

CHROMOSOME INVERSION POLYMORPHISMS IN *DROSOPHILA MELANOGASTER*. I. LATITUDINAL CLINES AND ASSOCIATIONS BETWEEN INVERSIONS IN AUSTRALASIAN POPULATIONS

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ABSTRACT

Nineteen Australasian populations of *Drosophila melanogaster* have been screened for chromosome inversion polymorphisms. All 15 of the inversion types found are paracentric and autosomal, but only four of these, one on each of the major autosome arms, are common and cosmopolitan. North-south clines occur, with the frequencies of all four of the common cosmopolitan inversions increasing toward the equator. These clines in the Southern Hemisphere mirror north-south clines in the Northern Hemisphere, where the frequencies of all four of the common cosmopolitan inversions again increase towards the equator.—While few of the Australasian populations show significant disequilibrium between linked common cosmopolitan inversions, those that do invariably have excesses of coupling gametes, which is consistent with other reports. We also find nonrandom associations between the two major autosomes, with the northern populations in Australasia (those with high inversion frequencies) tending to be deficient in gametes with common cosmopolitan inversions on both major autosomes, while the southern populations in Australasia (low inversion frequencies) tend to have an excess of this class of gametes.—The clines and the nonrandom associations between the two major autosomes are best interpreted in terms of selection operating to maintain the common cosmopolitan inversion polymorphisms in natural populations of *D. melanogaster*.

CHROMOSOME inversion polymorphisms are common in natural populations of many *Drosophila* species (see DOBZHANSKY 1970 for review). Geographic clines in inversion frequencies in *D. pseudoobscura*, *D. robusta*, *D. flavopilosa*, *D. packea* and *D. subobscura* (DOBZHANSKY 1948; STALKER and CARSON 1948; BRNCIC 1962, 1966; WARD *et al.* 1974; PINSKER, LANKINEN and SPERLICH 1978), as well as cyclic and long-term temporal changes in inversion frequencies in *D. flavopilosa* and *D. pseudoobscura* (BRNCIC 1972; ANDERSON *et al.* 1975; DOBZHANSKY 1943), have provided evidence that some inversions are subject to natural selection.

In *D. melanogaster*, there are four common cosmopolitan paracentric inversions in most natural populations so far screened (ASHBURNER and LEMEUNIER 1976); one occurs on each of the four major autosome arms; they are denoted

In(2L)t, *In(2R)NS*, *In(3L)P* and *In(3R)P*, with breakpoints at 22D-34A, 52A-56F, 63C-72E and 89C-96A, respectively (LINDSLEY and GRELL 1968). Several lines of evidence suggest that these inversion polymorphisms in *D. melanogaster* are also subject to natural selection.

In particular, significant clines for the frequencies of these four common cosmopolitan inversions occur over 20 degrees of latitude for 20 populations from the eastern United States (METTLER, VOELKER and MUKAI 1977). The frequencies of all four inversions and the total inversion frequency increase in populations sampled southwards to the equator. Data from three populations from the west coast of the United States on the two common cosmopolitan chromosome 2 inversions (VOELKER, MUKAI and JOHNSON 1977) and from five populations in Japan for all four (INOUE and WATANABE 1979) are also consistent with a north-south cline in inversion frequencies.

Another line of evidence suggesting the action of selection is the existence of two types of nonrandom associations among the inversions of *D. melanogaster*. First, there are cases of significant disequilibrium between linked inversions in both natural and laboratory populations. (ALAHOTIS, PELECANOS and ZACHARPOULOU 1976; CHOI 1977; LANGLEY, ITO and VOELKER 1977; INOUE and WATANABE 1979). Second, in some populations, there is a negative correlation between the occurrence in individuals of heterokaryotypic second and third chromosomes; this may, in turn, contribute to an excess of individuals with an intermediate number of inversions (STALKER 1976).

Further results suggestive of selection have been recorded by STALKER (1976, 1980). They include associations of inversion frequencies in at least some natural populations of *D. melanogaster* with food type, size of sperm load in females, yearly seasons, wing-load index and flying ability at low temperatures.

A critical test of the hypothesis that the latitudinal clines and the nonrandom associations among the inversions of *D. melanogaster* are due to natural selection is one that tests their repeatability in other widely separated populations. To achieve this, we have screened 19 Australasian collections of *D. melanogaster* for geographic clines in inversion frequencies. We have also analyzed these data for (a) linkage disequilibria between pairs of common cosmopolitan inversions, (b) associations between pairs of unlinked common cosmopolitans, (c) associations between the second and third autosomes (classed as inversion carrying or not), and (d) the distribution of the total number of common cosmopolitans per gamete.

MATERIALS AND METHODS

Wild *D. melanogaster* were collected in 19 localities from Sogeri, New Guinea (8.8° latitude south) to Cygnet, Tasmania (43° latitude south) (Table 1). Inseminated females from these collections were used to initiate isofemale lines. One male was taken from each isofemale line no more than 9 generations after establishment of that line (Table 1) and crossed with virgin females homozygous for standard-sequence chromosomes. A salivary gland preparation was made from a single 3rd-instar larva from each of the crosses (maintained at 18°), and lacto-acetic orcein was used to stain the chromosomes. Breakpoints of the inversions were established by

comparing photographs with photographic representations of BRIDGES' (1935) standard maps (LEFEVRE 1976).

It is known that the common cosmopolitan inversions are eliminated over generations in some laboratory cage populations (INOUE 1979). However, the evidence suggests that the decline in their frequencies is comparatively small when maintained in isofemale lines for the time periods used in this survey (INOUE, personal communication; KNIBB, unpublished data). Also, as the time of screening the 19 collections was random with respect to the latitudinal location of the collection sites, sampling error of this type should not contribute to the overall latitudinal variation in inversion frequencies.

The clines were quantified with correlation and regression analyses, in which southern latitudes and eastern longitudes were treated as positive values. The inversion frequencies were analyzed after angular transformation (except for the data on total inversion frequency per individual) and standardized for sample size (MULLEY, JAMES and BARKER 1979).

RESULTS

Geographic distributions of inversions

We detected a total of 15 different inversions, five on chromosome 2 and 10 on chromosome 3. All of the inversions are paracentric, and their breakpoints and demographic status (based solely on their Australasian frequencies) are:

Chromosome 2, left arm (2L)

A. 22D-34A, common cosmopolitan

Chromosome 2, right arm (2R)

B. 52A-56F, common cosmopolitan

C. 51D-59F/60A, unique endemic

D. 42A-53A/B, unique endemic

E. 50C-57A/B, unique endemic

Chromosome 3, left arm (3L)

F. 63C-72E, common cosmopolitan

G. 66C/D-70F/71A, recurrent endemic

H. 62C-68A, unique endemic

I. 62A/B-69A, unique endemic

Chromosome 3, right arm (3R)

J. 89C-96A, common cosmopolitan

K. 92D-100C, rare cosmopolitan

L. 85D-93B/C, unique endemic

M. 87C-95A, unique endemic

N. 93D-98F, unique endemic

O. 95D-98F, unique endemic

The four demographic classes are based on the scheme proposed by METTLER, VOELKER and MUKAI (1977). In that scheme, common cosmopolitans are those that occur in many populations, often at frequencies greater than 5%, while the rare cosmopolitans are those that are present in many populations, but at frequencies usually less than 5%. Recurrent endemics are those that occur in only a few individuals in the same or adjacent populations, while unique endemics are those that were recorded only once.

TABLE 1
Collection sites and dates with the frequencies of inversions for 19 Australasian populations, the corresponding common cosmopolitan inversion frequency per individual (T.C.C.) and total inversion frequency per individual (T.I.F.) values

Locality	Lat. °S	Long. °E	No. isolines	Collected	Generations elapsed†	$In(2L)I$	$In(2R)NS$	$In(3L)P$	$In(3R)P$	$In(3R)C$	Other inversions	T.C.C.	T.I.F.
Sogeri	8.8	148.3	22	Nov 1978	9	0.46	0.18	0.50	0.68	0.00	0.09:G	3.64	3.80
Snake Bay	11.4	130.7	11	Jan 1979	4	0.55	0.46	0.46	0.27	0.00		3.27	3.27
Darwin	12.5	130.8	12	Jan 1979	4	0.25	0.25	0.25	0.58	0.00		2.67	2.67
Innisfail	17.5	146.0	5	Jul 1979	3	0.40	0.20	0.00	1.00	0.00		3.20	3.20
Townsville	19.3	146.7	9	Nov 1978	6	0.33	0.44	0.22	0.55	0.00		3.11	3.11
Nambour	26.6	153.0	53	Nov 1978	7	0.21	0.06	0.06	0.45	0.00	0.02:D	1.56	1.60
Brisbane	27.5	153.0	76	Dec 1978	9	0.22	0.09	0.03	0.21	0.04	0.01:N	1.10	1.26
Stanthorpe	28.7	152.0	21	Feb 1979	6	0.10	0.10	0.00	0.19	0.05		0.78	0.86
Sandalford	31.9	116.0	27	Feb 1979	6	0.04	0.00	0.04	0.00	0.04		0.16	0.22
Westfield	31.9	116.0	13	Feb 1979	6	0.00	0.00	0.15	0.15	0.08		0.60	0.77
Craigmoor	32.6	149.6	44	Apr 1979	2	0.07	0.11	0.05	0.11	0.00		0.68	0.68
St. Peters	34.9	138.6	18	Jan 1979	8	0.22	0.00	0.00	0.06	0.00		0.56	0.56
Coriole	35.0	138.5	37	Jan 1979	8	0.03	0.00	0.00	0.00	0.00		0.05	0.05
Carberra	35.3	149.2	81	Apr 1979	4	0.09	0.04	0.01	0.10	0.01	0.01:E, H, L, O	0.48	0.67
Araluen	35.5	149.8	51	Feb 1979	3	0.10	0.16	0.12	0.43	0.02		1.62	1.65
Rutherglen	36.1	146.5	36	Jun 1979	2	0.03	0.06	0.08	0.03	0.00		0.39	0.39
Chateau Tahbilk	36.4	145.4	32	Jun 1979	1	0.03	0.00	0.00	0.06	0.06	0.03:I	0.18	0.38
Melbourne	37.7	144.8	59	Mar 1979	6	0.05	0.07	0.02	0.02	0.00	0.02:C, M	0.32	0.37
Cygnat	43.0	147.3	19	Dec 1978	7	0.00	0.05	0.00	0.00	0.00		0.11	0.11

† After initiation of lines to time screened.

Inversions *A*, *B*, *F*, *J* and *K* are interpreted to be the same as *In(2L)t*, *In(2R)NS*, *In(3L)P*, *In(3R)P* and *In(3R)C*, respectively (LINDSLEY and GRELL 1968), which are cosmopolitan in natural populations of *D. melanogaster* in Europe, Asia and North America (ASHBURNER and LEMEUNIER 1976). Of the nine endemic inversions, seven have not been recorded before in natural populations. Inversion *N* apparently has the same breakpoints as those of *In(3R)Mo* (LINDSLEY and GRELL 1968), which is a rare cosmopolitan on other continents (ASHBURNER and LEMEUNIER 1976). Also the breakpoints for inversion *G* are apparently the same as those of a unique endemic recorded by STALKER (1976).

Table 1 also presents the frequencies of all inversions for each of the 19 localities. In general, the frequencies of all four common cosmopolitan inversions, as well as the magnitude of the total inversion frequency per individual, are higher at latitudes nearer the equator and decline southwards. Over all populations, the frequencies of the common cosmopolitan inversions account for 89% of the average total inversion frequency per individual.

The simple correlations of the frequencies of the four common cosmopolitan inversions with latitude and longitude, as well as the partial correlations with latitude and longitude (corrected for the effects of longitude and latitude respectively), are given in Table 2, together with the results of the multiple-regression

TABLE 2
Correlation and regression analyses of transformed and standardized inversion frequencies with latitude and longitude

Analysis	<i>In(2L)t</i>	<i>In(2R)NS</i>	<i>In(3L)P</i>	<i>In(3R)P</i>	Total inversions
<i>Simple correlations</i>					
Latitude	-0.84†	-0.67†	-0.74†	-0.72†	-0.89†
Longitude	0.20	0.26	-0.21	0.26	0.07
<i>Partial correlations</i>					
Latitude, (controlling for longitude effect)	-0.92†	-0.77†	-0.72†	-0.82†	-0.93†
Longitude (controlling for latitude effect)	0.71†	0.55*	-0.08	0.61†	0.58*
<i>Multiple regressions</i>					
<i>F</i> value for latitude	88.97‡	23.06‡	20.00‡	32.48‡	97.87‡
<i>F</i> value for longitude	16.03‡	7.08*	0.10	9.25‡	7.90‡
<i>Multiple regression equations</i>					
Constant	-4.71	-7.00	5.67	-11.00	-1.65
Latitude coefficient	-0.23	-0.18	-0.20	-0.33	-0.39
Longitude coefficient	+0.08	+0.08		+0.15	-0.09
Simple <i>R</i> ² : latitude	0.71	0.45	0.54	0.52	0.79
Multiple <i>R</i> ² : latitude and longitude	0.85	0.62		0.69	0.86

The results of the multiple-regression analyses of the inversion frequencies on latitude and longitude are given in terms of the *F* ratio to enter latitude and longitude into the regression, the regression equation on latitude and longitude if their *F* ratios are significant, the simple *R*² for latitude only, and the multiple *R*² (proportion of the between-collection variance in genetic variables explained by the equation).

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

analyses. As latitude and longitude are themselves correlated over our collection sites ($r = +0.10$), the partial correlations give better estimates of the association between the inversion frequencies and latitude or longitude.

Overall, the correlation and regression analyses are consistent in that the variables with significant partial correlations are also the ones with significant F ratios in the multiple regressions.

For all four common cosmopolitan inversions, as well as for total inversion frequency per individual, there are significant latitudinal clines. These account for between 45 and 71% of the between-collection variance in the frequencies of the four common cosmopolitan inversions, and for 79% of the variance of total inversion frequency per individual.

None of the simple correlations of longitude with the five measures of inversion frequency are significant. However, after correcting for the effects of latitude, the partial correlations are all significant, except in the case of *In(3L)P*. Longitude, then, can account for a further proportion of the between-collection variance for all variables except for *In(3L)P*. Overall, there is a general, though slight, decline in inversion frequencies westwards.

In combination, latitude and longitude can account for 85%, 62% and 69% of the between-collection variance in the frequencies of *In(2L)t*, *In(2R)NS*, *In(3R)P* and for 86% of the variance of total inversion frequency per individual.

No obvious association exists for the rare cosmopolitan or the endemic inversions with either latitude or longitude, although formal analysis of this is not possible because of their low frequencies.

Associations between inversions

Preliminary analyses of the associations between inversions and between whole chromosomes in gametes showed that populations with relatively high inversion frequencies often showed contrasting patterns from those with relatively low inversion frequencies. The data were therefore sorted into two groups, those for populations with an average of more than 1.00 inversion per individual and those for populations with an average of fewer than 1.00 inversion per individual. For the following analyses, the rare cosmopolitan and the endemic inversions have been pooled with the standard sequences.

Linkage disequilibrium between pairs of common cosmopolitan inversions: For *In(2L)t* and *In(2R)NS*, in only one of the 13 populations was there a significant case of linkage disequilibrium (Table 3); this was in the Sogeri population ($P = 0.029$), which contained an excess of coupling gametes. For *In(3L)P* and *In(3R)P*, the disequilibria in both the Sogeri ($P = 0.004$) and the Brisbane populations ($P = 0.042$) were significant, while that in the Araluen population ($P = 0.072$) approached significance; these three cases also showed an excess of coupling gametes.

The comparison of these results with those in earlier reports (ALAHOTIS, PELECANOS and ZACHAROPOULOU 1976; CHOI 1977; INOUE and WATANABE 1979; LANGLEY, TOBARI and KOJIMA 1974; LANGLEY, ITO and VOELKER 1977; MUKAI and VOELKER 1977; MUKAI, WATANABE and YAMAGUCHI 1974; VOELKER, MUKAI and JOHNSON 1977) is not straightforward, due to the differences in both the

TABLE 3
 Correlation coefficients (R) for pair-wise combinations of common cosmopolitan inversions
 for populations with (a) > 1.00 inversion per individual and (b) < 1.00 inversion per individual

Populations	Linked inversions		Nonlinked inversions		
	$2Lx-2RNS$ R_{\dagger}	$3LP-3RP$ R	$2Lx-3LP$ R	$2Lx-3RP$ R	$2RNS-3RP$ R
(a) With > 1.00 inversion per individual					
Sogeri	+0.52*	+0.68**	-0.18	-0.16	+0.07
Snake Bay	+0.10	-0.15	-0.27	-0.26	+0.26
Darwin	+0.11	+0.10	-0.33	-0.29	+0.49
Innisfail	-0.41				
Townsville	-0.16	-0.06	-0.38	-0.79*	+0.35
Nambour	-0.13	-0.22	+0.28	-0.19	-0.06
Brisbane	-0.06	+0.32*	-0.09	-0.05	-0.05
Araluen	+0.04	+0.30	-0.12	-0.15	-0.05
A.	+0.00±0.10	+0.14±0.12	-0.16±0.08	-0.27±0.09*	+0.14±0.08

TABLE 3—Continued

Populations	Linked inversions		Nonlinked inversions	
	$\frac{2Lt-2RNS}{Rt}$	$\frac{3LP-3RP}{R}$	$\frac{2Lt-3RP}{R}$	$\frac{2RNS-3LP}{R}$
(b) With <1.00 inversion per individual				
Stanthorpe	+0.45		-0.16	+0.26
Sandalford				
Westfield	+0.19	-0.18	+0.19	+0.10
Craigmoor		-0.08	+0.45	
St. Peters	-0.09	-0.07	+0.07	+0.12
Canberra 1			+0.56	
Canberra 2	-0.04	-0.05	+0.37	-0.04
Rutherglen			-0.05	
Chateau Tahbilk	-0.06	-0.02	-0.03	-0.04
Melbourne	+0.09±0.10	-0.08±0.03*	+0.13±0.09	+0.20±0.16
B.			+0.28±0.21	+0.08±0.06

Values are not shown for cases that had fewer than three gametic classes. Mean R values with their standard errors are also given for all populations in group (a) (Line A), and for all populations in group (b) (Line B).
 * $P < 0.05$, ** $P < 0.01$.
 † To calculate R values ($e.g.$, for $In(2L)t-In(2R)NS$) gametes were sorted into 4 gametic classes: $g1$ ($std. 2L-std. 2R$), $g2$ ($std. 2L-2RNS$), $g3$ ($2Lt-std. 2R$) and $g4$ ($2Lt-2RNS$). D (disequilibrium index) = $f(g1) \cdot f(g4) - f(g2) \cdot f(g3)$. $R = D / \sqrt{pq(1-p)(1-q)}$, where $p = f$ ($std. 2L$) and $q = f$ ($std. 2R$).

source (isofemale lines or wild populations) and the methods of data analysis (pooling the rare cosmopolitan and the endemic inversions with the standard or with the inverted sequences). Wherever possible, we sorted the previously published data by pooling all inversions other than the common cosmopolitan inversions with standard sequence chromosomes and then calculated probabilities, using Fisher's exact test. In total, two of 16 populations for which there were suitable data had significant disequilibria for the chromosome 2 inversions (Ishigaki, Japan, 1976, $P = 0.029$, INOUE and WATANABE 1979; Cephalonia, Greece, 1973, $P = 0.014$, ALAHIOTIS, PELECANOS and ZACHAROPOULOU 1976), while for the chromosome 3 inversions, one population of 11 showed significant disequilibrium (Carpenter, U.S.A., 1975, $P = 0.001$, LANGLEY, ITO and VOELKER 1977). As in our data, all the populations with significant disequilibria had an excess of coupling gametes. It is noteworthy that those populations that had no significant disequilibria, but tended towards disequilibrium, (*i.e.*, $0.1 < P > 0.05$) for the chromosome 2 inversions (Akayu, Japan, 1977, $P = 0.071$, INOUE and WATANABE 1979; Raleigh, U.S.A., 1968, $P = 0.051$, MUKAI, WATANABE and YAMAGUCHI 1974); and the chromosome 3 inversions (Sapporo, Japan, 1977, $P = 0.054$ and Ishigaki, Japan, 1976, $P = 0.090$, INOUE and WATANABE 1979) invariably had an excess of coupling gametes.

Associations between pairs of unlinked common cosmopolitan inversions: Of the 50 individual comparisons of pairs of unlinked inversions in the Australasian collections, only two showed significant associations (Sandalford: excess of $In(2L)t$ with $In(3L)P$ gametes, $P = 0.037$; Townsville: deficiency of $In(2L)t$ with $In(3R)P$ gametes, $P = 0.048$, Table 3). Although two significant results among the 50 individual comparisons can be explained by sampling error, there is a suggestion of a systematic overall trend. R tends to be negative for the group of populations with more than 1.00 inversion per individual and positive for the group of populations with fewer than 1.00 inversion per individual, although this is not consistently reflected by individual populations. The mean R value for the 28 populations in the former group is -0.108 ± 0.051 , which is significantly different from zero ($P < 0.05$), and that for the 22 populations in the latter group is $+0.165 \pm 0.063$, which is again significantly different from zero ($P < 0.05$). These two means are also significantly different from each other ($P \ll 0.001$).

Associations between inversion-bearing second and third chromosomes: The gametic classes in Table 4 have been sorted into four groups: (1) those gametes with no common cosmopolitan inversions on either chromosome 2 or 3, (2) at least one common cosmopolitan on chromosome 2, but none on chromosome 3, (3) at least one common cosmopolitan on chromosome 3, but none on chromosome 2, and (4) at least one common cosmopolitan on both chromosome 2 and 3.

Correlation coefficients were calculated in the same manner as those for Table 3, so that positive R values represent excesses of gametes with common cosmopolitan inversions on both autosomes and/or inversion-free gametes, and negative R values represent deficiencies of these classes of gametes.

For each of the populations with more than 1.00 inversion per individual, there is a consistent (but nonsignificant) deficiency of gametes with inversions on both

TABLE 4

The gametic array for chromosome 2 and 3 types for population samples with (a) >1.00 inversion per individual and (b) <1.00 inversion per individual

Population	Gametic classes				R
	(a)2-,3-	(b)2+,3-	(c)2-,3+	(d)2+,3+	
(a) With >1.00 inversion per individual					
Sogeri	3	4	9	6	-0.16
Snake Bay	1	3	2	5	-0.04
Darwin	2	2	5	3	-0.12
Townsville	0	3	3	3	-0.50
Nambour	18	8	22	5	-0.14
Brisbane	41	19	12	4	-0.06
Araluen	20	8	19	4	-0.13
Mean R value with standard error					-0.16 ± 0.06*
(b) With <1.00 inversion per individual					
Stanthorpe	15	2	3	1	+0.15
Craigmoor	32	5	5	2	+0.15
St. Peters	14	3	0	1	+0.45
Canberra 1	30	4	5	3	+0.27
Canberra 2	36	2	0	1	+0.56
Rutherglen	31	1	2	2	+0.53*
Tahbilk	29	1	2	0	-0.05
Melbourne	50	7	2	0	-0.07
Mean R value with standard error					+0.25 ± 0.09*

Population samples with less than 3 gametic classes are not included.

* $P < 0.05$.

autosomes. The overall mean R value for these populations is negative and significantly different from zero ($P < 0.05$). By contrast, for populations with fewer than 1.00 inversion per individual, this pattern is reversed, with the mean R value positive and significant ($P < 0.05$) and with a trend toward an excess of gametes with inversions on both autosomes. The mean R values for the two population groups are significantly different from one another ($P \ll 0.001$).

Only the data of STALKER (1976) are from a sufficiently large number of populations to permit us to make a critical comparison with our results, and this is done in the DISCUSSION.

Overall, for the population group with more than 1.00 inversion per individual, the associations between all pairs of unlinked common cosmopolitan inversions (Table 3), except for $In(2R)NS$ with $In(3R)P$, contribute to the associations between whole chromosomes. For the population group with fewer than 1.00 inversion per individual, all four unlinked combinations contribute to the respective whole chromosome associations.

Total number of inversions in gametes: The five gametic classes in Table 5 represent those gametes with 0, 1, 2, 3 and 4 common cosmopolitan inversions, ir-

TABLE 5

Total number of inversions in gametes

		Inversion numbers per gamete				
		0 (a)	1 (b)	2 (c)	3 (d)	4 (e)
†(a) Populations with >1.00 inversion per individual	obs.	85	99	45	9	1
	exp.	87.40	96.72	42.45	11.04	1.30
‡(b) Populations with <1.00 inversion per individual	obs.	272	46	13	0	0
	exp.	264.99	60.26	5.52	0.23	0.00

† χ^2 for homogeneity = >0.95. χ^2 after pooling (d) and (e) = 0.45.

‡ χ^2 for homogeneity = >0.70. χ^2 after pooling (c), (d) and (e) = 11.23 ($P < 0.01$).

respective of their chromosome location. The observed distributions of the numbers of common cosmopolitans per gamete are compared with those expected given random associations between inversions and assuming no selection, inbreeding or assortative mating with respect to the different possible associations.

The expected frequencies in Table 5 are the sums of the expected frequencies calculated separately for each population. Data for Cygnet and Coriole were not pooled, as both contained only one inversion; thus, there is no difference between observed and expected values. For the populations with more than 1.00 inversion per individual, the observed gametic classes do not depart significantly from the expected values. However, for the populations with fewer than 1.00 inversion per individual, the result is significant ($P < 0.01$) and is due to the tendency in these populations for inversions to occur together in one gamete at greater than expected frequencies. This excess in the number of gametes with two inversions results in a corresponding excess of inversion-free gametes and a deficiency of gametes with only one inversion.

DISCUSSION

Geographic distributions of inversions

In general, the distributions of inversion frequencies and types in Australasia are consistent with those found in other surveys (*e.g.*, ASHBURNER and LEMEUNIER 1976; INOUE and WATANABE 1979; STALKER 1976) in that polymorphic inversions in *D. melanogaster* are almost invariably paracentric and confined to the two major autosomes. Also, only *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P* are generally found in populations at frequencies above 5%. The average frequency per individual of the common cosmopolitan inversions for all Australasian populations is 0.47; however, this figure is dependent on the location of the populations. Although endemic inversions comprise the majority of the inversion types, they, together with the rare cosmopolitan *In(3R)C*, contribute an average of only 0.06 inversion per individual for all populations. This is similar to the value for North American populations (see METTLER, VOELKER and MUKAI 1977) and varies little with the location of the populations. These data provide further evidence that most natural populations of *D. melanogaster*,

even though widely separated, have similar complements of available chromosome inversion variation.

The clear result from this survey is that the four common cosmopolitan inversions in Australasia are clinally distributed with latitude. This pattern is a mirror image of the inversion clines in North America, so that in both Hemispheres all four common cosmopolitan inversions decrease in frequency with increasing latitude. The simple correlations of inversion frequencies with degrees latitude in Australasia (Table 2) for *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P* correspond well to the respective *R* values for the North American populations (-0.92 , -0.90 , -0.74 and -0.80 ; METTLER, VOELKER and MUKAI 1977). Although data are not available from the North American continent for localities closer to the equator than 18.5° latitude, our data would suggest that the inversion clines probably continue past this latitude.

Considering that similar north-south clines in inversion frequencies occur for all four of the common cosmopolitan inversions in both Hemispheres, we conclude that stochastic processes such as random drift, differential migration without selection and founder effects are improbable causal agents of the clines.

Selection, which can account for these data, seems likely to be the major factor maintaining these clines. However, two different selection models might be involved in the maintenance of the clines. First, it is possible that all of these inversions had a tropical origin and had fitnesses greater than their respective standard sequences. Therefore, the observed distributions could represent a gradual selective spread away from the equator of the common cosmopolitan inversions as they replaced the standard-sequence chromosomes. Second, the inversions could be selectively maintained in populations as stable polymorphisms, with the equilibrium inversion frequencies varying clinally along the latitudinal gradient and being independent of the site of origin of the inversions.

This second selection model seems to us to be more likely, since it is improbable that migration of *D. melanogaster* in Australasia, North America and Asia was always directionally away from the equator. This species is associated with and transported by man, and these three regions have had different histories of settlement by man and, presumably, by *D. melanogaster*. Furthermore, the opportunity for long-distance migration within these regions would suggest that the exchange of genetic variation between populations would not be insubstantial, nor necessarily in a progressively north-south direction.

Associations between inversions

While earlier we argued that the maintenance conditions for our stocks would not significantly contribute to the results of the geographic distributions of the inversion frequencies, we do not know whether our maintenance conditions affected our results on the associations between inversions.

Nevertheless, our data for linkage disequilibrium between the common cosmopolitan inversions are consistent with previous studies. Most of the populations we sampled do not show significant linkage disequilibrium between either *In(2L)t* and *In(2R)NS* or between *In(3L)P* and *In(3R)P*. However, overall, there are more populations in which there is chromosomal linkage disequilibrium

than would be predicted by chance, and without exception those populations showing significant disequilibria contain an excess of coupling gametes. We therefore argue that the probability of migration, sampling error or random drift accounting for this pattern is small. However, because of reduced recombination between double-inversion chromosomes (LINDSLEY and GRELL 1968), founder effects may contribute to these cases of coupling associations. Alternatively, selection may operate to create an adaptive advantage for double-inversion chromosomes in some populations.

While survey data of this type cannot differentiate between these two explanations, LEVITAN (1955) concluded that the linkage disequilibrium found between chromosome 2 inversion groups in natural populations of *D. robusta* was adaptive. For this species, differences between the sexes in the strength of the linkage disequilibrium, as well as a cyclic behavior in the total amount of linkage disequilibrium through seasons, were recorded. This is one approach that should be applied to *D. melanogaster*.

As stated previously, STALKER (1976) found a negative correlation within individuals between heterokaryotypic second and third chromosomes. If we assume that our findings are not artefacts from using isofemale lines, and that the populations we sampled were in approximate Hardy-Weinberg equilibrium for individual inversions, so that the inversion compositions of gametes reflected those of individuals, then our demonstration of nonrandom associations between whole chromosomes corroborates that of STALKER (1976). However, this is true only for the populations (generally the northern ones) in our survey that have an average of more than 1.00 inversion per individual. For the rest of the populations (generally southern), there is a clear reversal, with a positive correlation between the respective chromosome types. As none of the populations described by STALKER (1976) had an average inversion frequency per individual less than 1.00, we are unable to compare his result with our data from our southern populations. For the Australasian populations, the interactions between the individual pairs of unlinked common cosmopolitan inversions tend to contribute to the observed nonrandom associations between whole chromosomes by having negative R values for the northern populations and positive R values for the southern populations.

Although the positive R values in these southern populations may represent adaptive associations, they could be explained simply as the result of migration between populations with different inversion frequencies and the consequent sampling of mixed populations (JACQUARD 1974). However, this explanation cannot account for negative R values, such as those we find in the northern populations.

One factor that could contribute to negative R values is a reduction in the fitness of multiple-inversion individuals compared with the fitness of the other inversion classes; there is some evidence consistent with this. COOPER, ZIMMERLING and KRIVSHENKO (1955) and REDFIELD (1957) have shown with laboratory stocks that *D. melanogaster* females that are heterokaryotypic for all four major autosome arms have a greater frequency of egg mortality than other females.

Further data (STALKER 1976), although limited to four females, but from natural populations, is consistent with this.

A prediction of the interactions between whole chromosomes is that populations in the north should tend to maximize, and those in the south minimize, the number of inversion-carrying individuals relative to the inversion frequency of a given population. While we find this to be true for the southern populations, in that there is an increase over the expected number of standard sequence gametes, it is not apparent for the northern ones, possibly because disequilibrium between linked inversions tends to counteract this net result.

To conclude, this paper provides critical evidence in support of the hypothesis that natural selection operates to maintain the common cosmopolitan inversion polymorphisms in *D. melanogaster* by establishing the presence of inversion clines in the Southern Hemisphere. Some systematic associations, both between inversions and between whole chromosomes, are also found that cannot be explained by stochastic processes and may well also have adaptive significance.

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