biotics[6], or to germ free rats[13] reduced or abolished the excretion of phenolic acids and phenylvalerolactones. Recently evidence has been presented[14] that the major biliary metabolite of (+)-catechin, 3′-O-methyl-(+)-catechin glucuronide is formed in liver. The conjugates observed in urine in the present studies may also be formed in liver but the possibility that formation also occurs in other tissues such as the intestine wall should not be excluded.

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Genetic variability of *Apis mellifera ligustica* Spin. in a marginal area of its geographical distribution

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Summary. Marginal populations of *Apis mellifera ligustica* differ from the central populations of this subspecies in allele frequencies at the *Mdh*-1 locus. The difference seems to be due to gene flow from French populations of *A. m. mellifera.*

Apis mellifera L. shows a remarkable variability in several morphological and behavioral properties[1,2] (e.g. hygienic, foraging, stinging behaviour). In turn the level of its biochemical variability has been reported to be very low as compared with other invertebrates[3–5]. Only the malate dehydrogenase (MDH), alcohol dehydrogenase (ADH) and esterase (Est) gene-enzyme systems and the P-3 protein are polymorphic, in some or all of the geographical strains studied[6–9]. The present work analyses the electrophoretic variability of different *A. m. ligustica* Spin. populations from western Piedmont (Italy), which is a marginal area in the geographical distribution of this subspecies. Our investigation was carried out by examining the Est system on polyacrylamide gel (7.5%) and MDH, PGI (phosphoglucose isomerase) PGM (phosphoglucomutase), IDH (isocitrate dehydrogenase), ME (malic enzyme), G6PD (glucose-6-phosphate dehydrogenase), LDH (lactate dehydrogenase) on cellulose acetate strips (Cellogel, Labometrics, Milano). Samples of 58–82 adult workers were randomly collected from 8 spatially separated apiaries, each consisting of 20–50 colonies. The thorax of every bee was homogenized in 0.1 ml of 0.0025 M $MgCl_2$ and centrifuged at 12,000 rpm for 5 min, then the supernatant was used for electrophoresis. The electrode buffers were modified from Shaw and Prasad[10] for MDH, IDH, ME, G6PD, LDH; from Badino[11] for PGI, from Spencer et al.[12] for PGM and from Ayala and Powell[13] for Est. Enzyme activities were demonstrated by classical histological methods[10,14]. With the exception of MDH, none of the examined gene-enzyme systems was polymorphic in the populations studied. Both PGI and ME showed 2 isoenzymatic bands, and PGM, IDH, G6PD, LDH and Est 1 band only.

The MDH electrophoretic pattern (fig. 1) showed 3 enzymatically active regions, one of which migrated to the cathode in our buffer system; only the more anodal region was polymorphic. There was either a 1-banded phenotype or a 3-banded one. The allozymes of this region are produced by 3 codominant alleles at 1 locus (*Mdh*-**1**). These alleles seem to be homologous with fast (F), medium (M), slow (S)[9] and with a, b, c[8,15] of other authors respectively. The allele frequencies we found are shown in the table.

Allele frequencies at the *Mdh*-1 locus in Piedmont populations of *A.m. ligustica.* F, fast; M, medium; S, slow

Apiaries	No. of workers	*Mdh*-1 allele frequencies F	M	S
1 Asti	66	0.265	0.023	0.712
Maira valley				
2 Paschero	58	0.043	0.112	0.845
3 S. Michele	76	0.243	0.020	0.737
4 Acceglio	70	0.301	0.070	0.629
Tanaro valley				
5 Bagnasco	72	0.083	0.174	0.743
6 Ormea	76	0.072	0.572	0.355
7 Upega	78	0.128	0.519	0.353
8 Monesi	82	0.219	0.549	0.232

Figure 1. Some MDH-1 phenotypes observed in *Apis mellifera ligustica.* F, fast; M, medium; S, slow.

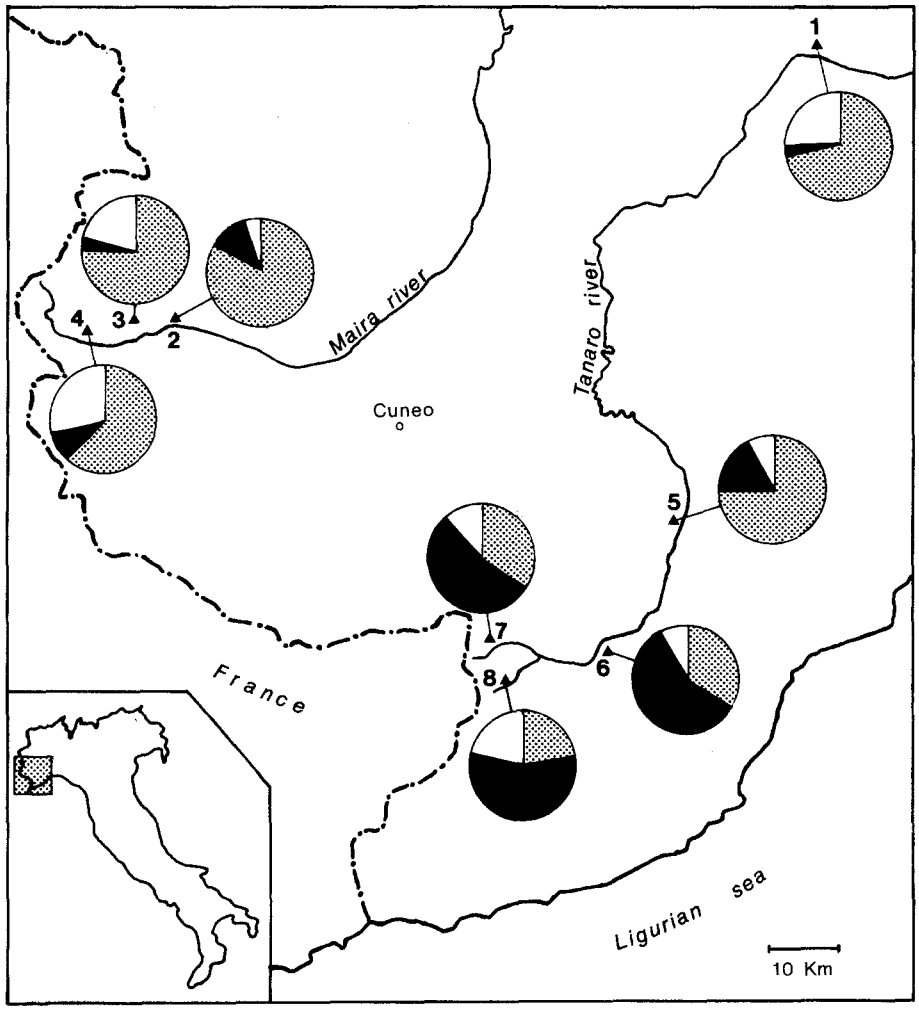

Figure 2. *Mdh*-1 allele frequencies in the Piedmont populations. F, white; M, black; S, gray.

The Piedmontese populations of *A. mellifera*, as well as all the others studied up to now, showed a low enzyme polymorphism level. In fact the *Mdh*-1 locus was the only variable one among 12 loci studied. As can be seen in figure 2, there is a clear difference in *Mdh*-1 allele frequencies between the 2 groups of populations from the 2 alpine valleys. Such a difference can be explained by the presence or absence of a gene flow between Italian and French populations. In fact the Maira valley populations, separated from French ones by a high (above 3000 m) mountain barrier, show high *Mdh*-1 S and minimal *Mdh*-1 M frequencies, as does the Asti population which lives in a plains area far from the French boundary. This allelic array can be considered typical of the Italian *A. m. ligustica* as confirmed by the rather scarce literature data about Italian stocks established in Brazil[8] and Australia[9]. In contrast to this situation, a gene flow may exist between French and Tanaro valley populations along the Ligurian sea coast and, perhaps, through the Marittime Alps, which are rarely more than 2000 m high. In fact we found a remarkable increase in the *Mdh*-1 M allele frequency with a corresponding reduction of the *Mdh*-1 S allele as the sampling localities were nearer the French boundary. Honey-bee strains of French descent, studied in Guadaloupe[15], actually showed a high *Mdh*-1 M allele frequency. If it is confirmed by direct investigations on original French populations, the Tanaro valley would be a hybridization area between *A. m. ligustica* and *A. m. mellifera* and the *Mdh*-1 allele frequencies could serve as useful markers for indicating the extent of gene flow.

By means of the *Mdh*-1 locus, *A. m. ligustica* could also be distinguished from the African subspecies, *A. m. adamsonii*, which is characterized by high *Mdh*-1 F and low *Mdh*-1 M and *Mdh*-1 S allele frequencies[8,16]; this would have great practical usefulness.

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