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Clonal Composition of the Parthenogenetic Gecko, *Lepidodactylus lugubris*, at the Northernmost Extremity of Its Range

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ABSTRACT—The mourning gecko, *Lepidodactylus lugubris*, is an all-female parthenogenetic species widely distributed in tropical and subtropical regions. We examined clonal diversity of *L. lugubris* in southern Japan, the northernmost extremity of the species' range. Results indicated that the assemblages of the Ogasawara Islands are composed solely of diploid clone A, which may have originated from artificial transportation after World War II. Assemblages of *L. lugubris* in the Ryukyu Archipelago were composed solely of triploid clone C. This, along with literature records, suggests that the gecko recently colonized the Ryukyu Archipelago on limited opportunities from limited sources. Samples from the Daito Islands included both diploid and triploid individuals representing one and 11 different clones, respectively. Except for one triploid clone (clone B), these clones were most likely to be endemic to the Daito Islands. Analyses of genotypic pattern suggest that most of the putative endemic triploid clones of the Daito Islands originated from iterative crosses between sympatric diploid clones and males of closely related bisexual species that were most likely extirpated subsequently.

INTRODUCTION

The mourning gecko, *Lepidodactylus lugubris*, is an all-female parthenogenetic species widely distributed in the tropical-subtropical Pacific and Indian Ocean islands and adjacent continental coasts (e.g., Bauer and Henle, 1994; Ineich, 1999). Several recent studies of samples from the Micronesian and Polynesian islands demonstrated that this nominotypical species actually consists of diploid (2n=2x=44) and triploid (2n=3x=66) strains (Moritz and King, 1985; Volobouev *et al*., 1993). Furthermore, each of these strains was found to include a number of genetically divergent clonal lineages, some of which were diagnosable on the basis of dorsal color pattern (Ineich, 1988, 1999; Moritz *et al*., 1993). It is considered that the diploid clones were derived from hybridizations between congeneric bisexual species, and that the triploid clones originated through back crosses between the diploid clones and males of parental species (Volobouev *et al*., 1993; Radtkey *et al*., 1995).

Japan is located at the northernmost extremity of the range of *L. lugubris*. Within this region, the gecko is distributed in the southern half of the Ryukyu Archipelago, the Daito Islands, and the Ogasawara Islands (Ota, 1989, 1994). Comparisons of clonal diversity and composition between assemblages in such border areas and other, more central areas of the current species' range are expected to be effective in testing several hypotheses relevant to the evolution of this gecko, such as those regarding the spatio-temporal dynamics of clones and their parental lineages (Ineich, 1999). However, except for one rather problematic karyological description of an Ogasawara specimen (Makino and Momma, 1949) and external overview of some specimens from the Ryukyus (Ineich, 1999) (see Discussion), no information is available regarding the genetic properties or genealogical affinities of individuals occurring in those Japanese territories. We thus conducted a detailed survey for the clonal composition of *L. lugubris* assemblages in Japan. Results indicated a great clonal diversity in this nominotypical species from this region.

STUDY METHODS

Samplings were carried out from 1997 to 1999 on the following islands: Yakabi-jima and Zamami-jima of the Okinawa Islands, Miyako-jima, Ogami-jima, Tarama-jima, and Kurima-jima of the Miyako

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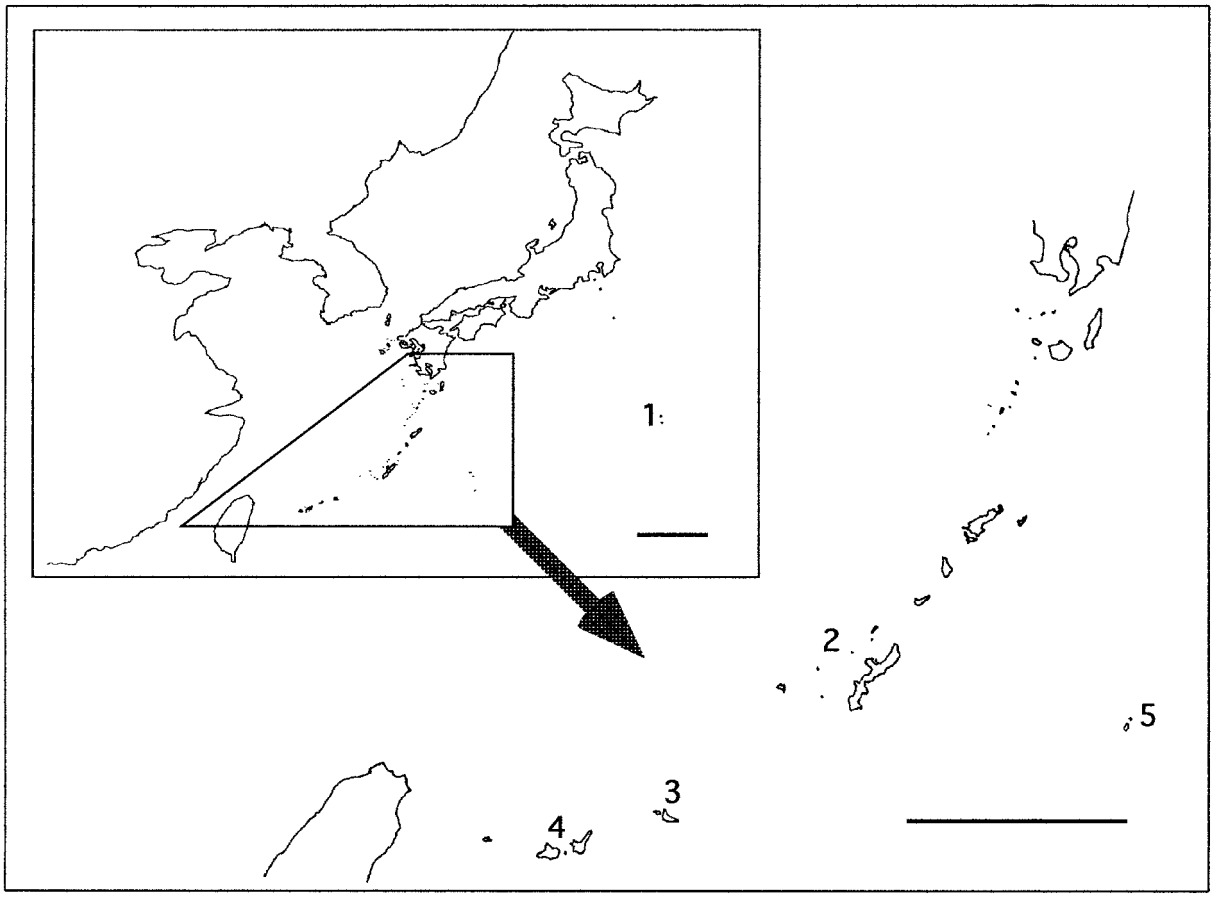

Fig. 1. Map of southern Japan and its vicinity, showing sampling localities of specimens examined. 1, Ogasawara Islands; 2, Okinawa Islands; 3, Miyako Islands; 4, Yaeyama Islands; 5, Daito Islands. Scales equal 300 km.

Table 1. Enzymes examined, buffer systems employed, and presumed loci.

Proteins	E. C. numbers	Buffer system*	Presumed loci
Aspartate aminotransferase	2.6.1.1	TBE8.7	*Aat*
Adenosine deaminase	3.5.4.4	TC7	*Ada*
Aconitate hydratase	4.2.1.3	TC7	*Acoh*
Esterase	3.1.1.1	TC8	*Est-1*
			Est-2
Fumarate hydratase	4.3.1.2	TC7	*Fumh*
Glycerate dehydrogenase	1.1.1.29	AC6	*Glydh*
Glycerol-3-phosphate dehydrogenase	1.1.1.8	TC7	*G3pdh*
Glucose-6-phosphate isomerase	5.3.1.9	TC8	*Gpi*
L-Iditol dehydrogenase	1.1.1.14	LiOH	*Iddh*
Isocitrate dehydrogenase	1.1.1.42	TC7	*Idh*
L-Lactate dehydrogenase	1.1.1.27	TC7	*Ldh*
Malate dehydrogenase	1.1.1.37	AC6	*Mdh*
Malate dehydrogenase ($NADP^+$)	1.1.1.40	TC7	*Mdhp*
Mannose-6-phosphate isomerase	5.3.1.8	LiOH	*Mpi*
Peptitase (Leucyl-glycyl-glycine)	3.4.–.–	TC8	*Pep-lgg*
Peptitase (Leucyl-valine)	3.4.–.–	TC8	*Pep-lv*
Purine-nucleoside phosphorylase	2.4.2.1	TBE8.7	*Pnp*
Phosphogluconate dehydrogenase	1.1.1.44	LiOH	*Pgdh*
Phosphoglucomutase	5.4.2.2	TC7	*Pgm-1*
			Pgm-2

* Abbreviation for buffer system employed are: AC6=Aminopropylmorpholine-Citrate pH. 6.0 (Clayton and Tretiak, 1972), TC7=Tris-Citrate pH. 7.0 (Shaw and Prasad, 1970), TC8=Tris-Citrate pH. 8.0 (Clayton and Tretiak, 1972), TBE 8.7=Tris-Borate EDTA pH. 8.7 (Boyer *et al.*, 1963), and LiOH= Lithium Hydroxide-boric acid pH. 8.1 (Ridgway *et al.*, 1970 modified).

Islands, and Ishigaki-jima, Iriomote-jima, Kuro-shima and Yonaguni-jima of the Yaeyama Islands, Ryukyu Archipelago; Chichi-jima and Haha-jima of the Ogasawara Islands; and Minamidaito-jima and Kitadaito-jima of the Daito Islands (Fig. 1). On each island, efforts were made to collect specimens from as many types of habitats as possible (e.g., illuminated houses, uninhabited constructions, and trees) to avoid sampling bias to particular clones, because different clones may have differential habitat preferences (Bolger and Case, 1994).

Most geckos collected were brought alive back to the laboratory, where they were subjected to the preparation of metaphase cells by the bone marrow air-dry method following Ota *et al.* (1987). Metaphase cells were stained in 2–3% Giemsa's solution for approximately 30 min, and then were photographed under a microscope to determine the standard karyotype for each individual.

Color pattern of each gecko was observed and photographed for a record before karyotyping. The reference name for each color morph basically followed Ineich (1988), and some other papers referring to the clonal variation in color pattern of *L. lugubris* (Ineich and Ota, 1992; Moritz *et al.*, 1993).

During the above mentioned process of karyotyping, fresh liver tissues were removed from those specimens, stocked under –80°C, and subjected to horizontal starch-gel electrophoresis. Enzymes examined are given in Table 1 along with their Enzyme Commission (E. C.) numbers, presumed loci, and buffer systems employed in the electrophoretic assay.

RESULTS

Results of field surveys, and overviews of karyotypic and allozyme variations

A total of 222 specimens were collected in the field, of which 58 were from the Ogasawara Islands, 90 from the Ryukyu Archipelago, and 74 from the Daito Islands.Among these field collected specimens, 142 individuals were successfully karyotyped (Table 2). All karyotypes examined exclusively

Table 2. Localities and sizes of samples examined in this study.

Locality	Sample size	
	Dorsal pattern and electrophoresis	Karyotype
Ogasawara Islands		
Chichi-jima	22	16
Haha-jima	36	25
Ryukyu Archipelago		
Okinawa Islands		
Yakabi-jima	3	0
Zamami-jima	14	11
Miyako Islands		
Kurima-jima	2	1
Miyako-jima	11	4
Ogami-jima	2	1
Tarama-jima	16	8
Yaeyama Islands		
Iriomote-jima	4	2
Ishigaki-jima	15	3
Kuro-shima	21	18
Yonaguni-jima	2	1
Daito Islands		
Kitadaito-jima	11	8
Minamidaito-jima	63	44

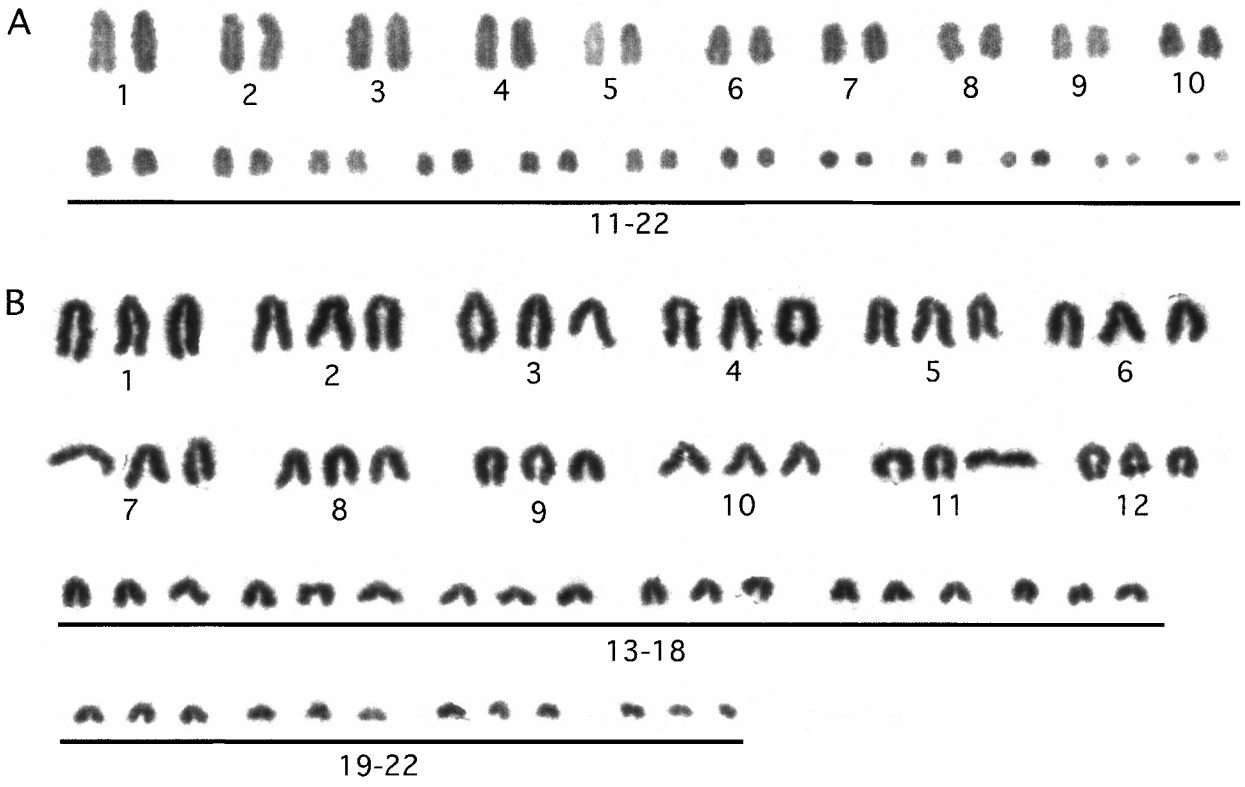

Fig. 2. Karyotypes of (A) diploid clone A of *Lepidodactylus lugubris* from Haha-jima of the Ogasawara Islands (KUZ 47053), and (B) triploid clone C from Iriomote-jima of the southern Ryukyus (KUZ 34716).

Table 3. Genotypes at polymorphic loci in samples of *Lepidodactylus lugubris* from Japan.

Clone	Ploidy	Locus												
		Aat	*Acoh*	*Glydh*	*G3pdh*	*Gpi*	*Ldh*	*Mdh*	*Pnp*	*Pgdh*	*Pgm-1*	*Pgm-2*	*Est-1*	*Est-2*
A	2x	*ab*	*ac*	*bb**	*bb**	*bb**	*bb**	*bb**	*ac*	*bb**	*bb**	*cc**	*bb**	*aa**
C	3x	*abb*	*abb*	*ab*	*aaa**	*abb*	*aaa**	*aaa**	*ac*	*abb*	*abb*	*ccc**	*acc*	*bbb**
Da	2x	*ab*	*ab*	*ab*	*aa**	*ab*	*aa**	*ab*	*ab*	*ab*	*ab*	*cc**	*cc**	*bb**
B	3x	*aab*	*abc*	*bbb**	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*aab*	*abb*	*acc*	*acc*	*bbb**
B'	3x	*aab*	*abc*	*bbb**	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*aab*	*abb*	*aaa**	*acc*	*bbb**
Bl-1	3x	*abb*	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*abb*	*abb*	*bcc*	*acc*	*bbb**
Bl-2	3x	*bbb**	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*abb*	*abb*	*bcc*	*acc*	*bbb**
Bl-3	3x	*aab*	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*bbb**	*abb*	*bcc*	*acc*	*bbb**
Bl-4	3x	*abb*	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*bbb**	*abb*	*bcc*	*acc*	*bbb**
Bl-5	3x	*aab*	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*abb*	*abb*	*bcc*	*acc*	*bbb**
Bl-6	3x	*abb*	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*aab*	*abb*	*ccc**	*acc*	*bbb**
Bl-7	3x	*abb*	*abc*	*ab*	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*abb*	*abb*	*bcc*	*acc*	*bbb**
Bl-8	3x	*abb*	*abc*	*bbb**	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*abb*	*abb*	*bcc*	*acc*	*bbb**
N	3x	*aab*	*abc*	?	*aaa**	*abb*	*aaa**	*bbb**	*ab*	*abb*	*abb*	*bcc*	*acc*	*bbb**

* Electromorphs consisting of single bands only are interpreted as indicative of two and three homozygous alleles for diploid and triploid clones, respectively, but one or (in triploid clones) two alleles may actually be suppressed (see text).

consisted of telocentric chromosomes forming a graded series, and except for the differences in the ploidy level (i.e., 2n=44 in diploids, and 2n=66 in triploids: Fig. 2), no variations were evident among those karyotypes. We then assumed the ploidy level in specimens not successfully karyotyped as being same with that in successfully karyotyped specimens with similar dorsal pattern from a same island group. Such an assumption did not conflict at all with results of electrophoretic investigations (i.e., verified *a posteriori*).

Genotypic variations were recognized at 13 out of 21 presumed loci examined. Putative double dosage or triallelism in band pattern for triploid specimens were recognized at seven loci. A total of 14 clones were recognized on the basis of the electrophoretic pattern, and this did not contradict classifications on the basis of dorsal coloration and ploidy level (Table 3).

Table 4. The clonal composition in each sampling site.

Site	Clone	Sample size
Ogasawara Islands		
Chichi-jima	clone A	22
Haha-jima	clone A	36
Ryukyu Archipelago		
Okinawa Islands	clone C	17
Miyako Islands	clone C	31
Yaeyama Islands	clone C	42*
Daito Islands		
Kitadaito-jima	clone Da	5
	clone B	4
	clone Bl-1	1
	clone Bl-8	1
Minamidaito-jima	clone Da	30
	clone B'	1
	clone Bl-1	23
	clone Bl-2	1
	clone Bl-3	1
	clone Bl-4	2
	clone Bl-5	2
	clone Bl-6	1
	clone Bl-7	1
	clone N	1

* Containing one male phenotype from Ishigaki-jima.

Clonal composition in each local assemblage

Ogasawara Islands

All specimens collected from Chichi-jima and Haha-jima during the present field survey were diploid, and had seven or eight pairs of small V-shaped markings along the middorsal line only (Fig. 3A). There were no genotypic variations at all among those specimens (Table 4). They were thus identified as the diploid clone A, which is widely distributed on tropical Pacific islands and is mentioned as being characterized by two rows of V-shaped markings on dorsum (Ineich, 1988, 1999).

Ryukyu Archipelago

Eighty-nine females from the Okinawa Islands, Miyako Islands, and Yaeyama Islands invariably had dorsolateral black bars on the neck and base of tail, and indistinct W-shaped marks along the middorsal line (Fig. 3B) like Ineich's (1988, 1999) triploid clone C from southern Pacific islands. Moreover, no genotypic variations were evident. We thus identified all females from the Ryukyus as clone C.

Besides those females, one sterile male-phenotype of unknown ploidy and with clone C-like dorsal pattern was found from Ishigaki-jima of the Yaeyama Islands (Yamashiro and Ota, 1998). Electrophoretic investigations revealed no genotypic differences at all between this specimen and the putative clone C females from Ishigaki-jima and other islands of the Ryukyus.

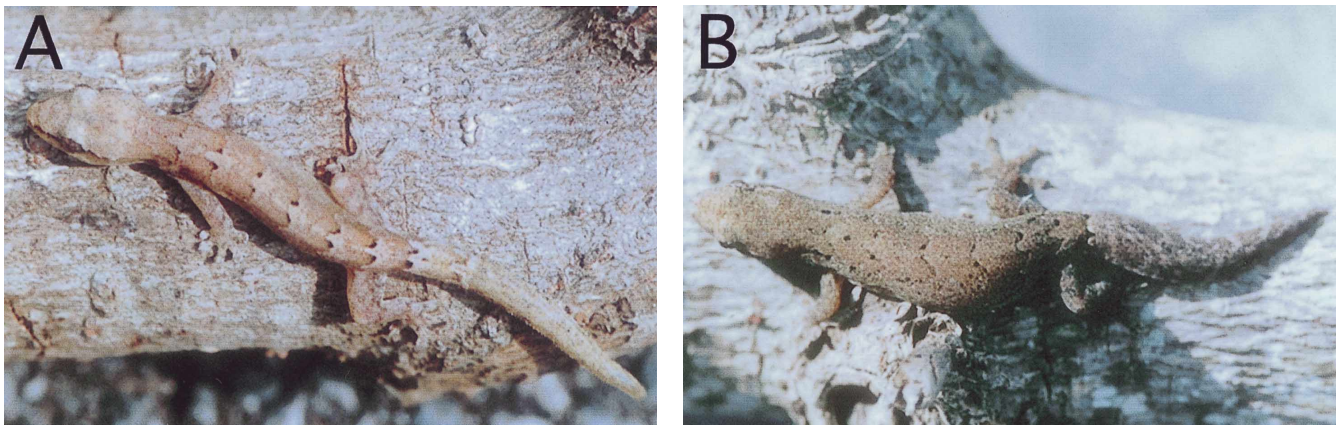

Fig. 3. Dorsal patterns of *Lepidodactylus lugubris* from the Ogasawara Islands and the Ryukyu Archipelago. A: clone A from Haha-jima (KUZ 47063). B: clone C from Zamami-jima, Okinawa Islands (KUZ 34867).

Fig. 4. Dorsal patterns of *Lepidodactylus lugubris* from the Daito Islands. A: clone Da from Minamidaito-jima (KUZ 48533). B: clone B from Kitadaito-jima (KUZ 48466). C: clone B' from Minamidaito-jima (KUZ 48482). D– J: clones Bl-1– Bl-7 from Minamidaito-jima (KUZ 48658, 48460, 48483, 48637, 34738, 48653, and 48638, respectively). K: clone Bl-8 from Kidataito-jima (KUZ 48530). L: clone N from Minamidaito-jima (KUZ 48463).

Daito Islands

Samples from the two islands surveyed included both diploid and triploid clones. Of these diploid individuals from both islands invariably shared a dorsal pattern consisting of lateral black bars on the neck and base of tail, chevrons and short bars alternating with each other along the middorsal line, and a relatively distinct W-shaped mark on the head (Fig. 4A). No genotypic variations were evident among these diploid individuals. On the other hand, they showed genotypic differences at 11 loci when compared with the diploid clone A from the Ogasawara Islands. Because of such a difference, as well as of their unique dorsal pattern, we henceforth refer to the diploids from the Daito Islands as clone Da. In contrast, triploids were highly variable in both dorsal pattern and genotypic composition (Table 3). Four individuals from Kitadaito-jima had a dorsal pattern consisting of lateral black bars on the neck and base of tail, a W-shaped mark on head, and two dorsolateral rows of bold crescent-shaped black spots on body (Fig. 4B). These individuals, identified as Ineich's (1988) triploid clone B from the French Polynesia and Micronesia on the basis of such a dorsal pattern, showed genotypic difference at seven loci when compared with clone C from the Ryukyu Archipelago. One individual from Minamidaito-jima had a clone B-like dorsal pattern but with additional transversal lines on the back. This individual was also highly similar to clone B in allelic composition, but differed from the latter in lacking allele *c* at *Pgm-2*. We thus refer to this individual as clone B' (Fig. 4C). Except for one individual, all of the remainder shared a color pattern similar to that of clone B but with less prominent dark spots (Figs. 4D–K). Electrophoretically, however, this colormorph was divided into eight distinct lineages that are henceforth referred to as clones Bl-1–Bl-8 (Table 3). Clone Bl-1, differing from clone B in genotypes at four loci (*Aat*, *Glydh*, *Pgdh*, and *Pgm-2*), was predominant in number among triploid clones on Minamidaito-jima (Table 4). Clones Bl-2–Bl-7, differing from each other and from Bl-1 in genotypes at one or two loci, and from clones B and B' at three or four loci, were found only from Minamidaito-jima, whereas clone Bl-8 was confined to Kitadaito-jima. This clone differed from clones Bl-1–Bl-7 in allele compositions at one or two loci, and from clones B and B' at three loci. The remaining one individual, collected from Minamidaito-jima had a unique color pattern, which consisted only of lateral black bars on the neck and base of tail (Fig. 4L). Electrophoretically this individual was identical with clone Bl-5 among the present materials except for *Glydh*: our experiment did not yield any allozyme bands for this locus in this specimen (Table 3). We henceforth refer to this individual as clone N.

DISCUSSION

Results of electrophoresis, showing a high frequency of heterozygotes in all clones, further confirm the hybrid origin of *L. lugubris* (Volobouev *et al.*, 1993; Radtkey *et al.*, 1995; Boissinot *et al.*, 1997). In our experiments, however, several presumptive allozyme loci of triploid clones exhibited electromorphs that can be interpreted by assuming two alleles only (Table 3). At *Glydh* of the triploid clone C, for example, two bands of equal densities emerged, and these can obviously be most reasonably interpreted as products of two different alleles. Pasteur *et al.* (1987) also reported a similar phenomenon in the allozyme electromorphs for triploid individuals from tropical Pacific islands. These may suggest a frequent suppression of one of the three alleles in a triploid genome.

Our surveys revealed a great clonal diversity of *L. lugubris* within Japan, which is largely attributable to the surprisingly high diversity in the Daito assemblages. Judging from results of field surveys, current assemblages of this gecko in the Ogasawara Islands are likely to be composed solely of diploid clone A (Table 4). However, Makino and Momma (1949) described karyotype of an individual from Chichi-jima as consisting of 63 uniarmed chromosomes. Interpretation of this chromosome number is controversial (Moritz and King, 1985), but we tentatively assume it as being derived from miscounting of 2n=3x=66 chromosomes rather than indicating the presence of a karyotypically divergent clone on Chichi-jima at that date. For, the gonadal-sectioning method, by which Makino and Momma (1949) made chromosome preparations from their Chichi-jima specimen, usually yield rather poorly spread cells, making it difficult to count chromosomes accurately (Gorman, 1973; Ota and Lue, 1994).

Ineich (1999), after examining numerous museum specimens of *L. lugubris* collected from a number of southern Pacific and Indian Ocean islands in various eras, surmised that clone A, originally having a rather limited range in New Guinea, New Britain and the Solomon Islands, rapidly spread out through artificial transportation during and after World War II. Based on the apparent changes in the clonal composition on some Micronesian and Polynesian islands before and after the arrival of clone A, he also suspected that this clone easily extirpates other clones through competition. Considering that there was active army traffic after World War II between the Ogasawara Islands and the Marianas where clone A was abundant (Ineich, 1999), it is probable that clone A colonized the Ogasawara Islands during that period, and that a triploid clone once occurring there was extirpated consequently.

All samples from the Ryukyu Archipelago were invariably identified as the triploid clone C, despite their relatively large geographic isolation from each other. In this archipelago, *L. lugubris* was first discovered in no earlier than 1971 (Shibata *et al.*, 1972). After the initial discovery, however, this gecko was recorded from most islands in the southern half of the archipelago in no more than two decades (Ota, 1989, 1999, unpublished data). Taking all these into consideration, it is likely that the current assemblages of *L. lugubris* in the Ryukyus were derived from recent artificial introductions of a small number of individuals, and even more recent inter-island transportations of their descendants within this archipelago.

Several previous authors reported occasional emergences of sterile male-phenotypes in assemblages of *L.*

lugubris on several southern Pacific islands (Ineich, 1988, 1999; Ota *et al*., 1995), and they have generally been considered as the consequences of crosses between clonal females and males of congeneric bisexual species (Ineich, 1988; Ineich and Ota, 1992; Boissinot *et al*., 1997). Based on this assumption, Ineich (1999) went so far as to regard the presence of a sterile male in an otherwise all-female *L. lugubris* sample as an indication of the presence of a sympatric bisexual congener. However, discovery of the sterile male-phenotype from Ishigaki-jima, on which no congeneric bisexuals occur, casts serious doubt on such an assumption, and Yamashiro and Ota (1998) surmised that this individual emerged from the normal clone C through a hormonal sex change. Results of the allozyme electrophoresis, showing identical genotypes at all loci examined between this "male" and clone C, lend an additional robust support to this hypothesis and further negate the hybrid origin of the former.

Contrary to other island groups where only single clones were found, the Daito Islands were shown to have one diploid clone and a total of no less than 11 triploid clones. Of these, nine and three triploid clones occurred sympatrically with the diploid clone on Minamidaito-jima and Kitadaito-jima, respectively. It is also interesting to note that except for clone B from Kitadaito-jima, all Daito clones appeared to be endemic to this island group. Such a great diversity and high endemicity of clones in the Daito islands are higher than those reported for a number of southern Pacific and Indian Ocean islands by some previous authors (Ineich, 1988, 1999; Ineich and Ota, 1992, 1993; Bolger and Case, 1994; Moritz *et al*., 1993; Hanley *et al*., 1994), and this is surprising when considering that both Minamidaito-jima and Kitadaito-jima are small, flat islands (30.74 and 12.71 km^2 in area, and 62 and 74.6 m in maximum height, respectively). For a great clonal diversity in parthenogenetic vertebrates of hybrid origins, two explanations can generally be hypothesized — (1) emergences through multiple hybridizations, and (2) mutations subsequent to the initial establishment of a clonal lineage (see Darevsky [1992] for review). Hypothesis (2) predicts that the genetic difference between resultant clones involves mutation-based alleles unique to one of them. Obviously this is not the case with clonal diversity in the Daito Islands detected here (Table 3). We thus suspect that the clonal diversity in the triploid assemblages of *L. lugubris* in the Daito islands is largely a consequence of multiple hybridizations between diploid clonal females and bisexual males.

For the high clonal diversity in a particular geographic area like the Daito Islands, two explanations would be also possible — (3) *in situ* emergences of diverse clones, and (4) multiple colonizations from outside. *Lepidodactylus lugubris* is generally considered as a "skillful colonizer", especially in association with human traffic activities (see the above discussion regarding the origins of the Ogasawara and Ryukyu assemblages), and this appears to favor the latter explanation. However, unlike those other island groups, the Daito Islands have had no direct traffic connection with possible sources of diverse clones since initial human colonization in 1900 (Minamidaito Village Board of Education, 1989): both vessels and airplanes have been connecting the Daito Islands only with the Ryukyu Archipelago where clone C alone occurs (see above). This, as well as its great geographic isolation from other ranges of *L. lugubris* (>1800 km) and the apparent absence of most Daito clones in other areas, much reduces the likelihood of (4). Such a consideration is also concordant with the almost complete absence of other possible colonizers from the south in the terrestrial fauna of this island group. On the other hand, the absence of bisexual *Lepidodactylus* in the Daito Islands may seem to preclude the possibility of multiple hybridizations within this area. However, Ineich (1999) demonstrated recent extinctions of bisexual *Lepidodactylus*, as well as some clones of *L. lugubris*, on several southern Pacific and Indian Ocean islands most likely through competition with other clones in artificially disturbed habitats. It is known that both Minamidaito-jima and Kitadaito-jima were originally covered by dense forests, and that deforestation has progressed rapidly and drastically on both islands after human colonization, forcing a number of terrestrial organisms including several birds into extinction (Minamidaito Village Board of Education, 1989; Kitadaito Village Board of Education, 1986). Recent extirpation of bisexual populations of *Lepidodactylus* in the Daito Islands thus appears not so unlikely as the multiple colonizations mentioned above. So, we tentatively hypothesize that the current diversity of triploid clones of *L. lugubris* in the Daito Islands were largely derived from multiple hybridizations of an extinct bisexual *Lepidodactylus* with clone Da and other possibly extinct diploid clones within this island group. The broadly distributed clone B (Ineich, 1988, 1999) on Kitadaito-jima may represent a recent colonization. Extensive comparative genetic surveys of *L. lugubris* assemblages and bisexual congeners in Micronesia and other regions are strongly desired to test this *ad hoc* hypothesis.

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APPENDIX. Localities and catalogue numbers of specimens examined in this study. These specimens were deposited in the herpetological collection of the Department of Zoology, Kyoto University (KUZ).

Ogasawara Islands: Chichi-jima, KUZ 47696, 47778, 47780–47787, 47799, 4782–47833; Haha-jima, KUZ 47048–47069, 47271, 47272, 47624–47627, 47723–47730. Ryukyu Archipelago: Yakabi-jima, Okinawa Islands, KUZ 47658–47660; Zamami-jima, Okinawa Islands, KUZ 34715, 34731–34733, 34736, 34867, 48511–48515, 48583–48585; Kurima-jima, Miyako Islands, KUZ 34622, 34623; Miyako-jima, Miyako Islands, KUZ 34615–34619, 34624, 34717–34720, 47648; Ogami-jima, Miyako Islands, KUZ 34620, 34621; Tarama-jima, Miyako Islands, KUZ 34567, 45885, 47451, 47470, 47472–47481, 47649, 47650; Iriomote-jima, Yaeyama Islands, KUZ 34716, 48481, 48586, 48587; Ishigaki-jima, Yaeyama Islands, KUZ 34885–34893, 34894 (male phenotype), 47511, 47512, 47651–47653; Kuro-shima, Yaeyama Islands, KUZ 47495–47510, 47655–47657, 49106, 49107; Yonaguni-jima, Yaeyama Islands, KUZ 47654, 47779. Daito Islands: Kitadaito-jima, KUZ 45793, 45794, 47849, 48466, 48528–48532, 48535, 48536; Minamidaito-jima, KUZ 34737–34742, 47800, 47803–47809, 48432–48437, 48441–48465, 48482–48485, 48533, 48534, 48541–48548, 48634–48640, 48651–48658.