EVOLUTION OF RESISTANCE IN THE PRESENCE OF TWO INSECTICIDES

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Manuscript received August 1, 1984
Revised copy accepted November 12, 1984

ABSTRACT

A two-locus model is used to analyze the effectiveness of a mixture of insecticides in delaying resistance, compared to the use of the insecticides singly. The effects of factors such as recombination, effective dominance, initial value of allele frequencies and initial value of linkage disequilibrium are considered. It is shown that the use of mixtures is always more effective in delaying the onset of resistance, often by many orders of magnitude. It is shown that there exists a threshold value of recombination fraction, above which the evolution of resistance is extremely slow. Resistance evolves very rapidly for values of recombination fraction below the threshold. Finally, the relevance of these results on resistance management is discussed.

IN the past two decades there has been extensive study of the properties of two-locus systems under selection. Most of the work has been concerned with symmetric viabilities with multiplicative or additive assumption (KARLIN and FELDMAN 1970, 1978; KARLIN 1975; BODMER and FELSENSTEIN 1967; FRANKLIN and FELDMAN 1977; FELSENTEIN 1965; KIMURA 1965). These theoretical studies, especially for two alleles at two loci, have shown the increase in the complexity of the polymorphic states as one goes from one locus to two loci problems. A few applications of two-locus models to problems of familial diseases can be found in the literature (MERRY, ROGER and CURNOW 1979; SKOLNICK, CARMELLI and TYLER 1977). In this paper the evolution of resistance in the presence of two insecticides is studied using a specific two-locus model. As shown later, the use of two insecticides simultaneously could, under suitable conditions, provide an effective method in the management of resistance. The rest of this section is devoted to a brief review of the present status in resistance management.

In his review on the "Management of resistance in arthropods," GEORGHIOU (1983) has listed three main modes of chemical strategies used or suggested for the management of resistance. These are (1) management by moderation, (2) management by saturation and (3) management by multiple attack. The first two modes involve the use of a single insecticide and the management is effected through factors such as control of effective dominance, preservation of "refugia" and suppression of detoxication mechanism by synergists. The

effects of these various factors on the evolution of resistance have been extensively discussed in the literature (SUTHERST and COMMINS 1979; GEORGHIOU and TAYLOR 1977; MARCH 1953; WOOD and MANI 1981; MANI and WOOD 1984). For mode 3 Georghiou gives two subdivisions, namely, the use of mixtures of chemicals and the alternation of chemicals either in space or in time. Spatial alternation of chemicals was first suggested by MUIR (1977) and has been theoretically studied by COMINS (1977) and by CURTIS, COOK and WOOD (1978) for some restricted types of migration. CURTIS and RAWLINGS (1980) have shown that the effectiveness of such a strategy depends on a high migration rate. Recently, CURTIS (1984; see report by WOOD 1981a) has shown that mosaic spraying with two compounds sprayed in alternate mosaics would be little different from the use of the two compounds sequentially over the whole area. The use of the strategy of rotation of chemicals in time has been considered both theoretically and experimentally (see GEORGHIOU 1983 for references). This mode of application of chemicals requires that the individuals resistant to one chemical have a lower biotic fitness than susceptible individuals so that their frequency declines during the period when that chemical is not used. The rate of decline depends both on the fitness depression of the resistance homozygotes and on the dominance relation for the heterozygotes. Also, in some instances it has been demonstrated that the fitness of resistant individuals could improve with continued selection through the process of coadaptation (ABEDI and BROWN 1960; MCENROE and NAEGELE 1968; GEORGHIOU 1972; KEIDING 1963, 1967).

The use of mixtures has been mentioned frequently in the literature (BROWN 1961, 1976; CROW 1952, 1960; BUSVINE 1957). GEORGHIOU (1983) has presented some experimental results on the effect of mixtures in the evolution of resistance. In this experimental work, LAGUNES and GEORGHIOU (GEORGHIOU 1983) have studied the changes in susceptibility to the three insecticides temephos, propoxur and permethrin in sibling strains of *Culex quinquefasciatus* when these compounds are applied singly or in various combinations. The results indicated that, in the case of temephos and permethrin, resistance was delayed when the chemicals were used in mixtures. On the other hand, resistance to propoxur seemed to develop when it is used as part of a mixture. Since these results were on the larval stages of the mosquito and since such laboratory experiments do not specifically allow for escape, the results obtained cannot easily be translated into the effect in the field. The importance of escape in dealing with mixtures has been stressed recently by CURTIS (1984).

In this paper a detailed analysis of the effect of a mixture of two insecticides on the evolution of resistance is investigated. The dependence of the rate of evolution of resistance on factors such as recombination, initial value of linkage disequilibrium, effective dominance and initial gene frequencies is discussed. For sake of clarity, the derivation of the equations and some of the analytic results are given in the APPENDIX.

THE MODEL

Two insecticides, labeled A and B, act on loci A and B, respectively, and produce no cross-resistance. There are two alleles A_1, A_2 and B_1, B_2 at these

loci, A_1, B_1 being susceptible and A_2, B_2 being resistant to the respective insecticides. The population is assumed large enough for stochastic effects such as genetic drift to be unimportant. Since it was shown in MANI and WOOD (1984) that density-dependent regulation of population size has minimal effect on the evolution of resistance, such regulation is not considered here. All matings are assumed to be random.

Any practical application of insecticides in the field would result in a fraction, α (defined as escape probability), of the insects escaping the effects of the insecticide. The escape probability is strictly an "effective" escape parameter which takes into account partial exposures that do not kill the insect but only make it less effective in mating and reproduction. Any genotype dependence on α arising from such a definition is ignored. Evolution of resistance in the presence of escape has been extensively studied in two earlier papers (WOOD and MANI 1981; MANI and WOOD 1984) and, thus, in this paper an arbitrary value of $\alpha = 0.1$ for escape is assumed.

The frequencies of the gametes A_1B_1, A_1B_2, A_2B_1 and A_2B_2 are denoted by X_1, X_2, X_3 and X_4, respectively. The frequencies of the alleles A_1, A_2, B_1 and B_2 are given by $p_1(n)$, $p_2(n)$, $q_1(n)$ and $q_2(n)$ in generation n. The linkage disequilibrium factor is given by $D = X_1X_4 - X_2X_3$ and the recombination factor by r.

The dosage of insecticide applied is assumed to be large enough to kill all susceptible homozygotes and a fraction $(1 - h_a)$ or $(1 - h_b)$ of the heterozygotes at locus A or B but no resistant homozygotes. Here, h_a and h_b represent effective dominance that can be varied by altering the dosage applied (see WOOD and MANI 1981). When h_a or $h_b = 0$, the resistance gene in the corresponding locus is recessive and, when h_a or $h_b = 1$, it is dominant.

Determination of differential biotic fitness in the field is subject to large uncertainities. Thus, very few such measurements have been attempted. CURTIS, COOK and WOOD (1978) have discussed some of these problems in the case of *Anopheles culicifacies* and *A. stephensi* exposed to DDT, dieldrin and malathion. In the case of *A. gambie* there are some indications in laboratory tests that the biotic fitness of the resistant homozygote is slightly lower than the susceptible homozygote in the absence of the insecticides DDT and dieldrin (EMEKA-EJIOFUR, CURTIS and DAVIDSON 1983). Such a small fitness difference would have a minimal effect on the evolution of resistance, in comparison with the effect due to the strong selective forces arising from the presence of insecticides. Thus, in the present investigation any difference in biotic fitness is neglected. Finally, two-locus selection arising from the application of the insecticides is assumed to be of the multiplicative form.

The following two modes of application of the insecticides are considered: Type 1: Both insecticides are applied simultaneously every generation. The dosage of each insecticide can be varied independently to change the effective dominance for that insecticide. Type 2: Insecticide A is applied for T_A generations until the resistance to it is established. In what follows, the resistance to an insecticide is assumed to have been established when the frequency of the corresponding resistant gene reaches a value of 0.9. The insecticide B is then applied until the resistance to it is established. The total number of genera-

tions, $t_2 = T_A + T_B$, defines the time taken for the population to become resistant to both of the insecticides. The variation of T_A (or T_B) with effective dominance, initial gene frequencies and escape has been extensively studied by MANI and WOOD (1984). Throughout this paper t_1 refers to the number of generations for resistance to evolve when mixtures are used and t_2 when the two insecticides are used sequentially as in type 2.

The explicit equations used in the calculations together with some approximate solutions are given in the APPENDIX. The program was set up to answer the following questions: (1) How does the evolution of resistance change with effective dominance? (2) What is the effect of initial allele frequencies on the evolution of resistance? (3) What difference does it make whether selection occurs before or after mating? (4) What effect does recombination and initial value of linkage disequilibrium have on the evolution of resistance. For type 2 application, questions (1) to (3) have already been discussed in earlier papers (WOOD and MANI 1981; MANI and WOOD 1984).

RESULTS AND DISCUSSION

The values of t_1 for various values of h_a and h_b are shown in Figures 1–3. Throughout this section it is assumed that the initial gene frequencies satisfy $p_2(0) = q_2(0)$. In Figures 1–3, the initial gene frequency of the resistant gene is taken to be 10^{-3}. These figures also show the effect of recombination on t_1. Two values of recombination were used, namely, $r = 0$ (tight linkage) and $r = 0.5$ (loose linkage). As shown in the APPENDIX (A.3), for a given value of $p_2(0)$ $(= q_2(0))$, the initial linkage disequilibrium, $D(0)$, has to lie within the interval $\{-p_2(0)q_2(0), +p_1(0)q_2(0)\}$. Figures 1 and 2 are for $D(0) = 0$ and $D(0) = p_1(0)q_2(0)$, its maximum value, in Figure 3. The broken lines in these figures are for the case in which selection acts only after mating (MS), and the full curves are for selection acting before mating (SM). Changing the initial values of the gene frequencies does not alter the shape of the curves but only changes the value of t_1. As the initial values of the frequencies of the resistant genes are decreased, the values of t_1 are correspondingly increased. From these results the following general conclusions can be made:

1. In almost all cases, as h_a increases from 0 to a small value, say 0.02, t_1 decreases by three to six orders of magnitude, depending on the initial allele frequencies and the value of the recombination fraction, r. The only exception is when $D(0)$ is large and positive and r is near 0 (Figure 3). For $r = 0$, as h_a increases from 0.02 to 1.0, t_1 decreases by a factor of 2–10, depending on the value of h_b. Thus, to obtain a large delay in the evolution of resistance, the dosage of at least one of the insecticides should be sufficiently high for the resistant allele to become recessive.

2. When r increases from 0 to its maximum value of 0.5, the evolution of resistance is delayed in all situations. For $r = 0.5$, the initial decrease in t_1 with increasing h_a is much less steep than for $r = 0$. Thus, in this case, the above stringent condition for recessiveness can be relaxed to some extent.

3. In general, in the tight linkage limit, t_1 for the case in which selection precedes mating is about half of t_1 when selection acts after mating. This

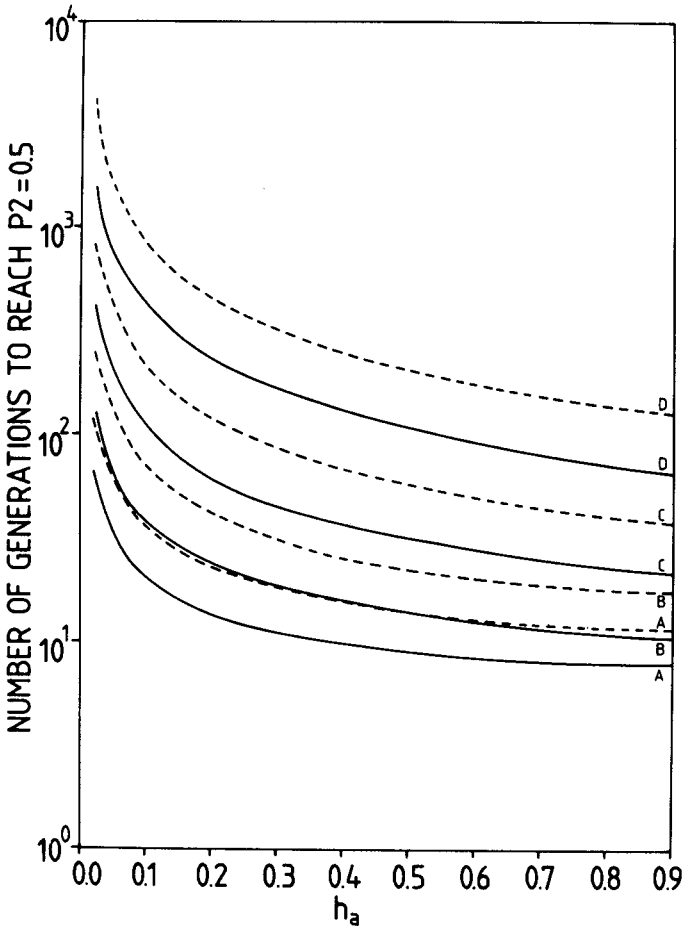

FIGURE 1.—The dependence of the number of generations for resistance to evolve on the effective dominance of one of the insecticides, while that of the other insecticide is fixed. Here, h_a and h_b denote the effective dominance of the two insecticides. The initial allele frequency is taken to be $p_2(0) = q_2(0) = 0.001$; the initial linkage disequilibrium $D(0) = 0$ and the recombination fraction $r = 0$. The escape probability $\alpha = 0.1$ and the insecticides are applied simultaneously. The curves marked A–D are for the following values of h_b: A, $h_b = 1.0$; B, $h_b = 0.4$; C, $h_b = 0.1$; D, $h_b = 0.02$. The broken line corresponds to mating before selection and the solid line to mating after selection.

difference between selection before and after mating arises from the fact that selection before mating (SM) affects genotype distribution of both sexes, before mating has occurred. On the other hand, for selection after mating, (MS), one need only consider the mated females. The selection on them would have the effect of reducing relatively more resistant homozygotes from the next generation than in the case of SM. Thus, the onset of resistance is delayed in the case of MS compared to SM. The reason for the factor of 2 in the case of tight linkage is discussed in the APPENDIX (*cf.* (A.33). This factor could be much larger than 2 as r increases toward 0.5. As can be seen in Figure 2, this

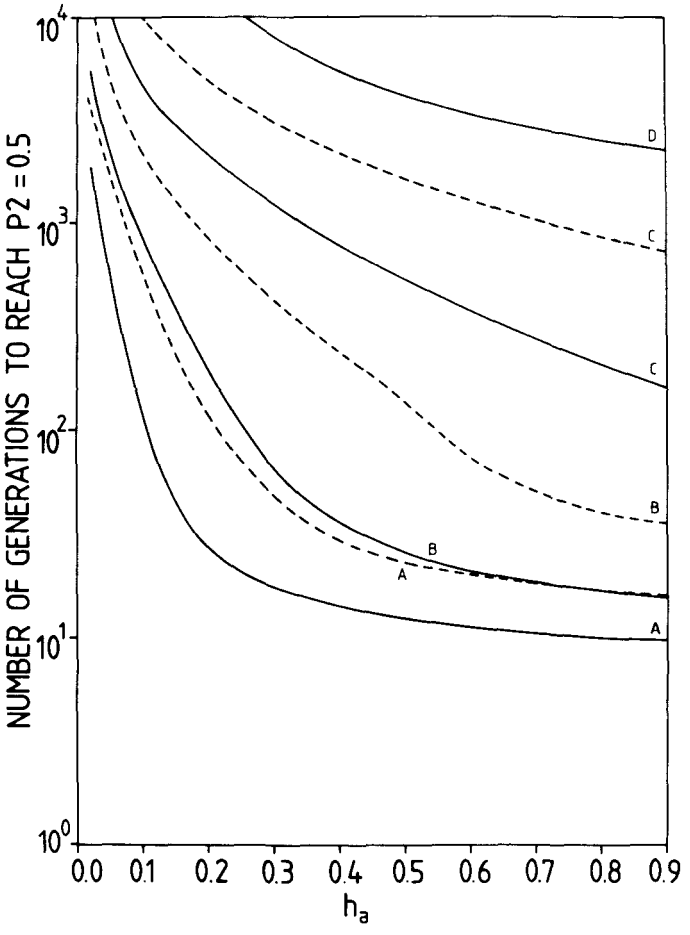

FIGURE 2.—The dependence of the number of generations for resistance to evolve on the effective dominance of the insecticides. All parameters are the same as in Figure 1, except the recombination fraction $r = 0.5$. Curves A–D have the same significance as in Figure 1. The broken and solid lines are for mating before and after selection, respectively.

factor could be as high as 10 for $r = 0.5$. Thus, in resistance management, it is always preferable to apply the insecticide, if possible, at the postmating stage of the life history rather than at the larval or premating adult stage.

The effect of recombination on the evolution of resistance: Figures 4 and 5 show the influence of recombination fraction on the evolution of resistance. In these figures the initial allele frequencies are varied between 10^{-2} and 10^{-4} and the effects are studied for various values of $D(0)$, h_a and h_b. The remarkable feature of these curves is the existence of a threshold value for r, above which t_1 is large and approximately constant and below which it is small and constant. The threshold value of r is strongly dependent on the initial allele frequencies, effective dominance and the initial value of linkage disequilibrium. The occurrence of a threshold in the value of r is not self-evident from the equations

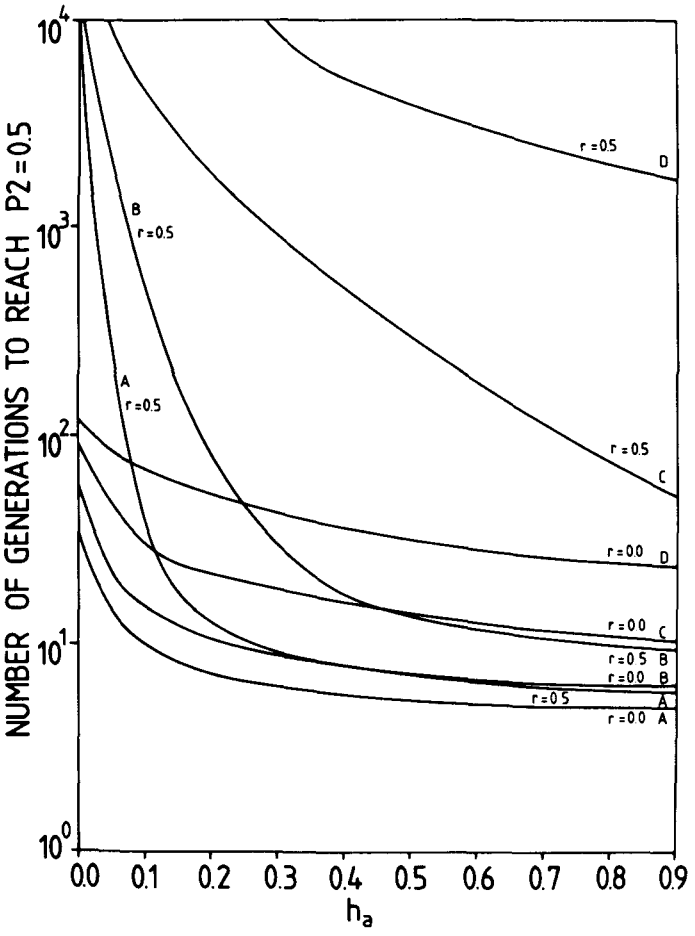

FIGURE 3.—The dependence of the number of generations for resistance to evolve on the effective dominance of the insecticides when $D(0) = 0.999 \times 10^{-3}$, its maximum value, and when $r = 0.0$ and 0.5. The curves are for selection before mating. The curves marked A–D have the same significance as in Figure 1.

for the evolution of resistance. A plausible reason for the threshold effect is discussed in the APPENDIX. In general, for r sufficiently greater than 0, and for $D(0) \neq 0$, the linkage disequilibrium goes rapidly to 0 initially and thereafter the dynamics are the same as for the case when $D(0) = 0$. This is the reason for the curves with different values of $D(0)$ to yield almost the same value of t_1 above the threshold. The threshold in r becomes less well defined as the effective dominance increases. Also, in this case the threshold is pushed toward a higher value of r. The threshold moves toward lower values of r with decreasing values of the initial allele frequencies and of $D(0)$.

The effect of initial value of linkage disequilibrium on the evolution of resistance: Figures 6–8 show the variation of t_1 with $D(0)$ for various values of initial allele frequencies, recombination fraction and effective dominance. Note that

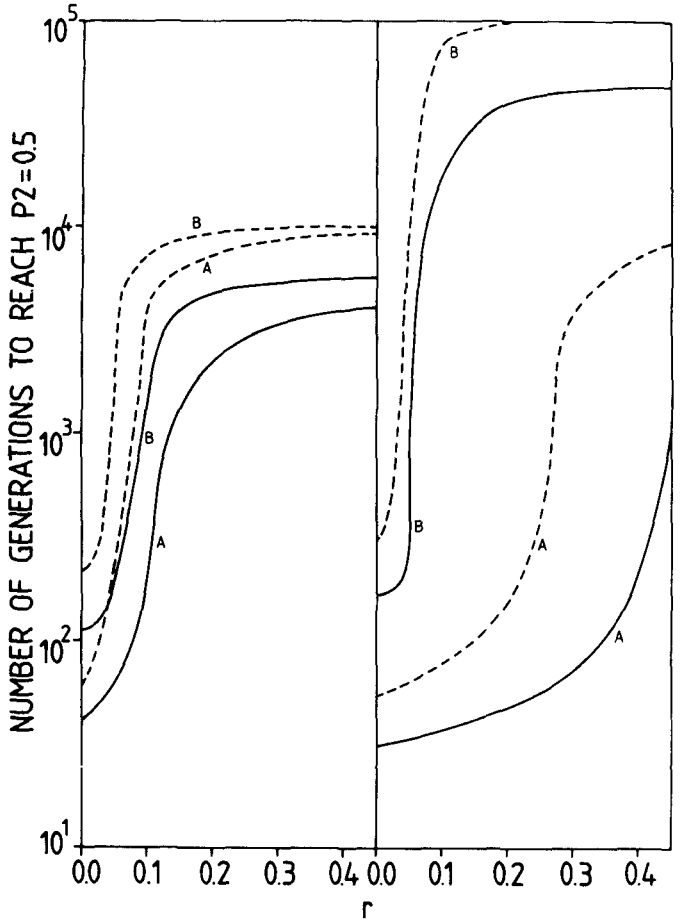

FIGURE 4.—The dependence of the number of generations for resistance to evolve on the recombination fraction r. The curves on the left are for $p_2(0) = q_2(0) = 10^{-3}$, $h_a = h_b = 0.1$ and $\alpha = 0.1$. The curves marked A and B are for $D(0) = 0.0$ and 0.999×10^{-3}. The curves on the right are for $p_2(0) = q_2(0) = 10^{-4}$, $D(0) = 0$ and $\alpha = 0.1$. The curves marked A are for $h_a = 0.4$ and $h_b = 0.2$ and the curves marked B are for $h_a = h_b = 0.1$. The broken lines show the case in which mating precedes selection and the solid line when selection precedes mating.

in Figure 6 the scale of $D(0)$ below 0 is expanded by a factor of 100. Since a new mutant would normally be expressed at first as a heterozygote, in real biological situations one would not expect a large positive value for $D(0)$. If the field population had already been exposed to one of the two insecticides so that the frequency of the resistant gene is higher than the mutation frequency, $D(0)$ can be positive. Thus, in actual situations $D(0)$ can be slightly positive or negative, depending on the type of selective pressure that the population has experienced. For $r < r_T$, where r_T is the threshold value discussed in the previous section, t_1 is extremely sensitive to the value of $D(0)$ and decreases rapidly with increasing value of $D(0)$. For $r > r_T$, t_1 becomes very insensitive to the variation of $D(0)$.

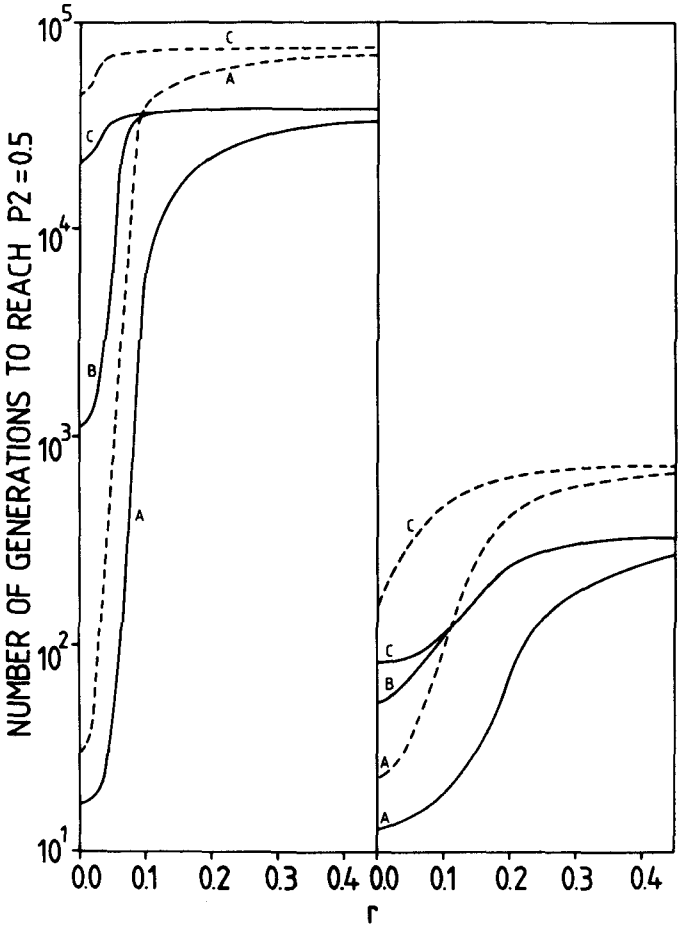

FIGURE 5.—The dependence of the number of generations for resistance to evolve on the recombination fraction r. The initial allele frequencies are $p_2(0) = q_2(0) = 0.01$. The curves marked A, B and C are for $D(0) = 0.009$, 0.0 and −0.000095, respectively. The broken lines are for the case in which mating precedes selection and the solid line when selection precedes mating. The curves on the left are for $h_a = h_b = 0$ and on the right are for $h_a = h_b = 0.1$.

Comparison of type 1 and type 2 applications: In the case in which the insecticide A is applied on its own, the alleles at locus B would naturally experience no selection. On the other hand, the frequency of the alleles at locus B could alter through the "hitch-hiking" effect (MAYNARD SMITH and HAIGH 1974). The theory of the hitch-hiking effect for the case of type 2 application is discussed in the APPENDIX. The effect of hitch-hiking on the frequency of the resistant allele at locus B is maximal when the recombination fraction r is close to 0 and $D(0)$ well removed from 0. When $D(0) < 0$, the frequency of the resistant allele at locus B would decrease from its initial value and, for $D(0) > 0$, it would increase. It is shown in the APPENDIX that the effect of hitch-hiking in altering the frequency of the resistant allele at locus B when the insecticide

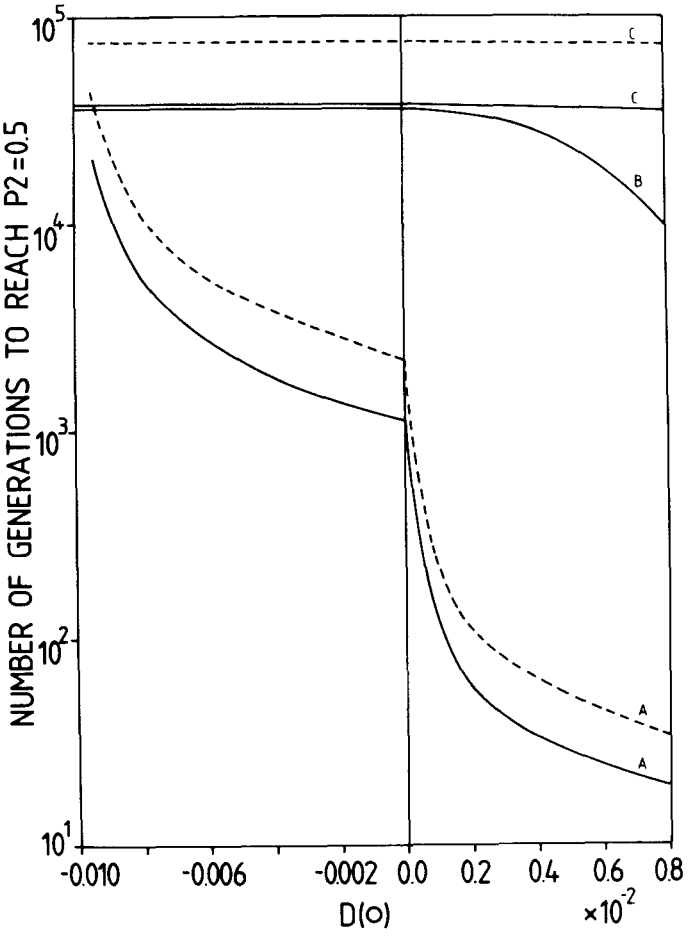

FIGURE 6.—The dependence of the number of generations for resistance to evolve on the initial value $D(0)$ of linkage disequilibrium. Here, $p_2(0) = q_2(0) = 0.01$, $h_a = h_b = 0$ and $\alpha = 0.1$. The curves marked A, B and C are for $r = 0$, 0.1 and 0.4, respectively. The broken and the solid lines represent mating before and after selection, respectively. Note the change in the scale for $D(0) < 0$ by a factor of 100.

A is applied on its own rapidly disappears as $D(0)$ moves away from its extreme values and as the value of r slightly increases above 0.

For a completely recessive resistant gene, when $r = 0$, it is seen from (A.20) and (A.41) in the APPENDIX that

$$t_2/t_1 = \{p_2(0)q_2(0) + D(0)\}\{1/p_2(0) + \eta/q_2(0)\}$$

where t_2 and t_1 are the number of generations required for resistance to both the insecticides to develop in the case of type 2 and type 1 applications, respectively. In this equation η is a factor that depends on both the value of $p_2(T_A)$ in type 2 application and on $D(0)$. The parameter η decreases from a value of approximately 10 to unity as $D(0)$ is increased from $-p_2(0)q_2(0)$ to 0.

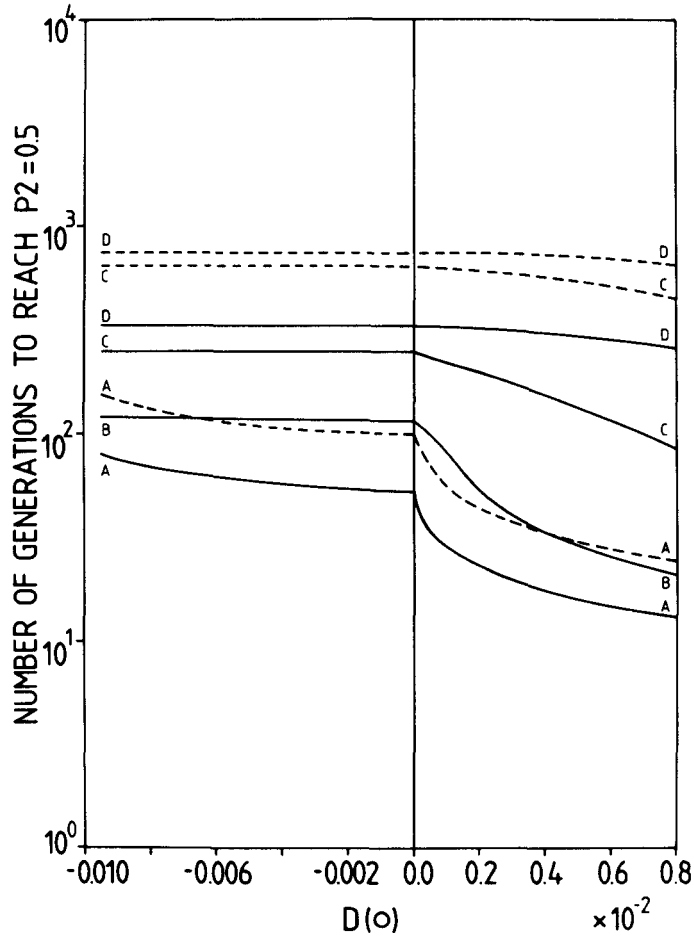

FIGURE 7.—The dependence of the number of generations for resistance to evolve on $D(0)$, for $p_2(0) = q_2(0) = 0.01$ and $h_a = h_b = 0.1$. The curves marked A, B, C and D are for $r = 0.0$, 0.1, 0.2 and 0.4, respectively. The broken and solid line curves represent mating before and after selection, respectively. The scale for $D(0) < 0$ is expanded by a factor of 100.

As $D(0)$ is increased from 0 to its maximum value, η goes to 0. The value of η is strongly dependent on the value of $p_2(T_A)$ and the value of 10 is for $p_2(T_A) = 0.9$. To a first approximation, for $D(0) \geq 0$, η is independent of $p_2(T_A)$. Thus, when both of the resistant genes are recessive, as $D(0)$ varies from 0 to its maximum value, t_2/t_1 increases from $p_2(0) + q_2(0)$ to unity. When both of the resistant alleles are at the frequency of, say, 10^{-5}, and when $D(0) = 0$, resistance can be delayed by more than five orders of magnitude by the use of mixtures. Even when the insecticide A has been applied on its own for some period so that the allele resistant to it has increased its frequency to approximately 1%, the use of mixtures would delay the evolution of resistance by a factor of 100 compared to the switching over from insecticide A to insecticide B.

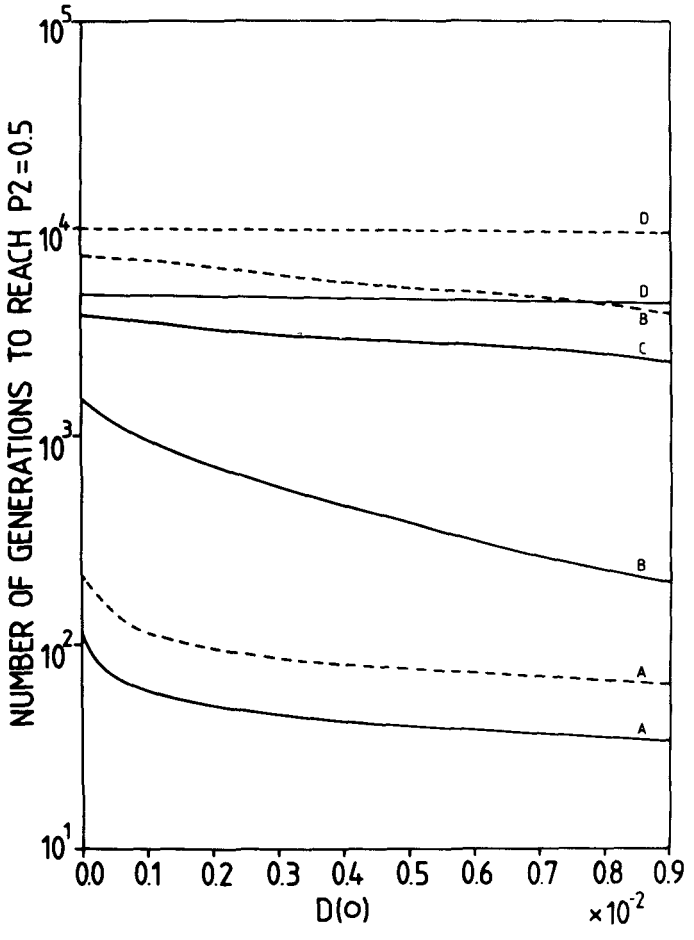

FIGURE 8.—The dependence of the number of generations for resistance to evolve on $D(0)$, for $p_2(0) = q_2(0) = 0.001$ and $h_a = h_b = 0.1$. The curves marked A, B, C and D are for $r = 0.0$, 0.1, 0.2 and 0.5, respectively. The broken and solid lines represent mating before and after selection, respectively. Since the range of $D(0)$ less than 0 is extremely narrow, it is not shown in this figure.

If one of the insecticides is effectively recessive and the other dominant, (say, $h_a = 0$ and $h_b = 1$), then from (A.28), (A.43), (A.44) and (A.46) in the APPENDIX we get

$$t_2/t_1 = -1/\ln q_2(0) \qquad : D(0) = 0$$

$$= 1 \qquad : D(0) = \text{max. value.}$$

Thus, even in this situation, the use of mixtures delays the evolution of resistance by a factor of 10 compared to the sequential application of insecticides.

A few examples of the ratio t_2/t_1 is shown in Table 1. The values quoted in this table were obtained by solving the exact equations numerically. In almost all situations, type 1 application is far more effective than type 2 application and in no case is it worse. The effectiveness increases by orders of magnitude

TABLE 1

Values of t_2/t_1 *for various values of* h_a, h_b, $p_2(0)$ *and* r

h_a	h_b	$p_2(0)$	r	t_2/t_1
0.0	0.0	0.001	0.0	0.002
0.0	0.2	0.001	0.0	0.045
0.0	0.6	0.001	0.0	0.114
0.0	1.0	0.001	0.0	0.186
0.2	0.2	0.001	0.0	0.333
0.2	0.2	0.001	0.5	0.002
0.0	0.0	0.0001	0.0	0.0002
0.0	1.0	0.0001	0.0	0.118
0.2	0.2	0.0001	0.0	0.407
0.2	0.2	0.0001	0.5	0.002

$D(0) = 0$ and $p_2(0) = q_2(0)$.

as the resistant alleles become recessive. Also, the effectiveness of type 1 mode improves by a few orders of magnitude as the value of r increases. There is very little to choose between the two if the values of both h_a and h_b increase beyond 0.5.

CONCLUSIONS

One of the earliest experimental works on the use of mixtures was carried out by MACDONALD (1959) who studied the evolution of resistance to insecticides by *A. gambiae* and *A. sundaicus*. His general conclusion was that the use of mixtures could prove unusually effective in the control of mosquitoes. The preliminary experiments by GEORGHIOU (1983) arrive at similar conclusions.

The main conclusion of this paper is that it is always desirable to use mixtures rather than insecticides sequentially. In the absence of any information regarding the initial allele frequencies, recombination fraction and initial linkage disequilibrium, the dosage of the mixture should be high enough to make both the resistant alleles as recessive as possible within the constraints of cost. This constraint can be relaxed if the two loci are relatively loosely linked. This is the second main conclusion of the present work, namely, that the evolution of resistance is delayed by orders of magnitude, provided there exists loose linkage between the two loci. A threshold effect for the recombination fraction has been shown to exist and resistance becomes considerably delayed for r above this value. Experimental evidence of linkage is rather sparse. In general one would expect the R genes to be randomly scattered over the chromosomes and, hence, the recombination to be large. On the other hand, in *A. arabiensis* there is indication that malathion and dieldrin resistance are closely linked ($r < 0.05$), whereas for DDT and dieldrin, r has been reported to be approximately 0.25, a value well above the predicted threshold (C. CURTIS, personal communication). This only underlines the need for detailed experiments both in the field and in the laboratory to establish the amount of recombination between the R genes and to estimate the frequency of occurrence of these genes in the field populations.

No model can hope to even approximately mimic field conditions. For example, nonuniform spraying (very common in practical situations), and background effects from agricultural spraying can drastically affect the effective dominance in programs for controlling vectors. The various parameters such as escape and effective dominance used in the present model are average values over space and time. In practical situations these values could fluctuate very rapidly over space and time, thus accelerating the evolution of resistance at some spatial region which could then spread rapidly through the whole population. Furthermore, in these calculations many factors such as migration, finite population size, stochastic effects and nonrandom mating have been disregarded. Thus, the present results could only provide a guide for further experiments in the laboratory and in the field and the final decision regarding resistance management strategy could arise only out of such experimental data.

Aside from the question of initial frequencies of the alleles, recombination, effective dominance, escape and linkage disequilibrium, the other major problem is the choice of the two insecticides. Such a choice of compounds for a mixture has to satisfy various constraints, some of which will be referred to later. In the first place, the two compounds must be insecticides for which there is a minimum possibility of cross-resistance. It would be inadvisable, for example, to mix a carbomate with an organophosphate (OP) because of the evolution of a mutant, producing the enzyme acetyl cholinesterase, insensitive to carbomates which would almost certainly confer resistance also to OPs (Wood 1981b). However, it would probably be safer to combine a carbomate with a pyrethroid because a common mechanism of resistance is less likely.

The widest selection of potential insecticides for mixtures is to be found among insecticides used against the immature stages because these include not only members of the conventional insecticide groups but also the growing variety of insect growth regulators. On the other hand the genetic arguments discussed earlier indicate that mixtures that select at the postmated adult are more favorable in delaying resistance.

It is known that some compounds in a mixture of insecticides could enhance the action of each other, whereas other classes of compounds in mixtures could reduce the action of each other. For example, GERA and GUPTA (1978), working with house flies, noted such enhancement when malathion was mixed with HCH, carbaryl and tetrachlorvinphos; on the other hand they found antagonism between malathion and DDT or malathion and dieldrin.

From a practical point of view, the constraints mentioned could restrict drastically the choice of compounds for mixtures. The widespread occurrence of resistance to most insecticides indicates the need for rethinking the concept of resistance management. There exists no simple answer to it. In this paper it has been shown that the use of mixtures could delay the onset of resistance by a very substantial factor. What is needed is detailed experimental investigation both in the laboratory and in the field, especially regarding many of the factors discussed.

My thanks to CHRIS CURTIS for bringing this problem to my attention and for various discussions and to ROGER WOOD and L. M. COOK for a critical reading of the paper and for many of the ideas discussed in the conclusion.

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Communicating Editor: M. NEI

APPENDIX

The equations relating to the use of type 1 and type 2 modes of application of insecticides are derived in this appendix. Approximate solutions for some limiting cases are given in the hope that these will be useful in deciding various resistance management strategies.

The two insecticides A and B affect loci A and B, respectively. There are two alleles A_1, A_2 and B_1, B_2 at these loci, A_1 and B_1 being susceptible and A_2 and B_2 being resistant to the insecticides A and B, respectively. The four gametes are denoted by $G_1(A_1B_1)$, $G_2(A_1B_2)$, $G_3(A_2B_1)$ and $G_4(A_2B_2)$ and their frequencies before selection in generation n by $X_i(n)\{i = 1, \ldots, 4\}$. Evidently,

$$\sum_{i=1}^{4} X_i(n) = 1 \tag{A.1}$$

Taking the allele frequencies of A_1, A_2, B_1 and B_2 to be p_1, p_2, q_1 and q_2, we get

$$p_1 = X_1 + X_2;\ q_1 = X_1 + X_3;\ p_1 + p_2 = q_1 + q_2 = 1$$

$$X_1 = p_1q_1 + D;\ X_2 = p_1q_2 + D;\ X_3 = p_2q_1 + D;\ X_4 = p_2q_2 + D \tag{A.2}$$

$$D = \text{linkage disequilibrium} = X_1X_4 - X_2X_3$$

The initial allele frequencies are denoted by $p_i(0)$, $q_i(0)$ $(i = 1, 2)$ and the initial linkage disequi-

librium by $D(0)$. For $p_1(0) = q_1(0)$, with $p_1(0) \gg p_2(0)$ and $q_1(0) \gg q_2(0)$, it is evident from (A.2) that, for the condition $X_i \geq 0$,

$$-p_2(0)q_2(0) \leq D(0) \leq p_1(0)q_2(0) \tag{A.3}$$

In what follows, r denotes the recombination fraction and $P_{ij}(n)$ the frequency of the genotype G_iG_j in generation n.

Let $R_{ij}^{(k)}$ denote the probability that the genotype G_iG_j, under segregation, produces the gamete G_k. For one-locus Mendelian segregation

$$R_{ij}^{(k)} = \tfrac{1}{2}(\delta_{ik} + \delta_{jk}) \tag{A.4}$$

where δ_{ij} is the Kronecker delta defined by

$$\begin{aligned} \delta_{ij} &= 0 \quad \text{if} \quad i \neq j \\ &= 1 \quad \text{if} \quad i = j \end{aligned} \tag{A.5}$$

For two loci with recombination r,

$$R_{ij}^{(k)} = \tfrac{1}{2}(\delta_{ik} + \delta_{jk}) + \tfrac{1}{2}r(1 - 2\delta_{ik} - 2\delta_{jk})\delta_{i+j,5} \tag{A.6}$$

Let $W_{ij}(n)$ be the selection acting on the genotype G_iG_j in generation n. $W_{ij}(n)$ is composed of two factors, one being selective differences in the absence of insecticides (Λ_{ij}) and the other arising from selection due to insecticides (w_{ij}).

$$W_{ij}(n) = \Lambda_{ij}w_{ij}(n) \tag{A.7}$$

The fitness of the two-locus system is assumed to obey the multiplicative model. For reasons discussed in the text, we shall in what follows assume that $\Lambda_{ij} = 1$ for all i and j. Thus, (A.7) becomes

$$W_{ij}(n) = w_{ij}(n) \tag{A.8}$$

Two modes of insecticide application are considered:

1. Both insecticides are applied simultaneously every generation. In this case the fitness matrix $|w_{ij}(n)|$ is denoted by $|w_{ij}^{(0)}|$.
2. Insecticide A is applied for a period T_A followed by insecticide B for a period T_B and the process repeated. Then

$$\begin{aligned} w_{ij}(n) &\equiv w_{ij}^{(1)} \qquad 1 \leq t_m \leq T_A \\ &\equiv w_{ij}^{(2)} \qquad \text{otherwise} \end{aligned} \tag{A.9}$$

where t_m is given by

$$n = t_m \bmod (T); \qquad T = T_A + T_B \tag{A.10}$$

Type 1 application implies $T_A = T_B = 0$ and type 2 implies $T_A, T_B > 0$.

For the case in which the heterozygotes have effective dominance h_a and h_b for the two loci A and B, respectively, and when all of the susceptible homozygotes are killed and when all of the resistant homozygotes survive, the matrices $|w_{ij}^{(k)}|$, $k = 0, 1, 2$ are given by

$$\begin{aligned} |w_{ij}^{(0)}| &= (1 - \alpha) \begin{vmatrix} 0 & 0 & 0 & h_ah_b \\ 0 & 0 & h_ah_b & h_a \\ 0 & h_ah_b & 0 & h_b \\ h_ah_b & h_a & h_b & 1 \end{vmatrix} + \alpha \mathbf{E} \\ |w_{ij}^{(1)}| &= (1 - \alpha) \begin{vmatrix} 0 & 0 & h_a & h_a \\ 0 & 0 & h_a & h_a \\ h_a & h_a & 1 & 1 \\ h_a & h_a & 1 & 1 \end{vmatrix} + \alpha \mathbf{E} \\ |w_{ij}^{(2)}| &= (1 - \alpha) \begin{vmatrix} 0 & h_b & 0 & h_b \\ h_b & 1 & h_b & 1 \\ 0 & h_b & 0 & h_b \\ h_b & 1 & h_b & 1 \end{vmatrix} + \alpha \mathbf{E} \end{aligned} \tag{A.11}$$

In the expressions α is the escape probability and **E** is a matrix with all its elements unity.

Two modes of selection will be discussed, namely, selection acting before mating and selection acting after mating.

Selection before mating

When selection precedes mating, it is easy to verify that

$$P_{ij}(n+1) = \left\{\sum_{km} W_{km}(n)P_{km}(n)R_{km}^{(i)}\right\}\left\{\sum_{km} W_{km}(n)P_{km}(n)R_{km}^{(j)}\right\} \Big/ \Delta^2 \tag{A.12}$$

$$\Delta = \sum_{km} W_{km}(n)P_{km}(n) \tag{A.13}$$

Since, $\sum_k R_{ij}^{(k)} = 1$, we get

$$X_i(n+1) = \sum_{km} W_{km}(n)P_{km}(n)R_{km}^{(i)}/\Delta \tag{A.14}$$

The main interest in the present investigation is to determine the number of generations it would take for the frequency of the resistant gene to approach a large value, say 0.5–0.9. The change in the frequency of the resistant gene is extremely slow until its frequency reaches a few percent. Thereafter, it rapidly increases to a value of 0.9. This is especially true when the escape parameter α is large (greater than a few percent) and when the resistant gene is effectively recessive. Under these conditions, since mating is assumed to be random, the genotype frequencies can be approximated by

$$P_{km} = X_k X_m \tag{A.15}$$

Extensive calculations indicate that such an approximation would yield at most an error of five to ten generations in the calculation of the time taken for resistance to evolve. When the resistant gene is dominant, this could produce an error of 25%, since for this case resistance evolves very rapidly even in the presence of large escape. Thus, from (A.14) and (A.15) we obtain the following approximate equations for the evolution of the gamete frequencies:

$$X_i(n+1) = \sum_{km} W_{km}(n)X_k(n)X_m(n)R_{km}^{(i)}/\Delta$$

$$\Delta = \sum_{km} W_{km}(n)X_k(n)X_m(n) \tag{A.16}$$

Equation (A.14) cannot be solved analytically to obtain the number of generations required for resistance to evolve. All of the results presented in this paper were obtained through computer simulation. Even the approximate equation (A.16) is not amenable to analytic treatment except for a few special cases. In what follows the approximate analytic solutions for some specific cases are discussed. In most situations these approximate solutions yield values that are within 10% of the exact solutions. These results are presented here in the hope that they will be of help to field workers engaged in the management of resistance.

The resistant genes are completely recessive ($h_a = h_b = 0$) *and the recombination factor,* $r = 0$ (*tight linkage*): When the insecticide dosage is such that the resistant genes to both the insecticides are completely recessive and $r = 0$, (A.16) simplifies to

$$\begin{aligned} X_i(n+1) &= X_i(n)\{\alpha + (1-\alpha)\delta_{i,4}X_4(n)\}/\Delta \\ \Delta &= \alpha + (1-\alpha)X_4^2(n) \end{aligned} \tag{A.17}$$

As discussed earlier, initially resistance evolves very slowly until the frequency of the resistant gene reaches a value of approximately a few percent; thereafter the resistance gene goes to near fixation very rapidly, in general, taking a few generations only to change its frequency from a few percent to, say 90%. This is especially true for recessive genes. In this situation the difference equation can be approximated to a differential equation to a good degree of accuracy. Defining $\beta = \alpha/(1-\alpha)$, we get

$$\begin{aligned} dX_i/dt &= -X_i X_4^2/\{\beta + X_4^2\} \qquad (i = 1, 2, 3) \\ dX_4/dt &= X_4^2(1 - X_4)/(\beta + X_4^2) \end{aligned} \tag{A.18}$$

The equation for $X_4(t)$ yields

$$t = \frac{\beta}{X_4(0)} - \frac{\beta}{X_4(t)} + \ln\left[\frac{X_4^{\beta}(t)(1 - X_4(0))^{1+\beta}}{X_4^{\beta}(0)(1 - X_4(t))^{1+\beta}}\right] \tag{A.19}$$

where ln represents natural logarithm. This approximate solution would yield an error of less than 10% provided $\alpha > 10\ X_4(0)$. In this case the logarithmic term in (A.19) can be neglected. Also, for $X_4(t)$ greater than a few percent, $X_4(0) \ll X_4(t)$. Thus, the time taken for resistance to evolve is approximately given by

$$t = \beta/X_4(0) = \beta/\{p_2(0)q_2(0) + D(0)\} \tag{A.20}$$

$$= \beta/\{p_2^2(0) + D(0)\} \quad \text{for} \quad p_2(0) = q_2(0) \tag{A.21}$$

It has already been demonstrated that for $p_2(0) = q_2(0)$,

$$-p_2^2(0) \leq D(0) \leq p_1(0)p_2(0)$$

Equation (A.21) shows that as $D(0) \to -p_2^2(0)$, $t \to \infty$. In the limit for $D(0)$, note that $X_4(0) = 0$ and, since $r = 0$, the resistant gene will not go to fixation and the population would eventually go extinct. When $D(0) = p_1(0)p_2(0)$, its maximum value, (A.21) yields

$$t = \beta/p_2(0) \tag{A.22}$$

Thus, as $D(0)$ increases from 0 to its maximum value, the evolution of resistance becomes more rapid, with t decreasing from $\beta/p_2^2(0)$ to $\beta/p_2(0)$. Note that for $\alpha = 0$, (A.19) yields t to be approximately given by $-\ln(1 - X_4(t))$ compared to the exact value of one generation for fixation to occur.

The resistant genes are completely recessive ($h_a = h_b = 0$) *and* $r > 0$: When $r > 0$, it is difficult to obtain even approximate analytic solutions. The equivalent differential equation for this case is given by

$$dX_i/dt = \epsilon_i rD - X_i X_4^2/\beta \qquad (i = 1, 2, 3) \tag{A.23}$$

where $\epsilon_i = +1$ for $i = 2, 3$ and -1 for $i = 1, 4$. Since resistance evolves very rapidly, once the frequency of the resistant gene attains a value of approximately 10%, in (A.23) X_4^2 is neglected in comparison with α, which in the present work is taken to be 0.1. Under the same approximation, we also have

$$\begin{aligned} dX_4/dt &= -rD + X_4^2(1 - X_4)/\beta \\ dD/dt &= -rD + X_4^2(X_1 - (r + 2)D)/\beta \end{aligned} \tag{A.24}$$

Consider first the case in which $D(0) = 0$. In the early generations, the rate of increase of X_4 is governed by the term $X_4^2(1 - X_4)/\beta$ as in (A.18). As X_4 increases, $D(t)$ becomes more positive and the rate of increase of X_4 is decelerated because of the term $-rD$. Thus, resistance evolves more slowly. Also, the rate of evolution of resistance decreases with increasing value of r due to the $-rD$ term in the equation. If $D(0) \neq 0$, then for sufficiently large values of r, initially the absolute value of $D(t)$ would decrease to 0 very rapidly within a few generations, the decrease being exponential, given by $\exp(-rt)$. Thus, for sufficiently large value of r, $D(t)$ would go to 0 in a time within which $X_4(t)$ has not changed appreciably from its initial value and, thus, resistance would evolve as in the case of $D(0) = 0$. This rapid decrease in the initial value of $D(t)$ for large enough value of r implies that there exists a sharp threshold in the value of r above which the rate of evolution of resistance is almost independent of the value of r for all values of $D(0)$. This is shown in Figures 4 and 5. Also, this implies that for large values of r, the evolution of resistance is insensitive to the initial choice of D as seen in Figures 6 and 7. Finally, for $r > 0$, resistance would always evolve at a rate slower than that given by (A.19).

The resistant gene at locus A *is recessive* ($h_a = 0$) *and at locus* B *is dominant* ($h_b = 1$) *and* $r = 0$: Within the approximation discussed, the differential equations for this case are given by:

$$\begin{aligned} dX_3/dt &= X_3X_4(1 - 2X_3)/\beta \\ dX_4/dt &= X_3X_4/\beta \end{aligned} \tag{A.25}$$

TABLE 2

Comparison of exact and approximate solutions for the evolution of resistance

Equation	$D(0)$	$p_2(0)$	h_a	h_b	t_{exact}	$t_{approximate}$
(A.21)	0.0	10^{-2}	0	0	1122	1111
(A.21)	0.8×10^{-2}	10^{-2}	0	0	19	14
(A.21)	0.8×10^{-3}	10^{-3}	0	0	147	139
(A.21)	0.8×10^{-4}	10^{-4}	0	0	1400	1389
(A.28)	0.0	10^{-2}	0	1	53	51
(A.29)	0.99×10^{-2}	10^{-2}	0	1	17	11
(A.28)	0.0	10^{-3}	0	1	716	768
(A.29)	0.999×10^{-3}	10^{-3}	0	1	123	111
(A.28)	0.0	10^{-4}	0	1	9609	10234
(A.29)	0.9999×10^{-4}	10^{-4}	0	1	1122	1111

These equations are valid for $D(0) \neq p_2(0)q_1(0)$. The approximation becomes progressively inaccurate as $D(0)$ moves toward its maximum value of $p_2(0)q_1(0)$. When $D(0) = p_2(0)q_1(0)$, the equivalent approximate equations are

$$X_3(t) = 0$$
$$dX_4/dt = X_4^2(1 - X_4)/\beta \tag{A.26}$$

The equation for X_4 is the same as in the first case (see (A.18)) when X_4^2 is neglected in comparison with α. Thus, as $D(0)$ increases to its maximum value, the evolution of resistance becomes independent of the values of the effective dominance at one locus, provided the resistant allele at the other locus is completely recessive. If, instead of being completely recessive, the heterozygotes have a small value of dominance, this independence with respect to the effective dominance at the other locus would be destroyed. This can be seen in Figure 3 for $h_b = 0.02$ and $r = 0$. For $D(0) = 0$, (A.25) yields the solution

$$t \cong \frac{\beta}{X_3(0)} \ln \frac{X_4(t)X_3(0)}{X_4(0)\{X_4(t) + X_3(0)\}} \tag{A.27}$$

Since $X_4(t) \gg X_3(0)$, this equation simplifies to

$$t \cong -(\beta/p_2(0))\ln(p_2(0)) \tag{A.28}$$

Similarly, for $D(0) = p_2(0)q_1(0)$, (A.26) yields

$$t \cong \beta/p_2(0) \tag{A.29}$$

The approximate solutions discussed are compared with exact calculations in Table 2. In this table the relevant approximate equations used are also given. It is seen from the table that the approximate solutions yield reasonable values over a wide range of parameters.

Mating before selection

In the case in which mating precedes selection

$$P_{ij}(n+1) = \frac{\left(\sum_{km} W_{km}(n)P_{km}(n)R_{km}^{(i)}\right)\left(\sum_{km} P_{km}(n)R_{km}^{(j)}\right) + \left(\sum_{km} W_{km}(n)P_{km}(n)R_{km}^{(j)}\right)\left(\sum_{km} P_{km}(n)R_{km}^{(i)}\right)}{2\sum_{km} W_{km}(n)P_{km}(n)} \tag{A.30}$$

For the approximation $P_{ij} = X_iX_j$, (A.30) reduces to

$$X_i(n+1) = \frac{X_i(n)\sum_k W_{ik}(n)X_k(n) + X_i(n)\Delta(n)}{2\Delta(n)} \tag{A.31}$$

where (n) is given by (A.16). In the limit $h_a = h_b = 0$ and $r = 0$, the approximate differential equations for the evolution of resistance are given by

$$\begin{aligned} dX_i(t)/dt &= -X_i(t)X_4^2(t)/2(\beta + X_4^2(t)) \qquad (i = 1, 2, 3) \\ dX_4(t)/dt &= X_4^2(t)(1 - X_4(t))/2(\beta + X_4^2(t)) \end{aligned} \tag{A.32}$$

Comparing (A.32) with (A.18) we get

$$t_{MS} = 2t_{SM} \tag{A.33}$$

where MS and SM refer to selection after and selection before mating, respectively. For selection before mating, resistance evolves at least twice as fast as when mating precedes selection. This factor of 2 can be exceeded, depending on the value of r and $D(0)$. The same factor of 2 occurs also for type 2 application when the resistant allele is recessive. In general, the effectiveness of type application over type 2 application is not dependent on whether selection acts before or after mating.

Sequential application of two insecticides

We shall be concerned with the case in which the insecticide A is applied first on its own for T_A generations until the frequency of the resistant gene, A_2, at locus A attains the value $p_2(T_A)$. Due to hitch-hiking effect, the frequency of the resistant gene B_2 at locus B would change from its initial value $q_2(0)$ to the value $q_2(T_A)$. The maximum deviation from $q_2(0)$ would occur for $r = 0$ and when $D(0)$ is at its maximum or minimum value. After T_A generations, the insecticide B is applied on its own for T_B generations until the frequency of the resistant gene B_2 attains the value $q_2(T_A + T_B)$. In practice, $p_2(T_A)$ and $q_2(T_A + T_B)$ wuld be 0.1–0.9, depending on the particular strategy employed in the management of resistance. The number of generations for the allele A_2 to reach a given frequency of $p_2(T_A)$ is independent of the initial linkage disequilibrium, $D(0)$, and of the recombination fraction, r. On the other hand, due to hitch-hiking, $q_2(T_A)$ and the time T_B required for B_2 to reach the given frequency $q_2(T_A + T_B)$ are dependent on both $D(0)$ and r. In this section this dependence is investigated. We need only consider the situation in which the insecticide A is applied first and, hence, the fitness matrix $|w_{ij}^{(1)}|$ in (A.11). Only the case of selection before mating is discussed since the case of mating before selection is similar, apart from a factor, as shown in the preceding section. For $r = 0$, from (A.16) we get

$$\begin{aligned} X_i(n + 1) &= X_i(n)\{\alpha + (1 - \alpha)h_a p_2(n)\}/\Delta; \qquad i = 1, 2 \\ X_i(n + 1) &= X_i(n)\{\alpha + (1 - \alpha)h_a(1 - p_2(n)) + (1 - \alpha)p_2(n)\}/\Delta; \qquad i = 3, 4 \\ \Delta &= \alpha + (1 - \alpha)\{2h_a(1 - p_2(n)) + p_2^2(n)\} \end{aligned} \tag{A.34}$$

where $p_2 = X_3 + X_4$ and $q_2 = X_2 + X_4$.

$r = 0$; $D(0) = -p_2(0)q_2(0)$: For this case, from (A.34) it is evident that $X_4(0) = 0$ for all n and, hence, the equations reduce to

$$\begin{aligned} p_2(n + 1) &= p_2(n)\{\alpha + (1 - \alpha)h_a(1 - p_2(n)) + (1 - \alpha)p_2(n)\}/\Delta \\ q_2(n + 1) &= q_2(n)\{\alpha + (1 - \alpha)h_a p_2(n)\}/\Delta \\ \Delta &= \alpha + (1 - \alpha)p_2(n)\{2h_a(1 - p_2(n)) + p_2(n)\} \end{aligned} \tag{A.35}$$

Transforming to differential equations, we get

$$\begin{aligned} dp_2/dt &= (1 - \alpha)p_2(1 - p_2)\{h_a(1 - 2p_2) + p_2\}/\Delta \\ dq_2/dt &= (1 - \alpha)p_2 q_2\{h_a(1 - 2p_2) + p_2\}/\Delta \end{aligned} \tag{A.36}$$

Hence,

$$dq_2/dp_2 = -q_2/(1 - p_2) \tag{A.37}$$

which yields the solution

$$\begin{aligned} q_2(t) &= (1 - p_2(t))q_2(0)/(1 - p_2(0)) \\ &\simeq (1 - p_2(t))q_2(0) \end{aligned} \tag{A.38}$$

TABLE 3

Hitch-hiking effect for $p_2(0) = q_2(0) = 10^{-3}$

t_r	t_d	$p_2(t)$	r	$D(0)$	$q_2(t)$
116	28	0.955	0.00	-1.0×10^{-6}	0.45×10^{-4}
			0.05	-1.0×10^{-6}	0.996×10^{-3}
			0.00	-0.75×10^{-6}	0.28×10^{-3}
			0.05	-0.75×10^{-6}	0.997×10^{-3}
120	2230	0.9995	0.00	-1.0×10^{-6}	0.5×10^{-6}
			0.05	-1.0×10^{-6}	0.996×10^{-3}
			0.00	-0.75×10^{-6}	0.25×10^{-3}
			0.05	-0.75×10^{-6}	0.997×10^{-3}

This equation shows the dependence of $q_2(t)$ on $p_2(t)$ due to hitch-hiking. As the allele A_2 approaches fixation, the allele B_2 goes to extinction. Note that for the case in which $D(0) = -p_2(0)q_2(0)$ and $r = 0$, the frequency $q_2(t)$ does not depend on the dominance relation of the heterozygotes at locus A. As the value of r is slightly increased above 0, $q_2(t)$ rapidly approaches $q_2(0)$. Similarly, as $D(0)$ moves toward 0, $q_2(t)$ moves toward $q_2(0)$. The effect of increasing r, $D(0)$ and $p_2(t)$ on the value of $q_2(t)$ is shown in Table 3. The number of generations required for the frequency of the allele A_2 to increase from $p_2(0)$ to $p_2(t)$, when A_2 is recessive and when it is dominant, is also given in Table 3 under the columns headed t_r and t_d, respectively.

The value of t_r and t_d can be estimated by solving equation (A.36) for $p_2(t)$ and we get

$$t_r = \beta\{1/p_2(0) - 1/p_2(t)\} + \ln\left[\frac{p_2^{\beta}(t)}{p_2(0)^{\beta}(1 - p_2(t))^{1+\beta}}\right](h_a = 0) \tag{A.39}$$

$$t_d = (\beta + 1)\left[\frac{p_2(t)}{1 - p_2(t)}\right] + \ln\left[\frac{p_2^{\beta}(t)}{p_2(0)^{\beta}(1 - p_2(t))^{1+\beta}}\right](h_a = 1) \tag{A.40}$$

For recessive gene, t_r is dominated by the first term in (A.39) and the approach to fixation is very rapid. For dominant gene, for $p_2(t) < 0.5$, the second term in (A.40) dominates and, when $p_2(t)$ increases above 0.5, t_d is determined by the first term. Thus, when the resistant gene is dominant, its frequency initially rises very rapidly (within five to ten generations) to a value of approximately 0.5–0.9 and thereafter it increases very slowly at a rate determined by the factor $1/(1 - p_2(t))$. This difference in the behavior between the recessive and the dominant gene can be seen in Table 3.

In practical resistance management, insecticide B would replace insecticide A once $p_2(t)$ has attained a value between 0.5 and 0.9. Taking $p_2(t) = 0.9$, from (A.39) and (A.40) we get the following approximate relations for the number of generations t_2 required for both the resistant genes in the population to reach a frequency ≥ 0.9 when $r = 0$ and $D(0) = -p_2(0)q_2(0)$:

$$\begin{aligned} t_2 &\simeq \beta\{1/p_2(0) + 10/q_2(0)\} && :h_a = 0;\ h_b = 0. \\ &\simeq 10\beta/q_2(0) && :h_a = 1;\ h_b = 0. \\ &\simeq \beta/p_2(0) && :h_a = 0;\ h_b = 1. \\ &\simeq 22(\beta + 1) - \beta \ln\{p_2(0)q_2(0)\} && :h_a = 1;\ h_b = 1. \end{aligned} \tag{A.41}$$

D(0) = 0: The gametic equations can be written as

$$X_i(n + 1) = \{w_{i\cdot}(n)X_i(n) + \epsilon_i w_{14} r D(n)\}/\Delta \tag{A.42}$$

where

$$w_{i\cdot} = \sum_k w_{ik}X_k$$

and Δ is given by (A.16). In (A.42), $\epsilon_i = -1$ for $i = 1, 4$ and $= +1$ for $i = 2, 3$. For the fitness matrix $|w_{ij}^{(1)}|$ given in (A.11)

$$w_{1\cdot} = w_{2\cdot} = h_a(1 - \alpha)p_2 + \alpha \equiv W_r$$

$$w_{3\cdot} = w_{4\cdot} = (1 - \alpha)\{h_a(1 - p_2) + p_2\} + \alpha \equiv W_s$$

Hence,

$$D(n + 1) = D(n)\{W_r(n)W_s(n) - W_r(n)p_2(n) - W_s(n)(1 - p_2(n))\}$$

Thus, if $D(0) = 0$, then $D(n) = 0$ for all n. Equation (A.42) thus reduces to

$$p_2(n + 1) = \alpha p_2(n) + (1 - \alpha)\{h_a(1 - p_2(n)) + p_2(n)\}p_2(n)/\Delta$$

$$q_2(n + 1) = q_2(n)$$

Thus, when $D(0) = 0$, $q_2(n) = q_2(0)$ for all n and for all r. In this case the number of generations required for both the resistant genes to get established in the population is given by:

$$\begin{aligned} t_2 &= \beta/p_2(0) + \beta/q_2(0) && :h_a = 0;\ h_b = 0 \\ &= \beta/q_2(0) && :h_a = 1;\ h_b = 0 \\ &= \beta/p_2(0) && :h_a = 0;\ h_b = 1 \\ &= 22(\beta + 1) - \beta \ln\{p_2(0)q_2(0)\} && :h_a = 1;\ h_b = 1 \end{aligned} \tag{A.43}$$

D(0) = *Maximum value*; r = 0: Two cases need to be considered when $D(0)$ has maximum possible value:

(1) $q_2(0) > p_2(0)$. In this case, $D(0) = p_2(0)q_1(0)$ and it can easily be demonstrated that

$$dq_2(t)/dp_2(t) = (1 - q_2(t))/(1 - p_2(t))$$

Thus,

$$1 - q_2(t) = (1 - p_2(t))(1 - q_2(0))/(1 - p_2(0))$$

For $p_2(0), q_2(0) \ll 1$ or for $p_2(0) = q_2(0)$ we have $p_2(t) = q_2(t)$, this relation being exact for the second condition. In this case, taking $p_2(t) = 0.9$, we get the following approximate expressions for t_2, the number of generations for resistance to both the insecticides to get established in the population. These relations are independent of the value of h_b.

$$\begin{aligned} t_2 &\simeq \beta/p_2(0) && :h_a = 0 \\ &\simeq 11(\beta + 1) - \beta \ln p_2(0) && :h_a = 1 \end{aligned} \tag{A.44}$$

(2) $p_2(0) > q_2(0)$. In this case $D(0) = p_1(0)q_2(0)$ and

$$dq_2/dp_2 = q_2/p_2$$

yielding

$$q_2(t) = p_2(t)q_2(0)/p_2(0) \tag{A.45}$$

and for $p_2(t) = 0.9$ we have the following approximate relations for t_2. Note that in this case, t_2 is dependent on the dominance relation for insecticide B.

$$\begin{aligned} t_2 &\simeq \beta/p_2(0) + 0.9\beta p_2(0)/q_2(0) && :h_a = 0;\ h_b = 0 \\ &\simeq 11(\beta + 1) - \beta \ln p_2(0) + 0.9\beta p_2(0)/q_2(0) && :h_a = 1;\ h_b = 0 \\ &\simeq \beta/p_2(0) + 11(\beta + 1) - \beta \ln\{q_2(0)/p_2(0)\} && :h_a = 0;\ h_b = 1 \\ &\simeq 22(\beta + 1) - \beta \ln q_2(0) && :h_a = 1;\ h_b = 1 \end{aligned} \tag{A.46}$$

As the value of r increases, $q_2(T_A)$ decreases toward $q_2(0)$ at a rate much slower than in the case in which $D(0) = -p_2(0)q_2(0)$. Also, in this case the value of $q_2(T_A)$ is strongly dependent on the dominance relation of A_2 for $r > 0$.