Myrmecological News	15	85-90	Vienna, May 2011

Complete mitochondrial genomes of normal and cheater morphs in the parthenogenetic ant *Pristomyrmex punctatus* (Hymenoptera: Formicidae)

Eisuke HASEGAWA, Kazuya KOBAYASHI, Norihiro YAGI & Kazuki TSUJI

Abstract

Complete sequences of the mitochondrial genome were determined for the normal and cheater morphs of the parthenogenetic ant *Pristomyrmex punctatus*. The circular genomes of both morphs were 16269 and 16180 bp in length, respectively, with each genome containing the standard gene complement of metazoan mitochondria, i.e., 16S and 12S ribosomal RNA subunits, twenty-two transfer RNA (tRNA) genes, and thirteen protein-coding genes. With the exception of several tRNA gene translocations, the organization of the morph genomes was similar to that found in vespid wasps. The nucleotide composition was AT-rich (79.6%), resulting from an AT bias at the third codon position in the protein-coding genes. The base composition bias in the control region (77.9% AT) was lower than previously reported for the Apocrita of the Hymenoptera (85.6-96.0% AT) due to the GC-rich composition of two direct-repeats located on both sides of a short AT-rich region (209 bp, 84.6% AT). Considerable substitutions between the two genomes were observed, illustrating that these are useful to show evolutionary history of the cheater morph within the species.

Key words: Mitochondria, complete genome, genome organization, mtDNA.

Myrmecol. News 15: 85-90 (online 20 April 2011)
ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 2 June 2010; revision received 16 September 2010; accepted 20 September 2010

Dr. Eisuke Hasegawa (contact author), Kazuya Kobayashi & Norihiro Yagi, Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Hokkaido, University, Kita-ku, Sapporo 060-8589, Japan. E-mail: ehase@res.agr.hokudai.ac.jp

Prof. Dr. Kazuki Tsuji, Faculty of Agriculture, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.

Introduction

Due to its maternal inheritance, small size, high rate of evolution, and highly polymorphic nature within a population, animal mitochondrial DNA (mtDNA) is well suited to studies of molecular evolution, population genetics and phylogeny (AVISE 1991, SIMON & al. 1994). In phylogenetic studies, problems arising from heterozygosity and the recombination of nuclear genes can be overcome by using mtDNA. Although the high rates of mtDNA evolution are suitable for phylogenetic investigations among recently differentiated species or populations (AVISE & al. 1987), this characteristic of mtDNA means that it is not well suited to resolving phylogenetically very deep nodes in the tree of life.

As of 9 April 2010, complete mitochondrial genome sequences for 35 insect species have been deposited in the DDBJ database. Most of the genomes are approximately 16 kb in length and contain the 37-mtDNA-gene pattern typical of metazoan taxa. Although the arrangement of the rRNA and protein-coding genes was generally well conserved (e.g., SHAO & al. 2001), the translocation of several tRNA genes, both with respect to position and direction, was observed among the genomes examined. Indeed, comparative analysis of these genomes revealed considerable divergence among taxa (DOWTON & al. 2009a).

Among hymenopteran insects, CROZIER & CROZIER (1993) were the first to report the complete mitochondrial genome sequence of the honeybee, *Apis mellifera*. Interestingly, while complete mitochondrial sequences have been determined for several hymenopteran taxa (summarized in Tab. 1), no comparable data exist for any ant species, even though ants comprise one of the largest hymenopteran taxa (WILSON 1971). Given the lack of mitochondrial sequence information as of 9 April 2010 in ants, sequencing the mitochondrial genome of an ant species is considered important for establishing phylogenetic relationships among hymenopteran taxa.

We selected the parthenogenetic ant *Pristomyrmex punctatus* (formerly *P. pungens*; Hymenoptera: Formicidae), because genetically differentiated cheaters are known in this species (DOBATA & al. 2009b, TSUJI & DOBATA 2011). The cheaters, which are larger than the normal morphs and bear ocelli, exhibit high fecundity and relatively low labor activity (SASAKI & TSUJI 2003). The widespread occurrence of similar phenotypes over a wide geographic area of Japan (H. Mori, pers. comm.) prompts several questions: Do the cheaters have a single origin? Have the cheaters differentiated from a population within the same species? How much genetic differentiation exists between the two morphs? Resolving these questions will require sequence data from both nuclear (HASEGAWA & al. 2001, DOBATA & al. 2009a) and mitochondrial genomes. In addition, *P. punctatus* is a queenless ant, which means that each worker produces daughter workers by parthenogenesis (ITOW & al. 1984). Consequently, since the mtDNA

Tab. 1: A list of hymenopteran insects for which the complete sequence of the mitochondrial genome has been reported.

Family	Species	Length of the mt genome	References
Apidae	*Apis mellifera*	16343 bp	CROZIER & CROZIER (1993)
Apidae	*Bombus ignitus*	16434 bp	CHA & al. (2007)
Vespidae	*Abispa ephippium*	16953 bp	CAMERON & al. (2008)
Formicidae	*Pristomyrmex punctatus*	16269 bp	This study
Ichneumonidae	*Diadegma semiclausum*	18728 bp	WEI & al. (2009)
Evaniidae	*Evania appendigaster*	17817 bp	WEI & al. (2010)
Vanhorniidae	*Vanhornia eucnemidarum*	16574 bp	CASTRO & al. (2006)
Orussidae	*Orussus occidentalis*	15947 bp	DOWTON & al. (2009a, b)
Cephidae	*Cephus cinctus*	19339 bp	DOWTON & al. (2009a, b)

Tab. 2: Primer pairs in mt genome and amplified regions for *Pristomyrmex punctatus*.

Primer pair	Forward primers	Reverse primers	Length	Region
CI13 - pmr8	5'-ATAATTTTTTTATAGTAATACC-3'	5'-ATTACGTCAACAAAATGTCA-3'	3943 bp	COI – COIII
pmf71n - pmr911	5'-TTGAAGCACCCTTTACTATTGCTG-3'	5'-TTAATAGAAAATCCCCCCCAAATTC-3'	5721 bp	COIII – Cytb
pmf81 - pmr22	5'-TCCATGAGGACAAATATCAT-3'	5'-AGAAGAGTTAAGGAGGGCGG-3'	6762 bp	Cytb – COI

lineage reflects the evolutionary history of individuals, descriptions of the mitochondrial genome of both normal and cheater morph could be applied to resolving the evolution of the two morphs in this species.

Material and methods

Individuals of normal and cheater *Pristomyrmex punctatus* morphs were collected at Nomoto and Kii-Nagashima in Kagawa and Mie prefectures in 2001, respectively. Voucher specimens are deposited at the Hokkaido University Museum. Total DNA was extracted from individual ants using a DNA extraction kit (DNeasy Blood and Tissue Kit, Qiagen, Germany). Several sets of universal primers were designed by comparing the mitochondrial genomes of the honeybee, *Apis mellifera* (see CROZIER & CROZIER 1993), and the fruit fly *Drosophila yakuba* (see CLARY & WOLSTENHOLM 1985). The entire mitochondrial genome was amplified using the three primer pairs shown in Table 2. The amplified fragments measured 4 - 7 kb in length and each fragment overlapped with adjacent fragments at both ends. We used long-PCR conditions and enzyme mixes (Ex-Taq™, Takara, Osaka) to amplify target regions. The PCR conditions consisted of 35 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 60°C for 5 min and a final extension step at 60°C for 7 min. The PCR products were purified using a DNA purification kit (QIAquick PCR Purification Kit, Qiagen). The purified fragments were then sequenced from both ends using a DNA sequencing kit (DTCS Quick Start Kit, Beckman-Coulter, CA) and genetic analyzer (CEQ 8000, Beckman-Coulter). Many internal primers were designed to obtain the complete sequence of one strand (primer information is available from E. Hasegawa). Both strands were read. At several nucleotide positions, nucleotide identity was ambiguous because of double peaks. In such cases, a third reading was conducted on the ambiguous strand.

The position of each gene was determined by alignment with the honeybee sequence using CLUSTALX for rRNA and protein coding regions. Positions of start and stop codon were estimated using the information from the honeybee sequence. The position of tRNA genes was searched by tRNAscan-SE (ver. 1.2.1). When tRNAscan-SE failed to assign the position, we determined the position by comparing with the honeybee sequence.

A phylogenetic analysis was conducted using amino acid sequence of 13 protein coding regions. We used amino acid sequences because *Vanhornia eucnemidarum* lacks several tRNA genes and evolutionary rate is very high between distant taxa in nucleotide sequence. We prepared the data matrix by CLUSTALX and analyzed it by two different methods, i.e., maximum parsimony (MP; by MEGA4) and Bayesian inference (by MrBayes 3.1.2). For the Bayesian inference, the best substitution model of amino acids was selected by MrModeltest 2.2.

Results and discussion

Genome organization and phylogenetic relationships among hymenopteran species

The complete mitochondrial genome sequences of the normal (16269 bp) and cheater (16180 bp) morphs were deposited in the DDBJ database (GenBank Accession Nos. AB556946, AB556947). The organization of the circular *Pristomyrmex punctatus* genome is presented in Table 3. The genomes, which consisted of 13 protein-coding genes, two ribosomal RNA genes and 22 tRNA genes, were typical of metazoan mtDNA (WOLSTENHOLME 1992). The start codon of ATP6 may be ATA, 7 bp after ATG (Tab. 3). We could not determine which is the true start codon because we did not check the mRNA sequence in this study. Reported organizations of gene sequences in hymenopteran insects are summarized in Table 4.

Tab. 3: Genome organization of the mitochondrial genome of normal and cheater morphs of *Pristomyrmex punctatus*. Genes are arranged such that the control region is at the end of the sequence. nc: non-coding intergenic space; CW and CCW: clockwise and counter clockwise, respectively;
Direct repeat I: TCAATTCCAAAATCAACACCCCCTTTAAAATATCCAGTAGAGTCA (45 bp);
Direct repeat II: GTGTTTTTGTTTCCAAATTCGG (21 bp); * the true start codon of this gene may be ATA starting from 7 bp later. Short abbreviations for tRNA genes given.

Normal morph			Cheater morph						
Start	**End**	**Length (bp)**	**Start**	**End**	**Length (bp)**	**Direction**	**Gene**	**Start**	**Stop**
1	74	74	1	74	74	CCW	tRNA-Val: V		
75	152	78	75	152	78		nc		
153	221	69	153	221	69	CW	tRNA-Met: M		
222	228	7	222	228	7		nc		
229	300	72	229	300	72	CW	tRNA-Ile: I		
301	301	1	301	301	1		nc		
302	371	70	302	371	70	CCW	tRNA-Gln: Q		
372	425	54	372	425	54		nc		
426	1418	993	426	1418	993	CW	ND2	ATT	TAA
1419	1480	62	1419	1480	62		nc		
1481	1550	70	1481	1550	70	CW	tRNA-Trp: W		
1551	1554	4	1551	1554	4		nc		
1555	1626	72	1555	1626	72	CCW	tRNA-Cys: C		
1627	1640	14	1627	1640	14		nc		
1641	1703	63	1641	1703	63	CCW	tRNA-Tyr: Y		
1704	1714	11	1704	1714	11		nc		
1715	3247	1533	1715	3247	1533	CW	COI	AGT	TAA
3243	3311	69	3243	3311	69	CW	tRNA-Leu(UUR): L1		
3312	3995	684	3312	3995	684	CW	COII	ATT	TAA
3996	4022	27	3996	4022	27		nc		
4023	4090	68	4023	4090	68	CW	tRNA-Asp: D		
4091	4121	31	4091	4121	31		nc		
4122	4193	72	4122	4193	72	CW	tRNA-Lys: K		
4194	4259	66	4194	4259	66		nc		
4260	4433	174	4260	4433	174	CW	ATP8	ATC	TAA
4424	5095	672	4424	5095	672	CW	ATP6	ATG*	TAA
5096	5103	8	5096	5103	8		nc		
5104	5886	783	5104	5886	783	CW	COIII	ATG	TAA
5887	5913	27	5887	5913	27		nc		
5914	5983	70	5914	5983	70	CW	tRNA-Gly: G		
5984	6334	351	5984	6334	351	CW	ND3	ATT	TAA
6335	6367	33	6335	6367	33		nc		
6368	6437	70	6368	6437	70	CW	tRNA-Ala: A		
6438	6452	15	6438	6452	15		nc		
6453	6511	59	6453	6511	59	CW	tRNA-Arg: R		
6512	6516	5	6512	6516	5		nc		
6517	6581	65	6517	6581	65	CW	tRNA-Asn: N		
6578	6637	60	6578	6637	60	CW	tRNA-Ser(AGN): S1		
6638	6660	23	6638	6660	23		nc		
6661	6730	70	6661	6730	70	CW	tRNA-Glu: E		
6723	6790	68	6723	6790	68	CCW	tRNA-Phe: F		
6791	6793	3	6791	6793	3		nc		
6794	8458	1665	6794	8458	1665	CCW	ND5	ATG	TAA
8459	8524	66	8459	8524	66	CCW	tRNA-His: H		
8525	8535	11	8525	8535	11		nc		

8536	9864	1329	8536	9864	1329	CCW	ND4	ATG	TAA
9865	9873	9	9865	9873	9		nc		
9874	10149	276	9874	10149	276	CCW	ND4L	ATT	TAA
10150	10167	18	10150	10167	18		nc		
10168	10234	67	10168	10234	67	CW	tRNA-Thr: T		
10235	10239	5	10235	10239	5		nc		
10240	10303	64	10240	10303	64	CCW	tRNA-Pro: P		
10304	10327	24	10304	10327	24		nc		
10328	10879	552	10328	10879	552	CW	ND6	ATT	TAA
10880	10889	10	10880	10889	10		nc		
10890	11993	1104	10890	11993	1104	CW	Cytb	ATA	TAA
11994	12068	75	11994	12068	75		nc		
12069	12139	71	12069	12139	71	CW	tRNA-Ser(UCN): S2		
12140	12151	12	12140	12151	12		nc		
12152	13111	960	12152	13111	960	CCW	ND1	ATA	TAA
13112	13121	10	13112	13121	10		nc		
13122	13182	61	13122	13182	61	CCW	tRNA-Leu(GAN): L2		
13183	13208	26	13183	13208	26		nc		
13209	14538	1330	13209	14535	1326	CCW	16S rRNA		
14504	15303	800	14500	15299	800	CCW	12S rRNA		
15304	15394	91	15300	15389	90		nc		
15395	15826	432	15390	15737	348		Direct repeat I		
15827	16035	209	15738	15946	209		AT-rich		
16036	16154	119	15947	16065	119		Direct repeat II		
16155	16269	115	16066	16180	115		nc		

Tab. 4: Mitochondrial gene order of nine hymenopteran insects for which the complete mitochondrial gene sequence has been reported. Abbreviations of tRNA genes as in Table 3. CR: control region; *1: The L gene repeats 4 times; *2: The genome lacks several tRNA genes and the control region in this species; *3: The block S-N-A repeats 4 times.

Taxon	**Gene order**
Apis mellifera (Apocrita, Apidae)	COI-L-COII-D-K-ATP8-ATP6-COIII-G-ND3-R-N-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-S-ND1-L-16S-V-CR-12S-E-S-M-Q-A-I-ND2-C-Y-W
Bombus ignitus (Apocrita, Apidae)	COI-L-COII-D-K-ATP8-ATP6-COIII-G-ND3-R-N-E-S-F-ND5-H-ND4-ND4L-P-T-ND6-Cytb-S-ND1-L-16S-V-12S-Q-CR-M-A-I-ND2-C-Y-W
Abispa ephippium (Apocrita, Vespidae)	COI-L-COII-K-D-ATP8-ATP6-COIII-G-ND3-A-R-N-S-E-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-S-ND1-16S-V-12S-CR-L-M-Q-I-ND2-W-C-Y *1
Pristomyrmex punctatus (Apocrita, Formicidae)	COI-L-COII-D-K-ATP8-ATP6-COIII-G-ND3-A-R-N-S-E-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-S-ND1-L-16S-12S-CR-V-M-I-Q-ND2-W-C-Y
Diadegma semiclausum (Apocrita, Ichneumonidae)	COI-COII-K-D-ATP8-ATP6-COIII-G-ND3-A-R-N-S-E-F-ND5-H-ND4-ND4L-P-T-ND6-Cytb-S-ND1-L-16S-V-12S-CR-L-M-I-Q-ND2-W-Y-C
Evania appendigaster (Apocrita, Evaniidae)	COI-L-COII-K-D-ATP8-ATP6-COIII-G-ND3-A-R-N-S-E-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-ND1-L-16S-V-12S-W-CR-C-M-I-S-Q-ND2-Y
Vanhornia eucnemidarum (Apocrita, Vanhorniidae)*2	COI-COII-L-K-D-ATP8-ATP6-COIII-ND3-S-N-A-E-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-S-ND1-M-16S-V-12S-L-ND2-W-C-Y *3
Orussus occidentalis (Symphyta, Orussidae)	COI-L-COII-K-D-ATP8-ATP6-COIII-G-ND3-A-R-N-S-E-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-M-S-ND1-L-16S-V-12S-CR-Q-I-ND2-W-C-Y
Cephus cinctus (Symphyta, Cephidae)	COI-L-COII-K-D-ATP8-ATP6-COIII-G-ND3-A-R-N-S-E-F-ND5-H-ND4-ND4L-T-P-ND6-Cytb-S-ND1-L-16S-V-12S-M-CR-Q-I-ND2-W-C-Y

Since ants along with several wasp families are members of Vespoidea (HÖLLDOBLER & WILSON 1990), we expected a close relationship between vespid wasp (*Abispa ephippium*) and ant (*Pristomyrmex punctatus*). However, phylogenetic analyses positioned ants at the base of *A. ephippium* + bees (Fig. 1a; MP) or showed monophyly of ants with *Evania appendigaster* (Fig. 1b; Bayes). Together with the findings of PILGRIM & al. (2008), our results highlight that more species and further analyses are required to resolve the phylogenetic relationship of ants and vespid wasps.

While the control regions in other hymenopteran species are extremely AT rich (85.6 - 96.0%), the AT content in *Pristomyrmex punctatus* was relatively lower (77.6%). In the normal morph, there were two direct repeats (45 and 21 bp; Tab. 3) at both ends of a short AT-rich region (209 bp,

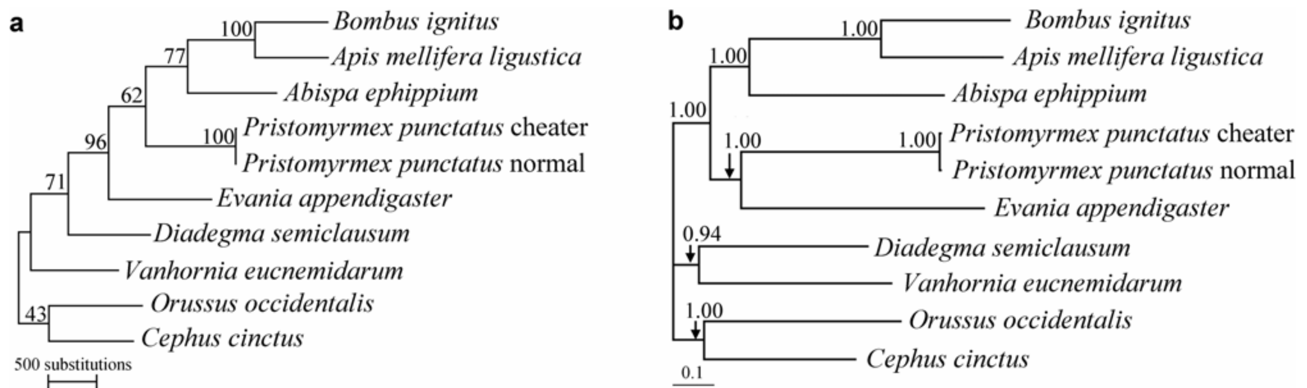

Fig. 1: Phylogenetic tree inferred by (a) maximum parsimony method and (b) Bayesian inference. Node support values indicated.

Tab. 5: Nucleotide substitutions and indels in the mitochondrial genomes of normal and cheater morphs. Syn: synonymous substitution; Non-syn: non-synonymous substitution; nc: non-coding region. *1: TATT; *2: AAAATCAACACCCCCT TTAAAATATCCAGTAGAGTCATCAATTCCAAAATCAATACCCCCTTTAAAATATCCAGTAGAGTCATC.

Morph	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Position	349	1203	1821	2276	4073	4196	4394	4433	5068	5359	6045	6666	7113	7266	7367	7584	8226	8468	9124	9203	9518	10037
Cheater		C	G	G	G	T	C	T	G	A	C	C	G	G	G	G	C	G	T	C	T	C	T
Normal		T	A	A	A	C	T	A	A	G	T	T	A	A	A	A	T	A	C	T	G	T	C
	Gene	ND2	nc	COI	COI	ATP6	ATP6	ATP6	ATP6	COIII	COIII	Arg	ND5	ND5	ND5	ND5	ND5	ND4	ND4	ND4	ND4	ND4L	ND6
	Codon	1	–	1	3	3	3	3	3	3	3	–	3	3	3	1	3	2	3	1	3	3	1
	Type of change	Non-syn	–	Non-syn	Syn	Syn	Syn	Syn	Syn	Syn	Syn	–	Syn	Syn	Syn	Syn	Syn	Non-syn	Syn	Syn	Non-syn	Syn	Non-syn
	No.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	
	Position	10427	10686	11822	12255	13333	13412	14075	14729	14945	14974	15292	15426	15430	15496	15499	15575	15611	15628	15649	15670	16034	
Cheater		A	A	G	C	A	A	4 indel	T	1indel	C	84 indel	A	T	A	C	A	A	A	A	A	A	
Normal		G	G	A	T	G	G	*1	C	T	T	*2	G	C	G	T	T	G	T	T	T	C	
	Gene	ND6	Cytb	ND1	ND1	16S	16S	16S	12S	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	Met	
	Codon	1	3	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	Type of change	Non-syn	Syn	Syn	Syn	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

84.6% AT) in the control region. Both of the repeats had a relatively low AT bias (64.4% and 66.6%), resulting in a relatively low composition bias in this region. The cheater morph had the same repeats, but the number of repeats differed from that of the normal morph. A preliminary investigation indicated that some individuals exhibited heteroplasmy in this region, probably due to differences in the number of repeats among mitochondria (E. Hasegawa, unpubl.). Thus, the difference in repeat number would not be a fundamental difference between the morphs.

Differences between normal and cheater morphs

Over the entire mitochondrial genome, normal and cheater morphs differed by approximately 40 substitutions and three indels (Tab. 5). As explained in section Material and methods, these differences were confirmed twice or three times. Thus, these differences are reliable. Of these 43 polymorphisms, 12 occurred in the control region. Similarly, of the 24 substitutions that occurred within protein-coding genes, 16 occurred at the third codon position and six were non-synonymous changes. A 4-bp deletion was observed in the 16S ribosomal (r) RNA gene of the cheater morph, and this deletion is commonly observed among cheater specimens collected in eastern Japan (S. Dobata, pers. comm.), suggesting a single origin of the cheater morphs from that area.

We also analyzed a specimen collected from Kagawa on Shikoku Island. Specimens with ocelli were collected from this area, their morphology being similar to that of the cheater morph investigated in this study (F. Ito, pers. comm.). A preliminary investigation revealed that the Kagawa specimen had just two substitutions in the region of approximately 5000 bp between ND2 and COIII; however, nine substitutions were observed in the same region in the normal and cheater morphs in this study (E. Hasegawa, unpubl.; see also Tab. 5). These preliminary results suggest that there may be multiple genetic lines of the morph with ocelli in *Pristomyrmex punctatus* and that the morph with ocelli may have arisen repeatedly in this species. Study-

ing the phylogenetic relationships among the different lines and local populations of the normal morph is thus considered important for resolving the evolutionary history and phylogeography of this ant species (TSUJI 1995, DOBATA & al. 2009b, TSUJI & DOBATA 2011). In addition, combining the results of this study with information from nuclear genetic assays (HASEGAWA & al. 2001, DOBATA & al. 2009a) could further our understanding of the evolution of social organisms.

Acknowledgements

E. Hasegawa expresses his appreciation to the late Ross H. Crozier for facilitating a sabbatical stay in Australia. We thank S. Dobata for helpful comments on the manuscript. This study was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS, No. 20370030) to E. Hasegawa. K. Kobayashi is a JSPS fellow.

References

AVISE, J.C. 1991: Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. – Annual Review of Genetics 25: 45-69.

AVISE, J.C., ARNOLD, J., BALL, R.M., BERMINGHAM, E., LAMB, T., NEIGEL, J.E., REEB, C.A. & SAUNDERS, N.C. 1987: Intraspecific phylogeography: the mitochondrial DNA bridge between populations genetics and systematics. – Annual Review of Ecology and Systematics 18: 489-522.

CAMERON, S.L., DOWTON, M., CASTRO, L.R., RUBERU, K., WHITING, M.F., AUSTIN, A.D., DIEMENT, K. & STEVENS, J. 2008: Mitochondrial genome organization and phylogeny of two vespid wasps. – Genome 51: 800-808.

CASTRO, L.R., RUBERU, K. & DOWTON, M. 2006: Mitochondrial genome of *Vanhornia eucnemidarum* (Apocrita: Vanhorniidae) and *Primeuchroeus* spp. (Aculeata: Chrysididae): evidence of rearranged mitochondrial genomes within the Apocrita (Insecta: Hymenoptera). – Genome 49: 752-766.

CHA, S.Y., YOON, H.J., LEE, E.M., YOON, M.H., HWANG, J.S., JIN, B.R., HAN, Y.S. & KIM, I. 2007: The complete nucleotide sequence and gene organization of the mitochondrial genome of the bumblebee, *Bombus ignitus* (Hymenoptera: Apidae). – Gene 392: 206-220.

CLARY, D.O. & WOLSTENHOLM, D.R. 1985: The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. – Journal of Molecular Evolution 22: 252-271.

CROZIER, R.H. & CROZIER, Y.C. 1993: The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. – Genetics 133: 97-117.

DOBATA, S., HASEGAWA, E. & TSUJI, K. 2009a: Isolation and characterization of eleven nuclear microsatellite loci for the parthenogenetic ant *Pristomyrmex punctatus*. – Molecular Ecology Resources 9: 1375-1379.

DOBATA, S., SASAKI, T., MORI, H., HASEGAWA, E., SHIMADA, M. & TSUJI, K. 2009b: Cheater genotypes in the parthenogenetic ant *Pristomyrmex punctatus*. – Proceedings of the Royal Society B-Biological Sciences 276: 567-574.

DOWTON, M., CAMERON, S.L., AUSTIN, A.D. & WHITING, M.F. 2009a: Phylogenetic approaches for the analysis of mitochondrial genome sequence data in Hymenoptera – a lineage with both rapidly and slowly evolving mitochondrial genome. – Molecular Phylogenetics and Evolution 52: 512-519.

DOWTON, M., CAMERON, S.L., DOWAVIC, J.I., AUSTIN, A.D. & WHITING, M.F. 2009b: Characterization of 67 mitochondrial tRNA gene rearrangements in the Hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral. – Molecular Biology and Evolution 26: 1607-1617.

HASEGAWA, E., SANADA, S., SATOH, T. & OBARA, Y. 2001: Microsatellite loci and genetic polymorphism among colony members in the parthenogenetic ant *Pristomyrmex pungens*. – Entomological Science 4: 399-402.

HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

ITOW, T., KOBAYASHI, K., KUBOTA, M., OGATA, K., IMAI, H.T. & CROZIER, R.H. 1984: The reproductive cycle of the queenless ant *Pristomyrmex pungens*. – Insectes Sociaux 31: 87-102.

PILGRIM, E.M., VON DOHLEN, C.D. & PITTS, J.P. 2008: Molecular phylogenetics of Vespoidea indicate paraphyly of the superfamily and novel relationships of its component families and subfamilies. – Zoologica Scripta 37: 539-560.

SASAKI, T. & TSUJI, K. 2003: Behavioral property of unusual large workers in the ant, *Pristomyrmex pungens*. – Journal of Ethology 21: 145-151.

SHAO, R., CAMPBELL, N.J.H. & BARKER, S.C. 2001: Numerous gene rearrangements in the mitochondrial genome of the wallaby louse, *Heterodoxus macropus* (Phthiraptera). – Molecular Biology and Evolution 18: 858-865.

SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LIU, H. & FLOOK, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. – Annals of the Entomological Society of America 87: 651-701.

TSUJI, K. 1995: Reproductive conflicts and levels of selection in the ant *Pristomyrmex pungens*: contextual analysis and partition of covariance. – The American Naturalist 146: 586-607.

TSUJI, K. & DOBATA, S. 2011: Social cancer and the biology of the clonal ant *Pristomyrmex punctatus* (Hymenoptera: Formicidae). – Myrmecological News 15: 91-99.

WEI, S.J., SHI, M., HE, J.H., SHARKEY, M. & CHEN, X.X. 2009: The complete mitochondrial genome of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) indicates extensive independent evolutionary events. – Genome 52: 308-319.

WEI, S.J., TANG, P., ZHENG, L.H., SHI, M. & CHEN, X.X. 2010: The complete mitochondrial genome of *Evania appendigaster* (Hymenoptera: Evaniidae) has low A+T content and a long intergenic spacer between atp8 and atp6. – Molecular Biology Reports 37: 1931-1942.

WILSON, E.O. 1971: The insect societies. – Belknap Press of Harvard University Press, Cambridge, MA, 548 pp.

WOLSTENHOLME, D.R. 1992: Genetic novelties in mitochondrial genomes of multicellular animals. – Current Opinion in Genetics & Development 2: 918-925.