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

M.M. Rahman1,2, S. Hosoishi1, K. Ogata1

**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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in Bangladesh need to be compared with the results of mtDNA sequences. The main objectives of this study were to analyse 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 Samehashi 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.

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 inconsistence, the comparison 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 opsins 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

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

|        |        |        |
|--------|--------|--------|
| LW Rh  | LW RhF | Forward |
|        | LW RhR | Reverse |
| LR 798 F |      | Forward |
| LR 1047 R |    | Reverse |
| LR482 FCR |    | Forward |
| LR 855 R |    | Reverse |

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 | Batiaghat | Khulna | Khulna | 03 Mar. 2014 | KY906981 |
| L04 | Batiaghat | 1 | Batiaghat | 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 | Nintali | 1 | Shirajdkhan | 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 | Seemangal | Moulovibazar | Sylhet | 14 Nov. 2014 | KY906968 |
| L12 | Lauchara National Park | 1 | Seemangal | 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 | Naichiti primary sc. field | 1 | Naichiti | Jhalokati | Barisal | 15Feb. 2016 | KY906988 |
| L19 | BRAC More | 1 | Jhalokati Sadar | Jhalokati | Barisal | 15 Feb. 2016 | KY906987 |
| L20 | Kawkhal Upz P chottor | 1 | Kawkhal | Piripur | Barisal | 16 Feb. 2016 | KY906982 |
| L21 | Panpati | 1 | Golachipa | Patuakhali | Barisal | 20 Feb. 2016 | MF345829 |
| L22 | Mohespur | 1 | Bakerganj | Barisal | Barisal | 10 Feb. 2016 | KY906975 |
| L23 | Bhaluka Bazar | 1 | Bhaluka | Mymensingh | Mymensingh | 12 Nov. 2016 | MF345831 |
| L24 | BAU campus | 2 | BAU sadar | Mymensingh | Mymensingh | 13 Nov. 2016 | MF345832 |

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 |

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| mtDNA type | LW Rh haplotype                        | Site 1 | Site 27 | Site 36 |
|------------|---------------------------------------|--------|---------|---------|
| SE Asian   | Smaragdina A *                        | ATGCCGC A A C A A G C G A A A A A A T G A A ATTTGCTTCC |
| SE Asian   | Smaragdina B (L11) **                 |        |         |         |
| SE Asian   | Smaragdina B (L13) **                 |        |         |         |
| Indian     | Smaragdina B *                        |        |         |         |
| Oecophylla longinoda |                                |        |         |         |
| Cataglyphis bombycinus |                               |        |         |         |
| Camponotus abdominalis |                                 |        |         |         |
| Bombus terrestris |                                 |        |         |         |

| mtDNA type | LW Rh haplotype                        | Site 37 | Site 72 |
|------------|---------------------------------------|---------|---------|
| SE Asian   | Smaragdina A *                        | T T G C G A T C G C C G A A T C A G A T G A C G G A A |
| SE Asian   | Smaragdina B (L11) **                 |        |         |
| SE Asian   | Smaragdina B (L13) **                 |        |         |
| Indian     | Smaragdina B *                        |        |         |
| Oecophylla longinoda |                                |        |         |
| Cataglyphis bombycinus |                               |        |         |
| Camponotus abdominalis |                                 |        |         |
| Bombus terrestris |                                 |        |         |

Fig 2: Sequence alignments 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, Camponotus abdominalis, 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.

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