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Original article

Esterase-6 locus, a new enzyme polymorphism in *Apis mellifera*

A Biasiolo *, A Comparini

Istituto di Difesa delle Piante dell'Università, p le M Kolbe n 4, 33100 Udine, Italy

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Summary — A new polymorphic enzyme locus, esterase-6, has been resolved by electrophoresis in *Apis mellifera*. Samples from Bologna (Emilia, Italy), representative of *A m ligustica*, and from North-East Friuli (Italy), regarded as hybrids between *A m ligustica* and *A m carnica*, were analyzed. A preliminary population survey shows this locus to be highly variable.

***Apis mellifera* / enzyme polymorphism / esterase-6**

INTRODUCTION

Electrophoretic investigations on gene-enzyme systems have shown a very low level of allozyme polymorphism in honey bee populations, possibly related to their haplodiploid condition and/or to eusociality (Sylvester, 1986).

In European populations of *Apis mellifera*, only 2 loci (malate dehydrogenase-1 and esterase) have shown fairly high variability to-date, and have been used for biochemical-genetic characterization of different races (Badino *et al*, 1983, 1984, 1985) and for studying their hybridization patterns (Badino *et al*, 1982; Marletto *et al*, 1984; Sheppard and McPheron, 1986).

The discovery of other highly variable loci would be very useful for improved genetic characterization of different races or populations of *A mellifera*.

Bitondi and Mestriner (1983) detected, by electrophoresis, 6 esterase loci in *A mellifera;* some of them showed a clear allozyme variability. Esterase-3 (Est-3) was used for some of the population genetic research cited above. The more cathodic esterase (Est-6) was perceived to be variable, but allozyme bands were not sufficiently differentiated for reliable genotype classification.

In the course of studies designed to identify markers for genetic characterization of *A mellifera* from Friuli, regarded as

* Correspondence and reprints

a zone of hybridization between *A mellifera ligustica* and *A mellifera carnica* (Bolchi Serini *et al*, 1982; Ruttner, 1988), we obtained a reliable electrophoretic separation of Est-6 allozyme bands. A preliminary population survey was carried out in samples of *A mellifera* from Friuli and, for comparison, in a sample of *A m ligustica*. The data show that Est-6 locus is a potential new marker for genetic population studies in *A mellifera*.

MATERIALS AND METHODS

Adult workers of *A mellifera* were sampled, in late summer 1987, from 2 apiaries near Tarvisio (North-East Friuli, Italy) and 1 near Bologna (Emilia, Italy). The latter were supplied by the Istituto Nazionale di Apicoltura, Bologna, as representative of *A m ligustica*. For each apiary, workers were captured near the entrance of several hives. The samples were stored at – 40 °C until needed. Five individuals from every hive were used for electrophoresis.

Thorax-head sections of individual bees were homogenized in 0.15 ml buffer (0.2 mol•l^{-1} Tris-HCl, pH 8; 0.25 mmol•l^{-1} 2-mercaptoethanol; 1 mmol•l^{-1} EDTA). Electrophoresis was conducted on the supernatants after homogenate centrifugation. Horizontal electrophoresis was performed in 11.5% starch gels using, as electrode buffer, 0.18 mol•l^{-1} Tris-citrate, pH 7.0, diluted 1:15 in the gel. Electrophoresis was carried out at 250 V and 5 °C for 5 h.

The best staining method for Est-6 was the following: the gel slices were immersed in the substrate solution (1 ml of 0.5% α-naphthylbutyrate in acetone added to 50 ml of 0.05 mol•l^{-1} Tris-HCl, pH 7.2); after 1 h of incubation at 38 °C, the substrate solution was removed and replaced with the staining solution (50 mg of Fast Blue BB salt in 50 ml of 0.05 mol•l^{-1} Tris-HCl, pH 7.2). Est-6 allozyme bands were clearly resolved after 30 min at 38 °C. Best results were obtained by staining the upper gel slice.

RESULTS AND DISCUSSION

In *Apis mellifera*, the esterase electrophoretic pattern is composed of 6 different regions, which have been attributed to the activity of 6 different loci (Bitondi and Mestriner, 1983). Est-6, the most cathodic, exhibited 3 co-dominant alleles in our samples. This enzyme is a monomer, based on heterozygote banding patterns (fig 1).

The Est-6 genotype distribution of the studied populations of *A mellifera* well fitted the Hardy-Weinberg expectations, supporting our genetic interpretation for this polymorphism. The allele frequencies are reported in table I. Comparison of allele distribution by contingency tables have shown significant differences among the 3 samples, whether considered together or 2 at a time.

Table I. Est-6 allele frequencies and heterozygosity values observed (expected) in the analyzed samples of *Apis mellifera*. H = heterozygosity; *N* = number of workers analyzed.

Apiary localities: alleles	*Camporosso*	*S Leopoldo (Friuli samples)*	*Bologna*
a	0.07	0.28	0.03
b	0.74	0.70	0.95
c	0.19	0.02	0.02
N	50	50	75
H	0.42 (0.41)	0.36 (0.43)	0.11 (0.10)

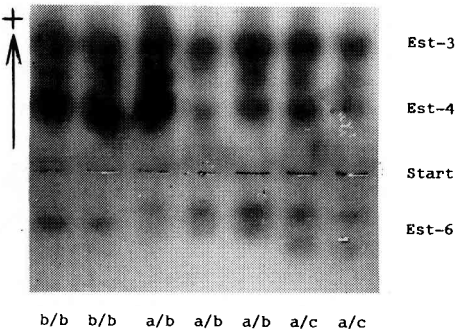

Fig 1. Photograph of some Est-6 electrophoretic patterns and corresponding genotypes, observed in workers of *Apis mellifera*. Under the staining conditions, overstained bands corresponding to Est-3 and Est-4 loci (Bitondi and Mestriner, 1983) also appear.

The 2 Friuli samples are clearly more variable at Est-6 than the Bologna sample (heterozygosity about 0.4 and 0.1, respectively). This agrees with the view that Friuli populations of *A mellifera* might represent hybridization between *A m carnica* and *A m ligustica* (Bolchi Serini *et al*, 1982; Ruttner, 1988).

The Est-6 polymorphism may aid in the better characterization of different races of *A mellifera*, particularly *A m carnica* and *A m ligustica*. This possibility should be ascertained by extensive studies of Est-6 variability in *A m carnica* and *A m ligustica* populations from areas of endemism.

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Résumé — Le locus esterase-6, nouveau cas de polymorphisme enzymatique chez *Apis mellifica*. Les populations de l'abeille domestique ont montré jusqu'à présent un très faible degré de polymorphisme allozymique. Un nouveau système enzymatique polymorphique, le locus esterase-6 (Est-6), a été mis en évidence chez *Apis mellifica* par électrophorèse.

Les analyses ont porté sur 15 ruches situées à Bologne (Emilie, Italie), représentatives de la race *Apis mellifica ligustica* et 20 ruches du nord-est du Frioul (Italie), considérées comme des hybrides de *A m ligustica* et *A m carnica*. Cinq ouvrières adultes ont été prélevées dans chaque ruche. L'électrophorèse horizontale sur gel d'amidon a été réalisée sur des coupes individuelles tête-thorax. Le tampon électrode était constitué de tris-citrate à 0,18 mol•l^{-1}, pH 7,0 dilué à 1/15 dans le gel. Les bandes d'Est-6 ont été mieux révélées en utilisant l'α-naphtylbutyrate comme substrat dans le bain de coloration.

L'Est-6 a présenté dans nos échantillons 3 allèles codominants (fig 1). Les 2 échantillons du Frioul se différencient nettement de celui de Bologne par un taux plus élevé de variabilité de l'Est-6 (tableau I). Le polymorphisme du locus de l'Est-6 peut contribuer à une meilleure caractérisation des différentes races d'*Apis mellifica*.

***Apis mellifica* / polymorphisme enzymatique / estérase-6**

Zusammenfassung — Der Esterase-6 Locus, ein neuer Enzympolymorphismus bei *Apis mellifera*. Die Populationen der Honigbiene haben bisher einen sehr niedrigen Grad von Allozym-Polymorphismen gezeigt. Ein neues polymorphes Gen-Enzym-System, der Esterase-6 Locus (Est-6) wurde durch Elektrophorese bei *Apis mellifera* eindeutig nachgewiesen.

Es wurden 15 Völker aus Bologna (Emilia, Italien), repräsentativ für *Apis*

mellifera ligustica, und zwanzig Völker aus Nordost-Friaul (Italien), die als Hybriden zwischen *A m ligustica* und *A m carnica* betrachtet werden können, analysiert. Von jedem Volk wurden fünf erwachsene Arbeitsbienen benutzt. An individuellen Thorax-Kopf-Schnitten wurde eine horizontale Stärkegel-Elektrophorese durchgeführt. Der Elektrodenpuffer war 0,18 M Tris-Citrat, pH 7,0, in dem Gel zu 1:15 verdünnt. Die Est-6-Banden wurden am deutlichsten dargestellt, wenn man α-Naphtyl-Butyrat als Substrat in der Färbelösung benutzte.

Est-6 zeigte in unseren Proben drei codominante Allele (Abb 1). Die zwei Proben aus Friaul sind von der Probe aus Bologna deutlich durch den höheren Grad der Variabilität von Est-6 unterschieden (Tabelle I). Der Polymorphismus im Est-6 Locus kann zu einer besseren Charakterisierung der verschieden Rassen von *Apis mellifera* beitragen.

Apis mellifera **/ Enzym-Polymorphismus / Esterase-6**

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