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Population differentiation of the endangered salt-marsh snail *Ellobium chinense* in Japan (Gastropoda: Ellobiidae)

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Abstract: *Ellobium chinense* is a red-listed snail species of the family Ellobiidae with a geographic distribution from Vietnam and south coast of China to South Korea and mainland Japan. This species is restricted to specialized habitats in a narrow upper-intertidal to lower-supratidal zone of salt marshes and thus particularly sensitive to environmental degradation through land reclamation and other human activities. Here, we first report the genetic diversity and population structure of *E. chinense* in Japan to evaluate the connectivity and conservation value of its local populations. Specimens were collected from seven localities (Tsu, Okayama, Yamaguchi, Usa, Imari, Saga and Izumi) that cover the species' present distribution in the country. Analyses of 612-bp sequences of the mitochondrial cytochrome *c* oxidase subunit I gene showed high genetic diversity within populations and a reasonable level of connectivity among populations. However, significant genetic differentiation was detected among distant geographic regions in Japan and South Korea, due potentially to the disjunct distribution of habitable salt marshes and a short pelagic larval period of the species. The population of the Ise–Mikawa Bay area, representing the eastern limit of the current distribution range, showed the highest level of genetic differentiation and deserve particular conservation efforts to avoid local extinction, which occurred in Tokyo Bay area in the last century.

Key words: conservation, disjunct distribution, larval dispersal, marginal population, mitochondrial COI gene

Introduction

Population genetics provides invaluable information, including genetic diversity and population connectivity, for the conservation and management of endangered species (Lande 1988, O'Brien 1994, DeSalle & Amato 2004). Genetic diversity is the raw material for evolutionary changes in response to environmental changes (Frankham 1996). Recent human-induced habitat loss and population fragmentation, however, often result in reduced genetic diversity and diminished resilience in rapidly changing environments (Williams 2001, Reusch et al. 2005). Geographically marginal populations would be more strongly affected by such habitat loss and fragmentation than central populations, especially with a low dispersal capability of individuals (Kawecki 2008).

The gastropod family Ellobiidae comprises nearly 300 described species in the tropical to temperate regions worldwide (Martins 1996, WoRMS 2021). Many species inhabit intertidal to supratidal zones of salt marshes, mangrove swamps and rocky shores (Morton 1955, Martins 1996), whereas at least two lineages have independently colonized the land and become fully terrestrial (Dayrat et al. 2011, Romero et al. 2016). Salt marshes and mangrove forests are vulnerable environments and decreasing in area through land reclamation and other human activities (Lotze et al. 2006). Loss and fragmentation of these habitats have also been extensive in Japan for several decades (Hanawa 2006). This has led a number of ellobiids designated as endangered species by the Japanese Association

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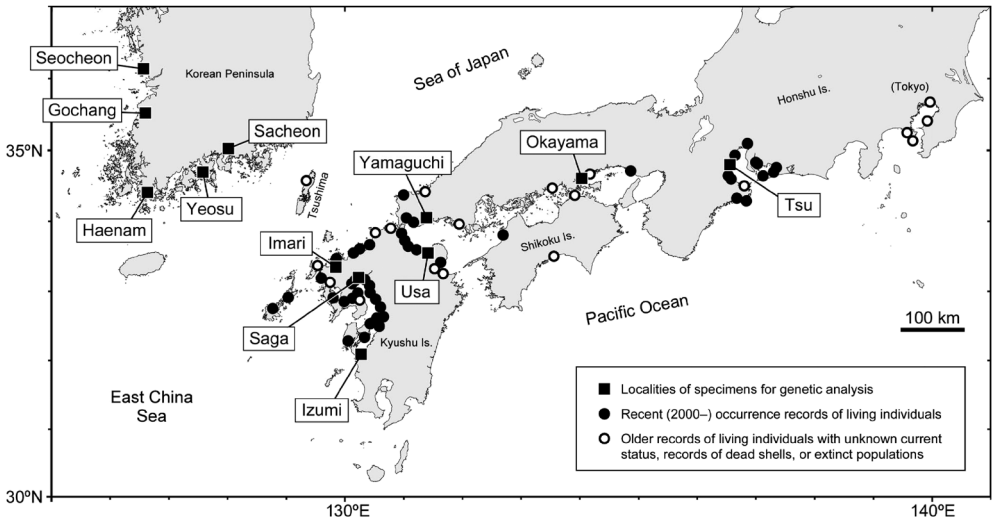

Fig. 1. Geographic distribution of *Ellobium chinense* in Japan. Solid squares denote collection sites of specimens used in present genetic analysis; Korean specimens were collected and studied by Yi et al. (2019). Solid and open circles indicate recent and old occurrence records, respectively (see Data S1 for details).

Table 1. Localities and numbers of specimens used in population genetic analysis of *Ellobium chinense.*

Population	Locality	Coordinates	N	Specimen voucher numbers, AORI_YK#
Japan				
Tsu	Tanaka R., Higashi-chisato, Kawage, Tsu, Mie	34°48′N, 136°34′E	15	4010–4014, 4050–4053, 4067–4072
Okayama	Yoshii R., Otogo, Okayama	34°38′N, 134°02′E	16	4218–4227, 4249–4254
Yamaguchi	Himiori R., Kagawa, Yamaguchi	34°04′N, 131°24′E	10	4228–4237
Usa	Yorimo R., Usa, Oita	33°34′N, 131°25′E	8	4054–4060, 4073
Imari	Susuya, Hatazu, Imari, Saga	33°21′N, 129°51′E	15	4018, 4019, 4075–4082, 4260–4264
Saga	Honjoe R., Nishiyoka, Saga	33°12′N, 130°15′E	15	4015–4017, 4061–4066, 4074, 4255–4259
Izumi	Takaono R., Sho, Izumi, Kagoshima	32°06′N, 130°17′E	8	4238–4245
South Korea*				
Sacheon	Gonyang-myeon, Sacheon, South Gyeongsang	35°03′N, 128°01′E	5	
Yeosu	Hwayang-myeon, Yeosu, South Jeolla	34°42′N, 127°35′E	5	
Haenam	Bukpyeong-myeon, Haenam, South Jeolla	34°25′N, 126°38′E	5	
Gochang	Buan-myeon, Gochang, North Jeolla	35°32′N, 126°36′E	5	
Seocheon	Seo-myeon, Seocheon, South Chungcheong	36°08′N, 126°34′E	5	

*Data from Yi et al. (2019).

of Benthology (Kimura 2012) and Japanese Ministry of the Environment (2020).

Ellobium chinense (Pfeiffer, 1855) is one of the red-listed ellobiid species with a geographic distribution from Northern Vietnam and south coast of China to South Korea and mainland Japan (Kurozumi 1997, Wu 2004, Xiutong 2004, Kimura 2012, Nam 2017, Ma et al. 2018, Li et al. 2020). Various morphological, ecological, environmental and genetic studies have been conducted for this species (e.g. Sumikawa & Miura 1978, Sumikawa 1983, Yamashita et al. 2002, Kimura 2011, Lim et al. 2015, Chen et al. 2017, Kang et al. 2018, Yi et al. 2019, Li et al. 2020, Shin et al. 2021). It occurs in a narrow upper-intertidal to lower-supratidal zone of coastal salt marshes and estuaries with reed beds or other types of vegetation (Yamashita et al. 2002, Kimura 2011). With such specialized habitat requirements, *E. chinense* seems to be particularly sensitive to environmental changes and degradation. The populations of Tokyo Bay area once represented the easternmost extension of the species' distribution (Kobelt 1879, Kuroda 1957) but became extinct sometime in the 20th century (Fig. 1; Kurozumi 1997, Ikeda et al. 2001). This species is currently listed as Vulnerable in the Japanese Red Lists (Japanese Ministry of the Environment 2020), Red Data Book of Seashore Benthos in Japan (Kimura 2012) and Korean Red List of Threatened Species (Suh et al. 2014), and Endangered or Critically Endangered in red lists of several prefectures in Japan (Aichi, Hyogo, Ehime, Saga, Nagasaki, Oita and Kagoshima).

Recent studies on *E. chinense* have revealed high genet-

ic diversity and panmixia along an approximately 400-km stretch of the southwest coast of South Korea, presumably through dispersal of pelagic larvae by the branches of the Kuroshio current (Yi et al. 2019, Shin et al. 2021). On the other hand, Japanese populations have not been the subject of a genetic survey despite the allegedly endangered status and conservation efforts. The patchy distribution of habitats in a wider geographic area (over 1,000 km along the coastline) suggests a potential of genetic differentiation in Japan, especially in the remote populations of Ise Bay and Mikawa Bay, central Honshu, which now represent the easternmost extension of the species' range (Fig. 1). Here, we report the genetic diversity and population structure of *E. chinense* in Japan to evaluate the connectivity and conservation value of its local populations.

Materials and Methods

A total of 87 specimens of *Ellobium chinense* were collected from seven localities in four regions, which span the current geographic range of the species in Japan (Table 1, Fig. 1, Data S1). The four regions include (1) Ise–Mikawa Bay area in central Honshu, herein represented by the population of Tsu, (2) eastern part of Seto Inland Sea including Okayama, (3) Suo-nada or the western part of Seto Inland Sea, which encompasses Yamaguchi and Usa populations, and (4) west coast of Kyushu Island including Imari, Saga and Izumi. These regions were set by referring to the patchiness of the populations (Fig. 1), oceanographic characteristics (Kawai 1972, Sakamoto et al. 2016), and genetic structures of other salt-marsh species (e.g. Kojima et al. 2004, Yuhara et al. 2014).

Field sampling was made by the authors in 2020 for all populations except Usa, which was sampled by YK in 2004. Collected snails were boiled in 70–90℃ water for 0.5–1 min, preserved in 99% ethanol and deposited at Atmosphere and Ocean Research Institute, The University of Tokyo (AORI), with voucher numbers AORI_YK#4010–4019, 4050–4082, 4218–4245 and 4249–4264 (Table 1). The shells of all sequenced specimens were measured for height and diameter to 0.1 mm precision with a digital caliper. All sequenced specimens, except for one from Usa, had a fully-grown shell with a more or less eroded surface and a thickened outer lip of the aperture (Fig. 2). These adult shells share the same characteristics of the species in shape and sculpture (see e.g. Kurozumi 1997), but the height and width varied among the populations (Table S1).

Total DNA was extracted from the foot tissue of the 87 specimens using DNeasy Tissue Extraction Kit (Qiagen); several samples were then purified with GeneReleaser (Bioventures). Fragments of 706 base pairs (bp) of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene were amplified by the polymerase chain reaction (PCR) using the primer pair LCO1490 and HCO2198 (Folmer et al. 1994). Each reaction mixture contained 15.8–17.3 μL DDW, 2.5 μL EX Taq buffer (10x), 2.0 μL dNTP mixture (2.5 μM each), 0.3 μL of each forward and reverse primer (20 μM), 0.13 μL Ex Taq or Ex Taq Hot Start Version (TaKaRa) and 2.5–4.0 μL genomic DNA. PCR reactions were carried out with an initial denaturation step at 94.5℃ for 2 min, followed by 35 cycles consisting of a denaturation step at 94.5℃ (30 s), an annealing step at 42℃ (40 s) and an extension step at 72℃ (60 s). Amplicons were purified by ExoSAP-IT or ExoSAP-IT express (Thermo Fisher Scientific) following the described protocol. Sequencing reactions were prepared using a Big Dye Terminator Cycle Sequence Kit 3.1 (Applied Biosystems). The reaction mixtures were purified with a Big Dye XTerminator Purification Kit (Applied Biosystems) and then analyzed on ABI PRISM 3130xl sequencers. Obtained sequences were aligned and trimmed to exclude the amplification primers in AliView (Larsson 2014). The final 655-bp sequences have been deposited in DDBJ/ENA/GenBank databases with accession numbers LC641717–LC641757 (see Data S2 for original data in fasta format).

In addition, 25 published COI sequences of *E. chinense* were retrieved from DDBJ/ENA/Genbank. These sequences, 631 bp in length, were determined by Yi et al. (2019) for five populations (five specimens each) in South Korea, and had a 612-bp overlapping region with our sequences from Japanese specimens. These allowed us to analyze the population genetic structure of *E. chinense* based on a total of 112 individuals from 12 populations in five regions in Japan and South Korea (Table 1).

The numbers of haplotypes and polymorphic sites were counted and haplotype diversity h and nucleotide diversity π (Nei 1987) were calculated for each of the 12 populations and five regions in ARLEQUIN 3.5 (Excoffier & Lischer 2010). Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was also performed in ARLEQUIN to partition the total genetic variation into (1) within populations, (2) among populations within regions, and (3) among regions, based on Kimura 2-parameter genetic distances (K2P; Kimura 1980); statistical significance of estimated variance components was assessed based on 10,000 permutations. Estimates of pairwise F_{ST} and exact test of differentiation (Raymond & Rousset 1995, Excoffier & Lischer 2010) were then conducted to assess the pattern of genetic differentiation among populations and regions, with 10,000 permutations (F_{ST}) or 100,000 Markov chain steps (exact test) for p-values. The five populations in South Korea were excluded from the estimates of pairwise F_{ST} and exact test of differentiation among populations because of small sample sizes. False discovery rate (FDR) q-values were calculated to account for multiple comparisons based on the Benjamini-Hochberg procedure (Benjamini & Hochberg 1995) in the *qvalue* package version 2.22.0 (Storey et al. 2020) in R version 4.0.4 (R Core Team 2021). Finally, a parsimony-based haplotype network was reconstructed using TCS method (Clement et al. 2002) in PopART (Leigh & Bryant 2015).

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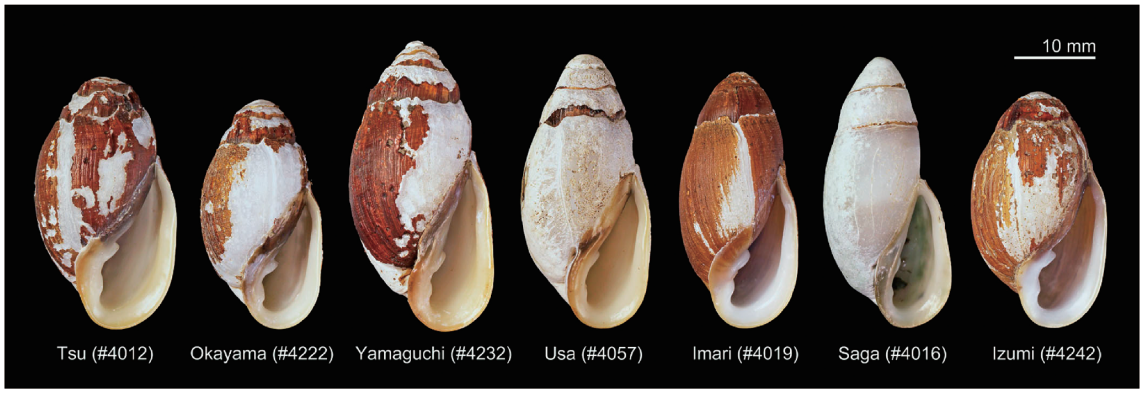

Fig. 2. Shells of *Ellobium chinense* in apertural view, representing seven Japanese populations compared in present genetic analysis. Numbers denote specimen voucher numbers (see Table 1).

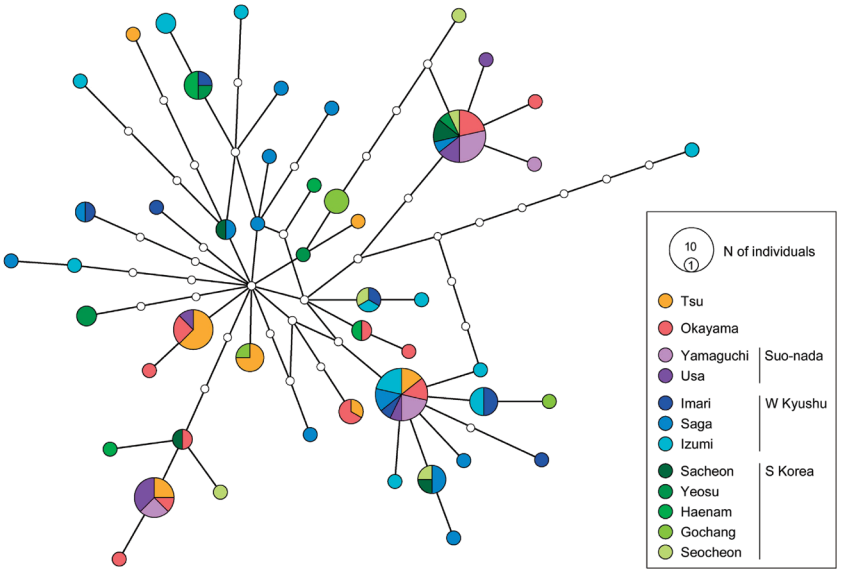

Fig. 3. Haplotype network for *Ellobium chinense* based on 612-bp sequences of mitochondrial COI gene. Circle sizes reflect haplotype frequency; colors denote geographic origins of haplotypes. Each line represents one mutational step; small open circles indicate undiscovered haplotypes.

sequences for 114 additional individuals became available in DDBJ/ENA/Genbank (Shin et al. 2021) for populations in the same stretch of South Korea's coastline as studied by Yi et al. (2019). These new sequences were even shorter (595 bp) and thus incorporated into separate analyses to verify that a resulting haplotype network, pairwise F_{ST} values and *p*-values of exact test do not contradict those of the main analyses regarding the differentiation of Japanese populations.

Results

The analysis of 612-bp mitochondrial COI sequences showed high genetic diversity in *E. chinense* (Table 2). A total of 48 COI haplotypes with 62 polymorphic sites were identified among 112 specimens from 12 populations in five regions. Each population showed a moderate to high haplotype diversity (h: 0.7–1) with a nucleotide diversity (π) ranging from 0.006 to 0.011 (Table 2). Haplotype diversity was relatively low in the populations of Tsu, Yamaguchi, Usa and Gochang ($h \leq 0.857$); the populations of Tsu and Gochang also showed low nucleotide diversity ($\pi = 0.006$).

The haplotype network consisted of 14 shared and 34 singleton haplotypes (Fig. 3). These haplotypes differed by one to 13 substitutions from each other. The two most common haplotypes, 14 individuals each, consisted of either all seven populations in Japan, or seven populations in Japan or South Korea. The two second most common haplotypes (8 individuals each) were obtained exclusively from the Suo-nada region and eastward.

The results of the hierarchical AMOVA showed that (1) 93.64% of the total genetic variation occurred within populations, (2) −0.49% occurred among populations within

Table 2. Genetic diversity in 612-bp mitochondrial COI sequences of *Ellobium chinense*.

Region: Population	*N*	*Nh*	*K*	*h*	π
Japan	87	40	56	0.944±0.013	0.009±0.005
Tsu	15	7	16	0.857±0.065	0.006±0.004
Okayama	16	11	18	0.950±0.036	0.008±0.005
Suo-nada	18	6	13	0.797±0.056	0.008±0.005
Yamaguchi	10	4	11	0.778±0.091	0.008±0.005
Usa	8	5	12	0.857±0.108	0.009±0.005
Western Kyushu	38	26	44	0.964±0.018	0.009±0.005
Imari	15	11	27	0.952±0.040	0.010±0.006
Saga	15	13	25	0.981±0.031	0.008±0.005
Izumi	8	7	16	0.964±0.077	0.008±0.005
South Korea*	25	16	25	0.953±0.024	0.008±0.005
Sacheon	5	4	11	0.900±0.161	0.009±0.006
Yeosu	5	4	10	0.900±0.161	0.008±0.005
Haenam	5	4	10	0.900±0.161	0.008±0.005
Gochang	5	3	8	0.700±0.218	0.006±0.004
Seocheon	5	5	14	1.000±0.127	0.011±0.007
All	112	48	62	0.955±0.009	0.009±0.005

N, number of individuals; *Nh*, number of haplotypes; *K*, number of polymorphic sites; *h*, haplotype diversity; π, nucleotide diversity. *Data from Yi et al. (2019).

Table 3. Sources of genetic variation in *Ellobium chinense* identified by hierarchical AMOVA.

Source of variation	d.f.	% of variation	Fixation indices	*p*
Within populations	100	93.64	—	—
Among populations within regions	7	−0.49	F_{SC}: −0.005	0.610
Among regions	4	6.85	F_{CT}: **0.069****	**0.003****

*$p<0.05$, **$p<0.01$.

Table 4. Genetic differentiation among seven Japanese populations. Lower left and upper right triangles show pairwise F_{ST} values and *p*-values of exact test, respectively; significant value (with false discovery rate *q*-value of <0.05) shown in bold. Figure in parentheses indicates number of individuals sampled from each population.

	Tsu	Okayama	Yamaguchi	Usa	Imari	Saga	Izumi
Tsu (15)	—	0.215	0.015	0.213	0.007	0.008	0.009
Okayama (16)	0.036	—	0.659	0.881	0.050	0.241	0.101
Yamaguchi (10)	**0.215***	0.005	—	0.624	0.045	0.284	0.032
Usa (8)	0.104	−0.035	−0.040	—	0.074	0.423	0.093
Imari (15)	0.095	0.049	0.093	0.106	—	0.245	0.810
Saga (15)	0.078	0.041	0.104	0.114	−0.017	—	0.646
Izumi (8)	0.085	0.045	0.118	0.128	−0.051	−0.039	—

*$q<0.05$.

regions, and (3) 6.85% occurred among regions (Table 3). Significant genetic differentiation was detected among all five regions (F_{CT}=0.069, p=0.003) and among all 12 populations (F_{ST}=0.064, p=0.002), but this was not the case among populations within regions (F_{SC}=−0.005, p=0.61).

Pairwise F_{ST} values among the seven populations in Japan were generally low (−0.051–0.215; Table 4). Eight out of the 21 comparisons showed significant *p*-values (Table S2), but only one pair (Tsu–Yamaguchi) was significantly different after FDR correction (F_{ST}=0.215, q=0.021; Table S3). Pairwise F_{ST} among the five regions of Japan and South Korea showed significant differentiation in six out of the 10 comparisons after FDR correction (F_{ST}: 0.052–0.164; Table 5, Supplementary Tables S4, 5). Western Kyushu differed significantly from all other regions (F_{ST}: 0.052–0.124); Tsu also differed from Suo-nada and

Table 5. Genetic differentiation among five geographic regions. Lower left and upper right triangles show pairwise F_{ST} values and p-values of exact test, respectively; significant values (with false discovery rate q-values of <0.05) shown in bold. Figure in parentheses indicates number of individuals sampled from each region.

	Tsu	Okayama	Suo-nada	W Kyushu	South Korea
Tsu (15)	—	0.206	**0.005***	**0.003****	**0.000****
Okayama (16)	0.036	—	0.194	0.041	0.070
Suo-nada (18: Yamaguchi, Usa)	**0.164***	−0.001	—	**0.006***	**0.002****
W Kyushu (38: Imari, Saga, Izumi)	**0.081***	**0.053***	**0.124****	—	**0.002****
South Korea (25)	**0.068***	−0.001	0.042	**0.052***	—

*q<0.05, **q<0.01.

South Korea (F_{ST}: 0.068–0.164).

The exact test of differentiation based on haplotype frequencies indicated genetic differentiation among populations or regions in a consistent manner with the pairwise F_{ST} estimates. Six comparisons among populations, including four with Tsu population, showed p-values of <0.05 (Table 4) but none was found significant after FDR correction (q>0.05; Table S3). At the regional level, Okayama was not significantly different from the other four (p>0.05; Table 5), whereas all remaining comparisons detected significant differentiation even after FDR correction (Table S5).

Separate analyses of 595-bp sequences from 226 individuals resulted in a haplotype network shown in Fig. S1. Pairwise F_{ST} values and p-values of exact test showed the same trend with the main analyses for the four Japanese regions, notably the differentiation of Tsu population (Table S6).

Discussion

The present study based on the mitochondrial COI sequences first revealed high genetic diversity in the Japanese populations of the endangered salt-marsh snail *Ellobium chinense* (Table 2). The overall haplotype and nucleotide diversity values were higher than those of many other salt-marsh and tidal-flat species in Japan, including batillariid snails (Kojima et al. 2003, 2004) and sesarmid crabs (Kawane et al. 2008, Yuhara et al. 2014). The low pairwise F_{ST} values among populations in Japan and South Korea (Table 4) suggest a relatively high dispersal capability of *E. chinense* thanks to its pelagic rather than direct development (Kimura 2011). The presence or absence of a pelagic period fundamentally determines species' dispersal capability and consequently the level of population differentiation in benthic invertebrates (Hedgecock 1986, Collin et al. 2001, Kojima et al. 2004). High dispersal capability also contributes to increased population persistence and genetic diversity within populations (Ellingson & Krug 2016, Bani et al. 2019).

However, the hierarchical AMOVA revealed significant genetic differentiation at the levels of population and region (Table 3). The pairwise F_{ST} and exact test also detected significant differentiation between six out of 10 pairs of regions (q<0.05; Table 5), although only one F_{ST} value was significant in comparisons among Japanese populations probably due to limited numbers of individuals sequenced (8–16 per population; Table 4). The size and proportion of the shell also varied among the populations (Fig. 2; Table S1), although no direct relationship was observed between the patterns of differentiation in the shell traits and mitochondrial haplotypes.

This relatively high level of genetic differentiation in Japan exhibits a stark contrast to the panmictic population structure along the southwest coast of the Korean Peninsula (Yi et al. 2019, Shin et al. 2021). A simple explanation for this discrepancy is the different sizes of species' distribution ranges in the two countries. Geographic distances between the sequenced populations in Japan reach up to 1,080 km along the coastline (Tsu–Saga; Fig. 1), which is about 2.5 times the distance between the two most remote populations in South Korea (Sacheon–Seocheon, 410 km). Another possibility is the patchy, disjunct distribution of populations in Japan (Fig. 1) and more continuous occurrences of the species between Sacheon and Seocheon (Fig. S1; Shin et al. 2021).

The patchy distribution in Japan, especially in the central part of Seto Inland Sea and eastward, seems to be attributable to both natural and anthropogenic causes. *Ellobium chinense* has a specific habitat requirement in coastal salt marshes and estuaries, occurring in a narrow upper-intertidal to lower-supratidal zone with reed beds or other types of vegetation. This zone is as narrow as 0.4–0.5 m in height, presumably to archive a balance between their aerial respiration as metamorphosed snails and deposition of eggs that hatch as swimming larvae (Yamashita et al. 2002, Kimura 2011). Another unique aspect of the species is obligate winter hibernation in the soil at a slightly higher level (Sugimura 1994; KI & YK, personal observation). These ecological conditions are met only on coasts in sheltered sea areas without degradation through land reclamation and other types of human activities.

The population of Tsu in the Ise–Mikawa Bay area showed significant genetic differentiation from all other regions except the geographically closest Okayama (Table 5). The Ise–Mikawa Bay area represents the eastern limit

of the species' current geographic range, with a distance of more than 300 km to the nearest known locality (Hyogo, Seto Inland Sea; Matsumura 2007) along the coast of Kii Peninsula (Fig. 1). Populations at margins of geographic ranges typically have low levels of genetic diversity and gene flow and are more vulnerable to habitat loss and fragmentation than central populations (Lesica & Allendorf 1995, Guo et al. 2005). *Ellobium chinense* has actually gone extinct from the Tokyo Bay area, which once represented the eastern margin of the species' range. The only clear record from the bay dates back to the 1870s (Kobelt 1879); mollusk collections in the National Museum of Nature and Science, Tokyo do not contain any specimens from this area (K. Hasegawa, personal communication), suggesting its rarity or absence already in the early 20th century. Similarly, no specimen has been collected alive in the nearby Miura Peninsula since the 1930s (Ikeda et al. 2001). *Ellobium chinense* in the Ise–Mikawa Bay area thus seems to deserve particular conservation efforts to avoid local extinction and further shrinking of the species' distribution range. The relatively high genetic diversity observed in Tsu population (Table 2), presumably reflecting higher abundance in the recent past in this Ise–Mikawa Bay area (Kanamaru 1967, Hanawa 2006), would enable future population recovery with indigenous genetic resources.

Summing up, the present study on *E. chinense* revealed not only high genetic diversity within its populations but also differentiation among geographic regions in Japan. The latter finding underscores the importance of conservation efforts at local and regional scales in the maintenance of connectivity and overall genetic diversity of the species. The details of larval development are also important but still missing information for *E. chinense*. The differentiation of populations in a few hundred kilometers, detectable with a mitochondrial marker, implies that the pelagic larval duration (PLD) of the species is not exceedingly long, presumably in the order of days or weeks, not months (see e.g. Kojima et al. 2005, Dawson et al. 2014). Similar implications have been found in population genetic studies of benthic animals on Japanese salt marshes and tidal flats. *Batillaria multiformis* and *Orisarma dehaani*, both with a PLD of some 20 days, showed no structure in mainland Japan (Baba & Miyata 1971, Kojima et al. 2003, Kawane et al. 2008), whereas *Clistocoeloma sinense* (10 days) and *Austruca lactea* (≥18 days) exhibited genetic differentiation at geographic scales equal to or even smaller than *E. chinense* (Muraoka 1976, Terada 1979, Yuhara et al. 2014, Tokuyama et al. 2020). The presence or absence of feeding (planktotrophy), swimming behavior and physiological constraints are also important aspects of larval dispersal and population connectivity for benthic invertebrates (see Morgan 1995, Yahagi et al. 2017, Pineda & Reyns 2018).

Lastly, there is no sequence data so far published for populations in Vietnam, Taiwan and mainland China. The southwestern limit of this species apparently occurs in Halong Bay (Nam 2017) and Hainan Island (Ma et al. 2018) and distribution range along the coast of China extends northward at least to the northern part of Zhejiang Province (Xiutong 2004). The continuity and genetic characteristics of populations along this 2,000-km stretch of coastline remain to be elucidated for a comprehensive understanding of evolutionary and demographic history of *E. chinense*.

Electronic supplementary material

The online version of this article (doi: 10.3800/pbr.17.66) contains supplementary material:

Fig. S1. Haplotype network for *Ellobium chinense* based on 595-bp sequences of mitochondrial COI gene. Circle sizes reflect haplotype frequency; colors denote geographic origins of haplotypes. Each line represents one mutational step; small open circles indicate undiscovered haplotypes. See Shin et al. (2021) for details of Korean individuals.

Table S1. Shell measurements for seven Japanese populations of *Ellobium chinense*. Figure in parentheses denotes number of individuals (see Table 1).

Table S2. *P*-values for pairwise F_{ST} among seven Japanese populations; *p*-values below 0.05 are shown in bold. Figure in parentheses indicates number of individuals sampled from each population.

Table S3. False discovery rates (*q*-values) for *p*-values of pairwise F_{ST} (lower left) and exact test (upper right) among seven Japanese populations; significant value ($q<0.05$) shown in bold. Figure in parentheses indicates number of individuals sampled from each population.

Table S4. *P*-values for pairwise F_{ST} among five geographic regions; *p*-values below 0.05 are shown in bold. Figure in parentheses indicates number of individuals sampled from each region.

Table S5. False discovery rates (*q*-values) for *p*-values of pairwise F_{ST} (lower left) and exact test (upper right) among five geographic regions; significant values ($q<0.05$) shown in bold. Figure in parentheses indicates number of individuals sampled from each population.

Table S6. Genetic differentiation among four Japanese and eight Korean regions based on 595-bp sequences of mitochondrial COI gene. Lower left and upper right triangles show pairwise F_{ST} values and *p*-values of exact test, respectively; significant values shown in bold. Figure in parentheses indicates number of individuals sampled from each region. See Shin et al. (2021) and Figure S1 for abbreviations for Korean regions and other details.

Data S1. References for additional occurrence records of *Ellobium chinense* in Japan. All websites were accessed on 31 January 2022.

Data S2. Fasta file containing 655-bp COI sequences from 87 Japanese specimens of *Ellobium chinense* with reference to specimen voucher numbers.

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