Population genetics of the metabolically related *Adh, Gpdh* and *Tpi* polymorphisms in *Drosophila melanogaster* : II. Temporal and Spatial Variation in an Orchard Population

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Summary

Seasonal and spatial variation in gene frequencies at 3 diallelic loci : alcohol dehydrogenase *(Adh)*, glycerophosphate dehydrogenase *(Gpdh)* and triosephosphate isomerase *(Tpi)*, have been studied in an orchard population of *D. melanogaster*. Gene frequency at the *Tpi* locus varied seasonally and was associated positively with total monthly rainfall measured both immediately prior to and concurrent with the month of collection. Temporal heterogeneity, not associated with the environmental parameters, was present at the *Adh* locus. *Gpdh*-F frequency was negatively associated with mean monthly maximum temperature measured prior to the time of collection.

Within the orchard site, spatial heterogeneity in gene frequency at the *Tpi* locus was observed within collections. A deficiency of *Gpdh* heterozygotes was observed in individual trap samples and among collections with traps pooled. Overall, this variation is interpreted as being due to sampling from a population of partially isolated subgroups, founded by few individuals, and dependent upon transient pockets of fruit resources.

Key words : Drosophila, *enzyme, polymorphism, orchard.*

Résumé

*Étude génétique du polymorphisme aux loci d'*Adh, Gpdh *et* Tpi
chez Drosophila melanogaster. *II. Variations temporelles et spatiales*
dans la population d'un verger

Les variations saisonnières et spatiales des fréquences géniques à 3 locus dialléliques, alcool déshydrogénase *(Adh)*, glycérophosphate déshydrogénase *(Gpdh)* et triosephosphate isomérase *(Tpi)* ont été étudiées chez *D. melanogaster* dans une population de verger. La fréquence génique au locus de *Tpi* varie avec la saison et est associée positivement à la pluviométrie mensuelle totale aussi bien pendant le mois de capture que durant celui qui précède la capture.

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Au locus d'*Adh,* on observe une hétérogénéité temporelle qui n'est pas liée aux para-
mètres environnementaux mesurés. La fréquence de l'allèle de *Gpdh* est corrélée négati-
vement à la température maximum moyenne du mois précédant la capture. Dans le verger,
on a observé une hétérogénéité spatiale (entre pièges intra-captures) de la fréquence
génique au locus de *Tpi*. On a également pu mettre en évidence un déficit d'hétérozygotes
au locus de *Gpdh* aussi bien au niveau des échantillons individuels qu'à celui de l'ensemble
des captures, tous les pièges étant réunis. Globalement cette variété est interprétée comme
l'incidence de l'échantillonnage dans une population subdivisée en groupes partiellement
isolés qui ont été constitués à partir d'un nombre réduit d'individus et qui doivent faire
face à des ressources fruitières temporaires et discontinues.

Mots clés : Drosophile, *enzyme, polymorphisme, verger.*

I. Introduction

Enzyme polymorphisms are ubiquitous in natural populations and have proven
to be useful tools in understanding the nature and intensity of natural selection
operating on single loci. This has been shown in recent studies on the *Pgi* locus in
Colias butterflies (WATT, 1983). Enzyme polymorphisms also provide a useful system
for understanding epistatic interactions, which are important components of the ge-
netic response of populations subject to environmental change (LEWONTIN, 1974 ; HE-
DRICK *et al.,* 1978). Studies on metabolically related enzymes in *D. melanogaster*
have made important contributions to this area (eg. BIJLSMA, 1978 ; CAVENER &
CLEGG, 1981 ; WILTON *et al.,* 1982). Also, enzyme polymorphisms may provide a
link between variation at the nucleotide level and variation at the phenotypic level
where the effects of selection can be detected. For example, the 2 common alleles
at the *Adh* locus of *D. melanogaster* differ by a single base substitution (KREITMAN,
1983). This difference has affected the ability of individuals to utilize ethanol-rich
environments, at least in the laboratory (VAN DELDEN *et al.,* 1978 ; OAKESHOTT *et al.,*
1980).

Field studies are essential in the detection of selective factors affecting enzyme
polymorphisms (CLARKE, 1975). We have initiated a field study of 3 metabolically-
related, polymorphic enzyme loci, with relatively high levels of heterozygosity, in an
orchard population of *D. melanogaster.* The enzymes chosen for study, alcohol dehy-
drogenase (ADH), glycerophosphate dehydrogenare (GPDH) and triosephosphate iso-
merase (TPI), are metabolically related and have the potential to influence rates of
triglyceride synthesis (CHIANG, 1972 ; GEER *et al.,* 1983, McKECHNIE & GEER, 1984).
Variation in enzyme activities may cooperatively influence metabolic flux (KASCER
& BURNS, 1981) and ultimately the phenotype and fitness of individuals. The study
of metabolically related enzymes has likely potential in detecting and understanding
epistasis and the forces which structure the genome.

Macrogeographic patterns of variation have been reported for all 3 of these
polymorphisms (BERGER, 1971 ; JOHNSON & SCHAFFER, 1973 ; PIPKIN *et al.,* 1973 ;
OAKESHOTT *et al.,* 1984) and latitudinal clines independent of chromosome inversion
associations have been established (OAKESHOTT *et al.,* 1982, 1984). Although these
geographic patterns have been correlated to climatic parameters, they give little insight
into causative environmental factors and their mode of action. In addition, when
such correlations are compared with those detected in temporal studies of single
populations, conflicting associations often occur. The frequency of the *Adh*-S allele,

for example, has been shown to be correlated both positively and negatively with temperature parameters (OAKESHOTT *et al.,* 1982 ; McKECHNIE & McKENZIE, 1983). Additional temporal studies of individual populations are required in order to establish any generality for the associations already reported for both *Adh* and *Gpdh* gene frequencies (or in the case of *Tpi,* to initiate such a study). Only then can we attempt to reconcile these data and identify causative environmental factors.

Microspatial patterns of variation at enzyme loci have recently been shown to occur in animal populations (SELANDER, 1970 ; RICHMOND, 1978 ; BURTON & FELDMAN, 1981 ; BARKER, 1981), often as a consequence of the breeding structure of the population. In *Drosophila,* microspatial variation has been shown to be associated with habitat type (TAYLOR & POWELL, 1977), and to be largely independent of habitat type (JAENIKE & SELANDER, 1979 ; MITTER & FUTUYAMA, 1979 ; LACY, 1983). It is important to establish the relative roles of gene flow and selective factors in determining the significance of spatial genetic variation in field populations.

Here, we describe a study of gene frequencies at the *Adh, Gpdh* and *Tpi* loci in an orchard population of *D. melanogaster.* Temporal patterns of variation and associations with environmental correlates are examined and our observations compared to the known patterns of geographic variation at these loci. Microspatial patterns of variation are also examined as the orchard carries a diversity of fruit resources. In addition, we look for evidence of gametic disequilibrium.

II. Materials and methods

A. *Collection of* Drosophila

Collections of *Drosophila* were made in an orchard at Wandin North, 35 km east of Melbourne, Australia (latitude 37.7° S, longitude 144.8° E). The orchard is planted with cherries (*Prunus cerasus*), apples (*Malus* spp.), plums (*Prunus* spp.) and peaches (*Prunus persica*). Collections were made over a 3 year period from January 1980 to December 1982. From January to May 1980, flies were aspirated directly from decomposing fruit. For all subsequent collections, banana bait traps were used. These were plastic boxes (23 cm × 30 cm × 10 cm) containing 2 decaying bananas. Funnels extending into the boxes provided entry for flies and minimised escape. Seventeen traps were placed in a grid pattern (50 m between traps) throughout the orchard (fig. 1). Collections were made at monthly intervals. From June 1980 to June 1981, traps were left in the orchard for 7 days. In order to boost winter sample sizes, traps were left for 14 days from July 1981. This procedure was continued for subsequent collections. The 2 week collection period was insufficient for eggs deposited on the baits to develop to eclosion due to low overnight temperatures.

Rainfall and temperature data, collected about 5 km from the orchard, were obtained from the Australian Bureau of Meteorology.

B. *Electrophoresis*

Flies of both sexes were individually ground in 10 µl distilled water, and their genotypes determined at the *Gpdh* and *Tpi* loci by starch gel electrophoresis (McKECHNIE *et al.,* 1981) and at the *Adh* locus by cellulose acetate electrophoresis
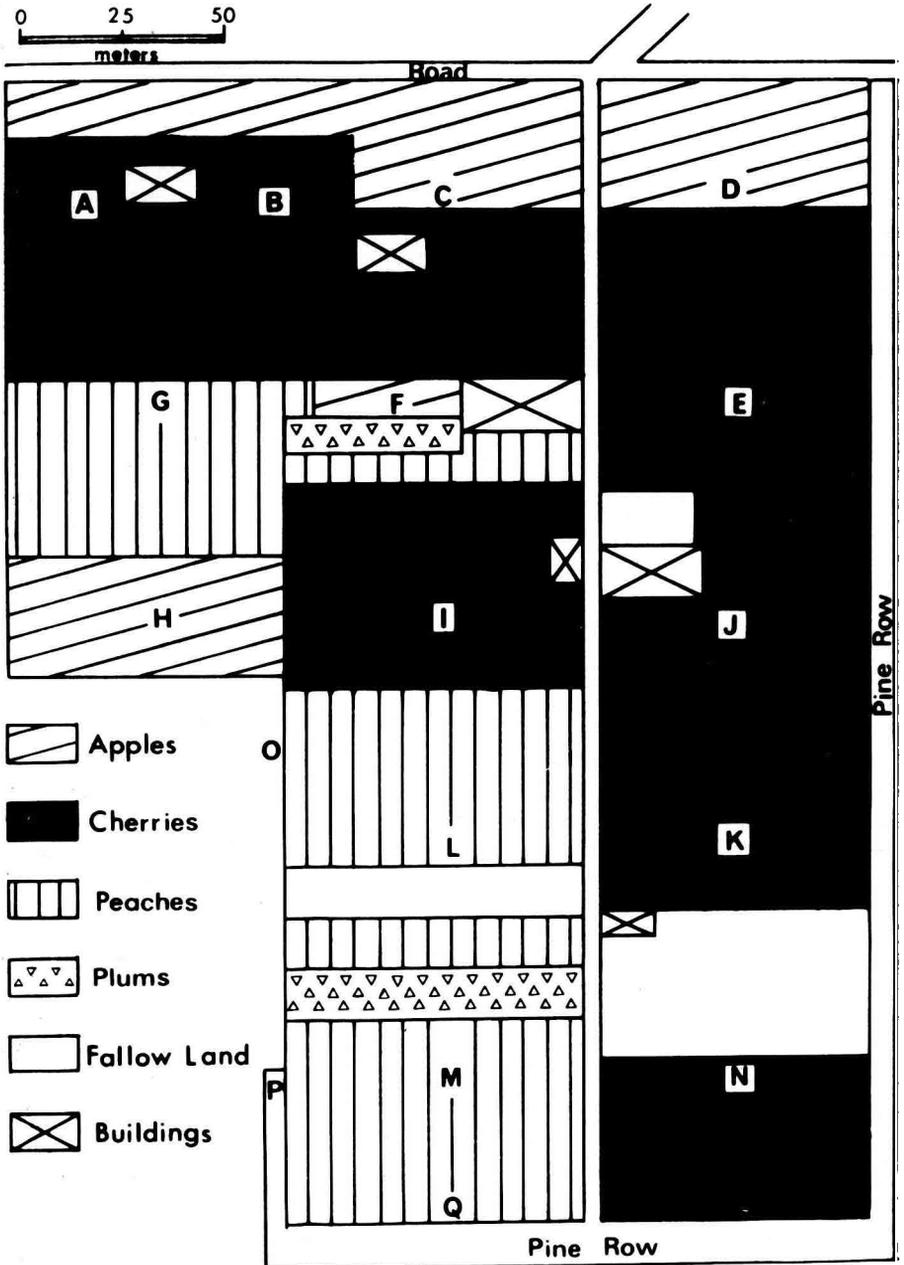
FIG. 1

Map of orchard site. Individual trap positions are indicated A → Q.

Carte du verger. Les emplacements des pièges sont indiqués par les lettres A à Q.

(Lewis & Gibson, 1978). Two alleles were discernible at each locus, designated fast (F) and slow (S) according to their relative anodal electrophoretic mobilities. Thermostability variants have been found at the *Adh* locus in Australian populations of *D. melanogaster* (Wilks *et al.*, 1980), however, the frequency of this allele is very low in Melbourne populations (Gibson *et al.*, 1982) and was not considered.

C. *Data Analysis*

Samples of less than 20 individuals were excluded from the analyses. Gene frequency associations with environmental variables were tested by Kendall rank correlation coefficients (Siegel, 1956). Comparisons made among samples were by Contingency X^2 tests on the number of genes sampled for each locus separately. The gene and genotype frequencies did not differ between the sexes at the 3 loci and these data were pooled. A Sign Test (Siegel, 1956) was used to test for heterozygote deficiency among trap samples within collections, and among collections with trap samples pooled. Gametic disequilibrium among the loci considered pairwise was investigated using correlation coefficients based on Burrow's $\triangle_{ij}$ (Langley *et al.*, 1978 ; Laurie-Ahlberg & Weir, 1979). The significance of the correlation coefficients was tested by a t-test.

III. Results

A. *Spatial Variation Within the Orchard*

The number of Fast and Slow alleles sampled at each locus was compared among the traps within each collection ; the X^2 values and their corresponding degrees of freedom being summed over all collections. Overall, significant heterogeneity was observed among traps at the *Tpi* (P < 0.001) and *Adh* (P < 0.05) loci (tabl. 1).

Since most fruit types are available in the orchard from January to early April, these collections were used to test the hypothesis that the heterogeneity among traps may be related to fruit type. Data were grouped according to the type of fruit trees in the immediate vicinity of each trap : apple (traps C, D and H), cherry (traps A, B, E, I, J, K and N) and peach (traps G, L, M and Q) (tabl. 2). Plum trees comprise only a small proportion of the trees in the orchard and were not included. Gene frequency differed among fruit types only in February 1981 at the *Adh* locus (P < 0.05), and in March 1981 at the *Gpdh* locus (P < 0.05). *Tpi* gene frequencies were homogeneous throughout, and all combined X^2 values were not significant. Hence, we conclude that there was no consistent association between fruit type and gene frequency.

At the *Adh* and *Gpdh* loci, deviations from Hardy-Weinberg expectations were investigated for each trap sample individually, and for each collection with traps pooled. Due to the low frequency of the *Tpi*-F allele, expected numbers of the FF homozygote were consistently less than 5, therefore this locus was not tested. Considering the traps separately over all collections, the number of traps deviating significantly from expected was not greater than would be expected by chance (tabl. 3).

TABLE 1

Tests for genetic heterogeneity among traps at each of the three loci for 14 collections.

Tests de l'hétérogénéité génétique entre pièges à chacun des 3 locus pour les 14 captures.

Month	Tpi		Gpdh		Adh	
	X² (Df)	Range (N)	X² (Df)	Range (N)	X² (Df)	Range (N)
1981						
J	—	—	0.17 (1)	0.65 (20) - 0.69 (32)	—	—
F	—	—	1.25 (1)	0.67 (71) - 0.69 (157)	—	—
M	2.49 (8)	0.02 (27) - 0.08 (64)	9.28 (9)	0.65 (39) - 0.86 (27)	23.66*** (9)	0.64 (48) - 0.81 (173)
A	2.19 (3)	0.01 (32) - 0.07 (38)	6.04 (7)	0.64 (50) - 0.76 (48)	5.66 (7)	0.69 (48) - 0.82 (47)
M	9.14 (5)	0.02 (67) - 0.09 (177)	23.20* (11)	0.60 (135) - 0.73 (184)	17.93 (11)	0.75 (188) - 0.80 (62)
J	0.49 (1)	0.04 (121) - 0.05 (51)	0.22 (1)	0.67 (71) - 0.69 (157)	0.17 (1)	0.76 (79) - 0.78 (163)
J-S						
O	5.79 (3)	0.02 (89) - 0.11 (36)	9.83 (6)	0.62 (73) - 0.77 (37)	16.00* (6)	0.67 (90) - 0.82 (37)
N	3.69 (5)	0.05 (39) - 0.13 (50)	9.48 (13)	0.59 (69) - 0.74 (49)	11.80 (13)	0.69 (40) - 0.82 (50)
D	30.8*** (8)	0.01 (39) - 0.19 (45)	15.76 (10)	0.53 (31) - 0.82 (47)	8.69 (10)	0.68 (31) - 0.82 (47)
1982						
J	19.77*** (2)	0.04 (230) - 0.14 (60)	2.21 (5)	0.67 (60) - 0.74 (210)	3.69 (5)	0.71 (29) - 0.78 (60)
F	0.07 (1)	0.02 (24) - 0.11 (23)	8.75 (6)	0.61 (42) - 0.78 (26)	5.67 (6)	0.70 (27) - 0.81 (42)
M	0.99 (2)	0.02 (41) - 0.08 (48)	2.84 (5)	0.06 (41) - 0.76 (61)	3.39 (5)	0.70 (62) - 0.78 (78)
A	1.55 (3)	0.06 (42) - 0.11 (31)	2.68 (6)	0.64 (24) - 0.77 (38)	9.09 (6)	0.59 (27) - 0.81 (42)
M-O						
N	1.45 (1)	0.03 (35) - 0.12 (21)	3.09 (3)	0.68 (56) - 0.80 (23)	2.78 (3)	0.68 (38) - 0.75 (38)
Total X² Among Traps	78.42*** (42)		94.80 (84)		108.53* (82)	

Included are total X² for heterogeneity among traps. Traps yielding less than 20 individuals were not considered. Contingency X² were calculated on the number of genes per trap. The table includes only those data where the expected values were greater than 5.
* P < 0.05 - *** P < 0.001.

Figurent les X² totaux d'hétérogénéité entre pièges et les valeurs extrêmes des fréquences de l'allèle F de chaque locus. Les pièges avec moins de 20 individus n'ont pas été considérés. Les X² ont été calculés sur le nombre de gènes par piège. Le tableau ne comporte que les données correspondant à des valeurs espérées supérieures à 5.

TABLE 2

Comparison of gene frequencies among fruit resource areas in the orchard at each of the three loci and eight collections.

Comparaison des fréquences géniques entre les zones de ressources fruitières du verger pour chacun des 3 locus et les 8 prélèvements.

Date	Locus	Apples	N	Cherries	N	Peaches	N	† X^2	Df
1981	*Tpi*-F								
J		0.08	25	—	10	0.09	69	0.11	1
F		0.05	200	0.06	229	0.06	178	0.50	2
M		0.04	157	0.04	163	0.04	89	0.38	2
A		0.07	243	0.05	290	0.07	428	2.98	2
1982									
J		0.04	50	0.08	44	0.07	420	1.49	2
F		0.10	25	0.04	135	0.07	60	0.84	1
M		0.07	83	0.10	192	0.08	72	0.95	2
A		0.13	64	0.09	91	0.09	52	1.16	2
Mean ± SD		0.07 ± 0.03		0.07 ± 0.02		0.07 ± 0.02		# 8.41	14
1981	*Gpdh*-F								
J		0.62	25	—	10	0.87	54	0.04	1
F		0.70	201	0.66	197	0.70	177	1.57	2
M		0.65	198	0.74	198	0.71	118	6.65*	2
A		0.64	343	0.69	304	0.72	431	3.04	2

TABLE 2 (suite)

Date	Locus	Apples	N	Cherries	N	Peaches	N	†X²	Df
1982									
J		0.73	47	0.66	43	0.70	423	1.09	2
F		0.68	25	0.71	135	0.64	61	2.26	2
M		0.77	83	0.70	192	0.69	71	2.84	2
A		0.71	64	0.68	91	0.75	51	1.30	2
Mean ± SD		0.69 ± 0.05		0.69 ± 0.03		0.72 ± 0.07		# 18.79	15
1981	Adh-F								
J		0.76	27	—	10	0.72	78	1.83	1
F		0.82	192	0.75	196	0.75	273	8.12*	2
M		0.73	181	0.76	189	0.76	99	1.28	2
A		0.76	353	0.77	304	0.75	436	0.68	2
1982									
J		0.78	51	0.77	44	0.76	423	0.27	2
F		0.76	25	0.74	136	0.74	75	0.08	2
M		0.75	83	0.75	191	0.74	72	0.13	2
A		0.67	64	0.68	92	0.62	54	1.31	2
Mean ± SD ...		0.75 ± 0.04		0.75 ± 0.03		0.73 ± 0.05		# 13.70	15

Data pooled across traps from apple (traps C, D and H), cherry (traps A, B, E, J, I, K and N) and peach (traps G, L, M and Q) areas. † : Contingency X² value of number of genes against fruit type. # : Combined X² values.
* P < 0.05.

Les données de différents pièges ont été réunies pour chacune des zones fruitières : pomme (pièges C, D et H), cerise (pièges A, B, E, J, I, K et N) et pêche (pièges G, L, M et Q). † : valeur du X² dans le tableau de contingence : nombre de gènes × type de fruit. # : Valeurs combinées du X².

TABLE 3

Investigation of Hardy-Weinberg equilibrium, heterozygosity and the application
of Wahlund's formula.

Etude de l'équilibre de Hardy-Weinberg, l'hétérozygotie et application
de la formule de Wahlund.

	Hardy-Weinberg Goodness-of-fit test		Sign Test	
	Number deviating significantly from expected	Combined X^2 (Df)	Number of cases of heterozygote deficiency	Number of cases
Among all traps				
Adh	3	86.88 (98)	45	98
Gpdh	7	106.05 (101)	63**	101
Traps pooled within collections				
Adh	2	23.78 (20)	12	20
Gpdh	0	22.14 (20)	17**	20
Traps pooled within collections after adjustment using Wahlund's formula				
Adh	1	21.75 (18)	6	18
Gpdh	0	11.48 (18)	12	18

** $P < 0.01$.

Heterozygosity at these loci was investigated by subtracting the number of hete-
rozygotes expected under Hardy-Weinberg from the number observed. This was carried
out (i) for all individual trap samples and (ii) for all collections with traps pooled
(tabl. 3). For the smaller samples (from the traps), expected values were corrected
for sampling error as described by CANNINGS & EDWARDS (1969). Analyses were by
Sign tests (SIEGEL, 1956). At the *Adh* locus, the number of heterozygotes was as
expected both within individual traps and among collections with traps pooled.
However, at the *Gpdh* locus, significant heterozygote deficiency was present among
both traps and collections. A deficiency of heterozygotes is expected when a subdi-
vided population is treated as a single panmictic unit (WAHLUND, 1928). Wahlund's
formula was applied to the trap samples in each collection for both loci. After adjust-
ment, only one collection significantly deviated from Hardy-Weinberg expectations,
and the number of cases of heterozygote deficiency was as expected by chance
(tabl. 3). Thus, the genotypic data at the *Gpdh* locus, and the allelic data at the *Tpi*

and *Adh* loci, suggest that there may be a tendency within the orchard for the adult population to consist of a number of genetically diverse and partially isolated sub-groups.

B. *Temporal Variation Within the Orchard*

Tpi-F frequency fluctuated seasonally, characterised by an increase in the F allele frequency in autumn and winter months (fig. 2). Total monthly rainfall, mean daily maximum and minimum temperature for each month and the availability of fruit resources are also presented. The observed annual increase in *Tpi*-F frequency appeared to coincide with the persistence of apples as the sole resource available. However, as noted above, no association of *Tpi*-F frequency with fruit type was apparent.
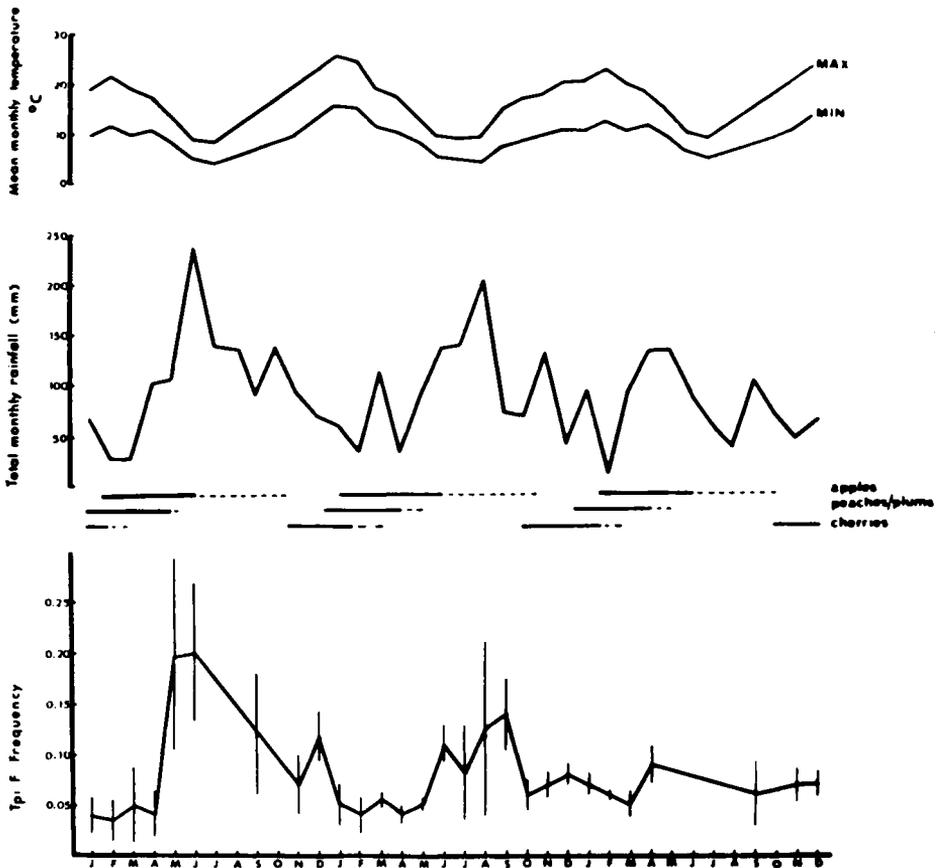
FIG. 2

Tpi-F *(± standard error), availability of fruit resources (- - - - - fruit persisting on ground) and climatic variables.*

Fréquence de Tpi-F *(± erreur standard), disponibilités fruitières (- - - - - persistance de fruits au sol) et variables climatiques.*

Environmental variables can influence the survival of individuals at all life cycle stages. It is therefore important to consider any effects of the environment on both adult and preadult stages of development. Environmental factors, for example rainfall affecting yeast flora on rotting fruit, may not influence adult gene frequencies for a number of weeks. Hence, gene frequencies at the adult stage may be influenced by previous environmental factors. In this analysis, we have therefore considered the environmental parameters of the month immediately prior to the month of collection as well as those of the collection month (tabl. 4).

TABLE 4

Kendall rank correlation coefficients for F allele frequency associations with climatic variables (A) concurrent with and (B) previous to the month of collection.

Coefficients de corrélation de rang de Kendall entre la fréquence de l'allèle F et des variables climatiques relatives au mois de capture (A) ou à celui qui la précède (B).

	Adh (n = 21)	Gpdh (n = 21)	Tpi (n = 28)
A. Concurrent			
Rf	0.07	0.22	0.38**
Tmin	0.05	— 0.21	— 0.41**
Tmax	0.07	— 0.25	— 0.33**
B. Previous			
Rf	0.10	0.21	0.50***
Tmin	— 0.01	— 0.22	— 0.38**
Tmax	0.03	— 0.29*	— 0.43***

Rf : Total monthly rainfall. Tmin : Mean monthly minimum temperature. Tmax : Mean monthly maximum temperature.
** P < 0.01 - *** P < 0.001.

There were no seasonal trends in gene frequency at the *Adh* or *Gpdh* loci and *Adh*-F frequency was independent of all climatic parameters considered (tabl. 4). *Gpdh*-F frequency was negatively associated with mean monthly maximum temperature (Tmax) for the month prior to collection. Heterogeneity among collections was detected at the *Adh* locus ($X^2 = 38.3$, Df = 20, P < 0.01) but was not present at the *Gpdh* locus ($X^2 = 31.2$, Df = 20, P > 0.05). Gene frequency estimates of natural populations are subject to sampling error, however no significant associations were apparent between sample size and gene frequency at these loci (*Adh*, r = 0.00, Df = 18, P = 0.50 ; *Gpdh*, r = — 0.17, Df = 18, P = 0.14 ; *Tpi*, r = — 0.21, Df = 28, P = 0.07).

Tpi-F frequency was positively associated with total monthly rainfall (Rf), and negatively associated with both temperature parameters for both the month of col-

lection and the previous collection month. The relationships among climatic variables indicated that the temperature and rainfall parameters were also significantly correlated. In order to determine which of these correlations were the most pertinent, Kendall partial correlation analysis was performed (tabl. 5). Unfortunately, an adequate test for the significance of Kendall partial rank coefficients is not available, therefore, the effect of controlling a variable (holding it constant) was determined by comparing the magnitude of the partial coefficient to that of the simple coefficient as described by SIEGEL (1956). Initially, the climatic variables at the time prior to collection were considered. The most clear correlation was with total monthly rainfall. Controlling for the effects of the temperature parameters did not appreciably reduce the Tpi-F : Rf association (tabl. 5 A), however controlling for Rf markedly reduced the correlations with mean monthly minimum temperature (Tmin) (by 76 p. 100 and 95 p. 100 respectively) and Tmax (by 47 p. 100 and 95 p. 100 respectively).

TABLE 5

Kendall partial rank correlation coefficients for Tpi-F *frequency associations with climatic variables (A) prior to the time of collection, (B) concurrent with the time of collection and (C) investigation of the effects of previous variables on associations with concurrent variables.*

Coefficients de corrélation partielle de rang de Kendall entre la fréquence de Tpi-F *et des variables climatiques précédant (A) la période de capture et concomitante (B) à celle-ci et étude de l'influence des variables antérieures (à la capture) sur les relations entre fréquence et variables climatiques concomitantes (C).*

	Rf	Tmin	Tmax
A.			
Simple coefficients	0.50***	— 0,38**	— 0.43***
Controlled variable			
Rf		— 0.09	— 0.23
Tmin	0.42		— 0.39
Tmax	0.36	0.25	
B.			
Simple coefficients	0.38**	— 0.41**	— 0.33**
Controlled variable			
Rf		— 0.19	— 0.19
Tmin	0.41		— 0.20
Tmax	0.37	0.04	
C.			
Control previous			
Rf	0.60	— 0.36	— 0.42

** $P < 0.01$; *** $P < 0.001$.

When considering the climatic variables concurrent with the collection month (tabl. 5 B), *Tpi*-F : Rf again was the strongest association. Controlling for Rf markedly reduced the *Tpi* associations with the temperature parameters and the *Tpi*-F : Rf coefficient was not reduced when controlling for Tmin or Tmax. Possibly, this association was a function of the *Tpi*-F correlation with previous Rf, however controlling for this variable (tabl. 5 C) did not reduce any coefficient for *Tpi*-F frequency with the concurrent variables. These patterns of association indicate that the significant correlations of *Tpi* gene frequency with the temperature parameters are a function of their association with total monthly rainfall. Thus, *Tpi*-F frequency is positively and significantly correlated with the total rainfall of the months both concurrent with and previous to the time of collection.

C. *Gametic Disequilibrium*

To assess gametic disequilibrium, the correlation coefficient R^B was calculated for each collection (tabl. 6). The *Adh* (2-50.1) and *Gpdh* (2-20.5) loci are unlikely

TABLE 6

Linkage disequilibrium values : R^B.

Valeurs du coefficient R^B de déséquilibre de linkage.

Collection	*Adh*-F : *Gpdh*-F	*Gpdh*-F : *Tpi*-F	*Adh*-F : *Tpi*-F
1980			
D	0.1084	0.0047	— 0.1747*
1981			
J	0.0819	0.1496	0.1046
F	— 0.1772	— 0.0217	— 0.0755
M	0.0074	— 0.0201	— 0.0219
A	0.0151	0.0506	— 0.0568
M	— 0.0168	— 0.0127	— 0.0212
J	— 0.0412	— 0.0225	— 0.0920
J	0.1613	0.1093	0.3317
A	0.5309	— 0.0433	0.0309
S	0.1085	— 0.0287	0.1076
O	— 0.0033	— 0.0745	— 0.0118
N	— 0.0038	0.0025	0.0087
D	0.0466	0.0136	— 0.0008
1982			
J	— 0.0599	0.0333	— 0.0418
F	0.0294	— 0.0245	0.016
M	—	—	—
A	— 0.0587	— 0.0203	0.0353
S	0.0242	— 0.2354	0.1176
N	0.0296	— 0.0373	0.0179
D	— 0.0619	— 0.0369	— 0.0479

* $P < 0.05$.

to be strongly associated with any chromosomal inversion as the frequency of
In(2L)t is low in Melbourne populations (KNIBB *et al.,* 1981). The *Tpi* locus (3-100.1)
is not physically linked to either the *Gpdh* or *Adh* loci. Only one test out of 57 was
significant, and the direction of the disequilibria was inconsistent across collections.
Although the values for *Adh*-F : *Tpi*-F from February to June 1981 were all negative,
this trend was not repeated in 1982. We therefore conclude that there is no evidence
for gametic disequilibrium among these 3 loci in this population.

IV. Discussion

We have found seasonal variation in gene frequency at the *Tpi* locus, observed
over at least a 2 year period (1980-1981). The available 1982 data also support this
trend although, as a consequence of drought conditions, no samples could be obtained
between May and August of that year. An initial increase in *Tpi*-F frequency was
observed however, and this trend has previously been observed in a neighbouring
orchard population (PHILLIPS, 1978). *Tpi*-F frequency correlated positively with total
monthly rainfall measured immediately prior to and concurrent with the time of col-
lection. This indicates that some factor or factors related to rainfall can affect gene
frequency at this locus, or of the chromosomal region encompassing this locus. The
chromosomal inversion In(3L)P occurs close to the *Tpi* locus and is present at low
levels in Melbourne populations (KNIBB *et al.,* 1981). Since *Tpi*-F frequency is also
relatively low, the possibility of some form of hitch-hiking selection with this in-
version cannot be excluded.

OAKESHOTT *et al.* (1984) described a positive association of *Tpi*-F frequency
with maximum temperature underlying the large scale latitudinal cline in Australasia.
The negative temperature association we observed is therefore in the opposite direc-
tion. Also, in the geographical survey, no association with rainfall was apparent,
contrary to our temporal pattern of gene frequency change.

In this study, associations between *Adh* gene frequency and environmental para-
meters, including seasonal trends, were not detected ; although temporal heterogeneity
over the collections was present. Other field studies of single populations have also
failed to establish any seasonal trend in *Adh* gene frequency (JOHNSON & BURROWS,
1976 ; GIONFRIDDO & VIGUE, 1978), or any association with environmental parameters
(GIONFRIDDO *et al.,* 1979). However, one report indicates that the *Adh*-S allele was
negatively associated with environmental temperature (McKECHNIE & McKENZIE,
1983). This association was in the opposite direction to the temperature association
established for *Adh*-S from studies of macrogeographic variation (PIPKIN *et al.,* 1973 ;
MALPICA & VASSALLO, 1980). Thus, the results of temporal studies of single popu-
lations show associations apparently conflicting with those of macrogeographic sur-
veys.

Gpdh-F frequency was negatively and significantly associated with mean monthly
maximum temperature (Tmax) of the month immediately prior to the time of collec-
tion. BERGER (1971) reported a decrease in *Gpdh*-F frequency during late summer
and autumn in apple orchard and woodland populations in North America — a result
consistent with the Wandin North temperature association. Macrogeographic asso-
ciations have also been reported at this locus with *Gpdh*-F decreasing in frequency

with increasing distance from the equator (JOHNSON & SCHAFFER, 1973 ; OAKESHOTT *et al.,* 1982) — a result consistent across continents at latitudes greater than 32° (OAKESHOTT *et al.,* 1984). Although the geographic and temporal associations for *Gpdh*-F frequency with temperature are in agreement, associations with other environmental variables are not consistent. OAKESHOTT *et al.* (1982) report on positive association of *Gpdh*-F frequency with maximum rainfall in Asia that was not apparent in Europe or North America. In the Wandin North population, *Gpdh*-F frequency was independent of rainfall.

Factors affecting genetic variation patterns within populations and at the geographic level may differ. Different populations will evolve distinct genetic backgrounds whether by chance or by selection. Hence, geographic variation in gene frequency is superimposed upon differences in genetic background among populations. The variation in associations observed among continents in geographic surveys also suggests that different selective parameters are important in different areas. Despite this, parallel clines on different continents at the *Adh* and *Gpdh* loci (OAKESHOTT *et al.,* 1982) suggest some association with large scale environmental variation. However, these selective forces may not be relevant as an influence on temporal variation in individual populations. Also, a greater understanding of how selection might work on such loci and of the causal basis behind environmental correlations is required.

The presence of nonrandom association of the alleles at all 3 loci was investigated, however we found no evidence for gametic disequilibria among these loci. This result is not surprising as recent studies (MUKAI, 1977 ; LANGLEY *et al.,* 1978) suggest that in outbreeding populations such as *Drosophila,* gametic disequilibrium is likely only over short map distances. As *Gpdh* and *Adh* are relatively distant (separated by about 30 map units), and with the *Tpi* locus on chromosome III, selection favouring a combination of alleles at these loci would have to be strong for disequilibria to be detected.

Significant spatial heterogeneity at 2 loci, especially *Tpi,* was found within the orchard site indicating that the orchard does not consist of a single panmictic population. In the Wandin North population, microspatial heterogeneity in *Gpdh* gene frequency occurs among emergents from fallen apple resources (NIELSEN, 1984). This occurred even when the apples were taken from an 80 m² grid. Each trap sample is likely to contain adults from a number of such heterogeneous patches, and result in a deficiency of heterozygotes when Hardy-Weinberg equilibrium is tested. This may explain the deficiency of heterozygotes at the *Gpdh* locus among trap samples (tabl. 3). Thus, the *Gpdh* genotype data is also consistent with sampling from a number of diverse subgroups.

Potential factors contributing to the heterogeneity are habitat selection, natural selection and random events. Habitat selection has been implicated in accounting for genetic microvariation in a number of studies (eg. TAYLOR & POWELL, 1977 ; CHRISTIENSEN, 1977 ; BARKER *et al.,* 1981 ; JONES, 1982). One difficulty in deciding between these alternatives is the estimation of gene flow. McINNIS *et al.* (1982) have carried out mark release recapture studies with *D. melanogaster* and found that marked flies moved an average of 150 m per day. However, this study was carried out at 2 forest sites, where *Drosophila* resources are not likely to be plentiful, as reflected by the low density of flies (up to 2-3 per 100 m²). Another mark release recapture study carried out by McKENZIE (1974) in a vineyard reported much lower rates of movement for *D. melanogaster* (less than 0.5 m per day in the pre-vintage period).

This site supported a much higher density of this species (an estimated 2,000 in the vicinity of the vineyard buildings). These numbers are more similar to those found in an equivalent area of the orchard. In general, *Drosophila* tend to remain in the vicinity of a favourable resource (WALLACE, 1970 ; McKENZIE, 1980) and during most of this study, fallen fruit resources were plentiful. Thus, we would expect movement within the orchard to be low.

One argument against the importance of habitat selection is that there was no consistent pattern to the heterogeneity across traps ; it occurred at the different loci at different collection times. The heterogeneity was not consistently associated with resource type, and there was little detectable heterogeneity in other environmental features of the orchard. Hence, there is no evidence for an association between frequencies at the enzyme loci and environmental heterogeneity. This heterogeneity is consistent with the population being substructured into a number of partially isolated, transient subgroups within the orchard.

The spatial genetic heterogeneity also emphasizes the importance of sampling technique in the estimation of gene frequencies from field sites. For example, the range in *Gpdh* gene frequency between traps in one collection (0.53-0.82) is nearly as great as the range observed in the entire Australasian cline (0.54-0.92). Geographic and seasonal fluctuations in gene frequency may be, at least in part, a function of the random fluctuations in subpopulation frequency differentially sampled over time.

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