Discovery of sympatric cryptic species within *Gekko hokouensis* (Gekkonidae: Squamata) from the Okinawa Islands, Japan, by use of allozyme data

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Accepted: 25 September 2000

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Introduction

A cryptic species refers to a reproductively independent unit that is not morphologically discernible from a recognized species. Such an entity offers potentially interesting material for the study of the causes of character evolution and divergence. However, in many cases, such cryptic species are undetectable by systematists dealing only with morphological characters (Mayr & Ashlock 1991).

Allozyme analysis has played an active part in the field of systematic biology because it can often detect taxonomic and phyletic diversity in groups of organisms showing little morphological divergence, or in groups exhibiting complicated patterns of morphological variation (e.g. Hillis et al. 1981; Hedges & Thomas 1991; Toda et al. 1998). This approach, coupled with various population genetic models, is an especially powerful tool for the elucidation of non-random reproductive systems, and thus is most useful in detecting sympatric cryptic species that may not be revealed by examining variation in morphological characters, or in maternally inherited mitochondrial DNA markers.

During an intensive allozyme survey on the intraspecific genetic variation in *Gekko hokouensis*, a gekkonid species broadly distributed from eastern continental China to southern Japan (Zhou et al. 1982), we found two genetically distinctive entities from several islands of the Okinawa Group, Ryukyu Archipelago, Japan. These entities, while genetically strongly differentiated from each other, exhibited extremely morphologically similar, sharing all of the character states that have been regarded as diagnostic of *G. hokouensis*. In this paper, we document this extreme example of discrepancy between morphological and genetic divergences, and discuss its taxonomic and evolutionary implications.

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![Map of East Asia showing localities of samples of *Gekko hokouensis* examined in this study: 1, Iheyajima; 2, Izenajima; 3, Iejima; 4, Benoki; 5, Katsu-u; 6, Nago; 7, Nakagunoku; 8, Chinen; 9, Agunijima; 10, Tokashikijima; 11, Akajima; 12, Tonakijima; 13, Kumejima; 14, Yamagawa; 15, Kuchinoshima; 16, Amamioshima; 17, Ishigakijima; 18, Taipei. The broken line and white circle indicate the known distributional range (slightly modified from Zhou *et al.* 1982) and the type locality of *G. hokouensis*, respectively.](image)

Zootaxa 53, 3, January 2001, pp1–11 • © The Norwegian Academy of Science and Letters
enzymes analysed, their presumptive loci and tissue sources, systems and one non-enzymatic protein were screened. The Kyoto University (KUZ: see Appendix for further details).

Monkeys after fixation in 10% formalin, and were deposited in gel electrophoresis. Voucher specimens were preserved in 70% men, and their extracts were subjected to horizontal starch populations from southern Kyushu, the northern Ryukyus, the Amami Group of the central Ryukyus, and the southern Ryukyus and Taiwan, were also examined for comparisons the large size of Okinawajima (1.192 km²). Groups of species belong to a single interbreeding population. Because of the large size of Okinawajima (1.192 km²), groups of specimens collected from five localities within this island (each <3 km²).

Table 1 Localities and sizes of the samples examined in this study. Sample numbers correspond to those in Fig. 1.

| Locality            | Size  |
|---------------------|-------|
| Okinawa Group       |       |
| 1. Iheyajima        | 40    |
| 2. Izenajima        | 36    |
| 3. Kajima           | 23    |
| 4. Beniski (Okinawajima) | 51 |
| 5. Katsusu (Okinawajima) | 6 |
| 6. Nago (Okinawajima) | 20  |
| 7. Nakagusuku (Okinawajima) | 39 |
| 8. Chinen (Okinawajima) | 20  |
| 9. Akiu (Okinawajima) | 20   |
| 10. Tokashikijima   | 20    |
| 11. Akajima         | 18    |
| 12. Tosashinjima    | 40    |
| 13. Kurijima        | 46    |
| Southern Kyushu     |       |
| 14. Yanna-Gawa      | 28    |
| Northern Ryukyus    |       |
| 15. Kuchinoshima    | 20    |
| Amami Group (central Ryukyus) |   |
| 16. Amamioshima     | 15    |
| Southern Ryukyus    |       |
| 17. Ishikujima      | 20    |
| Taiwan              |       |
| 18. Taiji           | 25    |

Materials and methods

A total of 379 specimens were collected from 13 localities in nine islands of the Okinawa Group, the central Ryukyus (Fig. 1, Table 1). All of these specimens were identified as G. hokouensis on the basis of diagnostic external characters (i.e. internasal as large as following scales; enlarged tubercules present on dorsal surface of body, but absent on limbs; single pair of cloacal spur; male pores only in the preanal region; apical caudal tubercles absent or present but only in proximal portion: Ota 1989). Specimens from each island, except those from Okinawajima, were treated as a single sample on the basis of the assumption that individuals occurring on a small island belong to a single interbreeding population. Because of the large size of Okinawajima (1.192 km²), groups of specimens collected from five localities within this island (each <3 km x 3 km) were designated as five separate samples for the analysis. Five other samples of G. hokouensis, representing populations from southern Kyushu, the northern Ryukyus, the Amami Group of the central Ryukyus, the southern Ryukyus and Taiwan, were also examined for comparisons (Fig. 1, Table 1).

Liver and skeletal muscles were removed from each specimen, and their extracts were subjected to horizontal starch gel electrophoresis. Voucher specimens were preserved in 70% ethanol after fixation in 10% formalin, and were deposited in the Herpetological Collection of the Department of Zoology, Kyoto University (KUZ: see Appendix for further details).

By-products of 30 presumptive loci encoding 23 enzyme systems and one non-enzymatic protein were screened. The enzymes analysed, their presumptive loci and tissue sources, and the buffer systems employed are listed in Table 2. Notations of loci and alleles follow Toda et al. (1997).

We conducted a significance test using exact probability (the ‘EXACTP’ option in BIOSYS-1: Swofford & Selander 1981) for each polymorphic locus to see whether the genotype frequency in each sample satisfied Hardy–Weinberg expectations. In these tests, we adopted a null hypothesis that each sample is composed of individuals from a single randomly mating population. When the hypothesis was rejected by significant deviation at one or more loci ($P < 0.05$), the sample was divided into two subsamples on the basis of the combination of alleles possessed by each individual (see below), and the genotype frequency in each subsample was again subjected to the testing procedure mentioned above.

To compare overall genetic differentiation among samples and subsamples from the Okinawa Group and those from other regions (see above), we calculated Nei’s (1978) unbiased genetic distance for all pairwise comparisons of samples and subsamples examined. The coefficient was clustered by the UPGMA algorithm (Sneath & Sokal 1973). All calculations were made using BIOSYS-1 (Swofford & Selander 1984).

Results

Significant deviations of genotype frequencies from Hardy–Weinberg expectations were detected for samples from Iheyajima, Izenajima, Tonakijima and Kumejima at 11–18 loci, apparently due to the heterozygote deficiencies. Heterozygotes were completely absent in these samples at six to eight loci, despite the presence of two kinds of homozygotes in each. Based on the differential patterns of alleles possessed at these loci, individuals in each of the four samples were grouped into two subsamples without any conflict: no individuals possessed a combination of alleles characteristic to both subsamples simultaneously, and genotype frequencies in each subsample satisfied Hardy–Weinberg expectations at all polymorphic loci. We thus considered these subsamples as corresponding to actual randomly mating populations and treated them separately in further analyses, designated by a ‘1’ or ‘2’ following the locality names.

Allele frequencies in all samples and subsamples at 26 polymorphic loci (95% criterion) are presented in Table 3. Due to remarkable allele frequency differences at many loci, the samples and subsamples from the nine islands of the Okinawa Group could be unequivocally classified into two groups. Of these, one, henceforth referred to as Group 1, consisted of 12 samples and subsamples, Iheyajima-1 (locality number 1 in Fig. 1), Izenajima-1 (2), Iejima (3), Katsu-u (5), Nago (6), Nakagusuku (7), Chinen (8), Agunijima (9), Tokashikijima (10), Kumejima (11), Tonakijima-1 and Kumejima-1 (13), all of which were characterized by possessing predominant alleles ‘b’, ‘a’, ‘a’, ‘d’, ‘b’, ‘c’, ‘b’, ‘t’ and ‘a’ or ‘c’ at AcoA, Acoh-2, Est, Gda, Idh-1, Idh-2, Mpi, Pnp, Pep-lgg and Pgm-1.
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respectively. The other, Group 2, consisted of five samples and subsamples, Iheyajima-2 (1), Izenajima-2 (2), Benoki (4), Tonakijima-2 (12) and Kumejima-2 (13), characterized by the predominant alleles 'a', 'b', 'e and f', 'b', 'b', 'e', 'd', 'd', 'c' and 'd' at the above loci, respectively. Thus, two sympatric subsamples from each of the four islands were assigned to different groups. Samples from outside of the Okinawa Group (i.e. those from Yamagawa, Kuchinoshima, Amamioshima, Ishigakijima and Taipei: locality numbers 14–18) showed allele frequencies similar to those of Group 1 at the above loci. Prominent genetic differences between the Group 1 and 2 samples and subsamples were also reflected by fairly large values of Nei's genetic distance ($D$), ranging from 0.489 (Katsu-u vs. Tonakijima-2) to 0.654 (Izenajima-1 vs. Izenajima-2) ($\bar{D} = 0.567$) (Table 4). On the other hand, $D$ values obtained within each group were fairly small (as small as, or smaller than, 0.052 for Group 1 and 0.085 for Group 2). $D$ values between the Group 2 samples and the samples from five other regions were considerably large (0.486–0.611, $\bar{D} = 0.553$), whereas the values between the Group 1 samples and the latter were much smaller (0.004–0.142, $\bar{D} = 0.083$).

**Table 2** Enzymes analysed, their presumptive loci and tissue sources, and buffer systems employed to examine allozyme variations among *Gekko* samples from the Okinawa Group and five other regions in the East Asian islands.

| Enzyme                         | E. C. Number | Locus | Tissue | Buffer system |
|-------------------------------|--------------|-------|--------|---------------|
| Aconitate hydratase           | 4.2.1.3      | Acoh-1| L      | CAPM6, TC8    |
| Aconitate hydratase           | 4.2.1.3      | Acoh-2| L      | CAPM6, TC8    |
| Adenosine deaminase           | 3.5.4.4      | Ada   | L      | TC7, TC9      |
| Aspartate amiontransferase    | 2.6.1.1      | Ast-1 | L      | CAPM6         |
| Aspartate amiontransferase    | 2.6.1.1      | Ast-2 | L      | CAPM6, TC7    |
| Adenylate kinase              | 2.7.3.2      | Ak    | M      | CAPM6         |
| Creatine kinase               | 2.7.3.2      | Cr    | M      | CAPM6         |
| Esterase                      | 3.1.1.1      | Est   | L      | CAPM6, TC7    |
| Fumarate hydratase            | 4.1.2.2      | Fumh  | L      | TC7           |
| General protein               | —             | Gp    | M      | CAPM6         |
| Glucose-6-phosphate isomerase | 5.3.1.9      | Gpi   | M      | CAPM6         |
| Glyceraldehyde dehydrogenase  | 1.1.3.1      | Ghdh  | L      | TC8           |
| Guanine deaminase             | 3.5.4.2      | Gda   | L      | TE8.7         |
| 3-Hydroxybutyrate dehydrogenase| 1.1.1.30     | Nhbd  | L      | TC7           |
| Isocitrate dehydrogenase      | 1.1.1.27     | Isdh-1| L      | CAPM6, TC7    |
| Isocitrate dehydrogenase      | 1.1.1.27     | Isdh-2| L      | CAPM6, TC7    |
| l-Lactate dehydrogenase       | 1.1.1.27     | Ldh-1 | L      | CAPM6         |
| l-Lactate dehydrogenase       | 1.1.1.27     | Ldh-2 | L      | CAPM6         |
| Malate dehydrogenase          | 1.1.1.37     | Mdh-1 | L      | M, CAPM6, TC8 |
| Malate dehydrogenase          | 1.1.1.37     | Mdh-2 | L      | M, CAPM6, TC8 |
| Malate dehydrogenase (NADP+)  | 1.1.1.40     | Mdhp  | M      | CAPM6         |
| Mannose-6-phosphate isomerase | 5.3.1.8      | Mpi   | M      | CAPM6         |
| Dipeptidase (leucyl-glycine)  | 3.4.–        | Pep-ig| L      | TC7           |
| Dipeptidase (leucyl-proline)  | 3.4.–        | Pep-il | L   | TE8.7         |
| Dipeptidase (leucyl-proline)  | 3.4.–        | Pep-il | L   | TE8.7         |
| Phosphoglucomutase            | 5.4.2.2      | Pgm   | L      | TC8           |
| Phosphoglucomutase            | 5.4.2.2      | Pgm   | L      | TC8           |
| Phosphogluconate dehydrogenase| 1.1.1.44     | Pgdh  | L      | CAPM6         |
| Purine-nucleoside phosphorylase| 2.4.2.1      | Pnp   | L      | TC8           |
| Superoxide dismutase          | 1.15.1.1     | Sod   | L      | CAPM6, TE8.7  |

Tissue: L, liver; M, muscle. Buffer systems: CAPM6, citrate-aminopropylmorpholine, pH 6.0 (Clayton & Tretiak 1972); TC7, tris-citrate, pH 7.0 (Oshawa & Prasad 1970); TC8, tris-citrate, pH 8.0 (Clayton & Tretiak 1972); TE8.7, tris-borate-EDTA, pH 8.7 (Boyer et al. 1963).

**Discussion**

Significant deficiencies of heterozygotes at a large number of polymorphic loci in the original samples from Iheyajima, Izenajima, Tonakijima and Kumejima indicate the occurrence of two genetic entities within each of the assemblages of *Gekko hokouensis* on these islands. Furthermore, these entities showed fixed differences at six to eight loci on each island.
Table 3  Allele frequencies at 26 polymorphic loci in all samples and subsamples of ‘Gekko hokouensis’ from the Okinawa Group and five other regions of the East Asian islands. Sample numbers (hyphenated numbers 1 and 2 indicate divided subsamples) correspond to those in Fig. 1. The size of each sample or subsample is provided in parentheses.

| Locus | Okinawa Group | Sample Group 1 | Sample Group 2 | Other regions |
|-------|---------------|----------------|----------------|---------------|
|       | (14)          | (26)           | (23)           | (6)           |
| 1-1   | (14)          |                |                | (28)          |
| 2-1   | (26)          |                |                | (23)          |
| 3     | (23)          |                |                | (23)          |
| 4     | (20)          |                |                | (20)          |
| 5     | (20)          |                |                | (20)          |
| 6     | (20)          |                |                | (20)          |
| 7     | (14)          |                |                | (14)          |
| 8     | (20)          |                |                | (20)          |
| 9     | (14)          |                |                | (14)          |
| 10    | (20)          |                |                | (20)          |
| 11    | (20)          |                |                | (20)          |
| 12    | (20)          |                |                | (20)          |
| 13    | (20)          |                |                | (20)          |
| 14    | (20)          |                |                | (20)          |
| 15    | (20)          |                |                | (20)          |
| 16    | (20)          |                |                | (20)          |
| 17    | (20)          |                |                | (20)          |
| 18    | (20)          |                |                | (20)          |

| Locus | Okinawa Group | Sample Group 1 | Sample Group 2 | Other regions |
|-------|---------------|----------------|----------------|---------------|
|       | (14)          | (26)           | (23)           | (6)           |
| 1-1   | (14)          |                |                | (28)          |
| 2-1   | (26)          |                |                | (23)          |
| 3     | (23)          |                |                | (23)          |
| 4     | (20)          |                |                | (20)          |
| 5     | (20)          |                |                | (20)          |
| 6     | (20)          |                |                | (20)          |
| 7     | (14)          |                |                | (14)          |
| 8     | (20)          |                |                | (20)          |
| 9     | (14)          |                |                | (14)          |
| 10    | (20)          |                |                | (20)          |
| 11    | (20)          |                |                | (20)          |
| 12    | (20)          |                |                | (20)          |
| 13    | (20)          |                |                | (20)          |
| 14    | (20)          |                |                | (20)          |
| 15    | (20)          |                |                | (20)          |
| 16    | (20)          |                |                | (20)          |
| 17    | (20)          |                |                | (20)          |
| 18    | (20)          |                |                | (20)          |

| Locus | Okinawa Group | Sample Group 1 | Sample Group 2 | Other regions |
|-------|---------------|----------------|----------------|---------------|
|       | (14)          | (26)           | (23)           | (6)           |
| 1-1   | (14)          |                |                | (28)          |
| 2-1   | (26)          |                |                | (23)          |
| 3     | (23)          |                |                | (23)          |
| 4     | (20)          |                |                | (20)          |
| 5     | (20)          |                |                | (20)          |
| 6     | (20)          |                |                | (20)          |
| 7     | (14)          |                |                | (14)          |
| 8     | (20)          |                |                | (20)          |
| 9     | (14)          |                |                | (14)          |
| 10    | (20)          |                |                | (20)          |
| 11    | (20)          |                |                | (20)          |
| 12    | (20)          |                |                | (20)          |
| 13    | (20)          |                |                | (20)          |
| 14    | (20)          |                |                | (20)          |
| 15    | (20)          |                |                | (20)          |
| 16    | (20)          |                |                | (20)          |
| 17    | (20)          |                |                | (20)          |
| 18    | (20)          |                |                | (20)          |
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**Table 3 Continued**

| Locus | Okinawa Group | Sample Group 1 | Sample Group 2 | Other regions |
|-------|---------------|----------------|----------------|---------------|
|       | (14) | (26) | (23) | (6) | (20) | (20) | (21) | (28) | (22) | (26) | (19) | (12) | (24) | (20) | (19) | (20) | (23) |
| G6a   | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.92 | 0.87 | 1.00 | 1.00 | 0.08 | 0.13 |
|       | 0.05 | 0.08 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Hbdh  | 0.35 | 0.25 | 0.12 | 0.10 | 0.55 | 0.25 | 0.62 | 0.53 |      |      | 0.41 | 1.00 | 0.13 |      |      |      |      |      |
|       | 0.65 | 0.75 | 0.88 | 1.00 | 1.00 | 1.00 | 0.90 | 0.45 | 0.75 | 1.00 | 0.38 | 0.47 |      | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Idh-1 | 0.02 | 0.03 |      |      |      |      |      |      |      |      | 1.00 | 0.97 | 1.00 |      |      |      |      |      |
|       | 0.98 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |
| Idh-2 | 0.04 | 0.05 |      |      |      |      |      |      |      |      | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
|       | 0.96 | 0.87 | 1.00 | 1.00 | 0.90 | 0.95 | 0.97 | 0.84 | 1.00 |      |      | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ldh-2 | 0.04 | 0.13 | 0.10 | 0.03 | 0.16 |      |      |      |      |      |      | 0.98 | 1.00 | 0.88 | 1.00 | 1.00 |      |      | 0.01 |
|       | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Mdh-1 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 0.82 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
|       | 0.02 | 0.18 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Mdh-2 | 0.05 | 0.01 | 0.01 | 0.02 | 0.02 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Mdhp  | 0.01 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Mpi   | 0.20 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.07 | 0.10 | 0.01 | 0.25 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Pep-1g | 0.02 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.06 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.33 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.06 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.05 | 0.01 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.45 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

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### Table 3  Continued

| Locus | Okinawa Group | Sample Group 1 | Sample Group 2 | Other regions |
|-------|---------------|----------------|----------------|---------------|
|       | 1-1 (14) | 2-1 (26) | 3 (23) | 5 (20) | 6 (41) | 7 (20) | 8 (20) | 9 (20) | 10 (20) | 11 (20) | 12-1 (26) | 13-1 (26) | 14 (28) | 15 (28) | 16 (28) | 17 (28) | 18 (28) |
| Pep-1gg | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.88 |
| b  | 0.98 | 0.08 |
| c  | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 |
| d  | 0.02 | 0.04 |
| Pep-1p | 0.02 | 0.05 | 0.02 |
| b  | 0.08 | 0.03 | 0.08 | 0.01 | 0.03 | 0.03 | 0.02 | 0.03 | 0.28 | 0.08 | 0.27 | 0.11 |
| c  | 1.00 | 0.98 | 0.92 | 1.00 | 0.99 | 1.00 | 0.60 | 0.97 | 0.94 | 0.48 | 0.81 | 0.05 | 0.60 | 0.62 | 0.19 | 0.74 | 1.00 | 0.73 | 0.87 |
| d  | 0.40 | 0.03 | 0.52 | 0.17 | 0.04 | 0.38 | 0.61 | 0.18 | 1.00 |
| e  | 0.03 |
| Pgm-1 | 0.02 | 0.01 | 0.35 | 0.62 | 0.61 | 0.61 | 0.79 |
| b  | 0.08 | 0.25 |
| c  | 1.00 | 0.90 | 1.00 | 0.75 | 1.00 | 0.99 | 1.00 | 0.65 | 0.38 | 0.39 | 0.21 | 0.02 | 0.01 | 0.98 | 1.00 | 0.97 | 0.97 | 0.96 |
| d  | 0.98 | 1.00 | 0.99 | 0.79 | 1.00 | 1.00 | 1.00 | 0.03 | 0.04 | 0.21 |
| e  | 0.02 | 0.03 |
| Pgm-2 | 0.17 | 0.03 | 0.47 | 0.05 |
| b  | 0.25 | 0.42 | 0.17 | 0.08 | 0.30 | 0.43 | 0.45 | 0.20 | 0.57 | 0.56 | 0.25 | 0.29 |
| c  | 0.08 | 0.08 | 0.03 | 0.25 | 0.05 | 0.03 | 0.11 | 0.14 | 0.04 | 0.05 | 0.10 | 0.08 | 0.15 | 0.25 | 0.53 | 0.03 | 0.37 | 0.02 |
| d  | 0.03 | 0.10 | 0.47 |
| e  | 0.02 | 0.46 | 0.59 | 0.47 | 0.01 | 0.20 | 0.31 | 0.13 | 0.19 | 0.90 | 0.90 | 0.88 | 0.71 | 0.85 | 0.32 | 0.83 | 0.08 | 0.48 |
| f  | 0.10 | 0.04 | 0.29 | 0.02 |
| g  | 0.75 | 0.48 | 0.20 | 0.25 | 0.20 | 0.31 | 0.30 | 0.30 | 0.32 | 0.22 | 0.11 | 0.42 | 0.08 |
| h  | 0.08 |
| Pgdh | 0.02 | 0.01 | 0.03 |
| b  | 0.05 |
| c  | 0.10 |
| d  | 0.93 | 0.90 | 0.87 | 0.52 | 0.92 | 0.91 | 0.50 | 0.95 | 0.92 | 0.92 | 0.89 | 0.93 | 0.06 | 0.86 | 1.00 | 1.00 | 0.09 | 0.77 | 0.92 | 0.90 |
| e  | 0.07 | 0.10 | 0.13 | 0.08 | 0.06 | 0.09 | 0.50 | 0.05 | 0.05 | 0.08 | 0.08 | 0.05 | 0.94 | 1.00 | 0.12 | 0.91 | 1.00 | 0.23 |
| f  | 0.09 | 0.02 | 0.01 |
| g  | 0.10 |
| Pnp | 0.06 | 0.03 | 0.05 | 0.05 | 0.02 | 0.38 | 0.03 | 0.20 |
| b  | 1.00 | 1.00 | 1.00 | 0.92 | 0.97 | 0.95 | 1.00 | 0.95 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| c  | 0.02 | 0.62 | 0.97 | 1.00 | 1.00 | 0.80 |
| d  | 0.05 | 0.13 |
| Sod | 1.00 | 1.00 | 0.93 | 0.87 | 1.00 |
| a  | 0.04 |
| b  | 0.96 | 0.90 | 0.56 | 0.92 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.70 |
| c  | 0.04 | 0.10 | 0.44 | 0.08 | 0.10 | 0.10 | 0.26 |
## Table 4

Matrix of Nei’s (1978) genetic distance values between samples and subsamples of *Gekko hokouensis* from the Okinawa Group and five extralimital regions in the East Asian islands.

| Population       | Okinawa Group (genetic Group 1) | Okinawa Group (genetic Group 2) | Other regions |
|------------------|----------------------------------|----------------------------------|---------------|
|                  | 1                                | 2                                | 3             |
| 1. Iwayajima-1    | —                                | 0.002                            | —             |
| 2. Izenajima-1    | 0.018                            | 0.013                            | —             |
| 3. Kiiijima       | 0.014                            | 0.009                            | 0.007         |
| 4. Katsujiko      | 0.015                            | 0.011                            | 0.011         |
| 5. Nago           | 0.009                            | 0.017                            | 0.009         |
| 6. Nakagusuku     | 0.013                            | 0.018                            | 0.012         |
| 7. Chinen         | 0.016                            | 0.015                            | 0.026         |
| 8. Aguniijima     | 0.003                            | 0.047                            | 0.036         |
| 9. Tokachikijima  | 0.004                            | 0.015                            | 0.024         |
| 10. Akajima       | 0.041                            | 0.039                            | 0.045         |
| 11. Tonakijima-1  | 0.026                            | 0.024                            | 0.043         |
| 12. Kumejima-1    | 0.644                            | 0.637                            | 0.599         |
| 13. Iwayajima-2   | 0.654                            | 0.647                            | 0.589         |
| 14. Kumejima-2    | 0.572                            | 0.568                            | 0.516         |
| 15. Benoki        | 0.538                            | 0.533                            | 0.489         |
| 16. Tonakijima-2  | 0.559                            | 0.555                            | 0.520         |
| 17. Kumejima-2    | 0.119                            | 0.122                            | 0.121         |
| 18. Yanagawa      | 0.029                            | 0.024                            | 0.016         |
| 19. Kuchinokijima | 0.096                            | 0.100                            | 0.110         |
| 20. Anamokijima   | 0.042                            | 0.040                            | 0.032         |
| 21. Shijuku       | 0.041                            | 0.041                            | 0.041         |
| 22. Taipei        | 0.051                            | 0.052                            | 0.053         |
(Table 3), indicating the absence of gene flow between them. Detailed sampling data showed that several specimens of both entities were collected together from the same microhabitats on Tonakijima and Kumejima (e.g. from the same sidewalk and the same small cottage along a forest road, and the same rocky cliff). This indicates that the two entities occur syntopically, at least in part, on these islands and thus potentially have chances to interbreed under natural conditions. Thus, we can safely conclude that certain reproductive isolation mechanisms have been established between the two entities and that they actually represent separate biological species.

On Okinawajima, the two species were collected from separate localities: a Group 2 sample was obtained exclusively from Benoki, the northern part, whereas Group 1 samples derived from the middle (Nago and Katsu-u) and southern (Nakagusuku and Chinen) parts (see Fig. 1). The northern part of Okinawajima is more humid and mountainous with evergreen broad-leaf forest being the predominant vegetation, whereas the middle and southern parts are drier and relatively flat with more open vegetation. It is thus probable that the two species have segregated on this large island on the basis of their habitat preferences.

Such an assumption for the difference in habitat preference between the two species is supported by field observations in some other islands. Although there are no quantitative data for statistical testing, it is obvious from observations in Iheyajima and Kumejima that the Group 2 species tends to occur more frequently in undisturbed habitats, such as dense forests, than in open habitats compared to the Group 1 species, and vice versa. However, such a tendency was not evident in Tonakijima, where the two species were observed in nearly equal frequencies in both forest and open habitats. Tonakijima is much smaller (3.5 km$^2$) than Iheyajima (20.6 km$^2$) and Kumejima (58.4 km$^2$), and thus may not be large enough for the two geckos to segregate their habitats.

Samples from southern Kyushu, the northern Ryukyus, the Amami Group, the southern Ryukyus and Taiwan did not show significant deviation from the Hardy–Weinberg expectation at any of the loci examined, and their genetic properties are essentially similar to those of the Group 1 samples of the Okinawa Group (Fig. 2). These results suggest that

Fig. 2 UPGMA phenogram for the samples and subsamples of the two genetic groups of Gekko hokouensis based on Nei's (1978) genetic distance.
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populations of ‘Gekko bokouensis’ in other regions of the East Asian islands are conspecific with the Okinawan Group 1 populations, and that the species represented by the Group 2 populations is geographically restricted to the Okinawa Group. Gekko bokouensis was originally described from Hok’ou (= Yanshan), Jiangsu Province, eastern continental China (Pope 1928; Zhao & Adler 1993) (Fig. 1). We have no direct information at present regarding the relationships among the present two cryptic species in the East Asian islands and the typical G. bokouensis due to the lack of available continental samples. As far as the currently available information is concerned, however, it is most likely that the species having a much broader range over the East Asian islands including Taiwan on the continental shelf (i.e. that represented by the Group 1 populations) also occurs in eastern continental China. Therefore, we tentatively regard the species represented by Group 1 as the nominotypical species and the other as a possible undescribed species endemic to the Okinawa Group. More extensive allozyme surveys incorporating the continental samples, along with detailed morphological comparisons, are strongly desired to resolve the taxonomic problem raised by the above results.

Nei’s genetic distance (D) values obtained between the two cryptic species within ‘Gekko bokouensis’ are 0.489–0.654. These values are much greater than those previously reported for many conspecific populations in various organisms (D < 0.16) (Thorpe 1982). This seems to negate the probability of their recent divergence from each other. On the other hand, the two species are seemingly identical externally, sharing a suite of character states diagnostic of G. bokouensis (see above). Similar examples of apparent morphological identity between genetically divergent species have been reported for other groups of reptiles (Good et al. 1996; Sadlier et al. 1999). Such a situation is attributable to the long parallel retention of primitive character states, or otherwise to independent acquisitions of derived states (i.e. convergence) (Mayr & Ashlock 1991). Further phylogenetic analyses of the relationships of these and other congeneric species, and the mapping of relevant character states on the resultant phylogenetic trees, are strongly desired to resolve the taxonomic problem raised by the above results.

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Appendix: catalogue numbers of the specimens used in this study

Identifications of the two genetic entities in the Okinawa Group samples were made *a posteriori* by allozyme analyses (see text).

**Okinawa Group (genetic Group 1)**
- Iheyajima-1: 30573-4, 32447, 32456-9, 32453-9, Izenajima-1: 34938-42, 47348-51, 47355, 47357-61, 47363, 47366-9, 47371-6. Iejima: 47385-95, 47403-6, 47396-402. Katsu-u: 47740-5. Nago: 32269-75, 32323-30, 33336-40. Nakagusuku: 30421-2, 30570-1, 31172-5, 31202-3, 32309-10, 32347-8, 33631-6, 34141-2, 45559-61, 45563-6, 48071-74, 48081-86. Chinen: 31176-85, 31238-42, 32257-61. Agunijima: 47527-46. Tokashikijima: 31204-15, 32484-7, 33352-5. Akajima: 3454-63, 34231-8. Tonakijima-1: 47554-60, 47561-5, 47567-8, 47570-1, 47573-84. Kumejima-1: 30416-20, 30471, 30473-6, 30514-21, 30600-1, 45368-9.

**Okinawa Group (genetic Group 2)**
- Iheyajima-2: 30572, 30575-7, 32443-6, 32448-52, 32454-5, 3269-70, 33540-1, 34147-50, 45325, 45327, 50137. Izenajima-2: 34934-6, 47370, 47377-82. Benoki: 30550-9, 31228-9, 32314-6, 33573-6, 34214-9, 34239-64, 34558-69, 45319-21, 45463, 47336-39. Tonakijima-2: 47390-3, 47585-9, 47594-6. Kumejima-2: 30415, 30472, 32158-61, 32263-8, 33297-300, 32995-8, 43265-6, 45310, 45371.

**Other regions of East Asian islands**
- Yamagawa (southern Kyushu): 30492-5, 30594-9, 31198-201, 33653-8, 45316-7, 45427, 45429, 47880, 47885, 47886, 47889. Kuchinoshima (the northern Ryukyus): 33293-6, 33301-4, 33520-5, 33687-92. Amamioshima (the central Ryukyus): 31259-64, 33530-3, 33620-3, 34025. Ichijokajima (the southern Ryukyus): 30409-14, 30578-81, 31004-7, 31170-1, 33629-30. Taipei (Taiwan): 31152-5, 31222-7, 32480-3, 33544-9, 45507-11.