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Distribution, Ploidy Levels, and Fruit Characteristics of Three *Actinidia* Species Native to Hokkaido, Japan

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The genus *Actinidia* includes widely-sold kiwifruit, and is thus horticulturally important. We investigated the distribution, ploidy levels, and fruit characteristics of the natural populations of three edible *Actinidia* species [*Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq., *Actinidia kolomikta* (Maxim. & Rupr.) Maxim., and *Actinidia polygama* (Siebold & Zucc.) Planch. ex Maxim.] in Hokkaido, the northern island of Japan. *Actinidia arguta* and *A. kolomikta* were common, and their habitat ranges overlapped. *Actinidia polygama* was less common, and its habitat was mostly limited to lowland deciduous forests. Flow cytometric analysis revealed that all wild collections of *A. kolomikta* and *A. polygama* were diploid, and that *A. arguta* was tetraploid, suggesting a lack of intraspecific ploidy variation. Fruit shape varied from round to ovoid in *A. arguta*, ranged from ovoid to ellipsoidal in *A. kolomikta*, and was ellipsoidal in *A. polygama*. The fruit skin of all species was glabrous, and skin color was orange in *A. polygama*, green to dark green in *A. kolomikta*, and light to dark green in *A. arguta*. The fresh weight of *A. kolomikta* fruit was less than that of *A. arguta*, and the soluble solids content (SSC) of the fruits varied widely within species. One sample of *A. arguta* had extremely high SSC (average Brix of 30.8%). The ascorbic acid content (AAC) was the highest in *A. kolomikta* (up to 805 mg per 100 g fresh weight). *Actinidia arguta* and *A. kolomikta* germplasm may be useful for breeding new kiwifruit varieties for cultivation in cold-temperate regions.

Key Words: *Actinidia arguta*, *Actinidia kolomikta*, *Actinidia polygama*, flow cytometry, kiwifruit relatives.

Introduction

The genus *Actinidia* comprises 76 species and approximately 120 taxa (Ferguson and Huang, 2007) distributed across a wide natural range from the tropics (lat 0°) to cold temperate regions (lat 50°N) in southeast to east Asia (Huang et al., 2004). Despite the diversity of *Actinidia*, only a few species have been used commercially until recently (Ferguson, 1999). Kiwifruit, *Actinidia deliciosa* (A. Chev.) C. F. Liang & A. R. Ferguson or *Actinidia chinensis* Planch., is a well-known commercial crop that is cultivated worldwide. Kiwifruit is a good source of vitamin C, potassium, folic acid, vitamin E, and vitamin K (Ferguson and Ferguson, 2003). The most widely grown commercial cultivar is 'Hayward' (*A. deliciosa*) developed in New Zealand. Recently, other cultivars such as 'Koryoku' (*A. deliciosa*), 'ZESH004' (*A. deliciosa*), 'Hort16A' (*A. chinensis*), 'Sanuki Gold' (*A. chinensis*), 'Rainbow Red' (*A. chinensis*), and 'ZESY002' (*A. chinensis*) have been on the market in Japan.

Originally, kiwifruit, native to southern China, was developed as commercial fruit crop after introduction into New Zealand at the beginning of last century. It is among the most recently domesticated of all fruit crops (Ferguson and Bollard, 1990). It needs a long frost-free period of about 270–300 days from budburst to commercial harvest, and it is therefore susceptible to late spring or early autumn frosts (Ferguson and Seal, 2008). Although it cannot withstand winter temperatures much below 0°C, it requires a period of winter chilling to break dormancy, and to ensure adequate flowering (Ferguson and Seal, 2008). In Japan, it is cultivated mainly in warm temperate regions on the main island, and in Shikoku, and Kyushu.

Four species of *Actinidia* occur in Japan: *A. arguta*, *A. kolomikta*, *A. polygama*, and *A. rufa*. *Actinidia rufa* is native to subtropical and warm-temperate regions, *A. arguta* and *A. polygama* are widely distributed throughout the country except for the Ryukyu Islands

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(the southernmost islands), and *A. kolomikta* is distributed primarily in the central to northern main island, and Hokkaido. These species also occur in other countries, including China, Korea, and eastern Russia (Ferguson and Huang, 2007).

Hokkaido is the second largest and northernmost island in Japan (lat 42°N–46°N), and is characterized by a relatively cold temperate climate. Three species related to kiwifruit are indigenous to Hokkaido: *A. arguta*, *A. kolomikta*, and *A. polygama*. These wild species have higher cold tolerance than *A. deliciosa*, or *A. chinensis* (Chat, 1995; Lawes et al., 1995), and are well adapted to the climate in Hokkaido. Therefore, they are of great interest as breeding materials for the production of kiwifruit cultivars suitable for cultivation in cold-temperate regions.

Fruits of these wild species are small berries with edible, smooth skins, and are rich in taste and flavor. *Actinidia kolomikta* is similar to *A. polygama* in general appearance, but differs in having rosy variegated leaves rather than white variegation during the flowering season. *Actinidia kolomikta* is similar to *A. arguta* in having green, sweet, flavorful berries, but differs from *A. polygama*, which has yellow berries, and an acrid and sweet taste. In Hokkaido, berries of *A. arguta* and *A. kolomikta* are sometimes both called Kokuwa, a local name that applies to *A. arguta*, possibly because of their abundance and resemblance in fruit appearance and characteristics.

The Ainu, indigenous people of Hokkaido, have used wild collected *A. arguta* and *A. polygama* for eating raw fruits (Haginaka et al., 1992; Nishiumi et al., 2012), and *A. arguta* for medicinal purpose (Mitsuhashi, 1976). Recently, some processed products of *A. arguta*, or possibly *A. kolomikta* such as fruit jam, juice, and wine, as well as fresh fruit, have been on the market in Hokkaido. These products are thought to be produced mainly using wild collected plants, or from cultivated plants on a small scale. Breeding based on evaluation of wild genetic resources is needed for the commercial cultivation of these wild *Actinidia* species. Interest in kiwifruit relatives such as *A. arguta* and *A. rufa* that are native to Japan has increased, and these genetic resources have been evaluated and incorporated into breeding programs (Arase and Uchida, 2009a, b, 2010; Kataoka et al., 2003, 2010, 2014; Kim et al., 2007, 2009, 2012; Matsumoto et al., 2011). Here, we investigated the distribution, ploidy levels, and fruit characteristics of *A. arguta*, *A. kolomikta*, and *A. polygama* to obtain general information applicable to the cultivation and breeding of these native Hokkaido species.

Materials and Methods

1) Plant materials

We conducted a field investigation from June to October 2014 to collect plant materials of *A. arguta*, *A. kolomikta*, and *A. polygama* in the Teshio, Nakagawa, and Tomakomai Experimental Forests, which form part of the Field Science Center for Northern Biosphere, Hokkaido University (Fig. 1). The Tomakomai Experimental Forest (2700 ha) is located in southern Hokkaido, and ranges in elevation from 5 to 95 m. The Teshio and Nakagawa Experimental Forests (22000 and 19000 ha respectively) are adjacent to one another and are in northern Hokkaido, a mountainous region in which the elevation ranges from 30 to 580 m in Teshio and 20 to 700 m in Nakagawa. The annual mean temperature in each experimental forest is 6.3°C (max. 31.2°C, min. −22.1°C) in Tomakomai (elevation 20 m), 5.7°C (max. 34.6°C, min. −32.9°C) in Teshio (elevation 15 m), and 5.4°C (max. 34.9°C, min. −34.9°C) in Nakagawa (elevation 40 m, the data obtained from the Japan Meteorological Agency, http://www.jma.go.jp/jma/index.html). The temperature data in Teshio and Tomakomai were provided by each Experiment Forest, Field Science Center for Northern Biosphere, Hokkaido University. Typical vegetation in Tomakomai is deciduous broad-leaved forest; mixed conifer and deciduous forests predominate in Teshio and Nakagawa. We also collected plant samples from natural forests in Sapporo, Kyowa, and Hidaka. Location data (latitude, longitude, and elevation) were recorded using a hand-held global positioning system (GPS) (Oregon 450TC; Garmin International Inc., Olethe, KS, USA) when samples were collected to investigate the distribution range and relationship between plant characteristics and location. From mid-June to early September, young leaves were collected from extending shoot tips of 48 *A. arguta* plants, 52 *A. kolomikta*, and 8 *A. polygama*, and flow cytometric analyses were performed. For comparison, one sample of *A. kolomikta* from Nagano and one sample of *A. polygama* from Tokyo on the main island were also collected for flow cytometric analysis. To evaluate fruit characteristics, mature fruits were collected from 19 *A. arguta*, 13 *A. kolomikta*, and one *A. polygama* in

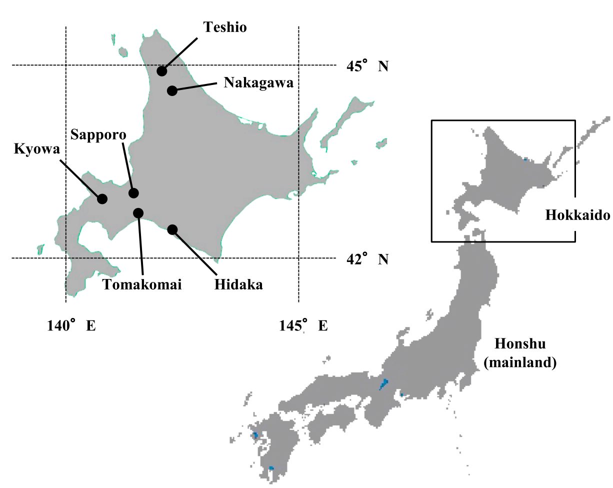

Fig. 1. Location of *Actinidia* collection sites in Hokkaido.

Tomakomai and Sapporo from early August to early October.

2) Flow cytometric analysis

Leaf segments (approximately 0.5 × 0.5 cm) were sliced with a razor blade in a plastic Petri dish containing 0.2 mL of ice-cold nuclei extraction buffer (solution A of a high-resolution DNA kit; Partec, Münster, Germany). After filtration through a 30 μm nylon mesh, crude nuclear samples were stained with 0.7 mL of DAPI (4',6-diamidino-2-phenylindole) solution containing 10 mM Tris, 50 mM sodium citrate, 2 mM $MgCl_2$, 1% (w/v) polyvinylpyrrolidone (PVP) K-30, 0.1% (v/v) Triton X-100, and 2 $mg \cdot L^{-1}$ DAPI (pH 7.5) (Mishiba et al., 2000). After incubation for 30 s at room temperature, nuclear fluorescence was measured using a ploidy analyzer (Partec PA; Partec). Fresh leaves of barley (*Hordeum vulgare* 'Aominori') were used as the internal standard. More than 5000 cells were analyzed for each measurement.

3) Fruit evaluation

The fresh weight (FW), length, and width of 10 fruits from individual plants were measured using a digital caliper (CD-S15C; Mitutoyo, Kawasaki, Japan), and the pH and soluble solids content (SSC) of the fruit juice was measured immediately after collection. The pH was measured using a digital pH meter (LAQUAtwin; Horiba, Kyoto, Japan); SSC was measured using a digital refractometer (Atago PAL-1; Atago, Tokyo, Japan). Ascorbic acid content (AAC) was quantified using a reflectometer (Merck RQflex 10; Merck, Darmstadt, Germany). Fresh fruit samples (~1 g) were mixed with 5% (w/v) metaphosphoric acid (Wako Chemicals, Tokyo, Japan) to a final concentration of 5% to 10%, homogenized for 30 s, and filtered. The filtrate was used for the quantitative analyses. For AAC, three replicates were performed for each collection. For comparison, kiwifruits (*A. deliciosa* 'Hayward') from New Zealand that were purchased from a local market were also used.

Means were compared using Tukey's test, with $P < 0.05$ considered significant. All analyses were performed using R software (version 3.1.1).

Results

1) Distribution

Actinidia arguta and *A. kolomikta* were collected in all three experimental forests investigated, whereas *A. polygama* was not found in Teshio (Fig. 2). Six *A. polygama* specimens were collected in Tomakomai, and one was collected in Nakagawa, which was considerably fewer than the numbers of *A. arguta* and *A. kolomikta* growing in the same areas. The vertical distribution of *A. arguta* and *A. kolomikta* mostly overlapped in all three experimental forests (Fig. 3A–C). In Nakagawa, *A. polygama* was found only in the lowland area, whereas both *A. arguta* and *A. kolomikta* had a wide vertical distribution from lowlands to nearly 500 m, suggesting that *A. arguta* and *A. kolomikta* are the dominant species at higher elevations in that area (Fig. 3C).

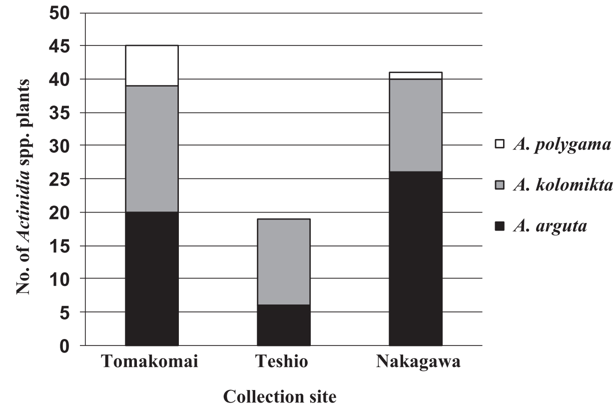

Fig. 2. Number of *Actinidia arguta*, *A. kolomikta*, and *A. polygama* collected in Teshio, Nakagawa, and Tomakomai, respectively.

2) Ploidy levels

Ploidy levels of wild *A. arguta*, *A. kolomikta*, and *A. polygama* collected in Teshio, Nakagawa, Tomakomai, Sapporo, Kyowa, and Hidaka were investigated. All *A. kolomikta* and *A. polygama* were diploid, and all *A. arguta* were tetraploid (Fig. 4A–C). One sample of *A. kolomikta* from Nagano, and one sample of *A. polygama* from Tokyo on the main island were diploid (Fig. 4D and E).

3) Fruit evaluation

Mature fruits, which had become soft and had specific flavor, were collected from wild *Actinidia* in Tomakomai and Sapporo from early August to early October (Table 1). The date of harvesting was determined by preliminary field observation at regular intervals. The fresh weight of *A. kolomikta* fruit ranged from 1.2 to 2.5 (total average 1.8) g, whereas the fresh weight of *A. arguta* fruit ranged from 3.1 to 7.4 (total average 4.5) g and was 5.3 g in *A. polygama*. Fruit shape was round to ovoid in *A. arguta*, ovoid to ellipsoidal in *A. kolomikta*, and ellipsoidal in *A. polygama* (Fig. 5). The fruit skin of all species was glabrous, and skin color was orange in *A. polygama*, green to dark green in *A. kolomikta*, and light to dark green in *A. arguta*. The fruit surface of some *A. arguta* and *A. kolomikta* samples had a rosy blush. The flesh color and skin color were comparable (Fig. 5). The fruit pH was 4.7 in *A. polygama*, and ranged from 3.6 to 4.8 (total average 4.2) in *A. kolomikta*, and from 3.5 to 4.8 (total average 4.0) in *A. arguta*; the pH of kiwifruit (*A. deliciosa*) was 3.7. The SSC varied widely: values of 20.1 were recorded in *A. polygama*, whereas values ranged from 7.4 to 18.9 (total average 13.3) in

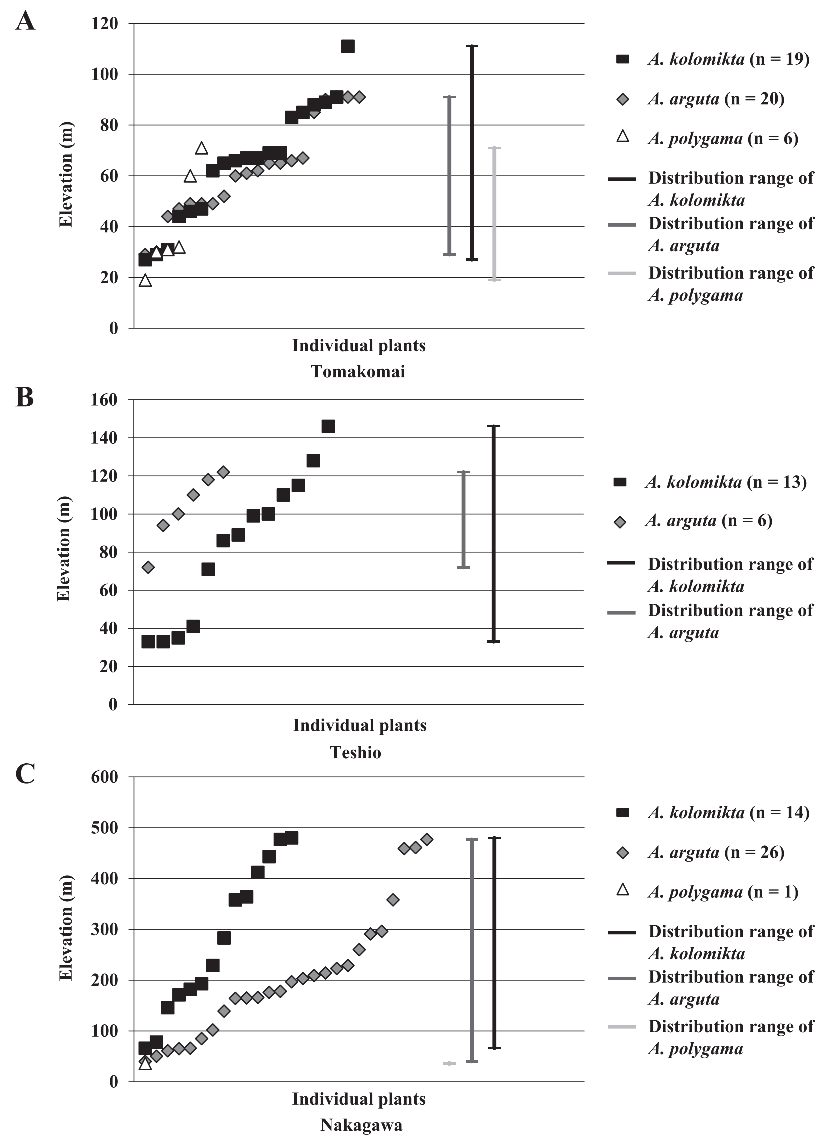

Fig. 3. Vertical distribution of *A. arguta*, *A. kolomikta*, and *A. polygama*. A, Tomakomai. B, Teshio. C, Nakagawa.

A. kolomikta and from 12.2 to 30.8 (total average 18.8) in *A. arguta*; the SSC of New Zealand kiwifruit was 15.7. The AAC was relatively high in *A. kolomikta*, ranging from 182 to 805 (total average 453) mg/100 g FW, whereas it ranged from 21 to 171 (total average 70) mg/100 g FW in *A. arguta*, and was 73 mg/100 g FW in *A. polygama* and 57 mg/100 g FW in kiwifruit.

Discussion

We investigated the distribution of *A. polygama*, *A. arguta*, and *A. kolomikta* in Hokkaido, northern Japan. Arase and Uchida (2009a, b, 2010) reported that the habitats of these species are primarily in mountainous regions (770–1400 m for *A. arguta*, 520–1330 m for *A. polygama*, and 1200–2000 m for *A. kolomikta*) in central and southern Nagano Prefecture on Japan's main island. These studies found differential distribution patterns of these species according to the elevation on the main island. We observed that the habitat range of *A. arguta* and *A. kolomikta* overlapped in Hokkaido, which indicates that *A. arguta* and *A. kolomikta* are distributed differently between the main island and Hokkaido. We observed different habitat patterns for *A. kolomikta* relative to those reported by Arase and Uchida, with the species occurring at high latitude and low elevation in Hokkaido, and at low latitude and high elevation in Nagano. These differential distribution patterns may be related with the climatic condition in each habitat, such as temperature. The annual mean temperature, average maximum temperature in the hottest month (August), and average minimum temperature in the coldest month (January or February) in three study sites in Hokkaido are as follows: 5.7°C, 25.7°C, −16.8°C in Teshio (elevation 15 m), 5.4°C, 25.0°C, −15.6°C in Nakagawa (elevation 40 m), 6.3°C, 24.8°C, −13.9°C in Tomakomai (elevation 20 m). For compari-

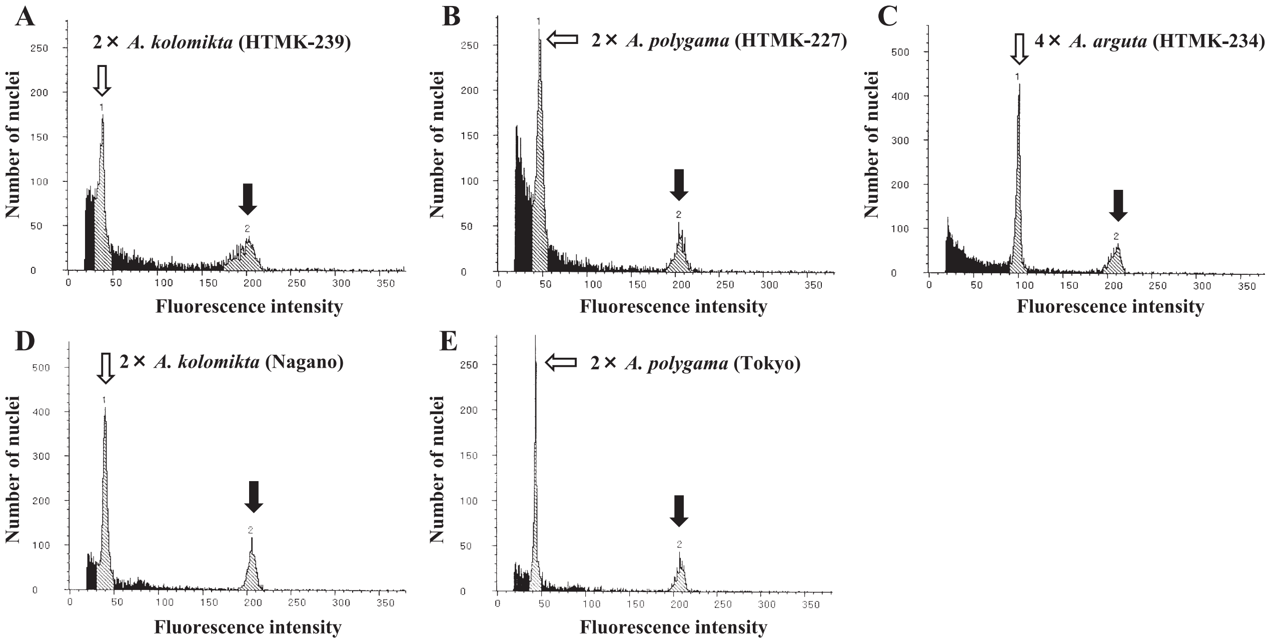

Fig. 4. Relative fluorescence intensity obtained from DAPI-stained nuclei by flow cytometry in *A. arguta*, *A. kolomikta*, and *A. polygama* collections. A, Diploid *A. kolomikta* from Tomakomai, Hokkaido (white arrow). B, Diploid *A. polygama* from Tomakomai, Hokkaido (white arrow). C, Tetraploid *A. arguta* from Tomakomai, Hokkaido (white arrow). D, Diploid *A. kolomikta* from Nagano, main island (white arrow). E, Diploid *A. polygama* from Tokyo, main island (white arrow). Fresh leaves of barley (*Hordeum vulgare* 'Aominori') were used as the internal standard (black arrows).

son, although it may not correspond to each habitat exactly, the approximate climatic data of the sites located near the lower limits of vertical distribution of *A. kolomikta*, *A. aruguta*, and *A. polygama* in Nagano, are estimated to be as follows with the same parameters above: 7.4°C, 26.4°C, −11.3°C in Kaidakogen (elevation 1120 m), 10.9°C, 29.3°C, −5.3°C in Iijima (elevation 728 m), 12.8°C, 31.1°C, −3.8°C in Iida (elevation 516 m) (all data obtained from the Japan Meteorological Agency, http://www.jma.go.jp/jma/index.html). The annual mean temperatures and the average maximum temperatures in the hottest month (August) in three areas in Hokkaido are slightly lower than that at the elevation of 1120 m in Nagano, which is estimated to be near the lower limit of vertical distribution range of *A. kolomikta*. Due to these similar condition in temperature, these three species could be sometimes found growing together even in the lowland areas in Hokkaido. Chat (1995) reported that these three species, withstanding −18°C in two-year-old vines, had higher cold tolerance than kiwifruit, which was severely damaged at the same temperature. Another study using dormant stem cuttings of *Actinidia* species (Lawes et al., 1995) suggested that the cold tolerance of *A. arguta* was slightly higher than that of *A. polygama*, with the median lethal temperatures (LT 50, temperatures required to kill half the population) of the buds being −18.6°C to −18.8°C in *A. arguta*, −13.8°C to −15.2°C in *A. polygama*, and −10.6°C to −14.1°C in kiwifruit (*A. deliciosa* 'Hayward'), although *A. kolomikta* was not used in the experiment. The average minimum temperature of the coldest month (February) in Nakagawa (elevation 40 m), where only one plant sample of *A. polygama* was collected in the present study, is −15.6°C, almost the same value as LT 50 of *A. polygama* (Lawes et al., 1995). Thus, the distribution and population size of *A. polygama* may be affected strongly by the winter cold in Hokkaido, resulting in its vertical distribution limited to low elevation and relatively small population compared to other two species. *Actinidia polygama* is not distributed in Sakhalin, just north of Hokkaido, and is rare in eastern Hokkaido; distribution in the Kuril Islands, just east of Hokkaido, is uncertain (Takahashi, 2015). Therefore, the habitat in Nakagawa may be near the northern limit of its distribution in Japan. On the other hand, *A. arguta* and *A. kolomikta* were distributed at higher elevations, as high as 500 m in the same area, and is also distributed in Sakhalin and the Kuril Islands. This suggests that these two species have strong cold tolerance.

Kataoka et al. (2010) described ploidy variations (diploid, tetraploid, hexaploid, heptaploid, and octaploid) of *A. arguta* on the main island of Japan; the tetraploid plants were distributed all over the country, whereas the diploid and hexaploid plants were geographically localized, in the warm Pacific hill areas of the south western part, and in the deep-snow region of the mid-northern part of the main island, respectively. They collected two plant samples from Hokkaido, both of which were tetraploid. Li et al. (2013) reported tetra-

Table 1. Fruit characteristics of *A. polygama*, *A. kolomikta* and *A. arguta* collected in Hokkaido.

Accession name	Collection site[z]	Harvest date	Color[y] Skin	Color[y] Flesh	FW (g)	Length (mm)	Diameter (mm)	L/D ratio[x]	Shape	pH	SSC[w] (%)	AAC[v] (mg/100 g FW)
A. polygama												
AP-HSP-1	Sp	Oct. 1	or	or	5.3±0.5 jklm[u]	35.2±1.7 n	15.9±1.2 ef	2.20 p	ellipsoidal	4.7±0.5 h	20.1±3.1 jkl	73.6±13.7 a
A. kolomikta												
AK-HSP-TY-758	Sp	Aug. 29	dg	g	1.8±0.2 ab	17.6±1.2 abcdef	13.0±0.8 bcd	1.34 ijk	ovoid	4.5±0.2 defgh	15.9±2.7 defghi	805.3±87.6 f
AK-HSP-TY-823	Sp	Aug. 29	g/r	lg	1.6±0.3 ab	20.2±1.3 defghijk	11.9±1.1 abc	1.68 mn	ovoid	4.4±0.3 cdefgh	12.6±1.3 bcde	206.6±27.1 abc
AK-HSP-TY-966	Sp	Aug. 29	g/r	g	2.5±0.2 abcde	23.2±1.2 klm	13.2±1.0 bcd	1.75 n	ovoid	4.8±0.2 h	13.2±2.2 bcdefg	260.5±72.9 bc
AK-HSP-TY-972-1	Sp	Aug. 29	dg	g	2.1±0.2 abc	17.4±0.6 abcde	14.2±1.0 cde	1.23 fghij	ovoid	4.4±0.2 defgh	7.9±1.0 a	604.6±228.2 def
AK-HSP-TY-972-2	Sp	Aug. 29	dg	g	1.8±0.1 ab	16.0±0.6 ab	13.7±0.5 bcde	1.16 defgh	ovoid	4.0±0.2 abcdefg	7.4±1.3 a	684.3±102.2 ef
AK-HSP-TY-972-3	Sp	Aug. 29	dg	g	1.5±0.3 a	17.6±1.8 abcdef	12.0±0.9 abc	1.46 kl	ovoid	4.5±0.2 efgh	10.9±2.6 abc	556.6±104.1 de
AK-HSP-TY-979	Sp	Aug. 29	g/r	g	2.1±0.2 abc	21.1±1.3 ghijk	12.6±0.7 abcd	1.67 mn	ovoid	4.7±0.3 gh	14.4±1.5 cdefgh	182.0±107.7 abc
AK-HSP-TY-990	Sp	Aug. 29	dg	g	2.1±0.4 abc	18.2±1.5 abcdefgh	13.6±1.1 bcd	1.34 ijk	ovoid	4.0±0.3 abcdefg	9.4±2.0 ab	769.3±118.2 ef
AK-HTKM-Y	Tkm	Aug. 31	g	g	1.5±0.3 a	17.4±2.2 abcde	12.4±0.8 abcd	1.39 jkl	ovoid	3.6±0.2 ab	16.0±1.3 defghi	737.3±52.7 ef
AK-HTKM-230	Tkm	Sep. 3	g	g	1.6±0.3 a	17.1±1.9 abcd	12.9±1.0 bcd	1.32 ghijk	ovoid	4.1±0.3 abcdefgh	15.8±1.8 defghi	248.3±12.0 abc
AK-HTKM-236	Tkm	Sep. 3	dg	g	1.4±0.1 a	18.0±0.6 abcdefg	11.6±0.6 ab	1.55 lm	ovoid	4.1±0.2 abcdefgh	12.9±1.9 bcdef	394.3±50.5 cd
AK-HTKM-237	Tkm	Sep. 3	dg	g	1.2±0.2 a	20.5±2.0 efghijk	10.3±1.0 a	1.97 o	ellipsoidal	3.9±0.3 abcdef	17.9±5.2 hijk	258.6±20.2 abc
AK-HTKM-238	Tkm	Sep. 3	dg	g	2.3±0.2 abcd	19.8±1.1 cdefghij	14.4±0.4 de	1.37 ijk	ovoid	3.9±0.3 abcdef	18.9±2.2 ijk	192.0±41.6 abc
A. arguta												
AA-HTKM-223	Tkm	Oct. 2	dg	dg	5.1±0.5 ijkl	22.6±1.0 jklm	20.5±0.8 ij	1.10 bcdef	ovoid	4.8±0.3 h	18.3±1.6 hijk	82.6±6.6 a
AA-HTKM-224	Tkm	Oct. 2	lg/r	lg	4.8±0.7 hijkl	19.3±1.3 bcdefghij	20.9±1.5 ijk	0.92 ab	round	3.4±0.1 a	15.6±1.8 defghi	79.3±5.5 a
AA-HTKM-225	Tkm	Oct. 2	dg	dg	5.3±1.8 jklm	20.9±3.3 fghijk	20.7±2.6 ijk	1.00 abcd	ovoid	3.5±0.4 a	15.4±1.9 defghi	48.8±10.6 a
AA-HTKM-226-1	Tkm	Oct. 2	lg	g	3.4±1.6 cdefgh	20.2±4.1 defghijk	17.0±2.3 fg	1.18 defgh	ovoid	4.0±0.6 abcdefgh	19.8±2.3 ijkl	171.0±33.3 abc
AA-HTKM-226-2	Tkm	Oct. 2	lg/r	g	6.6±0.5 mn	25.0±0.7 lm	22.0±1.0 jk	1.13 cdefg	ovoid	3.7±0.1 abc	16.5±1.7 efghij	73.6±14.1 a
AA-HTKM-234	Tkm	Oct. 2	g	g	3.8±0.7 efghi	22.5±1.3 jklm	17.0±1.4 fg	1.32 hijk	ovoid	4.4±0.8 defgh	13.3±2.2 bcdefg	21.0±9.0 a
AA-HTKM-240	Tkm	Oct. 2	lg	lg	6.2±0.7 lmn	25.3±1.5 m	21.8±0.9 jk	1.16 defgh	ovoid	3.8±0.6 abcd	15.9±2.5 defghi	90.6±18.3 ab
AA-HTKM-241	Tkm	Oct. 2	dg/r	dg	7.4±0.9 n	21.8±1.5 ijkl	23.1±0.8 k	0.94 ab	ovoid	3.5±0.5 a	12.2±2.0 bcd	26.6±6.6 a
AA-HTKM-242	Tkm	Oct. 2	dg	g	3.1±0.6 bcdef	17.6±2.0 abcdef	16.9±1.1 fg	1.03 abcde	ovoid	4.3±0.2 bcdefgh	23.9±1.8 lm	76.5±17.8 a
AA-HTKM-243	Tkm	Oct. 2	dg	dg	3.6±0.3 defghi	20.4±1.1 defghijk	18.6±0.8 ghi	1.09 bcdef	ovoid	3.8±0.1 abcde	19.4±1.1 ijk	97.8±6.0 ab
AA-HTKM-244	Tkm	Oct. 2	lg	g	4.8±0.7 hijkl	22.5±1.2 jklm	19.9±1.4 hij	1.13 cdef	ovoid	4.3±0.4 bcdefgh	25.9±2.5 m	94.6±6.5 ab
AA-HTKM-246	Tkm	Oct. 2	g	g	3.3±1.0 cdefg	18.7±2.7 abcdefghi	17.0±1.3 fg	1.10 bcdef	ovoid	4.0±0.2 abcdef	17.0±3.5 fghij	75.0±8.5 a
AA-HTKM-247-1	Tkm	Oct. 2	lg	g	4.0±0.3 fghij	20.1±1.3 defghijk	19.8±0.5 hij	1.01 abcd	ovoid	4.5±0.8 efgh	17.2±3.0 ghij	82.5±7.7 a
AA-HTKM-247-2	Tkm	Oct. 2	lg	g	4.6±0.4 ghijk	20.8±1.4 efghijk	20.3±0.9 hij	1.02 abcd	ovoid	4.8±0.6 h	17.9±1.7 hijk	74.0±17.7 a
AA-HTKM-248	Tkm	Oct. 2	g	g	6.0±0.6 klmn	25.1±1.3 lm	20.7±0.7 ijk	1.21 efghi	ovoid	3.6±0.2 abc	12.8±2.1 bcde	31.1±9.8 a
AA-HSP-TD	Sp	Sep. 30	dg	dg	3.6±1.0 defgh	18.2±2.5 abcdefgh	18.0±1.7 fgh	1.00 abcd	ovoid	4.1±0.3 abcdefgh	30.8±3.0 n	87.3±2.5 ab
AA-HSP-TO	Sp	Oct. 5	lg	lg	2.7±1.1 abcdef	15.9±2.7 a	17.5±1.8 fg	0.90 a	round	3.9±0.6 abcdef	25.8±3.2 m	56.3±4.8 a
AA-HSP-J-2	Sp	Oct. 5	dg	dg	4.8±2.5 hijkl	21.5±4.4 hijk	20.5±3.5 ij	1.04 bcde	ovoid	4.3±0.5 cdefgh	19.1±1.1 ijk	23.1±5.1 a
AA-HSP-JO	Sp	Oct. 5	dg	dg	2.6±0.8 abcdef	16.6±2.4 abc	17.1±1.8 fg	0.97 abc	ovoid	4.6±0.3 fgh	21.8±4.2 klm	52.1±13.7 a

[z] Sp: Sapporo, Tkm: Tomakomai.
[y] or: orange, r: red, g: green, dg: dark green, lg: light green.
[x] L/D ratio: Length/Diamter ratio of fruit.
[w] SSC: soluble solids content.
[v] AAC: ascorbic acid content.
[u] Values within a column with different letters are significantly different ($P<0.05$) by Tukey's test.
All data shown are average±SD (n=10, except for AAC, n=3).

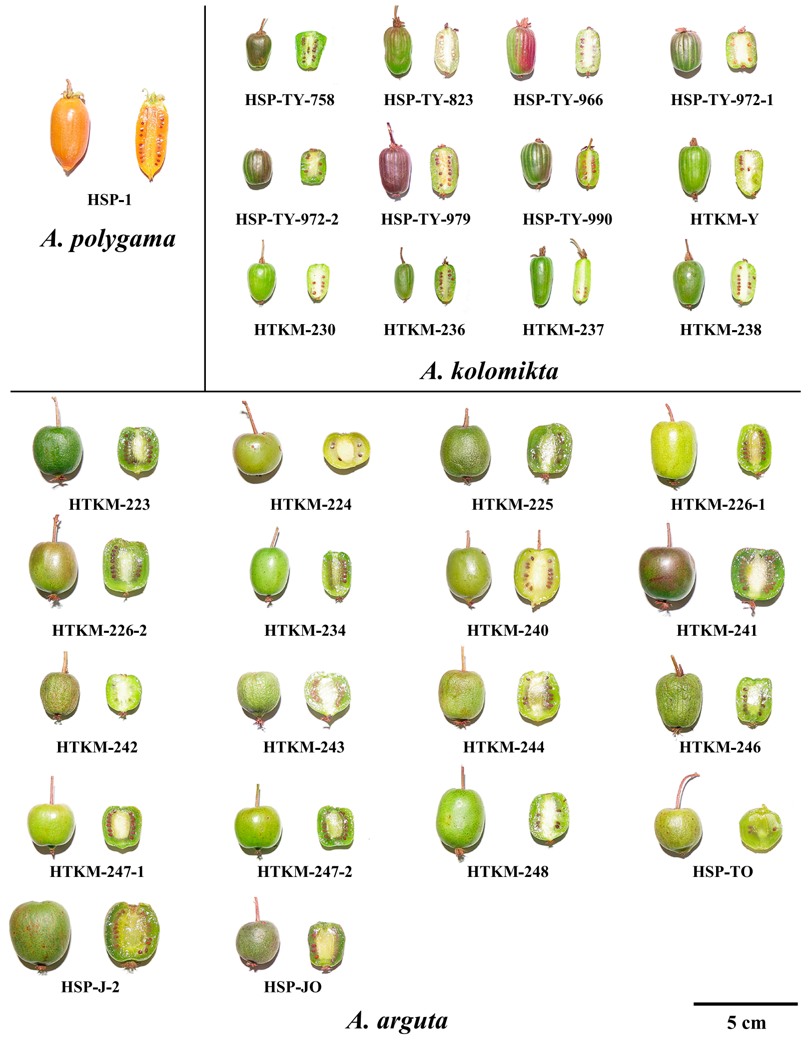

Fig. 5. Fruit morphological and color variation of *A. arguta*, *A. kolomikta*, and *A. polygama* collected in Tomakomai and Sapporo. Letter–number combinations below fruit are sample names. Bar = 5 cm.

ploid, hexaploid, octaploid, and decaploid populations of *A. arguta* on Daba Mountain in Shaanxi, China. These results suggest that the ploidy level of *A. arguta* could vary within a relatively narrow range of habitats. Here, all of the 48 wild-collected *A. arguta* in Hokkaido were tetraploid, consistent with the findings of Kataoka et al. (2010). This indicates that ploidy variation in *A. arguta* differed between Hokkaido and the main island of Japan.

In *A. kolomikta*, diploid plants were reported in Russia (Poyarkova, 1949), and tetraploid plants were observed in Japan (Nakajima, 1942). In *A. polygama*, diploid plants were found in Japan (Nakajima, 1942), and tetraploid plants were observed in the U.S. (Bowden, 1940, 1945). All of our *A. kolomikta* and *A. polygama* wild-collected in Hokkaido were diploid. One each sample of *A. kolomikta* and *A. polygama* collected from the main island was also diploid. Therefore, in Japan, both species may be mainly diploid.

We did not observe any triploid individuals estimated to be a ploidy level of interspecific hybrid between diploid *A. polygama* or *A. kolomikta* and tetraploid *A. arguta*. The three species examined here belong to taxonomic section Leiocarpae, and are closely related (Huang et al., 2002). However, the cross-compatibility and natural occurrence of hybrids is unknown. Several hybrid plants have been obtained between hexaploid *A. arguta* and diploid *A. polygama* by embryo rescue (Hirsch et al., 2001).

Few studies have investigated variation in fruit morphology and chemical characteristics in wild *A. arguta*, *A. kolomikta*, and *A. polygama*. In this preliminary evaluation of these fruits, simplified methods were used to screen potential elite accessions with respect to SSC and AAC as important parameters of fruit characteristics, using wild collected fruits in a single growing season. We found a large variation in fruit characteristics, especially in fruit size, SSC, and AAC, in *A. kolomikta* and *A. arguta* in Hokkaido, though we could not collect enough fruiting materials of *A. polygama* to compare fruit morphology and characteristics due to its scarcity of fruiting plants.

Unlike kiwifruit, which generally needs ethylene treatment for the fruit to attain full maturity after harvesting, fruit of all three species described here attain full maturity on the vines. Mature fruits of *A. kolomikta* were similar to those of *A. arguta* in general appearance, taste, and flavor, but were distinguished by their early maturity (Table 1), striped surface, smaller weight and size, and high AAC. In the present study, fruit maturity of *A. kolomikta* in natural conditions was estimated to be late August to early September in Tomakomai and Sapporo, one month earlier than that reported by Arase and Uchida (2010), who collected mature fruit samples of this species in October at elevations from 1420 to 1830 m in central Nagano. This delayed maturity in Nagano may come from the delayed time of flowering, as the elevation of the habitat in Nagano was considerably high. Compared to *A. kolomikta*, fruit maturity of *A. arguta*, or *A. polygama* was late September to early October, about one month later in the same study sites in Hokkaido. This suggests the relatively short growing season of *A. kolomikta* to achieve fruit maturity.

The relationship between fruit size or shape and ploidy level has been described for some *Actinidia* species. Kataoka et al. (2010) reported that the fruit size in diploid plants is relatively smaller than in the tetraploid and hexaploid ones, and the fruit shape of tetraploid *A. arguta* varies from round to ovoid or ellipsoidal, whereas the fruit of hexaploid plants tends to be ellipsoidal. In *A. rufa*, which is native to warm-temperate regions of Japan, the fruit shape of diploid plants varies from round to ovoid (Kim et al., 2009); fruit of tetraploid plants collected in the same habitat is smaller and ellipsoidal (Matsumoto et al., 2013). In our study, the fruit shape was mainly ovoid to ellipsoidal in diploid *A. kolomikta*, and mostly round to ovoid in tetraploid *A. arguta*, comparable to other previous studies. Total average fruit weight of 19 tetraploid *A. arguta* accessions collected in Hokkaido was 4.5 g. This value is almost the same as that from Nagano reported in Arase and Uchida (2009a), but is only about half of that of tetraploid plants reported in Kataoka et al. (2010). As for *A. kolomikta*, total average fruit weight of 13 diploid *A. kolomikta* in Hokkaido was 1.8 g, more than 1.5 times larger than that collected in central Nagano (Arase and Uchida, 2010).

The AAC was relatively high in *A. kolomikta*, but there was a more than four-fold difference between the lowest and highest values. Wide variation in AAC has also been observed among cultivars, with the following values reported on a mg/100 g FW basis: 'Lande', 700; 'Pavlovskaja', 600; and 'VIR-1', 850 (Chesoniene et al., 2004); 'Dr Szymanowski', 1008 (Latocha et al., 2010); and an unknown cultivar, 211 (Zuo et al., 2012). We found much larger variability in AAC in *A. arguta*, with a more than eight-fold difference between the lowest and highest values. Nishiyama et al. (2004) described varietal differences in ascorbic acid content in the fruits of kiwifruit and other related species with all the values reported on a mg/100 g FW basis; 65.5 in green fleshed kiwifruit (*A. deliciosa* 'Hayward'), and varied from 29 to 80 in other cultivars of *A. deliciosa*; 103.7 in yellow fleshed kiwifruit (*A. chinensis* 'Hort16A'), and varied from 73.7 to 205.8 in other cultivars of *A. chinensis*; 37.3 to 184.6 in five cultivars of *A. arguta*. This AAC variation of *A. arguta*, all from mainland, was comparable to those found in the present study.

Wide variation in SSC was also observed in *A. arguta* and *A. kolomikta*. Total average SSC of 19 tetraploid *A. arguta* accessions in Hokkaido was 18.8%, relatively higher than that of 13 diploid *A. kolomikta* accessions, which was 13.3%. One sample of *A. arguta*, AA-HSP-TD, had extremely high SSC (average of 30.8%). This value was much higher than that found by others: 14%–15% in an unknown cultivar (Huang et al., 2004), 10.5% in 'Mitsu-ko' (Okamoto and Goto, 2005), 11.1%–12.8% in wild collections from Japan (Kataoka et al., 2010), 16.6% in 'Ananasnaya' and 16.8% in 'Weiki' (Latocha et al., 2010), and 10.2%–17.0% in two cultivars and 14 local collections from Japan (Kim et al., 2012); kiwifruit 'Hayward' was 13.2%–14.6% (Tavarini et al., 2008). Our data probably represent one of the highest SSC levels reported to date in *A. arguta* and in the genus *Actinidia*.

Overall, some accessions of *A. kolomikta* and *A. arguta* collected in the present study were found to have interesting characteristics such as high SSC or AAC, as well as strong cold tolerance. These values of SSC and AAC are relatively much higher compared with other kiwifruit cultivars and horticulturally of interests. These values could change with the season, or locality, and therefore, it is important to study further to confirm that these fruit characteristics found in the present study are stable. Breeding of these species by using other species or kiwifruit having large fruit and long storage period will be necessary for commercial use, which may result in the improvement of fruit taste, nutritional values, and cold tolerance of kiwifruit, because these accessions have very small fruit, short storage period, and low fruit yield. All the species described here are dioecious as kiwifruit, and thus, in this case, kiwifruit will be used as a male parent. *Actinidia arguta* has cross compatibility with hexaploid kiwifruit (*A. deliciosa*), and several hybrid cultivars have been produced recently (Kokudo et al., 2003). As for *A. kolomikta*, several hybrid plants with kiwifruit (*A. deliciosa* and *A. chinensis*) have also been obtained recently by embryo rescue or protoplast fusion (Hirsch et al., 2001; Xiao et al., 2004). Xiao et al. (2004) reported that the interspecific somatic hybrids between *A. kolomikta* and *A. chinensis* obtained by protoplast fusion had potential cold tolerance. Another interesting approach is to use elite accessions themselves for further improvement, by the approach such as polyploidization, genetic transformation, and mutagenesis. Wu et al. (2012) reported that colchicine-induced autotetraploid plants of *A. chinensis* showed a significant increase in fruit size, by up to 50% to 60%. Including these approaches, further studies are needed for the improvement of these potential useful wild genetic resources abundant in Hokkaido, as well as additional evaluation of the plant and fruit characteristics.

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