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BIOSYSTEMATIC STUDIES OF THE *DUMORTIERA HIRSUTA* COMPLEX (DUMORTIERACEAE, HEPATICAE), 2. MONOPLOID AND DIPLOID DIVERSIFICATION IN THE HAWAIIAN ISLANDS

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Abstract. The *Dumortiera hirsuta* complex (Dumortieraceae, Marchantiales) in the Hawaiian Islands is shown to include monoploids and diploids. They differ in outer morphology, habitat preference (diploid populations were found at higher elevations than the monoploids), and genetic features detected by allozyme analyses. Judging from Nei's genetic distance values, Hawaiian monoploids are closely related to Japanese monoploids. The origin of the diploids is the subject of further study.

Key words: *Dumortiera hirsut*a complex*, D. trichocephal*a, Hawaiian Islands, polyploidy, speciation, allozymes

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INTRODUCTION

The genus *Dumortiera* Nees is the sole member of the Dumortieraceae classified in the thalloid liverworts, Marchantiales (Long 2006). The single species, *D. hirsuta* (Sw.) Nees, is almost cosmopolitan throughout the world and usually forms large populations in rather moist places, such as stream sides and small ravines from lower to upper elevations, but sometimes also in periodically desiccated, calcareous habitats or on mesic soils along trails in forests. The light to dark green thalli are one of the most distinguishing features of the genus. Such an appearance is caused by the total lack of photosynthetic layers, which are shared by almost all members of the Marchantiales species. *Dumortiera*, however, is often supplied with few to numerous papillae and low, whitish walls on the upper epidermis of the thallus, and they are thought to be remnants of photosynthetic filaments and chambers which are often found in the Marchantiales. One of the interesting characteristics previously reported from *Dumortiera* is the presence of a series of different (mono-, di-, and triploid) ploidy levels (Tatuno 1938; 1954, and many others). These ploidy levels have been recognized at infraspecific rank under *Dumortiera hirsuta*, i.e., monoploids as *D. hirsuta* subsp. *hirsuta* (or *D. hirsuta* var. *hirsuta*) (Evans 1919), diploids as *D. hirsuta* subsp. *nepalense* (Taylor) R. M. Schust. [or *D. hirsuta* var. *nepalense* (Taylor) Frye & L. Clark] (Horikawa 1951), and triploids as *D. hirsuta* subsp. *tatunoi* Horik. (Schuster 1992).

Several papers dealing with inter-relationships of populations of the *Dumortiera hirsuta* complex have been published, for example, Akiyama (1999, 2011), Akiyama *et al*. (2003), and Forrest *et al*. (2011). Using allozyme data Akiyama (1999, 2011) and Akiyama *et al*. (2003) suggested that a number of genetically distinct groups (corresponding to species) could be recognised in mono-, di-, and triploid populations distributed in Asia. This suggests that chromosome counting is important in studying diversity within the highly polymorphic *Dumortiera hirsuta* complex. Recently, Forrest *et al*. (2011) pointed out that there must be at least two 'genetically and geographically distinct clades', or biological entities, in the world, based on three chloroplast and one nuclear gene

sequences, though they did not confirm ploidy levels of the examined samples.

The Hawaiian Islands are geographically remote from other continents and island groups and thus well known for their unique phytogeographical position, even for bryophytes that can disperse over very long distances by small spores (Bartram 1933; Miller 1954). *Dumortiera* was first reported from the Hawaiian Islands by Hooker (1837) as *Marchantia trichocephala* Hook. and later as *D. trichocephala* Nees (Nees von Esenbeck 1838). The taxon is now usually recognized as *D. hirsuta* subsp. *nepalense* (Evans 1919; Miller *et al.* 1983, Staples & Imada 2006; Schuster 1992).

The main objectives of the present research were to understand the following issues regarding the Hawaiian *Dumortiera hirsuta* complex with regard to morphology, chromosome numbers, and allozyme analyses: (*i*) the number of biological entities in the Hawaiian Islands; (*ii*) the extent of genetic diversity within populations; and (*iii*) interrelationships between populations on different islands. We included Japanese and Taiwanese populations (both monoploids and diploids) in the allozyme analyses as described below, because preliminary study suggested their close affinity with Hawaiian populations (Akiyama 1999). The results of extended analyses, including other populations from all over East and Southeast Asia, will be presented elsewhere.

Materials and methods

Sampling sites and sampling methods

Field sampling was carried out at six sites on Maui Island and one on Oahu Island in 1998, and at seven

Table 1. Samples used in the analyses and their genetic indices. Localities A–N as in Fig. 1 and Appendix. Ns – sample size used in electrophoretic studies, P – percentage of polymorphic loci, Aa – number of alleles, Ap – number of alleles per polymorphic loci, He – gene diversity. Both monoploids and diploids are found in the two populations (A and D) and they are listed separately. Plants of A, D (monoploid), F, and K were checked only for their morphology and chromosome numbers.

No.	Locality		Ns	P	Aa	Ap	He
	Monoploids (n = 9)						
1	Hawaii, Hilo	A	5	–	–	–	–
2	Hawaii, Waikaea	B	19	0.461	1.46	2.00	0.200
3	Hawaii, Kahaulaea	C	5	0.000	1.00	–	0.000
4	Hawaii, Volcanos	D	3	–	–	–	–
5	Maui, Kopiliula	H	3	0.231	1.23	2.00	0.123
6	Maui, Wailua	J	17	0.357	1.36	2.00	0.151
7	Maui, Makamaole	K	1	–	–	–	–
8	Maui, Waikamoi	L	6	0.154	1.15	2.00	0.064
9	Oahu, Manoa	N	17	0.500	1.50	2.00	0.153
10	JAPAN, Kawara		29	0.500	1.64	2.28	0.175
11	JAPAN, Keisoku		26	0.571	1.71	2.25	0.194
12	TAIWAN, Tailuge	THS	45	0.429	1.50	2.17	0.096
	Diploids (n = 18)						
13	Hawaii, Hilo	A	7	–	–	–	–
14	Hawaii, Volcanos	D	11	0.286	1.43	2.50	0.148
15	Hawaii, Waimea	E	13	0.143	1.14	2.00	0.043
16	Hawaii, Makaula-ooma	F	5	–	–	–	–
17	Hawaii, Hakalau	G	12	0.286	1.34	2.25	0.108
18	Maui, Kosmer	I	20	0.385	1.62	2.60	0.159
19	Maui, Paliku	M	20	0.461	1.77	2.67	0.178
20	JAPAN, Ryugado		34	0.571	1.64	2.13	0.280
21	TAIWAN, Wushyken	TTW	41	0.785	1.93	2.18	0.371

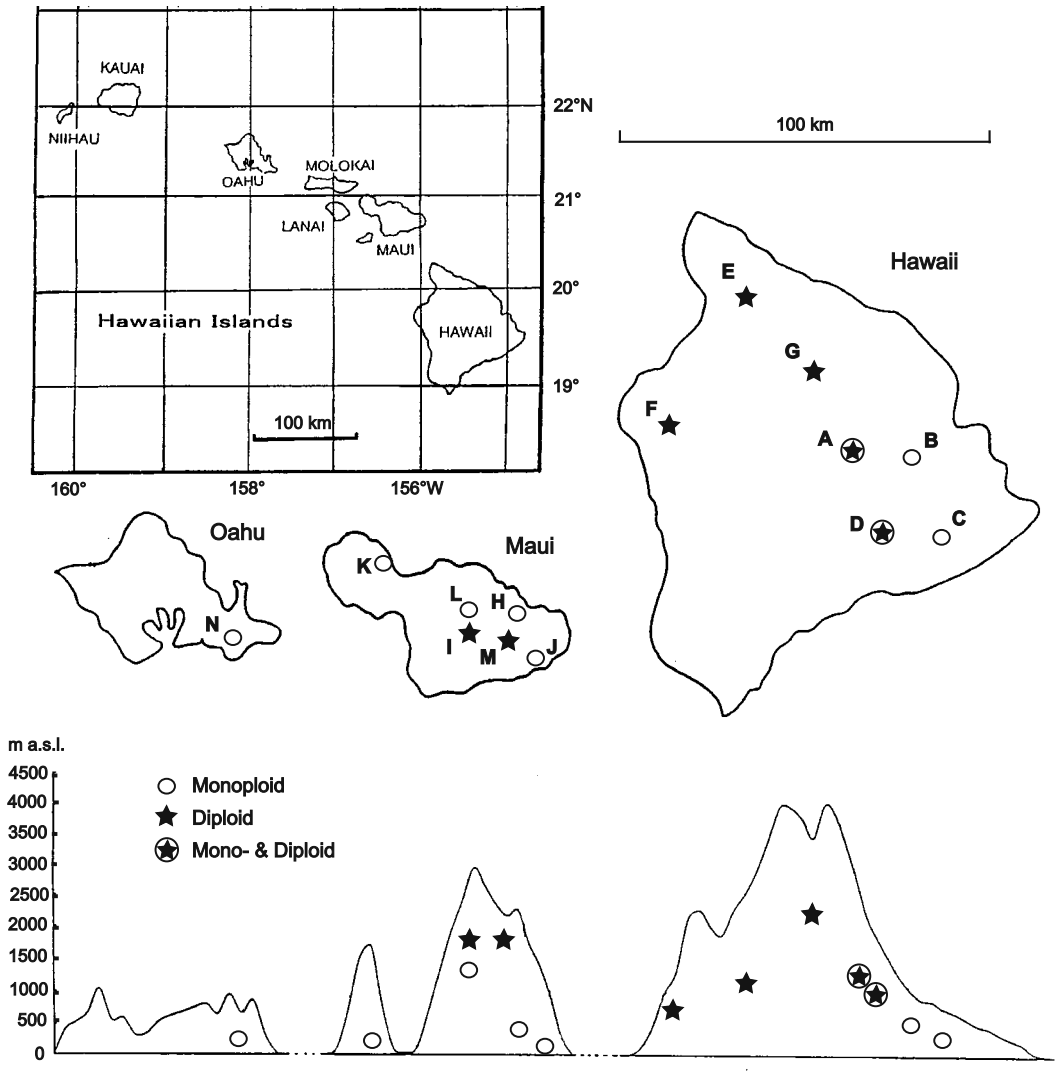

Fig. 1. Map showing horizontal and vertical distribution of sampling sites on Oahu, Maui and Hawaii Islands. Localities A–N as in Table 1 and APPENDIX.

sites on Hawaii Island in 1999 (Fig. 1 and Table 1). Hawaiian *Dumortiera* plants usually grew in moist habitats, such as along streams (Fig. 2). Three Japanese populations (two monoploid and one diploid) and two from Taiwan (one monoploid and one diploid; Akiyama *et al.* 2003) were included only for allozyme analyses. Description of the localities studied is given in the APPENDIX.

From each population we collected 5 cm × 5 cm patches, each separated by 1 m distance, using the same sampling method as for *Conocephalum conicum* (Akiyama & Hiraoka 1994) and Taiwanese *Dumortiera hirsuta* complex (Akiyama *et al.* 2003). This avoids sampling more than one ramet from a single genet. Because of the variations in population size and abundance of plants, the number of patches for each sampling site varied from 3 to 20 for Hawaiian populations.

All of the samples were transported to laboratories in Japan and then divided into three portions for chromosome counting, morphological features, and allozyme analyses with starch gel electrophoresis. The voucher specimens are kept in HYO.

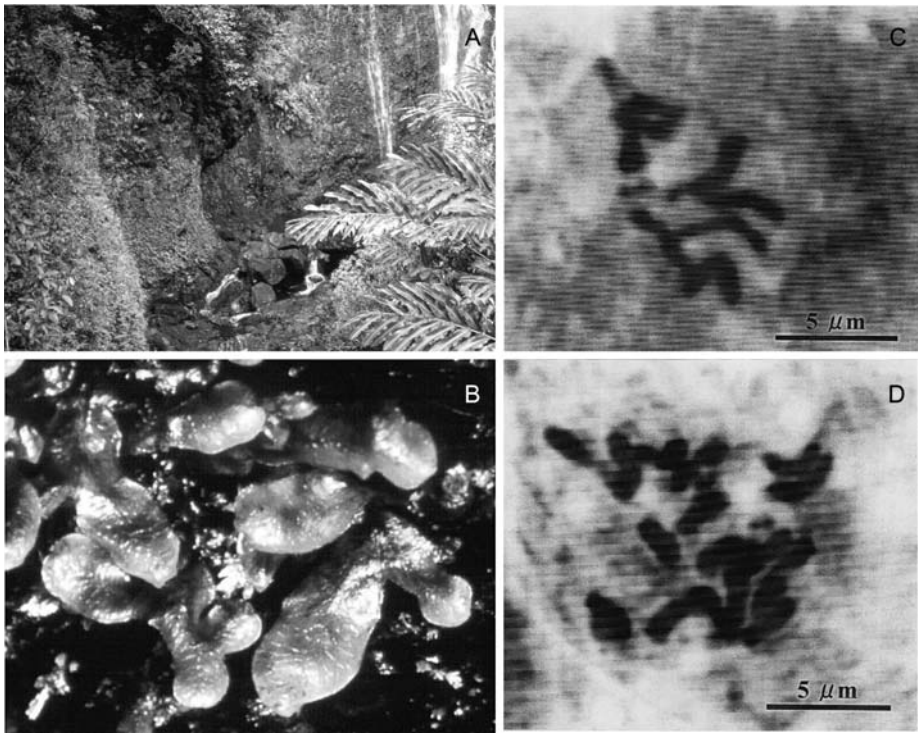

Fig. 2. A–B – Monoploid population of *Dumortiera hirsuta* at Wailua, Kanahualii Falls, Maui Island (150 m a.s.l.). C–D – Chromosomes of monoploid (C; n = 9) and diploid (D; n = 18). Note the whitish remnant walls developed on the upper surface of green thalli in Fig. 2B.

CYTOLOGY

Living material was cultured in moistened plastic pots kept at room temperature in the laboratory. When new shoots developed, their tips were excised and immediately fixed in a modified Carnoy's fluid (100% ethanol: chloroform: glacial acetic acid; 1:1:1 v/v) for three hours at 18°C. After fixation, they were soaked in 45% acetic acid for several minutes, and stained in 2% aceto-orcein for more than 10 hours at 15°C. The stained tips were dissected in 0.5% aceto-orcein on a glass slide, using two fine iron needles under a dissection microscope. The dissected tissues were covered with a cover glass and gently heated for a few minutes to *ca* 100°C. After heating, the cover glass was tapped several times with the handle of the iron needle to crush and spread the tissues. There were no obvious chromosomal differences detected, and only numbers of chromosomes were recorded. For each sample patch, no less than five tips from different thalli were examined. Different chromosome numbers (ploidy levels) were not detected from the same sample patches.

THALLUS MORPHOLOGY

Morphological features (Fig. 3), were measured as follows: (1) thallus width; (2) thickness of the costal region; (3) thickness at the middle part of thallus wings; (4) thickness of small-cell layers of costal region; (5) length from central part to the point of 1/2 thickness of costa; (6) length of dorsal epidermal cell, (7) width of dorsal epidermal cell; (8) length of ventral epidermal cell, (9) width of ventral epidermal cell. Features 1–5 were measured for 1–2 thalli for each sample, and features 6–9 were measured ten times at different places on each thallus. These measurements were summarized and categorized for each Island and ploidy level.

ALLOZYME ANALYSES

10 enzymes and 14 loci were examined: *acn*, *gdh*, *idh*, *lap*, *mdh-1*, *mdh-2*, *me-1*, *me-2*, *me-3*, *6pgd*, *pgm-2*, *skdh*, *tpi-1*, and *tpi-2*. The method used follows Akiyama and Hiraoka (1994) and Akiyama and Suzuki (1998), including buffer systems and loading conditions. The

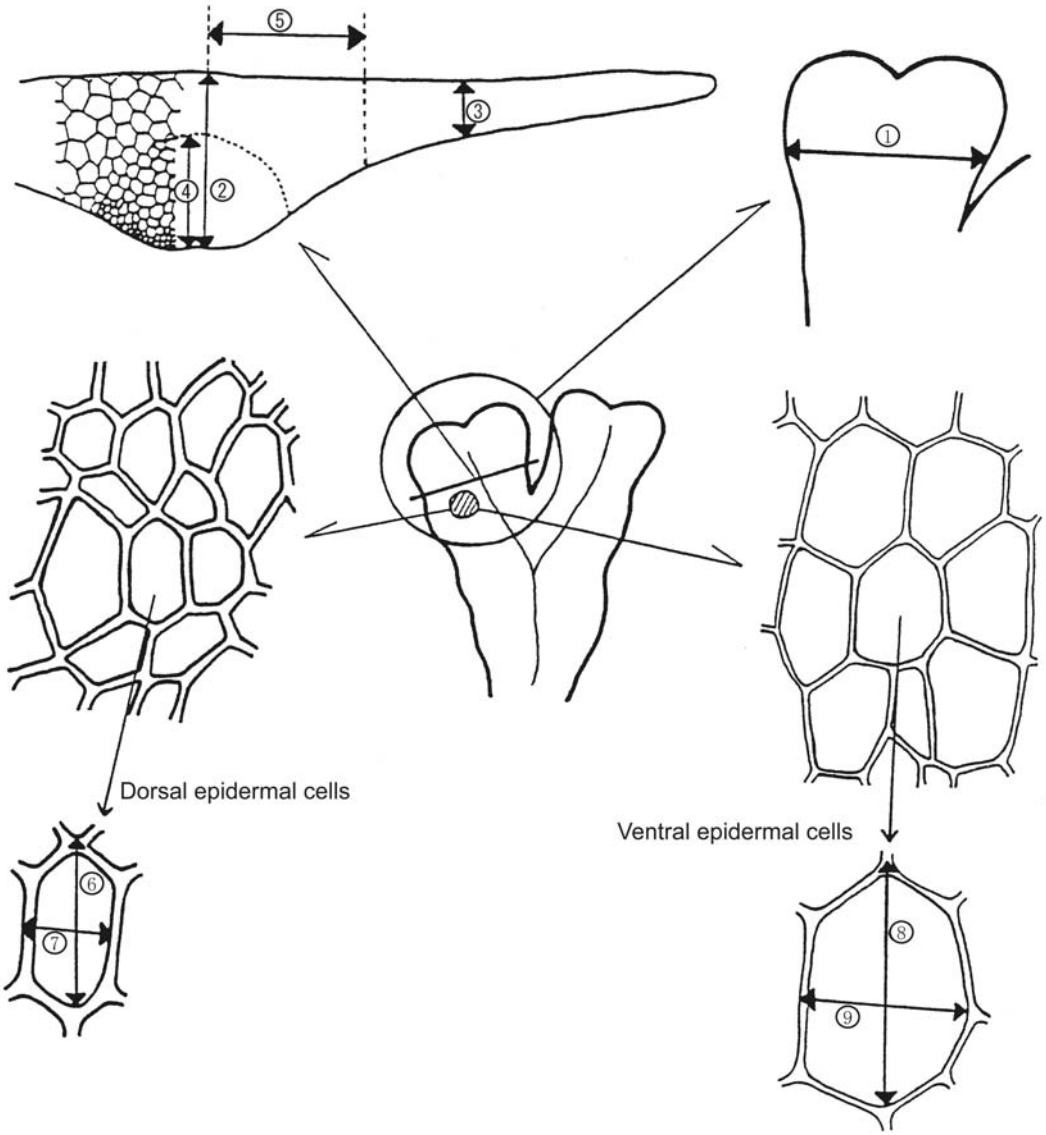

Fig. 3. Measurements of *Dumortiera*. 1 – thallus width, 2 – thickness of the costal region, 3 – thickness at the middle part of thallus wings (MPT), 4 – thickness of small-cell layers of costal region (SCL), 5 – length from central to the point of 1/2 thickness of costa (TPT), 6 – length of dorsal epidermal cells (DEC), 7 – width of dorsal epidermal cells, 8 – length of ventral epidermal cells (VEC), 9 – width of ventral epidermal cells.

data were analyzed with computer software GDA (Lewis & Zaykin 2001) and the following genetic indices calculated: average number of alleles per locus (Aa), average numbers of alleles per polymorphic locus (Ap), and gene diversity (He). Nei's genetic distances (Nei 1978) were also estimated to create UPGMA dendrograms for monoploids and diploids separately.

RESULTS

CHROMOSOMES

Results of chromosome counting are indicated in Table 1. All the individual samples that were used in allozyme analyses were confirmed for their

Table 2. Arithmetic means and standard deviations (in parenthesis) of morphological features 1–9 (as in Fig. 3) of monoploids and diploids of the *Dumortiera hirsuta* complex on Hawaii, Maui and Oahu Island. N – number of measurements for features 1–5 and 6–9 are the same, respectively.

Islands/ploidy	Features								
	1	2	3	4	5	6	7	8	9
HAWAII IS.									
monoploids	10.84 (±1.60) N=42	645.91 (±121.91)	229.77 (±39.91)	443.61 (±104.17)	1.17 (±0.31)	27.96 (±5.48) N=420	15.42 (±3.29)	61.99 (±10.11)	30.05 (±5.35)
diploids	12.65 (±2.29) N=79	776.71 (±147.14)	297.05 (±72.45)	469.47 (±101.03)	1.63 (±0.71)	34.26 (±6.48) N=790	18.58 (±3.08)	67.12 (±7.77)	34.41 (±6.39)
MAUI IS.									
monoploids	9.50 (±2.79) N=29	444.44 (±132.15)	182.96 (±44.88)	286.30 (±95.44)	1.40 (±0.51)	30.93 (±4.87) N=290	15.87 (±3.34)	55.22 (±10.31)	26.45 (±4.18)
diploids	13.28 (±2.21) N=26	891.92 (±156.56)	300.77 (±48.41)	542.00 (±109.96)	1.88 (±0.61)	39.79 (±6.89) N=260	18.97 (±2.82)	60.70 (±8.87)	30.35 (±3.69)
OAHU IS.									
monoploids	7.46 (±1.82) N=14	406.43 (±108.32)	187.14 (±46.97)	250.77 (±84.1)	1.46 (±0.43)	27.51 (±6.23) N=140	14.14 (±3.01)	51.21 (±10.76)	23.65 (±5.00)
TOTAL									
monoploids	9.87 (±2.39) N=85	542.47 (±163.12)	207.88 (±47.99)	354.74 (±129.25)	1.31 (±0.43)	28.82 (±5.55) N=850	15.35 (±3.28)	57.71 (±11.04)	27.66 (±5.45)
diploids	12.81 (±2.27) N=105	805.24 (±156.93)	297.98 (±67.05)	487.43 (±107.46)	1.71 (±0.69)	35.63 (±6.98) N=1050	18.68 (±3.01)	65.50 (±8.49)	33.39 (±6.058)

ploidy levels. Both monoploids and diploids were detected in Maui and Hawaii Island populations. Two populations from Hawaii Island included both monoploids and diploids. Monoploids in Maui and Hawaii Islands were found at lower elevation (150–1280 m a.s.l.) while diploids were found at higher elevation (950–1950 m a.s.l.) (Fig. 1).

MORPHOLOGY

Hawaiian *Dumortiera* populations totally lack papillae on the dorsal surface of the thallus in both mono- and diploids. Though most plants are also lacking remnant walls, seen as a distinct network of delicate ridges, and are often whitish in appearance (Schuster 1992), these remnant walls are sometimes weakly developed in both mono- and diploids. In contrast, both mono- and diploid populations of Japanese and Taiwanese plants always have conspicuous remnant walls on the dorsal surface of the thallus that are usually associated with dense papillae.

From values of the measured features in Table 2 it is evident that diploid plants have larger and thicker thalli and grew at higher elevations when compared to monoploid plants. However, differentiation in morphological features of the same ploidy level among the three islands is not clear, though plants from Oahu were somewhat smaller than those from Hawaii and Maui.

GENETIC VARIABILITY WITHIN AND AMONG POPULATIONS

We scored genetic variation at 14 enzyme loci. Allele frequency detected in each population is shown in Table 3. Names of alleles are the same as used in Akiyama *et al*. (2003). *Me-3* is monomorphic in all the populations examined, including mono- and diploids. In the Hawaiian populations 11 loci out of 14 enzymes examined were polymorphic in the monoploids, and 7 in the diploids.

In the Hawaiian populations, it is noteworthy that both mono- and diploids have a higher number of monomorphic loci compared to Japanese or Taiwanese populations, even though sample sizes were small for all examined populations. It is also notable that diploids show banding patterns similar to monoploids. This might suggest that diploids are of autopolyploid origin. In this respect, it is also notable that there are a number of characteristic alleles only found in either mono- or diploids among Hawaiian populations: five in monoploids (*tpi*1-c, *gdh*-c, *idh*-b', *skdh*-e, *lap*-a') and six in diploids (*tpi*1-a, *pgm*-b, *skdh*-a, *me*1-c, *mdh*1-a, *mdh*1-b'). These results suggest that (*i*) Hawaiian diploids have not been derived by ancient autopolyploidization of Hawaiian monoploids, and that (*ii*) monoploids and diploids have been genetically isolated from one another for a long period as expected from different species. Unfortunately, we could not examine allozyme features of the monoploid plants that co-existed with diploid ones in two populations (A and D) on Hawaii Island.

As for genetic diversity within populations for each ploidy level (Table 1), monoploid populations are shown to maintain rather high genetic diversity (mean He = 0.114) as in the case of *Plagiomnium ciliare* (Wyatt *et al*. 1989). Diploids also have a high level of genetic diversity within a population (mean He = 0.340); higher values are partly derived from fixed heterozygosity found in some loci (for example, *pgm*2).

UPGMA dendrograms showing inter-relationships among monoploid (Fig. 4) and diploid populations (Fig. 5), respectively, were inferred based on Nei's (1978) genetic distances (Table 4). UPGMA dendrogram analysis of monoploid populations suggests that all the monoploids are closely related ($D < 0.13$), although there is relatively high differentiation between the two populations from Hawaii Island and those on Maui and Oahu Islands. It is also notable that all the monoploid populations of the Hawaiian Islands appear closely related to the Japanese monoploids (Fig. 4).

There appears to be little genetic differentiation between diploid populations of Maui and Hawaii Islands, except for the Paliku population on Maui Island. Hawaiian diploids are genetically distantly related to Japanese and Taiwanese diploid populations (Fig. 5).

DISCUSSION

In this study, we confirmed that both monoploids and diploids of the *Dumortiera hirsuta* complex exist in the Hawaiian Islands. There is differen-

Table 3. Allele frequencies found in Hawaiian, Japanese, and Taiwanese populations of the *Dumortiera hirsuta* complex. Allele names follow Akiyama *et al*. (2003). x1 – monoploid, x2 – diploid.

Allele	Oahu – Manoa (x1)	Maui – Wailua (x1)	Maui – Kopiliula (x1)	Maui – Waikamoi (x1)	Hawaii – Waiakea (x1)	Hawaii – Kahaulaea (x1)	JAPAN – Keisoku (x1)	JAPAN – Kawara (x1)	TAIWAN – THS (x1)	Maui – Paliku (x2)	Maui – Hosmer (x2)	Hawaii – Volcanos (x2)	Hawaii – Waimea (x2)	Hawaii – Kalaoa (x2)	Hawaii – Hakalau (x2)	JAPAN – Ryugado (x2)	TAIWAN-TTW (x2)
tpi1	17	18	3	6	19	5	26	29	45	10	20	11	13	5	12	34	41
a								0.034	1.000	1.000	1.000	0.636	1.000	1.000	1.000	0.500	0.500
b	1.000	0.889	0.333	1.000	0.737	1.000	1.000	0.966				0.364				0.500	0.500
c		0.111	0.667	0.000	0.263												
tpi2	17	18	3	6	19	5	26	29	45	10	20	10	13	5	12	34	41
a							0.077	0.241	1.000								0.500
b	1.000	1.000	1.000	1.000	1.000	1.000	0.923	0.759		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.500
pgm2	17	18	1	6	19	5	26	29	45	10	20	10	13	5	12	34	44
a+					0.105												
a’					0.000												
a	0.800	1.000	1.000	1.000	0.895	1.000	1.000	1.000		0.500	0.500	1.000	1.000	0.500	0.500	0.500	
b’										0.500	0.500			0.500	0.500		
b”																0.500	
c																	0.976
d									1.000								0.024
gdh	17	18	3	6	19	5	26	29	45	10	20	10	13	5	12	34	41
a	1.000	1.000	0.666	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000		
c			0.333						1.000							1.000	1.000
idh	17	18	3	6	19	5	26	29	45	10	20	10	13	5	12	34	44
a									0.289								
b’					0.182			0.069									
b	1.000	1.000	1.000	1.000	0.818	1.000	0.730	0.897	0.711	1.000	1.000	1.000	1.000	1.000	1.000		
b-																0.500	0.500
c							0.270	0.034								0.500	0.500
skdh	17	17	3	6	17	5	26	29	45	10	20	10	13	5	12	34	41
a’							0.000	0.103	0.044								
a							0.231	0.483	0.956	0.900	0.250						
b’																0.114	
b					0.294		0.615	0.414		0.100	0.750	0.700	1.000	1.000	1.000	0.886	0.500
c’																	0.500
c							0.154										
d	0.300	0.333	0.667		0.706	1.000						0.300					
e	0.700	0.667	0.333	1.000													

me1	17	18	3	6	19	5	25	22	45	10	20	10	13	5	12	34	41
n							0.240	0.591	1.000								
a	0.600	0.333		0.667	1.000	1.000				0.500	1.000	1.000	1.000	1.000	1.000	0.500	0.500
b	0.400	0.667	1.000	0.333			0.760	0.409		0.400						0.500	0.500
c										0.100							
me2	0	0	0	0	19	5	26	29	45	0	0	10	13	5	12	34	41
a					1.000	1.000	0.115	1.000	0.113			1.000	1.000	1.000	1.000	0.500	
a-																	0.500
b’									0.864								
b							0.885		0.023								0.500
c																0.500	
me3	17	18	3	6	19	5	26	29	45	10	20	10	13	5	12	34	41
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mdh1	17	18	3	6	18	5	8	16	45	10	20	10	13	5	12	34	41
p					0.167					0.200	0.400						
a					0.833	1.000			1.000	0.300	0.100	0.400	1.000	1.000	1.000		0.500
b’										0.200	0.400	0.600					
b	1.000	1.000	1.000	1.000			1.000	1.000		0.300	0.100					1.000	0.500
mdh2	17	18	3	6	18	5	8	16	45	10	20	10	13	5	12	34	41
a					0.222			0.063		0.300	0.100	0.400	1.000	1.000	1.000	0.500	0.500
b	0.900	1.000	1.000	1.000	0.778	1.000	1.000	0.947	1.000	0.700	0.825	0.600				0.500	0.500
c	0.100										0.075						
acn	17	18	3	6	19	5	26	29	45	10	20	10	13	5	12	34	41
a	0.900	0.444	1.000	1.000	0.263		0.692	1.000	0.111	1.000							0.500
b	0.100	0.556			0.737	1.000	0.231				1.000	1.000	1.000	1.000	1.000	1.000	
c’									0.889								
c							0.077										0.500
6pgd	17	18	3	6	18	5	25	28	45	10	20	10	13	5	12	34	41
a	0.100	0.000	0.000	0.000	0.333	0.000	0.120	0.571	0.000	0.100	0.100			1.000	0.917		
b	0.900	1.000	1.000	1.000	0.667	1.000	0.880	0.429	0.111	0.900	0.900	1.000	0.500		0.083	1.000	0.049
b-																	0.451
c													0.500				0.049
c-									0.889								0.451
lap	17	9	3	6	11	5	26	5	45	10	20	10	13	5	12	0	41
a’					0.273		0.192										
a	1.000	1.000	1.000	1.000	0.727	1.000	0.808	1.000	0.889	1.000	1.000	1.000	1.000	1.000	1.000		0.500
b																	0.500
c									0,111								

Table 4. Nei's genetic identity (above diagonal) and distance (below diagonal) of monoploids and diploids of the *Dumortiera hirsuta* complex.

Locality	Features								
	1	2	3	4	5	6	7	8	9
MONOPLOIDS									
1 Oahu Manoa (N)		0.9402	0.8609	0.8783	0.7958	0.7680	0.8351	0.9442	0.3646
2 Maui Wailua (J)	0.0617		0.8960	0.9576	0.8603	0.8693	0.9040	0.9636	0.3585
3 Maui Kopiliula (H)	0.1498	0.1098		0.7784	0.7997	0.7941	0.8531	0.8956	0.3568
4 Maui Waikamoi (L)	0.1298	0.0433	0.2505		0.8843	0.9145	0.8277	0.8755	0.4017
5 Hawaii Waikaea (B)	0.2284	0.1504	0.2236	0.1229		0.9598	0.8238	0.7743	0.2753
6 Hawaii Kahaulaea (C)	0.2640	0.1400	0.2306	0.0894	0.0411		0.7804	0.7708	0.2873
7 JAPAN Kawara	0.1801	0.1009	0.1589	0.1891	0.1938	0.2480		0.8836	0.4141
8 JAPAN Keisoku	0.0574	0.0371	0.1103	0.1330	0.0574	0.037	0.1103		0.1330
9 TAIWAN THS	1.0090	1.0258	1.0306	0.9119	1.2900	1.241	0.8817	1.0987	
DIPLOIDS	1	2	3	4	5	6	7		
1 Maui Paliku (M)		0.8426	0.6510	0.7567	0.7891	0.5466	0.5082		
2 Maui Hosmer (I)	0.1713		0.8083	0.9417	0.9034	0.6661	0.4690		
3 Hawaii Waimea (E)	0.4292	0.2128		0.8782	0.9249	0.6205	0.4655		
4 Hawaii Volcanos (D)	0.2788	0.0600	0.1299		0.9332	0.7118	0.4537		
5 Hawaii Hakalau (G)	0.2368	0.1016	0.0781	0.0691		0.6967	0.4839		
6 JAPAN (Ryugado)	0.6040	0.4063	0.4772	0.3399	0.3614		0.6559		
7 TAIWAN (TTW)	0.6768	0.7571	0.7647	0.7903	0.7259	0.4217			

tiation in morphological features, for example in thallus width and thickness, found between monoploids and diploids in Hawaiian *Dumortiera hirsuta*. Thallus morphology in *D. hirsuta* is highly variable depending on environmental conditions (Campbell 1895; Evans 1919; Schuster 1992). In Hawaii, however, plants of both ploidy levels share similar outer morphology in all populations from the three islands.

According to Akiyama (1999, 2011), there are at least seven genetically different groups of monoploids within the *Dumortiera hirsuta* complex. Forrest *et al*. (2011) also recognized more than two 'lineages' based on sequence analyses of five genes from chloroplast and nuclear DNA. Hawaiian monoploids and diploids are two distinct entities, judging from genetic distances between each ploidy level. In addition, Hawaiian monoploids appear to be closely related to Japanese monoploids found growing on limestone outcrops. The relationships of these to other monoploid populations of the world is the subject of ongoing studies. However, the Hawaiian diploids do not show a close relationship to diploids from either Japan or Taiwan that we have examined in this study.

There is clear habitat segregation in altitude between monoploid and diploid populations in the Hawaiian Islands. In Taiwanese populations, no such altitudinal segregation has been reported between monoploids and diploids (Akiyama *et al*. 2003). However, from a preliminary study conducted on Yakushima Island (Kyushu District, Western Japan), diploids grow at lower altitudes than triploids (Akiyama, unpublished). These observations might suggest more adaptive features of plants of higher ploidy levels to the cooler environments. Nevertheless, it should be noted that monoploids and diploids have been gathering a number of unique alleles and thus differ from one another in genetic features. It suggests Hawaiian diploids might not have been derived by simple chromosome duplication in Hawaiian monoploids. Polyploids in bryophytes were assumed to be autopolyploids (Wyatt & Anderson 1984, and many others), but critical re-examination, mostly based

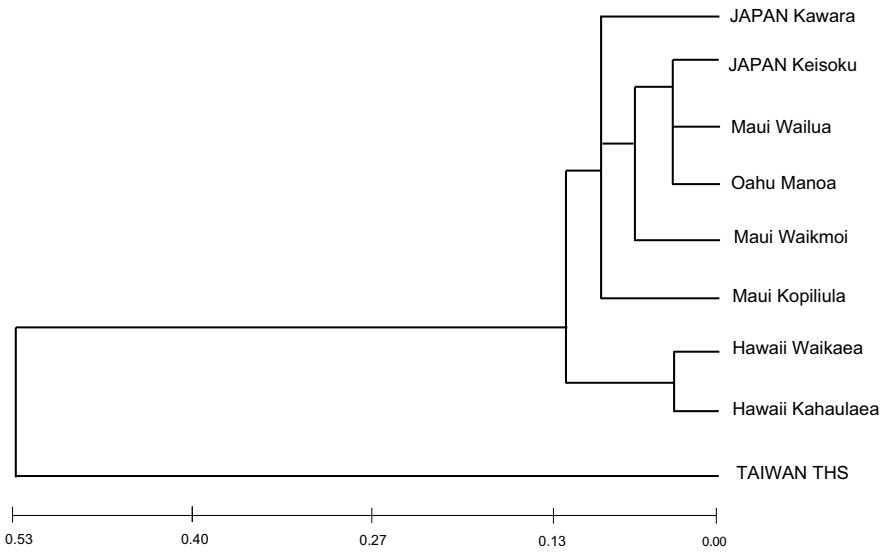

Fig. 4. UPGMA dendrogram of monoploids based on Nei's (1978) genetic distances (cf. Table 4).

on allozyme analyses, have revealed that there are many cases of allopolyploidy (Wyatt *et al*. 1988, and many others). To determine whether the diploids of Hawaiian *Dumortiera hirsuta* have been derived through autopolyploid events (i.e., selfing) or allopolyploidy (hybridization between different entities) will be a good case study to show evolution through chromosome duplication in bryophytes on isolated islands. Though only two samples have been examined [single monoploid plant from the Kopiliula population (H) and sigle diploid from the Hosmer population (I)], preliminary DNA sequence analyses of the *atp*B-*rbc*L intergenic region of Hawaiian *D. hirsuta*, show that both plants share the same sequence even though these regions are highly variable due to extensive insertions/deletions (Akiyama *et al.* 2003).

Campbell (1918) reported a totally smooth thallus from Hawaiian *Dumortiera* (as *D. trichocephala*). Our results confirm his observation

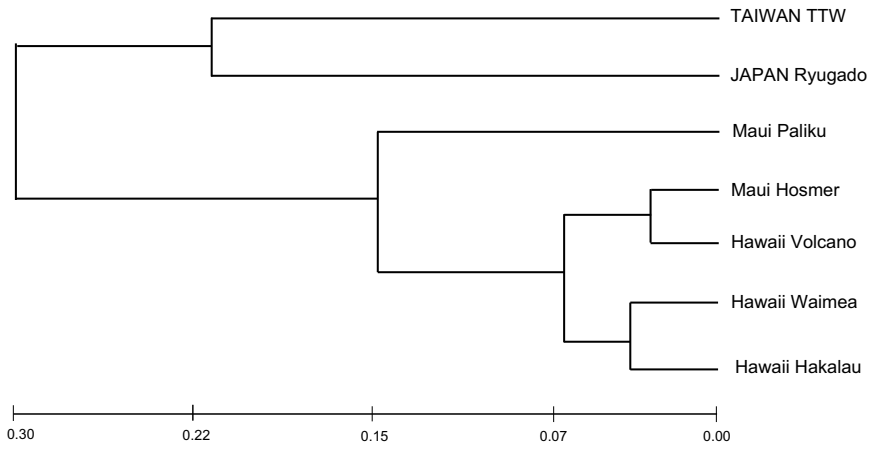

Fig. 5. UPGMA dendrogram of diploids based on Nei's (1978) genetic distances (cf. Table 4).

except for remnant walls which are sometimes observed on the dorsal surface of the thallus. None of the plants from the Hawaiian Islands show papillosity on their thallus surface. Judging from the definition by Evans (1919) and Schuster (1992) about categorization within the *Dumortiera hirsuta* complex, that is, *D. hirsuta* subsp. *hirsuta* as 'dorsal thallus surface smooth except for the occasional presence of faint ridges marking the vestigial air chambers', and *D. hirsuta* subsp. *nepalense* as 'dorsal surface with the vestigial air chambers ± well marked, the surface between the network of ridges bearing crowded, papilliform cells (at least locally)', *D. trichocephala* cannot be a synonym of *D. hirsuta* subsp. *nepalense* because of its smooth thallus without papillae, as well as difference in ploidy level. On the other hand, it may not be justifiable to refer Hawaiian monoploids to *D. hirsuta* subsp. *hirsuta*, because *D. hirsuta* subsp. *hirsuta* (= *Marchantia hirsuta* Ws.) was originally described based on plants collected from Jamaica, and although the ploidy level is unknown, Forrest *et al.* (2011) clearly showed that some of the plants collected from Central America are highly separated from those distributed in other regions.

Hawaiian *Dumortiera* plants were once described as a distinct species, *D. trichocephala*, which is now treated as a synonym of *D. hirsuta* subsp. *nepalense* (Schuster 1992; or as *D. hirsuta* var. *nepalense* in Evans 1919). Since the infraspecific categories of the *D. hirsuta* complex are tightly linked to ploidy levels, as well as morphological features (Schuster 1992), the Hawaiian monoploid cannot belong to *D. hirsuta* subsp. *nepalense*. In addition, Hawaiian diploids are revealed to be distantly related to Japanese and Taiwanese diploids, both of which are also regarded as *D. hirsuta* subsp. *nepalense* by many bryologists (Hattori 1951, and others). These findings, we consider, do not support the treatment of Hawaiian monoploids as *D. hirsuta* subsp. *hirsuta* and diploids as *D. hirsuta* subsp. *nepalense*. Though much more data are needed to settle the problem, we think it is better to apply the name *D. trichocephala* to either Hawaiian monoploids or diploids. Which is the more appropriate candidate for the scientific name is another issue to be solved but awaits critical examination of the type specimen of *D. trichocephala*. If it is shown that the Hawaiian monoploids belong to *D. trichocephala*, Japanese monoploids (distributed in calcareous regions) examined in this study may well be included in *D. trichocephala* because of their close genetic resemblance, even if they look very different due to the dense papillosity of the thallus surface.

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APPENDIX

Description of localities studied.

HAWAIIAN ISLANDS. HAWAII ISL. South Hilo Dist., Hilo Reserve, 1280 m alt. (A); South Hilo Dist., Waiakea Forest Reserve, 600 m alt. (B); Puna Dist., Kahaualea Natural Area Reserve, 600 m alt. (C); Kau Dist., Hawaii Volcanos National park, Thurston Lava Tube, 1140 m alt. (D); North Kohala Dist., 6 km N of Waimea, 1150 m alt. (E); North Kona Dist., Makaula-ooma Mauka Tract Forest Reserve, 3 km E of Kalaoa, 950 m alt. (F); North Hilo Dist., Hakalau Forest NWR, E slope of face of Mauna Kea, 1900 m alt. (G). MAUI ISLAND. Hana Dist., Kopiliula Stream, 400 m alt. (H); Makawao Dist., Haleakala, Hosmer Grove, 1950 m alt. (I); Hana Dist., Wailua, Kanahualii Falls , 150 m alt. (J); Wailuku Dist., Makamaole, 10 km SW of Wailuku, 250 m alt. (K); Makawao Dist., Waikamoi Stream, Olinda flume, 1300 m alt. (L); Makawao Dist., Haleakala, Paliku, 1950 m alt. (M). OAHU ISLAND. Honolulu Dist., Manoa Falls trail, 200 m alt. (N).

JAPAN. HONSHU, Hiroshima Pref., Keisoku Valley, 130 m alt.; KYUSHU, Fukuoka Pref., Mt. Kawara, 100 m alt.; SHIKOKU, Kochi Pref., Ryugado Limestone Cave, 200 m alt.

TAIWAN. HUALINE CO., Tailuge, Shen-Miku trail, 140 m alt. (THS); Taichung Co., Wushyken, 1000 m alt. (TTW).

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