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DETECTION OF ESTERASE PATTERNS ANDONTOGENIC GENETIC VARIABILITY IN HONEYBEES *Apis mellifera L.*

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ABSTRACT

Ontogenic variations of esterase isozymes and biochemical genetic polymorphisms were investigated on the Syrian and Carniolan races and their hybrid genotypes. Healthy and infested samples with *Varroa* mites showed similar pattern for the same developmental stage. Banding pattern differs according to developmental stages, where positive bands which were present in larval and pupal stages disappeared in the adult stage.

Comparisons between the two races and their F1 hybrid proved the presence of considerable pattern variations. The Carniolan race presented high levels of electrophoretic variability in all developmental stages and under different conditions of *Varroa* infestation, compared to the Syrian race and hybrid genotype Esterase patterns appeared along different stages in the Syrian race and hybrid genotype. Esterase patterns detected in the different stages of the Syrian, and Carniolan races and their hybrid genotypes showed that the great majority of isozymes were monomorphic.

INTRODUCTION

Isozymes (multiple molecular enzymes) may be generated in different ways. The causes of isozyme formation are classified into three main categories: (1) the occurrence of multiple gene loci coding for structurally distinct polypeptide chains of the enzyme, (2) : the occurrence of multiple allelism at a single locus with different alleles determining structurally different versions of a particular polypeptide chain and (3) : The occurrence of the so-called "secondary" isozyme formation due to post-translation modifications of the enzyme structure (Harris, 1969; Hopkinson and Harris, 1971).

Esterase isozymes of the worker honeybee, (adults and all developmental stages) and genetic polymorphism in four populations of *Apis mellifera* occurred in 6 esterase loci (esterase 1 to 6) according to (Bitondi and Mestriner (1983). Some of these isozymes were controlled by more than one allele. Esterase 1,2 and 4 did not exhibit developmental changes, but the electrophoretic profile of esterases 3,5 and 6 varied during ontogenic development. The variations in the esterases of honeybees are probably determined by 6 different gene loci (Bitondi and Mestriner, 1985).

Esterase-6 isozymes is present in *Apis mellifera* in all developmental stages during metamorphosis and in workers during the process of aging (Abou-Elenean, 1993). Electrophoretic mobility showed that the esterase-6 isozyme is a monomer having no structural subunits and it is controlled by one locus which may occur in two different forms coded by isoalleles "S" and "F" consequently. Three different patterns of mobility, generated by the genotypes SS, FF and SF were detected.

The effect of Varroa infestation on honeybee esterase isozyme was studied in two strains of honeybee, Carniolan- Egyptian and Italian- Egyptian by Morsy (1998). Esterase isozyme from three locations generally increased in both numbers and activities after one or two Varroa mites infestation. After infestation, the cathodal bands varied in numbers, while the anodal bands varied mainly in activity. Samples of the different honeybee strains varied in isozyme activities and number either collected from the same location or from different locations.

Electrophoretic variants of esterase-2 in *Apis mellifera* occurred using starch gel electrophoresis of abdomen extracts from adult drones (Ruvolo- Takasusuki *et al* ., 1998). Esterase-2 has the highest heat stability among all *Apis mellifera* esterases. Electrophoretic analysis of the worker pupae demonstrated that the heterozygous phenotype only presents an intermediate band between the fast and slow variants of this enzyme.

The aim of the present investigation is to compare two races of honeybees the Syrian race, *Apis mellifera syriaca* and Carniolan race, *Apis mellifera carnica* and their hybrid in relation to the esterase isozymes and ontogenic variations. This study was carried out on healthy and infested sealed brood by Varroa mites either worker or drone.

MATERIALS AND METHODS

The experiments were carried out in the Apiary and laboratory of Genetics of the Faculty of Agriculture, Alexandria University.

Five colonies of the Syrian and Carniolan races and their hybrid were established for the experiments. Syrian and newly mated queens were brought from the Syrian Beekeeping Development Project, Damascus, Syria. Carniolan queens were brought from the Egyptian Ministry of Agriculture and Land Reclamation. Hybrid genotype was obtained when Syrian virgin queens were naturally reared from mother colonies, then transported into Benton cages to an isolated region for Carniolan colonies in the North of the Delta.

The two races and their hybrid were compared with regard to the esterase isozymes and ontogenic variations. This study was carried out on healthy and infested sealed brood by Varroa mite either worker or drone. Worker and drone sealed brood in the last larval stage and pupal stage of different ages, white- eye (13-14 days), pink-eye (15-16 days), black- eye (17-18 days) and also newly emerged adults were used in the biochemical genetic studies. Each sample was kept in a vial in a deep feezer till using.

Starch gel electrophoresis was carried out according to El-Metainy *et al.* (1977). Electrode buffer and staining solution were prepared according

to Shaw, (1965) and Shaw and Keen, (1967). Samples of the pupal stage and last larval instars were homogenized in separate couples in 0.1 ml of distilled water, while the adult sample was homogenized in 0.3 ml distilled water according to Bitondi and Mestriner (1983). Each homogenate was absorbed on a 2 x 8 mm strip of filter paper, placed on the middle surface of a gel plate and kept at 4°C. for about one hour, and the filter papers were then removed. Electrophoresis was carried out with a constant current of 13-14 v/cm for 2 hours at 4°C. Then the gel plates were stained at room temperature with esterase staining solution for at least 30 minutes. The best plates were selected, photographed and their diagrams were drawn.

RESULTS AND DISCUSSION

The term isozyme (or iso-enzyme) refers to multiple forms of an enzyme occurring within a species as a result of the presence of more than one structural gene. Where such genetic variability is the result of multiple alleles at a single locus, these are referred to as allozymes (allelo- enzymes), which are inherited in a Mendelian fashion. Allozymes therefore are particularly useful for examining inheritance patterns in population genetics. Co-dominant expression of allozymes results in, for example, single bands for monomeric homozygotes (e.g. FF for a fast band, and SS for a slow band), but two bands for a heterozygote (FS) according to Menken and Ulenberg (1987). As enzymes are commonly expressed in polymeric forms (Richardson *et al.*, 1986), complex patterns can arise to provide diagnostic information. Further variability in banding patterns can arise from transposable elements within enzyme genes (Burkhart *et al.*, 1984) and post-translational modifications of enzymes caused by diet (Schwartz and Sofer, 1976), or the method of enzyme extraction (Ferguson, 1980) can also occur.

Although the direct study of differences in DNA sequences provides more detailed and precise information about the same genes, such detail is rarely required, and electrophoresis can often still provide a simpler and less expensive alternative (Hemingway *et al.*, 1996).

By comparing the electrophoretic pattern of each of the tested bees Syrian, Carniolan and their hybrid, it can be seen that banding pattern differs according to developmental stages, where positive bands which were present in larval and pupal stages disappeared in the adult stage. This variability is due to ontogenic variation in which allele expressivity varies according to developmental stages. From another point of view, it seems that Varroa infestation does not affect esterase isozyme pattern, where infested and healthy samples revealed a similar pattern for the same developmental stage.

By comparing the electrophoretic patterns of workers and drones from the Syrian race (Figs.1 and 2), it can be shown that the positive electrophoretic bands disappeared in drones in the pink-eye pupal stage, while these bands remained active in workers till the beginning of the black-eye pupal stage.

Regarding the electrophoretic patterns of the Carniolan drones (Fig.3), it is obvious that these samples exhibit much higher electrophoretic variability in all developmental stages under different conditions of *Varroa* infestation.Larval stages represented by slots from 1 to 5 show two negative bands , the faster band being more dense than the slower. In addition, one positive band in the larval stage is also present. Examination of the pink-eye pupal stage (slots from 6 to 10) reveals an additional band appearing at the extreme negative direction with the disappearance of the positive band which was present in the larval stage. Such a difference may be due to variable gene expressivity between larval and pink-eye pupal stages.

Concerning the black-eye pupal stage (slots from 11 to 15), it may be concluded that this stage exhibits a pattern which is similar to the pink-eye pupal stage with only one difference, i.e., a higher isozyme activity for the slow negative band. It is interesting to note that the black-eye pupal sample which is heavily infested by *Varroa* mites (slote 15) shows a rather different pattern where the additional extremely negative band was absent.

As the hybrid genotype is concerned (Fig. 4), the most obvious result revealed by the esterase isozyme electrophoretic pattern is the appearance of only one negative band which varies with respect to density according to the developmental stage and infestation level. Concerning the pink-eye pupal stage (slots from 1 to 5) the pattern shows a rather dense band in infested samples (slots, 3,4 and 5) compared with the healthy ones (slots 1 and 2). With regard to the black-eye pupal stage, a similar result could be again, concluded, i.e., healthy samples (slots 6 and 7) show lighter bands than infested ones (slots from 8 to 11). It can be mentioned that the infestation level does not affect the band activity within the infested samples patterns of the black-eye pupae infested with 1,2 and 4 mites represented in slots 8-9, 10-11 and 12-13, respectively. The light density bands of the black-eye pupal stage heavily infested (slots 12 and 13) may be attributed to the biochemical destruction of esterase isozymes, since no one could determine the viability of infested pupae at the time of sampling extraction.

In comparing the electrophoretic pattern of the hybrid genotype between Syrian and Carniolan races, it may be concluded that although no effect was observed for infestation level in the Syrian and Carniolan races, this factor plays an important role concerning the hybrid genotype, i.e., healthy samples exhibited lighter bands (smaller esterase activity) than infested ones. This difference could be attributed to the recombinant genetic structure of the hybrid leading to higher polymorphism in esterase isozymes loci. Positive bands present in the larval and pupal stages disappeared in adult stages of the races. This ontogenic variation was previously detected by Bitondi and Mestriner (1983). They examined the electrophoretic mobility of esterase 1-6 in different developmental stages of the honeybee, and found that while esterase 1,2 and 4 did not exhibit developmental changes, esterase 3,5 and 6 varied during ontogenic development. Variation in this study for esterase anodal bands may account to the latter isozymes.

In addition, comparing the pattern of workers and drones of the Syrian race, anodal bands disappeared in the drones during the pink-eye pupal stage, while these bands remained active in the workers till the black-

eye pupal stage. These results may be attributed to the presence of different roles played by different isozymes during early development, but become inactive in later stages, due to gene expression regulation acting according to an inducible system. The difference between workers and drones observed in the Syrian race is mainly due to the haplo - diploidy occurring in Hymenoptera, where two alleles are present in workers and only one is present in the drone. Such isozymes variation during different stages has been considered by Figueiredo *et al.* (1996).
As markers of specific stages of ontogenic development, since specific bands in the pattern were found to be related to determined stages.

Comparisons carried out between the two races and their hybrid proved the presence of considerable variations of pattern. Carniolan race presented high levels of electrophoretic variability in all developmental stages under different conditions of *Varroa* infestation, compared to Syrian and hybrid genotypes. This result is in accordance with the fact that Carniolan, as an European race, is generally subjected to a relatively higher levels of *Varroa* infestation (Moretto *et al.*, 1991; Medina and Martins, 1999) inducing a high adaptability as a response to this characteristic. The presence of a wide range of environmental variation due to susceptibility to *Varroa* facing the Carniolan race, higher levels of genetic variations should be acquired in order to challenge the variability of its environment.

Another important feature has been found in this study. Esterase patterns found along different stages in the tested races and their hybrid showed that the great majority of isozymes were monomorphic. This result repeatedly occurred as a characteristic in Hymenopteran populations. It was attributed to the haplodiploid genetic structure in these populations (Metcalf *et al.*, 1975, Pamilo *et al* ., 1975, 1978). This is in accordance with theoretical expectations, as both deterministic selection models and the hypothesis predict that genic variablitity is reduced in haplodiploid populations. The evolution of altruism is also an important factor, since the average of heterozygosity per locus is lower in eusocial than solitary species, which may indicate that either small effective
population sizes or stable conditions in the nests of eusocial species affect genic variations.

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تقدير طرز الأستيريز والتغيرات الوراثية التطورية فى نحل العسل

وفاء يعقوب – نبيل البربرى- اسامة الانصارى – محمد عباس عبد اللطيف و محمد خليل يوسف

يتناول البحث دراسة مقارنة بين سلالتين من نحل العسل ، هما سلالة النحل السورى وسلالة النحل الكرنيولى والهجين الناشىء بينهما الناتج من تلقيح ملكات نحل سورى عذراء بذكور نحل كرنيولى فى منطقة معزولة فى شمال الدلتا . من حيث تقدير النشاط للمشابهه الانزيمى الأستيريز فى أطوار النمو المختلفة تحت الظروف العادية وتحت ظروف الاصابة بطفيل الفاروا بكثافات مختلفة باستخدام تقنية التفريد الكهربائى باستخدام جيل . وقد اوضحت نتائج الدراسة مايأتى :

- ان نموذج الحزم كما يظهره التفريد الكهربائى كان مختلفا تبعا لاطوار النمو ، حيث ان الحزم الموجبه والتى كانت موجودة فى كل من طورى اليرقة والعذراء قد اختفت فى طور الحشرة الكاملة .
- ان المقارنة بين السلالات أظهر وجود اختلافات واضحة فى النموذج .
- ان السلالة الكرنيولى قد أظهرت مستويات عالية من اختلاف التفريد الكهربائى وذلك فى كل أطوار النمو بوجود مستويات مختلفة من الاصابة بالفاروا وذلك مقارنة بالسلالة السورى والهجين .
- ان نموذج الاستيريز المدروس خلال الاطوار المختلفة فى كل من السلالة السورى والكرنيولى والهجين قد أظهر بان غالبية مشابهات انزيم الاستيريز كانت متماثلة الطرز المظيرية .
- ان كلا من العينات السليمة والمصابة بالفاروا قد أظهرت نموذجا متماثلا بالنسبة لنفس الطور والعمر .