

Latitudinal clines of allelic frequencies in Mediterranean populations of *Ceratitis capitata* (Wiedemann)

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Summary – Collections of *Ceratitis capitata* from 6 different areas from 4 Mediterranean countries were analysed for genetic variation. Six out of the 25 loci tested were found to be polymorphic. Allelic frequencies were estimated and the populations were found to be panmictic for 4 out of those 6 loci. Two of the polymorphic loci showed a significant clinal pattern in gene frequency changes by Spearman rank correlation with latitude. At 4 loci, a steep gradient in allele frequency (in *Idh* from 1.00 to 0.63) is observed in the axis of the north–south transect.

Ceratitis capitata / allelic frequency / cline

Résumé – Clines latitudinaux de fréquences alléliques dans des populations méditerranéennes de *Ceratitis capitata* (Wiedemann). Des échantillons de la mouche des fruits, *Ceratitis capitata*, provenant de 6 régions différentes de 4 pays méditerranéens, ont été examinés pour leur polymorphisme génétique à 25 locus enzymatiques. Six des locus se sont avérés polymorphes et les fréquences génotypiques observées à 4 de ces locus sont conformes à l'hypothèse de panmixie. Un cline latitudinal significatif des fréquences alléliques a été mis en évidence pour 2 de ces gènes polymorphes par un test de corrélation de rang de Spearman. Pour 4 locus, les variations de fréquences alléliques dans l'axe nord-sud sont importantes, par exemple la fréquence de l'allèle 1.00 du gène *Idh* varie de 1,00 à 0,63 le long de cet axe.

Ceratitis capitata / fréquence allélique / cline

INTRODUCTION

The Mediterranean fruit fly *Ceratitidis capitata* is a polyphagous and multivoltine tropical and subtropical species, and one of the most serious pests of fruits and vegetables. During the last 150 years, medfly has been established in several countries including those of the Mediterranean basin from its proposed equatorial African origin (Fletcher, 1989). Even though the first report of this pest within the European Mediterranean area dates from the middle of the last century, the medfly has expanded throughout this basin because this region offers an increasing number of host fruits of different cultivars and/or species (De Breme, 1842; Martelli, 1910; Fimiani, 1989). Medfly is a serious threat to important fruit production centers throughout the world. However, the biology of this species is poorly known, especially its population genetics. Genetic variability might also provide information on the spread of *C capitata* (Kourti *et al*, 1990; Gasperi *et al*, 1991). Recent results showed large genetic differences between introduced populations and those postulated to be their ancestral African ones. The average heterozygosity of medfly populations within the Mediterranean basin is about 5% versus 22% in the African populations (Huettel *et al*, 1980; Kourti *et al*, 1990; Gasperi *et al*, 1991). This significant heterozygosity of African populations (22%) is comparable to that of other insect natural populations, *eg*, *Drosophila* (Ayala *et al*, 1972; Lewontin, 1974; Prakash, 1977; Hyytia *et al*, 1985). The similarity displayed in allele frequencies among medfly or *Drosophila* populations could be expected under the neutral theory, provided that some exchange of genetic material takes place between populations (Kimura and Marayama, 1971; Kimura and Ohta, 1971). In many cases, it was concluded that the subcosmopolitan status of medfly was recently achieved, due to human transport, and that any genetic divergence between geographically distant populations has occurred over a very short time. However, *C capitata* appeared to be adapted to different environmental conditions, being able to constitute large populations in both the tropics and the temperate regions. Since the polymorphism of the introduced populations is low compared with that of their African counterparts, we focus our effort on elucidating this discrepancy among populations originating from different Mediterranean areas. This study was designed to detect latitudinal clines in gene frequencies. For this purpose, 6 natural populations from distantly located areas were examined.

MATERIALS AND METHODS

Origin of populations

Six populations of *C capitata* from the Mediterranean basin were used in this study. The Spanish population was represented by a sample of pupae on peaches collected in October 1986 in the area of Castellon (S). The Greek populations were: a) from Attiki, where pupae were collected on peaches in July of 1986 (G1); and b) from the island of Crete, where pupae were also collected on peaches in July 1986 in the area of Chania (G2). The Israeli population was from the area of Best Dagan, where pupae were collected on apricots in June 1984 (I). The Egyptian populations were represented by 2 samples: a) pupae on apricots collected in May 1986 in the area of

Kalubia (E1); and b) pupae on apricots collected the same period in the area of E1 Fayum (E2). Collections of wild flies from these Mediterranean populations were made by harvesting infested fruits from the ground. An effort was made to collect samples from 1 food source: the stone fruits (peaches or apricots).

Electrophoretic studies

The preparation of samples, and electrophoretic and staining procedures have been described by Kourti *et al* (1990). For each individual, 25 enzymes' loci were tested in the 6 populations. These were: *Mpi*, *To*, *Diaph-1* and *Diaph-2*, *Adh*, *Ak*, *Odh*, *G-6-pd*, *6-pgd*, *Idh*, *Hk-1*, *Hk-2*, *Got-1*, *Got-2*, *Fum*, *Est*, *Lap*, *Me*, *Mdh*, *Phi*, *Pgm*, α -*Gpdh*, *Pep-1*, *Pep-2* and *Pep-3*. From these the following enzymes were polymorphic: MPI, G-6-PD, IDH, PEP-1, PEP-2 and PEP-3. In a limited number of collections EST was also polymorphic.

Statistics

Chi-square tests were performed to compare observed numbers with those expected under Hardy-Weinberg equilibrium. The Chi-square test of the correlation between genes (Barker *et al*, 1986) was applied for loci with more than 2 alleles. The degree of relationship between clinal patterns was determined by Spearman rank correlation (Statgraph Statistical Program). The strategy is to look at conditional frequencies and their correlations with latitude as clinal measures.

RESULTS

The allelic frequencies at the 6 polymorphic loci, the collection sites and the number of individuals analysed are listed in table I. Most loci have 2 alleles with the exception of *G-6-pd*. All the peptidases are unmapped while the position of the other 3 loci is known (Malacrida *et al*, 1987). The *Mpi* and *Pep-1* loci are polymorphic in all places, while the others were found monomorphic at least in 1 of the collection sites. In order to have a constant scoring and recording of data, the relative mobility of each allele was defined as previously described by Kourti *et al* (1990). The genotypes were pooled into 2 classes, homozygotes and heterozygotes in order to perform a uniform statistical comparison of observed *versus* expected genotypes. In table II, the numbers of homozygotes and heterozygotes observed are compared with the expected ones. Using the allelic frequencies determined at each locality, expected genotypic proportions (Hardy-Weinberg equilibrium) were compared with those observed (table II). The χ^2 value is never significant for the biallelic loci, whereas in the case of *G-6-pd* 2 samples showed significant deviation from Hardy-Weinberg expectation. The correlation coefficient r_s between the common allele frequency and latitude is given in table III. The Spearman rank correlation coefficient (r_s) is given in table III. This analysis showed that 2 loci *G-6-pd* and *Idh* exhibit significant correlation between the common allele of these loci with latitude. Recent results obtained from only a few populations regarding *Est* polymorphisms also showed significant correlation (data not shown).

Table I. Allelic frequencies of polymorphic loci in 6 Mediterranean populations of *C capitata* classified by location in degrees north latitude.

<i>Loci allele</i>	<i>Spain 40°</i>	<i>Greece 1 38°</i>	<i>Greece 2 35°</i>	<i>Israel 32°</i>	<i>Egypt 1 30°</i>	<i>Egypt 2 29°</i>
<i>Chrom II</i>						
<i>Mpi</i>						
1.00	0.162	0.052	0.085	0.208	0.317	0.375
0.87	0.838	0.948	0.915	0.792	0.683	0.625
<i>n</i>	136	148	136	178	112	120
<i>Chrom V</i>						
<i>G-6pd</i>						
1.02	0.031	0.034	0.040	—	—	—
1.00	0.802	0.925	0.942	1.000	1.000	1.000
0.98	0.167	0.041	0.018	—	—	—
<i>n</i>	96	148	136	80	80	80
<i>Chrom VI</i>						
<i>Idh</i>						
1.00	1.000	1.000	1.000	0.728	0.707	0.633
0.83	—	—	—	0.272	0.293	0.367
<i>n</i>	80	148	81	160	140	120
<i>Loci unmapped</i>						
<i>Pep-1</i>						
1.26	0.524	0.470	0.662	0.442	0.489	0.535
1.00	0.476	0.530	0.338	0.558	0.511	0.465
<i>n</i>	155	148	136	189	140	101
<i>Pep-2</i>						
1.22	—	—	0.129	—	0.031	0.043
1.00	1.000	1.000	0.871	1.000	0.969	0.957
<i>n</i>	118	148	136	189	80	81
<i>Pep-3</i>						
1.06	0.278	0.158	0.125	—	0.011	0.005
1.00	0.722	0.842	0.875	1.000	0.989	0.995
<i>n</i>	158	148	136	189	140	101

Alleles in order of increasing anodal mobilities; *n*, number of individuals analysed. Populations according to country of origin. The numbers in the row below the countries represent latitudinal positions.

At the 2 loci, *G-6-pd* and *Idh* showing significant correlations with latitude, the common allele frequencies exhibit a gradual change from different localities of collections, indicating the presence of a latitudinal cline (fig 1).

In all cases, there is a large frequency difference between the third site of collection (Crete) and fourth (Israel) (fig 1). The same difference in *G-6-pd* is smaller. In contrast, the frequency changes of the most common allele at the locus *Mpi* are rather smooth with the exception of the population collected from Spain (fig 1a). For loci showing the smallest frequency difference, *Pep-1* and *Pep-2*, these are 1.1 and 4.3%, respectively. The differences of loci that showed latitudinal clines,

Table II. Comparison of observed (Obs) and expected (Exp) homozygous and heterozygous genotypes in the populations sampled.

Locality	Homozygotes		Heterozygotes		n	χ^2
	Obs	Exp	Obs	Exp		
<i>Mpi</i>						
Spain	96	99.07	40	36.93	136	0.97
Greece-1	133	134.75	15	13.25	148	0.03
Greece-2	115	114.85	21	21.15	136	0.00
Israel	116	119.35	62	58.65	178	0.59
Egypt-1	67	63.51	45	48.49	112	0.58
Egypt-2	60	63.75	60	56.25	120	0.44
<i>Idh</i>						
Israel	95	96.63	65	63.37	160	0.11
Egypt-1	80	82	60	58	140	0.17
Egypt-2	60	64.25	60	55.75	120	0.70
<i>Pep-1</i>						
Spain	75	77.68	80	77.32	155	0.19
Greece-1	79	74.27	69	73.73	148	0.61
Greece-2	80	75.14	56	60.86	136	0.87
Israel	92	95.77	97	93.23	189	0.31
Egypt-1	69	70.04	71	69.96	140	0.02
Egypt-2	47	50.75	54	50.25	101	2.73
<i>Pep-2</i>						
Greece-2	105	105.44	31	30.56	136	0.04
Egypt-1	75	75.25	5	4.75	80	0.09
Egypt-2	74	74.33	7	6.67	81	0.17
<i>Pep-3</i>						
Spain	96	94.57	62	63.43	158	0.09
Greece-1	111	108.62	37	39.38	148	0.62
Greece-2	103	106.25	33	29.75	136	2.49
<i>G-6pd</i>						
Spain	74	64.5	22	31.48	96	11.32*
Greece-1	130	127.47	18	20.53	148	3.53
Greece-2	126	120.94	10	15.06	136	23.08*

Number of individuals = n . χ^2 test with 1 dF . * $p < 0.001$

G-6pd, *Mpi*, *Pep-3* and *Idh*, are 19.9, 21.3, 27.3 and 36.7% respectively. The *Mpi* frequency difference between natural populations collected from Egypt and from Attiki is 33% and is one of the highest observed.

DISCUSSION

An extensive analysis has shown that the polymorphism (at least 16 genes out of 25) found in the African populations of *C. capitata* was high. However, only 6 loci out of these 25 were found polymorphic in the introduced populations of Mediterranean

Table III. Spearman rank correlation coefficient r_s between the common allele frequency and degree of north latitude.

	r_s	Significance level
<i>Mpi</i>	0.829	0.064
<i>G-6-pd</i>	-0.941	0.035
<i>Idh</i>	0.941	0.035
<i>Pep-1</i>	0.086	0.848
<i>Pep-2</i>	0.516	0.248
<i>Pep-3</i>	-0.829	0.064

* $p < 0.001$

medfly (Kourti *et al*, 1990). It is interesting to note that these 6 loci, *Mpi*, *G-6-pd*, *Idh*, *Pep-1*, *Pep-2* and *Pep-3* maintain variants at substantial frequencies and from these, the frequencies of *Mpi*, *G-6-pd*, *Idh* and *Pep-3* change steadily with latitude. When neighboring populations of a species are compared, one finds that they usually differ from one another, slightly or appreciably, in a number of characteristics, *eg*, size, color or any other morphological or physiological character (Halkka *et al*, 1975; Rhomberg and Singh, 1989). Huxley (1942) introduced the term 'cline' for such a character gradient. The gradual change of the common allele frequencies for at least these 4 loci corresponds to the latitudinal cline definition. However, only 2 loci, *G-6-pd* and *Idh*, have shown a significant r_s . The cyclic behavior of *Mpi*, found by Malacrida *et al* (1992) in another Mediterranean region (Italy), corroborates the results of the latitudinal cline observed through the Mediterranean basin. Alternatively, observed variations (*ie Mpi*) in allelic frequencies could be the combined effect of latitude and season. Several loci have been reported as latitudinally clinal in other insect natural populations. The list includes *Est-6*, *Adh*, α -*Gpd*, *G-6-pd*, *Odh*, *6-Pgd*, *Aph*, *Est-C*, and *Mdh* (Voelker *et al*, 1977, 1978; Anderson, 1981; Oakeshott *et al*, 1981, 1982; David, 1982; Anderson and Oakeshott, 1984). The seasonal cycle in *Mpi* and the latitudinal clines of other loci suggest that alternative alleles have different optimal temperatures or other environmental variables (see also Tomiuk and Wohrmann, 1984). The working hypothesis is that the cline results from a geographically variable selective factor of the environment (Halkka *et al*, 1975). A concordance of clines for different characters is normally only found where ranges are essentially latitudinal and where the various environmental gradients (temperature and humidity for example) run more or less in a parallel way. The study of geographic variations has revealed that many of them are clinal. Clines are, ultimately, the product of 2 conflicting forces, selection, which would make every population uniquely adapted to its local environment, and gene flow, which would tend to homogenize all populations.

While some alleles sampled at high frequency in African populations are also found in Mediterranean populations, others, especially the uncommon ones, are locally or regionally lost (Kourti *et al*, 1990). This new pattern of allozyme frequency is maintained at a relatively stable level.

The contrast in polymorphism patterns between African and Mediterranean populations suggests that medfly polymorphism could be maintained by balancing

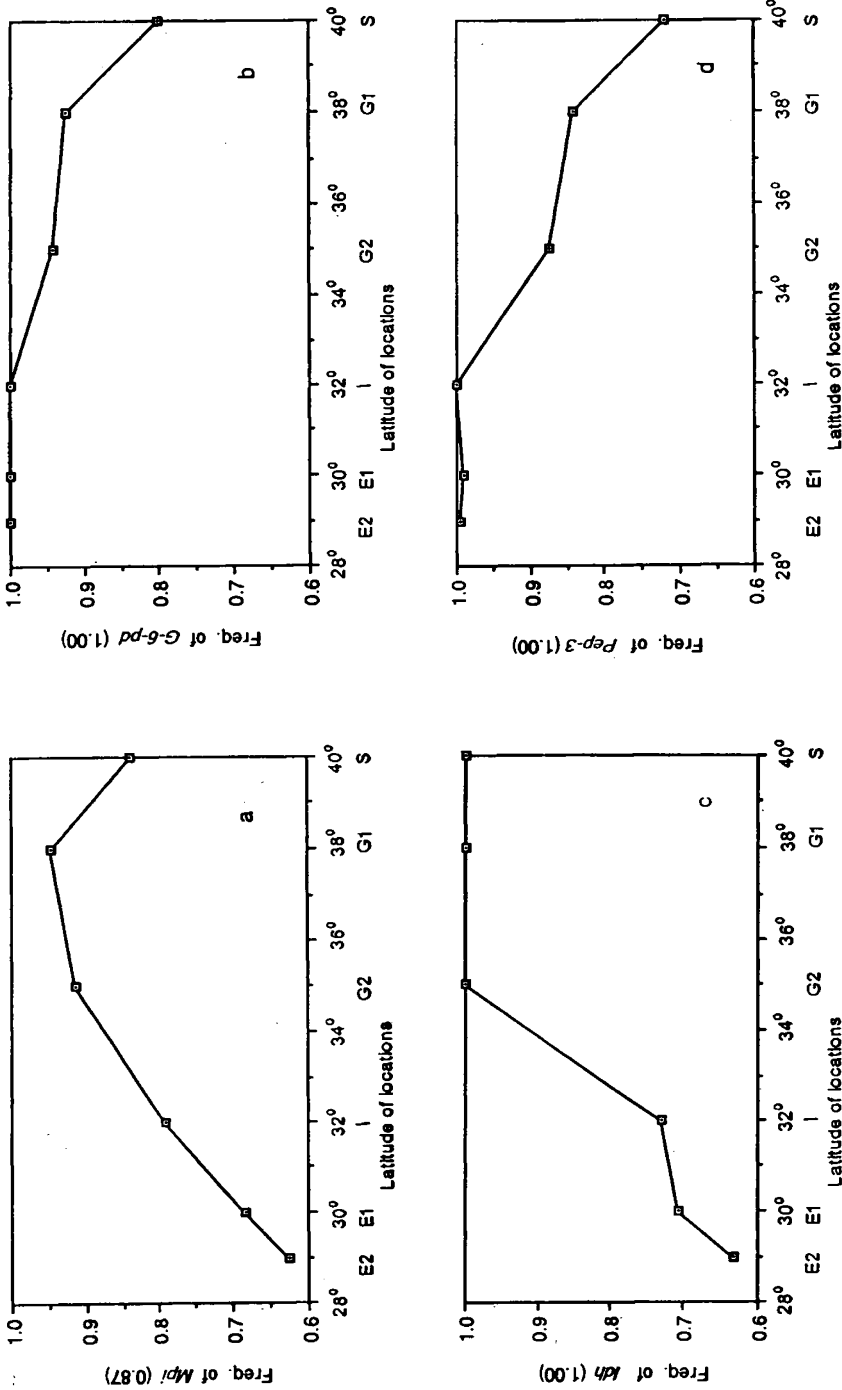


Fig 1. Allele frequencies at 4 polymorphic loci (a: *Mpi*, b: *G-6-pd*, c: *Idh*, d: *Pep-3*) versus latitude of collection sites. The populations are ordered by latitude, Spain (S): 40°; Greece 2 (G2): 35°; Israel (I): 32°; Greece 1 (G1): 38°; Egypt 1 (E1): 30° and Egypt 2 (E2): 20°. Each point represents the observed frequency for the most common allele at each locality.

selection for which the equilibrium shifts with climate. Therefore the adaptively neutral or nearly neutral variation is expected to be purged, most likely by the repeated passage of local populations through bottlenecks (Kourti *et al*, 1990). Moreover, the lower polymorphism of Mediterranean populations compared with the African populations resembles the general pattern of marginal populations of a species submitted to genetic drift. Loci that are under rather strong balancing selection manage to maintain their total variability while others become monomorphic. Identification of such loci, however small in proportion, as in the case of medfly, may be more important than performing generalized tests of the neutral theory (Tomiuk, 1987). This explanation of widespread monomorphism, the difficulty of establishing and maintaining alleles expected by balancing selection, is an alternative to the hypothesis of broad adaptability of 'general purpose genotypes' (Parker *et al*, 1977; Angus and Schutz, 1979; Jaenike *et al*, 1980; Lynch, 1983).

Environment (latitude and/or season) could exert its force on allozyme frequencies. Since it is reasonable to expect that some loci might be important selective targets, and that there is a relationship between genotype and environment (often confirmed), as previously discussed, neutrality could not satisfactorily explain the data. Similar results have been reported for other natural populations (Johnson *et al*, 1969; Johnson and Schaffer, 1973; Voelker *et al*, 1978). In most cases there are correlations between allelic frequencies and environmental variables clearly favoring the selection hypothesis (Schaffer and Johnson, 1974).

In conclusion, the genetic latitudinal variations of *C capitata* could be considered, as a whole, as a consequence of natural selection. Further studies, comparing tropical and temperate populations from other parts of the world, will determine whether such clines exist worldwide.

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