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Contrasting patterns of genetic variation in the two sympatric geckos *Gekko tawaensis* and *G. japonicus* (Reptilia: Squamata) from western Japan, as revealed by allozyme analyses

M Toda[1], T Hikida[2], S Okada[3] and H Ota[1]

[1]*Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan;* [2]*Department of Zoology, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan;* [3]*Department of Environmental Sciences, Faculty of Education and Regional Sciences, Tottori University, Tottori 680-8551, Japan*

Allozyme variation in two congeneric sympatric geckos, *Gekko tawaensis* and *G. japonicus*, from western Japan was examined. These species show similar densities and spatial arrangements of populations in this region, and their genetic structures are thus expected to have been formed under the influences of comparable geohistorical, environmental, and demographic factors. Results of the analyses, however, revealed strikingly different genetic patterns in the two species. Populations of *G. tawaensis* invariably showed a remarkably lowered heterozygosity (0–0.017) compared to *G. japonicus* (0.089–0.124). On the other hand, the genetic heterogeneity among populations is much greater in *G. tawaensis* ($F_{ST} = 0.726$) than in *G. japonicus* ($F_{ST} = 0.101$). The Mantel test failed to detect any significant correlations between log (estimated migration rate) and log (geographic distance) in either species, or between matrices of interpopulation pairwise F_{ST} for the two species. These results suggest that, in each species, formation of the current genetic structure in western Japan has been chiefly influenced by stochastic factors, rather than the geohistorical architecture of this region. The high F_{ST} and low heterozygosity in *G. tawaensis* suggest the effects of severe local fragmentation. On the other hand, the relatively low F_{ST} and high heterozygosity in *G. japonicus* imply extensive gene flow among populations. Absence of significant correlations between the estimated migration rate and geographic distance in *G. japonicus* may suggest that such gene flow is promoted by human-mediated transport of this primarily house-dwelling lizard.
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Introduction

A number of recent studies have demonstrated that in most, if not all, extant species, populations are genetically structured to some extent. In many of these studies, authors have attempted to specify major causal factors for observed genetic structures in the context of isolation history (Capula, 1996; Nielson *et al*, 2001), current status of populations (Reh and Seitz, 1990; Hitchings and Beebee, 1996), and ecological traits relevant to dispersal ability of the taxa in question (Ramirez and Haakonsen, 1999; Naihong *et al*, 2000). However, because population structures of real organisms are actually formed and maintained under the confounding effects of various biotic and abiotic factors, multiple interpretations are often inevitable for the evaluation of relative importance of a particular factor (Bossart and Prowell, 1998). Such a difficulty can be more or less circumvented by comparing closely related sympatric species sharing most historical and biological attributes (Bohonak, 1999). Such comparative studies have been made for several groups of marine organisms (eg, Waples, 1987; Planes *et al*, 1998), but for only a few terrestrial vertebrates (Matocq *et al*, 2000).

Two medium-sized geckos, *Gekko tawaensis* and *G. japonicus*, occur sympatrically in lowland areas of western Japan, including southwestern Honshu, Shikoku, northeastern Kyushu, and their adjacent islands. The former species is endemic to these areas, whereas the latter species occurs also in central and eastern Honshu, the remaining part of Kyushu, and eastern part of continental China as well (Shibata, 1983). Results of field observations and published information (HAPSE, 1996; Okada and Toda, 1998), indicating their comparable high population densities in suitable habitats and relatively low fecundity, suggest that the two species share similar demographic traits. The only prominent ecological difference between *G. japonicus* and *G. tawaensis* recognized so far resides in their habitat preferences (Okada and Toda, 1998). *Gekko* japonicus is primarily a house-dweller and is fairly ubiquitous in the human vicinities in western Japan, although it also occurs in a wide variety of lowland habitats outside urban areas. On the other hand, *G. tawaensis* is usually abundant in less disturbed habitats, such as natural and semi-natural outcrops, and primary and secondary vegetations in coastal and hilly environments. This species is also occasionally found in suburban environments, where it

Correspondence: M Toda, Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.
E-mail: gecko@zoo.zool.kyoto-u.ac.jp
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sometimes occurs syntopically with *G. japonicus* in the same building (Okada and Toda, 1998). Nonetheless, because both very and less disturbed landscapes are scattered throughout the lowland area of western Japan, interspersed with each other (JME, 1979), the pattern of geographic arrangement of populations is expected to be largely comparable between *G. tawaensis* and *G. japonicus* in this region.

In this study, we examined patterns and degrees of intra- and interpopulation genetic variations in *G. tawaensis* and *G. japonicus* from western Japan by means of protein electrophoresis. Results revealed prominent interspecific differences in these aspects. We discuss possible causal factors of such differential genetic patterns in the two species on the basis of an assumption that geological and demographic factors affecting their population structures are largely comparable.

Materials and methods

Samples analyzed

Sampling of the two *Gekko* species was carried out at 13 localities scattered almost throughout the whole range of *G. tawaensis* (Figure 1). The latter species was obtained from all sites, while *G. japonicus* were captured at five of the sites. We also collected *G. japonicus* from additional four localities in adjacent regions of Honshu and Kyushu (Figure 1). As a result, 388 geckos (245 *G. tawaensis* and 143 *G. japonicus*) were collected (Table 1).

These geckos were narcotized to death and dissected to remove livers and muscles. After dissection, the tissue samples were frozen in liquid nitrogen (in the field) or deep freezer (in the laboratory) as soon as possible and then were stored at -85°C until electrophoretic assay. Voucher specimens, after fixation in 10% formalin, were deposited in the Herpetological Collection of the Kyoto University Museum, Kyoto.

Electrophoresis

Tissues were minced in approximately equal volumes of distilled water, and the extracts were subjected to horizontal starch gel electrophoresis using a method following Murphy *et al* (1996) with slight modifications. Variations in 30 presumptive loci encoding 23 enzyme systems and one non-enzymatic protein were screened by use of four kinds of buffer systems. The enzymes analyzed and buffer systems employed were listed in Toda *et al* (2001a). Notations of presumptive loci and alleles followed Toda *et al* (1997).

Analysis

In order to estimate the degree of within-population genetic variabilities, we calculated mean number of alleles per locus (A), proportion of polymorphic loci (P: 99% criterion), and mean expected heterozygosity (H_{exp}: Nei, 1978) for each sample. To evaluate the

Table 1 Number of specimens examined for each local sample. Sample numbers correspond to those in Figure 1

Species	*Sample*	*N*
G. tawaensis	1. Wakayama	10
	2. Awajishima	27
	3. Takamatsu	20
	4. Tawa	6
	5. Shishikui	19
	6. Innoshima	38
	7. Hojo	37
	8. Sadamisaki	20
	9. Hofu	13
	10. Suzaki	6
	11. Ashizurimisaki	20
	12. Saganoseki	8
	13. Shimoaso	21
G. japonicus	14. Kyoto	20
	15. Wakayama	12
	16. Takamatsu	4
	17. Ako	4
	18. Innoshima	28
	19. Hojo	30
	20. Hofu	11
	21. Oumi	14
	22. Nagasaki	20

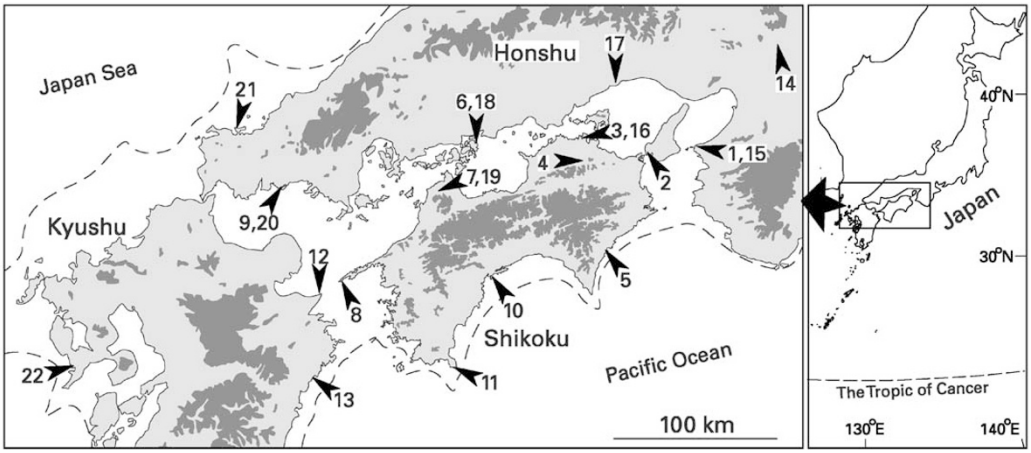

Figure 1 A map of western Japan, showing sampling localities of *G. tawaensis* and *G. japonicus*. 1, 15 = Wakayama; 2 = Awajishima; 3, 16 = Takamatsu; 4 = Tawa; 5 = Shishikui; 6, 18 = Innoshima; 7, 19 = Hojo; 8 = Sadamisaki; 9, 20 = Hofu; 10 = Suzaki; 11 = Ashizurimisaki; 12 = Saganoseki; 13 = Shimoaso; 14 = Kyoto; 17 = Ako; 21 = Oumi; 22 = Nagasaki. Dark stippled areas in dry lands and areas enclosed by dotted lines indicate areas higher than 600 m above sea level and those exposed during the latest glaciation (Quaternary Research Society, 1987).

magnitude of genetic heterogeneity among local samples within each species, Wright's (1965) F_{ST} was calculated. Statistical significance for each obtained F_{ST} value was tested following Workman and Niswander (1970). We also calculated Nei's (1978) unbiased genetic distance and Rogers' (1972) distance for all pairwise comparisons of the conspecific samples. Phylogenetic trees were inferred on the basis of the latter measure using the neighbor-joining (NJ) method (Saitou and Nei, 1985). All population genetic indices were calculated using BYOSIS-1 (Swofford and Selander, 1981) and the phylogenetic analysis (NJ) using Saitou's original program.

From the F_{ST} values, we further calculated migration rates (M) in pairwise comparisons of local samples within each species using the formula of Wright's (1931), and the Mantel test was performed by 10 000 permutations to determine significance of correlation between the log (M) and log (geographic distance) among samples (Slatkin, 1993) using the Arlequin ver. 2.000 (Schneider *et al*, 2000). For quantification of geographic distance between local samples, the following two measures were used: (1) linear distances ignoring the possible obstacles to gene flow, such as the sea and areas higher than 600 m above sea level where the geckos do not usually occur (Okada and Toda, 1998, unpublished field observations) (LGD); and (2) minimum distance by avoiding such obstacles (AGD). Values for AGD can thus be obtained only for pairs of samples from the same land mass, and we actually calculated them only for samples of *G. japonicus* and *G. tawaensis* from Honshu and Shikoku, respectively. In this analysis, we were concerned with geographic patterns of possible gene flows rather than the absolute estimates of migration rates. The Mantel test was also performed to determine the significance of the correlation between the pairwise F_{ST} matrices of the two species obtained on the basis of samples from five localities where both species were collected (Figure 1).

Results

Within-population variation

Nineteen and six presumptive loci were found to be polymorphic within *G. japonicus* and *G. tawaensis*, respectively. The sample of *G. japonicus* from Innoshima showed an extremely high percentage of polymorphic loci (53.3%: 16 out of 30) compared to the remaining conspecific samples (26.7–36.7%). This is mainly because of the presence of rare alleles at five out of the 16 polymorphic loci that are unique to the Innoshima sample within *G. japonicus* but are common to all *G. tawaensis* samples. Such an allelic pattern strongly suggests introgression from *G. tawaensis* to *G. japonicus* in this locality. In the preliminary survey, we detected several possible F_1 hybrids that possess marker alleles of both species heterozygous for all possible diagnostic loci (details to be reported elsewhere), and this circumstantially supports the above assumption. We thus excluded those rare alleles from the Innoshima sample of *G. japonicus*, and recalculated allele frequencies and other population genetic indices of this sample for further comparisons.

The three indices of within-population genetic variabilities, *P*, *A*, and *Hexp*, for samples of *G. japonicus* and *G. tawaensis* are presented in Table 2. Samples of *G. japonicus* collectively showed high values of *P* and *Hexp* (26.7–36.7 and 0.089–0.124, respectively), whereas samples of *G. tawaensis* showed much lower *P* and *Hexp* values (0–6.7 and 0–0.018, respectively). In the latter species, each of the Takamatsu, Tawa, Suzaki, Ashizurimisaki, and Shimoaso samples did not show any variation at all, and three other samples (Wakayama, Awajishima, and Saganoseki) showed only negligible variation that stems from the heterozygous possession of a rare allele at a single locus by a single individual. Even in the remaining five samples of *G. tawaensis*, polymorphisms were found at no more than two loci. Though less prominent, the genetic variability as expressed by *A* was also higher in the *G. japonicus* samples (1.2–1.4) than in the *G. tawaensis* samples (1.0–1.1).

Table 2 Mean proportion of polymorphic loci (*P*), number of alleles per locus (*A*), and expected heterozygosity (H_{exp}) in samples of *G. tawaensis* and *G. japonicus*. Standard errors are given in parentheses

Species	*Sample*	*P*	*A*	*Hexp*
G. tawaensis	1. Wakayama	3.3	1.0	0.003 (0.003)
	2. Awajishima	3.3	1.0	0.001 (0.001)
	3. Takamatsu	0	1.0	0.000
	4. Tawa	0	1.0	0.000
	5. Shishikui	3.3	1.0	0.017 (0.017)
	6. Innoshima	3.3	1.0	0.006 (0.006)
	7. Hojo	6.7	1.1	0.005 (0.004)
	8. Sadamisaki	6.7	1.1	0.018 (0.017)
	9. Hofu	3.3	1.0	0.007 (0.007)
	10. Suzaki	0	1.0	0.000
	11. Ashizurimisaki	0	1.0	0.000
	12. Saganoseki	3.3	1.0	0.004 (0.004)
	13. Shimoaso	0	1.0	0.000
G. japonicus	14. Kyoto	26.7	1.3	0.089 (0.032)
	15. Wakayama	33.3	1.3	0.107 (0.033)
	16. Takamatsu	23.3	1.2	0.094 (0.034)
	17. Ako	26.7	1.3	0.107 (0.036)
	18. Innoshima	36.7[a]	1.5[a]	0.113[a] (0.034)
	19. Hojo	33.3	1.4	0.114 (0.035)
	20. Hofu	36.7	1.4	0.124 (0.034)
	21. Oumi	33.3	1.4	0.124 (0.036)
	22. Nagasaki	33.3	1.4	0.108 (0.035)

[a]Values calculated after removing alleles possibly derived by the introgression (see text).

Among-population variation

Contrary to the remarkably higher intrapopulation genetic variabilities in *G. japonicus*, they showed relatively low levels of among-population genetic differentiation. Although F_{ST} values calculated for all nine samples of *G. japonicus* were significantly higher than zero at eight out of 14 polymorphic loci (Table 3), all samples shared an identical allele at all loci examined and the mean value of F_{ST} was relatively low (0.101). In *G. tawaensis*, on the other hand, allele frequencies at the *Pgm-1* locus were highly heterogeneous among samples, with fixed differences being present between several combinations of local samples (Appendix). Mean F_{ST} value calculated for the 13 *G. tawaensis* samples was very high (0.726).

Nei's genetic distance values (D) obtained between samples of *G. japonicus* were 0–0.031 ($\bar{x} = 0.009$), whereas those between samples of *G. tawaensis* were 0–0.034 ($\bar{x} = 0.014$). For samples from the five localities where both the species were collected (Figure 1), average D

Table 3 Summary of F_{ST} values for 13 local samples of *G. tawaensis* and nine samples of *G. japonicus*

	Acoh-1	*Est*	*Fumh*	*Gda*	*G3pdh*	*Hbdh*	*Idh-1*	*Ldh-1*	*Mdh-2*	*Pep-lg*	*Pep-lgg*	*Pep-lp*	*Pgdh*	*Pgm-1*	*Pgm-2*	*Sod*	*Mean*
G. tawaensis	0.023	—	0.037	—	—	—	—	0.107	0.058	—	—	—	—	0.787[a]	—	0.017	0.726
G. japonicus	0.083[a]	0.118[a]	—	0.148[a]	0.125[a]	0.058	0.057	0.098[a]	—	0.031	0.129[a]	0.107[a]	0.085	0.049	0.037	0.072[a]	0.101

[a]Significant ($P<0.01$).

values for *G. japonicus* and *G. tawaensis* were 0.006 and 0.018, respectively.

Correlation between genetic and geographic distances among local samples was obscure in each species. This is especially true for *G. tawaensis*, in which fixed allele difference at *Pgm-1* was observed even between samples from neighboring localities (Suzaki *vs* Ashizurimisaki), but no allele frequency differences were recognized between some geographically distant samples (eg, Hofu *vs* Ashizurimisaki, and Takamatsu *vs* Shimoaso). The Mantel test did not detect any significant correlation between matrices of $\log M$ and $\log$ LGD or $\log$ AGD in either species (with LGD and AGD as distance measures, $P>0.140$ ($n=13$) and $P>0.240$ ($n=7$) for *G. tawaensis*, and $P>0.439$ ($n=9$) and $P>0.692$ ($n=5$) for *G. japonicus*, respectively). The procedure also failed to detect significant correlation between the pairwise F_{ST} matrices for the five sympatric samples of the two species ($P>0.084$ ($n=5$)).

The contrasting patterns of geographic genetic variations in *G. tawaensis* and *G. japonicus* were also prominent in the NJ trees (Figure 2), which showed different topologies with respect to sympatric samples from the five localities. There was no evidence of a relationship between geographic and genetic patterns in either tree. The tree for the *G. tawaensis* populations is of an unusual shape, being more like a line rather than a tree (Figure 2a). This seems to be attributable to the predominance of the frequencies only of the two alleles at one locus (*Pgm-1*) in their whole variability, because in this 'linear' tree, populations monomorphic for the separate alleles occupy both ends and those polymorphic at this locus fall midway between them.

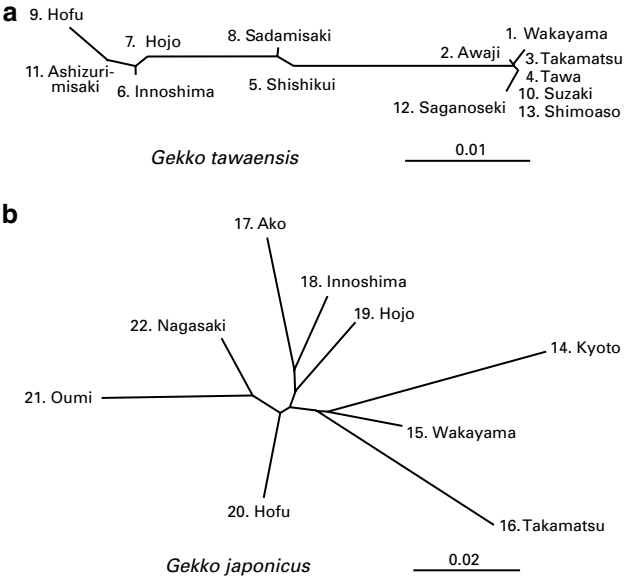

Figure 2 NJ trees for local samples of *G. tawaensis* (a) and *G. japonicus* (b) based on Rogers' (1972) distance. Note that the trees are not drawn in the same scale.

Discussion

Population structures in *G. tawaensis* and *G. japonicus*

The present results revealed that the western Japanese populations are genetically structured in both *G. tawaensis* and *G. japonicus*, but that patterns of genetic structures are quite different between the two species. At the within-population level, samples of *G. tawaensis* collectively showed low *Hexp* values (0–0.018), whereas those of *G. japonicus* showed much higher *Hexp* values (0.089–0.110). In contrast, among-population genetic heterogeneity was very high in *G. tawaensis* ($F_{ST}=0.726$), whereas much lower in *G. japonicus* ($F_{ST}=0.101$). In Table 4, we list values of the average heterozygosity and F_{ST} estimated from multi-locus allozyme data for populations of various other reptiles by previous authors. It is likely from this table that the heterozygosities within populations of *G. tawaensis* and *G. japonicus* represent some of the lowest and highest values described for reptiles, respectively. Conversely, F_{ST} values for these two species are nearly highest and lowest, respectively, among reptiles so far studied.

It is well substantiated that population structuring has often been induced by geographical and geohistorical isolations of local populations (Slatkin, 1985), and thus within-population genetic variation can vary depending on the insularity of populations (Gorman and Nevo, 1975). However, because most samples of the two species dealt with in the present study were from identical or contiguous localities, such a geographic factor does not seem to be responsible for the contrasting genetic patterns between *G. tawaensis* and *G. japonicus*. The absence of prominent geographically patterned population bifurcation also implies that geohistorical architecture of the study area has played little role, if any, in the formation and maintenance of the observed population structures in either species.

Gorman *et al* (1977) and Nevo and Beiles (1991), in the review of published data for genetic variability in reptiles and amphibians, argued that the main factors associated with levels of heterozygosity are habitat types (eg, fossorial, terrestrial, or arboreal) and life zones (eg, tropical, temperate, etc) at gross levels. However, these factors can be also excluded in the present case, because of the identity of habitats of the two species on these criteria.

Population structuring can also be induced solely by local population subdivisions owing to finite dispersal ability of individuals that prevent panmixing genetic exchanges throughout a given area (Slatkin, 1993). In this case, stochastic evolutionary forces (ie, mutation and drift) would act as major causes of population structur-

Table 4 Average heterozygosities and F_{ST} for the populations of several reptilian species estimated on the basis of multi-locus allozyme data

Species	*Number of populations sampled* (n)	*Average heterozygosity*	*Range*	F_{ST}	*Reference*
Gekko hokouensis	24[a]	0.054	0.012–0.102	0.575	Toda *et al* (1997)
G. yakuensis	9[a]	0.017	0.007–0.038	0.411[b]	Toda *et al* (2001b)
Gekko sp	5[a]	0.092[b]	0.065–0.119[b]	0.300[b]	Toda *et al* (2001a)
G. tawaensis	13	0.005	0.000–0.018	0.101	Present study
G. japonicus	9	0.110	0.089–0.124	0.726	Present study
Podarcis muralis	11	0.040	0.019–0.091		Capula (1997)
P. tiliguerta	14[a]	0.076	0.018–0.118	0.460	Capula (1996)
Lacerta caucasica	5	0.025	0.011–0.049	0.250	Fu *et al* (1995)
L. portschinskii	4[a]	0.043	0.011–0.071	0.313	MacCulloch *et al* (1997)
Phrynosoma cornutum	71	0.051			Sattler and Ries (1995)
23 lizard species	1–17	0.011–0.146			Gorman *et al* (1977)

[a]Populations on an island or fragmented habitat are included. [b]Calculated from the allele frequency data.

ing, and so the geographic pattern of genetic differentiation would not necessarily be compatible between two sympatric species. If this was the case here, the differential patterns of intraspecific genetic variation between *G. tawaensis* and *G. japonicus* could be attributable to the difference in their interpopulation migration rates. In the following sections, we extend our consideration along this line to seek possible causes for the characteristic genetic patterns in *G. tawaensis* and *G. japonicus* in western Japan separately.

Low genetic variability in the populations of *G. tawaensis*

The remarkably high F_{ST} value for *G. tawaensis* (0.726) suggests a severe restriction of gene flow among subdivided populations. This is compatible with the extremely low genetic variability in each of them (see Results), because such a genetic depauperation at within-population level can be caused by small effective size of populations. Many examples for such a severe genetic depauperation have been documented not only for small island populations (Gorman and Nevo, 1975; Capula, 1996), but also for mainland populations that have undergone some extent of habitat fragmentations (eg, MacCulloch *et al*, 1997; Sjogren-Gulve and Berg, 1999). Our results thus suggest that *G. tawaensis* is genetically fragmented into many populations occupying small patches.

The result of the Mantel test that indicates lack of significant negative correlations between expected migration rates and geographical distances in this species also supports the above view. If gene flow among subdivided populations was not highly restricted, this would have led to the isolation-by-distance pattern, in which neighboring populations show close genetic affinities than geographically distant populations. On the other hand, when gene flow is more restricted, subdivided populations would genetically diverge from each other irrespective of their geographic arrangements (Slatkin, 1985). One may argue that this is not necessarily true for the present result since the absence of significant correlations in *G. tawaensis* may have stemmed from standard error in the estimated gene flow rates. However, the view is further supported by the fixed differences at *Pgm-1* even between the neighboring populations in *G. tawaensis* (Appendix).

The present result further suggests that the possible local fragmentations have been initiated relatively recently. All of the populations of *G. tawaensis* examined share identical fixed allele at a huge proportion of loci. Moreover, only two alleles are found at the sole highly polymorphic locus, *Pgm-1*, at which many local populations are fixed for one or the other allele, apparently irrespective of their geographical allocations (Appendix). This cannot be easily explained by assuming long-term local isolation, because this would lead to fixation of populations to various different alleles under the effects of the mutation and drift (Slatkin, 1985). It is likely that the highly localized genetic subdivision in *G. tawaensis* has been driven by recent environmental alterations.

Relatively high genetic similarity among populations of *G. japonicus*

In contrast to *G. tawaensis* populations, those of *G. japonicus* contain a large amount of within-population genetic variation (Table 4). This, along with the low level of among-population genetic differentiation ($F_{ST} = 0.101$), suggests that there are substantial genetic exchanges over a broad area in this species. However, the result of the Mantel test did not support the presence of distance-dependent gene flow. *G. japonicus* chiefly occurs in urban environments, and is very liable to have opportunities for frequent dispersals by human-mediated transport, like some other house-dwelling geckos (eg, *Hemidactylus frenatus* and *Lepidodactylus lugubris*: Gibbons, 1985; Ineich, 1999). Because traffic networks have been highly developed in most parts of western Japan (even between separate islands), frequent human-mediated dispersals of *G. japonicus* without prominent distance effects are rather likely. We thus postulate that such 'stochastic' migrations are responsible for the formation of the observed genetic pattern in *G. japonicus*.

Even if the human-mediated dispersals have actually played an important role in the formation of the current genetic pattern in *G. japonicus*, it does not necessarily mean that the species outperforms *G. tawaensis* in its own dispersal ability. Significant F_{ST} values at several loci suggest that the ability of *G. japonicus* for long-distance dispersals is rather low. Therefore, it seems to be safer to assume that the primary cause for the striking difference in population structuring between *G. japonicus* and

G. tawaensis lies in their differential associations with human activities.

Present results suggest that the recent human activities have affected in different ways the formations of current patterns of genetic variation in the two sympatric geckos. Nevertheless, when and where such genetic alterations had initiated remain uncertain. In addition, it may be also possible to assume that the high within-population genetic variability in *G. japonicus* actually represents a non-equilibrium state, reflecting a past allopatric genetic divergence and subsequent progressive gene mixing by human transport. Such a condition would make it even more difficult to specify spatio-temporal factors responsible for the formation of the observed genetic patterns in this species. A number of recent studies have resolved such difficulties by analyzing variation in mtDNA, which provides degrees of divergences between haplotypes (eg, Cunningham and Moritz, 1998; Paulo *et al*, 2001). Thus, it is desired to test our hypothesis presented here by these approaches.

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Appendix

Allele frequencies at polymorphic loci (99% criterion) in local samples of *G. tawaensis* (a) and *G. japonicus* (b)

Locus		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
(a) *G. tawaensis*														
Acoh-1	*a*								0.025					
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000
Fumh	*a*	0.950	1.000	1.000	1.000	1.000	1.000	0.986	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.050						0.014						
Ldh-1	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.885	1.000	1.000	1.000	1.000
	c									0.115				
Mdh-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.938	1.000
	b												0.063	
Pgm-1	*a*					0.526	0.895	0.932	0.575	1.000		1.000		
	c	1.000	1.000	1.000	1.000	0.474	0.105	0.068	0.425		1.000		1.000	1.000
Sod	*a*	1.000	0.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b		0.019											

Reference numbers of samples correspond to those in Figure 1. Notation of alleles is made alphabetically in order of anodal mobilities throughout samples of both species.

Locus		*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
(b) *G. japonicus*										
Acoh-1	*c*	0.475	0.667	0.875	0.625	0.482	0.483	0.636	0.785	0.650
	d				0.125					
	e					0.018			0.036	0.125
	f	0.525	0.333	0.125	0.250	0.500	0.517	0.364	0.179	0.225
Est	*a*	1.000	0.958	1.000	0.625	0.839	0.833	0.864	0.679	0.800
	b		0.042		0.375	0.161	0.167	0.136	0.321	0.200
Gda	*a*		0.250	0.375	0.125	0.071	0.183	0.182	0.500	0.050
	b	1.000	0.750	0.625	0.875	0.929	0.817	0.818	0.500	0.950
G3pdh	*a*	0.425	0.625	0.625	0.875	0.821	0.917	0.727	0.857	0.868
	b	0.575	0.375	0.375	0.125	0.179	0.083	0.273	0.143	0.132
Hbdh	*a*	0.100				0.038	0.023			
	b	0.900	1.000	1.000	1.000	0.962	0.977	1.000	1.000	1.000
Idh-1	*a*	0.525	0.833	0.750	0.875	0.857	0.683	0.727	0.643	0.700
	b	0.475	0.167	0.250	0.125	0.143	0.317	0.273	0.357	0.300
Ldh-1	*a*							0.136	0.036	
	b	1.000	1.000	1.000	1.000	1.000	1.000	0.864	0.964	1.000

Continued

Locus		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Pep-lg	*a*							0.045		0.026				
	b	1.000	1.000	1.000	1.000	1.000	1.000	0.955	1.000	0.974				
Pep-lgg	*b*	0.675	0.500		0.375	0.518	0.367	0.273	0.429	0.325				
	c					0.093	0.050	0.091	0.107	0.200				
	d	0.325	0.500	1.000	0.625	0.389	0.583	0.636	0.464	0.475				
Pep-lp	*a*	0.025				0.089	0.033			0.025				
	b	0.925	0.792	0.500	0.625	0.572	0.500	0.727	0.893	0.800				
	c	0.050	0.208	0.500	0.375	0.339	0.467	0.273	0.107	0.175				
Pgdh	*a*				0.125	0.107		0.045	0.214	0.075				
	b	1.000	1.000	1.000	0.875	0.893	1.000	0.955	0.786	0.925				
Pgm-1	*a*	0.950	0.958	0.875	1.000	0.929	0.916	1.000	1.000	1.000				
	b	0.050		0.125		0.071	0.067							
	c		0.042				0.017							
Pgm-2	*a*	1.000	0.958	1.000	1.000	1.000	1.000	1.000	1.000	1.000				
	b		0.042											
Sod	*c*	0.850	0.667	0.875	1.000	0.780	0.650	0.682	0.714	0.667				
	d	0.150	0.333	0.125		0.220	0.350	0.318	0.286	0.333				