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Parapatric Distribution of the Lizards *Plestiodon* (Formerly *Eumeces*) *latiscutatus* and *P. japonicus* (Reptilia: Scincidae) Around the Izu Peninsula, Central Japan, and Its Biogeographic Implications

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The scincid lizard *Plestiodon latiscutatus* is found in the Izu Islands and Izu Peninsula of central Japan, whereas *P. japonicus*, a close relative, is found over the entire main island group of Japan, except the Izu Peninsula. The precise area of occupancy of these species was surveyed around the Izu Peninsula. Species identification was made through comparison of mitochondrial DNA partial sequences of specimens from the Izu Peninsula with those from the other regions, since morphological differences between these species have not yet been characterized. This study determined that these species are deeply diverged from each other in mitochondrial DNA sequence, and that the ranges of these species overlap only in a narrow zone. The results imply that gene flow between these species, if any, is restricted to a low level, without physical barriers. The boundary between the geographic ranges of these species was established as occurring along the lower Fuji River, Mt. Fuji, and the Sakawa River. This region is concordant with that of the old sea that is assumed to have separated the Izu Peninsula from other parts of the Japanese main island group until the middle Pleistocene. This pattern suggests that *P. latiscutatus* and *P. japonicus* were differentiated allopatrically before the connection of land areas of the Izu Peninsula and Honshu, the main island of Japan, and come into secondary contact through this connection. Thus, the species boundary is likely to have been maintained *in situ*, without physical barriers, since the secondary contact in the middle Pleistocene.

Key words: *Plestiodon latiscutatus*, *Plestiodon japonicus*, parapatry, secondary contact, Izu Peninsula, mitochondrial DNA

INTRODUCTION

The recent accumulation of phylogeographic studies using genetic markers has revealed occurrences of genetically differentiated entities that are difficult to recognize by morphological approaches alone. An array of studies has demonstrated that these entities have various geographic arrangements , for example in Japan, the allopatry of salamanders (Nishikawa *et al*., 2001, 2005), the parapatry of land snails (Hayashi and Chiba, 2000, 2004; Shimizu and Ueshima, 2000; Watanabe and Chiba, 2001) and ground beetles (Su *et al*., 1998), and the sympatry of geckos (Toda *et al*., 2001a, b) and salamanders (Tominaga *et al*., 2003). How such genetic differentiation is maintained despite a close relationship is an important question in the study of biodiversity. In cases of allopatry, there is virtually no interaction between isolated populations, and the occurrence and maintenance of genetic differentiation can be explained by the physical barrier. In cases of parapatry and sympatry, there can be various types of interactions between populations, some of which, such as assortative mating (*e.g*., McMillan *et al*., 1997) or selection against hybrids (*e.g*., MacCallum *et al*., 1995), should maintain genetic differentiation. Thus, the presence or absence of any interactions between particular populations is the most fundamental consideration in inferring maintenance mechanisms. This can be resolved by determining the precise area of occupancy of the populations.

Until recently, the common scincid lizard that occurs throughout the entire Japanese main island group had been recognized as *Eumeces latiscutatus* (Hallowell, 1861), whereas the populations on the Izu Islands had been recognized as a closely related species, *E. okadae* Stejneger, 1907 (Taylor, 1936; Nakamura and Uéno, 1963; Hikida, 1993; Kato *et al*., 1994; Hikida, 1996). By examining allozyme variation in *E. latiscutatus* throughout the entire Japanese main island group, Motokawa and Hikida (2003) found that the populations on the Izu Peninsula of central Honshu (Fig. 1-B) are distinct from the other populations on the Japanese main island group, and closely related to *E. okadae*. Based on this result, they concluded that *Eumeces* on the Izu Peninsula is a distinct species from those of the remaining part of the Japanese main island group, and conspecific

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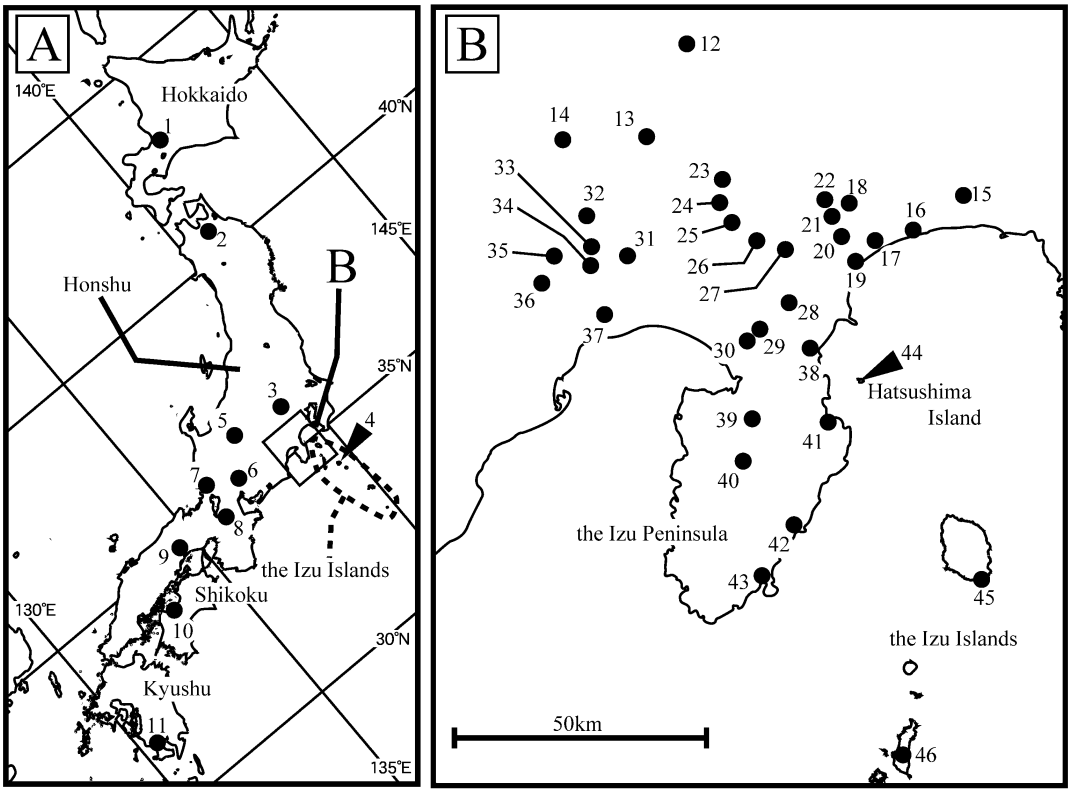

Fig. 1. Localities where the specimens were collected. **A)** The entire Japanese main island group. **B)** Izu Peninsula. See Appendix 1 for details.

with *E. okadae*. Because the type locality of *E. latiscutatus* is the southern Izu Peninsula (Shimoda: 43 in Fig. 1-B), they argued that *E. okadae* should be recognized as a junior synonym of *E. latiscutatus*, and that the previously suppressed name *E. japonicus* Peters, 1864 (type locality: Nagasaki, near 11 in Fig. 1A) should be resurrected for the other species distributed over the remainder of the Japanese main island group. In addition, recent phylogenetic studies (Griffith *et al.*, 2000; Schmitz *et al.*, 2004; Brandley *et al.*, 2005) and the taxonomic literature (Smith, 2005) have indicated that East Asian members of the genus *Eumeces* (*sensu lato*), including *E. latiscutatus* and *E. japonicus*, should be recognized as a separate genus, *Plestiodon*. In the present paper, we follow Motokawa and Hikida (2003), and Brandley *et al.* (2005) and Smith (2005) for the specific and generic classifications, respectively.

Motokawa and Hikida's (2003) findings indicated that the two closely related species are distributed within central Honshu. This situation prompted us to the question how their genetic differentiation has been maintained. To answer this question, an examination of the exact geographic distributions of *Plestiodon latiscutatus* and *P. japonicus* is critical. We surveyed the precise geographic distributions of these species on the basis of a number of specimens collected from the Izu Peninsula and adjacent regions. The Izu Peninsula population of *P. latiscutatus* is nearly identical to *P. japonicus* in external morphology (Motokawa and Hikida, 2003), and therefore identification without genetic markers is difficult. Thus, we first analyzed DNA sequence variation within and between these species to determine fixed differences between them, and then identified each specimen collected from the Izu Peninsula and adjacent regions on the basis of these differences.

MATERIALS AND METHODS

Samples examined

A total of 180 specimens of *Plestiodon latiscutatus* and *P. japonicus* were collected from 46 localities on the Japanese main island group and several adjacent islands, including some parts of the Izu Islands (Fig. 1; see Appendix 1 for details). To clarify whether the geographic ranges of these species overlapped, intensive surveys were made in the Izu Peninsula and adjacent regions (Fig. 1B). One specimen of *P. barbouri*, a closely related species (Hikida, 1993; Kato *et al.*, 1994), was used as an outgroup in the genealogical inference. Liver or skeletal muscle tissue was taken from each specimen and stored at –80°C or in 99% ethanol.

Identification of specimens from the Izu Peninsula

We identified specimens from the Izu Peninsula as *P. latiscutatus* or *P. japonicus* by comparing mitochondrial DNA (mtDNA) sequences. Prior to identification of these specimens, genetic variation within and between the two species was evaluated by inferring the gene genealogy among haplotypes from various localities. For this analysis, nucleotide sequences were determined for 26 specimens from 21 localities around the Izu Peninsula (12–17, 19, 23, 24, 27–30, 33, 36, 40, 43, 44 in Fig. 1) and Izu Islands (4, 45, 46 in Fig. 1), and 10 specimens from 10 other localities around the Japanese main island group (1–3, 5–11 in Fig. 1).

In the inferred genealogy, two distinct groups were evident: one consisted of haplotypes from the Izu Peninsula and Izu Islands, and the other consisted of those from the Japanese main island group (see Results). Because the geographic ranges of the two groups

agreed well with those of the two species delineated by a previous allozyme study (Motokawa and Hikida, 2003), these haplotypes were regarded as representative of *P. latiscutatus* and *P. japonicus*, respectively. By comparing nucleotide sequences between the two haplotype groups, fixed nucleotide differences between these species were detected. Using restriction enzymes recognizing some of the fixed differences, the remaining 144 specimens were identified without sequencing by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis (see below for further details).

Nucleotide sequencing and PCR-RFLP genotyping

Total DNA was extracted using the methods of Wada *et al*. (1992) and Honda *et al*. (1999). Tissues were frozen with liquid nitrogen, crushed, and lysed in lysis buffer (10 mM Tris-HCl [pH 8.0], 10 mM EDTA [pH 8.0], 150 mM NaCl, 0.1% SDS). After digestion of samples with proteinase K (100 μg/ml) at 50–55°C for three hours, DNA was extracted two or three times with phenol and once with 25:24:1 phenol/chloroform/isoamyl-alcohol, and precipitated in two volumes of 99% ethanol with one-tenth volume of 5.0 M sodium acetate (pH 5.2). Precipitated samples were dried and stored in TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0]). Part of the mitochondrial 12S and 16S rDNA regions were amplified with a PCR System GeneAmp 2400 or 2700 Thermal Cycler (Applied Biosystems, Lincoln, USA), using an Ex *Taq* Polymerase Kit (Takara Shuzo Co., Ltd., Otsu, Japan) and the primers L1091 (5'-AAACTGGGATTAGATACCCCACTAT-3') and H1478 (5'-GAGGGTGACGGGCGGTGTGT-3') for 12S rDNA (Kocher *et al*., 1989), and L2606 (5'-CTGACCGTGCAAAGGTAGCGTAATCACT-3') and H3056 (5'-CTCCGGTCTGAACTCAGATCACGTAGG-3') for 16S rDNA (Hedges *et al*., 1993; Honda *et al*., 1999). Both the 12S and 16S regions were sequenced directly; however, only 16S was used for RFLP analysis. The thermocycling regime was 30 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C, with an initial denaturation step of 5 min at 94°C and a final extension of 7 min at 72°C.

PCR products were purified by polyethylene glycol (PEG) precipitation using 0.6 volume of PEG solution (20% PEG 6000, 2.5 M NaCl) and then sequenced with a Big Dye Terminator Cycle Sequencing Ready Reaction Kit v1.0 and ABI PRISM 377 DNA Sequencer or ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Lincoln, USA), using the primers described above. The sequence data have been submitted to the GenBank database (Accession Nos. DQ173497–DQ173534).

For RFLP, aliquots of 16S rDNA PCR products were digested for three or four hours with two enzymes separately, *Dra* I and *Bsi*HKA I (Promega Corp., Madison, USA). These enzymes were selected to discriminate *latiscutatus* from *japonicus* sequences; the former cuts *latiscutatus* sequences only, whereas the latter cuts *japonicus* sequences only. The digested samples were subjected to 2% agarose gel electrophoresis in TAE buffer, stained by ethidium bromide (0.5 g/mL), and viewed with UV illumination.

Inference of gene genealogy of mtDNA

In the inference of genealogy, identical sequences were treated as a single unit. Sequences were automatically aligned in ClustalX version 1.81 (Thompson *et al*., 1997), and then adjusted by eye considering inferred secondary structures (not shown) of the 12S and 16S rRNA of *Plestiodon egregius* (GenBank Accession No. AB016606; Kumazawa and Nishida, 1999). The rRNA secondary structures of *P. egregius* were inferred by comparison with the hypothetical secondary structure of the homologous rRNAs of some other vertebrates available at the CRW site (Cannone *et al*., 2002). The mtDNA genealogy was inferred under the maximum likelihood (ML) criterion (Felsenstein, 1981), following a chi-square test to determine whether there was homogeneous equilibrium of base composition among sequences. Gaps inserted through alignment were treated as missing data. The optimal model (GTR with a gamma-distributed heterogeneous substitution rate among sites) of sequence evolution assumed in the ML inference was selected among the available models in TREEFINDER version March 2004 (Jobb, 2004) based on the Akaike information criterion (AIC: Akaike, 1974; Posada and Crandall, 1998). Support for the inferred topology was assessed by bootstrap probabilities (Felsenstein, 1985) with 1000 pseudoreplicates. The chi-square test for base composition bias, search for the ML tree, and bootstrapping were conducted with TREEFINDER version March 2004 (Jobb, 2004).

Table 1. Haplotype - locality correspondence. The designations of haplotypes (a–r) and the numbers for localities correspond to those in Fig. 2 and Fig. 1, respectively.

	haplotype	locality
	a	4
	b	46
P. latiscutatus	c	29
	d	19, 24 (two individuals), 27, 28, 33, 40, 43, 44, 45
	e	30
	f	11
	g	10
	h	8
	i	9
	j	6
	k	7
P. japonicus	l	23
	m	23
	n	2
	o	12, 13
	p	1, 5, 14, 15, 16, 24, 36
	q	17
	r	3, 24 (two individuals)

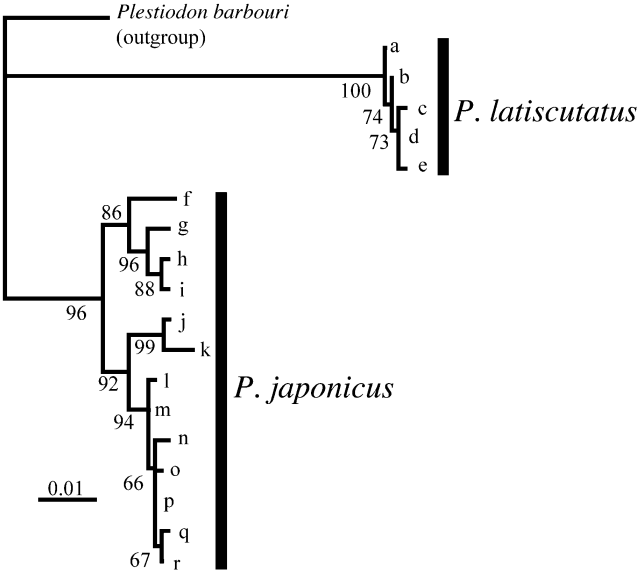

Fig. 2. Maximum likelihood tree showing genealogical relationships among 18 observed haplotypes of 12S+16S rDNA partial sequences (approximately 815 bp) from 36 specimens. Each unit (a–r) denotes one the 18 observed haplotypes. The localities of the specimens with each haplotype are presented in Table 1. The numbers at the internal edges indicate bootstrap probabilities (%). Only those greater than 50% are shown.

RESULTS

Genetic differentiation between species

In the 36 ingroup specimens sequenced, 18 haplotypes were observed (Table 1). No significant deviation from an assumption of homogeneous base composition equilibrium was found. The multiple alignment of the 18 sequences and the outgroup contained few gaps, none of which was longer than one base pair. The combined aligned sequences were 815 bp long (12S rRNA, 385bp; 16S rRNA, 430bp), with 92 and 80 variable sites for 12S and 16S, respectively.

The maximum likelihood tree (Fig. 2) showed two distinct groups. A deep divergence (6.43% mean pairwise *p*-distance) was observed between the two groups, compared to divergences within the groups (0.08% in the upper group in Fig. 2, 0.93% in the lower). The upper group included the specimens from the Izu Islands and Izu Peninsula and can be regarded as having haplotypes representing *Plestiodon latiscutatus*. The lower group included the specimens from the entire Japanese main island group except the Izu Peninsula, and can be regarded as having haplotypes of *P. japonicus*.

A comparison of 16S rDNA consensus sequences (not shown) between *P. latiscutatus* and *P. japonicus* showed fixed differences at 18 sites. In the subsequent PCR-RFLP analysis that incorporated a subset of these fixed differences, all 144 specimens were identified unambiguously as *P. latiscutatus* or *P. japonicus*.

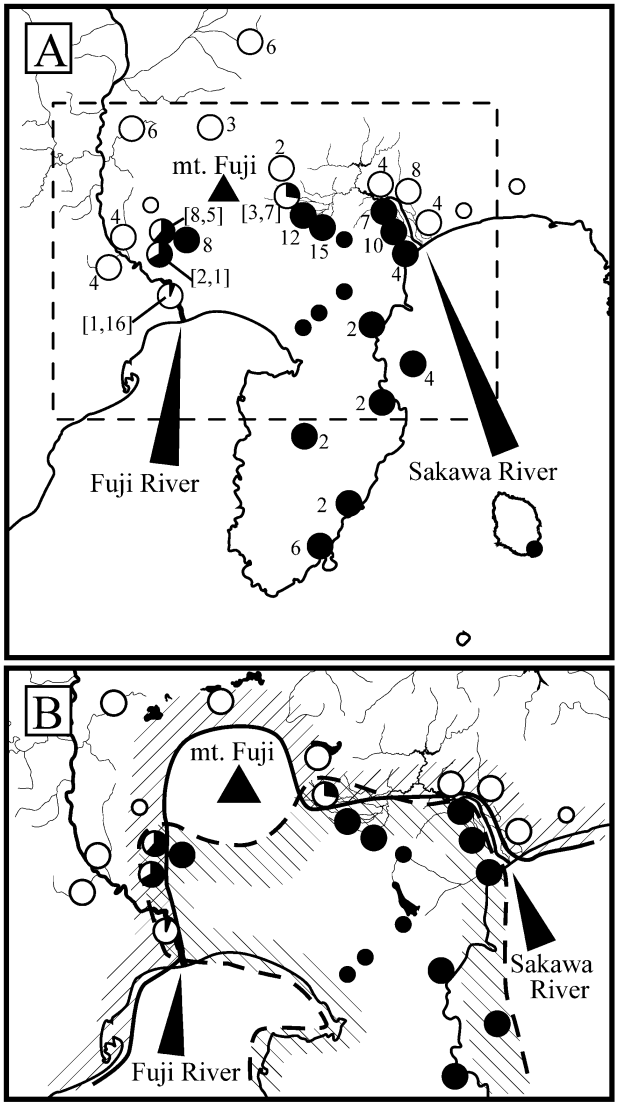

Fig. 3. A) Species composition of local samples. The small and large pie graphs indicate localities with one specimen and with two or more specimens, respectively. The solid and open sectors of each pie graph denote the fractions of *P. latiscutatus* and *P. japonicus*, respectively. The numbers adjacent to each pie graph are sample sizes. The numbers in brackets (left and right) indicate the number of *P. latiscutatus* and *P. japonicus*, respectively, at localities having both species. **B)** The ranges of *P. latiscutatus* (surrounded by a dashed line) and *P. japonicus* (surrounded by a solid line) around the Izu Peninsula, estimated from the data in A.

Geographic ranges of *Plestiodon latiscutatus* and *P. japonicus*

The species composition of each local sample around the Izu Peninsula is presented in Fig. 3A. Specimens identified as *P. latiscutatus* were found on and near the Izu Peninsula (19–21, 24–31, 33, 34, 37–43 in Fig. 1), Hatsushima Island (44 in Fig. 1), and the Izu Islands (4, 45, 46 in Fig. 1). In contrast, specimens identified as *P. japonicus* were found from the whole Japanese main island group except the Izu Peninsula (1–3, 5–18, 22–24, 32, 33, 34–37 in Fig. 1). The boundary between these two species was located along the lower Fuji River, the eastern and western sides of Mt. Fuji, and along the Sakawa River (Fig. 3-B). Along the boundary were four localities with both species (24, 33, 34, 37 in Fig. 1). Thus, these species are parapatrically distributed, with a narrow overlap zone (Fig. 3B).

At the localites along the boundary (24, 33, 34, 37 in Fig. 1), several specimens of both species were collected together at the same time and in the same microhabitat. For example, at locality 24 (Fig. 1), two individuals of *P. latiscutatus* (collection nos. KUZ R58316, 58319) and two individuals of *P. japonicus* (KUZ R58317, 58318) were collected from the same crack in a stone wall at the same time.

DISCUSSION

Current interspecific interactions

With respect to current interspecific interactions, three important aspects of our results should be addressed: (1) a deep divergence in mtDNA haplotypes exists between *Plestiodon latiscutatus* and *P. japonicus* (Fig. 2); (2) overlap of the geographic distribution of these species is limited to quite a narrow zone (Fig. 3); and (3) the two species were collected together at several localities along the narrow overlap zone.

The first and second aspects imply that gene flow between these species has been restricted to a low level for a considerable period. If there were a high or moderate level of gene flow between the two species, both species would either have become fixed to one of the two lineages, or the observed geographic ranges of the two mtDNA types would have overlapped greatly, by high spatial mobility and fast lineage sorting of mtDNA (Ferris *et al.*, 1983; Takahata and Slatkin, 1984; Avise, 2000). Instead, our results suggest that interspecific gene flow, if any, is at a low level. Concordance

between the geographic distribution of allozyme variants (Motokawa and Hikida, 2003) and mtDNA haplotypes additionally supports this conclusion. The third aspect, co-occurrences of individuals of these species, suggests that there is no physical barrier against interspecific interaction.

Together, these three aspects suggest that genetic differentiation between *P. latiscutatus* and *P. japonicus* is maintained by intrinsic reproductive isolation mechanisms, rather than a physical barrier. Although the boundary of the two species partly consists of the Sakawa River and the lower part of the Fuji River, the homogeneity of the mtDNA sequences within each species (Fig. 2), despite many other rivers in the region (such as upper part of the Fuji River: Fig. 3-A), suggests that a river itself is not a strict barrier to gene flow. Thus, we postulate that the genetic differentiation is maintained without any physical barriers. Nontheless, because our analyses are based on mtDNA data only, local hybridization along the boundary could have been overlooked. Further studies using nuclear genetic markers are needed to clarify this issue.

Origin of the parapatric distribution

A parapatric distribution of a pair of closely related species can arise in two ways: by primary intergradation through parapatric speciation, or by secondary contact preceded by allopatric differentiation (*e.g.*, Barton and Hewitt, 1985). Based on a theoretical study, Endler (1977) argued that without paleontological data it is difficult to distinguish between these two processes by interpreting a present geographic pattern. Nevertheless, independent geological evidence of a physical barrier in the past sometimes supports an origin by secondary contact of the parapatry of genetically differentiated entities. The parapatry of *Plestiodon* around the Izu Peninsula is such a case.

The Izu Peninsula is the current northern tip of the Philippine Sea Plate (Sugimura, 1972) and is assumed to have been separated from the remaining part of Honshu by the sea until the middle Pleistocene (Kitazato, 1997). Land areas of the Izu Peninsula and Honshu were connected in the middle Pleistocene (0.7 MYA) through the northward movement of the Philippine Sea Plate (e.g., Kitazato, 1997; Takahashi and Saito, 1997). Stratigraphic evidence (Kimura, 1985; Kitazato, 1997; Imanaga, 1999) indicates that the old sea covered only the region of the present-day Sakawa River and the lower part of the Fuji River (Fig. 4). Stratigraphic data for the same period is not available for the region around Mt. Fuji, because this region is covered with newer, thick volcanic material. In any case, the location of the old sea that had separated the Izu Peninsula from Honshu is concordant with the distributional boundary between *P. latiscutatus* and *P. japonicus*.

This concordance supports a hypothesis of parapatry by secondary contact between *P. latiscutatus* and *P. japonicus*. It appears that the distribution of these species around the Izu Peninsula is older than the land connection between the Izu Peninsula and Honshu; that is, before the land connection, the old islands of the Izu Peninsula and Izu Islands were already occupied by the ancestor of *P. latiscutatus*, whereas the side of the Japanese main island group was already occupied by the ancestor of *P. japonicus*. Thereafter, these species came into secondary contact through the connection of these land areas, which is assumed to have occurred at 0.7 MYA (Kitazato, 1997). If this was the case, the species boundary has been maintained without physical barriers *in situ* for a long time.

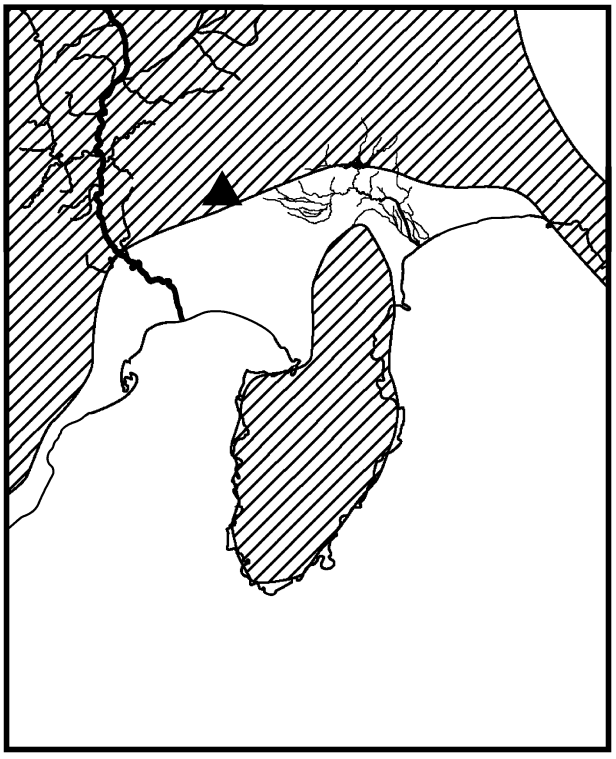

Fig. 4. Paleogeographic map of the Izu Peninsula in the early Pleistocene (modified from Kimura, 1985). Shaded parts indicate land areas.

Differentiation between populations of the Izu Peninsula and other parts of Honshu, and/or an affinity between the Izu Peninsula and the Izu Islands, has also been reported for several other organisms at the levels of faunas and populations. For example, there have been studies on the land-snail fauna (Habe, 1977) and the phylogeographic patterns of sea slaters (Itani, 1999, 2000) and land snails (Hayashi and Chiba, 2000, 2004; Shimizu and Ueshima, 2000; Watanabe and Chiba, 2001). The similarities in geographic differentiation patterns among these unrelated organisms implies isolation by a common extrinsic factor. This also supports our historical scenario of parapatry by secondary contact for *P. latiscutatus* and *P. japonicus*. Further study is required at both the faunal and population levels to better understand the origin of the fauna of the Izu Peninsula and the Izu Islands.

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Appendix 1. List of locality names and catalogue numbers (KUZ) of the specimens examined in this study. Numbers for the localities correspond to those in Fig. 1.

Plestiodon latiscutatus and *P. japonicus*

1, Sapporo, Hokkaido, KUZ R45154; 2, Mt. Hakkoda, Aomori, Aomori Pref., KUZ R30677; 3, Mt. Tenso, Okutama, Tokyo Metro., KUZ R58303; 4, Miyakejima Is., the Izu Islands, Tokyo Metro. KUZ R36495; 5, Azumi, Nagano Pref., KUZ R39024; 6, Kasahara, Gifu Pref., KUZ R45929; 7, Tsuruga, Fukui Pref., KUZ R51175; 8, Otsu, Shiga Pref., KUZ R58302; 9, Yamasaki, Hyogo Pref., KUZ R51930; 10, Saijo, Ehime Pref., KUZ R50633; 11, Kagoshima, Kagoshima Pref., KUZ R46921; 12, Yamato, Yamanashi Pref., KUZ R51139–51144; 13, Narusawa, Yamanashi Pref., KUZ R51137, 51138, 51162; 14, Shimobe, Yamanashi Pref., KUZ R51130–51133, 51135, 51136; 15, Samukawa, Kanagawa Pref., one uncataloged specimen; 16, Oiso, Kanagawa Pref., one uncataloged specimen; 17, Kouzu, Odawara, Kanagawa Pref., four uncataloged specimens; 18, Matsuda, Kanagawa Pref., KUZ R60271–60278; 19, Shiroyama, Odawara, Kanagawa Pref., four uncataloged specimens; 20, Tsukahara, Minami-ashigara, Kanagawa Pref., KUZ R60246–60255; 21, Nuda, Minami-ashigara, Kanagawa Pref., KUZ R60260–60266; 22, Yamakita, Kanagawa Pref., KUZ R60267–60270; 23, Yamanakako, Yamanashi Pref., KUZ R58313, 58314; 24, Subashiri, Oyama, Shizuoka Pref., KUZ R58315–58319, 59595, 59596, 60100–60103; 25, Tamaho, Gotenba, Shizuoka Pref., KUZ R60104–60114, one uncataloged specimen; 26, Higashitanaka, Gotenba, Shizuoka Pref., KUZ R60115–60129; 27, Sengokuhara, Hakone, Kanagawa Pref., KUZ R60130; 28, Hakone, Hakone, Kanagawa Pref., one uncataloged specimen; 29, Tsukaharashinden, Mishima, Shizuoka Pref., one uncataloged specimen; 30, Taisha, Mishima, Shizuoka Pref., one uncataloged specimen; 31, Yamamiya, Fujinomiya, Shizuoka Pref., KUZ R60137–60144; 32, Utsuno, Fujinomiya, Shizuoka Pref., KUZ R60407; 33, Kamijo, Fujinomiya, Shizuoka Pref., KUZ R51160, 60313, 60314, 60394–60402, one uncataloged specimen; 34, Aokidaira, Fujinomiya, Shizuoka Pref., KUZ R60403–60405; 35, Yagisawa, Nambu, Yamanashi Pref., KUZ R60311, 60312, 60315, 60316; 36, Fukushi, Nambu, Yamanashi Pref., KUZ R60310, 60411, 60412, one uncataloged specimen; 37, Iwabuchi, Fujikawa, Shizuoka Pref., KUZ R60317–60333; 38, Atami, Shizuoka Pref., KUZ R36328, 36340; 39, Shuzenji, Shizuoka Pref., KUZ R58274; 40, Amagiyugashima, Shizuoka Pref., KUZ R36363,36373; 41, Itou, Shizuoka Pref., KUZ R36400, 36403; 42, Inatori, Higashiizu, Shizuoka Pref., KUZ R36285, 36286; 43, Shimoda, Shizuoka Pref., KUZ R36318, 36323,36334–36336, one uncataloged specimen; 44, Hatsushima Is., Atami, Shizuoka Pref., KUZ R36048, 36140, 36141, one uncataloged specimen; 45, Oshima Is., the Izu Islands, Tokyo Metro., KUZ R51161; 46, Niijima Is., the Izu Islands, Tokyo Metro., one uncataloged specimen.

P. barbouri

Kakeroma Is., Amami Group, Kagoshima Pref., KUZ R35982.