Aust. J. Biol. Sci., 1988, **41**, 239–46

Polymorphic Inversion and Esterase Loci Complex on Chromosome 2 of *Drosophila buzzatii* II*. Spatial Variation

W. R. Knibb[A] *and J. S. F. Barker*

Department of Animal Science, University of New England, Armidale, N.S.W. 2351.
[A]Present address: Division of Entomology, CSIRO, Clunies Ross St, Canberra, A.C.T. 2601.

Abstract

The potential influence of linked inversions on allele frequency variation at the *Est-1* and *Est-2* loci among Australian populations of *D. buzzatii* was determined by statistical analyses of allele and inversion gametic frequencies. Most of the significant spatial and climatic associations found for all *Est-1* allele frequencies, and for one allele only of *Est-2* (*Est-2*$^{c+}$), were accounted for by their linkage disequilibria with the inversions, which covaried with environmental variables. Consistent with this result, the spatial and climatic associations for conditional *Est-1* and *Est-2* allele frequencies tended to be different from those for the respective unadjusted allele frequencies. In one important respect, the results for *Est-1* and *Est-2* were not altered by inversions. For both unadjusted and conditional *Est-1* allele frequencies, few climatic associations remain after correcting for geographic location, whereas for both unadjusted and conditional *Est-2* allele frequencies, climatic associations remain after correcting for geographic location. Thus, apparent selection affecting allele frequencies at the *Est-2* locus is not accounted for by linked inversions.

Introduction

Drosophila buzzatii has colonized Australia from South America, probably 50–55 years ago (Barker and Mulley 1976; Barker *et al.* 1985). It is specific to the cactus niche, and is now widely distributed in Australia concordant with scattered and isolated (island) stands of *Opuntia* cactus. Barker and Mulley (1976) found population samples from throughout eastern Australia to be consistently variable for six allozyme loci: *alcohol dehydrogenase (Adh-1)*, *aldehyde oxidase (Aldox)*, β -N-*acetyl-hexosaminidase* (*Hex*) (previously designated *pyranosidase*), *esterase-1* (*Est-1*), *esterase-2* (*Est-2*), and *phosphoglucomutase (Pgm)*. At all loci, allozyme frequencies varied significantly among the populations.

To investigate the evolutionary processes that have given rise to this population differentiation, Mulley *et al.* (1979) used canonical correlation and multiple regression procedures to test for associations of allozyme frequencies (and heterozygosities) with spatial and climatic variables. The rationale was that while associations with spatial variables could be due to drift and/or migration, any with climatic variables after correction for geographic location would indicate possible differential selection in different populations. Consistent associations of the former type were found for allozyme frequencies at all loci except *Hex*, while those of the latter type were found for *Adh-1, Est-2* and *Pgm*. Subsequently, Sokal *et al.* (1987) have reanalysed these, and additional, allozyme frequency data using spatial and directional autocorrelation procedures. They confirmed the substantial spatial structure previously evident for allozyme frequencies at all loci except *Hex*. Further, their analyses indicated that a number of alleles had continent-wide spatial patterns, but that these patterns were not correlated between loci. Thus drift and migration were excluded as factors determining the spatial structure. Selection then is implicated as underlying this observed spatial structure in Australian *D. buzzatii* populations. However, selection may not necessarily act directly on the enzyme loci, but on other linked loci or chromosome inversions that are in linkage disequilibrium with them.

*Part I, *Aust. J. Biol. Sci.*, 1987, **40**, 257–69.

0004-9417/88/020239$03.00

Chromosome 2 of *D. buzzatii* is polymorphic for three gene sequences (viz. 2 *ST, j, jz*^{3}) in Australian populations (Carson and Wasserman 1965; Knibb *et al.* 1987). *Aldox, Est-1* and *Est-2* are tightly linked to these inversions, being located within or adjacent to them (D. Schafer, personal communication), and are in strong linkage disequilibrium with the inversions (Knibb *et al.* 1987 and unpublished data). The present study tests whether the spatial patterns evident for alleles at the two esterase loci are due to hitch-hiking with the inversions.

Further, as negligible recombination occurs among *Est-1, Est-2* and the gene arrangements in heterokaryotypes (D. Schafer personal communication), the alleles in 2*ST* and in 2*j* can be viewed as different gene pools within populations. Thus, it is of interest to quantify and compare the spatial variation evident for conditional allele frequencies in 2*ST* with those in 2*j*. The hypothesis is put forward that if a given electromorph represents the same allele in 2*ST* and in 2*j*, and is subject to common selective factors in both arrangements, then its sets of conditional frequencies should be significantly correlated over populations.

Materials and Methods

As reported in Knibb *et al.* (1987), 19 collections of wild *D. buzzatii* were made from 17 eastern Australian sites extending over 14·56° latitude. For each sample, inversion, *Est-1* and *Est-2* gametic frequencies were estimated (given in Tables 1, 2, 4, 5, 7, 8 in Knibb *et al.* 1987).

To estimate the contributions of inversions to the associations of allele frequencies with spatial and climatic variables, we have adopted the protocol of Voelker *et al.* (1978). They have reported that the approximate covariance between the frequencies of a given allele (*A*) and a given spatial or climatic variable (*V*) can be estimated from its constituent parts:

$$\hat{C}_{AV} = (\bar{a}_2 - \bar{a}_1)\, C_{jV} + \bar{j} C_{a_2 V} + \overline{ST}\, C_{a_1 V} \qquad (1)$$

where a_1 and a_2 are the conditional allele frequencies in 2*ST* and 2*j*, respectively. The first term (i) = $(\bar{a}_2 - \bar{a}_1)\, C_{jV}$ can be attributed to the association of the inversion (2*j*) with the variable (*V*), while the remaining two terms, (ii) and (iii), are attributable to the associations of conditional allele frequencies (in 2*j* and 2*ST*) with the variable (*V*). Hence, the proportion of the original association between *A* and *V* due to the inversion can be calculated as:

$$p = \frac{\text{(i)}}{\text{(i)} + \text{(ii)} + \text{(iii)}} \qquad (2)$$

When the sign of (i) is opposite to that of the original association, we conclude p is zero. In these analyses, all 2*jz*^{3} chromosomes were ignored, and since 2*jz*^{3} occurs at a mean frequency of 1·5%, the resultant error is trivial. There was, in general, good correspondence between $\hat{C}_{AV}$ and direct estimates of such covariance. Here, untransformed and unstandardized allele and inversion frequencies were used, although their estimated correlation coefficients with the independent variables were very similar to those estimated using transformed and standardized frequencies (see below).

Partial-correlation and multiple-regression procedures were used to test for associations of allele and conditional-allele frequencies with spatial and climatic variables. Frequencies were angularly transformed and standardized for sample size (Oakeshott *et al.* 1982). To reduce the problems with such analyses of detecting spurious associations, we restricted the numbers of spatial variables to two, and climatic variables to four, selecting those variables which tended to be uncorrelated within each of the two groups (see Oakeshott *et al.* 1982; Knibb 1982). Thus, the two spatial variables were *Lat* (latitude) and *Dinland* (the distance in km from collection site to the coast) and the four climatic variables were *Tmax* (mean of the daily temperature maximum in °C for the hottest week), *Tmin* (mean of the daily temperature minimum in °C for the coldest week), *Rmax* (the total rainfall in mm for the wettest week) and *Rmin* (the total rainfall in mm for the driest week). *Dinland* was $\log_{10}$ transformed, *Rmax* and *Rmin* were square-root transformed. Climatological data for each locality were taken from the nearest Australian Bureau of Meteorology station, the average distance between collecting locality and meteorology station being 22·7 ± 27·6 km, and the maximum 112 km. The climate variables were estimated as weekly normals for periods of at least 30 years by methods as in Keig and McAlpine (1969), and were supplied by the CSIRO Division of Land Use Research.

For those analyses which quantified the contributions of inversions to allele frequency differences among populations, p (equation 2) was estimated for a wide variety of different associations to provide robust estimates of $\bar{p}$. Hence, additional spatial and climatic variables were used, namely *Long* (longitude), *Ele*

(elevation in m), *Avtmax* (mean of the daily temperature average in °C for the hottest week) and *Avtmin* (mean of the daily temperature average in °C for the coldest week). *Ele* was $\log_{10}$ transformed.

Results

Contributions of Inversions to Allele Frequency Differences among Populations

Mean chromosome 2 inversion, *Est-1*, *Est-2* and conditional *Est-1* and *Est-2* allele frequencies and the significance levels for χ^2 homogeneity tests over the 19 Australian collections are given in Table 1. Clearly, inversion and *Est-1* and *Est-2* allele frequencies show significant differences among the 19 collections, but there are also strong linkage disequilibria of the inversions with *Est-1* and *Est-2* alleles, as indicated by the mean conditional *Est-1* and *Est-2* allele frequencies in 2*ST* and in 2*j*.

Table 1. Means with standard deviations in parentheses[A] over 19 Australian populations of (i) chromosome arrangement frequencies, (ii) *Est-1* allele frequencies in all chromosomes, in 2*ST* and in 2*j*, (iii) *Est-2* allele frequencies in all chromosomes, in 2*ST* and in 2*j*

Significance levels indicate the probability of χ^2 homogeneity over the 19 collections for each inversion/allele. $2jz^3$ is monomorphic for *Est-1*, and nearly so for *Est-2*, $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$

(i)	2*ST*	2*j*	$2jz^3$		
	40·2 (16·5)***	58·4 (16·2)***	1·5 (2·4)***		
(ii)	*Est-1*^{a}	*Est-1*^{b}	*Est-1*^{x}	*Est-1*^{c}	
in all	17·9 (8·2)***	71·5 (10·4)***	6·3 (3·3)***	3·3 (2·6)***	
in 2*ST*	37·8 (13·6)***	45·2 (12·5)***	11·6 (7·8)***	4·2 (5·2)***	
in 2*j*	5·1 (5·4)***	90·4 (6·8)***	1·6 (1·9)*	2·4 (2·4)*	
(iii)	*Est-2*^{a}	*Est-2*^{b}	*Est-2*$^{c+}$	*Est-2*^{c}	*Est-2*^{d}
in all	39·2 (9·3)***	26·1 (6·9)***	13·1 (6·0)***	11·6 (5·7)***	9·6 (5·3)***
in 2*ST*	32·6 (15·5)***	25·1 (10·1)***	26·8 (11·0)***	10·4 (5·0)	4·2 (5·5)**
in 2*j*	45·9 (13·3)***	26·6 (9·0)***	3·0 (3·8)***	12·3 (8·4)***	11·8 (6·1)***

[A]Data abstracted from Knibb *et al.* 1987, excluding null and rare alleles.

If all *Est-1* and *Est-2* allele frequency differences among collections were due simply to hitch-hiking with the inversions, then we would expect the conditional allele frequencies within 2*ST* and within 2*j* to be invariant among the populations. This clearly is not the case (Table 1), indicating that the allele-inversion disequilibria vary among populations.

Since the contributions of inversions to the spatial variation of *Est-1* and *Est-2* allele frequencies were not complete, we need to examine the 'proportional' contributions (equation 2). This proportion can be estimated for any specified allele-spatial (or climatic) association, but some idea of the overall potential contributions of inversions is needed. Thus p has been calculated for all the associations with $P < 0{\cdot}1$ in the present data (Table 2). However, some allele frequencies at each locus are themselves correlated over populations (*Est-1*^{a} with *Est-1*^{b}, $r = -0{\cdot}91$, $P < 0{\cdot}001$; *Est-1*^{a} with *Est-1*^{c}, $r = 0{\cdot}67$, $P < 0{\cdot}01$; *Est-1*^{b} with *Est-1*^{c}, $r = -0{\cdot}78$, $P < 0{\cdot}001$; *Est-2*^{a} with *Est-2*^{b}, $r = -0{\cdot}59$, $P < 0{\cdot}01$; *Est-2*^{a} with *Est-2*^{d}, $r = -0{\cdot}53$, $P < 0{\cdot}05$; all coefficients calculated using untransformed and unstandardized allele frequencies). Therefore, a conservative estimate of p for a given allele-spatial (or climatic) association would be the largest p value of those estimated for the given allele and its significantly correlated alleles.

Hence, we conclude for *Est-1* that the inversions account for a large proportion of all the tabulated associations, except perhaps those of *Est-1*^{x} with *Lat*, and *Est-1*^{a} and *Est-1*^{b} with *Rmax*. For *Est-2*, however, p was consistently large for just one allele (*Est-2*$^{c+}$); this is most likely to have been a reflection of the greater disequilibrium for this allele with the inversions than that for the other *Est-2* alleles (Table 1). Thus, we conclude for this locus that the inversions account, on average, for about one half of each association for *Est-2*$^{c+}$, but for only a very small proportion for the other alleles.

Table 2. Simple correlations of untransformed and unstandardized inversion and gene frequencies with spatial and climatic variables

Only correlation coefficients with probabilities less than 0·1 are tabulated. The proportion of a given association attributed to gene-inversion disequilibrium is given in parentheses. $^{+}P < 0{\cdot}1$; $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$

	Lat	Long	Dinland	Ele	Tmax	Tmin	Avtmax	Avtmin	Rmax	Rmin
ST	−0·42+			0·40+	0·65**		0·62**			−0·66**
j		0·40+		−0·42+	−0·66**		−0·59**			0·70**
jz[3]	0·56*	−0·62**						−0·48*	−0·45+	
Est-1[a]		−0·57*							−0·42+	
		(0·40)							(0·23)	
x	−0·62**				0·50*		0·55*			−0·40+
	(0·27)				(0·62)		(0·50)			(0·72)
b		0·49*			−0·45+				0·41+	0·46*
		(0·48)			(0·92)				(0·27)	(0·91)
c										
Est-2[a]			−0·42+			0·43+			0·40+	
			(0·14)			(0·06)			(0·09)	
b		0·60**					0·44+			
		(0·03)					(0·00)			
c+		−0·50*	0·48*	0·58*	0·52*				−0·45+	−0·62**
		(0·43)	(0·41)	(0·39)	(0·75)				(0·21)	(0·65)
c			−0·49*	−0·55*	−0·53*		−0·42+			0·74***
			(0·05)	(0·05)	(0·00)		(0·08)			(0·05)
d	0·45+					−0·47*		−0·55*	−0·43+	
	(0·46)					(0·00)		(0·12)	(0·00)	

Conditional Allele Frequency Variation among Populations

As noted previously, *Est-1* and *Est-2* conditional allele frequencies varied among populations.

The partial correlations and multiple regressions of conditional *Est-1* allele frequencies with *Lat* and *Dinland* are given in Table 3, while those with climatic measures are given in Table 4.

Table 3. Partial correlations and multiple regressions of (A) unadjusted *Est-1* allele frequencies, (B) conditional *Est-1* allele frequencies in the *ST* chromosomes, and (C) conditional *Est-1* allele frequencies in the *j* chromosomes with spatial variables

$^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$

	Allele	Partial correlations[A]		Multiple regressions[B]		
		Lat	Dinland	Lat	Dinland	r^2
(A)	a					
	x	−0·60**		−0·60**		0·36
	b					
	c					
(B)	a					
	x	−0·66**		−0·66**		0·44
	b					
	c					
(C)	a	0·87***	−0·52*	0·81***	−0·27*	0·80
	x	0·60**		0·55*		0·30
	b	−0·78***		−0·79***		0·62
	c					

[A]For example, the partial correlations with *Lat* control for *Dinland*. Coefficients are given only if they are significant.

[B]Beta values are given only if they are significant. The multiple r^2 is the proportion of variance explained by the significant Beta values (similar tabulation procedures apply to Tables 4, 5, 6).

Table 4. Partial correlations and multiple regressions of (A) unadjusted *Est-1* allele frequencies, (B) conditional *Est-1* allele frequencies in the *ST* chromosomes, and (C) conditional *Est-1* allele frequencies in the *j* chromosomes with climatic variables
$^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$

	Allele	Partial correlations				Multiple regressions				
		Tmax	Tmin	Rmax	Rmin	Tmax	Tmin	Rmax	Rmin	r^2
(A)	a			−0·53*				−0·46*		0·21
	x									
	b		−0·58*	0·64**					0·46*	0·21
	c		0·57*	−0·64**						
(B)	a							−0·46*		0·21
	x									
	b							0·47*		0·22
	c		0·60*	−0·65**A						
(C)	a			−0·52*			−0·75***	−0·55**		0·59
	x									
	b			0·54*			0·67**	0·63**		0·57
	c									

[A]Remains significant after controlling for spatial variables. For the partial correlations, controlling was for three climatic variables and *Lat* and *Dinland*. Standardized residuals from the multiple regressions of allele frequencies on *Lat* and *Dinland* (forced) were used to re-evaluate the tabulated regression coefficients. (Similar procedures were used in Table 6).

Similar analyses for *Est-1* allele frequencies unadjusted for inversions are also tabulated to enable comparisons with the previous analyses of Mulley *et al.* (1979) and Sokal *et al.* (1987). As expected from the preceding analyses, there were many significant spatial and climatic associations for conditional *Est-1* allele frequencies that were not evident without adjustment for inversions. Following the rationale of Mulley *et al.* (1979) for inferring evidence of selection from climatic associations, we repeated the analyses of Table 4 after controlling for spatial variables. Then only one of the 17 associations originally significant in Table 4 remained so, and one case out of 17 could be accounted for by chance.

Table 5. Partial correlations and multiple regressions of (A) unadjusted *Est-2* allele frequencies, (B) conditional *Est-2* allele frequencies in the *ST* chromosomes, and (C) conditional *Est-2* allele frequencies in the *j* chromosomes with spatial variables
$^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$

	Allele	Partial correlations		Multiple regressions		
		Lat	Dinland	Lat	Dinland	r^2
(A)	a		−0·47*			
	b			−0·47*		0·22
	c+				0·46	0·21
	c					
	d					
(B)	a	−0·62**	−0·65**	−0·54**	−0·58**	0·55
	b					
	c+	0·65**	0·68**	0·56**	0·60**	0·58
	c					
	d					
(C)	a					
	b					
	c+					
	c		−0·53*		−0·53*	0·28
	d		0·55*		0·55*	0·29

The only evidence for related spatial associations for frequencies (of a given *Est-1* allele) in 2*ST* and in 2*j*, was that for *Est-1*^{x}, which in 2*ST* was negatively and significantly associated with *Lat*, whereas in 2*j* the same association was significantly positive. Two cases of concordance of climatic associations for frequencies (of a given *Est-1* allele) in 2*ST* and in 2*j* were evident, albeit only in the multiple regression analyses. They were, firstly, negative associations for *Est-1*^{a} in 2*ST* and in 2*j* with *Rmax*, and positive associations for *Est-1*^{b} in 2*ST* and in 2*j* also with *Rmax*. These two cases may not be independent, because of the lack of independence between the alleles themselves. Direct examination of simple correlation coefficients between frequencies (of a given *Est-1* allele) in 2*ST* and in 2*j* confirmed one significant association, which was for *Est-1*^{x} ($r = -0{\cdot}46$, $P < 0{\cdot}05$), though the correlation for *Est-1*^{a} approached significance ($r = 0{\cdot}41$, $P < 0{\cdot}1$).

For *Est-2*, there were significant spatial and climatic associations for conditional allele frequencies not previously evident without adjustment for the inversions (Tables 5 and 6). After controlling for spatial variables, eight of the 22 associations originally significant in Table 6 remained so; seven of the eight involved *Rmin*.

Table 6. Partial correlations and multiple regressions of (A) unadjusted *Est-2* allele frequencies, (B) conditional *Est-2* allele frequencies in the *ST* chromosomes, and (C) conditional *Est-2* allele frequencies in the *j* chromosomes with climatic variables

$^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$

	Allele	Partial correlations				Multiple regressions				
		Tmax	Tmin	Rmax	Rmin	Tmax	Tmin	Rmax	Rmin	r^2
(A)	a	−0·56*			−0·64**^{A}					
	b	0·52*								
	c^{+}								−0·60**	0·36
	c			−0·51*	0·71**^{A}				0·73***^{A}	0·60
	d						−0·47*			0·22
(B)	a							0·69**		0·48
	b									
	c^{+}	−0·52*			−0·62*^{A}			−0·78***		0·61
	c									
	d									
(C)	a	−0·66**			−0·82***^{A}	−1·11***^{A}			−1·37***^{A}	0·70
	b									
	c^{+}									
	c		0·68**	−0·68**	−0·73** ^{A}				0·71***	0·50
	d		−0·55*				−0·53*			0·28

^{A}Remains significant after controlling for spatial variables.

There was no evidence for a concordance of associations for given *Est-2* alleles in 2*ST* and in 2*j* in either Table 5 or Table 6. Direct examination of simple correlation coefficients between frequencies (of a given *Est-2* allele) in 2*ST* and in 2*j* revealed just one significant association, which was for *Est-2*$^{c+}$ ($r = -0{\cdot}54$, $P < 0{\cdot}05$).

Discussion

The significant spatial and climatic associations evident for all *Est-1* allele frequencies typically are accounted for by their linkage disequilibria with the inversions and the covariance of inversion frequencies with the respective environmental variables. This indicates that the linked inversions make major contributions to the precise nature of the spatial variation of *Est-1* allele frequencies. Consequently, the utility of using such spatial data, without consideration of inversions, to identify putative selective factors at this locus would seem rather limited.

On the contrary, the inversions make only minor contributions to the respective associations of *Est-2* alleles, with the exception of *Est-2*$^{c+}$. The exceptional status of *Est-2*$^{c+}$ appears to

be a direct consequence of the nature of its linkage disequilibrium with the inversions, being far stronger and more consistent in direction across populations than those for the remaining *Est-2* alleles (see Knibb *et al.* 1987).

It should be noted that *Est-2*$^{c+}$ has further exceptional features: conditional *Est-2*$^{c+}$ allele frequencies in 2*ST* (to which *Est-2*$^{c+}$ is largely restricted) show the strongest spatial and climatic associations of all *Est-2* alleles (Table 6), and it was the only *Est-2* allele to show a significant correlation between its frequences in 2*ST* and 2*j*. Additionally, unpublished data indicate that, of all *Est-2* allele frequencies, those of *Est-2*$^{c+}$ show the clearest regular seasonal patterns which recur over years and in different populations. Taken together, this is strong evidence for selection on *Est-2*$^{c+}$. However, *Est-2*$^{c+}$ has another exceptional property: its electromorph on cellulose-acetate gels has an unusual triple-banded appearance, with the relative staining intensities of each band varying ontogenetically, whereas the remaining *Est-2* alleles code for monomeric enzymes (Knibb *et al.* 1987 and references therein). This raises the possibility that *Est-2*$^{c+}$ is not an allele at the *Est-2* locus and could be a closely linked polymorphic duplication, an explanation that is consistent with the apparently large selection coefficients.

Considering all alleles at *Est-1* and *Est-2*, significant spatial variation does remain for their frequencies after accounting for inversions. This is evident as significant differences among populations in conditional allele frequencies (Table 1), i.e. the gene-inversion disequilibria tend to differ among populations. However, the significant spatial and climatic associations for conditional allele frequencies at both loci tend to be different from those for the respective unadjusted allele frequencies (Tables 3–6). Nevertheless, while they differ in these associations, unadjusted and conditional allele frequencies do show similarities in the following respects. Overall, both categories of *Est-1* allele frequencies show significant spatial and climatic associations, but essentially no climatic associations remain after correcting for geographic location. For *Est-2*, both categories of allele frequencies show significant spatial and climatic associations, and many climatic associations remain after correcting for geographic location.

These overall patterns are analogous to those detected previously by Mulley *et al.* (1979), who suggested that they indicate natural selection on *Est-2*. Their recurrence here, in spite of differences between the previous and present analyses in collection sites, in the classification of some alleles and in statistical procedures, indicates the consistency of the phenomenon, and that this apparent selection at the *Est-2* locus is not accounted for by linked inversions.

Finally, for all alleles (except *Est-1*^{x} and *Est-2*$^{c+}$), there is no evidence for a concordance of conditional allele frequencies in 2*ST* with those in 2*j*. Thus, alleles in the two gene arrangements are not subject to common selection, or given electromorphs in 2*ST* and in 2*j* and are not necessarily the same allele. The latter has been suggested previously, at least for *Est-2* (East 1984). Studies of cryptic variation using sequential gel electrophoresis are in progress to provide further data on this latter possibility.

Acknowledgments

We thank Annette Edmonds and Don Fredline for their valued technical assistance. The research was done while W. R. Knibb was a National Research Fellow, and was supported by a grant from the Australian Research Grants Scheme to J. S. F. Barker.

References

Barker, J. S. F., and Mulley, J. C. (1976). Isozyme variation in natural populations of *Drosophila buzzatii*. *Evolution* **30**, 213–33.

Barker, J. S. F., Sene, F. de M., East, P. D., and Pereira, M. A. Q. R. (1985). Allozyme and chromosomal polymorphism of *Drosophila buzzatii* in Brazil and Argentina. *Genetica (The Hague)* **67**, 161–70.

Carson, H. L., and Wasserman, M. (1965). A widespread chromosomal polymorphism in a widespread species, *Drosophila buzzatii*. *Am. Nat.* **99**, 111–15.

East, P. D. (1984). Biochemical genetics of two highly polymorphic esterases in *Drosophila buzzatii*. Ph.D. Thesis, University of New England, Armidale.

Keig, G., and McAlpine, J. R. (1969). Austclimdata: a magnetic tape with estimated mean weekly climatic data for the Australian continent. Aust. CSIRO Div. Land Res. Tech. Mem. No. 69/14.

Knibb, W. R. (1982). Chromosome inversion polymorphisms in *Drosophila melanogaster*. II. Geographic clines and climatic associations in Australasia, North America and Asia. *Genetica (The Hague)* **58**, 213–21.

Knibb, W. R., East, P. D., and Barker, J. S. F. (1987). Polymorphic inversion and esterase loci complex on chromosome 2 of *Drosophila buzzatii*. I. Linkage disequilibria. *Aust. J. Biol. Sci.* **40**, 257–69.

Mulley, J. C., James, J. W., and Barker, J. S. F. (1979). Allozyme genotype–environment relationships in natural populations of *Drosophila buzzatii*. *Biochem. Genet.* **17**, 105–26.

Oakeshott, J. G., Gibson, J. B., Anderson, P. R., Knibb, W. R., Anderson, D. G. and Chambers, G. K. (1982). Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in *Drosophila melanogaster* on different continents. *Evolution* **36**, 86–96.

Sokal, R. R., Oden, N. L., and Barker, J. S. F. (1987). Spatial structure in *Drosophila buzzatii* populations, simple and directional spatial autocorrelation. *Am. Nat.* **129**, 122–42.

Voelker, R. A., Cockerham, C. C., Johnson, F. M., Schaffer, H. E., Mukai, T., and Mettler, L. E. (1978). Inversions fail to account for allozyme clines. *Genetics* **88**, 515–27.

Manuscript received 15 July 1987, accepted 12 January 1988