RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 54 Issue 6: 787-791 (December 2020)

Discordance between Mitochondrial and Nuclear DNA Genes Suggests the Possibility of Hybridization of Indian and Southeast Asian Types of *Oecophylla smaragdina* (Fabricius) (Hymenoptera, Formicidae) in Bangladesh

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10.18805/IJARe.A-540

ABSTRACT

Background: *Oecophylla smaragdina* is distributed from India, SE Asia and Australia including many tropical Islands. A recent phylogenetic study based on mitochondrial DNA analysis reveals that Bangladesh is the overlapping zone of both Indian and Southeast Asian type of *O. smaragdina*. These two different lineages of Indian and SE Asian type have the opportunities of creating the zone of contacts, but no such data was found. In this study, shed light was given to reveal the chance of hybridized colony of *O. smaragdina* in Bangladesh.

Methods: To asses the hybridization scenario, 28 *O. smaragdina* colony from 27 localities in Bangladesh were analyzed using Longwave length Rhodopsin (LW*Rh*) nuclear gene sequences and was compared with the mtDNA sequences, which was collected from the same localities and deposited into NCBI GenBank.

Result: The inconsistency between mitochondrial and nuclear gene types was observed from two colonies of the overlapped zone of contact. These two colonies were identified as SE Asian type by mtDNA analysis however, by nuclear DNA analysis; it was identified as Indian type. These significant discrepancies within the colony suggested the possibility of hybridization of weaver ant in Bangladesh.

Key words: Mitochondrial DNA, Nuclear DNA, Indian type, Southeast Asian type, Hybridization, LW *Rh.*

INTRODUCTION

The weaver ant, *Oecophylla* (Hymenoptera, Formicidae) has only two extant species, *Oecophylla longinoda* (Latreille) distributed in tropical Africa and *O. smaragdina* (Fabricius) in southeastern Asia and Australia(Bolton, 1995).

Previous phylogeographic study on *O. smaragdina* based on mitochondrial cytochrome b (Cytb) and cytochrome c oxidase subunit I (CO1) genes identified two major clades where Indian types occurred mainly in India and Sri Lanka while the Southeast Asian (SE Asian) clades have been observed in most of the SE Asian countries including Bangladesh (Azuma *et al*. 2006). However, the occurrence of Indian type from Bangladesh has been recorded (Rahman *et al*. 2017b). The recent phylogenetic study based on mitochondrial CO1 and Cytbgenes revealed the overlapping distribution of both India and Southeast Asian clades of *O. smaragdina* in central Bangladesh (Rahman *et al*. 2017a). In Bangladesh, the occurrence of different types implies the chance of hybridization. Recently, for inferring the evidence of hybridization a comprehensive view of evolutionary history by analyzing nuclear and mitochondrial DNA was found effective and has been using extensively (Roos *et al*. 2011). The nuclear long-wavelength rhodopsin gene (LW *Rh*) belongs to a family of visual pigment genes and has been regarded as a useful marker for the between-species level phylogeny of insects, especially Hymenoptera (Ascher *et al*. 2001; Cameron and Williams, 2003). In the LW*Rh* analysis, Azuma (2006) categorized the Indian and SE Asian type of *O. smaragdina* population as Smaragdina B and Smaragdina A by comparing the nucleotide sequences. This analysis suggested that SE Asian are derived and monophyletic while the Indian type is the ancestor. Discordant genetic relationships between *mt* DNA and nuclear DNA results from mitochondrial introgression or the incomplete lineage sorting (Eto *et al*. 2013). Therefore, for confirming the validity of phylogenetic study LW*Rh* nuclear DNA sequence of *O. smaragdina* colonies from some randomly selected localities

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How to cite this article: Rahman, M.M., Hosoishi, S. and Ogata, K. (2020). Discordance between Mitochondrial and Nuclear DNA Genes Suggests the Possibility of Hybridization of Indian and Southeast Asian Types of *Oecophylla smaragdina* (Fabricius) (Hymenoptera, Formicidae) in Bangladesh. Indian Journal of Agricultural Research. 54(6): 787-791.

Submitted: 10-02-2020 **Accepted:** 02-05-2020 **Published:** 22-08-2020

in Bangladesh need to be compared with the results of *mt*DNA sequences. The main objectives of this study were to analyses the nuclear LW *Rh* gene for detecting possible hybridization by pointing out the inconsistency of nucleotide sequences.

MATERIALS AND METHODS

The experiments were conducted in the Institute of Tropical Agriculture laboratory of Kyushu University, Japan. Adult *Oecophylla smaragdina* workers from 28 colonies of 27 localities of Bangladesh were used for performing this study which was collected during 2013 to 2016 (Fig 1). Only one individual per colony was chosen for sequencing the nuclear gene. The specimens were preserved in 99% ethanol prior to DNA extraction. Genomic DNA was extracted from the legs of specimens that were preserved in alcohol by using QIAGEN DNeasy Blood and Tissue kit (Qiagen, Maryland, USA). Amplification of Nuclear DNA was done by polymerase chain reaction (PCR). The primers used for amplification are identical to primers reported by Crozier *et al*. (1994), Lunt *et al*. (1996), Azuma *et al*. (2002), and Azuma *et al*. (2006). The thermal cycling parameters for LW*Rh* basically followed the protocols established by Crozier and Crozier (1993) and Sameshima *et al*. (1999). For other primer pairs, the annealing temperature ranged from 60°C- 62°C has been maintained, accordingly. Illustra ExoProStar was followed according to the instruction of the manufacturer GE Healthcare. For cycle sequencing, ABI PRISM Big Dye Terminator v3.1. Cycle sequencing kits from Applied Biosystems were used in an automated sequencer. Sequencing reactions were performed by using ABI 3100 Avant DNA Sequencer (Applied Biosystems). For analyzing nuclear DNA by long wavelength rhodopsin, we used the several primers pairs as mentioned in Table 1.

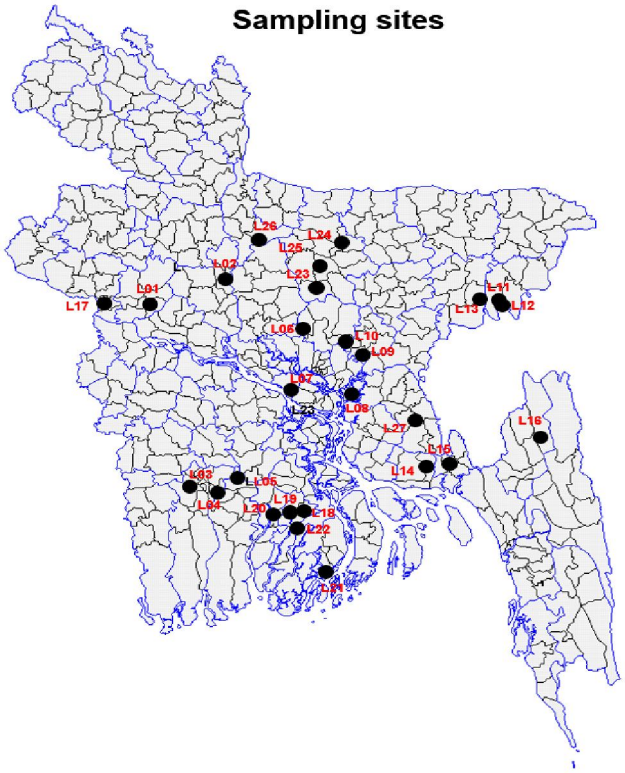

Fig 1: The sampling sites of *Oecophyllasmaragdina* in Bangladesh. The locality code corresponds to those in Table 2.

The sequencing result of Nuclear DNA analysis from 28 samples of 27 localities in Bangladesh have been deposited to GenBank. The locality information with accession number was shown in Table 2. For finding the inconsistency, thecomparison was done between the previously sequenced results of a phylogeographic study by mt DNA analysis (CO1 and Cytb genes) from (Rahman *et al*. 2017b) and nuclear DNA among those 27 localities. For the two localities (locality 11& locality 15) a total of 40 individuals were analyzed and the sequence data was deposited to GenBank. The nucleotide sequences of LW*Rh* Azuma *et al*. (2006) were followed by references. The intron region was identified by comparing the 528-bp sequence with LW Rh mRNA of the Saharan silver ant (*Cataglyphis bombycinus*, DDBJ accession no. U32501), carpenter ant (*Camponotus abdominalis*, U32502) and large earth bumblebee (*Bombus terrestris*, AF091722). Whereas insect opsin genes comprise many paralogous copies, the determined sequences of *Oecophylla* were more similar to the three LW *Rh* sequences than to any others based on a homology search using FASTA in DDBJ. This homology search also provided evidence that the amplified region was LW *Rh*. After identifying the intron regions, the introns were removed and the sequences were aligned in MEGA 7.0

RESULTS AND DISCUSSIONS

Among 27 localities, 16 locality samples were found as Indian type and 11 localities are of SE Asian type (Table 3). Hereafter, Indian haplotype is mentioned as Smaragdina Indian type and SE Asian haplotype as Smaragdina SE Asian type. Between these two haplotypes, there was only one

Table 1: List of primers used for analyzing nuclear DNA.

LW Rh	LW RhF	Forward	AATTGCTATTAYGARACNTGGGT[1]
	LW RhR	Reverse	ATATGGAGTCCANGCCATRAACCA[1]
	LR 798 F	Forward	GCH GCY CAY GAG AAG AAY ATG CG[2]
	LR 1047 R	Reverse	GG ATT RTA YAC RGC RTT GGC TTT BGC[2]
	LR482 FCR	Forward	ATA TGG ACG ATG ACR ATG ATC GC[2]
	LR 855 R	Reverse	GA TCG YAR VGA AGC RAC GTT CAT[2]

[1](Mardulyn and Cameron, 1999); [2](Blaimer, 2012).

substitution: site 27 contains thymine in Smaragdina SE Asian type and cytosine in Smaragdina Indian type (Fig 2). This substitution is in a coding region but is synonymous and transitional. Since all the other haplotypes, including *O. longinoda*, had acytosine at site 27, this thymine substitution is parsimoniously considered to be derived, suggesting strong monophyly and isolation South East Asian type.

From the results it is observed the inconsistent mitochondrial and nuclear DNA type in the colony located in L11 and L15 (Fig 3). Rahman *et al*. (2017b) in the mitochondrial DNA analysis reported that these two localities were identified as SE Asian type.

For further confirmation the nuclear DNA of additional 40 individuals from those two colonies, 24 individuals from L11 and 16 individuals from L15 were analyzed. Among those 24 individuals from L11, 1 individual is recognized as exactly sharing different nucleotide sequences from mtDNA of SE Asian type and 23 individuals are true to Smaragdina SE Asian type. However, the 16 individuals from the colony of locality 15 and identified 5 individuals as Indian and 11 individuals as SE Asian type, respectively. This finding indicated that in bothcolonies of thattwo localities have the mixture of both Indian and SE Asian type of *Oecophylla smaragdina*, which can be treated as the heterozygous colony often, used for the evidence of hybridization. There was not too many evidence of such heterozygous condition within the colony of *Oecophylla* in India or any other SE Asian country and it is the first report of such mixed colony in Bangladesh as well. These results are in collaboration with findings of Roos *et al*. (2011) about their study of tracing the evolution and hybridization of colobine monkey in the Asian continent.

They found several hybridization patterns by tasting the mitochondrial and nuclear DNA. This hybridization among

Table 2: Detailed locality information with GenBank accession number of nuclear DNA sequencing data.

Locality code	Locality Name	No. of colonies	Upazila	District	Division	Collection Date	Accession number LW*Rh*
L01	Bonpara	1	Baraigram	Natore	Rajshahi	19 Mar. 2014	KY934248
L02	w side of Jamuna Bridge	1	Sirajganj sadar	Sirajganj	Rajshahi	18 Mar. 2014	KY906977
L03	Khulna Univ. Campus	1	Batiaghata	Khulna	Khulna	03 Mar. 2014	KY906981
L04	Batiaghata	1	Batiaghata	Khulna	Khulna	15 Sep. 2013	KY906986
L05	Mollarhat Bazar	1	Mollarhat	Bagerhat	Khulna	29 Oct. 2014	KY906980
L06	Nurbag	1	Kaliakoir	Gazipur	Dhaka	22 Oct. 2014	KY906971
L07	Nimtali	1	Shirajdikhan	Munshiganj	Dhaka	21 Oct. 2014	KY906976
L08	Bejgaon	1	Sreenagar	Munshiganj	Dhaka	21 Oct. 2014	KY906978
L09	Panchdona	1	Norsingi	Norsingdi	Dhaka	20 Oct. 2014	KY906973
L10	Charpara	1	Kaliganj	Gazipur	Dhaka	20 Oct. 2014	KY906970
L11	Tea Resort Center	1	Sreemangal	Moulovibazar	Sylhet	14 Nov. 2014	KY906968
L12	Lauchara National Park	1	Sreemangal	Moulovibazar	Sylhet	15 Nov. 2014	KY906984
L13	Bahubal	1	Bahubal	Habiganj	Sylhet	14 Nov. 2014	KY906988
L14	Sebarhat	1	Senbag	Noakhali	Chittagong	13 Aug. 2015	MF345827
L15	Mohipal Primary School	1	Feni Sadar	Feni	Chittagong	14 Aug. 2015	KY934247
L16	Dighinala HRC	1	Dighinala	Khagrachari	Chittagong	12 Aug. 2015	MF345828
L17	Thanapara Sardah	1	Charghat	Rajshahi	Rajshahi	23 Nov. 2015	KY906985
L18	Nalchiti primary sc. field	1	Nalchiti	Jhalokati	Barisal	15Feb. 2016	KY906988
L19	BRAC More	1	Jhalokati Sadar	Jhalokati	Barisal	15 Feb. 2016	KY906987
L20	Kawkhali Upz P chottor	1	Kawkhali	Pirojpur	Barisal	16 Feb. 2016	KY906982
L21	Panpatti	1	Golachipa	Patuakhali	Barisal	20 Feb. 2016	MF345829)
L22	Mohespur	1	Bakerganj	Barisal	Barisal	10 Feb. 2016	KY906975
L23	Bhaluka Bazar	1	Bhaluka	Mymensingh	Mymensing	12 Nov. 2016	MF345831
L24	BAU campus	2	BAU sadar	Mymensingh	Mymensingh	13 Nov. 2016	MF345832 MF345833
L25	Nandail	1	Muktagacha	Mymensingh	Mymensingh	30 Oct. 2016	MF345834
L26	Sarishabari highschool	2	Sarishabari	Jamalpur	Mymensingh	02 Nov. 2016	MF345835
L27	Madhoyoa	1	Chandina	Comilla	Comilla	21 Oct. 2014	KY906972

Table 3: List of Nuclear DNA haplotypes corresponding to locality.

LW *Rh* Haplotypes	Locality No.
Smaragdina Indian types	L01, L02, L03, L04, L05, L06, L07, L08, L09, L10, L11, L15, L18, L21, L22, L27
Smaragdina SE Asian types	L12, L13, L16, L14, L19, L20, L17, L23, L24, L25, L26

mtDNA type	LW Rh haplotype	Site 1	Site 27	Site 36
SE Asian	Smaragdina A *	A T G C G C G A A C A A G C G A A A A A A A T G A A	T	G T T G C T T C C
SE Asian	Smaragdina B (L11) **	. . . . .	C	. . . . .
SE Asian	Smaragdina B (L15) **	. . . . .	C	. . . . .
Indian	Smaragdina B *	. . . . .	C	. . . . .
	Oecophylla longinoda	. . . . . G	C	. . . . .
	Cataglyphis bombycinus	. . . . . A . . . . . G	C	. . . . .
	Camponatus abdominalis	. . . . . G	C	. . C . . . . .
	Bombus terrestris	. . . . . C . . . . . G	C	. . A . . . . . T

mtDNA type	LW Rh haplotype	Site 37	Site 72
SE Asian	Smaragdina A*	T T G C G A T C G G C C G A G A A T C A G A G T A C C A G T G C C G A A
SE Asian	Smaragdina B (L11) **	. . . . .
SE Asian	Smaragdina B (L15) **	. . . . .
Indian	Smaragdina B*	. . . . .
	Oecophylla longinoda	. . . . .
	Cataglyphis bombycinus	. . . . . A . . C . . . . . C . . . . . C . . . . .
	Camponatus abdominalis	. . . . . C . . . . . C . . . . . C . . T . .
	Bombus terrestris	C . C . . . . . A T . T . . A . . . . . A . A . . . T . . . . .

Fig 2: Sequence allignments for 72bp of LW*Rh* for 6 hymenopteran species including *Oecophylla smaragdina* and *Oecophylla longinoda*. Smaragdina A indicates the *Oecophylla* haplotypes of SE Asian types (group 2) and Smaragdina B indicates the haplotypes of other groups including Indian type as mentioned by Azuma et al (2006). Asteric (*) marks on Smaragdina A and Smaragdina B indicated that it included the *Oecophylla* samples from Bangladesh with similar results. Double asteric (**) on L11 and L15 indicated that in mt DNA analysis it was identified as SE Asian type however in LW *Rh* analysis it grouped into Smaragdina B, *i.e*. as Indian type. Sequences of *Cataglyphis bombycinus*, *Camponotusabdominal*, *Bombus terrestris* and *Oecophylla longinoda* was retrieved from DDBJ GenBank. Site 27 was shown by shaded column. Dot identical with Smaragdina SE Asian type.

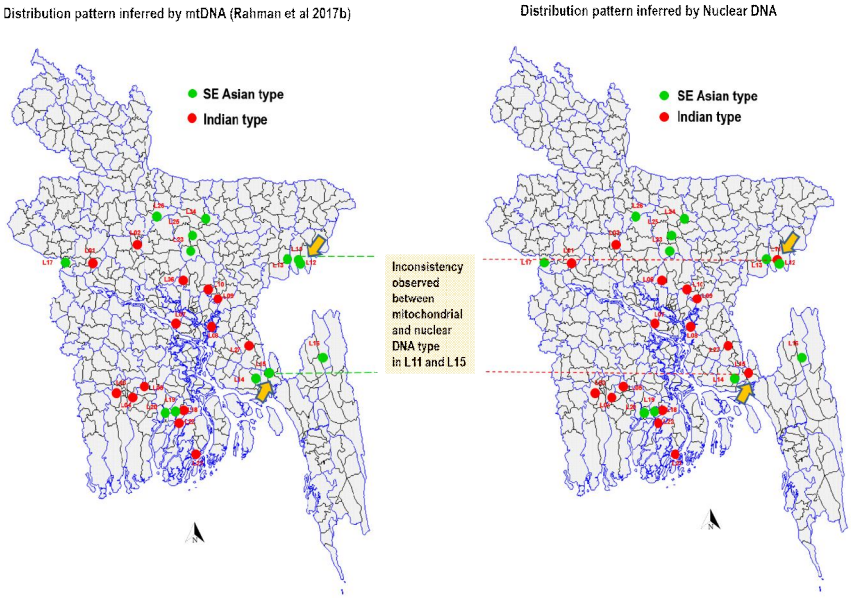

Fig 3: The inconsistency of the distribution pattern of Indian and SE Asian type of *Oecophylla smaragdina* in Bangladesh inferred from mitochondrial and nuclear DNA analysis. Distribution pattern inferred by mitochondrial DNA analysis were retrieved from (Rahman *et al*., 2017b) with modification of the locality numbers. The locality information is the same as mentioned in Table 1.

ancestral lineages most likely causes for the observed phylogenetic incongruences due to the presence of potential contact zones like present Bangladesh, Myanmar and the northeast of India, which is suggested as hybridization area (Karanth *et al*. 2008). However, several big mountains and big rivers in the border region of Myanmar, India and China might have been a possible diversification hotspot (Chakraborty *et al*. 2007) lead to develop such hybridization pattern.

ACKNOWLEDGEMENT

We are thankful to the authority of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Authority of Bangladesh Tea Research Institute, Sreemangal, Sylhet for helping us to collect samples. We express our sincere gratitude to Dr. Masaru Matsumoto, Institute of Tropical Agriculture of Kyushu University, for his technical support. We are grateful to Dr. Noriko Azuma and Ms. Yukiko Asaka, Sapporo, Japan for their valuable advice and suggestions.

We are also thankful to Dr. Akinori Ozaki, Institute of Tropical Agriculture for providing facilities during sampling in Bangladesh. This work was supported in part by JSPS KAKENHI (Grant- in-Aid for Scientific Research (B) Grant Number 26304014, MEXT, Japan.

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