Pest Management in Horticultural Ecosystems,
Vol. 20, No. 1 pp 55-58 (2014)

Qualitative and quantitative differences in DNA extracted from different body parts of *Apis* spp. (Hymenoptera:Apidae) and its validation using microsatellite markers

S. SUDHAGAR[2], P. V. RAMI REDDY[1], V. SRIDHAR[1], P. D. KAMALA JAYANTHI[1] and R. VANI[3]

[1]Division of Entomology and Nematology, Indian Institute of Horticultural Research, Bengaluru - 560089, India
[2] Ph. D Scholar, Department of Biotechnology, Jain University, Bengaluru, India
[3] Department of Biotechnology, Jain University, Bengaluru, India
E-mail: pvreddy2011@gmail.com

ABSTRACT : To study the adaptability and the genetic diversity in *Apis* species, the molecular analyses play vital role for which good quality/ quantity of DNA is an important component. Here we describe a simple and efficient method for isolating quality genomic DNA from different body parts of *Apis* species through modifying the previous protocols. Alteration was made in the extraction buffer (100 mM Tris-HCl, pH 8.0, containing 2% Cetyltrimethylammonium bromide (CTAB), 1.5 M NaCl, 10 mM Ethylenediaminetetraacetic acid (EDTA) and 2% â-mercaptoethanol added after grinding) and the developed protocol was successfully tested on two different species, *A. cerana* and *A. florea*. DNA isolated from thorax yielded good quality DNA compared to other body parts that was further validated using microsatellite markers.

Keywords : *Apis cerana*, *Apis florea*, DNA, honey bees

INTRODUCTION

Honey bees are one of the most important pollinator groups which pollinate 16% of flowering plant species in the world and nearly 400 species of agricultural plants (Crane and Walker, 1984). There are about 19,000 described species of bees in the world (Linsley, 1958). India isendowed with the greatest biodiversity as for as honey bees are concerned and is home to five species namely, *Apis cerana, A. florea, A. dorsata, A. andreniformis* and *A. laboriosa* (Thakur, 2012). There are four honey bee species which are widely used as pollinators *viz.*, *Apis mellifera, A. dorsata, A. cerana* and *A. florea* (Merti, 2003). Thus, individual foraging behaviour of a pollinator species and their population diversity in an area affects pollination efficiency and hence crop yield. Knowledge of these patterns is essential in any decision aiming at optimization of pollinator inundative releases or containment measures. Nevertheless, honey bees have been living under dissimilar geographical and environmental conditions and subjected to certain changes at molecular level, leading to adaptation to the specific environment. To study the adaptability and their genetic diversity, molecular analysis plays a vital role. These molecular studies help to understand the complex inter and intra-specific interactions and the demographic population changes through markers like RFLP, SSR's and others. In case of honey bees, these markers were used extensively to understand the population genetic diversity. For conducting such studies the quality and quantity of DNA is an important component; hence an efficient protocol has to be followed (Ji *et al.*, 2011 & Waldschmidt *et al.*, 1997). Though DNA extraction protocols were developed for *Apis* spp. information is lacking in respect of DNA quality/yield from different body parts.

Extraction of DNA was done in different countries from different *Apis* species like stingless bee in Brazil (Waldschmidt *et al.*, 1997), *A. cerana* in Philippines (Rua *et al.*, 2000), *A. mellifera in* UK (Châline *et al.*, 2004) and *Apis* spp. (single bee) based on phenol-chloroform method (Sheppard & McPheron, 1991). Attempts were also made to isolate DNA from wings, exuviae also in an attempt to develop a non-lethal sampling (Châline, *et al.*, 2004). The present study was aimed at developing a protocol for obtaining high quality yield of DNA from *A. cerana* and *A. florea* with particular reference to different body parts. Thus, the objective of the study is to see whether DNA extracted from different body parts of honey bees show any differential yield and purity of DNA.

MATERIALS AND METHODS

Insects

Adult worker bees of two honey bee species *Apis cerana* and *A. florea* were collected from the strong apiary located at Indian Institute of Horticultural Research, Bengaluru (13° 13' N and 77° 49' E) through

sweep net sampling method. The collected samples were preserved in 70% ethanol and used for the DNA extraction.

Sample preparation

Three different methods such as, H.P. method (as described by Hunt and Page, 1992 for *Apis* spp.), Waldschmidt method (as per Waldschmidt *et al.*, 1997 for stingless bee), CTAB method (Modified method in combination of H.P. and Waldschmidt) were tested. Different body parts of honey bee used for DNA extraction were head, thorax, abdomen and wings. All body parts were used singly, except wings for which 8 pairs were used.

DNA extraction

Three methods tested for DNA extraction utilized different concentrations of specific components of the extraction buffer which are presented below. In H.P. method: The extraction buffer contained 100 mM Tris-HCl, pH 8.0, with 2% CTAB (hexadecyl trimethyl ammonium bromide), 1.4 M NaCl, 20 mM EDTA and 100µg/ml proteinase K. In Waldschmidt method, the extraction buffer contained 50 mM Tris-HCl, pH 8.0, with 2% SDS (sodium dodecyl sulphate), 0.75 M NaCl, 10 mM EDTA and 100µg/ml proteinase K. In CTAB method, the extraction buffer contained 100 mM Tris-HCl, pH 8.0, along with 2% CTAB, 1.5 M NaCl, 10 mM EDTA and 2% â-mercaptoethanol (added freshly before grinding the sample).

Different body parts of bee samples used in the study (head, thorax, abdomen and wings) were ground finely by using mortar pestle and adding liquid N_2 as well as extraction buffer. The samples were then incubated at 60°C for 1 hour and deproteinized with equal volumes of phenol: chloroform (1:1), followed by centrifugation at 11,100 rpm for 10 mins. Further, samples were deproteinized with equal volumes of chloroform: isoamylalcohol (24:1) at 11,100 rpm for 10 mins. Nucleic acid was precipitated by addition of equal volume of ice cold absolute alcohol with 5µl of 3M sodium acetate and incubated at -20°C for 1 hour. After centrifugation at 12,000 rpm for 15 min, the pellets were washed twice with 70% (v/v) ethanol and allowed to air dry. The pellet was resuspended in 100 µl TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) and incubated with RNAse A (100 µg/ml) for 1 hour at 37°C. The integrity and purity of the DNA samples were checked by adding 5 µl on 0.8% (w/v) agarose mini-gels and quantified (Table 1) using Spectrophotometer (Thermo Scientific- JH Bio).

DNA quantification

The DNA isolated from different body parts were quantified using spectrophotometer and qualified using agarose gel electrophoresis. Absorbance at 230nm, 260nm and 280nm were measured and the following formula used to determine the DNA concentration (concentration of pure double-stranded DNA with an A260 of 1.0 is 50 mg/ml).

Unknown (mg/ml)/ Measured A260 = 50 (mg/ml) / 1.0 A260

Purity was calculated by the absorbance ratios, A260/A280 and A260/A230 for proteins and polyphenols/ polysaccharide compounds, respectively (Kamala Jayanthi *et al.*, 2012). Quality was checked by electrophoresis of 5µl of the isolated DNA in 0.8% agarose gel and bands were visualized under UV transmitter (SYNGENE, G:Box) (Fig. 1).

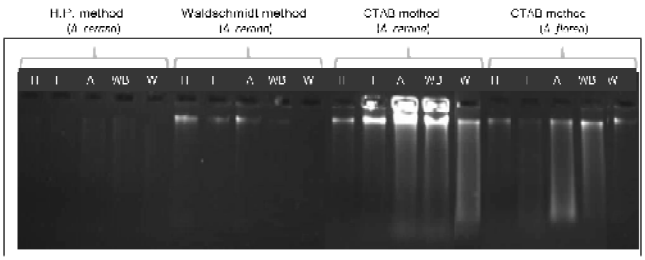

Fig. 1. DNA isolated by H.P. method (*A. cerana*), Waldschmidt method (*A. cerana*) and CTAB method (*A. cerana* and *A. florea* respectively), (H – Head, T – Thorax, A – Abdomen, WB – Whole body and W – Wing).

PCR amplification

The quality of isolated DNA from four different colonies of *A. cerana* at IIHR was further validated using four microsatellite markers AS45, AS46, AS10 and AS60 that were generated from the *A. mellifera* genome sequences (Table 2, Fig. 2). Standard PCR reaction mixture (15µl) contained 2µl of 10X buffer, 0.5µl $MgCl_2$ (50mM; 3B Black Biotech Pvt. Ltd.), 0.5µl dNTPs (10mM; Amnion Pvt. Ltd, India), 1µl primer (10pmol; Bio-serve Pvt. Ltd, India), 2µl template DNA (25ng), 8.8µl DEPC treated water and 0.2µl Taq DNA Polymerase (5U/µl; 3B Black Biotech Pvt. Ltd.). The PCR cycle conditions for the above microsatellite markers are as follows. Initial denaturation at 94°C for 4 min., followed by 35 cycles of 1 min. at 94°C for denaturation, 1 min. at 52°C annealing temperature (varies for different SSR primers) and 1 min. at 72°C for extension, followed by final extension 10 min. at 72°C in Eppendorf Mastercycler pro S. Bright and specific bands up to two alleles were

Table 1. DNA sample concentration of *A. cerana* and *A. florea* from different body parts of honeybee.

Sample	*Apis cerana*		*Apis florea*	
	DNA concentration (ng/µl)	A_{260}/A_{280} (O.D.)	DNA concentration (ng/µl)	A_{260}/A_{280} (O.D.)
Head	960	1.78	822	1.71
Thorax	973	1.82	820	1.80
Abdomen	1110	1.93	1020	1.82
Wings	920	1.65	600	1.58
Whole bee	1715	2.03	1260	1.87

Table 2. Details of SSR primers used for validation of isolated DNA from thorax of *A. cerana*.

Primer	Repeat	No. of repeats	Forward primer	Reverse primer	AT* (°C)	Product (bp)
AS45	TACA	5	CCGTTATACCCG CAACATTC	CCCAGATCGGAAG ATGTGTT	55	166-172
AS46	TTAT	3	CGCAATTCCTAT GTGCATGT	CGACGAGTGGTTG ATCAATTT	55	206-216
AS10	TA	22	CGACGACACTGA AAATGTGG	CGTCCCGAAAAGT CTCTCAC	54	317-366 400-430
AS60	AATT	3	GCAGTTAGGTGG GACCAAAA	TGATTCGTCATCGT CCTTGA	54	152-160

*AT: Annealing temperature

amplified using above mentioned protocol in different body parts (Fig. 2).

RESULTS AND DISCUSSION

Quantitative and qualitative differences were observed in isolated DNA samples following the three different methods and body parts (Fig. 1 & Table 1). The

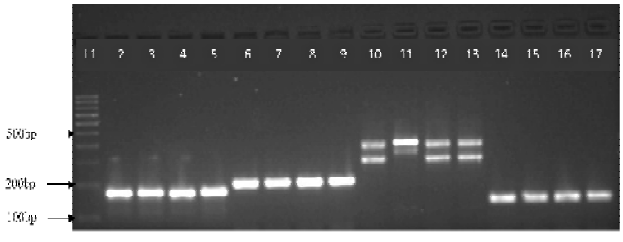

Fig. 2. PCR amplification of different population of *A. cerana* using microsatellite markers (L1 - 100bp ladder, Amplification of AS45 primer: 2-5, AS 46 primer: 6-9, AS10 primer: 10-13 and AS60 primer: 14-17).

body samples were weighed using precision balance (*A. cerana*: Head-2.04mg, thorax-14mg, abdomen-14.88mg and wings-1.25mg and *A. florea:* Head-1.48mg, thorax-5.14mg, abdomen-7.88 and wings -0.59mg). From H.P. method, there were no yields of DNA and Waldschmidt method yielded low quantity of DNA which is not sufficient for molecular studies. However, alteration of extraction buffer composition in CTAB method yielded enough DNA from different body parts of *A. cerana* (Fig. 1 CTAB method) and *A. florea* (Fig. 1 CTAB method) that was evident through intact bands. Hence, CTAB method was better for DNA extraction from *Apis* spp. compared to other methods.

Further, though the sample quantities taken for DNA extraction were <15 mg, all samples yielded good quality DNA sufficiently. Hence CTAB method is efficient and easier method when compared to chelex100 (Walsh *et al.*, 1991) and other commercial kit methods which are costly and yields less DNA (DNA is sufficient for only few PCR reactions compare to CTAB method).

Among different body parts used, large quantity of DNA (1715ng) was isolated from whole bee samples, followed by abdomen (1110ng), thorax (973ng), head (960ng) and wings (920ng). Nevertheless, the DNA from whole body and abdomen always suffers chance of cross contamination from associated symbionts and pollen when universal markers are used. The DNA isolated from wings showed lower optical density (O.D.) value when compared to other parts (Châline, *et al.*, 2004). Similarly, head also yielded low DNA quantity 960ng and also has chance of cross contamination. Though the DNA yield from thorax was low compared to abdomen, purity wise good with OD range considered to be good quality (Clark and Christopher, 2001). Further, DNA isolated from thorax will be free from associated symbionts and other contaminating sources like pollen (Raffiudin *et al.*, 2009, Gregory & Rinderer, 2004) can be fit for PCR amplification.

The validation of PCR quality DNA extracted from thorax and microsatellite regions were amplified on four apiaries of *A. cerana* using different microsatellite markers. Microsatellite primers (AS45, AS46, AS10 and AS60) were designed and their amplicon sizes were predicted as of 170, 210, 350 and 150bp respectively (Table 2) for four apiaries. Figure 2 suggested that the samples from different apiaries amplified in different size of alleles (polymorphism). These variations may be attributed to different ecological and biological parameters reflected at molecular level. From the results, we establish that better quality DNA can be obtained from thorax of Indian honey bee which can be used for genetic diversity analysis using microsatellite markers.

ACKNOWLEDGEMENTS

This paper is an outcome of the Ph.D. work of the senior author. Authors gratefully acknowledge the financial assistance extended by Indian Council of Agricultural Research (ICAR), New Delhi in the form of *National Initiative on Climate Resilient Agriculture* (*NICRA*) sponsored project. The facilities provided by the Director, Indian Institute of Horticultural Research (IIHR), Bengaluru are also acknowledged.

REFERENCES

Châline, N., Ratnieks, F. L. W., Raine, N. E., Badcock, N. S. and Burke, T. 2004. Non-lethal sampling of honey bee, *Apis mellifera*, DNA using wing tips. *Apidologie*, **35**: 311-318.

Clark, W. and Christopher, K. 2001. An Introduction to DNA: Spectrophotometry, Degradation, and the 'Frankengel' Experiment, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, CANADA, pp. 81-99.

Crane, E. and Walker, P. 1984. Directory of Important World Honey Sources. Int. Bee Res. Assoc., (IBRA) London, pp. 187.

Gregory, P. G. and Rinderer, T. E. 2004. Non-destructive sources of DNA used to genotype honey bee (*Apis mellifera*) queens. *Entomologia Experimentalis et Applicata*, **111**: 173-177.

Hunt, G. J. and Page, R. E. 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theoretical Applied Genetics*, **85**: 15-20.

Ji, T., Yin, L. and Chen, G. 2011. Genetic diversity and population structure of Chinese honey bees (*Apis cerana*) under microsatellite markers. *African Journal of Biotechnology*, **10**: 1712-1720.

Kamala Jayanthi, P. D., Rajinikanth, R., Sangeetha, P., Ravishankar, K. V., Arthikirubha, A., Devi Thangam, S. and Abraham Verghese. 2012. Rapid isolation of high molecular weight DNA from single dry preserved adult beetle of *Cryptolaemus montrouzieri* for polymerase chain reaction (PCR) amplification. *African Journal of Biotechnology*, **11**: 15654-15657.

Linsely, E. G. 1958. The ecology of solitary bees. *Hilgardia*, **27**: 543-599.

Merti, A. A. 2003. Botanical inventory and phenology in relation to foraging behaviour of the cape honey bees (*Apis mellifera capensis*) at a site in the Eastern Cape, South Africa, Thesis, Rhodes University.

Raffiudin, R., Bintar, A., Widjaja, M. C., Farajallah, A. and Purwantara, B. 2009. Rapid Detection of the Africanized honey bee: A tool for Indonesian animal quarantine. *Biotropia*, **16**: 38-44.

Rua, P. A., Simon, U. E., Tilde, A. C., Moritz, R. F. A. and Fuchs, S. 2000. Mt DNA variation in *Apis cerana* populations from the Philippines. *Heredity*, **84**: 124-130.

Sheppard, W. S. and McPheron, B. A. 1991. Ribosomal DNA diversity in Apidae. In: Diversity in the genus *Apis* (Smith, D.R., ed.). Westview Press, Boulder, pp. 89-102.

Thakur, M. 2012. Bees as Pollinators – Biodiversity and Conservation. *International Research Journal of Agricultural Science and Soil Science*, **2**: 1-7.

Waldschmidt, A. M., Salomão, T. M. F., Barros, E. G. E. and Campos, L. A. O., 1997. Extraction of genomic DNA from *Melipona quadrifasciata* (Hymenoptera: Apidae, Meliponinae). *Brazil Journal of Genetics*, **20**: 421-423.

Walsh, P. S., Metzger, D. A. and Higuchi, R. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**: 506-513.

MS Received : 5 March 2014
MS Accepted : 26 May 2014