

ALLOZYME VARIABILITY IN SOME CENTRAL ANATOLIAN BUMBLE BEE (*BOMBUS*, APIDAE, HYMENOPTERA) SPECIES

A. Murat Aytekin*, Neşe Çağatay*

Received: 20.10.2004

Summary: In this study some bumble bees from the genus *Bombus* Latreille, which were collected from different localities in Central Anatolia, were analysed by horizontal starch-gel enzyme electrophoresis. Of the eight species examined, only *Bombus* (*Thoracobombus*) *humilis* were found to be polymorphic with low heterozygosity (Mean heterozygosity = 0.19 ± 0.19), while the other species had distinct electromorphs fixed in different populations. The situation in *B. humilis* populations can be regarded as a possible reason for the confusion at subspecies level. The phenogram based on electrophoretic data showed the same systematic pattern proposed before by several authors. This supported the hypothesis that the subgeneric system in some bumble bees reflects the evolutionary pathway and may be accepted as natural.

Keywords: Hymenoptera, Apidae, *Bombus*, systematics, allozyme, starch-gel electrophoresis

Introduction

Low levels of genetic variation in Hymenoptera have been reported by various authors for 30 years¹⁻⁸. Several hypotheses have been suggested from the results of different studies based on allozymes⁷. These are based upon either the sex determination or ecological (mainly behavioral) characteristics of most of the electrophoretically studied species. There have been more biochemical studies on Apidae than on other families of Hymenoptera. This was probably because of their unique role in the world of arthropods and their extraordinary effect on many aspects of human culture, mythology, economy and agriculture⁹. Bumble bees have a specific place in the *corbiculate* world; they are wide spread and can be found in all the Palaearctic. Turkey is well known as the richest country in the West-Palaearctic region that is represented by 49 species¹⁰, but no electrophoretic

data study has been carried out yet on bumble bees of Turkey.

In the history of bumble bee taxonomy, the supraspecific classification model aroused great interest and was generally accepted after Richards¹¹ revised all so far described subraspecific groups of non parasitic bumble bees by recognizing 35 subgenera¹². This model has been tested by different methodologies including DNA studies¹³, allozyme based models³⁻⁶ pheromones¹⁴ and morphometry both in cladistic¹⁵ and traditional¹⁶ methods and revised several times. Today, there is general acceptance about most of the subgenera excluding some like *Thoracobombus* Dalla Torre and *Rhodobombus* Dalla Torre. Confusing situations are generally because of the great hair color variation among populations. These color differences caused taxonomists to name several confusing subspecies

* Hacettepe University Faculty of Science Department of Biology Beytepe 06532 Ankara, TURKEY

E-mail: ama@hacettepe.edu.tr

This study was part of the requirements for the PhD degree submitted to Hacettepe University.

or even some species¹⁷. Among these, *Bombus* (*Thoracobombus*) *humilis* can be found in Anatolia in four different subspecies¹⁰. There is no doubt about the existence of *B. humilis insipidus* Radoszkowski, 1884 and *B. humilis quasimuscorum* Vogt. But the taxonomical position of *B. humilis auranticus* Dalla Torre and *B. humilis nigrinus* Krüger is not clear at the moment. *B. humilis nigrinus* populations greatly overlap in Central and North Anatolia with those of *B. humilis auranticus* and were found in the same localities¹⁰.

This paper aimed to present electrophoretic information about variation in the bumble bees of Central Anatolia, Turkey. Three subgenera from the genus *Bombus* Latreille, 1802 were preferred in order to form an opinion about their systematics on subgenus level, which is widely accepted to use after Richards¹¹ in bumble bee systematics. We also aimed to test allozyme based models in bumble bee taxonomy, and if subspecific relationships could be detected by this approach by using different populations of *B. humilis*.

Materials and Methods

125 specimens of eight different species from the subgenera *Megabombus* (s. str.) Dalla Torre, 1880 (*B. argillaceus* 15 specimens; *B. hortorum* 11 specimens; *B. portschinskyi* 9 specimens); *Thoracobombus* Dalla Torre, 1880 (*B. zonatus* 18 specimens; *B. humilis* 26 specimens from both color pattern; *B. mlokosiewitzi* 8 specimens) and *Rhodobombus* Dalla Torre, 1880 (*B. armeniacus* 17 specimens; *B. mesomelas* 21 specimens) of the genus *Bombus* were collected from different localities in Central Anatolia between 1996-2000 and were analysed by horizontal starch-gel enzyme electrophoresis.

The specimens were all caught on plants while they were searching for nectar or pollen. The bumble bees were put in small labeled boxes and kept in a mobile-ice box in order to bring them to the laboratory alive. They were kept alive in Hacettepe

University Bumble bee Rearing Room until dissection. After determination of species level the thoraces of the females were ground and homogenates were kept at -80°C till needed for electrophoresis. The present nomenclature preferred here follows that used by Rasmont¹⁸ and Pawlikowski¹⁹.

Six enzyme systems: *Mdh* (malate dehydrogenase, EC 1.1.1.37), *Me* (malic enzyme, EC 1.1.1.40), *Pgm* (phosphoglucomutase, EC 5.4.2.2 formerly EC 2.5.7.11), *Pgi* (phosphoglucose isomerase, EC 5.3.1.9), *Est* (esterase, EC 3.1.1), and *Hk* (hexokinase, EC 2.7.1.1) were studied by horizontal starch-gel electrophoresis. Three enzyme systems (*Est*, *Pgi* and *Hk*) were studied using the Tris-citrate, pH 7.0 buffer system^{20,21}, two enzyme systems (*Mdh* and *Me*) were studied using the Tris-HCl, pH 8.6 buffer system²⁰ and *Pgm* was studied using the Tris-EDTA-maleate-magnesium, pH 7.4 buffer system²⁰. Gel and sample preparation and experimental conditions were as in Kandemir & Kence¹ and Kandemir et al.²

All allozymes were designated by using relative mobilities, with the most common allozyme used as standard² (relative mobility: 100). Gene frequencies, enzyme and population heterozygosities were calculated according to Nei²². A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. The suitable fitness test of gene frequencies to Hardy-Weinberg expectations was carried out, and an UPGMA phenogram was constructed from the genetic similarities between species by using BIOSYS-1²³.

Results

Among the species studied electrophoretically; in the subgenera *Megabombus* and *Rhodobombus* all the species are found to be monomorphic for the six loci examined. The only heterozygosity is in the subgenus *Thoracobombus*. *B. (Thoracobombus) humilis*, which is monomorphic in five enzyme loci except *Pgm*. The heterozygosity in *B. humilis* were low (Mean heterozygosity=0.19±0.19). Populations

of bumble bees were found to be in Hardy-Weinberg equilibrium with respect to all polymorphic enzymes ($P > 0.05$) except *B. humilis* ($\chi^2 = 15.873$, $P < 0.001$). Genetic similarities between species were calculated and the phenogram was constructed from the similarity matrix (Figure 1).

Discussion

B. humilis have different populations that have different color features in West-Palaeartic region. Some authors determined these populations as different subspecies^{10,24}. But there is an overlapping in the distribution of some subspecies of this species in Anatolia. *B. humilis nigrinus* and *B. humilis auranticus* were collected from overlapped provinces in Central Anatolia by different authors^{10,24}. The results of the electrophoretic analysis in the present study imply that these subspecies would not be natural and that the differences appeared possibly because of the allozyme variability in *Pgm* locus. Further studies would be needed especially on DNA level to test this hypothesis in the future.

The low heterozygosity in Hymenoptera is a general phenomenon^{1,2}. Haplodiploidy was suggested as a possible cause of low genetic diversity in bees³. Pekkarinen⁶ also observed different electromorphs

in bumble bees and obtained different dendrograms. The subgeneric model in bumble bee systematics has been discussed by several authors since it was proposed by Richards¹¹. In the present study, the phenogram based on electrophoretic data is in conformity with the study of Richards¹¹, which supports the natural hypothesis of at least these three subgenera. Further studies including all the species will be helpful to clarify the situation by using other alternative methods such as geometric morphometrics and DNA based models.

Acknowledgements

The authors wish to thank Dr. Aykut Kence and Dr. İrfan Kandemir for their great help.

References

1. Kandemir, İ., Kence, A., Allozyme variability in a Central Anatolian honeybee (*Apis mellifera* L.) population, *Apidologie*, 26, 503-510, 1995.
2. Kandemir, İ., Kence, M., Kence, A., Genetic and morphometric variation in honeybee (*A. mellifera* L.) populations of Turkey, *Apidologie*, 31, 1-14, 2000.
3. Pamilo, P., Varvio-Aho, S., Pekkarinen, A., Low enzyme gene variability in hymenoptera as a consequence of haplodiploidy, *Hereditas*, 88, 93-99, 1978.
4. Pamilo, P., Pekkarinen, A., Varvio, S., Clustering of bumble bee subgenera based on interspecific genetic relationships (Hymenoptera, Apidae: Bombus and Psithyrus), *Ann. Zool. Fennici*, 24, 19-27, 1987.

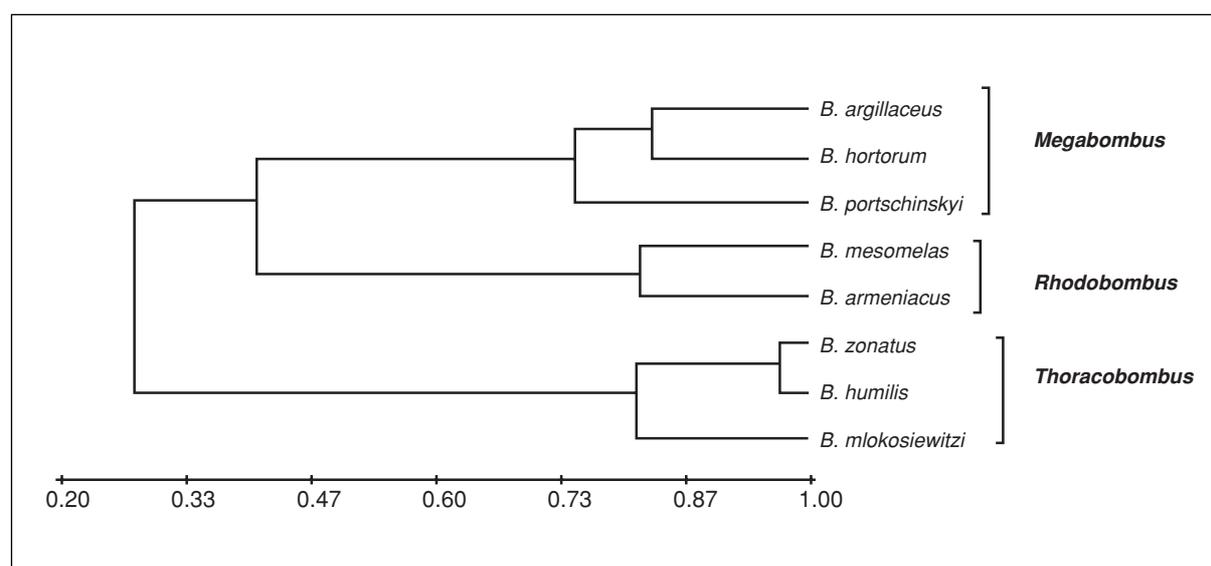


Figure 1. The UPGMA similarity phenogram in the examined species based on the allozyme data

5. Pamilo, P., Tengö, J., Rasmont, P., Pirhonen, K., Pekkarinen, A., Kaarnama, E., Pheromonal and enzyme genetic characteristics of the *Bombus lucorum* species complex in Northern Europe, *Entomologica Fennica*, Vol. 7, 187-194, 1997.
6. Pekkarinen, A., Morphometric, colour and enzyme variation in bumble bees (Hymenoptera, Apidae, *Bombus*) in Fennoscandia and Denmark, *Acta Zoologica Fennica*, No: 158, Helsinki, 1-60, 1979.
7. Thorpe, J. P., Solé-Cava, A. M., The use of allozyme electrophoresis in invertebrate systematics, *Zoologica Scripta*, Vol. 23, No: 1, 3-18, 1994.
8. Rosenmeier, L., Packer, L. A., Comparison of genetic variation in two sibling species pairs of haplodiploid insects, *Biochemical Genetics*, Vol. 31, Nos., 3/4 185-200, 1993.
9. Engel, M. S., A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera), *Bulletin of the American Museum of Natural History*, Number 259, New York, 192p, 2001.
10. Rasmont, P., Flagothier, D., Biogéographie et choix floraux des bourdons (Hymenoptera, Apidae) de la Turquie, NATO-OTAN TU Pollination Project Report, Belgium, 68 p, 1996.
11. Richards, O. W., The subgeneric divisions of the genus *Bombus* Latreille (Hymenoptera: Apidae), *Bull. Br. Mus. Nat. Hist. (Ent.)*, 22, 210-276, 1968.
12. Ito, M., Supraspecific classification of bumble bees based on characters of male genitalia, *Zoological Section, The Institute of Low Temperature Science Publ., Hokkaido University, Japan*, 143pp, 1985.
13. Koulianos, S., Schmid-Hempel, P., Phylogenetic relationships among bumble bees (*Bombus*, Latreille) inferred from mitochondrial cytochrome b and Cytochrome Oxidase I Sequences, *Molecular Phylogenetics and Evolution*, 14(3): March: p, 335-341, 2000.
14. Terzo M., Valterova, I., Urbanova, K., Rasmont, P., De la nécessité de redécrire les phéromones sexuelles des mâles de bourdons [Hymenoptera, Apidae, Bombini] publiées avant 1996 pour leur utilisation en analyse phylogénétique, *Phytoprotection*, 84(2) 39-51, 2003.
15. Williams, P. H., A preliminary cladistic investigation of relationships among the bumble bees (Hymenoptera, Apidae), *Systematic Entomology*, 10: 239-255, 1985.
16. Aytekin, A. M., Çağatay, N., A phenetic approach to the subgenera of bumble bees (Hymenoptera: Apidae), *Mellifera*, Vol: 2, No: 3, 60-64, 2002.
17. Williams, P. H., An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini), *Bulletin of the Natural History Museum, Entomology*, 67, 79-152, 1998.
18. Rasmont, P., Catalogue commenté des bourdons de la région ouest-paléarctique, *Notes Fauniques de Gembloux*, No. 7. Belgique, 71p, 1983.
19. Pawlikowski, T. A., Field guide to identification of bumble bees (Hymenoptera: Apidae: Bombini) in Poland, *Wydawnictwo Uniwersytetu Mikołaja Kopernika*, 30p, 1999.
20. Shaw, C. R., Prasad, R., Starch gel electrophoresis of enzymes, A compilation of recipes, *Biochemical Genetics*, Vol. 4, 297-320, 1970.
21. Hillis, D. M., Moritz, C., *Molecular systematics*, Sinauer Assc., Inc., Sunderland, Massachusetts, USA, 588p. 1990.
22. Nei, M., *Molecular evolutionary genetics*, Columbia University Press, New York, 634p, 1987.
23. Swofford, D., Selander, R. B., *Biossyst-1*, Department of Genetics and Development, Illinois, 65p, 1981.
24. Aytekin, A. M., Ankara ili ve ilçeleri Apidae (Hymenoptera) familyası üzerinde sistematik araştırmalar ve bunların ayçiçeği (*Helianthus annuus* L.) bitkisindeki pollinatör etkileri üzerine ön-çalışmalar, *Bilim Uzmanlığı Tezi, Hacettepe Üniversitesi Fen Bilimleri Enstitüsü, Ankara*, 83s. 1996.

Copyright of Mellifera is the property of Development Foundation of Turkey and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.