# DNA analysis of genetic variation in Asian honey bees


## H. Allen Sylvester

*USDA, ARS, Honey-Bee Breeding, Genetics & Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820*

## and Siriwat Wongsiri

*Bee Biology Research Unit, Chulalongkorn University, Bangkok 10330, Thailand.*


## Introduction

This research was conducted to determine whether DNA markers from European honey bees, *Apis mellifera*, could be used to identify and characterize genetic variation in Asian species of *Apis*. Being able to use the same DNA for all species would have the advantages of eliminating the expense of developing the technology for each species individually and would allow direct comparison of the results from different species. Clones of *Apis mellifera* DNA (probes) isolated from a genomic library have been isolated and found to detect variation in *A. mellifera* (unpublished results), or restriction fragment length polymorphisms (RFLPs). These probes were selected because they strongly hybridized to honey bee total genomic DNA, indicating that they may be fragments of DNA which are highly replicated in the honey bee genome. This is promising for use in honey bee population genetics and contrasts to the situation with isozymes, which have shown relatively little variation in honey bees (Sylvester 1986).

The RFLP patterns produced by these probes in *A. mellifera* are quite simple, similar to those seen in isozyme electrophoresis. None have yet produced the very complex patterns found in DNA fingerprinting. This produces results which are much easier to interpret and whose inheritance should be simpler to establish.

The fact that drones are haploid is a great advantage in using RFLPs in honey bee population genetics. Each drone produces a clone of genetically identical sperm (ignoring the rare occurrence of mutations), so all daughters of one drone have exactly the same arrangement of DNA from their father. This will make it easier to separate super-sisters (offspring from the same drone) from half-sisters (offspring

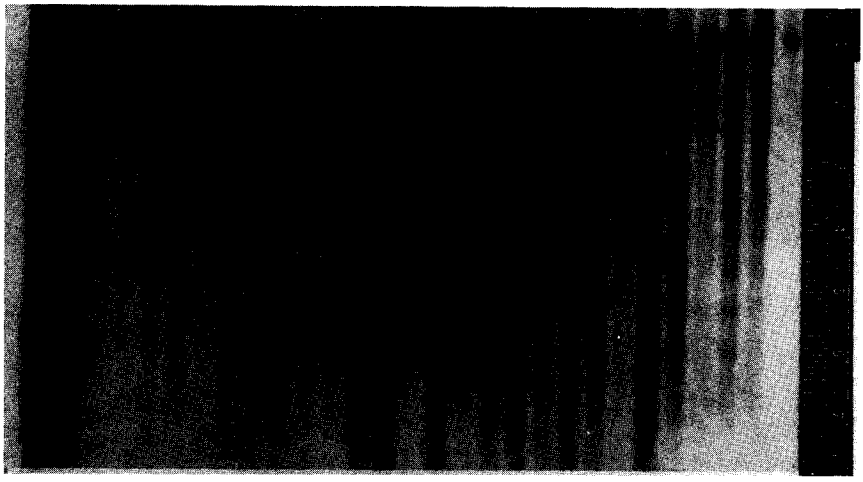

***Figure 1.*** *DNA from one colony each of* Apis cerana *(lanes 1-12),* A. dorsata *(lanes 13-24), and* A. florea *(lanes 25-28), hybridized to probe #47. The lanes are numbered from number 1 on the left and the origin is at the top.*

from different drones which are not brothers). Since drones are descended from unfertilized eggs, a small sample of drones can be used to determine their mother's genotype without having to sacrifice the queen.

## Methods and Materials

DNA was extracted from individual adult worker honey bees of *Apis andreniformis, A. florea, A. cerana,* and *A. dorsata* from Thailand, using a procedure developed for *A. mellifera* larvae and pupae - the details are available from HAS. Extracted DNA from each bee was then digested with *EcoR* I restriction enzyme, which cuts the DNA at specific base sequences, and then the fragments were separated according to size by horizontal electrophoresis in a 1% agarose gel. The DNA was transferred by Southern blotting onto Biosbrane®, nylon membrane and bound by UV irradiation. Two probes were individually labeled using the Genius, nonradioactive DNA labeling system from Boehringer Mannheim. Membranes were then hybridized to a probe and the position of the hybridized probe detected with the Genius®, nonradioactive detection system, which produces colored bands on the membrane.

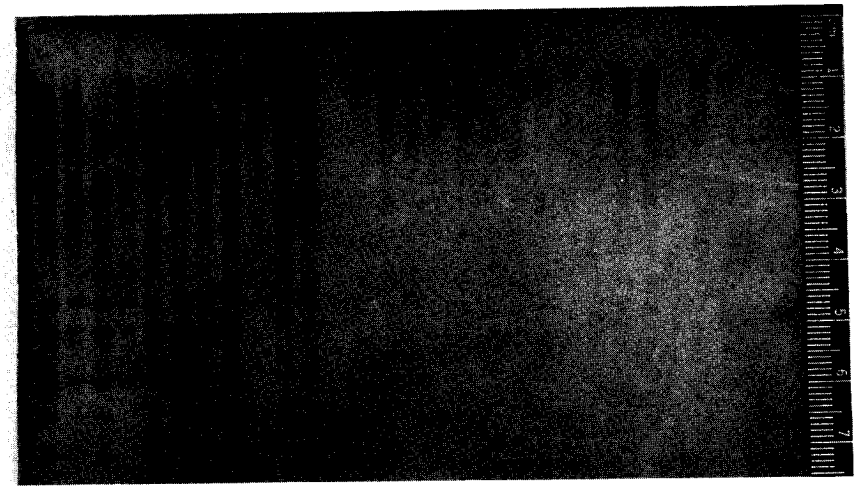

*Figure 2. DNA from one colony of* A. dorsata *(lanes 1-12) and one colony of* A.andreniformis *(lanes 14-25) hybridized to probe #44. The lanes are numbered from number 1 on the left and the origin is at the top.*

## Results and Discussion

Clear results were only obtained in a few of the lanes. Figure 1 shows a membrane which was probed with one probe. *A. cerana* workers from one colony show clear bands in lanes 1 and 11. The presence of 4 bands in lane 1 and 3 bands in lane 11 indicates a probable genetic difference between sister workers in this colony. An *A. dorsata* worker shows 5 bands in lane 16. An *A. florea* worker shows 6 bands in lane 26. Several of the bands in each species do not appear in those workers examined of the other species.

Figure 2 shows a different membrane which was probed with another probe. *A. dorsata* workers from another colony, in lanes 1-12, show 5 bands in lanes 1 and 3. An *A. andreniformis* worker shows 2 bands in lane 20.

The pattern of bands in *A. dorsata* is clearly distinct between Figures 1 and 2, showing that these probes hybridize to different areas of DNA in this species. The differences between the banding patterns of each species are great enough that they may be interspecies differences, rather than just intraspecies variants, but additional samples are needed to confirm this.

The amount of variation visible in Figure 1 indicates that it should be possible

to obtain single probes which will simultaneously be useful for detecting variation within colonies, among populations or races and among species.

Most of the lanes are uninterpretable because of too little DNA (nearly blank lanes) or because the DNA was not adequately prepared by the purification procedure (dark streaks).

These results, while preliminary, clearly show that it is feasible to use *A. mellifera* DNA probes to study genetic variation in other species of honey bees. It is also clear that detectable nuclear DNA variation does exist among *A. dorsata*, *A. cerana*, *A. florea*, and *A. andreniformis*. This system thus provides additional markers for comparing and quantifying genetic variation within and among honey bee species.

## Acknowledgments

We would like to thank Sandra Kleinpeter and Vivek Sriram for technical assistance. Travel grants to collect samples in Thailand were provided by the Research Affairs Division of Chulalongkorn University and USAID-CDR Project #936-5544GSS9082-00. Financial support for HAS to travel to Thailand and attend this meeting was provided by the Thai National Science and Technology Development Agency through the U. S. National Academy of Sciences. In cooperation with Louisiana Agric. Exp. Stn. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

## Literature Cited

Sylvester, H.A. 1986. Biochemical genetics. *In* Bee Genetics and Breeding, Rinderer, T.E., ed., Academic Press, N.Y.: 177-203.

## Abstract

This research demonstrated that nuclear DNA markers from European honey bees, *Apis mellifera*, could be used to identify and characterize genetic variation in Asian species of Apis. DNA clones isolated from a genomic DNA library were screened and those which showed variation in *A. mellifera* were selected. DNA was extracted from individual workers of *A. andreniformis*, *A. florea*, *A. cerana* and *A. dorsata*. This DNA was cut into fragments with a restriction enzyme, these fragments separated by size in an agarose gel and transferred to a nylon membrane by Southern blotting. The fragments were then hybridized to each probe individually and the probe binding sites visualized with a non-radioactive detection system.

The DNA of these species did hybridize to *A. mellifera* probes and there was variation among the species.

## Key Words:

Asian honey bees, *Apis mellifera*, DNA markers, DNA probes, restriction fragment length polymorphisms, isozyme electrophoresis, *Apis andreniformis*, *Apis florea*, *Apis cerana*, *Apis dorsata*, Thailand.

*Dr. H. Allen Sylvester received his Ph. D. in Genetics in 1976 from UC Davis and is currently working on developing molecular biology techniques to study honey bee population genetics, particularly relating to the process of Africanization in A. mellifera. Dr. Siriwat Wongsiri received his Ph. D. in Entomology in 1975 from UC Davis and is currently working on reproductive biology and behavior of Asian honey bees.*