PRELIMINARY STUDY ON MORPHOLOGICAL AND GENETIC CHARACTERISTICS OF SOME NATIVE HONEYBEE (*APIS CERANA*) POPULATIONS IN NORTHERN VIETNAM

Nguyen Van Niem, Le Quang Trung, Pham Hong Thai

Bee Research and Development Center

Trinh Dinh Dat, Nguyen Thi Minh Nguyet, Tran Thi Ton Hoai

Faculty of Biology, College of Natural Sciences - VNUH

Abstract: *Twelve samples of Apis cerana bees from three locations in Northern Vietnam were analyzed for morphological and genetic variations of these native honeybee populations.*

Based on discriminate morphological analysis, three bee populations are separated. Among them, the bees from Caobang are found to be a clearly distinct group from the bees of Catba and Laichau. The electrophoresis for enzymes esterase and isocitric dehydrogenase supports the morphological study. Among three bee populations, the bees from Caobang revealed genetic characteristics differing from the rest bee populations. The bees from Catba have a closer relationship with the bees from Laichau than those from Caobang. These findings provide a good potential for honeybee selection and breeding.

1. Introduction

The study on variations of the different natural subspecies and geographic ecotypes of the native honeybees (*Apis cerana*) in Vietnam is one of requirements for the development of the beekeeping industry and further genetic improvement of bee stocks.

Traditionally, morphometric methods are important research tools, which have been being used for the investigation of geographic variations of the honeybees. Morphometric studies have been made a great detail on the European honeybee, *A. mellifera*, (Ruttner, 1988). However, very little is known about the morphometry of the *A. cerana* bees in Vietnam (Le Dinh Thai et al., 1982; Ruttner, 1988; Nguyen Van Niem et al., 1992).

In several last decades, molecular techniques such as electrophoresis, polymerase chain reaction can provide strong supports for study on biodiversity and polygene of honeybee species. In this aspect, many publications reported on the enzyme variations in *Apis mellifera* species, including Sheppard and Berlocher (1984, 1985), Meixner et al. (1994), Schiff et al.(1994), Sheppard and McPheron (1986). While available data confirming enzyme variations in *A. cerana* bees was very little. Sheppard and Berlocher

(1989) analyzed 15 enzymes of four honeybee species in Sri Lanka and they found that *A. cerana* species was polymorphic for four enzymes. Lee (1994) electrophoretically analyzed 11 enzymes of the *A. cerana* bee populations at five locations of South Korea. He found only enzyme malate dehydrogenase was detected as polymorphic locus. Asco and Laude (1993) reported that both *A. mellifera* and *A. cerana* species in the Philippines were found polymorphic for alkaline phosphatase. In Viet nam, we have not found any publication reported on using electrophoretic techniques for genetic study of honeybee species.

This paper presents a morphometric study and an analysis of two enzymes (esterase and isocitric dehydrogenase) of the native honeybee (*A. cerana*) populations in three locations of Northern Vietnam. The objectives of this study were to determine geographic variations of the studied bee populations and to contribute basic scientific information into the assessment of the honeybee biodiversity in Vietnam.

2. Materials and methods

The studies were carried out at the laboratory of Bee Research and Development Center and the laboratory of Genetic Department, Hanoi University of Science. The bee samples were collected in the managed bee colonies from the locations of Catba (Cb), Caobang (Cg), and Laichau (Lc) in the North of Vietnam. In a location, four colonies were sampled. Each sample (about 150 - 200 adult worker bees) was separately collected from a single colony. Upon arrival in the laboratory, the bees were kept alive in the small cage and fed on honey. In the laboratory, these worker bees were maintained alive for electrophoresis. A subset of each sample was killed with hot water (90^0C) and then transferred to 70% ethanol for morphometric analysis.

Fifteen bees per sample were dissected and measured for 15 morphometric characters according to the method of Ruttner (1988). The measurement was done with a microscope and an ocular micrometer.

The statistical analysis of the data was performed with SPSS/PC, using program for cluster analysis and discriminant analysis.

Sixty to 100 individuals from each sample were analyzed for enzyme esterase and 30 to 60 individuals for enzyme izocitric dehydrogenase. Thorax section of each bee were homogenized in approximately equal volume of triss-HCl grinding buffer (0.1 g sucrose; 0.1 ml Trifon x-100; 0.01 ml 2- mercapto-ethanol; 10.0 ml H_2O) and electrophoresis was conducted in this crude homogenate.

The enzymes for electrophoresis were esterase (E.C.:3.1.1.1) and izocitric dehydrogenase (E.C:1.1.1.42). These enzymes were electrophoresed using polyacrylamide gel according to the methods of Steiner and Joslyn (1974) and Green (1990) with some modifications. Steiner and Joslyn run the electrophoresis under buffer condition on 12% starch gel in 6.00 hours. While in our study, electrophoretic analysis was carried out using polyacrylamide gel in 16.00 hours for enzyme esterase and 8.00 hours for enzyme izocitric dehydrogenase. The bees of all populations were run side-by-side lanes for comparison of

relative mobilities. Enzyme activities were visualized with standard histochemical techniques (Green, 1990) and gels were photographed. The determinations of alleles, allele movement distances and allele frequencies of the two enzymes were done using the Ferguson's (1980) method.

3. Result and discussion

1. Morphological analysis

Fifteen morphological characters of the worker body parts were measured. The obtained results are presented in table 1.

Table 1. Morphological data of the studied bee populations

#	Location / Character		Catba (A) n = 60	Caobang (B) n = 60	Laichau (C) n = 60	Significant difference		
						A-B	A-C	B-C
1	Length of proboscis	(mm)	4.98± 0.010	5.07± 0.012	4.97± 0.010	**	ns	**
2	Width of prementum	(mm)	0.75± 0.005	0.79± 0.004	0.76± 0.005	**	ns	**
3	Length of forewing	(mm)	8.15± 0.016	8.41± 0.023	8.25± 0.014	**	**	**
4	Width of forewing	(mm)	2.86± 0.007	2.95± 0.008	2.92± 0.008	**	*	*
5	Cubital index (a/b)		3.001± 0.077	2.748± 0.059	3.001± 0.068	**	ns	**
6	Length of femur	(mm)	2.33± 0.005	2.38± 0.007	2.35± 0.006	*	*	*
7	Length of tibia	(mm)	2.98± 0.009	3.05± 0.008	2.95± 0.010	*	*	**
8	Length of basitarsus	(mm)	1.92± 0.007	1.97± 0.006	1.91± 0.006	**	ns	**
9	Width of basitarsus	(mm)	1.08± 0.005	1.10± 0.005	1.07± 0.004	*	ns	*
10	Tergit 3, longitudinal	(mm)	8.35± 0.028	8.60± 0.024	8.16± 0.020	**	*	**
11	Tergit 3, transverse	(mm)	1.91± 0.007	1.97± 0.007	1.81± 0.005	**	**	**
12	Sternit 3, longitudinal	(mm)	4.47± 0.012	4.74± 0.012	4.40± 0.014	**	*	**
13	Sternit 3, transverse	(mm)	2.07± 0.006	2.15± 0.008	2.06± 0.005	**	ns	**
14	Wax plate of ster.3,longi.	(mm)	1.98± 0.006	2.09± 0.008	1.95± 0.007	**	*	**
15	Wax plate of ster.3, trans	(mm)	0.97± 0.005	1.01± 0.006	0.92± 0.006	*	*	**

* *: Significan differences at P≤0.01. *: Significant differences at P≤0.05.
ns: No significan dofferences.

The body size of bees from the Caobang population was bigger than that from Catba and Laichau ones. Of the15 measured characters, the values of 14 characters were significantly bigger, except cubital index. The differences are highly significant. Between the Catba and Laichau bee populations, some body parts of bees from Catba were bigger (1, 7, 8, 9, 10, 11, 12, 13, 14 and 15), while some others were smaller (2, 3, 4, 5 and 6). However, these differences were less significant than those compared to the Caobang bees.

The obtained data show that, three studied bee populations can be mophologically separated into 3 groups. Of which the Caobang group is clearly distinct from the Catba and Laichau populations ones.

Cluster and discriminant analysises (figure 1) also show that the Caobang bee cluster is highly differentiated from the bee clusters of Catba and Laichau. These differences are rather significant considering their separated mophological types. However, the differences between the Catba and Laichau bee populations are not strongly significant to support separated designation.

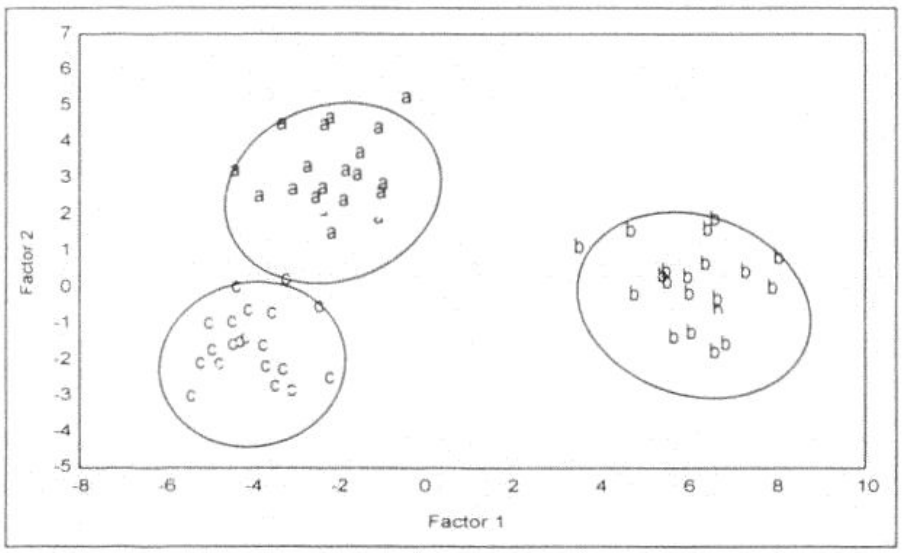

Figure 1. Discriminant analysis of the Catba (a), Caobang (b) and Laichau (c) bee populations. Factor 1 (88.7% of the variation). Factor 2 (11.3% of the variation). The ellipses of confidence (95%) are given.

2. *Enzyme analysis*

In order to test the results of the morphological measurements, an electrophoretic analysis for enzymes esterase and enzyme isocitric dehydrogenase of three bee populations was conducted. The summary results of the electrophoresis are presented in figures 2a, 2b, 2c, 2d, 3a, 3b and table 2.

Alleles of esterase locus

The results of the electrophoresis show that electrophoretic bands of isozyme esterase from Catba bee populations exhibited three band patterns: S/S, S/F and F/F with Rf: 1.8, 2.2 and 2.6 respectively (figure 2a). The Caobang bee population also exhibited three band patterns: S/S, S/F with Rf: 1.8, 2.2 respectively and F/F with Rf: 2.7 (figure 2b), while the Laichau bee population had two band patterns only: S/F and F/F with Rf: 2.2 and 2.6 (figure 2c).

Enzyme esterase from the Catba bee population is controlled by a locus gene having three codominant alleles Est^b, Est^c, Est^d with the homozygote genotypes Est^b/Est^b (Rf: 2.6), Est^c/Est^c (Rf: 2.2), Est^d/Est^d (Rf: 1.8) and two heterozygote genotypes Est^b/Est^c (RF: 2.6 and 2.2), Est^c/Est^d (Rf: 2.2 and 1.8). In this study, we did not detect heterozygote genotype Est^b/Est^d.

Locus esterase of the Caobang bee population represented three dominant alleles Est^a, Est^c, Est^d and their genotype types were Est^c/Est^c (Rf: 2.2), Est^a/Est^c (Rf: 2.7

and 2.2) and Est^c/Est^d (Rf: 2.2 and 1.8). Locus esterase of the Laichau bee population exhibited two codominant alleles Est^b, Est^c and their genotype types were Est^b/Est^b (Rf: 2.6), Est^c/Est^c (Rf: 2.2) and Est^b/Est^c (Rf: 2.6 and 2.2).

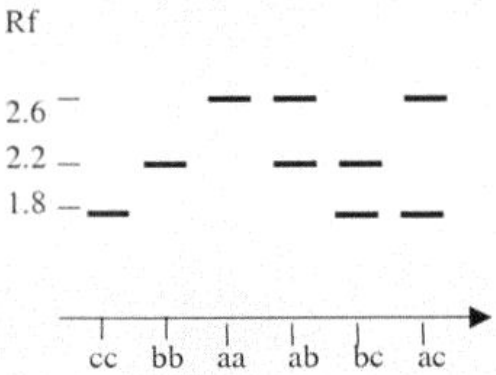

Figure 2a: Summary electrophoretic phenotype of esterase in bees from Catba

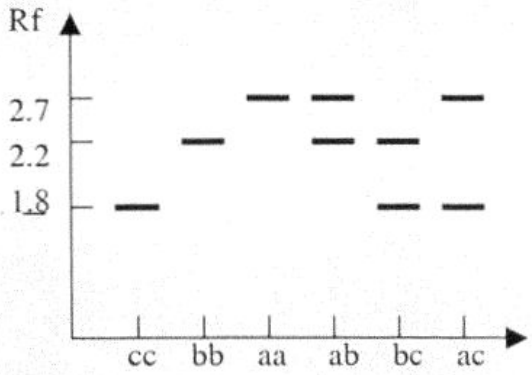

Figure 2b: Summary electrophoretic phenotype of esterase in bees from Caobang

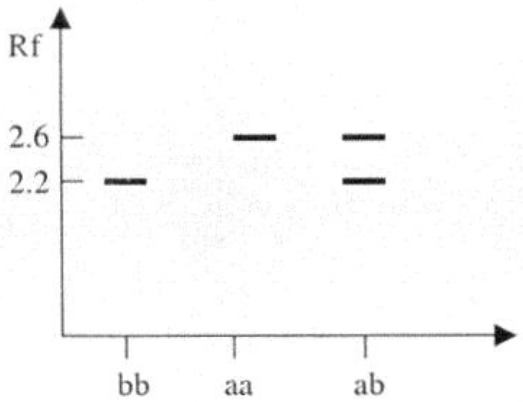

Figure 2c: Summary electrophoretic phenotype of esterase in bees from Laichau

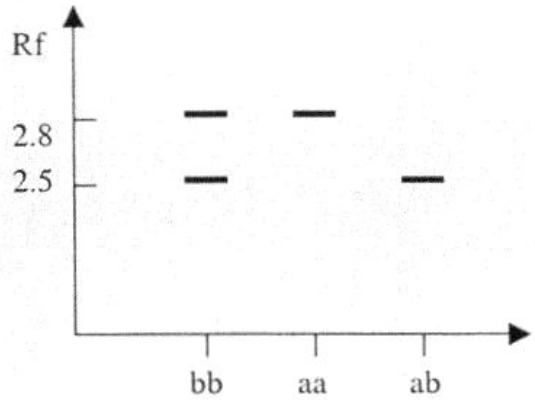

Figure 2d: Summary electrophoretic phenotype of Idh in bees from three locations

2. Allele frequencies of enzyme esterase

The results of allele frequencies in studied bee populations are presented in table 2.

Table 2. Allele frequencies of esterase and isocitric dehydrogenase in three bee populations

Place	Esterase					Isocitric dehydrogenase		
	n	Est^a	Est^b	Est^c	Est^d	n	Idh^a	Idh^b
Cb	291	-	0.0378	0.8591	0.1031	144	0.6667	0.333
Cg	248	0.1774	-	0.7230	0.0988	132	0.8523	0.1477
Lc	180	-	0.1500	0.8500	-	96	0.6875	0.3125

Among three bee populations, genetic characteristics of the Caobang bees were different from the other populations. In this bee population, allele Est^a was detected, but the allele Est^b was not found. The polymorphism of locus Est. in the Laichau bees was lower than that in other bee populations (two alleles Est^b, Est^c only), but its allele frequency was higher

Our obtained data support the report of Sheppard and Berlocher (1989), but not the same with the report of Lee (1993). While studying on allozymes of four honeybee species in Sri Lanka, these authors found that enzyme esterase in *A. cerana* bee populations exhibited polymorphism. In addition, they reported that the enzyme esterase exhibited two alleles polymorphic at Est^{57} and Est^{86}, while Lee (1993) couldn't detect polymorphism in any allele Est. of the Korean honey bee populations. In our study, the enzyme esterase showed higher degree of polymorphism. We found four alleles: Est^a, Est^b, Est^c and Est^d.

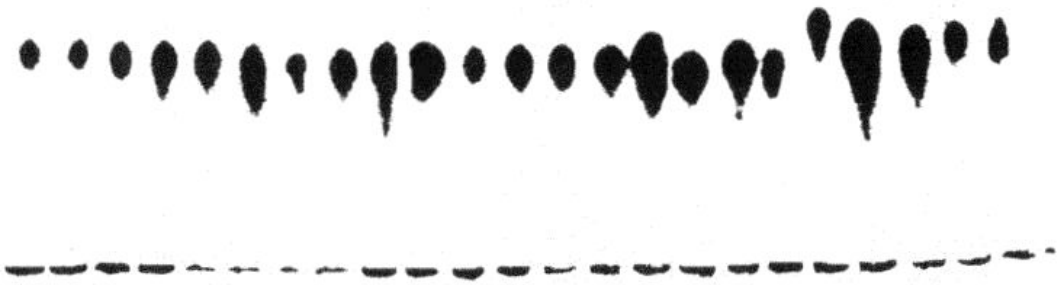

Figure 2a: Electrophoretic photograph of esterase of three bee populations
From left to right: *1 - 12: Lc, 13 - 18: Cg, 19 - 23: Cb*

Figure 2b: Electrophoretic photograph of IDH of the bee populations
From left to right: *1 - 12: Lc, 13 - 18: Cb, 19 - 23: Cg*

Enzyme isocitric dehydrogenase

The electrophoretic results of the enzyme isocitric dehydrogenase (Idh) of the three bee populations are presented in figure 2d and 3b.

The results show that in the studied bee populations, some individuals exhibited one banding pattern for Idh enzyme, and some others had two banding patterns. Locus of enzyme isocitric dehydrogenase had two codominant alleles: Idh^a and Idh^b. Their

genotype types were Idh^a/Idh^a (Rf: 2.8) and Idh^a/Idh^b (Rf: 2.5 and 2.8). In all analyzed samples, we could not detect genotype type Idh^b/Idh^b yet.

The allele frequencies of Idh from the Caobang bee population, as presented in table 2, were higher than those of other populations. This indicated that genetic characteristics of the Caobang bees are different from the Catba and Laichau ones. These results support the morphological studies reported above.

4. Conclusion

From the obtained results, it can be concluded that:

- The Caobang honeybees reveal the characteristics differing from the Catba and Laichau ones, in both morphological and genetic characteristics. These differences support a separation of the bee population from Caobang and the bee populations from Catba and Laichau as two geographical ecotypes.

- The results of this investigation confirm the diverse genpool of the native honeybees in Northern Vietnam and may be useful for bee breeders to select and hybrid better bee stocks for beekeeping industry.

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TẠP CHÍ KHOA HỌC ĐHQGHN, KHTN, t.XVII, n^{0}1 - 2001

NGHIÊN CỨU BƯỚC ĐẦU VỀ CÁC ĐẶC ĐIỂM HÌNH THÁI VÀ ĐẶC ĐIỂM DI TRUYỀN CỦA MỘT SỐ QUẦN THỂ ONG MẬT (*APIS CERANA*) Ở MIỀN BẮC VIỆT NAM

Nguyễn Văn Niệm, Lê Quang Trung, Phạm Hồng Thái
Trung tâm Nghiên cứu và Phát triển ong

Trịnh Đình Đạt, Nguyễn Thị Minh Nguyệt, Trần Thị Tôn Hoài
Khoa Sinh học, Đại học Khoa học Tự nhiên - ĐHQG Hà Nội

Mười hai mẫu ong nội, *Apis cerana*, thu thập từ 3 địa phương ở miền Bắc Việt Nam đã được phân tích về hình thái học và điện di enzym để tìm hiểu sự đa dạng sinh học của các quần thể ong nghiên cứu.

Trong 3 quần thể ong nghiên cứu, quần thể ong Cao Bằng biểu hiện sự sai khác rõ ràng so với các quần thể ong ở Cát Bà và Lai Châu về cả một số đặc điểm hình thái và đặc điểm di truyền của các enzym esterase và isocitric dehydrogenase. Có thể tách quần thể ong Cao Bằng với quần thể ong Cát Bà và Lai Châu thành 2 dạng sinh thái riêng biệt. Kết quả nghiên cứu này có ý nghĩa quan trọng trong công tác chọn lọc và lai tạo các giống ong có chất lượng cao hơn phục vụ cho người nuôi ong.