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False biogeographical pattern derived from artificial animal transportations: A case of the soft-shelled turtle, Pelodiscus sinensis, in the Ryukyu Archipelago, Japan.

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Abstract:

Genetic variation in the Ryukyu populations of the Chinese soft-shelled turtle, Pelodiscus sinensis, was examined by starch-gel electrophoresis. In order to collect information regarding their origins, questionnaires were also submitted to inhabitants of islands where the soft-shelled turtles occur. Electrophoretic investigations revealed that there is a relatively large genetic differentiation between populations of the Amami Group and the remainder ($D = 0.036-0.125$, $\bar{x} = 0.07$), and that they are genetically much closer to populations of the Main-islands of Japan and Taiwan, respectively than they are to each other. The questionnaire survey failed to obtain any evidence for the presence of original populations of P. sinensis on any Ryukyu island, but agreed with the electrophoretic results indicating recent introductions of the soft-shelled turtles to the Amami Group, and the Okinawa, Daito and Yaeyama Groups from the Main-islands of Japan and Taiwan, respectively.

Without information from the inhabitants, the geographic genetic pattern illustrated by the electrophoretic examination would be interpreted as indicative of an initial collapse of the East Asian landbridge between the Amami and Okinawa Groups, which involved vicariance of the soft-shelled turtles that had already widespread there, or blocked the dispersals of genetically differentiated races of the turtles more recently dispersed to Ryukyu region from the Main-islands of Japan and Taiwan. It is, however, almost certain from results of the questionnaire survey that the recognized geographic allozyme pattern actually reflects the commercial associations of areas through which the turtles had been introduced to each Ryukyu island. This case shows that the artificial transportation of animals can produce false biogeographical patterns, and that systematic questionnaires to inhabitants in relevant areas are sometimes highly effective in preventing erroneous conclusions.

1. Introduction

The geographic pattern of variation and distribution in an insular organismal lineage is generally expected to reflect more or less the paleogeographical events in and around its current range, such as formation and subsidence of landbridges. It is not unusual to find substantial variation between geographically proximate island populations that have nevertheless been isolated from each other for a long time. In recent years, it has become possible to detect and quantify genetic differentiation at the intraspecific level by using molecular and biochemical methods (Haig, 1998). However, when an organism is artificially transported within its range or introduced to areas where it does not occur naturally, subsequent genetic analyses of its populations may yield geographic patterns leading to an erroneous biogeographical

conclusion.

The Chinese soft-shelled turtle, *Pelodiscus sinensis* (Wiegmann, 1834), is widely distributed in East Asia including Japan (Iverson, 1992). The species has been also introduced to many other regions, such as Hawaii (McKeown and Webb, 1982) and Singapore (Lim and Lim, 1992), for the purpose of artificial breeding because of its high market value. Through the escape of some individuals from breeding ponds, and intentional releases, such an introduction has been followed almost always by the establishment of feral populations (see above literature, for example). This turtle is highly aquatic and inhabits rivers, lakes, ponds, and creeks with slow currents (Pope, 1935).

In Japan, *P. sinensis* commonly occurs over the Main-islands exclusive of Hokkaido and on several islands of the Ryukyu Archipelago (Sato *et al.*, 1997)(Figure1). Except for that on Ishigakijima Island (Takashima, 1935; Nakamura and Uéno, 1963), however, all Ryukyu populations had not been recorded before 1970s (Sengoku, 1979). Without any additional information, two opposite explanations would be possible for such a long absence of record from each island: 1) a native population had not been detected until recently; 2) the current population has derived from recent artificial introduction.

If the first possibility is actually the case, the Ryukyu populations should exhibit substantial genetic variations from each other, as well as from extralimital populations, as a result of long-standing geographic isolations (see Nishida [1990], Kato *et al.* [1994], and Toda *et al.* [1999] for examples of variations in other terrestrial vertebrates of this region). Considering the presence of numerous vernacular names for native animals on each inhabited island of the Ryukyus, it is expected that there is also a local name for the soft-shelled turtle on each of the

islands, where this animal occurs originally. If the second possibility is the case, inhabitants might have information regarding the dates and processes of introductions of the turtle into their islands.

Thus, we conducted an allozyme survey to examine genetic variations among the Ryukyu and adjacent populations of *P. sinensis*. We also carried out a questionnaire survey to inhabitants of the islands, where the soft-shelled turtle populations exist at present.

2. Materials and methods

2-1. Electrophoretic analysis

A total 17 samples, consisting of 211 specimens of *P. sinensis*, were obtained from the Ryukyu Archipelago, the Main-islands of Japan, Taiwan, and Hongkong (Figure 1, Table 1).

The field-captured samples were from Amamioshima, Kikaijima and Tokunoshima, of the Amami Group, Iheyajima and northern and southern parts of Okinawajima of the Okinawa Group, Minamidaitoujima of the Daitou Group, and Ishigakijima, Iriomotejima and Yonagunijima of the Yaeyama Group, Ryukyu Archipelago. Samples were also purchased from cultivation ponds on Amamioshima and Ishigakijima of the Ryukyus, and those in Sizuoka, Hiroshima, and Saga Prefectures on the Main-islands of Japan. Samples purchased from fishery markets in Hongkong and Taiwan were tentatively regarded as representing native populations in these regions, because people at each of those markets confirmed the local origin of animals sold there. Likewise, owners of the three cultivation ponds

on the Main-islands of Japan told us that their cultivations had first started with animals wild-captured at their vicinities. Voucher specimens will be deposited in the herpetological collection of Department of Zoology, Kyoto University (KUZ).

Liver and muscles, removed from each specimen, were homogenized, and extracts from these tissues were assayed by a horizontal starch gel electrophoresis. Enzymes analyzed and 23 presumptive loci used to estimate genetic distances among populations are given in Table 2. Enzyme nomenclature and E. C. number follow Murphy et al., (1996). Notations of presumptive loci were made by use of abbreviations of enzymes. In case that two or more loci control an enzyme system, we added numerical supplementaries, with "1" representing the most anodal locus. We made notations for loci encoding peptidase with hyphenated abbreviations of the substrates. Electromorphs were designated alphabetically in order of their anodal mobility.

In order to estimate overall genetic differentiation among samples, we calculated Nei's (1978) unbiased genetic distance for all pairwise comparisons of samples on the basis of their allele frequencies. The coefficient was subjected to the UPGMA algorithm (Sneath and Sokal, 1973). We also calculated expected heterozygosities (H_e : Nei, 1978) for each sample to estimate intrapopulational genetic variability. The computations were made by using BIOSYS-1 computer program (Swofford and Selander, 1981).

2-2. Questionnaire survey

A questionnaire survey was made from July 1993 to August 1997 on all islands of the Ryukyu Archipelago from which soft-shelled turtles had been reported. Efforts

were made to involve the present and past soft-shelled turtle breeders, their relatives, and public fishery officers on each island in the questionnaire as long as possible. The numbers and categories of respondents are shown at Table 3. On each island, the following three procedures were taken: 1) Town and village fishery officers were first contacted by telephone about their experiences instructing soft-shelled turtle breeding to inhabitants, and if their answers were affirmative, we asked them for further details by both telephone and questionnaire letters; 2) local inhabitants, especially farmers and town and sometimes village officers, were directly interviewed as to whether they had any information regarding the soft-shelled turtles on their island, e.g., when and where they occurred, whether they have local names; 3) present and past soft-shelled turtle breeders and their relatives were asked directly or by telephone for the dates of starts of their breeding, and sources and fates of animals used. In addition, we looked for references to this turtle in local publications such as historical records of towns and villages, local name dictionaries, old traditional stories, and formerly published newspapers.

3. Results and discussion

3-1. Electrophoretic survey

Of the 23 presumptive loci examined, seven (Fh, Gpd, Idh-2, Ldh-2, Mdh-2, Pgm and Sod) were monomorphic in all samples. Table 4 shows allelic compositions in the remaining 16 polymorphic loci, of which four (Ah, Ak, Ck, Gpi) were fixed in all samples but one. At locus Gda, the sample from Hongkong had a fixed unique allele. At locus Me-1, samples from the Main-islands of Japan and the Amami Group

were fixed or nearly fixed to one allele, whereas those from the remaining Ryukyu islands, Taiwan and Hongkong were fixed to another allele (Figure 2).

Results of pairwise comparisons of the samples are presented in Table 5. Nei's (1978) genetic distance (D) values were insignificant between samples from the Main-islands of Japan and the Amami Group, ranging from 0.000 to 0.020 (\bar{x} = 0.006), or between those from the remaining Ryukyus (i.e., Okinawa Prefecture islands) and Taiwan, ranging from 0.015 to 0.040 (\bar{x} = 0.023). Values were much larger between the Main-island-Amami samples, and samples from the Okinawa prefecture islands and Taiwan (\bar{x} = 0.073, 0.036-0.145). The Hongkong samples showed prominent divergence from the former (D : \bar{x} = 0.135, 0.112-0.162), and moderate divergence from the latter (D : \bar{x} = 0.055, 0.039-0.073)(Table 5).

Expected heterozygosity (H_e) for each sample examined is presented in the bottom of Table 4. The H_e values, largely low, were similar among samples, ranging from 0.104 (Yonagunijima sample) to 0.158 (sample from a cultivation pond on Ishigakijima)(\bar{x} = 0.125).

A distance phenogram generated by UPGMA method is presented in Figure 3. Two major clusters, one consisting of samples from the Main-islands of Japan and the Amami Group, and the other of those from the Okinawa Prefectural islands, Taiwan and Hongkong, were recognized. Within the former cluster, the Amami samples constituted a lower cluster with two of the Main-island samples. Within the latter major cluster, Taiwan sample was located within a subcluster consisting of samples from the Okinawa Prefecture islands.

Geographic patterns of both allelic displacements (Figure 2) and overall genetic similarity (Figure 3) consistently indicate that the border of the most prominent genetic differentiation exists between the Amami and Okinawa Groups, and that the

second divergence occurred between Taiwan and Hongkong. On the other hand, results of our analyses indicate the absence of discernible genetic variations among populations in the northern half of the Ryukyu Archipelago and the Main-islands of Japan, or those in the southern half of the archipelago and Taiwan.

It is almost certain that most East Asian islands have been subjected to complicated paleogeographical dynamics involving their landbridge connections to adjacent lands in various combinations, and that most, if not all, of native terrestrial lineages of animals in this region exhibit variations and distributions more or less influenced by such events (Nishida, 1990; Ota, 1998). From such a view point, the geographic pattern of genetic variation in the East Asian populations of *P. sinensis* could be interpreted as indicative of recent formation of a landbridge that connected Taiwan, Kyushu and most intervening islands of the Ryukyus, but with a primary or long-standing breakage somewhere between Okinawajima and Tokunoshima: *P. sinensis* might have been widespread over the landbridge and then diverged through isolation caused by its subsequent partial inundation, or, otherwise, originally two differentiated forms might have independently dispersed from Taiwan northeastward and from Kyushu southwestward, both to the long-standing Okinawajima-Tokunoshima straits.

3-2. Questionnaire survey

A total of 116 inhabitants, representing all Ryukyu islands where we obtained samples of *P. sinensis* for genetic analysis, responded to our questionnaire (Table 3). Information provided by these respondents and results of literature surveys for relevant information, already partially published in Sato et al. (1997), are

summarized in Table 6. In short, this survey confirmed the following six issues: 1) There are no local names for *P. sinensis* on any of the islands surveyed; 2) there are no respondents who know of, or argue for the presence of, original natural populations of *P. sinensis* on any of the islands surveyed; 3) all islands surveyed experienced introductions of *P. sinensis* from outside for commercial breeding and/or private consumption; 4) such introductions were made to islands of the Amami Group from the Main-islands of Japan directly or through a cultivation pond of Kikaijima, and to other islands of the Ryukyus from Taiwan directly or through a cultivation pond of Ishigakijima; 5) such introductions were made in or after the 1950s; and 6) first records of wild-captures and field-observations of *P. sinensis* appearing in publications were made in or after the 1970s for all islands but Ishigakijima, where two individuals were collected in 1933 (Takashima, 1935; Sato et al., 1997).

Absence of local names for *P. sinensis* on any of the Ryukyu islands where it current occurs makes a sharp contrast to situations related to the other Ryukyu reptiles, including three native turtles (*Geoemyda japonica*, *Cistoclemmys flavomarginata evelynae*, and *Mauremys mutica kami*), because most, if not all, of them have specific local names (e.g., "Yanbaru-ga-mi" for *G. japonica* on Okinawajima, and "yama-ga-mi" for *C. f. evelynae*, on Ishigakijima and Iriomotejima, and "mizu-ga-mi" for *M. m. kami* on Ishigakijima, Iriomotejima and Yonagunijima: information derived from questionnaire survey). One may argue that this is possibly attributable to the aquatic, cryptic, largely nocturnal nature of *P. sinensis* (Kaji, 1968), which might have prevented this turtle from being recognized by residents on each island. However, the fact that in our survey most of the correspondents did know of the recent "wild" occurrences of this species on their islands, offers a strong

counter-evidence to the above assumption. Such a fact also negates the explanation for the recency of discovery of each island population by assuming the difficulties that institutional collectors might have had in finding the turtle during the short period of their stays on each island, because many of the early biological explorers visiting the Ryukyu islands purchased specimens of native animals from residents (Ezaki, 1984).

As such, the most and probably the only plausible explanation for the results of our questionnaire and literature surveys seems to invoke an assumption that all of the current Ryukyu populations of *P. sinensis* are of recent artificial origins. Records of the soft-shelled turtle from Ishigakijima in 1933 (Takashima, 1935) and the absence of information regarding its introduction to this island before 1950s in the present questionnaire, may seem to indicate the occurrence of natural populations on Ishigakijima. Nevertheless, the presence of literature records, though rather anecdotal, of the soft-shelled turtle introduction to Ishigakijima from Taiwan for private consumption early in this century (Shokita, 1984; Chigira, 1991) and the absence of local names on Ishigakijima (see above: also see Sato *et al.* [1997]) seem still to favor the former explanation rather than the latter.

4. Conclusion

The hypothetical scenario, primarily deriving from the results of questionnaire and literature surveys, is also well concordant with results of electrophoretic surveys. It is especially noteworthy that all of the three genetic characteristics of the Ryukyu populations revealed by the electrophoretic surveys (i.e., high genetic uniformity among the populations of the Main-islands of Japan and the Amami Group; that

among the populations of remaining Ryukyu islands and Taiwan; and the presence of a distinct border of the two genotypes between Okinawajima and Tokunoshima: Figure 3) can be explained well by assuming the recent introductions of the turtles to the Amami and other Ryukyu islands from the Main-islands of Japan and Taiwan, respectively (Table 6).

By contrast, the hypothetical scenario deriving from the results of genetic surveys alone is not supported by results of the questionnaire and literature surveys (see above). Furthermore, that paleogeographical scenario, assuming a recent formation of the landbridge throughout the Ryukyu region with the only breakage between Okinawajima and Tokunoshima, is not supported by phylogeographical patterns in other terrestrial vertebrate lineages in this region (e.g., Ota, 1998). We thus consider the other hypothetical scenario, schematized in Figure 4, as being much more likely.

5. Lesson from the present case

In the case of *P. sinensis*, we have been lucky in several ways. First, the introduction took place quite recently, making it possible for us to confirm that fact through questionnaires. Secondly, the introduction was made intentionally and mostly for cultivation, making it fairly easy to specify people involved. Thirdly, human migration involving each of the relevant islands has not apparently been so active, making it easier to address the past events by addressing the questionnaire to current inhabitants. Consequently, we have been able to formulate a highly reliable scenario by assuming artificial transportations in detail. However, without even one of the above three factors, it would have been almost impossible to hypothesize the scenario with such a high certainty.

Recently, exotic animals introduced to islands have been causing various kinds of nasty problems, of which those related to conservational issues, such as elimination of native organisms and alteration of genetic diversity by introgression, are widely known of at present (e.g., Diamond and Case, 1986; Haig, 1998). The present case demonstrates the strong probability that such an artificial transportation of organisms may also lead to an erroneous biogeographical conclusion by exhibiting false geographic patterns of genetic variations.

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Table 1

Sampling localities and sample size for each population of *Pelodiscus sinensis*. The sample numbers correspond to those in Figure 1.

Region	Island	Population	Sample size
Main-islands of Japan			
	Sizuoka	1. Cultivation pond (CL)	23
	Hiroshima	2. Cultivation pond	10
	Saga	3. Cultivation pond	10
Amami Group			
	Amamioshima	4. Cultivation pond	15
		5. Wild	2
	Kikaijima	6. Wild	12
	Tokunoshima	7. Wild	5
Okinawa Group			
	Iheyajima	8. Wild	12
	Okinawajima	9. Wild	25
		10. Wild	8
Daitou Group			
	Minami-daitoujima	11. Wild	17
Yaeyama Group			
	Ishigakijima	12. Cultivation pond	25
		13. Wild	4
	Iriomotejima	14. Wild	8
	Yonagunijima	15. Wild	8
The Chinese of Taipei			
	Taiwan	16. Wild	20
Continental			
	Hongkong	17. Wild	7
Total			211

Table 2

Enzymes, loci, tissues and buffer systems used in the analysis of allozyme variations among populations of *Pelodiscus sinensis*.

Enzyme	E.C. number	Locus	Tissue	Buffer system
1. Aconitate hydratase	4.2.1.3	Ah	L	A,B,C
2. Asparate aminotransferase	2.6.1.1	Aat	L	A,E
3. Adenylate kinase	2.7.4.3	Ak	L	B
4. Creatine kinase	2.7.3.2	Ck	L	B
5. Fumalate hydratase	4.2.1.2	Fh	L	A,B,E
6. Guanine deaminase	3.5.4.3	Gda	L	C,D
7. Glycerol-3-phosphate dehydrogenase	1.1.1.8	Gpdh	M	A,C
8. Glucose-6-phosphate isomerase	5.3.1.9	Gpi	L,M	B,C,D
9. Isocitrate dehydrogenase	1.1.1.42	Idh-1	L,M	A,C
10. Isocitrate dehydrogenase	1.1.1.42	Idh-2	L,M	A,C
11. L-Lactate dehydrogenase	1.1.1.27	Ldh-1	L,M	A,B
12. L-Lactate dehydrogenase	1.1.1.27	Ldh-2	L,M	A,B
13. Malate dehydrogenase	1.1.1.37	Mdh	L,M	A,B
14. Malate dehydrogenase (NADP ⁺)	1.1.1.40	Me-1	L,M	B,C
15. Malate dehydrogenase (NADP ⁺)	1.1.1.40	Me-2	L,M	B,C
16. Mannose-6-phosphate isomerase	5.3.1.8	Mpi	L,M	A,B
17. Purine nucleoside phosphelase	2.4.2.1	Np	L	A,D,E
18. Peptidase (leucyl-glycyl-glycyl)	3.4.-.-	Pep-igg	L	A,D
19. Dipeptidase (leucyl-proline)	3.4.-.-	Pep-lp	L	D,E
20. Dipeptidase (leucyl-valine)	3.4.-.-	Pep-lv	L	A,D
21. Phosphogluconate dehydrogenase	1.1.1.42	Pgd	L	A
22. Phosphoglucomutase	5.4.2.2	Pgm	L,M	C,E
23. Superoxide dismutase	1.15.1.1	Sod	L	D

Tissue: L= liver, M= Muscle.

Buffer: A: CAPM6= Citrate-aminopropylmorphorine, pH 6.0 (Clayton and Tretiak, 1972); B: TC7= Tris-citrate, pH 7.0 (Show and Prasad, 1970); C: TC8= Tris-citrate, pH 8.0 (Clayton and Tretiak, 1972); D: TBE8.7= Tris-borate-EDTA, pH 8.7 (Boyer *et al.*, 1963); E LiOH: lithium hydroxide-borate, pH 8.1 (Ridgway *et al.*, 1970).

Table 3

Categories and numbers of respondents to the present questionnaire survey on each island. Categories are: A and B, persons who introduced the soft-shelled turtle into a given island, and relatives of those persons, respectively; C, town and village officers; D, soft-shelled turtle breeders; E, fishery officers of prefectural government; F, other inhabitants of a given island.

Islands		Respondents	
		Category	Number*
Amami Group	Amamioshima	B	2
		C	8
		D	2
		E	2
		F	8
	Kikaijima	A	1
		C	2
		D	3
		E	2
		F	8
	Tokunoshima	A	1
		B	1
		C	2
D		1	
E		2	
F		5	
Okinawa Group	Iheyajima	A	1
		C	1
		F	5
	Okinawajima	D	2
		E	1
		F	18
Daitou Group	Minamidaitoujima	B	1
		C	4
		F	7
Yaeyama Group	Ishigakijima	B	1
		D	2
		E	1
	Iriomotejima	F	6
		A	1
		F	6
	Yonagunijima	F	7
Total			116

*Excluding the numbers of respondents who did not know of the soft-shelled turtle at all.

Table 4

Allele frequencies at 16 polymorphic loci in 17 populations of *Pelodiscus sinensis*, with a measure of intrapopulational genetic variabilities, the expected heterozygosities (H_e , standard errors in parentheses). Sample numbers correspond to those in Table 1.

Locus	Main-islands of Japan						Ryukyu Archipelago		
	Amami Group						Okinawa Group		
	1	2	3	4	5	6	7	8	9
Aat									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.920
b	-----	-----	-----	-----	-----	-----	-----	-----	.080
Ah									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ak									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ck									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gda									
a	-----	.300	.200	.333	-----	-----	-----	.375	.480
b	1.000	.700	.800	.667	1.000	1.000	1.000	.625	.520
c	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gpi									
a	1.000	1.000	.950	1.000	1.000	1.000	1.000	1.000	1.000
b	-----	-----	.050	-----	-----	-----	-----	-----	-----
Idh-1									
a	1.000	.950	1.000	.967	1.000	1.000	1.000	1.000	1.000
b	-----	.050	-----	.033	-----	-----	-----	-----	-----
Ldh-2									
a	.391	.900	.800	.767	1.000	.958	.800	1.000	.880
b	.609	.100	.200	.233	-----	.042	.200	-----	.120
Mdh									
a	1.000	1.000	1.000	.933	.750	.958	1.000	1.000	1.000
b	-----	-----	-----	.067	.250	.042	-----	-----	-----
Me-1									
a	-----	-----	-----	-----	-----	.125	.200	1.000	1.000
b	1.000	1.000	1.000	1.000	1.000	.875	.800	-----	-----
Mpi									
a	.978	1.000	1.000	1.000	1.000	1.000	1.000	.950	1.000
b	.022	-----	-----	-----	-----	-----	-----	.050	-----
Np									
a	.348	.200	.250	.200	.500	.458	.400	.318	.650
b	.652	.800	.750	.800	.500	.500	.600	.682	.350
c	-----	-----	-----	-----	-----	.042	-----	-----	-----
Pep-Igg									
a	.087	.200	.100	.067	.000	.208	.300	.083	.140
b	.130	.250	.250	.100	.250	.167	.300	.250	.360
c	.370	.150	.200	.333	.500	.292	.200	.458	.380

d	.413	.400	.450	.500	.250	.333	.200	.208	.120
Pep-lp									
a	.870	1.000	1.000	1.000	.750	1.000	1.000	1.000	.980
b	.130	-----	-----	-----	.250	-----	-----	-----	.020
Pep-lv									
a	.283	.250	.200	.167	.500	.375	.400	.292	.240
b	.457	.550	.600	.600	.500	.208	.400	.417	.300
c	.261	.200	.200	.233	-----	.417	.200	.292	.460
Pgd									
a	-----	.100	.150	-----	-----	.208	-----	.150	.500
b	1.000	.900	.850	1.000	1.000	.792	1.000	.850	.500

<u>He</u>	.112	.115	.119	.112	.138	.119	.121	.118	.141
	(.047)	(.045)	(.045)	(.043)	(.057)	(.049)	(.052)	(.048)	(.050)

Table 4
Continued

		Daitou Group					Yaeyama Group		Taiwan	Hongkong
		10	11	12	13	14	15	16	17	

Aat										
a		1.000	1.000	.980	1.000	.750	1.000	1.000	.929	
b		-----	-----	.020	-----	.250	-----	-----	.071	
Ah										
a		1.000	1.000	.960	1.000	1.000	1.000	1.000	1.000	
b		-----	-----	.040	-----	-----	-----	-----	-----	
Ak										
a		1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	
b		-----	-----	-----	-----	-----	-----	.025	-----	
Ck										
a		1.000	1.000	.980	1.000	1.000	1.000	1.000	1.000	
b		-----	-----	.020	-----	-----	-----	-----	-----	
Gda										
a		.500	.500	.479	.375	.563	.563	.615	-----	
b		.500	.500	.521	.625	.438	.438	.385	-----	
c		-----	-----	-----	-----	-----	-----	-----	1.000	
Gpi										
a		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
b		-----	-----	-----	-----	-----	-----	-----	-----	
Idh-1										
a		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
b		-----	-----	-----	-----	-----	-----	-----	-----	
Ldh-2										
a		.938	.941	.580	.750	.938	.938	.500	.857	
b		.063	.059	.420	.250	.063	.063	.500	.143	
Mdh										
a		1.000	.971	1.000	1.000	1.000	1.000	1.000	1.000	
b		-----	.029	-----	-----	-----	-----	-----	-----	
Me-1										
a		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
b		-----	-----	-----	-----	-----	-----	-----	-----	
Mpi										
a		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
b		-----	-----	-----	-----	-----	-----	-----	-----	
Np										
a		.375	.469	.400	.500	.625	.600	.275	.571	
b		.625	.531	.600	.500	.375	.400	.475	.429	
c		-----	-----	-----	-----	-----	-----	.250	-----	
Pep-igg										
a		.250	.176	.340	.375	.188	.125	.025	-----	
b		.438	.500	.240	.500	.375	.438	.375	.429	
c		.250	.059	.340	.125	.375	.375	.325	.429	
d		.063	.265	.080	-----	.063	.063	.275	.143	
Pep-lp										

a	1.000	1.000	.940	1.000	1.000	1.000	.975	1.000
b	-----	-----	.060	-----	-----	-----	.025	-----
Pep-iv								
a	.250	.088	.340	-----	.063	.125	.125	.429
b	.250	.353	.460	.875	.125	.188	.525	.143
c	.500	.559	.200	.125	.813	.688	.350	.429
Pgd								
a	.375	.353	.400	.250	.875	-----	.700	.857
b	.625	.647	.600	.750	.125	1.000	.300	.143

He	.133	.127	.158	.126	.124	.104	.152	.109
	(.052)	(.048)	(.052)	(.047)	(.045)	(.046)	(.053)	(.045)

Table 5

Matrix of Nei's (1978) unbiased genetic distance between 17 samples of *Pelodiscus sinensis*.

population	1	2	3	4	5	6	7	8	9	10	11
1. Sizuoka CL	-----										
2. Hiroshima CL	.017	-----									
3. Saga CL	.010	.000	-----								
4. Amami CL	.012	.000	.000	-----							
5. Amamioshima	.007	.000	.000	.003	-----						
6. Kikaijima	.020	.009	.008	.016	.000	-----					
7. Tokunoshima	.007	.002	.001	.008	.000	.000	-----				
8. Iheyajima	.076	.050	.053	.055	.048	.045	.036	-----			
9. Okinawajima	.098	.075	.074	.083	.073	.056	.057	.011	-----		
10. Okinawajima	.091	.059	.063	.070	.069	.051	.046	.001	.000	-----	
11. Minamidaitoujima	.095	.062	.064	.072	.075	.055	.051	.008	.004	.000	-----
12. Ishigakijima CL	.076	.065	.064	.070	.072	.062	.048	.011	.009	.005	.014
13. Ishigakijima	.078	.056	.056	.065	.063	.061	.039	.009	.012	.006	.007
14. Iriomotejima	.145	.112	.112	.125	.125	.086	.101	.041	.008	.014	.018
15. Yonagunijima	.094	.070	.074	.074	.071	.058	.051	.007	.009	.003	.006
16. Taiwan	.101	.087	.082	.090	.107	.087	.082	.031	.015	.017	.019
17. Hongkong	.162	.135	.134	.148	.137	.112	.121	.065	.041	.047	.055

Table 5
Continued

population	12	13	14	15	16	17
12. Ishigakijima CL	-----					
13. Ishigakijima	.003	-----				
14. Iriomotejima	.033	.043	-----			
15. Yonagunijima	.022	.017	.033	-----		
16. Taiwan	.010	.019	.024	.040	-----	
17. Hongkong	.057	.073	.039	.072	.051	-----

Table 6

The origin and introduced year of *Pelodiscus sinensis*, calculated from the results of questionnaire surveys.

Region Island	Local name	Original population	the Year of first record	Introduced year	Source population
Amami Group					
Amamioshima	none	none	1997 ⁽¹⁾	1979	Main-islands of Japan*
Kikaijima	none	none	1997 ⁽¹⁾	1976	Main-islands of Japan
Tokunoshima	none	none	1997 ⁽¹⁾	1976	Main-islands of Japan
Okinawa Group					
Iheyajima	none	none	1997 ⁽²⁾	1979	Taiwan**
Okinawajima	none	none	1984 ⁽³⁾	1950's	Taiwan
Daitou Group					
Minamidaitoujima	none	none	1997 ⁽²⁾	1962	Taiwan**
Yaeyama Group					
Ishigakijima	none	none	1935 ⁽²⁾	1950's 1970's	Taiwan Taiwan
Iriomotejima	none	none	1979 ⁽⁴⁾	1970's 1980's	Taiwan** Taiwan**
Yonagunijima	none	none	1984 ⁽²⁾	1950's	Taiwan

⁽¹⁾: Sato unpublished data.

⁽²⁾: See Sato *et al.* (1997) for further details.

⁽³⁾: Shokita (1984).

⁽⁴⁾: Sengoku (1979).

*: Through a cultivation pond on Kikaijima.

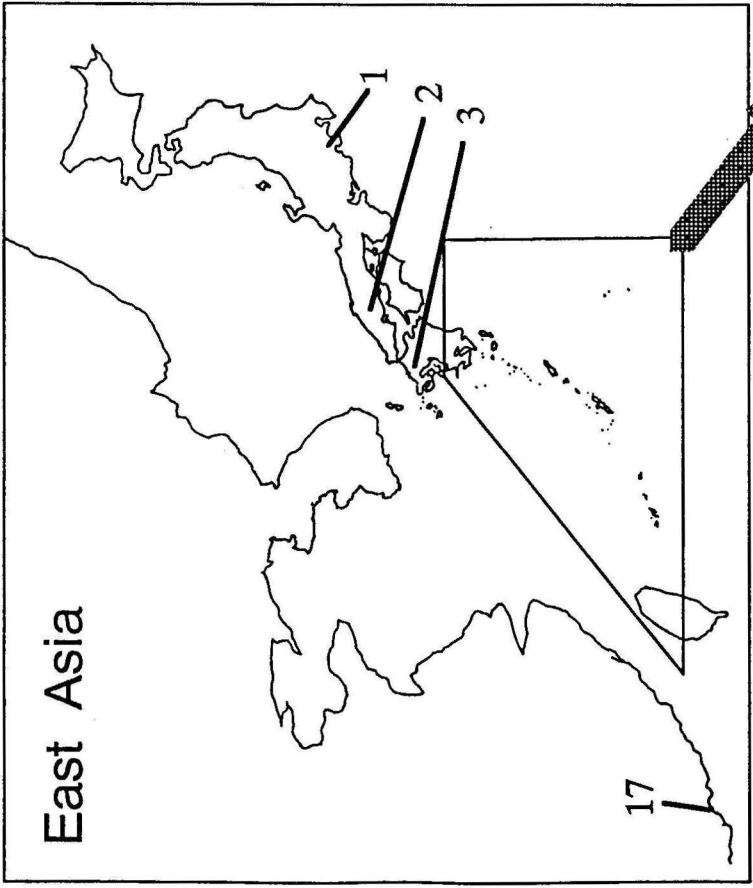
** : Through a cultivation pond on Ishigakijima.

Figure 1. Map of East Asia showing the location of the Ryukyu Archipelago. Locality numbers refer to those in Table 1. I, the Amami Group; II, the Okinawa Group; III, the Daito Group; IV, the Yaeyama Group.

Figure 2. Diagrams showing allele frequencies at locus Me-1 in 17 populations of *Pelodiscus sinensis* .

Figure 3. UPGMA dendrogram for 17 populations of *Pelodiscus sinensis* based on Nei's (1978) unbiased genetic distance data for 23 loci.

Figure 4. Schematic diagram representing the estimated timings and routes of introductions of *Pelodiscus sinensis* to the Ryukyu islands.



Main-islands of Japan

