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Research Article

Genetic variation among species, races, forms and inbred lines of lac insects belonging to the genus *Kerria* (Homoptera, Tachardiidae)

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Abstract

The lac insects (Homoptera: Tachardiidae), belonging to the genus *Kerria*, are commercially exploited for the production of lac. *Kerria lacca* is the most commonly used species in India. RAPD markers were used for assessing genetic variation in forty-eight lines of *Kerria*, especially among geographic races, infrasubspecific forms, cultivated lines, inbred lines, etc., of *K. lacca*. In the 48 lines studied, the 26 RAPD primers generated 173 loci, showing 97.7% polymorphism. By using neighbor-joining, the dendrogram generated from the similarity matrix resolved the lines into basically two clusters and outgroups. The major cluster, comprising 32 lines, included mainly cultivated lines of the *rangeeni* form, geographic races and inbred lines of *K. lacca*. The second cluster consisted of eight lines of *K. lacca*, seven of the *kusmi* form and one of the *rangeeni* from the southern state of Karnataka. The remaining eight lines formed a series of outgroups, this including a group of three yellow mutant lines of *K. lacca* and other species of the *Kerria* studied, among others. Color mutants always showed distinctive banding patterns compared to their wild-type counterparts from the same population. This study also adds support to the current status of *kusmi* and *rangeeni*, as infraspecific forms of *K. lacca*.

Key words: DNA fingerprinting, genetic variation, *Kerria*, lac insects, RAPD, Tachardiidae.

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Introduction

The lac insects (Coccoidea: Tachardiidae (= Kerriidae)) have been commercially harnessed to yield three useful products, *viz*., resin, wax and dye, which have found a remarkably wide range of applications in food, pharmaceuticals, cosmetics, perfumes, varnishes, paints, polishes, adhesives, jewellery and textile dyes, since ancient times (Dave, 1950; Sarkar, 2002; Ramani *et al.*, 2007). Lac is the only resin of animal origin. The importance of this commodity lies in its safety for human use, and as a renewable and eco-friendly resource. Lac production is confined to a few south, southeast and east Asian countries in the tropical forest region (Ramani *et al.*, 2007) with India is the leading lac-producer, with an annual production of about twenty thousand tons (Pal *et al.*, 2011). Lac insects are characterized by their resinous or horny protective secretion. They are phytosuccivorous and sessile; only the crawlers and adult males are free moving. They thrive well only on certain plant species known as lac hosts (Kapur, 1962; Varshney, 1985). Information on the taxonomy of lac insects is based on a monograph and its supplement (Chamberlin 1923, 1925), as well as subsequent works by Kapur (1958), Varshney (1977) and Kondo and Gullan (2007). Ninety species, under nine genera, have so far been reported worldwide (Varshney, 2009). Distribution is mainly restricted to tropical and subtropical regions between the latitudes 40° N and 40° S (Kapur, 1962).

Only species belonging to the genus *Kerria* produce true lac. In India, nearly all the production comes from the Indian lac insect *Kerria lacca*, represented by two infrasubspecific forms, viz., *kusmi* and *rangeeni*, which differ by host preference, life-cycle pattern, the quality and amount of lac produced, etc. (Kapur, 1962; Ramani, 2005). Other minor species are *K. sharda* (Mishra and Sushil, 2000) and *K. chinensis*. *Palas* (*Butea monosperma*), *ber* (*Ziziphus mauritiana*) and *kusum* (*Schleichera oleosa*) are the most common hosts used for lac production in India (Roonwal 1962), which is mainly restricted to the states of Jharkhand, Chhattisgarh, Madhya Pradesh, West Bengal, Maharashtra, besides a few others (Pal *et al.*, 2011). Notwithstanding, wild populations of *Kerria* are distributed

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throughout the length and breadth of the country, except in the colder regions (Varshney 1977).

The taxonomy of coccoids is based on adult-female morphology (Varshney, 1977; Kondo and Gullan, 2007). Even so, they are highly degenerate, and undergo tremendous changes in size and shape during the post-metamorphic stage. Differentiated populations, due to geographic separation and host-choice, and which have not diverged morphologically, pose an additional challenge to identification. Molecular approaches would therefore serve as useful complementary tools for characterizing such lac-insect taxa with greater reliability. A wide range of markers are employed for understanding insect-population genetics (Behura, 2006). RAPD-PCR is widely used for identifying cultivars, clones, natural populations, etc. Despite the limitation posed by reproducibility, unless reaction conditions are stringent (Baruffi *et al.*,1995 and Bertin *et al.*, 2007), this technique offers the advantages of simplicity, independence from prior DNA sequence information, and the evaluation of a large number of loci across the genome (Hadrys *et al.*, 1992; Lynch and Milligan, 1994; Weising *et al.*, 2005), besides providing the basis for developing more reliable SCAR (sequence characterized amplified regions) markers (Kethidi *et al.*, 2003). The technique has already been widely employed for assessing the genetic diversity of other insect populations (Reyes and Ochando, 1998; Castiglioni and Bicudo, 2005; Dvorak *et al.*, 2006; Lopes-Da-Silva and Vieira, 2007; Martins *et al.*, 2007; Magaña *et al.*, 2007; Karam *et al.*, 2007; Sosa-Gomez *et al.*, 2008; Sharma *et al.*, 2009).

This constitutes a report on genetic diversity in lac-insect populations belonging to the genus *Kerria*, the true lac-producing insects, from different parts of India, using the RAPD technique (Williams *et al.*, 1990; Welsh and McClelland, 1990). The study material included lac-insect populations collected from different parts of the country, both natural and cultured populations, besides inbred lines derived from *K. lacca.* The usefulness of RAPD primers for line-characterization is also examined. An attempt was also made to understand relationships among the lines studied.

Material and Methods

Lac-insect collection and culture

The insects used in the study were obtained from lac-insect cultures maintained at the Research Farm, IINRG campus, Ranchi (23°19'51" N 85°22'18" E; Elevation ~2080 ft). A few were also drawn from collections of natural field populations (Table 1; Figure 1). The lac-insect cultures were maintained on a common lac host *Flemingia macrophylla*, under potted conditions. The cultures, enclosed in synthetic mesh sleeves to exclude parasite and predator infestation, were regularly sprayed with fungicide (carbendazim, 0.01%) to maintain cleanness. Field-collected insects were carefully screened, in order to select only healthy ones. The lines studied mainly included the most commonly used species for lac production, *viz*., *K. lacca (kusmi* and *rangeeni* infrasubspecific forms*)*, a collection of *K. sharda*, and two collections of *K. chinensis* (India and Thailand), as well as inbred and crossbred lines,

Table 1 - Details of the 48 lines of *Kerria* studied, including coding and place of collection.

Group/Nature	Number used to indicate the location on the map	Code No.	Place of collection
I *K. lacca,* cultivated lines			
Rangeeni	1	LI003	Silli, Jharkhand
	2	LI011	Ranchi, Jharkhand
	3	LI019A	Bokaro, Jharkhand, yellow
	3	LI019B	Bokaro, Jharkhand, crimson
	4	LI032	Kirnapur, Madhya Pradesh*
	5	LI042	Mainpur, Chhattisgarh*
	6	LI044	Jhalda, West Bengal*
	7	LI048	Kalamati, Jharkhand
	8	LI087	Vardha (Guna), Madhya Pradesh
Kusmi	9	LI005	Ranchi, Jharkhand
	10	LI012	Purulia, West Bengal
	11	LI025	Putidih, West Bengal
	12	LI026	Chandai, West Bengal
	13	LI027	Nawadih, Jharkhand
	14	LI028	Kulajanga, West Bengal
	15	LI036	Kurubhatta, Chhattisgarh*
	16	LI075	Kalamati, West Bengal

Table 1 (cont.)

Group/Nature	Number used to indicate the location on the map	Code No.	Place of collection
	17	LI077	Hesadih, Jharkhand
II *K. lacca,* geographic races			
Northern	18	LI072B	Varanasi, Uttar Pradesh*
Northern	19	LI073	Bhathat, Uttar Pradesh
Eastern	20	LI029	Ashok Nagar, Ranchi, Jharkhand
Eastern	21	LI031	Rajendra Chowk, Ranchi, Jharkhand
Eastern	22	LI078	Orissa, *kusmi* yellow
Western	23	LI004	Simbalpani, Gujarat
Western	24	LI018	Manasarovar, Gujarat
Western	25	LI006	Pushkar, Rajasthan
Western	26	LI069	Chargaon, Maharashtra*
Western	27	LI015	Simbalpani, Gujarat, yellow
Western	28	LI013	Alsipur, Gujarat, yellow
Central	29	LI085	Guna, Madhya Pradesh*
Southern	30	LI009A	Thrissur, Kerala, yellow
Southern	30	LI009B	Thrissur, Kerala, crimson
Southern	31	LI079	Bangalore, Karnataka
Southern	32	LI082	Vishakapatnam, Andhra Pradesh*
Southern	33	LI010RR	Echoda, Andhra Pradesh
III *K. lacca*, experimental lines			
Inbred line 3,	-	LI007	Kundri, Jharkhand
Inbred line 8	-	LI008	Kundri, Jharkhand
Inbred line 1	-	LI014	Kundri, Jharkhand
Inbred line 6	-	LI020	Kundri, Jharkhand
Inbred line 9	-	LI022	Kundri, Jharkhand
Inbred line 13	-	LI024	Kundri, Jharkhand
Crossbred line 1 (13f x 8m)	-	LI058	Kundri, Jharkhand
Crossbred line 2 (13f x 3m)	-	LI061	Kundri, Jharkhand
Cream line, recombinant, crimson	-	LI001B	Kundri, Jharkhand
Cream line, recombinant, cream	-	LI001A	Kundri, Jharkhand
IV other species			
K. chinensis	33	LI002	Nangpoh, Meghalaya, India
K. sharda	34	LI066	Sarat, Orissa
K. chinensis	-	LI068	Thailand

* Field collected. The exact locations of certain collections are unavailable.

and collections from wild populations collected from all over of India. These lines were divided into four groups, depending on the nature (Table 1).

Insect processing and DNA isolation

Mature female insects were kept in 100% ethanol for 48 h at room temperature, so as to dissolve the resinous covering, whence they were individually cleaned with sable-hair brushes under a stereo-zoom microscope, and serially washed with alcohol to eliminate waxy secretions. The cleaned insects were kept in 200 µL absolute ethanol in 1.5 mL microcentrifuge tubes and stored in -80 °C freezer. DNA was extracted from mature females, adopting a phenol-chloroform procedure described by De Barro *et al.* (1995), with some modifications. The extracted DNA was individually quantified with a Shimadzu UV-VIS 1700 spectrophotometer using a DNA program pack, and checked by electrophoresis on 1% agrose gel together with 100 bp DNA ladder-plus (Fermentas, Germany).

RAPD amplification and gel electrophoresis

We screened 120 decamer primers (Operon Biotechnologies GmbH, Germany) for satisfactory amplification of products, using three selected lines. RAPD-PCR was carried out with 48 samples of pooled genomic DNA from three female insects, to shortlist primers exhibiting polymorphism and reproducibility. For each of these primers, annealing temperatures and other parameters were standardized by repeated experiments (Table 2). All the RAPD reactions were done in 25 µL of reaction mixtures containing 20 ng of template DNA, 1X *Taq* buffer [750 mM Tris-HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween 20; Fermentas GmbH, Germany], 2.5 mM of $MgCl_2$ (Fermentas GmbH, Germany), 0.2 mM of each dNTP mix (Fermentas GmbH, Germany), 20 pmol of each primer, and 1.5 units of *Taq* DNA polymerase (Fermentas GmbH, Germany). All the PCR reactions were carried out in a thermal cycler (BioRad iCycler, USA) programmed with the following cycling conditions: initial denaturation of template DNA was carried out at 95 °C for 5 min followed by 35 cycles programmed for denaturation step at 95 °C for 1 min,

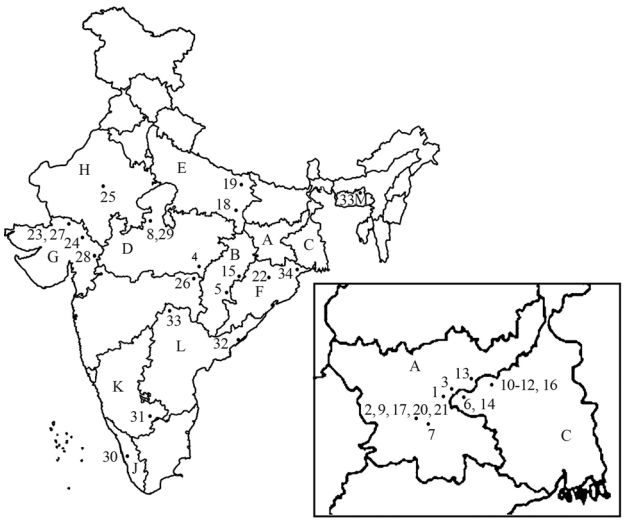

Figure 1 - Map of India, showing State boundaries, collection sites of the cultivated lines, and geographic races of *K. lacca* and other species of *Kerria* studied (A: Jharkhand, B: Chhattisgarh, C: West Bengal, D: Madhya Pradesh, E: Uttar Pradesh, F: Orissa, G: Gujarat, H: Rajasthan, I: Maharshtra, J: Kerala, K: Karnataka, L: Andhra Pradesh, M: Meghalaya). Inset – the states of Jharkhand and West Bengal.

Table 2 - The list of primers used, their sequences, T_m, number of bands generated, size-range and degree of polymorphism.

Primers	Primer sequence (5'-3')	T_m (°C)	Total number of scored bands	Band-size range (bp)	No. of polymorphic bands (%)
OPS9	TCCTGGTCCC	42	3	200-1000	2 (66.6%)
OPS10	ACCGTTCCAG	42	7	200-1500	6 (85.7%)
OPS12	CTGGGTGAGT	42	1	600	1 (100%)
OPS13	GTCGTTCCTG	42	10	300-1200	10 (100%)
OPS14	AAAGGGGTCC	42	6	400-1850	6 (100%)
OPS15	CAGTTCACGG	42	4	600-1500	4 (100%)
OPS16	AGGGGGTTCC	42	1	700	1 (100%)
OPS17	TGGGGACCAC	42	7	600-1500	7 (100%)
OPS19	GAGTCAGCAG	42	12	300-1200	11 (91.7%)
OPS20	TCTGGACGGA	42	8	500-2000	8 (100%)
OPT5	GGGTTTGGCA	37	10	200-1000	10 (100%)
OPT7	GGCAGGCTG	37	11	400-1600	11 (100%)
OPT15	GGATGCCACT	37	8	200-950	8 (100%)
OPT16	GGTGAACGCT	37	10	300-1700	9 (90%)
OPH5	AGTCGTCCCC	37	10	450-2000	10 (100%)
OPH9	TGTAGCTGGG	37	5	400-1300	5 (100%)
OPH12	ACGCGCATGT	37	9	400-1300	9 (100%)
OPH19	CTGACCAGCC	37	10	600-1900	10 (100%)
OPB4	GGACTGGAGT	37	4	600-1400	4 (100%)
OPB15	GGAGGGTGTT	37	4	600-1600	4 (100%)
OPB18	CCACAGCAGT	37	4	600-1300	4 (100%)
OPA2	TGCCGAGCTG	42	8	300-1500	8 (100%)
OPA10	GTGATCGCAG	42.5	3	800-1600	3 (100%)
OPA9	GGGTAACGCC	42	7	600-1250	7 (100%)
OPA18	AGGTGACCGT	42.5	4	350-1200	4 (100%)
OPA13	CAGCACCCAC	42	7	400-1400	7 (100%)

primer annealing step at specific T_m for the particular primer (Table 2) for 45 s, and extension step at 72 °C for 2 min. The final extension of the PCR products was carried out at 72 °C for 7 min. The reactions were carried out as described by Williams *et al.* (1990) and Nagaraja and Nagaraju (1995). All PCR amplified products were resolved on 2% agarose gel containing 0.5 µg/mL, ethidium bromide, prepared with 0.5 X TBE buffer [45 mM Tris-borate, 1.0 mM EDTA (pH 8.0)] and electrophoresed in 0.5 X TBE at 4 V cm^{-1} for 2 h in an Amersham submarine electrophoresis unit. Either Fermentas 100 bp ladder (100-1000) or 100 bp plus ladder (100-3000) were used as reference, depending on the band-size range. Sufficiently resolved DNA bands were documented using the Bioimaging system (Gene Genius, Syngene, U K), through GeneSnap.

Data analysis

Clear and unambiguous bands present across the DNA samples from 48 lac-insect lines at a particular locus (based on size) were scored as 1, whereas their absence or only a very faint outline were scored as 0, to so generate a binary matrix, to be used for analysis.

For line diagnosis and the analysis of marker discrimination power, the average band frequency obtained for each primer, marker index (MI) and resolving power (Rp), using band informativeness (Ib), were calculated (Prevost and Wilkinson 1999).

$$Ib = 1 - (2|0.5\text{-}pi|)$$

where pi is the proportion of lines showing the i^{th} band, and i = 1 to n where `n' is the total number of bands.

Primer resolving power (Rp) was also calculated using the following formula

$$Rp = \Sigma Ib$$

where Ib is band informativeness, as calculated above.

Marker index (MI) is the parameter for determining the utility of the marker in distinguishing different genotypes. The estimation of MI was by applying the following formula provided by Archak *et al.* (2003), based on band informativeness, as computed above.

$$MI = 1/n\ Ib \times EMR$$

where EMR (effective multiplex ratio) is the product of the number of polymorphic bands (*i.e.* a band absent in at least one genotype at a particular locus) per primer and the fraction of polymorphic bands.

In order to study the genetic relationships among *Kerria* lines, the scored binary data matrix was analyzed using the NTSYSpc version 2.02e software program (Exeter Software, New York, USA) (Rohlf, 1998). Data analysis was to obtain Jaccard's similarity coefficient. The dendrogram was generated by applying the neighbor-joining method (Saitou and Nei, 1987), using midpoint rooting. Only wild-type insects were considered for assessing the variation of similarity indices, in the case of mixed populations. The same software was used for principal coordinate (PCOORD) analysis (Sneath and Sokal, 1973) of the data. The confidence level for distinguishing genotypes, using the selected primers, was estimated through the analysis of probability of identical match by chance (P_{imc}), as proposed by Wetton *et al.* (1987) and Ramakishana *et al.* (1994).

$$P_{imc} = (\text{Mean the Jaccard similarity index})^n$$

where 'n' is the mean number of bands amplified per line.

Results and Discussion

Out of the 120 RAPD primers screened, 26 produced the satisfactory, clear and reproducible banding patterns used herein. These produced 173 loci in the 48 lines studied, of which 169 (97.7%) were found to be polymorphic. The size of the amplified products varied between ~200 bp and 2.0 kbp. The maximum size-range of amplified products for a single primer was obtained with OPH5 (450-2.0 kbp), whereas the minimum, *i.e.,* one single band (600 bp), was obtained with OPS12. The number of bands produced by the primers ranged from 1 (OPS 12 and OPS 16) to 12 (OPS 19), with an average of 6.7 per primer. Polymorphism in the bands produced by all the RAPD primers, except for OPS 9, OPS 10, OPS 19 and OPT 16, was 100%. The least was obtained with OPS9 (66.6%). The primers used, their sequences, T_m, number of scored bands, band-size range and the number of polymorphic bands, are given in Table 2. The total number of amplified bands in all the forty-eight lac-insect lines generated, when using all the twenty-six RAPD primers, was 3,380, with an average of 70.4 bands per line. The number of bands amplified through all the primers in all the lines ranged from 33 (LI036) to 95 (LI025).

The resolving power (Rp) of the primers screened ranged from 0.08 (OPS9) to 4.08 (OPA13), with a mean of 1.97. The lowest MI value was observed with OPS9 and the highest with OPS19. The mean MI of the primers was 1.82.

A total 33 unique bands were generated, with 15 RAPD primers (OPS9, OPS10, OPS13, OPS15, OPS17, OPS19, OPT7, OPT16, OPH5, OPH12, OPH19, OPB15, OPB18, OPA2 and OPA9) in eight lac-insect lines (LI002, LI010RR, LI09B, LI068, LI05, LI044, LI087 and LI069). The highest number of unique bands (21 out of 33), compared to the remainder, was in Line LI002, *Kerria chinensis* from India. Only three (LI002, LI003 and LI015) presented unique null bands with four RAPD primers, OPT16, OPS19, OPS10 and OPS9. In line LI003, there was a unique null band with three primers, viz., OPS19, OPS10 and OPS9. Out of the 48 lac-insect lines analyzed, nine, *viz*., LI009A , LI002, LI068, LI043, LI085, LI026, LI027, LI003 and LI028, could be identified by the presence of two bands by eight primers. With most of the primers, lines LI002 and LI068, *K. chinensis* lines, respectively from In-

dia and Thailand, were found to share a common pattern, by the presence or absence of two bands.

Pair-wise Jaccard's similarity indices varied from 0.15 (LI068, LI003) to 0.81 (LI012, LI015; LI029, LI032; LI048, LI032), with a mean of 0.58 for all the cultivated lines, geographic races of *K. lacca* and two other species (Groups I, II and IV in Table 1). The index varied between 0.27 (LI036, LI085) and 0.81 among the 33 cultivated lines and geographic races of *K. lacca* (Groups I and II in Table 1), with a mean of 0.61, indicative of significant intra-specific genetic diversity. The six inbred lines of *K. lacca* (*rangeeni*), derived from a single mother population, presented divergence with a mean similarity index of 0.77 (range: 0.62-0.92). Analysis of the above data showed that the probability of identical chance matching was 4.74×10^{-16}, thus indicative of a high level of reliability.

The dendrogram generated from the Jaccard's similarity matrix, by using the neighbor-joining method, appears in Figure 2. Basically, it resolves the lines into two major clusters (nodes A and B), as well as outgroups comprising eight lines under six branches (1 to 6). The cluster originating from node A, the major one comprising 32 lines, basically includes cultivated lines of the *rangeeni* form, geographic races and inbred lines of *K. lacca*. It is further differentiated into three subclusters (A1, A2 and A3). Subcluster A1 is a heterogeneous group of seven lines, comprised of two (LI003 and LI031) from Jharkhand State, two (LI42 and LI36) from Chhattisgarh State, two from Andhra Pradesh State, and a cream recombinant mutant line (LI001A). The subcluster from node A2 is comprised of 15 lines, mainly from the eastern region, and consists of six lines derived from cultivated populations of four adjoining lac-growing states (Jharkhand, West Bengal, Chhattisgarh and Madhya Pradesh), six inbred lines (also developed from a cultivated population from Jharkhand), two geographic races from northern and eastern India, and the wild-type color form of a recombinant line (LI001B). The subcluster of ten lines originating from A3, is mainly composed of those from western India (five from Gujarat and Rajasthan), one from the northern state of Uttar Pradesh (LI72B), two crossbred lines (LI058 and LI061), and the yellow mutant of a *kusmi* line from Orissa (LI078), whereas subclusters A2 and A3 mainly consist of lines from eastern and western regions of India, respectively.

Node B comprises eight lines, viz., seven of the *kusmi* form and one of the *rangeeni* of *K. lacca* from the southern state of Karnataka. These forms of *K. lacca* appear to have originated through a common ancestor at O. *Kusmi* insects are naturally distributed mainly in the eastern part of peninsular India, whereas the *rangeeni* are distributed country-wide. These two forms differ as to host preference - *rangeeni* insects are characterized by their ability to thrive well on *Butea monosperma*, and *kusmi* on *Schleichera oleosa* (Varshney, 1977). Based on crosses between the two, it has been shown that the *kusmi* form is genetically

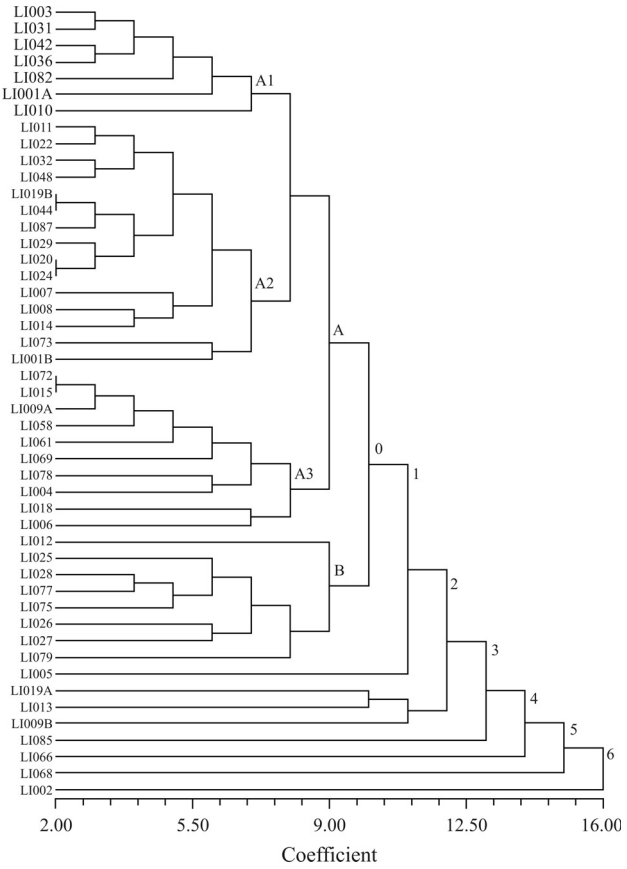

Figure 2 - Dendrogram showing phenetic relationship of 48 lines of *Kerria,* generated from Jaccard's similarity coefficients, based on RAPD data, and using the neighbour-joining method.

endowed for survival on *S. oleosa* (Chauhan and Mishra, 1970). This form of the Indian lac insect probably originated through host-shift. The remaining eight lines form a series of outgroups (branches 1-6), which include one of the *kusmi* form (LI005), one collection from the central state of Madhya Pradesh (LI085), and a group of three yellow mutant lines (9B, 19A and 13) of *K. lacca*, besides two lines of *K. chinensis* (LI068 and LI002) and one of *K. sharda*.

The dendrogram differentiated the species of *Kerria* studied, as well as LI085, represented as an outgroup, and which requires re-examination as to taxonomic status. Three color mutants in *K. lacca* (white, yellow and cream), which affect body and resin color, have been shown to be recessive (Chauhan, 1977; Chauhan and Mishra, 1977; Ramani, 2002). In the present study, the dendrogram generated always separated colour mutants (yellow and cream) from their wild-type counterparts in the same population, due to their distinctive RAPD banding profiles, indicative of their distinct genetic makeup.

Principal component analysis revealed that 27.2% of the variation could be attributed to the first three components. Figure 3 presents the 3-D plot of the principal coordinate analysis of the similarity matrix data of the lines studied. The *K. lacca* populations are well-spread over the

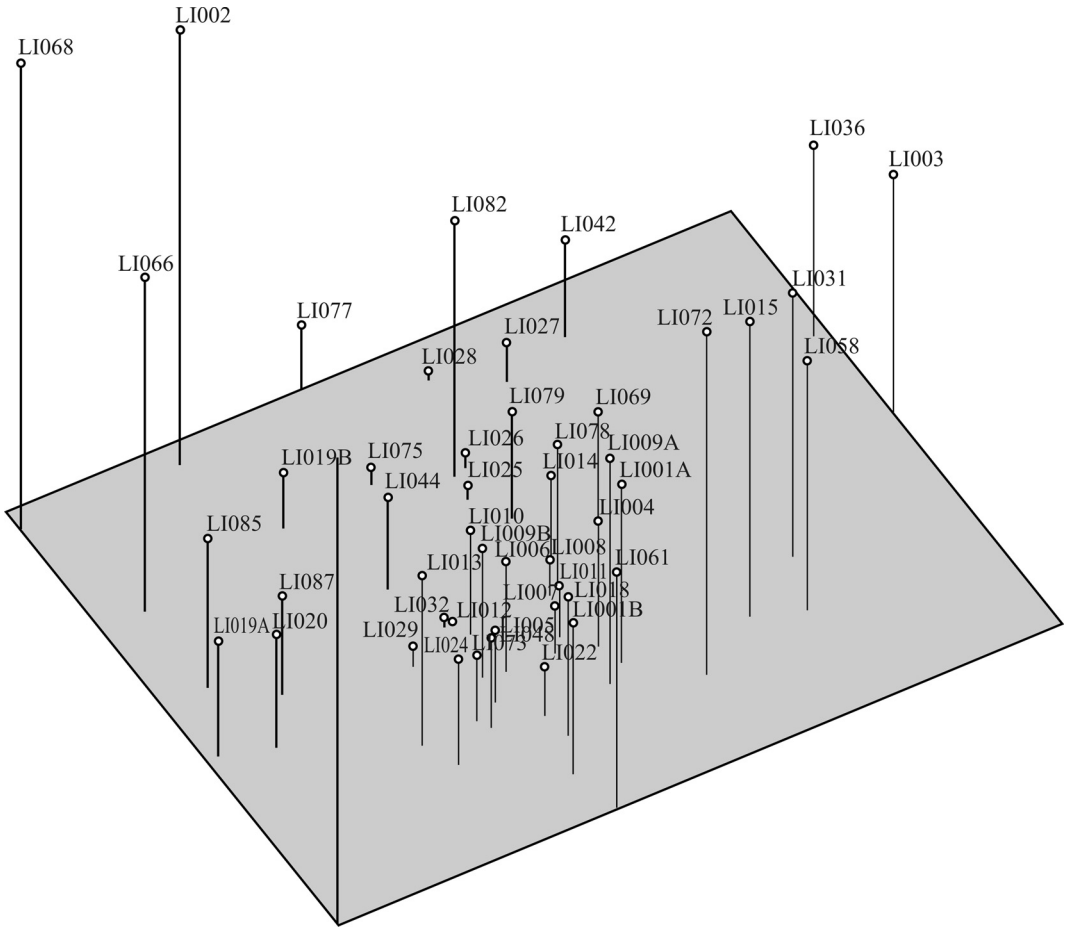

Figure 3 - 3-D plot generated from principal coordinate analysis of RAPD similarity matrix data from the forty-eight lines of *Kerria*.

dimensions, thereby indicating their diversity. Two lines of *K. chinensis* derived from different geographic locations (LI002 and LI068), as well as *K. sharda* (LI066), are well separated from those of *K. lacca*, thus consistent with the NJ dendrogram. According to PCOORD analysis, the seven *kusmi* lines of *K. lacca*, which formed a group at node B in this dendrogram, appeared to spread out. The lines LI075 (Kalamati, West Bengal) and LI077 (Hesadih, Jharkhand) appeared distinct. It is worth mentioning that spurious emergence was reported in the line from LI077. Spurious larval emergence, a phenomenon whereby some crawler emergence takes place during a non-typical period (Nov-Dec) in *kusmi* populations, is indicative of interbreeding in *kusmi* and *rangeeni* forms. Oviposition peaks in the segregating progenies of crosses of these two forms correspond to the parental types. Four lines of the *rangeeni* form of *K. lacca*, LI19A &B, LI020, LI085 and LI087, tended to form a cluster at variance with the dendrogram. These populations were from Jharkhand and Madhya Pradesh.

Lac insects, by depending on perennial trees for survival, are specialists in the preference for host-plants with limited dispersal. They may thus become locally adapted, thereby forming genetically distinct geographic and host races without morphological differentiation. Human intervention, with systematic lac-cultivation and the transport of insect populations across various regions, also exerts an influence on dispersal and interpopulation gene flow. Thus, divergence in *K. lacca*,can be expected, through being both the most commonly used species for lac-production and widely distributed in India. The above findings corroborate population divergence through geographic isolation and differences in host preference. The results also indicated that populations from geographically adjoining areas tend to be similar. Intermixing of cultivated populations is also expected in lac-producing areas, due to the transportation of insects within the cultivated areas, discernible in line-clustering in node A2 from the principal lac growing region. The six inbred lines derived from the same mother population also diverged from each other, with only LI024 and LI020 remaining similar.

The two forms of *Kerria lacca*, *kusmi* and *rangeeni*, commonly referred to as `strains' in lac-insect literature, are distinct as regards certain commercial and biological traits. Based upon morphological characteristics, they were allocated as infrasubspecific forms by Varshney (1977). Mishra *et al.* (1998) recorded the mutual morphometric differences. Even so, they hybridize freely under laboratory

conditions, thereby producing viable progeny. Premating barriers, such as differences in host preference and asynchrony in sexual-maturity periods, due to differences in life-cycle patterns, prevent interbreeding under natural conditions. Nevertheless, through occasional period-overlaps during the rainy-season generation of both forms, interbreeding is possible. A probable illustration is a subgroup of the A1 node cluster, comprising two collections from geographically close locations, *i.e*., LI042, a line of the *rangeeni* form, collected from Mainpur, Chhattisgarh, and LI036 a line of the *kusmi* form from Kurubhatta, also Chhattisgarh. Spurious crawler emergence in LI077, indicative of interbreeding in these two forms, has already been discussed.

The usefulness of RAPD-PCR for assessing genetic diversity in Indian lac insects has been demonstrated. Considering the general recommendation of fifty polymorphic markers to establish precise genetic distances (Nei, 1978), 26 RAPD primers having produced 169 polymorphic bands is sufficient for distinguishing species to complement pertinent taxonomic studies. Some of these can even be used for characterizing populations at the intraspecific level. The dendrogram generated from the similarity matrix also throws light on the interrelationships of the OTUs investigated. The above molecular evidence supports the current status of *kusmi* and *rangeeni*, as infraspecific forms of *K. lacca*. These forms appear to have descended from a common ancestor. Color mutants also need to be examined in greater detail, in order to understand the basis for their differentiation. Based on RAPD profiles, the lac-insect populations of *K. lacca* collected from different locales presented considerable variation. Further studies using other markers and DNA sequence variation, are likely to throw fresh light on these populations and their respective relationships.

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