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The Log Dosage-Probit Mortality Curve in Genetic Researches of Insect Resistance to Insecticides. Masuhisa Tsukamoto (Department of Genetics, Osaka University Medical School, Osaka, Japan). Received Oct. 31, 1963. *Botyu-Kagaku*, **28**, 91, 1963.

14. 殺虫剤抵抗性の遺伝と薬量-死亡率曲線 塚本増久(大阪大学医学部遺伝学教室) 38. 10. 31 受理

一般にミュータントを利用して詳細な遺伝学的分析をおこなうことができない種々の昆虫において殺虫剤抵抗性の遺伝をしらべる場合には，交配実験によつて生じた子孫の抵抗性レベルを測定し，その薬量死亡率関係から遺伝様式を推定しなければならないが，そのデータの解釈のしかたは従来必らずしも充分であるとはいえなかつた．すなわち，これらの子孫は種々の抵抗性レベルの異なつた個体が分離してくるはずであるので一種の混合集団であるとみなすことができる．このような観点から，種々の遺伝様式をモデルとして用いてその薬量死亡率プロビット曲線の形状を追求した．その結果，抵抗性遺伝子の数のみならず，抵抗性レベルに関しての遺伝子の性質や遺伝子相互間の交互作用などによつてもプロビット曲線の形は著しく影響をうけることが示された．このことから，逆に F_2 世代におけるプロビット曲線の形だけから簡単に遺伝様式を知ることは容易ではないことがいえる．
また野外の多くの昆虫集団の場合も，遺伝学的には不均質な混合集団であると考えられるので，その抵抗性レベルを表現するにはプロビット曲線全体を用いるのが望ましく，本来均質集団にしか適用できない回帰直線を安易に使用すべきではないことを強調したい．

The insecticide resistance is an invisible genetic character and is recognizable only after toxicological tests were performed with an appropriate dose of an insecticide which can separate resistant phenotypes from susceptible ones. In order to investigate the mode of inheritance of insecticide resistance in insects, therefore, somewhat indirect and inferential methods have usually been employed because susceptible individuals cannot produce their progeny after insecticidal treatment. The most familiar method is an interpretation of toxicological data, such as the dosage-mortality relation, obtained with progeny of crossing experiments between genetically unmarked strains of which insecticide susceptibilities are much different. When the difference between the degree of resistance of parental strains or between the resistant segregant and the susceptible segregant is large enough to be distinguished each genotype or phenotype for the resistance character among the F_2 progeny of the crosses between susceptible (S) and resistant (R) strains or backcross progeny of the F_1 hybrids to either parent strains, one might easily find out an appropriate diagnostic dose or doses. Log dosage-probit mortality regression lines (hereafter, briefly ld-p lines as proposed by Hoskins and Gordon[4]) for each genotype are usually more or less overlapping to each other in their ranges of dosage. When the ld-p line for the F_2 progeny of the S×R crosses was interpreted as if it were a straight line with a more gentle slope and no clear cut diagnostic dose was available, most of the toxicologists have used to infer that the resistance character was inherited as if a complicated multifactorial genetic system. Results based on increasing doses or scalar doses sometimes mislead investigators to an incorrect conclusion on the mode of inheritance, although

the necessity of re-examination of data was reviewed by Milani[6] to harmonize these confusing results.

The more reliable method is the use of morphological mutations as visible markers for whole or parts of chromosome set in crossing experiments. Thus this method is highly effective to get more accurate informations on the nature of resistant factor or factors and on the linkage relation to the marker genes used. In most insect pests of medical or agricultural importance, however, there were some difficulties to apply such an orthodox genetical analysis to investigations on insecticide resistance. Until various visible mutants have recently been available in some insect pests, *Drosophila melanogaster* was the only insect suitable for investigating genetics of resistance in detail. The author and his coworkers[8] have successfully showed, using a diagnostic dose, that some of major factors responsible for insecticide resistance, as one of the physiological characters, were located on particular regions of chromosomes as well as in other morphological characters. Recent progresses in the formal genetics of the housefly, *Musca domestica*, have made it possible to use visible mutant markers[1,2,5,7] in genetic researches of insecticide resistance as in *Drosophila*.

For these years, genetic analyses of insecticide resistance in the housefly are being carried out at the laboratory in Osaka using both the ld-p curve with scalar doses and the visible mutant markers with diagnostic doses. Prior to report practical results obtained from crossing experiments, some theoretical considerations are made on the methods which can be used in the investigation of resistance. The present paper is the first of the series of genetic studies on the insecticide resistance in the housefly. The actual examples of the experimental results will be reported in separate papers of this series.

The Log Dosage-Probit Mortality Curves in a Heterogeneous Population

When the per cent mortalities of a homogeneous insect population are continuously plotted on a graph paper against varying log doses, these are resulted in a sigmoid curve. Since this is an integral curve for the frequency in a normal distribution, it can be expressed as the following well-known equation:

$$p=\frac{1}{\sqrt{2\pi}\,\sigma}\int_{-\infty}^{X} e^{-\frac{(X-M)^2}{2\sigma^2}}\,dX \qquad (1)$$

where p is the per cent mortality in a homogeneous population,
X is log dosage of an insecticide,
M is the log LD_{50} of the insecticide used,
σ is the standard deviation of the normal distribution, and
π is the ratio of the circumference of a circle.

In figure 1, A and B show the frequency curves for each normal distribution and their integral curves, respectively. Such a relation between the dosage and the mortality in the homogeneous population might be expanded, with some modifications, to a heterogeneous mixed population which is constituted from several homogeneous sub-populations with different resistance levels. Actual examples of such a heterogeneous mixed population will be found easily in the progeny of the R×S crossing experiments and even in natural field populations of insects.

In the heterogeneous mixed population, the following equation might be applicable:

$$P=\sum_{i=1}^{n}\frac{q_i}{\sqrt{2\pi}\,\sigma_i}\int_{-\infty}^{X} e^{-\frac{(X-M_i^2)}{2\sigma_i^2}}\,dX \qquad (2)$$

where i is the number of constituent sub-populations ($i=1,2,3,\cdots,n$),
P is the per cent mortality for a whole mixed population,
M_i is the LD_{50} for each homogeneous constituent,
σ_i is the standard deviation for each normal distribution, and
q_i is the frequency of each constituent in the mixed population.

In figure 1D and 1E, a synthesized distribution curve and an integral curve are shown in solid lines, but curves for each constituent in dotted lines.

Thus whole per cent mortality at a given dose, x, will be calculated practically from the following rather simple equation:

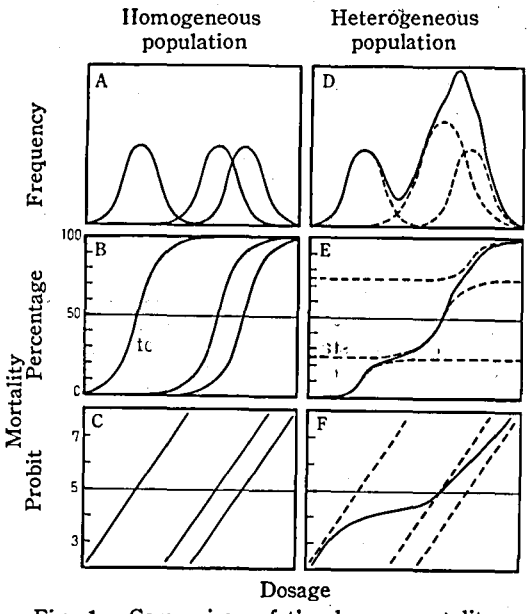

Fig. 1. Comparison of the dosage-mortality relation between homogeneous and heterogeneous populations.

$$P_x=\sum_{i=1}^{n} p_i q_i \qquad (3)$$

where P_x is the total per cent mortality in the mixed population at a given dose x, and p_i is the per cent mortality of each constituent at the dose x.

Since the last equation has just the same meaning with that described by Hoskins[3], the author would like to propose to call the "Hoskins' formula" for the last equation though he used another expression.

In the homogeneous population, per cent mortality curve which is expressed by the equation (1) can be transformed into a straight probit line as shown in figure 1C. In the heterogeneous population, however, the per cent mortality curve which is defined by the equation (2) does not give rise to a straight line even after the probit transformation (figure 1F). The probit transformation thus becomes invalid for the mixed population because the initial purpose of this transformation is to get the straight regression line for the homogeneous population. The probit or per cent mortality plotted on the probit scale, however, is used in the present paper because the probit scale is now one of the most familiar expressions to toxicologists and because the response of each constituent sub-population can be expressed by the straight ld-p line.

The Shape of the ld-p Curve and The Mode of Inheritance

Assuming the well-known mode of Mendelian inheritance, some considerations were made on the shape of synthesized probit curves for segregants among the F_2 progeny of the S×R cross or those among the backcross progeny. In these considerations, an imaginary resistant strain is used as a model where the resistance level is, for the convenience, 100 times as resistant as the susceptible one and the same slopes of the ld-p line for both the original strains.

Monofactorial inheritance:

(1) When the resistance is completely recessive to the susceptibility, 3 portions of the susceptible (+*r* and *r*+ genotypes) and 1 portion of the resistant (*rr*) segregants may be expected to appear in the F_2 generation. If the range of ld-p lines for each segregant does not overlap with each other, the shape of the synthesized ld-p curve may have a distinct "plateau" around 75% mortality or probit 5.675 (figure 2A). Such a plateau means a range of effective diagnostic or discriminating doses. When the resistance level is not so high in the resistant phenotype, and hence when the ld-p lines for the segregants are overlapping with each other, no distinct plateau will be observed.

(2) When the resistance is completely dominant over the suceptibility, a distinct plateau may be expected around 25% mortality or probit 4.326 suggesting a typical segregation of two phenotypes, i.e., 1 susceptible (++) and 3 resistant (*R*+ and *RR*). The shape of the ld-p line in such a case is shown in figure 2C.

(3) When the resistance is incompletely dominant over the susceptibility, 1 susceptible homozygote(++), 2 heterozygote (*R*+) and 1 resistant homozygote (*RR*) will be expected in the F_2 generation. If the ld-p lines for these segregants are not overlapping with each other, a wavy ld-p curve with two distinct plateaux around 25% and 75% mortalities may be expected for the F_2 progeny (figure 2B, curve 1). In most cases of practical

data in crossing experiments, however, degree of resistance in heterozygous hybrids may take any intermediate value, which depends upon the intensity of dominancy, from that of the resistant parent to that of the susceptible parent. Therefore, when the ld-p line for the hybrids and that for one of parent strains are overlapping to each other, only one plateau may be recognizable (figure 2B, curve 2)

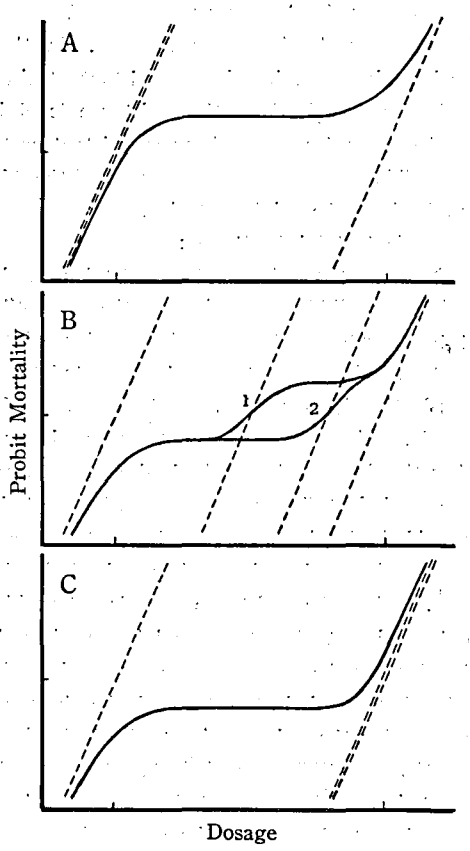

Fig. 2. Schematic ld-p curves for the F_2 progeny of the R×S cross expected from single recessive (A), incomplete dominant (B), and dominant (C) genetic systems.

When the dose range of the ld-p lines for all the three genotypes (i. e., ++, *R*+ and *RR*) are overlapping to each other, and/or when the number of observed mortality plots is not enough (for example, only 4 or 5 plots), no distinct plateau may be recognized even though the resistance is inherited in a simple monofactorial system. Thus the shape of the synthesized ld-p curve sometimes

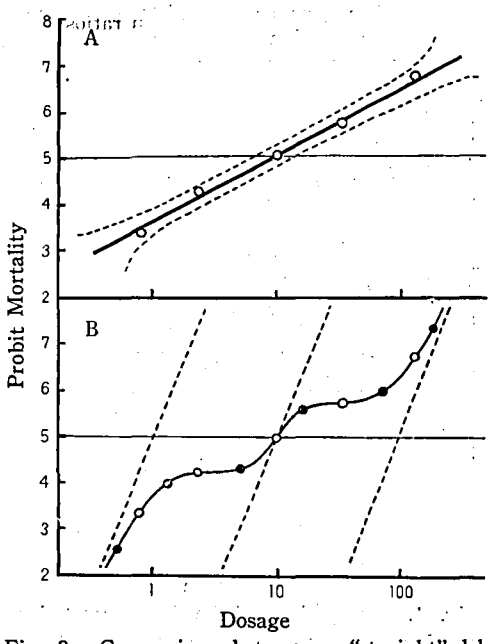

Fig. 3. Comparison between a "straight" ld-p line with the 95% confidence interval inferred from insufficient numbers of plots (A) and a wavy ld-p curve confirmed by additional plots (B).

misleads the toxicologists' interpretation as if it were a straight ld-p line. Figure 3 represents an extreme sample of such a confusable case where 5 plots are arranged on an almost straight ld-p line suggesting a complicated multifactorial system. However, these plots can be presumed to be extracted from an ld-p curve for the mixed population which is constituted from three segregant groups with a ratio of 1:2:1.

(4) Similarly, when the dose range of ld-p lines for two genotypes are not overlapping to each

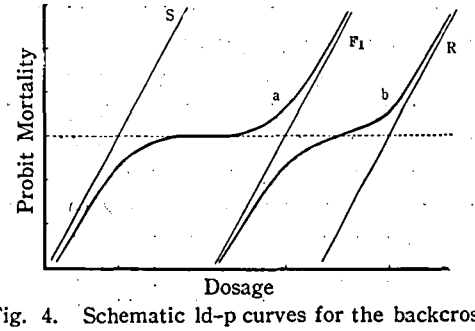

Fig. 4. Schematic ld-p curves for the backcross progeny showing the typical 1:1 segregation with (a) or without (b) distinct diagnostic doses.

Table 1. Assumed segregation ratios and the level of resistance in the F_2 progeny of the R×S cross by the two-dominant system.

Genotype	Frequency	Level of resistance (Phenotype)							
		Complete dominant				Incomplete dominant			
		R : ×90 R' : ×10	Ratio	R : ×50 R' : ×50	Ratio	R : ×45 R' ×: 5	Ratio	R : ×25 R' : ×25	Ratio
RR ; $R'R'$	1	×100		×100		×100	1	×100	1
RR ; $R'+$	2	×100		×100		× 95	2	× 75	
RR ; $++$	1	× 90	9	× 50	9	× 90	1	× 50	4
$R+$; $R'R'$	2	×100		×100		× 55	2	× 75	
$R+$; $R'+$	4	×100	3	×100	6	× 50	4	× 50	6
$R+$; $++$	2	× 90		× 50		× 45	2	× 25	
$++$; $R'R'$	1	× 10		× 50		× 10	1	× 50	4
$++$; $R'+$	2	× 10	3	× 50		× 5	2	× 25	
$++$; $++$	1	× 1	1	× 1	1	× 1	1	× 1	1
Shape of the ld-p curves in fig. 5		a		b		c		d	

Table 2. Assumed segregation ratios and the level of resistance in the F_2 progeny of the R×S cross by the one dominant and one recessive system.

Genotype	Frequency	Level of resistance (Phenotype)					
		R : ×10 rr : ×90	Ratio	R : ×50 rr : ×50	Ratio	R : ×90 rr : ×10	Ratio
RR ; rr	1	×100		×100		×100	
RR ; $r+$	2	× 10	3	× 50	3	× 90	3
RR ; $++$	1	× 10		× 50		× 90	
$R+$; rr	2	×100	9	×100	10	×100	9
$R+$; $r+$	4	× 10		× 50		× 90	
$R+$; $++$	2	× 10		× 50		× 90	
$++$; rr	1	× 90	1	× 50		× 10	1
$++$; $r+$	2	× 1	3	× 1	3	× 1	3
$++$; $++$	1	× 1		× 1		× 1	
Shape of the ld-p curves in fig. 6		e		f		g	

Table 3. Assumed segregation ratios and the level of resistance in the F_2 progeny of the R×S cross by the two-recessive system.

Genotype	Frequency	Level of resistance (Phenotype)					
		rr : ×10 $r'r'$: ×90	Ratio	rr : ×50 $r'r'$: ×50	Ratio	rr : ×75 $r'r'$: ×25	Ratio
rr ; $r'r'$	1	×100	1	×100	1	×100	1
rr ; $r'+$	2	× 10		× 50		× 75	
rr ; $++$	1	× 10	3	× 50		× 75	3
$r+$; $r'r'$	2	× 90		× 50	6	× 25	
$r+$; $r'+$	4	× 1	3	× 1		× 1	3
$r+$; $++$	2	× 1		× 1		× 1	
$++$; $r'r'$	1	× 90	9	× 50	9	× 25	9
$++$; $r'+$	2	× 1		× 1		× 1	
$++$; $++$	1	× 1		× 1		× 1	

other, a distinct plateau of the ld-p curve will be observed around 50% mortality in progeny from the backcross of the F_1 heterozygote to one of either parent strains. On the other hand, when degrees of resistance in two genotypes close to each other, the ld-p curve shows no typical segregation. Figure 4 represents two schematic examples of the ld-p curve for the backcross progeny.

Difactorial inheritance:

In this case, there are many expectable combination of two genes: for example, two complete dominants, two incomplete dominants, one dominant and one recessive, two recessives, two major genes or one major gene and one accessory gene, with or without gene interactions, etc. The shape of the ld-p curve for these various situations are not uniform because the theoretical segregation ratios are quite different from each other as far as the resistance level is concerned.

(1) Only a few simple cases where these genes are belonging to different linkage groups from each other will be considered here as model systems for the mode of inheritance. Tables 1, 2, and 3 represent some typical samples of the "without interaction" system by two dominants, one dominant and one recessive, and two recessives, respectively. Some of the ld-p curves for these cases are also illustrated schematically in figures 5 and 6.

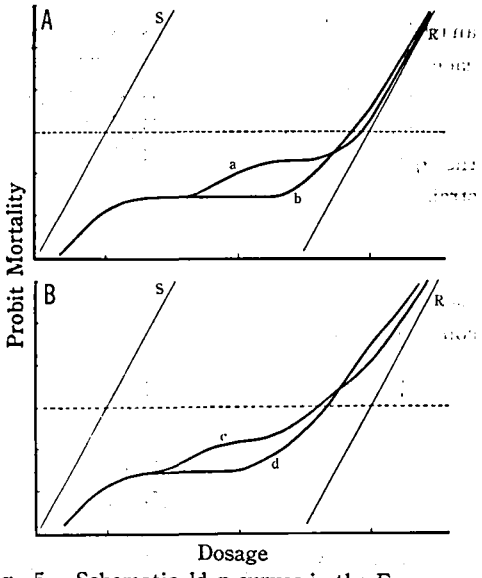

Fig. 5. Schematic ld-p curves in the F_2 progeny expected from the two-complete-dominant (A) and two-incomplete-dominant (B) systems. The symbols, a, b, c and d, correspond to those used in table 1, respectively.

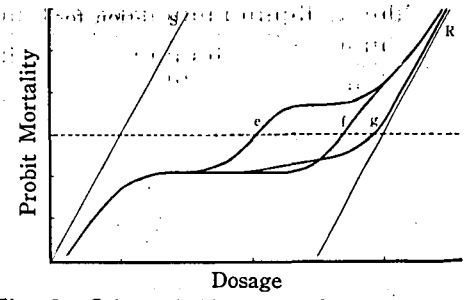

Fig. 6. Schematic ld-p curves for the F_2 progeny expected from the one dominant and one recessive system. The symbols, e, f and g, correspond to those used in table 2, respectively.

(2) When some interactions exist between the genes, however, the effects of resistance factors are not additive but synergistic to each other, and the shape of the ld-p curve is more diverse than that expected from the "without interaction" system. For example, even in such a case where only two resistant factors are involved, the shape of the ld-p curve in the F_2 generation of the R×S cross may become almost straight as if suggesting a complicated multifactorial inheritance of resistance.

Multifactorial inheritance:

The more the number of resistant factors increases, the more the complicated segregations are expected to occur in the F_2 generation. However, the shape of the ld-p curves may not always become a straight line when the effect of gene dosage on the degree of resistance is merely additive (i. e., the without interaction system). For example, if each of 5 recessive genes corresponds to ×20 resistance level, 32 kinds of combination of phenotypes with various resistance levels may result in the F_2 progeny of the $+;+;+;+;+ \times r_1;r_2;r_3;r_4;r_5$ cross as shown in table 4.

The ld-p curve for this model system has a plateau around probit 4.28 or 23.7% mortality and then the curve does increase gradually. Namely, the shape of the ld-p curve is not straight and is similar to that presumed by the monofactorial

Table 4. Expected segregation for 5 recessive resistant gene system in the F_2 progeny.

Number of homozygous *rr* genes	Resistance level	Segregation ratio	Kind of combination	Per cent frequency
5	×100	1	1	0.10
4	× 80	3	5	1.47
3	× 60	9	10	8.79
2	× 40	27	10	26.37
1	× 20	81	5	39.55
0	× 1	243	1	23.73

dominant system although the resistance character is controlled by a multifactorial system. Figure 7 shows the ld-p curves expected from the model systems where 1, 2 and 5 recessive genes are equally responsible for resistance, respectively.

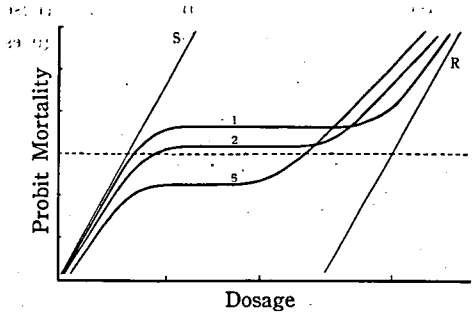

Fig. 7. Schematic ld-p curves for the F_2 progeny expected from the recessive genetic system where the resistance is due to 1, 2 and 5 genes, respectively.

In the case where resistant genes are incompletely dominant and there are some complicated interactions among these resistant genes, the ld-p curve may sometimes approach to a straight line.

General Considerations

When the toxicological experiments for testing the degree of resistance are carried out on a basis of the same physiological condition as far as possible, the levelling-off of the ld-p curve (i. e., plateau) above a certain dose of an insecticide indicates two possible cases: (1) where increasing doses of the insecticide, especially of the chlorinated hydrocarbons, are not effective at higher doses even in a genetically homogeneous population of insects because physiologically effective amounts of the insecticide at a site of action are not proportional with applied doses of the insecticide at a site of application, and (2) where the sample tested is not homogeneous genetically. The latter case is, of course, the subject of the present paper.

From the genetical viewpoint, almost all the natural field populations of insects or usual laboratory strains of the housefly, such as CSMA or NAIDM, should be considered not to be homogeneous but to be heterogeneous as far as resistance level is concerned. In fact, segregations of resistant and non-resistant individuals in field or laboratory populations of insects have been reported by various investigators, or, at least, these segregations can be detected, by careful re-examinations, from the data illustrated as figures in their papers. If a population is consisted of a mixture of a number of various phenotypes whose responses to an insecticide differ slightly and continuously from each other, the whole response of such a mixed population may be similar to that of a single normal distribution. In such an extreme case of the quantitative characters, estimation of the straight ld-p line does not always represent the true nature of resistance.

From these considerations using the model of the ld-p curve for the genetic segregation, it is concluded that the resistance level of heterogeneous insect populations should be expressed, or be compared, by the shape of the whole ld-p curve which is based on a number of observed plots, but not by the straight regression line or by the LD_{50} value alone which are based on only a few observed plots or are effective merely to the homogeneous normal distribution. Analytical examination of toxicological data and the shape of the ld-p curves are often highly effective to detect the heterogeneity of the population tested, and furthermore, in some cases where the

insecticide resistance is due to a simple genetic system, it is possible to estimate the gene frequency of the resistant factor involved in the population by the Hardy-Weinberg law. Some investigators have used a terrace-like combination of straight ld-p lines for showing genetic segregation of resistance levels in a heterogeneous mixed population, but it is now obvious that such an expression is not correct (figure 8).

Finally, the author would like to emphasize again that the straight ld-p line in the progeny of the cross between resistant and susceptible strains does not always represent the multifactorial inheritance of resistance, and vice versa; and that the straight ld-p line should be used more strictly and more correctly.

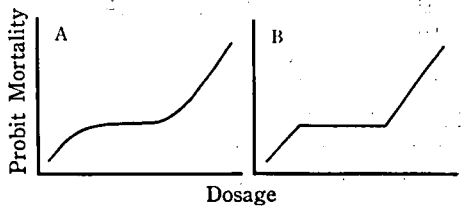

Fig. 8. Correct (A) and incorrect (B) expressions of the ld-p curve in heterogeneous populations.

Summary

Using the Mendelian mode of inheritance, some theoretical considerations were made on the shape of the log dosage-probit mortality curve in a heterogeneous population such as the progeny of crosses between resistant and susceptible strains.

The shape of the ld-p curve is largely influenced not only by the number of resistant genes but also by the level of resistance exhibited by these genes, dominancy, gene interactions, etc. The straight ld-p line in the F_2 progeny of the R×S cross does not always represent the multifactorial inheritance of resistance.

From the viewpoint of population genetics, almost all the natural field populations of insects should be considered not to be homogeneous but to be heterogeneous as far as the resistance level is concerned. The resistance level of heterogeneous populations should therefore be expressed by a whole ld-p curve, but not by the straight regression line or by the LD_{50} value alone which are based on and effective merely to the homogeneous normal distribution.

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Biological Differences between Resistant and Susceptible Strains of the House Fly. Kazuo Buéi (Public Health Research Institute, Osaka-Fu, Osaka) Received Oct. 31, 1963. *Botyu-Kagaku*, 28, 98, 1963 (with English résumé, 103).

15. 殺虫剤抵抗性および感受性イエバエにおける生態学的な諸性質の比較* 武衛和雄 (大阪府立公衆衛生研究所) 38. 10. 31 受理

感受性の異なる6系統のイエバエについて，生態的な諸性質の相異点を比較観察した．抵抗性の増大したイエバエは，感受性系統にくらべて卵-幼虫期間が有意的に長くなり，また羽化率が低かつた．抵抗性系統の蛹の体重は感受性系統のそれにくらべてより重い傾向を示した．

* この報告の一部は，1960年6月25日第12回日本衛生動物学会大会(札幌)において発表．