GENETIC VARIATION IN NATURAL POPULATIONS OF *DROSOPHILA OBSCURA*[1]

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THE geographic distribution area of *Drosophila obscura* Fallén extends from southern Europe to central Finland and Sweden; it also has another area of occurrence, isolated by at least 500 kilometers from the former in northern Lapland and on the northwestern coast of Norway. This latter area coincides with that of the northern unicentric species group that is assumed to have survived the Pleistocene glaciation on the northwestern coast of Norway and on the adjacent islands. This study describes enzyme polymorphism of *D. obscura* in northernmost Europe. We have studied genetic variation in 33 loci in material collected from natural populations of the species in Finland and northern Sweden and Norway.

MATERIALS AND METHODS

Electrophoresis and assay techniques: The horizontal starch technique described earlier (LAKOVAARA and SAURA 1970a) was used for alcohol dehydrogenase (Adh), octanol dehydrogenase (Odh), tetrazolium oxidase (To)—this enzyme is variably called indophenol oxidase, achromatic regions (BREWER 1970) or "oxidase" (PRAKASH, LEWONTIN and HUBBY 1969)—glucose-6-phosphate dehydrogenase (G-6-pdh), esterase-7 (Est-7) and alkaline phosphatases 2, 3, 6 and 7 (Aph). For isocitrate dehydrogenase (Idh), xanthine dehydrogenase (Xdh), malate dehydrogenase (Mdh), malic enzyme (Me), α-glycerophosphate dehydrogenase (α-Gpdh), aldehyde oxidase (Ao), leucine aminopeptidase (Lap), Aph-1, -4, and -5 and Est-1–6 the procedure described by AYALA *et al.* (1972) was followed.

The staining methods were in general those given by SHAW and KOEN (1968) with the following exceptions: Ao activity was assayed according to PRAKASH *et al.* (1969) with only one third of the amount of acetaldehyde (2.5 ml/100 ml staining solution). Instead of KOH-containing solution, hypoxanthine was dissolved directly into the staining buffer and Odh was detected by adding 1.5 ml of 1:3 ethanol-octanol solution to 100 ml stain. After staining the gels were either photographed or fixed with a mixture of acetic acid and methanol.

Populations studied: Drosophila obscura is the most common Drosophila species in the south of Finland, growing scarcer towards the north and very rare or absent beyond 65° lat. N. It reappears, however, in northernmost Lapland beyond 69° lat. N.—this is connected with a continuous, though fragmentary, distribution along the cost of Norway. Within this area *D. obscura* is again the most common Drosophila species. The flies were trapped by the malt bait method of LAKOVAARA, HACKMAN and VEPSÄLÄINEN 1969. Drosophilids were trapped also in the region extending from 65° lat. N. to northern Lapland but no *D. obscura* were recorded. For practical purposes the study area is divided into three parts: southern Finland, extending from the Gulf

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of Finland (60° lat. N.) to 62° lat. N.; central Finland, the area, where *D. obscura* grows progressively scarcer from 62° lat. N. to 65° lat. N.; Lapland, extending on the coast of Norway from the Arctic Circle to the northernmost tip of Europe or about 70° lat. N., including the Lofoten and Vesterålen Islands. In the interior Lapland this area comprises the northernmost parts of Finland and Sweden from 60° lat. N. to 70° lat. N. We have collected flies from 32 populations in southern Finland, from 12 populations in central Finland and from 13 populations in Lapland. Seven, six and eight laboratory stocks, respectively, were secured of these populations. For each of the three major areas these laboratory stocks represent at least one hundred founder females collected in the wild. The data for Adh, Odh, To, G-6-pdh, Est-7 and Aph-2, -3, -6 and -7 are obtained from wild-caught flies from the 57 localities mentioned. The collections were made in the summer of 1970 and many localities were visited several times in the course of June–August. *D. obscura* has in the north very likely one generation a year and in the south no more than two or three. The rest of the enzymes were studied in laboratory stocks, no later than about the eighth generation after capture. In most cases over 25 flies were studied for each enzyme per stock. The gene frequencies observed for each stock were weighted by the number of inseminated females used to start the stock. The gene frequencies for each major geographic region are the weighted mean frequencies of all the stocks from that region.

RESULTS

The individual loci of each enzyme are designated by a hyphenated number. The numbering starts from the cathodal end, i.e. the most cathodal isoenzyme is called one, and the isoenzyme migrating farthest to the anode has the highest number. The most common allozyme is given the value 1.00 and the others are nominated according to their average migration difference from this one. In other words, Aph-$4^{.98}$ migrates two mm less towards the anode than Aph-$4^{1.00}$ and Aph-$4^{1.03}$ three mm farther than the latter, respectively. In most cases this a fitting description, since most allozymes show only one band in the gels in homozygous condition. Even though Adh consists of a pattern of at least four isoenzymes (LAKOVAARA and SAURA 1970a,b), it is described similarly, so that the migration difference of patterns is measured from the most cathodal, heat-sensitive and strongly staining band of the pattern.

Among the 33 loci studied, no variation has been detected in fourteen (or 42%) loci. They are *Ao-1*, *Aph-1*, *Aph-2*, *Est-1*, *Est-2*, *Est-3 Est-4*, *G-6-pdh*, *Lap-1*, *Lap-2*, *Lap-3*, *Mdh-2*, *Odh* and *Xdh-1*.

Sixteen polymorphic loci can be traced accurately in the gels. These are *Adh*, *Ao-2*, *Ao-3*, *Aph-3*, *Aph-4*, *Aph-5*, *Aph-7*, *Est-5*, *Est-6*, *Est-7*, *α-Gpdh*, *Idh*, *Lap-4*, *Me*, *To-1* and *Xdh-2*. *Mdh-1* and *To-2* are obviously polymorphic but the method used did not discriminate reliably between alleles. *Aph-6* shows erratic behavior, appearing here and there and yielding no clear ratios in crosses. In general it behaves like *Aph-6* of *D. pseudoobscura* (HUBBY and LEWONTIN 1966). If we regard these latter three as polymorphic, 58% of the loci studied are polymorphic.

The allele frequencies for most loci appear to be uniform over the area studied; minor differences between localities can be attributed to sampling errors. The local populations are therefore combined together and only the data for the total area are presented. In the southernmost part of our area, as well as in the central—though to a lesser extent—migration between populations can happen freely, but the Lappish populations are geographically isolated from the former

and are therefore treated separately. The data for the three major areas are given separately. Table 1 gives for fourteen loci the frequencies of alleles in the area studied, the number of genes sampled and the proportion of individuals expected to be heterozygotes, on the assumption of Hardy-Weinberg equilibrium. The observed heterozygosities do not differ significantly from those expected in the cases, where both were obtained for wild-caught flies. In *Ao-3* and *α-Gpdh* the frequency of the most common allele is .99. *Ao-3*$^{.98}$ has the frequency of .01 both in the North and in the South, but it has not been encountered in the intervening area. *α-Gpdh*$^{.95}$ has been found only in two populations in central Finland. It has there the frequency of .01. As these two loci are almost monomorphic, they are not included in Table 1.

Loci with frequencies of the most common allele below .95 can be divided into three categories according to the geographic distribution of the alleles in populations:

Geographically undifferentiated polymorphisms: To this category belong *Adh, Aph-3, Aph-5, Idh* and *Xdh-2*. A sample taken from any locality within our area of study can be expected to contain the major alleles of these loci in almost fixed frequencies. In this category can—with due reservation—be included loci with a frequency of the most common allele between .95 and .99. Loci, in which the frequency of the most common allele exceeds .95, namely *Lap-4, Me, Ao-2, To-1* and *Aph-7* show widespread polymorphism too. The percentage of heterozygotes varies widely in this category. The rare alleles are also widespread in populations.

Clinal polymorphism: A significant north–south cline can be seen in the most common alleles of *Aph-4* (*1.00* and *1.03*) and *Est-7* (*.94* and *1.00*). A sample from populations on a given latitude fits the allele frequencies characteristic of that latitude; e.g. the frequency of *Est-7*$^{.94}$ of wild-caught flies on the same latitude in northern central Finland fluctuated only within limits .52–.55 in seven populations taken from the west to east transect Vaasa-Jyväskylä-Kuopio.

Geographically differentiated polymorphism: Esterases 5 and 6 are highly polymorphic enzymes, the allele frequencies of which vary significantly from population to population. *Est-6*$^{1.00}$ is by and large the most common allele throughout the three major areas and has been found in all populations studied. *Est-6*$^{.97}$ is uniformly present in all samples from central Finland, but has been encountered in three populations only in the south, where in one case it has a frequency of .55 (number of flies analyzed: 65). In Lapland it has been found in one population, where it has a frequency of .07 (number of flies: 20). *Est-5*$^{1.00}$ has again been found in all populations studied, but it is replaced as the most common allele in central Finland by *Est-5*$^{1.01}$. *Est-5*$^{1.01}$, though being the most common and widespread allele in central Finland, has not been encountered in Lapland. *Est-5*$^{1.02}$ has been encountered in northernmost Lapland only; i.e., not in the populations on the Norwegian coast or the Lofoten and Vesterålen islands below 69° lat. N. In the South it has a frequency of .14 in one locality (number of flies: 65), but is not encountered elsewhere.

DISCUSSION

The enzymes coded by the loci *Adh, Odh, To, G-6-pdh, Est-7, Aph-2, -3, -6,*

TABLE 1

Allele frequencies at different loci of Drosophila obscura

ALCOHOL DEHYDROGENASE

Area	Number of populations	Genes sampled	Alleles .80	.90	1.00	1.10	Proportion of heterozygotes
S. Finland	32	944	.002	.21	.79	.002	.327 ± .015
C. Finland	12	370	—	.23	.765	.005	.370 ± .022
Lapland	13	260	.01	.23	.75	.01	.341 ± .033

ALKALINE PHOSPHATASE-3

Area	Number of populations	Genes sampled	Alleles 1.00	1.03	Proportion of heterozygotes
S. Finland	26	584	.64	.36	.424 ± .020
C. Finland	8	234	.68	.32	.429 ± .027
Lapland	9	102	.59	.41	.459 ± .013

ALKALINE PHOSPHATASE-5

Area	Number of populations	Genes sampled	Alleles .97	.99	1.00	1.01	Proportion of heterozygotes
S. Finland	7	252	.004	.01	.98	.004	.081 ± .066
C. Finland	6	324	—	.09	.91	—	.072 ± .046
Lapland	8	222	.004	.08	.90	.01	.169 ± .037

ISOCITRATE DEHYDROGENASE

Area	Number of populations	Genes sampled	Alleles .98	1.00	1.03	Proportion of heterozygotes
S. Finland	7	252	—	.996	.004	.036 ± .036
C. Finland	6	324	—	.87	.13	.050 ± .050
Lapland	8	222	.02	.93	.05	.093 ± .059

XANTHINE DEHYDROGENASE-2

Area	Number of populations	Genes sampled	Alleles .99	1.00	1.02	Proportion of heterozygotes
S. Finland	7	252	.09	.78	.13	.153 ± .099
C. Finland	6	324	.19	.77	.04	.304 ± .077
Lapland	8	222	.08	.91	.005	.185 ± .067

ALKALINE PHOSPHATASE-4

Area	Number of populations	Genes sampled	Alleles .93	.98	1.00	1.03	Proportion of heterozygotes
S. Finland	7	252	.004	.01	.54	.45	.357 ± .104
C. Finland	6	324	.015	—	.65	.33	.447 ± .050
Lapland	8	222	—	.01	.75	.24	.283 ± .091

ESTERASE-7

Area	Number of populations	Genes sampled	Alleles .88	.94	.97	1.00	Proportion of heterozygotes
S. Finland	30	772	.003	.38	.10	.51	.533 ± .021
C. Finland	12	296	.01	.48	.105	.41	.559 ± .023
Lapland	11	230	.02	.56	.07	.34	.506 ± .024

ESTERASE-5

Area	Number of populations	Genes sampled	Alleles .96	.98	.99	1.00	1.01	1.02	1.05	Proportion of heterozygotes
S. Finland	7	252	.02	.07	.004	.68	.12	.07	.04	.479 ± .054
C. Finland	6	324	.003	.01	—	.42	.56	.003	—	.392 ± .087
Lapland	8	222	.004	.11	.08	.71	—	.10	—	.311 ± .083

TABLE 1—Continued

Allele frequencies at different loci of Drosophila obscura

ESTERASE-6

Area	Number of populations	Genes sampled	Alleles .87	.93	.95	.97	1.00	1.02	Proportion of heterozygotes
S. Finland	7	252	.015	.004	.17	.31	.50	.004	.529 ± .053
C. Finland	6	324	.015	.01	.05	.25	.68	—	.488 ± .048
Lapland	8	222	—	.01	.42	.01	.56	—	.368 ± .069

ALDEHYDE OXIDASE-2

Area	Number of populations	Genes sampled	Alleles .99	1.00	1.02	Proportion of heterozygotes
S. Finland	7	252	.04	.96	—	.065 ± .043
C. Finland	6	324	—	.97	.03	.079 ± .079
Lapland	8	222	—	.99	.01	.062 ± .062

LEUCINE AMINOPEPTIDASE-4

Area	Number of populations	Genes sampled	Alleles .97	1.00	1.03	Proportion of heterozygotes
S. Finland	7	252	.04	.95	.01	.154 ± .061
C. Finland	6	324	.01	.99	—	.046 ± .031
Lapland	8	222	.08	.92	—	.182 ± .067

TETRAZOLIUM OXIDASE-1

Area	Number of populations	Genes sampled	Alleles .80	.94	1.00	Proportion of heterozygotes
S. Finland	32	944	.002	.015	.98	.042 ± .022
C. Finland	12	370	.005	.005	.99	.019 ± .015
Lapland	13	260	.01	.04	.95	.076 ± .034

MALIC ENZYME

Area	Number of populations	Genes sampled	Alleles .98	1.00	Proportion of heterozygotes
S. Finland	7	252	—	1.00	— —
C. Finland	6	324	.06	.94	.190 ± .070
Lapland	8	222	.005	.995	.063 ± .063

ALKALINE PHOSPHATASE-7

Area	Number of populations	Genes sampled	Alleles 1.00	1.01	Proportion of heterozygotes
S. Finland	32	944	.98	.02	.045 ± .013
C. Finland	12	370	.97	.03	.043 ± .018
Lapland	13	260	.985	.015	.027 ± .027

and -7 were recorded in wild-caught flies with total numbers of genes sampled for each enzyme between 920–1574. As for the loci studied in the offspring of wild-caught flies maintained in the laboratory, the frequencies observed do not necessarily coincide with the ones in the wild. The effect on the average heterozygosity of maintaining the flies in stock cultures is, however, negligible according to PRAKASH *et al.* (1969). We assume that some rare alleles may have been lost in our study during the first generations in the laboratory, but that the results otherwise accurately reflect the allele frequencies in the wild. We have checked the allele frequencies of some rare alleles (e.g. of *Adh*) in our stocks at eighth generation and found them to have conserved the original frequency.

In most loci studied by us the polymorphism appears to be uniform within and between populations. The numbers of alleles vary from two to four and the proportion of heterozygotes from .092 to .588. This suggests a balancing selection that maintains the alleles at fixed frequencies. It is noteworthy that the frequency of $Xdh\text{-}2^{1.00}$ is uniform in the southern and central areas and again uniform but significantly higher in Lapland. The clinal variation in *Est-7* and *Aph-4* would, according to this hypothesis, indicate that the selective value of alleles varies in different environments. It should be pointed out that the populations in northern central Finland live on the margin of the species under presumably rigorous ecological conditions. As *D. obscura* seems to be unable to inhabit the area between 65° lat. N. and 69° lat. N. in Finland and Sweden—in other words, the area with a rigorous continental climate compared to the Norwegian coast and the area further to the south—the migration between the Lappish and Central-Finnish populations is rare or absent. The drosophilid species inhabiting the area, from which *D. obscura* is absent, are represented there mostly by univoltine races. The closely related and univoltine *D. bifasciata* replaces *D. obscura*, which seems to be always multivoltine, as the dominant Drosophila species within this region (LAKOVAARA *et al.*, in press). The allele frequencies can therefore be balanced in Lapland freely without migration from the south. We consider the continuity of the cline over the gap in distribution to be most easily explained by a balancing selection.

The degree of polymorphism in Lapland is on the same level as in southern and central Finland. This indicates that the Lappish *D. obscura* populations do not show founder effect like the Bogotá population of *D. pseudoobscura* does (PRAKASH *et al.* 1969). The only common allele absent from Lapland is $Est\text{-}5^{1.01}$. This does not give any evidence for or against of Lappish *D. obscura* being a glacial relict.

The loci showing differentiation between populations, *Est-5* and *Est-6*, are the ones with the highest number of alleles, 7 and 6 alleles, respectively. The proportion of heterozygous individuals is also highest in them. The geographically differentiated polymorphism in *Est-5* and *Est-6* may be due either to differential selection or to random drift. Est-5 is the most variable enzyme also in *D. pseudoobscura* (YAMAZAKI 1971). When as many as seven alleles are in an equilibrium some of them can be expected to fluctuate in different populations. Esterases as an enzyme category do not conform to the isoallelic hypothesis by being in general highly (and randomly) variable. We have checked e.g. *Est-4* in various groups of the subgenus Sophophora and it has turned out to be the least variable locus we know. The metabolic reasons for variation versus stability in esterases remain therefore unknown.

JOHNSON (1971) has suggested that high-frequency polymorphisms can be expected for loci coding for enzymes, which catalyze physiologically irreversible reactions. He cites Adh, Ao and Xdh as such enzymes. Our data for *D. obscura* seem to support his hypothesis (it may be noted that as many as six separate loci can be detected in Ao gels), but while *Adh* is polymorphic in *D. obscura*, it is nearly monomorphic in other related and sympatric species. We have found the

frequencies of the most common *Adh* allele to be .978 in *D. subobscura* (LAKOVAARA and SAURA 1971), .996 in *D. alpina* and .994 in *D. bifasciata*. KOJIMA, GILLESPIE and TOBARI (1970) have observed low degrees of polymorphism for enzymes involved in energy metabolism. Our observed data show a similar tendency (e.g. *Idh, Me, α-Gpdh, G-6-pdh, Mdh*). They have an average heterozygosity of .034 ± .016 per locus as contrasted with the other loci with a corresponding value of .133 ± .014 per locus. The latter category is far from homogenous. It is evident that some enzymes vary more than others and that the degree of polymorphism for each individual locus is often species-specific. The average heterozygosity per locus is remarkably uniform over a wide range of species. PRAKASH *et al.* (1969) give an average heterozygosity of .123 per locus per individual *D. pseudoobscura* fly. The average heterozygosity per individual *D. obscura* is .109 ± .014 for the loci studied by us. This approaches the corresponding value in *D. pseudoobscura* even though the polymorphisms observed were largely different.

SUMMARY

The genetic variability of 33 enzyme loci of *Drosophila obscura* Fallén has been studied by starch gel electrophoresis. Samples were collected from 57 populations in Finland and northern Sweden and Norway. The data are presented separately for southern Finland, central Finland and Lapland. Fifty-eight percent of the loci are polymorphic, with an average heterozygosity per fly of 11% for the loci studied. The patterns of distribution of the polymorphisms and the causes underlying them are discussed. The results suggest balancing selection as the main cause maintaining the polymorphism.

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