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GENETIC ASPECTS OF THE DEVELOPMENT OF RESISTANCE TO CHEMICAL INSECTICIDES

Riassunto. — Lo studio genetico della mosca domestica è stato iniziato per fornire le basi necessarie all'analisi dei fenomeni di natura ereditaria connessi con la insorgenza di ceppi resistenti agli insetticidi. Sono stati presi in particolare considerazione caratteri polimorfi e mutazioni presenti in popolazioni naturali. Si è constatato che il polimorfismo è geneticamente controllato, che popolazioni naturali sono affette da un notevole numero di mutazioni recessive portate allo stato eterozigote e che all'incrocio consegue rapido declino della vitalità e della fecondità. Inoltre reperti di ginandromorfi in laboratorio con incidenza familiare e in natura indicano l'esistenza di condizioni ereditarie capaci di determinare profondo scompenso genetico nell'organismo senza che ne consegua grave declino della vitalità.

Questi fatti suggeriscono che il sistema genetico della mosca sia tale da assicurare grande plasticità alla specie attraverso il mantenimento di una elevata eterogeneità genetica.

Molte delle mutazioni isolate hanno espressività variabile e penetranza incompleta il che determina incostanza nella manifestazione dei caratteri e nelle segregazioni mendeliane. Queste osservazioni possono aiutare ad interpretare la capricciosità spesso osservata nel grado di resistenza di ceppi selezionati.

La formazione di ceppi resistenti propone problemi biologici e di metodologia che interessano tutte le branche della genetica e che vengono discussi richiamando l'attenzione soprattutto sulla necessità di analizzare la resistenza nei suoi vari aspetti, di esprimerne la valutazione in modo esauriente e di estendere le ricerche genetiche su popolazioni naturali seguendo in esse le fluttuazioni del patrimonio genetico e in particolare il destino dei fattori di resistenza.

Infine vengono considerati i rapporti genetici tra le forme di resistenza ad insetticidi diversi.

Résumé. — L'étude génétique de *Musca domestica* a été commencée pour fournir les bases nécessaires à l'analyse des phénomènes héréditaires se rapportant à l'apparition de souches résistantes aux insecticides. On a pris particulièrement en considération les caractères polymorphes et les mutations existant dans les populations naturelles. On a constaté que le polymorphisme est génétiquement contrôlé, que les populations naturelles sont capables d'un nombre considérable de mutations régressives portées à l'état étérozygote et que le croisement produit un déclin rapide de la vitalité et de la fécondité. En outre le repérage de xynandromorphes dans le laboratoire avec incidence familiale et dans la nature, indique l'existence de conditions héréditaires capables de déterminer un déséquilibre génétique profond dans l'organisme sans que s'en suive une grave diminution de vitalité.

Ces faits suggèrent que le système génétique de la mouche est tel qu'il assure à l'espèce une très grande plasticité au moyen de la conservation d'une haute étérogénéité génétique.

Souvent les mutations isolées représentent seulement des expressions variables et ont une pénétration incomplète ce qui cause l'inconstance des manifestations des caractères et des ségrégations mendeliennes. Ces observations peuvent aider à interpréter l'aspect capricieux souvent relevé dans le degré de résistance des souches sélectionnées.

La formation de souches résistantes pose des problèmes biologiques et de méthodologie qui intéressent toutes les branches de la génétique et qui sont discutés en attirant l'attention surtout sur la nécessité d'analyser la résistance dans ses différents aspects, d'exprimer leur importance d'une façon complète et d'amplifier les recherches génétiques sur les populations naturelles en suivant chez elles les fluctuations du patrimoine génétique et en particulier le destin des facteurs de résistance.

On considère enfin les rapports génétiques entre les formes de résistance aux différents insecticides.

Summary. — Genetic study of the house fly was begun by formulating the necessary basis for analysis of phenomena of an hereditary nature connected with the appearance of strains resistant to insecticides. Polymorph characters and the mutations present in natural populations were particularly taken into consideration. It was established that polymorphism is genetically controlled, that natural populations are affected by a considerable number of recessive mutations carried to the heterozygous state, and that in-breeding is followed by a rapid decline of vitality and fertility. Moreover, the discovery of

gynandromorphs in the laboratory and in nature with a familial incidence, argue the existence of hereditary conditions capable of determining instability in the organism, not followed by a serious decline in vitality.

These facts suggest that the genetic system of the fly, by maintaining a raised genetic heterogeneity, ensures great adaptability for it as a species.

Many of the mutations isolated have a variable expressivity and incomplete penetration, resulting in inconstancy in display of characters and in Mendelian segregations. These observations may help to interpret the same capriciousness which has been observed in the degree of resistance of selected strains.

The formation of resistant strains sets biological and methodological problems which are of interest to all branches of genetics; discussion of them calls attention especially to the need for analysing resistance in its various aspects, of evaluating it satisfactorily, and of extending genetic research on natural populations, following in these the fluctuations of genetic inheritance and in particular the fate of resistance factors.

Finally, genetic reports concerning forms of resistance to various insecticides are considered.

Zusammenfassung. — Es wurde die genetische Nachforschung der Hausfliege unternommen um die nötigen Analysen der Vererbungserscheinungen, die mit dem Erscheinen der gegen Insektiziden resistenten Stämme verbunden sind, durchführen zu können.

Speziell wurden die polymorphische Charaktere und Mutationen bei natürlichen Bevölkerung in Anbetracht genommen. Es wurde dabei bemerkt dass das Polymorphismus genetisch kontrolliert wird, dass die natürlichen Bevölkerung einer beträchtlichen Anzahl von rezessiven Mutationen unterzogen werden, die zum heterozygotischem Stadium mitgeführt werden und bei der Kreuzung einen raschen Abfall der Vitalität und Fruchtbarkeit hervorrufen. Gynandromorphische Befunde im Laboratorium, mit Abschweifungen in Familien und in Natur, weisen ausserdem auf der Anwesenheit von Vererbungsverhältnisse die im Stande sind im Organismus eine gründliche genetische Störung zu verursachen, wobei die Vitalität dadurch keine allzu schwere Minderung leidet.

Diese Sachlagen deuten darauf hin, dass das genetische System der Hausfliege deartig ist, dass es eine grosse Plastizität, durch das

Instandehalten einer hohen genetischen Heterogeneität, versichern kann.

Viele einzelne Mutationen erweisen veränderliche Ausdrucksweise und unvollständige Eindringung die in den Charakter-Erscheinungen und in den mendelianischen Segregationen eine Unstetigkeit bestimmen. Diese Äusserungen können dazu dienen dass oft bemerkte launische Verhalten der Resistenzstärke der selektionierten Stämme zu erläutern.

Das Zustandewerden der resistenten Stämme hebt biologische und methodologische Probleme hervor, die sämtliche Zweige der Genetik umfassen und hier besprochen werden, wobei die Aufmerksamkeit hauptsächlich auf der Notwendigkeit gelenkt wird, die Resistenz von allen Gesichtspunkte aus zu analysieren, dessen Wert gründlich zu erklären und die genetischen Untersuchungen auf natürlichen Bevolkerungen zu verbreitern, und bei diesen die genetischen Schwingungen und besonders das Schicksal der Resistenz-Faktoren zu erforschen. Schliesslich werden die genetischen Verhältnisse zwischen Resistenzformen und den verschiedenen Insektiziden in Anbetracht genommen.

The development of insecticide resistance has provided us with challenging as well as attractive problems. Physiological differences genetically controlled are liable to undergo selection and lead to the development of strains, differing from the original stock in properties which enable them to cope with a new ecological situation, suddenly introduced by man; i.e. the presence of toxic agents in their environment.

These strains are endowed with defence mechanisms specific to the selective (toxic) agents. We do not know very much about these defence mechanisms or their level of action; the tolerance to a given insecticide can have different physiological control in different strains (Busvine, 1951). We know that the factors for resistance must be present in nature before the introduction of the insecticides, and I think we can safely assume that, in the absence of the toxic agents, they do not increase the fitness of their carriers, and possibly decrease it: strains partially selected for resistance undergo a progressive loss of tolerance if left alone for several generations; moreover, the mere fact that resistance has not been reached under the pressure of natural selection implies that the factors for resistance are not a bonus for their

carriers, and are possibly disadvantageous in the absence of the toxic agent.

Resistance is a physiological property; its inheritance is a genetical attribute; the development of resistant strains is a problem of evolution. Their study requires highly specialized techniques; this fact has a rather unfortunate consequence — it tends to split the main problem into separate sections with independent development.

It is certainly possible to begin a study of the inheritance of inborn properties — say, different degrees of tolerance to a given drug — while ignoring the physiological processes controlling them; but in this case we are forced to confine the analysis to the ultimate effect of unknown processes — a result which can be reached through quite different paths. Dealing with flies and toxic drugs, it is not feasible to determine the tolerance of single individuals: we are forced to deal with batches, and all we can do is to correlate dose with response of groups of animals of which the origin is known; this is a serious limitation to careful study.

A genetic study is possible when inherited individual differences exist and can be analysed as alternative or measurable characters. It is important to recognize the elementary constituents of the differences.

There is no point in asking if what we see as « resistance » and « sensitivity » are alternative and measurable characters; however, it might be well worth asking if they are *elementary* characters: that is to say, if they cannot be split into smaller units.

Busvine and Harrison, I think, were the first to prove that the two properties of DDT resistance to knock-down and DDT resistance to kill have distinct genetical control. If we consider the killing effect we refer to a property which we measure by the incidence of death in a given batch of insects, and we say that the survivors have a higher « tolerance » than those killed.

The estimate of tolerance is just what is needed for any practical purpose; but, alone, is too crude to be taken as a substitute for missing data in an attempt to define the reactions to a given insecticide of samples of a population. This definition is essential as a basis for a biological and, particularly, a genetic study.

The genetic investigation of the properties which control tolerance covers many aspects which may be arranged under many headings: phenogenetics, physiogenetics, formal genetics, population genetics, evolution genetics, and comparative genetics. That is to say, all the specialized branches of genetics must unite in the effort to clarify properties which present us with the gross problem of resistance.

It is obvious that the factors controlling tolerance are a part of the genetical make-up of a species, and it is impossible to analyse or understand them properly if we lack a broad knowledge of the framework within which they are set up. For this reason, I think it is well worth while to give a survey of the work which has been carried out in this laboratory on the genetics of the housefly.

The aspects which have been considered are polymorphism, mutants and natural heterozygosis; the observations have been made mostly on flies from Latina.

Polymorphism.

The study of morphological characters shows great heterogeneity in natural populations. The colour of the abdomen, and the chaetotaxy show discontinuous variations of a degree which can properly be called polymorphism.

Colour of the abdomen: The sternites are yellow-brown and/or black. Individual differences can be easily observed both among wild flies and in laboratory strains. The colour patterns range, for the females, from self-brownish (« orange » in freshly hatched flies observed under artificial light) to self-black. The intermediate phenotypes are the result of different degrees and modalities of the expansion of a median longitudinal black band. The sexes show pigmentation differences:

(a) males always have a central band;

(b) the band is much more sharply drawn in males;

(c) the shape of the black marks differs to some extent;

(d) the relative frequencies of comparable phenotypes do not correspond in the two sexes.

True breeding lines have been started for three phenotypes: full black, linear band and orange abdomen. Crossing between the two extreme phenotypes results in intermediate F_1. A statistically significant shortage of females has been a peculiarity of this cross.

Selection experiments which were started from intermediate phenotypes have resulted in shifting and reduction of variability in both directions.

Chaetotaxy: The presence and development of the two prescutellar bristles are not fixed characters. Among both wild and laboratory

flies it is easy to find specimens with one or both bristles missing, reduced, or displaced. Practically all the females collected in the Latina district gave, either in F_1 or in F_2, a few abnormal flies of these phenotypes. By inbreeding a penetrance of 90%-95% has been reached and with it, sterility. This character seems to be controlled by a single main gene, but its penetrance is erratic. It has been observed in *Musca domestica* of various localities, and in *M. vicina*.

Reduction of the anterior dorsomedian bristles has occasionally been found with evidences of inheritance (familial incidence) in the Latina population. Absence, reduction or disarrangement was common in a *vicina* stock from Tripoli, and persisted for at least three generations; by the ninth generation such changes had become an exception.

Reduction of the anterior dorsomedian bristles is one of the characters of diagnostic value for *M. domestica cuthbertsoni*.

Mutants.

Several mutants have been found, sometimes among stock strains but mainly arising from inbreeding from fertilized wild females. Twenty-three are listed; four are not from my material; 15 affect the wings and at least thirteen of them can be assumed to be almost certainly not biased by selective recording; one is lethal and affects the larvae before hatching.

The degree of natural heterozygosis for recessive mutants seems to be very high. The wild flies which have been tested for mutants were 18 fertilized females, five from Tripoli and 13 from Latina. Four distinct recessive genes were found, segregating in the F_2 of the five from Tripoli (*M. domestica vicina*); only one out of the 13 from Latina failed to show recessive genes in its descent. In the following table the numbers of mutations recovered in the descent of each female are compared with their incidence:

Number of mutations	*Number of females**
0	2T; 1L
1	2T; 4L
2	1T; 3L
3	1L
4	1L
5	2L
6	1L

* T = Tripoli, L = Latina

The offspring of two females have been found heterozygous for the same five mutations, or at least for five mutations of similar phenotype. Many mutations have been found repeatedly, not only in flies from the same locality, but also in flies of geographically distinct origin; four, or perhaps five, were already well-known to the workers acquainted with this material, having been found several times during a period of several years. The persistence of these genes over a long period in a population subject to drastic contractions with a yearly rhythm should not be overlooked.

The description of the mutants and their history is given in Appendix 1.

Gynandromorphs: Several gynandromorphs have been found among samples of wild populations, among laboratory stocks, and among flies inbred for experimental purposes. Referring to the last group, 21 examples have been found, among about 10^4 flies. Two peculiarities deserve attention: (a) none has been obtained from flies bred from parents kept as single pairs; (b) the incidence is typically familial, as is shown by the following table:

Family	*Number*
$F_2$3	2
$F_2$5	2
$F_4$6	3
$F_4$21	3
$F_2$26	2
$F_3$26	7
Orange strain	2
	21

In addition to these, one has been found in the Tripoli stock and one in a mass-culture cross between Tripoli ♀♀ × Roma ♂♂. In almost every collection in the Latina district some gynandromorphs are found. I have records of one collection of 700 flies showing the following sexual distribution: ♀♀ 410, ♂♂ 287, ♀̂♂ 3. The distribution of heterosexual tissues can be perfectly unilateral, anteroposterior, or sectional. Frontal width, legs, pulvilli, wings, pigmentation of the tergites, sternites and sternal bristles, and external genitalia are sexually differentiated, making it possible to recognize the segmental distribution of the abnormality. The smaller portion of heterosexual tissues observed involves unilater-

ally two abdominal segments. Double external genitalia of both sexes have been observed, and also incomplete double internal genitalia, sexually differentiated, without duplication of gonads. Two examples, with anteroposterior distribution and female abdomen, have been seen in copulation with normal males, one in nature, by Dr. G. Saccà, — unpublished — and one in captivity by the author.

Effect of inbreeding on fertility.

Fertility is affected by inbreeding. The number of single pairs which are fertile, and the number of their offspring, decreases very quickly; it is often impossible to have F_4 flies after three generations of close brother-sister breeding; however, no reduction of the number of eggs laid in single batches has been observed.

This preliminary survey on the genetics of the housefly leads us to the conclusion that its genetical make-up includes (and perhaps is based on) great genetical heterogeneity, which expresses itself (a) directly, through the existence of polymorphic characters and the great number of recessive mutations carried by wild flies, and (b) indirectly, through the difficulties of keeping stocks homozygous for some genes, and of successful close inbreeding.

The recognition of the features of any genetically controlled character is a necessary preliminary to its study. This involves formal description, and analysis of expressivity and penetrance. These two terms, being technical, may require explanation. Expressivity is the intensity of the expression of a character in the individual: a given genotype can give phenotypes differing very little (poor expressivity) or very much (good expressivity) from the normal, and individuals of the same genetic constitution can be very alike (uniform expressivity) or not very alike (variable expressivity). On the other hand, mutations exist which can affect only some of their carriers. The penetrance is the ratio of the abnormal individuals to the total number of individuals with corresponding genetic constitution. These two concepts are very important, because expressivity and penetrance give a measure of the intensity and regularity of gene expression.

Let us consider first the formal description referring to « tolerance ». It is usual to express the tolerance by indicating the median lethal dose (LD_{50}). This is quite insufficient to describe the properties of a given stock, as it is just a point of a line. Moreover, as is well known, its value is greatly influenced by the conditions of development of the flies and experimental conditions. The standard deviation of this datum is perhaps more indicative, because it gives not only an estimate of

the exactitude of estimation, but expresses the spreading of the data, and thus indicates the distribution of individual tolerances in the population.

Samples of the same population should have constant proportions of individuals with different tolerances. Disturbing factors with generalized effect are likely to affect all the members of a sample, and so the LD_{50} will be affected, but its standard deviation will not. However, any disturbing factor with selective action will affect also the slope of the regression line. Repeated treatments of samples of several populations suggest that the slope of the probit/log concentration line is much less erratic than the actual value of the LD_{50}.

Moreover, if we want to describe the properties of a stock (and this must be very accurate if the data are to be used for comparative purposes) we must refer to other elements. We know that the paralysing effect is distinct from the killing action; we could more properly say that knockdown-tolerance and kill-tolerance are distinct phenomena with different genetic control (Busvine, Harrison). A survey of several stocks kept in this laboratory has shown that for every stock repetitions give results in good agreement; the stocks with lower « knockdown » tolerance are from Tripoli, Iran, and India; the Tripoli and India stocks have also low toxicity tolerance. Two Italian stocks, one susceptible to kill (Roma sensibile) and the other resistant to kill (Latina), gave similar « knockdown » curves, and included a small fraction of « knockdown »-resistant flies. A Danish stock is « knockdown »-resistant and kill-resistant.

The differences between the stocks become much more apparent if the doses per surface unit are not very high; increase of the dose apparently affects the « knockdown » curve of the « knockdown »-resistant stock much more than in the case of the « knockdown »-susceptible stock (Fig. 1).

The study of the effect of doses on « knockdown » could give very valuable results not only for comparative purposes, but also for the understanding of the underlying phenomena.

The kill-tolerance of individuals within the same population with different « knockdown » tolerance is another interesting element, which can help to throw light not only on the attributes of a given population, but also on the relations between « knockdown »-and kill-tolerance. Our stocks differ greatly in this respect.

It is no mystery that the results of independent experiments in this field are often difficult to compare, and sometimes contradictory. The tolerance appears to be somehow erratic, even in selected strains. Is

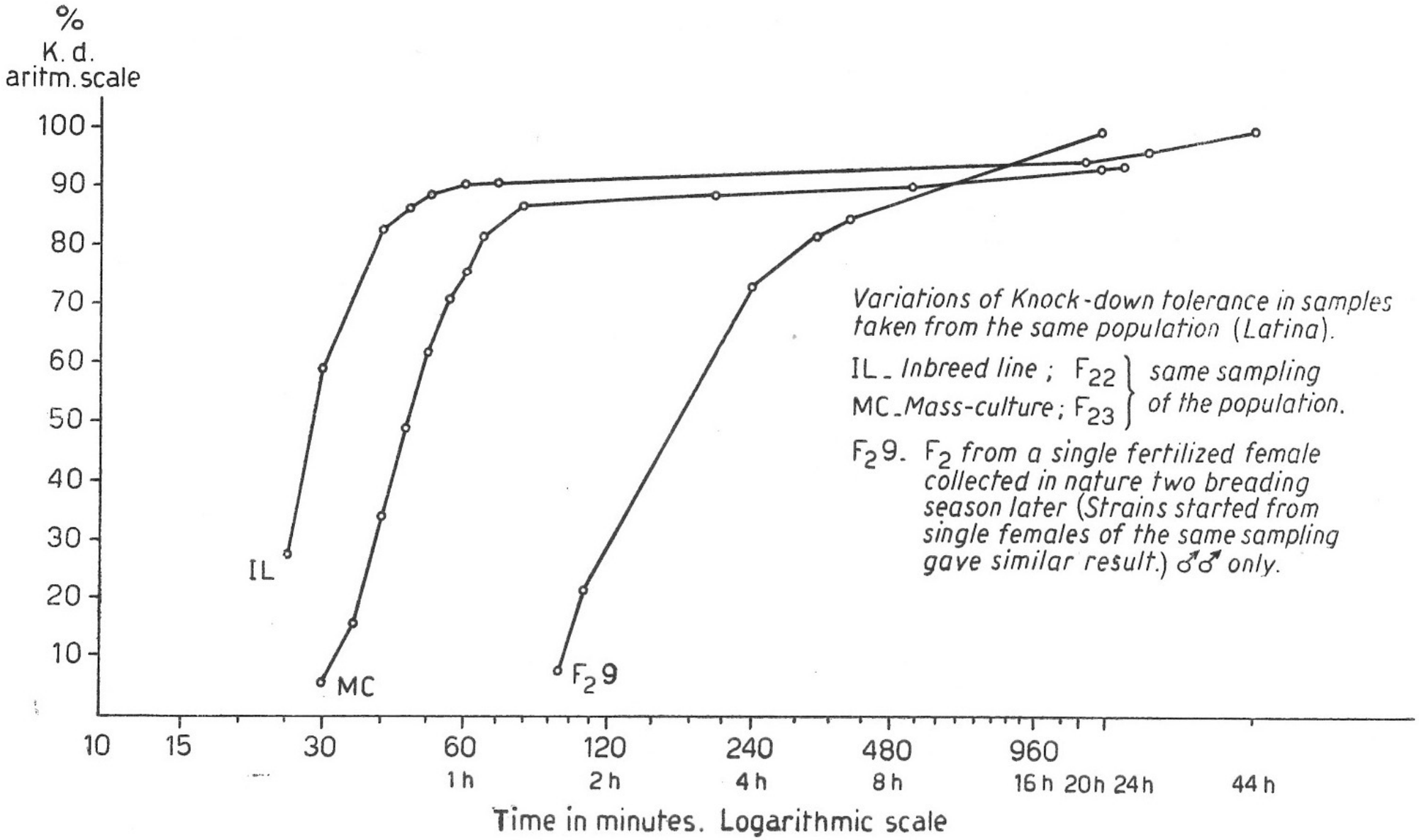
%
K. d.
aritm. scale
100
90
80
70
60
50
40
30
20
10
IL
MC
F2 9
Variations of Knock-down tolerance in samples taken from the same population (Latina).
IL _ Inbreed line ; F22
MC _ Mass-culture ; F23
same sampling of the population.
F2 9. F2 from a single fertilized female collected in nature two breading season later (Strains started from single females of the same sampling gave similar result.) ♂♂ only.
10
15
30
60
1 h
120
2 h
240
4 h
480
8 h
960
16 h
20h
24 h
44 h
Time in minutes. Logarithmic scale

Fig. 1.

this a peculiarity of the tolerance-controlling factors, or rather of the genetical make-up of the fly?

If we go through the list of mutants we realize that most of them have irregular expressivity and incomplete penetrance. This is not exceptional: even in the geneticists' favourite genus (Drosophila) there are species in which the « good mutants » are rare. This means that the abnormality introduced by a mutated gene is easily overcome by the residual genotype, perhaps with the help of environmental conditions and/or that of secondary genes. So far as we know, that is how things are in *M. domestica* for mutants which we recognize through some morphological effect: we have no reason to expect anything different for genes of which the main action may be recognized through a physiological property,

I have stressed before the importance of defining the toxicity tolerance in terms both of effective dose *and* of standard deviation. This is important also on account of the methodology used when genetic researches are involved. The polygenic characters are studied mainly by comparing the spread of distribution, measured by the standard deviation, in subsequent generations. It is now accepted that kill-resistance to DDT is a property genetically controlled by polygenic systems. This consideration leads us into formal genetics, the branch of study mainly concerned with modality of inheritance, mutability, and genetic systems.

Formal genetics is primarily based on the study of the Mendelian behaviour of mutants. The mutants affecting morphological characters may obviously be handled more easily than those affecting physiological traits, and an accurate study of such mutations is essential if we are to recognize the linkage groups and the amount of recombination introduced by crossing-over. We know that in the housefly the chromosomes are six, and so we must expect six linkage groups. We have no information about the closeness of the linkage, either by direct observation or by indirect evidence of a cytological nature. A knowledge of linkage is essential for the interpretation of the genetical behaviour of polygenic characters, because the reshuffling of the elements which are integrated in a polygenic system is greatly affected by intrachromosomal recombination. We know that inbreeding is considerably handicapped by great decrease in vitality and fertility. Lethal genes and sterility genes are certainly involved, and some have already been discovered. When genetical maps are available, it will be easy to recognize not only which chromosomes carry these genes, but also if, and how, they interfere with recombination and so with the spread

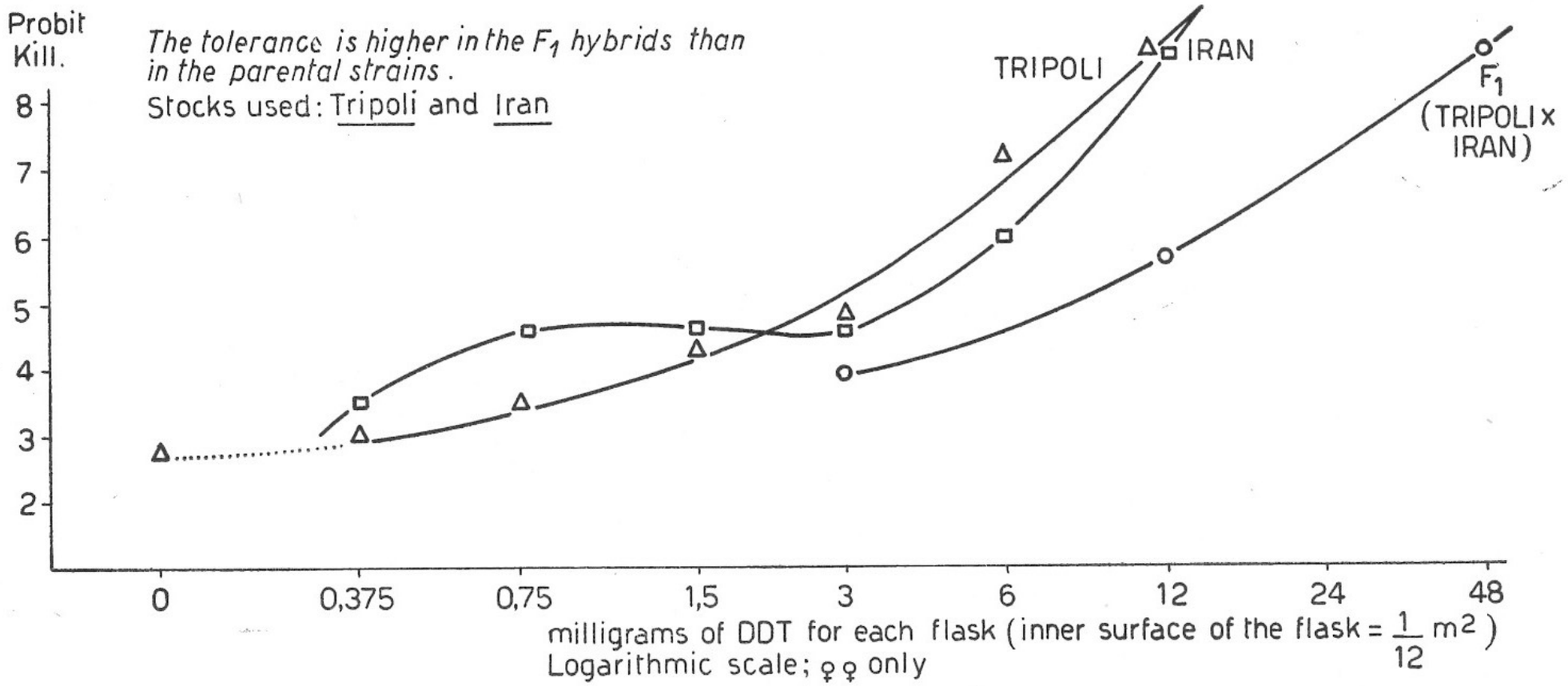
Probit
Kill.
The tolerance is higher in the F_1 hybrids than in the parental strains.
Stocks used: Tripoli and Iran
TRIPOLI
IRAN
F_1 (TRIPOLI x IRAN)
8
7
6
5
4
3
2
0
0,375
0,75
1,5
3
6
12
24
48
milligrams of DDT for each flask (inner surface of the flask = $\frac{1}{12}$ m2)
Logarithmic scale; ♀♀ only

Fig. 2.

of genes in the population and with the building up of gene complexes.

To obtain a rough idea of the linkage groups, we need some 20 fairly good mutants. At present, efforts have been made to collect and keep in stock mutants extracted by inbreeding from wild flies, and during this work observations have been made on their mode of inheritance. A peculiar feature is that no evidence of criss-cross inheritance has been obtained: it seems unlikely that out of 23 genes distributed over six chromosomes not one should show sex-linked inheritance. However, we must remember that genes showing criss-cross inheritance are subject to drastic selection when they come into the heterozygous sex, and that absence of typical criss-cross inheritance does not disprove sex-linkage: if the X and the Y chromosomes have homologous, genetically active, parts, the genes included do not follow a typical criss-cross schedule, in spite of the fact of being included in the sex chromosomes.

Another peculiarity which deserves attention is that several recessive mutations have been repeatedly recovered by inbreeding from strains of quite distinct geographical origin; moreover, some of them have been observed several times during a period of years in one limited population: these are common too.

These facts may have two explanations: (a) the genes involved are unstable; (b) the mutant genes are very old and may increase the fitness of their carriers, at least in some circumstances. The resistance-controlling genes have apparently similar properties, as their ubiquity suggests.

A topic pertinent to formal genetics is dominance. We say that a character is dominant when it is shown both in the homozygous and in the heterozygous state. However, « good » dominant characters may appear as intermediate ones if the study is carried to a deeper level than external morphology. Dr. La Face, exposing flies to contact in cages treated with 1 g per m^2 of DDT for 24 hours found DDT-resistance to be recessive. My experiments are indicative of heterosis (fig. 2, 3) and of (incomplete) dominance: (Fig. 4). I exposed flies for one hour to regularly increasing scalar doses, the highest being equal to the one used by Dr. La Face. The mortality was measured 24 hours later. The results are not necessarily contradictory, because the factor « time of exposure » is very important and, I think, deserves more attention than it has hitherto received. In nature, flies are rarely forced to remain in contact with treated surfaces for long and the nervous excitation which is the first sympton of intoxication might be quite efficient in limiting the actual contact with the treated surface. The

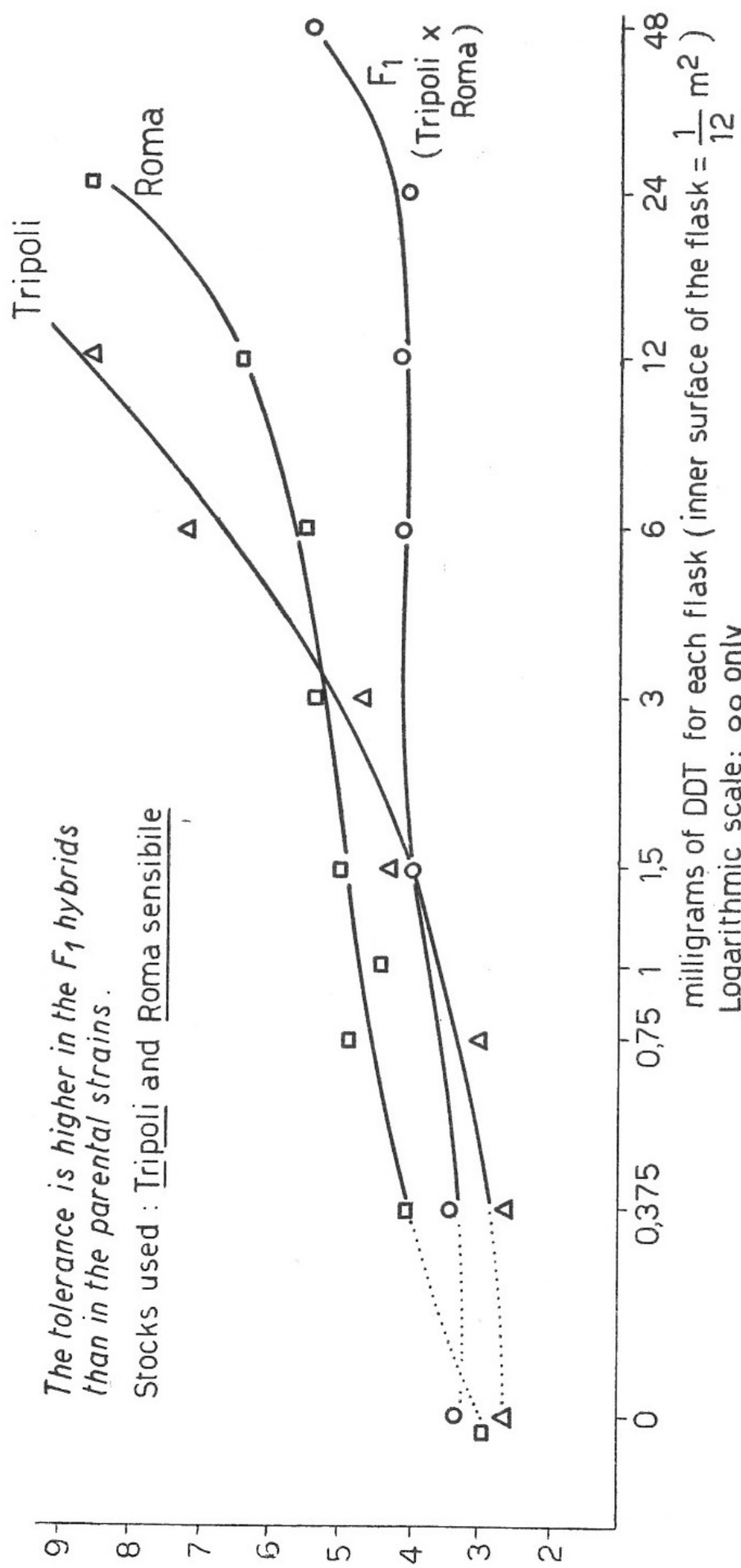
Probit Kill.
9
8
7
6
5
4
3
2
0
0,375
0,75
1
1,5
3
6
12
24
48
milligrams of DDT for each flask (inner surface of the flask = 1/12 m²)
Logarithmic scale; ♀♀ only
Tripoli
Roma
F1
(Tripoli x Roma)
The tolerance is higher in the F1 hybrids than in the parental strains.
Stocks used : Tripoli and Roma sensibile

Fig. 3.

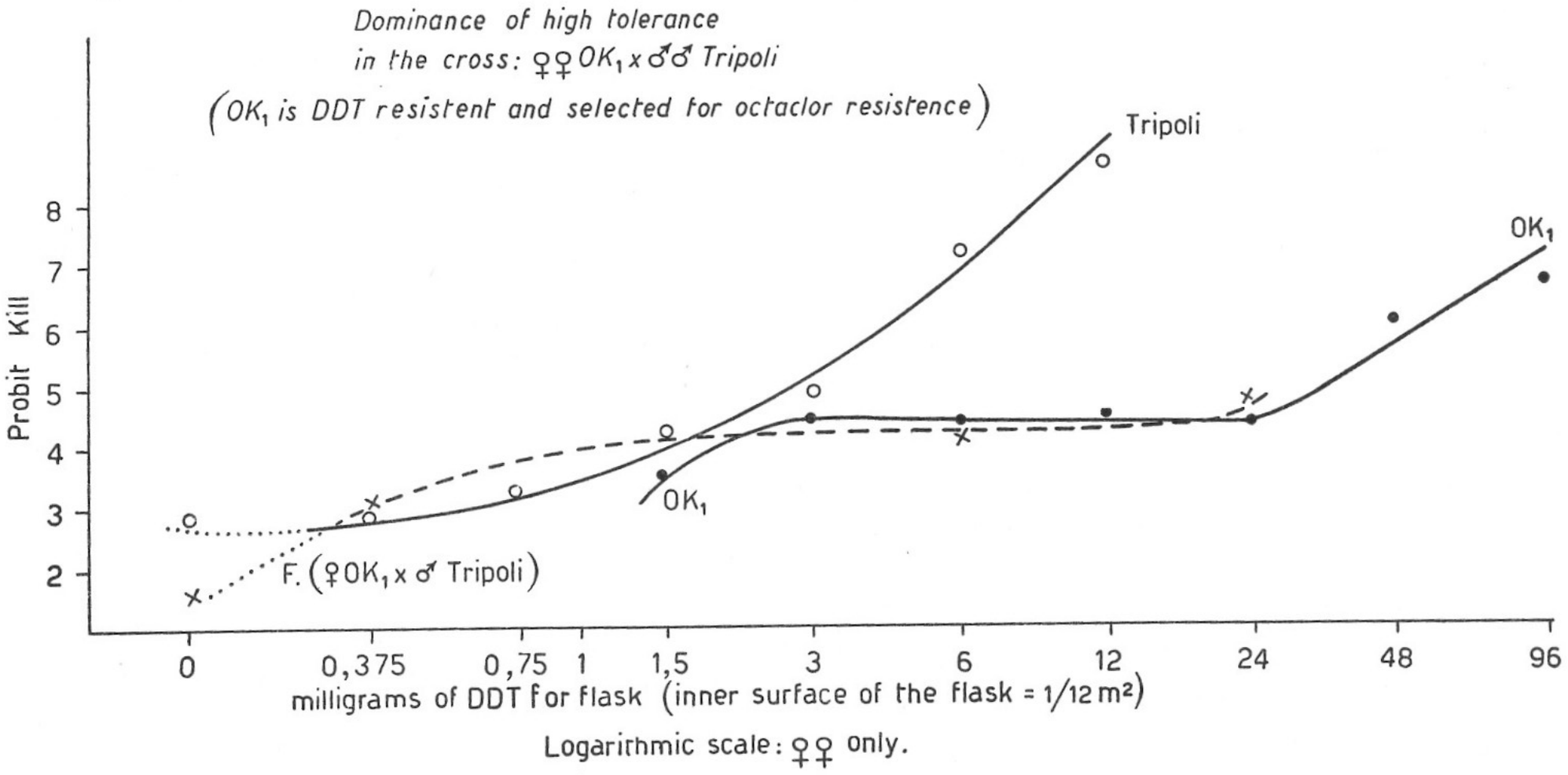
Dominance of high tolerance
in the cross: ♀♀ OK1 x ♂♂ Tripoli
(OK1 is DDT resistent and selected for octaclor resistence)
Tripoli
OK1
OK1
F. (♀OK1 x ♂ Tripoli)
Probit Kill
8
7
6
5
4
3
2
0
0,375
0,75
1
1,5
3
6
12
24
48
96
milligrams of DDT for flask (inner surface of the flask = 1/12 m²)
Logarithmic scale: ♀♀ only.

Fig. 4.

biological advantage which partial or complete dominance of resistance offers in the development of resistant strains, and the bearing that its recognition has on any study concerned with population and the evolutionary aspects of resistance, are obvious. Any form of intermediate inheritance is liable to misinterpretation as a consequence of the modality of estimation.

We have seen before that polymorphic characters are present in nature and that natural populations are affected by an enormous number of heterozygous mutations. We do not know at present whether these two forms of genetical heterogeneity are primarily concerned with the survival of the species, or whether this heterogeneity is a by-product of a more complex phenomenon. However, these findings have a direct bearing on our problem: they help to throw light on the existence of that special form of genetical heterogeneity which permits of the development of resistance.

A high degree of genetical heterogeneity implies primarily selection for heterozygosis, but it should imply also a high mutation rate. Species differ for tendency to mutate and the genes themselves have different stability; this makes it difficult to foresee the bearing that the appearance of new mutations has on the genetic plasticity of any organism; however, we must remember that mutations provide the rough material for selection, and that chemicals can increase the mutation rate.

When mutations are present in natural populations, the recognition of their frequency permits of inferences on the survival value of the genes involved, or of genes closely linked to them; their special distribution gives a measure of the genetical differentiation of populations; and their seasonal and yearly variations in frequency allow of the estimation of the dynamics of gene complexes. For our main problem, research should be based on morphological mutants and on factors for resistance. The first line will provide us with general knowledge, the second bears directly on the problem. It has been proved that resistant flies of different geographical origin differ in resistance mechanisms. Other forms of resistance-determining factors might be discovered. There is, however, another point which I should like to stress. We do not know anything about the fate, in nature, of the resistance-determining genes, which reach a high concentration in the areas treated with insecticides. Are they likely to permeate the neighbouring original populations or will they be merged and eliminated? Indirect evidence suggests that they are subject to elimination but this requires proof.

I have some experimental data which may be of interest. A DDT-resistant strain from Latina was knockdown-sensitive and an inbred line derived from it was shown to have been selected for great knockdown sensitivity; occasional samplings of the original wild population showed the same property. However, two F_2 obtained from wild females collected at the beginning of the present breeding season showed the highest knockdown resistance which I have as yet observed (Fig. 5). I am not going to advance theories based on occasional data, but I give this instance as an example of the fact that natural populations are by no means genetically uniform and stable. During an experiment of selection for light body colour (orange line) I started a collateral strain with sister flies, showing some incipient pigmentation, not arranged in the form of a central band, and limited to the posterior abdominal segments. Now after six unselected generations examples can be found which have reached a great degree of melanism; the main genes for melanism had been excluded, but similar morphological effects have apparently been reached through the selection of genes with additive effect. A similar thing happened in a strain of wild flies collected near Tripoli; originally the colour was rather light, with variations; now after nine generations self-black samples have been found, and the colour distribution seems to be similar to that of the local flies. A comparable phenomenon has been repeatedly observed by several authors on *M. domestica vicina* and *M. domestica nebulo* kept in the laboratory. The frontal width, very narrow in these species, has been observed to increase progressively and to reach the value which is typical of the local flies. The characters involved are different — the effect is the same, and shows how quickly a change in the ecological conditions may be followed by genetical adaptation.

Now for the practical aspect of our problem it is essential to know if a given susceptible population is gifted with genes for resistance; how quickly resistance can be developed, if it can spread over, or if it is likely to disappear and within what length of time.

These questions can only be answered by research in population genetics. Comparative study of resistance to different insecticides has already been undertaken (Busvine, 1953; Keiding, 1953; La Face, personal communication), and has given information of great interest. Busvine, using stocks of Italian and Sardinian origin, found that resistance to BHC, chlordane, and dieldrin are linked together, and are quite independent of DDT-resistance. Keiding's finding with Danish flies indicate a complete independence of DDT, BHC and chlordane resistance. Dr. La Face's observations on the specificity of resistance

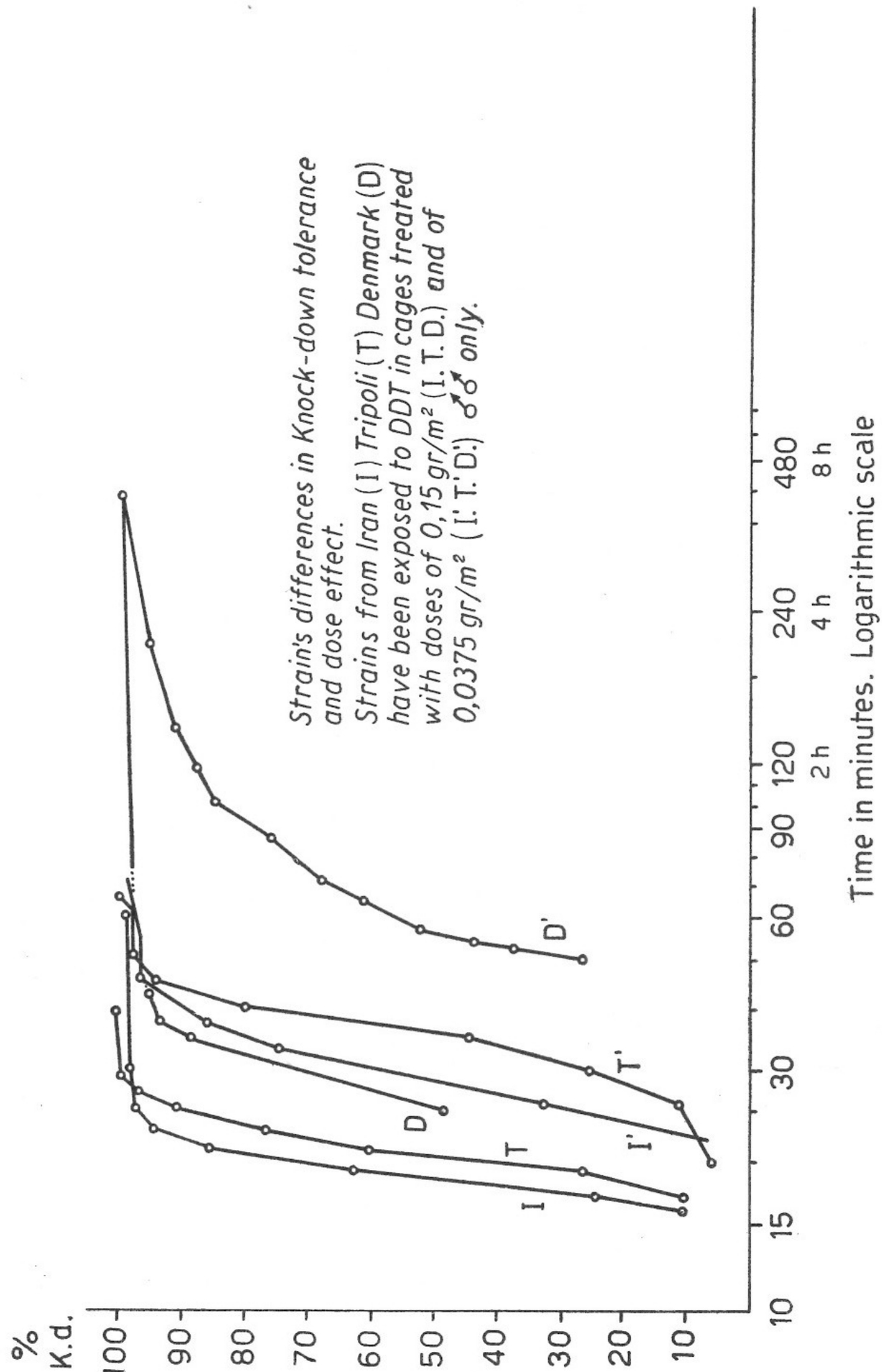
Strain's differences in Knock-down tolerance and dose effect.
Strains from Iran (I) Tripoli (T) Denmark (D) have been exposed to DDT in cages treated with doses of 0,15 gr/m² (I. T. D.) and of 0,0375 gr/m² (I'. T'. D'.) ♂♂ only.
% K.d.
100
90
80
70
60
50
40
30
20
10
I
T
D
I'
T'
D'
10
15
30
60
90
120
240
480
2 h
4 h
8 h
Time in minutes. Logarithmic scale

Fig. 5.

are in agreement with these findings. Moreover, Dr. La Face controlled genetically this specificity with crosses between a Sardinian strain selected for Octa-Klor resistance and an Italian (Torre di Pietra) strain selected for DDT resistance. A great decrease of resistance toward both insecticides in the F_1 confirmed the independence of the genetical control, and is indicative of recessivity, or, as Dr. La Face suggests, the resistance-controlling factors could have some kind of antagonistic action. Laboratory (Bruce and Decke, Busvine) and field observations (Keiding) show that resistance to one compound is reached much more quickly if resistance to other unrelated compounds is already present; specificity of resistance factors and easier development of resistance in stocks already resistant to unrelated compounds appear somehow contradictory. The two facts may be explained in many ways. It is, for example, possible that some unspecific key-genes exist, the effect of which is not shown at the level of resistance (we might find an analogous condition to the one suggested in the normal allele of the gene for albinism in vertebrates: no colour gene can manifest itself if this key-gene is not present); another genetical possibility is that a previous selection leads as a by-product, to the organization of gene-complexes which can be directly utilized by the subsequent selection. It might also be that competition between susceptible and resistant individuals is less strong in these already selected stocks. The genetic analysis of stocks showing differences such as those observed by Busvine and Keiding can be very enlightening; a physiological analysis of the properties of these differences might show whether a common background exists for the various forms of resistance.

I have deliberately not considered the physiological aspect until now because I have no personal experience on this point; however, it is not an aspect which I need to stress. It is obvious that only physiological research offers the possibility of distinguishing between stocks in terms of alternative characters, and of providing information on their additiveness or complementarity, or on their antagonism. We may expect that defence barriers might arise at quite different levels, affecting intake, storage, degradation, and/or blockage of the toxic agents. The researches of Bruce and Decker have already shown that, even in flies living in similar ecological conditions, the defence mechanisms can be quite different: Busvine has been able to demonstrate that « knockdown »-resistance and one of these special forms of defence are associated. These successes show clearly how important is the recognition of the constituents of *resistance* for the advance of our research at all levels.

APPENDIX I.

In the present list the definitions « recessive » and « autosomal » are provisional and based on records of stocks. No proper genetic test has been done; only the gene *ocra* has been properly studied (La Face; unpublished). In particular the term « autosomal » is liable to revision, as it is based on absence of typical criss-cross inheritance; a part of the Y chromosome of the housefly is genetically active (Perrje A. M., personal communication) and this introduces a variant in the schedule of sex-linked inheritance.

List of mutants.

bulging head (*blg*): recessive, autosomal. The head bulges frontally; the frons is enlarged, and the eyes look bigger than normal; both sexes are fertile; the expressivity is irregular. Found as F_2 segregant from two out of 13 Latina parental females. Recovered in F_2 from the cross: *blg* × ±.

ocra (*ocra*): recessive, autosomal. Eyes ochre or orange. Three similar mutants have been reported from laboratory stocks of Italian flies possibly related. Very good Mendelian segregations (La Face).

eyeless (*ey*): recessive, autosomal. One eye missing; slight abnormalities of the head. Found as two ♀♀ in the F_2 of a wild fly from Tripoli (*M. Domestica vicina*).

prescutellarless (pre): recessive, autosomal. One or both prescutellar bristles can be reduced, displaced and/or absent; absence is the commonest feature. It has been observed in wild and laboratory flies. All females from Latina which have been tested genetically gave either in F_1 or in F_2 a few prescutellar-less flies. The penetrance is erratic and can be improved by selection.

cuthbertsoni (cuth): (recessive, autosomal [?]. One or more anterior dorso-median bristles can be reduced or absent, often symmetrically; overlaps the same character, diagnostic for *M. domestica cuthbertsoni*, found repeatedly in flies from Latina and Tripoli, with clear evidences of familial incidence.

broad crossvein (brcr); autosomal, semidominant. Anterior crossvein enlarged; common in laboratory stocks; found twice out of 12 wild females from Latina. Expressivity very variable; the heterozygotes may overlap both homozygotes.

confluent (cnfl): autosomal, recessive. Second and third longitudinal veins bent towards the costa, with enlargment of a delta type at junction with margin; the delta affects also the first longitudinal vein, which results confluent with the second - and occasionally with the third - longitudinal vein. The shape of the wings can become irregularly narrowed and reduced; found as F_2 segregants of both sexes from ♀ showing thickened posterior crossvein.

fused (fu): autosomal, recessive. Third and forth longitudinal veins fused at the crossvein and at the distal ends by enlargements; the distal part of the fifth longitudinal vein may be similarly affected. Found as a single male in broad crossvein stock; recovered in F_2 after outcross. Female sterile.

gap (gap): autosomal, recessive. Vein L4 is weak or has a section missing anterior to the first-cross-vein; occasionally asymmetrical; the wings can be irregular in shape. Found repeatedly in a line selected for light (*orange*) body colour; twice from 13 wild females from Latina, once from Tripoli flies.

abruptex (ax): autosomal, recessive. Posterior cross vein has a section missing; occasionally asymmetrical. Found segregating in the F_2 of five out of 13 wild ♀♀ from Latina.

plexus (px): endemic in laboratory stocks, carried by the F_1 of six out of 13 wild females from Latina. Recessive, autosomal; incomplete penetrance, variable expressivity. Posterior cross vein bent and/or thickened, and/or extra veins; similar abnormality may involve the distal parts of veins IV and V.

scalloped (scl): very common among preserved examples of a plexus stock now discarded; no records about its inheritance; referred as erratic.

fringed (*fr*): autosomal, recessive. Wings narrower than normal; small cuts at the tip and internal margin of the wings; marginal bristles somewhat scattered. Found as F_2 segregant from one Tripoli female.

taxi (*tx*): recessive, autosomal; expressivity and penetrance good; viability poor. The wings are spread 75°-90° from the body axis, arc-like downward in old flies; does not allow of flight. Appeared in the F_2 of four out of 13 Latina wild ♀♀

divergent (*dv*): recessive autosomal. Expressivity and penetrance not very good; the wings spread at about 75° from the body axis, have normal texture; allows of flight; appeared in the F_2 of one Latina ♀

teasing (*tsg*): incomplete recessive; autosomal. The wings are outspread, often asymmetrically; occasionally some flies rest in normal positions, particularly under narcosis. Recurrent in laboratory stocks; found once from Latina flies.

balloon (*ba*): recessive autosomal. Blisters in the wings, presumably left by blood vesicules. Found as F_2, F_3 segregants from two out of 13 fertilized ♀♀ from Latina No offspring obtained. The specimens had the wings spoiled at death.

abnormal abdomen (*ab*): autosomal, recessive. Tergite raggedly incomplete; frequently a split median to the second visible sternite, with the dark band forming an x; minimal detectable expression is absence of michrochetae along the median line of some segments. Found as F_2, F_3 of two out of 13 wild fertilized females from Latina.

still-born (*stb*): recessive, autosomal. Lethal homozygous, affects the larvae before hatching; the yolk is incompletely enclosed, and the larvae eventually assume a brownish appearance, possibly due to melanization of haemorrhagic haemolymph. Found twice from Latina flies; possibly endemic in laboratory stocks.

(*short legs*): found as a single ♀ F_2 from a wild Tripoli ♀

Information has been received about the following mutants:

outspread wings (Ricciardi, Brazil), *yellow body* (Saccà, Italy), *curled wings* (Battaglia, Italy).

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