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Selection of effective microsatellite DNA locus and genetic structure analysis of the pre-tsunami wild population of sea urchin *Strongylocentrotus nudus* in Sanriku, Japan

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Abstract — The sea urchin *Strongylocentrotus nudus* is one of the important marine products of fishing industry in Sanriku, Iwate, Japan. The Tohoku-Pacific Ocean Earthquake decreased the population of adult sea urchin in the Sanriku area, which is reflected in the decreased genetic diversity in this population. This study examined the extent of genetic variability in 14 microsatellite DNA loci and estimated genetic structure of *S. nudus* population established before the tsunami in Touni Bay (Iwate Prefecture) by using these genetic markers. Some loci used in this study showed high null allele frequency and a low number of alleles, which hampers the estimations of the genetic structure of *S. nudus*. The selection of effective microsatellite DNA locus in the estimation of genetic structure would improve our understanding of the changes in genetic diversity of *S. nudus* population.

Key words: Great East Japan Earthquake, population genetics, reef burning, allele frequency, bottleneck

Introduction

The sea urchin *Strongylocentrotus nudus* is one of commercially important marine organisms in Japan. The deeply indented coastline (rias coastline) area in the Pacific northeastern Japan called Sanriku has been traditionally one of the regions with thriving fishery industry of this species. Increased population density of sea urchin in a habitat is one of the factors causing a decrease in seaweed community that provide habitat, food, and nurseries for a wide range of organisms (Arakawa et al. 2014), a process called rocky shore denudation or *isoyake*. Rocky shore denudation, in turn, results in poor growth and disappearance of resource organisms occupying the surf zone, such as abalone, juvenile fish, and kelp, eventually affecting coastal fishery industry. Therefore, the resource management, including the release of land-cultured seeds of sea urchin into natural waters, had been performed. However, the mega earthquake of moment magnitude 9.0 (Tohoku-Pacific Ocean Earthquake) happened on March 11, 2011, immediately followed by enormous tsunami. This natural disaster significantly affected marine ecosystems through tectonic subsidence, decrease of seaweed bed, change in sea currents, and decrease in available organic matter. These physical effects have caused mass death of individual marine organisms, divided some populations, and relocated others. Thus, the genetic structure of natural *S. nudus* populations was altered. For example, the rapid decrease in population numbers due to mass death of individuals and division of the population reduced genetic diversity within the population. This natural disaster also eliminated the natural enemy of the sea urchin, which in turn increased the density of sea urchin without increasing genetic diversity of its population, eventually leading to reef burning. The decrease of genetic diversity reduces adaptability to environmental changes (Allendorf 1986) and increases the chances of extinction. Therefore, genetic study is required for strategic planning of resource management protocols in natural populations.

Microsatellite DNA (msDNA) has been used as genetic markers to estimate the genetic structure in natural populations. Obtained information can then be used for resource management. Li and Li (2008) and Yan et al. (2010) reported 70 msDNA loci for *S. nudus*, but only a few studies investigated the genetic structure of this species. Nam et al. (2014) estimated genetic structure of *S. nudus* using 10 of the 70 msDNA loci, but reported that four msDNA loci could not be amplified reliably. Furthermore, they detected high frequency of null alleles at one locus (*SN 225*) of even amplified six loci (Nam et al. 2014). Therefore, the existing effective msDNA loci may not be adequate to estimate the genetic structure of *S. nudus*. In addition, these loci may incorrectly identify a change in genetic structure of sea urchin population established after the tsunami.

This study revealed the extent of genetic variability in 14 msDNA loci that have higher number of alleles and/or do not shown deviation from Hardy-Weinberg equilibrium (*HWE*) (Li and Li 2008, Yan et al. 2010); they included some loci used in a previous study (Nam et al. 2014). Using these genetic markers, we estimated genetic structure of *S. nudus* population that hatched before the tsunami of the Great East Japan Earthquake in the Touni Bay (Iwate Prefecture).

Materials and Methods

A previous study in Miyagi Prefecture showed that shell width of sea urchins in crustose coralline habitats reaches up to 60 mm in 5–6 years, and even in seaweed beds inhabiting, the width can reach to about 50 mm for 2 years (Agatsuma et al. 2005). Therefore, *S. nudus* specimens with shell width 50 mm were collected from the Touni Bay (Iwate Prefecture) in summer of 2012 and 2013.

The gonads were removed from the collected sea urchins and placed in 1.5-mL microcentrifuge tubes with an RNAlater stabilization reagent (Qiagen, Venlo, Netherlands), and the tubes were stored at −80°C. Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Netherlands) and cleaned using a QIAquick PCR Purification Kit (Qiagen, Netherlands). The extracted DNA concentration was measured with a NanoDrop 2000 (Thermo Fisher, Waltham, MA, USA) and then adjusted to 1 ng μL^{-1} by adding sterilized water for polymerase chain reaction (PCR) amplification.

For msDNA analysis, 14 loci reported by Li and Li (2008) and Yan et al. (2010) were amplified using primers labeled with fluorescent dye and a Taq PCR Master Mix Kit (Qiagen, Netherlands) according to the manufacturer's instructions in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) or 2720 Thermal Cycler (Applied Biosystems, Carlsbad, CA, USA). PCR protocol for six loci (*SN 73*, *SN 135*, *SN 153*, *SN 164*, *SN 178*, and *SN 199*) consisted of initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing at primer-specific temperature for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The protocol used for PCR amplification of eight loci (*HD-1*, *HD-7*, *HD-10*, *HD-12*, *HD-19*, *HD-26*, *HD-34* and *HD-59*) included initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at primer-specific temperature for 30 s, and exten-

Table 1. Primers of microsatellite DNA loci used in this study.

Locus	Repeat sequences (5′–3′)	Primer sequences (5′–3′)	Fluorescence label	Ta (°C)	Reference
SN 73	$(AG)_{13}$	F: ψ CGC AGG ATG CAG TGA TAC C R: ATT CCA CCA GTA TCC CAG CT	HEX	55	Li and Li 2008
SN 135	$(AG)_{11}$	F: ψ CCT TAG AAC AGC AGC AAA R: GTA ACA TTT CAC CCA TTC AC	HEX	49	Li and Li 2008
SN 153	$(AC)_7TCGT(CA)_5$	F: ψ ACC TAA CCC ATG CTC CCT A R: ATA CCT CGG TCC ATC TTG TCT	6-FAM	54	Li and Li 2008
SN 164	$(CT)_{18}N_{10}(CT)_5$	F: ψ GCG CTT AAT CTT TGG ATA ATT G R: CTG TAG TCG CTC CGC ATG T	HEX	52	Li and Li 2008
SN 178	$(TC)_{13}TT(TC)_5$	F: ψ GTC TTT CTT TTT GTA GTC CCA R: GGG GTA AAT CTG ATT GCA T	NED	47	Li and Li 2008
SN 199	$(CT)_{11}N_9(TC)_5$	F: ψ CCG TCA GGG CTC GTT CAT T R: AGT TCA TGC TTA TCG TGC TTA CCA	6-FAM	52	Li and Li 2008
HD-1	$(AG)_{60}$	F: ψ TGC TGT GCC TGT AAC ACT T R: CTG CCA ATC CAC AAC CTC C	6-FAM	56	Yan et al. 2010
HD-7	$(TG)_{34}$	F: ψ GTT GCG GTT ATG TAT TCG TTC R: GCA TCG CTA TGT TGA GGG TC	NED	50	Yan et al. 2010
HD-10	$(AG)_{20}$	F: ψ TTT CCG TTG AAG ATC CCT A R: CAA ATC ATT CGG TGT TGC T	6-FAM	56	Yan et al. 2010
HD-12	$(CT)_{10}$	F: ψ ACA TCT TTC CTC CGT TTC T R: CAG TCC CAG TGT TCT ATT T	NED	50	Yan et al. 2010
HD-19	$(GA)_{22}$	F: ψ ACA TCA AAG GAC ATT GAC TC R: AGA CTG GCG TAG CAT AGG T	NED	56	Yan et al. 2010
HD-26	$(AG)_{25}$	F: ψ CTC TTT GAT CGC TGA GAA R: TAG CCA GAA TGG TTA CGC	6-FAM	58	Yan et al. 2010
HD-34	$(CT)_{19}$	F: ψ GAG CCA ACT AAC TGG GAC T R: AGG AGA TAT GGA GCG AGA	6-FAM	50	Yan et al. 2010
HD-59	$(GA)_{12}$	F: ψ ATC TTA CCA GCC TCC TTT R: ATC TCA CTC CTC CAC AAC	HEX	45	Yan et al. 2010

Ta, annealing temperature; ψ, fluorescence label

sion at 72°C for 30 s, and a final extension at 72°C for 5 min. Primer sequences and annealing temperatures are presented in Table 1. PCR products were analyzed with a GeneScan 400HD ROX dye Size standard (Applied Biosystems, USA) in an ABI PRISM 3130*xl* genetic analyzer (Applied Biosystems, USA). Different allelic types were determined using Peak Scanner Software 1.0 (Applied Biosystems, USA).

The number of alleles (A_N), allele frequency, and observed (H_o) and expected heterozygosity (H_e) were calculated using GENEPOP 4.2 (Raymond and Rousset 1995). The significant deviation from *HWE* for each locus was evaluated by Fisher's exact test with a Markov chain method (Markov-chain length = 100,000; dememorization = 10,000) using ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010). Inbreeding coefficient (F_{is}) was calculated using FSTAT 2.9.3.2 (Goudet 1995). The null allele frequency in each msDNA locus was calculated with Cervus 3.0.7 (Marshall et al. 1998).

Results and Discussion

This study estimated genetic structure of *S. nudus* that was established before the natural disaster in the Touni Bay using 14 msDNA loci. The clear allele types were obtained for all the loci except *SN 178* and *HD-34*. The genetic diversity of *S. nudus* for the 12 loci is shown in Table 2. The variation of A_N ranged from 2 (*SN 199*) to 25 (*HD-10*); allele frequency of each locus is shown in Figure 1. The H_e of each locus ranged from 0.088 (*SN 199*) to 0.959 (*HD-10*). *SN 153* and *SN 199* loci were not suitable for genetic structure analysis because these loci had low A_N and H_e and biased allele

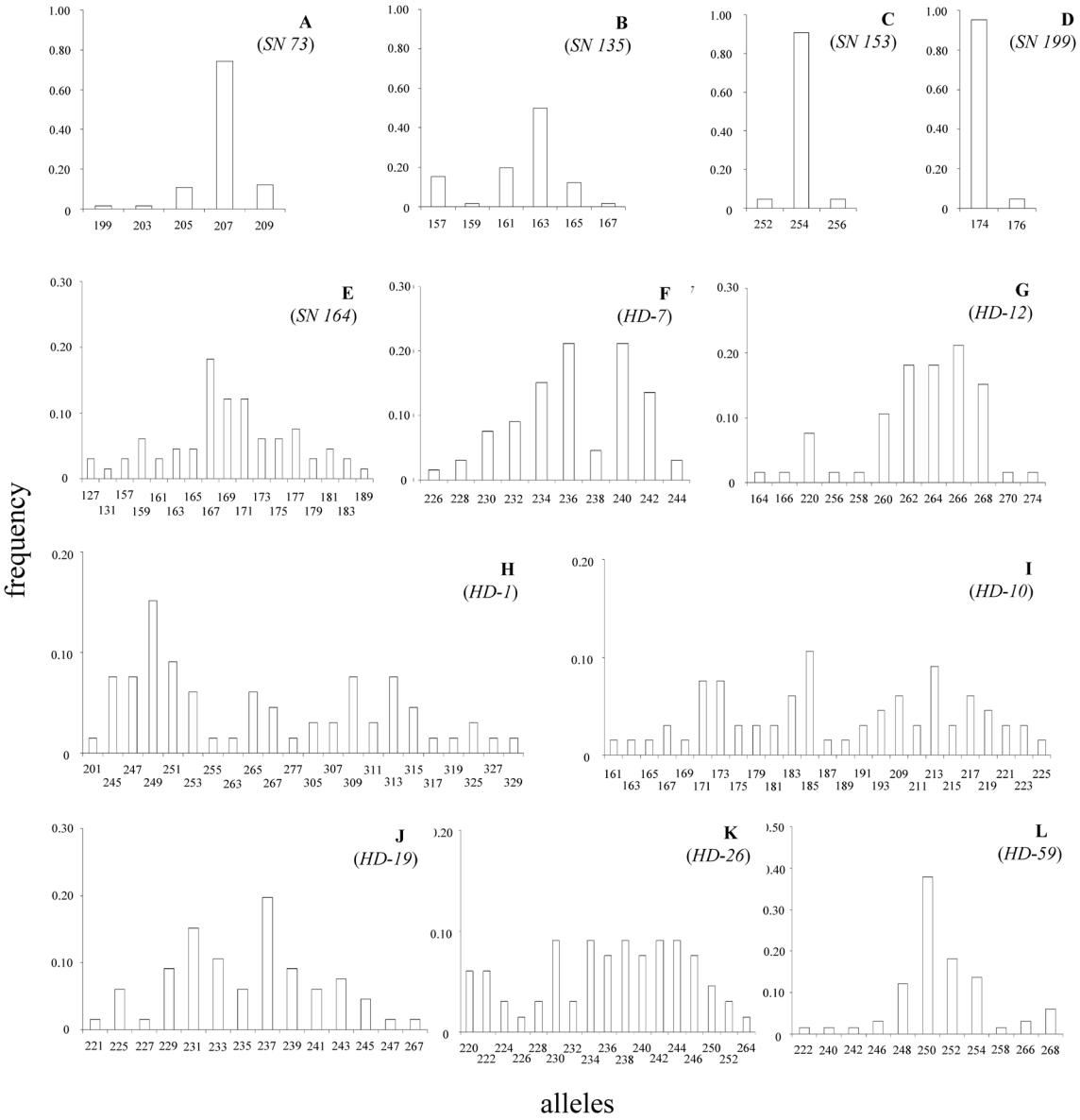

Fig. 1. Allele frequencies in 12 microsatellite DNA loci of *Strongylocentrotus nudus* in Touni Bay. Vertical bars show alleles frequency of (A) *SN 73*; (B) *SN 135*; (C) *SN 153*; (D) *SN 199*; (E) *SN 164*; (F) *HD-7*; (G) *HD-12*; (H) *HD-1*; (I) *HD-10*; (J) *HD-19*; (K) *HD-26*; (L) *HD-59*.

Table 2. Number of alleles (A_N), observed heterozygosity (H_o), expected heterozygosity (H_e), deviation from Hardy-Weinberg equilibrium (*HWE*), inbreeding coefficient (F_{is}) and null alleles frequency (A_{null}) of *Strongylocentrotus nudus* in Touni Bays for each of the 12 microsatellite DNA loci.

Loci	*N*	A_N	H_o	H_e	*HWE*	F_{is}	A_{null}
SN 73	33	5	0.455	0.429	0.487	−0.061	−0.025
SN 135	33	6	0.606	0.683	0.574	0.115	0.063
SN 153	33	3	0.182	0.172	1.000	−0.058	−0.039
SN 164	33	17	0.939	0.924	0.655	−0.002	−0.017
SN 199	33	2	0.091	0.088	1.000	−0.032	−0.014
HD-1	33	22	0.758	0.943	0.000*	0.199	0.100
HD-7	33	10	0.788	0.863	0.694	0.089	0.033
HD-10	33	25	0.818	0.959	0.019*	0.149	0.148
HD-12	33	12	0.788	0.861	0.402	0.086	0.036
HD-19	33	14	0.636	0.904	0.001*	0.300	0.167
HD-26	33	17	0.788	0.942	0.061	0.166	0.082
HD-59	33	11	0.636	0.796	0.007*	0.203	0.105

N, number of samples; * $P < 0.05$

frequency. In addition, high null allele frequency (0.05 or more) was detected at six loci (*SN 135*, *HD-1*, *HD-10*, *HD-19*, *HD-26*, and *HD-59*); of these, the null allele frequency of four loci (*HD-1*, *HD-10*, *HD-19* and *HD-59*) was greater than 0.10. Four loci significantly deviated from *HWE*: *HD-1* ($p = 0.000$), *HD-10* ($p = 0.019$), *HD-19* ($p = 0.001$), and *HD-59* ($p = 0.007$) (Table 2). The positive value of F_{is}, which indicates high degree of inbreeding, in these four loci was caused by high null allele frequency but not by homozygote excess. The high frequency of null allele in the four loci hindered the estimation of genetic structure of *S. nudus*.

Other studies investigated *S. nudus* populations in the Oshika Peninsula (Miyagi Pref.) and the Otsuchi Bay (Iwate Pref.) after the tsunami (Takami et al. 2013, Kawamura et al. 2014). Although significant decrease in population density of the sea urchin was detected in both areas after the tsunami, the recovery processes differed between the two areas. The recovery in the Otsuchi Bay was fast as indicated by apparent increase in large-sized sea urchin individuals, return to the shallow coastal areas of individuals that were transported away to deeper areas, whereas the populations in the Oshika Peninsula showed low level of recovery and did not follow the scenario observed in the Otsuchi Bay (Kawamura et al. 2014). Since the bottleneck was caused by the decrease of large-sized adult sea urchins and the extent of their offspring, the difference in number of survived large-sized sea urchins or those that returned from deeper areas and the recovery processes after the natural disaster have influenced the future genetic structure of *S. nudus*.

In conclusion, the msDNA loci that can effectively estimate genetic structure of this species are not provided, although this study selected loci that have higher number of alleles and/or do not shown deviation from *HWE*. However, the four loci (*SN 73*, *SN 164*, *HD-7*, and *HD-12*) that Nam et al. (2014) used for genetic structure analysis of *S. nudus* could be utilized as effective genetic markers. The selection of effective msDNA loci to estimate genetic structure of *S. nudus* populations is important, because it will help us reveal the changes in genetic diversity of the population.

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