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MULTI-LOCUS POLYMORPHISM AND SELECTION IN A POPULATION OF *DROSOPHILA MELANOGASTER*

I. LINKAGE DISEQUILIBRIUM ON CHROMOSOME III

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SUMMARY

Linkage disequilibrium was detected between three polymorphic loci (octanol dehydrogenase, esterase-6 and pink) on 3rd chromosomes extracted from a population of *Drosophila melanogaster*. Over the chromosome segment of 12·4 centimorgans, the alleles at all three loci were not associated at random. Evidence is presented suggesting that the observed linkage disequilibria are maintained by selection.

1. INTRODUCTION

AT least 30 per cent of the electrophoretically detected proteins within populations of many species are polymorphic (Harris, 1966, 1969; Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Selander and Yang, 1969; Ayala and Powell, 1972). As yet the organisation of this wealth of genetic variation within the genome has received little attention from experimental population geneticists. Studies in theoretical population genetics show that a great deal of linkage disequilibrium may be the norm within populations; selection generally acts upon groups of highly correlated genes rather than upon individual loci (Franklin and Lewontin, 1970). For populations of outbreeding organisms only small amounts of linkage disequilibrium have been reported between the alleles at individual loci. In populations of *Drosophila melanogaster*, Mukai *et al.* (1971) did not find any linkage disequilibrium between three protein loci on chromosome II, and Charlesworth and Charlesworth (1971) report that only four out of 30 tests for non-random association between alleles at loci on chromosome III were statistically significant. In contrast, there is striking evidence for selectively maintained linkage disequilibria in populations of the inbreeding cereals, *Hordeum vulgare* (Clegg, Allard and Kahler, 1972) and *Avena barbata* (Allard, Babbel, Clegg and Kahler, 1972).

In the present study, linkage disequilibrium was studied between three polymorphic loci on chromosome III in a long established population of *D. melanogaster*.

2. Materials and methods

The population of *D. melanogaster*, " Texas ", used in this study was maintained in a population cage, and details of the origin and maintenance of this population are given by Barnes and Kearsey (1970). The three polymorphic loci whch were studied are *Esterase-6* (*Est-6*), Octanol dehydrogenase (*Odh*) and the eye mutant, *pink* (*p*). The spindle-fibre of chromosome III is located at 46·0 and the map positions of the three loci are *Est-6*—36·8, *pink*—48·0 and *Odh*—49·2 (Lindsley and Grell, 1967). The population had been polymorphic at the *pink* locus for at least 5 years and details of the stability of this polymorphism have been kindly made available by Drs Kearsey and Barnes of this department. The electrophoretically distinguishable allele products of the *Est-6* and *Odh* loci are designated 1 for the slowest and 2 for the fastest migrating bands on a zymogram (*i.e.* $Est\text{-}6^1$, Odh^1, $Est\text{-}6^2$, Odh^2).

Samples of flies were raised at high density by removing food vials from the population cage after egg-laying had stopped (3 days). The adults which emerged from these food vials were collected. Flies were raised at low density from eggs collected from the cage population and allowed to hatch in petri dishes containing 3 per cent agar and several drops of live yeast. Twenty larvae were transferred to a standard food vial and allowed to develop into adults.

Individual male flies were crossed to a " tester " line homozygous for pink, $Est\text{-}6^1$ and Odh^1, which had been extracted from the " Texas " population. From every male family a single female fly was taken at random and scored for its genotype at the *Est-6*, pink and *Odh* loci. The genetic constitution of the gamete therefore provided by the male parents could be deduced for both high and low density samples.

Starch-gel electrophoresis was used to identify the *Est-6* and *Odh* genotypes. An 11 per cent starch (Connaught Medical Research Laboratories) gel was used. The gel and electrode buffers were the same as those described by Poulik (1957), and the staining procedures for esterase and octanol dehydrogenase were the same as those described by Ayala *et al.* (1972).

3. Results

The frequencies of the eight types of gamete, from flies raised at high and low densities, are given in table 1. The data were analysed by using a multiway contingency χ^2 test (Kendall and Stuart, 1961). In this analysis the four dimensions of the $2 \times 2 \times 2 \times 2$ contingency table are *Est-6*, pink, *Odh*, and density. The analysis (table 2) tests for non-random association between alleles at both the di- and trigenic levels, and for the interaction of these di- and trigenic associations with density.

It is seen that the allele frequencies of all three loci are insensitive to density. All of the digenic interactions ($Odh \times pink$, $Odh \times Est\text{-}6$, and $Est\text{-}6 \times pink$), are significant. There is also a highly significant χ^2 for the trigenic interaction, $Est\text{-}6 \times pdh \times pink$.

Of all these intergenic associations, only the disequilibrium between alleles at the *Odh* and *pink* loci is affected by density.

The strengths of the linkage disequilibria have been estimated by calculating the correlation coefficient (r) between alleles at any two loci (table 3). The correlation was calculated as

$$r = \frac{(g_1 g_4 - g_2 g_3)}{(p\ q\ r\ s)^{\frac{1}{2}}} \quad \text{(Franklin and Lewontin, 1970)}$$

where p, q, r and s are the frequencies of alleles A,a and B,b respectively, and g_1, g_2, g_3, g_4 are the frequencies of the four types of gamete AB, Ab, aB, and ab

TABLE 1

The observed types of gamete in high and low density samples

	Density			
	High		Low	
Gametes	Observed No.	Proportion	Observed No.	Proportion
Est-6^1 *p Odh*^{1}	28	0·0557	20	0·0424
Est-6^1+ *Odh*^{1}	12	0·0239	15	0·0318
Est-6^1 *p Odh*^{2}	25	0·0497	34	0·0720
Est-6^1+ *Odh*^{2}	290	0·5765	267	0·5657
Est-6^2 *p Odh*^{1}	24	0·0477	17	0·0360
Est-6^2+ *Odh*^{1}	3	0·0060	4	0·0085
Est-6^2 *p Odh*^{2}	19	0·0378	15	0·0318
Est-6^2+ *Odh*^{2}	102	0·2028	100	0·2119
Total chromosomes	503		472	

TABLE 2

2 × 2 × 2 × 2 contingency χ^2 analysis for gametic association

Item	d.f.	χ^2	P
(i) Allele frequency × Density			
Est-6 × Density	1	0·04	80%
pink × Density	1	0·12	70%
Odh × Density	1	0·47	50%
(ii) Digenic association			
Odh × *pink*	1	267·26	0·001%
Est-6 × *pink*	1	15·82	0·01%
Est-6 × *Odh*	1	6·68	1%
(iii) Digenic association × Density			
Odh × *pink* × Density	1	6·53	1%
Est-6 × *pink* × Density	1	1·53	20%
Est-6 × *Odh* × Density	1	0·19	65%
(iv) Trigenic association			
Est-6 × *Odh* × *pink*	1	29·86	0·001%
(v) Trigenic association × Density			
Est-6 × *Odh* × *pink* × Density	1	0·21	65%

respectively. It is noted that the significance test for linkage disequilibrium was given by the χ^2 tests shown in table 2.

Some insight into the nature of these disequilibria may be gleaned from the gametic proportions shown in table 3.

In the digenic associations, the following two types of gamete were in excess of their expected (assuming random association of alleles) frequencies.

Locus pairs	Gametes in excess
Odh—*pink*	Odh^1p, Odh^2+
Est-6—*pink*	$Est\text{-}6^1+$, $Est\text{-}6^2p$
Odh—*Est-6*	$Est\text{-}6^1\ Odh^2$, $Est\text{-}6^2\ Odh^1$

The interaction *Odh* × *pink* × density is due to a fall in the excess of Odh^1p and Odh^2+ gametes at low compared with high density. The form of the

TABLE 3

Gametic frequencies. The expected gametic frequencies assuming random combination of alleles between the two pairs of loci are shown in brackets

	Types of gamete				
Odh × *pink*	Odh^1p	Odh^1+	Odh^2p	Odh^2+	*r*
High density	0·10338	0·02982	0·08747	0·77932	−0·58
	(0·02542)	(0·10778)	(0·16543)	(0·70137)	
Low density	0·07838	0·04025	0·10381	0·77754	−0·46
	(0·02162)	(0·09702)	(0·16058)	(0·72078)	
Est-6 × *pink*	$Est\text{-}6^1p$	$Est\text{-}6^1+$	$Est\text{-}6^2p$	$Est\text{-}6^2+$	
High density	0·10538	0·60040	0·08548	0·20874	+0·16
	(0·13470)	(0·57107)	(0·05615)	(0·23808)	
Low density	0·11440	0·59745	0·06779	0·22033	+0·09
	(0·12970)	(0·58216)	(0·05250)	(0·23564)	
Est-6 × *Odh*	$Est\text{-}6^1\ Odh^1$	$Est\text{-}6^1\ Odh^2$	$Est\text{-}6^2\ Odh^1$	$Est\text{-}6^2\ Odh^2$	
High density	0·07952	0·62624	0·05368	0·24056	+0·09
	(0·09401)	(0·61176)	(0·03919)	(0·25504)	
Low density	0·07415	0·63771	0·04449	0·24364	+0·07
	(0·08445)	(0·62741)	(0·03418)	(0·25396)	
Est-6 × *Odh* × *pink*	Odh^1p	Odh^1+	Odh^2p	Odh^2+	
$Est\text{-}6^1$	0·06946	0·03907	0·08538	0·80607	−0·47
	(0·01681)	(0·09173)	(0·13804)	(0·75342)	
$Est\text{-}6^2$	0·14436	0·02464	0·11971	0·71126	−0·60
	(0·04463)	(0·12438)	(0·21945)	(0·61154)	

trigenic interaction *Odh* × *pink* × *Est-6* is such that the correlation between alleles at the *Odh* and *pink* loci is greater when the gametes carry an $Est\text{-}6^2$ allele (+0·60) than it is when the $Est\text{-}6^1$ allele (+0·47) is present.

The gametes produced by the adults in the cage population will show the effects of intrachromosomal recombination in the *Drosophila* female. Knowing the map locations of the three loci it is possible to calculate expected frequencies of the four types of gamete, produced by adults in the cage population, from the observed gametic frequencies in the high density sample, by using the following relationship:

$$\begin{aligned} g_1' &= g_1 + \tfrac{1}{2}RD \\ g_2' &= g_2 - \tfrac{1}{2}RD \\ g_3' &= g_3 - \tfrac{1}{2}RD \\ g_4' &= g_4 + \tfrac{1}{2}RD \end{aligned}$$

modified from (Lewontin and Kojima, 1960)

where the frequencies of the four types of gamete (*AB*, *Ab*, *aB* and *ab*) are, at low density, g_1', g_2', g_3' and g_4' and at a high density g_1, g_2, g_3 and g_4.

R is the proportion of crossovers and D the coefficient of linkage disequilibrium. The value of R is divided by $\frac{1}{2}$ because there is no intrachromosomal recombination in the male *D. melanogaster*. The proportion of crossovers was calculated for each two locus pair from the map distances given in Lindsley and Grell (1967). The expected frequencies may be compared with the observed frequencies of the four types of gamete in the low density sample by a χ^2 test for three degrees of freedom. The three degrees of freedom were partitioned into two comparisons each with one degree of freedom comparing the observed and expected allele frequencies of the two loci, and the remaining orthogonal comparison, which tests the observed linkage disequilibrium at low density with that expected to be produced by the cage population before selection. As recombination does not alter the allele frequencies only the $\chi^2_{(1)}$ for the comparison of linkage disequilibrium is shown below for each locus pair

Locus pair	$\chi^2_{(1)}$	P
Odh—pink	3·02	10 %
Est-6—pink	0·66	40 %
Odh—Est-6	0·03	85 %

The χ^2 tests show that the linkage disequilibrium in the low density sample does not differ from that expected to be produced by recombination from the sample of high density flies. Selection is not effective at low density.

Polymorphism at the *pink* locus was first observed in September 1968. The frequency of the pink homozygote has been monitored for 3 years. The samples (table 4) are large, and do show a fluctuation in the frequency

TABLE 4

Frequency of the pink homozygote in the " Texas " population

	Phenotypes		
Sample date	*p*	+	% pink homozygote
26.1.71	33	1217	2·64
10.12.71	69	1428	4·61
17.9.73	137	5037	2·65

of the pink homozygote ($\chi^2_{(2)} = 15{\cdot}99$, P $\simeq$ 0·1 per cent), but, there has been no tendency for the frequency of the *pink* homozygote consistently to increase or decrease in the population.

Some idea of the stability of the linkage disequilibrium between the *Odh* and *pink* loci is known for a sample of 94 chromosomes from flies grown at low density 9 months prior to the main experiment reported in this paper.

The frequencies of the four types of gamete Odh^1p, Odh^1+, Odh^2p and Odh^2+, were compared with those of the low density sample, and gave a $\chi^2_{(3)}$ of 1·79, P $\simeq$ 20 per cent. There is no evidence to suggest that the linkage disequilibrium between these two loci is transient.

4. DISCUSSION

In this study the maximum separation between any two of the three loci was about 12·4 centimorgans (*Odh* and *Est-6*). The two most tightly linked loci (*Odh* and *pink*), showed the strongest linkage disequilibrium and the two

most loosely linked loci (*Odh* and *Est-6*) the least linkage disequilibrium. The strong trigenic interaction means that none of the linkage disequilibria observed at the digenic level is independent; a highly correlated gene-complex constitutes part of the genetic architecture of this chromosome segment. Although only the disequilibrium between the *Odh* and *pink* loci was affected by density, the linkage disequilibria observed at low density between these two loci did not differ from those which would be expected to be produced by a sample of flies raised at high density. In addition, the correlation between alleles at any two of the three loci, was always greatest at high density. These observations are in agreement with the hypothesis that the disequilibria are maintained by natural selection. Further analyses of the behaviour of these polymorphisms are being made with the " Texas " population.

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