Journal of the Marine Biological Association of the United Kingdom, 2013, 93(3), 789–795.
doi:10.1017/S0025315412001178

Genetic structure of the wide-ranging fiddler crab *Uca crassipes* in the west Pacific region

MISUZU AOKI AND KEIJI WADA

KYOUSEI Science Center for Life and Nature, Nara Women's University, Kitauoya-higashimachi, Nara 630-8506, Japan

The genetic relationship between fiddler crab Uca crassipes *populations from the continental coast, continental islands, and oceanic islands in the west Pacific was investigated using 1039 bp (base pairs)-long combined 12Sr-RNA–16Sr-RNA sequences and a 504-bp mitochondrial DNA control region. The combined 12Sr-RNA–16Sr-RNA sequences indicated that the Vietnamese population, located along the continental coast, and the Chichi-jima population, which is located on an oceanic island north of the Northern Mariana Islands, formed different clades than populations from the other Ryukyu Islands and Moorea Island. Conversely, the Ryukyu Islands and Moorea Island populations exhibited a close genetic relationship, although the mtDNA control region indicated significant differentiation between the Ryukyu Islands and Moorea Island populations. The isolated Vietnam and Chichi-jima populations exhibited higher genetic diversity in the control region than the other populations.*

Keywords: genetic structure, control region, fiddler crab, west Pacific

Submitted 27 May 2012; accepted 25 June 2012; first published online 24 September 2012

INTRODUCTION

Fiddler crabs of the genus *Uca* in the family Ocypodidae inhabit the intertidal to supratidal zone of tropical to temperate regions worldwide. Most are distributed in marginal seas, but species ranging from the continental coast to oceanic islands are limited (*Uca tetragonon* (Herbst) and *U. crassipes* (Adams & White)) (Crane, 1975). Given their wide range, they are suspected to have greater dispersal during the larval stage and higher survival rates after settlement than other *Uca* spp. However, no studies have explored the cause of their wide distribution. Genetic structure analysis is useful for estimating larvae dispersal among populations based on gene flow. Several studies have examined the genetic structure of marine invertebrates with wide distributions (Benzie & Williams, 1995; Lavery *et al.*, 1996; Palumbi, 1996; Benzie, 1999; Gopurenko *et al.*, 1999), some of which have suggested great larvae dispersal in the Pacific Ocean (Benzie & Williams, 1995; Lavery *et al.*, 1996; Benzie, 1999; Gopurenko *et al.*, 1999). Some researches have addressed the intraspecific genetic structure of marine invertebrates from the continental coast through the continental islands, and to oceanic islands (Williams & Benzie, 1998; Meyer *et al.*, 2005), but such research has not been conducted for intertidal brachyuran crabs.

Uca crassipes is distributed from the western Pacific Ocean to the Indian Ocean (Crane, 1975). In Japan, *U. crassipes* is distributed only in the continental Ryukyu Islands and the oceanic Bonin Islands, which are separated by about 1500 km. Fukuda (1993, 1994) compared the species composition of marine gastropods in the Bonin Islands with those in three other island groups: the Izu Islands, 700 km north; the Ryukyu Islands, 1500 km west; and the Northern Mariana Islands, 1000 km south of the Bonin Islands. These studies showed that most species from the Bonin Islands were common to the Ryukyu Islands. This implies long larvae dispersal along the oceanic current running from the Ryukyu to the Bonin Islands; however, marine invertebrate genetic relationships between the two island groups have not been studied.

Mitochondrial DNA sequencing, particularly of the most rapidly evolving and highly variable control region (CR), has proven useful in population genetic studies of many terrestrial and aquatic organisms (Avise, 1994). Little is known regarding the structure and evolution of marine invertebrate mitochondrial control regions; however, in shrimp this region is divided into three polymorphic domains separated by two stretches with no intraspecific variability (Grabowski & Stuck, 1999). The CR has been used successfully to study decapod population genetics (Grabowski & Stuck, 1999; McMillen-Jackson & Bert, 2003, 2004; Diniz *et al.*, 2005; Babbucci *et al.*, 2010). Furthermore, the hypervariable domain in the mtDNA CR could help elucidate the genetic structure of a population with high resolution.

This study aimed to clarify the genetic relationship between continental coast, continental island and oceanic island populations in the west Pacific region using 12Sr-RNA, 16Sr-RNA, and the control region of mitochondrial DNA in *U. crassipes*. Using these results, we examined the connectivity among these populations.

MATERIALS AND METHODS

Sample collection and DNA extraction

Uca crassipes specimens were collected from four localities in Japan (Chichi-jima and the Ryukyu Islands Okinawa-jima,

Corresponding author:
M. Aoki
Email: mjiro4203@goo.jp

Miyako-jima, and Iriomote-jima), one in Vietnam (Hoi An), and one in French Polynesia (Moorea Island) (Figure 1). For each specimen, muscle tissue from the third leg was fixed in 99% ethanol. Total DNA was extracted from the muscle tissue using proteinase K and sodium dodecyl sulphate (SDS)-phenol-chloroform extraction.

Mitochondrial DNA amplification and sequencing

The target DNA fragments of the small subunit of ribosomal RNA (12Sr-RNA) and the large subunit of ribosomal RNA (16Sr-RNA) were amplified via polymerase chain reaction (PCR) using the primers H2492i (5′CAG ACA TGT TTT TAA TAA ACA GGC-3′; modified from Palumbi *et al.*, 1991), L1496i (5′-GTA CAT ATC GCC CGT CGC TT-3′; Kitaura *et al.*, 1998), H3059i (5′-CCG GTC TGA ACT CAG ATC ATG T-3′), H3062 (5′-CCG GTC TGA ACT CAG ATC A-3′), and L2510C (5′-CGC CTG TTT AAC AAA GAC AT-3′; modified from Palumbi *et al.*, 1991). The PCR was performed in a total reaction volume of 25 μl containing 0.2 μl of TaKaRa Ex *Taq* (5 units/μl), 2.5 μl of 10× Ex *Taq* buffer, 2.0 μl of dNTP mixture (2.5 mM each), 0.4 mM of each primer, and 1.0 μl of template. The PCR consisted of 25–30 cycles of 94°C for 30 seconds, 46–50°C for 30 seconds, and 72°C for 60 seconds using a GeneAmp PCR System 2400 (Applied Biosystems) or TaKaRa PCR Thermal Cycler Dice Version 3 Model TP600 (TaKaRa).

The CR portion of the mitochondrial DNA gene, located between the 12Sr-RNA and the isoleucine transfer RNA (tRNA-Ile) gene, was amplified by PCR using primers that Kitamura *et al.* (2005) designed for the mtDNA genome of the Japanese spiny lobster *Panulirus japonicus* (Von Siebold) (GenBank Accession No. NC004251; Yamauchi *et al.*, 2002) Pjapo13350 (CCT TTA AGT TTA ACC GCA GAT GC) and the swimming crab *Portunus trituberculatus* (Miers) (GenBank Accession No. NC005037; Yamauchi *et al.*, 2003) Pjapo14471 (ACG GGG TAT GAG CCC ATT AGC). The PCR consisted of 30 cycles of 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 60 seconds. The amplification products were purified using ExSAP-IT (USB) and sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) with an automated 3500/3500xL Genetic Analyzer (Applied Biosystems) DNA sequencer. The sequencing reactions followed the manufacturer's protocol. The partial mitochondrial 12Sr-RNA and 16Sr-RNA genes were sequenced to determine the phylogenetic relationships among haplotypes. In addition, the partial mitochondrial CR gene was sequenced and used to analyse genetic diversity, genetic structure and gene flow.

The DNA sequences were aligned using the program ClustalW (Thompson *et al.*, 1994) and inspected visually with a chromatogram viewer editor. Polymorphic sites were assessed visually on the original chromatogram and alignments were refined manually when necessary.

Data analysis

PHYLOGENETIC RELATIONSHIPS, GENE DIVERSITY AND GENETIC STRUCTURE

Some consensus sequences of the 611-bp 12Sr-RNA–16Sr-RNA and 428-bp 16Sr-RNA were obtained for 18

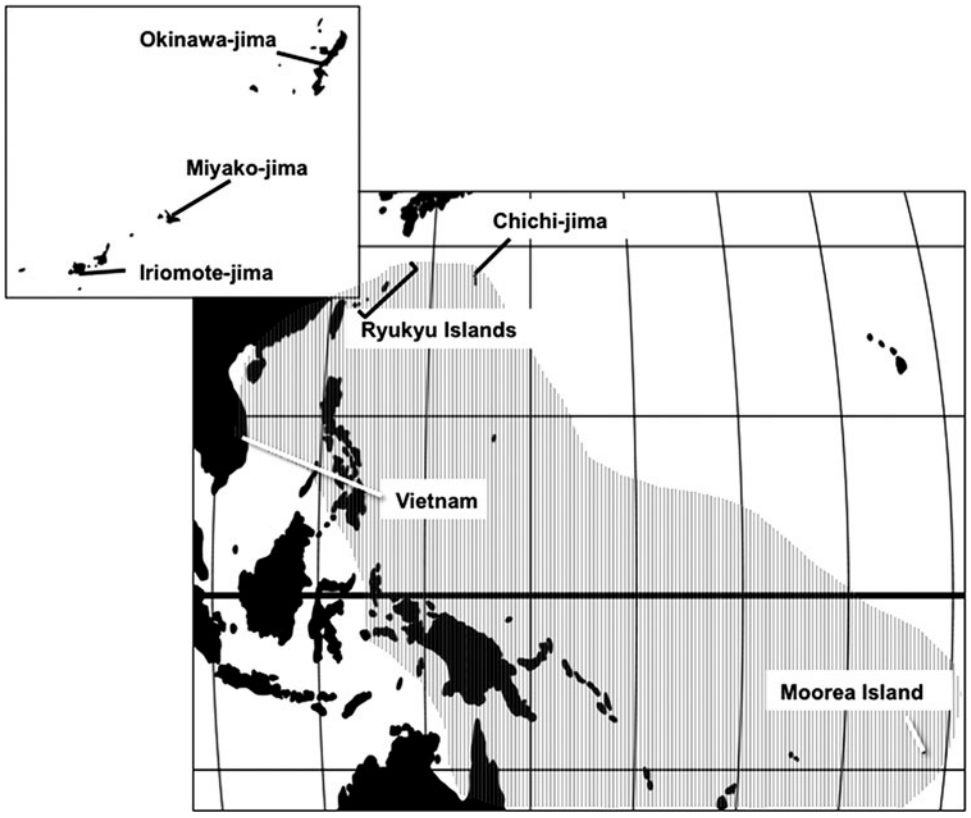

Fig. 1. Locations of the six *Uca crassipes* sampling sites. The shaded region indicates the distribution of *U. crassipes*.

https://doi.org/10.1017/S0025315412001178 Published online by Cambridge University Press

individuals, which represented all of the sample populations. The sequences were first analysed with the software Modeltest (Posada & Crandall, 1998) to identify the evolutionary model that best fit the data. The 12Sr-RNA–16Sr-RNA and 16Sr-RNA sequence data were combined because Modeltest selected the same evolutionary model, HKY, for each sequence.

The combined sequences were analysed with the maximum likelihood (ML) and neighbour-joining (NJ) methods using PAUP version 4.0b10 (Swofford, 2001). The ML analysis was performed using a heuristic search algorithm, setting the parameters to the values calculated by Modeltest. For both tests, bootstrap analyses as heuristic searches were applied, with 100 replications for ML and 1000 replications for NJ. The tree was visualized and drawn using Fig Tree Version 1.3.1 (Rambaut, 2009).

Gene diversity at the mitochondrial CR was described as haplotype (h) and nucleotide (π) diversities (Nei, 1987) using ARLEQUIN 3.1 (Excoffier *et al.*, 2005). To test for regional subdivisions in the sequences, we used an analysis of molecular variance (AMOVA), and pairwise F_{ST} values between populations were calculated in ARLEQUIN ver. 3.1. All *F*-statistics were computed using the method of Weir & Cockerham (1984), and *F* values significantly different from zero were identified by comparison with the results of 10,000 data permutations (Raymond & Rousset, 1995). We adjusted the critical *P* values for these tests using Bonferroni corrections. Gene flow (*Nm*) was estimated as $Nm = (1 - F_{ST})/2F_{ST}$ (Hudson *et al.*, 1992). The haplotype network using statistical parsimony (Templeton *et al.*, 1992) was built with TCS ver.1.21 (Clement *et al.*, 2000). However, it was difficult to estimate the connections between all of the haplotypes from Vietnam and several from Iriomote-jima (H75, H76 and H77) and the other haplotypes by TCS because these haplotypes differed greatly from those from the Ryukyu Islands and Moorea Island (Supplementary Appendix 2). Therefore, we drew the haplotype network with 80 haplotypes, excluding these haplotypes.

RESULTS

The combined 1039-bp 12Sr-RNA–16Sr-RNA sequences were obtained for 18 individuals in six populations. We identified eight haplotypes using the 46 variable sites, including 12 gaps, in the combined sequences (Supplementary Appendix 1). The forty of 46 variation sites were found in the Vietnamese samples. The HKY model was selected for DNA substitution by hLRT. The parameter values under the selected model were as follows: base frequencies A = 0.3801, C = 0.1573, G = 0.0702, T = 0.3924; substitution model, Ti/Tv ratio = 7.9768; proportion of invariable sites = 0.0000; and variable sites (G) = equal rates for all sites. The NJ analysis produced the same topology as ML. Two major clusters were supported strongly: the Vietnamese population and the other populations (Figure 2). In addition, in the non-Vietnamese population cluster, the haplotypes from Chichi-jima (H2 and H3) were local haplotypes, whereas haplotype H1 was shared with the Ryukyu Islands, Okinawa-jima, Miyako-jima, Iriomote-jima and the Moorea Island populations.

A 504-bp portion of the CR sequence was obtained from 133 individuals in six *Uca crassipes* populations. We identified

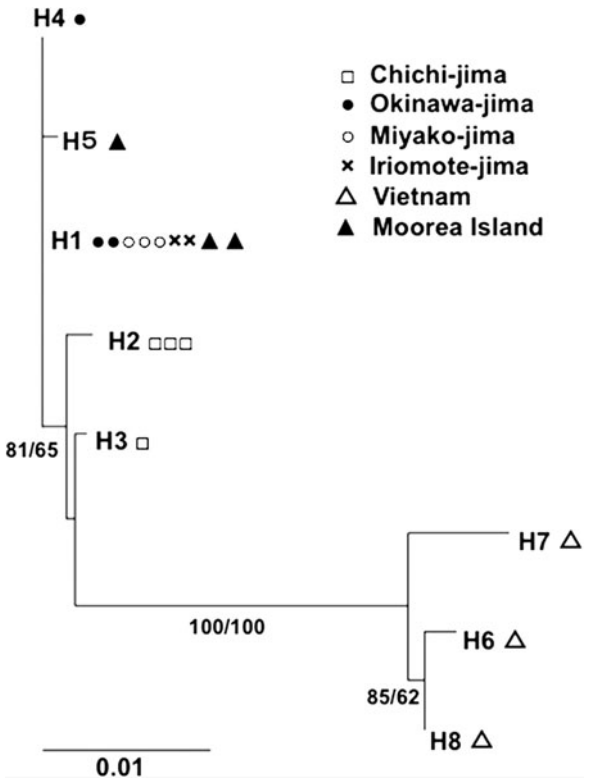

Fig. 2. Maximum-likelihood (ML) tree for the combined sequence of mtDNA 12Sr-RNA and 16Sr-RNA (1039 base pairs) haplotypes using the HKY model. Eight haplotypes are shown in 6 sampling sites. The numbers are bootstrap values from 100 replications with ML (the prior value) and 1000 replications with neighbour-joining (the posterior value). Bootstrap values higher than 50% are shown in the tree.

94 haplotypes using the 166 variable sites, including seven gaps, with 107 informative sites (Supplementary Appendix 2).

The h and π diversities were lowest in the Okinawa-jima population (h = 0.8719, π = 0.0034), whereas those of the Vietnamese population (h = 1.0000, π = 0.0603) had the highest values (Table 1). The AMOVA revealed significant overall population differentiation ($\Phi_{ST} = 0.5807$, $P < 0.001$). In the pairwise F_{ST} values (Table 2), all combinations of populations, excluding two combinations, Miyako-jima and Okinawa-jima and Miyako-jima and Iriomote-jima, showed significant genetic differentiation ($F_{ST} = 0.0929–0.8527$; $P < 0.0033$; Table 2).

The haplotype network by TCS, excluding the Vietnamese haplotypes and some from Iriomote-jima, indicated that the three Ryukyu Islands populations shared four haplotypes: H1, H2, H3 and H12 (Figure 3). A common haplotype, H1, occurred in the three populations and was designated ancestral by TCS. Private haplotypes that were unique to one locality and were connected to the shared haplotypes by a few steps did not form a cluster for each locality. All of the Moorea Island haplotypes were private (Figure 3) and were connected with the Miyako-jima haplotype H70 within ten steps. Haplotypes from Chichi-jima formed two clusters many steps apart and connected with the Moorea Island haplotypes by >19 steps (Figure 3). All of the Chichi-jima haplotypes were private and separated by many steps from each other and the Ryukyu and Moorea Islands haplotypes.

https://doi.org/10.1017/S0025315412001178 Published online by Cambridge University Press

Table 1. Haplotype and nucleotide diversities of six populations of *Uca crassipes*.

	Chichi-jima	Okinawa-jima	Miyako-jima	Iriomote-jima	Vietnam	Moorea
Number of samples (n)	14	29	22	22	11	35
Number of haplotype s	13	14	14	19	11	28
Haplotype diversity ($h \pm$ SD)	0.9890 ±0.0314	0.8719 ±0.0433	0.9264 ±0.0388	0.9740 ± 0.0276	1.0000 ±0.0388	0.9782 ±0.0159
Nucleotide diversity ($\pi \pm$ SD)	0.0477 ±0.0251	0.0034 ±0.0023	0.0042 ± 0.0027	0.0387 ±0.0198	0.0603 ± 0.0322	0.0079 ±0.0045

DISCUSSION

Vietnamese population

The pairwise F_{ST} and relationship of some sequences from the regions of 12Sr-RNA to 16Sr-RNA and the mtDNA CR revealed that the Vietnamese population exhibited high genetic differentiation from the other Pacific Ocean populations (Table 2; Figure 2). In addition, the Vietnamese population contained higher gene and nucleotide diversity than the other populations, suggesting that the Vietnamese population has maintained its large population longer than the other populations. Hoi An is located in Vietnam on the South China Sea, which, with an average depth of about 1500 m, is known to have been reduced in size as recently as the last glacial maximum (Li *et al.*, 2009). Over 2 Mya, the connections between the South China Sea and Pacific Ocean were limited by low sea levels (Tjia, 1980). Therefore, the limited connection of the South China Sea with the Pacific Ocean could have caused the remarkable differentiation of the Vietnamese *Uca crassipes* population. Similar genetic differentiation of South China Sea populations has been detected in the Kuruma shrimp *Penaeus japonicus* (Bate) (Tsoi *et al.*, 2005) and the oval squid *Sepioteuthis* cf. *lessoniana* (Lesson) (Aoki *et al.*, 2008).

The differentiation of the Vietnamese population may be observed at an intraspecific level, as the haplotypes H75, H76 and H77 found on Iriomote-jima were similar to haplotypes from the Vietnamese population. However, more taxonomic studies (morphological feature, breeding feature and nucleus marker) will be needed to resolve this issue.

Connections between the Ryukyu Islands and Moorea Island populations

The private haplotypes from the Ryukyu Islands were connected with the shared haplotypes by several steps (Figure 3). In addition, the pairwise F_{ST} showed no significant difference between Miyako-jima and Okinawa-jima or Iriomote-jima (Table 2), indicating that gene flow has occurred within the Ryukyu Islands. However, two lineages were found in the Iriomote-jima population, one connected to the populations of the Ryukyu Islands and the other with the Vietnamese population, suggesting that the Iriomote-jima population was formed by the ingression of different gene pools.

The Moorea Island population is located in the Society Islands and exhibited significant differentiation from all other populations, although the haplotypes showed a close relationship with the Ryukyu Islands haplotypes. The tree topology created from the combined 12Sr-RNA – 16Sr-RNA sequences also showed a close genetic relationship between the Ryukyu Islands and Moorea Island populations. Therefore, the Moorea and Ryukyu Islands populations were likely either derived from the same historical gene pool or there was great gene flow that spanned almost 10,000 km between the Ryukyu Islands and Moorea Island via oceanic currents.

Chichi-jima population

Chichi-jima is an oceanic island of the Bonin Islands, 1000 km north of the Northern Mariana Islands. The Bonin Island biota has been influenced greatly by oceanic currents. Fukuda (1993, 1994) compared the composition of marine gastropod species of the Bonin Islands with the three surrounding island groups: the Izu Islands, Ryukyu Islands, and Northern Mariana Islands; 70% of the species were common to the Ryukyu Islands, 45% to the Izu Islands and 20% to the Northern Mariana Islands. Furthermore, there is a sister relationship between the Bonin Islands and Ryukyu Islands in the intertidal gastropod *Lunella coronata* (Gmelin) group (Williams *et al.*, 2011). However, our results did not identify shared haplotypes between the Ryukyu and Bonin Islands, suggesting little recent transport of larvae between the two island groups.

If a population was isolated from other populations during a long period, the isolated population could show low genetic diversity by the effect of inbreeding. However, our results are in contrast with this; the oceanic island haplotypes of the Chichi-jima population had higher variability, with two different lineages (Figure 3), than the continental Ryukyu Islands populations. This implies that larvae have been transported from Chichi-jima from a large gene pool, possibly the Mariana Islands, as both groups of islands share the same

Table 2. Pairwise F_{ST} values between populations (below diaonal) and *Nm* (above the diagonal) for the mtDNA control region among six populations of *Uca crassipes*, as by Arlequin version 3.1.

	Chichi-jima	Okinawa-jima	Miyako-jima	Iriomate-jima	Vietnam	Moorea
Chichi-jima		0.26	0.32	0.75	0.29	0.32
Okinawa-jima	0.6587**		47.05	4.88	0.09	0.58
Miyako-jima	0.6117**	0.0105		6.53	0.11	0.69
Iriomote-jima	0.4008**	0.0929**	0.0711		0.30	1.67
Vietnam	0.6368**	0.8527**	0.8250**	0.6266**		0.09
Moorea	0.6063**	0.4616**	0.4218**	0.2301**	0.8417**	

Significance was tested at the 5% level with a Bonfeferroni-corrected P for multiple test ($^{*}P < 0.05$,$^{**}P < 0.01$).

https://doi.org/10.1017/S0025315412001178 Published online by Cambridge University Press

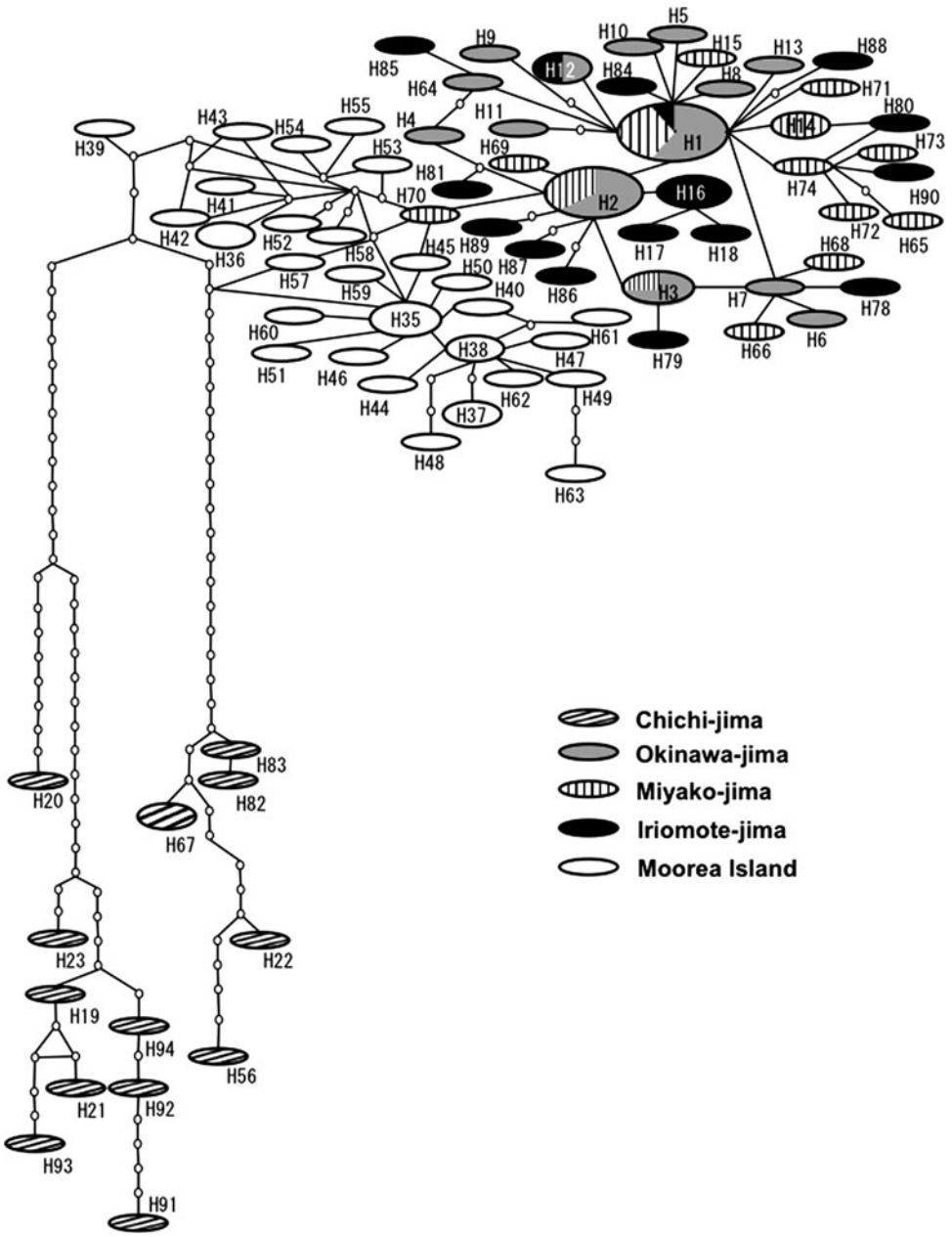

Fig. 3. Statistical parsimony cladogram for the mtDNA control region haplotypes using TCS. The individual oval sizes in the diagram are proportional to the number of individuals possessing each haplotype. Branches correspond to single mutations and additional circles on the branches represent additional inferred mutations. Haplotype 1 is the ancestral haplotype inferred by TCS.

geological history (Seno & Maruyama, 1984). Further genetic study of *U. crassipes*, including the Mariana Island population, will determine the origin of the Chichi-jima population.

The *U. crassipes* population in the Bonin Islands occurs only in Chichi-jima. The habitat area, which is limited to a few sites, and the population size (<50 individuals) are extremely small, suggesting the possibility of extirpation. Considering the genetic lineage of the Chichi-jima population, their conservation status should be amended.

ACKNOWLEDGEMENTS

We thank Dr M. Tsuchiya, Dr M. Izawa, and Dr T. Naruse (University of the Ryukyus) and Mr T. Tannai (Ogasawara Subprefecture, Tokyo) for their cooperation with specimen sampling. We thank Dr H. Imai (University of the Ryukyus), Professor Y. Yusa, and the members of the Laboratory of Population and Community Ecology, Nara Women's University, for their invaluable input and support. This study was partly supported by the Mikimoto Fund for Marine Ecology in Japan, and by Nara Women's University Intramural Grant for Young Women Researchers to M.A.

Supplementary materials and methods

The supplementary material refered to in this paper can be found online at journals.cambridge.org/mbi.

https://doi.org/10.1017/S0025315412001178 Published online by Cambridge University Press

REFERENCES

Aoki M., Imai H., Naruse T. and Ikeda Y. (2008) Low genetic diversity of oval squid, *Sepioteuthis* cf. *lessoniana* (Cephalopoda: Loliginidae), in Japanese waters inferred from a mitochondrial DNA non-coding region. *Pacific Science* 62, 403–411.

Avise J.C. (1994) *Molecular markers*. London: Natural History and Evolution, UK, and Chapman & Hall.

Babbucci M., Buccoli S., Cau A., Cannas R., Goñi R., Díaz D., Marcato S., Zane L. and Patarnello T. (2010) Population structure, demographic history, and selective processes: contrasting evidences from mitochondrial and nuclear markers in the European spiny lobster *Palinurus elephas* (Fabricius, 1787). *Molecular Phylogenetics and Evolution* 56, 1040–1050.

Benzie J.A.H. and Williams S.T. (1995) Gene flow among giant clam (*Tridacna gigas*) populations in Pacific does not parallel ocean circulation. *Marine Biology* 123, 781–787.

Benzie J.A.H. (1999) Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution* 53, 1782–1795.

Clement M., Posada D. and Crandall K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9, 1657–1660.

Crane J. (1975) *Fiddler crabs of the world*. Princeton: Princeton University Press.

Diniz F.M., Maclean N., Ogawa M., Cintra I.H.A. and Bentzen P. (2005) The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. *Marine Biotechnology* 7, 462–473.

Excoffier L., Laval G. and Schneider S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1, 40–50.

Fukuda H. (1993) Marine Gastropoda (Mollusca) of the Ogasawara (Bonin) Islands, Part 1: Archaeogastropoda and Neotaeniglossa. *Ogasawara Research* 19, 1–86.

Fukuda H. (1994) Marine Gastropoda (Mollusca) of the Ogasawara (Bonin) Islands, Part 2: Neogastropoda, Heterobranchia and fossil species, with faunal accounts. *Ogasawara Research* 20, 1–126.

Gopurenko D., Hughes J.M. and Keenan C.P. (1999) Mitochondrial DNA evidence for rapid colonisation of the Indo-West Pacific by the mud crab *Scylla serrata*. *Marine Biology* 134, 227–233.

Grabowski M. and Stuck K.C. (1999) Structure and intraspecific variability of the control region mtDNA in the pink shrimp, *Farfantepenaeus duorarum* (Decapoda, Penaeidae). In Schram F.R. and von Vaupel Klein J.C. (eds) *Crustaceans and the biodiversity crisis. Volume I*. Leiden, The Netherlands: Brill Academic Publishers, pp. 333–344.

Hudson R.R., Slatkin M. and Maddison W.P. (1992) Estimation of level of gene flow from DNA sequence data. *Genetics* 132, 583–589.

Kitamura T., Nitta A., Wakao H. and Oda T. (2005) Population structure in mitochondrial DNA of fiddler crab. In Japanese Society for DNA Polymorphism Research (ed.) *DNA polymorphism. Volume 13*. Tokyo: Toyo Shoten, pp. 140–144.

Kitaura J., Wada K. and Nishida M. (1998) Molecular phylogeny and evolution of unique mud-using territorial behavior in ocypodid crabs (Crustacea: Brachyura: Ocypodidae). *Molecular Biology and Evolution* 15, 626–637.

Lavery S., Moritz C. and Fielder D.R. (1996) Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. *Molecular Ecology* 5, 557–570.

Li Q., Zhong G. and Tian J. (2009) Stratigraphy and sea level changes. In Wang P. and Li Q. (eds) *The South China Sea, paleoceanography and sedimentology. Volume 13*. Dordrecht, The Netherlands: Springer Science + Business Media B.V., pp. 75–170.

McMillen-Jackson A.L. and Bert T.M. (2003) Disparate patterns of population genetic structure and population history in two sympatric penaeid shrimp species (*Farfantepenaeus aztecus* and *Litopenaeus setiferus*) in the eastern United States. *Molecular Ecology* 12, 2895–2905.

McMillen-Jackson A.L. and Bert T.M. (2004) Genetic-diversity in the mtDNA control region and population structure in the pink shrimp *Farfantepenaeus duorarum*. *Journal of Crustacean Biology* 24, 101–109.

Meyer C.P., Geller J.B. and Paulay G. (2005) Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. *Evolution* 59, 113–125.

Nei M. (1987) *Molecular evolutionary genetics*. New York: Columbia University Press.

Palumbi S.R., Martin A., Romano S., McMillan W.O., Stice L. and Grabowski G. (1991) *The simple fool's guide to PCR. Version 2*. Honolulu: University of Hawaii.

Palumbi S.R. (1996) What can molecular genetics contribute to marine biogeography? An urchin's tale. *Journal of Experimental Marine Biology and Ecology* 203, 75–92.

Posada D. and Crandall K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.

Rambaut A. (2009) *FigTree: Tree Figure Drawing Tool, Version 1.3.1*. Edinburgh, Scotland: Institute of Evolutionary Biology, University of Edinburgh.

Raymond M. and Rousset F. (1995) An exact test for population differentiation. *Evolution* 49, 1280–1283.

Seno T. and Maruyama S. (1984) Paleogeographic reconstruction and origin of the Philippine Sea. *Tectonophysics* 102, 53–84.

Swofford D.L. (2001) *PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.0*. Sunderland, MA: Sinauer Associates.

Templeton A.R., Crandall K.A. and Sing C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132, 619–633.

Thompson J.D., Higgins D.G. and Gibson T.J. (1994) ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–4680.

Tjia H.D. (1980) The Sunda Shelf, Southeast Asia. *Zeitschrift für Geomorphologie* 24, 405–427.

Tsoi K.H., Wang Z.Y. and Chu K.H. (2005) Genetic divergence between two morphologically similar varieties of the kuruma shrimp *Penaeus japonicus*. *Marine Biology* 147, 367–379.

Weir B.S. and Cockerham C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1358–1370.

Williams S. T. and Benzie J.A.H. (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by color morphs, mtDNA, and allozyme data. *Evolution* 52, 87–99.

Williams S., Apte D., Ozawa T., Kaligis F. and Nakano T. (2011) Speciation and dispersal along continental coastlines and island arcs in the Indo-West Pacific turbinid gastropod genus *Lunella*. *Evolution* 65/66, 1752–1771.

Yamauchi M., Miya M. and Nishida M. (2002) Complete mitochondrial DNA sequence of the Japanese spiny lobster, *Panulirus japonicus* (Crustacea: Decapoda). *Gene* 295, 89–96.

and

Yamauchi M., Miya M. and Nishida M. (2003) Complete mitochondrial DNA sequence of the swimming crab, *Portunus trituberculatus* (Crustacea Decapoda: Brachyura). *Gene* 311, 129–135.

Correspondence should be addressed to:
M. Aoki
KYOUSEI Science Center for Life and Nature
Nara Women's University
Kitauoya-higashimachi,
Nara 630-8506, Japan
email: mjiro4203@goo.jp

https://doi.org/10.1017/S0025315412001178 Published online by Cambridge University Press