日本産トキワススキの遺伝的多様性

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Yasushi Ibaragi[1*], Takahisa Tanaka[2], Koji Yonekura[2], Chang-Sheng Kuoh[3] and Mitsuo Suzuki[2]: **Genetic variation in *Miscanthus floridulus* (Labill.) Warb. ex K. Schum. et Lauterb. populations in Japan revealed by nuclear DNA markers**

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Miscanthus floridulus (Labill.) Warb. ex K. Schum. et Lauterb. is a large tussock grass of the tribe Andropogoneae (Panicoideae in the Poaceae) that is closely related to sugarcane (Sasaki 1937; Adachi et al. 1955; Adachi 1958; Clayton and Renvoize 1986). This perennial grass is widespread in temperate and tropical regions from New Guinea to the Pacific side of Japan (Lee 1964a, b; Koyama 1987; Osada 1989). It has been used by local people for agriculture and has recently generated interest as a potential biofuel energy crop (Bullard 1996; Bullard et al. 1997; Christian et al. 1997). The biological and agricultural aspects of this grass have been well studied. *M. floridulus* is diploid (Sasaki 1937; Adachi et al. 1955; Adachi 1958), and most species of *Miscanthus* are outbred, with reduced seed sets from selfing (Hirayoshi et al. 1955, 1959; Deuter 2000).

While researching endangered plants in Japan, we noticed that local populations of *M. floridulus* were exponentially declining. The plant is becoming rare, especially in eastern Japan and the southern part of Shikoku Island, with only a few remaining tussocks in Chiba, Okinawa, and Tokushima prefectures (Okinawa-ken 1996; Tokushima-ken 2001).

To explore causes for the decline of this species, we conducted field surveys and genetic analyses using microsatellite markers. We also examined seed fertility to estimate successful reproduction in the wild. This information is important for developing conservation strategies for this species and to provide a basis for germplasm collection.

Materials and methods

SSR analysis

For DNA analysis, 110 samples were gathered from five populations and a few individuals in three regions to cover the complete distribution of *Miscanthus floridulus* in Japan (Fig. 1, Table 1). For comparison, 44 samples were collected from two populations and a few additional individuals distributed in Taiwan (Fig. 1, Table 1). All DNA samples were collected in 2007.

Within each population, we sampled leaves from more than 20 tussocks separated by a minimum distance of 2 m to reduce the risk of redundantly sampling the same individual.

To investigate genetic diversity, total genomic DNA was isolated from 20 mg of dried leaf tissue using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA), following the manufacturer's protocol. Eight primer pairs detecting simple sequence repeats (SSRs) were used (ZCA176, ZCT439, ZCT155, ZCT434, ZCT339, ZCT653, ZCT182, ZCT603), which were originally designed for *Zea mays*. The PCR primer sequences were obtained from the MaizeGDB Web site (Schaeffer et al. 2011; MaizeGDB Working Group 2012). Polymerase chain reaction (PCR) was performed using Premix *taq* (Ex Taq™ Version; TaKaRa Bio, Shiga, Japan). Amplification reactions were performed according to Hernández et al. (2001). Cycling conditions were as follows: an initial denaturation at 94℃ for 10 min, followed by 30 cycles of 30 s denaturation at 95℃, 1 min annealing at

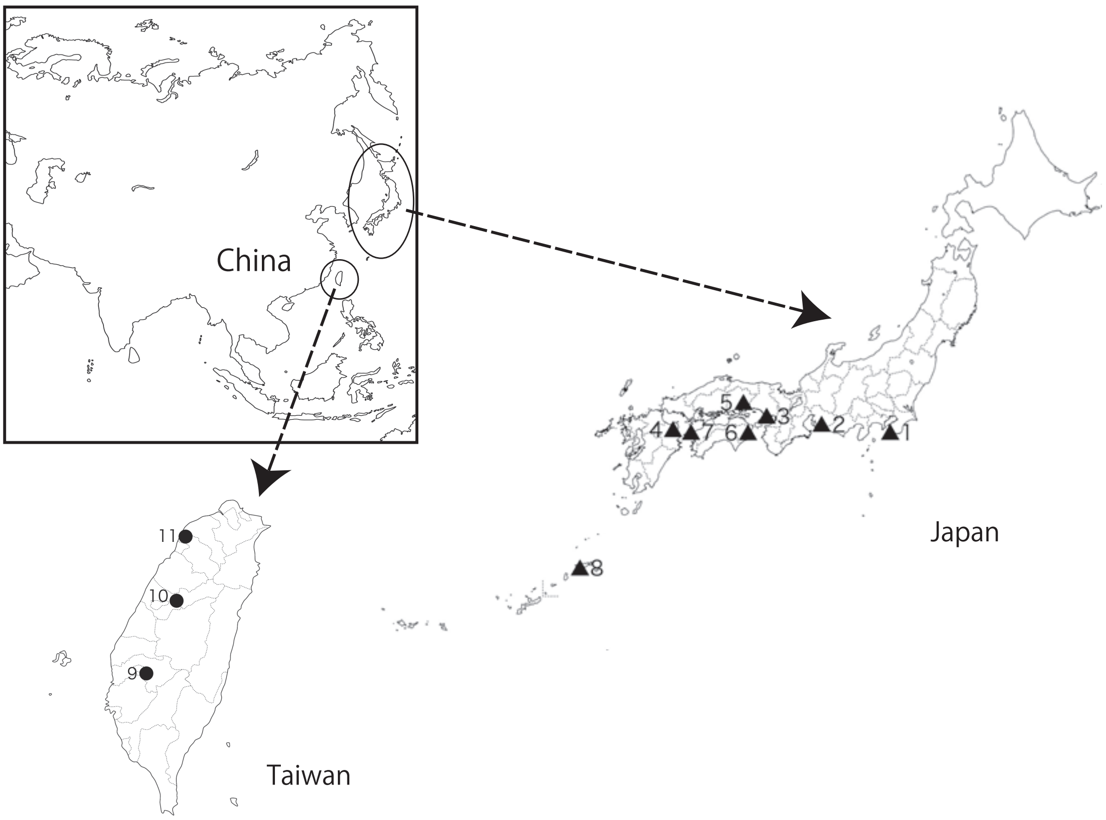

Fig. 1. Locations of the studied populations of *Miscanthus floridulus* in Japan and Taiwan. Numbers represent each population (1. Chiba, 2. Aichi, 3. Hyogo, 4. Ohita, 5. Okayama, 6. Tokushima, 7. Ehime, 8. Kagoshima, 9. Chiayi, 10. Taichung, 11. Hsinchu).

55℃, and 1 min extension at 72℃, and a final extension for 5 min at 72℃. The genotypes for each microsatellite marker were determined using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA), along with Peak Scanner™ Software ver. 1.0 and GeneMapper™ Software ver. 4.0 (Applied Biosystems).

For each nuclear microsatellite locus, the number of alleles per locus (K), the observed heterozygosity (H_O), and the unbiased expected heterozygosity (H_E; Nei 1987) were calculated using CERVUS ver. 2.0 (Marshall et al. 1998). The inbreeding coefficient (F_{IS}) per population and Nei's estimation of heterozygosity were calculated using FSTAT ver.2.93 (Goudet 1995). F_{IS} calculations for each locus were conducted with GenePop (Raymond and Rousset 1995).

Seed fertility

To explore reproductive characteristics and seed dispersal, we investigated seed fertility. We collected 145 specimens from Ohita, Saga, Nagasaki, Kumamoto, Kagoshima, Miyazaki, Hyogo, Okayama, Hiroshima, Tokushima, Ehime, Aichi, and Chiba prefectures in Japan. For comparison, 55 specimens were collected from Taiwan, including Tainan, Chiayi, Taichung counties, and Hsinchu City (Table 2). Seed samples were gathered primarily in 2005, but some additional samples were collected in 2007. Fertile spikelets that were easy to separate from the pedicel were gathered. One hundred spikelets were randomly chosen from each sample for removal of the caryopsis.

Specimen examination and field observation

Specimens borrowed from the following herbaria were used to investigate morphological differences among specimens from different countries and determine the worldwide distri-

Table 1. Location of samples used for DNA analysis.

Population	locality	latitude	longitude	Number of indivuduals investigated	Collection number	nationality
Aichi	Ikawazu-cho, Tawara-shi	34.38	137.09	3	184-186	Japan
	Ikejiri-cho, Tawara-shi	34.36	137.11	1	170	Japan
	Ishigami-cho, Tawara-shi	34.38	137.08	3	179,180, 183	Japan
	Kumihara, Tawara-shi	34.39	137.19	5	165-169	Japan
	Koshido, Koshido-cho, Tawara-shi	34.35	137.09	7	171-177	Japan
	Takagi-cho, Tawara-shi	34.38	137.07	1	178	Japan
	Higashiakazawa-cho, Toyohashi-shi	34.39	137.21	1	164	Japan
Hyogo	Around Awaji castle, Ozaki, Awaji-shi	34.29	134.51	5	038,044,045,048,051	Japan
	Ezaki, Awaji-shi	34.36	134.59	2	011,012	Japan
	Ozaki, Awaji-shi	34.30	134.52	2	036,037	Japan
	Kareki, Awaji-shi	34.30	134.51	3	052,065,081	Japan
	Nagase, Awaji-shi	34.35	134.58	2	025,026	Japan
	Ohtanigawa, Awaji-shi	34.36	135.00	4	007,008,009,010	Japan
	Murotu, Awaji-shi	34.31	134.53	2	027,028	Japan
Ehime	Ikata-cho, Nishiuwa-gun	33.24	132.10	3	142-144	Japan
Ohita	Katano, Kitsuki-shi	33.24	131.37	5	129-133	Japan
	Nonaka, Kitsuki-shi	33.23	131.35	6	123-128	Japan
	Hirouchi, Ohita-shi	33.10	131.42	1	134	Japan
	Hatano, Kamiura-machi, Minamiamabe-gun	33.03	131.56	1	135	Japan
	Miyanouchi, Kmiura-machi, Minamiamabe-gun	33.00	131.54	1	136	Japan
	Miyanouchi bus stop, Kmiura-machi, Minamiamabe-gun	33.00	131.53	4	138-141	Japan
	Koura, Beppu-shi	33.21	131.29	6	115-119,122	Japan
Okayama	Iwatani, Tamashimakurosaki, Kurashiki-shi	34.29	133.37	3	110,112,113	Japan
	Gokan, Tamano-shi	34.31	133.58	2	092,096	Japan
	Ohyabu, Tamano-shi	34.31	133.58	2	087,091	Japan
	Hachihama-cho, Tamano-shi	34.32	133.56	1	105	Japan
	Muneage, Tamano-shi	34.33	134.00	4	097-099,101	Japan
	Mt.Washu, Tanoura, Shimotsui, Kurashiki-shi	34.26	133.48	1	114	Japan
	Tai, Tamano-shi	34.30	133.57	2	082,086	Japan
Kagoshima	Is.Yoro, Setouchi-cho, Ohshima-gun	28.02	129.09	1	210	Japan
	Kaneku-cho, Naze, Amami-shi	28.23	129.28	1	213	Japan
	Around Amami Habu center, Kaneku-cho, Naze, Amami-shi	28.23	129.28	1	211	Japan
Chiba	Otohama, Shirahama-cho, Minamiboso-shi	34.54	139.55	6	203-208	Japan
	Nemoto, Shirahama-cho, Minamiboso-shi	34.54	139.50	14	189-202	Japan
Tokushima	Ohmata, Ichiba-cho, Awa-shi	34.06	134.15	1	209	Japan
	Nagahara, Matsushige-cho, Itano-gun	34.07	134.36	2	001,003	Japan
	Yokoo, Mima-cho, Mima-shi	34.03	134.02	1	214	Japan
Chiayi	at the foot of Mt. Alishan, Fanlu-xiang, Zhongpu-xiang	23.26	120.33	18	3009-3018,3030-3034,3037-3039	Taiwan
Taichung	at the foot of Mt. Daxueshan, Danankeng, Dongshi-xiang,	24.12	120.52	24	3041-3058,3066-3071	Taiwan
Hsinchu	Lianhuasi, Hsinchu-shi	24.52	120.57	2	3072,3073	Taiwan

bution (abbreviations according to Holmgren et al. 1990): BISH, CAS, CBM, KYO, LAE, MBG, P, TI, TNS, TUS, and the herbarium of the Fukui City Museum of Natural History. Specimens were also examined at KATH, MAK, PNH, TKPM, KPM, PE, RYU, and the herbarium of Tamagawa University.

Field observations were conducted in the Philippines, Taiwan, and southern China, as well as Ohita, Saga, Nagasaki, Kumamoto, Kagoshima, Miyazaki, Hyogo, Okayama, Hiroshima, Tokushima, Ehime, Aichi, and Chiba prefectures of Japan. Interviews were also conducted with local inhabitants to gather information regarding planting, seedlings, and usage of the grass.

Voucher specimens for genetic SSR analysis and seed fertility were deposited at the herbarium of the Tokushima Prefectural Museum (TKPM).

Results

Nuclear DNA microsatellite variation

A single amplicon was obtained by PCR using all primer pairs, excluding ZCT 339 and 653 (in which two amplicons were obtained). We used 110 DNA samples from Japan and 44 from Taiwan as PCR templates (Tables 3 & 4), but no variations were observed in the number of amplicons according to DNA templates.

Among the Japanese individuals, 107 had identical genotypes, although a few primer pairs did not yield amplicons in some samples (Table 3). Genotypes of the remaining three samples differed from other Japanese individuals in one to five alleles. In contrast, the Taiwan individuals showed marked variation (Table 3).

The average expected heterozygosity (H_E) ranged from 0.205 to 0.397 for Japanese populations and from 0.509 to 0.633 for Taiwanese

Table 2. Location of samples for seed fertility tests.

nationalit	Locarity	latitude	longitude	Date	Collector	Collection number
Japan	Around Tsurusaki station, Oita Pref., Kyushu.	33.14	131.41	2005/7/2	Y.Ibaragi	t-1
Japan	At the entrance of Matsugao tunnel, Kunimi-cho, Higashikunisaki-gun, Oita Pref., Kyushu.	33.29	131.38	2005/7/3	Y.Ibaragi	t-9,t-11
Japan	Kunisaki-machi, Higashikunisaki-gun, Oita Pref., Kyushu.	33.38	131.41	2005/7/3	Y.Ibaragi	t-12
Japan	Mikawa, Kitsuki City, Oita Pref., Kyushu.	33.24	131.37	2005/7/3	Y.Ibaragi	t-17
Japan	Mitukawa, Hiji-machi, Hayami-gun, Oita Pref., Kyushu.	33.21	131.29	2005/7/3	Y.Ibaragi	t-25
Japan	Around Houyou junior hischool.,Usuki City, Oita Pref., Kyushu.	131.49	33.10	2005/7/4	Y.Ibaragi	t-34
Japan	Around Saganoseki primaly school, Saganoseki-machi, Kitaamabe-gun, Oita Pref., Kyushu.	33.14	131.52	2005/7/4	Y.Ibaragi	t-38,t-40
Japan	on a bank along a rail road, Nishikamiura, Saiki City, Oita Pref., Kyushu.	33.00	131.54	2005/7/4	Y.Ibaragi	t-42, t-46
Japan	Shinkiba, Hizen-cho, Higashimatsuura-gun, Saga Pref., Kyushu.	33.25	129.51	2005/7/5	Y.Ibaragi	t-60
Japan	Konagai-cho, Kitatakaki-gun, Nagasaki Pref., Kyushu.	32.56	130.11	2005/7/6	Y.Ibaragi	t-67
Japan	Isakina, Konagai-cho, Kitatakaki-gun, Nagasaki Pref., Kyushu.	32.56	130.11	2005/7/6	Y.Ibaragi	t-72,73,74,75
Japan	At the front of Matushima city centor, Matsushima, Amakusa-gun, Kumamoto Pref., Kyushu.	32.30	130.25	2005/7/7	Y.Ibaragi	t-92,93,94
Japan	Around Ohsakihana, Makurazaki City, Kagoshima Pref., Kyushu.	31.15	130.20	2005/7/8	Y.Ibaragi	t-95
Japan	Minamibeppu, Chiran-cho, Kawanabe-gun, Kagoshima Pref., Kyushu.	31.15	130.24	2005/7/8	Y.Ibaragi	t-96
Japan	Around Misoko river, Kiire-cho, Ibusuki-gun, Kagoshima Pref., Kyushu.	31.15	130.24	2005/7/8	Y.Ibaragi	t-97
Japan	Ohsako, Ibusuki-gun, Kagoshima Pref., Kyushu.	31.15	130.33	2005/7/8	Y.Ibaragi	t-98
Japan	Nakahama, Ei-cho, Ibusuki-gun, Kagoshima Pref., Kyushu.	31.14	130.30	2005/7/8	Y.Ibaragi	t-99,100
Japan	Kanatoguchi, Higashiichiki-cho, Hioki-gun, Kagoshima Pref., Kyushu.	31.38	130.19	2005/7/8	Y.Ibaragi	t-101
Japan	Ono, Fukiage-cho, Hioki-gun, Kagoshima Pref., Kyushu.	31.33	130.20	2005/7/8	Y.Ibaragi	t-102
Japan	near the Amagao beach, Hiyoshi-cho, Hioki-gun, Kagoshima Pref., Kyushu.	31.36	130.20	2005/7/8	Y.Ibaragi	t-103
Japan	Kukino, Kaseda City, Kagoshima Pref., Kyushu.	31.21	130.16	2005/7/8	Y.Ibaragi	t-104,106
Japan	At road side, near Furue primary school, Kanoya City, Kagoshima Pref., Kyushu.	31.23	130.46	2005/7/9	Y.Ibaragi	t-107
Japan	Nishihama, Kushima City, Miyazaki Pref., Kyushu.	31.27	131.12	2005/7/9	Y.Ibaragi	t-108,109
Japan	Near the Tarumizu port, Tarumizu City, Kagoshima Pref., Kyushu.	31.27	130.42	2005/7/9	Y.Ibaragi	t-110-113
Japan	near the Ohsaki Kannonzaki, Tarumizu City, Kagoshima Pref., Kyushu.	31.34	130.46	2005/7/9	Y.Ibaragi	t-114-116
Japan	near the Kanagasaki beach, Hiyuuga City, Miyazaki Pref., Kyushu.	32.21	131.37	2005/7/10	Y.Ibaragi	t-120-126
Japan	at Ohyabu bus stop, Tamano City, Okayama Pref., Honshu.	34.31	133.58	2005/7/26	Y.Ibaragi	t-144
Japan	Near the Muneage Hokkoh Kagaku iriguchi bus stop, Tamano City, Okayama Pref., Honshu.	34.33	134.00	2005/7/26	Y.Ibaragi	t-146
Japan	Iwaya, Yorishima-cho, Asakuchi-gun, Okayama Pref., Honshu.	34.29	133.37	2005/7/27	Y.Ibaragi	t-156
Japan	Keikoya, Kure City, Hiroshima Pref., Honshu.	34.11	132.33	2005/7/28	Y.Ibaragi	t-157
Japan	Myohjinhara, Takehara City, Hiroshima Pref., Honshu.	34.20	132.58	2005/7/28	Y.Ibaragi	t-161
Japan	Etajima-cho, Etajima city, Hiroshima Pref., Honshu.	34.12	132.28	2005/7/28	Y.Ibaragi	t-165
Japan	Kohzaki-cho, Mihara City, Hiroshima Pref., Honshu.	34.19	133.01	2005/7/28	Y.Ibaragi	t-169
Japan	North side of Nagahara primary school, Matsushige-cho, Itano-gun, Tokushima Pref., Shikoku.	34.07	134.56	2005/8/4	Y.Ibaragi	t-171-173
Japan	Nishino-kubo, Mima-cho, Mima-gun, Tokushima Pref. Shikoku.	34.03	134.02	2003/8/6	K.Manabe	manabe-1
Japan	Cyohba, Tai icchohme, Tamano City, Okayama Pref., Honshu.	34.30	133.57	2007/7/25	Y.Ibaragi	84
Japan	at Ohyabu bus stop, Tamano City, Okayama Pref., Honshu.	34.31	133.58	2007/7/25	Y.Ibaragi	89,91
Japan	Gokan, Tamano City, Okayama Pref., Honshu.	34.31	133.58	2007/7/25	Y.Ibaragi	92-96
Japan	Muneage, Tamano City, Okayama Pref., Honshu.	34.33	134.00	2007/7/25	Y.Ibaragi	98,101
Japan	Mizushima, Kurasiki City, Okayama Pref., Honshu.	34.31	133.42	2007/7/26	Y.Ibaragi	107
Japan	Sami beach, Kurosaki, Tamashima, Okayama Pref., Honshu.	34.29	133.37	2007/7/26	Y.Ibaragi	108,109
Japan	Iwaya, Yorishima-cho, Asakuchi-gun, Okayama Pref., Honshu.	34.29	133.37	2007/7/26	Y.Ibaragi	110,112
Japan	Koura, Beppu City, Ohita Pref., Kyushu	33.21	131.29	2007/8/6	Y.Ibaragi	115-122
Japan	Nonaka, Kitsuki City, Ohita Pref., Kyushu	33.23	131.35	2007/8/6	Y.Ibaragi	123-125,127-128
Japan	Kitsuki City, Ohita Pref., Kyushu	33.24	131.37	2007/8/6	Y.Ibaragi	129,130,132,133
Japan	Hirouchi, Ohita City, Ohita Pref., Kyushu	33.10	131.42	2007/8/6	Y.Ibaragi	134
Japan	At the mouth of Tsui tunnel, Hatano, Kamiura-machi, Minamiamabe-gun, Ohita Pref., Kyushu	33.03	131.56	2007/8/6	Y.Ibaragi	135
Japan	at the bus stop, Miyanoura, Kamiura-machi, Minamiamabe-gun, Ohita Pref., Kyushu,	33.00	131.53	2007/8/6	Y.Ibaragi	137,140,141
Japan	Natori, Misaki-cho, Nishiuwa-gun, Ehime Pref. Shikoku.	33.24	132.10	2007/8/6	Y.Ibaragi	142,143
Japan	Ohhisa tunnel, Misaki-cho, Nishiuwa-gun, Ehime Pref. Shikoku.	33.24	132.10	2007/8/6	Y.Ibaragi	146,148,149
Japan	Around Seto-Nougyo Park, Misaki-cho, Nishiuwa-gun, Ehime Pref. Shikoku.	33.26	132.15	2007/8/6	Y.Ibaragi	151,154,156-158
Japan	Near the Michino-eki-Kirara, Misaki-cho, Nishiuwa-gun, Ehime Pref. Shikoku.	33.28	132.18	2007/8/6	Y.Ibaragi	161,162
Japan	Higashi-Akasawa-cho, Toyohashi City, Aichi Pref. Honshu.	34.39	137.21	2007/8/11	Y.Ibaragi	163
Japan	Kumihara, Tahara City, Aichi Pref. Honshu.	34.39	137.19	2007/8/11	Y.Ibaragi	165-169
Japan	Akabane fishing port, Ikejiri-cho,Tahara City, Aichi Pref. Honshu.	34.36	137.11	2007/8/11	Y.Ibaragi	170
Japan	Otto, Otto-cho,Tahara City, Aichi Pref. Honshu.	34.35	137.09	2007/8/11	Y.Ibaragi	172-177
Japan	Takagi-cho, Tahara City, Aichi Pref. Honshu.	34.38	137.07	2007/8/11	Y.Ibaragi	178
Japan	Ishigami-cho, Tahara City, Aichi Pref. Honshu.	34.38	137.08	2007/8/11	Y.Ibaragi	180,181,183
Japan	Ikawadu-cho, Tahara City, Aichi Pref. Honshu.	34.38	137.09	2007/8/11	Y.Ibaragi	184-188
Japan	Nemoto, Shirahama-cho, Minami-bousou City, Chiba Pref. Honshu.	34.54	139.50	2007/8/13	Y.Ibaragi	189-197,202
Japan	Otohama, Shirahama-cho, Minami-bousou City, Chiba Pref. Honshu.	34.54	139.55	2007/8/13	Y.Ibaragi	203,205-208
Taiwan	Tainan: Zuojhen xiang	23.04	120.22	2007/12/20	Y.Ibaragi, C.S.Kuoh and M.H.Chuang	3003-3005,3007
Taiwan	Chiayi: at the foot of Mt. Alishan, Fanlu-xiang, Zhongpu-xiang	23.26	120.33	2007/12/21	Y.Ibaragi, C.S.Kuoh and M.H.Chuang	3009,3012,3015-3018,3020,3021,3023-3028,3030,3032,3034-3036,3038,3039
Taiwan	Taichung:at the foot of Mt. Daxueshan, Danankeng, Dongshi-xiang	24.12	120.52	2007/12/22	Y.Ibaragi, C.S.Kuoh and M.H.Chuang	3041,3042,3044-3071

Table 3. Geological information related to the studied population and population genetic parameters revealed by nuclear microsatellite markers.

Population	No.of individuals	number of genotypes	K	Ho	He	Fis	P-value
Japan: Chiba	20	1	1.40	0.4	0.207	-1.000	1.0000
Japan: Aichi	21	1	1.40	0.4	0.205	-1.000	1.0000
Japan: Hyogo	20	1	1.40	0.4	0.207	-1.000	1.0000
Japan: Ohita	24	2	1.50	0.405	0.210	-0.978	1.0000
Japan: Okayama	15	2	1.40	0.393	0.208	-0.964	1.0000
Japan: Tokushima	4	1	1.40	0.4	0.274	-1.000	1.0000
Japan: Ehime	3	1	1.40	0.4	0.247	-1.000	1.0000
Japan: Kagoshima	3	2	2.00	0.417	0.397	-0.087	0.6614
Taiwan: Chiayi	18	18	4.70	0.432	0.509	0.158	0.0036
Taiwan: Taichung	24	24	5.40	0.276	0.522	0.476	0.0005
Taiwan: Hsinchu	2	2	2.50	0.550	0.633	0.185	0.2195

K:mean number of alleles per locus
Fis: Fis per population
He:mean expected heterozygosity,
Ho:mean observed heterozygosity
P-value:P-value for Fis within samples

Table 4. Genetic diversity measures estimated at 10 microsatellite loci across the 11 populations.

Locus	number of alleles	size range (bp)	Ho	He	Null frequency
ZCA176	4	152-156	0.093	0.231	+0.4477
ZCT439	4	208-224	0.734	0.482	–0.2166
ZCT155-1	4	115-157	0.023	0.346	+0.8776
ZCT155-2	9	200-295	0.794	0.677	–0.1227
ZCT434-1	3	73-78	0.969	0.517	–0.3147
ZCT434-2	7	80-93	0.093	0.152	+0.2284
ZCT339-1	16	109-134	0.096	0.451	+0.6734
ZCT339-2	14	120-164	0.153	0.429	+0.5209
ZCT653-1	5	134-142	0.908	0.624	–0.2151
ZCT653-2	15	140-173	0.087	0.389	+0.6708

Ho:observed heterozygosity
He:expected heterozygosity

populations. The observed heterozygosity (H_O) ranged from 0.393 to 0.417 for Japanese populations and 0.276 to 0.55 for Taiwanese populations. The F_{IS} values were close to –1.0 (range, –1 to –0.087, average –0.879) for the Japanese populations, indicative of heterozygotic individuals. In contrast, the value ranged from 0.158 to 0.476 (mean = 0.273) for the Taiwanese populations, which was similar to Hardy–Weinberg equilibrium. The number of alleles per locus (K) ranged from 1.4 to 2.0 for Japanese plants and from 2.5 to 5.4 for Taiwanese plants. Although the genetic diversities were much higher in Taiwanese populations than Japanese populations, these values did not exceed the general ranges for a perennial grass (Sun et al. 2002; Hung et al. 2008).

Seed fertility

The average seed fertility of Japanese specimens was very low at only 1.39% (Table 5). Almost all individuals were sterile, with 0–3% fertility. Only three individuals from Takehara-cho, Takehara-shi, Hiroshima (collection no. t-161: 28%), Saizaki-cho, Mihara-shi, Hiroshima (collection no. t-169: 64%), and Otto-cho, Tahara-shi, Aichi (collection no. 172: 60%) had high seed fertility. While the seed fertility of Taiwanese specimens was apparently higher than that of Japanese specimens, the average fertility of inflorescences was 62% (range, 2–96%; Table 5). The spikelets of Japanese plants were almost sterile, whereas the Taiwanese plants showed high seed fertility.

Discussion

Our research showed that Japanese *Miscanthus floridulus* had very little genetic variation and limited production of viable seeds, indicating that the population was declining to extinction. This situation contrasts markedly with that in Taiwanese populations, in which wide genetic diversity and high seed fertility were retained. High genetic diversity of *Miscanthus* species in Taiwan has also been reported using isozyme and other molecular markers (Chou et al. 1987; Chou and Chang 1988; Chou and Chen 1990).

Only three samples from Amami Island in Kagoshima (collection no. 0211), Kitsuki-shi in Ohita (collection no. 133), and Tamano-shi in Okayama (collection no. 082) differed from each other by one to five genes. This likely represents somatic cell mutation of the alleles, rather than crossing with related species or an individual with a different genotype, as the differences were too small. In other words, the Japanese population of *M. floridulus* became virtually a single clone. However, it is possible that these variations were generated prior to establishment of asexual propagation in *M. floridulus*, and multiple lines of clones were introduced artificially or non-artificially to Japan.

Table 5. Seed fertility of Japanese and Taiwanese *Miscanthus floridulus*.

JAPAN

Collection number	Seed fertility (%)	Collection number	Seed fertility (%)	Collection number	Seed fertility (%)	Collection number	Seed fertility (%)	Collection number	Seed fertility (%)
t-1	0	t-104	0	84	0	130	0	174	3
t-9	1	t-106	0	89	1	132	0	175	1
t-11	0	t-107	1	91	1	133	1	176	1
t-12	0	t-108	0	92	1	134	1	177	2
t-17	0	t-109	0	93	2	135	0	178	0
t-25	0	t-110	0	94	1	137	0	180	0
t-34	0	t-111	0	95	0	140	2	181	0
t-38	0	t-112	0	96	0	141	2	183	2
t-40	0	t-113	0	98	0	142	0	184	0
t-42	0	t-114	0	101	0	143	0	185	0
t-46	0	t-115	0	107	1	146	0	186	0
t-60	1	t-116	1	108	1	148	0	187	0
t-67	0	t-120	0	109	1	149	0	188	0
t-72	0	t-121	0	110	3	151	0	189	0
t-73	0	t-122	0	112	0	154	0	190	3
t-74	0	t-123	0	115	0	156	0	191	0
t-75	0	t-124	1	116	0	157	0	192	0
t-92	0	t-125	0	117	1	158	0	193	0
t-93	0	t-126	0	118	0	161	2	194	0
t-94	0	t-144	0	119	0	162	0	195	2
t-95	1	t-146	1	120	1	163	1	196	0
t-96	0	t-156	0	121	0	165	1	197	0
t-97	0	t-157	0	122	0	166	0	202	0
t-98	0	t-161	28	123	0	167	0	203	0
t-99	1	t-165	0	124	1	168	1	205	0
t-100	0	t-169	64	125	1	169	0	206	1
t-101	0	t-171	0	127	0	170	0	207	0
t-102	0	t-172	0	128	0	172	60	208	0
t-103	0	t-173	0	129	0	173	0	manabe-1	0

sample number =145, average seed fertility:1.39%

TAIWAN

Collection number	Seed fertility (%)	Collection number	Seed fertility (%)
3003	76	3045	50
3004	35	3046	15
3005	64	3047	68
3007	75	3048	65
3009	57	3049	87
3012	72	3050	64
3015	93	3051	50
3016	85	3052	67
3017	93	3053	30
3018	74	3054	46
3020	53	3055	74
3021	90	3056	68
3023	59	3057	18
3024	96	3058	47
3025	75	3059	16
3026	60	3060	39
3027	67	3061	38
3028	82	3062	25
3030	64	3063	73
3032	84	3064	94
3034	51	3065	74
3035	92	3066	80
3036	90	3067	62
3038	2	3068	76
3039	24	3069	90
3041	28	3070	57
3042	45	3071	80
3044	72		

sample number =55, average seed fertility:62%

Fig. 2. *Miscanthus floridulus* used as protection around a crop field (photo: Tarumizu-shi, Kagoshima Prefecture; July 09, 2005).

M. floridulus is likely a transplanted species due to its common use. In New Guinea, its culms have been used as arrow shafts (Henty 1969), and in Japan, the leaves and culms have been used as a roofing material and to make cow reins (Fujimoto 1995). Fujimoto (2001) also reported the use of *M. floridulus* in strengthening riverbanks, protecting orange fields, and making brooms. To explore the usage of this grass, we interviewed local people claiming that the plants were cultivated after World War II for use in daily life. We also confirmed that many tussocks had been planted around riverbanks and crop fields (Fig. 2). Considering these reports and comments from local people, some *M. floridulus* populations in Japan likely originated from intentional transplanting and cultivation by farmers.

Seed fertility of *Miscanthus* species is typically high. For example, Yoshida et al. (1958) examined the seed fertility of *M. sinensis* and reported an average seed set of 22.8–58.1%. The average percentage seed set (± S.D.) for *M. sacchariflorus*, *M. sinensis*, and *M. tinctorius* is 71.8 ± 21.59%, 47.6 ± 12.89%, and 30.1 ± 10.78%, respectively (Matsumura and Yukimura 1975), and a seed set ratio of 22.4-31.1% has been reported for *M. sinensis* (Nishiwaki et al. 1996). We also observed high seed fertility for *M. floridulus* in Taiwan (at 62%), while Japanese populations of *M. floridulus* had very low seed fertility (1.39%), which is likely a major cause of the decline of this species.

The reasons for low seed fertility of Japanese *M. floridulus* remain unclear, but may be related to the breeding system of this species. *Miscanthus* species produce their offspring mostly by outcrossing (Hirayoshi et al. 1955; Hirayoshi et al. 1959; Deuter 2000). As discussed above, the majority of Japanese *M. floridulus* individuals are virtually clones. Therefore, seed fertility would decline at rates similar to those of selfing individuals, even if outcrossings are realized between functional female and male gametes.

M. floridulus is widely distributed and grows in many habitats, from tropical areas of New Guinea to temperate regions of Japan, and has wide morphological variation. This study on *M. floridulus* in Japan and abroad shows that while *M. floridulus* is polymorphic in China, Taiwan, New Guinea, the Philippines, and Indonesia, it is homomorphic in Japan. This was confirmed by both genetic SSR analysis and field and herbaria specimens. Japanese *M. floridulus* homogeneously have very small spikelets less than 3.5 mm long. Furthermore, we found that plants with such small spikelets are distributed only in Japan, Taiwan, and southern provinces of China, such as Zhejiang, Guangdong, Fujian, Anhui, Jiangxi, and Hubei. Since *M. floridulus* is used by the local people for agricultural purposes as noted above, this similarity between Japanese and southern Chinese individuals suggests that Japanese *M. floridulus* may have been transplanted mainly from south China. However, we do not have sufficient information to discuss the origin and history of Japanese *M. floridulus*. Further genetic comparison between Japanese and Chinese *M. floridulus* populations could increase our understanding of the biological characteristics of Japanese *M. floridulus*.

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茨木靖[1]・田中孝尚[2]・米倉浩司[2]・郭長生[3]・鈴木三男[2]：日本産トキワススキの遺伝的多様性

日本産および台湾産のトキワススキについて，遺伝的多様度を解析した。その結果，3個体については，遺伝子に変異が見いだされたが，日本のトキワススキは殆どがクローンであった。これに対して台湾のトキワススキは，多様な遺伝子型よりなる集団であった。もし日本のトキワススキが在来種だとすると，現在では，その殆どが1クローンになってしまっていることになる。しかし野外での観察や聞き取り結果などもふまえると，日本のトキワススキは移入個体が人為的に増殖された可能性がある。後者の場合は，絶滅危惧種として保全する必要はなくなるだろう。また，前者の場合も，現在の分布域の全部ないし一部が，本来の自生地ではなく移入されたものである可能性が残る。いずれにせよ，日本の個体群の起源については，他のアジア地域の個体群との比較を含めた再検討が必要となる。また，遺伝的な差異の見られた日本産の3個体については，いずれも少数の遺伝子しか異なっておらず，近縁種はおろか，別遺伝子型他個体との交雑も考えにくく，該当遺伝子座の突然変異の可能性が高いと考えられる。

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