References and Notes

1. M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.* **11**, 423 (1962).
2. J. J. Furth and S. S. Cohen, *Cancer Res.* **28**, 2061 (1968); A. P. Kimball and M. J. Wilson, *Proc. Soc. Exp. Biol. Med.* **127**, 429 (1968).
3. M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.* **14**, 333 (1965); **17**, 752 (1968); S. Silagi, *Cancer Res.* **25**, 1446 (1965).
4. W. F. Benedict, N. Harris, M. Karon, *Cancer Res.* **30**, 2477 (1970).
5. E. H. Y. Chu, *Mutation Res.* **2**, 75 (1965).
6. M. Karon and S. Shirakawa, *Cancer Res.* **29**, 687 (1969).
7. ———, *J. Nat. Cancer Inst.* **45**, 861 (1970).
8. P. C. Hanawalt and R. B. Setlow, *Biochim. Biophys. Acta* **41**, 283 (1960); A. Kelner, *J. Bacteriol.* **65**, 252 (1953).
9. R. E. Rasmussen and R. B. Painter, *J. Cell Biol.* **29**, 11 (1966); J. E. Cleaver, *Nature* **218**, 652 (1968).
10. R. B. Painter and J. E. Cleaver, *Radiat. Res.* **37**, 451 (1969); J. E. Cleaver and R. B. Painter, *Biochim. Biophys. Acta* **161**, 552 (1968).
11. R. B. Painter and J. E. Cleaver, *Nature* **216**, 369 (1967).
12. C. F. Brunk and P. C. Hanawalt, *Radiat. Res.* **38**, 285 (1969).
13. J. E. Cleaver, *ibid.* **37**, 334 (1969).
14. ———, *Int. J. Radiat. Biol.* **16**, 227 (1969).
15. N. R. Draper and H. Smith, *Applied Regression Analysis* (Wiley, New York, 1966); W. G. Cochran, *Biometrics* **10**, 417 (1954).

16. Supported by grants CA-11050 from the PHS and T-493 from the American Cancer Society. We acknowledge the expert technical assistance of Natalie Harris and Paul Saarinen and the statistical advice of Joseph Garfinkel. M.K. is a Leukemia Society of America Scholar. Send reprint requests to M.K.

* Present address: Section on Developmental Enzymology, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development, Bethesda, Maryland. 20014.

14 August 1970

Insect Control by Genetic Manipulation of Natural Populations

Abstract. *The possible use of chromosome rearrangements is considered as a means for introducing genes into insect populations for their own control. The release of laboratory-constructed strains differing from the field population for a number of chromosome interchanges should create an unstable situation leading to the rapid replacement of the field population. This replacement should allow introduction of genes for insecticide susceptibility, cold sensitivity, or the like. The process would produce sterile hybrids while the genetic displacement occurs which itself will contribute to a reduction in pest numbers.*

The ability of insects to evolve resistance to insecticides continues to pose a major problem in controlling many pest species. Furthermore the absence of selective forces to reduce sufficiently the frequency of "resistance" genes in natural populations after insecticide application prevents reuse of discarded insecticides. The often limited life of insecticides has led to attempts to modify the outmoded insecticide by altering those portions of the molecule subject to attack by the resistance mechanisms (*1*), or else by synthesizing new, and sometimes more potent, compounds. Little attention has been given to the possibility of manipulating the insect population to remove the resistance genes in a limited number of generations and thus permitting the reuse of otherwise suitable insecticides.

Meiotic drive, one suggested candidate for such a task (*2*), has become less attractive with the discovery that it may invariably cause recessive sterility (*3*), thus preventing it, along with the susceptibility genes that it would carry, from becoming fixed in a population. I now suggest a possible system using homozygous chromosomal interchanges (translocations) which allows the rapid removal of insecticide resistant genes, while, at the same time, permitting the infusion of other "useful" genes into natural populations. Since the system can engender additionally a high level of inherited sterility which by itself may reduce population numbers (*4, 5*), it is suggested that adequate control may be provided for some species of insects by a period of insecticide application until resistance evolves, followed by a period where synthetic strains are released. Thus these releases provide the twofold service of direct control via high zygotic mortality while the appropriate gene substitutions occur. The whole cycle can then be repeated once the resistance genes have been removed and effectiveness is restored to the insecticide.

The production and isolation of translocations are routine procedures, particularly in the higher Diptera—such as the housefly, the screwworm fly, the Australian sheep blowfly, and *Drosophila*—where marker genes are available and the absence of genetic crossing-over in males makes translocations easier to detect. Since some 30 percent of translocations are viable and fertile as homozygotes (*6*) and do not show any visible phenotypic effects, the collection of homozygous translocations should be quite feasible for some important pest species (*7*). By appropriate backcrossing it should be possible to have available two strains, each with similar genomes but differing for one homozygous interchange. The backcrossing (i) eliminates recessive lethals that may have been carried by the two chromosomes involved in the translocation and (ii) restores the initial variability that was present before the translocation was isolated.

A serial repetition of the procedure should allow the synthesis of a strain homozygous for several translocations but, once again, differing in no other respect to the base strain. Although this multiple translocation strain can be expected to equal the base strain in fitness in that it contains a similar spectrum of genetic variability, a translocation hybrid resulting from a cross between the two strains should be nearly sterile if sufficient translocations have been incorporated.

We can formally equate the situation where a multiple translocation strain and the base strain are mixed to the single locus case of two alleles, A and T, representing the normal and translocated sequences. Let w_1, w_2, and w_3 be the relative genetic fitness of AA, AT, and TT, respectively. Since it is assumed that w_1 and w_3 have values near 1 while w_2 is near zero, because AT is nearly sterile though viable, we have a sufficient condition for an unstable equilibrium (*8*). Thus if q is the frequency of T, we have for $q > \hat{q}$, $q \to 1$; for $q < q$, $\hat{q} \to 0$, where $\hat{q}$ is the equilibrium frequency of T and is given by

$$\hat{q} = (w_1 - w_2)/(w_1 - 2w_2 + w_3)$$

Of particular interest is the rapidity of the replacement of A by T. Suppose the frequency of T exceeds the equilibrium by as little as 0.05. Within six or seven generations A is eliminated from a finite population after a single release of the same size order as the native population (Table 1). It can also be noted from Table 1 that $\hat{q}$ depends primarily on the fitness of TT relative to AA, while the rate of displacement of A is largely a function of the level of sterility of AT. It is important to observe that a genotype can be displaced by a less fit genotype provided that the less fit genotype begins in sufficient frequency.

Thus, if we can induce a set of homozygous translocations in a strain taken from the field and then return this strain in a higher frequency to the native population, we can expect that portion of the genome of the native

population involved in the translocations to be replaced by the synthetic strain (*9*). With hybrids virtually sterile there would be little opportunity for a "wild" nontranslocation chromosome to transfer over to the displacing strain. It is possible to accelerate the rate of displacement of the native strain by increasing the relative numbers of the released strain. For example, releases in the ratio of 2:1, 4:1, and 10:1 to the native strain reduce by one, two, and three, respectively, the number of generations for frequency of A to reach 10^{-8}. This would give a frequency of the native form as low as 10^{-16}.

By ensuring that the synthetic strain carries the appropriate insecticide susceptibility genes at the outset—this may involve a short selection program with the use of field collections—its displacement of the native population should allow the reuse of insecticides after some five generations.

One objection is that the release of synthetic strains entails an initial increase in population size because of the release of fertile individuals. However this increase is more than offset in ensuing generations by the presence of sterile individuals (*4, 5*). If $(n-1)$ multiple translocation strains are developed and each is released in equal frequency to the native population, it can easily be shown that only $1/n$ individuals of the resulting generation will be fertile while as few as $1/n^2$ matings among these will produce any offspring. Serebrovskii (*5*) made a significant error in supposing that the release of a single strain could give a zygotic mortality or genetic load of 100 percent if a sufficient number of translocations are included in the released strain. A 75 percent load represents the theoretical maximum with the release of one strain because that is the level obtained when the hybrid is fully sterile; but if more strains are liberated, a greater proportion of matings in the following generations will involve one or both parents being sterile. However, this level of sterility will not persist indefinitely because the least frequent strain will be at a selective disadvantage; thus one by one each strain will be eliminated until finally only one strain remains.

Table 2 gives an indication of the rate of elimination of the native strain after the simulated release of one and four synthetic strains in a set of computer trials. One of the synthetic strains is given a sufficient numerical advantage, empirically determined at about 10 percent, to ensure its ultimate fixation. A random number generator for the selection of genotypes each generation, together with finite population size, introduces a stochastic element into the model. Sampling error, due to small population size, tends to retard the rate of fixation of the synthetic strain below the predictions of the deterministic model used for Table 1.

Table 3 indicates the genetic load (each generation in a parallel set of simulation runs after the release of one, four, and eight synthetic strains). For simplicity it has been assumed that each strain is equal in fitness to the native strain but as was mentioned above this condition is not essential. The genetic load remains quite constant for four or five generations as predicted by Serebrovskii (*5*) until most of the substitution has taken place. It then drops rapidly in the final stages. The initial weighting of one of the released strains has several important implications. First, where several strains are released it ensures the fixation of one of the synthetic strains. Second, in a control program covering a large area it can be used to fix different synthetic strains in neighboring areas. Thus, the initial release should trigger off a population collapse probably leading to the formation of pockets each

Table 1. The rate of substitution of a gene when its frequency exceeds the unstable equilibrium value ($q = \hat{q} + 0.05$).

Relative fitness			Equilibrium value	No. of generations for frequency of A to equal:		
(AA) w_1	(AT) w_2	(TT) w_3	$\hat{q}$	10^{-3}	10^{-6}	10^{-8}
1	0	1.0	0.50	5	6	7
1	0	0.9	0.5263	5	6	7
1	0	0.8	0.5556	5	6	7
1	0	0.7	0.5882	5	6	7
1	0.2	1.0	0.50	8	11	15
1	0.2	0.9	0.5333	8	11	15
1	0.2	0.8	0.5714	9	12	15
1	0.2	0.7	0.6154	9	13	17
1	0.5	1.0	0.50	16	23	29
1	0.5	0.9	0.5556	17	25	33
1	0.5	0.8	0.6250	20	30	39
1	0.5	0.7	0.7143	24	38	51

Table 2. Estimated percentage of populations* where the native strain would be eliminated after the release of multiple translocation strains. Forty populations of 2000 individuals each were used to provide these percentages.

Strains released* (No.)	Generations (No.):					
	5	6	7	8	9	10
1	2.5	55.0	90.0	100.0		
4	17.5	60.0	77.5	85.0	95.0	100.0

* One of the released strains was given a numerical advantage of 12 percent; $w_1 = w_3 = 1$ and $w_2 = 0$.

Table 3. Estimates from simulation studies of the genetic load after release of varying numbers of multiple translocation strains. Each strain is assumed equally fit, and all hybrids are assumed sterile. One of the released strains was given a numerical advantage of 8.4 percent. Figure in parentheses in indicates the number of populations studied. Each population contained 2000 individuals.

Generation	Genetic load (%) from release of:		
	One strain (40)	Four strains (15)	Eight strains (5)
1	74.8	95.9	98.7
2	74.5	96.1	98.8
3	73.4	95.7	98.6
4	68.2	94.8	98.1
5	52.6	89.1	91.8
6	26.2	64.0	74.2
7	6.9	24.6	57.7
8	2.2	7.7	36.5

fixed for a different translocation complex. As these isolates expand and integrate, new translocation heterozygotes arise. Further genetic load is produced, which should lead to a second population decline, though of lesser magnitude. In theory this method could be used to replace an insecticide-resistant native strain with a susceptible released strain. Once this was achieved there are alternative courses open for future action. Insecticides could be reintroduced until resistance redevelops and so the cycle repeated.

For a variety of reasons we may prefer to manipulate the population in other directions as well. For example, the introduction of recessive cold-sensitive mutants (*10*) might merit consideration, provided that their pleiotropic effects are found to be suitable. If a multivoltine native insect population could be virtually replaced during a single season by a synthetic strain homozygous for a cold-sensitive mutant, the overwintering stages would succumb and a very high proportion of the population would be eliminated.

If we consider the release of more than one synthetic cold-sensitive strain, the idea becomes feasible for certain dipteran populations. For a species such as the Australian sheep blowfly with six or more generations a year, if we release, early in spring, four synthetic strains each carrying the same recessive lethal which acts only at low temperatures, then we might expect a load in excess of 90 percent over the next five generations while the lethal is being spread through the population. At the onset of winter when the lethal would have its effect, its frequency should exceed 0.999; the resulting genotypic frequencies would be AA, 0.000001; AT, 0.001998; and TT, 0.998; where T is carrying the temperature lethal. Consequently, less than one in a million flies entering winter will both survive the winter and be fertile. This rate of gene substitution is significantly more rapid than any meiotic drive mechanism could achieve.

The release of several translocation strains in which heterozygotes are sterile, once they are available, would appear to be more effective with less effort than the sterile male method of control and may therefore prove economical for some species where cost factors preclude the use of sterile males. For example, a single release of four synthetic strains, in equal frequency to the native population, can be shown to cause a population reduction equivalent to a 20:1 sterile-to-normal-male release repeated for five generations. It provides a further advantage in allowing direct manipulation of the genetic composition of the insect population.

Such a program would seem suited to some higher Diptera, potential candidates including the Australian sheep blowfly, the housefly, and various fruit flies. The presence of crossing-over in male mosquitoes and the availability of only three pairs of chromosomes that would affect the ease with which multiple translocation strains are produced appear to make these important vectors of disease unsuited. However, complete heterozygote sterility is not essential. If the genes to be fixed are included in inversions prior to the induction of translocations it may be possible to prevent their replacement by undesirable alleles from the native strain during the period of displacement, which is the major problem arising from incomplete sterility of the heterozygote. In those instances where there has been leakage of genes through the heterozygote, it may be necessary to make a succession of releases until adequate gene substitution is obtained. Mosquito species have been the subject of a special study (*11*) that indicates it should be possible to substitute genes in less than ten generations while providing a permanent genetic load of 50 percent.

Once a set of suitable strains has been developed and the system has been shown to work over the first cycle of releases, its permanence is ensured since natural selection cannot oppose it; rather, natural selection is an essential ingredient of the program. The prospects of an indefinite life for an insecticide, made possible by genetic manipulation, may provide incentive for the development of better insecticides whose synthesis might otherwise have been economically unattractive.

M. J. WHITTEN

Division of Entomology,
CSIRO, P.O. Box 109,
Canberra, Australia

References and Notes

1. G. Holan, *Nature* **221**, 1025 (1969).
2. G. Craig and W. Hickey, in *Genetics of Insect Vectors of Disease*, J. Wright and R. Pal, Eds. (Elsevier, London, 1967); L. Sandler and E. Novitski, *Amer. Natur.* **91**, 105 (1957).
3. D. Hartl, *Proc. Nat. Acad. Sci. U.S.* **63**, 782 (1969); Y. Hiraizumi and S. S. Watanabe, *Genetics* **63**, 121 (1969).
4. C. Curtis, *Bull. Entomol. Res.* **57**, 509 (1968).
5. A. S. Serebrovskii, *Zool. Zh.* **19**(4), 618 (1940).
6. C. Burnham, *Discussion in Cytogenetics* (Burgess, Minneapolis, 1962); J. P. Patterson, W. Stone, S. Bedichek, M. Luche, *Amer. Natur.* **68**, 359 (1934). Patterson *et al.* isolated 1992 translocations and examined 332 for viability and fertility. They found that 40 percent were viable as homozygotes and 90 percent of these were fertile, so that 36 percent were both viable and fertile.
7. The spontaneous generation and fixation of translocation during the course of evolution is a very common phenomenon, indicating that translocations need not be associated with a reduction in fitness. See M. J. Whitten, "The genetics of pest species in their management," in *Concepts in Pest Management*, R. Rabb and F. Gruthrie, Eds. (North Carolina State Univ. Press, Raleigh, 1970).
8. C. C. Li, *Amer. Natur.* **89**, 281 (1955).
9. C. Curtis [*Nature* **218**, 368 (1968)] has suggested the use of translocations to fix desirable genes in insect pest populations. He has restricted consideration to single translocations which does not fully reveal the power of the technique. It would be difficult to include more than one translocation in a strain of tsetse fly but serious problems should not be encountered in many other pest species.
10. D. Suzuki, L. K. Piternick, S. Hayashi, M. Tarasoff, D. Baillie, U. Erasmus, *Proc. Nat. Acad. Sci. U.S.* **57**, 907 (1967); D. Suzuki *et al.*, *ibid.*, in press; D. Suzuki, *Science* **170**, 695 (1970).
11. M. J. Whitten, The use of chromosome rearrangements for mosquito control is discussed in "*Sterility Principle for Insect Control or Eradication*" (Joint FAO/IAEA Symposium, Athens, September, 1970).
12. I thank Drs. R. Hughes and D. F. Waterhouse for criticism of this manuscript.

18 August 1970; 19 October 1970

Hydrocarbon Sex Pheromone in Tiger Moths (Arctiidae)

Abstract. *2-Methylheptadecane is a sex pheromone compound in many sibling species of the* Holomelina aurantiaca *complex, in* Holomelina laeta, *and in* Pyrrharctia isabella, *which are all arctiids. Habitat preference, temporal distribution, and differing diurnal cycles help effect reproductive isolation among the species, but secondary sex pheromone chemicals are also suggested by the field studies.*

Males of many species in the families Gelechiidae, Noctuidae, Pyralidae, and Tortricidae have been attracted under natural field conditions to specific monounsaturated alcohols or acetates (*1*). Species from many other lepidopterous families have been conspicuously absent in these field screening tests, presumably because monounsaturated fatty alcohols and acetates represent only one of several classes of compounds used as attractants in the Lepidoptera. We have now found that in the family Arctiidae a saturated aliphatic hydrocarbon, 2-methylheptadecane, is a sex pheromone for at least nine species.

The pheromone was obtained by extracting the abdominal tips of 50 *Homomelina nigricans* (*2*) females