperature preference of certain strains of the eulophid parasitoid, *D. fuscipennis*, for oviposition; one strain selected 9° C and another 25° C. Later Wilkes (1947) was able to improve his laboratory strain of *D. fuscipennis* by doubling the mean number of progeny per female, decreasing the number of sterile males produced from 35 to 2%, and reducing the variability in development, oviposition, and adult life span.

Simmonds (1947) achieved similar success by selecting out a strain of the ichneumonid, *Mastrus (= Aenoplex) carpocapsae* (Cushman), which produced a high proportion of female progeny.

D. fuscipennis

Urquijo (1951) selected a strain of *T. minutum* (Riley) with superior host-finding ability, whereas Allen (1954) elegantly broadened the host preference range of an

T. minutum

ichneumonid, *Diadegma (= Horogenes) molestae* (Uchida), beyond the normal host, *Grapholitha molesta* (Busck), to include a more easily reared laboratory host, the potato tuberworm, *P. operculella* (Zeller), for improved mass production. The resulting strain of *D. molestae* was 24 times as efficient on *P. operculella* as the initial stock.

The development of a strain of parasitoid resistant to insecticides, for release in the field, should have great practical value, and was first attempted by Pielou and Glasser (1951) with *Macrocentrus ancylivorus* Rohwer. By the F_{19} generation of selection, a maximum resistance ratio of 12 times the normal was attained, but this declined gradually in succeeding generations, and resistance disappeared altogether when the selective pressure of DDT treatment was discontinued (Robertson, 1957).

A more recent attempt to develop pesticide-resistant strains in a boll weevil parasitoid, B. *mellitor* Say, was even less successful (Adams and Cross, 1967). The best known and, thus far, only successful field application of the principle of using pesticide-resistant biotic agents involves two species of phytoseiid mites, *Typhlodromus occidentalis* Nesbitt and *Amblyseius fallacis* (Garman), both of which are specialized predators of tetranychid spider mites on deciduous fruit trees (Croft and Brown, 1976). In these species, resistance arose through natural selection in the field, but both have been moved about and re-colonized widely (Croft and Barnes, 1972; Croft and Brown, 1975).

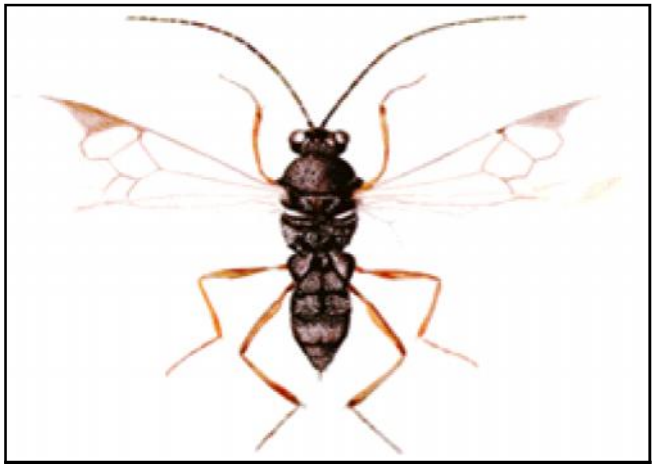
Until now, we have discussed breeding improvements made by selecting from single species populations; however, a number of workers have advocated interspecific crosses for increasing germ plasm diversity and adding useful characters not available intraspecifically. Handschin (1932) successfully crossed two species of parasitic wasps, *Spalangia orientalis* Graham and *Spalangia sundaica* Graham, to produce a fecund, long-lived, fertile hybrid which was better adapted to the environment of North Australia than either parent. Box (1956) improved the host-preference behavior of the tachinid parasitoid, *Paratheresia claripalpis* (Wulp), by hybridizing two geographically isolated strains of the species which would not normally interbreed in nature.

Hodek (1973) cites a Russian study in which increased viability (heterosis) was gained through intraspecific crossbreeding of coccinellid beetles from different climatic regions. Hoy (1975) hybridized various geographic strains of *Apanteles melanoscelus* (Ratzeburg) to improve diapause response. White *et al.* (1970) tried irradiation as another means of increasing genetic diversity in their breeding stock of *Aphytis*, but no benefit was evident.

One final point to consider in regard to the genetics of laboratory or insectary reared beneficial organisms, is the possibility of deterioration of the desirable qualities originally displayed by the initial strain or those qualities selectively bred into it. For example, arrhenotokous stocks of *Trichogramma* egg parasitoids were unaffected by continuous laboratory rearing, but a deuterotokous stock, similarly reared, deteriorated significantly in host finding efficiency over a period of 4 years (Ashley *et al.*, 1973).

***Aphytis* sp.**

Paratheresia claripalpis



***Apanteles* sp.**

***Trichogramma* sp.**

Arthropod transformation techniques currently being developed often involve microinjection of embryos. Embryo microinjection will likely require the development of new methods for egg collection and processing for each new species and is not feasible for some species, such as parasitoids and viviparous insects. An alternative gene delivery method, maternal microinjection, was developed to transform the western predatory mite, *Metaseiulus occidentalis* (Presnail and Hoy, 1992). This species is a member of the Family Phytoseiidae. a group of mites that are mass-reared for the control of spider mites and that are often wiped out by pesticides sprayed to control other pest species.

Hence, transforming mites of this family with genes conferring pesticide resistance may enhance their pest control capacity. Efforts to transform *M. occidentalis* by microinjection of eggs resulted in substantial mortality and little success. To bypass the difficulties involved in injecting eggs of this

M. occidentalis

species, gravid females were injected (Presnail and Hoy, 1992). *M. occidentalis* eggs are developed one at a time and are visible through the cuticle. These properties allowed for the injection of DNA directly into or close to the maturing eggs within the female mite.

Maternal microinjection of mites proved to be easier than microinjection of mite eggs. For the first reported maternal microinjection experiment, gravid female mites II were injected with a plasmid that contained the *lacZ* gene (that encodes p-galactosidase) under control of the *Drosophila mekmogaster hsp70* promoter (Presnail and Hoy, 1992). However, these lines did not persist long enough to provide the amount of DNA required to detect chromosomal integration of the plasmid.

Maternal microinjection was also attempted with a braconid parasitoid wasp *Cardiochiles diaphaniae* (Presnail and Hoy, 1996). This species was imported into the United States to control lepidopteran pests of the genus *Diaphania*. Because the cuticle of this wasp is pigmented and opaque, it was necessary to carry out dissection and preliminary injections to determine where to insert the microinjection pipette in order to deliver DNA into the ovaries. Wasps were microinjected with a plasmid containing the paraoxon resistance (parathion hydrolase) gene and selected for transgenic G_1 progeny with parathion. This procedure yielded one survivor with DNA that displayed a hybridization pattern with a plasmid probe that was consistent with chromosomal integration of plasmid sequences.

Field releases of transformed *M. occidentalis* at a Florida research plot were carried out in the spring and fall of 1996. The goal of these releases was to determine the persistence of the plasmid-derived *lacZ* gene in the predatory mites under field conditions, the capacity of transgenic mites to control spider mites, and the ability of transgenic mites to disperse from the release site. For the initial trial (in April 1996), spider mites and transgenic predatory mites were released. Both predatory and spider mite populations declined rapidly due to heavy rains and low temperatures, forcing the termination of the experiment. By this time, few of the predatory mites contained the plasmid sequences, indicating that the sequence was unstable in the field. This was unexpected, because the plasmid sequence had persisted in the transgenic mites for more than 150 generations under laboratory condition, and no differences in several fitness parameters were detected between transgenic and wild-type mites (Li and Hoy, 1996). The plasmid sequence was also rapidly lost during a second trial carried out in October 1996 with mites from six additional transgenic lines. Little to no dispersal of the transgenic mites was observed *M. occidentalis* is not adapted to the climate is Florida, and held trials in the western United States, which it the native habitat of this species, may have yielded different results.

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