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Genetic variability in Indian populations of banana corm weevil [*Cosmopolites sordidus* (Coleoptera: Curculionidae)] assessed by RAPDs and AFLPs

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Abstract. *Cosmopolites sordidus* (Germar), commonly known as banana corm weevil, is an important economic pest in Asia that can cause severe yield loss depending upon the stage at which infestation occurs. In spite of its economic importance, little is known about the population structure of this pest in India. Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) were used to characterize the population genetic structure of *C. sordidus* collected from five hot spot locations in India. Nineteen RAPD primers and five selective AFLP primer combinations generated 147 and 304 amplification products, respectively. UPGMA dendrograms generated with both marker systems failed to reveal populations clustered based on geographic distance, which was confirmed by the Mantel test, which did not show a strong correlation between genetic distance and geographic distance. Values of indices of genetic identity showed that the populations were closely related. Though the gene flow estimate (Nm) between the populations was 0.469, suggesting restricted gene flow, the populations are not genetically distinct. These observations suggest that the range expansion of this banana pest in India has taken place through transport of infested corms and plant material, resulting in genetically close populations that are geographically distinct. These results provide important information on the population structure of this pest in India, which will aid in designing suitable strategies for its control and management, especially with respect to insecticide resistance.

Keywords: *Cosmopolites sordidus*, RAPDs, AFLPs, genetic diversity, restricted gene flow

Introduction

Banana is the fourth most important crop in the developing world, and India is the world's largest producer of banana and plantain, accounting for almost one-fifth of global production (Padmanaban *et al.*, 2001). In India, the banana rhizome weevil/banana corm weevil, i.e. *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), is the most serious pest of banana (Ostmark, 1974; Rukazambuga *et al.*, 1998). This weevil belongs to the sub-family Rhynchophorinae, an economically important sub-family that includes pests of stored grains and other members that damage soft-trunked plants, such as aloe, banana, palm and sisal. The weevil is specific to *Musa* (banana and plantain), as well as to *Ensete* (Gold *et al.*, 2001), and is distributed

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in all the world's major banana growing areas (Ostmark, 1974). The adults, as well as larvae, cause severe damage affecting the production of banana in several tropical and sub-tropical regions (Ostmark, 1974; Gold *et al.*, 2001; Kiggundu *et al.*, 2003). Almost 100% loss of the banana crop can occur depending on the stage of plant growth at which pest infestation occurs, and also the efficiency of the management and cultivation practices followed (Rukazambuga *et al.*, 1998; Gold *et al.*, 1999*a*; 1999*b*; 2001; 2004). Use of conventional breeding in banana to transfer resistance is restricted, because of parthenocarpy (non-seeded nature and male/female sterility) of the banana plants. Organophosphorus insecticides, such as prothiofos, chlorpyrifos, pirimiphos-ethyl and ethoprophos, are applied to the corm, since the larvae and adults are cryptic in habit. Use of these insecticides gives rise to several environmental issues and has also resulted in the emergence of insecticide-resistant weevil strains (Collins *et al.*, 1991; Gold *et al.*, 1999*a*).

A broad integrated pest management (IPM) strategy would provide the best strategy for the successful control of this pest. Important components of a successful IPM programme would include identifying host plant resistance, cultural control, biological control and a study of the population structure of the pest. Important factors that contribute to the population structure of a pest species include genetic diversity, gene flow, migration, dispersal ability, population size, and life history. In spite of *C. sordidus* being a major pest of banana in India, to date, there have been no studies to assess the genetic diversity of Indian populations of the pest. In the present work, the genetic diversity within and between five populations of *C. sordidus* was assessed using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) analyses, because these molecular approaches require no prior sequence information.

RAPD and AFLP are PCR-based DNA fingerprinting techniques that have been used to study genetic diversity in different organisms. RAPD primers generate amplification products by annealing to homologous target sites on the template DNA, which are randomly distributed in the genome (Williams *et al.*, 1990; Welsh and McClelland, 1990). AFLP is based on selective PCR amplification of restriction fragments from a total genomic DNA digest. As dominant markers, co-migrating RAPD and AFLP amplification products need not necessarily represent the same locus or alleles. RAPDs require stringent standardization of protocols, to ensure reproducibility (Black, 1993). AFLP detects polymorphism at several hundreds of independent loci. Both these markers require no prior sequence information and have the potential to assess genome-wide variation. A few examples of their use in genetic diversity studies in insects are summarized in Tables 1a and 1b.

Assessment of the genetic variation between the five Indian populations of *C. sordidus* would help to determine their population structure, the level of gene flow between them, and whether or not they are genetically distinct. Such information is vital to draw conclusions about dispersal of banana corm weevil, since dispersal of the pest is an important factor to be considered while designing and implementing management programmes for its effective control. Knowledge about such aspects is important for the success of pest management practices, especially to understand the occurrence of resistance to insecticides and to devise suitable control strategies to prevent its spread (Aylor and Irwin, 1999; Byrne, 1999).

Materials and methods

Sample collection

A total of 29 adult weevils of *C. sordidus* were collected from infested banana corms in banana fields from each of the following five locations: Kamrup (Assam), Calicut (Kerala), Narayangaon (Maharashtra), Nanjangud (Karnataka) and Trichy (Tamil Nadu) (Fig. 1). In each location, the individuals were collected within an area of 500 m^2.

DNA extraction

Total genomic DNA was extracted from individual beetles using the protocol of Dellaporta *et al.* (1983) with modifications as follows: The entire beetle was crushed to a fine powder in liquid nitrogen using a mortar and pestle. The powder was suspended for 15 min at 65 °C in pre-warmed extraction buffer [100 mM Tris (pH8), 50 mM EDTA, 500 mM NaCl, 1.25% SDS, 10 mM β-mercaptoethanol]. This was followed by the addition of 400 µl of chilled 5M potassium acetate per ml of the suspension. The suspension was then kept on ice for 1 h followed by centrifugation at 10,000 rpm for 10 min at 10 °C. An equal volume of Sevag's phase [chloroform and isoamyl alcohol (24:1)] was added to the supernatant followed by centrifugation at 10,000 rpm for 10 min at 10 °C. The DNA was precipitated from the aqueous layer by the addition of 750 µl of iso-propyl alcohol per ml of the aqueous layer. The tubes were kept overnight at room temperature to allow the DNA to precipitate. The precipitated DNA was collected by centrifugation at 10,000 rpm for 10 min at 10 °C and given a wash with 70% chilled alcohol. The DNA was air dried and dissolved in $T_{10}E_1$ buffer (pH 8.0). RNase treatment (50 µg/ml) was given to the

Table 1a. Examples of insect pests where RAPDs have proved useful in genetic diversity studies

Name of the insect pest	Study	Reference
Ceratitis capitata	Detection of geographical origin of the populations in Spain	Reyes and Ochando (1998)
Scirpophaga incertulas (Walker)	Study of genetic diversity of Indian populations	Kumar *et al*. (2001*a*; 2001*b*)
Leptinotarsa decemlineata	Determination of microevolutionary factors affecting the genetic structure of Colorado potato beetle from Kiev, Ukraine	Sidorenko and Berezovska (2002)
Anthonomus grandis (Boheman)	(a) Inferring the magnitude and pattern of genetic differentiation among boll weevil populations from eight US states and north east Mexico; (b) Distinguishing populations from large-scale intensive farming from areas of small-scale farming	Kim and Sappington (2004) Martins *et al.* (2007)
Haematobia irritans	Study of the genetic variability within and between one population originating from America and four from Brazil	Castiglioni and de Campos Bicudo (2005)
Rhynchophorus ferrugineus (Olivier)	Distinguishing populations of red palm weevil	Gadelhak and Enan (2005)
Phlebotomus sergenti (Parrot)	Differentiating specimens according to geographical origin	Dvorak *et al*. (2011)
Psyttalia concolor	Ability to reveal the Mediterranean population structure and development of potential markers to monitor performance in biological control programmes	Karam *et al.* (2008)
Metopolophium dirhodum (Walker)	Ability to reveal existence of host races within *M. dirhodum*	Lopes-da-Silva and Vieira (2007)
Cosmopolites sordidus (Germar)	Ability to reveal existence of banana weevil biotypes	Ochieng (2001), Gold *et al.* (2001), Ochieng *et al.* (2002), Magaña *et al.* (2007)
Ostrinia nubilalis	Ability to reveal gene flow between populations	Krumm *et al.* (2008)
Lac insects (Genus: Kerria)	Study of genetic diversity in Indian lac insects	Ranjan *et al.* (2011)

Table 1b. Examples of insect pests where AFLPs have proved useful in genetic diversity studies

Name of the insect	Study	Reference
Spodoptera frugiperda (J. E. Smith)	(a) Ability to distinguish two host-associated strains in combination with other markers; (b) Detection of minor gene flow among 23 populations from Mexico, Continental US, Brazil, Argentina, and Puerto Rico, suggesting that *S. frugiperda* in the western hemisphere is an interbreeding population	McMichael and Prowell (1999), Clark *et al*. (2007)
Lymantria dispar Linnaeus	Genetic variability between European gypsy moth populations based on their geographic origin	Reineke *et al.* (1999)
Tuta absoluta (Meyrick)	Distinguishing tomato leafminer populations based on their response to insecticides and host plants	Suinaga *et al.* (2004)
Bemisia tabaci (Gennadius)	Grouping of biotypes	Cervera *et al.* (2000)
Orseolia oryzae (Wood Mason)	Revealing the origin of gall midge biotypes; detecting sexual dimorphism; differentiation from its major parasite *Platygaster oryzae*	Katiyar *et al.* (2000)
Meligethes aeneus	Low level of gene flow among 14 populations from six European countries and their clustering by origin	Kazachkova *et al.* (2008)
Grapholita molesta	Low level of dispersal among fruit moth populations from six regions in South Africa	Timm *et al.* (2008)
Glossina morsitans morsitans	Distinction between laboratory and field populations of tsetse flies	Lall *et al.* (2010)
Cosmopolites sordidus (Germar)	Identification of biotypes	de Graaf (2006)

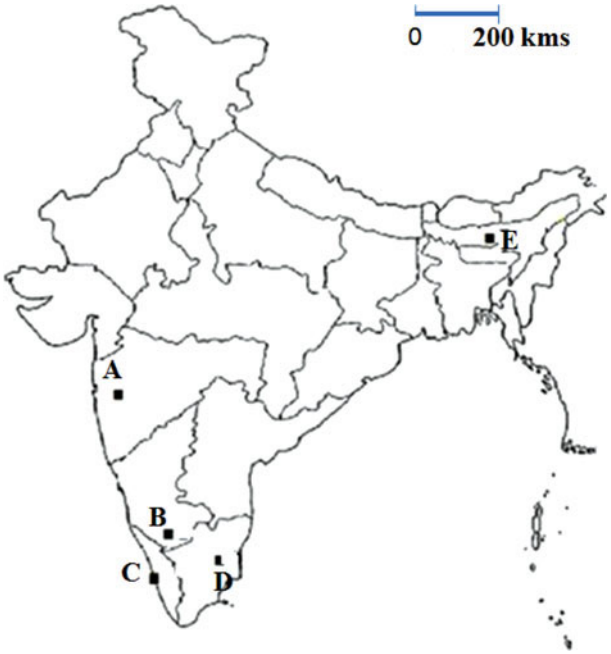

Fig. 1. Geographic distribution of the populations of *Cosmopolites sordidus* (Germar) used in the present study. [On the map, the solid squares refer to the collection sites. In the key below, the states are shown in parentheses and the geographical coordinates are shown in square brackets. n = the number of individuals used for RAPD analysis.]
A – Narayangaon (Maharashtra) [19°7′22.45″N; 73°58′37.65″ E] (n = 6)
B – Nanjangud (Karnataka) [12°7′N; 76°44′E] (n = 6)
C – Calicut (Kerala) [11°14′52″ N; 75°46′49″E] (n = 6)
D – Trichy (Tamil Nadu) [19°53′35.28″ N; 73°43′52.63″ E] (n = 6)
E – Kamrup (Assam) [26°18′57.89″ N; 91°35′54.22″ E] (n = 5)].

DNA solution and the solution was deproteinized with equal volumes of phenol, chloroform: isoamyl alcohol mix and precipitated as described above. The DNA was dissolved in $T_{10}E_1$ (pH 8.0). The concentration of the extracted DNA was determined by agarose gel electrophoresis using λ DNA of known concentration.

RAPD-PCR amplification

A total of 68 decanucleotide RAPD primers of the series A, B, G (20 primers per series) and E (8 primers) obtained from Operon Technologies Inc. (Alameda, CA, USA) were initially screened for identifying primers that would give clear amplification products. Of these primers, 19 RAPD primers gave clear amplification patterns and hence were selected for inter- and intra-population analyses (Table 2). RAPD-PCR was standardized with respect to the concentration of DNA, RAPD primer, temperature of annealing, Taq DNA Polymerase and spermidine concentration. The PCR reactions were carried out in a total volume of 25 µl containing 25 ng of genomic DNA, 0.1 mM dNTPs, 1 µM of primer, 1X Taq buffer, 1.5 U Taq DNA Polymerase (Bangalore Genei, India), and 0.1 mM/0.4 mM spermidine. The PCR cycle conditions for RAPD-PCR included an initial denaturation at 94 °C for 5 min followed by 35 cycles each of a denaturation step at 94 °C for 30 s; annealing at 32 to 40 °C (depending upon the primer) for 1 min; extension at 72 °C for 2 min followed by a final extension at 72 °C for 5 min. The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml).

Amplified fragment length polymorphism (AFLP) analysis

The AFLP protocol consists of the restriction of the DNA, ligation of the restricted DNA with adapters and a two-step amplification (pre-amplification and selective amplification) (Vos *et al.*, 1995). AFLP analysis was performed on pooled DNA from 10 Kamrup individuals and 20 individuals from each of the other populations. An initial screening using 60 pre-selective primer combinations was performed on the pooled DNA from the five locations. The following five selective primer combinations (*Eco*RI–*Mse*I) with selective nucleotides, which gave clear and reproducible electrophoretic patterns and showed variation within and between populations, were chosen for further analyses: *EcoRI*-AA + *MseI*-AA, *EcoRI*-AA + *MseI*-CC, *EcoRI*-AA+ *MseI*-CG, *EcoRI*-AA + *MseI*-CT and *EcoRI*-AA + *MseI* CA (Table 3). Selective amplification products were separated with an Applied Biosystems automated sequencer. Fragments were scored automatically by the program Genemapper v3.7. For each combination, all loci showing a clear and unambiguous banding pattern were scored, whereas uncertain peaks were considered as missing data.

Data scoring and analysis

A binary matrix was generated for the RAPD and AFLP data by scoring the presence of clear and unambiguous bands across the DNA samples at a particular locus as 1 and the absence of the band as 0. For each individual (for RAPDs) and each population (for AFLPs), the resolving power of the primer (Rp), polymorphic information content (PIC), effective multiplex ratio (EMR) and marker index (MI) values were calculated as follows: (i) Band informativeness (Ib) $= 1-(2 \times \left|0.5-p\right|)$, where p is the proportion of genotypes having

Table 2. Indices describing the polymorphism in the five populations of *Cosmopolites sordidus* (Germar) revealed by the 19 RAPD primers

RAPD primers	Spermidine concentration	Annealing temperature	No. of bands studied	Range of fragment sizes amplified (bp)	Resolving power (Rp)	PIC	EMR	MI
OPA-05	–	32 °C	4	400–650	2.827	0.313	2.12	0.663
OPA-12	0.1 mM	33 °C	11	300–1340	5.757	0.636	3.65	2.32
OPA-20	0.1 mM	33 °C	9	300–1040	4.93	0.355	3.83	1.35
OPB-05	–	34 °C	11	200–1000	6.37	0.311	4.63	1.44
OPB-08	0.1 mM	32 °C	7	400–950	4.34	0.285	3.1	0.883
OPB-19	0.1 mM	32 °C	6	350–930	3.128	0.295	2.61	0.77
OPE-04	–	40 °C	11	150–1000	7.344	0.248	6.67	1.65
OPE-06	–	40 °C	12	280–1630	6.274	0.21	4.7	0.988
OPE-08	–	40 °C	8	350–1220	5.242	0.262	4.29	1.12
OPE-11	–	40 °C	7	400–1200	4.034	0.225	2.88	0.648
OPE-13	–	40 °C	8	300–1150	4.793	0.237	3.59	0.851
OPG-06	–	34 °C	5	390–990	3.345	0.156	2.0	0.314
OPG-08	0.1 mM	33 °C	8	350–950	5.24	0.294	3.93	1.15
OPG-09	–	34 °C	9	300–1150	4.793	0.308	3.75	1.15
OPG-11	0.4 mM	33 °C	6	340–1180	4.586	0.244	2.29	0.559
OPG-12	0.1 mM	34 °C	4	250–850	2.896	0.28	2.17	0.61
OPG-14	0.1 mM	34 °C	7	250–900	4.069	0.214	2.32	0.496
OPG-15	0.1 mM	35 °C	7	250–1100	5.274	0.265	3.79	1.004
OPG-16	0.1 mM	34 °C	7	400–900	4.137	0.336	3.54	1.19
Average					4.704	0.288	3.466	1.008

Table 3. Indices describing the polymorphism in the five populations of *Cosmopolites sordidus* (Germar) revealed by AFLPs

Primer pair + SelectiveExt		No. of bands	Fragment size	Resolving			
EcoR-I	*Mse-I*	studied	range (bp)	power (Rp)	PIC	EMR	MI
AA-JOE	AA	51	66–278	18	0.429	0.567	0.243
AA-JOE	CT	62	60–278	17.6	0.375	0.688	0.258
AA-JOE	CC	56	71–372	10	0.350	0.474	0.165
AA-JOE	CG	67	60–427	24	0.384	0.744	0.286
AA-JOE	CA	68	66–180	11.6	0.284	0.531	0.151
Average		304		16.24	0.364	0.600	0.221

the band. Rp of a primer is the sum of the band informativeness of all the bands amplified by that primer, i.e. Rp = ΣIb (Prevost and Wilkinson, 1999; Guasmi *et al.*, 2012); (ii) PIC for each locus = 2fi (1−fi), where fi is the frequency of the genotypes having the band (Anderson *et al.*, 1993). The PIC of a primer is the average of the PIC values of all the loci amplified by that primer; (iii) EMR is defined as the product n × β, where n = average number of bands detected per genotype for that marker and $\beta = n_p/(n_p + n_{np})$, where n_p = number of polymorphic loci and n_{np} is the number of non-polymorphic loci; (iv) Marker Index (MI) is a parameter that determines the utility of the marker for distinguishing the different genotypes (Powell *et al.*, 1996; Archak *et al.*, 2003); (v) expected heterozygosity (Hn) for a genetic marker was calculated from the sum of the squares of allele frequencies as Hn = 1– (p^2+ q^2), where p is the frequency of individuals having the allele and q is the frequency of individuals lacking the allele. The arithmetic mean heterozygosity Hav for RAPDs was calculated as the arithmetic mean heterozygosity Hav, i.e. Hav = Σ*Hn*/*n*, where n is the number of markers or loci analyzed (Powell *et al.*, 1996). The binary matrix was analyzed by POPGENE v 1.3 using a dominant marker data set assuming Hardy–Weinberg equilibrium for estimating diversity parameters, such as the percentage of polymorphism, heterozygosity (H), number of alleles (Na), number of effective alleles (Ne), Shannon's information index (I), coefficient of gene differentiation (G_{st}) and gene flow (N*m*) (Nei, 1973, 1978; Nevo, 1978; Yeh *et al.*, 1999). The coefficient of gene differentiation, i.e. G_{ST} values were estimated for the RAPDs data set. Gene flow was estimated from G_{ST} values and expressed as $Nm = 0.5\ (1-G_{ST})/G_{ST}$ (McDermott and McDonald, 1993). Geographical distances of

Table 4. Gene diversity indices among *Cosmopolites sordidus* (Germar) populations from five locations in India based on RAPD analysis

Population	No. of alleles (Na)	No. of effective alleles (Ne)	Number of polymorphic loci	Proportion of polymorphic loci (%)	Shannon's Information index (I)	Gene diversity of the single population (Hs)
Kamrup	1.2381	1.1582	35	23.81	0.1368	0.0925
Calicut	1.3401	1.2405	50	34.01	0.2001	0.1368
Nanjangud	1.2925	1.2207	43	29.25	0.1778	0.1228
Narayangaon	1.3061	1.2105	45	30.61	0.1772	0.1206
Trichy	1.415	1.2569	61	41.50	0.2299	0.1538

Table 5. Genetic distance (below diagonal) and genetic identity (above diagonal) indices[+] for the five *Cosmopolites sordidus* (Germar) populations estimated using RAPDs

Population	Kamrup	Calicut	Nanjangud	Narayangaon	Trichy
Kamrup	–	0.8812	**0.7453**[+]	0.7485	0.8489
Calicut	0.1264	–	0.7581	0.7647	0.9176
Nanjangud	**0.2940**[+]	0.2770	–	**0.9178**[+]	0.7563
Narayangaon	0.2896	0.2682	**0.0858**[+]	–	0.7641
Trichy	0.1638	0.0860	0.2793	0.2690	–

[+]The highest and lowest genetic distances and identities are indicated in bold.

pairs of populations for the Mantel test were calculated using the latitude and longitude of each population. The Mantel test was used to find out the correlation between geographical distances and genetic distances (Mantel, 1967). The Principal Co-ordinate Analysis (PCA) and Analysis of Molecular Variance (AMOVA) were carried out using the GenAlEx software v 6.1 (Peakall and Smouse, 2006). For AMOVA analysis, total variance of the RAPD data set was partitioned at three hierarchical levels: (1) an among-population component; (2) a regional or five-population component; and (3) a within-population component.

Results

RAPD analysis

The 19 selected RAPD primers amplified a total of 147 bands ranging in size from 150 bp to 1630 bp, of which 111 (75.5 %) were polymorphic and 36 (24.4 %) were monomorphic (Table 2). The number of amplified bands ranged from 4 to 12 with primer OPE-06 giving the maximum of 12 bands and primers OPA-05 and OPG-12 giving the minimum number of 4 bands. Rp values ranged from 2.8 (OPA-05) to 7.3 (OPE-04) with an average of 4.704. The average PIC, EMR and MI values were 0.288, 3.466 and 1.008, respectively (Table 2). OPE 04 amplified a band of 150 bp only in the individuals from Kamrup and this band was absent in the other populations. Population-specific bands were not amplified by the other 18 primers.

Results of the population diversity analysis using POPGENE are summarized in Table 4. The average number of observed alleles varied from 1.23 in the Kamrup population to 1.41 in the Trichy population, while the effective number of alleles varied from 1.16 (Kamrup) to 1.25 (Trichy). The intra-population genetic diversity was highest for the Trichy population (0.1538) and lowest for the Kamrup population (0.0925) with an average gene diversity within sub-populations (Hs) of 0.1253 (Table 4). The total genetic diversity (Ht) was 0.258. Shannon's information index varied from 0.1368 (Kamrup) to 0.2299 (Trichy). The percentage polymorphism varied from 23.81% (Kamrup) to 41.50% (Trichy), with an overall average polymorphism of 31.84% among the five populations.

Pair-wise comparison of the populations showed that the genetic distance varied from 0.0858 between Narayangaon and Nanjangud to 0.2940 between Nanjangud and Kamrup (Table 5), suggesting that these populations were closely related. The total genetic differentiation coefficient (G_{ST}) among the populations was 0.515 and the gene flow estimate (Nm) among the populations was 0.469, suggesting little evidence of population sub-structure, because gene flow is considered significantly high when $Nm > 1$ (Table 6). AMOVA analysis and a PhiPT value of 0.493, which was significant at a probability of 0.001, revealed that of the total genetic diversity, 51% variance occurred within populations and 49% variance occurred among populations (Table 7).

An UPGMA dendrogram generated based on the distance matrix expressed as similarity coefficients clustered all individuals into two major groups

Table 6. Comparison of different polymorphism and gene diversity indices of the RAPDs and AFLPs marker systems in the five populations of *Cosmopolites sordidus* (Germar)

Parameter	RAPDs	AFLPs	*P* value of Student's 't' test
Total number of bands	147	304	
Total number of polymorphic bands	111	135	
% polymorphic bands	75.5	89.3	
Average number of bands per primer	7.73	60.8	
Average number of polymorphic bands per primer	5.84	27.6	
Rp	4.74	16.24	
Shannon's Information Index	0.387	–	
Total Heterozygosity (Ht)	0.258	–	
Average Heterozygosity (Hs)	0.124	–	
G_{ST}	0.515	–	
Gene Flow (Nm)	0.469	–	
PIC	0.288	0.364	ns
EMR	3.46	0.600	ns
MI	1.008*	0.221	0.0013*

*Significant; $P = 0.001$; ns = non-significant.

Table 7. Hierarchical analysis of molecular variance (AMOVA) for the five populations of *Cosmopolites sordidus* (Germar)

Source	df	SS	MS	Est. Var.	%
Among Pops	4	297.741	74.435	10.911	49
Within Pops	24	269.501	11.229	11.229	51
Total	28	567.241		22.140	100

PhiPT value = 0.493 ($P = 0.001$).

(Fig. 2). Within each group individuals from each location formed a sub-cluster. Group I had individuals from Calicut, Kamrup and Trichy, while group II clustered individuals from Nanjangud and Narayangaon. The UPGMA dendrograms did not reveal populations clustered solely based on geographic distance, i.e. populations that were situated close to each other geographically were not necessarily closely related genetically. For example, the populations of the Trichy and Calicut group were in the same cluster, which is to be expected as they are close to each other in geographical space. However, the Kamrup population is also included in this cluster. The PCA ordination based on the first two components (factors) confirmed cluster analysis with the Eigen values for first and second components being 33.79% and 9.68%, respectively. The first two components of PCA explained 36.23% of the total variation, and the first three components explained 10.38% of the total variation (Fig. 3). The Mantel test did not reveal a strong correlation (r = 0.53) between genetic distance and geographic distance (Fig. 4).

AFLP analysis

For the AFLP analysis, the DNA of 10 individuals from Kamrup and 20 individuals from each of the other populations were pooled separately, because pooled samples have been reported to give consistent results in AFLP analysis for detecting differences among populations, though variation among individuals would be sacrificed while pooling the DNA (Katiyar *et al.*, 2000). Five AFLP primer combinations were used for studying the genetic diversity among the five populations (Table 3). A total of 304 bands representing 66 alleles were amplified by the five primer combinations in the five populations of which 135 (44.41%) were polymorphic. The maximum number of bands (69) was amplified in the Calicut population and the least number of bands (52) was amplified in the Nanjangud population. The size of the amplified bands varied from 60 to 427 bp. The resolving power (Rp) of the primer pairs screened ranged from 10 to 24 with a mean of 16.24. Pair-wise comparison of the populations showed that the genetic distances varied from 0.1374 between Calicut and Trichy to 0.2927 between Calicut and Kamrup (Table 8).

In the UPGMA dendrogram generated by AFLPs, the populations of Calicut, Trichy and Nanjangud formed a sub-cluster conforming to their geographical positions, but the populations of Kamrup and Narayangaon formed a sub-cluster though they are distantly located (Fig. 5). This observation was also confirmed by the Mantel test, which did not reveal strong correlation between genetic distance and geographic distance (r = 0.34) (Fig. 6).

Table 8. Pair-wise Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between five populations of *Cosmopolites sordidus* (Germar) using AFLPs

Population	Kamrup	Narayangaon	Trichy	Calicut	Nanjangud
Kamrup	–	0.8418	0.791	**0.7463**$^{+}$	0.7791
Narayangaon	0.1722	–	0.7821	0.7791	0.7761
Trichy	0.2344	0.2458	–	**0.8716**$^{+}$	0.791
Calicut	**0.2927**$^{+}$	0.2496	**0.1374**$^{+}$	–	0.806
Nanjangud	0.2496	0.2534	0.2344	0.2157	–

$^{+}$The highest and lowest genetic distances and identities are indicated in bold.

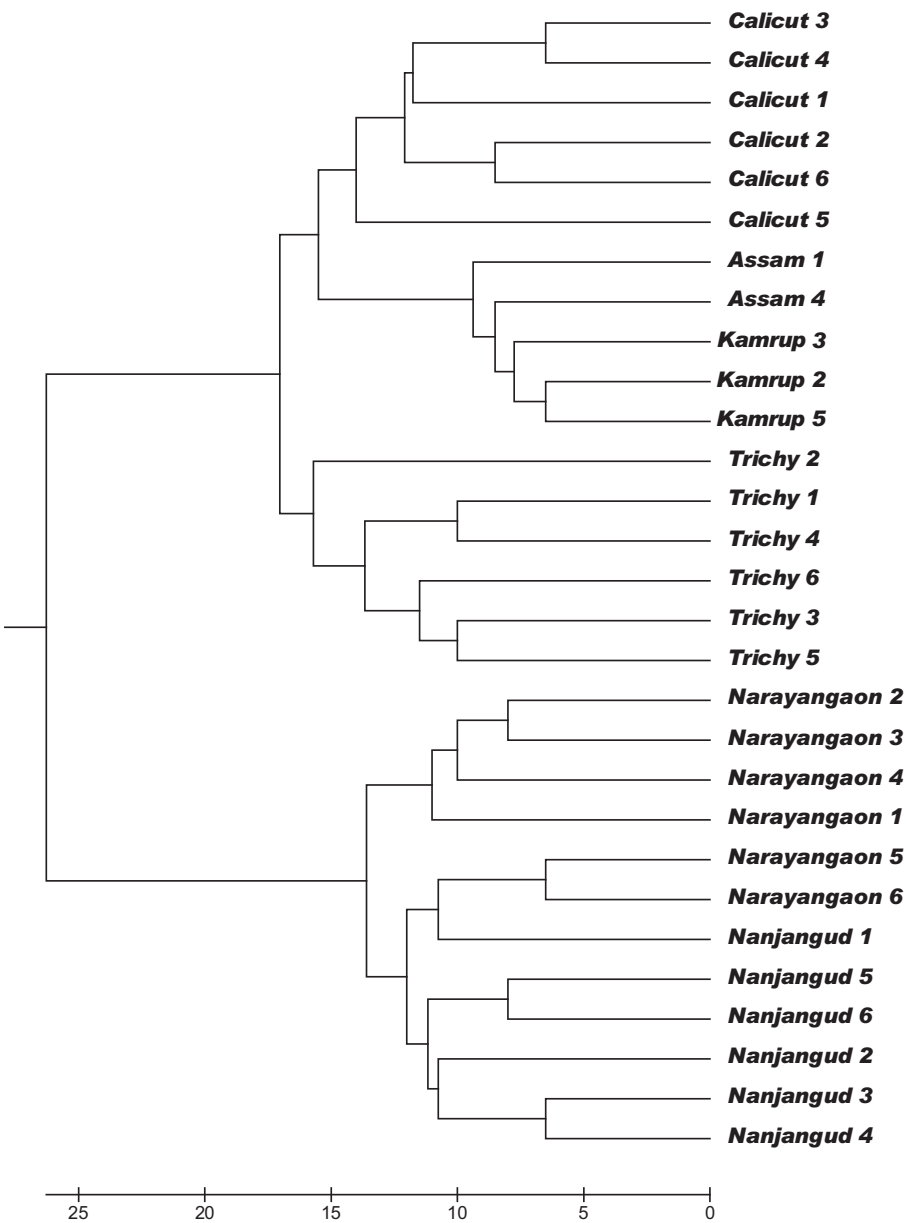

Fig. 2. Dendrogram of 29 individuals of *Cosmopolites sordidus* (Germar) constructed from the genetic distance matrix estimated from the RAPD data and clustered using UPGMA.

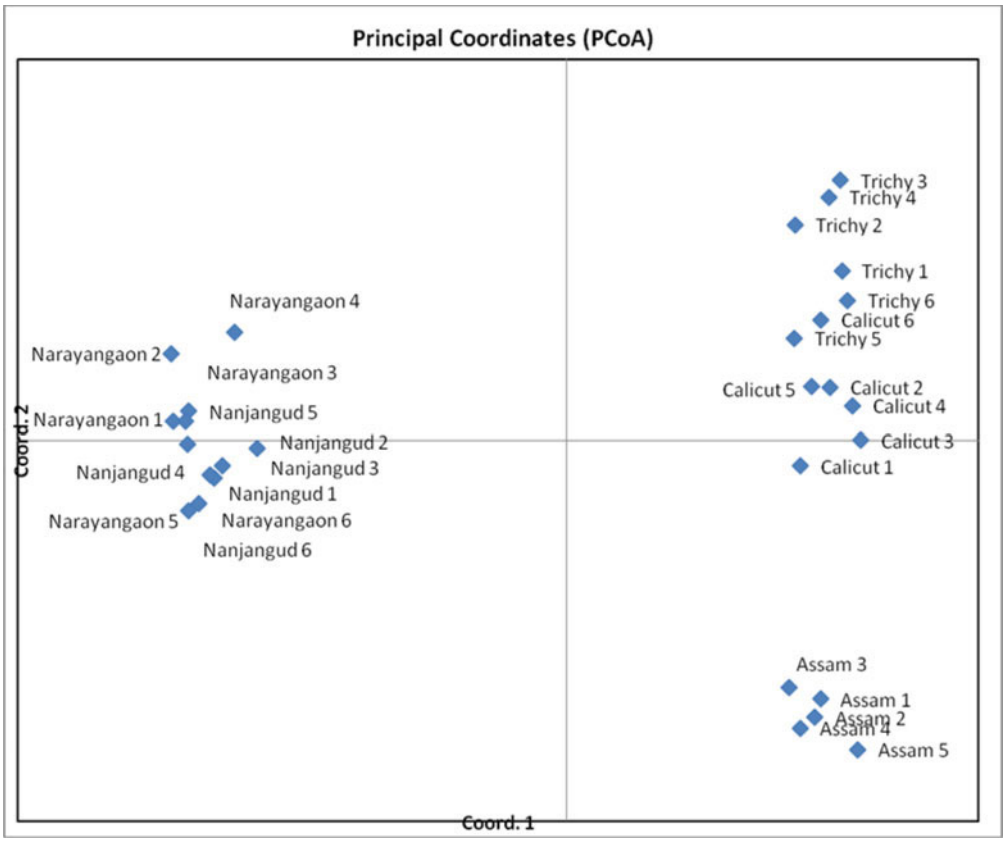

Fig. 3. Principal Component Analysis (PCA) of five populations of *Cosmopolites sordidus* (Germar) showing three distinct clusters.

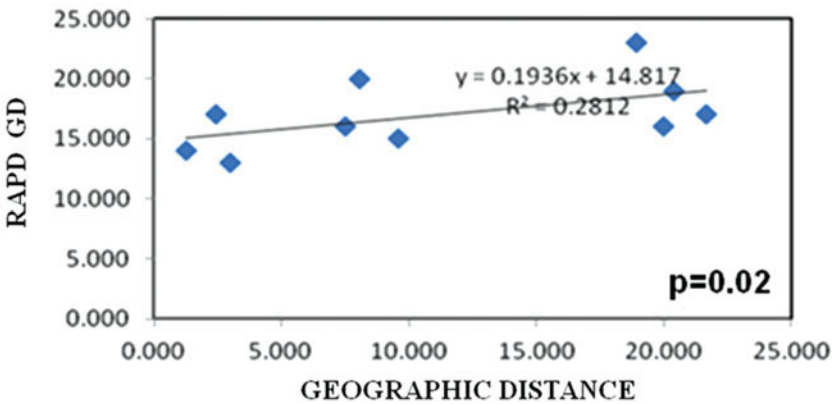

Fig. 4. Mantel test for correlation between genetic and geographical distance for the RAPDs data.

One hundred and eleven (111) polymorphic bands were generated by RAPDs and 135 by AFLPs, satisfying the general recommendation of having at least 50 polymorphic bands for a precise estimation of genetic distances (Nei, 1978). The different marker parameters derived using each of these marker systems are summarized in Table 6. Values of parameters such as average number of bands per primer, percentage of polymorphic bands, average number of polymorphic bands per primer, Rp, Hav and PIC were higher for AFLPs than for RAPDs, but estimates for the percentage of polymorphic bands, EMR and MI were higher for RAPDs than AFLPs. The MI value, which determines the utility of a marker system, was significantly higher for RAPDs than

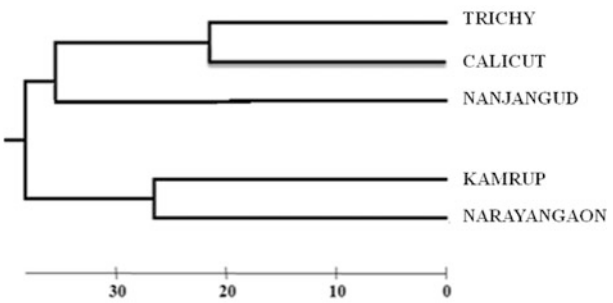

Fig. 5. Dendrogram of five *Cosmopolites sordidus* (Germar) populations constructed using the UPGMA method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The evolutionary distances are in the units of the number of base substitutions per site.

AFLPs ($P = 0.001$), as determined by the standard two-sample '*t*' test, suggesting that RAPDs are more useful than AFLPs in revealing polymorphism in these populations. However, based on the level of polymorphism detected by AFLPs, which was higher than RAPDs, it can be concluded that both marker systems are useful in the genetic diversity analysis of this banana pest.

Discussion

RAPD and AFLP analyses of five Indian populations of *C. sordidus* has revealed that though there is restricted gene flow between them, these populations are not genetically distinct. This conclusion is supported by the sedentary nature of these weevils, which limits their dispersal capacity. Adult weevils have been observed not to disperse more than 50 m in three months (Gold *et al.*, 1999*b*). Though the adult weevils have functional wings, their flight is uncommon and the weevils are relatively sedentary. It is a monophagous pest of bananas, i.e. it has a narrow host range and also has a low reproductive potential. These two factors limit its capacity to disperse. Hence, new infestations by this weevil mainly occur through transport or exchange of infested planting material (Gold et al., 1999*b*), which could have contributed to the range expansion of this pest, resulting in populations that are genetically related closely but geographically distinct. The UPGMA dendrogram derived from RAPDs clusters the individuals according to the sampling locations and AMOVA analysis shows that almost half of the observed genetic variation occurs within the populations. These results indicate that the banana corm weevil had formed localized populations, due to limited dispersal. Pest species can establish large local populations that maintain genetic variation for local adaptation, reflecting a successful ecological strategy.

The present strategies that are being followed for the control of banana corm weevil mainly include cultural practices and use of pesticides. Cultural practices, i.e. selection of clean planting material, systematic trapping of adult weevils with pseudostem traps and mulching, field sanitation, etc., require a high labour input and material requirements, which are not economical (Gold *et al.*, 2001; Gold and Messiaen, 2000). Biological control methods include use of biological control agents such as arthropods, entomopathogenic nematodes, fungi and organisms such as *Beauveria bassiana*, *Steinerma* and *Heterorhabditis* spp., are still in their preliminary stages (Pena *et al.*, 1993; Godonou *et al.*, 2000; Gold and Messiaen, 2000). Moreover, their economic cost and efficacy limits their application for large-scale use. Hence, presently, organophosphorus insecticides, such as prothiofos, chlorpyrifos, pirimiphos-ethyl and ethoprophos, are being used to control weevil infestations. However, use of these insecticides has resulted in the emergence of insecticide-resistant weevil strains (Collins *et al.*, 1991; Gold *et al.*, 1999*a*). The potential of an insect pest depends upon its ability to invade, reproduce and establish itself in new habitats. Hence, knowledge about gene flow and dispersal in insect pests is instrumental to understanding the factors that enable or prevent local adaptation, the evolution of insecticide resistance and prevention of its spread. Reliable information on gene flow, i.e. pest dispersal and movement and its relationship to pest management practices, environmental factors and ecosystem is important, as it can aid in predicting pest infestations (Loxdale *et al.*, 1993; Aylor and Irwin, 1999; Byrne, 1999). This information is important to understand the occurrence of resistance and prevention of its spread, to assess the spatial scale at which the management practices should be implemented and how fast the resistance to a given control method could evolve and how broadly, in a geographical sense, a control method should be applied (Roush and Daly, 1990; Nahrung and Allen, 2003; Labbe *et al.*, 2005). It is important to minimize selection pressure to keep susceptible insects alive, which can be possible by use of appropriate dosages of insecticide, stringent use of control thresholds and insecticides with different modes of action in rotation. As RAPDs and AFLPs have been shown to be useful in studying the genetic diversity in *C. sordidus*, they can be used to monitor population structure reflecting local adaptation. Dispersal by insects is also important for IPM, especially when they carry genes for resistance to insecticides. Markers specific for susceptible and resistant insects can be identified, which would help in timely monitoring of the populations (Behura, 2006).

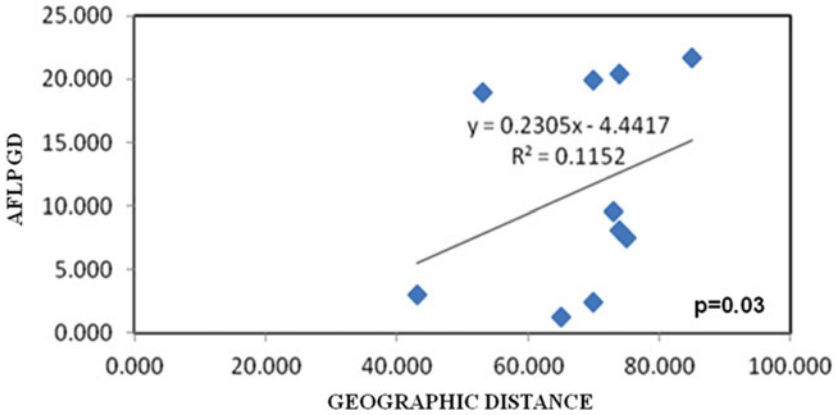

Fig. 6. Mantel test for correlation between genetic and geographical distance for AFLPs.

Conclusion

RAPDs and AFLPs are useful marker systems to study genetic variation in Indian populations of *C. sordidus* (Germar), as they reveal sufficient polymorphisms. Though the four populations from central and south India and the population from Kamrup, Assam, are geographically distinct, they are genetically closely related to each other, suggesting that the dispersal of this banana pest in India has taken place by exchange and transport of planting material. Limited dispersal has resulted in the pest forming localized populations with a high level of within-population variation, thereby revealing an ecological strategy for adaptation to the local environment. These results provide important information that could be used to design suitable strategies for the control of spread of this pest and in the management of resistance to organophosphorus insecticides.

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