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RESEARCH ARTICLE

Genetic population structure of the masked palm civet *Paguma larvata*, (Carnivora: Viverridae) in Japan, revealed from analysis of newly identified compound microsatellites

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Abstract The masked palm civet *Paguma larvata* (Carnivora: Viverridae) in Japan has been phylogeographically considered an introduced species from Taiwan. To reveal the population structures and relationships among the *P. larvata* populations in Japan, seven compound microsatellite loci were isolated from the genome and genotyped for 287 individuals collected from the field. STRUCTURE analysis and factorial correspondence analysis of genotyping data revealed that animals from Japan were divided into four genetic clusters. Geographic distribution of the genetic clusters partly referred to sampling areas, indicating multiple introductions into distinct areas of Japan or independent founding events leading to the generation of different genetic clusters within introduced populations in Japan. The large genetic differentiation of populations in the Shikoku District from those in other areas within Japan suggests that there were at least two introduction routes into Japan, and a possibility that some founders from areas other than Taiwan were also involved in the introduction into Japan. The genetic variation within Japanese populations were not markedly reduced compared with that of Taiwan. The results indicated that the Japanese populations of *P. larvata* could have retained moderate genetic diversity during founding events, because of multiple introductions, or a large number or high genetic diversity of founders. Although some individuals in Japan showed a sign of admixture between different clusters, there is no evidence that such an admixture markedly increased the genetic diversity within Japanese populations.

Keywords Compound microsatellite · Factorial correspondence analysis · Founder effects · Introduced species · Masked palm civet · *Paguma larvata* · STRUCTURE analysis

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Introduction

Since the beginning of human migration throughout the world, many animal and plant species have been introduced into their non-native ranges. Some of the introduced species have successfully established new habitats and populations, resulting in the disturbance of native ecosystems. To control such introduced species, information about their population biology and genetics is important (Sakai et al. 2001). Population genetic data may predict invasiveness to reduce the occurrence of new invasions or to improve the efficiency of control efforts for conservation of the native ecosystem (Sakai et al. 2001).

The masked palm civet *Paguma larvata* (Carnivora: Viverridae) is the only viverrid species in Japan and is considered an introduced species because of their discontinuous distribution and lack of fossil records (Torii 2009). *P. larvata* matures sexually at around two years old (Gao et al. 1987; Torii 2009). This species is basically nocturnal and arboreal, and they live in families comprising a mother and her cubs (Torii 2009). Although their natural range is western, southeastern and eastern parts of Asia (Torii 2009), in Japan this species was first reported in Shizuoka Prefecture (in Chubu District: Fig. 1) in 1943 (Nawa 1965). Since the first record in Japan, their range has become wider, and currently they are found in most areas of central and eastern Honshu Island and Shikoku Island (Fig. 1) (Torii 2009). As their range has expanded, damage to crops by *P. larvata* has also increased. Furthermore, their diet overlaps with those of native carnivore species in Japan, such as the raccoon dog (*Nyctereutes procyonoides*), badger (*Meles anakuma*) and Japanese marten (*Martes melampus*) (Matsuo et al. 2007; Matsuo and Ochiai 2009) and competition for food between them occurs. It is also reported that, in Chiba Prefecture (Kanto District: Fig. 1), *P. larvata* eats the endangered Tokyo salamander (*Hynobius tokyoensis*) (Matsuo et al. 2007).

The origins and introduction periods of Japanese *P. larvata* have long been unclear (Kuroda 1955; Nakamura 1994; Torii 2009). Masuda et al. (2008, 2010) analyzed the phylogeography of the mitochondrial DNA (mtDNA) cytochrome *b* gene of *P. larvata* in Japan, Southeast Asia and Taiwan, and they revealed that the Japanese populations have some haplotypes common to those in Taiwan. Taiwan has been thought to be one of the origins of Japanese *P. larvata* without any scientific evidence (Furuya 1973; Miyashita 1977). Masuda et al. (2010) suggested the introduction of at least three lineages of *P. larvata* into Japan from Taiwan, although further genetic study is necessary to clarify the introduction routes as well as the expansion history after the introduction in Japan.

Meanwhile, Masuda et al. (2010) reported founder effects in the Japanese populations of *P. larvata*: mtDNA genetic diversity much lower than those of natural populations of Taiwan and Southeast Asia, and broad distribution patterns of particular mtDNA haplotypes. In general, the genetic diversity of introduced populations is expected to be lower than that of natural populations (Dlugosch and Parker 2008) because of the small number of founders and genetic drift.

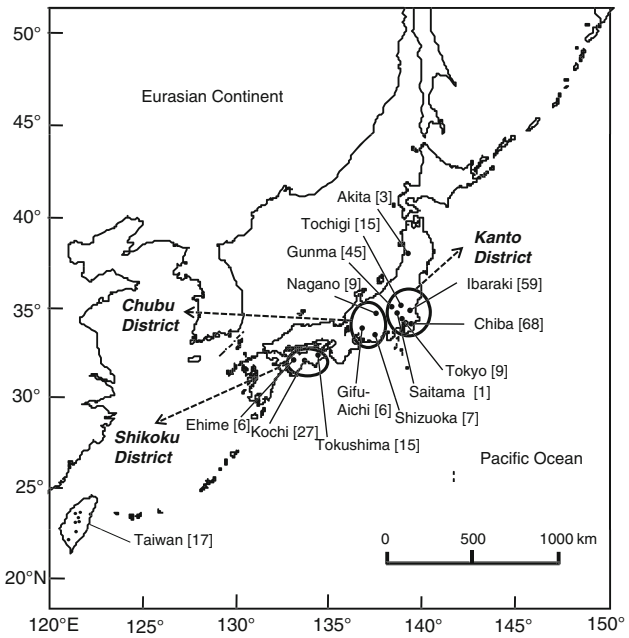

Fig. 1 Sampling locations (*dots*) and the numbers of specimens of *P. larvata* in Japan and Taiwan. The sampling locations in Japan, referring to geographic populations, were grouped into three geographic areas (Kanto, Chubu and Shikoku Districts), as shown by *circles*. In Taiwan, animals were collected at eight locations

On the other hand, multiple introductions may increase genetic diversity in populations of introduced species (Dlugosch and Parker 2008; Zalewski et al. 2010). Although there is a possibility of multiple introductions in Japanese *P. larvata* (Furuya 1973; Nakamura 1994; Harada and Torii 1993), Masuda et al. (2010) demonstrated low genetic diversity in the Japanese populations of *P. larvata* as a sign of founder effects, by analysis of mtDNA, which reflects only the female's migration history after introduction. Therefore, as the next step, to further understand the current population structure and introduction history of Japanese *P. larvata*, population genetic studies using biparentally inherited genes as genetic markers are necessary.

In the present study, compound microsatellites were newly isolated from the genome of *P. larvata*, using a simplified method. Then, genotypes of the compound microsatellite loci of *P. larvata* from Japan and Taiwan were determined, and the detailed population structure, genetic diversity and founder effects are discussed on the basis of the genotyping data. Information on the genetic structure in local areas can be applied to the management

strategy of introduced *P. larvata* populations in Japan and the conservation of native populations in Taiwan. In addition, the present (microsatellite) and previous (mtDNA) genetic studies contribute to the identification of effective management units and to detection of contact zones among populations in Japan.

Materials and methods

Samples and DNA extraction

Specimens of *P. larvata* were collected from roadkills and ecological surveys at various locations in Japan (270 individuals from 13 locations) and Taiwan (17 individuals from eight locations) (Fig. 1). Total DNA was extracted from muscle tissues (about 3 × 3 × 3 mm) or total bloods (about 100 µl) using the DNeasy Blood & Tissue Kit (Qiagen), eluted in 200 µl of TE buffer and stored at 4 °C until use. Total DNA from hair samples (20–30 hairs per animal) was extracted using the QIAamp DNA Micro Kit (Qiagen), eluted in 100 µl of TE buffer and stored at 4 °C until use.

Development of compound microsatellite markers

Compound microsatellite loci (Lian et al. 2006) were newly isolated from the *P. larvata* genome using the intercompound microsatellite (ICSSR) method (Wu et al. 2008; Nishida and Koike 2009). Using polymerase chain reaction (PCR), the ICSSR regions were amplified directly from extracted DNA of one Japanese *P. larvata*, with a compound simple sequence repeat (SSR) primer, $(AC)_6(AG)_5$ or $(TC)_6(AC)_5$ (Lian et al. 2006), or a mixture of both primers (Nishida and Koike 2009). The PCR amplifications were performed in 20 µl of the reaction mixture: 2.0 µl of 10× reaction buffer (Takara), 1.6 µl of dNTP (2.5 mM), 0.4 µl of the single compound SSR primer (25 pmol/µl) or 0.4 µl of a mixture of both primers (0.2 µl each), 0.1 µl of r*Taq* DNA polymerase (5 U/µl, Takara) and 1.0 µl of the DNA extracts. After denaturing at 94 °C for 3 min, 40 cycles were performed using a thermal cycler (TP600; Takara) with the following program: denaturing at 94 °C for 1 min; annealing at 56–58.6 °C for 1 min; extension at 72 °C for 1 min; and the reaction completion at 72 °C for 10 min. To check PCR amplification, 9 µl of each product was electrophoresed on a 2 % agarose gel, stained by ethidium bromide, and visualized under an ultraviolet illuminator.

The PCR products showing multiple bands or smear were cloned using the TA Cloning Kit (Invitrogen). Positive colonies were selected and incubated in the liquid medium (LB broth, Invitrogen) at 37 °C overnight. Plasmid DNA was isolated with the QIAprep Miniprep Kit (Qiagen) and sequenced using an automated sequencer (SQ5500; Hitachi). Because the cloned nucleotide sequences included compound microsatellite sequences at both ends, site-specific primers were designed within the regions between the two compound microsatellites.

To test the polymorphisms of compound microsatellite loci identified in the present study, 20 individuals of *P. larvata* from various locations in Japan were analyzed. The PCR amplifications were performed in 10 µl of the reaction mixture: 1.0 µl of 10× reaction buffer (Takara), 0.8 µl of dNTP (2.5 mM), 0.1 µl of the compound SSR primer labeled with Texas Red (5 pmol/µl), 0.1 µl of the site-specific primer (5 pmol/µl), 0.1 µl of r*Taq* DNA polymerase (5 U/µl, Takara) and 1.0 µl of the DNA extract. After denaturing at 94 °C for 3 min, 35–45 cycles were performed using a thermal cycler (TP600; Takara) with the following program: denaturing at 94 °C for 1 min; annealing at 55–57 °C for 1 min; extension at 72 °C for 1 min; and the reaction completion at 72 °C for 10 min. The PCR products (2.5 µl) or 2.5 µl of the Fluorescent Ladder CXR (Promega) for molecular size marker were mixed with 2.5 µl of Bromophenol Blue Loading Solution (Promega), heated at 95 °C for 2 min, and cooled down on ice immediately. Mixtures of 2.5 µl were electrophoresed using an automated sequencer (SQ5500, Hitachi). The molecular sizes were determined by FRAGLYS version 3 (Hitachi). The newly isolated compound microsatellite loci, which were polymorphic in the 20 individuals, were PCR-amplified and genotyped for all 287 samples in the same manner as above.

Data analysis

For all 287 samples, the observed (*Ho*), expected (*He*) heterozygosities, deviations from Hardy–Weinberg equilibrium and allele frequencies of each locus were calculated by ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010). The samples were then divided into 14 geographic populations based on sampling locations (Fig. 1), and standard genetic variations within each population were examined. In addition to *Ho* and *He*, allelic richness (*R*) was calculated by FSTAT 2.9.3.2 (Goudet 2001). To evaluate genetic differentiations between the geographic populations, pairwise *Fst* values and Nei's standard genetic distances (*Ds*, Nei 1978) were calculated using ARLEQUIN 3.5.1.2 and SPAGeDi 1.3 (Hardy and Vekemans 2002), respectively. Using the two values, neighbor-joining trees (Saitou and Nei 1987) were constructed by MEGA4 (Tamura et al. 2007). GENETIX 4.05 (Belkhir et al. 1996–2004) was also used to visualize the pattern of genetic relationships among geographic populations by factorial correspondence analysis (FCA) (Benzecri 1973). The

above analyses of genetic variations and population differentiations were performed in 12 populations, except for individuals in Saitama and Akita Prefectures, Japan, because of their small sample sizes (Fig. 1). Analysis by BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) was performed in three geographic groups in Japan (Kanto, Chubu and Shikoku Districts; Fig. 1) to detect evidence of recent bottleneck effect as heterozygosity excess. In this analysis, two-phase mutation model (TPM) with 95 % stepwise mutation model (SMM) as mutation model of microsatellites and Wilcoxon sign-rank test (Piry et al. 1999) were used.

To investigate the population structures of *P. larvata* in the 287 samples from Japan and Taiwan, Bayesian clustering method implemented in STRUCTURE 2.3 (Pritchard et al. 2000) was applied. In this analysis, the admixture model and correlated allele frequencies model were used. STRUCTURE 2.3 was run with five repetitions of 10,000 iterations of Markov chain Monte Carlo, following a burn-in of 10,000 iterations at $K = 1$–10. Although analyses with more precise conditions (10 repetitions of 100,000 iterations of Markov chain Monte Carlo, following a burn-in of 100,000 iterations at $K = 1$–10) were also performed, the results were almost the same as that with the shorter run. To estimate the real number of the subpopulation (K), the values of the log-likelihood ratio [Ln$P(D)$] and ΔK (Evanno et al. 2005) were calculated. Because the value of ΔK usually detects the uppermost hierarchical level of the genetic structure (Evanno et al. 2005), the samples were divided into two geographic groups, and further analyses were performed at $K = 1$–6 to investigate the detailed genetic structures respectively, after the first analysis. When individuals had q values (estimated membership in clusters) of $\geq$0.7, they were assumed to be members of that particular cluster.

General genetic information (*Ho*, *He* and *R*) on each cluster inferred by STRUCTURE 2.3 was calculated in the same manner as geographic populations, and indicators of genetic differentiations (pairwise *Fst* and *Ds*) between the clusters were also calculated.

Results

Genetic characteristics of compound microsatellite loci

In the development of compound microsatellite markers, 68 TA-clones were selected and sequenced. Of 40 site-specific primers designed, 34 worked successfully for PCR. Of the 34 loci, nine were polymorphic, of which seven were usable as compound microsatellite markers. The remaining two loci were not usable, because one of them showed multiple ambiguous bands in electrophoresis and the other indicated a deviation from Hardy–Weinberg equilibrium. The successful seven loci were named PLAMSA-1, PLAMSA-3, PLAMST-9, PLAMST-16, PLAMST-18, PLAMST-20 and PLAMST-26, respectively (Table 1). Two to twelve alleles were identified from the seven loci, and the average number was 6. The observed heterozygosity (*Ho*) ranged from 0.195 (PLAMST-18) to 0.581 (PLAMSA-1): 0.447 (0.137 SD) on average. The expected (*He*) ranged from 0.193 (PLAMST-18) to 0.688 (PLAMST-26): 553 (0.177 SD) on average (Table 1).

The sequences of the seven loci were deposited in DDBJ/EMBL/GenBank nucleotide databases with the following accession numbers: AB619599–AB619605 (Table 1).

Genetic diversities and the relationships among geographic populations

Table 2 shows the average values of *Ho*, *He* and *R* for each geographic population. The highest *Ho* value (0.548) was obtained from the Gifu-Aichi population and the lowest (0.274) was from the Tokyo population, both of which were populations from Japan. The highest *He* value (0.590) was from the Nagano population, whereas the lowest (0.307) was from the Tokyo population. For average values of allelic *R*, the highest value (3.423) was obtained from the population native to Taiwan, whereas the lowest (2.344) was from the Tokyo population. Allele distributions of each locus in geographic populations were shown in Table 3. Of 42 alleles identified from all individuals genotyped, 28 (66.7 %, 28/42 alleles) were shared by both Japan and Taiwan. Four alleles were private to Taiwan, but not to Japan, whereas 10 alleles were private to Japan, but not to Taiwan (Table 3). Of the 10 alleles private to Japan, one allele (#1 at PLAMST-20) was found only in the Kochi population of Shikoku Island. The pairwise *Fst* and Nei's standard genetic distance (*Ds*) values among the populations showed that the Kochi, Ehime and Tokushima populations, all of which are located in Shikoku District (Fig. 1), were more highly differentiated from the other populations (Table 4). In the neighbor-joining trees based on pairwise *Fst* and *Ds* (Fig. 2), the Japanese populations were classified into three groups: Kanto group (Tokyo, Tochigi, Gunma, Ibaraki and Chiba), Chubu group (Nagano, Shizuoka and Gifu-Aichi) and Shikoku group (Kochi, Ehime and Tokushima), in concordance with geographic distances among them (Fig. 1). The pairwise *Fst* values were not significant ($P > 0.05$) within Shikoku group, Kanto group (between "Tochigi and Gunma" and "Tochigi and Ibaraki") and Chubu group (between "Gifu-Aichi and Nagano" and "Gifu-Aichi and Shizuoka") (Table 3). In addition to the neighbor-joining trees (Fig. 2), FCA analysis showed that individuals in Japan were largely

Table 1 Genetic characteristics of seven compound microsatellite loci developed in the present study

Locus	Primer sequence (5′–3′)	Annealing temperature (°C)	Number of samples genotyped	Allele size range (bp)	Number of alleles	Heterozygosities		Accession no.[a]
						Ho	*He*	
PLAMSA-1	F: $(AC)_6(AG)_5$	57	284	223–239	5	0.581	0.672	AB619599
	R: AAGCCGTTCATGGCTGTTAA							
PLAMSA-5	F: $(AC)_6(AG)_5$	55	287	250–255	4	0.341	0.459	AB619600
	R: TCCTGGAGCTTCTGTTCTAC							
PLAMST-9	F: $(TC)_6(AC)_5$	55	286	226–241	4	0.448	0.581	AB619601
	R: GTGTAGATATGCCTGAAGTCA							
PLAMST-16	F: $(TC)_6(AC)_5$	55	286	167–200	12	0.490	0.616	AB619602
	R: CTGTTTGTTGTGTTAGAATTAC							
PLAMST-18	F: $(TC)_6(AC)_5$	55	287	181–182	2	0.195	0.193	AB619603
	R: CATTCATTAGCTCAACTGTGG							
PLAMST-20	F: $(TC)_6(AC)_5$	55	287	149–164	7	0.502	0.664	AB619604
	R: AAAGCAGGGCTCGTGCTCA							
PLAMST-26	F: $(TC)_6(AC)_5$	55	286	253–272	8	0.570	0.688	AB619605
	R: CATTGAGTCACCTGTGATCC							
Average					6	0.447	0.553	
Standard deviation					3.317	0.137	0.177	

F, forward (compound microsatellite); R, reverse (site specific primer)

[a] Sequences including the primers were deposited in DDBJ/EMBL/GenBank nucleotide databanks with those accession numbers

Table 2 Genetic characteristics within each geographic population

Geographic population	Number of samples	Average value of allelic richness (R)[a]	Heterozygosities	
			Ho	*He*
Tokyo	9	2.344	0.274	0.307
Tochigi	15	2.658	0.352	0.432
Gunma	45	2.912	0.453	0.487
Ibaraki	59	2.855	0.488	0.507
Chiba	68	2.556	0.404	0.451
Nagano	9	3.307	0.524	0.590
Shizuoka	7	2.403	0.531	0.473
Gifu-Aichi	6	2.857	0.548	0.569
Kochi	27	2.907	0.529	0.533
Ehime	6	2.857	0.476	0.537
Tokushima	15	2.778	0.437	0.533
Taiwan	17	3.423	0.399	0.504

Ho, observed; *He*, expected

[a] Based on the minimum sample size $n = 6$

separated into three main groups (Kanto, Chubu and Shikoku groups), and overlaps of individuals between Kanto and Chubu groups (Fig. 3). Individuals from Taiwan were distributed between Kanto and Shikoku groups. In BOTTLENECK analysis, significant heterozygosity excesses were detected from Chubu District ($P < 0.005$) and Shikoku Districts ($P < 0.05$).

Population structure analysis

The simulations in STRUCTURE 2.3 clarified the population structures in Japan and Taiwan. The value of the log-likelihood ratio [Ln$P(D)$] was largest at $K = 4$, although ΔK was highest at $K = 2$. Whenever K was set to 2, 3 or 4, most of the individuals from Shikoku District were assigned to one common cluster, and the other individuals from other areas were assigned to other clusters (Fig. 4). Because ΔK often detects the uppermost hierarchical level of the genetic structure (Evanno et al. 2005), the result (Fig. 4b) could reflect large genetic differences between the populations in Shikoku District and those from other areas, as demonstrated by *Fst* and *Ds* values (Fig. 2; Table 4). When further analyses were performed, samples were divided into two groups according to their sampling locations, Shikoku District and other areas. In Shikoku District, the value of log-likelihood ratio [Ln$P(D)$] was largest at $K = 1$, and when K was larger than 1, most individuals were assigned to each cluster with an equal proportion of membership. In other areas, the value of the log-likelihood ratio [Ln$P(D)$] and ΔK was largest at $K = 3$; therefore, $K = 4$ was chosen as an appropriate number of clusters for all samples (Fig. 4a). The characteristics of the four genetic clusters are summarized in Table 5. These clusters were partly in concordance with geographic populations. Most individuals from Chubu District (Nagano,

Table 3 Distribution of microsatellite alleles among geographic populations of *P. larvata*

Group	Population	PLAMSA-1	PLAMSA-5	PLAMST-9	PLAMST-16	PLAMST-18	PLAMST-20	PLAMST-26	Total
Kanto	Tokyo	# 1, 3, 4	# 2, 3	# 1, 3	# 3, 8, 10	# 1	# 2, 4, 5	# 1, 3, 5, 7	
	Tochigi	# 1, 3, 4	# 2, 4	# 1, 3	# 3, 8	# 1, 2	# 2, 3, 4, 5, 7	# 2, 3, 4, 5, 6, 7, 8	
	Gunma	# 1, 3, 4, 5	# 2, 3	# 1, 3, 4	# 2, 3, 4, 8, 10, 12	# 1, 2	# 2, 3, 4, 5	# 1, 3, 4, 5, 6, 7, 8	
	Ibaraki	# 1, 3, 4, 5	# 1, 2, 3, 4	# 1, 3	# 2, 3, 4, 6, 8	# 1, 2	# 2, 3, 4, 5, 7	# 3, 4, 5, 6, 7, 8	
	Chiba	# 1, 3, 4	# 2, 3	# 1, 3	# 3, 4, 8	# 1, 2	# 2, 3, 4, 5, 7	# 3, 5, 6, 7, 8	
Chubu	Nagano	# 3, 4, 5	# 1, 2, 3	# 1, 3	# 2, 3, 6, 8, 10, 11, 12	# 1, 2	# 2, 3, 4, 5	# 1, 3, 6, 7	
	Shizuoka	# 3, 4	# 2, 3	# 1, 3	# 2, 3, 10	# 1, 2	# 2, 4, 5	# 1, 3, 5	
	Gifu-Aichi	# 3, 4, 5	# 2, 3	# 1, 3	# 2, 3, 8, 10, 12	# 1, 2	# 2, 4	# 3, 4, 6, 7	
Shikoku	Kochi	# 3, 4, 5	# 2, 4	# 1, 3	# 1, 3, 6, 8, 10, 11	# 1	# 1*, 2, 3, 5, 6	# 2, 3, 5, 7	
	Ehime	# 3, 4, 5	# 2, 4	# 1, 2, 3	# 1, 3, 6, 8, 10	# 1	# 2, 3, 5	# 3, 5, 7	
	Tokushima	# 3, 4, 5	# 2, 4	# 1, 2, 3	# 1, 3, 6, 8, 10, 11	# 1	# 2, 3, 5	# 2, 5, 7	
	Akita	# 3	# 2	# 1, 3	# 3, 6, 8	# 1	# 5	# 4, 5, 7	
	Saitama	# 4	# 2	# 1, 3	# 3, 10	# 1	# 2, 5	# 4, 7	
	Taiwan	# 1, 2*, 3, 4, 5	# 1, 2, 4	# 1, 2, 3, 4	# 1, 3, 4, 5*, 6, 7*, 8, 9*, 10	# 1	# 2, 3, 5, 6	# 2, 3, 4, 5, 6, 7	
Total no. of alleles		5	4	4	12	2	7	8	42
No. of alleles common between Japan and Taiwan		4	3	4	6	1	4	6	28
No. of alleles private to Japan		0	1	0	3	1	3	2	10
No. of alleles private to Taiwan		1	0	0	3	0	0	0	4

Numbering of alleles was done independently at each locus. Asterisks indicate alleles private to the geographic populations

Table 4 *Fst* (lower matrix) and *Ds* (upper matrix) values among geographic populations

	Tokyo	Tochigi	Gunma	Ibaraki	Chiba	Nagano	Shizuoka	Gifu-Aichi	Kochi	Ehime	Tokushima	Taiwan
Kanto group												
Tokyo		0.086	0.034	0.167	0.132	0.163	0.087	0.131	0.386	0.429	0.470	0.160
Tochigi	0.094		0.019	0.009	0.021	0.297	0.132	0.174	0.322	0.303	0.407	0.062
Gunma	0.035	0.020*		0.047	0.032	0.153	0.075	0.101	0.323	0.318	0.416	0.080
Ibaraki	0.135	0.008*	0.045		0.016	0.257	0.146	0.147	0.353	0.308	0.431	0.095
Chiba	0.122	0.024	0.037	0.021		0.244	0.140	0.161	0.362	0.295	0.443	0.088
Chubu group												
Nagano	0.212	0.235	0.148	0.198	0.219		0.107	0.012	0.451	0.446	0.452	0.320
Shizuoka	0.191	0.195	0.117	0.182	0.189	0.103		0.014	0.502	0.467	0.580	0.185
Gifu-Aichi	0.196	0.178	0.111	0.144	0.174	0.010*	0.043*		0.419	0.295	0.390	0.248
Shikoku group												
Kochi	0.283	0.240	0.227	0.231	0.250	0.252	0.288	0.246		0.020	0.015	0.198
Ehime	0.332	0.236	0.220	0.205	0.221	0.233	0.280	0.187	0.010*		−0.005	0.232
Tokushima	0.321	0.268	0.255	0.246	0.275	0.252	0.320	0.241	0.012*	−0.007*		0.295
Taiwan	0.159	0.067	0.069	0.085	0.090	0.204	0.179	0.170	0.159	0.168	0.198	

* Not significant ($P > 0.05$); the other values of *Fst* were all statistically significant ($P < 0.05$)

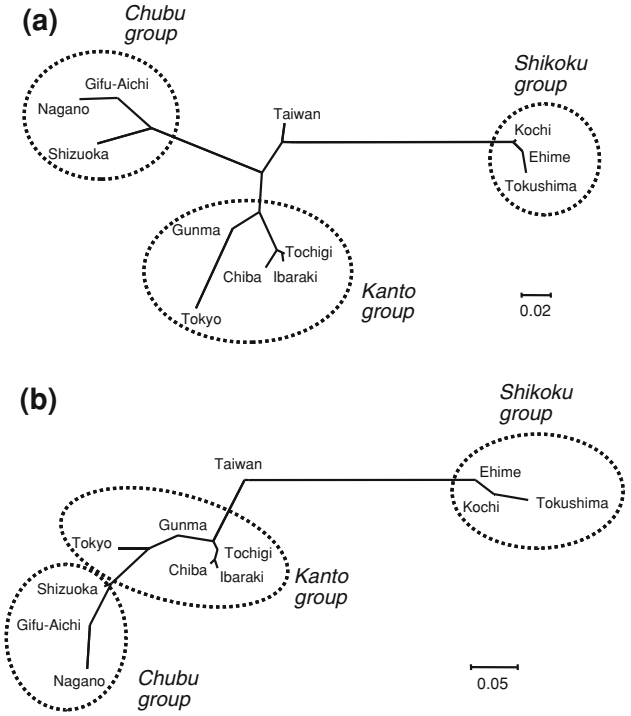

Fig. 2 Neighbor-joining relationships among geographic populations, constructed using values of pairwise *Fst* (**a**) and *Ds* (**b**). *Broken circles* show districts (Fig. 1), in which geographic populations are located

Shizuoka and Gifu-Aichi: Fig. 1) were assigned to cluster 3, and those from Shikoku District to cluster 4 (Fig. 4a; Table 5). Individuals assigned to cluster 3 were also found in Kanto District (Tokyo, Tochigi, Gunma, Ibaraki and Chiba: Fig. 1); those from Kanto District were assigned to any of clusters 1–3 (Fig. 4a; Table 5). Some individuals from single sampling locations were assigned to different genetic clusters (for instance, individuals from the Ibaraki population were divided into three clusters: Fig. 4a; Table 5). In addition, about 48 % (94/196) of individuals in Kanto District were not assigned to any clusters with $q \geq 0.7$ (Fig. 4a; Table 5). A single cluster consisting of only specimens from Taiwan was not formed, but individuals from Taiwan were assigned to any of three clusters 2–4, all of which were common to Japanese individuals (Fig. 4a; Table 5).

Discussion

Multiple introductions and range expansions indicated by population structures of Japanese *P. larvata*

In the present study, *P. larvata* populations in Japan and Taiwan were divided into four genetic clusters. The finding of genetic clusters and alleles common between individuals from both regions (Fig. 4a; Tables 3, 5) supports the previous study of mtDNA (Masuda et al. 2010) reporting that one of the origins of Japanese *P. larvata* was Taiwan. Identification of 10 alleles private to Japan and four private to Taiwan in the present study (Table 3), however, does not neglect *P. larvata*'s immigration to Japan from other countries, in addition to Taiwan. Masuda et al. (2010)

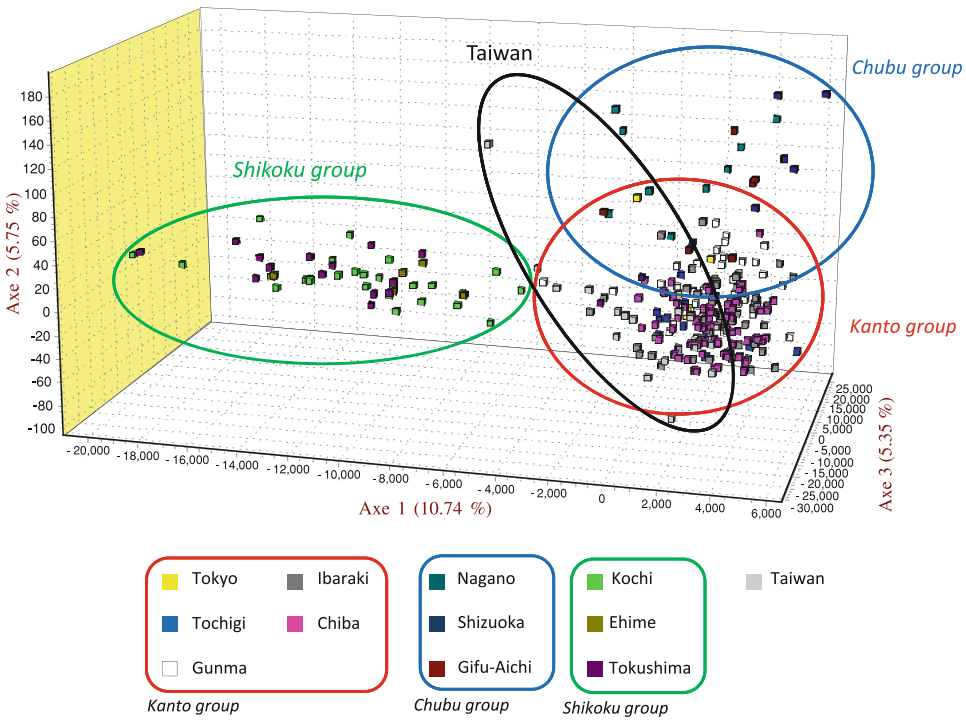

Fig. 3 FCA of *P. larvata* in Japan and Taiwan. Each individual is represented by one *cube*. *Colors* of the cubes show geographic populations, to which each individual belongs. Clustering of individuals corresponds with the geographic populations

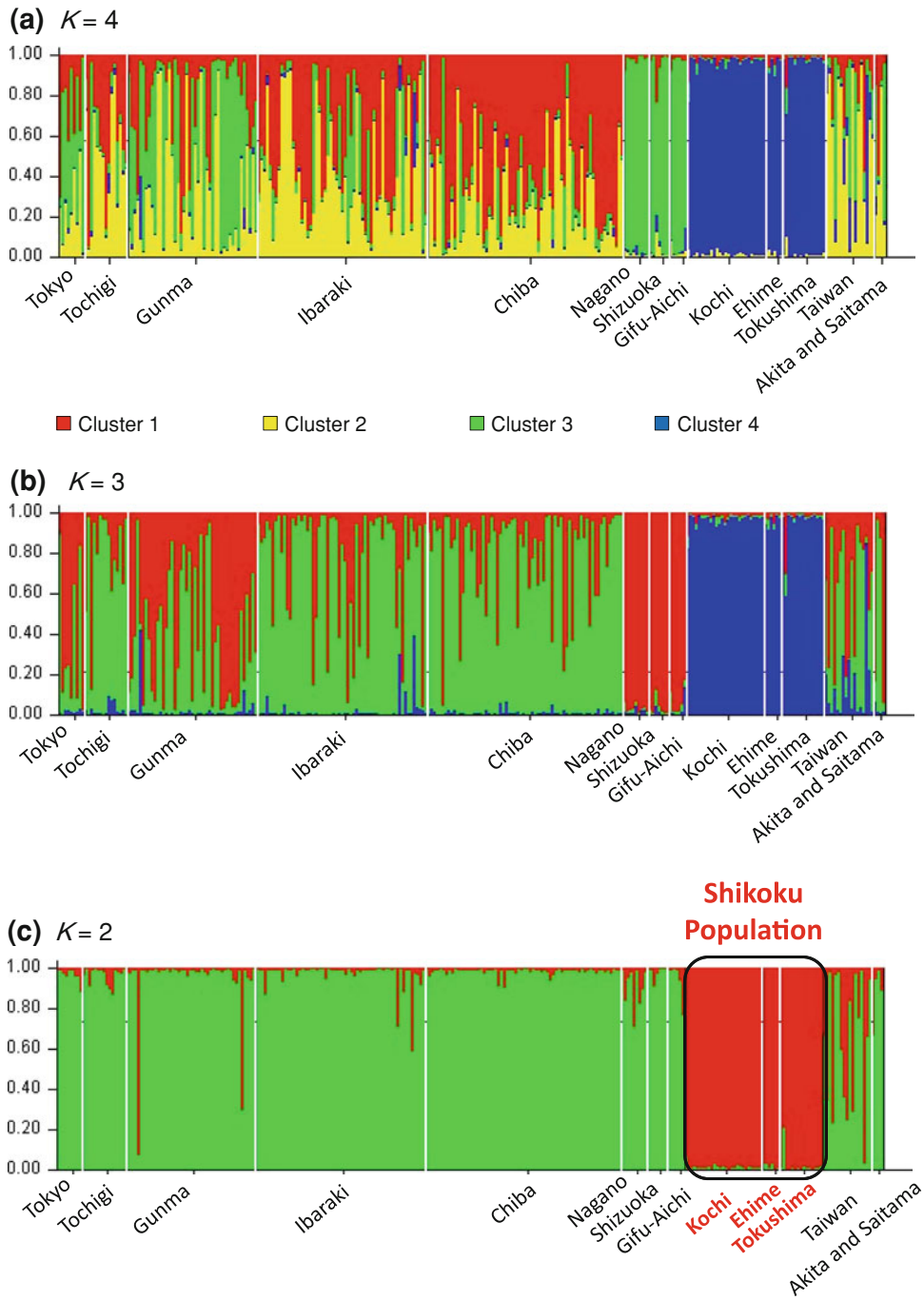

Fig. 4 Population structures of *P. larvata* in Japan and Taiwan inferred by STRUCTURE analysis. Each individual is represented by one *vertical thin line* partitioned into *K colored segments*, and arranged according to geographic populations. The proportion of each *color in the line* shows the individual's estimated membership for each cluster. The log-likelihood ratio [Ln$P(D)$] was largest at $K = 4$ (**a**), and ΔK was largest at $K = 2$ (**c**). For a comparison, the result at $K = 3$ (**b**) is also shown

actually reported three mtDNA haplotypes specific to Japan, not yet found in Taiwan. Since the numbers of samples and sampling locations from Taiwan in the present study as well as Masuda et al. (2010) are not enough to conclude whether Taiwan is only an origin, it is necessary to further survey genotypes in the populations of Taiwan and the rest of the natural range of this species.

Because *P. larvata* has been sporadically found in various areas of Japan, some researchers supposed that this species was introduced into multiple locations within Japan (Furuya 1973; Harada and Torii 1993; Nakamura 1994; Masuda et al. 2008, 2010). The present study revealed that different genetic clusters are distributed in distinct areas of Japan. For instance, cluster 3 is mainly distributed in Chubu District (Nagano, Shizuoka and Gifu-Aichi: Fig. 1), whereas cluster 4 is located in Shikoku District (Kochi, Ehime and Tokushima: Fig. 1) (Fig. 4a; Table 5). This result suggests that individuals from different lineages were introduced into different areas in Japan, and supports the previous inferences about multiple introductions.

Table 5 Frequencies of individuals which were assigned to the four clusters grouped by STRUCTURE analysis

Geographic population	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Number not assigned ($q < 0.7$)	Total number
Kanto District						
Tokyo	1	0	4	0	4	9
Tochigi	2	4	1	0	8	15
Gunma	2	7	16	0	20	45
Ibaraki	15	12	4	0	28	59
Chiba	29	4	1	0	34	68
Chubu District						
Nagano	0	0	9	0	0	9
Shizuoka	0	0	6	0	1	7
Gifu-Aichi	0	0	6	0	0	6
Shikoku District						
Kochi	0	0	0	27	0	27
Ehime	0	0	0	6	0	6
Tokushima	0	0	0	14	1	15
Taiwan						
Taiwan	0	7	1	1	8	17
Other areas						
Akita	0	1	0	0	2	3
Saitama	0	0	1	0	0	1
Total number	49	35	49	48	106	287
Ho	0.496	0.431	0.448	0.488		
He	0.508	0.472	0.515	0.537		
R	2.770	4.130	4.054	3.375		

Ho observed heterozygosity; *He* expected heterozygosity

R, average value of allelic richness over seven loci based on $n = 34$ (because one individual had missing data, its data was eliminated for calculation)

Masuda et al. (2010) reported that *P. larvata* in Shikoku District had a single mtDNA haplotype common to some individuals in Chubu District, and proposed that animals introduced into Chubu District brought the haplotype to Shikoku District, or that animals were introduced independently into Shikoku District from Taiwan. In the present study, compared between Shikoku and Honshu Islands (Table 3), four microsatellite alleles (allele #2 at locus PLAMST-9; #1 at PLAMST-16; and #1 and #6 at PLAMST-20) were private to Shikoku, but not to Honshu. Conversely, 13 alleles (#1 at PLAMST-1; #1 and #3 at PLAMST-5; #2, #4 and #12 at PLAMST-16; #2 at PLAMST-18; #4 and #7 at PLAMST-20; #1, #4, #6 and #8 at PLAMST-26) were specific to Honshu, but not to Shikoku. Thus, the allele distribution is different between the two main islands of Japan. The populations in Shikoku District were largely genetically differentiated from other Japanese populations (Fig. 2; Table 4); Therefore, the present study supports the latter scenario for independent introduction between Honshu and Shikoku Districts. In addition, it may suggest that the founders were introduced to Shikoku Island from areas other than Taiwan.

From their discontinuous distribution patterns, Suzuki (1985) reported that Japanese *P. larvata* expanded from four locations: Miyagi and Fukushima Prefectures (both prefectures are located north of Kanto District), Shizuoka Prefecture (Chubu District: Fig. 1) and Kochi Prefecture (Shikoku District: Fig. 1). Suzuki (1985) suggested that *P. larvata* in Miyagi and Fukushima Prefectures reached the northern part of Kanto District (Gunma, Tochigi and Ibaraki: Fig. 1), and that they expanded from Shizuoka Prefecture to Nagano and Aichi in Chubu District (Fig. 1) and western parts of Kanto District (including Tokyo and Saitama: Fig. 1). This inference is almost consistent with the distribution patterns of the four genetic clusters found by STRUCTURE and FCA analyses in the present study. On the other hand, *P. larvata* could have immigrated from northern to southern regions within Ibaraki Prefecture, and then expanded to adjacent Chiba Prefecture (both prefectures are located in Kanto District: Fig. 1) (Ochiai 1998;

Yoshitake 1998; Yamazaki et al. 2001). The lower values of *Fst* and *Ds* showed that *P. larvata* in Ibaraki and Chiba are closely genetically related (Fig. 2; Table 4), supporting the previous inference (Ochiai 1998; Yoshitake 1998; Yamazaki et al. 2001) about their range expansions in Kanto District. These results suggest that *P. larvata* introduced into multiple locations of Japan quickly expanded their range toward adjacent areas.

Occurrence of four different lineages in Japan and Taiwan

The present study revealed that individuals from wide areas of Taiwan (Fig. 1) were assigned to three genetic clusters (clusters 2–4; Fig. 4a; Table 5). In addition, the Taiwan population showed deviations from Hardy–Weinberg equilibrium at two loci. These results indicate that there are multiple lineages in the native populations of Taiwan, in agreement with the previous study of mtDNA variations (Masuda et al. 2010). Masuda et al. (2010) suggested that populations in Taiwan were geographically separated by high mountain ranges, resulting in uneven distribution patterns of mtDNA haplotypes. Although specimens from Taiwan in the present study included individuals from populations separated by such high mountain ranges, they did not form genetic clusters in concordance with the mtDNA lineages reported by Masuda et al. (2010). Some individuals having haplotypes from separate mtDNA lineages were included in the single genetic clusters detected by the present microsatellite analysis. Because alleles in microsatellite loci are biparentally inherited, the genetic clusters shown by the present study could reflect migration patterns within Taiwan, different from those obtained by maternal mtDNA data. Since there are few studies about the sexual differences of home ranges or migration patterns of *P. larvata*, it is unclear which behaviors affect the distribution differences of lineages detected from maternal (mtDNA) and biparental (microsatellites) genetic data. Further ecological studies of this species may give more insight into the issue.

Alternatively, the four genetic clusters detected in the present study could have been formed after their introduction into Japan. Although the distribution patterns of mtDNA haplotypes in Taiwan are separated by geographic barriers such as high mountains, the differences between mtDNA sequences were relatively small (Masuda et al. 2010). In addition, Patou et al. (2009) suggested a lack of clear genetic structures in native continental Chinese *P. larvata* populations. Thus, because *P. larvata* in natural ranges may not be well genetically differentiated among populations, the four genetic clusters detected in the Japanese populations could have been structured from founder populations due to genetic drift during their introduction, rather than originating from natural lineages.

Contact and admixture between different genetic lineages in Japan

The present study revealed four genetic clusters in Japanese *P. larvata*. Most individuals from single sampling locations in Chubu and Shikoku Districts were assigned to single clusters, respectively, whereas individuals from single sampling locations in Kanto District (Tokyo, Tochigi, Gunma, Ibaraki and Chiba: Fig. 1) were divided into three clusters (Fig. 4a; Table 5). This result suggests that frequent contacts among sampling locations (geographic populations) in Kanto District could have occurred. Although there were three clusters in Kanto District, about 48 % (94/196) of individuals from this district were not assigned to any clusters with $q \geq 0.7$ (Fig. 4a; Table 5). Because those individuals had admixture characteristics of different genetic clusters (for instance, some had almost equal values of q to two or three clusters), it is also suggested that an admixture of genetic clusters has occurred in Kanto District.

On the other hand, although individuals assigned to cluster 3 were found in all sampling locations in Kanto District, it is notable that many individuals (20/26) in cluster 3 were especially found in the Tokyo and Gunma populations (Fig. 4a; Table 5) located in western Kanto District, close to Chubu District (Fig. 1). In addition, FCA analysis (Fig. 3) showed the closely relatedness between Kanto and Chubu Districts, suggesting contacts of these two lineages around Gunma Prefecture. Compared between Kanto and Chubu Districts, seven alleles (#1 at PLAMST-1; #4 at PLAMST-5; #4 at PLAMST-9; #4 at PLAMST-16; #7 at PLANST-20; and #2 and #8 at PLAMST-26) were private to Kanto, whereas only one allele (#11 at PLAMST-16) was private to Chubu (Table 3). This indicates that the two lineages have different origins and the variation in Kanto is higher. The high frequency of cluster 3 in the Tokyo and Gunma populations, overlaps of individuals between Kanto and Chubu Districts, and still different distribution of private alleles suggest the ongoing migration (and breeding) from Chubu District to Kanto District or via the counter route. Although there are higher mountain ranges, such as Mt. Fuji and the Kanto Mountain Range including elevations of >2,000 m, which cannot be habitats of *P. larvata*, they could have migrated through valleys and low lands connecting Gunma Prefecture (Kanto District) with Nagano Prefecture (Chubu District). Since Chubu District is one of the earliest areas where the existence of *P. larvata* was reported (Nawa 1965), the former migrating direction (from Chubu to Kanto) might be more reasonable. Masuda et al. (2010) reported that mtDNA haplotypes found in Chubu and Shikoku Districts were also identified from individuals of the Gunma population, in addition to other haplotypes found in Kanto District. In the

present study, 36 % (16/45) of individuals from the Gunma population were assigned to cluster 3 (Table 5). The data of the present (microsatellites) and previous (mtDNA) studies demonstrate that Gunma is a contact zone between the populations from Kanto District and those from Chubu District.

Genetic diversity of *P. larvata* in Japan, compared with Taiwan

Because Masuda et al. (2010) reported a sign of founder effects in the Japanese *P. larvata* population based on mtDNA phylogeography, it was expected that they had lower polymorphisms at microsatellite loci than those of Taiwan. Despite this assumption, the heterozygosities in some populations in Japan were higher than in Taiwan, whereas those of other populations in Japan were lower than those in Taiwan (Table 2). Average values of *Ho* (0.450) and *He* (0.553) in all samples from Japan were higher, compared with those (0.399 for *Ho* and 0.504 for *He*) of Taiwan. Although this may be ascribed to the relatively small sample size (17 individuals) of the Taiwan population, if there are different genetic lineages in Taiwan as detected by mtDNA (Masuda et al. 2010), the specimens collected from wide areas of Taiwan should have showed higher diversities of microsatellites, compared with a population (such as the Shikoku population) consisting of one single lineage. Alternatively, if there are no clear genetic structures in Taiwan based on microsatellites, four genetic clusters detected in the present study could have been generated due to genetic drift during the introduction of small founder populations into Japan, and a population consisting of a single cluster (such as the Shikoku population) should have showed lower genetic diversities than native Taiwanese populations. However, the Shikoku population, which consisted of single genetic lineages of microsatellites (present study) and mtDNA (Masuda et al. 2010), respectively, indicated higher heterozygosities than those in Taiwan (Table 2). On the other hand, the allelic richness of the Japanese populations was slightly lower than the Taiwanese (Table 2). It is reported that allelic richness is more sensitive to founder effects than heterozygosity values (Dlugosch and Parker 2008). Furthermore, BOTTLENECK analysis in the present study showed significant heterozygosity excess in populations from Chubu and Shikoku District, suggesting that these populations could have experienced bottleneck recently. Considering these results, it is possible that the Japanese populations still retain the genetic diversity throughout introduction. Thulin et al. (2006) investigated microsatellite diversities in native and introduced populations of the small Indian mongoose (*Herpestes auropunctatus*), and reported that the Jamaica population originating from the native population had moderate genetic diversity, whereas some populations originating from the Jamaica population lost diversity. Although the genetic diversity of introduced populations is expected to be lower than that of natural populations (Dlugosch and Parker 2008), in some case, the degree of reduction of diversity by single founding events from natural populations may not be so large. Furuya (1973) reported that distributions of *P. larvata* in Shizuoka Prefecture (Chubu District: Fig. 1) were discontinuous, and suggested multiple introductions into different locations within Shizuoka. Due to such multiple introductions, if animals from the same lineage independently immigrated into geographically close areas, they might continue to retain some original genetic diversity. Alternatively, the number of founders in the introductions of *P. larvata* into each area of Japan may not have been so small. Nawa (1965) reported that a considerable number of animals were farmed in Shizuoka Prefecture before World War II. Extensive introduction into some areas of Japan in the past might have resulted in maintaining moderate genetic diversities of current animal populations. Shimatani et al. (2010) investigated introduced American mink (*Neovison vison*) populations in Nagano Prefecture in Chubu District, and reported that some microsatellite loci showed low genetic variations probably resulting from the low genetic diversity of founder individuals from farms, compared with other native and introduced animal populations. In *P. larvata* populations introduced into Japan, relatively higher genetic diversities of founders could have brought the present moderate variations.

Although the present study suggested admixtures among different genetic clusters in Kanto District, genetic diversity seen in this district was not so high compared with other areas (Table 2). The multiple introduction and admixture of populations originating from different sources sometimes increased the genetic diversity of introduced populations (Dlugosch and Parker 2008; Zalewski et al. 2010), but such cases are rare (Dlugosch and Parker 2008). It is likely that the admixture between different genetic clusters found in Kanto District has not increased the genetic diversity of the introduced *P. larvata* populations.

Implications for management of Japanese *P. larvata*

Because of agricultural damage, it is considered that effective reduction of the number of *P. larvata* in Japan is important (Furuya 2009). In addition, they may have competed with native species for foods (Matsuo et al. 2007; Matsuo and Ochiai 2009). Management of their population size is needed to protect crops and native ecosystems.

The present study revealed that most individuals from different districts were assigned to distinct genetic clusters (Fig. 4a; Table 5). This result indicates that animals from

each of the three districts (Kanto, Chubu and Shikoku Districts: Fig. 1) may be treated as independent management units. Although higher mountains are located between Kanto and Chubu Districts, some valleys and low lands may be their migration routes, promoting gene flow, which was detected in the present study. This indicates that even if the population size of *P. larvata* is successfully reduced in one district, migrants from an other district may supply individuals for reduced populations. In addition, although the present study showed that admixtures between different genetic clusters did not increase genetic diversity in introduced populations, in the future, the continuous admixture may increase the genetic diversity of *P. larvata* in Japan. For effective management of populations in Kanto and Chubu Districts, the separation of migration routes between them could be important. Because the present microsatellite and previous mtDNA (Masuda et al. 2010) studies revealed that the contact zone between lineages from two districts is around Gunma, eradication efforts in this area may help to manage population sizes in Kanto and Chubu Districts.

On the other hand, the present study indicated that most individuals from Shikoku District were included in a single genetic cluster (Fig. 4; Table 5). No signs of genetic contact or migrants between populations in Shikoku District and the other districts show that *P. larvata* in Shikoku District have not met any other populations since their introduction. Since Shikoku District consists of one island and is geographically separated from other areas by the sea (Fig. 1), there are no factors to increase the genetic variations by additional founders within the island; therefore, concentrated trapping could reduce the population size in Shikoku District more effectively, rather than Kanto and Chubu Districts.

Efficiency of microsatellite marker development using the ICSSR method

Although microsatellite markers are highly useful tools for many kinds of population genetics studies, their development is sometimes hard work (Lian et al. 2006). The ICSSR method (Wu et al. 2008; Nishida and Koike 2009) is an easy method for developing microsatellite markers. In this method, to detect microsatellite loci in genome, only three steps (PCR, cloning and sequencing) are needed. In the present study, the ICSSR method was applied to marker development from mammal species for the first time. Consequently, nine of 40 designed primer sets (22.5 %) were polymorphic and seven were able to be used as actually usable markers. Chen et al. (2008) isolated microsatellite loci from the *P. larvata* genome by constructing a dinucleotide-enriched library (Kandpal et al. 1994; Karp et al. 1998), and reported that six of 38 loci containing simple repeat motifs (about 15.8 %) were polymorphic. Thus, there are no large differences in efficiency between the present study and Chen et al. (2008). The method used in the present study is more simplified for the isolation of effective markers, and shows high potential for wide application to the development of genetic markers for population genetics studies of even introduced animals.

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References

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, populations, interactions. CNRS UMR 5000, Université de Montpellier II, Montpellier (France)

Benzecri JP (1973) L'analyse des Donnees: T.2, L'analyse des correspondences. Dunoud, Paris

Chen JP, Andersen DH, Veron G, Randi E, Zhang SY (2008) Isolation and characterization of polymorphic microsatellite markers for the masked palm civet (*Paguma larvata*). Biochem Genet 46:392–397

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449

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

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Furuya Y (1973) On *Paguma larvata* in Shizuoka Prefecture 1. Geographical distribution. J Mamm Soc Jpn 5:199–205 (in Japanese)

Furuya M (2009) The masked palm civet and raccoon, their interesting behaviors and smart ways to prevent them. Rural Culture Associate Japan, Tokyo (in Japanese)

Gao Y, Wang S, Zhang M, Ye Z, Zhou J, Ma Y, Li G, Wu J, Zheng Y, Guo F, Xu L, Peng H, Wang Y, Sheng H, Li C, Fu Y (1987) Fauna Sinica. Mammalia, vol 8: Carnivora. Science Press, Beijing (in Chinese)

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version2.9.3). http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995)

Harada M, Torii H (1993) Karyological study of the masked palm civet *Paguma larvata* in Japan (Viverridae). J Mamm Soc Jpn 18:39–42

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. Proc Natl Acad Sci USA 91:88–92

Karp A, Isaac PG, Ingram DS (1998) Molecular tools for screening biodiversity. Champman & Hall, London

Kuroda N (1955) The present status of the introduced mammals in Japan. J Mamm Soc Jpn 1:13–18

Lian C, Wadud MA, Geng Q, Shimatani K, Hogetsu T (2006) An improved technique for isolating codominant compound microsatellite markers. J Plant Res 119:415–417

Masuda R, Kaneko Y, Siriaroonrat B, Subramaniam V, Hamachi M (2008) Genetic variations of the masked palm civet *Paguma larvata*, inferred from mitochondrial cytochrome *b* sequences. Mamm Study 33:19–24

Masuda R, Lin L, Pei KJ, Chen Y, Chang S, Kaneko Y, Yamazaki K, Anezaki T, Yachimori S, Oshida T (2010) Origins and founder effect on the Japanese masked palm civet *Paguma larvata* (Viverridae, Carnivora), revealed from a comparison with its molecular phylogeography in Taiwan. Zool Sci 27:499–505

Matsuo R, Ochiai K (2009) Dietary overlap among two introduced and one native sympatric carnivore species, the raccoon, the masked palm civet, and the raccoon dog, in Chiba Prefecture, Japan. Mamm Study 34:187–194

Matsuo R, Kaneshiro Y, Ochiai K (2007) Comparative analyses on food habits of five carnivore species in the Chiba Prefecture, Japan. Bull Biol Soc Chiba 57:73–80 (in Japanese with English abstract)

Miyashita K (1977) Ecology of introduced animals. Kodansha, Tokyo (in Japanese)

Nakamura K (1994) Story on introduced animals. Gihodo, Tokyo (in Japanese)

Nawa A (1965) Notes on *Paguma larvata* in Shizuoka Prefecture. J Mamm Soc Jpn 2:99–105 (in Japanese)

Nei M (1978) Estimation of average heterozygosity and genetic distance for small number of individuals. Genetics 89:583–590

Nishida S, Koike K (2009) Isolation and characterization of compound microsatellite loci in the tri-spine horseshoe crab (*Tachypleus tridentatus*). Conserv Genet Resour 2:31–33

Ochiai K (1998) Distribution and invading process of the masked palm civet, *Paguma larvata*, in Chiba prefecture, central Japan. J Nat Hist Mus Inst Chiba 5:51–54 (in Japanese with English abstract)

Patou ML, Chen J, Cosson L, Andersen DH, Cruaud C, Couloux A, Randi E, Zhang S, Veron G (2009) Low genetic diversity in the masked palm civet *Paguma larvata* (Viverridae). J Zool 278:218–230

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

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

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughmen S, Cabin RJ, Choen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Shimatani Y, Fukue Y, Kishimoto R, Masuda R (2010) Genetic variation and population structure of the feral American mink (*Neovison vison*) in Nagano, Japan, revealed by microsatellite analysis. Mamm Study 35:1–7

Suzuki K (1985) Notes on *Paguma larvata* in Saitama Prefecture. Nat Anim 15(1):30–33 (in Japanese)

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol 24:1596–1599

Thulin GC, Simberloff D, Barun A, Mccracken G, Pascal M, Islam MA (2006) Genetic divergence in the small Indian mongoose (*Herpestes auropunctatus*), a widely distributed invasive species. Mol Ecol 15:3947–3956

Torii H (2009) *Paguma larvata* (Smith, 1827). In: Ohdachi SD, Ishibashi Y, Iwasa MA, Saitoh T (eds) The wild mammals of Japan. Kyoto, Shoukadoh, pp 267–268

Wu B, Shiokawa A, Matsushita N, Hogetsu T (2008) Isolation of compound microsatellite loci in the herbaceous perennial *Cirsium purpuratum* (Maxim.) Matsum. Mol Ecol Resour 8:814–817

Yamazaki K, Koyanagi K, Tsuji A (2001) List of mammals found in Ibaraki prefecture, central part of Japan. Bull Ibaraki Nat Mus 4:103–108 (in Japanese with English abstract)

Yoshitake W (1998) Miscellaneous notes of animals in Ibaraki Prefecture: Requiem for roadkilled animals. Ibaraki Seibutsu 18:33–57 (in Japanese)

Zalewski A, Michalska-Parda A, Bartoszewicz M, Kozakiewicz M, Brzezinski M (2010) Multiple introductions determine the genetic structure of an invasive species population: American mink *Neovison vison* in Poland. Biol Conserv 143:1355–1363