Chromosome inversion polymorphisms in *Drosophila melanogaster* IV. Inversion and *Adh* allele frequency changes under selection for different development times

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Replicate lines from two wild-caught populations of *D. melanogaster* were selected for fast, intermediate or slow development times. After 36 generations of selection, the average time to the first emergence of adults was about half a day less in lines selected for fast development than it was in the other lines. Polymorphisms for *Adh* and six chromosome inversions were monitored during the course of selection. Average *Adh* *F* allele frequencies become higher over the 36 generations in lines selected for fast development than in other lines. Collating this result with earlier reports, an association with development time has now been found for three enzyme polymorphisms in laboratory *D. melanogaster* populations selected for rapid development. Allele frequencies at all three loci show latitudinal clines in natural populations, as does development time, and for each of the three polymorphisms the allele associated with rapid development in the laboratory selection lines is also associated with rapid development in the latitudinal clines. This suggests that development time selection may contribute to the latitudinal clines for the enzyme polymorphisms. The frequencies of four inversions showing latitudinal clines generally decreased during the experiment, while two inversions not showing latitudinal clines generally persisted or increased in frequency. Only one inversion, *In(3R)Mo*, showed clear differences in response among different development time regimes and this inversion does not show a latitudinal cline. There was thus no evidence that geographic patterns in inversion frequencies are the result of development time selection.

INTRODUCTION

Large scale latitudinal clines have been reported in the frequencies of four common chromosome inversions and alleles at six polymorphic enzyme loci in natural populations of *D. melanogaster* (e.g., Knibb, 1982; Oakeshott *et al.*, 1984b). It is difficult to explain the consistency of these clines across continents except by the action of natural selection (Anderson and Oakeshott, 1984; Anderson *et al.*, 1987). However, laboratory experiments have generally failed to elucidate specific mechanisms of selection operating on such polymorphisms

(Oakeshott *et al.*, 1985; Eanes *et al.*, 1985 and references therein).

These laboratory experiments commonly involve monitoring experimentally perturbed inversion or allele frequencies in wild-caught populations kept in different environments. Two patterns of results often obtained are the rapid elimination of the inversions from the populations and the restabilisation of the enzyme allele frequencies around values characteristic of the original wild-caught populations (e.g., Lewontin, 1958; Inoue, 1979; Carfagna *et al.*, 1980; Palabost-Charles, 1982). Moreover, essentially the same results are often obtained in all the environments tested, despite the imposition of some relatively

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severe stresses, including, in relation to the enzymes, regimes which should impinge directly on the functions of the specific enzymes tested (e.g., extremes of substrate concentration; Zera *et al.*, 1983 for a review). Despite the different results for the inversions and enzymes, it seems that both patterns may be due to selection, but not to selection imposed directly by the specific external environments compared.

Various alternative explanations have been offered for the laboratory behaviour both of the inversions and of the enzyme polymorphisms. Although differing in other respects, one proposition common to most of these explanations is that the interactions of genes with one another may be as influential as their interactions with the external environment in determining the selective pressures on polymorphism. The important genetic interactions proposed may derive from gametic disequilibrium among linked genes (Lewontin, 1958; Mukai and Yamazaki, 1980; Yamazaki *et al.*, 1983) or from biochemical interactions among gene products, in so much as the physiological environment in which each gene's product functions depends closely on the products of other genes (Oakeshott *et al.*, 1984a, 1985).

Many studies have now documented selective effects of the genetic background on particular polymorphisms due to their gametic disequilibrium with linked genes (e.g., Powell, 1973; Barnes, 1983). This paper is more concerned with background effects due to physiological interactions among gene products, an area which has so far received less attention. Our approach, based on experiments with *D. melanogaster* by van Delden and Kamping (1979) and Cavener (1983), has been to monitor changes in inversion and allozyme frequencies during selection on a major fitness character. The character chosen for study was development time, because it is the outcome of many metabolic and developmental processes, selection on which may significantly affect the physiological environment (Church and Robertson, 1966; Cluster *et al.*, 1987). Moreover, the character displays polygenically inherited latitudinal variation in both *D. melanogaster* and *D. subobscura*; development in standard laboratory conditions is slower in populations collected from close to the equator than it is in populations from higher latitudes (McFarquar and Robertson, 1963; Cavener, 1983).

Van Delden and Kamping (1979) and Cavener (1983) have already presented some evidence that selection for faster development may affect allele frequencies for three enzyme polymorphisms in *D.*

melanogaster (*Adh*, *Gpdh* and *6Pgd*). Furthermore, in all three cases the allele associated with fast development was also the allele found at higher frequencies in natural populations living at higher latitudes. The directions of these effects are thus consistent with the hypothesis that selection on these three polymorphic enzymes is related to their physiological environments as assessed by development time.

Our experiment further tests this hypothesis by monitoring the frequencies of several inversions and alleles at one enzyme locus in populations of *D. melanogaster* selected for fast, intermediate and slow development times. Some of the inversions monitored show latitudinal clines while others do not (Knibb, 1982). The enzyme polymorphism monitored is *Adh* (alcohol dehydrogenase), allele frequencies for which were associated with development time in the experiment of van Delden and Kamping (1979) but not in that of Cavener (1983).

MATERIALS AND METHODS

Over 400 *D. melanogaster* were collected from each of two localities, *Cleveland* and *Ipswich*, situated about 55 km apart at 28°S, 153°E in eastern Australia. Thirty six isofemale lines were established from wild-caught females from each locality. About 300 other wild-caught flies from each population were scored for the polymorphic *F* and *S* *Adh* allozymes by the methods of Lewis and Gibson (1978). About 50 gametes from each population were screened for chromosomal inversions by scoring the polytene karyotypes of the progeny of wild-caught males mated to females of a standard sequence laboratory stock (Knibb, 1982).

The 36 isofemale lines from each locality were combined by adding 12 inseminated first generation females from each line to a large population cage. Twelve selection lines were then founded by leaving six smaller cages open in each of the two large cages. Each smaller cage measured 21 × 21 × 8 cm high and five 50 ml food cups were attached to each (food recipe as in Oakeshott *et al.*, 1983) except that agar concentration was 0.8 per cent and yeast 1.2 per cent). After 5 days each smaller cage was emptied of adults, sealed and removed. Of the six selection lines for each locality founded in this way, two each were randomly assigned to Fast, Intermediate and Slow development time selection regimes.

The three selection regimes were established by only using progeny emerging as adults during the first 3, 4th through 6th, and 7th through 9th

days of emergence, respectively, as parents for the following generation. These parents were transferred to fresh small cages as soon as they were collected and removed 5 days later. Such selection was continued for 36 consecutive generations at $22 \pm 2^\circ\text{C}$. The average numbers of parents used in each selection line in each generation after the first were 742 for the Fast, 550 for Intermediate and 530 for the Slow selection regimes. *Adh* allele and inversion frequencies were estimated after 12, 24 and 36 generations of selection, with an average of 625 alleles scored for *Adh* and 53 gametes for inversions in each line at each scored generation.

The statistical methods of Wilson and Oakeshott (1985) were applied to the frequency data to estimate the various selection coefficients operating on the *Adh* and inversion genotypes. In the analysis below each selection line is denoted by a three symbol code (*l, d, r*) defining the locality from which it originated (*l* = *Cleveland*, *Ipswich*), the development time regime to which it was assigned (*d* = Fast, Intermediate, Slow), and a replicate number (*r* = 1, 2). Selection coefficients were estimated by maximum likelihood methods for each line and for various groups of lines defined by *l, d* or *r* values. Differences among the coefficients for different lines and their groups were assessed by analysis of deviance.

RESULTS

Selection response could be assessed from the time to emergence of the first adult progeny in each line at each generation. During the course of selection this time dropped by 0.4 ± 0.3 days in the Fast selection lines and rose by 0.1 ± 0.3 and 0.3 ± 0.4 days in the Intermediate and Slow lines respectively. The changes in the Intermediate and Slow

selected lines were not significantly different from each other but the eventual difference of 0.6 ± 0.3 days between the Fast selected lines and the others was statistically significant ($P < 0.05$ on a 1-tailed *t*-test). This compares with an average difference of 0.9 days between the lines Cavener (1983) selected for fast and slow development.

The changes in *Adh* allele frequencies in the 12 selection lines over the 36 generations of selection are shown graphically in fig. 1. The frequency of the *Adh F* allele rose consistently in three Fast lines and one Intermediate line, fell consistently in one Slow line, while remaining relatively close to initial values in the remaining lines. *Adh* genotype frequencies did not differ significantly from Hardy-Weinberg expectations in either of the initial *Cleveland* and *Ipswich* samples or in 32 of the 36 subsequent samples from the selection lines. (The exceptions involved one heterozygote excess, in a *Cleveland* Fast line, and three heterozygote deficits in a *Cleveland* Fast, an *Ipswich* Fast and an *Ipswich* Slow line). However, sign tests revealed a significant trend across lines for deviations in heterozygote frequencies from Hardy-Weinberg expectations. Thus, there was no consistent direction to the deviations among the 12 samples from the Fast selection lines (six excesses, six deficits), but a significant majority of samples from the other lines (18 of 24, $P < 0.05$) showed heterozygote excesses.

The initial *Cleveland* and *Ipswich* collections were each polymorphic for the four common cosmopolitan paracentric inversions, *In*(2*L*)*t*, *In*(2*R*)*NS*, *In*(3*L*)*P* and *In*(3*R*)*P*, which show world-wide latitudinal clines. The frequencies of all four inversions declined steadily in all 12 selection lines and in 31 of 48 cases had become zero after 36 generations (fig. 2). There were no obvious differences in the dynamics of these inversions

Figure 1 Changes in *Adh F* frequency (Y axis, range 0–100%) over the 36 generations of selection (X axis) in the *Cleveland* and *Ipswich* Fast, Intermediate and Slow development time selection regimes. The two lines on each graph represent the two replicate populations.

among lines from the two localities or three selection regimes.

Two other paracentric inversions were recorded at polymorphic frequencies (>1 per cent) in either or both of the initial *Cleveland* and *Ipswich* collections. These were the rare cosmopolitan *In(3R)C* and *In(3R)Mo* inversions, which have been reported at low frequencies in many populations world-wide and do not show latitudinal clines (Knibb, 1982). The dynamics of these inversions during the experiment differed markedly from those of the four common cosmopolitans (fig. 3). After 36 generations the frequencies of *In(3R)C* and *In(3R)Mo* were higher than initial values in 9 out of 18 cases and had only declined to zero in 5 cases. The largest increases occurred in the *Ipswich* Fast lines, in both of which *In(3R)Mo*

frequency rose rapidly from about 5 per cent initially, to stabilise around 50 per cent. *In(3R)Mo* was not found in *Cleveland* but other smaller increases were distributed across both inversions, both localities and all three selection regimes.

Maximum likelihood methods were applied to the frequency data to estimate the selection coefficients, s and t operating on the *Adh* and inversion polymorphisms. Analyses of deviance were carried out to test the significance of differences in the coefficients, first from zero and then among localities, development time regimes and replicate lines. The procedures are described in full in Wilson and Oakeshott (1985) and their applications to analogous data sets are given in Oakeshott *et al.* (1984a, 1985) and references therein. In the present experiment the selection

Figure 2 Changes in the frequencies (%) of the four common cosmopolitan inversions over the 36 generations of selection. Format and scales are as in fig. 1.

Figure 3 Changes in the frequencies (%) of the two initially rare inversions *In(3R)C* and *In(3R)Mo* over the 36 generations of selection. *In(3R)Mo* was never recorded in the *Cleveland* lines. Format and scales are as in fig. 1.

coefficients defined relative fitnesses of

$$\begin{array}{ccc} 1 & : & 1+t & : & 1+s \\ \text{for the } S/S & : & F/S & : & F/F \end{array}$$

Adh genotypes, and

$$\text{for the } St/St : In/St : In/In$$

inversion genotypes (*St* = standard sequence, *In* = inversion). Four inversion polymorphisms were analysed, one for each major autosomal arm. The polymorphisms on the 2L, 2R and 3L arms each comprised just two arrangements, *St* versus *In(2L)t*, *In(2R)NS* and *In(3L)P* respectively. However, it was necessary to pool the frequencies of the three 3R inversions (*In(3R)P*, *In(3R)C* and *In(3R)Mo*) because there were insufficient data to estimate individual selection coefficients for all ten 3R genotypes possible. In view of the differences in the dynamics of the 3R inversions, it was difficult to interpret this particular analysis.

Deviance values were calculated for six different models of selection on each of the five polymorphisms analysed. The deviance indicated the goodness of fit of each model to the observed frequency dynamics. The difference in deviance between two models differing by only a single factor indicated the improvement in fit due to fitting different *s* and *t* values according to that factor. The significance of the improvement in fit was straightforward to assess because the deviance difference was distributed as a χ^2 with degrees of freedom given by the difference in the numbers of separate *s* and *t* values fitted in the two models.

The simplest model was a neutral one (0, 0, 0) in which *s* and *t* were set at zero in all lines. The

difference in deviance from this model provided a test of the second ($\cdot, \cdot, \cdot$), which fitted common non-zero *s* and *t* in all selection lines. The third and fourth models, ($l, \cdot, \cdot$) and ($\cdot, d, \cdot$), allowed *s* and *t* to vary among localities and development times respectively and were each tested against the second. The fifth model ($l, d, \cdot$) allowed *s* and *t* to vary among the different combinations of localities and development time regimes and was tested against the fourth. The sixth model (l, d, r) was fully parameterised, allowing *s* and *t* to vary across all selection lines, and was tested against the fifth.

The magnitudes of the deviances and their differences were positively correlated with the effective population size assumed in the model fitting (because random effects were less able to explain the observed frequency dynamics). However, the observed population sizes were known (see Materials and methods) and the ratio *f*, of effective to observed population size, was estimated by the Monte Carlo simulation methods of Wilson and Oakeshott (1985). Simulations were carried out separately for two polymorphisms, *Adh* and *In(2L)t*, and both sets suggested that the 95 per cent confidence limits of *f* were 0.05 and 0.30. The analyses below are carried out for both *f* = 0.05 and *f* = 0.30, as well as for their mean, *f* = 0.175.

For *Adh*, table 1 shows that no model gave a significant deviance decrease at *f* = 0.05 but two models, ($\cdot, \cdot, \cdot$) and ($\cdot, d, \cdot$), did so at *f* = 0.175 and a third ($l, d, \cdot$) was also significant at *f* = 0.30. The ($\cdot, \cdot, \cdot$) model represented selective effects on *Adh* common to all lines but was difficult to interpret because of the significance of the other two, more complex models. Thus, the ($\cdot, d, \cdot$) model

indicated that the selection on *Adh* differed among development time regimes while the $(l, d, \cdot)$ model further implied interaction effects, in the form of variation in selection among the six combinations of localities and development times. Inspection of component deviances indicated that most of the development time effect was due to the increase in *Adh* *F* frequency in three of the four fast development time lines. The fact that one *Cleveland* fast development line did not show this increase (fig. 1) could account for the weak locality-development time interaction effect.

Fig. 4 shows the 95 per cent confidence contours of the *s* and *t* estimates for *Adh* in each of the six locality- development time combinations. The contour for the *Ipswich* Fast lines was relatively tightly defined and indicated significant positive *s*, whereas the other five contours were less defined, although generally indicating positive, and in some cases significant *t*. Thus, at $f=0.30$ at least, the *Ipswich* Fast data were explained by the selective advantage of *F/F* homozygotes, while the data for the other lines were generally consistent with *F/S* heterozygote advantage.

Fitting $(\cdot, \cdot, \cdot)$ models produced highly sig-

nificant deviance decreases for all four inversion polymorphisms at all three *f* values (table 1). No other model was significant at all three *f* values for any of the four polymorphisms. The major form of selection on the inversions thus involved common non-zero *s* and *t* values for each polymorphism in all selection lines. The 95 per cent confidence contours for the estimates of these *s* and *t* values are given in fig. 5 and as expected from the analyses of deviance, none of the contours overlap the origin (where $s=t=0$). All four contours are almost entirely in regions of negative *t* and most of each contour also lies in regions of negative *s*. Except for the *3R* polymorphism (see below) we therefore conclude that the inversions were selected against, certainly as heterozygotes and probably as homozygotes as well, in lines from both localities under all three selection regimes.

For *In(2L)t* and *In(3L)P*, the only significant model apart from $(\cdot, \cdot, \cdot)$ was (l, d, r) , which implied some variation in selection among replicate lines. However this variation was only significant at $f=0.30$. For *In(2R)NS*, the $(l, \cdot, \cdot)$ and $(l, d, \cdot)$ models were significant at both $f=0.175$ and at $f=0.30$. These effects could be attributed

Figure 4 The 95 per cent confidence contours for the maximum likelihood estimates of the selection coefficients *s* and *t* operating on *Adh* in each of the six combinations of localities and development time regimes. Values of *s* apply to the fitness of *F/F* relative to *S/S* and values of *t* to the fitness of *F/S* relative to *S/S*. Best estimates of *s* and *t* along with origins (*s* and *t* = 0) are indicated with crosses. All contours are calculated assuming $f=0.30$ (see text).

Table 1. Deviance differences (ΔD) for testing a series of models of selection on the Adh and inversion polymorphisms. f is the ratio of effective to observed population size assumed during model fitting. $(\cdot, \cdot, \cdot)$ = common non-zero s and t in all lines; $(l, \cdot, \cdot) = s$ and t vary between the two localities; $(\cdot, d, \cdot) = s$ and t vary among the three development time regimes; $(l, d, \cdot) = s$ and t vary among the six combinations of localities and development time regimes; $(l, d, r) = s$ and t vary between replicate lines within locality-development time combinations

Model tested	df	0.05	Adh ΔD at $f =$		$In(2L)t$ ΔD at $f =$	0.05	0.30	$In(2R)NS$ ΔD at $f =$	0.05	0.175	0.30	$In(3L)P$ ΔD at $f =$	0.05	0.175	0.30	$In(3R)$ ΔD at $f =$
$(\cdot, \cdot, \cdot)$	2	2.6	7.7*	12.5**	84.2***	29.3***	134.0***	14.6***	42.7***	67.5***	6.9*	19.8***	31.3***	14.1***	33.3***	50.0***
$(l, \cdot, \cdot)$	2	0.5	1.5	2.5	3.1	1.2	4.0	4.5	9.2*	12.1**	1.1	2.0	2.5	1.5	4.6	7.5*
$(\cdot, d, \cdot)$	4	4.3	11.4*	18.1**	4.7	2.1	6.3	2.5	6.2	8.9	0.4	1.1	1.6	2.9	12.0*	19.0***
$(l, d, \cdot)$	6	3.2	8.8	14.0*	6.3	2.9	8.8	5.7	12.7*	17.4**	1.5	3.1	4.3	6.8	11.9	15.0*
(l, d, r)	12	4.6	12.5	19.6	20.6	9.3	28.4**	3.8	8.9	13.0	8.5	20.3	28.8**	3.4	5.3	8.2

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

Figure 5 The 95 per cent confidence contours of s and t estimates for the four inversion polymorphisms calculated for the total data over all 12 selection lines. Values of s apply to the fitness of In/In relative to St/St and values of t to the fitness of In/St relative to St/St . All contours are calculated assuming $f = 0.05$. Otherwise the format is as in fig. 4.

to the *Ipswich* Fast development time data, since these data contributed about half the deviance difference in each case and their exclusion from the analysis left both models non-significant.

For the 3R polymorphism, the $(\cdot, d, \cdot)$ model was significant at $f = 0.175$ and this and the $(l, \cdot, \cdot)$, and $(l, d, \cdot)$ effects were all significant at $f = 0.30$. At least in part this complexity resulted from the necessity of analysing three different 3R inversions together, even although their frequency dynamics differed. As detailed earlier, $In(3R)P$ was initially at frequencies around 40 per cent but rapidly declined to values averaging about 5 per cent, while $In(3R)C$ and $In(3R)Mo$ frequencies were initially less than 5 per cent but generally persisted or increased. In particular, $In(3R)Mo$, which was only found in *Ipswich* lines, rose to around 50 per cent in the Fast development time lines. If the *Ipswich* Fast data were omitted from the analysis, then the only model still significant was $(\cdot, \cdot, \cdot)$.

Lastly, it was possible that *Adh* frequency changes were in part the result of "hitch-hiking" with the linked $In(2L)t$ (Knibb, 1983). However, even assuming the most extreme case, that all $In(2L)t$ sequences carried *Adh S*, comparisons of *Adh* and $In(2L)t$ frequency changes (figs. 1 and 2) revealed that $In(2L)t$ could have made only minor contributions to *Adh* dynamics before generation 24, and thereafter $In(2L)t$ frequencies were generally negligible. Nor was there any obvious correlation between *Adh* and $In(2L)t$ frequency changes across different selection lines. We conclude that $In(2L)t$ did not contribute substantially

to the observed *Adh* frequency changes, although of course the possibility of effects due to gametic disequilibrium with other, unscored elements cannot be excluded (Yamazaki *et al.*, 1983).

DISCUSSION

Wild-caught populations of *D. melanogaster* were subject to selection for fast, intermediate and slow development times. After 36 generations of selection the average time to the first emergence of adults was about half a day less in the lines selected for fast development than it was in the other selection lines. This confirms earlier evidence of genetic variation for development times in wild-caught populations (Cavener, 1983 and references therein) and suggests that development times in natural populations are subject to balancing selective forces. The nature of the component forces is unclear but the balance apparently varies with latitude; clinal variation has been reported in the laboratory development times of populations collected from different latitudes (McFarquar and Robertson, 1963; Cavener, 1983). It has been the aim of the present analysis to determine whether these effects are associated with the latitudinal clines also recorded for several allozyme and inversion polymorphisms.

This study provided several indications that selection operated on the *Adh* polymorphism and, in *Ipswich* in particular, that the form of selection varied with the development time regime. Thus, *Adh F* frequency increased markedly in both *Ipswich* and one *Cleveland* Fast development lines and in one *Cleveland* Intermediate line, while it remained relatively steady in all other lines, except for a marked decrease in one *Cleveland* Slow line. Moreover, results of an analysis of deviance on the *F* frequency changes indicated variation in the selection among development time regimes, with *F/S* heterozygote advantage apparent in most lines except the Fast lines, particularly from *Ipswich*, in which the *F/F* homozygote was the fittest genotype.

The differences in selective effects of *Adh* among development time regimes were statistically significant in the analysis of deviance if the upper 95 per cent confidence limit, or a mean value for effective population size was assumed. However, they were not significant if the lower 95 per cent limit of effective population size was assumed in the analysis. Nevertheless, the results of the analysis were corroborated by inspection of genotype frequencies; there was a slight but consistent excess of heterozygotes over Hardy-Weinberg expectations in all except the Fast lines. Furthermore, the

advantage to *F/F* homozygotes under selection for fast development was consistent with the results of van Delden and Kamping (1979), albeit not with those of Cavener (1983), while *F/S* heterozygote advantage has been reported previously in several laboratory populations not deliberately selected for fast development (Oakeshott *et al.*, 1984a and references therein).

With the inclusion of the present *Adh* results, there is now evidence of associations with development time for all three enzyme polymorphisms in *D. melanogaster* tested for such relationships. The other two polymorphisms are *Gpdh* and *6Pgd* (Cavener, 1983). All three systems show latitudinal clines and in each case the allele associated with faster development becomes more frequent at higher latitudes (Oakeshott *et al.*, 1984b). The directions of the clines are thus consistent with the relationships between the particular alleles and development time, given that populations collected at high latitudes show faster development times in standard laboratory conditions than do those from lower latitudes (McFarquar and Robertson, 1963; Cavener, 1983). The present *Adh* results therefore support the proposition put forward in the Introduction that the developmental time and enzyme clines may be causally associated.

We suggest that in some part the physiological basis for such a causal connection may relate to known differences in the respective enzyme activity levels among the *Adh*, *Gpdh* and *6Pgd* genotypes (Oakeshott and Gibson, 1981; Cavener and Clegg, 1981; Palabost-Charles, 1982). Cluster *et al.* (1987) have recently shown that selection for rapid development leads to increased ADH and GPDH enzyme activity levels (they did not study 6PGD). This concurs with the increased frequencies of the high activity ADH F and GPDH S allozymes associated with rapid development in the studies of van Delden and Kamping (1979), Cavener (1983) and ourselves. ADH, GPDH and 6PGH activity levels all affect *in vivo* rates of fatty acid synthesis (Cavener and Clegg, 1981; Geer *et al.*, 1983, 1985, 1986) and such a central metabolic pathway may well be relevant to variation in development time.

There was unequivocal evidence for selection on the four common cosmopolitan inversions in the selection lines. The frequency of each inversion decreased steadily and analyses of deviance showed statistically significant selection in favour of standard sequence homozygotes, even at the lower 95 per cent confidence limit of effective population size. However, there was little evidence that the selection on the common cosmopolitans differed according to development time regime.

Unlike the four common cosmopolitans, the other two polymorphic inversions found in the selection lines do not show latitudinal clines in natural populations (Knibb, 1982). And these two inversions, *In(3R)C* and *In(3R)Mo*, also differed markedly from the common cosmopolitans in their dynamics during the course of our experiment. While initially rarer than the common cosmopolitans, *In(3R)C* and *In(3R)Mo* generally persisted or increased in frequency over the 36 generations of selection. Thus both the field and laboratory evidence indicate qualitatively different forms of selection operating on the two types of inversion.

Although there are no latitudinal clines for *In(3R)C* and *In(3R)Mo* to explain in terms of development time selection, the dynamics of *In(3R)Mo* in fact varied under different development time regimes. *In(3R)Mo* stabilised at higher frequencies in lines selected for fast development than it did in other lines. Clearly the laboratory behaviour of inversion frequencies under development time selection cannot explain the geographic distributions, either of the four common cosmopolitans, or of the two rarer inversions. This contrasts the evidence available for the three enzyme polymorphisms discussed above; allele frequencies at all three loci responded differently to development time selection and in all three cases the directions of the differences are consistent with the directions of their latitudinal clines.

It is not clear why the enzyme and inversion polymorphisms should differ in this respect but it is clear that further work is needed to substantiate the difference. It will be important to screen for development time associations among other enzyme polymorphisms either showing latitudinal clines (e.g., *Est6*, *G6pd*, *Odh*) or latitudinal homogeneity of allele frequencies (e.g., *Pgm*; Anderson and Oakeshott, 1984; Oakeshott *et al.*, 1984b). If the enzyme clines are indeed the result of latitudinally varying development time selection, then development time associations should not be found for the latitudinally invariant systems. However, they should be observed for the clinally varying polymorphisms and in particular, as has been found for *Adh*, *Gpdh* and *6Pgd*, the alleles which increase in frequency further from the equator should be associated with 'more rapid development'.

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