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Determination of esterase enzyme polymorphism in house fly (*Musca domestica* L.) populations in Turkey

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Abstract: The objective of this study was to determine esterase enzyme polymorphism in 16 natural *Musca domestica* L. populations from the Aegean and Mediterranean regions of Turkey by using the native polyacrylamide gel electrophoresis (PAGE) technique. The high level of genetic variation among populations and species make esterase enzymes an important tool for analyzing genetic differentiation and evolutionary relationships in insects. In the present study, 22 α-esterase bands were detected by using α-naphthylacetate as a substrate. The frequencies of these bands were variable within and among populations. Neither regional nor population specific esterase bands were detected. The origin of speciation of *M. domestica* is unknown, but the southern Palearctic region, particularly the Middle East, has been suggested as the likely candidate location. Studies on genetic diversity in *M. domestica* are important for providing knowledge that will better enable us to understand the origin of this species. This preliminary study is important because it is the first in the literature to illustrate the esterase isozymes and their frequencies for this part of the world. For a better evaluation and understanding of the ongoing situation of *M. domestica* populations in Turkey as a Palearctic country, however, further research is needed to screen not only populations from these 2 regions but also from other regions for many other enzyme and DNA loci.

Key words: Esterase patterns, *Musca domestica*, genetic variation, electrophoresis, PAGE

Türkiye'deki karasinek (*Musca domestica* L.) populasyonlarında esteraz enzim polimorfizminin belirlenmesi

Özet: Bu çalışmanın amacı, Türkiye'nin Ege ve Akdeniz Bölgeleri'ne ait 16 doğal *Musca domestica* L. populasyonundaki esteraz enzim polimorfizminin doğal polyakrilamid jel elektroforezi (native PAGE) yöntemiyle saptanmasıdır. Populasyonlar ve türler arasında yüksek düzeylerde genetik varyasyona sahip olmaları, esteraz enzimlerini böceklerdeki genetik farklılaşmaların ve evrimsel ilişkilerin analiz edilmesinde önemli bir araç yapmaktadır. Bu çalışmada α-naftil asetatın substrat olarak kullanılmasıyla 22 α-esteraz bandı tespit edilmiştir. Bu bantların frekansları populasyonlar içinde ve arasında değişmektedir. Herhangi bir bölge ya da populasyona özgü bant(lar) saptanmamıştır. *M. domestica*'nın orijini bilinmemektedir. Ancak, güney Palearktik Bölge, özellikle de Orta Doğu, bu türün orijini için en olası aday yer olarak önerilmektedir. *M. domestica*'nın genetik çeşitliliğine ilişkin çalışmalar, bu türün orijinini anlamaya yönelik bilgi edinilmesinde önemlidir. Literatürde bir ilk olan ve dünyanın bu bölgesi için esteraz izoenzimlerini ve bunların frekanslarını gösteren bu ön çalışma önemlidir. Ancak, bir Palearktik Bölge ülkesi olan Türkiye'nin *M. domestica* populasyonlarındaki durumun daha iyi değerlendirilmesi ve anlaşılması için sadece bu iki bölgeden değil, diğer bölgelerden de populasyonların daha fazla enzim ve DNA lokusu bakımından taranmaları gerekmektedir.

Anahtar sözcükler: Esteraz desenleri, *Musca domestica*, genetik varyasyon, elektroforez, PAGE

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Introduction

The house fly, *Musca domestica* Linnaeus, is a synanthropic insect of medical and veterinary importance. It is distributed throughout most of the world and especially in temperate and tropical climates, reproduces year round, and produces a large number of offspring (Marquez and Krafsur, 2003). A variety of molecular markers, such as allozymes (Black and Krafsur, 1985; Black and Krafsur, 1986; Krafsur et al., 1992), microsatellites (Krafsur et al., 2005), and mitochondrial DNA (Krafsur et al., 2000; Marquez and Krafsur, 2002; Marquez et al., 2003; Cummings and Krafsur, 2005), have been used in order to determine the genetic diversity within and among North American, European, Asian, and African house fly populations.

Esterase enzymes, which preferentially hydrolyze the esters of carboxylic acids, have been a major part of insect biochemical research for many years. The classification of esterase enzymes is primarily based on sensitivities to diagnostic concentrations of 3 groups of inhibitors: sulfhydryl reagents, organophosphates, and eserine sulfate. As a result, 4 classes of enzymes have been identified (Oakeshott et al., 1993). In insects, these enzymes are thought to play a role in reproductive behavior, pheromone and hormone metabolism, digestion, neurotransmission, and resistance to insecticides (Oakeshott et al., 2005). Many esterases can be visualized by electrophoresis; with multiple isozymes and high levels of genetic variation among populations and species, these enzymes are important tools for the analysis of genetic differentiation and evolutionary relationships in insects, especially for biochemical and ecological studies (Lima-Catelani et al., 2004; Oakeshott et al., 2005). It has been argued that gene duplication followed by the divergence of duplicated genes might be responsible for at least part of this variability (Nascimento and Bicudo, 2002).

The genetic and biochemical characteristics of esterase enzymes have previously been studied in some strains of Japanese house flies using gel electrophoresis techniques (Ogita, 1962; Ogita and Kasai, 1965). To the best of our knowledge, however, there have not been any publications in the literature on enzyme polymorphism, including esterase enzymes, in field populations of *M. domestica* in Turkey. The objective of our research was to determine the variation of esterase enzyme patterns by using polyacrylamide gel electrophoresis (PAGE) on *M. domestica* populations collected from 16 different cities in the Aegean and Mediterranean regions of Turkey. These regions are the most important agricultural, industrial, and touristic areas in the country and are under the continual influx of both organic and inorganic xenobiotics.

Materials and methods

House fly strains

The house fly samples were collected in the summer of 2005 from farms and garbage disposal sites in 16 different cities in the Aegean and Mediterranean regions of Turkey (Figure 1). Samples taken from 3 different locations in each city were used for the analysis. The field-collected samples were reared as described previously (Taşkin and Kence, 2004). They were bred in laboratory conditions for 1 generation and, after freezing, stored at –80 °C until used.

Analysis of esterases

In our preliminary experiments, α- and β-esterase bands totally overlapped in the gels, indicating that the α- and β-esterases might have not been differentiated enough yet in the house fly populations studied. Only α-esterases were analyzed, due to their higher intensity of staining. The α-esterase patterns of the flies were determined by using 4% stacking and 8% separating polyacrylamide gels (Sousa-Polezzi and Bicudo, 2005). In the analysis, 156 flies from the Aegean region and 148 flies from the Mediterranean region were used. The procedure was in brief: individual flies of 4-6 days old from each population were homogenized in 50 μL of the homogenization buffer (1.5 M Tris-HCl, pH = 8.8, 10% glycerol). Next, 25 μL of supernatant was subjected to electrophoresis at a constant 200V electrical current for 4 h at 4 °C by connecting the system to a cooling circulatory water bath in 0.1 M Tris-glycine (pH = 8.3) running buffer. The gels were stained in the dark at room temperature for 1 h with a solution containing 90 mg of α-naphthylacetate (α-NA), 180 mg of Fast Blue RR salt, and 5 mL of n-propanol in 150 mL of sodium phosphate buffer (pH = 6.2).

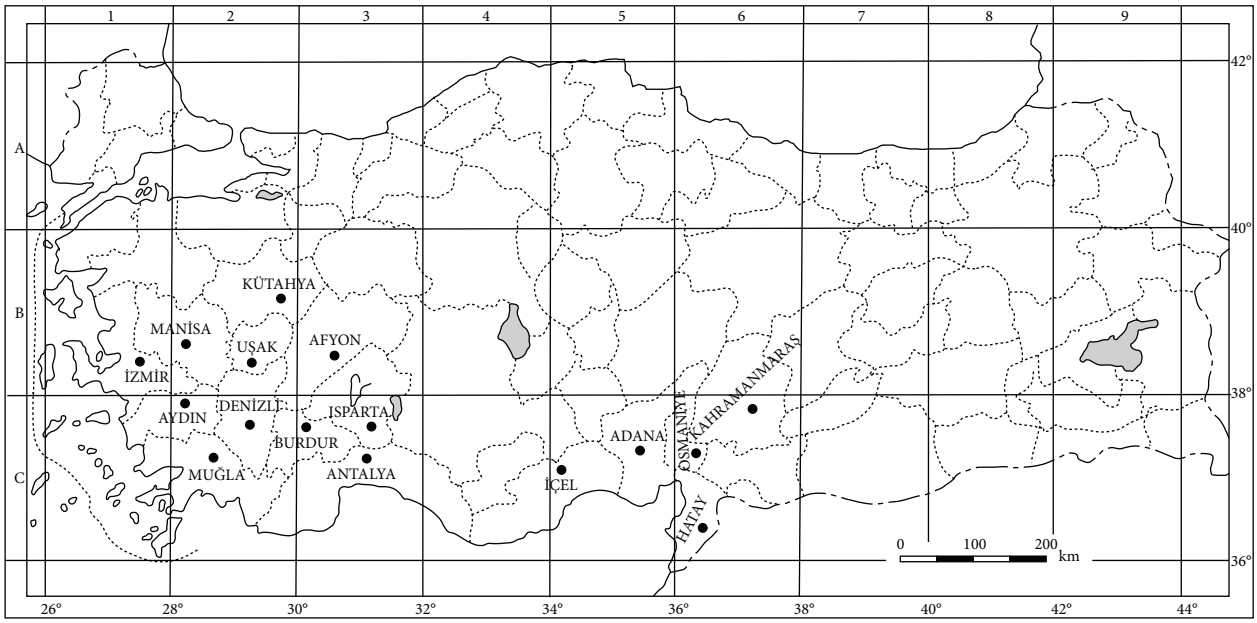

Figure 1. Map of the *M. domestica* sampling area.

Evaluation of esterases

The α-esterase bands separated by electrophoresis on gels were characterized by their position due to their relative mobility (Rm). After determining the band pattern of each individual in the populations, the zymograms were constructed and the frequencies of each band were calculated to evaluate the degree of variations within and among the populations in terms of α-esterases.

Statistical analysis

Differences among populations and regions as to the esterase patterns were statistically analyzed with POPGENE 1.31 (Yeh et al., 1999). In this analysis, the presence of the band was scored as "1" and the absence of the band was scored as "0."

Results and discussion

In all of the *M. domestica* populations from the Aegean and Mediterranean regions of Turkey screened in this study, a total of 22 α-esterase bands were detected, with Rm values ranging from 0.07 to 0.85. These bands were numbered successively according to their position in the gel, increasing the number with an increase in negative charge (Figure 2). A photographic illustration of the esterase band patterns is provided in Figure 3.

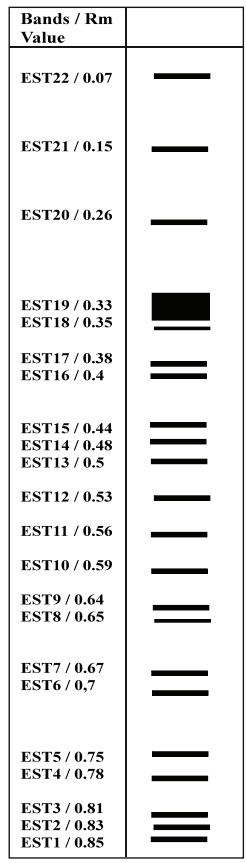

Figure 2. The 22 α-EST bands with their Rm values detected in field-collected populations of *M. domestica* from the Aegean and Mediterranean regions of Turkey.

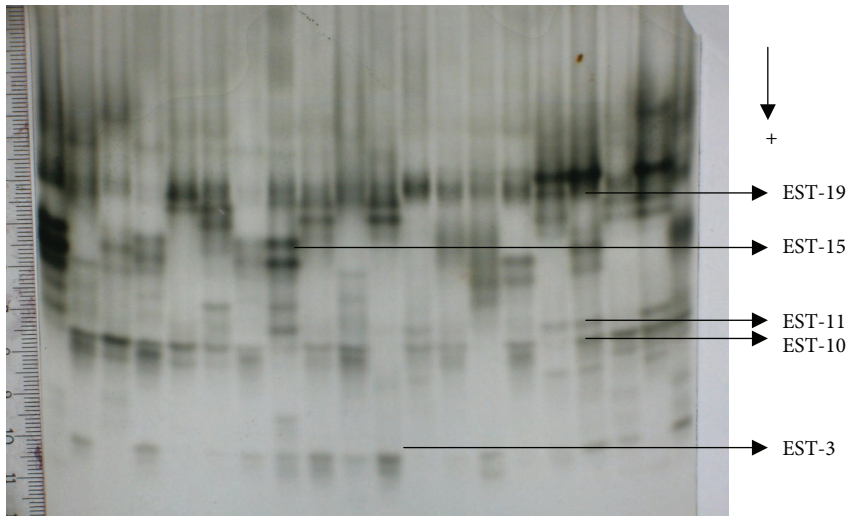

Figure 3. A photographic illustration of esterase band patterns for the Burdur population.

Among 22 bands, EST-19 (Rm = 0.33) showed the greatest activity in general, as indicated by the thickness and intensity of staining; it was also detected in all populations, with a frequency of 1.00 (Figure 2, Table 1). This reinforces the indispensable role of EST-19 for the species. On the other hand, EST-8 (Rm = 0.65) was the rarest band observed in the populations, with a mean frequency of 0.12 for Aegean populations and only 0.01 for Mediterranean populations. This band was detected in 4 out of 8 Aegean populations, with the frequencies varying between 0.05 (in the Afyon population) and 0.60 (in the Uşak population); among the Mediterranean populations, it was specific to Isparta with a frequency of 0.05. Another rare band observed was EST-18 (Rm = 0.35), found exclusively in the Mersin population from the Mediterranean region, with a frequency of 1.00; in the Muğla and Manisa populations of the Aegean region, meanwhile, it was found in frequencies of 0.50 and 0.45, respectively. The bands EST-1, EST-2, EST-7, EST-10, EST-14, EST-15, EST-18, EST-19, and EST-21 were present at similar mean frequencies in both regions. The bands EST-4, EST-6, EST-8, EST-9, and EST-22 were found in higher frequencies in the Aegean region than in the Mediterranean region. However, the frequencies of bands EST-3, EST-11, EST-12, EST-13, EST-16, EST-17, and EST-20 were higher in the Mediterranean region (Table 1).

The differences in frequencies of esterase bands detected within and among populations may suggest that the alleles are under different selection pressures. Climatic patterns, breeding media, the use of insecticides, and different pest management strategies can be considered as environmental selection factors. Sousa-Polezzi and Bicudo (2005) noted that the genes responsible for the esterase isozymes are affected by insect-control procedures. Previously, the partial sequence analysis of *MdαE7* (Gacar and Taşkın, 2009) and *Ace* (unpublished results) genes in the populations used in this study were carried out, and it was detected that the frequencies of organophosphate-associated mutations were very high in all the populations. Additionally, in another study (unpublished results), the frequencies of pyrethroid resistance-associated mutations of *Vssc1* and *CYP6D1* were found to be very low in the same populations. Based on these studies, it can be concluded that the insecticide pressures on these screened populations were similar to each other, and, therefore, the esterase polymorphism detected in this study is important for the genetic characterization of these populations.

According to Nei's (1987) analysis of gene diversity (Table 2), the total genetic variation (H_T) for

Table 1. The frequency of esterase bands for each population; number of individual flies analyzed from each population is shown in parentheses (Aege.: Aegean populations, Medit.: Mediterranean populations).

Band	Muğla (20)	İzmir (20)	Aydın (16)	Denizli (20)	Uşak (20)	Manisa (20)	Kütahya (20)	Afyon (20)	Hatay (16)	Osmaniye (20)	Isparta (20)	Antalya (20)	Adana (20)	Burdur (20)	Mersin (16)	K.Maraş (16)	Aege. (156)	Medit. (148)
EST-1	15	30	50	20	0	15	10	10	31	5	15	30	35	20	0	0	18	18
EST-2	25	45	19	30	40	10	15	50	19	40	25	75	25	30	6	6	30	30
EST-3	35	100	50	40	45	45	25	80	69	65	75	95	65	65	44	19	53	64
EST-4	20	20	0	25	20	10	0	0	0	0	0	15	15	5	13	0	12	6
EST-5	35	35	0	40	20	10	20	0	0	5	15	45	35	5	19	0	21	16
EST-6	30	5	75	5	30	10	15	5	0	0	0	30	15	5	13	0	21	8
EST-7	50	10	0	10	60	0	0	0	0	5	15	0	20	30	63	38	17	20
EST-8	15	0	0	0	60	10	0	5	0	0	5	0	0	0	0	0	12	1
EST-9	80	65	81	75	80	80	65	75	25	50	60	10	0	80	63	100	75	47
EST-10	80	80	94	75	40	95	65	80	44	50	75	90	95	85	100	63	76	76
EST-11	20	70	38	35	35	30	30	70	69	50	65	45	35	45	94	44	41	55
EST-12	0	0	0	0	0	5	20	30	50	40	25	20	0	25	63	0	7	27
EST-13	10	35	0	0	0	20	25	15	50	55	35	20	20	40	38	0	14	32
EST-14	45	60	69	80	40	50	65	55	38	65	80	85	45	50	50	56	58	60
EST-15	45	60	81	80	55	40	55	55	50	65	80	85	50	60	50	38	58	61
EST-16	5	15	31	10	0	30	15	10	0	55	35	0	10	35	0	44	14	23
EST-17	30	15	31	10	0	25	10	20	25	45	65	0	20	65	56	94	17	45
EST-18	50	0	0	0	0	45	0	0	0	0	0	0	0	0	100	0	12	11
EST-19	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
EST-20	5	100	19	80	50	50	20	30	25	35	90	35	35	30	69	100	45	51
EST-21	10	5	63	80	45	0	0	5	25	15	20	25	20	0	25	100	25	27
EST-22	15	10	44	0	40	0	0	5	0	0	10	0	25	0	6	0	14	6

Table 2. An analysis of gene diversity for Aegean populations, Mediterranean populations, and all populations studied (H_T: total expected heterozygosity, H_S: within-population genetic variation, GST: mean of genetic differentiation coefficient, Nm: gene-flow value estimated from G_{ST}).

EST bands	Aegean populations					Mediterranean populations					All populations				
	Sample size	H_T	H_S	G_{ST}	Nm*	Sample size	H_T	H_S	G_{ST}	Nm*	Sample Size	H_T	H_S	G_{ST}	Nm*
EST-1	156	0.184	0.170	0.077	5.970	148	0.167	0.156	0.065	7.205	304	0.176	0.163	0.072	6.476
EST-2	156	0.272	0.259	0.050	9.493	148	0.276	0.238	0.137	3.155	304	0.274	0.248	0.094	4.836
EST-3	156	0.463	0.321	0.307	1.29	148	0.486	0.425	0.126	3.469	304	0.476	0.373	0.216	1.810
EST-4	156	0.118	0.112	0.048	9.947	148	0.066	0.063	0.048	9.903	304	0.092	0.087	0.052	9.089
EST-5	156	0.201	0.186	0.076	6.065	148	0.155	0.139	0.102	4.380	304	0.179	0.163	0.090	5.075
EST-6	156	0.225	0.179	0.201	1.985	148	0.079	0.073	0.075	6.154	304	0.156	0.126	0.189	2.149
EST-7	156	0.173	0.134	0.222	1.751	148	0.221	0.188	0.147	2.898	304	0.197	0.161	0.182	2.247
EST-8	156	0.122	0.094	0.226	1.711	148	0.006	0.006	0.022	21.996	304	0.066	0.050	0.239	1.592
EST-9	156	0.499	0.489	0.021	23.376	148	0.458	0.274	0.400	0.748	304	0.487	0.382	0.216	1.817
EST-10	156	0.499	0.443	0.112	3.950	148	0.485	0.379	0.219	1.787	304	0.494	0.411	0.168	2.471
EST-11	156	0.373	0.336	0.099	4.563	148	0.462	0.404	0.126	3.482	304	0.424	0.370	0.127	3.428
EST-12	156	0.071	0.064	0.097	4.626	148	0.283	0.252	0.112	3.982	304	0.186	0.158	0.152	2.793
EST-13	156	0.131	0.122	0.068	6.808	148	0.299	0.278	0.068	6.826	304	0.221	0.200	0.095	4.767
EST-14	156	0.436	0.420	0.038	12.794	148	0.470	0.434	0.077	6.030	304	0.454	0.427	0.061	7.696
EST-15	156	0.441	0.420	0.048	9.939	148	0.471	0.436	0.073	6.379	304	0.457	0.428	0.063	7.407
EST-16	156	0.148	0.142	0.045	10.539	148	0.282	0.248	0.118	3.730	304	0.219	0.195	0.109	4.071
EST-17	156	0.171	0.164	0.037	13.068	148	0.411	0.221	0.462	0.583	304	0.310	0.193	0.378	0.824
EST-18	156	0.136	0.104	0.237	1.609	148	0.219	0.000	1.000	0.000	304	0.179	0.052	0.710	0.204
EST-19	156	0.000	0.000	****	****	148	0.000	0.000	****	****	304	0.000	0.000	****	****
EST-20	156	0.433	0.251	0.419	0.694	148	0.469	0.296	0.369	0.856	304	0.452	0.274	0.395	0.766
EST-21	156	0.272	0.194	0.289	1.229	148	0.333	0.152	0.543	0.420	304	0.304	0.173	0.431	0.659
EST-22	156	0.145	0.127	0.126	3.473	148	0.045	0.041	0.090	5.055	304	0.097	0.084	0.131	3.304
Mean	**156**	**0.251**	**0.215**	**0.142**	**3.024**	**148**	**0.282**	**0.216**	**0.234**	**1.640**	**304**	**0.269**	**0.215**	**0.200**	**1.996**
St. Dev.		**0.023**	**0.018**				**0.028**	**0.020**				**0.023**	**0.017**		

*Nm is the estimate of gene flow from G_{ST}: $Nm = 0.5(1 - G_{ST})/G_{ST}$.

the esterases analyzed was determined to be 0.251 ± 0.023 for the Aegean populations, 0.282 ± 0.028 for the Mediterranean populations, and 0.269 ± 0.023 for all of the populations screened. The higher proportion of these variations for Aegean populations [0.215 ± 0.018 (85.66%)], for Mediterranean populations [0.216 ± 0.020 (76.60%)], and for the overall tested populations [0.215 ± 0.017 (79.93 %)] was due to within-population genetic variation (H_S). In terms of the 22 α-EST bands, the mean of the genetic differentiation coefficient (G_{ST}) was 0.142 and 0.234 for the Aegean and Mediterranean populations, respectively. When considering all of the studied populations, this value was found to be 0.200. All of the 22 α-EST bands were differentiated among all tested populations, except for the EST-19 band, which was not differentiated among populations. The G_{ST} values of other bands in the Aegean populations varied between 0.021 (for the EST-9 band) and 0.419 (for the EST-20 band). For Mediterranean populations, the least differentiated band was EST-8, with a G_{ST} value of 0.022, and the most differentiated band was EST-18, with a G_{ST} value of 1.000. When both regions' populations were evaluated together, the EST-4 band was found to be the least differentiated and, again, the EST-18 band was identified as the most differentiated band, with G_{ST} values of 0.052 and 0.710, respectively. The level of gene flow (Nm) within a generation among all of the 16 populations studied was calculated to be, on average, 1.996; it was 3.024 among Aegean populations and 1.640 among Mediterranean populations.

Nei's genetic distance coefficient (D_N) (Nei, 1978) ranged from 0.0079 to 0.2694 among all of the possible population pairs (Table 3). Minimum distance was detected between the Osmaniye and Hatay populations, while maximum distance was detected between the populations collected from Kahramanmaraş and Antalya.

It has been stated that the house fly in nature is vagile and highly fecund, has a short life cycle, and demonstrates boom-and-boost dynamics (Krafsur et al., 2005). It cannot tolerate subfreezing temperatures in the long term and therefore must overwinter in restricted habitats by slow, continuous reproduction. This reproduction pattern results in annual population bottlenecks, in which genetic variation is lost by genetic drift. However, house flies are highly vagile and readily "hitchhike" in commercial vehicles and other forms of transport, and they are among the most common insects found in aircrafts. Over the long term, then, the forces of migration would seem likely to strongly neutralize the forces of drift and local regimes of natural selection. In our study, the lack of any region- or population-specific esterase band findings might be the result of great migration rates of this species between the cities and regions of Turkey.

Gel electrophoresis techniques have been widely used to examine the genetic variation in populations of many species. Though the bands on gels are electrophoretic phenotypes rather than alleles, these bands have been frequently evaluated as genes and alleles (Nascimento and Bicudo, 2002). It was reported that esterase enzymes were associated to 3 anodal and 2 cathodal genetic loci in a Nearctic *M. domestica* population from central Iowa, USA (Krafsur et al., 1992). When considering cosmopolitan house fly populations, the Nearctic region has the least mitochondrial DNA (mtDNA) diversity of 6 zoogeographical regions (Marquez and Krafsur, 2003). In the present study, the detected 22 anodal α-esterase bands can most probably be distributed to 13 genetic loci in Turkish house flies. The great difference observed between the numbers of anodal esterase loci can be explained by the origin of the organism. As cited in Marquez and Krafsur (2002), both Skidmore (1985) and Pont (1991) suggested that *M. domestica* originated in the Southern Palearctic region, and the Middle East in particular is viewed as the most likely candidate location for the origin of this species.

In the future, more extensive studies should be carried out in order to clarify the exact number of *esterase loci* in these populations. Studies determining the genetic diversity in *M. domestica* populations are important for understanding the origin of this species. The present study is the first to be carried out using esterase isozymes as biochemical markers in house fly populations in Turkey. However, for better evaluation and understanding of the ongoing situation in *M. domestica* populations in Turkey, a

Table 3. Estimates of Nei's genetic distance (D_N) coefficients (Nei, 1978) among the *M. domestica* populations studied.

Population	Muğla	İzmir	Aydın	Denizli	Uşak	Manisa	Kütahya	Afyon	Hatay	Osmaniye	Isparta	Antalya	Adana	Burdur	Mersin	K. Maraş
Muğla	****															
İzmir	0.1162	****														
Aydın	0.0389	0.1212	****													
Denizli	0.0368	0.0678	0.0472	****												
Uşak	0.0219	0.0928	0.0545	0.0272	****											
Manisa	0.0124	0.0799	0.0398	0.0310	0.0465	****										
Kütahya	0.0125	0.0957	0.0349	0.0312	0.0280	0.0123	****									
Afyon	0.0301	0.0539	0.0440	0.0387	0.0354	0.0228	0.0163	****								
Hatay	0.0448	0.0760	0.0671	0.0466	0.0464	0.0355	0.0182	0.0130	****							
Osmaniye	0.0395	0.0712	0.0548	0.0466	0.0453	0.0282	0.0109	0.0130	**0.0079**	****						
Isparta	0.0667	0.0299	0.0600	0.0441	0.0627	0.0385	0.0394	0.0266	0.0368	0.0186	****					
Antalya	0.0713	0.0632	0.0587	0.0728	0.0827	0.0569	0.0525	0.0344	0.0483	0.0436	0.0484	****				
Adana	0.0282	0.0768	0.0423	0.0368	0.0505	0.0142	0.0228	0.0282	0.0259	0.0326	0.0457	0.0275	****			
Burdur	0.0206	0.0779	0.0399	0.0446	0.0454	0.0161	0.0153	0.0120	0.0268	0.0122	0.0251	0.0582	0.0289	****		
Mersin	0.0957	0.1631	0.1458	0.1294	0.1528	0.0699	0.1229	0.1084	0.1295	0.1267	0.1154	0.1603	0.1187	0.1122	****	
K.Maraş	0.1765	0.1847	0.1550	0.0942	0.1583	0.1656	0.1843	0.1820	0.2097	0.1728	0.1158	**0.2694**	0.2104	0.1419	0.2464	****

country in the Palearctic region, there is a need to screen other isozymes and DNA loci, not only in populations from the 2 regions examined here but also from other parts of the country.

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