Ecol Res (2006) 21:126–136
DOI 10.1007/s11284-005-0101-6

ORIGINAL ARTICLE

N. Azuma · K. Ogata · T. Kikuchi · S. Higashi

Phylogeography of Asian weaver ants, *Oecophylla smaragdina*

Received: 21 January 2005 / Accepted: 6 July 2005 / Published online: 3 September 2005

Abstract The phylogeography of *Oecophylla smaragdina* was studied using the mitochondrial cytochrome *b* gene (Cytb), cytochrome oxidase subunit I (COI), and nuclear long-wavelength opsin gene (LW *Rh*). Weaver ants were collected from 35 localities and from one to nine colonies per locality. Neighbor-joining trees inferred from 647 bp of Cytb and 1,026 bp of COI using *Oecophylla longinoda* as an outgroup indicated that the haplotypes of *O. smaragdina* were clearly separated into seven groups: group 1 of India excluding West Bengal; group 2 of Bengal, Indochinese Peninsula, Malay Peninsula and Greater Sunda Islands, including Lombok and Sumbawa; group 3 of the Philippines; group 4 of Flores; group 5 of Sulawesi; group 6 of Halmahera; and group 7 of New Guinea and Australia. This grouping was also supported by a strict consensus tree derived from maximum parsimony and maximum likelihood trees. In addition, two haplotypes of LW *Rh* were found in *O. smaragdina*: one in group 2 and another in all the other groups. Comparison to haplotypes in other hymenopteran species suggests that group 2 is younger than other groups of *O. smaragdina*. The clustering of the seven groups was coincident with geological evidence of the distribution of continents, islands, and seas during glacial periods.

Keywords Mitochondrial DNA · *Oecophylla* · Phylogeography · Weaver ant

N. Azuma (✉)
Laboratory of Breeding Science, Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041-8611, Japan
E-mail: anoriko@fish.hokudai.ac.jp
Tel.: +81-138-408864
Fax: +81-138-408864

K. Ogata
Institute of Tropical Agriculture, Kyushu University, Fukuoka 812-8581, Japan

T. Kikuchi
Faculty of Agriculture, University of the Ryukyus, Okinawa 903-0213, Japan

S. Higashi
Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

Introduction

The weaver ant genus *Oecophylla* (Hymenoptera, Formicidae) is a relatively old genus that prospered especially during the Oligocene and Miocene. Two species are extant: *O. longinoda* (Latreille), distributed in tropical and subtropical Africa, and *O. smaragdina* (Fabricius) in southeastern Asia and Australia (Wheeler 1922; Bolton 1995). Weaver ants are arboreal and play an important role in rainforest ecosystems as a keystone predator of small animals, establishing aggressive and territorial colonies that sometimes dominate a wide range across forest canopies (Hölldobler 1979). *Oecophylla smaragdina* is widespread from southern Asia to northern Australia, including many tropical western Pacific islands (Cole and Jones 1948; Lokkers 1986). The species exhibits a high degree of variation in worker body color; they are light to dark brown in Southeast Asia, but in Australia they are known as "green tree ants" due to the intense green color of the abdomen.

Presently six "subspecies" are nominated including the nominal one, *fuscoides, gracilior, gracillima, selebensis, subnitida* and *smaragdina*, based on the morphological variations and limited distributions of the ants (Bolton 1995), but the relationship between the subspecies categorization and intraspecies phylogeny remains ambiguous. We previously reported three monophyletic local groups in this species (the Asian, Sulawesian, and Australian groups), which were not consistent with morphological grouping or current taxonomy (Azuma et al. 2002). The objective of the present study was to complete the intraspecies phylogeny of *O. smaragdina* using additional samples and molecular markers.

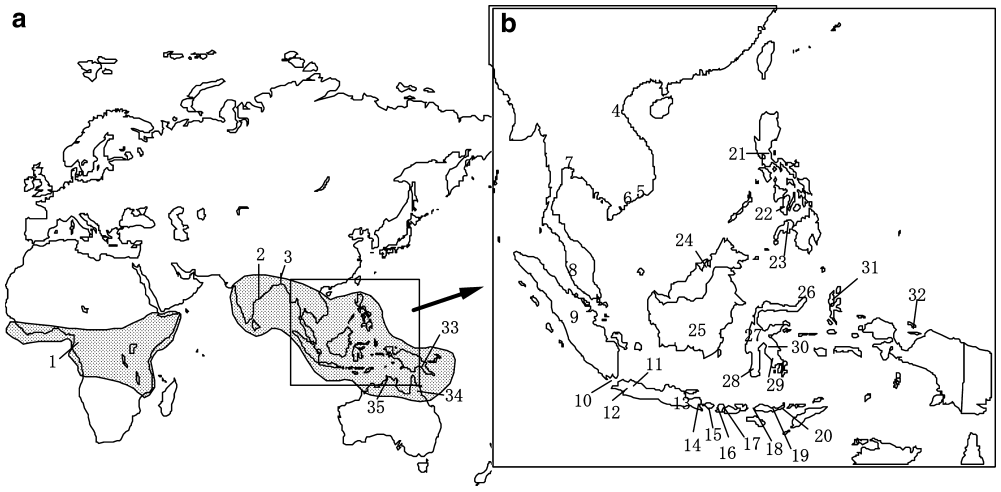

Fig. 1 Maps of Europe, Africa, and Asia (**a**) and Southeast Asia (**b**) showing collection sites. Numbers correspond to locality codes given in Table 1. The current distribution areas of *Oecophylla longinoda* (Africa) and *O. smaragdina* (Asia and Oceania) are enclosed by a *solid line* in (**a**)

Mitochondrial DNA (mtDNA) is one of the most useful genetic markers for phylogeographic studies of animals because of its maternal transmission, extensive intraspecific variation, and lack of recombination (Avise 2000). In this study we examined the mtDNA cytochrome oxidase subunit I gene (COI) and the cytochrome *b* gene (Cytb), which have been used successfully for phylogenetic analysis of *O. smaragdina* (Azuma et al. 2002). Some insect-specific oligonucleotide primers were designed previously for these regions (Crozier et al. 1994; Lunt et al. 1995; Sameshima et al. 1999), and these genes appear to have a proper substitution rate for analyzing the phylogeny among closely related species or conspecific populations of Hymenoptera (Jermiin and Crozier 1994; Wetterer et al. 1998; Sameshima et al. 1999; Chiotis et al. 2000; Leys et al. 2000; Tsutsui et al. 2001; Feldhaar et al. 2003; Johnson et al. 2003).

The nuclear long-wavelength opsin gene (LW *Rh*) belongs to a family of visual pigment genes studied by researchers interested in the molecular evolution of different members of a multiple-gene family (Mollon et al. 1984; Yokoyama and Yokoyama 1990; Neitz et al. 1991; Yokoyama 1995; Chang et al. 1996). Since Mardulyn and Cameron (1999) tested the utility of LW *Rh* as a genetic marker for phylogeny among bee species, however, LW *Rh* also has been regarded as a useful marker for between-species level phylogeny of insects, especially Hymenoptera (Ascher et al. 2001; Cameron and Williams 2003). *Oecophylla smaragdina* is widespread in Southeast Asia and Australia, and the gene LW *Rh* appears to be useful for analyzing phylogeny among geographically distant populations.

Methods

Adult *Oecophylla* workers were collected from 152 colonies at 35 localities (Fig. 1) by sampling one to nine colonies per locality (Table 1), except in Bali and its neighboring islet Nusa Lembongan, where 26 colonies were collected within a limited area. Samples include *O. longinoda* collected from one colony in Cameroon.

Total genomic DNA was extracted from alcohol-preserved specimens by the CTAB method of Hillis et al. (1990) and Navarro et al. (1999) with slight modification. Each specimen was torn and suspended in 0.7 ml of 2-CTAB buffer [100 mM Tris–HCl, pH 8, 1.4 mM NaCl, 20 mM EDTA, 2% hexadecyltrimethyl-ammonium bromide (CTAB)] with 200 mg polyvinylpolypyrrolidone and 0.2 mg of proteinase-K; samples were incubated at 55°C for 2h and at 37°C overnight after vortex mixing. The supernatant was twice extracted with chloroform, then ethanol or isopropanol precipitation was performed, and the pellet was resuspended in 20 µl distilled water. The colony mates of the specimens used for DNA analyses were deposited in the Hokkaido University Museum after DNA extraction.

DNA amplification using the polymerase chain reaction (PCR) was performed with the AmpliTaq Gold PCR Kit (Applied Biosystems) according to the manufacturer's instructions. Reaction mixtures (25–50 µl) containing 2 U of *Taq* polymerase, a pair of oligonucleotide primers (0.5 µM each; Table 2), dNTP (0.2 mM), Tris–HCl pH 8.4 (20 mM), KCl (50 mM), $MgCl_2$ (2.5 mM), and the total genomic DNA as a template (0.5–55 µl) were run in an automated thermal cycler (PCR thermal cycler SP, Takara). The thermal cycling parameters for Cytb and COI basically followed Crozier et al. (1994) and Sameshima et al. (1999), respectively, including 95°C for 5 min for hot start, 35 cycles of dissociation (92°C, 1 min), annealing (50°C for Cytb and 54°C for COI, 1 min), and extension (70°C, 2 min). For LW *Rh*, the PCR parameters were the same as for mitochondrial genes; thermal cycling parameters were nearly the same, except the annealing temperature was 60°C for this region. The sequences and positions for mitochondrial DNA of the primers for PCR and sequencing are shown in Table 2 and Fig. 2. Purified PCR products were sequenced using the Dye Terminator Cycle Sequence Kit or Big Dye Terminator Cycle Sequence Kit V1.1 (Applied Biosystems) and an auto-

Table 1 Sampling sites and the numbers of colonies collected

Species	Code	Locality	Country (island or state)	No. of colonies
Oecophylla longinoda	1	Campo Forest Reserve	Cameroon	1
Oecophylla smaragdina	2	Vishakhapatnam	India (Andra-Pradesh)	5
	3	Nurbag Gapzier	Bangladesh	3
	4	Hung Loc	Vietnam	2
	5	Hat Lot	Vietnam	2
	6	Ho Chi Minh	Vietnam	3
	7	Bangkok	Thailand	5
	8	Kuala Lumpur	Malaysia	6
	9	Solok	Indonesia (Sumatra)	4
	10	Krakatau	Indonesia (Krakatau)	3
	11	Jakarta	Indonesia (Java)	4
	12	Bogor	Indonesia (Java)	5
	13	Surabaya	Indonesia (Java)	5
	14	Banyuwangi	Indonesia (Java)	3
	15	Bali	Indonesia (Bali)	26
	16	Mataram	Indonesia (Lombok)	4
	17	Sunbawa besar	Indonesia (Sunbawa)	3
	18	Lewoleba	Indonesia (Flores)	3
	19	Maumera	Indonesia (Flores)	3
	20	Larantuka	Indonesia (Flores)	2
	21	Manila	Philippines (Luzon)	3
	22	Moalboal	Philippines (Negros)	1
	23	Dipolog	Philippines (Mindanao)	3
	24	Poring	Malaysia (Kalimantan)	3
	25	Palangkaraya	Indonesia (Kalimantan)	6
	26	Manade	Indonesia (Sulawesi)	2
	27	Mangkutana	Indonesia (Sulawesi)	2
	28	Ujung Pandang	Indonesia (Sulawesi)	5
	29	Lanowulu	Indonesia (Sulawesi)	2
	30	Soroako	Indonesia (Sulawesi)	2
	31	Ternate	Indonesia (Halmahera)	7
	32	Biak	Indonesia (Biak)	3
	33	Port Moresby	Papua New Guinea	3
	34	Cairns	Australia	12
	35	Darwin	Australia	6
Total				151

Table 2 Primers for amplifying and sequencing mitochondrial Cytb and CO I genes and nuclear LW *Rh* gene. The positions of primers for mitochondrial genes follow the complete sequence of mitochondrial DNA of *Apis mellifera* (Crozier and Crozier 1993)

Region	Name	Direction	Sequence (5′–3′)[a]	Position
Cytb	Cb 1[d]	Forward	TATGTACTACCATGAGGACAAATATC (1)	11,400–11,425
	tRs[c]	Reverse	TATTTCTTTATTATGTTTTCAAAAC (1)	12,250–12,226
	Cb1.5 F[b]	Forward	GAGATTTATATAAAATTCCT	11,596–11,616
	Cb1.5R[b]	Reverse	AGGAATTTTATATAAATCTC	11,596–11,616
	Cb 3[b]	Forward	CCAATTCATATTCAACC	11,777–11,794
	Cb 4R[d]	Reverse	CTCATATTTTTATAATTAGAAATGAT	12,100–12,126
COl	COl 1–3[d]	Forward	ATAATTTTTTTTATAGTTATACC (2)	1,981–2,002
	COl 2–1[b]	Forward	CTTTATCAACATTTATTTTGATTTTT (2)	2,481–2,499
	COl 2–4[d]	Reverse	TCCTAAAAAATGTTGAGGAAA (2)	3,063–3,083
	COl 660R[b]	Reverse	GCTGAAGTAAAATAAGCTCGTG	2,688–2,710
LW *Rh*	LW *Rh*F[d]	Forward	AATTGCTATTAYGARACNTGGGT (3)	
	LW *Rh*R[d]	Reverse	ATATGGAGTCCANGCCATRAACCA (3)	

[a](1) Crozier et al. 1994, (2) Lunt et al. 1995, (3) Mardulyn and Cameron 1999
[b]Used only for sequence
[c]Used only for PCR
[d]Used for both PCR and sequence

mated sequencer (Genetic Analyzer models 310 and 3100, Applied Biosystems).

For preliminary tests of the sequence variation in each colony, we sequenced 5–10 individuals from each of several colonies for the Cytb and COI regions. In each case, all colony mates shared an identical haplotype; therefore, we used only one individual per colony as a representative for these loci. Only one individual per colony was chosen for sequencing the nuclear gene LW *Rh*, which generally evolves much more slowly than

mtDNA. In total, 152 individuals from 152 colonies were examined for the three loci. The sequence data of mitochondrial genes are deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers 185853–185884 (Cytb) and 185453–185491 and 185818–185830 (COI).

Haplotypes of Cytb and COI were aligned, and the numbers of transversions and transitions between pairwise sequences were counted separately at first and second or third codon positions to determine the weight for a maximum parsimony tree (Nei and Kumar 2000) and to check saturations with substitutions. The number of variable or informative sites and nucleotide frequency were also checked to decide whether the two regions could be validly concatenated.

In the analysis of the intraspecies phylogeny of *O. smaragdina*, *O. longinoda* was used as an outgroup. Phylogenetic trees inferred from concatenated sequences of Cytb (647 bp) and COI (1,026 bp) were reconstructed in PAUP*4.0 Beta Version 8 (Swofford 2002) using neighbor-joining (NJ; Saitou and Nei 1987), weighted maximum parsimony (MP), and maximum likelihood (ML) methods. The best model of DNA substitution was identified by a hierarchical likelihood-ratio test using the programs MODELTEST 3.6 (Posada and Crandall 1998) and PAUP*. In the MP method, 100 MP trees having equal total branch length were chosen and reconstructed into a >50% strict consensus tree. Bootstrap values (Felsenstein 1985) were estimated to provide measures of relative support for each branch using 1,000, 300 and 300 replications in NJ, MP and ML, respectively.

For estimation of a molecular clock, pairwise sequence diversity was calculated by Kimura's (1980) two-parameter method by examining substitutions at all sites and only at first and second codon positions of Cytb.

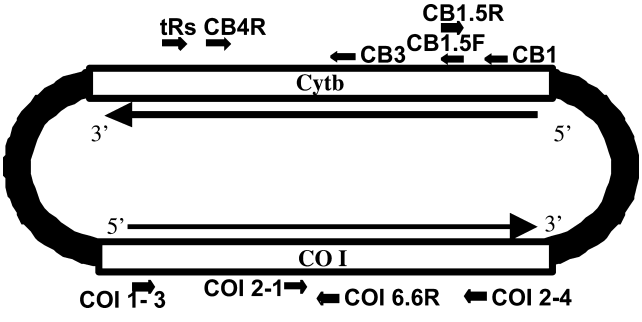

Fig. 2 Positions of the primers for PCR and sequencing of the Cytb and COI genes. Directions of primers and transcription are shown by *short arrows* and *long arrows*, respectively

Results

Nucleotide substitutions in mitochondrial genes

In addition to 26 Cytb haplotypes found in our previous study (Azuma et al. 2002), in this study we discovered 30 and 52 haplotypes in Cytb and COI, respectively.

In Cytb and COI, 128 and 176 sites were variable and 103 and 121 informative, respectively. In both genes, transitions (Ti) were proportional to transversions (Tv) with the ratio of Ti:Tv of ca. 3.0 (Fig. 3), suggesting no saturation with substitutions; the substitutions occurred more than twice as frequently in third codon positions than in first or second positions (Fig. 3). Both genes also had similar nucleotides frequencies (adenine: 32–33%, cytosine: ca. 13%, guanine: 9–12%, thymine: 42–45%). These similarities in Cytb and COI suggest that the concatenated sequences of the two regions may be used as operational taxonomic units (OTUs) in phylogenetic trees.

Fifty-six concatenated sequences were completed; in Fig. 4 and Fig. 6 each sequence is represented by country or island name with a locality code in parentheses, for example, India (2). When two or more different sequences were detected from one locality, they were given different numbers. In India, for instance, there were two sequences, India (2)1 and India (2)2.

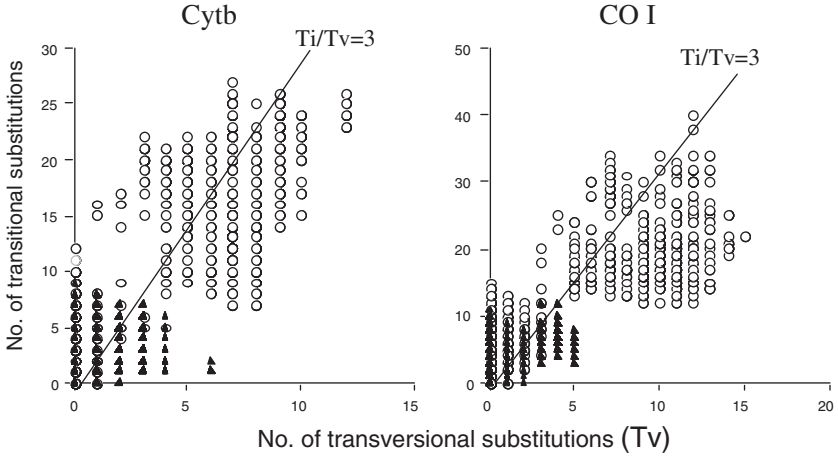

Fig. 3 Transitions plotted against transversions for all pairwise sequences of Cytb and COI of *Oecophylla smaragdina*. *Circle* third codon position, *triangle* first or second codon positions

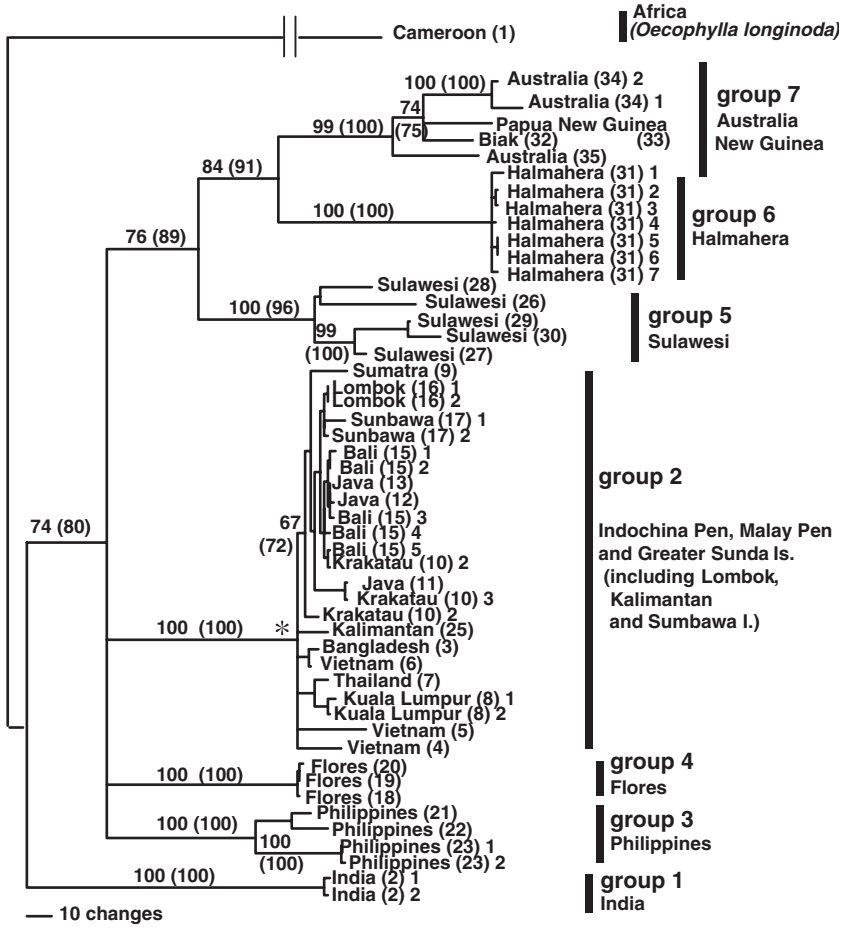

Fig. 4 Neighbor-joining tree using all substitutions based on the GTR + I + R model inferred from 56 of the concatenated sequences of the COI gene (1,026 bp) and the Cytb gene (647 bp) in *Oecophylla smaragdina*, with *O. longinoda* as an outgroup. *Numbers above or below branches* are bootstrap probability values derived from 1,000 replications; *adjacent numbers in parentheses* are bootstrap values (%) for the same nodes based on weighted maximum parsimony analyses from 300 replications

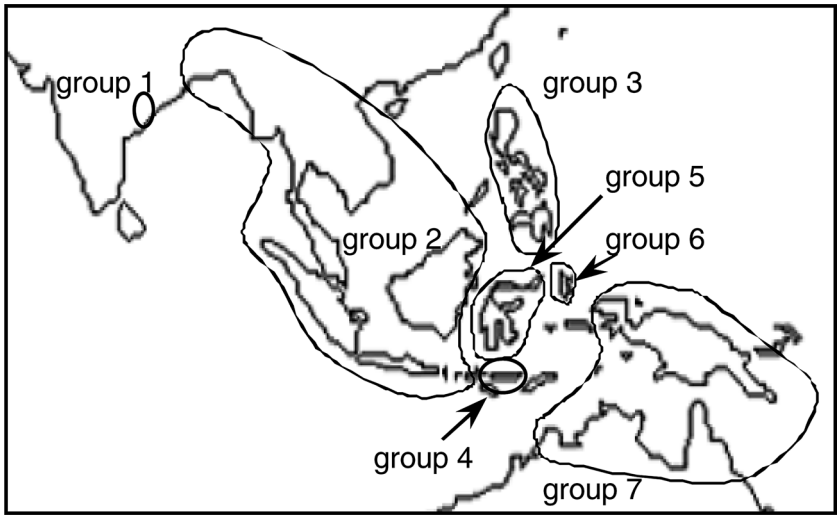

Fig. 5 Seven regions based on haplotype grouping of the mitochondrial genes Cytb and COI. Group 1 of India excluding West Bengal; group 2 of Bengal, Indochinese Peninsula, Malay Peninsula and Greater Sunda Islands, including Lombok and Sumbawa; group 3 of the Philippines; group 4 of Flores; group 5 of Sulawesi; group 6 of Halmahera; and group 7 of New Guinea and Australia. This grouping is based on the analysis of DNA samples collected from only 35 localities; the borders are arbitrary to some extent, as it is unknown which genetic types are distributed outside the borders

Molecular phylogenetic trees for mitochondrial DNA

A NJ tree was reconstructed using the GTR + I + G substitution model (Lanave et al. 1984; Swofford et al. 1996), which was suggested as the best fit for the concatenated sequence data by MODELTEST. The tree (Fig. 4) shows that *O. smaragdina* is separated deeply into seven phylogenetic groups: group 1 of India excluding West Bengal; group 2 of Bengal, Indochinese Peninsula, Malay Peninsula and Greater Sunda Islands, including Lombok and Sumbawa; group 3 of the Philippines; group 4 of Flores; group 5 of Sulawesi; group 6

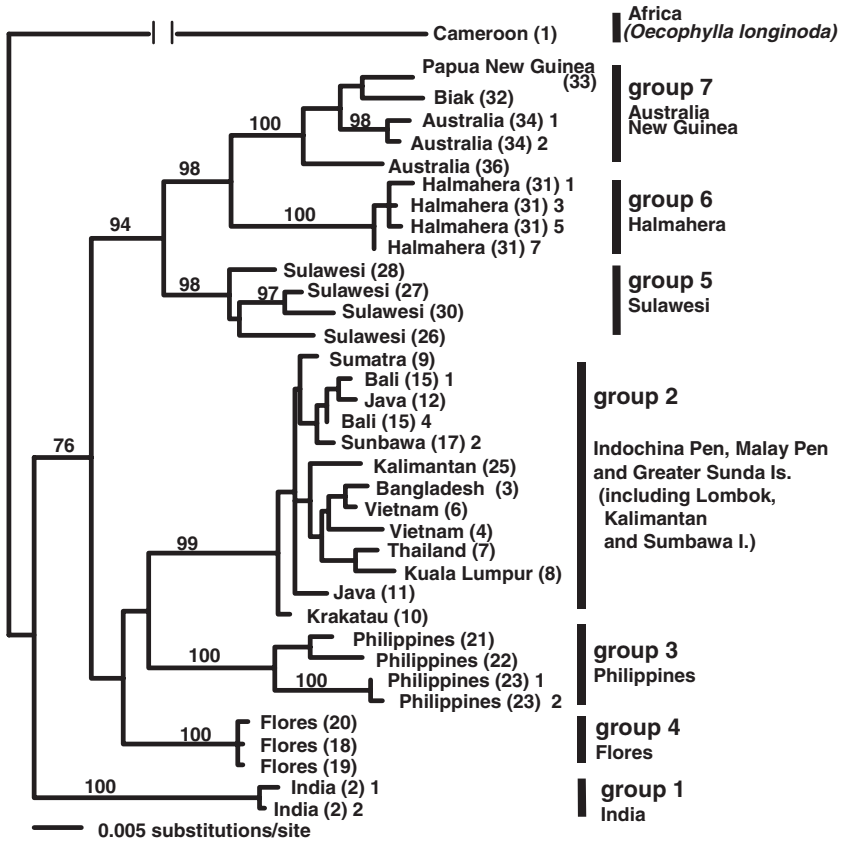

Fig. 6 Maximum-likelihood tree based on the GTR + I + R model inferred from 35 of the concatenated sequences of the COI gene (1,026 bp) and the Cytb gene (647 bp) in *Oecophylla smaragdina*, with *O. longinoda* as an outgroup. *Numbers above or below branches* are bootstrap probability values derived from 300 replications

of Halmahera; and group 7 of New Guinea and Australia. Geographic distribution of the seven groups is shown in Fig. 5. MP trees were searched using the weighting scheme 1:3 for Ti:Tv and 2:1 for the first or second codon position versus the third codon position. A strict consensus tree from 100 weighted MP trees (not shown) had a topology similar to the NJ tree except for some branches within groups. Monophyly of each *O. smaragdina* group is supported by high bootstrap values in both methods.

The bootstrap values were not high enough to resolve hierarchical relationships among groups, but some information was available. Group 1 appears to have separated from other groups first, and groups 5, 6, and 7 form a clade, supported with relatively high bootstrap values.

To decipher the detailed relationships among groups, a ML tree was also reconstructed for concatenated sequences (Fig. 6). We used only representative OTUs chosen from members of each group based on the NJ and MP analyses of the entire dataset, to facilitate a calculation of the bootstrap values. The monophylies of groups 5, 6 and 7, and of groups 6 and 7, were supported with higher bootstrap values than in NJ and MP trees.

The internal branches (e.g., the lateral branches from the nodes with asterisks to each tip of the group 2 OTUs in Fig. 4) were shorter in group 2 than in group 5 or group 7, indicating that the degree of genetic divergence among the group members is lower within group 2 than in group 5 or group 7 (Figs. 4, 6). The short branches within group 2 suggest that the radiation into the present populations began relatively recently, and that only one lineage survived and diverged into the present populations. The internal branches are remarkably short in the Greater Sunda Islands (Sumatra, Bali and Java), Lombok, and Sumbawa, where many islands are scattered across a large area, strongly supporting the theory of recent radiation in this region.

Cytb sequence divergence among lineages and estimation of a molecular clock

Table 3 shows between-group mean sequence divergences of Cytb estimated by Kimura's two-parameter method. In this calculation, the Cytb sequence of *O. longinoda* from Kenya (DDBJ accession no. AB056074) was also used. Using all substitutions, the genetic distance was 7.3–9.0% between *O. smaragdina* and *O. longinoda* and 3.0–6.3% between groups within *O. smaragdina*. The genetic distance was also calculated using only substitutions at the first and second codon positions (Table 3) in accordance with the method of Crozier et al. (1997), who estimated the average evolutionary rate of ant Cytb to be 0.165% per million years at the first and second codon positions. When this substitution rate was adopted, *O. smaragdina* and *O. longinoda* were estimated to have diverged 13.3–11.3 Ma

Table 3 Sequence divergence (%) according to Kimura's two-parameter estimation for Cytb gene (647 bp) among one *Oecophylla longinoda* group and seven *O. smaragdina* groups. *Upper right* All positions, *lower left* only first and second codon positions

	O. longinoda	*O. smaragdina*						
	Africa	India	Australia, New Guinea	Halmahera	Sulawesi	Solor Island	Phillipine	Indochina Pen., Malay Pen., Greater Sunda Island
O. longinoda								
Africa								
Mean		9.04	7.50	7.81	7.34	7.50	7.81	8.12
SD		0.11	0.11	0.33	0.33	0.54	0.54	0.54
O. smaragdina								
India								
Mean	2.20		5.98	6.37	5.27	4.59	6.25	5.27
SD	0.37		0.36	0.16	0.23	0.15	0.13	0.29
Australia, New Guinea								
Mean	2.04	1.28		3.82	3.89	3.77	3.97	5.46
SD	0.28	0.19		0.35	0.24	0.30	0.25	0.37
Halmahera								
Mean	2.04	0.78	0.90		4.08	4.16	4.52	6.03
SD	0.33	0.00	0.12		0.19	0.18	0.16	0.29
Sulawesi								
Mean	2.23	1.12	1.15	0.81		3.19	3.45	4.77
SD	0.34	0.07	0.20	0.06		0.17	0.27	0.30
Solor Island								
Mean	1.88	0.93	0.97	0.68	0.90		2.98	3.87
SD	0.36	0.10	0.25	0.08	0.14		0.21	0.28
Phillipine								
Mean	1.72	0.78	0.59	0.62	0.65	0.62		3.87
SD	0.36		0.12	0.00	0.07	0.15		0.22
Indochina Peninsula, Malay Peninsula, Greater Sunda Island								
Mean	1.87	0.90	1.13	1.06	1.24	0.91	0.75	
SD	0.34	0.13	0.17	0.12	0.13	0.19	0.12	

(million years ago), in the late Miocene, with subsequent divergence among the seven groups of *O. smaragdina* between 7.8 and 3.6 Ma, corresponding to the late Miocene and middle Pliocene. The diversification within each group began 4.7 Ma for group 7, 3.7 Ma for group 4, 2.1 Ma for group 5, and 1.6 Ma for group 2, from the middle Pliocene to early Pleistocene. The estimation was not possible for group 3 and group 6, because all substitutions between haplotypes were at third codon positions.

Sequences of LW *Rh*

Three LW *Rh* haplotypes (Longinoda, Smaragdina A, and Smaragdina B) were determined in *Oecophylla* from a 528-bp sequence including a 93-bp intron (Fig. 7). 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*.

All samples from group 2 had the haplotype Smaragdina A, whereas all the other *O. smaragdina* groups had Smaragdina B. Between these two haplotypes, there was only one substitution: site 466 contains thymine in Smaragdina A and cytosine in Smaragdina B. This substitution is in a coding region but is synonymous and transitional. Since all the other haplotypes, including Longinoda, had cytosine at site 466, this thymine substitution is parsimoniously considered to be derived, suggesting strong monophyly and isolation of group 2.

Discussion

Phylogeography of *Oecophylla smaragdina*

Based on this and our previous study (Azuma et al. 2002), we propose the following evolutionary scenario of intraspecies divergence in *O. smaragdina*. In the early Miocene, several species of *Oecophylla* prospered, per-

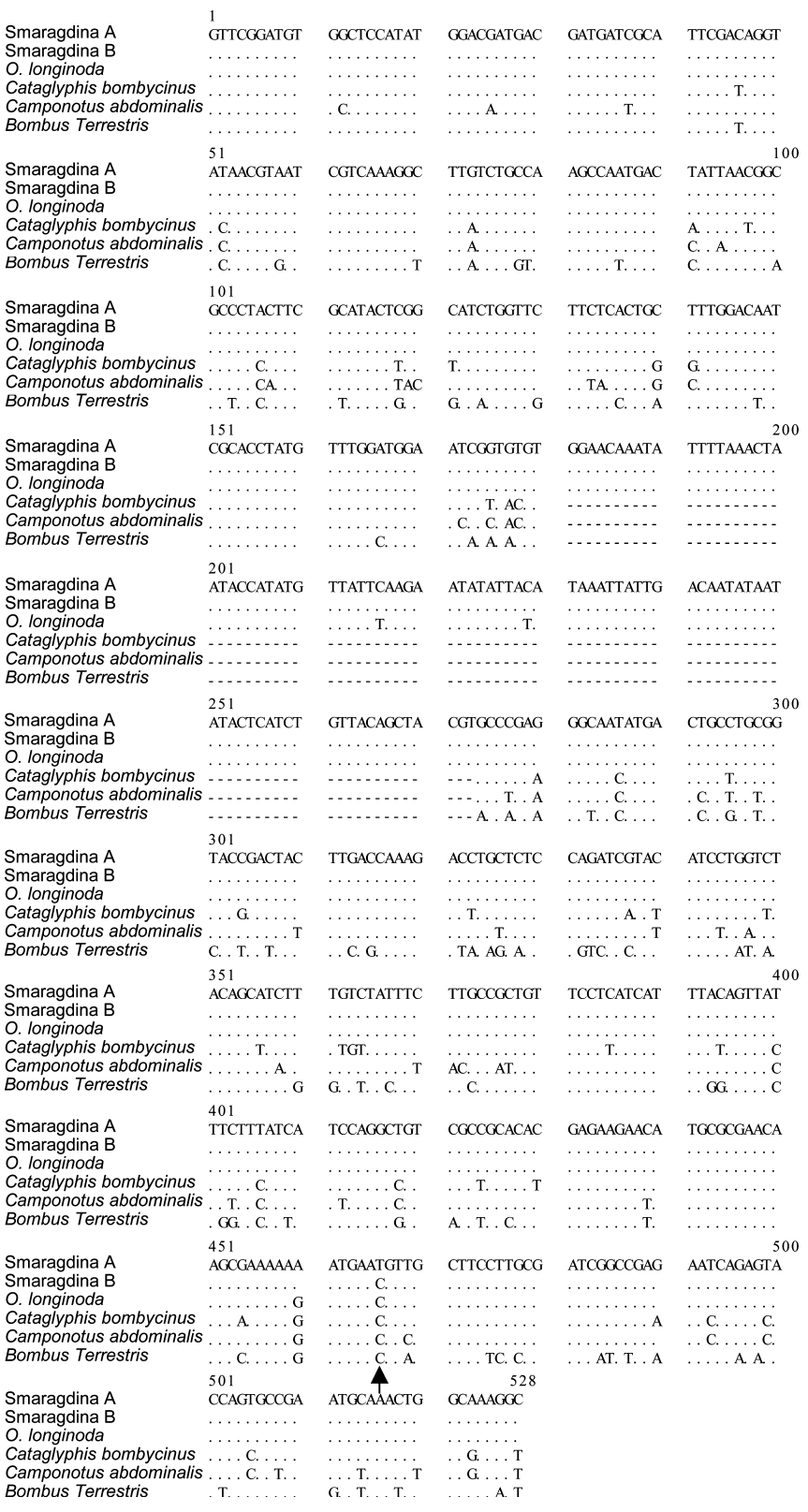

Fig. 7 Sequence alignments for 528 bp of LW *Rh* for six hymenopteran species including *Oecophylla smaragdina* and *O. longinoda*. The haplotype Smaragdina A was found only in group 2 of *O. smaragdina*, while all the other groups of this species had haplotype Smaragdina B. Sequences of *Cataglyphis bombycinus*, *Camponotus abdominalis*, and *Bombus terrestris* are cited from the DDBJ database (see text for accession nos.). Site 466 is shown by an *arrow*. *Dot* Identical with Smaragdina A, *hyphen* intron and no sequence data

haps including *O. sicula* discovered from a Miocene deposit in Sicily (Emery 1891; Bolton 1995), which is regarded as a common ancestor of extant *Oecophylla* species (Wilson and Taylor 1964). *O. longinoda* and *O. smaragdina* were established in the middle or late Miocene. Temperatures were relatively high and reached a peak in the middle Miocene; thereafter, temperatures continued to fall through the following Pliocene into the glacial periods of the Pleistocene (Hall and Holloway 1998; Morley 2000). *O. sicula* was likely divided into

several isolated populations by the middle or late Miocene, and two of them evolved into the extant species *O. longinoda* and *O. smaragdina*. Following the Pliocene cooling, a desert expanded over North Africa to the Middle East (Morley 2000) and completely isolated *O. smaragdina* from *O. longinoda*. The first intraspecific divergence of *O. smaragdina* occurred in the late Miocene or early to middle Pliocene.

Our phylogenetic analyses suggest that the group 1 was established first, followed by the dispersal from Asia to Australia through Sulawesi and New Guinea but perhaps not through Lesser Sunda Islands. After the ancestral colonies reached Australia and New Guinea, settled in, and started to disperse, *O. smaragdina* diverged into the present populations in the area of group 2. Although the detailed relationships among haplotypes of group 2 are not strongly supported by bootstrap values (Figs. 4, 6), probably because the radiation was too recent and rapid to be detected by these DNA markers, the NJ and MP trees agreed that the haplotypes from Greater Sunda Islands belong to the same cluster and seem to have diverged more rapidly and recently than those from the Indochina and Malay Peninsulas. The radiation in group 2 is estimated to have started in the middle Pleistocene, and this dating is consistent with the transgression and regression of the land area in Asia. In the glacial periods of the Pleistocene, the sea level was about 200 m lower than today. Most of Greater Sunda Islands and Kalimantan were probably connected to the Asian continent as Sundaland (Heaney 1986). The current continental shelves are considered to have delineated the boundaries of landmasses in the glacial periods. The Asian landmass was isolated from the Australia–New Guinea landmass throughout the Pleistocene; and Sulawesi, Halmahera, Flores, and the Philippines were independent from the two landmasses even during the glacial periods. In the trees, group 6 of Halmahera appeared as a sister group of group 7, suggesting vicariance occurred between the populations of group 6 and group 7 at a relatively recent stage. Five million years ago, when the main islands of Indonesia, including New Guinea, and the Asian and Australian continents were in their present locations, the island of Halmahera had not yet formed. At that time, the small rock islets that would eventually combine to form Halmahera between 3 and 1 Ma were moving westward in the northern sea of New Guinea (Hall and Nichols 1990; de Jong R 1998; Hall and Holloway 1998). This geological history was probably an important factor in the close relationship between groups 6 and 7.

The molecular phylogenetic trees suggest little diversity among the members of group 2 (Figs. 4, 6). The genetic divergence within group 2 should be higher than that in group 5 or group 7, however, for two reasons. First, members of group 2 were distributed across a much wider area than those of group 5 or group 7. Second, considering geographic locations, Asian populations that must have included ancestors of group 5 or group 7 members, should have settled and started to disperse into present populations earlier than in group 5 or group 7. Therefore, an extraordinary event such as a bottleneck may have caused a population decline or replacement by only one lineage in group 2 before the recent divergence into the present populations.

Population decline is more likely than replacement by newcomers without an initial decline of the original settlers. *Oecophylla smaragdina* exhibits extremely aggressive territorial behavior (Hölldobler and Wilson 1977, 1990; Hölldobler 1979). If an area is inhabited by a stable population, newcomers rarely succeed in founding their colonies at a nearby site. Thus, some force likely depopulated or weakened the earlier inhabitants in the Indochina Peninsula, Malay Peninsula, and Greater Sunda Island regions before the radiation into present populations.

Several factors may have caused this population decline, including diseases, predation, or competition within or among species. Environmental changes, such as submergence of lowlands, volcano eruptions, or the contraction and fragmentation of rainforests under the cold and dry climate in glacial periods, are also plausible reasons for the decline. According to Brandon-Jones (1996), evidence of mammals on Java is absent before the first major sea-level recession 2.4 Ma, suggesting that before that time Java may have been largely submerged. Our studies suggest that *O. smaragdina* in the Indochina Peninsula, Malay Peninsula, and Greater Sunda Islands started its radiation around 1.6 Ma. Brandon-Jones (1996) also suggested that contraction and fragmentation of rainforests caused extinction of some species of primates around 0.19 Ma. Similar events may have occurred at the end of the Pliocene or early Pleistocene when temperatures were falling. According to Brandon-Jones (1996), during such contraction of rainforests in glacial periods, two refuges existed on the Asian continent, a large one in northern Indochina and a small one near the southern tip of India. The existence of an Indian refuge would also explain the independence of group 1 from group 2; the latter included samples from Nurbag Gazipur in Bangladesh, located only 1,200 km away along the Bengal Gulf from Vishakhapatnam, where Indian specimens were sampled. If the Indian population originated from the Indian refuge and the Bangladesh population originated from the Indochina refuge, the deep genetic separation between them is reasonable. The pattern of divergence in group 2 might be due to a combination of submergence of lowlands or islands, deforestation caused by cold and drought in inland Asia, and other environmental changes.

How did *Oecophylla smaragdina* disperse across the waters?

Of all ant species, *O. smaragdina* is the most widespread over ocean islands, except such tramp species as *Linepithema humile*, *Solenopsis invicta*, and *Pheidole megacephala*, which are rapidly expanding their distribution

through human activity (Hölldobler and Wilson 1990; Passera 1994; Wild 2004). The dispersal of *O. smaragdina* without human intervention indicates that the species has the ability to disperse across wide water barriers.

Ants generally disperse through nuptial flights of winged queens; however, most ant species using nuptial flight are restricted to terrestrial migration because the alate queens shed their wings as soon as they have been inseminated by males (Hölldobler and Wilson 1990). Passive dispersal by wind can transport an inseminated queen to another island. Because *O. smaragdina* is an arboreal species, inseminated queens may be more likely to be lifted by the wind than terrestrial ants.

Rafting has been considered effective for between-island dispersal of several species of insects (Ikehata 1977; Kimoto 1979; Thornton 1996), and weaver ants construct light and waterproof nests of leaves that appear to be a preadaptation for rafting dispersal. Life strategies in ants are sometimes so plastic that we cannot explain the ancient means of dispersal by present behaviors. However, *O. smaragdina* practices pleometrosis, or colony founding by more than one female (Peeters and Andersen 1989; Peng et al. 1998), suggesting that rafting might be more effective than aerial dispersal in this species. Pleometrotic organisms require cooperation to establish new colonies (Peng et al. 1998), and it would be unlikely that wind would transport multiple queens to the same destination over the sea. Rafting, however, could transport many workers and broods with the queen or reproductive pupae or larvae with workers, making reconstruction of their colony easy.

In the Krakatau Islands, which are 15 and 35 km away from Sumatra and Java, respectively, the flora and fauna were totally destroyed by the great eruption in 1883. Weaver ants were reported on the islands in 1908, only 25 years after the eruption (Jacobson 1909). On Anak Krakatau Island, which emerged above sea level in August 1930, we found unique COI and Cytb haplotypes genetically close to the haplotypes in neighboring islands, suggesting the relatively frequent colonization by rafting that was described by Thornton (1996).

Genetic clusters and infraspecific taxonomy

Since J.C. Fabricius first named the Indian and Australian weaver ants *Formica smaragdina* and *F. virescens*, respectively, in 1775, the taxonomic classification of Asian and Australian weaver ants has undergone many changes. The current taxonomy classifies all of them as belonging to one species, *O. smaragdina* (Bolton 1995). Although Bolton (1995) listed six subspecies, a valid practice according to the ICZN rules, many ant taxonomists are doubtful about ranking subspecies of ants (Wilson and Brown 1953; Bolton 1995; Brown 2000). The present study demonstrates that the classification of subspecies is not consistent with the genetic clusters in *O. smaragdina*. In addition, we cannot find any new morphological characters that distinguish the seven genetic clusters from each other. More detailed comparisons of type materials will be needed to examine the validity of subspecies in *O. smaragdina*, something that is beyond the scope of the present study.

Acknowledgements We express our sincere thanks to Dr. A.N. Andersen, CSIRO Tropical Ecosystems Research Centre, Australia; Dr. K Tsuji, University of the Ryukyus; Dr. Sih Kahono, The Indonesian Institute of Science; and our colleagues Dr. H. Miyata, Dr. H. Hirosawa, and Mr. T. Shimamura for providing samples. Cordial thanks are also due to Dr. H. Suzuki, GSEES, Hokkaido University, for technical support and useful suggestions. This study was primarily supported by a JSPS Grant-in-Aid (no. 13640623) and in part by a Grant-in-Aid for Overseas Research Projects (nos.14255016 and 17255001) from the Ministry of Education, Science, Sports and Culture, Japan, and by Hokkaido University JSPS Core University Program on Environmental Conservation and Land Use Management of Wetland Ecosystems in Southeast Asia.

References

Ascher JS, Danforth BN, Ji S (2001) Phylogenetic utility of the major opsin in bees (Hymenoptera: Apoidea): a reassessment. Mol Phylogenet Evol 19–1:76–93

Avise JC (2000) Empirical roots of mitochondrial DNA reserach. In: Phylogeography. Harvard University Press, Cambridge, pp 9–22

Azuma N, Kikuchi T, Ogata K, Higashi S (2002) Molecular phylogeny among local populations of weaver ant *Oecophylla smaragdina*. Zool Sci 19:1321–1328

Bolton B (1995) A new general catalogue of the ants of the world. Harvard University Press, London

Brandon-Jones D (1996) The Asian Colobinae (Mammalia: Primates: Cercopithecidae) as indicators of quaternary climatic change. Biol J Linn Soc 59:327–350

Brown WL Jr (2000) Diversity of ants. In: Agosti D, Majer JD, Alonso LE, Schultz T (eds) Ants: standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press, Washington DC, pp 45–79

Cameron SA, Williams PH (2003) Phylogeny of bumble bees in the new world subgenus *Fervidobombus* (Hymenoptera: Apidae): congruence of molecular and morphological data. Mol Phylogenet Evol 28:552–563

Chang BSW, Ayers D, Smith WC, Pierce NE (1996). Cloning of the gene encoding honeybee long-wavelength rhodopsin: a new class of insect visual pigments. Gene 173:215–219

Chiotis M, Jermiin LS, Crozier RH (2000) A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. Mol Phylogenet Evol 17–1:108–116

Cole AC Jr, Jones JW (1948) A study of the weaver ant, *Oecophylla smaragdina* (Fab.). Am Midl Nat 39:641–651

Crozier RH, Crozier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 113:97–117

Crozier RH, Dobric N, Imai HT, Graur D, Cornoet J-M, Taylor RW (1994) Mitochondrial-DNA sequence evidence on the phylogeny of Australian jack-jumper ants of *Myrmecia pilosula* Complex. Mol Phylogenet Evol 4–1:20–30

Crozier RH, Jermiin LS, Chiotis M (1997) Molecular evidence for a Jurassic origin of ants. Naturwissenschaften 84:22–23

Emery C (1891) Le formiche dell'ambra Siciliana nel Museo Mineralgico dell'Universita di Bologna. Memorie della R. Accademia delle Scienze dell' Istituto di Bologna 1(5):141–165

Feldhaar H, Fiala B, Gadau J, Mohamed M, Maschwitzd U (2003) Molecular phylogeny of *Crematogaster* subgenus *Decacrema-ants* (Hymenoptera: Formicidae) and the colonization of

Macaranga (Euphorbiaceae) trees. Mol Phylogenet Evol 27:441–452
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Gathorne-Hardy FJ, Jones DT, Mawdsley NA (2000) The recolonization of the Krakatau islands by termites 8Isoptera), and their biographical origins. Biol J Linn Soc 71:251–267
Hall R, Holloway JD (1998) SE Asian geology and biogeography: an introduction. In: Biogeography and geological evolution of SE Asia. Buckhuys Publishers, Leiden, pp 120
Hall R, Nichols GJ (1990) Terrane amalgamation in the Philippine Sea margin. Tectonophysics 181:207–222
Heaney L (1986) Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. Biol J Linn Soc 28:127–165
Hillis DM, Larson A, Davis SK, Kimmer EA (1990) Nucleic acids III: sequencing. In: Hillis DM, Moritz C (eds) Molecular systematics. Sinauer, Sunderland, pp 318–370
Hölldobler B (1979) Territoriality in ants. Proc Am Phil Soc 123:211–218
Hölldobler B, Wilson EO (1977) Weaver ants. Sci Am 237:146–154
Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge, p 399
Ikehata K (1977) ''Top'' and ''bottom'' of the river—an essay on the lotic animal community in the Ryukyu Island (in Japanese). Sizen 32(5):42–53
Jacobson ER (1909) Die nieuwe fauna van Krakatau. Jaarverslag von des Topographiscen Dienst in Nederlandsch-Indie 4:192–211
Jermiin LS, Crozier RH (1994) The cytochrome *b* region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. J Mol Evol 38:282–294
Johnson RN, Agapow PM, Crozier RH (2003) A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. Mol Phylogenet Evol 29(2):317–330
de Jong R (1998) Halmahera and Seram: different histories, but similar butterfly faunas. In: Biogeography and geological evolution of SE Asia. Buckhuys Publishers, Leiden, pp 315–325
Kimoto S (1979) The creatures in south islands (in Japanese). Kyoritsu Shuppan
Kimura M (1980) A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
Lanave C, Preparata G, Saccone G, Serio G (1984) A new method for calculating evolutionary substitution rate. J Mol Evol 20:86–93
Leys R, Cooper SJB, Schwarz MP (2000) Molecular phylogeny of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae), based on mitochondrial DNA sequences. Mol Phylogenet Evol 17–3:407–418
Lokkers C (1986) The distribution of the weaver ant, *Oecophylla smaragdina* (Fabricius) (Hymenoptera: Formicidae) in Northern Australia. Aust J Zool 34:683–687
Lunt DH, Zhang D-X, Szymura JM, Hewitt GM (1995) The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol Biol 5:153–165
Mardulyn P, Cameron SA (1999) The major opsin in bees (Insecta: Hymenoptera): a promising nuclear gene for higher level phylogenetics. Mol Phylogenet Evol 12:282–294
Mollon JD, Bowmaker JK, Jacobs GH (1984) Variations of color vision in a New World primate can be explained by a polymorphism of retinal photopigment. Proc R Soc Lond B 222:373–399
Morley RJ (2000) Origin and evolution of tropical rain forests. Wiley, New York
Navarro E, Jaffre T, Gauthier D, Gourbiere F, Rinaudo G, Simonet P, Normand P (1999) Distribution of *Gymnostoma* spp. microsymbiotic *Frankia* strains in New Caledonia is related to soil type and to host-plant species. Mol Ecol 8:1781–1788
Nei M, Kumar S (2000) Phylogenetic inference: maximum parsimony methods. Molecular evolution and phylogenetics. Oxford University Press, New York, pp 115–145
Neitz M, Neitz J, Jacobs GH (1991) Spectral tuning of visual pigments underlying red-green color vision. Science 252:971–974
Passera L (1994) Characteristics of tramp species. In: Williams DF (eds) Exotic ants: biology, impact, and control of introduced species. Westview, Boulder, pp 23–43
Peeters C, Andersen A (1989) Cooperation between dealate queens during colony foundation in the green tree ant, *Oecophylla smaragdina*. Psyche 96:39–44
Peng RK, Christian K, Gibb K (1998) How many queens are there in mature colonies of green ant, *Oecophylla smaragdina* (Fabricius)? Aust J Entomol 37:249–253
Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817
Saitou N, Nei M (1987) The neighbor-joining method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
Sameshima S, Hasegawa E, Kitade O, Minaka N and Matsumoto T (1999) Phylogenetic comparison of endosymbionts with their host ants based on molecular evidence. Zool Sci 16–6:993–1000
Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4. Sinauer Associates, Sunderland
Swofford DL, Olsen GL, Waddell PJ, Hillis DM (1996) In: Hillis M, Moritz C, Mable BK (eds) Molecular systematics, 2nd edn. Sinauer, Sunderland, p 407
Thornton I (1996) Krakatau—the destruction and reassembly of an island ecosystem. Harvard University Press, Cambridge
Tsutsui ND, Suarez AV, Holway DA, Case TJ (2001) Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations. Mol Ecol 10:2151–2161
Wetterer JK, Schultz TR, Meier R (1998) Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. Mol Phylogenet Evol 9–1:42–47
Wheeler WM (1922) Ants of the American Museum Congo Expedition. A contribution to the myrmecology of Africa. Bulletin of The Museum of Natural History XLV (1):224–236
Wild AL (2004) Taxonomy and distribution of the Argentine ant, *Linepithema humile* (Hymenoptera : Formicidae). Ann Entomol Soc Am 97:1204–1215
Wilson EO, Brown WL Jr (1953) The subspecies concept and its taxonomic application. Syst Zool 2:97–111
Wilson EO, Taylor RW (1964) A fossil ant colony: new evidence of social antiquity. Psyche 7182:93–103
Yokoyama S (1995) Amino acid replacements and wavelength absorption of visual pigments in vertebrates. Mol Biol Evol 12:53–61
Yokoyama S, Yokoyama R (1990) Convergent evolution of the red- and green-like visual pigment genes in fish, *Astyanax faciatus*, and human. Proc Natl Acad Sci USA 87:9315–9318