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Genetic Polymorphism in Honey bee *Apis cerana indica* (F.) (Hymenoptera: Apidae) by Random amplified polymorphic DNA (RAPD) Markers

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Abstract
For pollination purpose and apiary by-products production *Apis cerana indica* is highly demanded honey bee in India. Honey bee is a polymorphic bee having three casts queen, workers and drones. In *Apis cerana indica* queen honey bee undergoes multiple mating with drones to gather genetic diversity in colony. Genomic DNA sample of adult drone and worker honey bees was used to study genetic polymorphism by Random amplified polymorphic DNA (RAPD) markers. Ten Primers of each containing different 10mer oligonucleotide sequence were used to generate RAPD profile. Present study observed the banding pattern for presence and absence of characters and find out the polymorphism information content (PIC) value generated by each primer. Low level of genetic polymorphism was found between workers and drones with 0.34984 PIC value.

Keywords: Drone, honey bee, polymorphism, RAPD, worker

Introduction
Genetic variation plays important role in evolution of social behavior in Hymenoptera (Hamilton 1964; Pamilo and Aho, 1984) [10, 28]. During nuptial flight queen of *Apis cerana indica* undergoes multiple mating with more than 30 drones (Woyke, 1975) [42] as compared to *Apis mellifera* which undergoes multiple mating with more than 17 drones (Adams *et al.*, 1977) [1] to gather more genetic diversity in colony. Various study in honey bee *Apis mellifera* confirmed the importance of genetic diversity for long term productivity and fitness, for colony resistance to disease (Tarpy and Seeley, 2006) [37], communication by waggle dancing (Mattila *et al.*, 2008) [21].

Haplodiploidy is common reproductive mechanism found in hymenopterans species. Kin selection hypothesis of Hamilton (1964) [10] regarding evolution of Eusociality in hymenopteran insects gives importance to male haploidy and observed high genetic relatedness among sibling.

To detect genetic relationship between individual populations several molecular methods like allozyme marker detection, DNA fingerprinting, RFLP, RAPD, Hormones are in use (Woyke, 1975; Pamilo *et al.*, 1978b; Welsh and Mccleland, 1990; Mortiz *et al.* 1991; Infante and Espin 1995; Barsagade and Jiwatode, 2021) [42, 26, 40, 25, 14, 2]. To study genetic variation in insects, RAPD is suitable technique as compared to other molecular marker technique due to low requirement of DNA sample, no previous knowledge of primer sequence and no hazards of radioactivity (Williams *et al.*, 1990) [41].Various researchers found that RAPD markers are convenient tools to study genetic variation in Hymenopteran insects (Hasegawa 1995; Lu and Rank 1996) [11, 17] and in other organisms (Welsh and Mccleland, 1990; Williams *et al.*, 1990) [40, 41]. RAPD marker was used to study paternity analysis population structure and genetic relatedness (Queller and Goodnight, 1989; Fondark *et al.*, 1993; Hadry *et al.*, 1993) [29, 7, 9].

The lower level of heterozygosity in haplodiploidy insects is due to smaller population size (Metcalf *et al.*, 1975; Pamilo *et al.*, 1978b) [22, 26], more significant sampling variance and stronger hitchhiking effect (Lester and Selander,1979) or stable condition in nest (Snyder 1974) [34]. Haplodiploidy leads to increased interpopulation variation (Helle and Oremeer, 1973) [12] and reduce intrapopulation variation (Crozier 1977a; Pamilo *et al.*, 1978b) [5, 26]. Ward (1980) observed low heterozygosity in intrapopulation of hymenopteran *Rhytidoponera*

impress ants. Landry *et al.*, in 1993 [15] used RAPD marker to study intra and interpopulation genetic variation in parasitic wasp. The present study is focused on identification of suitable primers sequence and find out polymorphism information content (PIC) between worker and drone caste of honey bee *Apis cerana indica* on the basis of presence and absence of banding pattern generated by RAPD profile.

Material and methods

For commercial and research purpose apiary of honey bee *Apis cerana indica* is maintained in our home filed at Warora, District Chandrapaur, Maharashtra, India (20º16'53"N79º01'21"E).

Fig 1: Google map showing the apiary site. 20º16'53"N79º01'21"E

Fig 2: Apiary and bee box of *Apis cerana indica*

Adult workers and drones from a colony of *Apis cerana indica* were collected. Genomic DNA sample from male drones and female workers honey bees was extracted separately using CBTA methods with minor modification (Saghai- Maroof *et al.*, 1984; Hunt and Page, 1992) [32, 13].

Adult drone honey bees were placed in a mortar and homogenized with 1 ml of extraction buffer. In 2ml-microfuge tube, homogenate and equal volume of Phenol: Chloroform: Isoamlyalcohol (25:24:1) were added and mixed well for centrifugation. At 14,000 rpm, tubes were centrifuge for 15 min. After centrifugation upper aqueous phase was separated in new tube and in it equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed. Then new tubes were centrifuge for 10 min at 14,000 rpm. To precipitated the DNA 0.1 volume of 3 M Sodium acetate pH 7.0 and 0.7 volume of Isopropanol were added in tubes for 15-20 min. After 15 min of incubation at room temperature the tubes were centrifuged at 4ºC for 15 min at 14,000 rpm. Obtained DNA pellet was washed twice with 70% ethanol and then washed with 100% ethanol and allowed to air dried.Genomic DNA quantified with flurometer and diluted to 5ng/µl in 10 mMTris, Ph 7.5, 0.3 mM EDTA. Same procedure was followed for genomic DNA collected from worker honey bees.

PCR amplification reactions were carried out according to Williams *et al.*, (1990) [41] using 10 mer oligonucleotides primers at Biokart Ind. Pvt. Ltd Bangalore. Detail of PCR condition and primers are given in table 1 and 2 respectively. DNA amplification products were observed by gel electrophoresis in 1.4% agarose gel with 0.8 x TBE buffer and stained with ethidium bromide. DNA Bands were visualised under UV light.

Table 1: PCR condition

Reaction mixture (20 µl)		**Cycling Condition**		
Template DNA	25 ng	Initial Denaturation	2 Minutes at 95 ^{0}C	40 Cycles
Primer	1 µM	Denaturation	1 Minutes at 95 ^{0}C	
dNTPs	0.5mM	Annealing	1 Minutes at 34 ^{0}C	
Mgcl2	2.5mM	Extension	2 Minutes at 72 ^{0}C	
		Final Extension	5 Minutes at 72 ^{0}C	

Table 2: Primer details used in RAPD-PCR amplification reaction.

Sr. No.	**Oligo name**	**Sequence 5'-3'**	**Tm (^{0}C)**	**GC – content**
1	OPA1	CAGGCCCTTC	27	70%
2	OPA2	TGCCGAGCTG	34	70%
3	OPA3	GGGTCCAAAG	32	60%
4	OPE02	GTCGTAGCGG	27	70%
5	A16	AGCCAGCGAA	45	60%

Result

Genomic DNA was amplified by using RAPD-PCR to find out the polymorphism between worker and drone honey bees in a colony. Ten Different primers were used to score polymorphic and monomorphic bands in gel image. For each primer we used the same samples obtained from male drone and female worker honey bees. Five primers produce detectable bands. Total 24 bands were generated by 5 primers with average 4.8 bands per primer (Fig: 1). Numbers of total monomorphic bands were 19 and polymorphic bands were 5. Present study finds out the polymorphism information content (PIC) between drone and workers honey bees. PIC value for each primer is given in table 4.4. Low level of polymorphism (0.208) was found for presence and absence of bands generated by RAPD markers with 0.34984 average value of PIC.

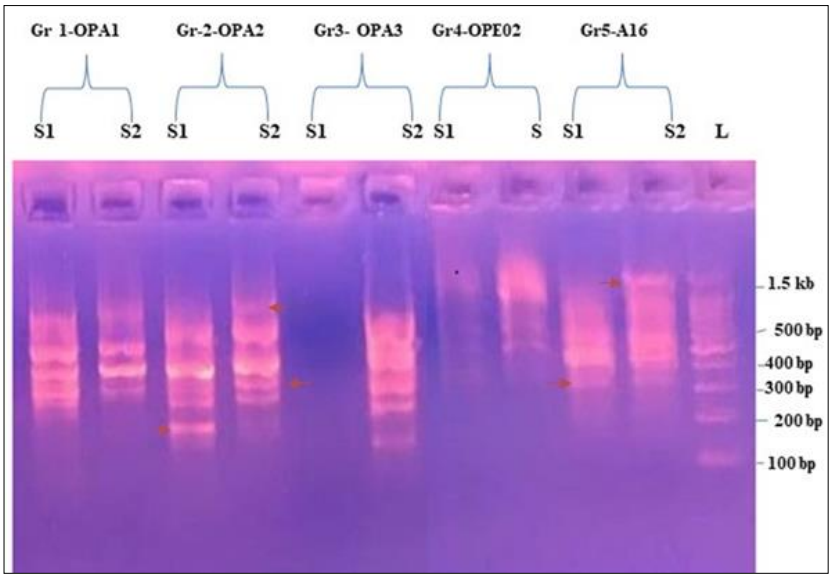

Fig 1: RAPD banding pattern of genomic DNA of honey bee *Apis cerana indica*. (S1-drone sample, S2-worker sample, L-ladder containing DNA fragment in size ranges from 100 bp – 500 bp and additional band at 1.5 kb. (OPA1, OPA2, OPA3, OPE02, A16 are primer marker and arrow-indicate the monomorphic bands). Each group (Gr) contained the same sample obtained from drone (S1) and worker (S2) and amplified by different primers.

Table 3: Band score (matrix) generated by 10mer oligonucleotides primer having different sequence.

		1	2	3	4	5	6	7	8	9	10
bp	**Ladder**	**S1OPA1**	**S2OPA1**	**S1OPA2**	**S2OPA2**	**S1OPA3**	**S2OPA3**	**S1OPE02**	**S2OPE02**	**S1A16**	**S2A16**
1.5kb	1	0	0	0	0	0	0	0	1	0	1
	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
500bp	1	1	1	0	1	0	1	0	1	1	1
400bp	1	1	1	1	1	0	1	0	0	0	0
300bp	1	1	1	0	1	0	1	0	0	1	0
200bp	1	0	0	1	1	0	1	0	0	0	0
	0	0	0	1	0	0	1	0	0	0	0
100bp	1	0	0	0	0	0	0	0	0	0	0

Table 4: Allele and Polymorphism Information Content (PIC)

Marker	Present	Absent	Present Frequency	Absent Frequency	Allele Frequency	PIC
OPA1	6	12	0.33	0.66	0.99	0.44
OPA2	7	11	0.38	0.61	0.99	0.4753
OPA3	5	13	0.27	0.72	0.99	0.2907
OPE02	2	16	0.11	0.88	0.99	0.1975
A16	4	14	0.22	0.77	0.99	0.3457

Calculation of polymorphism frequency

$$\textbf{Frequency of polymorphism} = \frac{Total\ number\ of\ polymorphic\ bands}{Total\ number\ of\ bands\ (Polymorphic\ bands + Monomorphic\ bands)}$$

The polymorphism information content (PIC) was calculated using the online tool named Gene-Calc.

Discussion

Unrelated individuals would expect a pattern of similarities of 75% for super sister and 25% for half-sister in honey bees (Pamilo and Crozier 1982; Mortiz *et al.* 1991) [27, 25]. Genetic diversity in honey bee colony has drastic effect on behaviors of colony members (Mortiz, 1987) [24]. Genetic diversity in honey bee colony increases the fitness and production (Mattila and Seeley 2007) [20] and communication by dancing (Mattila *et al.*, 2008) [21]. Low gene variability in haplodiploid hymenopteran species than diploid insects is due to the small population size or stable condition in nests (Snyder, 1974; Metcalf *et al.*, 1975; Pamilo *et al.*, 1978b) [34, 22, 26]. Dawson *et al.*, in 1993 concluded that genetic diversity within and between populations *of H. sponlaneum*is associated with ecosystem factor.

Hunt and Page in 1992 [13] detected four types of polymorphism in haploid drone and diploid queen parents and its progeny of *Apis mellifera* colony and concluded that two RAPD markers for presence /absence of bands and for band brightness polymorphism show mendelian mode of inheritance (Dominance) but fragment length polymorphism and heteroduplex bands show co-dominance mode of inheritance. Paternity analysis was carried out in dragonflies by Hadry *et al.*, 1993 [9] and in *Apis mellifera* by Fondark *et al.*, 1993 [7]. In haplodiploid ants *Colobopsis nipponicus*

Hasegawa (1995) [11] observed 44.7% average polymorphism in workers and queen's sperm and concluded the father's information is important to identify the true parents- offspring relationship by RAPD. To study relatedness among and within the honey bee colony mitochondrial DNA diversity was studied in *Apis* species (Cornuet and Garney, 1991; Magnus *et al*., 2014) [4, 19] and in Screwworm by Infante and Espin 1995 [14].

Black *et al.,* (1992) [3] found lesser and greater genetic variation among *Aphid pisum* individuals of different colour by using different primers in RAPD. Macgowan *et al.,* (1993) [18] observed different banding pattern in *Listeria* species by different primers used in RAPD. For RAPD present study found OPA2 is suitable primers having sequence 5' TGCCGAGCTG 3' which produce detectable bands to study genetic polymorphism in honey bees.

Mestriner (1969) [23] reported only two biochemical polymorphisms in *Apis mellifera* suggesting reduce level of variability. While studying on paternity in social ants *Colobopsis nipponicus* Hasegawa (1995) [11] found 34.9% polymorphic bands in total 166 bands generated by 16 primers and concluded that 20 polymorphic bands are sufficient to detect parents-offspring relationship. Metcalf (1975) [22] found average frequency of polymorphic loci in seven hymenopterans species. Mortiz in 1987 [24] found variability in DNA size region from 250-1300 bp with 3 to 8 polymorphic bands in worker larvae of *Apis mellifera* while discriminating the super and half-sister in naturally mated queen. Present study observed the 24 bands after RAPD-amplification reaction off which five band were polymorphic.

Tunka and Kenec (2011) [38] found 0.033 to 0.175% mean genetic diversity value with percentage of polymorphic loci ranges between 37.14-64.76 in larvae of workers honey bee *Apis mellifera* collected from different parts of Turkey and concluded the importance of genetic diversity in management and conservation of insects. Lu and Rank (1996) [17] while studying on hymenopterans alfalfa leaf cutting bee, *Megachile rotundata*, used 16 primers to study the genetic parameters in haploid male and found 130 polymorphic and 31 monomorphic bands. They concluded hymenopteran have highest level of genetic diversity at DNA level (H= 0.32-0.35) oppose to the conclusions of lack or low heterozygosity in haplodiploid hymenopterans at enzyme level (Snyder 1974; Metcalf *et al.,* 1975; Pamilo *et al.,* 1978b) [34, 22, 26], at intrapopulation level (Ward 1980). In present study low level of polymorphism for presence and absence of RAPD bands between drone and worker honey bee was found. Present study found the 0.208 (20%) frequency of polymorphism and 0.34984 PIC value between drone and worker of *Apis cerana indica.* Present preliminary study concluded the RAPD markers are suitable tools to estimate the genetic polymorphism within the population of insect species.

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