Genet. Res., Camb. (1966), 7, *pp.* 149–158
With 2 text figures
Printed in Great Britain

Chromosomal polymorphism in western Mediterranean populations of *Drosophila subobscura*

By ANTONIO PREVOSTI

Laboratory of Genetics, Faculty of Sciences,
University of Barcelona, Spain

(*Received* 9 *October* 1964)

INTRODUCTION

Since the earliest papers dealing with chromosomal polymorphism in *Drosophila pseudoobscura* (i.e. Dobzhansky & Epling, 1944), abundant evidence has accumulated suggesting an adaptive significance for such polymorphism in several species of the genus *Drosophila*. In particular, the existence of latitudinal and altitudinal clines in the frequencies of some chromosomal types has been given an adaptive interpretation.

The structural alteration of the chromosomes by inversions, by reducing or eliminating crossing-over within the inverted segments, allows these inverted segments to evolve as 'supergenes', in which coadapted sets of genes may accumulate by natural selection to form integrated units of crossing-over.

The first reports on chromosomal polymorphism in the European *D. subobscura* (Stumm-Zollinger, 1953) expressed some doubt as to the adaptive significance of the chromosomal types in this species. However, more recent data suggest the existence of adaptive clines in some of these types. Several chromosomal orders of *D. subobscura* show regular geographical variation. A latitudinal cline has been found in several of these orders (Sperlich & Kunze-Mühl, 1963; Sperlich, 1964; Prevosti, 1964*a*, 1964*b*). Until recently most data have come from rather widely separated populations, i.e. Vienna (Kunze-Mühl *et al.*, 1958), Israel (Goldschmidt, 1958), Edinburgh (Knight, 1961), several Italian populations, all except one from small islands (Sperlich, 1961; Kunze-Mühl & Sperlich, 1962; Sperlich & Kunze-Mühl, 1963), Barcelona (Prevosti, 1964), Dröbak (Norway) (Sperlich, 1964) and Lagrasse in the south of France (Prevosti, 1964*b*). From these data we are able to recognize only the most general trends in the geographical variability of the chromosomal types in this species, but, perhaps with the exception of the Vienna–Italy area, we know nothing about the regularity and details of these general trends. In order to get fuller information on this point, data have been collected in a rather limited area of the western Mediterranean region of Europe. These data will also be useful for comparison with those from the Vienna–Italy area.

* This work was carried out with the aid of a grant from the Department of Education of the Spanish Government, to the Chair of Genetics (University of Barcelona).

L

In addition, the comparison of the data from Lagrasse and Barcelona (Prevosti, 1964*b*) seems to indicate an influence of the Pyrenean barrier on the steepness of the cline in the frequencies of some chromosomal types. This is specially true in the chromosomal types where the frequency decreases northward. The frequencies of these types in the Lagrasse area are more akin to those from the distant population of Vienna than to the frequencies found in the neighbouring population of Barcelona, only 200 km. south of Lagrasse, but on the other side of the Pyrenean range. The comparison of the data from Barcelona with those from the more southern populations will contribute to the understanding of this effect of the Pyrenees.

Thus, the data about the western Mediterranean populations which are presented in this paper may contribute to an understanding of the evolutionary significance of the changes in the structure of the chromosomes by inversion, as a mechanism controlling the adaptation of the populations.

MATERIAL AND METHODS

Data from four western Mediterranean populations are compared in this paper. These populations are from Lagrasse in the south of France (30 km. west of Narbonne), and Barcelona, Ribarroja (Valencia) and Malaga (on the Mediterranean shore of Spain). The data from Lagrasse and Barcelona have been described in earlier publications (Prevosti, 1964*a*, *b*), but are included here with those from the more southern populations of Ribarroja and Malaga, since the fuller comparisons are informative.

The ecological conditions at the sites where the flies from Lagrasse and Barcelona were trapped have been described in the papers referred to above. The site where the population of Ribarroja was trapped is a riverside meadow, with cane shrubs and some sparse trees (*Populus* and *Salix*). It lies by the Turia river, some 20 km. west from Valencia, at about 50 m. above sea level. The Malaga site lies at sea level some 10 km. south of the town, and is also a meadow near a channel, with rather densely planted trees, specially Eucalyptus. Both samples were caught in April 1963.

The chromosome structure was determined on 56 males from Ribarroja (112 autosomal sets and 56 X chromosomes) by crossing the wild males to virgin females of the Küssnacht strain, which is homozygous for the standard order in all the chromosomes. From the progeny of these seven crosses seven larvae were analysed to ensure a high probability of observing both members of each pair of homologous chromosomes from the tested males.

The males trapped in Malaga were also crossed with Küssnacht females, but only the chromosome structure of one larva from each progeny was analysed. With this procedure only one set of chromosomes from each wild male was determined, thereby saving much tedious work. Altogether 135 larvae were analysed, corresponding to 135 sets of autosomes and 77 X chromosomes.

The chromosomes were stained with acetic orecin (60%) and lactic acid (40%).

REGULARITIES IN THE GEOGRAPHICAL VARIATION OF SOME CHROMOSOMAL ORDERS

The frequencies of several chromosomal types show a clear latitudinal cline in the western Mediterranean region (Fig. 1). The data from Edinburgh (Knight, 1961), a population located at about the same parallel as the western Mediterranean populations, supports the existence of these clines, with very few exceptions. Some of these clines are an expression of a general trend found in the *D. subobscura* populations so far examined. This is true of the north–south decrease in the frequency of

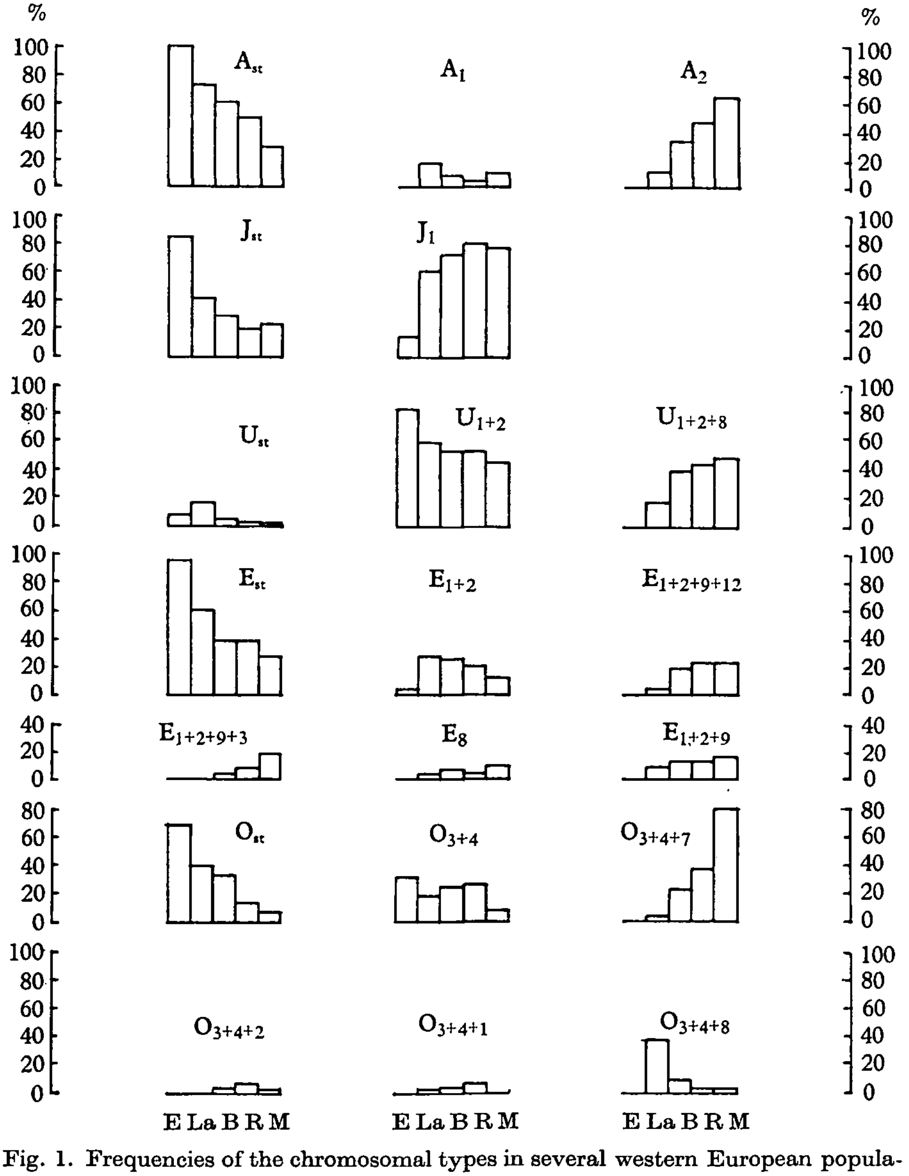

Fig. 1. Frequencies of the chromosomal types in several western European populations. In each histogram the populations are ordered north–south (left to right in the figure): E = Edinburgh, La = Lagrasse, B = Barcelona, R = Ribarroja (Valencia), M = Malaga.

the standard orders in the five large chromosomes. This trend was already pointed out by Sperlich & Kunze-Mühl (1963) and by Prevosti (1964*a*, *b*), and emerges more regularly in the comparison of the western European populations. Also the southward-decreasing frequency of the U_{1+2} order (Prevosti, 1964*b*) is more clearly confirmed in the western Mediterranean area. In this region, the low frequency of the order in Vienna does not break the regularity of the cline.

Table 1. *Frequency* (%) *of the chromosomal types of* D. subobscura *in several populations*

	Lagrasse $n=76$	Barcelona $n=252$	Ribarroja (Valencia) $n=112$	Malaga $n=135$
A_{st}	72·5	60·4	50·9	28·6
A_1	17·5	7·4	3·6	6·5
A_2	10·0	32·1	45·5	64·9
J_{st}	42·1	27·6	19·6	22·2
J_1	57·9	72·3	80·4	77·8
U_{st}	15·8	4·7	2·7	0·7
U_{1+2}	57·9	52·3	52·7	47·4
U_{1+2+8}	18·4	40·8	43·8	50·4
U_{1+2+3}	—	0·4	—	—
U_{1+2+6}	1·3	1 ·2	0·9	—
U_1	3·9	0·4	—	1·5
U_{1+2+7}	1·3	—	—	—
U_{1+2+4}	1·3	—	—	—
E_{st}	57·9	37·2	37·5	27·4
E_{1+2}	26·3	25·6	20·5	12·6
E_{1+2+9}	9.2	12·5	12·5	13·3
$E_{1+2+9+12}$	3·9	17·9	21·4	22·2
$E_{1+2+9+3}$	—	1·5	5·4	17·0
E_8	2·6	5·1	2·7	7·4
O_{st}	40·8	34·3	14·3	6·7
O_{3+4}	17·1	24·1	26·0	6·7
O_{3+4+7}	2·6	21·3	38·4	80·0
O_{3+4+8}	36·8	10·6	2·7	3·0
O_{3+4+2}	—	2·7	6·3	1·5
O_{3+4+1}	1·3	2·7	5·4	—
O_{3+4+22}	—	2·0	3·6	—
O_{3+4+17}	—	0·4	—	—
$O_{3+4+2+16}$	—	0·4	—	—
O_7	—	1·2	4·5	2·2
O_{3+4+12}	1·3	—	—	—

A general latitudinal cline has also been reported for A_2, J_1, E_{1+2} and E_{1+2+9} but in this case with the frequencies decreasing northwards (Prevosti, 1964*b*). These four orders exhibit in the western European populations the same trend, but in E_{1+2+9} the decline in frequency is very slight in the Mediterranean area.

Besides the above-mentioned clines which reflect a general trend in the geographical variation of the chromosomal types of the species in western Europe, there are also other local regularities, peculiar to the western European region. Among these, E_{1+2} and O_{3+4+8} show a stepped decrease in frequency in the north–south direction in the Mediterranean area, but the data from Edinburgh do not fit in with this local Mediterranean cline. Instead, the frequencies of U_{1+2+8}, $E_{1+2+9+3}$ and O_{3+4+7} increase southwards. All these three orders are typical of southern populations and are lacking in Edinburgh. However, O_{3+4+7} deserves a special comment, since this order shows appreciable frequencies only in the western Mediterranean populations, or more specially the Iberian populations. O_{3+4+7} is infrequent in the

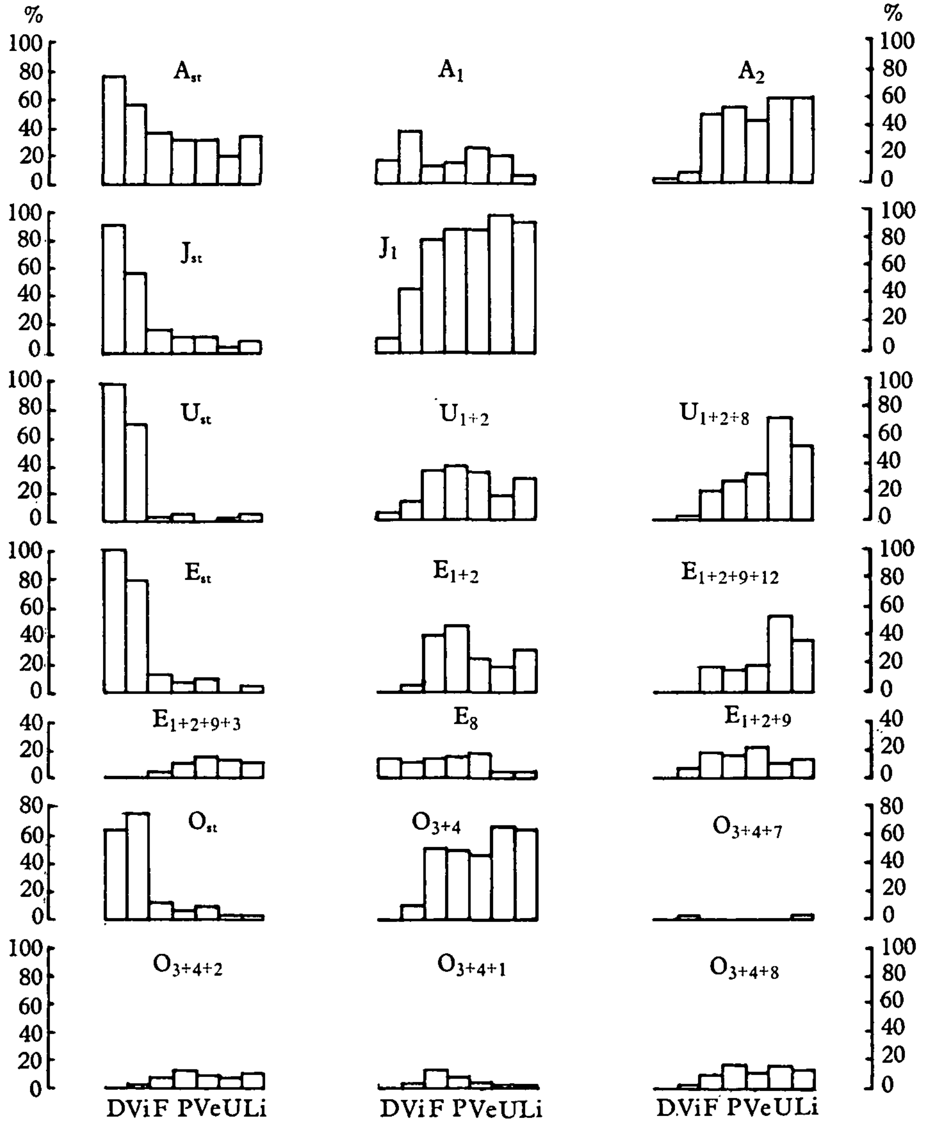

Fig. 2. Frequencies of the chromosomal types in several populations situated in a more eastern meridian than the populations in Fig. 1. In each histogram the populations are ordered north–south (left to right in the figure); D = Dröbak (Norway), Vi = Vienna, F = Formia, P = Ponza, Ve = Ventonene, U = Ustica, Li = Lipari.

south of France, in Lagrasse, only some 80 km. north of the Spanish border. Nevertheless, in Spain, at 100 km. south of the French border (in Barcelona), its frequency is considerable, and exceeds 20%. This steep increase in a rather short distance continues southwards, since in Malaga (about 740 km. south from Barcelona) O_{3+4+7} is the dominant order of the O chromosome, with a frequency of 80%.

The north–south clines found in the western Mediterranean area are, in general, similar to those found along a more eastern meridian, from Norway to Italy. These clines are illustrated in Fig. 2. The histograms of this figure correspond to data published in several papers by Sperlich and Kunze-Mühl (see Introduction), and were commented on in a previous paper (Prevosti, 1964*a*). Here these data are put together with those from Ustica (Sperlich & Kunze-Mühl, 1963) and from Dröbak (Norway) (Sperlich, 1964), which fit well with the clines found between the populations discussed previously.

The comparison of the Edinburgh–western Mediterranean clines with the Scandinavian–Central Europe–Central Mediterranean ones shows, as a common trait of all the northern populations, a high frequency of the standard orders which decreases southwards. In the southern populations the prevailing order is always a complex inversion type, but different in diverse geographical areas. The details of these differences are omitted here because they were already pointed out in a previous paper (Prevosti, 1964*a*).

THE EFFECT OF THE PYRENEAN RANGE ON THE DISTRIBUTION OF THE CHROMOSOMAL TYPES OF *D. SUBOBSCURA*

The comparison of the data from the populations of Lagrasse and Barcelona and those of Valencia and Barcelona enable us to examine the effect of the Pyrenean range as a barrier in the distribution of the chromosomal types of *D. subobscura* (see Prevosti, 1964*b*).

Lagrasse lies some 200 km. northwards of Barcelona and Valencia about the same distance southwards of Barcelona. Thus, any differentiation due to distance alone should be similar in both cases. But the Pyrenean range lies between Barcelona and Lagrasse, constituting a barrier that prevents migration and gene flow between the populations on opposite sides. Moreover, the Pyrenees are an ecological barrier as well. The climatic conditions are very different on the north and south slopes. On the north side the Atlantic influence favours humid and cool conditions; on the south the climate is much warmer and drier, since the mountain range acts as a wall, which shuts off the oceanic influence.

Between Barcelona and Valencia, however, there is no important barrier. The mountain ranges between these sites are lower and with north–south orientation instead of east–west as in the Pyrenees. The climatic conditions are also rather similar in both sites.

In order to evaluate the possible effects of the Pyrenees as a barrier, χ^2 tests have been calculated to compare the distributions of the chromosomal types in each element of the caryotype, between Barcelona and Lagrasse, and Valencia and

Barcelona. In these tests, the classes with theoretical expectation lower than 5 have been lumped together.

As can be seen in Table 2, the significance of the differences is high between Barcelona and Lagrasse, but the differences between Barcelona and Valencia, with the exception of the O element, are small and statistically insignificant. We can conclude therefore that the population from Barcelona differs much more from the Lagrasse than from the Valencia population, and this contrast is probably due to the Pyrenean barrier.

Table 2. *Significance of the differences between the distributions of the chromosomal types observed in Barcelona and those observed in Valencia and Lagrasse*

	Barcelona × Valencia		Barcelona × Lagrasse	
	χ^2	D.F.	χ^2	D.F.
Chromosome A	3·45	2	9·44**	2
Chromosome J	2·63	1	5·70*	1
Chromosome U	1·39	3	24·37***	3
Chromosome E	2.01	4	16·07**	4
Chromosome O	31·94***	4	40·57***	4

*, **, *** indicate statistical significance at $P = 0.02$, 0.01 and 0.001, respectively.

THE LEVEL OF HETEROZYGOSITY

Carson's index of free recombination (IFR) (Carson, 1955) reaches some of the lowest values found in the species in the Valencia population, in the populations immediately north and south of the Pyrenees and in some Italian populations. However, in the more southern population from Malaga, the value of this index is

Table 3. *Index of free recombination and average number of inversions in heterozygous condition per individual*

	IFR		Average number of inversions in heterozygous condition, per individual	
Locality	(1)	(2)	All chromosomes (3)	Autosomes
Lagrasse (France)	82·2	81·3	4·9	4·7
Barcelona (Spain)	80·3	79·8	5·1	4·5
Valencia (Spain	80·7	79·4	4·9	4·3
Malaga (Spain)	84·3	83·8	4·1	3·7

(1) IFR calculated from the relative length of the inversions according to Stumm-Zollinger & Goldschmidt (1959).

(2) IFR calculated from the relative length of the inversions according to Kunze-Mühl & Sperlich (1962).

(3) This figure has been obtained by adding to the observed frequency of heterozygosis in the autosomes the expected frequency of heterozygosis in the X chromosomes of the females based on the frequencies of the chromosomal orders of the X chromosome.

higher, and approaches the figures found in Central Europe (see Table 3 and, for comparative data, Prevosti, 1964*a*).

In agreement with the data on IFR, the average number of heterozygous inversions per individual shows some of the highest values in Valencia and is lower in Malaga (see Table 3 and, and for comparative data, Prevosti, 1964*a*).

The pattern of the contribution of the different chromosomes to the heterozygosity of the population is very similar in all the Iberian populations; Barcelona and Valencia almost coincide (compare the data of Table 4 with those published in Prevosti, 1964*a*). The features of this pattern, pointed out for Barcelona and Lisbon (Prevosti, 1964*a*), are also found in Valencia and Malaga. In all the Iberian populations we find a high contribution of chromosome E, a moderate contribution of the element O and low contributions of the U, A and specially the J chromosome. In Malaga, the high contribution of chromosome E is accentuated, while that of chromosome O is reduced.

Table 4. *Contribution of the different chromosomes to the heterozygosity of the population*

	Chromosomes				
Locality	A (%)	J (%)	U (%)	E (%)	O (%)
Lagrasse	9·6	9·6	15·0	33·7	32·1
Barcelona	12·1	7·1	13·3	40·1	27·4
Valencia	11·6	6·4	12·5	39·8	29·6
Malaga	10·2	8·3	13·4	50·6	17·3

DISCUSSION

In a former paper (Prevosti, 1964*a*) it was suggested that environmental conditions seem to be more important than spatial separation as determinants of the characteristics of chromosomal polymorphism in populations of *D. subobscura*. The data reported in this paper confirm this conclusion and lead to the following more precise inferences.

Firstly, in the western Mediterranean populations the adaptive significance of the chromosomal polymorphism in *D. subobscura* is also supported by aspects of its geographical distribution. On a rather limited regional scale there are significant differences between the populations of Lagrasse and Barcelona, north and south of the Pyrenees respectively. However, the frequencies of the chromosomal types found in Barcelona do not differ significantly from those of Valencia, which is the same distance from Barcelona as is Lagrasse, but located to the south and showing no important ecological differences.

The similarity of the clines found in western Europe and the Central Mediterranean and Central European region also supports the adaptive significance of these regularities in the geographical distribution of the chromosomal types.

Another point which emerges from the present data and which merits some comment is the value of the index of free recombination found in the population from Malaga. The index in this population from the south end of Spain is higher than in either Valencia or Barcelona. Its value resembles the values found among the populations of Central Europe and Central France. Thus, from the northern Mediterranean region, both northwards and southwards, there is a decrease in the values of this index, in agreement with the findings of da Cunha & Dobzhansky (1954) and Carson (1955) about the higher level of structural heterozygosity in the central area of its distribution, in the species with inversion polymorphism of the chromosomes. Also, recent data of Sperlich (1964) relating to populations from Norway strongly support the view that *D. subobscura* is not an exception to this rule.

SUMMARY

1. The frequency of the chromosomal types of several western Mediterranean populations of *D. subobscura*, distributed in a zone running north–south, is analysed and compared with that from an Edinburgh (Knight, 1961) site at a similar meridian as the Mediterranean populations.

2. North–south clines are found in the frequencies of several chromosomal types. Some types are more frequent in the north, decreasing gradually southwards; others show the reverse trend of variation.

3. The comparison with a similar array of populations from Central Europe and the Central Mediterranean area, indicates that the chromosomal types more frequent in the northern populations are mostly the same, i.e. the standard orders. But chromosomal types with complex inversion orders are the most frequent in southern populations: in some chromosomes, different orders are predominant in Israel, southern Italy and southern Spain.

4. The Pyrenees, acting as an ecological barrier, strongly influence the diversity of the populations north and south of the range. This result and the latitudinal clines support the adaptive significance of the chromosomal polymorphism in *D. subobscura*.

5. The index of free recombination of the population from Malaga (in the south of Spain) is higher than in the populations from the northern Mediterranean area. *D. subobscura* apparently supports the claims of da Cunha & Dobzhansky (1954) and Carson (1955) that a higher level of chromosomal polymorphism occurs in the central areas of the distribution of a species.

The author wishes to express his sincere thanks to Dr F. W. Robertson, who has assisted him with the preparation and criticism of the manuscript.

REFERENCES

Carson, H. L. (1955). The genetic characteristics of marginal populations of *Drosophila*. *Cold Spring Harb. Symp. quant. Biol.* **20**, 276–287.

Cunha, B. da & Dobzhansky, Th. (1954). A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. *Evolution Lancaster, Pa.* **7**, 119–134.

Dobzhansky, Th. & Epling, C. (1944). Contribution to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. *Carnegie Institution of Washington Publication* 554.

GOLDSCHMIDT, E. (1958). Polymorphism and coadaptation in natural populations of *Drosophila subobscura*. *Proc. Xth Int. Congr. Ent., Montreal*, **2**, 821–828.

KNIGHT, G. R. (1961). Structural polymorphism in *Drosophila subobscura* Coll. from various localities in Scotland. *Genet. Res.* **2**, 1–9.

KUNZE-MÜHL, E. *et al.* (1958). Qualitative, quantitative und Jahreszeitliche Untersuchungen über den chromosomales Polymorphismus natürlicher Populationen von *Drosophila subobscura* Coll., in der Umbegung von Wien. *Z. indukt. Abstamm- u. VererbLehre*, **89**, 636–646.

KUNZE-MÜHL, E. & SPERLICH, D. (1962). Vergleichende Untersuchungen über den chromosomalen Strukturpolymorphismus in Insel- und Festland-populationnen von *Drosophila subobscura*. *Z. indukt. Abstamm.- u. Vererb Lehre*, **93**, 237–248.

PREVOSTI, A. (1964a). Chromosomal polymorphism in *Drosophila subobscura* populations from Barcelona (Spain). *Genet. Res.* **5**, 27–38.

PREVOSTI, A. (1964*b*). Tipos cromosómicos de *Drosophila subobscura* en una población de Lagrasse (Francia). *Genet. Iber.* (in press).

SPERLICH, D. (1961). Untersuchungen über den chromosomalen Polymorphismus einer Population von *Drosophila subobscura* auf den liparischen Inseln. *Z. indukt. Abstamm.- u. VererbLehre*, **92**, 74–84.

SPERLICH, D. (1964). Chromosomale Strukturanalyse und Fertilitätsprüfung an einer Marginal population von *Drosophila subobscura*. *Z. indukt. Abstamm.- u. VererbLehre*, **95**, 73–81.

SPERLICH, D. & KUNZE-MÜHL, E. (1963). Der chromosomale Polymorphismus einer Population von *Drosophila subobscura* auf der Insel Ustica in Vergleich mit anderen Insel- und Festlandstandorten. *Z. indukt. Abstamm.- u. VererbLehre*, **94**, 94–100.

STUMM-ZOLLINGER, E. (1953). Vergleichende Untersuchungen über die Inversionshaufigkeit bei *Drosophila subobscura* in Populationen der Schweiz and Südwest-Europa. *Z. indukt. Abstamm- u. VererbLehre*, **85**, 382–407.

STUMM-ZOLLINGER, E. & GOLDSCHMIDT, E. (1959). Geographical differentiation of inversion systems in *Drosophila subobscura*. *Evolution, Lancaster, Pa.* **13**, 89–98.