Genet. Res., Camb. (1987), **50**, *pp.* 187–193 *With 2 text-figures Printed in Great Britain*

Linkage disequilibrium in experimental populations of *Drosophila simulans*: a test of the random drift hypothesis

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(Received 16 April 1987 and in revised form 7 July 1987)

Summary

Linkage disequilibrium between five polymorphic enzymic loci of the third chromosome (Esterase-6, Phosphoglucomutase, Esterase-C, Aldehyde Oxidase and Acid Phosphatase) was studied in experimental populations of *Drosophila simulans*. Gametic data were obtained by mating sampled males with homozygous females at the five loci. Four cage populations were initiated with flies caught from natural populations. Extensive linkage disequilibrium was detected after 25 or 34 generations. The effective size of these populations was estimated about 400. Monte-Carlo simulations were performed in order to determine whether the observed disequilibria could be due to genetic drift. The observed probability distribution of the experimental values of r (the gametic correlation coefficient) was consistent with the distribution expected under random genetic drift. Our results are thus in accordance with the neutralist hypothesis.

1. Introduction

Genetic drift can create linkage disequilibrium between genes selectively neutral in populations of finite size. Weir & Hill (1980) gave an approximate expression for the variance of the gametic correlation coefficient r, produced by random genetic drift in dioecious populations with random mating. If n gametes are sampled in such populations the expected variance of r is given by:

$$E(r)^2 = \frac{(1-c)^2+c^2}{2N_e c(2-c)} + \frac{1}{n} \qquad (1)$$

(c = recombination frequency and N_e = effective size).

This expression, which describes an equilibrium state, can be used in order to analyse observed disequilibria if the conditions for this equilibrium to be reached are realized.

Experimental results collected during the last 15 years show that significant linkage disequilibrium between enzymic loci is seldom found in natural populations of *Drosophila* (see Hedrick, 1983, for a review). When a significant disequilibrium is observed its interpretation requires an accurate knowledge of the concerned population dynamics (Montchamp-Moreau & Katz, 1986). Such information is rarely available.

In contrast to the situation in natural populations, large amounts of linkage disequilibria have been found in experimental populations (O'Brien & McIntyre, 1971; Birley, 1974; Beardmore & Ahmad, 1976; Alahiotis *et al.*, 1976; Langley *et al.* 1978; Laurie-Alberg & Weir, 1979).

In spite of the possibility of controlling the dynamics of experimental populations, the interpretation of observed disequilibria using formula (1) encounters several difficulties. First, the species usually studied, *Drosophila melanogaster* and *Drosophila subobscura*, show an inversion polymorphism. When an enzymic locus is linked with a polymorphic inversion, the effective recombination frequency between this locus and another one of the same linkage group, and consequently $E(r^2)$, depend on the inversion frequency and on the linkage disequilibrium between the allozymes and the inversion (Laurie-Ahlberg & Weir, 1979). Secondly, when loci are tightly linked, $E(r^2)$ reaches its equilibrium value after a long time (more than 45 generations when $c = 0{\cdot}05$) (Montchamp-Moreau & Katz, 1986). Third, when loci are loosely linked, the sampling variance (inversely proportional to the sample size) becomes large relative to the drift variance.

In order to overcome these difficulties we studied experimental populations of *Drosophila simulans*, a species which is devoid of inversion polymorphism. Large samples (600 gametes or more) were analysed to avoid too high sampling variances. The effective sizes of the populations having been previously estimated from the allelic drift, the expected values of $E(r^2)$

Table 1. *Allelic frequencies at five loci of the third chromosome*

Locus	Allele	La Sirole (G 0)	SI1 (G 34)	SI2 (G 25)	Barcelona (G 0)	BA1 (G 34)	BA2 (G 25)
		n	n	n	n	n	n
Est-6		674	596	630	849	611	690
	0·60	—	—	—	0·002	—	0·002
	0·87	0·003	—	0·022	—	—	—
	0·90	0·313	0·317	0·348	0·303	0·142	0·117
	1·00	0·628	0·683	0·546	0·621	0·689	0·746
	1·12	0·055	—	0·079	0·074	0·169	0·135
	Nul	0·001	—	0·005	—	—	—
Pgm		680	596	631	856	612	692
	0·75	0·121	0·049	0·139	0·085	0·047	0·123
	0·80	0·091	—	0·063	0·070	0·082	0·124
	1·00	0·753	0·945	0·740	0·842	0·871	0·753
	1·25	0·035	0·007	0·058	0·002	—	—
Est-C		669	596	631	855	610	690
	0·81	0·006	0·037	0·002	0·004	—	0·007
	1·00	0·229	0·178	0·250	0·143	0·405	0·238
	1·08	0·099	0·171	0·119	0·144	0·161	0·109
	1·12	0·102	0·039	0·073	0·088	0·144	0·193
	1·18	0·184	0·070	0·021	0·273	0·015	0·158
	1·23	0·209	0·195	0·258	0·246	0·152	0·192
	1·31	0·143	0·299	0·249	0·056	0·007	0·030
	1·40	0·025	0·012	0·029	0·043	0·116	0·073
	1·46	0·003	—	—	0·005	—	—
Aldox		674	596	625	856	610	687
	0·76	0·001	—	—	—	—	—
	1·00	0·499	0·441	0·586	0·572	0·426	0·541
	1·06	0·022	0·002	0·099	0·053	—	0·039
	1·12	0·233	0·198	0·101	0·121	0·328	0·189
	1·18	0·239	0·329	0·214	0·252	0·246	0·230
	1·24	0·003	0·030	—	—	—	—
	Nul	0·003	—	—	0·001	—	—
Acph		679	596	629	856	612	690
	0·80	0·244	0·250	0·259	0·145	0·265	0·254
	1·00	0·739	0·683	0·741	0·845	0·735	0·746
	1·35	0·016	0·067	—	0·011	—	—

n is the number of chromosomes sampled.

Table 2. *Values of r and* χ^2 1 D.F. *for each pair of loci (two allelic classes at each locus)*

Loci	c		La Sirole (G 0)	SI1 (G 34)	SI2 (G 25)	Barcelona (G 0)	BA1 (G 34)	BA2 (G 25)
		n	670	596	625	850	610	690
1 = Pgm/Est-6	0·06	*r*	−0·006	0·039	−0·040	−0·008	−0·027	−0·095
		χ^2	0·023	0·900	0·981	0·053	0·447	6·289*
2 = Est-C/Aldox	0·07	*r*	0·059	−0·175	0·213	0·014	0·039	0·025
		χ^2	2·323	18·200***	28·302***	0·166	0·932	0·422
3 = Pgm/Est-C	0·09	*r*	−0·043	0·074	0·043	0·019	0·036	0·019
		χ^2	1·264	3·284	1·184	0·297	0·783	0·262
4 = Est-6/Est-C	0·13	*r*	0·022	0·006	−0·002	0·015	−0·212	0·092
		χ^2	0·309	0·020	0·003	0·183	27·403***	5·851*
5 = Pgm/Aldox	0·13	*r*	−0·043	−0·110	0·061	0·024	0·007	−0·067
		χ^2	1·256	7·198**	2·320	0·503	0·027	3·115
6 = Est-6/Aldox	0·16	*r*	−0·017	0·119	−0·007	0·000	0·056	0·084
		χ^2	0·193	8·453**	0·027	0·000	1·906	4·846*
7 = Acph/Aldox	0·19	*r*	−0·018	−0·041	−0·049	0·015	0·208	0·035
		χ^2	0·214	0·985	1·481	0·195	26·328***	0·852
8 = Acph/Est-C	0·21	*r*	−0·007	−0·126	0·026	0·001	−0·086	0·106
		χ^2	0·037	9·495**	0·416	0·001	4·536*	7·761**
9 = Acph/Pgm	0·25	*r*	0·025	−0·086	0·004	0·026	0·100	0·005
		χ^2	0·422	4·424*	0·011	0·592	6·168*	0·016
10 = Acph/Est-6	0·25	*r*	0·072	−0·123	−0·055	0·008	−0·075	0·008
		χ^2	3·531	9·085**	1·888	0·055	3·447	0·040

n = number of chromosome sampled. c = recombination frequency.
* Significant at the 5% level; ** significant at the 1% level; *** significant at the 0·1% level.

produced by genetic drift at each generation were obtained using Monte-Carlo simulations. Simulated distributions of *r* were used to determine whether observed disequilibria could be due to genetic drift.

2. Material and methods

(i) *The populations*

These were established from several hundred wild flies, collected in September 1982. One collection was made outside a wine cellar near Barcelona (Spain), the other in an orchard in La Sirole (France). The collected males were used for estimation of allelic frequencies and linkage disequilibria in natural populations. Each collected female was placed in an individual vial in order to set up 150 isofemale lines from each collection. Three F_1 virgin females and three F_1 males were extracted from each line; the 900 flies obtained in this manner from each population were placed in a cage and were allowed to lay eggs on 16 cups of fresh medium (Pearl *et al.* 1926) for 6 days. The cups were then separated into two batches of eight cups, in order to set up two replicate populations for each collection: Barcelona 1 and 2, (BA1 and BA2), and La Sirole 1 and 2 (SI1 and SI2). The populations were maintained at 20 °C with overlapping generations by supplying cups of fresh medium every two days. Under these conditions there was strong competition among larvae. The effective generation length was estimated as 23 days (Montchamp-Moreau, 1985).

(ii) *Electrophoresis*

Populations were analysed by sampling at least 300 males. Each male was mated with females homozygous for the five studied loci (*st pe* stock from Turku, Finland). Electrophoresis was carried out on each sampled male and one F_1 fly in order to obtain the allelic composition of each chromosome III. Electrophoresis was performed in horizontal starch gels; the Tris-citrate II buffer system of Selander *et al.* (1971) was used. Five enzymes of the third chromosome were surveyed: Est-6 (esterase 6, 25·2), Pgm (phosphoglucomutase, 38·1), Est-C (esterase C, 59·6), Aldox (aldehyde oxidase, 75·4), and Acph (acid phosphatase, 134·0). The procedures for staining were adapted from Ayala *et al.* (1972).

(iii) *Tests for linkage disequilibrium*

χ^2 tests were performed to test the allelic associations between loci. A first group of tests was made considering two allelic classes at each locus: the more frequent allele and all the others pooled. In a second group, we took all alleles into acccount, except that we grouped alleles supplying less than two gametic classes with an expected size greater than five.

3. Results

Allelic frequencies and gametic correlation coefficients *r* were measured in males extracted from cages BA2 and SI2 at generation 25, and from cages BA1 and SI1

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Table 3. *Values of χ^2 for each pair of loci when all the alleles are considered* (D.F. *in parentheses*)

Loci	c		La Sirole (G 0)	SI1 (G 34)	SI2 (G 25)	Barcelona (G 0)	BA1 (G 34)	BA2 (G 25)
		n	670	596	625	850	610	690
1 = Pgm/Est-6	0·06		2·08 (4)	0·90 (1)	47·92*** (6)	4·65 (3)	13·42** (4)	13·11* (4)
2 = Est-C/Aldox	0·07		9·73 (13)	117·38*** (10)	70·58*** (12)	18·19 (17)	98·42*** (8)	37·44** (15)
3 = Pgm/Est-C	0·09		15·37 (12)	24·35*** (5)	53·94*** (10)	9·21 (9)	32·69*** (8)	59·06*** (11)
4 = Est-6/Est-C	0·13		22·29* (10)	61·43*** (5)	37·31*** (10)	7·47 (11)	41·34*** (8)	21·32* (12)
5 = Pgm/Aldox	0·13		7·85 (6)	7·20* (2)	29·93*** (8)	12·33* (5)	7·05 (4)	24·95*** (6)
6 = Est-6/Aldox	0·16		3·10 (5)	8·46* (2)	5·75 (6)	5·68 (5)	22·55*** (4)	19·44** (6)
7 = Acph/Aldox	0·19		0·81 (3)	43·53*** (4)	2·44 (3)	0·92 (4)	39·08*** (2)	1·92 (3)
8 = Acph/Est-C	0·21		9·79 (6)	153·21*** (10)	5·93 (5)	4·93 (6)	7·62 (4)	28·64*** (6)
9 = Acph/Pgm	0·25		2·10 (3)	5·10 (2)	2·24 (3)	1·01 (2)	6·47* (2)	0·11 (2)
10 = Acph/Est-6	0·25		6·12 (3)	14·91*** (2)	3·09 (2)	0·73 (2)	5·39 (4)	0·70 (2)

n = number of chromosomes sampled. c = recombination frequency.
* Significant at the 5% level; ** significant at the 1% level; *** significant at the 0·1% level.

at generation 34. Allelic frequencies and *r* values measured in La Sirole and Barcelona natural populations (see material and methods) were taken as initial values (generation 0) for the corresponding experimental populations.

The five loci were found to be polymorphic in all the samples. Table 1 shows the allozymic frequencies for each locus and each sample. Alleles are numbered according to their migration distance, expressed as a proportion of that of the most common allele, which was given a value of 1·00. The 1·00 allele is the same in all the samples except for the Est-C locus.

Table 2 shows the gametic correlation coefficients *r* and the corresponding χ^2 values ($\chi^2 = nr^2$, with n = sample size) in the six samples and the ten pairs of loci, when two allelic classes are considered at each locus. The pair of loci are ordered according to increasing effective recombination frequency *c*, which is half the recombination frequency in females (assuming no crossing-over in *Drosophila* males). No significant correlation was found in the two samples from natural populations, in accordance with other results on natural populations of *Drosophila simulans* (Montchamp-Moreau & Katz, 1987). On the contrary, extensive linkage disequilibrium was found in the four experimental populations after 25 or 34 generations; 15 χ^2 values (38%) are significant, of which six have $P < 0{\cdot}05$, five have $P < 0{\cdot}01$, and four have $P < 0{\cdot}001$. Highly significant values correspond mainly to tightly linked loci.

Table 3 shows the values of χ^2 when all alleles are considered. One significant linkage disequilibrium was found in each natural population: between Est-6 and Est-C ($P < 0{\cdot}05$, D.F. = 10) in La Sirole, and between Pgm and Aldox ($P < 0{\cdot}05$, D.F. = 5) in Barcelona. But two significant values among 20 can be due to sampling error. In samples from the experimental populations, 28 tests (70%) were significant, with $P < 0{\cdot}05$ for six, $P < 0{\cdot}01$ for three and $P < 0{\cdot}001$ for nineteen. The power of the test of linkage disequilibrium is greater if all the gametic classes are considered. Consequently, as linkage disequilibria do exist in our experimental populations, the number of significant cases in table 3 (all gametic classes taken into account) is expected to be higher than in table 2 (only four gametic classes considered). Particularly, such a result is expected when linkage disequilibrium is induced by random drift, because $E(r^2)$ depends on N_e, n, and c, and is only slightly influenced by allelic frequencies (Hill & Robertson, 1968). Consequently $E(\chi^2$ with k D.F.$) = knE(r^2)$ for each pair of loci.

4. Test of the drift hypothesis

The effective size of each experimental population was estimated from allelic drift, using the method of Pollak (1983). Table 4 shows that this size is about 400 except in BA1 where it is smaller. These estimates are in accordance with biological data (sex ratio, productivity, size fluctuation) measured in the populations

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Table 4. *Effective size N_e of experimental populations estimated by Pollak's method (K = number of alleles, F_K = Pollak's index, σ_N = standard deviation of N_e)*

Locus	$K-1$	$F_K.10^2$	N_e
		SI 1	
Est-6	4	3·066	
Pgm	3	10·861	
Est-C	8	4·787	
Aldox	5	1·973	
Acph	2	3·398	
All loci	22	4·537	403 ($\sigma_N = 131$)
		SI 2	
Est-6	4	1·450	
Pgm	3	0·809	
Est-C	8	4·407	
Aldox	5	4·661	
Acph	2	1·639	
All loci	22	3·185	428 ($\sigma_N = 141$)
		BA 1	
Est-6	3	6·728	
Pgm	3	0·927	
Est-C	8	11·842	
Aldox	4	8·487	
Acph	2	5·378	
All loci	20	8·100	217 ($\sigma_N = 71$)
		BA 2	
Est-6	3	7·039	
Pgm	3	1·929	
Est-C	8	3·164	
Aldox	4	0·972	
Acph	2	4·694	
All loci	20	3·268	416 ($\sigma_N = 143$)

(Montchamp-Moreau, 1985). In populations of such size, random drift can produce extensive linkage disequilibrium (Weir & Hill, 1980). To determine whether the disequilibria observed between the common alleles in the cages after 25 or 34 generations could be due to random drift, simulations were performed.

(i) *Simulation procedure*

In the simulations we assumed the following:

(1) The initial disequilibrium (D_0) is zero, because there is no significant disequilibrium in samples from natural populations.

(2) The population size (N_e) is 400.

(3) The number of chromosomes sampled (n) is 600.

(4) There is no recombination in males.

(5) There are two alleles at each locus: allele 1·00 and other alleles grouped together. Initial allelic frequencies (p_0 and q_0) are those of the natural populations.

For each population and each pair of loci, 1000 runs were performed. We then obtained the distribution of r expected among the segregating populations after 25 generations in La Sirole 2 and Barcelona 2 populations, or after 34 generations in La Sirole 1 and Barcelona 1 populations.

(ii) *Results*

We give in Fig. 1 an example of a simulated distribution of r obtained for the pair of loci Pgm/Est-6 after 25 generations with $N_e = 400$, when the initial allelic frequencies are those observed in the population La Sirole. The mean and variance of the 1000 simulated values were used to test the normality of the distribution. χ^2 tests (17 D.F.) for normality were not significant for the 20 simulated distributions. For each experimental value of r (r_0) the associated normal reduced value (ϵ) is obtained from the parameters of the corresponding distribution of simulated values (r_s):

$$\epsilon = \frac{|r_0 - \bar{r}_s|}{\sigma(r_s)}.$$

For the pair of loci Pgm/Est-6 in the cage La Sirole 2, $r_0 = -0{\cdot}040$. The corresponding value of ϵ is 0·421. Therefore, under the hypothesis of linkage disequilibrium created by genetic drift, the significance level of r_0 is 67%.

Figure 2(*a*) shows the distribution of the significance levels of linkage disequilibria observed between 1·00 alleles in each of the four experimental populations under the null hypothesis of genetic drift produced by a finite size of 400.

In Table 2, 15 cases are significant, but only five remain significant in Fig. 2(*a*): in sample BA1 between Est-6 and Est-C ($r = -0{\cdot}212$, $P < 0{\cdot}01$) and between Aldox and Acph ($r = 0{\cdot}208$, $P < 0{\cdot}01$), in sample SI1 between Acph and Est-C ($r = -0{\cdot}126$, $P < 0{\cdot}05$) and between Acph and Est-6 ($r = -0{\cdot}123$, $P < 0{\cdot}05$), in sample SI2 between Est-C and Aldox ($r = 0{\cdot}213$, $P < 0{\cdot}05$). There is no significant disequilibrium in sample BA2. Results for the four samples are pooled in Fig. 2(*b*).

4. Discussion

When genetic drift is taken into account, only five r values are significant ($P < 0{\cdot}05$) in the cage samples of which two have $P < 0{\cdot}01$. This result is consistent with sampling error because for a risk $\alpha = 5\%$, we expect up to five significant values with $P < 5\%$ and up to two values with $P < 1\%$.

Under the hypothesis of random drift as the unique cause of disequilibrium, the 40 observed values are expected to be uniformly distributed into equiprobable classes of significance level (Fig. 2(*b*). In order to test the uniformity of the observed distribution, a χ^2 test was performed taking into account five equiprobable

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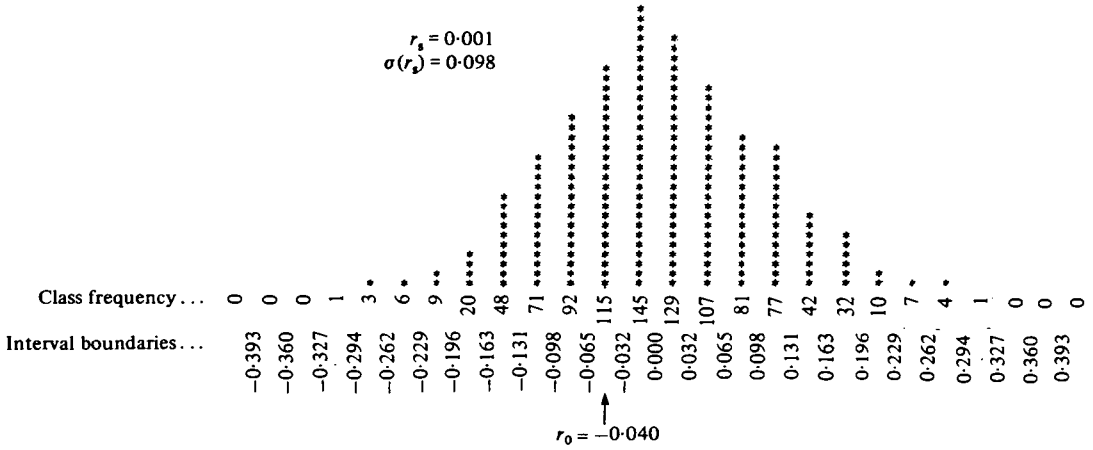

Fig. 1. Distribution of simulated values (r_s) of the gametic correlation coefficient for the pair of alleles Pgm 1·00/Est-6 1·00 in sample SI2 ($N_e = 400$, $n = 600$, $p_0 = 0{\cdot}753$, $q_0 = 0{\cdot}638$, r_0 = observed value of r in sample SI2).

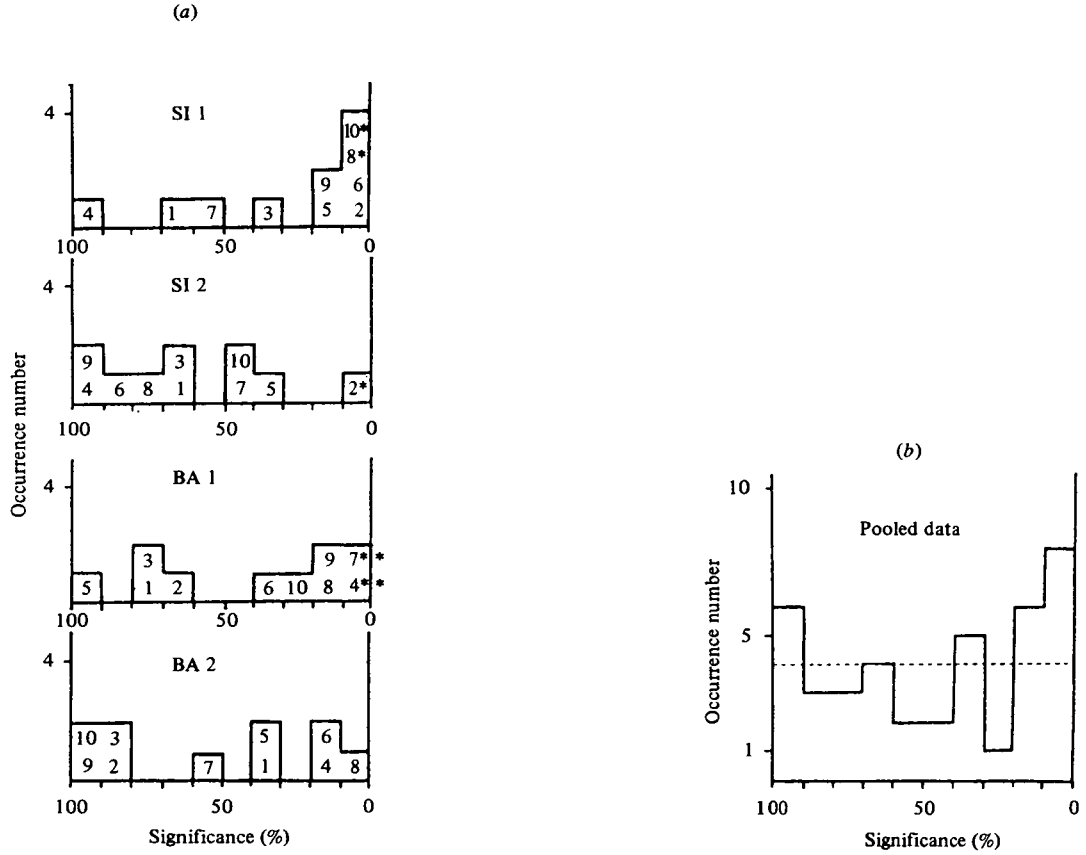

Fig. 2. Significance (%) of the linkage disequilibria observed in experimental populations, under the null hypothesis of linkage disequilibrium produced by genetic drift if $N_e = 400$. (*a*) For each sample, the pair of loci are numbered according to increasing recombination frequencies (from 1 = Pgm/Est-6, to 10 = Est-6/Acph). * Sign. 5%, ** sign. 1%, *** sign. 0·1%). (*b*) Data from the four samples is pooled (---- = uniform distribution expected under the null hypothesis).

classes (class interval = 20%). Its value is not significant ($\chi^2 = 7{\cdot}25$, D.F. = 4).

Moreover the following facts support the stochastic hypothesis.

(i) The effective sizes calculated from allelic drift are respectively 403 in La Sirole 1, 428 in La Sirole 2, 416 in Barcelona 2, but only 217 in Barcelona 1. Linkage disequilibria induced by random drift would thus be expected to be higher in Barcelona 1. In fact, the two disequilibria which are significant at the 1% level were found in this population. This tends to confirm our estimates of effective size, despite the high standard errors of these estimates.

(ii) A bottleneck during one or two generations induces a large relative increase of $E(r^2)$ when loci are loosely linked, but not when loci are tightly linked (Montchamp-Moreau & Katz, 1986). This may explain the 3 significant values observed between

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loosely linked loci ($c > 0{\cdot}19$) in the SI1 and BA1 samples: Aldox/Acph in BA1, Acph/Est-6 and Acph/Est-6 in SI1, as these samples were extracted from cages not long after a fortuitous bottleneck. The critical values used in our tests were obtained from the mean effective size during 25 or 34 generations and hence underestimated the bottleneck effect.

(iii) Two-locus models generally demonstrate that there will be stable linkage disequilibrium if there is considerable epistasis or very close linkage between loci. Consequently, the tighter two loci are linked, the larger the expected disequilibrium. In our experiment, the observed significant linkage disequilibrium cases between loosely linked pairs of loci which do not present evident functional relation (Aldox/Acph, Acph/Est-C and Acph/Est-6) cannot be explained by such models. But Franklin & Lewontin (1970) showed that two-locus models seriously underestimate the magnitude of linkage disequilibrium which can be produced between loosely linked loci by small epistatic interactions all along the chromosome. Nevertheless, despite strong larval competition in our experimental populations, a selective explanation does not fit the results of this experiment because the five significant disequilibria we observed occurred in five different pairs of loci.

We pointed out in the introduction that large amounts of linkage disequilibrium are currently found in experimental populations of *Drosophila*. This fact is confirmed by the disequilibrium values obtained in this experiment. However, when those values are interpreted taking into account random drift, the distribution of the significance levels of these values and the number of significant cases become compatible with sampling error. Consequently our results do not support the hypothesis that linkage disequilibria observed in *Drosophila* experimental populations are due to epistatic interactions.

The authors are grateful to Dominique Anxolabéhère for his critical comments during the preparation of the manuscript.

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