Journal of Insect Science, (2018) 18(3): 3; 1–8
doi: 10.1093/jisesa/iey042
Research

Field Populations of Wild *Apis cerana* Honey Bees Exhibit Increased Genetic Diversity Under Pesticide Stress Along an Agricultural Intensification Gradient in Eastern India

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Subject Editor: Daniel Swale

Received 15 February 2018; Editorial decision 19 April 2018

Abstract

Pesticides have been reported to be one of the major drivers in the global pollinator losses. The large-scale decline in honey bees, an important pollinator group, has resulted in comprehensive studies on honey bee colonies. Lack of information on native wild pollinators has paved the way for this study, which highlights the underlying evolutionary changes occurring in the wild honey bee populations exposed to pesticides along an agricultural intensification landscape. The study reports an increased genetic diversity in native *Apis cerana* Fabricius (Hymenoptera: Apidae) populations continually exposed to pesticide stress. An increased heterozygosity, evidenced by a higher electrophoretic banding pattern, was observed in the pesticide-exposed populations for two isozymes involved with xenobiotic metabolism—esterase and glucose-6-phosphate dehydrogenase. Differential banding patterns also revealed a higher percentage of polymorphic loci, number of polymorphic bands, Nei's genetic distance, etc. observed in these populations in the Randomly Amplified Polymorphic DNA–Polymerase Chain Reaction (RAPD–PCR) experiments using three random decamer primers. Higher heterozygosity, being indicative of a more resistant population, implies population survival within the threshold pesticide stress. This study reports such changes for the first time in native wild Indian honey bee populations exposed to pesticides and has far-reaching implications on the population adaptability under pesticide stress.

Key words: agricultural intensification, wild population, genetic diversity, isozyme study, RAPD–PCR study

Increased pesticide stress has become a major global concern for the loss in important pollinator populations (Henry et al. 2012, Whitehorn et al. 2012, Chakrabarti et al. 2015a,b). Though these harsh chemicals are targeted towards pests, nontarget beneficial insects are often exposed to such toxicity and are relentlessly incapacitated due to the sublethal continual exposure (Desneux 2007). Foraging honey bees are not only directly exposed to these toxicants in fields (Colin et al. 2004) but residues of the pesticides may also be brought back to the hive and fed to the brood—thereby imposing an additional threat to the population (Chauzat et al. 2006, Claudianos et al. 2006).

Despite pesticide stress, some honey bee colonies do survive in the intensive agricultural field sites—even though in dwindling numbers. It is possible that they are able to cope with the environmental stressors by developing genetic heterozygosity. Metals, as well as organic and inorganic pollutants such as pesticides, are reported to cause physiological poisoning by becoming attached to or by being absorbed by the cellular components that may lead to various effects, including alterations of genetic system, that are related to inhibition or alteration of enzyme alleles (Dix 1981). Various studies have reported the effects of stress on flora and fauna such as in the pathogen *Candida albicans* (Forche et al. 2011), drought stress on oak (Vrancks et al. 2014) and metal stress on *Daphnia magna* (Hochmuth et al. 2014). Environmental stress has been regarded to be a major force in population evolution (Hoffmann and Hercus 2000). Assessing genetic diversity at both protein and DNA levels can prove to be beneficial for testing genetic diversity between populations. With this realization, the genetic diversity of the wild native *Apis cerana* L. Fabricius (Hymenoptera: Apidae) honey bee populations, exposed to pesticides in intensive agricultural landscape, was compared to their less intensive colony counterparts as a possible underlying reason for such survival. The studied landscape was chosen in Odisha, India, comprising a high-intensity cropping (HIC) area or high pesticide use site and a low-intensity cropping (LIC) area or the control site based on the previous studies reported from this laboratory (Chakrabarti et al. 2015a,b).

Evidence of isozymes variations (enzymes with similar catalytic properties but products of separate genes; Ferguson 1980) being adaptive in both plants and animals have gradually accumulated (Stone et al. 1968, Lewontin 1974, Nevo 1976). In the

electrophoretic system, lack of dominance at the protein phenotypic level (i.e., genotype is equal to phenotype), makes it an appropriate system for identifying all the homo and heterozygous gene products in the population (Ferguson 1980). Successful isozyme studies have been previously done on honey bees (Del Lama et al. 1988, Kandemir and Kence 1995) but only with respect to determining the interspecific variations. Enzyme polymorphism in honey bees has been functional in various developmental studies, population genetics studies and in honey bee classification (Hamrick 1989).

Different methods of DNA analyses have been fractionally studied to clarify the race characteristics of the local honey bees (Ivanova et al. 1998, Ivanova et al. 2010). For ecologists, estimation of genetic diversity and gene flow between species is assisted by studies on DNA markers which essentially provide the supportive information (Speight et al. 2005, Yogesh and Khan 2014). A greater level of polymorphism may be obtained by using DNA markers than by protein markers (Richardson et al. 1986). Randomly Amplified Polymorphic DNA (RAPD) markers can be a very efficient tool for checking genetic diversity among populations because they produce multiple bands fragments (Williams et al. 1993).

Drones in the honey bee colony exhibit a haploid chromosome set of 16 whereas the queen and workers (sterile females) contain a diploid chromosome set of 32 (Petrunkewitsch 1901) and a total size of about 180 megabase pairs (Jordan and Brosemer 1974). The haplo–diploid nature of honey bees facilitates RAPD analyses (Hunt and Page 1992). Numerous reports have previously cited differences in honey bees based on RAPD studies (Suazo et al. 1998, El-Bermawy et al. 2012, Yogesh and Khan 2014). However, meager studies on the genetic diversity of stress imposed populations and large information gap on the responses of native wild pollinators (Osborne 2012, Chakrabarti et al. 2015a) have paved the way for this study.

It was thus hypothesized that wild populations of pesticide-exposed native *A. cerana* honey bees will show a greater heterozygosity both at protein and DNA levels compared to the wild populations of the same surviving in less intensive/ pesticide-free zones. At the physiological level, heterozygotes are favored especially because energy demands needed to adapt to stress are high (Parsons 1996). Hence, the present study aims to look at the possible adaptive mechanism at play in these populations that are trying to survive under such pesticide imposed stress.

Material and Methods

Study Sites

Study sites and honey bee sampling protocols to check the genetic diversity in *A. cerana* populations, exposed to pesticides at DNA and protein levels, are based on the previous studies (Chakrabarti et al. 2015a,b). The field sites were chosen based on extensive farmer surveys, pesticide residue analyses results from soil and honey bee bodies, mean cropping intensity values of the agricultural plots and mapping the land use/ land coverage of the agricultural intensification plots as has been previously reported (Chakrabarti et al. 2015a,b). Two field sites were chosen, in the Indian state of Odisha, based on the degree of agricultural intensification—control or the LIC site and pesticide use or HIC site. Each HIC and LIC site comprised of three nodes (sampling locations) that were approximately 2 km apart from each other. As endosulfan was a major pesticide used in the state, the nodes reporting soil pesticide residue values for endosulfan ≥30 ppm were considered part of the HIC site. Use of more than three types of pesticides, and at quantities ≥10 ml of pesticides per 10 liters water, defined our selected HIC site. Adjacent areas of the Kuldiha Wildlife Sanctuary were chosen as the LIC site, where farming is usually restricted to subsistence home gardens and cropping is restricted to only rainy season. HIC area is predominantly the large-scale vegetable production hub in the state. When studying the landscape maps, an area of ≥1.5 square kilometers for agriculture, was considered a threshold for a node being designated as part of the HIC site. The honey bee colonies were present in the study sites for at least 1 yr prior to their sampling in January 2015.

Isozyme Studies to Determine Honey Bee Population Heterozygosity at Protein Level

Before checking the variations in between the control (LIC) and pesticide-exposed (HIC) honey bee populations from field sites, 10 random individuals from each of the three colonies from each site were checked for banding patterns. It was observed that for a particular site, all three colonies showed a similar banding pattern. However, the banding pattern differed between the LIC and HIC sites for each of the isozymes. One hundred individual honey bees each from LIC and HIC sites were used in the isozyme studies. α-esterase and glucose-6-phosphate dehydrogenase (G6PD) polymorphism was checked in natural populations of *A. cerana* honey bees.

Protein Preparation

Protein was extracted from the whole honey bee bodies. The individual honey bees were homogenized using a Dounce homogenizer (Sigma, United States) in 0.5 (M) Tris-HCl buffer. The homogenate was centrifuged for 15 min at 15,000 rpm at 4°C and the supernatant was collected for electrophoresis. Protein was extracted and isozymes were separated in native polyacrylamide gel electrophoresis as described by Karmakar et al. (2008). 7.5 and 5.5% gels were prepared for α-esterase and G6PD, respectively. Gels were stained for α-esterase and G6PD based on the protocols by Brewer and Singh (1970). Immediately after visualization the bands were washed in distilled water and photographed by Gel Documentation XR system and Quantity One software version 4.6.3 (Hercules, Bio-Rad, CA, USA).

Staining Protocol

The staining was done following the protocols of Brewer and Singh (1970) with slight modification.

α-Esterase

After completion of the electrophoresis, the gel was incubated in sodium phosphate buffer solution at room temperature, after which, α-naphthyl acetate, dissolved in acetone, was added to the gel in dark. Fast Blue RR salt was next added to the gel and the gel was constantly shaken in dark until dark brown to reddish brown colored bands of α-esterase was visible.

Glucose-6-Phosphate Dehydrogenase

20 mg of glucose-6-phosphate sodium salt, 10 mg of NADP and 2 ml of 0.5 (M) $MgCl_2.6H_2O$ were dissolved in 10 ml of 0.2 M Tris-HCl buffer (pH 8.0). Before staining, 50 mg Nitrotetrazolium blue, 50 mg Thiazolyl blue and 5 mg Phenazine methosulfate were added to it. The gel was incubated in this solution for at least 15 to 30 min in dark until blue bands of G6PD appeared.

Calculation of the Heterozygosity Index

The heterozygosity index was calculated based on the Hardy–Weinberg Equilibrium Theory (Karmakar et al. 2008). The slow-migrating allele was considered as p and the faster moving allele was considered as q. The frequency of slower allele was calculated by

the formula $f(p) = (2 * H_o + H_e) / 2 * N$ and the frequency of faster allele was deduced by the formula $f(q) = (2 * H_o + H_e) / 2 * N$. The heterozygosity index was assessed by the formula $H = 1 - (p^2 + q^2)$. In each, H_o = number of homozygotes, H_e = number of heterozygotes and N = total number of individuals.

Genetic Diversity of the Natural Honey Bee Populations at DNA Level Studied by Randomly Amplified Polymorphic DNA–Polymerase Chain Reaction (RAPD–PCR)

Like isozyme experiments, 10 random individuals from each of the three colonies from each site were checked for banding patterns before their comparative bands were checked by RAPD–PCR analyses based on the presence or absence of the amplified products.

DNA Isolation and Estimation From the Individual Honey Bees

DNA was extracted based on the method by Sambrook et al. (1989). The lysis buffer used was composed of 10 mM Tris (pH 8.0), 1 mM EDTA, 0.1 (M) NaCl, and 0.5 % SDS. The DNA concentration was quantitated at 260 nm using Shimadzu UV-1700 UV–VIS spectrophotometer.

PCRs Using RAPD Primers

For RAPD analysis, ten 10-*mer* random DNA oligonucleotide primers (IDT, San Diego, CA) of arbitrary sequences (Suazo et al. 1998, Yogesh and Khan 2014) were independently used in PCR reactions as described by Williams et al. (1990) of which three primers (Primers 1, 2 and 3) showed amplified products. Sequences of these primers are listed in Table 1.

Each 25 µl PCR reaction mix contained 250 ng DNA, 4 mM each of dATP, dCTP, dTTP and dGTP, 100 pico moles of primer, 1 unit of Taq DNA polymerase and 1× PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ and 0.001% gelatine). Amplifications were performed based on the protocols of El-Bermawy et al. (2012) in Eppendorf thermocycler (Eppendorf AG 6321, Hamburg, Germany) for 40 cycles of 30 s denaturation at 94°C, annealing at 21°C for 2 min and elongation of the complementary strand of the template DNA at 72°C for 3 min. One cycle of heating was given initially for 5 min at 94°C and one final cycle was given at 72°C for 15 min.

Gel Electrophoresis

1× Tris-Boric acid- EDTA buffer (TBE) was used for electrophoresis. A volume of 12 µl of the RAPD products were electrophoresed in 1.2% agarose gel stained with ethidium bromide (1% w/v) and run was performed at 100 V by LKB Pharmacia power supply for about 60 min. The bands were visualized on UV trans-illuminator (Bio-Rad) and photographed by Gel Documentation XR system and Quantity One software version 4.6.3 (Bio-Rad).

Table 1. The decamer primers used for RAPD studies in honey bees

Primer number	Primer sequence
1	CTTACGTCAC
2	CGGTTAGACG
3	CCCAACACAC
4	CGTTACATCC
5	CCCAACATCC
6	CTTACAGACG
7	CCTGAGGTCA
8	CAATGCCTTA
9	AATTGCAGGC
10	CCTGAGGCAG

Scoring of RAPD bands

For all three colonies in any particular site, the individuals showed a similar banding pattern which differed from the banding pattern of the individuals from the other site. The bands were marked as '1' or '0' indicating presence or absence of the band. The band intensity was not taken into consideration. The band sharing between the wild populations of HIC and LIC *A. cerana* honey bees were estimated. The dissimilarity index of the populations was calculated as the mean of all pair wise comparison of the banding pattern in the honey bees (Nei and Li 1979, Wetton et al. 1987, Kuhnlein et al. 1989) by the formula $S = 2\ N_{AB} \div (N_A + N_B)$ where, S = similarity index; N_{AB} = number of bands shared by A (HIC) and B (LIC) populations both; N_A = number of bands in population A (HIC); N_B = number of bands in population B (LIC). The dissimilarity index (D) was estimated by the formula $D = 1 - S$.

Fifty individual honey bees from each study site were used in these studies and the data were collected and analyzed. Nei's genetic distance, percentage of polymorphic loci, number of polymorphic loci and Nei's overall genetic diversity, H, was tested by Popgen32 version 1.31 (Chen et al. 2013) and GeneAlEx version 6.501 (Teixeira et al. 2014) software.

Results

Population Heterozygosity at Protein Level in Wild *A. cerana* Honey Bees Exposed to Pesticides

Polymorphism of α-Esterase Enzyme

A total of six zones were observed for the enzyme α-esterase (Fig. 1A). It was observed that zones I and II reflected heterozygosity in the HIC honey bees whereas, the honey bees from LIC, showed monomeric bands. Bands for zones III were observed to be monomeric for both the HIC and LIC honey bee individuals. Bands for zones IV, V, and VI were exclusively present in the HIC honey bees. HIC honey bees were showed a heterozygosity index of 0.5 each for zones I and II over the LIC honey bees. The overall population heterozygosity was observed to be 0.49 for α-esterase zone I and heterozygosity index of 0.375 for α-esterase zone II. The allele frequencies calculated for zone I and zone II are shown in Table 2 as calculated from the zymogram profiles of the electrophoretic pattern of esterase expression (Fig. 1B). The slow alleles are indicated as 's' bands and the fast-moving alleles are indicated as 'f' bands for each locus. Supplementary Fig. 1 entails the detailed calculations of the heterozygosity index.

Polymorphism of Glucose-6-Phosphatase (G6PD) Enzyme

Five zones were observed for G6PD (Fig. 2A). Zones I and II were observed to be monomeric for both LIC and HIC honey bees. Zone III was observed to be polymorphic in the HIC honey bees whereas, the LIC honey bees showed monomeric bands for zone III. Bands for zones IV and V were exclusively present only in the HIC honey bees. Overall population heterozygosity was observed to be 0.375 for G6PD zone III. HIC honey bees showed heterozygosity index of 0.5 for G6PD zone III over LIC honey bees. The allele frequencies calculated for zone III are shown in Table 2 as calculated from the zymogram profiles of the electrophoretic pattern of G6PD expression (Fig. 2B). The slow alleles are indicated as 's' bands and the

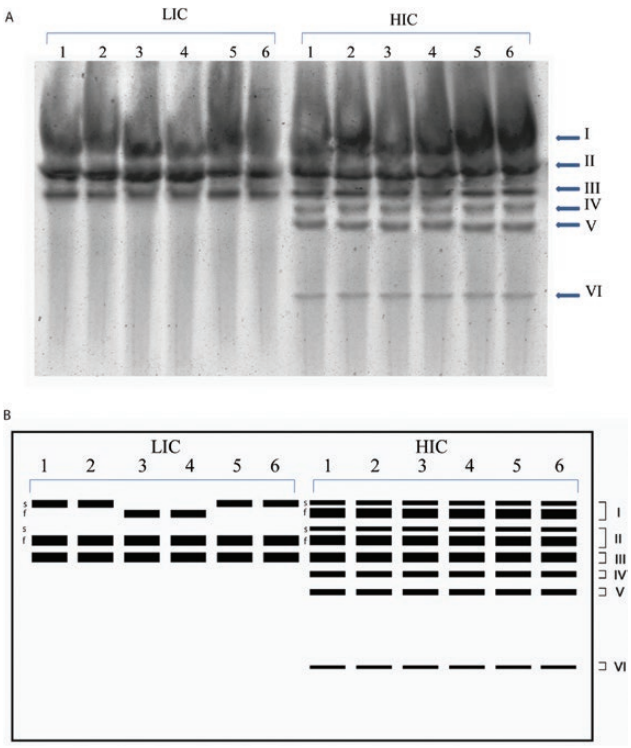

Fig. 1. (A) Representative α-esterase electrophoretic pattern observed in the LIC and HIC honey bees; (B) Zymogram of the comparative α-esterase isozyme profile. LIC: Low-intensity cropping site; HIC: High-intensity cropping site; s: slow-moving allele; f: fast-moving allele; I–VI: six observed zones; 1–6: honey bee individuals.

Table 2. The allele frequencies and heterozygosity index (H) from polymorphic zones of the two isozymes within the wild native honey bee populations

Isozyme		*f*(p)	p^2	*f*(q)	q^2	*H*
α–esterase	Zone I	0.58	0.3364	0.42	0.1764	0.49
	Zone II	0.25	0.0625	0.75	0.5625	0.375
G6PD	Zone III	0.25	0.0625	0.75	0.5625	0.375

fast-moving alleles are indicated as 'f' bands for each locus as has been done for α-esterase enzyme. The detailed calculations of the heterozygosity index have been incorporated in Supp. Fig. 2.

Genetic Diversity of Wild *A. cerana* Honey Bee Populations Exposed to Pesticides at DNA Level as Observed From the RAPD–PCR Studies

RAPD Results of Primer 1

Fifteen bands were amplified by Primer 1. The HIC honey bees were observed to have 15 bands compared to nine bands in LIC honey bees. Six additional unique bands were observed to be present in the HIC honey bees compared to the LIC honey bees and the dissimilarity index was observed to be 0.33 among the two populations (Fig. 3). Nei's genetic distance was observed to be 0.511. The percentage of polymorphic loci was calculated to be 40% and the number of polymorphic loci was 6. Nei's overall genetic diversity, H, was found to be 0.2.

RAPD Results of Primer 2

It was observed that 31 amplified products were present in HIC honey bees for Primer 2 including 16 unique bands (Fig. 4). The honey bees sampled from the LIC site were observed to have 14 bands of which

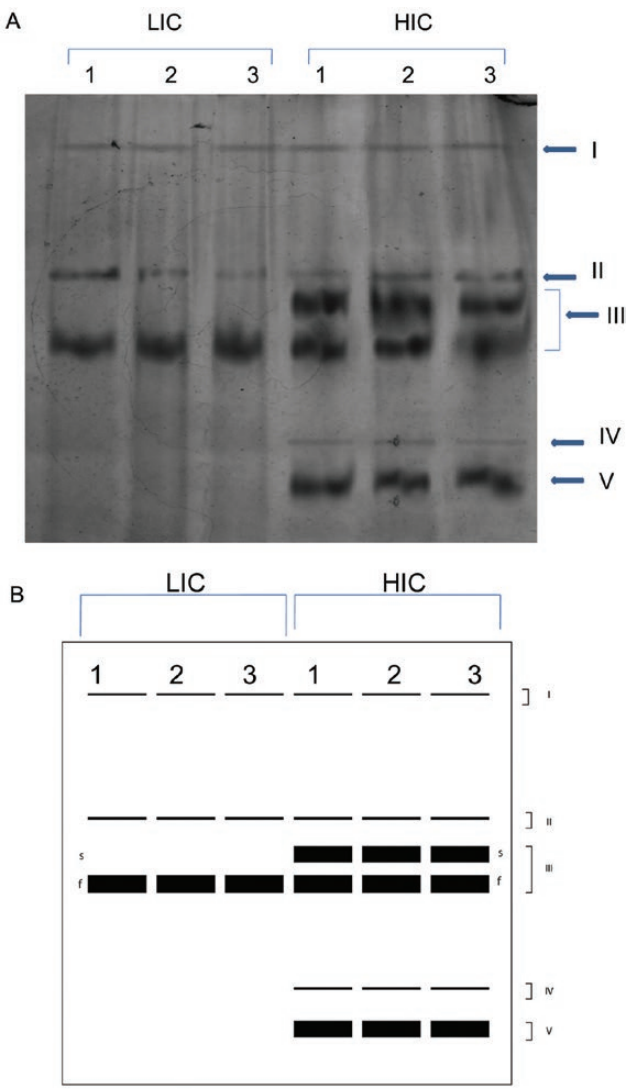

Fig. 2. (A) Representative G6PD electrophoretic pattern observed in the LIC and HIC honey bees; (B) Zymogram of the comparative G6PD isozyme profile. LIC: Low-intensity cropping site; HIC: High-intensity cropping site; s: slow-moving allele; f: fast-moving allele; I–V: five observed zones; 1–3: honey bee individuals.

1 was unique and was absent in the HIC honey bees. The dissimilarity index was calculated to be 0.45. Number of polymorphic loci was observed to be 18 and the percentage of polymorphic loci was found to be 58.06%. Nei's genetic distance was calculated to be 0.869 and H, Nei's overall genetic diversity was found to be 0.29.

RAPD Results of Primer 3

Similarly for primer 3, 14 bands were visualized in the HIC honey bees, of which 4 unique bands were absent in the LIC honey bees (Fig. 5). Only 10 bands were amplified in LIC honey bees. The dissimilarity index was observed to be 0.17. The number of polymorphic loci was observed to be 4 and 28.57 % of the loci was found to be polymorphic. Nei's genetic distance was observed to be 0.337 and H, Nei's overall genetic diversity was found to be 0.143.

Discussion

The effects of pesticides on honey bees may range from physiological alterations to behavioral and anatomical changes

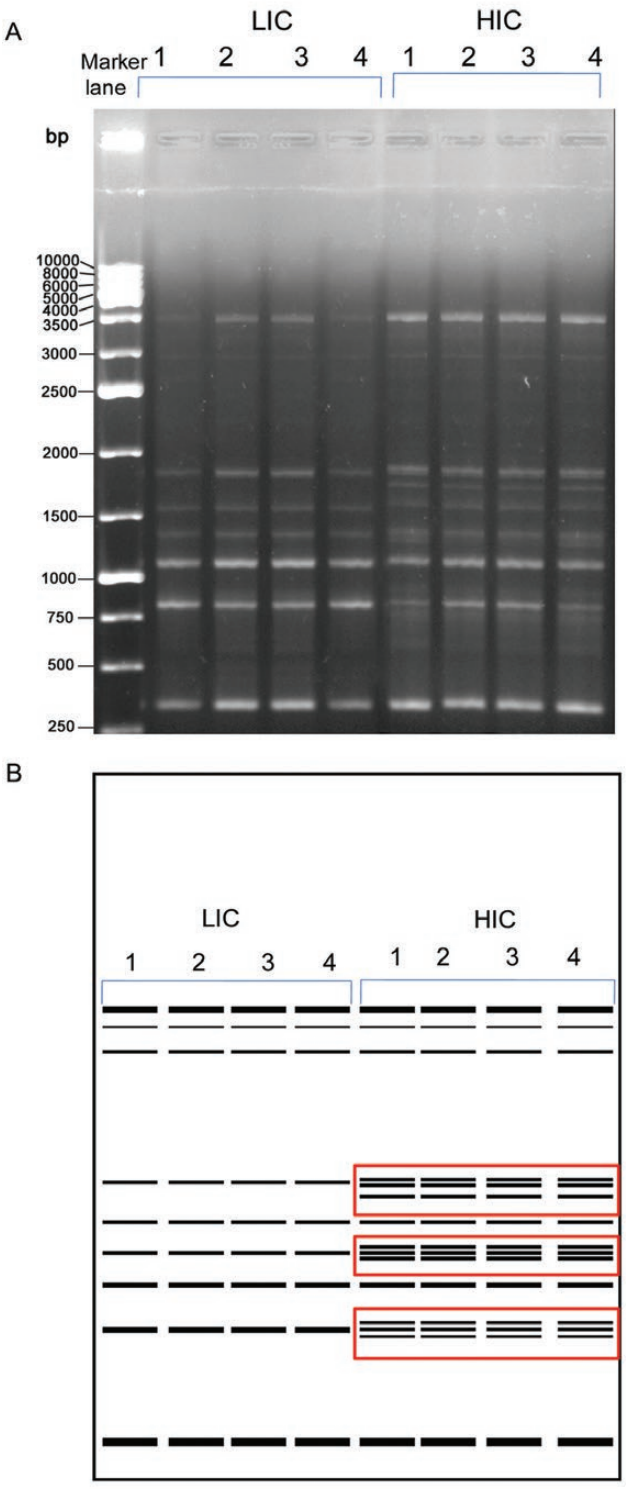

Fig. 3. (A) RAPD–PCR results of the representative LIC and HIC honey bees for Primer 1 on agarose gel; (B) Schematic representation of the banding pattern highlighting the regions where changes were observed. LIC: Low-intensity cropping site; HIC: High-intensity cropping site; 1–4: honey bee individuals.

(Chakrabarti et al. 2015a,b). The overall population heterozygosity is hence a determining factor to conclude the effects of such pesticide imposed stress. The analysis of genetic diversity and/or relatedness between or within species, population and individuals is a prerequisite to obtain information on individual identity (Ferguson 1980). The occupants living under various organic and inorganic stressors (e.g., pesticides) which are capable of altering its genetic system (Dix 1981) may eventually perish due to natural selection processes as a subsequent failure to adjust or adapt themselves for survival under such stress of lethal or sublethal doses of toxicants (Mount and Stephen 1967). An underlying natural selection process may occur, in which higher pesticide concentrations may select for individuals with greater ability to endure the deleterious effects of pesticide exposures, due to the heterozygosity of the alleles in question. Underlying this adaptive change of the participating organism is the differentiation of the molecular structure, reflected in the genetic diversity at the protein and DNA levels which drive the basic molecular mechanism of the organism (Nevo et al. 1987). In species with relatively short generation times (like honey bees) sudden shifts in allele frequencies may occur in populations (Hoffmann and Willi 2008). Changes in candidate loci responsible for adaptive responses and the DNA regions, which control their expressions, have been reported to be associated with environmental stressors such as pollution, global warming, etc. and these shifts may also occur in neutral genetic markers when there is a decline in such populations under stress (Hoffmann and Willi 2008). Their genetic variability thus would provide useful information on identity, relatedness and variations within populations under stress (Schierwater et al. 1995, El-Bermawy et al. 2012).

Esterases have varied functions in insects which include proteolysis, hormone and xenobiotic metabolism (Aldridge 1993). A greater capacity to detoxify insecticides, due to an increase in the expression or activity of three major enzyme families, also known as metabolic resistance, is one major resistance mechanisms in insects (Montella et al. 2012). Studies of insecticide resistance have signified the importance of esterases with respect to metabolism of xenobiotics in numerous insect species (Scharf et al. 1997). The additional presence of three zones in HIC honey bees with respect to α-esterase polymorphism may indicate activation of these alleles under stress conditions in the HIC honey bees. Also, the HIC honey bees exhibited a higher number of polymorphic bands compared to the LIC honey bees, further reinforcing the previous results.

Monooxygenases detoxified insecticides and increased G6PD, in turn, activates more monooxygenases in the body (Hemingway et al. 1998, Ganesh et al. 2003)—thus playing an important role in detoxification of insecticides. Hence, the presence of two additional G6PD zones in HIC honey bees may similarly indicate activation of these alleles under pesticide stress. The HIC honey bees also exhibited polymorphic bands in zone III compared to the presence of monomeric bands observed in zone III in the LIC honey bees.

RAPD technique, on the other hand, is a simpler and efficient technique for generating DNA polymorphism, using PCR amplification of the genomic DNA isolated from the tissues of living organism (Mullis et al. 1986, Welsh and McClelland 1990, Williams et al. 1990). This being a simple PCR-based technique can be used along with isozymes studies to discern the genetic diversity in natural populations of honey bees using single oligonucleotide primers of arbitrary sequences.

In the RAPD experiments, the HIC honey bees exhibited a more diverse banding pattern which was also reflected in the dissimilarity index values and the percentage of polymorphic loci for the HIC honey bees. Multiple bands fragments are produced through RAPD that are generated from different regions of the genome and hence multiple loci may be examined very quickly (Edwards 1998). The present study clearly showed a higher number of polymorphic bands in field populations of honey bees exposed to pesticides for all three primers. The band intensity was not taken into consideration because this may occur due to copy number differences (Lowe et al. 2004).

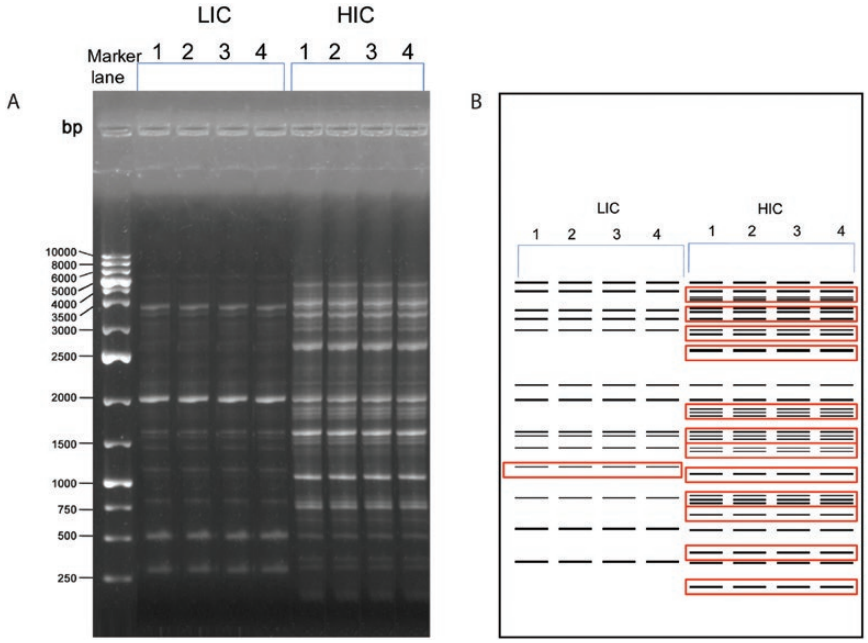

Fig. 4. (A) RAPD–PCR results of the representative LIC and HIC honey bees for Primer 2 on agarose gel; (B) Schematic representation of the banding pattern highlighting the regions where changes were observed. LIC: Low-intensity cropping site; HIC: High-intensity cropping site; 1–4: honey bee individuals.

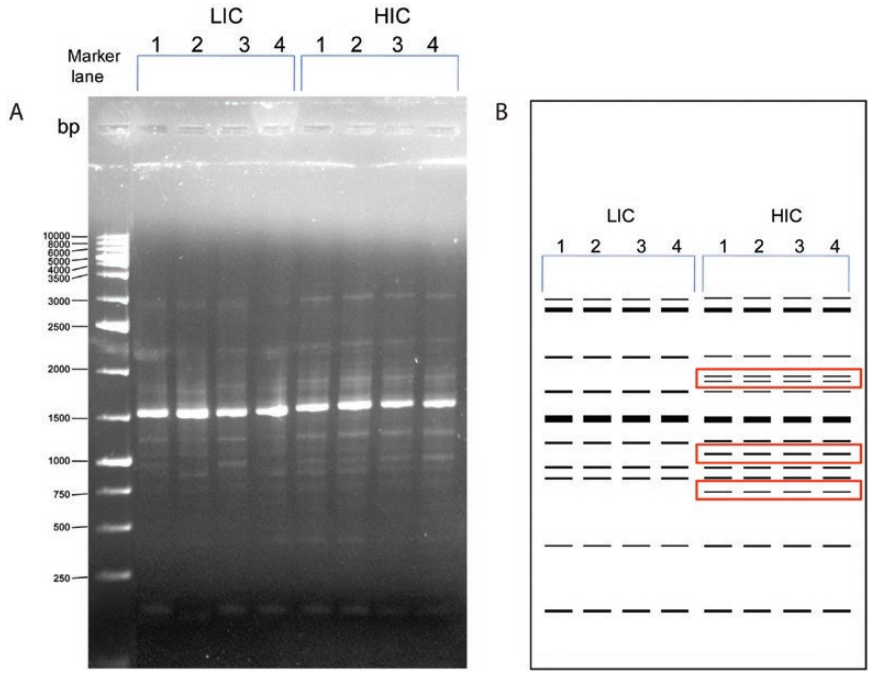

Fig. 5. (A) RAPD–PCR results of the representative LIC and HIC honey bees for Primer 3 on agarose gel; (B) Schematic representation of the banding pattern highlighting the regions where changes were observed. LIC: Low-intensity cropping site; HIC: High-intensity cropping site; 1–4: honey bee individuals.

Increased genetic diversity of the foragers within a honey bee colony may result in higher colony productivity and survival due to significant adaptive changes (Tarpy et al. 2013). It has also been reported that increased overall genetic diversity of honey bees will lead to healthier and hardier bees that can better fight off parasites, pathogens, and pests (Cobey et al. 2012). Thus the natural populations of honey bees, exhibiting a higher genetic diversity, may indicate underlying natural selection processes under such serious anthropogenically induced environmental threats.

This study reports for the first time any evidence for genetic heterozygosity in natural populations of native Indian honey bees exposed to pesticides. Previous studies have reported the stress imposed on these populations due to sublethal exposure to pesticides (Chakrabarti et al. 2015a,b) and this study may additionally indicate underlying adaptive processes under such stress. However, further studies are required to look into this aspect in the future. The study thus suggests a higher polymorphism in the pesticide-exposed wild populations of *A. cerana* honey bees with respect to isozymes

and DNA polymorphism analyses. This is indicative of a possible adaptive mechanism in a pesticide-laden environment and would justify the persistence of honey bee colonies in such an environment. However, it must be mentioned that beyond a threshold limit, the population would inevitably succumb to the pesticide stress and an absolute loss, i.e., crash in the colony is unavoidable.

It may be argued that the colonies might show similar patterns if they are genetically related. Although it cannot be totally ruled out, the probability of genetic mixing between colonies can be assumed to very little since the sampling locations (each node) were at least 2 km apart. This is well beyond the swarming range of *A. cerana* reproductives, which is usually less than 1 km (Koetz 2013).

Increased agricultural intensification, especially in the developing countries, has resulted in a surge of pesticide use (Schreinemachers and Tipraqsa 2012). An immediate estimation is required of the native honey bee responses to such elevated pesticide exposures and would be significant to assess such effects on the nontarget beneficial pollinators. It is in this context that the present study holds added importance. The challenge lies in formulating strict pesticide regulations to monitor the indiscriminate use of pesticides in agricultural fields. Loss of beneficial pollinators including the honey bees will have serious adverse impacts not only on the environment but also on the agricultural productivity (Kremen et al. 2002), thereby seriously affecting the global livelihoods of small and marginal farming communities.

This study observes that honey bees in the HIC area exhibit polymorphism with respect to the alleles in question. Since we do not have data to show that the honey bees in the HIC area began (at some time before the high intensity of cropping) as homozygous populations, and then progressed to heterozygous populations, we cannot definitively infer that exposure to pesticides causes genetic diversification of these genes. However, we may suggest that this process is occurring, as higher concentrations of pesticides would act as a selection force creating for better stress tolerant heterozygotes. Further investigations are warranted, in which two genetically similar populations are separated and placed in two different pesticide intensity sites, with one being exposed to HIC conditions and one group exposed to LIC conditions, and in which the changes in genetic diversity from the beginning of exposure period to the end of the exposure period are studied. In addition, further studies are also required over multiple high- and low-intensity sites in other geographical regions of the country.

Supplementary Data

Supplementary Data are available at *Journal of Insect Science* online.

Acknowledgments

The study was supported by an INSPIRE fellowship from the Department of Science & Technology, Government of India to P.C. (DST/INSPIRE Fellowship/2011/166 B); the Department for Environment, Food and Rural Affairs (DEFRA), Government of UK Darwin Initiative grant (1662) to P.B. and a research grant (37(1616)/13/EMR-II dated 07 October 2013) to P.B. and S.S. from the Council of Scientific and Industrial Research (CSIR), Government of India.

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