New Entomol., 56(1,2) : 1~7 2007

Phylogeography of Japanese alpine butterfly *Erebia niphonica* (Lepidoptera : Nymphalidae : Satyrinae) inferred from mitochondrial gene sequences

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Abstract : *Erebia niphonica* is one of the most widely distributed alpine butterflies in Japan. By using the mitochondrial COI and ND5 genes, we reconstructed the molecular phylogeography of *E. niphonica* in Japan. The Hokkaido clade was highly divergent from the Honshu clade suggesting a history of ancient divergence. Within Honshu, there were region-related, phylogenetically significant structures. Especially, the division between the central and eastern Honshu populations was evident, indicating multiple immigration events from the Asian continent and/or divergence in Honshu.

Key words : COI, divergence in high-altitude regions, Honshu-Hokkaido divergence, mtDNA phylogeny, ND5

Introduction

Isolated distribution of animal populations in high-altitude areas promotes genetic divergence of a species. Alpine butterflies offer a model to study the historical divergence of alpine organisms (Nice and Shapiro, 2001).

There are a lot of discontinuous mountain ranges in Japan. Pleistocene glacial cycles resulted in significant elevational shifts in vegetation zones in each mountain range and have generated intraspecific genetic differentiation in alpine plants (Fujii *et al.*, 1999). For animals, phylogeographic studies for alpine species are scarce, although Nice and Shapiro (2001) detected a considerable extent of geographic genetic variations in the alpine satyrinid *Oeneis chryxus* complex in the Sierra Nevada mountain ranges.

Erebia niphonica (Lepidoptera, Nymphalidae, Satyrinae) is one of the most widely distributed alpine butterfly species in Japan. Although their distribution is limited to areas above 1000 m in altitude in Honshu, central Japan, they inhabit lowlands and mountains in Hokkaido, northern Japan.

In his monograph, Warren (1936) treated *E. niphonica* (in Honshu and Hokkaido) as a separate species from *E. neriene* in Far East. Ono (1973) and Kogure (1975), contrarily, proposed that *E. niphonica* in Hokkaido originated via dispersal from Sakhalin, where *E. neriene* inhabits,

(Received May 11, 2007 ; Accepted: May 25, 2007)

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and that *E. niphonica* in Hokkaido and *E. neriene* are thus conspecifics. They also proposed that *E. niphonica* in Honshu is a Japanese endemic species, based on the geographic variation of male scent patches and other sexual characters. Sekiguchi *et al.* (2000) partially confirmed Ono and Kogure's hypothesis by showing the monophily of *E. neriene* in Sakhalin and *E. niphonica* in Hokkaido, although they did not characterize the divergence time of the Honshu and Hokkaido clades. They also suggested some phylogenetic structures of intra-Honshu populations.

In the present study, we used more samples than Sekiguchi *et al.* (2000) and reconstructed the phylogeography of *E. niphonica* populations in Japan based on both ND5 and COI genes. Here, we examine (1) the phylogenetic relationship and the divergence time flame between the Honshu and Hokkaido populations of *E. niphonica*, and (2) intraspecific phylogeography of *E. niphonica* in Honshu, central Japan.

Materials and Methods

Collection of samples

A total of 34 adult *Erebia niphonica* were collected during 1994 to 2001 from 23 localities in Japan (Table 1 and Fig. 1), and stored in 100% ethanol at −20°C until DNA extraction. *E. ligea* (obtained at Daimon-sawa, Yamanashi) was used as an outgroup.

DNA extraction, amplification and sequencing

We dissected the thorax muscles and digested them with 475μl of 0.6% SDS and 25μl of 20 mg/ml proteinase K at 55°C for 45 minutes. The solution containing DNA was treated twice with phenol, twice with phenol/chloroform/isoamyl alcohol (25:24:1), and twice with chloroform/isoamyl alcohol (24:1). The DNA was precipitated with ethanol, washed with 70% ethanol, dried, and dissolved in 100μl 10mM TE. The 439bp region of mitochondrial cytochrome oxidase subunit I (COI) gene and 487-bp region of NADH dehydrogenase subunit 5 (ND5) gene were amplified by the polymerase chain reaction (PCR) with the primers : 5'-ATAATTTTTTTTATAGTTAT-3' (CI13) and, 5'-GTTTCTTTTTTTCCTCTTTC-3' (CI14) for COI sequences (Itino *et al.*, 2001), and 5'-CCTGTTTCTGCTTTAGTTCA-3' (V1), 5'-AT CYTTWGAATAAAAYCCAGC-3' (C2), 5'-TTCGAATTTAGCTTTATGTGG-3' (A3) and 5'-GTTCTAATATAAGGTATAAATCATAT-3' (KA1L) for ND5 sequences (Yagi *et al.*, 1999), and using the following temperature profile : 30 cycles of 94°C for 1.5 min, 45°C for 1 min and 72°C for 1 min (COI), 35 cycles of 95°C for 1 min, 45°C for 1 min and 72°C for 2 min (ND5). The amplified DNA fragments were treated with SAP (shrimp alkaline phosphatase) and exonuclease I. The nucleotide sequences of both strands were determined by using the Big Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems) with an ABI Prism 377 DNA Sequencer.

Phylogenetic analyses

The combined sequence of COI and ND5 for each sample was aligned using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic analyses were conducted with Maximum-likelihood (ML) method in PAUP* 4.08b (Swofford, 1998). ML analysis of mtDNA assumed the TrN+I model of

Table 1 Data for the butterfly samples. The acronym represents the location or mountain range of each sample. HO : Hokkaido region, TO : Tohoku region, JO : Joetsu region, KI : Kita Alps mountain range, CH : Chuou Alps mountain range, MI : Minami Alps mountain range, YA : Yatsugatake mountain range. For locations, see Fig. 1.

Acronym	Locality	No. of samples	Accession No.	
			COI	ND5
	Erebia niphonica			
HO1	Tokachi Mitsumata, Hokkaido	1	AB303874	AB303898
HO2	Shiretoko-dake, Hokkaido	1	AB303875	AB303899
HO3	Nissho-toge, Hokkaido	4	AB303876	AB303900
TO1	Yakeishi-dake, Iwate	1	AB303877	AB303901
TO2	Ryumon-zan, Yamagata	2	AB303878	AB303902
JO1	Hakkai-san, Niigata	1	AB303879	AB303903
JO2	Hiuchi-yama, Niigata	2	AB303880	AB303904
JO3	Yunomaru-san, Gunma	1	AB303881	AB303905
JO4	Sajiki-yama, Gunma	2	AB303882	AB303906
JO5	Hafu-dake, Nagano	1	AB303883	AB303907
KI1	Kasagatake, Gifu	1	AB303884	AB303908
KI2	Nakafusa-gawa, Nagano	1	AB303885	AB303909
CH1	Utsugi-dake, Nagano	1	AB303886	AB303910
CH2	Hinokio-dake, Nagano	1	AB303887	AB303911
CH3	Minami-Komagatake, Nagano	1	AB303888	AB303912
CH4	Kosumo-yama, Nagano	3	AB303889	AB303913
MI1	Kamanashi-yama, Nagano	2	AB303890	AB303914
MI2	Hase-Minamisawa, Nagano	3	AB303891	AB303915
MI3	Hijiri-dake, Nagano	1	AB303892	AB303916
MI4	Iro-dake, Nagano	1	AB303893	AB303917
MI5	Osawa-dake, Nagano	1	AB303894	AB303918
YA1	Natsuzawa kosen, Nagano	1	AB303895	AB303919
YA2	Futago-yama, Nagano	1	AB303896	AB303920
	Erebia ligea			
OUT	Daimon-sawa, Yamanashi	1	AB303897	AB303921

substitution. Model and parameter values were estimated by using MODELTEST 3.7 (Posada and Crandall, 1998). One thousand bootstrap replicates were conducted with the heuristic search option, with maxtrees set to 10000 and random addition sequence in effect.

Results

The ML tree (Fig. 2) indicates that *E. niphonica* populations in Hokkaido are highly divergent from those in Honshu. When an evolutionary rate of 1.5% sequence divergence per million years in arthropod COI gene is applied (Quek *et al.*, 2004), the estimated date of divergence between the Honshu and Hokkaido clades is 1.55–2.35 Ma (million years ago, calculated by using COI data only : sequence divergence of COI gene between the Hokkaido and Honshu populations=0.023–0.035). Furthermore, the phylogeography of *E. niphonica* suggests that there are two major clades in Honshu, one in central Honshu (clade A in Figs 1 and 2) and

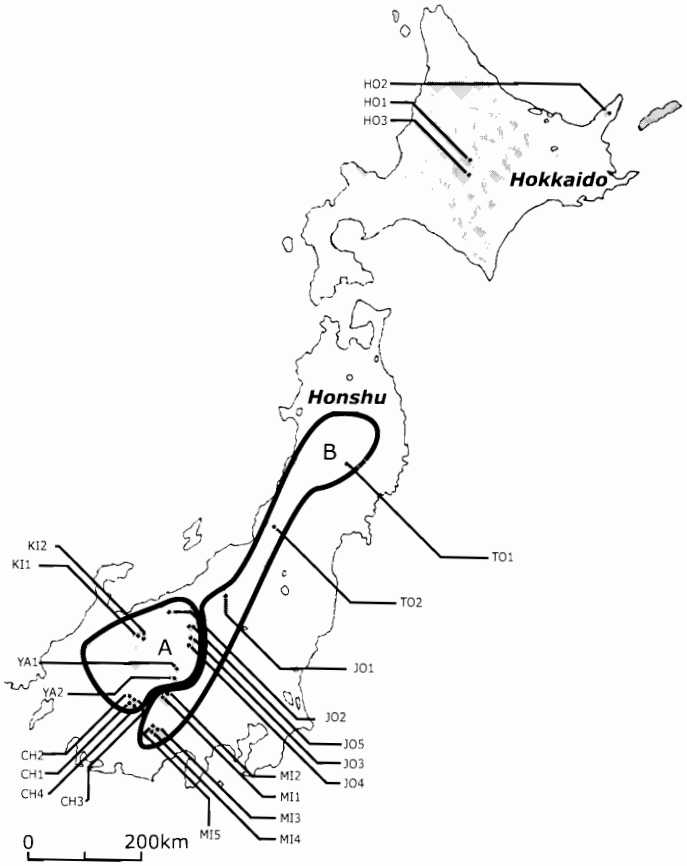

Fig. 1 The distribution of *Erebia niphonica* in Japan (shaded) and the sampling locations (dots). For details of the locations, see Table 1. Regions A and B indicate the distribution ranges of clades A and B, respectively (see Fig. 2).

the other (clade B) in eastern Honshu. Within each clade, several area-related subdivisions are observed.

Discussion

The evident divergence of the Honshu and Hokkaido clades of *Erebia niphonica* (Fig. 2) is consistent with the conclusion of Sekiguchi *et al.* (2000) that *E. niphonica* of Hokkaido constitutes a monophyletic clade together with *E. neriene* of Sakhalin, and the relationship between the clade and *E. niphonica* of Honshu is not even a sister clade. The time flame (1.55-2.35 Ma) of the disjunction between the Honshu and Hokkaido clades coincides with the periods of glacial advances and retreats in the late Pliocene or the early Pleistocene. This suggests a possibility that *E. niphonica* of Honshu diverged from some ancestral continental *Erebia* species

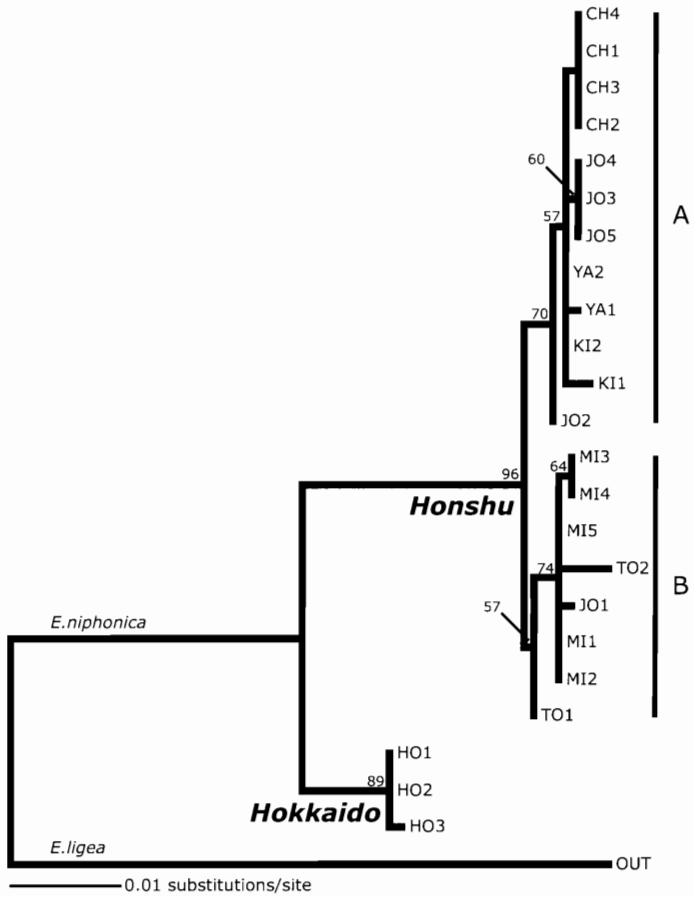

Fig. 2 Maximum likelihood tree for *Erebia niphonica* populations inferred from mitochondrial COI (439bp) and ND5 (487bp). Bootstrap values>50% from 1000 replicates are shown above branches. The sampling locations are shown (see Table 1).

during this period. A more exhaustive phylogeny including *E. niphonica* of Honshu, *E. niphonica* of Hokkaido, and other continental *Erebia* species is necessary to elucidate the history of divergence of Japanese *E. niphonica*.

E. niphonica of Honshu is highly structured in the phylogeny (Fig. 2). Especially, the subdivision between the central and eastern Honshu populations is significant (Fig. 2), suggesting multiple immigration events from the continental populations, or divergence in Honshu.

In the phylogeographic study of Japanese alpine plant *Primula cuneifolia*, Fujii *et al.* (1999) detected three major clades in Japan (Northern Hokkaido, Southern Hokkaido and Honshu), and showed that the Honshu clade is especially highly structured. They proposed that *P. cuneifolia* in Honshu diversified due to the contraction of their distribution in the periods of the glacial retreat in Pleistocene. The highly structured phylogeny of *E. niphonica* in Honshu strongly suggests a scenario similar to the one proposed by Fujii *et al.* (1999).

Acknowledgments

We thank H. Fujii, M. Suzuki, T. Matsumura, K. Maruyama, Y. Fukuda, K. Inami and K. Kobayashi for the collection of specimens. We are grateful to S. Ueda for technical support and T. Yagi for helpful discussion and critical reading of the manuscript.

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和文摘要

ベニヒカゲ *Erebia niphonica* は日本の高山蝶の中では最も広く分布する種である．本研究では国

内の23地点からベニヒカゲ34個体を採集し，それらについてミトコンドリアDNAのCOI遺伝子（439bp）およびND5遺伝子（487bp）を用いて分子地理系統樹を作成した．その結果，国内産ベニヒカゲの北海道クレードは本州クレードと大きく分かれた．本州内においても，系統学的に有意に異なる，地域ごとの集団構造がみられた．特に本州中央部および東部の集団はよりはっきりした構造を示しており，大陸からの多重侵入，または本州内において集団の分岐が起こったことを示唆した．このことは他の高山性生物の国内での分岐パターンと類似しており，同様の現象が並行して起こった可能性が考えられる．

＜採集・観察記録＞

長野県松本平におけるアオサナエの初記録

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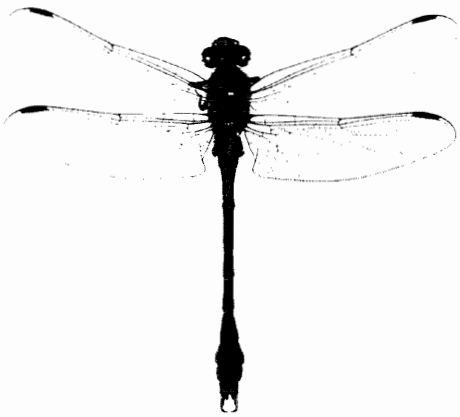
アオサナエ *Nihonogomphus viridis* Ogumaは，長野県中信地方では大町市の木崎湖や農具川が多産地として有名であるが，松本市周辺の松本平からは全く知られていなかった．しかし筆者の一人枝は今年（2006年）7月4日に塩尻市田川で，流れから突き出た石に静止している本種1♂を発見し，慎重にこれを採集することが出来た（写真）．松本平での初記録である．その後，筆者の一人森井は同年7月15日に開催された“信州大学自然誌科学館（女鳥羽川の生きもの観察会）”の際に，同所で本種1♂を捕獲した．松本市から初めてで，松本平2番目の記録である．枝は過去から女鳥羽川をかなり調査しているが，ミヤマサナエやオナガサナエなどを得ているものの，アオサナエは目撃さえも出来なかったのである．



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