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Structural polymorphism in *Drosophila subobscura* Coll. from various localities in Scotland

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INTRODUCTION

Philip, Rendel, Spurway & Haldane (1944) emphasize the almost universal presence of inversion loops in the salivary chromosomes of larvae obtained from wild parents of *Drosophila subobscura*. Structural rearrangements are found on all of the five long acrocentric elements of this species, in contrast to *D. pseudoobscura* where the bulk of chromosomal polymorphism is carried by the third chromosome (Dobzhansky, 1944). Because of this widespread polymorphism in *D. subobscura*, attempts have been made by many investigators to establish racial differentiation in the type and frequency of chromosomal arrangements.

Basden (1954) states that *D. subobscura* is the most widely and commonly distributed species of the genus throughout Scotland, and that its varied habitats extend over the whole of the mainland. It has been trapped at many sites inland and on the coast, as well as on some of the western isles, including the small island of Iona. This ubiquity of habitat in Scotland renders *D. subobscura* suitable, as it has been in other European countries and in Israel, for the study of potential geographic races within the species (Stumm-Zollinger & Goldschmidt, 1959).

Workers investigating salivary gland chromosomes of *D. subobscura* have adopted the Küsnacht stock as their standard. It was the first stock found structurally homozygous in all its chromosomes.

There has been in the past some diversity in the nomenclature used but the system devised by Mainx and his collaborators has now become accepted (Mainx *et al.*, 1953; Kunze-Mühl & Müller, 1958) and will be used here. The five long chromosomes are given the letters A, J, U, E and O respectively.

The structural arrangements have been summarized by Kunze-Mühl & Müller (1958), where a total of thirty-nine inversions are listed. In the present paper a new inversion from the north-central region of Scotland is recorded, and, added to the existing roll, makes, so far as is known, forty inversion types.

METHOD

Miss Mary M. Gunson, a research worker from Melbourne, Australia, while on a visit to the Institute of Animal Genetics, Edinburgh, made a preliminary study of samples of populations of *D. subobscura* from widely separated parts of Scotland.

The samples of flies were supplied by E. B. Basden (1954), who established in the laboratory a series of strains each from a separate locality. In every case the strain was derived from a single fertilized female captured in the wild. They were examined by Miss Gunson a few generations afterwards.

Miss Gunson (1952) merely recorded the occurrence of inversion loops on the separate chromosomes and roughly described their position. In our work about half of her slides were examined (the remainder having been accidentally lost) and the chromosome orders identified in both homozygotes and heterozygotes in accordance with the Mainx nomenclature. Slizynski's modified Feulgen technique was used to stain the salivary gland squashes (Gunson, 1952).

It was further arranged by the author to collect samples of two populations of *D. subobscura* within the county of Midlothian, Scotland. The sites chosen at Dalkeith and Heriot were ten miles apart. Both habitats were in mixed woodland and near to a river. The Heriot site was about 860 feet above sea-level amidst hill country south-south-east of Dalkeith.

The Dalkeith site in this experiment is at 160 feet above sea-level. It is also one mile north of the Dalkeith site in Gunson's investigation, with a built-up area intervening. Any differences in chromosomal frequencies found between samples trapped at those two sites will be interesting, remembering, however, that there was a period of several years between collections.

Traps as used by Basden (1954) were put out at the Dalkeith and Heriot sites in the month of October 1957. These were collected after 6 days' exposure during mild weather, and flies caught were segregated into species. Ultimate numbers of *D. subobscura* fertilized females were ten from Dalkeith and seven from Heriot. The resulting cultures used for investigation were therefore designated D10 and H7. From the larvae of the F_2 generation of these cultures a few diploid salivary preparations were made and male adults used for crossing with virgin ♀♀ of the standard Küsnacht stock. Twenty ♂♂ from each culture were used, each male being put into a vial along with one Küsnacht female. From the progeny of each pair-mating three larvae were sampled and permanent preparations made of salivary gland squashes (Slizynski 1952). Sixty haploid chromosome sets were examined from each site. The maximum numbers of chromosomes that could be tested from Dalkeith and Heriot were 40 and 28 respectively. In actual fact, however, these numbers would be less, due to the probability of some males contributing more than others to the progeny in the first two generations bred in the laboratory.

RESULTS

In Gunson's investigation the initial samples of *D. subobscura* from various localities in Scotland are small. Of the sixty-four individuals examined, thirty-seven were found to be structurally homozygous on all chromosomes. Table 1 lists the chromosome orders identified, and the sketch map (Fig. 1) shows the places of origin of those flies carrying particular orders.

Excluding standard, which was the order of all the A and J chromosomes, there

were identified in the other three elements only five different structural rearrangements, one of which, namely U_{1+2}, was found as a homozygote at several of the sites. Two exceptions occur. At Upper Loch Linnhe on the west-central coast, the U-chromosome in all the larvae except one was homozygous for standard. The odd larva carried the order $U_{1/1+2}$. At Stranraer, on the south-west coast, all larvae carried the rearrangement U_{1+2} except one which was standard.

The nature of these exceptions suggests that the two slides may have been accidentally interchanged in labelling. This has been assumed in the table and map. From the small western-island site of Iona, only a few chromosome sets

Table 1. *Chromosome orders present in* D. subobscura *sampled in Scotland in Gunson's investigation*

	Order					
Chromosome	Ullapool (north-west coast)	Stranraer (south-west coast)	Dalkeith (south-east)	Upper Loch Linnhe (west-central coast)	Drumnadrochit (north-central inland)	Iona (western island)
A	A_{ST}	A_{ST}	A_{ST}	A_{ST}	A_{ST}	A_{ST}
J	J_{ST}	J_{ST}	J_{ST}	J_{ST}	J_{ST}	J_{ST}
U	U_{1+2}	U_1 U_{1+2}	U_1 U_{1+2}	U_{ST}	U_{1+2}	U_{1+2}
E	E_{ST}	E_{ST}	E_{ST}	E_{ST}	E_{ST} *E_{14}	E_{ST}
O	O_{ST} O_{3+4}	O_{ST} O_{3+4} O_{3+4+8}	O_{ST}	O_{ST} O_{3+4}	O_{ST}	O_{ST}

* New inversion.

were available. The order was standard for all elements except in the U, which was homozygous for the inversion 1 + 2.

Gunson found only one inversion loop on the E-chromosome, at the Drumnadrochit locality. Its breakage points—proximal 67A, distal 70A/B on the new chart (Kunze-Mühl & Müller, 1958)—do not coincide with any already known inversions so far as can be ascertained by the author. It is therefore recorded here as new and named E_{14}. Fig. 2 shows the inversion loop formed with E_{ST}.

In the O element, the longest of *D. subobscura* salivary chromosomes, on which nineteen different gene arrangements are known, there were found the orders O_{ST}, O_{3+4} and O_{3+4+8}. The second was present at Ullapool, Stranraer and Upper Loch Linnhe. The Stranraer sample also carried the order O_{3+4+8}, which was not found elsewhere.

The 'dot' chromosome exhibited no discernible structural abnormality in any of the specimens.

Table 2 shows the results for *D. subobscura* trapped at Dalkeith and Heriot. The A-chromosome from both localities is entirely of the standard order, and this

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chromosome at Dalkeith is the only one of the set to be entirely free from inversions. Heriot, in addition to the standard order of its sex chromosome, shows no heterozygosity on the E-chromosome, which, at Dalkeith, has the order E_{1+2} at a frequency of 8·3%. There is little difference at both sites between the frequencies of the orders J_1, U_{1+2} and O_{3+4}, though, in each case, the non-standard order is slightly more frequent at Dalkeith.

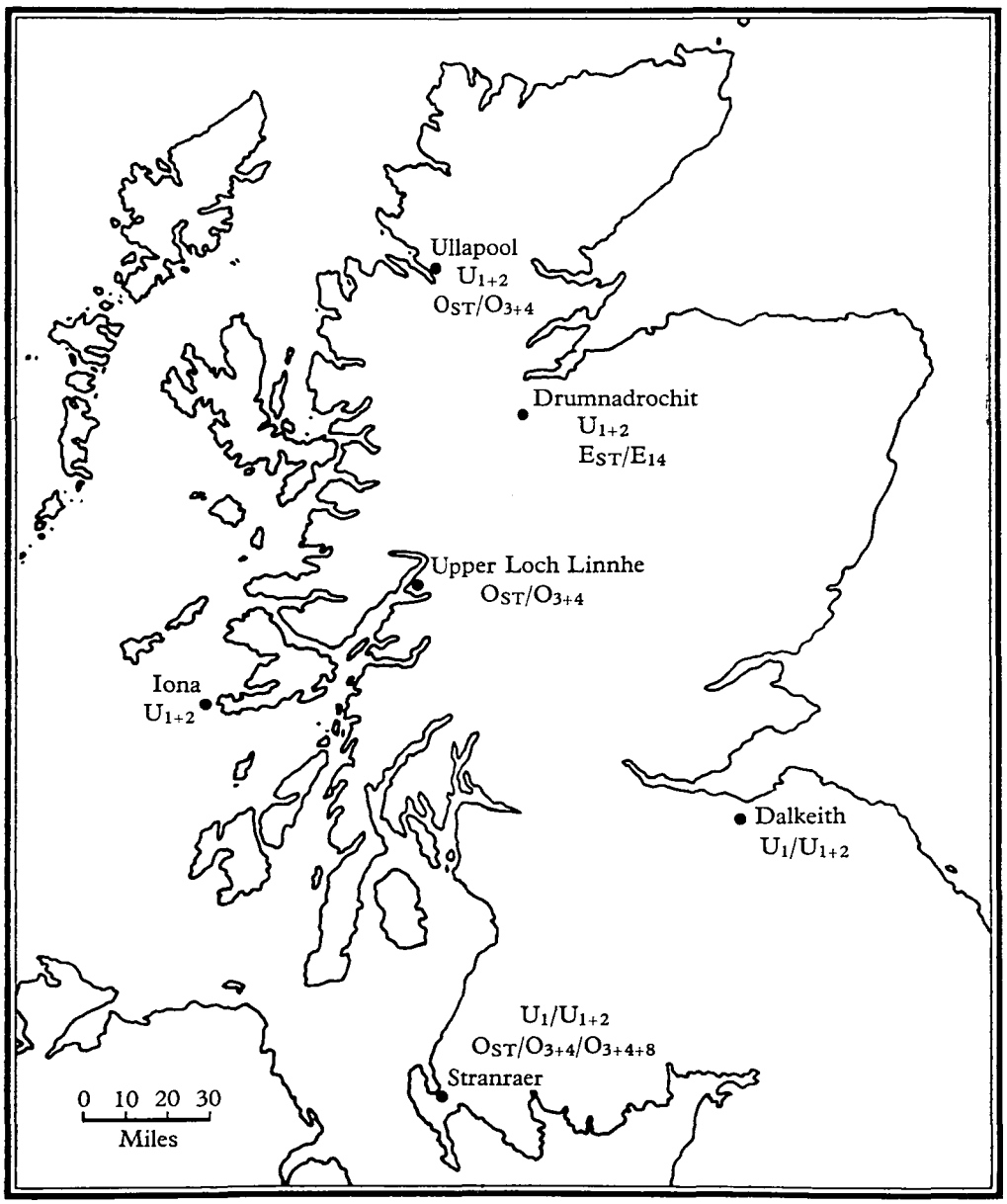

Fig. 1. *D. subobscura* chromosome orders found at various Scottish localities in Gunson's material. For the chromosomes not mentioned at a particular locality on the map only the standard order was found.

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Table 2

A. *Analysis of haploid chromosome sets of* D. subobscura *showing frequency of orders found at Dalkeith and Heriot, based on 120 larval offspring of crosses: Küs. ST* ♀♀ × *D. & H.* ♂♂

Dalkeith (60 larvae sampled)				Heriot (60 larvae sampled)			
Chromosome	Order	No.	Frequency (%) of order	Chromosome	Order	No.	Frequency (%) of order
A	A_{ST}	60	100·0	A	A_{ST}	60	100·0
J	J_1	11	18·3	J	J_1	7	11·7
	J_{ST}	49	81·7		J_{ST}	53	88·3
U	U_1	—	—	U	U_1	11	18·3
	U_{1+2}	51	85·0		U_{1+2}	49	81·7
	U_{ST}	9	15·0		U_{ST}	—	—
E	E_{1+2}	5	8·3	E	E_{1+2}	—	—
	E_{ST}	55	91·7		E_{ST}	60	100·0
O	O_{3+4}	21	35·0	O	O_{3+4}	16	26·7
	O_{ST}	39	65·0		O_{ST}	44	73·3

B. *Analysis of larvae from females caught in the wild. Only the structural heterozygotes are detailed. Of the homozygotes, all chromosomes except the U were of the standard Küsnacht order, the latter having the order* U_{1+2}

(6 larvae)	(6 larvae)
1. Homozygous for all chromosomes	1. U_1/U_{1+2}
2. E_{ST}/E_{1+2}	2. J_{ST}/J_1
3. O_{ST}/O_{3+4}	3. O_{ST}/O_{3+4}
4. Homozygous for all chromosomes	4. U_1/U_{1+2}
5. U_{ST}/U_{1+2}, O_{ST}/O_{3+4}	5. U_1/U_{1+2}, O_{ST}/O_{3+4}
6. U_{ST}/U_{1+2}, O_{ST}/O_{3+4}	6. J_{ST}/J_1, U_1/U_{1+2}, O_{ST}/O_{3+4}

There are then structural rearrangements on four of the chromosomes at Dalkeith and on three at Heriot. The two samples differ slightly in the U- and E-chromosomes. The U-chromosome shows heterozygosity at both locations, but U_1, present at Heriot, is replaced by U-standard at Dalkeith. Fig. 3 summarizes the inversion types at both localities.

DISCUSSION

Analyses of structural polymorphism in populations of *D. subobscura* so far sampled from Scotland are characterized by the simplicity of their inversions when compared with populations of this same species in Europe (Stumm-Zollinger, 1953) and Israel. Investigations from populations in the latter country have shown exceptionally complicated rearrangements (Goldschmidt, 1956, 1958).

The predominant rearrangement of the U-chromosome in Scotland is U_{1+2}. In

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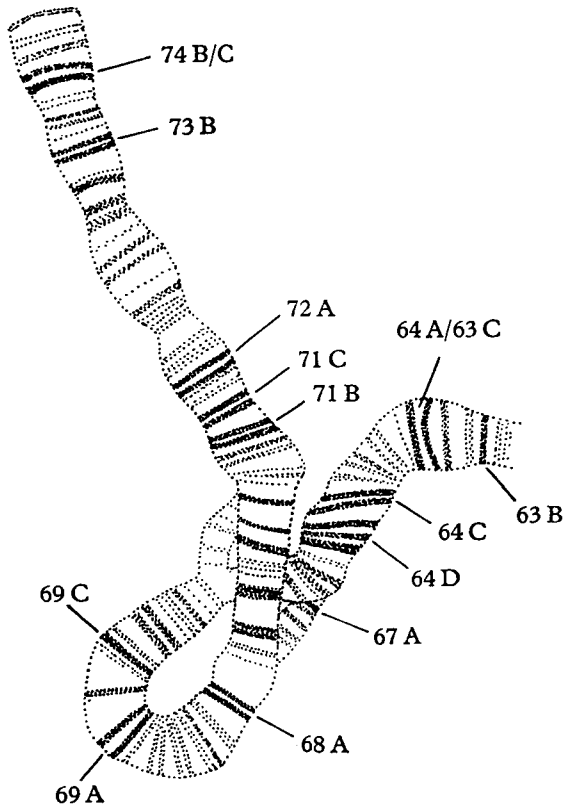

EST/E14

Fig. 2. Inversion loop of E_{14} formed with E standard.

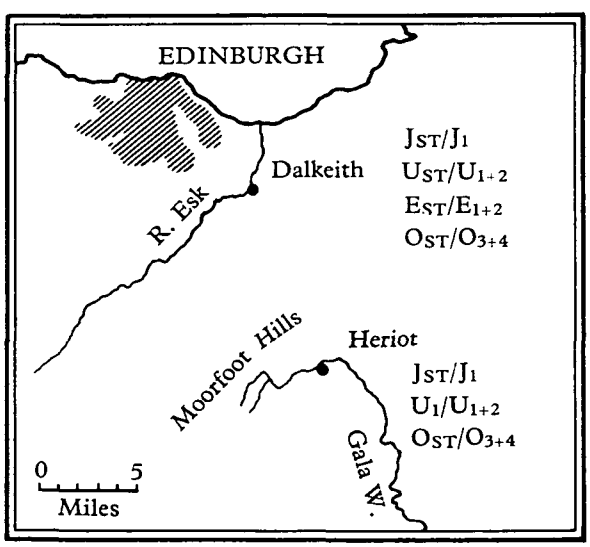

Fig. 3. Chromosome orders found at two adjacent localities in Midlothian, Scotland.

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England that order and U_{ST} were found about equally distributed amongst a sample of flies from the New Forest (J. Maynard Smith, written communication). U_{1+2} rarely occurs by itself in Israel. There it is replaced by much more complex orders U_{1+2+7} and U_{1+2+3} (Goldschmidt, 1958). In France and Switzerland U_{1+2} is very common.

U_1 and U_{ST} were found at low frequency at Heriot and Dalkeith respectively.

The orders E_{1+2}, recorded only at the Heriot site, and O_{3+4}, present at most other Scottish localities, are found in similar frequencies in Europe.

The J_1 inversion which was present at Heriot and Dalkeith at low frequency has been recorded around 50% at European localities, and 29% from Israel (Goldschmidt, 1956).

Throughout this preliminary investigation of *D. subobscura* in Scotland, the outstanding feature has been the high proportion of homozygous types found. In particular, the seven larvae sampled from Iona showed a homozygous chromosome set, which, when compared with 'Kusnacht', was found to be standard in all its elements except the U. This chromosome carried the U_{1+2} inversion in homozygous order. It must be emphasized, however, that Gunson's samples were bred each from one fertilized female from the wild.

The method of measuring the degree of polymorphism of *D. subobscura* by Stumm-Zollinger & Goldschmidt (1959) in their European and Israel populations was that adopted by Carson (1955) using his 'index of free recombination' (I.F.R.) as a yardstick in his work with *D. robusta*. This criterion necessitates the estimate of the average lengths of euchromatin not involved by inversions. By this method Carson demonstrated successfully an increase from 65% in the centre of the distribution area of *D. robusta* to 85% in the marginal areas.

It is not possible to estimate accurately the index of free recombination, but from the frequencies of the inversions it would be expected to be around 92% for the Dalkeith population and 95% for Heriot. Stumm-Zollinger & Goldschmidt (1959) found an average of 85·0% for the continent of Europe. They found an average of only 4% of all females homozygous. The estimated figures for Dalkeith and Heriot are 24% and 32% respectively. In fact, in the samples discussed by Gunson which had been inbred for one generation after capture, the actual proportion of homozygotes was 58%.

Stumm-Zollinger & Goldschmidt (1959) found that the I.F.R. of populations of *D. subobscura* inhabiting various localities differed significantly. Whilst these authors agree that by using this standard a more delicate indicator of geographic differentiation is obtained, they are of the opinion that it by no means conveys the complete picture. They add that the most significant difference between European and Eastern populations lies in the number of elements involved in polymorphism. Differences in inversion frequencies and/or diversity of structural types suffice largely to characterize widely divergent populations. For instance, the chromosome order O_{3+4} is found to be common in Europe and rare or even absent in Israel, whilst the configuration J_{3+4} is common in Israel and scarce in Europe (Goldschmidt, 1958).

As Scotland and Israel can reasonably be assumed to be geographical marginal areas of *D. subobscura*, then analysis of chromosomal polymorphism from these two extreme localities might be expected to conform to the working hypothesis of Da Cunha & Dobzhansky (1954). Those authors, from the results of their investigations into structural polymorphism in *D. willistoni*, found a decrease in the degree of heterozygosity towards the margin of the species area. Though this is not the case according to results of analysis of *D. subobscura* from Israel, it does appear that the Scottish findings at present tend to agree with this theory.

This Scottish investigation is mainly exploratory, but results recorded here do give some indication of the diversity of inversion types to be found and warrant further research. Having this aim in view, samples of three *D. subobscura* populations were collected from regions separated from each other by two natural barriers, and analyses of their chromosome sets will be recorded later.

SUMMARY

1. Gunson's salivary chromosome preparations of *Drosophila subobscura* from widely separated sites in Scotland have been re-examined and inversions recorded according to the Mainx nomenclature.

2. Sixty-four diploid sets only were available. Of these, thirty-seven sets were found to be structurally homozygous on all chromosomes.

3. From Drumnadrochit in the north-central area of Scotland, the inversion found on the E-chromosome, so far as is known, has not previously been described. Its break-points have been noted, and the inversion is named E_{14}.

4. A strain of *D. subobscura* from the small western island of Iona was the only one found to be completely homozygous in the five long arms of the chromosome set.

5. Samples of *D. subobscura* from two closely related localities in Midlothian, Scotland, also have been examined. Results are based on the analysis of 120 haploid sets in hybrids between the local race and the standard Küsnacht stock.

6. A slight difference in type and frequency of inversions has been noted between the two populations. The inversion E_{1+2} was recorded from Dalkeith, but was absent at Heriot, while U_1, present at Heriot, was replaced by U_{ST} at Dalkeith.

7. The A-chromosome was structurally homozygous throughout.

8. Scottish samples of *D. subobscura* are characterized by their qualitative simplicity of polymorphism, the variety of inversion types being small. Chromosome orders analysed have been compared with those occurring in Western Europe and Israel.

It is a pleasure to thank Miss M. M. Gunson, of Melbourne, Australia, without whose salivary chromosome preparations of *Drosophila subobscura* the first part of this work could not have been carried out. To Dr Alan Robertson I wish to express my gratitude for helpful criticism and guidance throughout this investigation. I am indebted to Mr E. B. Basden for his assistance with the trapping and identification of the flies. And to Mr E. D. Roberts, my thanks are due for the preparation of the diagram and maps.

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