

## MOLECULAR SYSTEMATICS OF BIRDWING BUTTERFLIES (PAPILIONIDAE) INFERRED FROM MITOCHONDRIAL ND5 GENE

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**ABSTRACT.** Birdwing butterflies including three genera, *Trogonoptera*, *Troides* and *Ornithoptera*, were subjected to molecular systematic analysis using sequences of the mitochondrial gene ND5. All three genera descend from a common ancestor and were monophyletic. *Trogonoptera* might have emerged from an ancestral species perhaps in the Miocene, from which *Troides* and *Ornithoptera* were also originated. *Ornithoptera* was further split in two subclusters, one totally corresponding to the subgenus *Schoenbergia* which lacks male sex marks in the forewing. The other subcluster includes species having sex marks. Green *O. priamus*, orange *O. croesus*, and blue *O. urvillianus* are regarded as an example of intraspecific variety of *O. priamus* by some authors, but they were totally different phylogenetically. *Trogonoptera* is limited to the Sundaland, but *Troides* is distributed across the Wallace line. It may be that *Troides* arose in Sundaland, but *Ornithoptera* probably arose in old Wallacea and migrated eastwards producing the various species we see today.

**Additional key words:** birdwing butterflies, molecular systematics, ND5 gene, phylogenetic tree, Wallacea.

Numerous publications were made on birdwing butterflies. Zeuner (1943) united their taxonomy with the geohistory of the Indo-Australian archipelago, considering continental drift. His work was called paleontology without fossils.

Larvae of troidine butterflies feed on various Aristochoiaceae which contain toxins. Even adult butterflies are toxic, therefore being protected from predation. Troidini is highly varied and widely distributed. According to Hauser (<http://www.insects-online.de/gartfron.htm>), a total of 10 genera are recognized in the tribe Troidini from all over the world except Africa, of which three genera are collectively referred to as “birdwing butterflies” for their beauty and birdlike size. These brilliant butterflies live in tropical rainforests encompassing the Oriental and Australian faunal regions.

Recent progress in DNA systematics opened a new era in lepidopterology, especially to trace evolutionary process in the light of geohistory, and to reevaluate traditional classification (Brower 1994, Sperling 1993, Sperling & Harrison 1994, Yagi et al. 1999). With respect to birdwing butterflies, Morinaka et al. (1999) and Morinaka et al. (2000) reported DNA-based systematic analyses for various troidine butterflies. In the latter study, one species of *Trogonoptera*, six of *Troides* and all of *Ornithoptera* were analyzed.

In our study presented here, one *Trogonoptera*, three *Troides* and all *Ornithoptera* were analyzed. We therefore provide an independent test for Morinaka et al. (1999, 2000) studies of birdwing butterflies. Fur-

thermore *Ornithoptera croesus*, *urvillianus*, and *euphorion* were sometimes treated as subspecies of *O. priamus* by some authors, but we tentatively regarded them as separate, and evaluated whether this is true.

### MATERIALS AND METHODS

**Samples.** Butterflies listed in Table 1 were preserved in 100% alcohol except for four species. Flight muscles from one each of single adult individuals are used to extract DNA. Muscles were digested with AL buffer and proteinase K according to QIAGEN Dneasy Tissue Kit. In four species, *O. alexandrae*, *O. victoriae*, *O. urvillianus* and *O. euphorion*, legs were removed from old dried museum specimens. They were crushed in a 1.5 µL tube and homogenized thoroughly with AL buffer. The DNA was washed according to the QIAGEN protocol. The DNA was dissolved in 400 µL of PE buffer.

**DNA analyses.** Primers V1, #A1 and KA2 for amplification of a part of mitochondrial ND5 gene (873 bases) were designed (Yagi et al. 1999, Su et al. 1996). The most conserved region of ND5 nucleotide sequences of *Drosophila melanogaster*, *D. yakuba*, *Carabus japonicus* and *Anopheles gambiae*, which are included in the EMBL data base were used. The polymerase chain reaction (PCR) was carried out in 50 µL of solution comprised of 130 ng template DNA, 0.2 µM each primer, and 2.5 units of ExTag DNA polymerase and dNTPs and PCR buffer according to Takara protocol. The amplification protocol was 30 cycles of

Table 1. Samples analyzed in this study and GenBank database.

No.	Species	Sampling place	DDBJ numbers
1	<i>Papilio memnon</i>	Kagoshima, Japan	ABO84426
2	<i>Atrophaneura varuna</i>	Cameron Highland, Malaysia	ABO84427
3	<i>Trogonoptera brookiana</i>	Cameron Highland, Malaysia	ABO84428
4	<i>Troides hypolitus</i>	Sulawesi, Indonesia	ABO84429
5	<i>Troides helena</i>	Bali, Indonesia	ABO84430
6	<i>Troides amphrysus</i>	Sumatra, Indonesia	ABO84431
7	<i>Ornithoptera tithonus</i>	Irian Jaya, Indonesia	ABO84432
8	<i>Ornithoptera goliath</i>	Irian Jaya, Indonesia	ABO84433
9	<i>Ornithoptera rothschildi</i>	Irian Jaya, Indonesia	ABO84434
10	<i>Ornithoptera paradisea</i>	Irian Jaya, Indonesia	ABO84435
11	<i>Ornithoptera chimaera</i>	Aseki, PNG**	ABO84436
12	<i>Ornithoptera meridionalis</i>	Aseki, PNG**	ABO84437
13	<i>Ornithoptera croesus</i>	Halmahera, Indonesia	ABO84438
	<i>Ornithoptera aesacus*</i>	Obi, Indonesia	
14	<i>Ornithoptera victorae</i>	Bougainville, PNG**	ABO84439
15	<i>Ornithoptera priamus</i>	Wau, PNG**	ABO84440
16	<i>Ornithoptera urvillianus</i>	Bougainville, PNG**	ABO84441
17	<i>Ornithoptera euphorion</i>	Cairns, Australia	ABO84442
18	<i>Ornithoptera alexandrae</i>	Popondetta, PNG**	ABO84443

\* DNA amplification was unsuccessful

\*\* Papua New Guinea

94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min in the PCR Thermal Cycler PK2400. The PCR product was separated by 1.0% agarose gel electrophoresis. The gel containing 873 base-DNA fragment was cut out, and the DNA fragment was extracted and purified by the QIAquick gel extraction Kit.

In *O. aesacus*, DNA was not successfully amplified, and this species was eliminated from further analyses.

For nucleotide sequencing of the ND5 DNA fragment, primers A3, C2 as well as V1, A1 and KA2 were used. Nucleotide sequences of both strands of the DNA fragment were determined with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Nucleotide sequences of primers were as follows:

V1: 5'-CCTGTTTCTGCTTTAGTTCA-3'  
 A1: 5'-AATADTAGGTATAAATCATAT-3'  
 A3: 5'-TTCGAATTTAGCTTTATGTGG-3'  
 C2: 5'-ATCYTTWGAATAAAAYCCAGC-3'  
 KA2: 5'-GTATAATATATTGTTAAACCTGTAG-3'

**Sequencing and phylogenetic study.** Nucleotide sequences were edited and aligned using Sequencher DNA Sequencing Software. A part of the ND5 nucleotide sequences (813 bases) accurately determined in all species was subjected to phylogenetic analysis and registered in the Genbank, as listed in Table 1.

Phylogenetic trees were constructed with the Neighbor-Joining (NJ) method. NJ method with the Bootstrap test was performed using the CLUSTAL X program (Felsenstein 1985, Thompson et al. 1997). Evolutionary distances were computed by the Kimura's two-parameter method (Kimura 1980). Max-

imum Parsimony method (MP) and UPGMA were also applied using standard default procedures in PAUP 4.0 (Swofford 1993).

#### Scanning electron-microscopy of sex marks.

Sex marks were dissected with the ordinary wing portion in the male forewings involving the Cu veins, kept in 99.5% alcohol, ultrasonically cleaned for 30 min (OMRON HU-10,46KHz), and dried for scanning electron-microscopy attached to a carbonized sticky tape. The samples were gold-spattered (200 nm) and a Hitachi T300 scanning electron microscope was used for observation.

#### RESULTS

**DNA phylogeny.** Fig. 1A, B and C show phylogenetic trees for 16 species studied plus three outgroup species. Besides the NJ method, MP and UPGMA produced basically similar trees with some differences. *O. aesacus* was not included in which DNA sequencing was not successful. Our analyses show that:

(1) The three birdwing butterfly genera *Trogonoptera*, *Troides* and *Ornithoptera* combined, were monophyletic.

(2) An ancestral species gave rise to *Trogonoptera*, and the ancestor of *Troides* plus *Ornithoptera*.

(3) *Ornithoptera* evolved in two subclusters. One totally corresponded to the subgenus *Schoenbergia*, lacking sex marks in the male forewings like *Trogonoptera* and *Troides*, and is almost completely endemic to main island of New Guinea. *Schoenbergia* appeared to split into two species groups; the *rothschildi* group and the *paradisea* group in NJ.

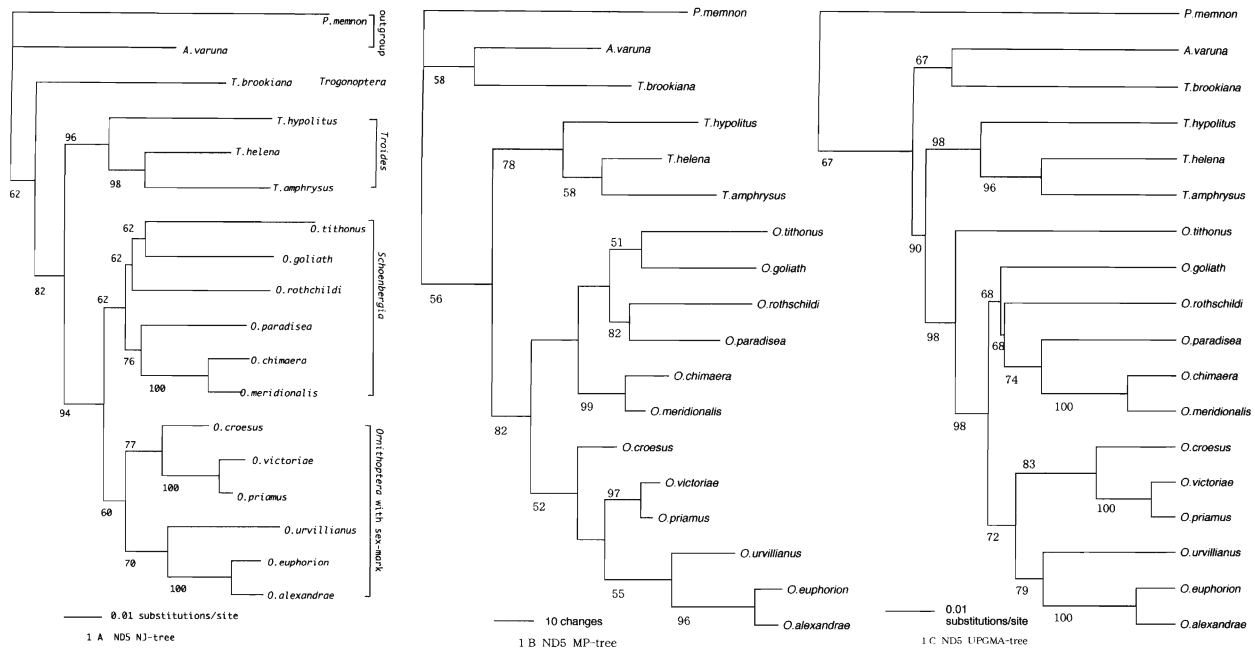


FIG. 1. Genealogical trees based on the base sequences of the ND5 gene, 813 base pairs, birdwing butterflies. A, B and C are based on NJ, MP and UPGMA methods, respectively. Numbers below branches are bootstrap values. *Trogonoptera*, *Troides* and *Ornithoptera* were monophyletic, *Trogonoptera* being most primordial, which yielded the other two. Of three *Troides* species for which permissions were obtained and analyzed, *T. hypolitus* lives west of the Wallace line. *Ornithoptera* was split in two subclusters; *Schoenbergia*, and all other species with sex mark in male forewing.

The other subcluster included all species having striking velvet-black male sex marks, and residing in islands from the Moluccas to the Solomons except for three species that live in mainland New Guinea and the north-east coast of Australia; *O. priamus*, *O. alexandrae* and *O. euphorion*. It also split in two species groups each represented by *O. croesus* and *O. urvillianus*. Unfortunately, *O. aesacus* was not included in the analyses, but it apparently belongs to this subcluster, because of the presence of a sex mark of the same structure. *O. priamus*, *O. croesus*, *O. urvillianus*, *O. euphorion* were totally paraphyletic.

Table 2 shows genetic distance analyzed using Kimura's 2 parameter method. Species numbers in Tables 1 and 2 coincide.

**Sex marks.** The basal scales found in the sex marks were uniformly black, while they had refraction lattice in their surface that express iridescent color in the other areas of the wing. The cover scales which are specific to the sex marks were cylindrical with falcate tips, and their radical sockets were enlarged and arranged in tandem with those of the basal scales (Fig. 2).

#### DISCUSSION

**Previous studies.** So-called birdwing butterflies involve three genera; *Trogonoptera*, *Troides* and *Ornithoptera*. The following three subgenera are recognized in the genus *Ornithoptera* based on morphological

evidence (D'Abrera 1975, Haugum & Low 1978, Scriver et al. 1995):

*Schoenbergia* (Pagenstecher 1893), including *O. goliath*, *O. rothschildi*, *O. tithonus*, *O. paradisea* and *O. meridionalis*.

*Aetheoptera* (Rippon, 1894), including *O. victoriae* and *O. alexandrae*.

*Ornithoptera* (Boisduval, 1832), including *O. croesus*, *O. aesacus*, *O. urvillianus* and *O. priamus*, involving its subspecies which live in various areas from Seram, entire New Guinea and adjacent islands to York peninsula, and *O. euphorion* which lives in the northern Queensland.

There are two successive reports (Morinaka et al. 1990, Morinaka et al. 2000) which are the only published studies on DNA phylogeny of birdwing butterflies. Our results are different from theirs in two important ways:

(1) In their study *Trogonoptera* shares common ancestor with all other *Troidine* butterflies but is paraphyletic with *Troides* plus *Ornithoptera*. Ours suggested monophyly of all three genera; i.e., *Trogonoptera* shares a common ancestor with *Troides* plus *Ornithoptera*;

(2) Their results are not parsimonious with respect to the sex mark. According to Morinaka et al. (2000), *O. alexandrae* with a sex mark is monophyletic with

TABLE 2. Kimura 2-parameter distance matrix (%) of the birdwing butterflies.

	1	2	3	4	5	6	7
1 <i>P. memnon</i>	—						
2 <i>A. varuna</i>	12.861	—					
3 <i>T. brookiana</i>	14.921	9.424	—				
4 <i>T. hypolitus</i>	15.831	10.841	11.126	—			
5 <i>T. helena</i>	13.884	9.844	10.975	7.400	—		
6 <i>T. amphrysus</i>	16.134	10.690	11.978	8.916	5.640	—	
7 <i>O. tithonus</i>	15.827	13.301	13.155	12.176	10.434	11.718	—
8 <i>O. goliath</i>	14.921	11.696	11.403	9.876	9.162	11.576	7.982
9 <i>O. rothschildi</i>	14.323	11.121	11.277	11.879	10.879	11.426	8.543
10 <i>O. paradisea</i>	15.221	11.691	11.710	10.427	9.716	10.133	8.111
11 <i>O. chimaera</i>	13.731	10.409	10.267	10.841	9.565	10.125	8.360
12 <i>O. meridionalis</i>	14.621	11.263	10.985	10.434	9.152	10.129	8.111
13 <i>O. croesus</i>	14.175	9.853	9.148	9.172	8.339	9.016	8.370
14 <i>O. victoriae</i>	15.082	9.998	9.012	9.881	9.742	9.856	10.364
15 <i>O. priamus</i>	14.476	9.714	8.872	9.595	9.457	9.856	9.493
16 <i>O. urvillianus</i>	15.678	11.413	12.000	11.916	10.155	10.708	10.749
17 <i>O. euphorion</i>	16.141	12.155	11.748	11.924	10.169	11.743	10.372
18 <i>O. alexandrae</i>	15.676	10.985	11.448	12.201	10.155	11.727	106.58
	8	9	10	11	12	13	14
8 <i>O. goliath</i>	—						
9 <i>O. rothschildi</i>	7.586	—					
10 <i>O. paradisea</i>	7.276	7.014	—				
11 <i>O. chimaera</i>	7.674	7.549	5.904	—			
12 <i>O. meridionalis</i>	6.742	6.899	5.259	1.995	—		
13 <i>O. croesus</i>	6.852	7.854	6.298	6.564	6.320	—	
14 <i>O. victoriae</i>	9.082	8.117	7.661	7.515	7.549	3.293	—
15 <i>O. priamus</i>	8.229	7.976	7.800	7.240	7.271	3.293	0.994
16 <i>O. urvillianus</i>	9.896	9.914	8.906	8.476	7.949	6.461	5.919
17 <i>O. euphorion</i>	9.246	6.748	6.723	8.246	7.172	6.627	7.038
18 <i>O. alexandrae</i>	8.809	6.339	7.549	8.099	7.445	7.445	7.579
	15	16	17	18			
15 <i>O. priamus</i>	—						
16 <i>O. urvillianus</i>	5.109	—					
17 <i>O. euphorion</i>	6.483	5.503	—				
18 <i>O. alexandrae</i>	7.299	5.500	1.620	—			

Outgroup status changed:  
 2 taxa transferred to outgroup  
 Total number of taxa now in outgroup = 2  
 Number of ingroup taxa = 16

*Schoenbergia* having no sex mark. In many of their trees, sex-marked *O. victoria* shares a common ancestor with all others including mixed species with and without sex mark.

The sex mark is a conspicuous inherited synapomorphy, and may be used to validate any attempt of systematics of birdwing butterflies. Namely, trees based on DNA should be consistent with the dichotomy of the species by presence/absence of the mark. The stated inconsistencies suggest some confusion with their results, and we do not quote their reports except their data on *O. aescacus*, which we do not have.

The new phylogenetic classification of the tribe *Troidini* using immature characteristics is fundamentally different from those based on adult morphology (Parsons 1996). In Parsons' study, origin of *Ornithoptera* was distinct from *Troides*, and the author assumed that the former has evolved in northward-drifting Australia,

while the latter evolved allopatrically on landmasses on the Eurasian plate. Two successive reports by Morinaka et al. (1999, 2000) indicated monophyly of *Troides* and *Ornithoptera*, and totally rejected Parsons' (1996) ideas, but the position of *Trogonoptera* in their study is obscure. We also rejected Parsons' (1996) views and demonstrated the monophyly of all three genera, unlike Morinaka et al. (1999, 2000).

**Origin of birdwing butterflies based on our study.** A bar of 0.01 in Fig. 1 corresponds to one million years required for 1% of base replacements. Based on the studies of ground beetles (*Carabus*, Carabidae, Coleoptera) in Japan and European Alps, Su et al. (1998) indicated a value of  $4 \pm 0.5$  million years necessary for this magnitude of base replacements. They proposed a new figure of 3.6 million years more recently (Su pers. com. 1999). It is therefore possible that ancestral *Trogonoptera* gave rise to the

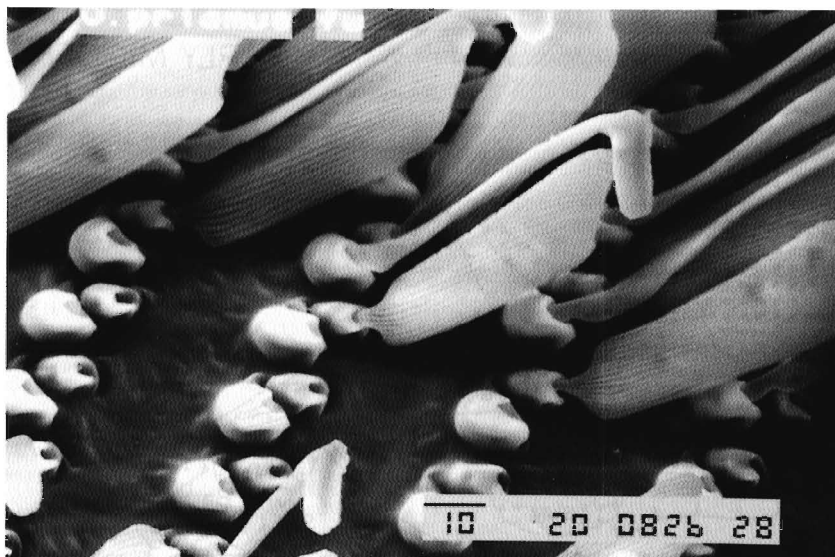


FIG. 2. Scanning electronmicrograph of the male sex mark, *Ornithoptera priamus*. The basal (ordinary) scales and the cover scales which are specific to the sex mark area are shown. They are arranged alternatively and in tandem. The latter lost a fan-like appearance as the basal scale, club-like with a falcate tip, and have enlarged radical sockets which probably emit scent. Most scales were removed to show sockets clearly. The scale bar is 10 microns.

ancestor of *Troides* plus *Ornithoptera* in the early or middle Miocene, if linearity and identical rate of nucleotide evolution are assumed as the stated beetles. More studies are necessary to solve this problem more accurately, however.

In the late Mesozoic Era, angiosperm trees started to form rainforests. In Sundaland, they stably existed for 130 million years and represented a cradle for biodiversity. Sundaland is the only place where *Trogonoptera* lives today, and it may be that *Trogonoptera* arose from an ancestral troidine butterfly in Sundaland. *Troides* is most varied there, decreasing in numbers of species towards surrounding areas north to Taiwan, west to India and east to Papua New Guinea. Only one each species is found in these extremes of the territory of the genus *Troides*. Possibly, *Troides* also arose in rainforests of the Sundaland.

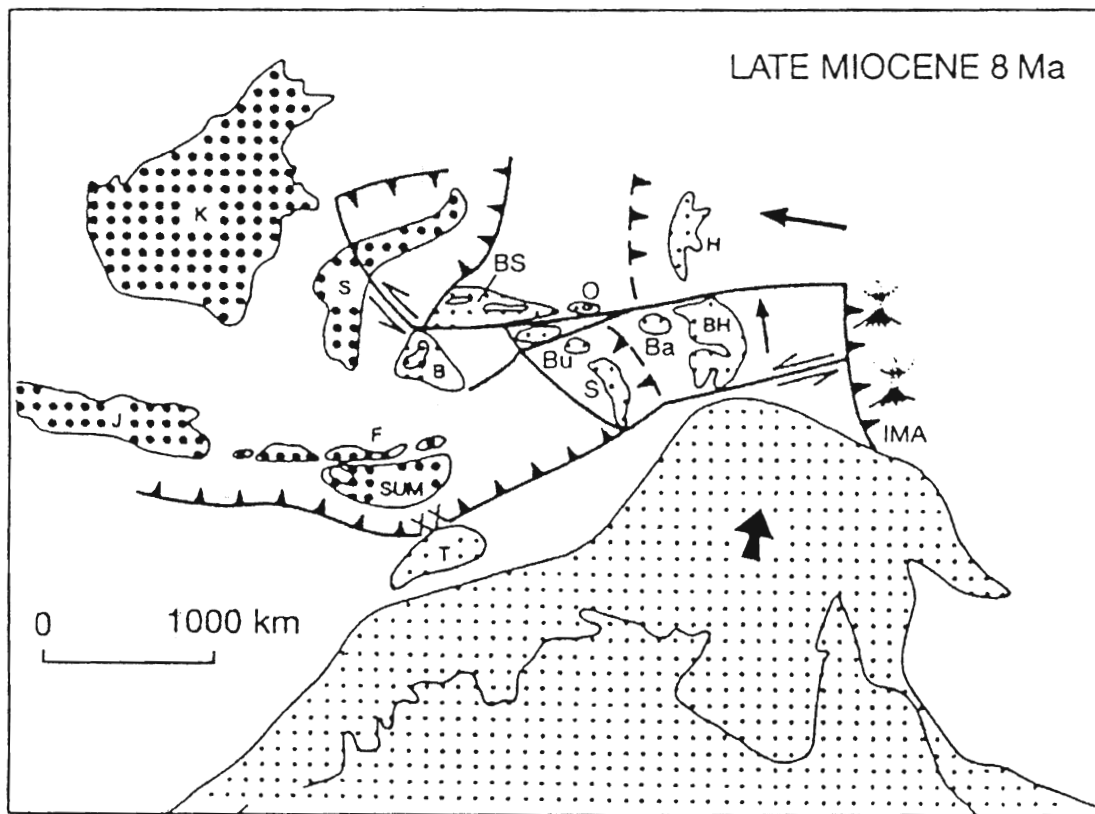
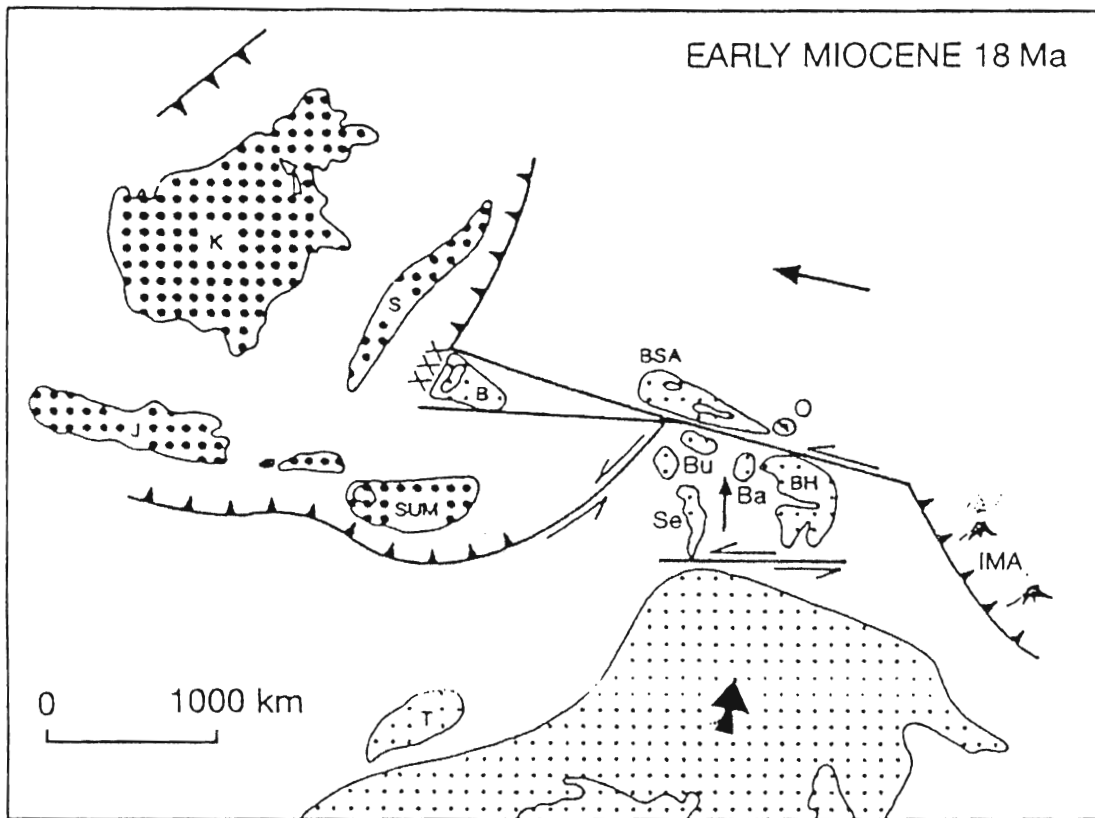
The diversification of *Ornithoptera* and *Troides* possibly took place in a landmass corresponding to today's Wallacea. We think this happened east of the Wallace line in any way, because Fig. 1 showed that *Troides hypolitus*, which lives east of the stated line, is basal within *Troides* species analyzed and therefore more closely related to the ancestor of *Ornithoptera*. Many more species of *Troides* are necessary of course, to evaluate this important hypothesis.

**Diversification of *Ornithoptera*.** There are two discrete monophyletic subclusters in the genus *Ornithoptera*. One group, corresponding to the subgenus *Schoenbergia*, diversified in two species groups repre-

sented by *O. rothschildi* and *O. paradisea* as indicated in Fig. 1 based on NJ, but other methods gave somewhat different results, although the monophyly of *Schoenbergia* was supported in all three trees.

*Schoenbergia* was thus natural but *Aetheoptera* appeared unnatural according to our results. Therefore, *Aetheoptera* is a synonym of *Ornithoptera*. Subgenus *Ornithoptera* plus Aetheopteran species appeared monophyletic, however, and all species share a striking synapomorphy, a male sex mark, which characterizes this subcluster. NJ and UPGMA gave the same result, but MP gave some doubts that this subcluster consists of *croesus* group and *urvillianus* group.

Origin of such diversification of *Ornithoptera* is unknown, but we propose a hypothesis based on the geof ormation of the area where *Ornithoptera* lives today. Owing to a complex tectonophysics of the oceanic plates, old Wallacea is much different from today's land masses. Australia was still far south during the Miocene. New Guinea was not yet formed (Van Bemmelen 1949), but it later rose as the Indo-Australian plate collided with the Pacific plate and initiated orogenic movements. The Bird's head peninsula (BH) of the western end of today's New Guinea was still an isolated island and located far west, just east of Halmahera which were being formed out of a group of islands (Hall & Nichols 1990, Burrett et al. 1991). Volcanic island arcs extended south to the line where the Pacific plate disappeared beneath of Indo-Australian plate. Owing to elevation and northward



drift of New Guinea, these island arcs were later roughly separated in eastern and western groups of islands (Fig. 3).

We hypothesize that the ancestral *Ornithoptera* arose somewhere in old Wallacea and reached an area corresponding to old BH which was still an island, and produced ancestral *Schoenbergia* species before or after it fused with New Guinea main island which was being formed by the northward drift of Australia. Mt. Arfak of today's BH is a home of many rare birdwing butterflies, including *O. rothschildi*, which is unique to this mountain. This species may represent the most primordial patterns of *Schoenbergia*.

On the other hand, we further assume that a separate group reached volcanic island arcs, evolved there and migrated towards east via island arc, and produced *Ornithoptera* plus *Aetheoptera*. Sex mark was probably produced at an early stage of this evolutionary process, perhaps around the time it departed from Wallacea to migrate eastwards and eventually became a hallmark of all species descendant to an ancestral species which entered to the island arc.

**Distribution.** While *Schoenbergia* is almost confined to mainland New Guinea, sex-marked species are found in islands from the Malucca to the Solomons with three exceptions; *O. euphorion*, *O. alexandrae* and *O. priamus* which live in Australia and New Guinea. The south-west corner of main island of Papua is regarded as a part of Australia, unlike the rest of New Guinea (Ollier & Bain 1994). We showed that *O. euphorion* is not a subspecies of *O. priamus*. It belongs to sex-marked species group and probably, arrived at an ancient landmass which corresponds to today's Queensland and South-east New Guinea. *O. alexandrae* which is closely akin to *O. euphorion* according to Fig. 1 is endemic to main island New Guinea, being confined to a small area near Popondetta. We assume that *O. alexandrae* was also a species originally evolved in the volcanic island arc. It has a velvet-black sex mark and probably evolved in isolated islands that once existed off the northeast coast of old Papua, which later became a part of today's New Guinea due to the gross elevation of land. Eastern Papua, especially Huon Peninsula area, is known for a large-scale land elevation, proven by coastal shelves containing corals and sea shells found even in the altitudes of 200 m (Bloom et al. 1974).

The origin of *O. priamus* is obscure. It is interesting to note that Morinaka et al. (2000) showed a tree indicating that *O. aesacus* and *O. priamus* share a close common ancestor. This is good circumstantial evidence that *O. priamus* arose in old Wallacea and migrated eastwards and finally invaded entire Papua New Guinea with neighboring island and northern Australia. *O. aesacus* occurs only in a small island of Obi, closely south to Halmahera, and its bluish-green coloration suggests a kinship with *O. priamus*. Possibly *O. aesacus* is a surviving relic of the ancestral species of *O. priamus*. This question can be solved when various subspecies of *O. priamus* were analyzed along with *O. aesacus* and *O. croesus*.

**Further studies.** Many puzzles remained unsolved.

(1) A complete study of *Troides* is necessary. *Troides* is the only birdwing butterfly genus containing species which live on the both sides of the Wallace line. The eastern margin of Sundaland is marked by a deep ocean ditch formed by disappearance of the Pacific plate beneath the Eurasian plate, thus stably existed since Mesozoic Era, forming a strong barrier against migration of animals; i.e., the Wallace line. It may be that *Troides* arose in Sundaland and perhaps migrated across the Wallace line with trade wind.

Which particular *Troides* species is most basal remains a puzzle. We suspect if *Troides rhadamantus dohertyi* is a candidate of the relic because of its simplistic yellow patterns, small size compared with other subspecies of *T. rhadamantus*, strong tendency to produce a melanic form and its delimited distribution in the Talaud Islands of Indonesia. Yellow pigment formation may be still weak in this species. We still do not know whether *Ornithoptera* arose west of the Wallace line, but a thorough study of *Troides* species may give a clue to this interesting question by evaluating how *Ornithoptera* is related with various *Troides* species residing west and east of the line.

(2) A complete study of various subspecies of *O. priamus* is necessary. This species is most widely spread and probably tells about routes of expanding of *Ornithopteran* distribution, not only *O. priamus*.

(3) Questions in the species level are many. For example, *Troides magellanus* and *T. prattorum* are similarly patterned sharing pearly shine in the upper side of female hindwing. *O. magellanus* is common in the Philippines, but *O. prattorum* is restricted to elevated

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FIG. 3. Southern Pacific landmasses in the Miocene (Burrett et al. 1991). Heavy-dotted landmasses are on the Asian plate, but thin-dotted landmasses are on the Indo-Australian plate. A thick arrow indicates drifting direction of Australia, and a thin arrow drifting of Asian islands. Plates submerged at the barbed lines towards the direction of the barbs. Ma, million years; K, Kalimantan; S, Sulawesi; B, Bachan; BS, Banggai-Sula; O, Obi; Bu, Buru; Se, Seram; H, Halmahera; BH, later Bird's head peninsula of Irian Jaya; IMA, Inner Melanesian arc.

altitudes in small island of Buru far south in the Banda sea. Whether both are close in term of DNA is very interesting and perhaps a complex tectonophysical movements of the area may shed some lights on this strange distribution of two sister species.

These are few examples of puzzles. To solve them, we attempted to obtain fresh alcohol specimens in vain. Birdwing butterflies are protected fauna. Probably, a comprehensive international collaborative project is necessary to persuade Governments of the countries where these lovely butterflies live.

#### ACKNOWLEDGMENTS

We are obliged to Dr. Aoki, Invertebrate Laboratory 1, The Research Institute of Evolutionary Biology, The Tokyo University of Agriculture, who supplied specimens of four species, *O. alexandrae*, *O. victorae*, *O. urvillianus* and *O. euphorion*, from which he removed legs for us. Time of collection of these species were all earlier than 1967. For all species other than the 4 species stated above, copies of certificates were submitted to the editorial board which are dated, stamped, signed, and issued by each respective Government authorities, according to the rules of the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

We are indebted to Mr. Hiromichi Makita in various ways. Also, we are obliged to Ms. Yoko Sugita for preparation of the manuscript.

Although S. Morinaka was an undergraduate student of the University of the Air, his work was carried out elsewhere without our awareness prior to publication.

#### LITERATURE CITED

- D'ABRERA, B. 1975. Birdwing butterflies of the world. Landsdowne, Melbourne, Australia. 42 pp.
- VAN BEMMELLEN, R. W. 1949. The geology of Indonesia, Vol 1A, General Geology of Indonesia and Adjacent *Archipelagoes*. Martinus Nijhoff, The Hague, Holland. 719 pp.
- BLOOM, A. L., W. S. BROECKER, J. M. A. CHAPPELL, R. K. MATTHEWS & K. J. MESOLELLA. 1974. Quaternary sea level fluctuations on a tectonic coast; new  $^{230}\text{Th}/^{234}\text{U}$  date from the Huon Peninsula, New Guinea. *Quaternary Res.* 4:85–205.
- BROWER, A. V. Z. 1994. Phylogeny of *Heliconius* butterflies inferred from mitochondrial DNA sequences (Lepidoptera; Nymphalidae). *Mol. Phylogenet. Evol.* 3:159–174.
- BURRETT, C. D., R. BERRY & R. VERNE. 1991. Asian and South-western Pacific continental terranes derived from Gondwana, and their biogeographic significance. *Aust. Syst. Bot.* 4:13–24.
- FELSENSTEIN, J. 1985. Confidence limits of phylogenies: an approach using bootstrap. *Evolution* 39:783–791.
- HALL, R. & G. J. NICHOLS. 1990. Terrane amalgamation in the Philippine Sea margin. *Tectonophysics* 181:207–222.
- HAUGUM, J. & A. M. LOW. 1978–1985. A monograph of the birdwing butterflies, 1–5. Scandinavian Science Press Ltd., Klampenborg.
- KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 32:128–144.
- MORINAKA, S., T. MAEYAMA, K. MAEKAWA, D. ERNIWATI, S. N. PRIJONO, I. K. GINARSA, T. NAKAZAWA & T. HIDAKA. 1999. Molecular phylogeny of birdwing butterflies based on the representatives in most genera of the tribe *Troidini*. *Entomol. Sci.* 2:374–358.
- MORINAKA, S., N. MINAKA, M. SEKIGUCHI, ERNIWATI, S. N. PRIJONO, I. K. GINARSA & T. MIYATA. 2000. Molecular phylogeny of birdwing butterflies of the Tribe *Troidini* (Lepidoptera: Papilionidae) using all species of the genus *Ornithoptera*. *Biogeography* 2:103–111.
- OLLIER, C. D. & J. H. C. BAIN. 1994. Geology. In Ryan, P. (ed.), *Encyclopedia of Papua and New Guinea*. Vol 1. Melbourne University Press, Melbourne, Australia. 478 pp.
- PARSONS, M. J. 1996. Gondwanan evolution of the *Troidine* swallowtails (Lepidoptera: Papilionidae): cladistic reappraisals using mainly immature stage characters, with focus on the birdwing *Ornithoptera* Boisduval. *Bull. Kitakyushu Mus. Nat. Hist.* 15:43–118.
- SCRIBER, J. M., Y. TSUBAKI & R. C. LEDERHOUSE (EDS). 1995. Swallowtail butterflies; their ecology and evolutionary biology. Scientific Publishers, Gainesville.
- SPELRLING, F. A. H. 1993. Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. *Heredity* 71:227–233.
- SPELRLING, F. A. H. & R. G. HARRISON. 1994. Mitochondrial DNA variation within and between species of *Papilio machaon* groups of swallowtail butterflies. *Evolution* 48:408–422.
- SU, Z.-H., T. OHAMA, T. S. OKADA, K. NAKAMURA, R. ISHIKAWA & S. OSAWA. 1996. Phylogenetic relationships and evolution of the Japanese Carabinae ground beetles based on mitochondrial ND5 gene sequences. *J. Mol. Evol.* 42:124–129.
- SU, Z.-H., O. TOMINACA, M. OKAMOTO & S. OSAWA. 1998. Origin and diversification of hindwingsless *Damaster* ground beetles with the Japanese Islands as deduced from mitochondrial ND5 gene sequences (Coleoptera, Carabidae). *Mol. Biol. Evol.* 15:1026–1039.
- SWOFFORD, D. L. 1993. PAUP (phylogenetic analysis using parsimony) version 3.1.1. Smithsonian Institution, Washington.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAC, F. JEANMOUGIN & D. G. HIGGINS. 1997. The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. *Nucleic Acids Research* 24:4876–4882.
- YAGI, T., G. SASAKI & H. TAKEBE. 1999. Phylogeny of Japanese papilionid butterflies inferred from nucleotide sequences of the mitochondrial ND5 gene. *J. Mol. Evol.* 48:42–48.
- ZEUNER, F. E. 1943. Studies in the systematics of *Troides* Huebner (Lepidoptera, Papilionidae) and its allies; distribution and phylogeny in relation to the geological history of the Australian Archipelago. *Trans. Zool. Soc. Lond.* 25:107–184.

Received for publication 7 June 2000; revised and accepted 14 August 2002.