Indian Journal of Entomology, **80**(4): 1327-1330 (2018) DoI No.: 10.5958/0974-8172.2018.00282.1

REPRODUCTIVE ISOLATION IN LAC INSECTS- *KERRIA LACCA* AND *KERRIA CHINENSIS*

A. Mohanasundaram*, R. Ramani, K.K. Sharma and Thamilarasi, K.

Lac Production Division
ICAR-Indian institute of Natural Resins and Gums, Ranchi 834010
**Email: mohaniinrg@gmail.com*

ABSTRACT

Lac insects belongs to the family Tachardiidae (=Kerridae) of the order Hemiptera, and superfamily Coccoidea. In this family, there are 99 species under 9 genera, of which 26 species under two genera occur in India. Natural populations of *Kerria* spp. are distributed throughout India and *K. chinensis* in the northeastern states is also cultivated to a certain extent. Cross breeding study done between lac insects, *K. lacca* and *K. chinensis* revealed that female cell weight was more in mated *K. chinensis* (14.5 and 29.6 mg) as compared to virgin *K. chinensis* (5.7 and 8.8 mg) in summer and rainy seasons, respectively. The physical growth of fertilized female indicated successful mating between the two species. Mated females secreted more resin compared to virgins; however, no embryonic development was observed in their ovaries and hence no young ones emerged from mated *K. chinensis*, indicating reproductive isolation. Therefore, it is inferred that *K. lacca* and *K. chinensis* are distinct species, which is corroborated with analyses using molecular markers. The ecological speciation is perhaps involved in *K. chinensis* during evolution.

Key words: *Kerria lacca, K. chinensis,* cross breeding, mating, summer, rainy seasons, physical growth, ovaries, reproductive evolution

Lac insects belong to the family Tachardiidae (=Kerridae), order Hemiptera and superfamily Coccoidea. This family includes 99 species under ten genera, with 26 species representing 4.7% of the total Coccoidea in India (Morales et al., 2016). Six more species have been added from India recently (Ahmad eta l., 2013a,b). In India, lac is cultivated mainly in Jharkhand, Chhattisgarh and parts of Madhya Pradesh, Maharashtra and West Bengal using mainly *Kerria lacca.* However, natural populations of *Kerria* spp are distributed throughout India and *K. chinensis* is also cultivated to a certain extent in the northeastern states of India. Chinese commercial species had also been named as *K. chinensis* (Chen et al., 1992, 1998). This is also the major one cultivated in Thailand (Chen et al., 2011).

Cross breeding done earlier between *K. lacca* (yellow bivoltine) female and *K. sharda* (crimson trivoltine) male were successful resulting in a viable crossbred trivoltine yellow insect (Anon., 2002; Personal communication K K Sharma). Mishra et al. (2004) studied reproductive compatibility of four species of *Kerria* viz., *K. lacca* (Kerr), *K. indicola* (Kapur), *K. ebracheata* (Chamberlin) and *K. chamberlini* (Varshney) with reciprocal crosses; these produced normal viable progenies at least for three generations. All these four species are neither geographically nor reproductively isolated, hence might be considered only as subspecies of *K. lacca.*

Varshney (1976) morphologically differentiated the lac insect species- *K. lacca lacca* and *K. chinensis. K. lacca* was distinguished with its higher number of dimples on branchial plates, bulging long pedicel and considerable distance between the anterior spiracle and branchial plate (Negi, 1954). And *K. chinensis* gets separated from *K. lacca* by its size and long sclerotized trailings below the anterior spiracle (Varshney, 1976).

The study on reproductive isolation is important to assess the gene flow during evolution of species. To know the reproductive compatibility of two commercially important lac insect species cultivated in India is essential, and the present study focuses on this. Although these are geographically isolated, there are chances of mixing of populations due to human activities. Hence, this study undertook crosses between *K. lacca* and *K. chinensis* and analysed their reproductive compatibility.

MATERIALS AND METHODS

Lac insects *K. lacca* and *K. chinensis* were inoculated on potted plants of *Flemingia macrophylla*

at the National Lac Insect Germplasm Center (NATLIGEC) during two consecutive years. It included summer and rainy season populations at the ICAR-Indian Institute of Natural Resins and Gums, Ranchi, Jharkhand. Lac cultures were covered with synthetic net sleeves (60 mesh) to protect the insects from their parasitoids and predators. Six potted culture of *F. macrophylla* were used, and the two cultures of *K. chinensis* (female) were crossed with *K. lacca* (male). All males were removed from *K. chinensis* culture and remaining population of females thinned manually to obtain single females.

At sexual maturity crosses were performed by releasing males of *K. lacca* on *K. chinensis* culture in the ratio of 4 (@&): 1(B&) for fertilization. Mating between these was observed with naked eye., and embryonic development assessed with microscope after maturation. In another set of two cultures of *K. chinensis* males were removed but, no crosses were made with the remaining two cultures remaining as control. Female cell weight from samples of ten cells were observed from each culture- for cross mated, virgin *K. chinensis* and selfed *K. chinensis* females at the time of maturation. These were compared using one-way ANOVA, significance of differences was calculated at the $p< 0.05$, and the means were compared with Duncan Multiple Range Test (DMRT) in software AGRES. Details of the parents used include: Summer crop (2012-13 & 2013-14), rainy season crop (2013 & 2014) with parent and COI accession numbers being- *K. chinensis* LIK0031(@&), HQ323772.1 and *K. lacca* LIK 0045(B&), HQ323761.1.

RESULTS AND DISCUSSION

Crosses done between *K. lacca* and *K. chinensis* revealed that the female cell weight was significantly more in self-fertilized *K. chinensis* (26.2 mg) followed by cross mated (14.5 mg) and the least in virgins (5.7 mg) in summer (Table 1). But in cross mated *K. chinensis,* it was higher compared to its virgin. Also, self-fertilized *K. chinensis* (31.5 mg) and cross mated *K. chinensis* (29.6 mg) were on par with each other but significantly different from their virgins (8.8 mg) with rainy season crop (Table 1). No significant difference was observed for female cell weight between crossed (*K. chinensis* × *K. lacca*) and self-fertilized *K. chinensis* whereas, virgin *K. chinensis* recorded less cell weight with rainy season generation.

Cell weight was more in self-fertilized *K. chinensis* with embryonic development and young ones emerging Cross mated females of *K. chinensis* also secreted more resin compared to their virgins (Figs. 1, 2). The physical growth of fertilized female indicated successful mating, however, no embryonic development was observed in the ovaries (Fig. 3); and hence no young ones (first filial generation) emerged from mated *K. chinensis*, indicating reproductive isolation.

The present study corroborates with earlier ones by Chauhan et al. (1990) wherein reciprocal crosses

Table 1. Cell weight in cross bred, virgin and selfed females of *K. lacca* and *K. chinensis*

Lac insect species	Growth pattern	F1	Cell weight (mg)*	
			Summer season	Rainy season
K. chinensis × *K. lacca* (crossed)	Physical growth of fertilized female however, no embryonic development	None	14.5^{b}	29.6^{a}
K. chinensis (Virgin)	No physical growth as compared to crossed lac insects	None	5.7^{c}	8.8^{b}
K. chinensis × *K. chinensis* (selfed)	Physical growth of fertilized female and observed embryonic development	Produced young ones and continued to next generation	26.2^{a}	31.5^{a}
		SED	0.79	0.98
		CD	1.56	1.95

*Means followed by different letters differ significantly at $p< 0.05$

Fig. 1. Mated female- *K. chinensis*

Fig. 2. Virgin female- *K. chinensis*

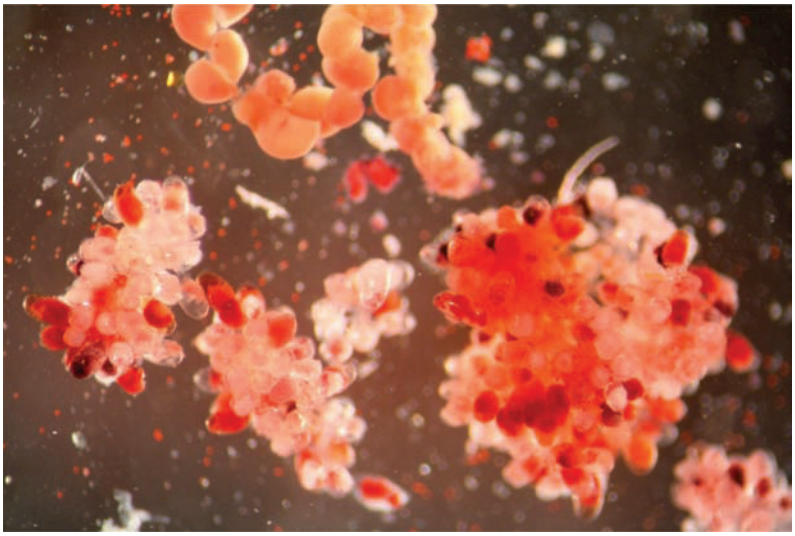

Fig. 3. Ovaries of cross (*K .chinensis* × *K. lacca*)

between lac insect stock obtained from Meghalaya (*K. chinensis*) and those of Bihar, Orissa and Punjab (*K. lacca*) showed that the Meghalaya stock was reproductively isolated. Chen et al. (2011) studied the status of two species of lac insects in the genus *Kerria* from China based on morphological, cellular and molecular evidence. It was shown with hybridization tests that mating between *K. lacca* and *K. sindica* produced first filial generation, indicating their close genetic relationship. However, crossbreeding between *K. yunnanensis* (grouped majority of the time with *K. chinensis*) and *K lacca*, did not produce any first filial generation, which indicated *K. yunnanensis* had distant relationship to *K. sindica* and *K. lacca.*

Our study reveals that *K. lacca* and *K. chinensis* are distinct species which also gets corroborated observations through molecular markers. Morphology and molecular biology evidences point out that *K. chinensis* is distantly related to *K. lacca*. The anonymous molecular markers such as RAPD markers (Ranjan et al., 2011) and ISSR markers (Saha et al., 2011) revealed that the *K. chinensis* is separated from *K. lacca* and always fall in separate clusters of the dendrogram. The more recent study using EST-SSR markers also revealed that this inference is indeed valid (Kandasamy et al., 2016). The phylogenetic tree based on the combined data of three genes namely, elongation factor 1 alpha, mitochondrial cytochrome c oxidase subunit 1 and small subunit ribosomal RNA gene also revealed that *K. lacca* belonged to Indian subcontinent clade and *K. chinensis* belonged to Eurasian clade; also

K. lacca could be the most primitive of lac insect and *K. chinensis* could be the recently evolved one (Chen et al., 2013). *Kerria chinensis* always fall in a separate branch compared to others based on morphology, cytology and molecular differences (Chen et al., 2011).

Mitochondrial cytochrome oxidase (mtCOI) divergence is a good indicator of species delimitation in most cases. The mtCOI sequences of the parents used in the study are available in the Gen Bank database. (HQ323761.1 for male and HQ323772.1 for female). These sequences of male parents are 99.2 to 99.7% similar to each other, whereas the ones of female parent (*K. chinensis*) is similar to an extent of 90.9 to 91.5% to the male parent *i.e.*, *K. lacca*. In whitefly, *Bemisia tabaci*, whenever the mtCOI divergence was >8% between the putative species, they exhibited complete reproductive isolation (Qin et al., 2016). Our results also show a similar trend because the divergence of mtCOI sequence observed between the parents is ~8%.

The reproductive isolation observed in this study between *K. chinensis* and *K. lacca* seems to be post zygotic isolation rather than prezygotic isolation since mating occurred but not the emergence of young ones. If the reciprocal crosses are carried out, there is further scope of exploring the mechanisms of post zygotic isolations whether there is any nucleus cytoplasmic interaction between these parents. Since viable progenies obtained between the reciprocal crosses of the species, *K. lacca* (Kerr), *K. indicola* (Kapur), *K. ebracheata* (Chamberlin) and *K. chamberlini* (Varshney) (Mishra et al., 2004), all of these could be incipient species. Since there is a reproductive isolation between *K. chinensis* and *K. lacca*, the speciation of *K. chinensis* might be attributed to ecological reasons.

ACKNOWLEDGEMENTS

The authors thank Shri P.A. Ansari for technical assistance in lac insect maintenance and crossing.

REFERENCES

Ahmad, A., Ramamurthy, V. V., Sharma, K. K., Mohanasundaram, A., Vidyarthi, A. S. and Ramani, R. 2013a. Three new species of *Kerria* (Hemiptera: Coccoidea: Tachardiidae) from India. *Zootaxa*, 3734 (4): 442-452.

Ahmad, A., Sharma, K K., Ramamurthy, V V., Vidyarthi, A S. and Ramani, R. 2013b. Three new species of *Kerria* (Hemiptera: Sternorrhyncha: Coccoidea: Tachardiidae), a redescription of *K. yunnanensis* Ou & Hong, and a revised key to species of *Kerria*. *Zootaxa*, 3620 (4): 518-532.

Anonymous. 2002. Lac insect breeding. Annual report, Indian Lac Research Institute, Ranchi. p. 14.

Chauhan, N. S., Ramani, R. and Sharma, K. K. 1990. Survey of genetic variation in lac insects - study of crosses, Annual report, Indian Lac Research Institute, Ranchi. p. 6.

Chen, H., Chen, X., Feng, Y., Yang, H., He, R., Zhang, W. and Yang, Z. 2013. Molecular phylogeny and biogeography of lac insects (Hemiptera: Kerriidae) inferred from nuclear and mitochondrial gene sequences. *Molecular Biology Reports*, 40: 5943-5952.

Chen, X. M., Wang, S. Y., Mao, Y. F. and Feng, Y. 1992. On the male aedeagus of four species of lac insects and preliminary cross breeding test. *Forest Research*, 5(2): 236-238.

Chen, X., Chen, H., Feng, Y., He, R. and Yang, Z. 2011. Status of two species of lac insects in the genus *Kerria* from China based on morphological, cellular and molecular evidence. *Journal of Insect Science* 11:106 retrieved from https://jinsectscience.oxfordjournals.org/ content/jis/11/1/106.full.pdf

Chen, X.M. 1998. The resource conservation of lac insects in *Kerria* and its utilization. *Chinese Biodiversity*, 6(4): 289-292.

Kandasamy, T., Mishra, V. K., Toppo, N. N., Ranjan. S. K., Mohanasundaram, A., Sharma, K. K. and Ramani, R. 2016. Genetic association of lac insect (genus: *Kerria*) genotypes using EST-microsatellite markers. Proceedings. International conference on natural resources management- an ecological perspectives Jammu. p. 857.

Mishra, Y. D., Chakrabarty, P. K., Bhattacharya, A. and Kumar, K. K. 2004. Reproductive compatibility of four species of the genus *Kerria* (Homoptera : Tachardiidae). New horizon of animal sciences. pp. 32-36. Pandy, B. N., Natarajan, P., Arumugam, N. and Premjith, S (eds.). Zoological Society of India, Kolkata.

Morales, G. M., Denno, B. D., Miller, D. R., Miller, G. L., Ben-Dov, Y. and Hardy, N. B. 2016. ScaleNet: A literature-based model of scale insect biology and systematics. Database. doi: 10.1093/database/bav118. http://scalenet.info.

Negi, P. S. 1954. Dimorphism in lac insects. *Indian Forester*, 80 (8), 423-426.

Qin, L., Pan, L. and Liu, S. 2016. Further insight into reproductive incompatibility between putative cryptic species of the *Bemisia tabaci* whitefly complex. *Insect Science* 23: 215–224, DOI 10.1111/1744-7917.12296.

Ranjan, S. K., Mallick, C. B., Saha, D., Vidyarthi, A. S. and Ramani, R. 2011. Genetic variation among species, races, forms and inbred lines of lac insects belonging to the genus *Kerria* (Homoptera, Tachardiidae). *Genetics and Molecular Biology*, 34: 511-519.

Saha, D., Ranjan, S K., Mallick, C B., Vidyarthi, A S. and Ramani, R. 2011. Genetic diversity in lac resin-secreting insects belonging to *Kerria* spp., as revealed through ISSR markers. *Biochemical Systematics and Ecology,* 39: 112-120.

Varshney, R. K. 1976. Taxonomic studies on lac insect of India. *Oriental Insects*, 5 (Suppl. No.): 1-97.

(Manuscript Received: January, 2018; Revised: August, 2018;
Accepted: August, 2018; Online Published: September, 2018)