Selection of Physiologic Strains of *Oncopeltus* and its Relation to Insecticide Resistance

Raimon L. Beard

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Introduction

Extensive physiologic and genetic studies have contributed much to an understanding of the development of resistance to insecticides in many species of insects. The problem is complex, and few generalizations can be made. One conclusion is clear. This is expressed by Crow (1957), in his review of the subject, as follows: "Insecticide resistance is an example of evolutionary change, the insecticide acting as a powerful sieve for concentrating resistant mutants that were present in the original population."

The variation in the population that permits the "sieve" to operate, and the "concentrating mechanism" of the "sieve" must be appreciated if the practical problem is to be attacked effectively. The current study is directed to this end, with the following objectives: (1) to identify variation among insects with the type of selection responsible for the most rapid segregation of physiologic strains, (2) to compare variation in response to one chemical with variation in response to a second chemical having different action, and (3) to compare the segregation of physiologic strains when these different chemicals are used separately, in combination, or alternately.

Variation in insects

Beard (1952a) called attention to three levels of variation in the response of an insect species to chemical treatment. The highest level is the variation found among groups separated by time, space, or condition. This has been studied extensively in ecology and genetics, but comparative studies in toxicology have been infrequent. Geographic isolation, for example, acting on this type of variation has potently influenced evolution (see Hubbell, 1956). However, group variation is not so important to the type of evolution discussed here. The second level of variation is that in responses by different individuals in a test group. This variation is usually represented by a normal distribution. It is generally characteristic for the response of a given insect species to a given chemical under the conditions of test. This is the variation studied by most insect toxicologists. Its expression by dosage-response curves or time-response curves is used so frequently that knowledge of this type of variation can be taken for granted. The third level of variation is to be found in each individual. The response of an individual to a chemical is not predictable, even though the distribution of responses of its group is known. This is because the response of an individual fluctuates in a seemingly random fashion and so the individual does not maintain a fixed position relative to other members of its test group. This was amply demonstrated by Beard (1952b) and Bliss and Beard (1953). They suggested that this fluctuation in an individual's response could account for the fact that survivors of an insecticide treatment were just as susceptible and as variable as the original population. This phenomenon was observed by Beard (1952b) and is evident in the work of Tattersfield and Kerridge (1955) and Hadaway (1956). Any individual has a tendency to respond characteristically, but a range of response surrounds this tendency. The relative magnitudes of the variance components (1) between individuals and (2) within individuals, were examined statistically by Bliss and Beard (1953). These components were called static and dynamic, following the designation of variation in responses of organisms to drugs by Gray (in Clark, 1933). These terms have proved to be misleading, so are not used in this discussion. It was speculated that the rate at which an insecticide-resistant race can be developed may be affected by the range of random responses in the different individuals,—that is, the intra-individual variation. This speculation is here explored.

Experimental techniques

Fluctuating responses in an individual cannot be studied with lethal doses of insecticides, as repeated treatments and independent responses are required. Sub-lethal doses of insecticides having no cumulative effect could be used, but none is known that elicits a definitive response with a precise end point, has no deleterious after-effects, and is convenient to handle. The anesthetizing gases carbon dioxide and nitrous oxide were found to be admirably suited to the requirements, as in the studies of Bliss and Beard (1953). The techniques used were suggested by the earlier work of Broadbent and Bliss (1936) who showed the relationship between recovery from sub-lethal doses and the toxicity of HCN gas and who developed the recovery-time curve as a graphic measure of group response to an anesthetic.

Adults of the large milkweed bug *(Oncopeltus fasciatus* (Dall.)) served as the test insect. The bugs were reared in ventilated plastic boxes on dried milkweed seed. Water was supplied in containers with dental cotton rolls as wicks. Both rearing and testing were done in a room maintained at 76°F. The bugs were of a laboratory culture that had been maintained for several years but which had been augmented occasionally by the addition of wild bugs to reduce the possibility of deleterious inbreeding. The age of the bugs after becoming adult seems to have little effect on the response. This is indicated by similar responses in a single group of bugs tested repeatedly over a period of 2 to 3 weeks, and in groups of different aged bugs tested at the same time under the same conditions. With few exceptions, bugs were used which matured in from 1 to 7 days prior to testing. This assured testing before the normal mating period of the bugs began. The sexes were separated soon after the final molt whenever the tests required controlled mating.

In previous studies most of the tests were made on individual bugs; those made in this study chiefly involved groups. In tests requiring individual identification, typewritten numbers or letters were affixed to the backs of the bugs by an adhesive. This treatment did not modify the response.

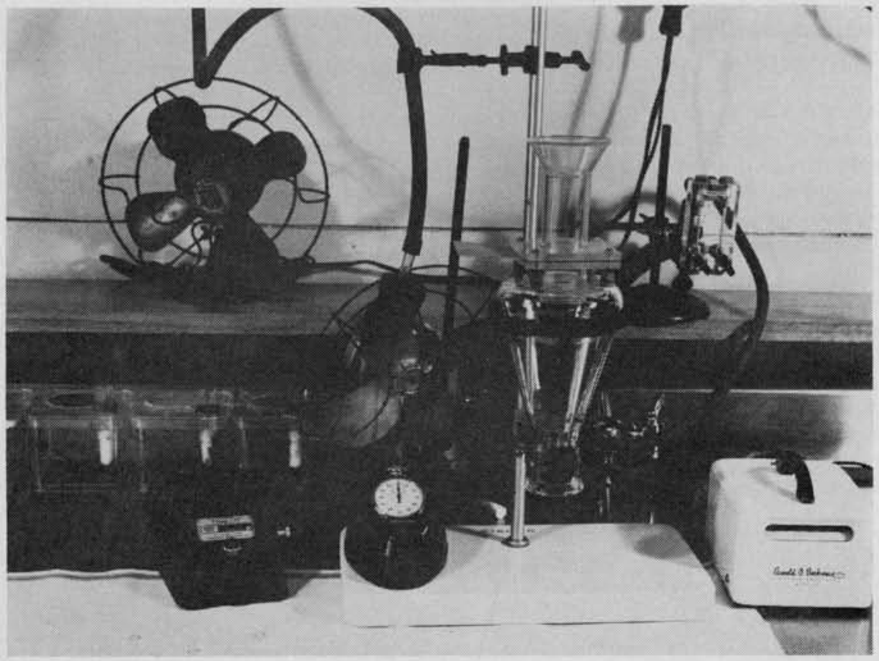


Figure 1. Laboratory arrangement of test equipment.

The standard test procedure was to introduce a group of bugs into the exposure chamber by means of a trapdoor device. (Figures 1, 2). This consisted of two plastic cylinders fastened to two plastic plates spaced so as to accommodate a paper slide. The top cylinder had a funnel opening. With the device positioned in the top of the exposure chamber, bugs were placed in the top cylinder. The paper slide was withdrawn, and the bugs dropped into the chamber. An oil film in the cylinder insured a rapid drop of the group.

The exposure chamber consisted of a one-liter Ehrlenmeyer filtering flask in the bottom of which was drilled a hole 1½ inches in diameter. The flask was used inverted, and the side arm served as the gas inlet. The excess gas escaped through the drilled hole, which also was the admittance port for the test bugs. When the trapdoor device was not in position, the drilled hole was closed with a rubber stopper and tubing to waste the excess gas to an exhaust. The gas flow was maintained at 2 liters per minute as metered by a manometer flow meter or rotameter. The actual rate of flow was probably unimportant provided the flask was completely filled with the test gas and the flow was not so rapid as to cool the bugs.

Fans were used to blow any waste gas away from the area of observation and to provide a constant gentle stimulus of moving air over the test bugs.

A stopwatch timed the length of treatment. The test bugs were removed at the end of an exposure through the neck of the flask, which was normally closed with a rubber stopper. The bugs were then placed on their backs, well spaced from each other, on a suitable surface. Too smooth a surface interferes with the righting response, which is the end-point criterion. Too rough a surface permits the righted bugs too firm a footing, and their removal disturbs the remaining individuals. A cotton-linen towel served as a satisfactory surface.

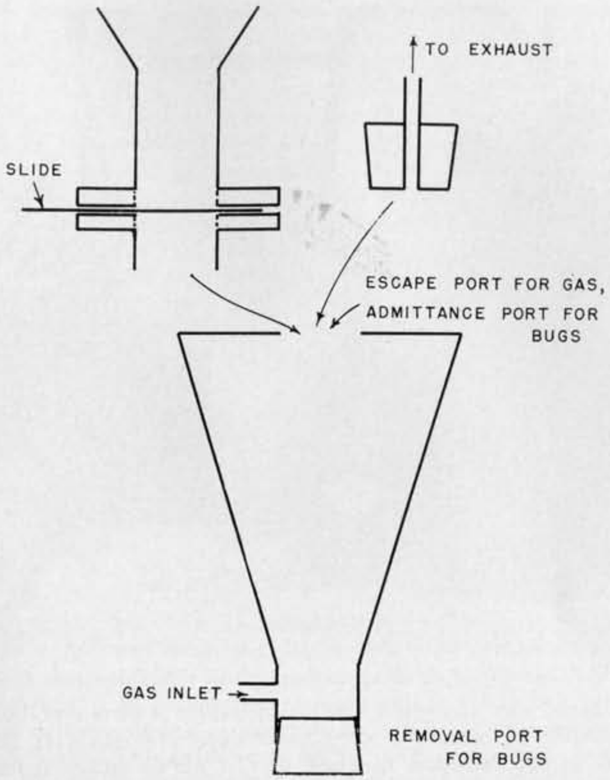


Figure 2. Details of exposure flask and trapdoor device.

The righting response provides a well-defined end-point for precise timing, although it does not necessarily represent complete recovery from the effects of carbon dioxide. (Bliss and Beard, 1953). The response is a reflex, operating even in a decapitated bug. In these tests the righting response appeared to function only after adequate bodily ventilation. For example, occasionally some bugs try to turn over, but appear physiologically unable to do so. The reflex seems to vary in sensitivity. Occasionally some bugs are physiologically able to right themselves, but a stronger-than-normal stimulus is required to induce the reflex. These unusual responses were infrequent, the majority of the responses being definitive. The interval from the end of an exposure until a bug turned over is called here its "response time." An electric timer indicating seconds measured this interval.

The exposure time was varied for the different test gases in order to equalize response time. Thus bugs were exposed 30 seconds to carbon dioxide, 90 seconds to nitrous oxide, and 60 seconds to a mixture of carbon dioxide and nitrous oxide in equal amounts. In a few special tests with a mixture of 80 per cent carbon dioxide and 20 per cent oxygen, a 90 second exposure was used. These exposure periods are approximately six times the customary knock-down time for the milkweed bug.

The gases used were all administered from the usual commercial pressure cylinders. As both carbon dioxide and nitrous oxide have the same density (specific gravity with respect to air being 1.53) they can be manipulated interchangeably. Oxygen, having a different density, required special consideration. Its concentration in mixtures was determined by means of a Beckman oxygen analyzer.

Bliss and Beard (1953) demonstrated the importance of the rest interval between exposures, and in the current tests none was repeated without allowing adequate rest time. In the former study some effort was made to determine the effects of one exposure upon the response to the next, especially when the gases were changed, but no conclusive results were indicated apart from the rest requirement. In a few tests it has been observed that in bugs exposed repeatedly, the response to the first test was slower than to subsequent tests. A possible induction of tolerance is considered unlikely. This has been observed too infrequently to affect the results presented and too unpredictably to permit special study. The reproducibility of group responses in different tests was definitely superior to most insecticidal tests.

Although carbon dioxide and nitrous oxide were used almost exclusively in these studies because of their ease of application and the clear-cut response of the bugs, the principles illustrated should apply to other toxicants. Certainly, insofar as tested, the toxicants nicotine, pyrethrum, and cyanide, applied as vapors, indicated nothing to the contrary.

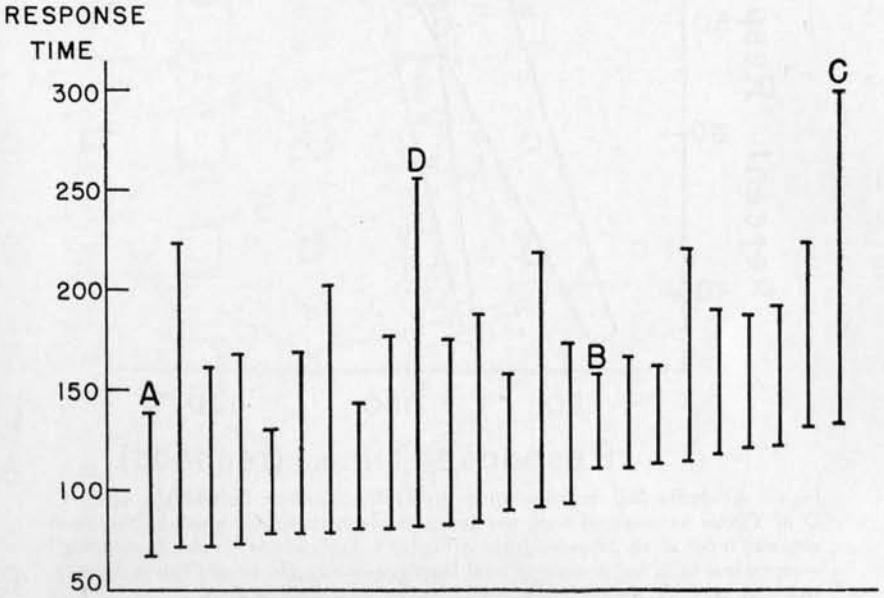


Figure 3. Range of response time (in seconds) in 24 bugs, each tested 14 times. Individuals A, B, C, D are otherwise compared in Figure 4.

Variation in responses of individual bugs and its effect on selection

Expression in group responses of fluctuating individual responses

The ranges within which individuals respond when tested repeatedly are illustrated in Figure 3. Twenty-four bugs were individually identified by number and exposed to carbon dioxide. The response time for each individual was recorded. The test was repeated for a total of 14 times. The range in response time—from the minimum to the maximum—in the 14 observations is illustrated for each of the 24 bugs. It is obvious that the ranges and their levels vary considerably. These differences can be more fully appreciated if individual response-time curves are compared—a device demonstrated by Bliss and Beard (1953). The data in Figure 3 for bugs A, B, C, and D show extreme ranges and differences in level. These are presented in Figure 4 as individual response curves (A, B, C, D) for comparison with the group response curve (G). Obviously the curves for A, B, and C have steep slopes (showing narrow ranges of response or small standard deviations). The curve for D has a flat slope (showing a wide range of response or large standard deviation). Clearly if single tests are considered, bug D would sometimes respond like bug A; at other times it would respond like bug C. Bug A would almost always respond more rapidly than C. It is also clear that individual mean response times and individual slopes are different in different individuals, and to a large extent, the means and slopes differ independently.

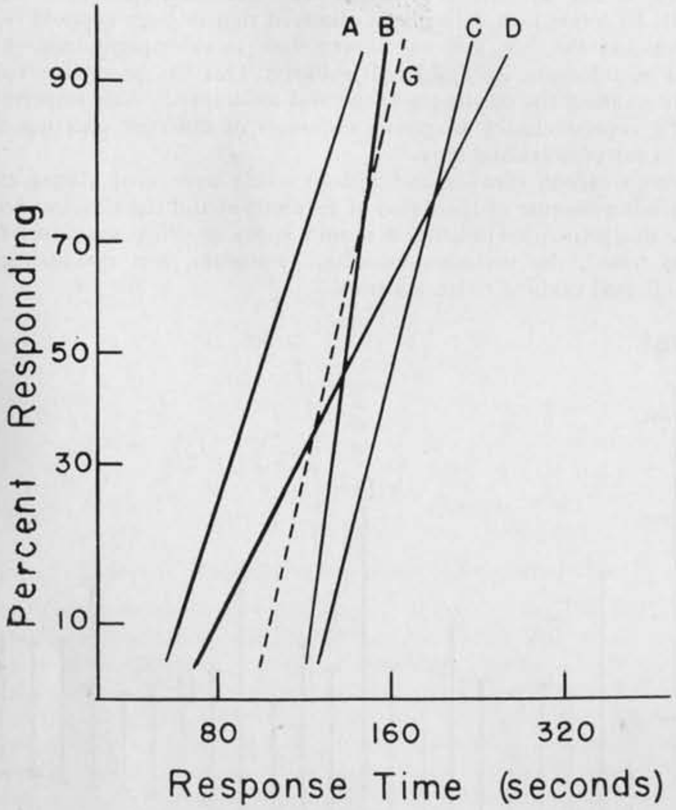


Figure 4. Individual response time curves for the four individuals A, B, C, D of Figure 3 compared with the group response curve, G, based on the mean response times of all 24 individuals in Figure 3. Ordinate—"Percent Responding"—represents in *G* the percent of total bugs responding (in terms of their means), and in A, B, C, D, the percent of responses for the individual bugs.

The assumption is made that bugs like A in Figure 4, responding more consistently at the low end of the time scale, would most likely carry the genes responsible for the physiologic conditions permitting fast recovery. On the other hand, bugs responding like C would most likely carry the genes thus responsible for delayed recovery. In selection tests based on single treatments, isolates of the A-type and C-type would be diluted with individuals like D. The problem, then,

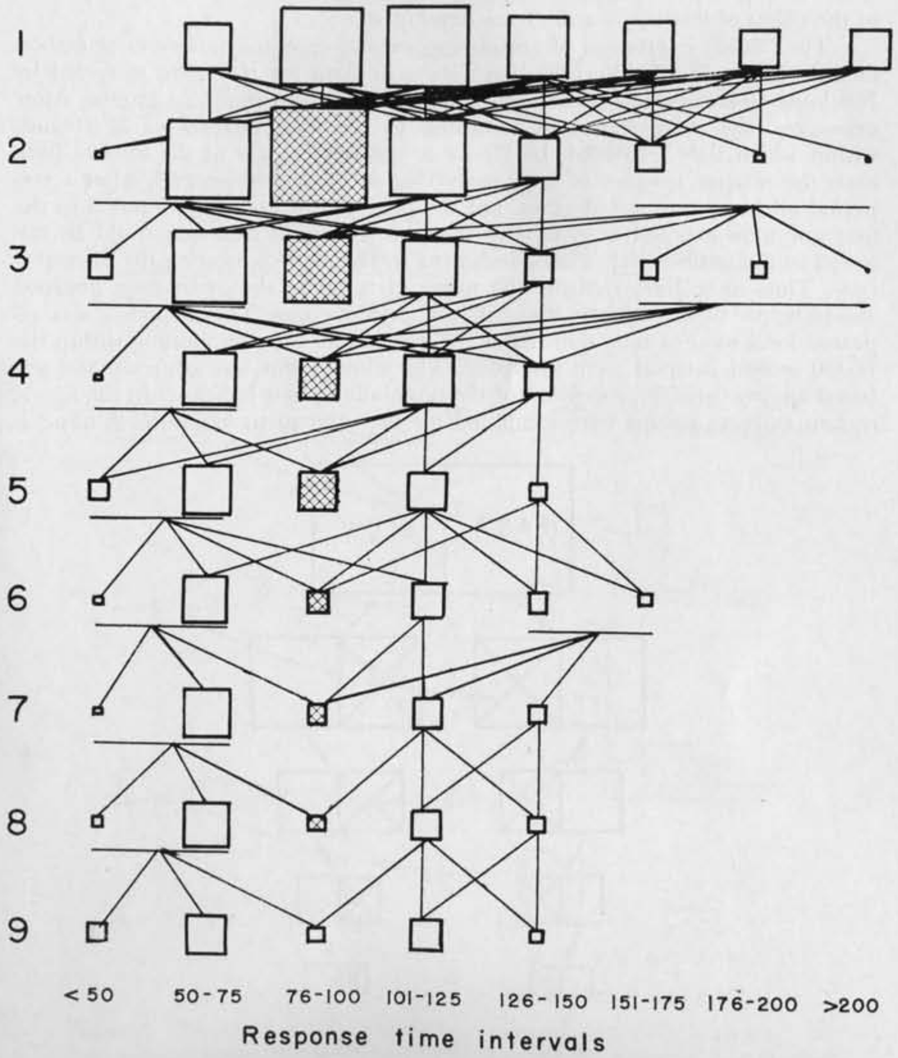


Figure 5. Distribution of bug responses grouped by response time intervals in nine successive tests on the same individuals except for those eliminated as responding in the 76-100 second interval (cross-hatched squares). Lines from each group connect the succeeding groups to which contribution is made. The size of the squares represents the relative numbers of insects.

is one of comparing isolates which include D-type individuals with isolates which exclude D-type individuals.

The most obvious approach to isolating A-type and C-type bugs, undiluted with the D-type, is to test repeatedly and consider the data as was done in Figures 3 and 4. This is very time-consuming and impracticable on a large scale. A modification of this was attempted, in which individually identified bugs were tested three times, and the consistency of response was the basis for selection. Three tests proved inadequate, as became evident when a more complete picture of the effects of shifting responses was developed.

The "fluid" expression of group responses to repeated exposures of carbon dioxide is graphically illustrated in Figure 5. Data for this were provided by 788 bugs of both sexes (essentially equal), which were tested in groups. After exposure, bugs were separated according to the time interval of 25 seconds within which they recovered. In Figure 5, the relative size of the squares indicates the relative number of bugs recovering in each time interval. After a rest period all bugs were tested again, but the bugs within each time interval in the first test were exposed as groups so their respective distributions could be followed in the second trial. This is indicated by the lines connecting the successive tests. Thus these lines indicate the range of response shown by bugs grouped according to their response times in the previous test. This sequence was repeated for a total of nine tests. After the first test all bugs responding within the 76-100 second interval were discarded. This elimination was arbitrary and not based on any definite proportion of the population. As is indicated in the figure, certain extreme groups were combined for the subsequent exposure. A trend is

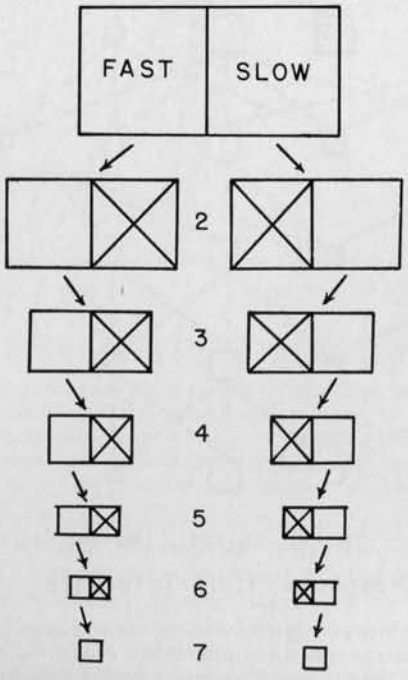


Figure 6. Scheme illustrating selection method based on repeated testing and successive elimination.

evident, at least in the first three tests, for the general response time to be diminished. The significance of this is not known. Most obvious is the redistribution of the bugs in test groups, united by response time in one test, ranging throughout the time scale in the next test. It is not until the seventh test that successive elimination of a central group has resulted in a kind of segregation of two distinctive groups.

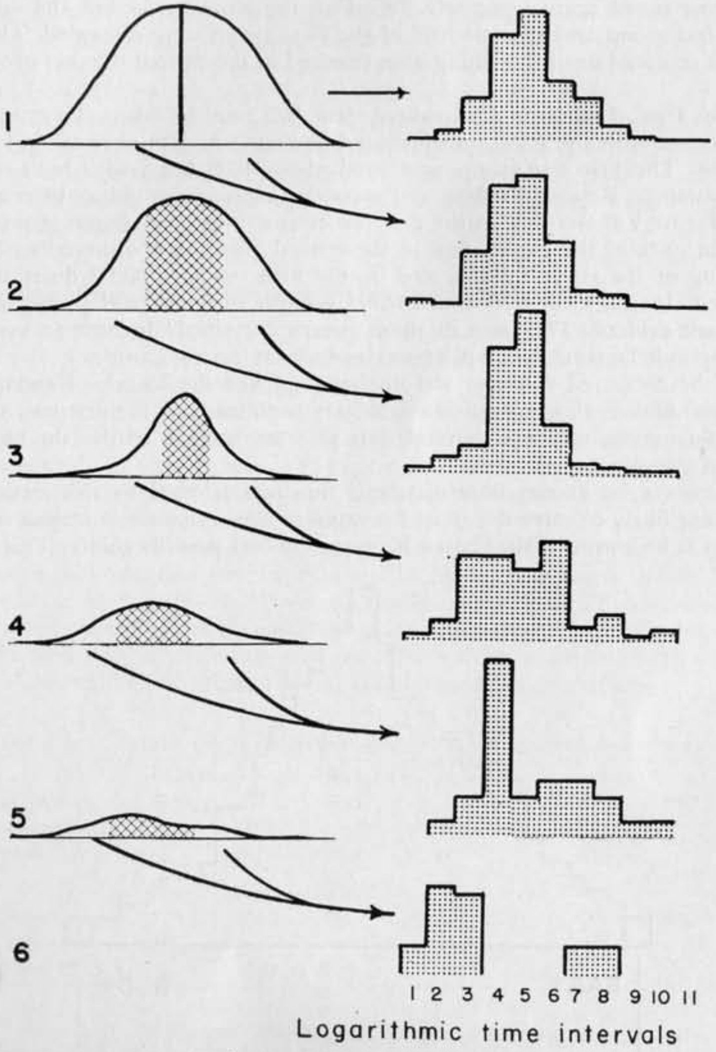


Figure 7. Frequency distributions of bug response times in six successive tests, with elimination according to the scheme illustrated in Figure 6. The diagrammatic curves on the left suggest the general form of the distribution and indicate the portions eliminated. The bar graphs on the right are based on experimental data, the height of the bars being doubled each time in the 3rd to 6th tests to compensate for the loss in numbers of individuals. Time (in seconds) in log. intervals of .0600.

The successive elimination of bugs responding in the 76-100 second interval was not rigorous enough to segregate two groups without overlap, even in nine tests. A more rigorous—and much simpler—procedure disregards the time values and considers only the responses of the bugs relative to each other. This is diagrammed in Figure 6. An initial large population was equally divided into the more rapidly responding half and the more slowly responding half. These were then tested again separately. Based on the second response, the slow half of the fast group and the fast half of the slow group were discarded. This process was repeated until the elimination resulted in the desired number of selected bugs.

This type of selection is expedient; few data need be taken. To compare its performance with the selection illustrated in Figure 5, data were taken for each exposure. The first test group numbered about 1000 bugs, with both sexes in equal numbers. Repeated testing and successive elimination reduced the number to 32. Figure 7 shows that in the first few tests a dominant central grouping remains in spite of the elimination of the central group in the preceding tests. A flattening of the curve follows, and by the fifth test a bimodal distribution is suggested. In the sixth test, two distinct groups, without overlapping response times, are evident. The bugs in these groups "survived" because in every test they responded among the 50 per cent chosen from the test group.

If the measured response was unchanging, like the length of antennae or eye facet number, this separation could have been made in the first test. Because of the fluctuating responses, several tests were required to resolve the bugs into uniform groupings.

It cannot be known immediately if the bugs selected by this method are those most likely to carry the genes for rapid or slow response to carbon dioxide. Nor can it be known if the chosen bugs are the best possible choice. This can be judged in a relative way if a standard is available for comparison. Such a standard is the typical selection in a single test. This is based on the usual frequency distribution of individuals. The selection process is simple. In a single test, the fastest bugs to recover and the slowest bugs to recover are separately saved for further testing or for reproduction. The proportion saved is determined by the desired selection pressure, the number required for reproduction, or the test in question. The selected individuals represent both extremes of the frequency distribution, as illustrated in Figure 8. Such a single selection test ignores the fluctuating responses within individuals. In other words, bugs responding like *D* in Figure 4 could sometimes occur in both selected groups.

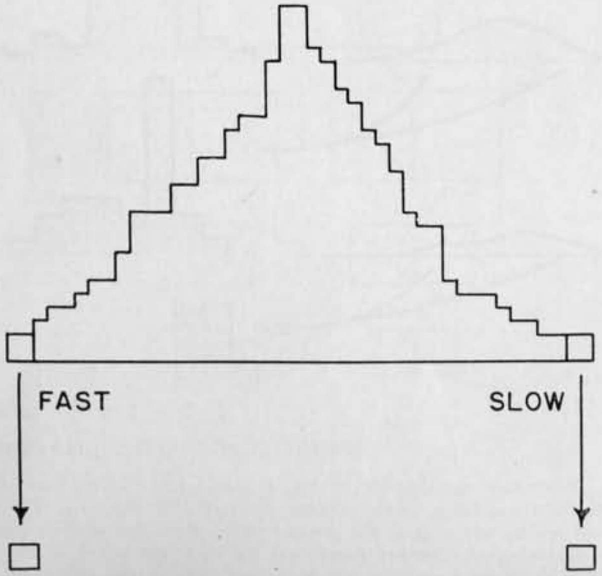


Figure 8. Diagrammatic frequency distribution illustrating selection of extreme responses in a single test.

Another method can be used in selecting individuals with due regard to their changing responses. This requires data on response times of all individuals in each of a series of several exposures. The mean response time for each individual is calculated, and bugs with minimum and bugs with maximum mean response times are selected.

Comparison of individuals selected by three procedures

The extent to which three selecting procedures might select the same bugs, and the variation in response of the selected bugs in different tests, can be judged from the results of the following experiment.

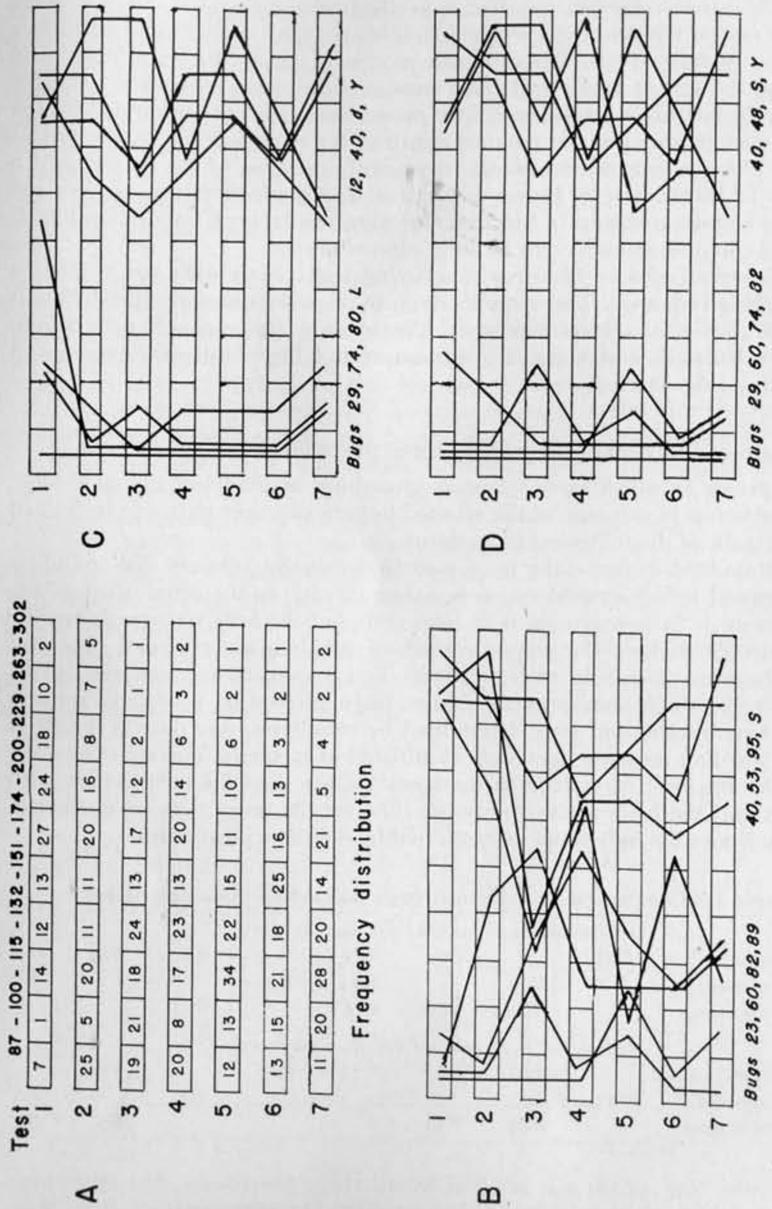
One hundred twenty-eight bugs were individually marked with numbers and letters and tested seven times with carbon dioxide in the usual manner. Exposure was made in four groups of 32 bugs each, but the bugs were pooled at the conclusion of each test. The response time of each bug was recorded. The first and last bugs to recover in each group in Test 1 were those considered to be selected by the single exposure procedure. Bugs selected by repeated exposure and successive elimination were determined by considering the data as if half of the bugs, based on response time, were eliminated in each test. This could be done because the response of each individual was known. And finally the bugs with the lowest and the highest mean response time for the seven tests were selected. Table 1 indicates the individual bugs selected by the three procedures.

Table 1. Comparison of individual bugs selected by three procedures

	Individual bugs selected for fast recovery							
Single exposure	#23		#60			#82	#89	
Repeated exposure		#29		#74	#80			#L
Mean response time		#29	#60	#74		#82		
	Individual bugs selected for slow recovery							
Single exposure		#40		#53	#95		#S	
Repeated exposure	#12	#40				#d		#Y
Mean response time		#40	#48				#S	#Y

Only one bug (#40) was selected by all three procedures. No other bugs were selected by both the single and the repeated exposure methods. Bugs #60, #82, and #S were selected by both the single exposure and the mean response time criteria, and bugs #29, #74, and #Y were selected by both the repeated exposure and the mean response time criteria. Certainly each procedure selects a different aggregation.

The performance of the selected bugs in the seven tests provides a measure of their variation. This is shown in Figure 9. Each box represents the group of



Test										
1	7	1	14	12	13	27	24	18	10	2
2	25	5	20	11	11	20	16	8	7	5
3	19	21	18	24	13	17	12	2	1	1
4	20	8	17	23	13	20	14	6	3	2
5	12	13	34	22	15	11	10	6	2	1
6	13	15	21	18	25	16	13	3	2	1
7	11	20	28	20	14	21	5	4	2	2

Figure 9. A. Frequency distribution of 128 bug responses in each of 7 successive tests. B. Positions in the frequency distribution of A occupied by eight bugs selected for fast and slow response on the basis of single exposure. C. Likewise, the positions occupied by eight bugs selected on the basis of repeated tests and successive elimination. D. Likewise, the position occupied by eight bugs selected on the basis of mean response time.

bugs responding in the logarithmic time intervals indicated. In *A*, the numbers indicate the total bugs responding in each interval in each test. In *B, C, D,* each line connects the group position occupied by one of the selected bugs, indicated in Table 1 in each test. *B* illustrates those bugs selected by the single exposure procedure; *C* illustrates those bugs selected by repeated exposure and successive elimination, and *D* those bugs selected on the basis of mean response time. Obviously the fast and slow bugs selected by single exposure overlap considerably in their responses, whereas the bugs selected by the other two procedures exhibit smaller ranges of response. The fast and slow bugs in *C* and *D* constitute distinctly different groups.

This performance of selected bugs indicated no particular advantage in the use of mean response time as a criterion of selection. Hence no further selections were made on this basis. The much simpler method of repeated exposure with successive elimination achieved the desired end.

Stability of group response in selected bugs

A comparison was made between bugs selected by single exposure and bugs selected by repeated testing in terms of the reproducibility of their group responses. From a total of 600 bugs, 24 were selected for rapid recovery and 24 for slow recovery as evident in a single test. From another group of 1000 bugs similar numbers were selected by repeated tests with successive elimination. The difference between the two population sizes arises from the fact that the data were taken for other purposes, but may appropriately be applied here. The group responses of the selected bugs in the selecting tests are graphically illustrated as curves F (fast) and S (slow) in Figure 10, *A* (single exposure) and *B* (repeated exposure). The same selected bugs were then tested again under the same conditions. The group responses are illustrated as curves F′ and S′ in the same figure. The selecting response in *B* is essentially reproduced by the second trial, but in *A*, the F′ and S′ curves are intermediate between F and S and show considerable overlap. This is just a group expression of the stability or

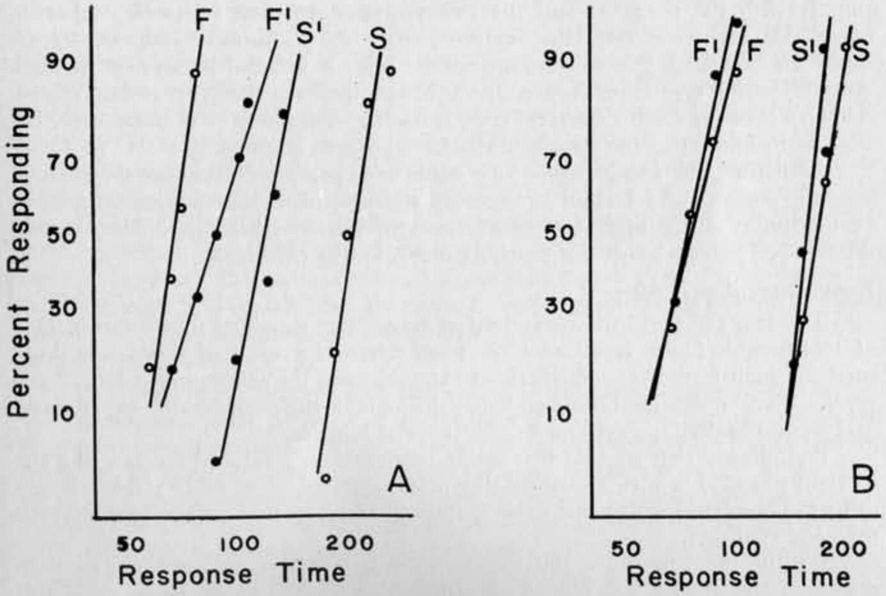


Figure 10. Group responses of bugs selected in A on basis of single exposure and in B on basis of repeated tests and successive elimination. Curves F and S, responses of fast and slow groups in the selecting tests. F′ and S′ responses of fast and slow groups in subsequent tests.

instability of response in the individuals comprising the group. The stability in response of the group selected by repeated exposure is greater than that of the group selected by single exposure.

Selection within a generation by means of different gases

Many of the experiments reported above on bugs of one generation tested with carbon dioxide were repeated with nitrous oxide and in some cases with mixtures of carbon dioxide and nitrous oxide. Inasmuch as the results in all cases were essentially the same, presentation of data seems superfluous. The conclusion is inescapable that by suitable techniques, individual bugs may be selected for extreme response within a single generation, by either carbon dioxide, nitrous oxide, or a mixture of the two.

Selective breeding and the genetic development of physiologic strains

From the foregoing it can be seen that in a laboratory population a small group of bugs can be selected for extreme response by single exposure which include bugs with wide ranges of responses. Bugs selected by repeated testing and successive elimination are much more homogeneous, and the responses for the two extremes do not overlap appreciably. The next question to be answered is whether these differences are also evident in the genetic development of fast responding and slow responding strains of bugs. The assumption here is that the response involves a genetically controlled physiologic system. If this is true, a direct analogy exists with resistance to insecticides, for it is assumed that resistance is conferred by genetically controlled physiologic systems varying in complexity with the chemical and the insect species. In most selection studies on insecticide resistance only the survivors (i.e., the "resistant" individuals) are saved for breeding. Selection of susceptible lines is unusual because of the difficulty of getting eggs from the test insects before their susceptibility is determined. The selection of both extremes (fast and slow responses) as is done here, has the effect of doubling the opportunities for segregating strains.

As before, the comparison to be made is between selection on the basis of single exposure and selection by repeated testing and successive elimination, but evaluation is made after ten generations of selective breeding. Also, carbon dioxide and nitrous oxide were each used as selecting chemicals.

Experimental procedure

The two gases and the two selection procedures provided four combinations of treatment.[1] Three replicate lines were started for each of these. The bugs used for each replicate were taken at random from the laboratory colony. Each replicate was maintained independently throughout the course of the experiment, and separate selections were made of males and females.

Preliminary tests showed that single pair matings produced too few progeny to yield practical results. Four breeding pairs could be expected to yield sufficient offspring maturing within the desired time interval, so this number was selected for each replicate.

Within the replicates, the insects were handled in test groups. In each replicate, the initial test groups consisted of four lots of 32 males each and similar lots of females. In single test selections, the first male to recover in each group and the first female to recover in each group were placed together to breed the next generation. Similarly the last bugs to recover served as breeding stock for the slow line. As selection was based on the first (or last) bug in each test group rather than on absolute recovery time, it was possible for a bug in one group to be selected over one or more faster (or slower) bugs in a different group. When feasible, the 32-bug test group was used in later generations, but frequently this proved impractical. As selection proceeded, the fast-recovering bugs could not be accurately timed in groups this large, and the slow-recovering bugs could be handled more efficiently in still larger groups. Therefore, the size of the test group was modified to suit the occasion. As the results were pooled for calculation, this procedure did not prejudice the outcome of the tests.

In repeated test selections with successive elimination, the scheme illustrated in Figure 6 was practiced. The initial population numbered 128 of each sex. Elimination continued until four bugs of each sex remained.

The number of bugs available for testing was sometimes a limitation that could not be controlled. The four breeding pairs did not always produce sufficient offspring, and some sterility was observed. A peculiar pathology developed sporadically in a number of instances. The cause of this could not be determined, but it resulted in considerable mortality when it occurred. It was not associated with any particular line of selection, and the surviving bugs in an affected culture were apparently normal.

Results

The response time in seconds was recorded for each test bug of the sample population in each generation. Consideration of the data was simplified by grouping the responses within the time intervals corresponding to the antilogarithm of .060. The number of bugs responding within each interval was tabulated in cumulative order, converted into percentages of the total, and plotted on logarithmic-probability paper. Lines were fitted by eye, and the response time for 50 per cent of the group (RT_{50}) was interpolated. The slope functions of the regression lines were calculated by the short method of Litchfield (1949). Without doubt the use of more refined statistical methods would change some of the values assigned, but it is unlikely that significant changes would result or if any conclusions would be modified. In a study of this kind major trends alone are important. The time required to calculate these and other data on more than 1200 regression lines was not justified. Even the summary data for this study are too extensive for publication. Data for one replicate in one selection are given for male and female bugs in Table 2. Similar data for all lines of selection are on file at The Connecticut Agricultural Experiment Station, and copies are available for study.

The range of response in each test group, as measured by the slope function, can be compared only from the complete data. The ranges varied somewhat, but in no way consistent with the type of selection with one exception. This was the selection for slow response to the single exposure by nitrous oxide. In the three male lines in this series the slope functions reached maxima (within ten generations) of 2.77, 2.98, and 3.90. In the three female lines, the maxima reached 1.99, 4.42, and 4.24. In none of 60 other lines did the slope function come to exceed 2.00. In arithmetic terms, some bugs selected for slow response by single exposure to nitrous oxide ranged in response time from about 100 seconds—well within the range of unselected bugs—to over three hours. In many of the individuals exhibiting long delayed recoveries, the righting reflex, rather than bodily ventilation, was probably affected. In some of these instances an extra stimulus induced prompt turning.

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[1] For convenience in later reference, these are given code designations as follows: (C)S—carbon dioxide, single test selection; (C)R—carbon dioxide, repeated tests with successive elimination; (N)S—nitrous oxide, single test selection; (N)R—nitrous oxide, repeated tests with successive elimination.

Table 2. Summary of response data for one line of milkweed bugs selectively bred for 10 generations for fast and slow response to carbon dioxide, single test selection

Gener.	Male bugs							Female bugs						
	RT_{50}		Slope*		Difference			RT_{50}		Slope		Difference		
	Fast	Slow	Fast	Slow	Fast†	Slow‡	Total§	Fast	Slow	Fast	Slow	Fast	Slow	Total
Orig.	135							158						
1	120	150	1.32	1.33	.05	.05	.10	122	175	1.32	1.17	.11	.04	.15
2	98	183	1.52	1.24	.14	.13	.27	86	183	1.28	1.24	.26	.06	.33
3	77	211	1.35	1.31	.24	.19	.43	78	250	1.42	1.24	.31	.20	.51
4	69	187	1.41	1.14	.29	.14	.43	70	200	1.61	1.16	.35	.10	.45
5	63	255	1.33	1.18	.33	.28	.61	66	255	1.35	1.18	.38	.21	.59
6	60	310	1.32	1.51	.35	.36	.71	68	300	1.28	1.25	.37	.28	.65
7	74	335	1.57	1.36	.26	.39	.65	74	335	1.57	1.27	.33	.33	.66
8	60	335	1.45	1.33	.35	.39	.74	61	315	1.31	1.30	.41	.30	.71
9	68	330	1.20	1.55	.30	.39	.69	65	380	1.33	1.35	.39	.38	.77
10	66	355	1.19	1.61	.31	.42	.73	66	312	1.18	1.23	.38	.30	.68

* Slope function $= \dfrac{\dfrac{RT_{84}}{RT_{50}} + \dfrac{RT_{50}}{RT_{16}}}{2}$

† Fast diff. = Difference in log. time between RT_{50} for bugs being selected for fast recovery and the RT_{50} for the original population sample.

‡ Slow diff. = Difference in log. time between RT_{50} for bugs being selected for slow recovery and the RT_{50} for the original population sample.

§ Total diff. = Difference in log. time between Fast RT_{50} and Slow RT_{50}.

A comparison of the degree of segregation obtained in the various lines of selection can be aided by graphic means. If no selection occurred, the RT_{50} would be the same for the "fast" and " slow" bugs in all generations. Any departure from this in the direction of selection would be a measure of the segregation. This principle was applied for all lines after 10 generations of selection by plotting the RT_{50} data on a grid to the scale illustrated in Figure 11. The vertical line O, representing the RT_{50} of the original population sample serves as the point of reference. To the left of this line is indicated, for each generation, the difference between the log RT_{50} for the strain selected for fast recovery and the original log RT_{50}. To the right of this line is indicated the log difference of the RT_{50} for the slow strain. All selected lines for both sexes were represented in this way, but only data for the males are illustrated in Figure 12. The more prompt the segregation and the more extreme it became, the greater would be the area subtended by the lines diverging from the point of origin. This area, measured by planimeter, is thus considered to be an index of segregation. In Table 3 segregation of each genetic line is represented by two comparative figures—one index for each sex. A mean value for each type of selection procedure is warranted on the basis that both sexes follow the same selection pattern, actually differing less than the replicates in each series.

Segregation into strains of fast and slow recovering bugs is obvious in all of these selections. Although not apparent from the data presented here, the range of response of the segregates, except in the (N) S groups already noted, lies outside the response range of unselected bugs. In other words, bugs of the

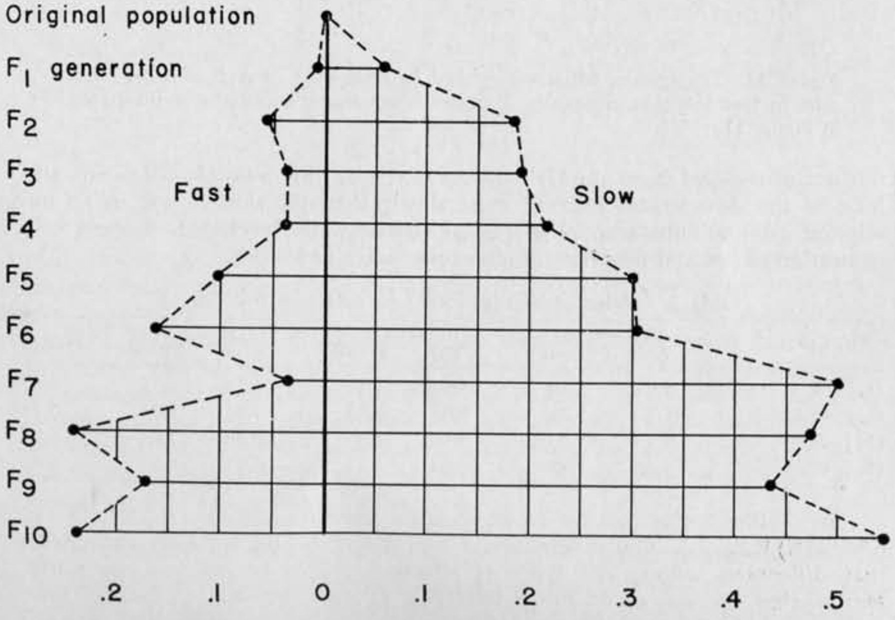

Figure 11. Method of plotting data to illustrate the patterns of segregation in Figure 12 and to calculate the index of segregation presented in Tables 3 and 13. The abscissa is scaled in logarithms; the position of the population response in each generation being the difference between the logarithms of the RT_{50} of the original population and the RT_{50} of the fast or slow group.

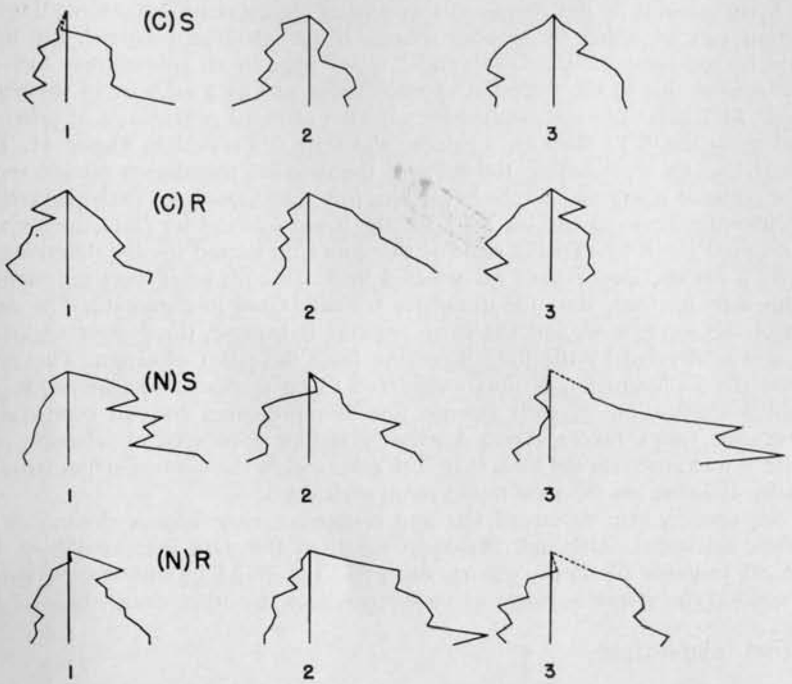

Figure 12. Segregation patterns exhibited by male bugs in each of three replicates in four selection procedures. Patterns drawn according to scheme illustrated in Figure 11.

fast strains respond more quickly than the fastest bug in an unselected group, and bugs of the slow strains respond more slowly than the slowest bug in an unselected group. Thus true physiological strains were developed. A mere concentration of fast and slow individuals cannot account for this.

Table 3. Index of segregation; four selection procedures

	Replicate 1		Replicate 2		Replicate 3		
	Male	Female	Male	Female	Male	Female	Mean
(C) S	4.01	3.60	4.34	4.38	6.34	6.55	4.87
(C) R	5.70	5.23	6.62	6.83	5.04	4.94	5.73
(N) S	6.96	5.48	5.98	6.32	8.40	7.08	6.70
(N) R	5.05	5.42	8.84	8.11	6.87	6.62	6.82

In Table 3 the indexes of segregation vary from 3.60 ((C) S,1, ♀) to 8.84 ((N) R,2, ♂). The differences among the replicates are sufficiently great that differences among the types of selection procedure are less impressive. Nevertheless, nitrous oxide has significantly greater "segregating ability" than carbon dioxide. Of more importance here, however, are the differences between single exposure selection and selection by repeated testing and successive elimination. Although a definite trend exists for a greater segregation resulting from selection by repeated testing, as predicted, the differences are not quite statistically significant. Certainly they are less than might have been indicated by the

consistency of response in one generation as illustrated in Figure 10. Undoubtedly, the variance within individuals is affecting selection, but it is not a dominating factor.

The chance factor, of course, favors selection of genotypes for extreme response by the single exposure technique, even though the chance was presumed to be less than by repeated exposure technique. It will be recalled, with reference to Figure 4, that it was postulated that the A-type and C-type bugs would be segregated by both selection procedures, but they would be diluted by D-type when selection was made by single exposures.

One other comparison suggests that the inconsistent response of individuals may be more influential in modifying selection than is apparent. This concerns the fixity of the genetic selection. In one replicate of each of the selected lines, breeding was continued beyond the tenth generation without further selection. A relatively large number of bugs in each generation were allowed to mate at will, and eggs of different ages and clutches were saved to insure a mixture representative of the total group. Only the even-numbered generations were tested, and bugs of both sexes in equal numbers constituted the test groups. Table 4 records the available data in terms of the RT_{50} for fast and slow strains of the designated lines.

Table 4. RT_{50} of fast and slow strains unselected beyond the tenth generation

	(C)S, 1 RT_{50}		(C)R, 1 RT_{50}		(N)S, 2 RT_{50}		(N)R, 3 RT_{50}	
Generation	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
F_{10}	83	500	72	350	55	750	64	460
F_{12}	105	430	76	410	70	620	64	485
F_{14}	114	184	62	370	85	799	64	405
F_{16}	122	232	86	375	69	181	66	403
F_{18}	155	253	lost	417	72	122	67	413
F_{20}	181	204		355	94	130	67	381
F_{22}	discontinued			342			66	350

The line (C)S,1 was discontinued after the 20th generation (10 generations without selection) because the fast and slow strains had converged to the area well within the range of normal unselected bug response. After 10 generations without selection (F_{20}) the slow line of (N)S,2 showed unmistakable reversion. The fast line of this series is less definite. On the other hand, lines (C)R,1 and (N)R,2 show little if any reversion to date. Insofar as these data go, reversion did not occur in the two lines selected on the basis of repeated exposure and successive elimination. This is good evidence that selection without regard to the varying responses in individuals (as with single exposure) leads to less firmly fixed genetic material than selection in which bugs with consistent responses are chosen for breeding.

Correlation of responses to carbon dioxide and nitrous oxide

Comparisons between selections by carbon dioxide and nitrous oxide are meaningful with respect to the objectives of this study only if important differences exist between the actions of the two gases.

The actions of the two gases compared

The anesthesia induced by both gases is of a general nature, but minor differences exist. The knockdown effect of carbon dioxide is rapid; bugs drop within four or five seconds. The effect of nitrous oxide is slower, being initially excitatory. Exposed bugs attempt to climb the sides of the vessel, and a few are able to maintain their grip on the surface even after they are immobilized. Bugs anesthetized with carbon dioxide are completely relaxed and can be handled easily. Those treated with nitrous oxide are not so completely relaxed. Their tarsal claws catch on forceps or other bugs and are more difficult to place.

The righting response represents complete recovery from the effects of nitrous oxide. This is not true with carbon dioxide. Bugs tested repeatedly with inadequate rest periods between tests show cumulative effects of carbon dioxide.

Nitrous oxide presumably exerts its action by excluding oxygen from the physiological environment. Evidence for this is that small amounts of oxygen greatly reduce the anesthetic action of nitrous oxide. If the percentage of oxygen exceeds 2 or 2½ per cent, the anesthetic action is completely lost. In most respects nitrogen can be substituted for nitrous oxide to achieve the same results. Carbon dioxide, on the other hand, has an anesthetic action even in the presence of oxygen. This is lost only when the oxygen in a carbon dioxide-oxygen mixture exceeds 60 per cent. As the oxygen in the atmosphere approximates 21 per cent, carbon dioxide clearly does not act by excluding oxygen except possibly at the cellular level. To be sure, 20 per cent oxygen in a carbon dioxide-oxygen mixture delays knock-down and shortens recovery time as compared with carbon dioxide alone. Increased time of exposure can compensate for the reduced carbon dioxide content in effecting similar response times. Nitrous oxide cannot be substituted for a part of the carbon dioxide in a carbon dioxide-oxygen mixture without correspondingly reducing the response time. This is evident in Table 5, which tabulates response times for groups of bugs exposed to gas mixtures in which the oxygen concentration is kept constant, but the proportion of carbon dioxide and nitrous oxide is varied. These results emphasize a specific action of carbon dioxide not shared by nitrous oxide.

Table 5. Response times, in seconds, of bugs exposed for 90 seconds to gas mixtures

O_2 20% CO_2 80	O_2 20% N_2O 27 C_2O 53	O_2 20% N_2O 53 CO_2 27	O_2 20% N_2O 80
123	56	0*	0
130	61	0	0
136	63	0	0
140	65	0	0
173	65	0	0
173	71	0	0
183	73	0	0
197	83	10	0
209	123	15	0
239	141	30	0

* 0 = no knock-down.

This is evidently a different situation from the altered behavior in honeybees following anesthesia as studied by Ribbands (1950). He concluded that carbon dioxide and nitrogen had similar effects because of their common property of excluding oxygen. It is similar, however, to the findings of Brooks (1957) that the toxic effects of carbon dioxide on the development of the German cockroach were not shared by nitrogen.

Another important difference in the action of the two gases is evident at low temperatures. At 40°F., bugs exposed to nitrous oxide are not anesthetized before they become sluggish with the cold. On the other hand bugs exposed to carbon dioxide at this temperature are anesthetized promptly and fail to recover before they become inactive with the cold.

Even though these real differences in action between the two gases exist, it may happen that at the cellular level the result is the same. Possibly vital centers are sensitive to oxygen loss resulting directly because of an unusual affinity for carbon dioxide or indirectly because of the gross exclusion of oxygen by nitrous oxide or nitrogen.

Sears and Fenn (1957) present evidence that even at the cellular level the action of the two gases may be different. In offering an explanation for narcosis on the basis of phase changes in the nerve membrane resulting from accumulations of the inert gases in the lipide in excess of that in the watery components, they found that carbon dioxide easily reverses the phases of an oil in water emulsion. Nitrous oxide on the other hand failed to cause reversals even at the maximum pressures permitted by their technique.

Thus, most of the evidence indicates distinct differences in the modes of action of carbon dioxide and nitrous oxide. The only real point of similarity is the state of anesthesia induced by them.

Comparison of individual bugs responding to carbon dioxide and nitrous oxide

Because of the inconsistent responses of individuals, indiscriminate testing of bugs chosen at random is of little value in comparing responses to the two anesthetizing gases. By successive elimination of bugs exposed repeatedly to the test gas, it is possible to select from a sample population groups of fast and slow recovering bugs so that within each group the individuals respond consistently for the group, though varying with respect to each other. In other words, bugs characteristically fast or slow in response to one gas can be obtained for testing with both gases.

A method of testing pooled segregates provides a striking demonstration of the presence or absence of group differences, and is especially useful in this instance. Bugs selected by one gas for fast response and for slow response are differentially marked in some way—by number, with paint, or by clipping wings. Individuals from both groups are exposed together to the test gas. Thus there can be no question of differential treatment. The same individuals are tested with both the selecting gas and the alternate gas, with suitable rest periods intervening. The results given in Table 6 are those obtained in a representative test of eight males and eight females selected out of 500 males and 500 females by carbon dioxide, and seven males and seven females selected from similar numbers by nitrous oxide.

One could read into these data the conclusion that among the carbon dioxide selected bugs, five were tolerant (fast) of CO_2 but sensitive (slow) to N_2O (F-s); five were tolerant of N_2O but sensitive to CO_2 (S-f); three were tolerant of both gases (F-f); and three were sensitive to both gases (S-s). Or, among the nitrous oxide selected bugs, that four were tolerant of N_2O but not of CO_2 (F-s); three were tolerant of CO_2 but not of N_2O (S-f); four were tolerant of both gases (F-f); and three were sensitive to both gases (S-s). In this particular test it is clear that the assemblage sensitive to (or tolerant of) CO_2 is different from the assemblage sensitive to (or tolerant of) N_2O. In other words, these susceptibilities to the two gases are not correlated. The correlation is not negative, however. If it were negative, the segments of the population selected by

Table 6. Response time, in seconds, of individuals identified as to Fast (F) or Slow (S)

CO$_2$ selected		N$_2$O selected	
CO$_2$ tested	N$_2$O tested	N$_2$O tested	CO$_2$ tested
66 F	FCO$_2$ 71 fN$_2$O	81 F	FN$_2$O 83 fCO$_2$
69 F	S 76 f	85 F	F 95 f
73 F	S 79 f	88 F	F 95 f
74 F	S 82 f	90 F	S 103 f
76 F	F 83 f	93 F	F 106 f
83 F	S 86 f	94 F	S 108 f
85 F	S 86 f	95 F	S 109 f
91 F	F 87 f	102 F	F 109 s
121 S	F 88 s	103 S	F 115 s
151 S	S 89 s	112 S	S 128 s
153 S	F 90 s	118 S	S 131 s
154 S	S 91 s	144 S	F 141 s
157 S	F 93 s	212 S	S 143 s
189 S	F 94 s	230 S	F 169 s
238 S	F 99 s		
275 S	S 104 s		

The bugs identified by f or s, that is in terms of the gas alternate to that used in selection, are so identified by virtue of the single test indicated; the bugs identified by F or S, that is in terms of the selecting gas, are so identified by repeated tests, although the data given are from one test. The same general results are obtained when the test is repeated with both gases and the same bugs.

CO$_2$ would be those rejected by N$_2$O. Such a possibility of negative correlation in insecticide susceptibilities has been postulated by Plackett and Hewlett (1948).

Results as indicated in the above test are not an isolated example, as similar results have been observed in several groups of the same sort. Nevertheless this pattern is not consistently obtained, and some other tests show more nearly similar selection by the two gases, particularly where the segregation by the selecting gas is less complete. In the absence of *negative* correlation, this is not surprising. The above data show that some individuals were fast (or slow) in their responses to both gases, and others were not. It is to be expected that the proportion of these would vary with different populations.

Table 7. Mean response time of bugs selected by CO$_2$, tested repeatedly with CO$_2$ and N$_2$O

Order* number of bugs ranked by CO$_2$	CO$_2$ mean response time (seconds)	N$_2$O mean response time (seconds)	Order number of bugs ranked by N$_2$O
1	88 F	83 Ff	5
2	91 F	68 Ff	1
3	93 F	84 Ff	6
4	97 F	104 Fs	10†
5	102 F	74 Ff	2
6	102 F	76 Ff	4
7	111 F	86 Ff	7
8	115 F	90 Ff	8
9	141 S	76 Sf	3†
10	206 S	159 Ss	15
11	234 S	139 Ss	13
12	235 S	117 Ss	12
13	241 S	93 Ss	9
14	250 S	145 Ss	14
15	253 S	109 Ss	11
16	280 S	162 Ss	16

* Bugs 1-8 selected initially for fast recovery to CO$_2$. Bugs 9-16 selected initially for slow recovery to CO$_2$.

† These two individuals are misplaced from their respective groups; i.e., they do not show cross-selection.

Another test can be represented somewhat differently, and a closer correlation in response can be seen between the two gases. As in the previous test, from approximately 500 bugs of each sex, four males and four females were selected for rapid response to carbon dioxide and the same number were selected for slow response to carbon dioxide. The bugs were individually marked, pooled, and tested seven times with carbon dioxide and seven times with nitrous oxide, with rest periods between tests. Individual response times were tabulated, and the mean response time for each bug to each gas was calculated. A similar test was conducted on another group of 1000 bugs with nitrous oxide as the selecting gas. The results are given in Tables 7 and 8.

Table 8. Mean response time of bugs selected by N$_2$O, tested repeatedly with N$_2$O and CO$_2$

Order* number of bugs ranked by N$_2$O	N$_2$O mean response time (seconds)	CO$_2$ mean response time (seconds)	Order number of bugs ranked by CO$_2$
1	66 F	95 Ff	1
2	71 F	109 Ff	4
3	71 F	112 Ff	5
4	72 F	120 Ff	7
5	75 F	97 Ff	2
6	76 F	125 Ff	8
7	79 F	115 Ff	6
8	96 F	100 Ff	3
9	116 S	230 Ss	15
10	117 S	125 Ss	9
11	160 S	157 Ss	12
12	183 S	154 Ss	11
13	193 S	127 Ss	10
14	209 S	177 Ss	14
15	241 S	159 Ss	13
16	245 S	273 Ss	16

* Bugs 1-8 selected initially for fast recovery to N$_2$O. Bugs 9-16 selected initially for slow recovery to N$_2$O.

The order number of individuals ranked by the alternate gas obviously differs from that ranked by the original segregating gas. This, however, is less important than the fact that the fast and slow groups are maintained essentially the same by the alternate as by the segregating gas. Only two individuals, both indicated in Table 7 as †, are misfits in the groups' responses. Other comparable results, too, have been observed in other groups, so the opposite conclusions to be drawn from Table 6 *vs.* 7 and 8 do not reflect the differences in presentation. Nevertheless, these two sets of data were chosen to illustrate both extremes. Intermediate conditions are found more frequently.

It may be concluded then, that from a representative population, some individual bugs physiologically selected by one chemical may be similarly selected for a different chemical to which they have not been exposed previously, whereas other individual bugs may show less or no cross-selection of this sort. Although group response is determined by the distribution of individuals showing complete, intermediate, or negligible cross-selection, this distribution may not be constant because of the variation in range of responses in different individuals. Selected bugs tend to respond consistently to the selecting chemical, but not necessarily to the alternate chemical. Thus, different data, obtained by the same techniques, may demonstrate completely diverse results. The significance of this is considered in the next section.

Cross-selection and cross-resistance

Cross-resistance to insecticides means that insects resistant to an insecticide are also resistant to a different insecticide to which they have not previously been exposed. The genetic elements responsible for the development of cross-resistance must be the same for both insecticides or at least have common characteristics. The selecting mechanism acting on the common elements to result in cross-resistance may be termed cross-selection. The common characteristics have often been associated with the mode of action of the insecticide. Opinion has favored the generalization that cross-resistance will easily be developed to insecticides having similar modes of action by the continued use of one of the insecticides. Cross-resistance should not develop to an insecticide by the use of a dissimilar one. Conversely, opinion has favored the generalization that when cross-resistance does develop, the modes of action of the insecticides concerned are likely to be the same; when cross-resistance does not develop, the modes of action are different. In spite of a great deal of work having been done, knowledge of comparative toxicology is inadequate, and these generalizations have arisen largely from knowledge of the chemical structure of the insecticides. As reviewed by Metcalf (1955a), many examples seem to justify the generalizations, but there are some inconsistencies and some definite unexpected observations. Thus, although resistance to DDT is also associated with a similar resistance to the related chemical analogues of DDT, the ratio of effectiveness by different analogues is not always constant against different strains of flies. Methoxychlor resistant flies may be susceptible to DDT. Cross-resistance to DDT and lindane is not at all consistent. These anomalies suggest that the generalizations based on chemical structure are inadequate, or as will be proposed, that the mode of action concept as it relates to cross-resistance has been too narrowly interpreted.

How do carbon dioxide and nitrous oxide fit into the generalizations regarding cross-selection? If these two chemicals had the same mode of action, cross-selection would be expected. If the modes of action are different, cross-selection should not occur. Conversely, if cross-selection did result, the modes of action might be assumed to be the same. In the preceding section evidence was presented that carbon dioxide and nitrous oxide do indeed differ in several respects. Also, individual bugs may or may not be similar in their responses to the two gases. The similarities of group responses to the two gases depends on the constituent individuals. The following discussion relates these considerations to genetic selection.

Of the 12 genetic lines so far considered (three replicates of each of the four selection procedures), 11 can be compared in the F_{10} generation. Data were recorded for responses to the alternate gas as well as to the selecting gas. Also data are presented for six genetic lines at the F_{15} generation. These are principally of value in confirming the trends already established by the tenth generation. The data are summarized in Table 9.

Table 9. Responses (RT_{50}) to different gases of 11 lines of bugs selectively bred for 10 generations, and of 6 lines for 15 generations

		Male						Female						Ratio L. T. D. Select, gas / L. T. D. Alternate gas	
		CO_2			N_2O			CO_2			N_2O				
		RT_{50}			RT_{50}			RT_{50}			RT_{50}				
		Fast	Slow	L.T.D.*	Fast	Slow	L.T.D.	Fast	Slow	L.T.D.	Fast	Slow	L.T.D.		
							Tenth Generation							♂	♀
(C)S	1	**83†**	**500**	**.78**	80	125	.19	**100**	**520**	**.71**	86	98	.06	.24	.08
	2	**56**	**250**	**.64**	84	110	.12	**90**	**315**	**.54**				.19	—
	3	**66**	**355**	**.73**	64	115	.25	**66**	**312**	**.68**	72	157	.34	.34	.50
(C)R	1	**72**	**350**	**.69**	72	365	.71	**83**	**335**	**.60**	72	365	.71	1.03	1.18
	3	**70**	**203**	**.47**	77	136	.25	**70**	**230**	**.51**	70	200	.46	.53	.90
(N)S	1	105	150	.15	**67**	**670**	**1.00**	107	170	.20	**76**	**380**	**.69**	.15	.29
	2	79	120	.18	**55**	**750**	**1.14**	67	125	.27	**62**	**750**	**1.08**	.16	.25
	3	78	190	.39	**77**	**760**	**.99**	84	202	.48	**103**	**720**	**.84**	.39	.57
(N)R	1	94	142	.18	**68**	**320**	**.67**	89	160	.26	**69**	**320**	**.67**	.27	.39
	2	110	155	.15	**65**	**725**	**1.04**	—	180	—	**62**	**640**	**1.02**	.14	—
	3	102	173	.23	**64**	**460**	**.86**	114	190	.22	**70**	**420**	**.78**	.27	.28
							Fifteenth Generation								
(C)S	3	**59**	**460**	**.89**	62	170	.44	**59**	**725**	**1.09**	64	250	.59	.49	.54
(C)R	1	**51**	**770**	**1.18**	66	920	1.14	**63**	**760**	**1.08**	76	710	.97	.97	.90
(N)S	2	71	111	.19	**62**	**1200**	**1.29**	62	150	.38	**62**	**940**	**1.18**	.15	.32
	3	92	170	.27	**70**	**730**	**1.02**	98	189	.28	**82**	**475**	**.76**	.27	.27
(N)R	2	87	166	.28	**60**	**1540**	**1.41**	96	189	.29	**77**	**580**	**.85**	.20	.34
	3	86	234	.43	**58**	**1050**	**1.26**	84	234	.44	**60**	**1090**	**1.26**	.34	.35

* L.T.D. = Log. total difference = Log. Slow RT_{50} — Log. Fast RT_{50}.
† Data for responses to the selecting gas are shown in boldface.

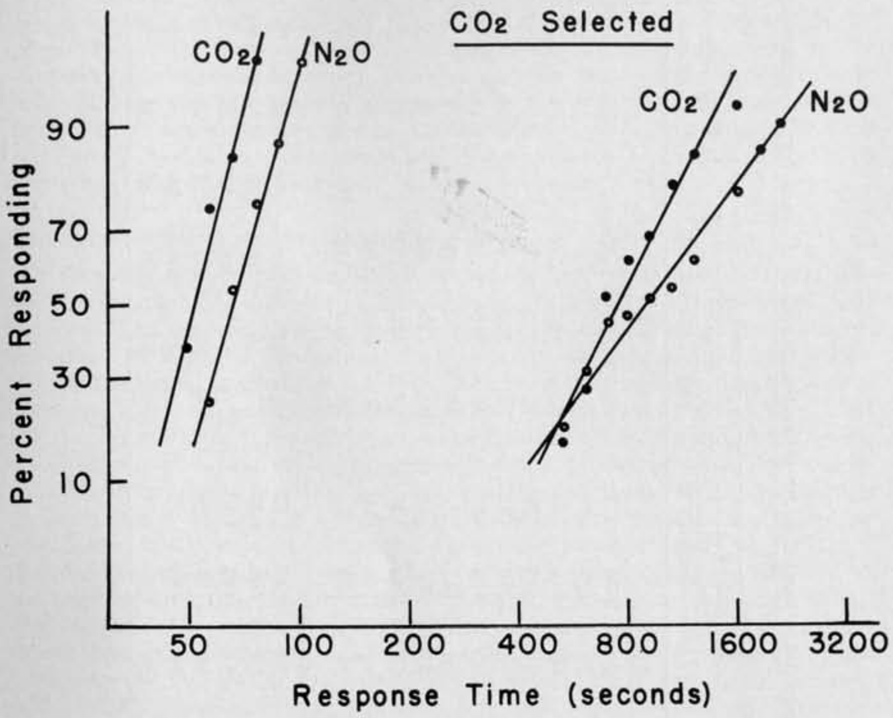

Figure 13. Response time curves showing extreme cross-selection found in Series (C)R, 1, F_{15}. Curves for responses by fast and slow selected groups to the selecting gas CO_2 and the alternate gas N_2O.

These data clearly indicate that in these 11 genetic lines, with the two sexes tested separately, a wide range of cross-selection exists. Cross-selection is complete in (C)R, 1. The fast and slow groups were just as distinctive when tested with the alternate gas N_2O as with the selecting gas CO_2. On the other hand a few groups such as (N)S,2 and (N)R,2 show very little cross-selection. Such diverse results as seen in (C)R,1 and (N)R,2 are graphically illustrated in Figures 13 and 14. Most of the data show various degrees of cross-selection between these chosen examples. The expression of cross-selection as a ratio of the L.T.D. by the selecting gas to the L.T.D. by the alternate gas as is done in Table 9 serves to illustrate the diversity of results, but it obscures the fact that in some cases cross-selection is practically complete on the fast side, but negligible on the slow side (i.e. (N)S, 2 , ♀ , F_{15}; (C)S, 3, ♂ , F_{15}). The converse of this does not appear evident.

It was shown above that in a single generation some individual bugs physiologically selected by one chemical may be similarly selected for response to a different chemical to which they have not been exposed previously; other individual bugs may be dissimilarly selected. Here is shown that in populations derived by selective breeding, cross-selection is relative and cannot be expressed as being present or absent. Cross-selection may be complete so that bugs selected for fast or slow response to one gas are just as fast or slow in response to the other. On the other hand, cross-selection can be considerably less or negligible.

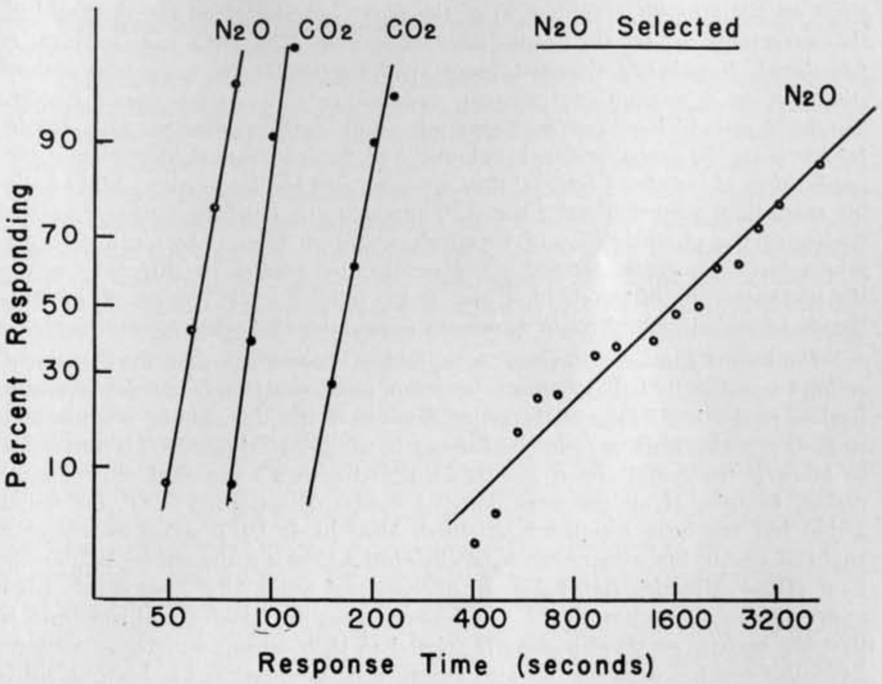

Figure 14. Response time curves showing limited cross-selection found in series (N)R, 2, F_{15}. Curves for responses by fast and slow selected groups to the selecting gas N_2O and the alternate gas CO_2.

How can this be explained by the "mode of action" concept? On the basis of data such as shown in Tables 7 and 8 and those obtained for the genetic line (C)R,1, it might be concluded that carbon dioxide and nitrous oxide have the same mode of action. On the basis of data such as shown in Table 6 and those obtained for the genetic line (N)R,2, it might just as well be concluded that the two gases differ in their mode of action. These anomalous results obtained with the same two chemicals under similar conditions raise questions on the mode of action concept that have greater ramifications than pertain to this study alone. A critique of the mode of action concept thus seems in order.

"Mode of action" concept as it relates to cross-selection

Some of the apparent anomalies in cross-resistance as related to "mode of action" of insecticides may hinge upon what is meant by the term "mode of action." Metcalf (1955b) has commented that "modern toxicological theory almost exclusively relates the mode of action of poisons to specific interference with biochemical systems, largely enzyme in nature." This may be true in theory and certainly represents the desired end result of many toxicological studies, but in practice and in many current writings, "mode of action" as a term is ill-defined and loosely applied. In some cases, to be sure, "mode of action" implies a very specific chemical action-insect reaction system at the molecular level—as may be the case with the anti-cholinesterases represented by physostigmine and some of the organophosphate insecticides. In other cases the term is used to cover a functional disturbance, the chemistry of which is not known, such as an effect on the synaptic transmission of the nerve impulse or on the threshold of chemoreception or on the contractility of muscle. The term may apply to a functional disturbance detected more symptomatically, as a nervous system stimulant or depressant, gut irritant, or even as a paralyzing agent. Finally, "mode of action" has been used to apply to an entire syndrome. Although in former years the terms "stomach poisons" and "contact insecticides" carried the implication of "mode of action," they are now used less frequently and do nothing more than suggest effective routes of administration or intoxication. In many studies of the pharmacology of chemicals acting on insects the terms used are relatively unimportant, because it is clear that the studies are directed towards the understanding of "mode of action" at the cellular level. The use of the term "mode of action" may lead to erroneous implications in other types of study.

Confusion can arise because it is seldom known whether the "mode of action" is a directly lethal action or merely a primary action in a series of events leading to death. As long as the action leads to death, this may be unimportant in most considerations of insect toxicology. In toxicological studies it is important to know if the lethal action can be identified with a recognized physiological action. In spite of all the work done on modes of action of DDT, the lethal action has not been completely clarified. Most likely DDT serves to trigger a chain of events, but a more direct biochemical action is suspected by Winteringham (1956). Metcalf's stated concept of "mode of action" has most closely been approximated in studies on the organophosphorus insecticides, and consensus is that the toxicity of these is directly related to their anti-cholinesterase activity. Even this conclusion is not without controversy, however, as has been ably discussed by Spencer and O'Brien (1957).

Confusion may also arise because similarity (or dissimilarity) of "mode of action" so determined by one criterion may not be confirmed by a second criterion. It has been noted that a series of chemicals, presumably having the same "mode of action," as determined by *in vitro* studies, has a disproportionate effect on two unrelated insects or their tissues (Beard 1951). This indicates that, in certain situations, interfering factors can mask something that in other situations indicates similar "modes of action." There is no doubt that in some systems a series of chemicals may act similarly at the symptomatic level and at the functional level of an organ system, but dissimilarly at the molecular level.

Caution needs to be exercised especially in indirect studies of "mode of action." Such methods have been employed as a hopeful approach to "mode of action" information and not as a substitute for the physiological and biochemical techniques that have lagged in development. Thus, statistical procedures have been used to differentiate responses to chemicals in terms of similar or independent action, synergistic action, and variations of these. Such procedures have, for the most part, been theoretical and have not been put to critical test because of lack of specific physiological-chemical information on "mode of action." A technique of this sort is to be found in the problem of resistance to insecticides and cross-resistance. Reference has already been made to the thought that cross-resistance offers a technique for studying similarity of "mode of action," or conversely, that a knowledge of "mode of action" will permit the prediction of cross-resistance. Bliss (1954) has expressed the reasoning thus: "If a strain has been made resistant to one chemical by selective breeding and is then found to be partially resistant to a second chemical, to which it rapidly develops full resistance under selection, the two poisons might well be expected to act similarly." This is a very reasonable expectation, but the situation does not appear to be this simple.

The indirect study of mode of action supposes that two chemicals acting similarly will select the same individuals in a population and that two chemicals acting dissimilarly will select different individuals; further, that these distinctions are retained through generations of selective breeding. From what has been reported above, it is obvious that populations are too fluid and dynamic for this to happen in just this way. Resistance or tolerance of an individual to an insecticide is not an all-or-none phenomenon, but may vary in degree depending upon the insect's physiological state at the moment the chemical is acting. This is a chemical action-insect reaction system that involves far more than the biochemical system at the cellular enzyme level. It involves such toxicological features as receptivity at the site of initial contact of the toxicant with the insect, permeability of cells and tissues, adsorption, transport of the toxicant by diffusion and circulation, differential solubilities in various tissue fluids, activating or toxifying systems, and detoxifying, competitive or other antagonistic mechanisms of different sorts, and secondary and side-effects—in addition to mode of action in the restrictive sense. Thus it is not to be expected that for two toxicants all of these features would be selected in the same way, unless, inconceivably, all features were identical for the two toxicants. The exception to this would be if the restrictive mode of action for a chemical was such a dominant, limiting action that it alone determined the selectivity of the chemical.

From the above data on responses of the milkweed bug to carbon dioxide and nitrous oxide it is clear that sometimes selection by the two gases is similar and sometimes it is different; sometimes genetic cross-selection is negligible and sometimes it is complete. Therefore, very little can be inferred about the similarity or dissimilarity of the mode of action of the gases by this technique. Moreover, it is unrealistic to assume that the differences in selection point to dissimilar "modes of action" at the cellular level—too many physiological variables are involved in the selection by a chemical.

That with different lines different degrees of cross-selection occur is not surprising because one would expect different genic combinations to be selected in somewhat the same way in which King (1955) found different genic constellations responsible for DDT resistance in *Drosophila*. Cross-resistance may result from concomitant selection, whether because of common gene-controlled physiological variables, gene linkages, or other mechanisms, and may be no different from the parallel selection of such features as DDT resistance and length of life cycle (in house flies) (Pimentel *et al.* 1951) or "peripheral pupation" in *Drosophila* (Sokal and Hunter, 1954)—which probably have nothing at all to do with the "mode of action" of the selecting agent. This thinking is in line with the conclusion of Barbesgaard and Keiding (1955) that specific resistance mechanisms exist for certain chemicals as well as that some mechanisms common to two or more chemicals occur and that of Van Asperen (1956) to the effect that there can be positive correlation of susceptibilities to two poisons which have independent action.

A more specific reference can be made to illustrate the point being made. At this writing the situation must be considered hypothetical, but evidence will be presented elsewhere to provide some basis for it. It is generally agreed that DDT unstabilizes sensory elements or otherwise affects the nervous system so that volleys of impulses induce abnormal and excessive muscular activity. It is also generally agreed that some of the phosphate insecticides inhibit cholinesterase in nerve ganglia or possibly have a direct effect on muscle. The result of this may be a similar uncoordinated muscle excitation. Thus the two types of chemicals act on different sites in different ways. But if the muscle's excitatory mechanism reacts similarly to nervous activities of different origin or if the energy metabolism of the muscle is affected in the same way in both cases, the ultimate cause of death could be the same. If this situation does exist, it is easy to see how selection directed primarily at variation in the nervous elements would not likely result in cross-selection. If, however, selection was directed at variation in the muscular elements, cross-selection would be almost certain. The genetic factors controlling these elements are likely to be very complex, so it is not surprising that all degrees of cross-selection can be observed in the use of two chemicals.

In short, these observations and this interpretation cast serious doubt on the validity of using cross-resistance as an indicator of mode of action in the restrictive sense and on the possibility that the use of insecticides with apparently dissimilar modes of action will insure against the development of cross-resistance. It seems much more likely that the attributes of a chemical that are responsible for selecting a certain assemblage from a population include the whole complex of its selective activity on the biological system. The "mode of action" in a restrictive sense is not enough. If all activities are different from those of an alternate chemical, or if certain activities having dominant selective value are different, then cross-selection will be minimized. If, however, the alternative chemicals share any of the activities having selective value, there will be cross-resistance—and the more activities common to the two chemicals, the greater, or the more frequent, will be the cross-resistance. This concept is not new, but is supported by this study.

A possible physiological basis for cross-selection

Even though the indirect evidence of cross-selection is not reliable in evaluating modes of action of carbon dioxide and nitrous oxide, one aspect of the action of these gases might explain cross-selection. As has been stated, the anesthetic action of nitrous oxide is nullified by the presence of small amounts of oxygen, whereas carbon dioxide is effective even when the atmospheric amount of oxygen is greatly exceeded. Thus nitrous oxide might select on the basis of its gross exclusion of oxygen. On the other hand because in these studies the concentrated gas was used, carbon dioxide might select either on the basis of the gross exclusion of oxygen or on its more specific action. If this is so, nitrous oxide selected bugs should not show their characteristic segregation when tested with carbon dioxide-oxygen mixtures. On the other hand, carbon dioxide selected bugs might or might not show segregation when tested with carbon dioxide-oxygen mixtures. If they did, it could be reasoned that selection was made primarily on the basis of the specific action, and these bugs should not show segregation when tested by nitrous oxide. If they did not, it could be reasoned that selection was made primarily on the basis of the general action, and similar segregation could be expected with nitrous oxide. These combinations and results are schematically indicated in Table 10.

All of these possibilities cannot be tested completely as data were not obtained in a systematic way for this purpose. Nevertheless a number of experiments provided data for examining this concept.

In Table 10 the most definitive possibility would be that of bugs selected by nitrous oxide tested with carbon dioxide-oxygen mixtures. No segregation should be evident. Because oxygen modifies the exposure-time, response-time relationship of carbon dioxide, segregation can be judged best by comparing the segregate strain with unselected control bugs tested at the same time under the same conditions. In Table 11, five selected lines are compared with companion controls.

Table 10. Expected segregation resulting from general and specific selection by gases

Supposed basis for selection	Expected segregation when tested with		
	N_2O	CO_2	$CO_2 + O_2$
Nitrous oxide (General anoxic action)	+	+ or –	–
Carbon dioxide (General anoxic action)	+	+	–
Specific action	–	+	+

Table 11. Response of N_2O selected bugs and unselected bugs to $CO_2 + O_2$

	N_2O segregates, RT_{50}	Unselected controls, RT_{50}
(N)S, 2 Slow , F10	146	146
(N)S, 1 Slow , F9	170	170
(N)R, 3 Fast , F13 (Revert)	108	117
(N)S, 2 Fast , F10	98	146

Of the four test groups in Table 11 the first three certainly show no segregation, as expected. The other one shows possible segregation, but this is much less than when the bugs were tested with the selecting gas, nitrous oxide. On the other hand a definite inconsistency was evident in a mixed group of fast and slow bugs of the (N)R,2, line. An incomplete segregation was observed when the bugs were tested with carbon dioxide plus oxygen, but the segregation was greater than when carbon dioxide was used alone.

Bugs selected on the basis of the specific action of carbon dioxide should not show segregation when tested with nitrous oxide. Single exposure selection of this kind, with carbon dioxide and oxygen was made in one series for ten generations. Comparative data for the tenth generation are given in Table 12.

Table 12. Response of bugs selected for 10 generations with $CO_2 + O_2$

	Males, RT_{50} tested by		Females, RT_{50} tested by	
	$CO_2 + O_2$	N_2O	$CO_2 + O_2$	N_2O
Fast	48	86	58	94
Slow	308	157	320	155

Clearly, segregation is much less in these bugs when tested with nitrous oxide than when tested with the selecting gas $CO_2 + O_2$. This approaches the expected results.

It is difficult to see how carbon dioxide used alone as the selecting agent could select exclusively on the basis of general action in some lines and exclusively on the basis of specific action in others. It is more likely that different individuals would be affected by one or the other action, the population being a resultant mixture. Evidence for this can be seen in male bugs selected for slow response by carbon dioxide in the line (C) R,1,F_{14}. Twelve bugs tested with carbon dioxide plus oxygen ranged in response time from 109 to over 1000 seconds, indicating a very heteogeneous group. Other similar data, or very peculiar response curves, could be presented as evidence of aberrant distributions of response characteristics within the selected strain. On the other hand selection by the specific action of carbon dioxide is suggested in the line (C) S,1,F_{13} slow males in which the RT_{50} for carbon dioxide plus oxygen is 351 seconds as compared with 120 seconds for unselected control bugs. When tested with nitrous oxide, the RT_{50} for the selected bugs was 114 seconds as compared with the RT_{50} of 120 in the controls, indicating negligible segregation. On the other hand, the fast males in this series did not respond in like fashion. With carbon dioxide plus oxygen, the RT_{50} was 55 for the selected strain and 120 for the control. With nitrous oxide, the RT_{50} was 73 for the selected strain and 120 for the control—a difference too great to be ignored.

It was pointed out in a previous section that the line (C) R,1 showed complete cross-selection and was equally responsive to carbon dioxide and nitrous oxide. It might be assumed in this case that all of the selection had been on the basis of the general anoxic effect, and if so, the bugs should not show segregation when tested with carbon dioxide plus oxygen. A test group of slow, F_{13}, males was tested and found to have an RT_{50} of 188 seconds as compared with an RT_{50} of 135 seconds in a similar group of unselected bugs. Although this difference is not extreme, some segregation is clearly evident.

From these examples it may be concluded that many of the data are in line with the scheme summarized in Table 10 and hence go a long way in suggesting an explanation for the diverse range of cross-selection. Nevertheless the data are not conclusive enough to attribute the inter-relationships to selection on the basis of the general anoxic effect or the specific carbon dioxide effect in a mutually exclusive way. The inconsistencies, then, only serve to emphasize the conclusion drawn in the previous section that all the attributes of a chemical in its action are likely to be involved in its selective potentialities.

Joint selection by two chemicals

Applied entomology makes regular use of combinations of insecticides against insect pests having different sensitivities to the components of the mixture. Synergistic mixtures are also widely used. Most toxicological studies on insecticide combinations have been of possible interactions of the components (see for example Sakai, 1951 and other papers, and Turner 1955). The practical end in view is synergism or other form of enhanced effect. Little experimental work has been done on the use of combinations of insecticides having different actions as a means of delaying or preventing the development of resistance. This has been widely discussed (see for example Demerec, 1952) on theoretical grounds. From the medical field has come some encouragement by a measure of success in the use of antibiotic mixtures for controlling infectious microorganisms. Crow (1952), on the other hand, theorized that nothing would be gained (in the absence of synergism) by the joint or alternate use of two insecticides. He pointed out the need for experimental work on this point.

The experimental system using carbon dioxide and nitrous oxide is especially applicable to evaluating different selective procedures involving both gases. Different types of action are a necessary requirement in this approach on the basis that susceptibilities among insects to two such chemicals are not likely to be correlated. It has already been demonstrated that carbon dioxide and nitrous oxide differ in their actions. This does not insure a lack of correlated susceptibility, however.

With the techniques already described, the following selective programs were followed for 10 generations. As with the separate gases, three replicates of each procedure were maintained, with selection of both fast and slow groups. Code symbols identifying each type of selection are given for later reference.

(CN) S *Carbon dioxide-nitrous oxide mixture; single exposure.*

The selecting gas was a mixture of equal parts of carbon dioxide and nitrous oxide. Selection was based on single exposure tests as previously described.

(CN) R *Carbon dioxide-nitrous oxide mixture; repeated exposure.*

Mixture of equal parts of carbon dioxide and nitrous oxide was the selecting gas, as (CN) S, but selection was based on repeated exposure and successive elimination as schematized in Figure 6.

(C-N) R *Repeated exposure, alternating carbon dioxide and nitrous oxide within each generation.*

Selection of bugs was based on repeated exposure, alternating between carbon dioxide and nitrous oxide for each generation. This does not necessarily mean that carbon dioxide and nitrous oxide were used equally. If five tests were required to eliminate all but four bugs of each sex, three tests would be with one gas and two with the other. The effect was essentially equalized by using carbon dioxide for the initial test in the even-numbered generations and nitrous oxide for the first test in the odd-numbered generations.

(C) - (N) S *Alternating carbon dioxide and nitrous oxide between generations; single exposure.*

Carbon dioxide and nitrous oxide were used as the selecting gases, alternating between generations. Selection of bugs was based on a single exposure each generation. Carbon dioxide was the selecting gas for the initial population and the successive even-numbered generations. Nitrous oxide was the selecting gas for the odd-numbered generations.

(C) - (N) R *Alternating carbon dioxide and nitrous oxide between generations; repeated exposure.*

Similar to (C) - (N) S above, but the selection of bugs was based on repeated tests and successive elimination.

(CRNR) *Separate selection to carbon dioxide and nitrous oxide and interbreeding; repeated exposure.*

In this procedure, the population was divided into two portions. One portion was repeatedly tested with carbon dioxide, with successive elimination, until two males and two females remained. The other portion was similarly treated with nitrous oxide until two males and two females remained. All eight bugs were then united to interbreed at will. This simulates, but in a more methodical way than could occur in nature, the field situation in which one area was treated with one insecticide, another area treated with a different insecticide, and the surviving insects in both areas were free to interbreed.

The original population and each generation through the 10th were tested for responses to the respective selecting gas. The trends of segregation developing through the 10 generations are illustrated in Figure 15, in the same manner as previously illustrated for the separate gases. The indexes of segregation in these hereditary lines are given in Table 13 (as was done in Table 3 for the single gases). The bugs of the 10th generation in all series are compared in their responses to both carbon dioxide and nitrous oxide. These data are presented in Table 14.

Segregation obviously occurred in all series. No selection procedure prevented nor delayed appreciably the separation of fast and slow strains. These

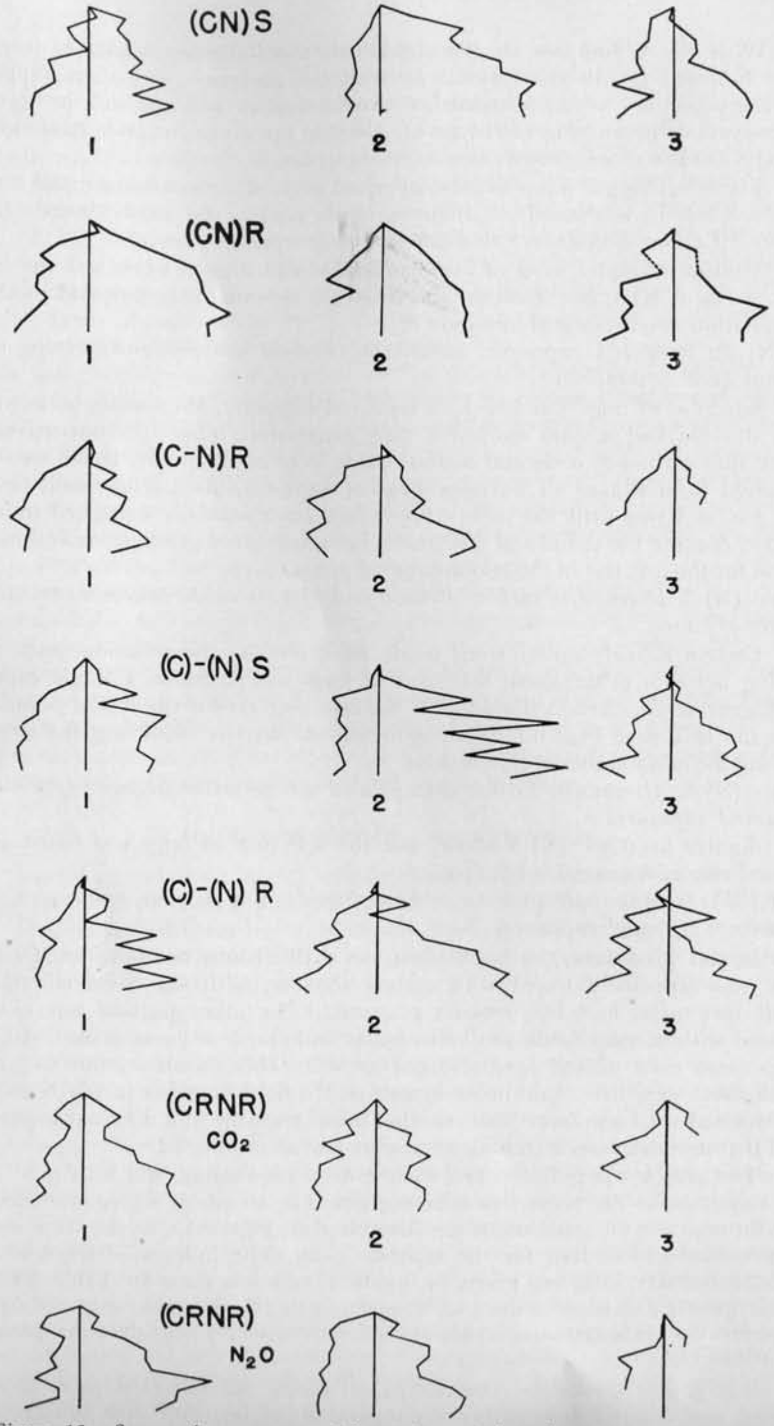

Figure 15. Segregation patterns exhibited by male bugs in each of three replicates in six selection procedures. Patterns drawn according to scheme illustrated in Figure 11.

Table 13. Index of segregation; six selection procedures

	Replicate 1		Replicate 2		Replicate 3		
	Male	Female	Male	Female	Male	Female	Mean
(CN) S	4.23	4.40	6.95	6.81	3.86	4.30	5.09
(CN) R	7.56	7.70	Incomplete		4.44	4.23	5.98
(C - N) R	4.23	3.65	4.95	4.82	Incomplete		4.42
(C) - (N) S	5.02	4.62	6.68	5.79	3.58	3.73	4.90
(C) - (N) R	3.88	4.35	5.86	6.22	3.96	3.78	4.68
(CRNR) CO_2	4.52	4.77	5.55	4.18	Incomplete		4.75
(CRNR) N_2O	5.33	5.58	5.60	5.11	Incomplete		5.32

data thus support Crow's view that mixtures of chemicals or alternate applications of different chemicals are not likely to retard the evolutionary processes leading to resistance.

No well defined trends reveal specific contributions of the two gases in their joint selection. In the series (CN) S and (CN) R, the mixed gas selects strains of bugs that are somewhat more widely separated in response time by the gas mixture than by each component, and somewhat more by carbon dioxide than nitrous oxide. This could mean that the gas mixture acts as a third type of gas. This was not studied physiologically. Dominant, exclusive action by one component is not evident.

In all other series the selection procedures resulted in more or less equal segregation in response to carbon dioxide and nitrous oxide; this in spite of the greater segregating effect of nitrous oxide evident in the procedures using the separate gases (see Table 3). The pronounced saw-toothed figures for (C) — (N) S, 2, and (C) — (N) R, 1 (Figure 10) suggest different contributions by the two gases. This trend is not consistent in the other replicates of these series. On the other hand, some less obvious saw-toothed trends are seen in other lines, even when only one gas is used (see Figure 12). Some inter-generation variation by chance apparently may be registered as an alternation in degree of segregation.

Direct comparisons between single and repeated selecting tests are made difficult by the interaction of the other variables in these procedures. Nevertheless, the results show nothing to suggest a modification of the conclusions drawn from comparisons based on the separate gases used alone.

Several of the diagrams in Figures 12 and 15 are more or less symmetrical. This indicates equal selection for fast and slow response. A few show greater selection for fast response than for slow response. In about half the cases, slow response is selected more prominently. This presumably reflects the biological possibilities. The potentiality for selection in the fast direction is finite; the physiologic limit of fast response is zero response time. The potentiality for selection in the slow direction—physiologically if not genetically—is infinite. A practical limitation is that at some indistinguishable point the condition of anesthesia merges into death. Of chief importance is that given an opportunity, selection can be made in the direction of sensitivity or susceptibility as well as tolerance or resistance. The genetic evidence mentioned in the next section demonstrates that the fast and slow responses to anesthesic gases are opposite expressions of the same physiological phenomena, and not two independent events.

Table 14. Responses (RT_{50}) to different gases of 16 lines of bugs selectively bred for 10 generations

		Male									Female								
		CO_2			N_2O			$CO_2 + N_2O$			CO_2			N_2O			$CO_2 + N_2O$		
		RT_{50}			RT_{50}			RT_{50}			RT_{50}			RT_{50}			RT_{50}		
		Fast	Slow	L.T.D.*	Fast	Slow	L.T.D.	Fast	Slow	L.T.D.	Fast	Slow	L.T.D.	Fast	Slow	L.T.D.	Fast	Slow	L.T.D.
(CN) S	1	50	140	.45	71	140	.29	63	200	.50	51	152	.47	76	173	.46	72	260	.56
	2	79	420	.73	78	144	.27	75	470	.80	82	602	.86	90	240	.48	95	860	.96
	3	76	210	.44	65	85	.12	80	312	.59	76	275	.56	67	92	.14	83	360	.64
(CN)R	1	58	490	.93	59	760	1.11	53	423	.90	59	560	.98	62	330	.73	62	520	.92
	2		338			280			255			340			255			330	
	3	70	225	.51	87	178	.31	68	295	.64	80	215	.43	98	170	.24	92	290	.49
(C·N)R	1	77	195	.40	64	285	.65				80	295	.56	56	295	.72			
	2	82	300	.56	64	290	.66				90	360	.60	64	280	.64			
(C)·(N)S	1	76	280	.57	60	220	.56				84	235	.45	69	165	.38			
	2	84	543	.81	67	530	.90				100	770	.89	82	480	.77			
	3	84	245	.46	74	240	.51				98	275	.45	68	280	.61			
(C)·(N)R	1	93	220	.37	65	335	.72				92	250	.43	66	490	.88			
	2	67	570	.93	63	480	.88				66	725	1.03	66	480	.87			
	3	76	340	.65	70	350	.70				100	340	.53	79	440	.75			
(CRNR)	1	78	380	.69	62	250	.60				78	460	.78	65	290	.65			
	2	80	230	.46	67	260	.59				86	240	.45	85	280	.52			

* L.T.D. = Log. total difference = Log. Slow RT_{50} — Log. Fast RT_{50}.

Genetics of selection

An understanding of the genetics involved was not a primary consideration of this study. It was important to demonstrate that the response time phenomenon was under genetic control in order to justify its use as a criterion in these selection studies, but beyond this it was considered that a genetic study, if undertaken, should be done so independently.

The crossing experiments that were done were admittedly opportunistic, but the data obtained were straightforward and seemed to justify fully this technique as a model system representing population responses to applied chemicals. The offspring of fast bugs mated with slow bugs resulted in an intermediate response closely resembling the response of unselected bugs. Reciprocal matings showed the same thing, as would be expected in view of the similar trends in selection exhibited by the two sexes. Reciprocally back-crossing these hybrids with fast bugs yielded bugs with somewhat faster response times. Reciprocal back-crosses of the hybrids with slow bugs yielded offspring with somewhat slower response times. The F_2 offspring of the fast-slow hybrids also showed an intermediate response-time curve which differed little from that of the F_1 hybrids among the carbon dioxide selected bugs, but which was much flatter in slope for the bugs selected by N_2O.

These observations confirm that the phenomenon studied is indeed under genetic control and suggest that the fast response and the slow response are opposite extremes of the same physiological situation, that there is no sex linkage, and that multiple factors are doubtless involved.

Discussion and practical conclusions

The results of this study can now be expressed in terms of the objectives stated in the introduction.

1. *To identify variation among insects with the type of selection responsible for the most rapid segregation of physiological strains.*

Geographic (or time, space) variation was not considered. Although this is important in evolution, it did not seem critical to the systems under study. Variation among individuals and variation in responses by single individuals were examined by Bliss and Beard (1953) with the insect test system used here. These two sources of variation were separated statistically, and the present study extends the knowledge of their biological expression. The practical stimulus for this inquiry was the hypothesis that variation in responses by single individuals might account for less resistance developing to the quick-acting transient insecticides than to the slower acting persistent insecticides. An insecticide like pyrethrum might act much as a "single exposure" in that the survivors of one treatment could be just as susceptible to a second treatment as the original group was to the first. By inference, some of the survivors would be genotypically resistant, but they would be diluted by other survivors which were "resistant" only at time of treatment (possibly phenotypically resistant). An insecticide like DDT, on the other hand, by its residual properties could act repeatedly on the same population, gradually eliminating those which were only apparently resistant. All the ultimate survivors would be presumed to be genotypically resistant. In the current study, repeated exposure with successive elimination took account of the variation in response by single individuals. Single exposure selection disregarded this variation. A comparison of these two selection procedures revealed that although this variation is involved, it is not a dominant influence. The differences were not great enough to guarantee that resistance cannot be developed by the continued use of transient insecticides. The chief difference seems to be that with single exposure selection genic elements are retained that permit selected strains to revert to normal when selection is stopped. In other words, strains selected by single exposure are less fixed genetically than strains selected by repeated exposure and successive elimination.

Variation among individuals is indeed the principal variation acted upon by the selecting "sieve" of insecticides.

2. *To compare variation in response to one chemical with variation in response to a second chemical having different action.*

Evidence was presented that carbon dioxide and nitrous oxide produce anesthesia in different ways. Correlation of responses to the two gases varies with individual bugs. Group responses reflect the proportion of bugs responding similarly or dissimilarly to the two gases. Hence groups in selective samplings of a population or derived by selective breeding may show negligible, complete, or intermediate correlation of response to carbon dioxide and nitrous oxide. The significance of this in cross-selection is that mode of action of a chemical in a restrictive sense may not determine the course of cross-selection. Rather, all toxicological features of a chemical may participate in its selective action. It is, therefore, suggested that the use of insecticides having different modes of action in the restrictive sense is no insurance against the development of cross-resistance; and cross-resistance cannot be depended upon as a criterion of similar modes of action in a restrictive sense.

3. *To compare the segregation of physiologic strains when different chemicals are used alone, in combination, or alternately.*

A total of 60 genetic lines was maintained by selective breeding. This included three replicates of 10 selection procedures for both fast and slow response. Except for a few lost through sterility, all lines were carried to the tenth generation. In all paired lines, the fast and slow strains became physiologically differentiated. The selection procedures differed somewhat in degree of selection, but none was outstanding in minimizing segregation.

If the experimental system is a satisfactory model—as it appears to be—having application to the broader field of insecticides, it is clear that mixtures of insecticides dissimilar in action cannot be depended upon to forestall development of resistance to either their components or the mixtures. Alternate use of different chemicals likewise offers no advantage.

Summary

As a contribution to the understanding of the development of resistance in insects, these studies were concerned with the variations that occur in the insect population and the selecting mechanisms that act on the variable population to result in segregation of physiologic strains by inheritance.

Special attention was given to that type of variation in responses of single individuals on different occasions. Study of this requires the use of chemicals that can be used repeatedly without ill effects.

The chemicals used were carbon dioxide and nitrous oxide. These have the same density and symptomatically similar anesthetic action, but otherwise their actions differ in several important ways. The adult of *Oncopeltus fasciatus* (Dall.) was the test insect. The time required for the occurrence of a righting reflex after anesthesia was the criterion of response.

From a sample population of bugs, individuals can be selected for consistently fast or consistently slow response. This is achieved by a test system of repeated exposures and successive elimination. The range of reponse in such selected individuals is at a minimum. Individuals selected for fast or slow response on the basis of single exposure tests are much less consistent in their performance.

Because of these differences, it was hypothesized that selective breeding based on repeated testing and successive elimination should lead to the inheritance of segregated physiologic strains more rapidly than selective breeding based on single tests.

These two types of selection were practiced in three replicates with each gas. Comparisons were made after 10 generations, and in some cases after 15 generations. After 10 generations some lines were continued without further selection to test the fixity of the genetic segregation.

Segregation of true physiologic strains showing extreme response times was obtained by all selecting procedures. Strains selected with nitrous oxide were somewhat more extreme than those selected with carbon dioxide. Segregation was only slightly greater with repeated tests than with single exposures. When selection was relaxed, however, the repeated test-selected strains retained their segregation whereas the single test-selected strains reverted to normal. It appears that single test selection permits the dilution of genetic elements responsible for extreme response even though this does not retard the segregation of strains if selection is maintained.

If carbon dioxide and nitrous oxide are identical in their selective mechanisms, the use of one should cross-select for response to the other. Actually a full range of cross-selection from negligible to complete was observed among the several lines. This puts in question the mode of action concept as it relates to cross-resistance. This is critically discussed. A partial physiological explanation of the diverse results obtained with the two gases is offered. This is based on the general anoxic action shared by nitrous oxide and carbon dioxide and a specific action of carbon dioxide alone.

Selective breeding was also done for 10 generations using mixtures of carbon dioxide and nitrous oxide and each gas alternately, both within generations and between generations. This was done on the theory that susceptibilities to differently acting chemicals are probably not correlated and that the use of combinations or alternate applications of different chemicals would retard the concentration of genic combinations responsible for resistance. This theory was not substantiated. Susceptibilities to the differently acting gases were not necessarily uncorrelated, and physiologic strains of fast and slow responding bugs were obtained with all selecting procedures. The alternate use of carbon dioxide and nitrous oxide resulted in somewhat less segregation than the other selecting procedures, but the differences were not great enough to suggest a practical program of insect control that, with assurance, would forestall the development of resistance.

Nothing unusual in the genetics of selection appeared. Crossing tests indicated straightforward inheritance, with no sex linkage. Multiple genic factors are doubtless responsible for the responses observed.

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