Doctoral Thesis

Ecological and genetic differentiation between clones of the parthenogenetic gecko *Lepidodactylus lugubris*

Yuki Murakami

Department of Biology
Tokyo Metropolitan University
Tokyo
Japan

February in 2018

Contents

Abstract

Vertebrates basically reproduce sexually in which males and females contribute their offspring genome and produce genetically diverse offspring. However, some of them are asexual without genetic contribution from males. Asexual reproduction lacks genetic diversity but is predicted to be advantageous for dispersal and for increasing abundance relative to sexual reproduction because of no cost to mate between the sexes.

The nocturnal small gecko, *Lepidodactylus lugubris*, is all female and reproduces parthenogenetically. This gecko is known to consist of diploid and triploid clones in the tropical and subtropical regions, which can be identified by their dorsal marking patterns, ploidy, or protein polymorphism. This gecko is also distributed in the southern parts of Japan, and several clones have been reported particularly in the Daito Islands. Moreover, the southern parts of Japan are often inhabited by this parthenogenetic gecko and the sexually reproductive *Hemidactylus frenatus*. This situation offers a unique opportunity to examine the relationships among sexuality and the abundance, distribution, and genetic diversity of the two species. Therefore, in this study, the three aims are addressed: (1) clonal discrimination using microsatellite analysis to examine the origins and genetic diversity of Japanese *L. lugubris*, (2) comparisons of distribution patterns and genetic population structures between asexual *L. lugubris* and sexual *H. frenatus* to assess how benefit of asexual reproduction to dispersal, and (3) behavioral observation in aggressive interactions for food among clones of *L. lugburis* to know how clonal competition affects their microscale distribution patterns.

First, microsatellite and mitochondrial DNA analyses clarified the clonal compositions of *L. lugubris* across Japan. A total of 748 individuals were collected from 21 islands of five island groups (Ogasawara, Okinawa, Miyako, Yaeyama, and Daito Islands) and 17 clones (Clones O1, O2, M, T, and D1-D13) were distinguished genetically. Mitochondrial cyt *b* sequences of these clones suggested that they were all closely related and differentiated recently. Clone diversity was much higher (14 clones) in the Daito Islands than in the other island groups (1 or 2 clones there). Judging from the dorsal marking patterns and ploidy, Clones O1, O2, D12 and D13 were the cosmopolitan Clones A, C, and Bs, and Clones M and T were considered to be colonized from the outsides. However, Clones D1 to D11 were endemic to the Daito

Islands and explained by hybridization between the female diploid Clone D1 (H1 in mtDNA) and male diploid Clone D2 (H2) because all Clones D3 to D11 were triploid and had the combinations of polymorphic alleles of D1 and D2 in eight microsatellite loci and haplotype H1. Although the males have never been found in Daito, there is a possibility that the male existed or accidentally appeared in the past.

Next, a total of 445 geckos of parthenogenetic *L. lugubris* and bisexual *H. frenatus* were collected across nine islands of the Ogasawara Islands. The population genetic structures estimated using microsatellite markers revealed that *L. lugubris* which was distributed on all nine islands, consisted of two clones, but *H. frenatus*, which was limited to four islands, exhibited a great variation in population genetic structure among islands, probably with some bottleneck effects. Thus, successful dispersal may be more frequent in *L. lugubris* than in *H. frenatus*, and therefore the asexual reproductive strategy of *L. lugubris* appears to have contributed to its dispersal success and increased abundance among the small oceanic islands.

In the last, we observed aggressive behaviors for food between the two individuals with different combinations of two clones (Clones O1, O2) of *L. lugubris* and male and female *H. frenatus*. As a result Clone O2 was much less aggressive than Clone O1 and also against *H. frenatus*. This behavioral tendency could help to explain the distribution patterns of these geckos in the Ogasawara Islands where the habitats of Clone O1 of *L. lugubris* and *H. frenatus* were biased towards the artificial environments such as house walls, electric poles, and road guardrails, whereas those of Clone O2 were in natural area such as forests, rock crevices, grasslands, and beaches.

These findings contribute to understand clonal diversity and dynamics of asexually reproducing animals. If diploid parthenogenetic geckos can produce triploid clones by mating the males, clonal diversity would increase rapidly in a small region and newly produced clones expand widely. However, there may be behavioral interference and competition among clones, which affect the clonal compositions and microhabitat segregation.

1. General introduction

1-1. Background and aims

Parthenogenesis is an asexual reproduction in which development of embryos occurs without fertilization (Beukeboom and Vrijenhoek, 1998). Parthenogenesis occurs naturally in some invertebrate species but a few vertebrates such as some fish (Chapman et al., 2007), amphibians (Bogart et al., 2007), reptiles (Ineich, 1989; Yamashiro et al., 2000) and very rarely birds (Olsen, 1965). Nearly all arose from interspecific matings which lead to hybrid genotypes sustained by non-recombinant reproductive processes including true parthenogenesis, gynogenesis, and hybridogenesis (Vrijenhoek et al., 1989).

All-female parthenogenetic species present a unique opportunity to test hypotheses regarding the nature and evolution of sexuality. Although vertebrates are generally gonochoristic, at least 27 species of reptiles are known to consist mostly or entirely of females and to reproduce only clones of female offspring (Cole, 1975). In reptiles, most species reproduce sexually, but parthenogenesis has been known to occur naturally in certain species of whiptails, some geckos, rock lizards (Macculloch et al., 1997), and Komodo dragons (Watts et al. 2006). Among them, the lizards belonging to the genera *Cnemidophorus* and *Lacerta* have been well studied and much is known concerning the genetics and ecology of parthenogenetic species (Cuellar, 1976; Mitchell, 1979). For example, the whiptails of the genus *Cnemidophorus* have genetic or ecological diversity despite that 15 species reproduce by parthenogenesis. All these asexual species appear to have arisen through the hybridization of two or three sexual species in the genus, which leading to polyploid individuals (Lutes et al., 2011). Because multiple hybridization events can occur, each parthenogenetic species consists of multiple independent asexual lineages. Different lineages have different genotypes.

Geckos also include relatively large number of parthenogenetic species such as *Lepidodactylus lugubris*, *Hemidactylus garnotii* and *Indotyphlops braminus*, and several studies have revealed their clone diversity and phylogenetic relationship with sexually reproductive relatives (Kluge and Eckardt 1969; Ineich, 1989, 1999; Moritz et al., 1993; Volobouev et al., 1993; Radtkey et al., 1995; Yamashiro et al., 2000; Kearney et al.,

2006; Roberts et al., 2012). However, such clonal polymorphisms have been still unclear because little information is available regarding the modern genetics, ecology and behavior of these clones.

Sexual reproduction is a potentially costly process for animals, although resulting in the production of genetic diversity among the progeny of an individual. Since the rate of adaptive evolution is a function of this diversity, the recombination of genes in sexual lineages provides an obvious longterm benefit in a changing environment (Fisher, 1930; Müller, 1932). On the other hand, asexuality provides an immediate two-fold advantage due to all female reproduction (Maynard Smith, 1978). Once an all-female lineage arises and all other things being equal, it should replace its bisexual ancestors due to the cost of producing males in the latter (Williams, 1975; Maynard Smith, 1978). Parthenogenesis avoids not only the two-fold cost of sexual reproduction but also the cost in failing dispersal to the place where the mate does not exist because all-female species to produce offspring independently (Maynard Smith 1978; Neaves and Baumann 2011). This strategy enables every member of the population to establish in a new habitat. Thus, parthenogenetic reproduction is predicted to be advantageous in increasing abundance and in dispersal.

However, all-female lineages have not completely replaced their bisexual ancestors on broad geographical scales (Vrijenhoek 1989). This is because bisexual reproduction has longterm benefits such as removing the accumulation of deleterious mutations from the genome and preventing extinction of offspring from environmental changes and diseases owing to their genetic diversity (Maynard Smith 1978).

In this study, the following three questions are addressed. The first is concerned to the origins and genetic diversity (Chapter 2). Sexual populations have usually a greater genetic diversity than clonal populations. If parthenogenetic lineages are produced at a high rate and from various sources, their genetic diversity may reach levels comparable to those of sexual populations (Simon et al. 2003). Therefore, determining distribution patterns of each clone and genetic diversity among clones of a parthenogenetic species is important. The second is concerned to the factors assessing costs and benefits of a parthenogenetic species (Chapter 3). The parthenogenetic species is expected to expand its distribution area more rapidly than the sexual species, because all individuals have potential to reproduce without mating at newly colonized places. Therefore, the

comparisons of distribution patterns and genetic population structures between asexual and sexual species in the same study area. The final question is whether behavioral differences among clones affect the population dynamics and distribution patterns of a parthenogenetic species (Chapter 4). This could in turn influence the outcome of clonal competition with their sexual counterparts inhabiting the same area.

1-2. Parthenogenetic gecko *Lepidodactylus lugubris*

Lepidodactylus lugubris (Duméril et Bibron, 1836) is a nocturnal small gecko and feeds mainly on arthropods but sometimes on nectar and ripe fruit (Nafus, 2012) (Fig. 1-1). The lectotype of this species was derived from Tahiti, French Polynesia (see Torres-Carvajal, 2001). This species is all females and reproduces parthenogenetically, consisting of diploid (2n = 2x = 44) and triploid (2n = 3x = 66) clones (Moritz and King, 1985; Volobouev et al., 1993; Ineich, 1999; Yamashiro et al., 2000). Each strain includes a number of genetically divergent clones, some of which were identifiable based on the dorsal color marking patterns (Ineich, 1988, Ineich 1999; Moritz et al., 1993; Yamashiro et al., 2000). The diploid clones of *L. lugubris* were derived from hybridizations between congeneric closely related bisexual species, and that the triploid clones originated through back crosses between the diploid clones and males of parental species (Moritz et al., 1993; Volobouev et al., 1993; Radtkey et al., 1995; Ineich, 1999; Yamashiro et al., 2000). The parental species cohabited only on Arno Atoll of Marshall Islands and therefore this place is thought to be the origin of *L. lugubris* (Radtkey et al., 1995). This species expanded its distribution areas widely by accidental introduction by human being or naturally occurred migration by driftwoods, and now is found on many islands located all over Indian and Pacific Oceans and also on continental Asia and middle America (Fig. 1-2). The eggs show salinity tolerance (Brown and Duffy, 1992). At least five major clones of *L. lugubris* differing in ploidy and coloration (dorsal marking pattern) are recognized by Ineich (1988, 1999). In Geckos, some species have a XY or ZW sex determination system, but other species have temperature-dependent sex determination (review by Gamble, 2010). *Lepidodactylus* is known to have ZW system (Volobouev and Pasteur, 1988; Gamble, 2010).

Fig. 1-1. *Lepidodactylus lugubris* on Anijima in the Ogasawara Islands.

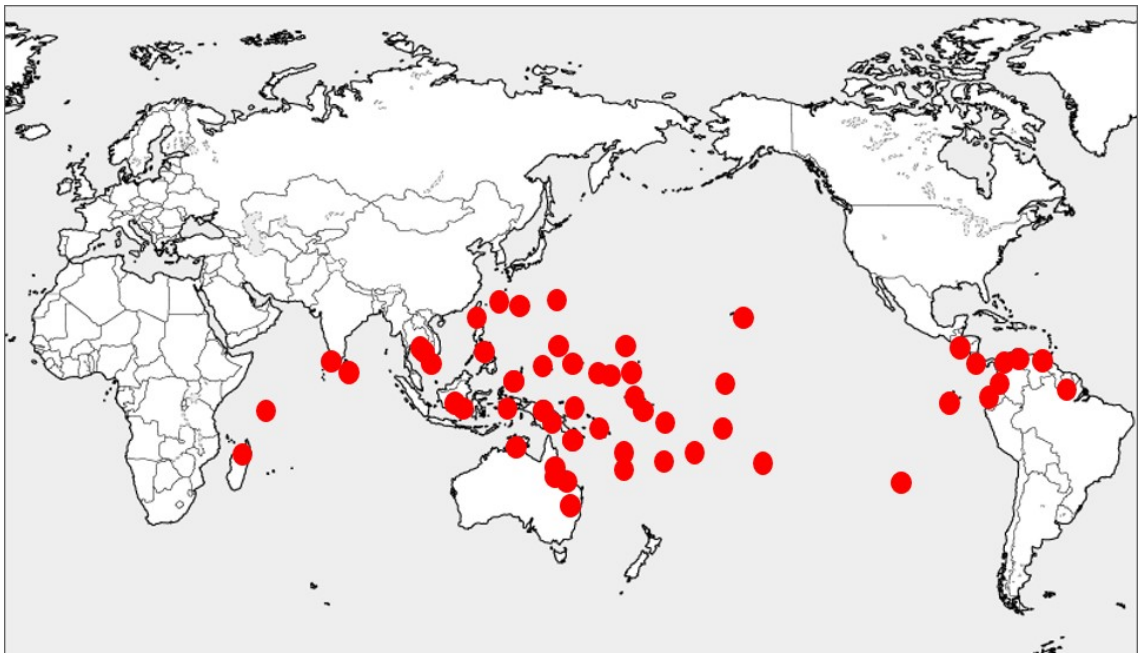

Fig. 1-2. Distribution (red circles) of *Lepidodactylus lugubris* (Duméril and Bibron, 1836; Girard, 1858; Bleeker, 1859; Fitzinger, 1861; Tytler, 1865; Stoliczka, 1870; Ferguson, 1877; Macleay, 1877; Peters and Doria, 1878; Boulenger, 1885, 1887; Garman, 1908; Werner, 1913; Taylor, 1918, 1953; Deraniyagala, 1929; Mertens, 1929; Cagle, 1946; Brongersma, 1948; Gibson, 1950; Smith and Grant, 1961; Daan S and Hillenius, 1966; Schauenberg, 1968; Cuéllar and Kluge, 1972; Henderson et al., 1976; Brown and Parker, 1977; Mau, 1978; Cuellar, 1984; Gardner, 1985; Müller, 1895; Cheng, 1987; Jarecki and Lazell, 1987; Ota, 1987, 1989; Pasteur et al., 1987; Bauer and Vindum, 1990; Henle, 1990; Case and Bolger, 1991; Zug, 1991; Rösler, 1992, 1995; Gill, 1993; Ineich and Ota, 1993; Volobouev et al., 1993; Bauer and Sadlier, 1994, 2000; Hanley et al., 1994; Radtkey, 1995; Irschick et al., 1996; Mckeown, 1996; Turner and Green, 1996; Barnett and Emms, 1997; Boissinot, et al., 1997; Manthey, 1997; Sadlier and Bauer, 1997; Sengoku, 1998; Crombie and Gregory, 1999; Das, 1999, 2004; Kikukawa, 1999; Cogger, 2000, 2014; Ferner et al., 2000; Ota et al., 2000; Swash and Still, 2000; Grismer et al., 2002; Röll, 2002; Savage, 2002; Morrison, 2003; Van, 2003; Goris and Maeda, 2004; Cooper, 2005; Rösler et al., 2005; Yamashiro and Ota, 2005; McCoy, 2006, 2015; Zerbe, 2006; Bauer et al., 2007; Ziesmann et al., 2007; Buden,

2008, 2014, 2015a, b; Castro and Vargas, 2008; Köhler, 2008; Röll and Düring, 2008; Amarasinghe et al., 2009; Fujita and Moritz, 2009; Ineich, 2009, 2011, 2015; Sang et al., 2009; Somaweera and Somaweera, 2009; Henderson, 2010; Venugopal, 2010; Gaulke, 2011; Grismer, 2011a, b; Lorvelec et al., 2011, 2017; Mcleod et al., 2011; Palacio et al., 2011; Zug et al., 2011, 2012; Castro et al., 2012; Daza et al., 2012; Koch, 2012; Niewiarowski et al., 2012; Brown et al., 2013; Gomes and Ibene, 2013; Parmentier et al., 2013; Sunyer, 2014; Valencia et al., 2014; Zug and Hinrich, 2014; Chandramouli, 2015; Hoogmoode et al., 2015; Jayaneththi et al., 2015; Jayaneththi, 2015; Jiménez and Abarca, 2015; Sosa and Reyes, 2015; Trifonov et al., 2015; Buden and Taboroši, 2016; Demangel, 2016; Jablonski, Ángel, 2016; Krysko and MacKenzie, 2016; Supsup et al., 2016; Señaris et al., 2017; Bosch and Páez, 2017).

2. Molecular discrimination and phylogeographic patterns of clones across the Japanese Archipelago

2-1. Introduction

Lepidodactylus lugubris is now distributed widely in most islands and continental parts of Indian and Pacific Oceans. In Japan, it is distributed in the Ryukyu Archipelago, the Daito Islands, and the Ogasawara Islands (Ota, 1989, 1994; Yamashiro et al., 2000). These Japanese areas are the northern limit of this gecko (Fig. 1-2 in Chapter 1) and there the breeding is limited in the warm seasons (Sakai, 2016). Yamashiro et al. (2000) revealed a great clonal diversity of *L. lugubris* in Japan, which is largely attributable to the surprisingly high diversity in the Daito Islands. However, in the Ogasawara Islands, only two islands were studied and the genotypes were discriminated using 13 loci of allozymes. In this study, we surveyed more islands than those studied by Yamashiro et al. (2000) and the clones were identified by modern genetic analysis using both mitochondrial DNA sequences and nuclear microsatellite markers. Such a detailed survey for the clonal compositions of *L. lugubris* enables us to compare clonal diversity and distribution patterns of each clone across Japan, and to discuss their spatio-temporal dynamics not only in Japan but also in Pacific Ocean using morphologically identified clones of this species.

2-2. Materials and methods

We collected *L. lugubris* on 21 islands (Fig. 2-1): Anijima and Chichijima in March 2012, Anijima, Chichijima, Hahajima, Hirashima, and Iwoto in June and July 2012, Anijima in August 2012, Nishijima and Chichijima in December 2012, Kitanoshima, Mukojima, and Chichijima in July 2013, Kitadaitojima and Minamidaitojima in October 2013, Mukojima in December 2013, Kitanoshima in July 2014, Okinawa Island, Iriomotejima, Kuroshima, Taketomijima and Ishigakijima in August 2014, Yomejima, Mukojima, Nakoudojima and Chichijima in July 2015, Hahajima in August 2015, Yonagunijima in October 2015, and Yomejima, Mukojima, Nakoudojima, Chichijima, and Hahajima in August 2016, Miyakojima in February 2017, Zamamijima, Mukojima,

Nakoudojima, and Hyoutanjima in July 2017. On each island, efforts were made to collect samples 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). Individual dorsal color patterns of field-caught *L. lugubris* were observed and recorded as photographs, and later distinguished using the reference name for each color morph described by Ineich (1988), Ineich and Ota (1992), Moritz et al. (1993), and Yamashiro et al. (2000). To extract the total genomic DNA, the tail tips were cut and preserved in 99.5% ethanol. Soon after these procedures, individuals were all released at the captured points.

Microsatellite DNA analysis

Total genomic DNA was extracted from the preserved tail tips using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Eight microsatellite loci, Ll01, Ll02, Ll05, Ll06, Ll07, Ll08, Ll09 and Ll10, were amplified using the primer sets of Wilmhoff et al. (2003) (Table 2-1). Microsatellite regions were amplified by T100TM thermal cycler (Bio-Rad, Hercules, CA, USA) using ExTaq®(Takara, Tokyo, Japan). The PCR reaction mix (total volume, 10 μl) contained of 1.0 μl 10× Extaq Buffer, 0.8 μl 25 mM dNTP mix, 0.5 μl fluorescent (6-FAM) forward primer (10 pM), 0.5 μl reverse primer (10 pM), 0.05 μl Taq polymerase, 6.15 μl distilled deionized water, and 1.0 μl template DNA. PCR conditions were as follows; an initial denaturation of 94°C for 3 min, 35 cycles of 95°C for 30 s, 54°C (in Ll09), 56°C (in Ll01, 02, 05, 06, 08 and 10), or 62°C (in Ll07) for 40 s, and 72°C for 40 s. An initial single step of 94°C for 4 min and a final single step of 72°C for 5 min were also included. Then, 1 μL product was added to 9 μL loading mix containing a GeneScan^{TM} 500 Liz$^{®}$ Size Standard (Applied Biosystems, Foster City, CA, USA) and Hi-Di Formamide (Applied Biosystems). This mixture was analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems). Allele lengths were scored using Peak Scanner version 1.0 (Applied Biosystems).

Mitochondrial DNA analysis

The 448-bp fragments of the mitochondrial cytochrome *b* (cyt *b*) DNA were amplified using Ex Taq® (TaKaRa) with primers: L15175

5’-GTGCAACYGTTATTACTAA-3’and H15725 5’-CATCCAATCCATAATAAAGCAT-3’ (Ricklefs et al., 2002). The PCR reaction mix (total volume 10 μL) contained 1.0 μL 10× Ex Taq Buffer, 0.8 μL 25 mM dNTP mix, 0.5 μL each of the forward and reverse primers (10 pM), 0.05 μL Taq polymerase, 6.15 μL distilled deionized water, and 1.0 μL template DNA. Using a T100™ thermal cycler (Bio-Rad), the PCR protocol was as follows: an initial 10-min denaturing step at 95°C, 30 cycles of 60 s at 95°C, 60 s at 95°C, 60 s at 53°C, and 120 s at 72°C, with a final 10-min extension at 72°C. The PCR products were purified with Illustra™ ExoStar™ 1-Step (GE Healthcare, Buckinghamshire, UK) and sequenced using BigDye Terminator ver. 3.1 (Applied Biosystems) on an ABI 3130xl Genetic Analyzer (Applied Biosystems).

Direct sequencing data were aligned with the sequences of other species of the family Gekkonidae deposited in GenBank (Table 2-2), using MEGA6 (Tamura et al., 2013). Phylogenetic analyses of the aligned sequences were performed with the neighbor-joining (NJ) method based on p-distance and the maximum likelihood (ML) estimation based on Tamura-Nei model (Tamura and Nei, 1993) using MEGA 6 (Tamura et al., 2013). The best-fit nucleotide substitution model was estimated based on the Bayesian Information Criterion (BIC: Schwarz, 1978) using MEGA 6 (Tamura et al., 2013).

2-3. Results

Genetic discrimination of clones

A total of 748 individuals were collected on 21 islands across Japan, 548 of which were from the Ogasawara Islands, 12 from the Okinawa Islands, 101 from the Yaeyama Islands, one from the Miyako Islands, and 86 from the Daito Islands (Table 2-3). These individuals were all successfully genotyped by 8 loci of microsatellite DNA, and 17 clones named O1, O2, M, T, and D1-D13 were discriminated by the combinations of these microsatellite alleles (Table 2-4). The maximum number of alleles observed in each microsatellite locus suggested that Clones O1, D1 and D2 are diploid and the others are triploid (Table 2-4). Dorsal marking patterns were compared for 656 individuals obtained on 19 of 21 islands, because only the tissue samples of tail tips

were available for some individuals on five islands in Ogasawara (Table 2-3).

Mitochondrial cyt *b* sequences were conducted for a total of 331 individuals covering all islands (Table 2-3). Base substitutions occurred only at five positions of 448 bp, producing five haplotypes H1-H5 (Table 2-5). These haplotypes were very close to each other in the phylogenetic tree of species within the subfamily Gekkoninae, although no sequence data were available for other *Lepidodactylus* species and clones (Fig. 2-3). Clones O2, D1, D3-D13 shared the same haplotype H1, but others had each specific haplotype; H2 in Clone D2, H3 in Clone O1, H4 in Clone T, and H5 in Clone M (Table 2-4).

Dorsal marking patterns differed among clones and seemed to be stable within the same clone (Fig. 2-2). Clone O1 had the dorsal marking pattern characterized by two simple rows of V shaped markings on the dorsum. Clone O2 had two pairs of additional large dark markings at the lateral sides compared with Clone O1. Clone M had black dots on the dorsal body, but Clone T lacked dorsal markings other than dorsolateral black dots on the neck and the basal part of tail. In Clone D1, the dorsal marking pattern consisted of lateral black bars from the neck to the basal part of tail, chevrons and short bars alternating with each other along the mid-dorsal line, and a relatively distinct W-shaped mark on the neck. This pattern was same in Clone D2, but Clone D2 had V-shaped marking instead of second chevron. Clones D3 to D5 had basically the same dorsal pattern; relatively large black bars from the neck to the basal part of tail and a W-shaped mark on the neck. However, there were the two dorsolateral rows of bold crescent shaped black spots on the body in Clone D3 and four rows in Clones D4 and D5. The angles of these dark spots differed between Clones D4 and D5. Clone D6 was characterized by the horizontally arranged black dots on the body. Clones D7 to D10 had also black markings from the neck to the basal part of tail, but no W-shaped mark on the neck. Asymmetrical black markings on the dorsal body were also characteristic to these four clones, but distinguished to each other by clone-specific positions of these markings. Clone D11 was unique in its marking patterns consisting of only of lateral black bars from the neck to the basal part of tail. Clones D12 and D13 had two rows of black spots on the mid-dorsal side of the body, but asymmetric markings differed between them.

Clonal compositions in five island groups

(1) The Ogasawara Islands

Only two clones were obtained; 405 (73.91%) of 548 were Clone A, and the remainings (26.09%) were Clone C (Table 2-6). Clone A was dominated on the two large human-inhabited islands, Chichijima and Hahajima, whereas Clone C was slightly dominated on the non-human-inhabited islands (Fig. 2-1).

(2) The Okinawa Islands

All 10 individuals from Okinawajima and 2 individuals from Zamamijima were all identified Clone C (Table 2-6, Fig. 2-1).

(3) The Miyako Islands

Only one individual was collected on Miyakojima. She was Clone M endemic to this island (Table 2-6, Fig. 2-1).

(4) The Yaeyama Islands

Two clones were obtained in this island group; 99 (98.02%) of 101 individuals were Clone C and only 2 (1.98%) of them were Clone T endemic to Taketomijima (Table 2-6, Fig. 2-1).

(5) The Daito Islands

A total of 28 individuals on Kitadaitojima and 58 on Minamidaitojima were distinguished into 14 clones, C and D1 to D13 (Table 2-6, Fig. 2-1). Clones D1 to D13 were endemic to this island group.

2-4. Discussion

2-4-1. Clone identification

Our genetic analyses showed that there are 17 clones of all-female parthenogenetic *L. lugubris* in Japan. Clone O1 was limited to the Ogasawara Islands and most dominant there (Table 2-6, Fig. 2-1). This clone was identified as previously known Clone A

because it was diploid (Table 2-4) and the dorsal marking pattern was characterized by two simple rows of V shaped markings on dorsum (Fig. 2-2) (Ineich, 1988, 1999; Yamashiro et al., 2000). This Clone A is widely distributed in Pacific and Indian Oceans (Moritz et al., 1993; Radtkey et al., 1995; Ineich, 1999).

Clone O2 was most widely distributed in Japan (Table 2-6, Fig. 2-1). This was triploid and had two pairs of additional large dark markings at the lateral sides, one was on the lateral neck and the other was near the hindlegs (Fig. 2-2). This is identical to well-known Ineich's (1999) Clone C.

Clone M, obtained from one individual on Miyakojima, was triploid (Table 2-4). Yamashiro et al. (2000) reported that only Clone C was distributed on this island based on the dorsal marking patterns and allozyme band positions. However, Clone M differed clearly from Clone C in microsatellite and mitochondrial DNA analyses, and also from any other clones known in the world (Ineich, 1988). Our sample is only one individual and therefore Clones C and M may coexist on this island.

Clone T obtained from Taketomijima in the Yaeyama Islands was also triploid (Table 2-4) and had no clear dorsal markings other than dorsolateral black dots on the neck and the basal part of tail (Fig. 2-2), suggesting it is peculiar among any known clones.

Clones D1 to D13 were all endemic clones to the Daito Islands (Table 2-6), two (D1, D2) of which was diploid but all others triploid (Table 2-4). These clones shared the same mitochondrial haplotype H1, excluding D2 with the haplotype H2 (Table 2-4). The dorsal marking pattern of Clone D1 (Fig. 2-2) seems to be the same as the pattern of diploid Clone Da described from the Daito Islands by Yamashiro et al., (2000). Clone D11 was unique in its marking patterns consisting of only of lateral black bars from the neck to the base of tail (Fig. 2-2). This pattern is similar to Clone N described from the Daito Islands by Yamashiro et al., (2000). Clones D12 and D13 had similarly two rows of black spots on the mid-dorsal side of the body, one of which may be identical to Ineich's (1988) triploid Clone B. However, it is difficult to identify other clones found from the Daito Islands by Yamashiro et al. (2000) and us based on the dorsal marking patterns. In the Daito Islands, the clone diversity may be much higher as expected by our examination.

2-4-2. Origin and diversification processes of clones

The phylogenetic analyses based on mitochondrial cyt *b* sequences suggested rather recent events of clonal diversification and distributional expansion in *L. lugubris*, compared with evolution in other species of Gekkoninae (Fig. 2-3). However, genetic analysis by eight microsatellite loci was much useful to identify clones.

In the Ogasawara Islands, *L. lugubris* consisted of only two clones, Clones O1 (=A) and O2 (=C), suggesting that at least two times of successful colonizations occurred in this archipelago probably from the southern Pacific source populations of this species. On Hahajima, Yamashiro and Ota (2005) recorded only Clone A in their field surveys in 1997 and 1998 (N = 36), which was confirmed in this study. Therefore, until now, there is no evidence of Clone C on this island (Yamashiro et al. 2000). However, the reason why this island is occupied completely by Clone A is unknown.

All individuals from Okinawajima and Zamamijima in the Okinawa Islands, and Yonagunijima, Iriomotejima, Kuroshima and Ishigakijima in the Yaeyama Islands were identified as Clone O2 (=C), despite of their relatively large geographic isolation from each other. In these areas, *L. lugubris* was first discovered in 1971 (Shibata et al., 1972), and now expanded across these islands (Ota, 1989; Yamashiro et al., 2000). Therefore, the current clone types may be derived from their recent introductions. Although one individual on Miyakojima and two on Taketomijima had unique dorsal patterns, microsatellite genotypes (Clones M and T, respectively), and mitochondrial haplotypes (H5 and H4, respectively), these are considered to colonize recently to these islands from unknown outsides of southern areas.

In contrast, a great clonal diversity was observed in the Daito Islands as reported previously by Yamashiro et al. (2000). Moreover, at least 11 of 14 clones were endemic to this island group and Clone O2 (=C), D12 (=B?) and D13 (=B?) was probably cosmopolitan. Such a great diversity and high endemicity of clones in the Daito Islands are higher than those reported for several southern Pacific and Indian Oceanic islands in the previous studies (Ineich, 1988, 1999; Ineich and Ota, 1992, 1993; Bolger and Case, 1994; Moritz et al., 1993; Hanley et al., 1994), and this is surprising that it occurred on two closely located small and flat islands, Kitadaitojima and Minamidaitojima.

In parthenogenetic *L. ligubris*, the diploid lineages may be derived from

hybridization between diploid bisexual species, and the triploids may have resulted from backcrosses of asexual diploid clonal females with males of one of the two bisexual species, and also with males of other and now extinct bisexual species (Ineich 1988, 1999; Moritz et al., 1993, Boissinot et al., 1997; Yamashiro et al., 2000). If so, clonal diversification in diploid lineages may occur by multiple or repeated hybridizations of the diploid species, and in triploid lineages by reapeated crosses between the diploid all-female clones and the male of the diploid bisexual species. In the Daito Islands, endemic Clones D1 to D11 had common microsatellite alleles of different combinations in all examined loci, whereas Clones D12 and D13 had unique alleles in two and three loci, respectively (Table 2-4), suggesting the common origin in Clones D1 to D11. Because Clones D1 and D2 were diploid but Clones D3 to D11 were triploid, it is hypothesized that these triploid clones are drived by the two diploid clones. Here, the mitochondrial haplotype was H2 in Clone D2, but H1 in all other clones (Table 2-4). Therefore, if the triploid clones are derived from the diploid clones, Clone D1 should be female, and Clone D2 should be male (Fig. 2-4). However, male *L. lugubris* have never been found in the Daito Islands (Yamashiro et al., 2000; this study) and Clone D2 is all-female parthenogenetic clone. Therefore, Yamashiro et al. (2000) suggested the recent extinction of bisexual diploid *L. lugubris* to explain a great clonal diversity in this island group. Recent extinctions of bisexual *Lepidodactylus*, as well as clones of *L. lugubris*, are thought to occur on several southern Pacific and Indian Ocean islands (Ineich, 1999). The reason(s) why they extincted probably through competition with other clones in artificially disturbed habitats (Ineich, 1999). Both Minamidaitojima and Kitadaitojima were originally covered by dense forests, and that deforestation has progressed rapidly and drastically on both islands after human colonization (Yamashiro et al., 2000). Thus, recent extinction of bisexual populations of *Lepidodactylus* in these islands is likely to occur.

Our genetic analysis also suggest another hypothesis that diversification of the triploid clones may be caused by the cross of most dominant females of Clone D1 with the unusual phenotypic male(s) derived from Clone D2 (Fig. 2-4). The phenotypic males have been found on few occasions in all-female parthenogenetic clones reared in the mass culture (Röll and von During, 2008) and in the field (Brown and Murphy-Walker, 1996; Yamashiro and Ota, 1998). Once such a male appeared in Clone

D2 in the Daito Islands, all triploid Clones D3 to D11 are explained by mating by this D2 male with D1 females without genetic contradiction. Microsatellite genotypes of triploid Clones D3 to D11 had common two alleles of diploid Clone D1 and one of two alleles of Clone D2 at all of eight loci (Table 2-4). This hypothesis is likely. At present, however, morphologically normal males known so far lack fully mature sperm and seem to be functionally infertile, despite spermatogenesis occurs in males of diploid Clone A (Röll and von During, 2008) and those of triploid Clone C (Yamashiro and Ota, 1998). Therefore, our hypothesis still needs evidence that fertile males appear in all-female diploid clones.

Another explanations would be also possible for the diversified clones in the Daito Islands. Multiple introductions and colonizations from the outsides would result in such a clonal diversity. Parthenogenetic reproduction is predicted to be advantageous in increasing abundance and in dispersal because parthenogenesis avoids the two-fold cost of sexual reproduction by making no investment in males and enabling each individual in all-female species to produce offspring independently (e.g., Maynard Smith 1978; Neaves and Baumann 2011). In fact, Clones O2 (=C), D12 (=B?) and D13 (=B?) cannot be explained genetically by our hypothesis of inter-clone hybridization (Table 2-4). These three clones are known to distribute widely (Ineich, 1988, 1999), and therefore may be migrated from the outsides. However, we cannot find out any peculiar situation that so many colonizations were successful in this island group, unlike those other island groups to which only one or two clones colonized (Table 2-6, Fig. 2-1).

Table 2-1. Primer sequences, repeat motifs, size ranges, optimized PCR temperatures, and references in eight microsatellite loci of *L. lugubris*.

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Anealing temperature (°C)	Reference
Ll01	F: [FAM]ATGTTGTTTTTCCCCCATGT	$(CT)_{25}$	121-133	56	Wilmhoff et al. (2003)
	R: AGAGACACAGGCATGTTTACG				
Ll02	F: [FAM]CAAAGGCATCTATGCAGACG	$(GT)_{8.5}$	144-152	56	Wilmhoff et al. (2003)
	R: CCTGCACACCAGCTTATGAAG				
Ll05	F: [FAM]ACAAGGGAGTATGGTAAGTTC	$(GT)_{13}(AT)_8(GT)_8$	134-180	56	Wilmhoff et al. (2003)
	R: GCATCATGCAATTAGGTTCCA				
Ll06	F: [FAM]CCCAAGTCTGCAGGAAAATC	$(CA)_{14}$	184-200	56	Wilmhoff et al. (2003)
	R: CCAGATGAAAAGTGGCAGGT				
Ll07	F: [FAM]ATGGCTCAGAAACACCCTGT	$(CA)_{12}(GT)_{15}$	236-262	62	Wilmhoff et al. (2003)
	R: GCAGGAAAAGAGGAAGACTCA				
Ll08	F: [FAM]GGACACCTGAACAGGTGAG	$(CA)_{21.5}$	264-284	56	Wilmhoff et al. (2003)
	R: TCTTTTCCCTGGGTGTTTTG				
Ll09	F: [HEX]AGGGTTTGGGTTTTCAGCTT	$(AC)_{18}$	105-119	54	Wilmhoff et al. (2003)
	R: GGTCACAGCTGATTTTTGGTG				
Ll10	F: [HEX]GATCCCCAAGGGGATTGGTG	$(GT)_{11.5}$	162-200	56	Wilmhoff et al. (2003)
	R: ATCGCTCCGTAGTGCATTG				

Table 2-2. Mitochondrial cyt *b* sequences used for phylogenetic analysis of the subfamily Gekkoninae and the outgroup.

Species	GenBank accession number	References
Gekko auriverrucosus	GQ857615	Yan et al. (2010)
Gekko japonicus	GQ857616	Yan et al. (2010)
Gekko swinhonis	GQ131373	Yan et al. (2010)
Hemidactylus dawudazraqi	HQ833758	Moravec et al. (2011)
Hemidactylus lavadeserticus	HQ833746	Moravec et al. (2011)
Hemidactylus mindiae	HQ833748	Moravec et al. (2011)
Hemidactylus smithi	KC818870	Smid et al. (2013)
Hemidactylus turcicus	HQ833740	Moravec et al. (2011)
Hemiphyllodactylus aurantiacus	FJ971012	Huang et al. (2009)
Hemiphyllodactylus harterti	FJ971013	Huang et al. (2009)
Hemiphyllodactylus typus	FJ971011	Huang et al. (2009)
Hemiphyllodactylus yunnanensis longlingensis	FJ971010	Huang et al. (2009)
Lepidodactylus lugubris Haplotype 1		This study
Lepidodactylus lugubris Haplotype 2		This study
Lepidodactylus lugubris Haplotype 3		This study
Lepidodactylus lugubris Haplotype 4		This study
Lepidodactylus lugubris Haplotype 5		This study
Sphaerodactylus vincenti	FJ404649	Surget and Thrope (2013)

Table 2-3. The number of individuals collected, used for microsatellite genotyping, mitochondrial haplotype analysis, and morphological observations.

Island group	Island	Number of individuals			
		Collected	Microsatellite genotyping	Mitochondrial haplotype	Dorsal marking patterns
The Ogasawara Islands	Kitanoshima	2	2	2	2
	Mukojima	56	56	20	56
	Nakoudojima	20	20	20	20
	Yomejima	10	10	10	10
	Anijima*	61	61	20	16
	Hyoutanjima	10	10	10	10
	Chichijima*	200	200	20	177
	Nishijima	6	6	6	6
	Hahajima*	180	180	20	159
	Hirashima*	2	2	2	0
	Iwoto*	1	1	1	0
	All islands	548	548	131	456
The Okinawa Islands	Okinawajima	10	10	10	10
	Zamamijima	2	2	2	2
	All islands	12	12	12	12
The Miyako Islands	Miyakojima	1	1	1	1
	All islands	1	1	1	1
The Yaeyama Islands	Yonagunijima	20	20	20	20
	Iriomotejima	21	21	21	21
	Taketomijima	20	20	20	20
	Kuroshima	20	20	20	20
	Ishigakijima	20	20	20	20
	All islands	101	101	101	101
The Daito Islands	Kitadaitojima	28	28	28	28
	Minamidaitojima	58	58	58	58
	All islands	86	86	86	86
Total		748	748	331	656

* Some samples were only the tail tips for DNA analyses and the dorsal marking pattern was not observed.

Table 2-4. Discriminated 17 clones of *L. lugbris* based on alleles in 8 microsatellite loci with possible diploidy, mitchondrial haplotypes and total number of individuals.

Locus	Ll01	Ll02	Ll05	Ll06	Ll07	Ll08	Ll09	Ll10	Ploidy	Haplotype	Total number
Motif	$(CT)_n$	$(GT)_n$	$(GT)_n(AT)_n(GT)_n$	$(CA)_n$	$(CA)_n(GT)_n$	$(CA)_n$	$(AC)_n$	$(GT)_n$			of individuals
Clone O1	111/133	147/155	136/182	204	242/250	264/272	105/109	182	2X	H3	405
Clone O2	111/121	147/151/159	136/158/178	194	242/252	268/270/272	105/111/123	180/196/208	3X	H1	257
Clone M	111/121/127	147/153/161	136/158	194	242/250	272/276	105/111/123	194/208	3X	H5	1
Clone T	115/121	149/151/157	136/164/176	192/208	240/270	264/274	109/111/123	180/198	3X	H4	2
Clone D1	111/121	147/159	136/178	194	242/252	262/264	105/111	180/208	2X	H1	41
Clone D2	103/121	147/151	136/158	194	252/256	262/264	105/123	180/196	2X	H2	5
Clone D3	103/111/121	147/159	136/178	194	242/252	262/264	105/111/123	180/196/208	3X	H1	5
Clone D4	103/111/121	147/159	136/178	194	242/252	262/264	105/111	180/196/208	3X	H1	2
Clone D5	103/111/121	147/159	136/158/178	194	242/252/256	262/264	105/111/123	180/196/208	3X	H1	1
Clone D6	103/111/121	147/151/159	136/158/178	194	242/252	262/264	105/111/123	180/196/208	3X	H1	2
Clone D7	111/121	147/159	136/158/178	194	242/252	262/264	105/111/123	180/196/208	3X	H1	4
Clone D8	103/111/121	147/151/159	136/178	194	242/252/256	262/264	105/111/123	180/196/208	3X	H1	3
Clone D9	103/111/121	147/151/159	136/178	194	242/252/256	262/264	105/111/123	180/208	3X	H1	6
Clone D10	103/111/121	147/151/159	136/158/178	194	242/252/256	262/264	105/111/123	180/196/208	3X	H1	3
Clone D11	111/121	147/151/159	136/178	194	242/252/256	262/264	105/111/123	180/208	3X	H1	1
Clone D12	103/111	147/151/157	136/158/180	194	242/252/256	262/264	105/111/123	180/196	3X	H1	4
Clone D13	103/111	147/151/157	136/158/180	194	242/252/256	262/266	105/111/123	180/196	3X	H1	6

Table 2-5. Five mitochondrial cyt *b* haplotypes in *L. lugubris*.

Haplotype	Position of 448-bp of cyt *b* sequences				
	34	95	115	293	429
H1	C	G	A	G	C
H2	-	A	-	-	-
H3	-	-	G	-	A
H4	-	-	-	C	-
H5	A	-	-	-	-

Table 2-6. The number of individuals of each clone of *L. lugubris* on each island.

Island group	Island	Clones																	Total
		O1	O2	M	T	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	
The Ogasawara Islands	Kitanoshima	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Mukojima	26	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	56
	Nakoudojima	6	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
	Yomejima	3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
	Anijima	26	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61
	Hyoutanjima	4	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
	Chichijima	155	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	200
	Nishijima	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
	Hahajima	180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	180
	Hirashima	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Iwoto	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
The Okinawa Islands	Okinawajima	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
	Zamamijima	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
The Miyako Islands	Miyakojima	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
The Yaeyama Islands	Yonagunijima	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
	Iriomotejima	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21
	Kuroshima	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
	Taketomijima	0	18	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	20
	Ishigakijima	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
The Daito Islands	Kitadaitojima	0	2	0	0	13	1	2	1	1	0	2	1	4	1	0	0	0	28
	Minamidaitojima	0	1	0	0	28	4	3	1	0	2	2	2	2	2	1	4	6	58
Total		405	257	1	2	41	5	5	2	1	2	4	3	6	3	1	4	6	748

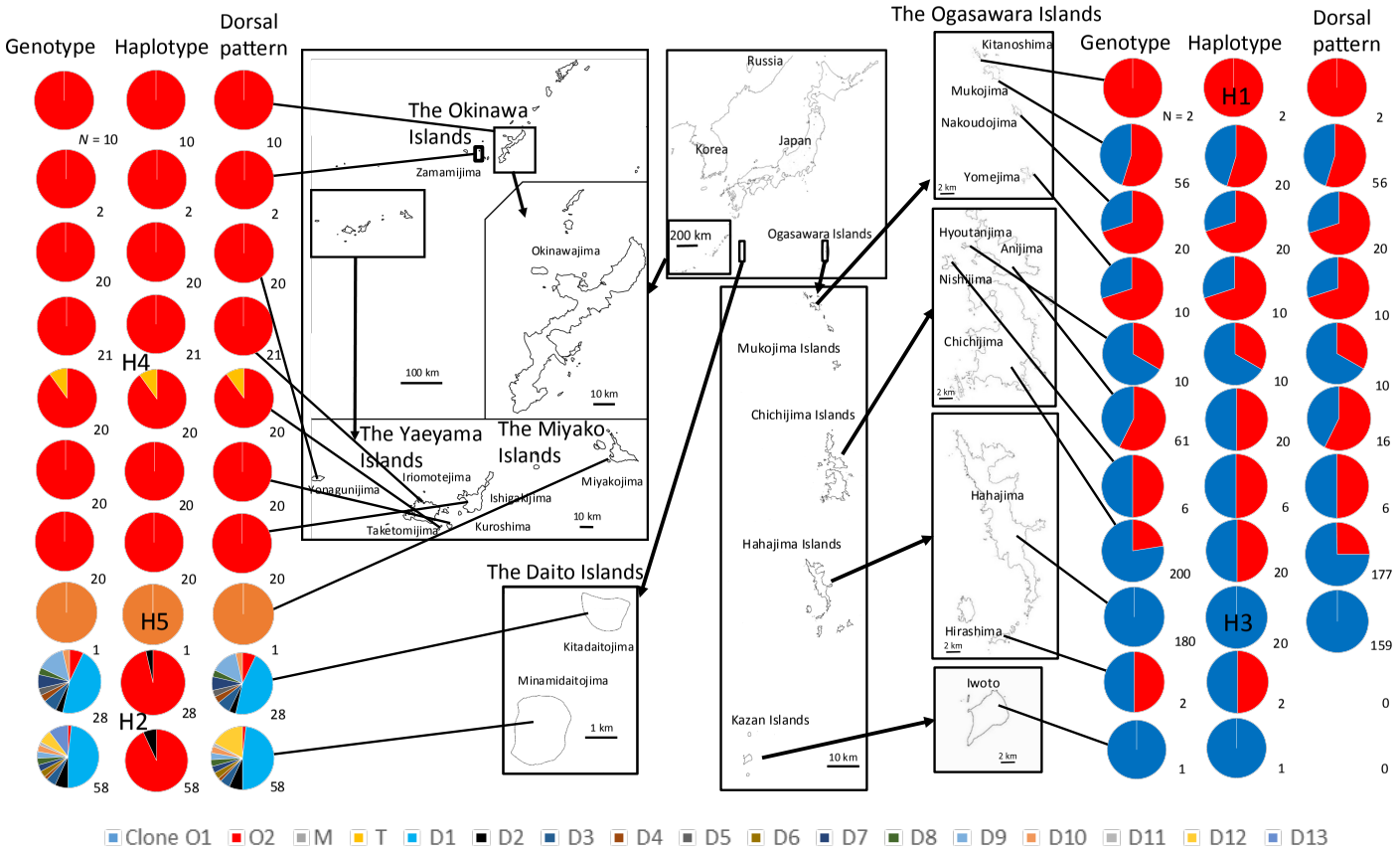

Fig. 2-1. Frequencies of the microsatellite genotypes, mitochondrial cyt *b* haplotypes, and dorsal marking patterns of 17 clones of *Lepidodactylus lugubris* on 21 islands in the Ogasawara, Okinawa, Miyako, Yaeyama, and Daito Islands, southern Japan. N shows the number of individuals examined (also see Table 2-3). 17 clones were distinguished by microsatellite genotypes (see Table 2-4). Five mitochondrial haplotypes (H1 to H5) were divided based on the cyt *b* sequences (see Table 2-5). Dorsal marking patterns were corresponded to microsatellite genotypes (see Fig. 2-2).

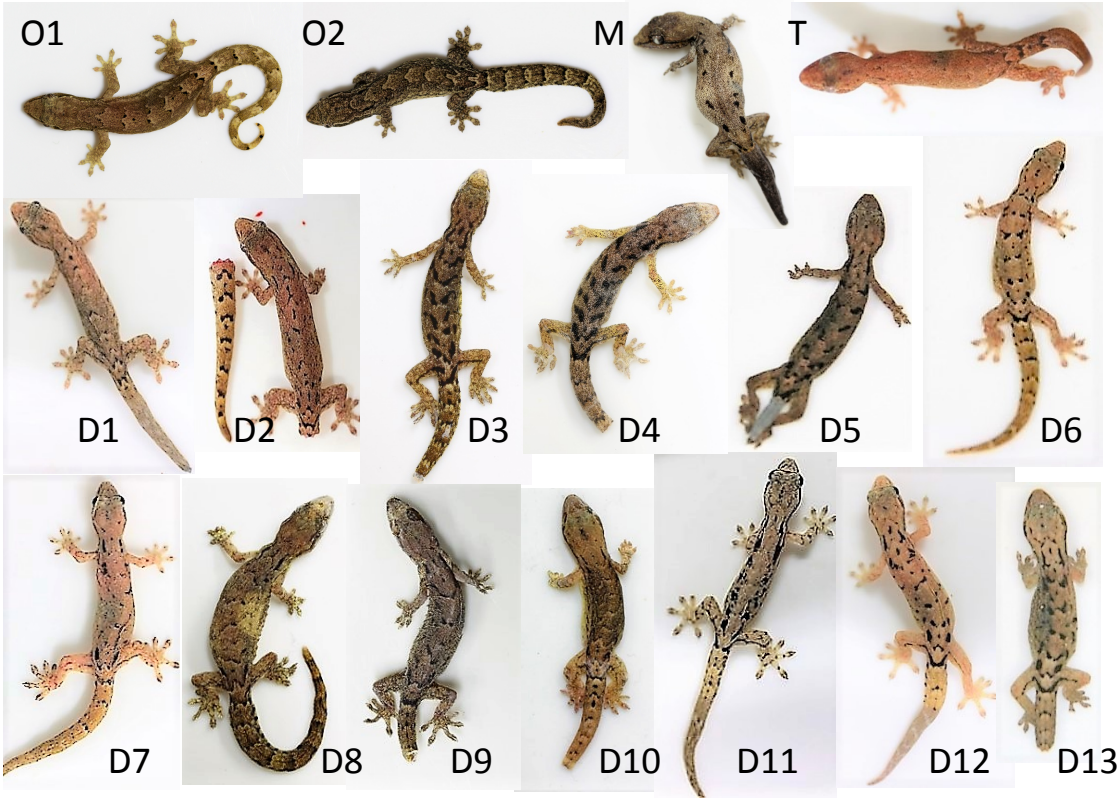

Fig. 2-2. Dorsal marking patterns of 17 clones (O1, O2, M, T, and D1 to D13) of *Lepidodactylus lugubris* in southern Japan. Magnifications differe among photographs.

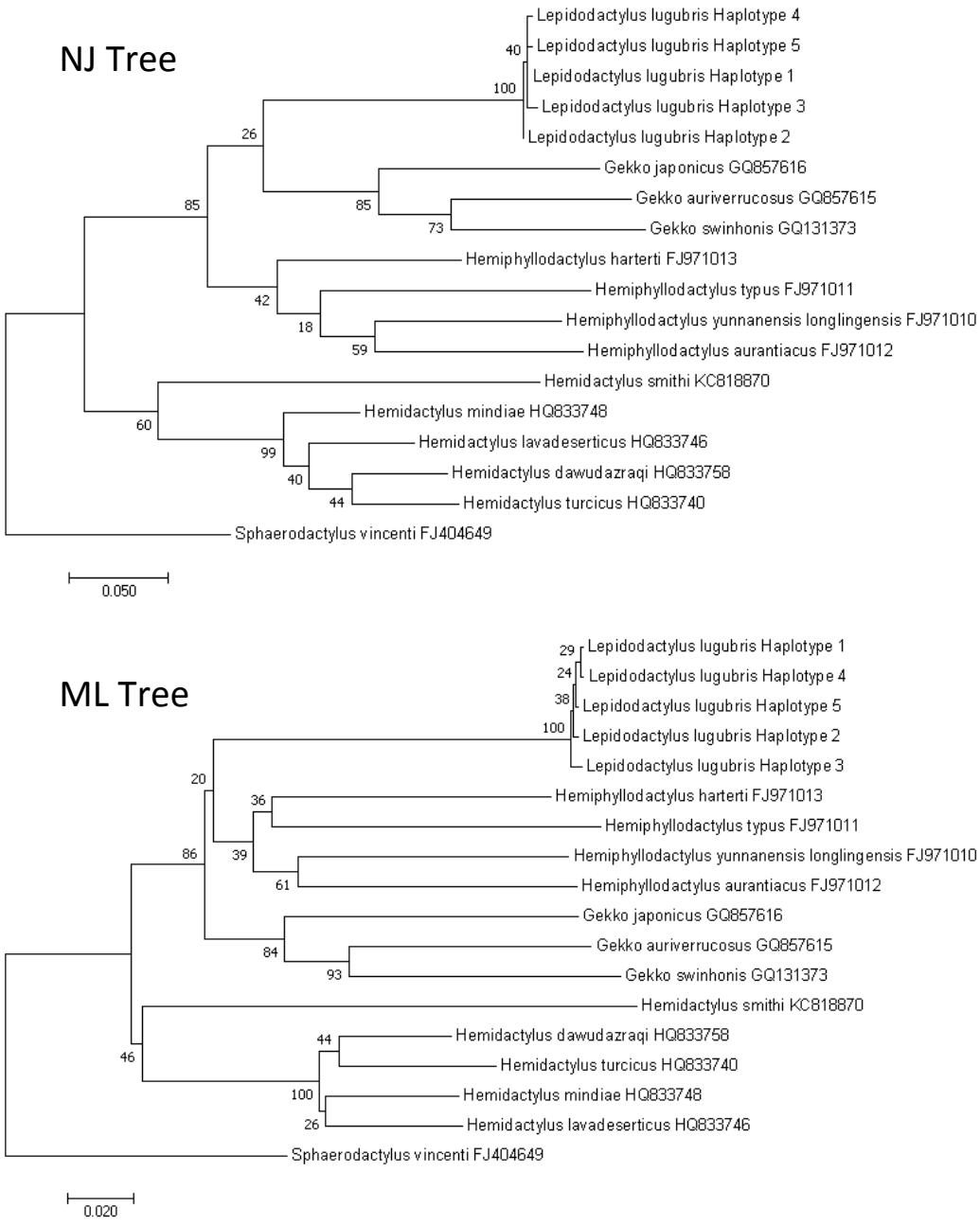

Fig. 2-3. Phylogenetic trees based on 448-bp cyt *b* sequences among species belonging to the subfamily Gekkoninae including the five haplotypes obtained for Japanese *Lepidodactylus lugubris*. *Sphaerodactylus vincenti* (Sphaerodactylidae) is used as the outgroup. For GenBank accession numbers, see Table 2-2. Bootstrap probability is based on 1,000 replications in the Neibor-Joining (NJ) tree and in the Maximum Likelyhood (ML) tree.

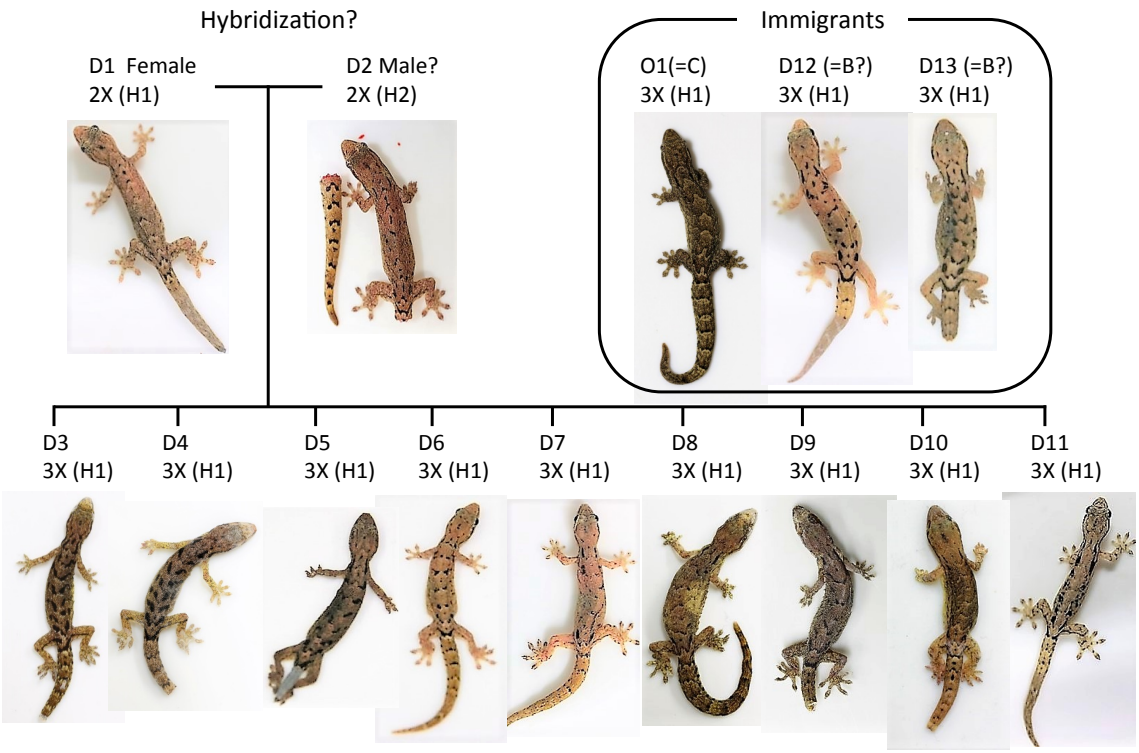

Fig. 2-4. Hypothetical clone diversification of *Lepidodactylus lugubris* in the Daito Islands. Three Clones O2, D12 and D13 may be colonized from the outsides because they are similar to widely distributed Clones B and C. In other endemic Clones D1 to D11, all triploid clones D3 to D11 can be explained to be produced by hybridization between diploid D1 female and diploid D2 male based on their allele combinations in eight microsatellite loci and also mitochondrial cyt *b* haplotypes (see Table 2-4). However, Clone D2 is all-female parthenogenetic and the existence of males of this clone has not be confirmed.

3. Comparisons of population genetic structures with the coexisting sexual gecko species *Hemidactylus frenatus* in the Ogasawara Islands

3-1. Introduction

Sexual reproduction has occurred widely in multicellular organisms; however, several species in various lineages of 19 of 34 phyla in the Animal Kingdom have secondarily lost this reproductive strategy and instead reproduce exclusively by parthenogenesis (Simon et al., 2003). Parthenogenesis avoids the two-fold cost of sexual reproduction by making no investment in males and enabling each individual in all-female species to produce offspring independently (e.g., Maynard Smith, 1978; Neaves and Baumann, 2011). This strategy enables every member of the population to establish in a new habitat. Thus, parthenogenetic reproduction is predicted to be advantageous in increasing abundance and in dispersal.

Among vertebrates, reptiles, and particularly geckos, include unexpected numbers of asexual (parthenogenetic) all-female species (Cole, 1984). One such species, the gecko *Lepidodactylus lugubris*, is distributed on the small oceanic Ogasawara Islands located approximately 1,000 km south of Tokyo, Japan (Takada and Ohtani, 2011; Fig. 3-1). *Lepidodactylus lugubris* is an all-female parthenogenetic species that is widely distributed in tropical-subtropical Pacific and Indian Ocean islands and adjacent continental coasts (Ineich, 1999). Specimens from Micronesian and Polynesian islands consist of diploid ($2n = 44$) and triploid ($3n = 66$) strains. Each strain includes a number of genetically divergent clonal lineages, some of which are diagnosable on the basis of dorsal color pattern (Ineich and Ota, 1992; Moritz et al., 1993; Hanley et al., 1994). The diploid clones are estimated to have derived from hybridizations between congeneric bisexual species, while the triploid clones originated via back crosses between the diploid clones and males of the parent species (Radtkey et al., 1995). In Ogasawara, two clones of *L. lugubris* have been recorded based on their dorsal color patterns (Yamashiro et al., 2000; Yamashiro and Ota, 2005).

Another gecko species, *Hemidactylus frenatus* Duméril et Bibron, 1836, is also widely distributed in tropical and subtropical regions (including the Ogasawara Islands), but this species reproduces sexually (Moritz et al., 1993; Takada and Ohtani, 2011).

Both *H. frenatus* and *L. lugubris* are so-called "house geckos," often coexisting on artificial substrates (Moritz et al., 1993). They are nocturnally active insectivores, with snout-vent lengths (SVLs) of less than 50 mm in females and of 60 mm in males (Moritz et al., 1993; Ota, 1994).

The Ogasawara Islands consist of four island groups, the Mukojima Islands, Chichijima Islands, Hahajima Islands, and Kazan Islands, as well as some other isolated islandsuch as Minamitorishima (Marcus Island). These island groups have never been connected to each other, as each is surrounded by deep sea waters; thus, overwater dispersal or artificial transportation was the route of colonization of these island groups (e.g., Hayashi et al., 2009). Only three islands, Chichijima, Hahajima, and Iwoto, are currently inhabited by humans. Although most islands of Ogasawara may have been temporarily inhabited, detailed information on such historical events is unavailable. Asexual *L. lugubris* and sexual *H. frenatus* on these islands may share a similar geographic history and climate. This situation offers a unique opportunity to study the relationship between reproductive strategy and the abundance, distribution, and genetic diversity of these two species. In the present study, we first monitored the distribution and abundance of the two species of geckos across nine islands. To document microhabitat selection of the two species, substrates upon which they were found (hereafter, perch substrates) were also recorded. Second, genetic diversity and population genetic structure were compared between the two species using microsatellite markers selected specifically for detecting intraspecific variation.

3-2. Materials and methods

Geckos were collected on nine islands (Fig. 3-1) that could be approached safely: Anijima and Chichijima in March 2012; Anijima, Chichijima, Hahajima, Hirashima, and Iwoto in June and July 2012; Anijima in August 2012; Nishijima and Chichijima in December 2012; Kitanoshima, Mukojima, and Chichijima in July 2013; Mukojima in December 2013; and Yomejima in July 2014. The area and maximum altitude of each island are 0.2 km^2 and 52 m for Kitanoshima, 2.6 km^2 and 88 m for Mukojima, 0.8 km^2 and 67 m for Yomejima, 7.9 km^2 and 254 m for Anijima, 0.5 km^2 and 88 m for Nishijima, 23.8 km^2 and 326 m for Chichijima, 20.8 km^2 and 462 m for Hahajima, 2.1

km^2 and 62 m for Hirashima, and 22.4 km^2 and 169 m for Iwoto. Sampling was conducted to cover as much of each island as possible. Field-caught geckos were identified to species, and the dorsal stripe patterns were recorded for *L. lugubris*. We also recorded the perch substrates of geckos, which were separated into seven categories: tree trunks and branches, rock crevices or under stones, grasses, sandy beaches, house walls and windows, electrical poles, and road guardrails. On Chichijima and Hahajima, all collection locations were plotted on maps using a global positioning system (GPSMAP® 62SJ, Garmin Ltd., Hampshire, UK).

Total genomic DNA was extracted from tail tips preserved in 99.5% ethanol using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For *L. lugubris*, four microsatellite loci were amplified using the primer sets of Wilmhoff et al. (2003): Ll01, 5'-ATGTTGTTTTTCCCCCATGT-3' and 5'-AGAGACACAGGCATGTTTACG-3'; Ll02, 5'-CAAAGGCATCTATGCAGACG-3' and 5'-CCTGCACACCAGCTTATGAAG-3'; Ll05, 5'-ACAAGGGAGTATGGTAAGTTC-3' and 5'-GCATCATGCAATTAGGTTCCA-3'; and Ll06, 5'-CCCAAGTCTGCAGGAAAATC-3' and 5'-CCAGATGAAAAGTGGCAGGT-3'. For *H. frenatus*, five microsatellite loci were amplified using the two primer sets of Li and Zhou (2007): di004, 5'-TGTAACCTGTGTGTGAAAGAA-3' and 5'-GCCTCAGAACCAAGAGTATG-3' and di005, 5'-CAAGAGAAGTGTTGTCAGAGG-3' and 5'-GGCTGAATAAACAAGAATAA; and three primer sets of Owusu et al. (2012): Gs112, 5'-CTGGTGCGGTGGTTATT-3' and 5'-AGGAGGTGCCTGTTGCAAATC-3'; Gs131, 5'-CTATGAGGGACACGGACC-3' and 5'-TCAACACAAGAAACGCTTATT-3'; and Gs133, 5'-AAATTTGCAAGGTGCTTAGG-3' and 5'-TTCAGCGGAAAATGTAAATG-3'.

Microsatellites were amplified in a T100™ thermal cycler (Bio-Rad, Hercules, CA, USA) using ExTaq®(Takara, Tokyo, Japan). The PCR reaction mix (total volume, 10 μL) contained 1.0 μL 10× of Extaq Buffer, 0.8 μL 25 mM dNTP mix, 0.5 μL fluorescent (6-FAM) forward primer (10 pM), 0.5 μL reverse primer (10 pM), 0.05 μL Taq polymerase, 6.15 μL distilled deionized water, and 1.0 μL template DNA. For *L. lugubris*, PCR conditions were as follows: an initial denaturation of 94°C for 3 min, 35

cycles of 95°C for 30 s, 56°C for 40 s, and 72°C for 40 s. An initial single step of 94°C for 4 min and a final single step of 72°C for 5 min were also included. For *H. frenatus*, the PCR conditions of two loci, di004 and di005, were as follows: an initial denaturation of 94°C for 5 min, 30 cycles of 94°C for 30 s, 56°C for 45 s, and 65°C for 45 s, and then eight cycles of 94°C for 30 s, 53°C for 45 s, 65°C for 45 s; and a final elongation of 65°C for 10 min. The PCR conditions for Gs112, Gs131, and Gs133 were an initial denaturation of 95°C for 3 min, 35 cycles of 95°C for 30 s, 52°C (but 54°C in Gs131, 49°C in Gs133) for 30 s, and 72°C for 30 s; and a final elongation of 72°C for 10 min. Then, 1 μL product was added to 9 μL loading mix containing a GeneScan™ 500 Liz® Size Standard (Applied Biosystems, Foster City, CA, USA) and Hi-Di Formamide (Applied Biosystems). This mixture was analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems). Allele lengths were scored using Peak Scanner version 1.0 (Applied Biosystems).

Observed heterozygosity (H_O) and expected heterozygosity (H_E) in each population were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). Deviation from Hardy-Weinberg equilibrium and linkage disequilibrium were estimated using Genepop'007 (Rousset, 2008). The significance of inbreeding coefficients was determined using FSTAT ver. 2.9.3.2 (Goudet, 1995). Tests of significant genetic differentiation among populations were conducted using *F*-statistics (Weir and Cockerham, 1984) with each parameter tested against zero by a bootstrapping method using FSTAT ver. 2.9.3.2. The genetic variation among and within populations was subjected to analysis of molecular variance (AMOVA) using Arlequin ver. 3.5 (Excoffier and Lischer, 2010). Assessment of current genetic structure was conducted using the program Structure ver. 2.3.3 (Pritchard et al., 2000). Ten runs were set with a burn-in length of 100,000 and an MCMC of 200,000 for each *K* (1 to 8). To make a precise estimate of population structure, the LOCPRIOR model was utilized (Hubisz et al., 2009). ΔK was calculated to examine the true *K* number (Evanno et al., 2005). To detect molecular signatures of bottlenecks, we used a method implemented in Bottleneck ver. 1.2.02 (Piry et al., 1999). For Bottleneck, three mutation models were used: the infinity allele model (IAM), two-phase mutation model (TPM), and stepwise mutation model (SMM) with 80% single- and 20% multiple-step mutations.

3-3. Results

3-3-1. Asexual *Lepidodactylus lugubris*

A total of 322 *L. lugubris* were collected and analyzed for their four microsatellite DNAs. This gecko was found on all nine study islands and consisted of only two microsatellite genotypes: Clone O1 (111/133 in Ll01, 147/155 in Ll02, 136/182 in Ll05, and 204 in Ll06) and Clone O2 (111/121 in Ll01, 147/151/159 in Ll02, 136/158/178 in Ll05, and 194 in Ll06) (Fig. 3-1). Clone O1 had two alleles, while Clone O2 had three alleles at a maximum, suggesting diploid and triploid clones, respectively. Dorsal stripe patterns were stable within the same clone but clearly differed between clones. Clone O2 had two pairs of additional large dark markings on the lateral sides (Fig. 3-2). Both clones were widely distributed throughout the Ogasawara Islands, although Hahajima lacked Clone O2 despite having collected a sufficient number of samples (Figs. 3-1, 3-3b). On Chichijima, both clones were mixed (Fig. 3-3a). Most individuals, i.e., 103 (84.4%) of 122 individuals on Chichijima and 70 (80.5%) of 87 on Hahajima, were collected on artificial substrates such as house walls and windows, electrical poles, and road guardrails (Table 3-1). On islands not inhabited by humans, however, they usually perched on trees and rocks (Table 3-1). Although the sample size was small ($N = 7$), all Clone O2 individuals were found on natural substrates, even on human-inhabited Chichijima (Table 3-1).

3-3-2. Sexual *Hemidactylus frenatus*

A total of 123 *H. frenatus* were collected. This gecko was found on four of the nine study islands, of which three are presently inhabited by humans (Fig. 3-1). On Chichijima and Hahajima, the collection sites were limited to areas of towns and along roadways (Fig. 3c, d), and almost all individuals were collected on artificial substrates: 46 of 48 individuals (95.8%) on Chichijima and 56 of 56 (100%) on Hahajima (Table 3-1). The proportion of geckos that preferred artificial perches differed between *H. frenatus* and *L. lugubris* ($\chi^2 = 4.14$, df = 1, $P < 0.05$ on Chichijima; $\chi^2 = 12.42$, df = 1, $P < 0.001$ on Hahajima). On Anijima, which lacks humans, all 18 individuals were found

on the trunks of trees (Table 3-1).

The numbers of alleles observed from all 123 *H. frenatus* were five for locus di004, two for di005, three for Gs112, four for Gs131, and three for Gs133. Observed and expected heterozygosities ranged from 0.32 to 0.69 and 0.44 to 0.74, respectively (Table 3-2). After Bonferroni correction ($\alpha = 0.03$), no significant linkage disequilibrium or deviation from Hardy-Weinberg equilibrium were identified. Allele frequencies appeared to be similar among northern, central, and southern parts of Chichijima and among these same areas of Hahajima (Table 3-2). However, allele frequencies differed among Anijima, Chichijima, and Hahajima (Iwoto, with only one sample, was excluded). Allele 245 of locus Gs131 was only found in the Chichijima population. Allele 319 of locus Gs133 was only found in the Hahajima population. In the Anijima population, all 18 individuals shared only two alleles/locus. AMOVA suggested that most of the variance (74%) could be explained by within-individual variation, and the variance explained by differences among populations (4.3%) was also significant (Table 3-3). Population pairwise F_{ST} values were 0.016 between Anijima and Chichijima ($P = 0.0003$), 0.093 between Anijima and Hahajima ($P = 0.0003$), and 0.071 between Chichijima and Hahajima ($P = 0.0003$). Moreover, the population genetic structure differed among the three islands at $K = 3$, when ΔK was highest (2.5) among all other values (0–2.2) at $K = 2$ to 7 (Fig. 3-4). A significant excess of heterozygosity compared to the expected equilibrium was obtained using Wilcoxon tests implemented in Bottleneck: H_{EQ} was 0.232 in the IAM, 0.262 in the TPM, and 0.278 in the SMM in the Anijima population ($P < 0.05$ in all cases); H_{EQ} was 0.327 in the IAM, 0.381 in the TPM, and 0.438 in the SMM in the Chichijima population ($P < 0.05$ in all cases); and H_{EQ} was 0.327 in the IAM, 0.381 in the TPM, and 0.449 in the SMM in the Hahajima population ($P < 0.05$ in all cases). These results suggest significant bottleneck effects in these three populations.

3-3-3. Microdistribution on small islands

A total of 107 *L. lugubris* samples were collected on six uninhabited islands of the Ogasawara Islands, and all of individuals were successfully genotyped by microsatellite DNA analysis and were observed their dorsal color patterns. A total of two clone types

(Clone A and C) were recognized on the basis of the microsatellite genotypes. Microsatellite genotypes of these clones did not contradict classifications on the basis of dorsal coloration. On the all of six islands, A-clones were distributed clearly higher frequency in coastal region, and C-clones were higher in inland areas (Fig. 3-2). Moreover, Clone A individuals were found only on or under the rocks, while C-clones were seen on the flowers or trees (Table 3-1).

3-4. Discussion

3-4-1. Clone diversity of asexual *L. lugubris*

In the Ogasawara Islands, asexual *L. lugubris* consisted of two clones, diploid Clone O1 and triploid Clone O2. A previous survey also documented two clone types on the islands, Clones A and C (Yamashiro et al., 2000; Yamashiro and Ota, 2005). Judging from the dorsal marking patterns and ploidy level, Clone O1 is the same as Clone A and Clone O2 is Clone C, as described in Yamashiro et al. (2000) and Yamashiro and Ota (2005). More specifically, Clone A is diploid and Clone C is triploid, and the dorsal dark markings are essentially similar between these clones (compare Fig. 3-2a in this study and Fig. 1A in Yamashiro and Ota, 2005, for Clone A and Fig. 3-2b in this study and Fig. 1B in Yamashiro and Ota, 2005, for Clone C). Yamashiro and Ota (2005) suggested that Clone C individuals may soon disappear from the Chichijima population based on the following sequence of specimen records on this island: Okada's (1930) first recorded specimen was identified as Clone C. An additional 25 museum specimens collected from 1968 to 1978 were identified as Clone A (21) and Clone C (3) individuals (one was unidentified). Specimens obtained from 1997 and 1998 were all Clone A (N = 22). Ineich (1999) also noted the decline of several clone types, sometimes to the point of complete disappearance, on tropical Pacific and Indian Ocean islands where Clone A individuals are common. However, we confirmed that Clone C individuals are still distributed on Chichijima, although their relative abundance was slightly lower than on other islands (Fig. 3-1). On Hahajima, Yamashiro and Ota (2005) recorded only Clone A during their surveys in 1997 and 1998 (N = 36), which was confirmed in the present study. Therefore, to date, there has been no

evidence of the existence of Clone C on Hahajima, although why this island is only occupied by Clone A remains unknown.

The Takapoto Atoll, French Polynesia, harbors both asexual *L. lugubris* and its sexual congener *L.* sp.; the former is distributed across the entire atoll, which consists of several small lands each separated by sea, but the latter is confined to a single southern land (Hanley et al. 1994). This distribution pattern is similar to our results. At this atoll, displacement between asexual and sexual species had not occurred during 1986 to 1991, and any significant aggression between the two species was not detected during laboratory observations. Among clones of *L. lugubris*, individuals of one clone-type are superior foragers compared to individuals of the other clone-type, suggesting that the former individuals monopolize limited prey items within the structurally simple human landscape (Short and Petren 2008). Inter-clonal differences in thermal preference may also explain the altitudinal distribution patterns of *L. lugubris* clones on Fiji (Bolger and Case 1994). To better understand the temporal and spatial population dynamics of *L. lugubris* in the Ogasawara Islands, more information is needed on such inter-clone competitive interactions.

3-4-2. Effects of sexuality on dispersal

In the Ogasawara Islands, asexual *L. lugubris* had widely expanded across the islands and was mixed genetically, although genetic variation was low (only two clones). In contrast, the distribution of sexual *H. frenatus* was limited to a few islands, forming genetically different insular populations accompanied by some bottleneck effects. Such differences in distribution and genetic population structure between species might be explained by variation in colonization success. In general, inter-island dispersal can be quite extensive in asexual species, as asexual organisms avoid the two-fold cost of sexual reproduction by not investing in males and enabling each individual in the all-female species to independently produce offspring (e.g., Maynard Smith, 1978; Neaves and Baumann, 2011). Frequent dispersal of asexual species contributes to the expansion of their distribution ranges and to the genetic homogenization of insular populations. In our study system, however, the colonization histories of the two species have not yet been examined, and we cannot rule out a more recent colonization of

sexual *H. frenatus* relative to asexual *L. lugubris*. Lizards have weak dispersal abilities among oceanic islands compared to flying animals; therefore, gene flow between individual islands may be greatly reduced by oceanic barriers. In the Ogasawara Islands, *L. lugubris* consists of only two clone types, suggesting at least two successful colonizations of this archipelago, likely from the southern Pacific source population of this species. We also cannot rule out the possibility that the two clone types colonized at the same time. Based on their genetic diversity in microsatellite loci, colonization of *H. frenatus* to Ogasawara may have occurred multiple times. However, we cannot identify how and when colonization actually occurred.

In addition to reproductive strategy, variation in dispersal success rate may also be caused by different microhabitat preferences of the two species. Both species are considered house geckos, and the two often coexist on artificial substrates (Moritz et al., 1993). Sexually reproducing *H. frenatus* exhibits a closer association with human habitation (Newbery and Jones, 2007). Our observations also indicated that *H. frenatus* collection sites were more confined to man-made structures in towns and along roadways compared to *L. lugubris* collection sites on Chichijima and Hahajima. This finding may partially explain the lack of *H. frenatus* on small islands without recent human habitation or may suggest that it is a recent colonizer brought by humans, probably accidentally, to human-inhabited islands. However, on Anijima, which lacks humans, *H. frenatus* was found on tree trunks, suggesting that they have the potential to live in natural habitats.

In general, asexual reproducers lack genetic diversity in offspring and therefore are thought to more easily succumb to parasites, diseases, and predation due to their negligible ability to adapt to changing environments (e.g., Neaves and Baumann, 2011). In Ogasawara, terrestrial reptiles other than *L. lugubris* and *H. frenatus* number very few: one other gecko, *Perochirus ateles* known only from Minami-iwoto, the southernmost island of the Kazan Islands, and Minamitorishima (Marcus Island); the skink *Cryptoblepharus nigropunctatus*; the human-introduced anole *Anolis carolinensis*; and the parthenogenetic blind snake *Ramphotyphlops braminus* (Horikoshi, 2008; Takada and Ohtani, 2011). No native terrestrial amphibians and mammals exist on the islands, except bats. Thus, at present, the islands may be free of effective parasites, diseases, and predators of the two study gecko species, which may realize

dispersal and abundance advantages for the asexual species.

Table 3-1. Substrates from which two species of geckos, *Lepidodactylus lugubris* (*L. l.*) and *Hemidactylus frenatus* (*H. f.*), were collected on nine of the Ogasawara Islands. Numerals indicate the number of geckos collected, and those in parentheses represent the number of Clone O2 individuals among all *L. lugubris* (Clones O1 and O2). Artificial substrates were unavailable on islands not inhabited by humans and are represented with dashes.

Islands	Natural substrates								Artificial substrates						Not recorded		Total	
	Tree trunk		Rock crevice		Grass		Sandy beech		House wall		Electric pole		Road guardrail					
	L. l.	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*	*L. l.*	*H. f.*
Kitanoshima			1 (1)		1 (1)				–	–	–	–	–	–			2 (2)	
Mukojima	4 (2)		20 (12)		2 (2)		16 (8)		–	–	–	–	–	–			42 (24)	
Yomejima			3 (2)						–	–	–	–	–	–			3 (2)	
Anijima	55 (36)	18	2 (0)						–	–	–	–	–	–			57 (36)	18
Nishijima			6 (3)						–	–	–	–	–	–			6 (3)	
Chichijima	10 (4)	2					9 (3)		53 (0)	22	20 (0)	6	30 (0)	18			122 (7)	48
Hahajima	17 (0)								42 (0)	32	6 (0)	8	22 (0)	16			87 (0)	56
Hirashima									–	–	–	–	–	–	2 (1)		2 (1)	
Iwoto															1 (0)	1	1 (0)	1
All islands	86 (42)	20	32 (18)	0	3 (3)	0	25 (11)	0	95 (0)	54	26 (0)	14	52 (0)	34	3 (1)	1	322 (75)	123

Table 3-2. Allele frequencies in five microsatellite loci of *Hemidactylus frenatus* on four Ogasawara islands. N = number of individuals sampled, N_A = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity. Frequencies on the northern, central, and southern parts of Chichijima and Hahajima are also shown separately (see Fig. 3-3).

Locus	Allele	Anijima	Chichijima				Hahajima				Iwoto
(motif)	in size		North	Central	South	Total	North	Central	South	Total	
di004	187 bp	0	12	14	1	28	5	8	8	21	0
$(TG)_n(GA)_m$	193 bp	22	15	7	10	31	7	30	7	44	0
	207 bp	14	7	4	9	20	13	10	0	23	1
	215 bp	0	7	5	2	14	2	0	0	2	0
	221 bp	0	3	0	0	3	5	12	5	22	1
	N_A	2	5	4	4	5	5	4	3	5	2
	H_O	0.66				0.50				0.69	
	H_E	0.47				0.74				0.73	
di005	167 bp	14	17	10	10	37	10	23	10	43	1
$(TC)_n$	175 bp	22	27	20	12	59	23	37	10	69	1
	N_A	2	2	2	2	2	2	2	2	2	2
	H_O	0.44				0.35				0.44	
	H_E	0.47				0.47				0.47	
Gs112	176 bp	20	13	9	12	34	8	26	8	42	2
$(GT)_n$	182 bp	16	6	3	1	11	15	3	5	23	0
	200 bp	0	25	18	9	51	9	31	7	47	0
	N_A	2	3	3	3	3	3	3	3	3	1
	H_O	0.32				0.41				0.57	
	H_E	0.49				0.57				0.64	
Gs131	245 bp	0	6	6	2	13	0	0	0	0	0
$(CT)_n$	247 bp	12	14	2	4	24	7	25	14	46	1
	249 bp	0	5	7	2	12	7	12	1	20	0
	265 bp	24	19	15	14	47	18	23	5	46	1
	N_A	2	4	4	4	4	3	3	3	3	2
	H_O	0.44				0.43				0.46	
	H_E	0.44				0.66				0.63	
Gs133	301 bp	15	23	15	9	47	10	28	11	49	2
$(AC)_n$	317 bp	21	21	15	13	69	13	25	9	47	0
	319 bp	0	0	0	0	0	9	7	0	16	0
	N_A	2	2	2	2	2	3	3	3	3	1
	H_O	0.38				0.40				0.45	
	H_E	0.49				0.50				0.62	
N		18	22	15	11	48	16	30	10	56	1

Table 3-3. Analysis of molecular variance (AMOVA) of five microsatellite loci in the three populations of *Hemidactylus frenatus*.

Source of variation	Sum of squares	Variance components	Percentage variation (%)	*P*-value
Among populations	13.79	0.07	4.34	< 0.001
Among individuals within populations	218.01	0.34	21.82	< 0.001
Within individuals	140.50	1.15	73.84	< 0.001
Total	372.30	1.56		

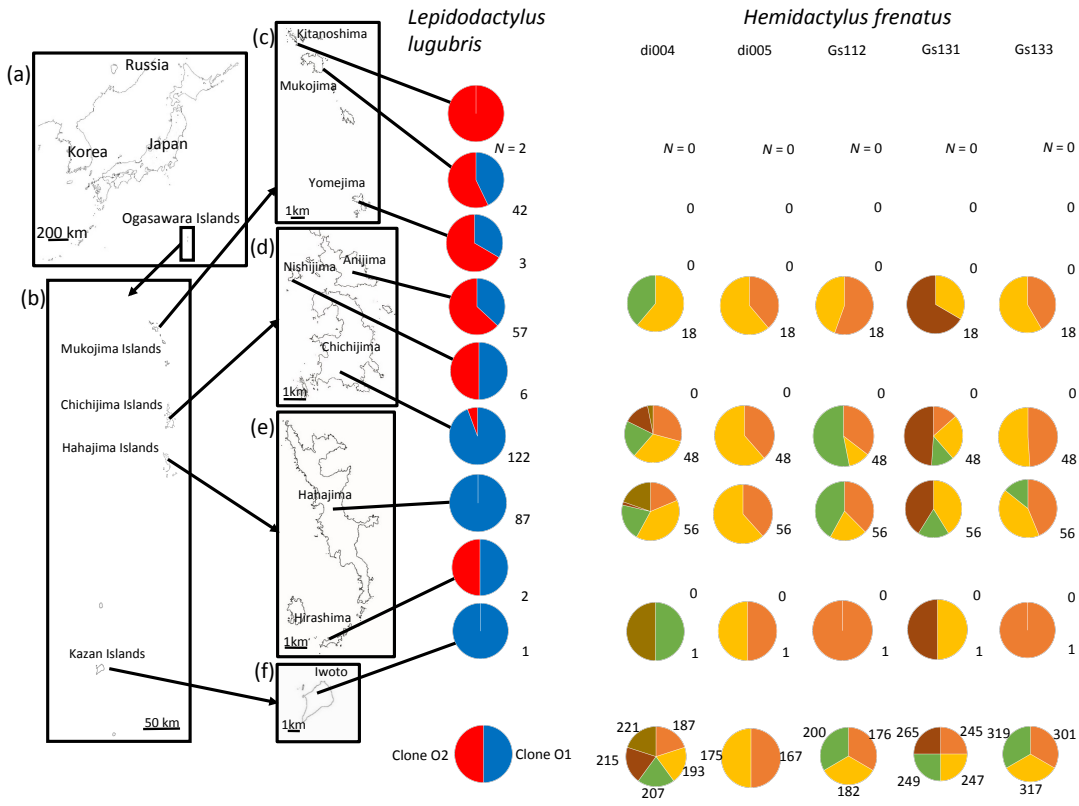

Fig. 3-1. Distributions and allele frequencies of microsatellite DNA of the two sympatric gecko species, *Lepidodactylus lugubris* and *Hemidactylus frenatus*, on nine small oceanic islands: Kitanoshima, Mukojima, and Yomejima in the Mukojima Islands group (c); Anijima, Nishijima, and Chichijima in the Chichijima Islands group (d); Hahajima and Hirashima in the Hahajima Islands group (e); and Iwoto in the Kazan Islands group (f) in the Ogasawara Islands (b) located about 1,000 km south of the Japanese mainland (a). In asexual *L. lugubris*, only two genetic clones, Clones O1 and O2, were found. In sexual *H. frenatus*, allele frequencies of five microsatellite loci are shown: five alleles (187, 193, 207, 215, and 221) of di004, two alleles (167 and 175) of di005, three alleles (176, 182, and 200) of Gs112, four alleles (245, 247, 249, and 265) of Gs131, and three alleles (301, 317, and 319) of Gs133. *N* = number of individuals examined on each island.

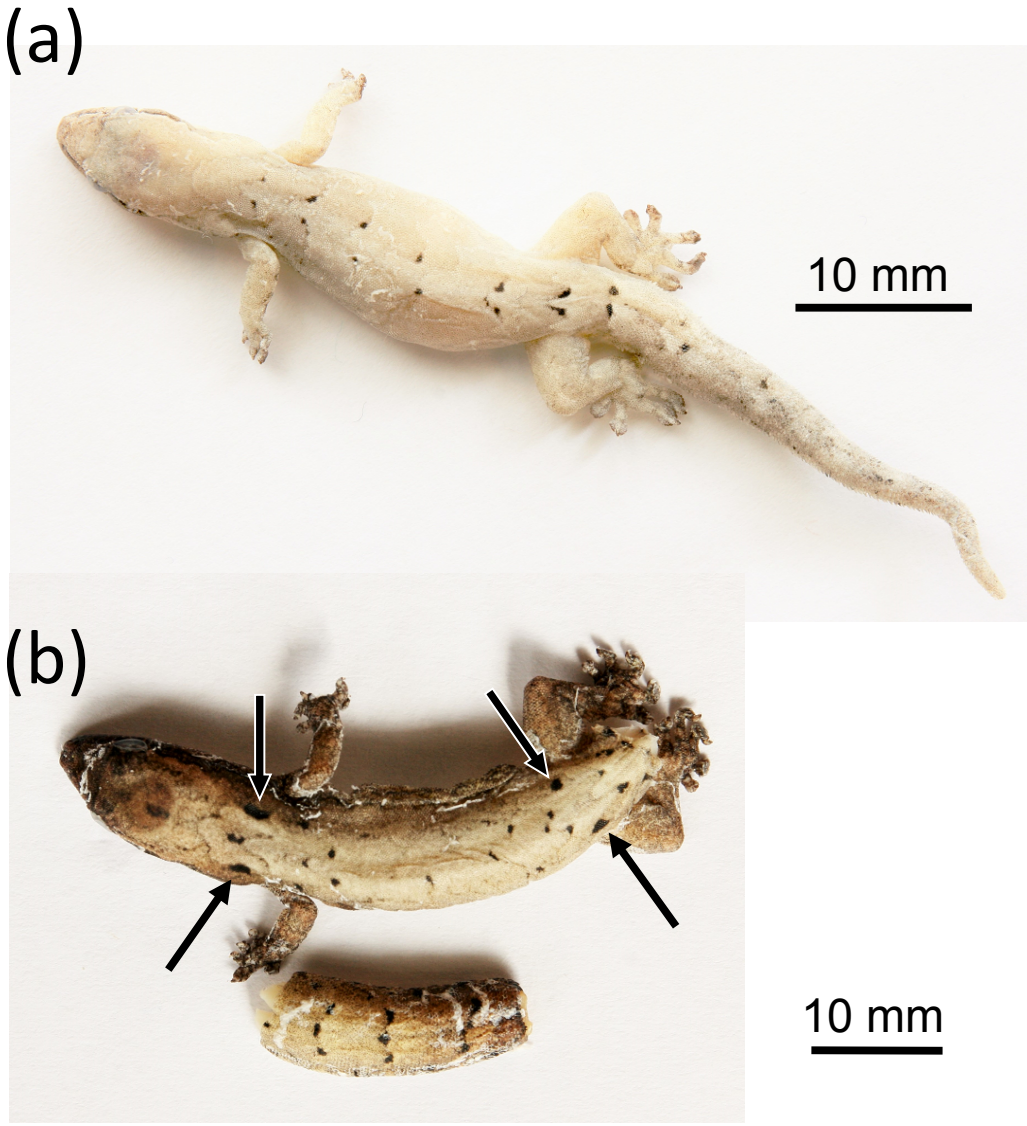

Fig. 3-2. Dorsal views of Clones O1 (a) and O2 (b) of *Lepidodactylus lugubris* found in the Ogasawara Islands. Two pairs of dark markings indicated with arrows clearly differ between the two clones. Clones O1 and O2 are identified as already known Clones A and C, respectively (also see Chapter 2).

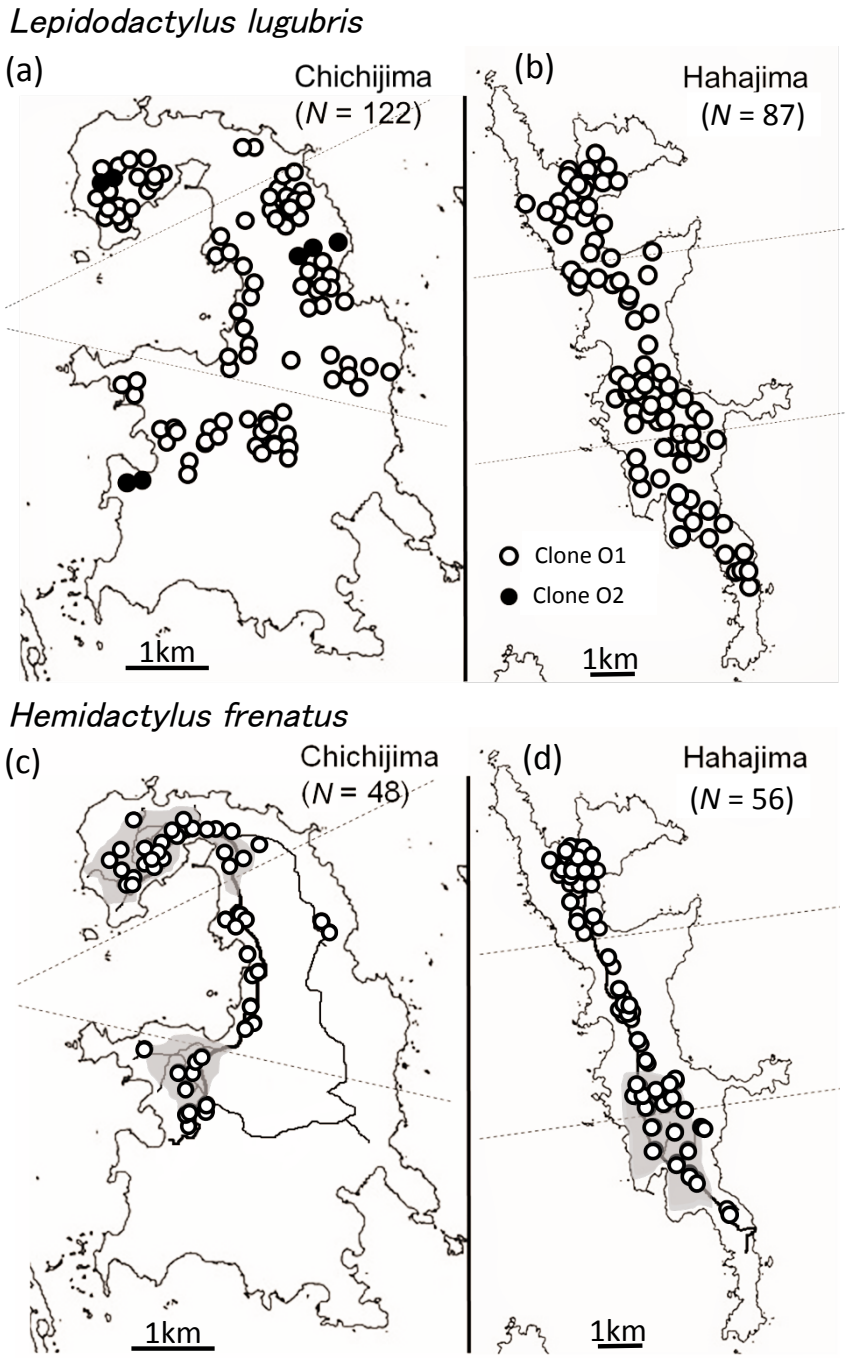

Fig. 3-3. All collection sites of Clones O1 and O2 of *Lepidodactylus lugubris* (a, b) and individuals of *Hemidactylus frenatus* (c, d) on Chichijima and Hahajima. In c and d, gray areas and lines drawn within each island represent towns and roadways, respectively. *N* = number of individuals examined on each island. Dashed lines show the northern, central, and southern parts on each island, which were categorized separately in this study.

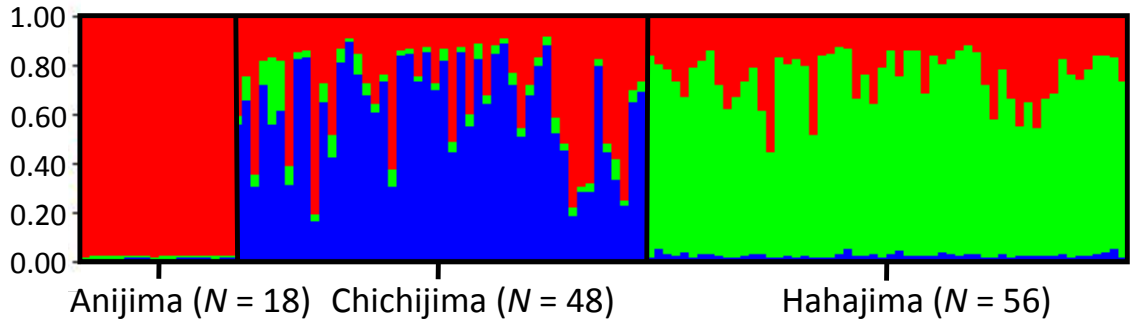

Fig. 3-4. Population genetic structure among three island populations of *Hemidactylus frenatus* drawn using the software STRUCTURE. Vertical columns represent the assignment probabilities for each of the inferred clusters identified at $K = 3$ when ΔK was highest for $K = 2$–7 calculations. The single sample obtained from Iwoto was excluded. N = number of individuals examined on each island.

4. Clonal differences in aggressive behavior

4-1. Introduction

Ecological differentiation between clones of parthenogenetic species is necessary for co-existence of multiple clones in narrow habitats such as islands and ponds, because overlap of their niches intensifies inter clonal competition. All-female parthenogenetic *Lepidodactylus lugubris* is a small gecko being widespread over Indian and Pacific Ocean islands and continental Asia and America (see Fig. 1-2 in Chapter 1). Multiple clones are reported genetically and morphologically in this gecko, and several clones co-exist on some islands (Ineich, 1988; Ineich and Ota, 1992; Hanley et al., 1994; Ota et al., 1995; Radtkey et al., 1995, 1996). However, the mechanisms responsible for the spread and establishment of a clone to a habitat already occupied by other clones are unclear (Bolger and Case 1994). In this study, to examine behavioral differences among *L. lugubris* clones, we observed the behavioral interactions between the two clones, Clones O1 and O2, inhabiting the Ogasawara Islands.

In the Ogasawara Islands, a sexually reproducing gecko, *Hemidactylus frenatus*, is also distributed in some islands (Takada and Ohtani, 2011). This gecko has also a wide distribution range in tropical and subtropical oceanic regions and often co-exists with *L. lugubris*, both of which are found even in the human habitats (Moritz et al., 1993; Takada and Ohtani, 2011). Therefore, *H. frenatus* was also included in the experiment of behavioral interactions among *L. lugubris* clones.

4-2. Materials and methods

A total of 111 females of Clone O1 and 92 females of Clone O2 of *L. lugubris*, and 67 males and 68 females of *H. frenatus* were collected from Chichijima in July and August of two years, 2016 and 2017. In addition, 10 females of Clone O2 were collected from Mukojima in July 2017. Snout to vent length (SVL) of all captured individuals were measured, and the state of their tail (complete tail, regenerated tail, or no tail) were recorded.

In the contest experiments, we used enclosures (50 × 50 cm^2 at the top, 10 × 10 cm^2

at the bottom, 30 cm high) made with wooden panels in which one small hole (1 cm in diameter) opens on only one side of walls (Fig. 4-1). Geckos to use for experiments were collected in the field before 7 to 15 h of video recording. They were kept together in the experimental enclosures and allow them move around there. In darkness, two individuals selected from Clones O1 and O2 of *L. lugubris*, and male and female *H. frenatus* (hereafter written as Clone O1, Clone O2, H-M, and H-F, respectively) were put into the empty enclosure, and the top of it was covered with a transparent acrylic board. Then, the behavior of geckos within it was recorded using the night-shot function of the video cameras (SONY FDR-AX55 or SONY DCRA-C160, Tokyo, Japan). After starting of video recording, one living small-sized mealworm (larvae of the beetle *Tenebrio molitor* Linnaeus, 1758) was thrown in the narrow pipe opening at the wall where the gecko ate it. If aggressive interactions competing for this bait were observed between the two individuals, the following seven behavioral categories were discriminated by referring Dame and Petren (2006); arches (warping body), lunges, wags (shaking the tail when the enemy was behind), clicks, bites, wrestles, and no clear behavior. The individual successfully feeding the mealworm was judged as a winner. This feeding trial using the single mealworm (hereafter called as "round") was repeated three times at >1 h intervals for the same pair, and then they were replaced to another cage to release at the captured sites in the next morning. All individuals were not used twice and more. These experiments were all conducted at the Ogasawara Field Research Station of Tokyo Metropolitan University on Chichijima. Geckos were maintained at 25–29°C and 50–60% in relative humidity.

All analyses were conducted using free programming language R and α was set to 0.05 for all hypothesis testing. Generalized linear model (McCullagh, 1984) was used to compare the incidence of struggle between two clones of *L. lugubris* and male and female *H. frenatus*. Furthermore, the Bradley-Terry model (Bradley and Terry, 1952) was also used to quantify and compare the strength in conflict between them using the 'Bradley Terry 2' package (Firth, 2005).

4-3. Results

Hemidactylus frenatus was larger than *L. lugubris* in SVL (Table 4-1). The

proportion of individuals with complete tail was 40.5% in Clone O1, 94.1% in Clone O2, 52.2% in H-M, and 58.8% in H-F (Table 4-1). Thus, most individuals of Clone O2 had a complete tail in the field (χ^2 test, $\chi^2 = 69.4$, df = 3, $P < 0.0001$).

A total of 522 trials by 174 pairs were observed (Table 4-2). Clones O1 and O2 differed in aggressive behaviors for resource acquisition, despite being the same species. Clone O2 showed little aggressive behaviors such as arches, wags, clicks, bites, or lunges and did never wrestle in any combinations with Clones O1, O2, H-M, and H-F (Fig. 4-2). Clicks were the most frequent response in aggressive interactions among Clone O1, H-M, and H-F (Fig. 4-2).

The contests (feeding of a given prey item with some aggressive behaviors) occurred usually in 66.7 to 100% of the rounds in the combinations of Clone O1, H-M, and H-F, whereas much less of the combinations including Clone O2; 17.8% with Clone O1, 9.3% with the same clone, 7.1% with H-M, and 10.6% with H-F (Table 4-2, Fig. 4-3). Although body size of *L. lugubris* was smaller than *H. frenatus*, Clone O1 fought with *H. frenatus*. The results of GLM analysis indicated that the contest rate of Clone O2 was significantly lower than Clone O1 and *H. frenatus* (Clone O2 vs O1; Estimate = -2.43, SE = 0.47, $P < 0.001$. Clone O2 vs H-M; Estimate = 1.93, SE = 0.49, $P < 0.001$. Clone O2 vs H-F; Estimate = 1.77, SE = 0.48, $P < 0.001$), whereas no significant difference was detected between Clone O1 and *H. frenatus* (Clone O1 vs H-M; Estimate = -0.50, SE = 0.32, $P > 0.1$. Clone O1 vs H-F; Estimate = -0.48, SE = 0.31, $P > 0.1$).

The quantifying the fighting ability using the Bradley-terry model that the strength of Clone O1 was set to the reference value 0 suggested a superiority/inferior relationship of two clones of *L. lugubris* and *H. frenatus* (H-M > Clone O1 > H-F > Clone O2) (Fig. 4-4). Clone O2 was found to have significantly lower fighting ability (ability = -1.204, SE = 0.189, $P < 0.001$) but no significant differences were detected between Clone O1 and *H. frenatus* (H-M; ability = 0.188, SE = 0.1801, P = 0.297. H-F; ability = -0.286, SE = 0.192, P = 0.136).

4-4. Discussion

Clones of *L. lugubris* differed in aggressiveness. Clone O2 was much less aggressive than Clone O1. This behavioral tendency could help to explain the

distribution patterns of these two clones in the Ogasawara Islands. Artificial habitats in urban areas consist of typically flat building walls with lights that attract insects because of their phototaxis. Therefore, insects, their main food, are abundant in a small area on the wall around the light, where competition to get these insects is severe. In this case, more aggressive individuals can eat more insects and therefore less aggressive individuals would be excluded from such an artificial area via longterm strong competition for food.

In the Ogasawara Islands, the distribution of Clone O1 was biased towards the artificial environments such as house walls, electric poles, and road guardrails, whereas all individuals of Clone O2 were found in natural area such as forests, rock crevices, grasslands, and beaches (see Table 3-1 in Chapter 3). Moreover, the detailed microdistribution study of these two clones suggested that Clone O1 tends to be distributed in the coastal regions whereas Clone O2 in the inland areas on non-human-inhabiting Mukojima, Nakoudojima, and Yomejima in the Ogasawara Islands (Murakami, 2017). Coastal regions are dominated by open habitats like the artificial walls where individuals may often compete for food. On the other hand, the inland areas are natural and covered by vegetation.

As shown in Chapter 2, Clones O1 and O2 are identical to Clones A and C, respectively, both of which are known widely in Pacific Ocean (Moritz et al., 1993; Radtkey et al., 1995; Ineich, 1999). Our results suggest that aggressiveness is one of the important processes that lead to the displacement of resident Clone C by introduced Clone A.

For Clone C of *L. lugubris*, another cosmopolitan gecko *H. frenatus* may have the same impact as conspecific Clone A. In the combinations between Clone C and *H. frenatus*, the former was much less aggressive to the latter. Aggressiveness including physical contacts may result in injury. The fact that most individuals of Clone C in the Ogasawara Islands had complete tails may be explained by less aggressiveness of this clone. Therefore, if Clone C co-exists with *H. frenatus*, the similar distribution shift would be expected as in the case of coexisting with Clone A. In the Okinawa and Yaeyama Islands, Clone A (=O1) is not distributed but *H. frenatus* is known from these Islands with Clone C (=O2) (Takada and Ohtani, 2011). At present, however, the effect of *H. frenatus* on the microhabitat shift of Clone C has not been examined.

In the Daito Islands, Japan, however, 14 clones of *L. lugubris* and *H. frenatus* cohabited two small, flattened islands (see Fig. 2-4 in Chapter 2). One of the clones is Clone C (=O2) and others (Clones D1 to D11) are endemic to these islands although the two are thought to be the widely distributed Clone B (=D12/D13). The mechanisms underlying the coexistence of so many clones remain unknown. If aggressiveness differs among clones, habitat segregation would occur as seen between Clones A (=O1) and C (=O2) in the Ogasawara Islands. This phenomenon will be examined in the future in the Daito Islands.

Table 4-1. The number of individuals with different state of tail conditions collected in the field and the mean snout to vent length (SVL) ± SE.

Species/Clone (sex)	Number of individuals with			Total	SVL
	complete tail	regenerated tail	no tail		(mm)
Lepidodactylus lugubris Clone O1 (female)	45	63	3	111	39.8 ± 0.14
Lepidodactylus lugubris Clone O2 (female)	96	4	2	102	39.7 ± 0.18
Hemidactylus frenatus (male)	35	26	6	67	53.5 ± 0.11
Hemidactylus frenatus (female)	40	23	5	68	54.0 ± 0.17

Table 4-2. The results of interactions between two individuals (players 1 and 2) in a box cage.

Combinations		Number	Number	Number of victories			Frequency of
Player 1	Player 2	of pairs	of rounds	Player 1	Player 2	No contests	contests (%)
Clone O1	Clone O1	18	54	27	18	9	83.3
Clone O1	Clone O2	30	90	15	1	74	17.8
Clone O2	Clone O2	18	54	3	2	49	9.3
H-M	Clone O1	24	72	31	17	24	66.7
H-F	Clone O1	21	63	26	29	8	87.3
H-M	Clone O2	14	42	2	1	39	7.1
H-F	Clone O2	22	66	7	0	59	10.6
H-M	H-M	9	27	14	13	0	100
H-F	H-M	11	33	16	9	8	75.8
H-F	H-F	7	21	9	10	2	90.5
Total		174	522	150	100	272	47.9

Clone O1: *Lepidodactylus lugubris* Clone O1

Clone O2: *Lepidodactylus lugubris* Clone O2

H-M: *Hemidactylus frenatus* (male)

H-F: *Hemidactylus frenatus* (female)

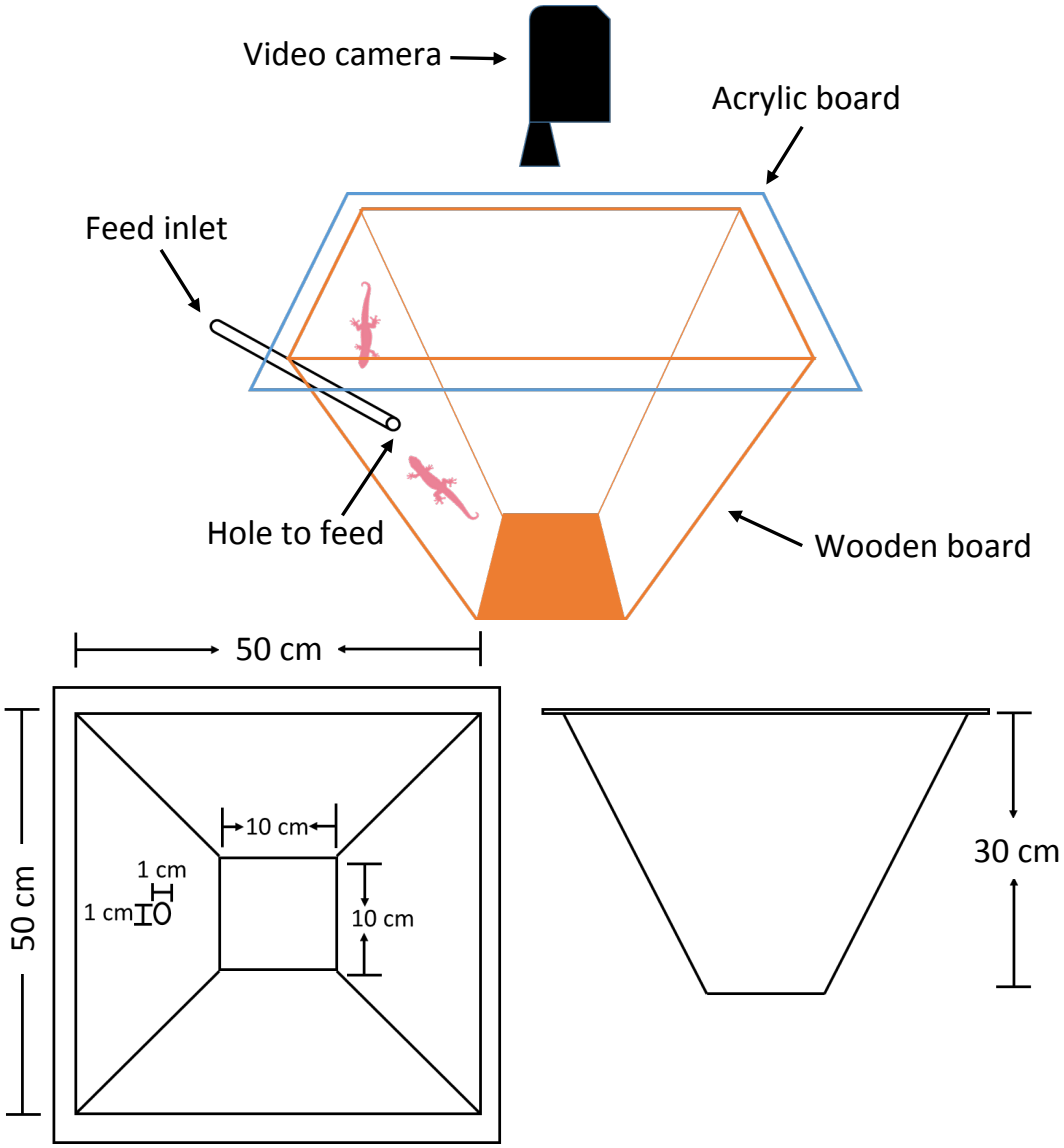

Fig. 4-1. Schematic drawings of the observation box for aggressive interactions between two individuals of Clones O1 and O2 of *Lepidodactylus lugubris* and male and female *Hemidactylus frenatus*. For the methods of observation, see the text.

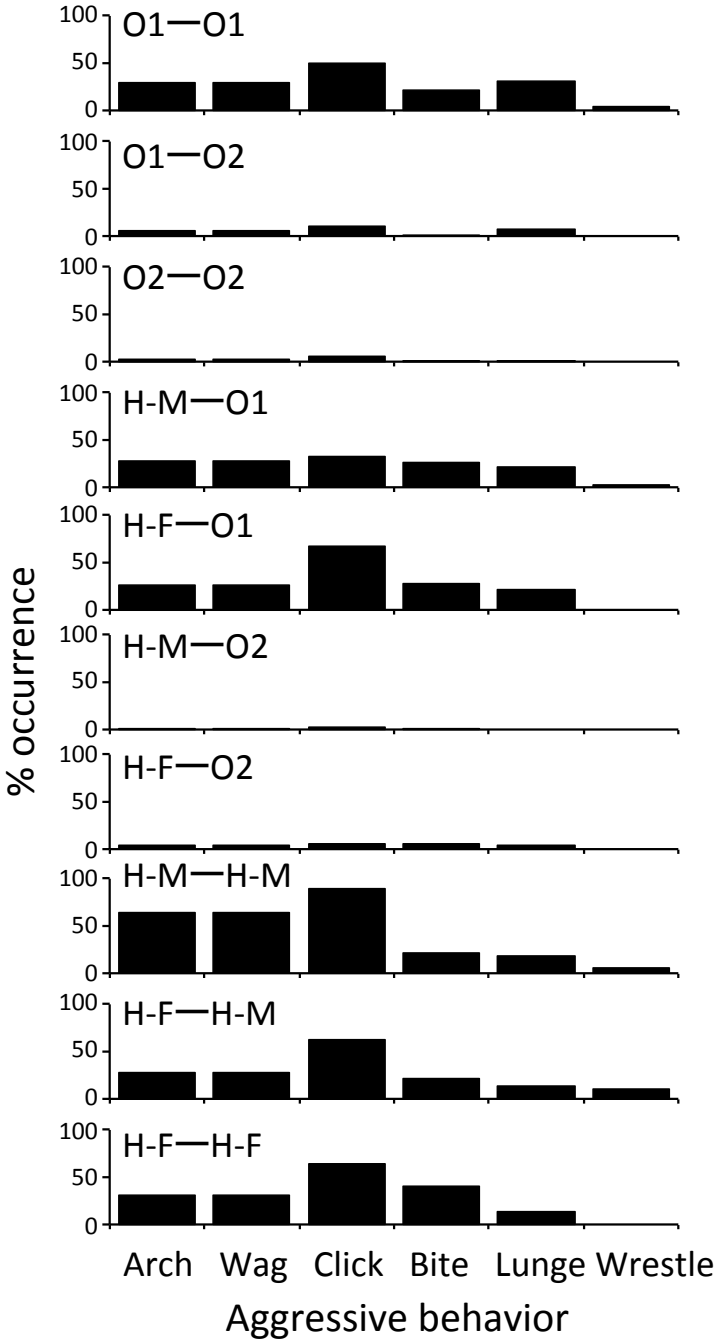

Fig. 4-2. Aggressive behaviors when two individuals of Clones O1 and O2 of *Lepidodactylus lugubris* and male and female *Hemidactylus frenatus* interact for a given prey item. Aggressive behaviors are shown in % occurrence of arches, wags, clicks, bites, lunges, or wrestles in the total number of rounds × two individuals.

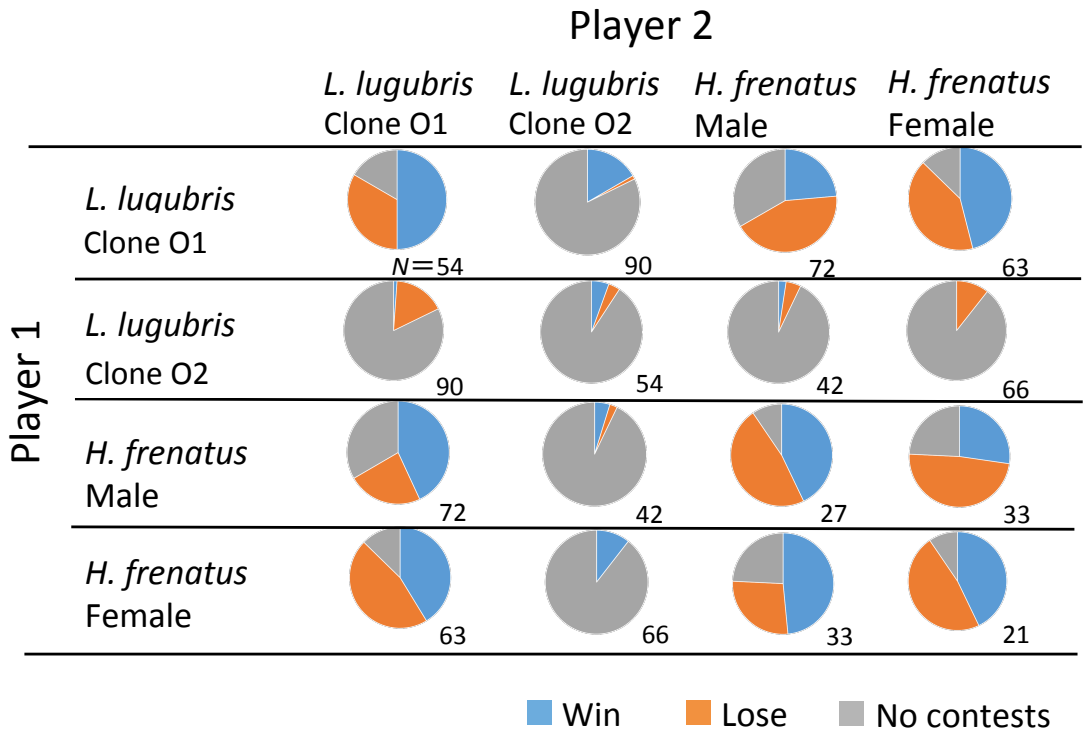

Fig. 4-3. The results of the interactions to feed prey items between two individuals of Clones O1 and O2 of *Lepidodactylus lugubris* and male and female *Hemidactylus frenatus*. If Player 1 fed a given prey item with some interactions with Player 2, we scored Player 1 wins and Player 2 loses. If players fed a given prey without any interactions between them, we scored both no contests.

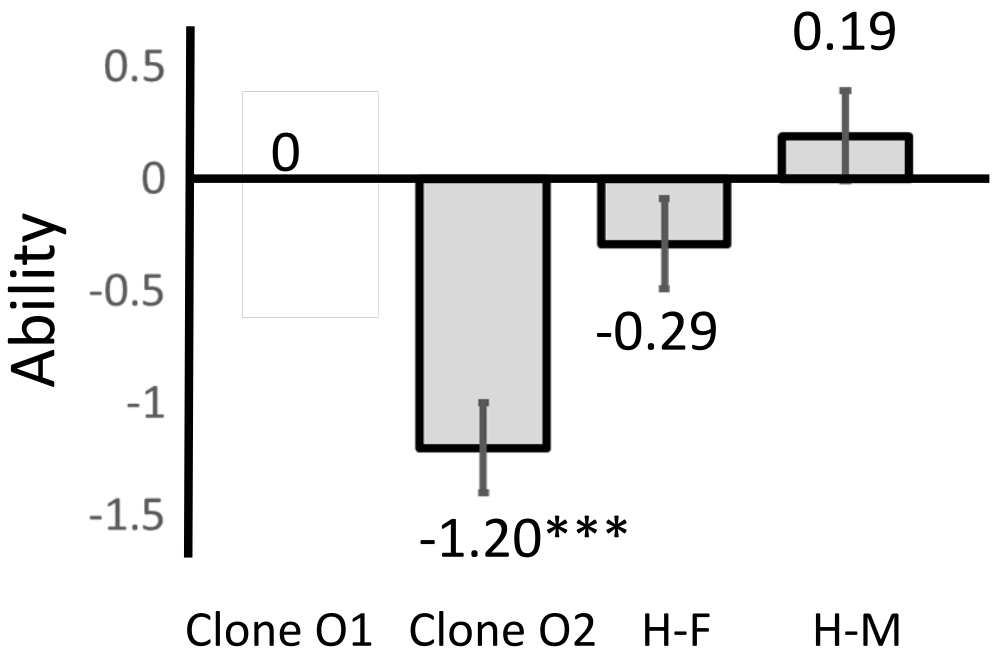

Fig. 4-4. The quantifying the fighting ability between two individuals of Clones O1 and O2 of *Lepidodactylus lugubris* and male and female *Hemidactylus frenatus*, using the Bradley-terry model that the strength of Clone O1 was set to the reference value 0. *** $P < 0.001$.

5. General discussion

5-1. Origin and genetic differentiation of clones

All-female parthenogenetic *Lepidodactylus lugubris* is considered to have the hybrid origin between congeneric closely related bisexual species, *L. moestus* and undescribed *L*. sp. (Moritz et al., 1993; Volobouev et al., 1993; Radtkey et al., 1995; Ineich, 1999; Yamashiro et al., 2000). These parental species cohabit only on Arno Atoll of Marshall Islands, and therefore this place is thought to be the origin of *L. lugubris* (Radtkey et al., 1995). If *L. lugubris* had the hybrid origin, the first parthenogenetic clone may have diploid, and the triploid clones originated through back crosses between the diploid clones and males of one of the two parental species (Moritz et al., 1993; Volobouev et al., 1993; Radtkey et al., 1995; Ineich, 1999; Yamashiro et al., 2000). Table 5-1 shows morphological characteristics and distribution areas of all known clones of *L. lugburis* and the possible parental species, *L. moestus* and undescribed *L*. sp.The main clones are Clones A, B, C, D, E, and F. Clone A is diploid and distributed widely in the Pacific Ocean areas and middle America. Clone B is triploid and distributed widely in the Pacific Ocean areas. Clone C is triploid and distributed widely in Sri Lanka, Pacific Ocean, and the middle of America. Clones D and E are little known clones distributed in the southern Pacific regions. Clone F is triploid and known only from Easter and Takapoto in the southern Pacific. Other minor will be re-examined in the future.

In our study, *L. lugubris* of Japan included 17 microsatellite genotypes. These genotypes had different dorsal marking patterns, and therefore all are thought to be different clones named as Clones O1, O2, M, T, D1 to D13. Furthermore, the ploidy estimated by the number of alleles at eight microsatellite loci was diploid in three clones O1, D1 and D2 and triploid in all other clones. In the Ogasawara, Okinawa, Miyako, and Yaeyama Islands, only one or two clones of Clones O1, O2, M, and T were found. Clone O1 was identified as Clone A known in the Pacific Ocean because of its diploidy and the dorsal marking pattern with two simple rows of V shaped markings on the dorsum. Clone O2 was identified as Clone C, also known widely as in Clone A, because it is triploid and dorsal pattern with two pairs of large dark markings at the lateral sides

of neck and waist in addition to two simple rows of V shaped markings on the dorsum. Clones M and T had a peculiar dorsal marking and at present are endemic to Miyakojima and Taketomijima, respectively. However, these Clones M and T may be colonized to each island from the outsides as in the cases of Clones O1 (=A) and O2 (=C).

However, in the Daito Islands, 14 clones were found. Such high clonal diversity was already pointed out by Yamashiro et al. (2000). The clonal diversity of *L. lugubris* was also known to be high in a particular geographic area. On Takapoto Atoll (the Tuamotu group in French Polynesia), a total of seven morphologically different clones (Clones A, B, C, D, E, F and A/B) of *L. lugubris* were distributed with a bisexual *L*. sp. which is considered to be a parent species of some clones (Ineich, 1988; Ineich and Ota, 1992; Hanley et al., 1994; Ota et al., 1995; Radtkey et al., 1996). On Arno Atoll (the Marshall Islands), three clones (Clones A, B and A/B) of *L. lugubris* are distributed with its possible parental species, *L. moestus* and *L*. sp. (Radtkey et al., 1995). Moreover, four clones of *L. lugubris* are also reported several islands: Clones A, B, C and D in the Society Islands (Pasteur, 1987; Moritz et al., 1993) and the Australs (Ineich and Ota, 1993; Moritz et al., 1993); Clones A, B, C and A/B in Hawaii (Moritz et al., 1993; Radtkey et al., 1995; Radtkey et al., 1996) and Cook Islands (Pasteur, 1987; Radtkey et al., 1995; Radtkey et al., 1996; Gill, 1998). Three clones are cohabited in the following islands: Clones A, B and C in the Marquesas Island (Pasteur, 1987; Ineich and Ota, 1993; Radtkey et al., 1996), Moorea Island (Boissinot et al., 1997; Ineich, 2015), Rangiroa Atoll (Boissinot et al., 1997), the Gambier Islands (Ineich and Ota, 1993), and Fiji (Pasteur, 1987; Moritz et al., 1993; Radtkey et al., 1996); Clones A, B and D on Maiao (Boissinot et al., 1997).

Clonal diversity was thus much higher in the Daito Islands. The two explanations may be possible for much higher clone diversity. One is that clones diversified within the islands and the other is that colonization and establishment occurred multiply from the outsides (Yamashiro et al., 2000). At least Clones O2, D12, and D13 are considered as the widely distributed clones, which may be colonized to this island group over the Pacific Ocean. Clone O2 was Clone C as shown above. Clones D12 and D13 may be Clone B distributed over the Pacific Ocean, because it is triploid and two rows of black spots exist on the mid-dorsal side of the body, although asymmetric markings differ

slightly between them. In contrast, the other 11 clones in the Daito Islands might have diversified within islands because they shared common microsatellite alleles in all examined microsatellite loci and all different dorsal marking patterns from the previously known clones. Our hypothesis is that diversification of the triploid clones may be caused by the cross of females of Clone D1 with the unusual phenotypic male(s) derived from Clone D2. The phenotypic males have been found on a few occasions in all-female parthenogenetic clones reared in the mass culture (Röll and von During, 2008) and in the field (Brown and Murphy-Walker, 1996; Yamashiro and Ota, 1998). Once such a male appeared in Clone D2 and fertile, all triploid Clones D3 to D11 with mitochondrial haplotype H1 are explained by mating by this D2 male with H2 with D1 females with H1.

5-2. Asexual and sexual reproduction and species concept

It is generally considered that parthenogenetic species avoid the two-fold cost of sexual reproduction by making no investment in males and enabling each individual in all-female species to produce offspring independently (Maynard Smith, 1978; Neaves and Baumann, 2011). This strategy enables every member of the population to colonize in a new habitat. Thus, parthenogenetic reproduction is predicted to be advantageous in increasing abundance and in dispersal. Our study of comparing distribution patterns and population genetic structure between parthenogenetic *L. lugubris* and sexual invasive species, *H. frenatus*, supported this hypothesis. In the Ogasawara Islands, successful dispersal may be more frequent in Clones O1 and O2 of *L. lugubris* than in *H. frenatus*, and therefore the asexual reproductive strategy of *L. lugubris* appears to have contributed to its dispersal success and increased abundance among the small oceanic islands.

Recent studies clarify that hybrid speciation is more common in animals than previously thought (Lutes et al., 2011). Moreover, it has been believed that hybridization combined with parthenogenesis produced parthenogenetic lizards (Radtkey et al., 1995; Neaves and Baumann, 2011). Subsequent hybridizations between diploid parthenogenetic females and males of sexual species can produce triploids (Radtkey et al., 1995). If this type of hybridization occurs, the triploid clones have all

diploid mother genome although the gene flow may not occur between the offspring clones. Such a situation was supposed in the Daito Islands in our study. Although at present it is not evident whether or not the males of Clone D2 were present in the past, all nine triploid clones endemic to this island group could be explained by the repeated hybridization between female Clone D1 and male D2 (see Fig. 2-4 in Chapter 2).

The absence of gene flow both within and between unisexual taxa has promoted debate on the taxonomic treatment of parthenogenetic species (Lutes et al., 2011). In our study, multiple clones can be discriminated morphologically and genetically in Japanese *L. lugubris*. In addition to genetic and morphological differentiation, ecological and behavioral characteristics such as aggressiveness between clones were also detected in this study. However, classifying each clone to different species based on their reproductive isolation, morphological and ecological differentiation, and unique combination of microsatellite alleles may be unrealistic because biological species concept (Mayr, 1963) is difficult to adapt to parthenogenetic species. On the other hand, based on ecological species concept (Van Valen, 1976), the clones currently being named *L. lugubris* might be divided into multiple species. At present, separating parthenogenetic *L. lugbris* into multiple species may important in contribution to conservation of rare endemic clones. Some cosmopolitan clones with a wide distribution range are colonizing to other islands and areas naturally and artificially. In this respect, these clones are the invasive alien species (Short and Petren, 2008). Some invasive clones have introduced to the Daito Islands, and therefore, if nobody takes care of conservation, various clones endemic to this island group might extinct in near future. It is a great loss to decrease clonal diversity of *L. lugubris*. Descriptions of clones as new species may be a pioneer of conserving these clones.

Table 5-1. Clones of *Lepidodactylus lugubris* (Duméril et Bibron, 1836), and bisexual *L. moestus* (Peters, 1867) and *L.* sp. (undescribed species) so far known in the world.

Species	Clone	Dorsal marking pattern	Genetic marker	Ploidy	Distribution	Reference
L. lugubris	Clone A		allozyme	2n	Marquesas, Society, Cook, Fiji	Pasteur (1987)
L. lugubris	Clone A	seven double points dispeosed on lateral medio-dorsal bands			Takapoto (French Polynesia)	Ineich (1988)
L. lugubris	Clone A	seven double points dispeosed on lateral medio-dorsal bands			Takapoto (French Polynesia)	Ineich and Ota (1992)
L. lugubris	Clone A		allozyme, mtDNA	2n	Belau, Rota, Solomon, Vanuatu, Fiji, Samoa, Cook, Tuamotu, Society, Hawaii	Moritz et al. (1993)
L. lugubris	Clone A	seven double points dispeosed on lateral medio-dorsal bands			Society, Tuamotu, Gambier, Australs, Marquesas	Ineich and Ota (1993)
L. lugubris	Clone A	seven double points dispeosed on lateral medio-dorsal bands			Pitcairn Islands	Gill (1993)
L. lugubris	Clone A		mtDNA	2n	Takapoto (French Polynesia)	Hanley et al. (1994)
L. lugubris	Clone A		mtDNA (cyt *b*)		Takapoto (French Polynesia)	Radtkey et al. (1995)
L. lugubris	Clone A	seven double points dispeosed on lateral medio-dorsal bands			Arno, Takapoto	Hanley et al. (1995)
L. lugubris	Clone A		mtDNA (cyt *b* fragment)		Cook, Hawaii, Vanuatu, Fiji	Radtkey et al. (1996)
L. lugubris	Clone A		allozyme		Maiao, Moorea, Rangiroa, Takapoto	Boissinot et al. (1997)
L. lugubris	Clone A	underside of the tail having dark markings			Cook Islands	Gill (1998)
L. lugubris	Clone A	seven or eight pairs of small V-shaped markings along the middorsal line only	allozyme	2n	Ogasawara (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone A	double points dispeosed on lateral medio-dorsal bands		2n	Ogasawara (Jaapn)	Yamashiro and Ota (2005)
L. lugubris	Clone A				Galápagos*	Torres-Carvajal and Tapia (2016)
L. lugubris	Clone A	seven pairs of small dark spots straddling the dorsal midline		2n	Kosrae (Micronesia)	Buden et al. (2014)
L. lugubris	Clone A	double points dispeosed on lateral medio-dorsal bands		2n	Bahama	Krysko and Krysko (2016)
L. lugubris	Clone A				Colombia*	Vanegas-Guerrero et al. (2016)
L. lugubris	Clone A	pattern of spots that form part of a series of light brown W-shaped chevrons		2n	Cuba	Bosch and Páez (2017)
L. lugubris	Clone A			2n	Venezuela	Señaris et al. (2017)

L. lugubris	Clone A/B				Takapoto (French Polynesia)	Hanley et al. (1994)
L. lugubris	Clone A/B	similar to back pattern of Clone A, but with larger paired spots over the thorax and pelvis.			Arno, Takapoto	Hanley et al. (1995)
L. lugubris	Clone A/B1		mtDNA (cyt *b*)		Takapoto, Belau, Guam, Hawaii, Rota	Radtkey et al. (1995)
L. lugubris	Clone A/B2		mtDNA (cyt *b*)		Cook Islands	Radtkey et al. (1995)
L. lugubris	Clone B		allozyme	3n	Marquesas, Society, Cook, Fiji	Pasteur (1987)
L. lugubris	Clone B	fixed black dorsal marks			Takapoto (French Polynesia)	Ineich (1988)
L. lugubris	Clone B	lacking the regular aired mid dorsal spots			Takapoto (French Polynesia)	Ineich and Ota (1992)
L. lugubris	Clone B		allozyme, mtDNA	3n	Fiji, Samoa, Cook, Tuamoto, Society, North Australia	Moritz et al. (1993)
L. lugubris	Clone B	fixed black dorsal marks			Society, Tuamotu, Gambier, Australs, Marquesas	Ineich and Ota (1993)
L. lugubris	Clone B	lateral black bars on the neck and base of tail			Pitcairn Islands	Gill (1993)
L. lugubris	Clone B				Takapoto (French Polynesia)	Hanley et al. (1994)
L. lugubris	Clone B		mtDNA (cyt *b*)		Arno, Hawaii, Fiji, Takapoto	Radtkey et al. (1995)
L. lugubris	Clone B		mtDNA (cyt *b* fragment)		Marquesas	Radtkey et al. (1996)
L. lugubris	Clone B		allozyme		Maiao, Moorea, Rangiroa, Takapoto	Boissinot et al. (1997)
L. lugubris	Clone B	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	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone B'	similar to Clone B but with less prominent dark spots	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-1	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-2	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-3	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-4	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-5	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-6	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-7	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone Bl-8	similar to Clone B	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)

L. lugubris	Clone C		allozyme	3n	Marquesas, Society, Cook, Fiji	Pasteur (1987)
L. lugubris	Clone C	dorsolateral black bars on the neck and base of tail, and indistinct W-shaped marks along the middorsal line			Takapoto (French Polynesia)	Ineich and Ota (1992)
L. lugubris	Clone C	dorsolateral black bars on the neck and base of tail, and indistinct W-shaped marks along the middorsal line			Society, Tuamotu, Gambier, Australs, Marquesas	Ineich and Ota (1993)
L. lugubris	Clone C	a pair of thin longitudinal stripes in the neck region			Pitcairn Islands	Gill (1993)
L. lugubris	Clone C		mtDNA	3n	Takapoto (French Polynesia)	Hanley et al. (1994)
L. lugubris	Clone C		mtDNA (cyt *b*)		Fiji	Radtkey et al. (1995)
L. lugubris	Clone C		mtDNA (cyt *b* fragment)		Fiji, Cook, Hawaii	Radtkey et al. (1996)
L. lugubris	Clone C		allozyme		Moorea, Rangiroa, Takapoto	Boissinot et al. (1997)
L. lugubris	Clone C	dorsolateral black bars on the neck and base of tail, and indistinct W-shaped marks along the middorsal line	allozyme	3n	Okinawa, Miyako and Yaeyama Islands (Japan)	Yamashiro et al. (2000)
L. lugubris	Clone C	dorsolateral black bars on the neck and base of tail		3n	Ogasawara (Japan)	Yamashiro and Ota (2005)
L. lugubris	Clone C				Colombia*	Daza et al. (2012)
L. lugubris	Clone C	the typical lateral fine stripes in the neck area.			Moorea Island (French Polynesia)	Ineich (2015)
L. lugubris	Clone C				Sri Lanka*	Karunarathna et al. (2015)
L. lugubris	Clone C	dorsolateral black bars on the neck and base of tail, and indistinct W-shaped marks along the middorsal line		3n	Okinawajima (Japan)	Sakai (2016)
L. lugubris	Clone C	dorsolateral black bars on the neck and base of tail, and indistinct W-shaped marks along the middorsal line			Takapoto (French Polynesia)	Ineich (1988)
L. lugubris	Clone D	less than seven spots restricted to the anterior medio-dorsal band			Takapoto (French Polynesia)	Ineich (1988)
L. lugubris	Clone D	less than seven spots restricted to the anterior medio-dorsal band			Society, Australs	Ineich and Ota (1993)
L. lugubris	Clone D				Takapoto (French Polynesia)	Hanley et al. (1994)
L. lugubris	Clone D		allozyme		Maiao	Boissinot et al. (1997)
L. lugubris	Clone Da	chevrons and short bars alternating with each other along the middorsal line, and a relatively distinct W-shaped mark on the head	allozyme	2n	Daito Islands (Japan)	Yamashiro et al. (2000)

L. lugubris	Clone E	no fixed black spots or stripes and only the basic labile pattern is descernable			Takapoto (French Polynesia)	Ineich (1988)
L. lugubris	Clone E	no fixed black spots or stripes and only the basic labile pattern is descernable			Takapoto (French Polynesia)	Ineich and Ota (1992)
L. lugubris	Clone E				Takapoto (French Polynesia)	Hanleyet al. (1994)
L. lugubris	Clone E		mtDNA (cyt *b*)		New Guinea	Radtkey et al. (1995)
L. lugubris	Clone F	prominent dark spots in the posterior part of the body and slightly greater count in the number of scansors under the fourth toe			Easter	Ineich and Ota (1993)
L. lugubris	Clone F		mtDNA	3n	Takapoto (French Polynesia)	Hanley et al. (1994)
L. lugubris	Clone N	only lateral black bars on the neck and base of tail	allozyme	3n	Daito Islands (Japan)	Yamashiro et al. (2000)
L. moestus	bisexual	pale yellowish gray, usually without markings, but occasionally with longitudinal rows of dark spots in the dorsolateral region, or with mid-dorsal row of dark spots			Arno Atoll, Kosrae, Pohnpei, Parau, Belau	Ota et al. (1995)
L. moestus	bisexual				Arno Atoll, Kosrae, Kiribati, Pohnpei, Yap, Carolin	Radtkey et al. (1995)
L. moestus	bisexual				Arno Atoll	Radtkey et al. (1996)
L. moestus	bisexual				Palau	Crombie and Pregill (1999)
L. moestus	bisexual				Pohnpei	Buden (2000)
L. moestus	bisexual	brown or pale yellowish brown (honey-colored) unpatterned dorsa			Caroline	Buden (2015b)
L. moestus	bisexual				Kosrae (Micronesia)	Buden et al. (2014)
L. sp.	bisexual	silver-grey dorsal slin color and less regular markings			Takapoto	Hanley et al. (1994)
L. sp.	bisexual				Arno Atoll, Kiribati, Cook, Society	Radtkey et al. (1995)
L. sp.	bisexual				Arno Atoll, Takapoto	Radtkey et al. (1996)

* judged from color photographs.

6. Acknowledgements

We thank Fumio Hayashi for useful advise throughout this study, Tamotsu Kusano for his suggestion in statistical tests, Hirotaka Sugawara for his help in molecular analyses, Hiroo Takahashi for providing useful material in Ogasawara, Naoki Kachi and Aya Takahashi for their improvement of the early version of the manuscript, Hidetoshi Ota and Mamoru Toda for providing relevant information and literature on *Lepidodactylus lugubris*, and Hidetoshi Kato, Takeshi Osawa, Syuntaro Hiradate, Kosei Hashimoto, Suguru Wakikaido, Ai Takaoka, Akinobu Imamura, Masahide Kubota, and Hisanori Okamiya for their help with the field work. Samples were collected from protected areas with permission of the Ministry of the Environment, Japan. All procedures of rearing and experiments of geckos were made after approved by the Tokyo Metropolitan University Animal Ethics Committee (A28-26 in 2016, A29-22 in 2017). This study was partially supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (JP16J05260).

7. References

Amarasinghe AAT, Bauer AM, Ineich I, Rudge J, Bahir MM, Gabadage DE (2009) The original descriptions and figures of Sri Lankan gekkonid lizards (Squamata: Gekkonidae) of the 18th, 19th and 20th centuries. Taprobanica 1: 83–106.

Barnett LK, Emms C (1997) Herpetological observations in the Chagos Archipelago, British Indian Ocean Territory. British Herpetological Society Bulletin 59: 6–12.

Bauer AM, Jackman TR, Greenbaum E, Papenfuss TJ (2007) First record of *Lepidodactylus lugubris* in Suriname. Applied Herpetology 4: 84–85.

Bauer AM, Sadlier RA (1994) The terrestrial herpetofauna of the Ile des Pins, New Caledonia. Pacific Science 48: 353–366.

Bauer AM, Sadlier RA (2000) The herpetofauna of New Caledonia. Contributions to Herpetology, 17; Society for Study Amphibians and Reptiles, Ithaca, New York, 358 pp.

Bauer AM, Vindum JV (1990) A checklist and key to the herpetofauna of New Caledonia, with remarks on biogeography. Proceedings of the California Academy of Sciences 47: 17–45.

Beukeboom LW, Vrijenhoek RC (1998) Evolutionary genetics and ecology of sperm-dependent parthenogenesis. Journal of Evolutionary Biology 11: 755–782.

Bleeker P (1859) Reptiliën en visschen van Bintang, aangeboden door E. Netscher, e.f. Meijer en G. Raat. Natuurkundig Tijdschrift voor Nederlandsch Indie, 16: 45–47.

Bogart JP, Bi K, Fu J, Noble DWA, Niedzwieki J (2007) Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. Genome 50: 119–136.

Boissinot S, Ineich I, Thaler L, Guillaume CP (1997) Hybrid origin and clonal diversity in the parthenogenetic gecko, *Lepidodactylus lugubris* in French Polynesia. Journal of Herpetology 31: 295–298.

Bolger DT, Case TJ (1994) Divergent ecology of sympatric clones of the asexual gecko, *Lepidodactylus lugubris*. Oecologia 100: 397–405.

Bosch RA and Páez RB (2017) First record from Cuba of the introduced mourning gecko, *Lepidodactylus lugubris* (Duméril et Bibron, 1836) BioInvasions Records 6: 297–300.

Boulenger GA (1885) Catalogue of the Lizards in the British Museum (Nat. Hist.) I. Geckonidae, Eublepharidae, Uroplatidae, Pygopodidae, Agamidae. London: 450 pp.

Boulenger GA (1887) Catalogue of the Lizards in the British Museum (Nat. Hist.) III. Lacertidae, Gerrhosauridae, Scincidae, Anelytropsidae, Dibamidae, Chamaeleontidae. London: 575 pp.

Bradley RA, Terry ME (1952) Rank analysis of incomplete block designs 1: The method of paired comparisons. Biometrika 39: 324–345.

Brongersma LD (1948) Lizards from the island of Morotai (Moluccas). Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen Series C 51: 486–495.

Brown R, Cameron S, Carl O, Luke W, Ashley R, John S, Merlijn VW, Jonah VB, Dominic R, Edmund J, Arvin D (2013) The amphibians and reptiles of Luzon Island, Philippines, VIII: the herpetofauna of Cagayan and Isabela Provinces, northern Sierra Madre Mou ntain Range. ZooKeys 266 (Special Issue): 1–120.

Brown SG, Duffy PK (1992) The effects of egg-laying site, temperature, and salt-water on incubation time and hatching success in the gecko *Lepidodactylus lugubris*. Journal of Herpetology 26: 510–513.

Brown SG, Murphy-Walker S (1996) Behavioural interactions between a rare male phenotype and female unisexual *Lepidodactylus lugubris*. Herpetological Journal 6: 69–73.

Brown WC, Parker F (1977) Lizards of the genus *Lepidodactylus* (Gekkonidae) from the Indo-Australian Archipelago and the islands of the Pacific, with description of new species. Proceedings of the California Academy of Sciences 41: 253–265.

Buden DW (2000) The Reptiles of Pohnpei, Federated States of Micronesia. Micronesica 32: 155–180.

Buden DW (2008) The reptiles of Nauru. Pacific Science 62: 499–507.

Buden DW (2015a) Reptiles of Lukunor Atoll, Mortlock Islands, Chuuk State, Federated States of Micronesia. Pacific Science 69: 117–124.

Buden DW (2015b) Reptiles of Uman District Islands (Southeastern Chuuk Lagoon and Kuop Atoll), Federated States of Micronesia. Pacific Science 69: 271–279.

Buden DW, Cianchini C, Taborosi D, Fisher RN, Bauer AM, Ineich I (2014)

Photographic evidence of interspecies mating in geckos of the *Lepidodactylus lugubris* unisexual-bisexual complex (Squamata: Gekkonidae). Phyllomedusa 13: 133–136.

Buden DW, Taboroši D (2016) Reptiles of the Federated States of Micronesia. Island Research and Education Initiative, University of Hawaii Press, Honolulu. 359 pp.

Cagle FR (1946) A lizard population on Tinian. Copeia 1946: 4–9.

Case TJ, Bolger DT (1991) The role of introduced species in shaping the distribution and abundance of island reptiles. Evolutionary Ecology 5: 272–290.

Castro-Herrera F, Anyelet VA, Diego VM (2012) Diversidad de Anfibios y Reptiles del Parque Nacional Natural Isla Gorgona Universidad del Valle, Santiago de Cali, Valle del Cauca, 112 pp.

Castro-Herrera F, Vargas-Salinas F (2008) Anfibios y reptiles en el departamento del Valle del Cauca, Colombia. Biota Colombiana 9: 251–277.

Chapman DD, Shivji MS, Louis E, Sommer J, Fletcher H, Prodöhl PA (2007) "Virgin birth in a hammerhead shark". Biology Letters 3: 425–427.

Chandramouli SR (2015) Neue Nachweise nicht einheimischer Geckos (Sauria: Gekkonidae) aus städtischen Bereichen auf den Andamanen, Indien Sauria 37: 16–22.

Cheng HY (1987) The record of a gekkonid lizard *Lepidodactylus lugubris* (Duméril and Bibron, 1836) from Taiwan. Journal of Taiwan Museum 40: 85–89.

Cogger HG (2014) Reptiles and Amphibians of Australia, 7th ed. CSIRO Publishing, Melbourne, 1033 pp.

Cogger HG (2000) Reptiles and Amphibians of Australia, 6th ed. Ralph Curtis Publishing, Sanibel Island, 808 pp.

Cole CJ (1975) Evolution of parthenogenetic species in reptiles. Intersexuality in the Animal Kingdom, Springer-Verlag, Berlin pp. 340–355.

Cole CJ (1984) Unisexual lizards. Scientific American 250: 94–100.

Cooper JWE (2005) Duration of movement as a lizard foraging movement variable. Herpetologica 61: 363–372.

Crombie RI, Pregill GK (1999) A checklist of the herpetofauna of the Palau Islands (Republic of Belau), Oceania. Herpetological Monographs 13: 29–80.

Cuellar O (1976) Intraclonal histocompatibility in a parthenogenetic lizard: evidence of

genetic homogeneity. Science 193: 150–153.

Cuellar O (1984) Histocompatibility in Hawaiian and Polynesian populations of the parthenogenetic gecko *Lepidodactylus lugubris*. Evolution 38: 176–185.

Cuéllar O, Kluge AG (1972) Natural parthenogenesis in the gekkonid lizard *Lepidodactylus lugubris*. Journal of Genetics 61: 14–26.

Daan S, Hillenius D (1966) Catalogue of the type specimens of amphibians and reptiles in the Zoological Museum, Amsterdam. Beaufortia 13: 117–144.

Dame EA, Petren K (2006) Behavioural mechanisms of invasion and displacement in Pacific island geckos (*Hemidactylus*). Animal Behaviour 71: 1165–1173.

Das I (2004) Lizards of Borneo. Natural History Publications, Kota Kinabalu, Borneo.

Das I (1999) Biogeography of the amphibians and reptiles of the Andaman and Nicobar Islands, India. In: Ota,H. (ed) Tropical Island herpetofauna, Elsevier, pp. 43–77.

Daza JD, Travers SL, Bauer AM (2012) New records of the mourning gecko *Lepidodactylus lugubris* (Duméril and Bibron, 1836) (Squamata: Gekkonidae) from Colombia. Journal of Species Lists and Distribution 8: 164–167.

Demangel D (2016) Reptiles en Chile. Fauna Nativa Ediciones, Santiago, 619 pp.

Deraniyagala PEP (1929) A gecko hitherto unrecorded from Ceylon. Ceylon Journal of Science 16: 157–158.

Duméril AMC, Bibron G (1836) Erpetologie Générale ou Histoire Naturelle Complete des Reptiles. Vol. 3. Libr. Encyclopédique Roret, Paris, 528 pp.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

Excoffier L, Lisher HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analysis under Linux and Windows. Molecular Ecology Resources 10: 564–567.

Ferguson W (1877) Reptile fauna of Ceylon. Letter on a Collection Sent to the Colombo Museum, Ceylon, pp. 1-42.

Ferner JW, Brown RM, Sison RV, Kennedy RS (2000) The amphibians and reptiles of Panay Island, Philippines. Asiatic Herpetological Research 9: 1–37.

Firth D (2005) Bradley-Terry models in R. Journal of Statistical software 12: 1–12.

Fisher I (1930) The Theory of Interest. Macmillan Press, New York pp. 1-35.

Fitzinger LJ (1861) Die Ausbeute der österreichischen Naturforscher an Säugethieren und Reptilien während der Weltumsegelung Sr. Majestät Fregatte Novara. Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Klasse der Kaiserlichen Akademie der Wissenschaften, Wien 42: 383–416.

Fujita MK, Moritz C (2009) Origin and evolution of parthenogenetic genomes in lizards: current state and future directions. Cytogenet Genome Research 127: 261–272.

Gamble T (2010) A review of sex determining mechanisms in geckos (Gekkota: Squamata). Sexual Development 4: 88–103.

Gardner AS (1985) An identification key to the geckos of the Seychelles, with brief notes on their distributions and habits. The Herpetological Journal 1: 17–19.

Garman S (1908) The reptiles of Easter Island. Bulletin of the Museum of Comparative Zoology at Harvard College 52: 3–13.

Gaulke M (2011) The herpetofauna of Panay Island, Philippines. Edition Chimaira, 390 pp.

Gibson HCA (1950) A note on the reptiles occurring on the Cocos-Keeling Islands. Bulletin of the Raffles Museum 22: 206–211.

Gill BJ (1993) The land reptiles of western Samoa. Journal of the Royal Society of New Zealand 23: 79–89.

Gill BJ (1998) Records of Cook Islands Lizard. Tubinga 10: 151-157.

Girard C (1858) Descriptions of some new reptiles, collected by the US. Exploring Expedition under the command of Capt. Charles Wilkes, U.S.N. Third Part. Proceedings of the Academy of Natural Sciences of Philadelphia 9: 181–182.

Gomès R, Ibéné B (2013) *Lepidodactylus lugubris* (mourning gecko). Distribution. Caribbean Herpetology, 44, 1.

Goris RC, Maeda N (2004) Guide to the Amphibians and Reptiles of Japan. Krieger, Malabar, 285 pp.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. Journal of Heredity 86: 485–486.

Grismer LL (2011a) Amphibians and reptiles of the Seribuat Archipelago. Edition Chimaira, Frankfurt, pp. 239.

Grismer LL (2011b) Lizards of Peninsular Malaysia, Singapore and their adjacent

archipelagos. Edition Chimaira, Frankfurt, 728 pp.

Grismer LL, McGuire JA, Sosa R, Kaiser H (2002) Revised checklist and comments on the terrestrial herpetofauna of Pulau Tioman, Peninsular Malaysia. Herpetological Review 33: 26–29.

Hanley KA, Bolger DT, Case TJ (1994) Comparative ecology of sexual and asexual gecko species (*Lepidodactylus*) in French Polynesia. Evolutionary Ecololgy 8: 438–454.

Hanley KA, Fisher RN, Case TJ (1995) Lower mite infestations in an asexual gecko compared with its sexual ancestors. Evolution 46: 418-426.

Hayashi F, Shima A, Horikoshi K, Kawakami K, Segawa RD, Aotsuka T, Suzuki T (2009) Limited overwater dispersal and genetic differentiation of the snake-eyed skink (*Cryptoblepharus nigropunctatus*) in the oceanic Ogasawara Islands, Japan. Zoological Science 26: 543–549.

Henderson CL (2010) Mammals, Amphibians, and Reptiles of Costa Rica - A field guide. University of Texas Press, Austin, 198 pp.

Henderson RW, Villa J, Dixon JR (1976) *Lepidodactylus lugubris* (Reptilia: Gekkonidae). A recent addition to the herpetofauna of Nicaragua. Herpetological Review 7: 173.

Henle K (1990) Beobachtungen an Reptilien auf den Gesellschaftsinseln. Salamandra 26: 45–49.

Hoogmoode MS, Teresa CS, Avilla P (2015) *Lepidodactylus lugubris* (Duméril & Bibron 1836) (Reptilia: Gekkonidae), an introduced lizard new for Brazil, with remarks on and correction of its distribution in the New World. Zootaxa 4000: 90–110.

Horikoshi K (2008) Lizards in Minami-Iwo-To Island. Ogasawara Research 33:129–134. (in Japanese)

Huang H, Zhou K, Feng J (2009) Cryptic species diversity in *Hemiphyllodactylus yunnanensis*. Unpublished in GenBank.

Hubisz MJ, Falush D, Stephens M, Prichard JK (2009) Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9: 1322–1332.

Ineich I (1988) Mise en évidence d'un complexe unisexué-bisexué chez le Gecko

Lepidodactylus lugubris (Sauria, Lacertilia) en Polynésie française. Comptes Rendus de l'Académie des Sciences, Série III, 307: 271–277.

Ineich I (1999) Spatio-temporal analysis of the unisexual-bisexual *Lepidodactylus lugubris* complex (Reptilia, Gekkonidae). In: Ota H (ed) Tropical island herpetofauna: origin, current diversity and conservation. Elsevier, pp. 199–228.

Ineich I (2009) The terrestrial herpetofauna of Torres and Banks Groups (northern Vanuatu), with report of a new species for Vanuatu. Zootaxa 2198: 1–15.

Ineich I (2011) Amphibians and reptiles. In: Bouchet P, Le Guyader H, PascaL O (eds), The Natural History of Santo, Paris, pp. 187–236.

Ineich I (2015) New data about the triploid clone C of the unisexual-bisexual *Lepidodactylus lugubris* (Duméril & Bibron, 1836) complex (Gekkonidae) Herpetology Notes 8: 165–168.

Ineich I, Gardner AS (1989) Qualitative analysis of the development of endolymphatic sacs by a Gecko (*Lepidodactylus lugubris*) in French Polynesia. Journal of Herpetology 23: 414-418.

Ineich I, Ota H (1992) Additional remarks on the unisexual-bisexual complex of the gecko, *Lepidodactylus lugubris*, in Takapoto Atoll, French Polynesia. Bulletin of the College of Science, University of the Ryukyus 53: 31–39.

Ineich I, Ota H (1993) Morphological variation and distribution of the unisexual-bisexual complex of the gecko, *Lepidodactylus lugubris*, in French Polynesia and Easter Island. Bulletin of the College of Science, University of the Ryukyus 56: 113–120.

Irschick DJ, Austin CC, Petren K, Fisher RN, Losos JB, Ellers O (1996) A comparative analysis of clinging ability among pad-bearing lizards. Biological Journal of the Linnean Society 59: 21–35.

Jablonski DDH, Ángel SB (2016) Distribution Notes. *Lepidodactylus lugubris* (Duméril & Bibron, 1836). Mesoamerican Herpetology 3: 181–182.

Jarecki L, Lazell JD (1987) Zur Grösse und Dichte einer Population von *Lepidodactylus lugubris* (DUMÉRIL & BIBRON 1836) in Aiea, Hawaii (Sauria: Gekkonidae). Salamandra 23: 176–178.

Jayaneththi HB (2015) Vertebrate fauna of Morankanda-Mukalana secondary forest patch in Sri Lanka: A checklist reported from 2004-2008 survey. Ruhuna Journal

of Science 6: 21–41.

Jayaneththi HB, Madurapperuma PL, Velauthem AV, Balasuriya NH, Jablonski D (2015) Bewertung der Verbreitung des Schuppenfingergeckos *Lepidodactylus lugubris* (Dumeril & Bibron, 1836) in Sri Lanka. Sauria 37: 17–26.

Jiménez RR, Abarca JG (2015) Records for the Mourning Gecko (*Lepidodactylus lugubris*) and its expansion in Costa Rica. Mesoamerican Herpetology 2: 214–217.

Karunarathna1 DMSS, Surasinghe TD, Madawala1 MB, Wellappulli-Arachchi SM, Kandambi HKD, Kusuminda TGT, Priyadarshana TMTS and Perera BNH (2015) Distribution, natural history and the conservation status of Hemiphyllodactylus typus and *Lepidodactylus lugubris* (Reptilia: Gekkonidae) in Sri Lanka. Sri Lanka Naturalist 8: 1-2.

Kearney M, Blackett MJ, Strasburg JL, Moritz C (2006) Waves of parthenogenesis in the desert: evidence for the parallel loss of sex in a grasshopper and a gecko from Australia. Molecular Ecology 15: 1743–1748.

Kikukawa A (1999) Geographic distribution. *Lepidodactylus lugubris*. Herpetological Review 30: 52.

Kluge AG, Eckardt MJ (1969) *Hemidactylus garnotii* Duméril and Bibron, a triploid all-female species of gekkonid lizard. Copeia 1969: 651–664.

Koch A (2012) Discovery, diversity, and distribution of the amphibians and reptiles of Sulawesi and its offshore islands. Edition Chimaira, Frankfurt, 374 pp.

Köhler G (2008) Reptiles of Central America. 2nd ed. Herpeton-Verlag, Offenbach, 400 pp.

Krysko KL, MacKenzie-Krysko C (2016) First report of the mourning gecko, *Lepidodactylus lugubris* (Duméril & Bibron 1836), from The Bahamas. Caribbean Herpetology, 54, 1–2.

Li J, Zhou K (2007) Isolation and characterization of microsatellite markers in the gecko *Gekko swinhonis* and cross-species amplification in other gekkonid species. Molecular Ecology Notes 7: 674–677.

Lorvelec O, Barré N, Bauer AM (2017) The status of the introduced Mourning Gecko (*Lepidodactylus lugubris*) in Guadeloupe (French Antilles) and the high probability of introduction of other species with the same pattern of distribution.

Caribbean Herpetology 57: 1–7.

Lorvelec O, Levesque A, Bauer AM (2011) First record of the mourning gecko (*Lepidodactylus lugubris*) on Guadeloupe, French West Indies. Herpetology Notes 4: 291–294.

Lutes AA, Baumann DP, Neaves WB, Baumann P (2011) Laboratory synthesis of an independently reproducing vertebrate species. Proceedings of the National Academy of Sciences 24: 9910–9915.

MacCulloch RD, Robert W, Murphy LW, Kupriyanova IA, Darevsky S (1997) The Caucasian rock lizard Lacerta rostombekovi: a monoclonal parthenogenetic vertebrate. Biochemical Systematics and Ecology 25: 33-37.

Macleay W (1877) The lizards of the Chevert Expedition. Proceedings of the Linnean Society of New South Wales, 2: 60-69, 97-104.

Manthey U, Grossmann W (1997) Amphibien and Reptilien Südostasiens. Natur und Tier Verlag (Münster), Nordrhein-Westfalen, 512 pp.

Mau KG (1978) Nachweis natürlicher Parthenogenese bei *Lepidodactylus lugubris* durch Gefangenschaftsnachzucht (Reptilia: Sauria: Gekkonidae). Salamandra 14: 90–97.

Maynard Smith J (1978) The evolution of sex. Cambridge University Press, Cambridge, 236 pp.

Mayr E (1963) Animal Species and Evolution. Belknap Press, Cambridge, 797 pp.

McCoy M (2006) Reptiles of the Solomon Islands. Pensoft Series Faunistica 57, Sophia, 212 pp.

McCoy M (2015) A Field Guide to the Reptiles of the Solomon Islands, Kuranda, 137 pp.

McCullagh P (1984) On the elimination of nuisance parameters in the proportional odds model. Journal of the Royal Statistical Society: Series B 46: 250-256.

Mckeown S (1996) A Field Guide to Reptiles and Amphibians in the Hawaiian Islands. Diamond Head Publishing, Inc., Los Osos, CA, 172 pp.

Mcleod DS, Silar CD, Diesmos AC, Diesmos ML, Garcia VS, Arkonceo AO, Balaquit KL, Charlene CUY, Villaseran MM, Yarra EC, Brown RM (2011) Amphibians and reptiles of Luzon Island, V: The herpetofauna of Angat Dam Watershed, Bulacan Province, Luzon Island, Philippines. Asian Herpetological Research 2:

177–198.

Mertens R (1929) Zwei neue Haftzeher aus dem Indo-Australischen Archipel (Rept.). Senckenbergiana, 11: 237–241.

Mitchell JC (1979) Ecology of southeastern Arizona whiptail lizards (*Cnemidophorus*: Teiidae): population densities, resource partitioning, and niche overlap. Canadian Journal of Zoology 57: 147–149.

Moravec J, Kratochvil L, Amr ZS, Jandzik D, Smid J, Gvozdik V (2011) High genetic differentiation within the *Hemidactylus turcicus* complex (Reptilia: Gekkonidae) in the Levant, with comments on the phylogeny and systematics of the genus. Zootaxa 2894: 21–38.

Moritz C, Case TJ, Bolger DT, Donnellan S (1993) Genetic diversity and the history of pacific island house geckos (*Hemidactylus* and *Lepidodactylus*). Biological Journal of the Linnean Society 48: 113–133.

Moritz C, King D (1985) Cytogenetic perspectives on parthenogenesis in the Gekkonidae. In "Biology of Australasian frogs and reptiles" Ed by G Grigg, R Shine, H Ehmann, Royal Zoological Society of New South Wales, Sydney, pp. 327–337.

Morrison C (2003) A Field Guide to the Herpetofauna of Fiji. Suva, Fiji: Institute of Applied Sciences, University of the South Pacific, pp. 121.

Müller F (1895) Reptilien und Amphibien aus Celebes. Verhandlungen der Naturforschenden Gesellschaft in Basel 10: 862–869.

Müller HJ (1932) Some genetic aspects of sex. American Natural 66: 118–138.

Murakami Y (2017) Clonal composition and distribution pattern of *Lepidodactylus lugubris* in the Mukojima Group of the Ogasawara Islands. Ogasawara Kenkyu Nenpou 40: 53-58. (in Japanese)

Nafus MG (2012) Natural history notes: *Lepidodatylus lugbris*. Foraging movement. Herpetological Review 43: 135.

Neaves WB, Baumann P (2011) Unisexual reproduction among vertebrates. Trends in Genetics 27: 81–88.

Newbery B, Jones DN (2007) Presence of Asian house gecko *Hemidactylus frenatus* across an urban gradient in Brisbane: influence of habitat and potential for impact on native gecko species. In: Lunney D, Eby P, Hutchings P, Burgin S (eds) Pest or

guest: the zoology of overabundance. Royal Zoological Society of New South Wales, Mosman, pp. 59–65.

Niewiarowski PH, McClung ASB, Chambers B, Sullivan T (2012) Faster but not stickier: invasive house geckos can out-sprint resident mournful geckos in Moorea, French Polynesia. Journal of Herpetology 46: 194–197.

Okada Y (1930) Notes on the herpetology of Chichijima, one of the Bonin Islands. Bulletin of the Biogeographical Society of Japan 1: 187–194.

Olsen M (1965) Twelve year summary of selection for parthenogenesis in Beltsville small white turkeys. British Poultry Science 6: 1–6.

Ota H (1987) A new species of *Lepidodactylus* (Gekkonidae: Reptilia) from Lanyu Island, Taiwan. Copeia 1987: 164–169.

Ota H (1989) A review of the geckos (Lacertilia: Reptilia) of the Ryukyu Archipelago and Taiwan. In: Matsui M, Hikida T, Goris RC (eds) Current herpetology in East Asia. Herpetological Society of Japan, Kyoto, pp. 222–261.

Ota H (1994) Female reproductive cycles in the northernmost populations of the two gekkonid lizards, *Hemidactylus frenatus* and *Lepidodactylus lugubris*. Ecological Research 9: 121–130.

Ota H, Darevsky IS, Ineich I, Yamashiro S (2000) Reevaluation of the taxonomic status of two *Lygodactylus* species (Squamata: Gekkonidae) from the Lesser Sunda Archipelago, Indonesia. Copeia 2000: 1109–1113.

Ota H, Fisher RN, Ineich IC, Case TJ (1995) Geckos of the genus *Lepidodactylus* (Squamata : Reptilia) in Micronesia: description of a new species and reevaluation of the status of *Gecko moestus* Peters, 1867. Copeia 1995: 183–195.

Palacio-Sierra JD, Guitiérrez OA, Castro-Herrera F (2011) Ampliación del rango de distribución de la salamanqueja *Lepidodactylus lugubris* (Duméril & Bibron, 1836) en Colombia. Herpetotropicos 7: 5–6.

Parmentier P, Ibene B, Gomes R (2013) *Lepidodactylus lugubris* (Mourning Gecko). Distribution. Caribbean Herpetology 47: 1.

Pasteur G, Agnese JF, Blanc CP, Pastuer N (1987) Polyclony and low relative heterozygosity in a widespread unisexual vertebrate, *Lepidodactylus lugubris* (Sauria). Genetica 75: 71–79.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population

genetic software for teaching and research - an update. Bioinformatics 28: 2537–2539.

Peters WHC, Doria G (1878) Catalogo dei retilli e dei batraci raccolti da O. Beccari, L. M. D'Alberts e A. A. Bruijn. nella sotto-regione Austro-Malese. Annali del Museo Civico de Storia Naturale di Genova. ser. 1, 13: 323–450.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredety 90: 502–503.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Radtkey RR, Donnellan SC, Fisher RN, Moritz C, Hanley KA, Case TJ (1995) When species collide: the origin and spread of an asexual species of gecko. Proceedings of the Royal Society of London B 259: 145–152.

Radtkey RR, Becker B, Miller RD, Riblet R and Case TJ (1996) Variation and evolution of Class I Mhc in sexual and parthenogenetic geckos. Proceedings of the Royal Society of London B 263: 1023–1032.

Ricklefs RE, Fallon SM (2002) Diversification and host switching in avian malaria parasites. Proceedngs of the Royal Society B 269: 885–892.

Roberts JA, Vo HD, Fujita MK, Moritz C, Kearney M (2012) Physiological implications of genomic state in parthenogenetic lizards of reciprocal hybrid origin. Journal of Evolutionary Biology 25: 252–263.

Röll B (2002) *Lepidodactylus lugubris* (Duméril & Bibron). Sauria (Suppl.) 24: 545–550.

Röll B, von Düring MUG (2008) Sexual characteristics and spermatogenesis in males of the parthenogenetic gecko *Lepidodactylus lugubris* (Reptilia, Gekkonidae). Zoology 111: 385–400.

Rösler H (1992) Beobachtungen an drei Männchen des parthenogenetischen Geckos *Lepidodactylus lugubris* (Duméril & Bibron, 1836). Sauria 14: 25–26.

Rösler H (1995) Geckos der Welt - Alle Gattungen. Urania, Leipzig, 256 pp.

Rösler H, Glaw F, Günther R (2005) Aktualisierte Liste der Geckos von Neuguinea (Sauria: Geckonidae: Gekkoninae) mit vorläufiger Charakterisierung von neun Formen aus den Gattungen *Cyrtodactylus* Gray, 1827, *Gehyra* Gray, 1834 und

Nactus Kluge, 1983. Gekkota 5: 33–64.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8: 103–106.

Sadlier RA, Bauer AM (1997) The terrestrial herpetofauna of the Loyalty Islands. Pacific Science 51: 76–90.

Sakai O (2016) Size distribution suggests a seasonal effect on reproduction of *Lepidodactylus lugubris* on Okinawajima Island, Japan, the northernmost distributional area. Current Herpetology 35: 59-63

Sang NV, Ho TC, Nguyen QT (2009) Herpetofauna of Vietnam. Chimaira, Frankfurt, 768 pp.

Savage JM (2002) The Amphibians and Reptiles of Costa Rica: A Herpetofauna Between Two Continents, Between Two Seas. University of Chicago Press, Chicago, 934 pp.

Schauenberg P (1968) Sur la presence de *Lepidodactylus lugubris* (Dumeril et Bibron, 1836) (Reptilia, Gekkonidae) en Equateur. Revue Suisse de Zoologie 75: 415–417.

Schwarz G (1978) Estimating the dimension of a model. Annals of Statistics 6: 461–464.

Sengoku S (1998) *Lepidodactylus lugubris* (Mourning Gecko). Ecuador: Galapagos Islands: Isla Santa Cruz in Puerto Ayora. Herpetological Review 29: 110.

Señaris C, Runjaic FJMR, Aristeguieta MM, Señaris GG (2017). Second record of the invasive gecko *Lepidodactylus lugubris* (Duméril & Bibron, 1836) (Squamata: Gekkonidae) from Venezuela. Journal of Species Lists and Distribution 13: 1–4.

Shibata Y, Kubota M, Ishimura M (1972) Mourning gecko (*Lepidodactylus lugubris*) from Okinawa and Yonaguni, Ryukyu Archipelago. Japanese Journal of Herpetology 5: 11–12.

Short KH, Petren K (2008) Boldness underlies foraging success of invasive *Lepidodactylus lugubris* geckos in the human landscape. Animal Behavour 76: 429–437.

Simon JC, Delmotte F, Rispe C, Crease T (2003) Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. Biological Journal of the Linnean Society 79: 151–163.

Smid J, Carranza S, Kratochvil L, Gvozdik V, Nasher AK, Moravec J (2013) Out of Arabia: A complex biogeographic history of multiple vicariance and dispersal events in the gecko genus *Hemidactylus* (Reptilia: Gekkonidae). PLoS ONE 8(5): e64018.

Smith HM, Grant C (1961) The mourning gecko in the Americas. Herpetologica 17: 68

Somaweera R, Somaweera N (2009) Lizards of Sri Lanka: a colour guide with field keys. Chimaira, Frankfurt, 304 pp.

Sosa BA, Reyes LR (2015) Distribution notes and comments on the predation of the Mourning Gecko, *Lepidodactylus lugubris* (Duméril & Bibron, 1836), in Panama. Mesoamerican Herpetology 2: 375–376.

Stoliczka F (1870) Observations on some Indian and Malayan Amphibia and Reptilia. The journal of the Asiatic Society of Bengal 39: 134–228.

Sunyer J (2014) An updated checklist of the amphibians and reptiles of Nicaragua. Mesoamerican Herpetology 1: 186–202.

Surget GY, Thorpe RS (2013) A likelihood framework analysis of an island radiation: phylogeography of the Lesser Antillean gecko *Sphaerodactylus vincenti*, in comparison with the anole Anolis roquet. Jornal of Biogeography 4: 105–116.

Supsup CE, Nevong MP, Augusto AA, Bernard RR, Panaguinit MFG, Guint FM, Rico EB, Diesmos AC, Brown RF, Mallari NAD (2016) Amphibians and reptiles of Cebu, Philippines: the poorly understood herpetofauna of an island with very little remaining natural habitat. Asian Herpetological Research 7: 151–179.

Swash A, Still R (2000) Birds, Mammals and Reptiles of the Galapagos Islands. Yele University Press, Connecticut, 168 pp.

Takada E, Ohtani T (2011) Keys to the illustrated manual of Japanese reptiles and amphibians in natural color. Hokuryukan Co., Ltd., Tokyo, 292 pp. (in Japanese)

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Taylor EH (1918) Reptiles of the Sulu Archipelago. Philippine Journal of Science 13:

233–267.

Taylor EH (1953) A review of the lizards of Ceylon. The University of Kansas Science Bulletin 35: 1525–1585.

Torres-Carvajal O (2001) Lizards of Ecuador: Checklist, distribution and systematic references, Smithsonian Herpetological Information Service 131: 1–35.

Torres-Carvajal O, Tapia W (2016) First record of the common house gecko *Hemidactylus frenatus* Schlegel, 1836 and distribution extension of *Phyllodactylus reissii* Peters, 1862 in the Galápagos. Journal of Species Lists and Distribution 7: 470–472.

Trifonov VA, Paoletti A, Caputo Barucchi V, Kalinina T, O'Brien PCM, Ferguson SMA (2015) Comparative chromosome painting and NOR distribution suggest a complex hybrid origin of triploid *Lepidodactylus lugubris* (Gekkonidae). PLoS ONE 10: e0132380.

Turner G, Green D (1996) Notes on the mourning gecko *Lepidodactylus lugubris* in the Daintree region. Herpetofauna (Sydney) 26: 5–7.

Tytler RC (1865) Observations on a few species of geckos alive in the possession of the author. Journal of the Asiatic Society of Bengal 33: 535–548.

Valencia ZA, Jaramillo MAF, Echeverry BA, Viáfara VR, Hernández CO, Cardona BVE, Gutiérrez ZJ, Castro HF (2014) Conservation status of the herpetofauna, protected areas, and current problems in Valle del Cauca, Colombia. Amphibian & Reptile Conservation 8: 1–18.

Van SHM (2003) Onze ervaringen met *Lepidodactylus lugubris*. Lacerta 61: 28–31.

Van Valen L (1976) Ecological species, multispecies, and oaks. Taxon 25: 233–239.

Vanegas-Guerrero J, Fernandez C, Buitrago-Gonzalez W, Vargas-Salinas F (2016) Urban remnant forests: are they important for herpetofaunal conservation in the central Andes of Clombia? Herpetological Review 47: 180–185.

Venugopal PD (2010) An updated and annotated list of Indian lizards (Reptilia: Sauria) based on a review of distribution records and checklists of Indian reptiles. Journal of Threatened Taxa 2: 725–738.

Volobouev V, Pasteur G (1988) Presumptive sex chromosomes of a unisexual homomorphic species of lizards, *Lepidodactylus lugubris*. Heredity 60: 463–467.

Volobouev V, Pasteur G, Ineich I, Dutrillaux B (1993) Chromosomal evidence for a

hybrid origin of diploid parthenogenetic females from the unisexual-bisexual *Lepidodactylus lugubris* complex (Reptilia, Gekkonidae). Cytogenetics and Cell Genetics 63: 194–199.

Vrijenhoek RC, Dawley RM, Cole CJ, and Bogart JP (1989) A list of the known unisexual vertebrates, pp. 19-23.

Watts PC, Buley RK, Sanderson S, Boardman W, Ciofi C, Gibson R (2006) Parthenogenesis in Komodo dragons. Nature 444: 1021–1022.

Weir BS, Cockerham CC (1984) Estimating *F*–statistics for the analysis of population structure. Evolution 38: 1350–1370.

Werner F (1913) Neue oder seltene Reptilien und Frösche des Naturhistorischen Museums in Hamburg. Reptilien der Ostafrika-Expedition der Hamburger Geographischen Gesellschaft 30: 1–51.

Williams GC (1975) Sex and evolution. Princeton University Press, New Jersey, 167 pp.

Wilmhoff CD, Csepeggi CE, Petren K (2003) Characterization of dinucleotide microsatellite markers in the parthenogenetic mourning gecko (*Lepidodactylus lugubris*). Molecular Ecology Notes 3: 400–402.

Yamashiro S, Ota H (1998) Discovery of a male phenotype of the parthenogenetic gecko, *Lepidodactylus lugubris*, on Ishigakijima Island of the Yaeyama Group, Ryukyu Archipelago. Japanese Journal of Herpetology 17: 152–155.

Yamashiro S, Ota H (2005) On the clone type of *Lepidodactylus lugubris* (Duméril and Bibron, 1836) corresponding to *Gehyra variegata ogasawarasimae* Okada, 1930 (Reptilia: Gekkonidae). Current Herpetology 24: 95–98.

Yamashiro S, Toda M, Ota H (2000) Clonal composition of the parthenogenetic gecko, *Lepidodactylus lugubris*, at the northernmost extremity of its range. Zoological Science 17: 1013–1020.

Yan J, Wang Q, Chang Q, Ji X., Zhou K (2010) The divergence of two independent lineages of an endemic Chinese gecko, *Gekko swinhonis*, launched by the Qinling orogenic belt. Molecular Ecology 19: 2490–2500.

Zerbe P (2006) Schlupf trotz zerbrochener Eischale bei *Lepidodactylus lugubris*. Elaphe 14: 25–26.

Ziesmann S, Janzen P, Klaas P (2007) Die Vielfalt der Geckos [Sri Lankas]. Draco 7:

38–44.

Zug GR (1991) The lizards of Fiji: Natural history and systematics. Bishop Museum Bulletins in Zoology 2: 1–136.

Zug GR, Alison MH, Christfer CA (2011) A new *Emoia samoensis* group lizard (Squamata: Scincidae) from the Cook Islands, South-central Pacific. Zootaxa 2765: 47–57.

Zug GR, Hinrich K (2014) A new species of four-toed skink (Squamata: Scincidae: Carlia peronii species group) from Pulau Sukur, Indonesia, and biogeographic notes on the herpetofauna of Flores and Komodo. Proceedings of the Biological Society of Washington 126: 379–392.

Zug GR, Ineich I, Pregill G, Hamilton AA (2012) Lizards of Tonga and a description of a new Tongan treeskink (Squamata: Scincidae: *Emoia samoensis* Group). Pacific Science 66: 225–237.

8. Appendices

8-1. Publications

Murakami, Y., Sugawara, H., Takahashi, H. and Hayashi, F. (2015) Different population genetic structure between sexual and asexual gecko species co-existing in the Ogasawara Islands. Ecological Research 30: 471-478.

村上勇樹（2017）小笠原諸島聟島列島におけるオガサワラヤモリのクローン多型とその分布．小笠原研究年報 40: 53-58.

8-2. Title and summary in Japanese

学位論文要旨（博士（理学））

単為生殖種であるオガサワラヤモリの生態的，遺伝的クローン間変異（英文）

村上勇樹（首都大学東京 理工学研究科 生命科学専攻）

オガサワラヤモリ（*Lepidodactylus lugubris*）は、太平洋、インド洋の島々に広く分布するヤモリ類で、雌のみで単為生殖を行う。ポリネシアやフィジーの集団を中心に、染色体数や背面の斑紋パターンに基づき、２倍体と３倍体のクローンタイプが識別されている。日本においても、主に沖縄の大東諸島において、染色体数、斑紋、酵素多型解析が行われ、そこには多くのクローン多型が存在することが知られている。本研究では、これら大東諸島だけでなく、日本全国のオガサワラヤモリのクローン多型の実態を明らかにするため、新たにマイクロサテライト DNA による分子生物地理学的解析を行った。また、小笠原諸島において、本種と有性生殖種であるホオグロヤモリ（*Hemidactylus frenatus*）の分布、集団遺伝構造、闘争行動を比較することによって、単為生殖種の各クローンおよび有性生殖種の間での移動分散能の差異、微生息環境の差異、闘争行動の差異について検討した。

日本全国（小笠原諸島、大東諸島、沖縄諸島、宮古諸島、八重山諸島）の 21 島からオガサワラヤモリ 748 個体を捕獲し、8 遺伝子座のマイクロサテライト解

析を行った結果、17 種類の遺伝的に異なるクローンが区別され、それぞれクローン O1、O2、T、M、D1～D13 と命名した。一方、cyt *b* 領域のミトコンドリア DNA 解析を行った結果、5 ハプロタイプが検出された。クローン O1、O2、D12, D13 は南太平洋の島々で広く分布が確認されているクローンと体の斑紋および倍数性が同じであったので、南方からの移入によるものと考えられた。一方、クローン D1～D11 は大東諸島のみに見られ、共通のマイクロサテライトアリルおよびミトコンドリアハプロタイプをもっており、大東諸島内で独自に分化した可能性が高いと考えられた。クローン T および M は、それぞれ竹富島および宮古島から見つかったが、その由来については不明である。

小笠原諸島の 11 島における調査では、オガサワラヤモリの 2 種類のクローン（クローン O1，O2）はほぼ全島に分布していたのに対し、ホオグロヤモリは 4 島のみに分布していた。捕獲したホオグロヤモリ 125 個体について、5 遺伝子座のマイクロサテライト解析を行った結果、ホオグロヤモリでは、島ごとに異なる集団遺伝構造を示し，小さい島では遺伝的多様性が低下していた。つまり、有性生殖種の方が，単為生殖種に比べて移動分散能が小さく、分布域が限定されるとともに島嶼間の遺伝的差異が生じる傾向があった。オガサワラヤモリの 2 種類のクローンおよびホオグロヤモリの観察箱内での餌をめぐる闘争行動を解析した結果、クローン O1 はクローン O2 より攻撃的であった。また、ホオグロヤモリもクローン O1 と同様に攻撃的であった。ヤモリ類では、こうした餌をめぐる競争が野外において起こることが知られており、クローン O2 の微生息場所が他とやや異なるのは、こうした餌をめぐる闘争の強弱の影響を受けている可能性が示唆された。