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Allozyme variation in the butterfly, *Maniola jurtina* (Lepidoptera: Satyrinae) (L.): evidence for selection

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This paper describes patterns of allozyme variation in the butterfly, *Maniola jurtina* (L.). Twelve loci, of which four were polymorphic (*PGM, PGI, IDH-1* and *IDH-2*), were screened across 14 populations in south-east England. The patterns described are not in agreement with expectation for a sedentary butterfly in which alleles are neutral to selection, for geographically distant populations differed very little in gene frequencies. Geographic homogeneity is compatible with either a higher degree of vagility than expected for this species or similar selection pressures maintaining allele frequencies across the area sampled. Associations between patterns of variation at two loci and behavioural, morphological and environmental variables suggested that selection may be acting upon them. In particular, the genotype at the locus for *PGM* affected the length of time for which individual butterflies could fly continuously, while *PGM* allozyme frequencies varied according to the altitude of the site. The frequency of allozymes at *IDH-2* was correlated with the mean size of individuals in both sexes when comparing sites. Possible causes of these associations are discussed. I conclude that patterns of variation in the loci studied are probably not good estimators of population structuring, but are more likely to be the result of selection.

Keywords: altitude, morphology, mutase, phosphoglucose, polymorphism, selection, temperature.

Introduction

The meadow brown butterfly, *M. jurtina* (Lepidoptera: Satyrinae) (L.), the subject of numerous papers spanning 30 years by E. B. Ford, W. H. Dowdeswell and others (summarized in Dowdeswell, 1981), and more recently by P. Brakefield (Brakefield, 1984; Brakefield & Noordwijk, 1985), provides one of the cornerstones of early research in population genetics. Most of this work has concentrated on the genetics and spatial and temporal variation of black spotting on the hind wing. The species is ubiquitous and abundant in grassland and scrub habitats throughout the western palearctic, and is considered to be relatively sedentary (Pollard, 1981).

This study describes patterns of allozyme variation in *M. jurtina* within and among sample sites in south-east England, and attempts to evaluate whether the observed patterns are best explained by neutrality or selection at the loci concerned. The use of both allozyme and morphological variation enables information inferred from one source to be cross-checked against the other. To quote Singh & Long (1992) 'The use of both molecules and morphology allows us to understand the causes of geographic variation in more detail than by studying either of them in isolation. Yet it is remarkable how few (such) species .... have been done during the past 25 years'. Despite the lack of any clear consensus as to the relative importance of selection and stochastic processes in controlling allozyme frequencies (Mani, 1984), electrophoretic data are often used to infer information on population structure (e.g. Grant & Little, 1992; Bilton, 1992) with the implicit assumption that selection pressures on the loci used are weak or absent.

An assessment of population structure in *M. jurtina* using allozyme variation is compared with that expected for a sedentary species. I also examine evidence for selection influencing allozyme frequencies by testing for associations with butterfly morphology, physiology and altitude at which they were sampled. These variables were chosen on the basis of associations with electrophoretic data in other studies of Lepidoptera (see discussion).

Materials and methods

Five hundred and sixty-five adult butterflies were hand-netted at 14 sites in south-east England (Fig. 1) during the flight season in 1990. Sample sizes are given in Table 1. The sampling sites were divided geographically into two clusters, one in Oxfordshire containing 10 sites, and the other in East Sussex containing four sites. This sampling design enabled detection of local geographic variation (< 1 km) and larger scale patterns between sites sufficiently distant from each other to prevent any likelihood of direct dispersal in a sedentary species (maximum distance between sites 165 km) (it should be noted that there were undoubtedly unsampled populations between these distant sites). Within each cluster, sites were chosen to represent each of the main habitat types of *M. jurtina*, namely lowland meadow (sites 1, 3, 4, 7 and 8) woodland clearings (sites 2, 5 and 6) and chalk grassland (sites 9 to 14).

Butterfly abdomens were removed and stored at − 70°C until electrophoresis could be carried out. The remainder of the butterfly was retained for morphological analysis. Individual numbering of specimens and electrophoretic material enabled associations between genotype and morphology to be examined. Abdomens were individually homogenized in numbered centrifuge tubes with 0.3 ml of extraction buffer (Pasteur *et al.*, 1988).

Proteins were separated using horizontal starch gel electrophoresis following the method described by Pasteur *et al.* (1988). A variety of gel and electrode buffer systems were used and 16 enzymes screened. The two most suitable buffer systems proved to be continuous Tris–citrate at *p*H 8 (Ahmad *et al.*, 1977) and discontinuous citrate histidine at *p*H 7 (Shaw & Prasad, 1970). Gels were run at 60 mA for 4.5 h.

Enzyme staining protocols are given in Pasteur *et al.* (1988). Twelve enzymes were clearly resolved, of which four proved to be polymorphic: phosphoglucose

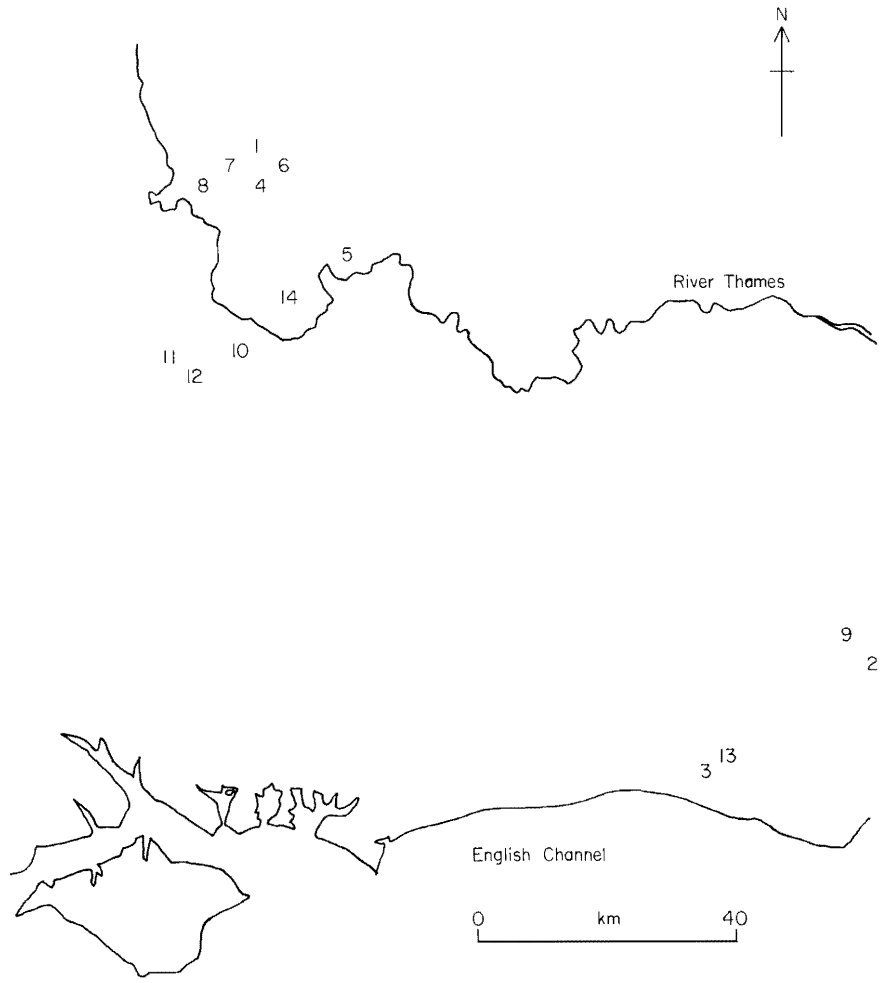

Fig. 1 Study sites in south-east England.

Table 1 Sample allele frequencies for the four polymorphic loci studied. * = missing values. Only the two most common alleles are shown

		PGI		*PGM*		*IDH-1*		*IDH-2*		
Site No.	Site N.G.R.	PGI^{100}	PGI^{150}	PGM^{100}	PGM^{85}	$IDH\text{-}1^{100}$	$IDH\text{-}1^{65}$	$IDH\text{-}2^{100}$	$IDH\text{-}2^{68}$	(*N*)
1	SP607112	0.823	0.069	0.795	0.151	0.895	0.105	0.956	0.044	144
2	TQ582275	0.793	0.069	0.733	0.200	*	*	0.897	0.103	29
3	TQ450086	0.857	0.071	0.841	0.114	0.864	0.136	1.000	1.000	21
4	SP601096	0.865	0.014	0.829	0.092	0.838	0.149	0.986	0.014	37
5	SP724958	0.837	0.083	0.738	0.086	0.878	0.130	0.969	0.033	58
6	SP612104	0.800	0.067	0.897	0.129	0.870	0.167	0.967	0.018	30
7	SP589095	0.883	0.091	0.806	0.148	0.833	0.000	0.964	0.000	30
8	SP540060	0.864	0.071	0.759	0.096	1.000	0.019	0.955	0.019	22
9	TQ565305	0.839	0.121	0.750	0.034	0.981	0.125	0.944	0.018	28
10	SU546862	0.759	0.051	0.914	0.069	0.875	0.082	0.982	0.069	29
11	SU530850	0.867	0.043	0.853	0.214	0.918	0.196	0.922	0.000	49
12	SU535850	0.783	0.057	0.738	0.129	0.804	0.000	1.000	0.029	23
13	TQ445088	0.900	0.117	0.800	0.150	*	*	0.971	0.000	35
14	SP675915	0.783	0.077	0.717	0.194	0.942	0.125	1.000	0.022	30

mutase (*PGM*), phosphoglucose isomerase (*PGI*), isocitrate dehydrogenase 1 (*IDH-1*) and isocitrate dehydrogenase 2 (*IDH-2*).

Malate dehydrogenase, glucose-6-phosphate dehydrogenase, adenylate kinase, aldolase and glyceraldehyde phosphate dehydrogenase were resolved and found to be monomorphic in all samples analysed.

Other variables screened

Flying ability. To test whether flying ability varied according to genotype at the four loci under study, 34 male butterflies from site 1 were captured and stored in the dark. They were then removed individually, placed in a brightly lit flight cage 1 m^3 at 29°C and stimulated into flight with a gentle tap. As soon as they settled they were stimulated again. Eventually this failed to provoke further flight. The total time from initial stimulation to this point was recorded.

Morphology. The sex, the length of the forewing from base to apex and the number of hindwing spots were recorded for all butterflies sampled.

Altitide. The altitude of study sites was obtained from Ordnance Survey maps.

Statistical analyses

Agreement with Hardy–Weinberg proportions was tested using both *F*-statistics and a χ^2 test for goodness of fit with Levene's (1949) correction for small samples. As the χ^2 test is likely to be unreliable when expected values are low (Sokal & Rohlf, 1981), the χ^2 test was repeated with the genotypes pooled into the three classes (i) homozygotes for the most common allele; (ii) heterozygotes for the most common allele; and (iii) other genotypes. Departures from Hardy–Weinberg were only considered significant if both χ^2 tests were significant.

Statistical significance was adjusted throughout to account for the increase in type I errors expected when carrying out multiple tests, using Cooper's (1968) modification where the critical probability for rejection of the null hypothesis is α/n, and where n is the number of loci tested.

Heterozygosities were calculated from the expected number of heterozygotes in each sample assuming Hardy–Weinberg equilibrium.

F_{is}, (inbreeding in the individual relative to the sample), F_{st} (the standardized variance of allele frequencies among samples), and F_{it} (inbreeding in the individual relative to the whole population), were calculated for 15 alleles at the four polymorphic loci (Nei, 1977; Wright, 1978). Null hypothesis ($F_{is}=0$, $F_{it}=0$ and $F_{st}=0$) were tested using:

$$\chi^2 = NF_{is}^2; \qquad \text{d.f.} = 1$$

$$t = |F_{IT}\sqrt{N}|; \qquad \text{d.f.} = \text{infinity}$$

$$\chi^2 = 2NF_{st} \qquad \text{d.f.} = \text{number of sites} - 1,$$

where N = total number of individuals (565).

The island model of migration was used to estimate the number of individuals dispersing between sites (m)

according to $F_{st} = 1/(1+4m)$ (adapted from Wright, 1951).

Genetic distances between samples were calculated using Nei's (1978) unbiased distance (Wright, 1978, provides an excellent discussion of these and other measures). Wright's modification of Rogers' (1972) distance, and Nei's (1972) distance were also calculated and gave similar results.

Results

Gel banding patterns were in accordance with the dimeric nature of enzymes *PGI* and *IDH*, in which heterozygotes exhibited clear hybrid bands equidistant between the bands for each allele. PGM is a monomer and displayed no hybrid bands in heterozygotes. Average heterozygosities for all polymorphic loci ranged from 0.148 to 0.280, with a mean of 0.218.

The frequency of the most common allozymes of *PGM, PGI, IDH-1*, and *IDH-2* at each site, and sample sizes are shown in Table 1. Interpretation of the data as allelic frequencies is further supported by a χ^2 goodness of fit test for deviation from Hardy–Weinberg expectations (combining classes where expected values were less than five). Of the 56 sample/locus combinations only one departed significantly from expectation. In sample 6 at the locus for *PGI* there was a lack of heterozygotes (unpooled $\chi^2 = 28.49$, d.f. = 10, $P = 0.002$, $F = 0.223$).

F statistics for 14 alleles (Table 2) describe a high degree of geographic uniformity and suggest random mating between individuals both within and between samples. Values of F_{is} vary from -0.061 to 0.035 (mean -0.005), values of F_{it} vary from -0.051 to 0.054 (mean -0.010), and values of F_{st} vary from 0.005 to 0.021 (mean 0.015). All are non-significant. These generally low values of F_{st} result in high estimates of dispersal between sites using the island model of migration. Estimates for individual alleles vary from 11.7 to 49.8 individuals immigrating into each sample site per generation, with a mean of 16.4.

Table 2 F-statistics for the 14 most common alleles (values of F_{is} and F_{it} are mean values for all 14 sites sampled)

Allele	Average frequency	Sample variance	F_{st}	F_{is}	F_{it}
PGM^{100}	0.794	0.002	0.017	−0.004	0.013
PGM^{75}	0.018	0.000	0.011	−0.026	−0.015
PGM^{85}	0.132	0.002	0.019	−0.011	0.008
PGM^{110}	0.028	0.000	0.005	−0.033	−0.028
PGM^{130}	0.027	0.000	0.005	0.024	0.029
PGI^{100}	0.833	0.002	0.009	−0.013	−0.004
PGI^{10}	0.023	0.000	0.019	−0.041	−0.021
PGI^{30}	0.046	0.001	0.010	−0.061	−0.051
PGI^{70}	0.008	0.000	0.005	−0.015	−0.010
PGI^{130}	0.015	0.000	0.015	−0.030	−0.015
PGI^{150}	0.072	0.001	0.008	0.014	0.022
$IDH\text{-}1^{100}$	0.904	0.001	0.021	0.031	0.051
$IDH\text{-}1^{65}$	0.095	0.001	0.020	0.035	0.054
$IDH\text{-}2^{100}$	0.965	0.000	0.018	−0.057	−0.039
$IDH\text{-}2^{68}$	0.026	0.000	0.021	−0.054	−0.032

The significance of allele frequency differences between sites was also tested using a χ^2 contingency test for heterogeneity (combining frequencies where expected values were low). *IDH-2* was the only locus at which allozyme frequencies differed significantly between sites ($P < 0.0001$).

Nei's genetic distance (D) varied from 0 to 0.013 for the possible 91 between-site comparisons. There was no apparent association between geographic distance and genetic heterogeneity (linear regression, $r^2 = 0.022$, ns): some sites separated by 165 km, such as 3 and 4, where genetically identical ($D < 0.0005$). Conversely other site pairs separated by only 1 km were towards the upper range of values of D found (for example, sites 10 and 11, $D = 0.008$).

There was no increase in genetic similarity between samples taken from similar habitats, for genetic distances were not significantly smaller when comparing within habitats than between (mean values of D, between habitats, 0.003; within meadows, 0.002; woodlands, 0.006; chalk grasslands, 0.004).

Evidence for selection upon allozyme frequencies

Evidence for selection upon allozyme frequencies suggested by associations between allozymes and other variables is summarized in Table 3.

Isocitrate dehydrogenase 2. Allozyme frequencies of *IDH-2* are correlated with size when comparing sites. The frequency of allozyme $IDH\text{-}2^{100}$ is negatively correlated with mean wing length in both sexes, (Pearson product-moment correlation coefficient $r = -0.481$, $P < 0.005$ in males, $r = -0.648$, $P < 0.001$ in females).

Phosphoglucose mutase. I. In between-site comparisons the frequency of the most common allozyme (PGM^{100}) declines with increasing altitude (linear regression, $P = 0.014$, $r^2 = 0.406$). This relationship is not linear (Fig. 2). At low altitudes sites occur with a wide range of frequency of PGM^{100}, while at higher altitudes (above 120 m) all sites have a comparatively low frequency.

This result appears to contradict the finding that *PGM* allozyme frequencies do not differ between sites.

Table 3 Summary of relationships between allele frequencies and morphological and environmental variables

Enzyme	Associated variable	Description of relationship	Statistical test and significance level
PGM	Altitude	Frequency of PGM^{100} declines with altitude	Linear regression, $r^2 = 0.406$, $P = 0.014$
	Stamina	At 29°C the stamina of individuals homozygous for PGM^{100} is greater than that of other genotypes (combined)	t-test, $P = 0.002$
IDH-2	Wing length	Comparing site means the frequency of $IDH\text{-}2^{100}$ is negatively correlated with mean wing length	Correlation, $r = -0.481$, $P < 0.005$ for males, $r = -0.648$, $P < 0.001$ for females

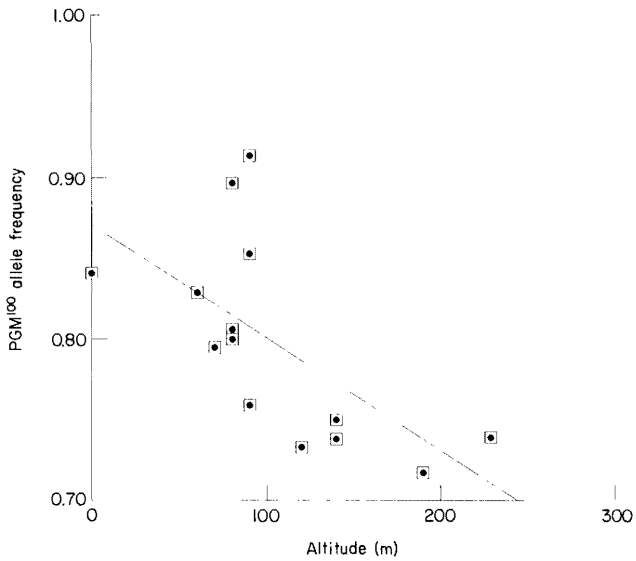

Fig. 2 Changes in PGM^{100} allele frequency with altitude of the sample site.

From Table 1 it can be seen that frequencies of PGM^{100} are similar at all sites, varying from 0.717 at site 14 to 0.914 at site 10. Although these differences are not significant, when ordinated according to altitude the relationship is significant.

II. The ability to sustain continuous flight at 29°C is significantly higher in individuals homozygous for PGM^{100} (t-test, $P = 0.002$). The mean length of flight for individuals homozygous for PGM^{100} is 10.47 s ($n = 17$), and for other genotypes combined is 2.21 s (six individuals of genotype PGM^{100}-PGM^{85}, and one of PGM^{100}-PGM^{75}).

Isocitrate dehydrogenase I and phosphoglucose isomerase. These loci were not significantly correlated with any morphological, behavioural or site variable measured.

Sex and hindwing spot number are not associated with genotype at any of the loci studied.

Discussion

If dispersal is low, and allele frequencies neutral to selection, then founder effects and genetic drift should result in genetic divergence between sample sites which is proportional to the degree of isolation (Sokal & Wartenberg, 1983). Even allowing for the undoubted existence of stepping stone populations between the more distant populations in this study, the remarkable genetic uniformity between and within all samples is not consistent with expectations for a sedentary species. Estimates of dispersal between samples based on the mean value of F_{st} suggest that sample sites are subject to a constant influx of immigrants. Comparison with mean F_{st} values from other studies on Lepidoptera and a small selection of other organisms (Table 4) suggests that *M. jurtina* is highly vagile; its value of F_{st} (0.015) is close to that for the known migrant, *Pieris rapae* ($F_{st} = 0.014$), and is far lower than that of known sedentary species such as *Euphydryas editha* ($F_{st} = 0.118$).

This estimate assumes that patterns of allele frequency are not subject to selection: if they are, then estimates of population structuring will be wrong. Selection may maintain similar gene frequencies in isolated populations provided that the selection pressures are the same at all sites.

Table 4 Summary of F_{st} values from studies on Lepidoptera and a small selection of other organisms (*)

Name	F_{st}	No. of loci	Sampling area	Reference
Spodoptera exempta	0.006	6	Kenya, Tanzania, Zimbabwe	Den Boer (1978)
Alabama argillacea	0.007	13	Mexico, Brazil	Pashley (1985)
Danaus plexippus	0.009	6	Eastern U.S.A.	Eanes & Koehn (1978)
Papilio glaucus	0.013	13	New York State	Hagen & Lederhouse (1985)
Pieris rapae	0.014	4	U.S.A.	Eanes & Koehn (1978)
M. jurtina	0.015	4	S.E. England	Present study
Anticarsia gemmatalis	0.021	19	Eastern U.S.A., Mexico	Pashley (1985)
Danaus plexippus	0.032	6	Australia	Hughes & Zalucki (1984)
*Drosophila melanogaster**	0.044	7	Eastern U.S.A.	Eanes & Koehn (1978)
Heliothios virescens	0.048	10	Southeast and Western U.S.A., Mexico	Sluss & Graham (1979)
Cydia pomonella	0.066	4	Africa, Europe, U.S.A., Australia	Pashley (1980)
Spodoptera frugiperda	0.084	13	Caribbean, Mexico, Eastern U.S.A.	Pashley *et al.* (1985)
Euphydryas chalcedona	0.090	8	Central California	McKechnie *et al.* (1975)
Euphydryas editha	0.118	8	Central California	McKechnie *et al.* (1975)
*Homo sapiens**	0.148	9	Worldwide	Cavalli-Svorza (1966)
Hydroporus glabriusculus (Coleoptera)*	0.199	9	Britain, Sweden	Bilton (1992)
Rumina decollata (Mollusca)*	0.294	5	Approx. 5,400 m^2	Selander & Hudson Hudson (1976)

Two of the four loci studied are significantly associated with other variables measured. Such associations may be attributed to 'hitchhiking' via close linkage to other loci (Thomson, 1977), but linkage disequilibrium between a neutral and selected locus is transitory (Clegg, *et al.*, 1980; Asmussen & Clegg, 1981; 1982). Thus although a possible cause of associations within a single population, hitchhiking is not a plausible explanation for patterns of geographic variation in gene frequency.

Previous studies on butterflies have rarely combined electrophoresis with morphological or behavioural studies, but those that have concluded that selection acts upon allozyme frequencies (McKechnie *et al.*, 1975; Watt, 1977; Eanes & Koehn, 1978; Hughes & Zaluki, 1984; Watt *et al.*, 1986).

Variation in isocitrate dehydrogenase 2

There are several possible causes of the observed relationship between size and genotype. It may be due to a direct effect of genotype at IDH-2 upon size, for example via larval growth rate. Alternatively it may be a non-causative effect of linkage to other genes involved in growth. Given the relationship between female size and fecundity reported in numerous studies of Lepidoptera, for example Lederhouse (1981) and Jones *et al.* (1982), the relationship between *IDH-2* and size is likely to result in powerful selection pressures on this loci.

Variation in phosphoglucose mutase

The frequency of PGM^{100} declined with altitude of the site. Examination of data published by Masetti & Scali (1976, 1978) reveals a similar trend in five populations of *M. jurtina* from Italy, for two mountain populations possessed lower frequencies of the most common allele than populations sampled near sea level. Similarly, data presented by Bullini *et al.* (1975) describe a decline in frequency of the most common allele with decreasing latitude in samples from Switzerland and Italy. However, it is unknown whether the most common allele is the same in each case.

Variation in the ability of butterflies to maintain flight at 29°C provides a possible explanation for this observation, for individuals homozygous for PGM^{100} could maintain flight for far longer than individuals of other genotypes tested. Why then do alleles other than PGM^{100} persist at this locus? Presumably at some time

during the life cycle, or at temperatures other than 29°C selection is reversed. The increase in frequency of rare alleles in samples taken at higher altitudes suggests temperature may be the variable which balances selection. During cool conditions heterozygotes may be more able to fly than individuals homozygous for PGM^{100}. Clearly further research is needed to establish whether this is so.

Associations between altitude, weather and flight activity have been recorded with regard to *PGI* allozyme frequencies in *Colias* sp. (Watt, 1983; Watt *et al.*, 1983, 1985), and *PGM* and *PGI* frequencies in *Danaus plexippus* (Carter *et al.*, 1989). Hovanitz (1948) found that the different wing colour morphs of *Colias* sp. are active at different air temperatures, so that samples of active individuals from the same site varied in morph frequency according to the weather. If the fitness of different morphs varies according to the weather then short-term temporal heterogeneity of weather conditions could maintain polymorphisms, with fluctuations in morph frequencies from year to year depending upon the weather conditions during the flight period.

In conclusion, the low F_{st} obtained for *M. jurtina* is probably not indicative of a high dispersal rate, but is more likely the result of selection acting upon at least some of the polymorphic loci studied.

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