♂) in addition to these four one-armed autosomes, two one-armed autosomes were found in pair No. 5 (2N = 23 and 24) (table 1, fig. 3).

Shrews from the Lake Drużno area have completely different karyotypes. The number of chromosomes in the eight individuals examined varies between 27 and 28 (table 1, figs 4 and 5). In addition to pairs Nos 1, 2 and 9 (always two-armed) two-armed autosomes occur only in pairs 3–5 and consist of the following elements: 3*i/h*, 4*l/j*, 5*o/k*, while elements *g*, *m*, *n*, *p*, *q* and *r* remain one-armed (fig. 5). Pair No. 5 consists of either four one-armed (fig. 4) or one two-armed and two one-armed chromosomes (fig. 5).

The two-armed chromosomes of pairs 1, 2 and 9 and X chromosomes are composed in all the *S. araneus* populations so far examined of the same one-armed elements. The metacentric pair No. 3, on the other hand, composed of arms *i* and *h*, is typical of the Northwestern European populations: chromosomes *i/h* have been identified in all three British races[6], three Scandinavian[4] and in the 'Ulm' race[7], metacentrics *i/h* also occur in the 'Altaj' race (Chaldejevo)[12]. In the 'Białowieża' race, however, element *i* is connected with element *k*, and element *h* with element *n*. The heterozygotic pair No. 5 in populations from the Lake Drużno area is formed of *o/k* elements. This combination occurs in two British race ('Hermitage' and 'Aberdeen'), and in the 'Skåne' race, whereas in the 'Białowieża' race element *o* remains one-armed ('pair' No. 8) (table 2).

The 'Drużno' race is most similar to the following races: South Sweden ('Skåne) and two British races ('Hermitage' and 'Aberdeen'); two-armed *i/h* and *o/k* chromosomes occur in all these races. It differs from these races, however, by the absence of two-armed chromosomes *m/g* (table 2). The fusion of elements *m* and *g* is typical of all the West European, British and Scandinavian populations so far examined. In shrews from the most northern parts of Sweden (Lapland) elements *m* and *g* are usually not fused, while a Robertsonian polymorphism is present in shrews of the same race in NE Finland[4,5].

Siberian shrews from the Novosibirsk district are most similar to the 'Białowièza' race. The similarity applies to four pairs of autosomes with combinations of arms *l/j*, *k/i*, *p/m* and *n/h*; while the differences are the fusion of elements *r/g* and elements *o* and *q* remaining one-armed in the 'Białowieża' race, and fusion of *r/q* in the 'Novosibirsk' race, with elements *o* and *g* remaining one-armed (table 2).

The boundary line between shrews of the 'Drużno' and 'Białowieża' races is at present unknown. It must be assumed that these two chromosome races originate from different refuges from the Ice Age. Most probably shrews of the 'Drużno' race had a common origin with West European shrews, as is indicated by the similarity to the 'Ulm', British and Scandinavian races (with the exception of these shrews from Lapland). On the other hand the 'Białowieża' race probably had a common ancestry with some Siberian shrew populations ('Novosibirsk' race).

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Enzyme polymorphism in the Sicilian honeybee[1]

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Summary. In Sicily we can identify two genetically differentiated groups of local honeybee populations, on the basis of the only two polymorphic loci, *Mdh-1* and *Est*; the western one, whose genetic characteristics are probably those of the native honeybee (*Apis mellifera sicula*) and the eastern one affected by recent large-scale importation of Italian bees from northern Italy.

Key words. Honey bee, local population; *Apis mellifera ligustica*; enzyme polymorphism; genetic variability; genetic marker; *Est*-S allele.

Since the early 19th century, honeybees whose morphological and behavioral characteristics were distinct from those of the Italian bee (*Apis mellifera ligustica* Spinola, 1806), were known to occur in Sicily[2]. The first systematic investigations confirmed that they were a peculiar taxon[3] which was later called *A. m. sicula* Montagano, 1911[4]. The workers of the Sicilian bee have a dark abdomen[3–5], and both tongue length and cubital index are different from those of *A. m. ligustica* and *A. m. mellifera* Linnaeus, 1758[6]. Although Sicilian beekeepers have occasionally imported the more productive Italian bee ever since the last century[7], stocks of *A. m. ligustica* from northern Italy have been introduced on a large scale only during the last 10 years and only in the eastern part of the island; from there they are now spreading into other parts, thus hybridizing with the local bee.

This work aims to investigate the genetic variability of the Sicilian honeybee and its relationships with neighbouring populations, by using two gene-enzyme polymorphic systems, *Mdh-1* and *Est*. Also the eventual effects on its gene pool due to the *A. m. ligustica* introduction will be taken into account.

During 1983 and 1984, worker bees were randomly sampled in

10 Sicilian localities (table 1) from as many apiaries. Most of these apiaries are moved for short distances (less than 30 km) during the year. Some have indigenous queens only (Castelvetrano, Salaparuta, Corleone, Melilli, Caltagirone), and others have also recently (1977–1981) introduced Italian queens (Sortino, Giarre, Zafferana Etnea, Randazzo, S. Teresa di Riva). Only the Melilli apiary is wholly managed in the traditional way. The bees were transported alive into the laboratory and their thoraces immediately used for electrophoretic analyses on cellulose acetate strips (cellogel, Labometrics, Milano). The following enzyme systems were examined: malate dehydrogenase (MDH), esterase (EST), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase (IDH), and lactate dehydrogenase (LDH). The adopted methods were reported in a preceding paper[8]: only the EST Tris-Glycine electrode buffer was modified to $pH = 7.6$.

With the exception of MDH and EST, all the examined enzyme systems were monomorphic and their electrophoretic bands showed the same mobility as the *A. m. ligustica* ones. Also in the Sicilian bee MDH is controlled by three loci, only one resulting polymorphic, and EST by a single polymorphic one. *Mdh-1* alleles were S, M, F, F_1 and those of the *Est* locus were S, M, F (fig. 1), both named in order of increasing mobility. Their frequencies are shown in table 1.

Mdh-1 alleles are the same as in *A. m. ligustica*[9], but *Mdh-1*-F is the most common, *Mdh-1*-M appears only, and with low frequencies, in the samples 1 and 7 and *Mdh-1*-S is present in seven localities, usually with reduced frequencies. Only the locality number 9 shows the *Mdh-1*-F_1 allele, which is typical of Calabria, the southern part of the Italian peninsula (fig. 2 and table 2).

Among the esterase alleles, *Est*-M frequencies decrease from eastern to western areas, while *Est*-S frequencies increase in parallel. The former is the most common allele in the Italian bee (table 2), along with the *Est*-F allele, which is fairly common in Calabria but is found only in two localities of eastern Sicily (samples 8 and 10).

Allele frequency distribution in Sicily at the level of these polymorphic loci allows us to distinguish a western area from an eastern one. In the former, *Est*-S and *Mdh-1*-F alleles are almost fixed and consequently the homozygosity level is very high (table 1). On the contrary in the eastern area, where more than 10,000 queens from northern Italy have been imported recently (in the whole island there are about 80,000 colonies), the frequencies of *Mdh-1*-S and *Est*-M, typical alleles of the Italian bee, are usually high as well as the heterozygosity level. Actually such alleles are carried almost exclusively by heterozygous individuals.

This distribution pattern could be explained as the result of a high gene flow level across the Messina channel (4 km wide), separating eastern Sicily from Calabria. Whether the Messina channel is an efficient geographical barrier to the natural migration of honeybees or not is difficult to say on the basis of the

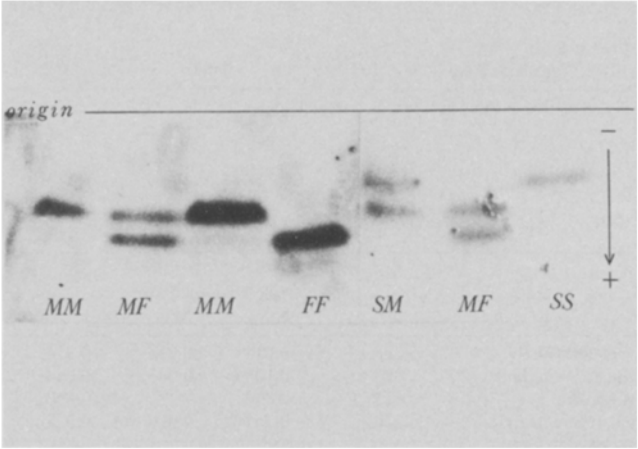

Figure 1. *Est* phenotypes observed in Sicilian honeybees.

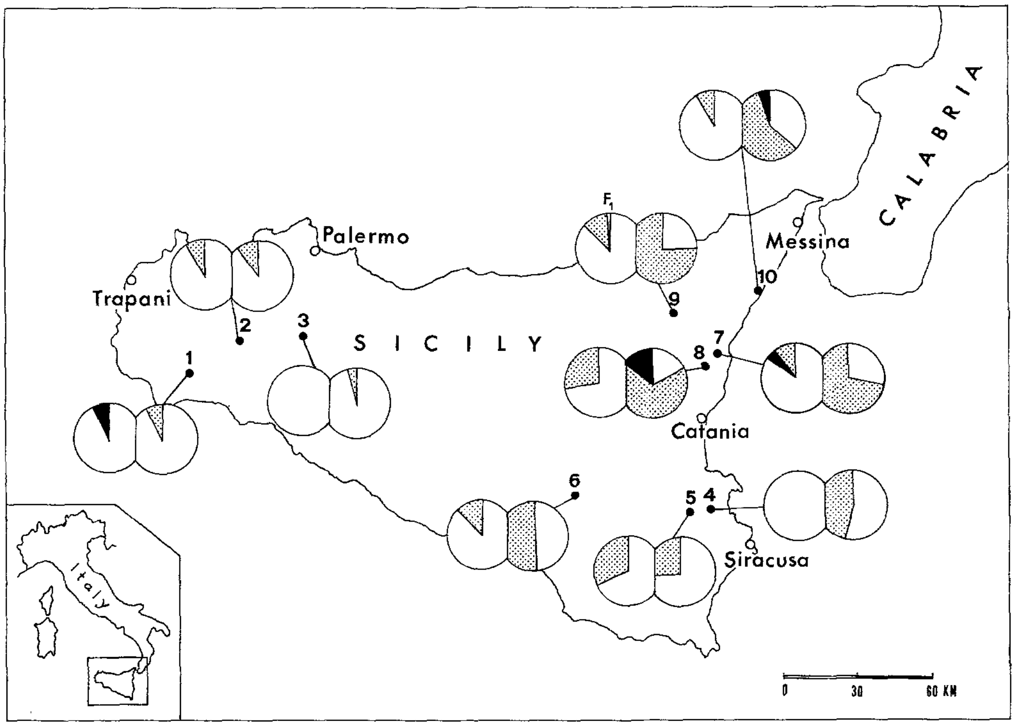

Figure 2. Allele frequencies at the polymorphic loci of Sicily honeybees. Left: *Mdh-1* (F, white; M, black; S, dotted). Right: *Est* (F, black; M, dotted; S, white).

Table 1. *Mdh-1* and *Est* allele frequencies in Sicily honeybee populations

Locality	N_e	*Mdh-1* locus							*Est* locus					
			Allele frequencies							Allele frequencies				
		N	S	M	F	F_1	H_0	H_e	N	S	M	F	H_0	H_e
Trapani														
1) Castelvetrano	21.43	92	–	0.071	0.929	–	0.141	0.131	46	0.935	0.065	–	0.130	0.122
2) Salaparuta	*	48	0.083	–	0.917	–	0.167	0.153	48	0.906	0.094	–	0.104	0.170
Palermo														
3) Corleone	42.86	88	–	–	1.000	–	0.000	0.000	40	0.975	0.025	–	0.050	0.049
Siracusa														
4) Melilli	287.57	124	–	–	1.000	–	0.000	0.000	116	0.539	0.461	–	0.491	0.497
5) Sortino	257.14	42	0.310	–	0.690	–	0.619	0.427	42	0.738	0.262	–	0.429	0.387
Catania														
6) Caltagirone	514.29	40	0.100	–	0.900	–	0.200	0.180	39	0.487	0.513	–	0.154	0.500
7) Giarre	75.00	45	0.111	0.044	0.845	–	0.244	0.273	43	0.267	0.733	–	0.488	0.392
8) Zafferana Etnea	53.57	42	0.274	–	0.726	–	0.548	0.398	50	0.160	0.700	0.140	0.600	0.465
9) Randazzo	107.14	44	0.102	–	0.875	0.023	0.250	0.223	46	0.239	0.761	–	0.435	0.364
Messina														
10) S. Teresa di Riva	107.14	50	0.080	–	0.920	–	0.160	0.147	48	0.364	0.594	0.042	0.792	0.513

N_e, effective population size according to Kerr[12]; H_e, expected heterozygosity; H_0, observed heterozygosity; *wild sample.

Table 2. *Mdh-1* and *Est* allele frequencies in honeybee populations from different parts of Italy

	Mdh-1 locus						*Est* locus				
		Allele frequencies						Allele frequencies			
	N	S	M	F	F_1	H_0	N	S	M	F	H_0
Western Sicily	228	0.018	0.028	0.954	–	0.092	134	0.936	0.063	–	0.097
Eastern Sicily	387	0.108	0.005	0.884	0.003	0.225	384	0.418	0.558	0.024	0.487
Calabria	413	0.092	–	0.860	0.048	0.271	380	0.004	0.685	0.311	0.421
Northern Italy	220	0.755	0.016	0.229	–	0.391	188	–	0.971	0.029	0.058

H_0 = observed heterozygosity.

population genetics data, since the North-Italian bee importations make the situation complicated. However, we think that between Sicily and the peninsula there is probably a gene flow restriction, as the differentiated distributions of some alleles at the polymorphic loci show (table 2), i.e.: a) *Est*-S, which shows a high frequency in the whole of Sicily, is virtually absent in Calabria; b) *Est*-F, which is frequent in Calabria, on the contrary is rare in eastern and absent in western Sicily; c) the whole of Sicily virtually lacks the *Mdh-1*-F_1 allele, which is typical of Calabrian honeybees.

Therefore we suppose that the genetic continuity between Calabrian and East-Sicilian honeybees is only an apparent phenomenon due to the activities of man. Moreover, east–west gradual variation of some allele frequencies (*Mdh-1*-F, *Mdh-1*-S, *Est*-S and *Est*-M) could represent a local geographic cline, caused by a simple diffusion of North-Italian genes from the Catania area. On this basis, we think that the genetic characteristics of the native Sicilian bee were similar to those of the present western populations at the level of the polymorphic loci examined, i.e.: a) *Est*-S, as a typical allele, highly frequent or probably fixed; b) *Mdh-1*-F highly frequent; c) *Mdh*-1-M rare.

Such genetic differentiation joins to the morphometric one shown by Giavarini[6], supporting the idea that *A.m.sicula* is a good subspecies, of which the present western populations are the last representatives. The *Mdh-1*-F allele is almost fixed in *A.m.intermissa* Buttel-Reepen, 1906 also[10], but this subspecies shows an equally fixed *Est*-M allele (unpublished data), like *A.m.ligustica* and *A.m.mellifera*[11]. This means that the *Est*-S allele, being typical of the Sicilian samples, can be used as a genetic marker to identify the Sicilian honeybee at the level of population, and can be employed eventually in selective programs.

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