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Distribution and morphological description of *Ilex rugosa* Fr. Schmidt from eight Japanese mountains

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Summary

This paper describes the distribution and morphology of *Ilex rugosa* Fr. Schmidt, an evergreen shrub native to Japan. The aims of this investigation were to determine if *Ilex rugosa* specimens from eight Japanese mountains could be segregated into the varieties accepted by YAMAZAKI (1987; 1989) and whether populations of *I. rugosa* differ morphologically based on mountain of origin, altitude, or plant gender (male or female). The specimens were found on seven of eight investigated mountains in Hokkaido and Honshu.
There was no statistically significant evidence for segregating the populations of *I. rugosa* from different mountains on the basis of leaf length or width. Internode length differed significantly from mountain to mountain. Leaf width decreased significantly with increasing altitude; leaf length displayed no such pattern.
Internode length varied more in male than in female plants, but no other morphological differences between male and female *I. rugosa* were found. We also examined growth habits, lateral shoot rooting ability, and leaf colour of the plants in the study area. These characteristics varied little between populations.
Plants of *I. rugosa* grown for four years in Denmark showed significantly larger leaves and internode length than plants in Japan.

Introduction

Ilex rugosa Fr. Schmidt is an evergreen shrub belonging to the Aquifoliaceae. Native to Japan, it grows on the mountains of Hokkaido and central and northern Honshu in woods mostly dominated by conifers (OHWI, 1965; 1984; YAMAZAKI, 1987; 1989; GALLE, 1997). The morphology of this species has been described by several authors (SCHMIDT, 1868; OHWI, 1965; KITAMURA and OKAMOTO, 1972; OHWI, 1984; BAUERS, 1986; GALLE, 1997; HUXLEY, 1999; IWATSUKI et al., 1999), but only YAMAZAKI (1987; 1989) recognizes three varieties and has described morphological variation in relation to native distribution within a number of Japanese districts.

Fig. 1: Male flowers of *Ilex rugosa*

I. rugosa is a long-creeping, spreading, and sometimes prostrate evergreen shrub with angular glabrous twigs. The species is dioecious, flowering from June to August. Flowers are tetra- or pentamerous with white petals, 2-5 mm across, arranged in cymose inflorenscences (OHWI, 1965; 1984; YAMAZAKI, 1989; GALLE, 1997; HUXLEY, 1999; IWATSUKI et al., 1999); 1-8 male flowers are clustered at the leaf axils (Fig. 1), while the female plants have 1-3 flowers per leaf axil (Fig. 2).
The ripe fruits are red, globular drupes, less than 6 mm in diameter. Typically only one or two fruits mature per inflorescence. The fruit contains 4, occasionally 5, triangular oblong pyrenes each containing one seed (OHWI, 1965; 1984; BAUERS, 1986; YAMAZAKI, 1989; GALLE, 1997; HUXLEY, 1999; IWATSUKI et al., 1999).

Fig. 2: Female flower of *Ilex rugosa*

Fig. 3: Map of Japan showing the districts. The arrows locate the eight mountains where the distribution and morphology of *Ilex rugosa* was investigated in this study. The names of the mountains and their latitudes appear in Tab. 1.

Generally, leaves are mostly lanceolate to ovate-oblong with attenuated obtuse bases and obtuse or acute apices. The margins have blunt serrations or shallow crenations. The midrib, lateral nerves, and veinlets are impressed on the upper side, and slightly raised beneath (OHWI, 1965; 1984; BAUERS, 1986; YAMAZAKI, 1989; GALLE, 1997; HUXLEY, 1999; IWATSUKI et al., 1999). Authors differ in their morphological descriptions of the shape and size of *I. rugosa* leaves (OHWI, 1965; 1984; BAUERS, 1986; YAMAZAKI, 1989; GALLE, 1997). For example, YAMAZAKI (1987; 1989) recognizes three varieties of *I. rugosa* endemic to various districts of Japan classified according to leaf size (Fig. 3 gives a district map of Japan).

The descriptions are as follows.

The *I. rugosa* var. *rugosa,* distributed in Hokkaido, the southern Chishima Islands, and Sakhalin Island, has large wide leaves, ranging from elongate-elliptic to elliptic, 2.5-4 cm long and 1-2 cm wide (YAMAZAKI, 1987; 1989). This variety is called *maruba-tsuru-tsuge* in Japanese, which means „creeping *Ilex* with round leaves", but in spite of its Japanese name the leaves of this variety are rather elongated (YAMAZAKI, 1987).

I. rugosa var. *hondoensis* (Yamazaki) T.R. Dudley is distributed in forests in the sub-alpine and alpine zones in Tohoku, Kanto, and in Chubu District. The leaves are elongate-elliptic to narrowly elongate-elliptic, 1.5-4 cm long and 0.5-1.3 cm wide (YAMAZAKI, 1987; 1989). This variety is called *tsuru-tsuge* in Japanese, which means „creeping *Ilex*". The leaves of this variety are smaller than those of *I. rugosa* var. *rugosa* found in Hokkaido (YAMAZAKI, 1987).

I. rugosa var. *stenophylla* (Koidz.) Sugimoto is mainly distributed in the southern part of Chubu District and in the Kinki and Shikoku Districts. The leaves are narrow lanceolate, 2.5-5 cm long and 0.3-0.7 cm wide (YAMAZAKI, 1987; 1989). This variety is called *hosoba-tsuru-tsuge* in Japanese, which means „creeping *Ilex* with narrow leaves" (YAMAZAKI, 1987). The distribution of *I. rugosa* var. *stenophylla* is largely restricted to the above districts; however, individuals with similar narrow leaves are found in the area extending from the north of Chubu District to the south of Tohoku District (YAMAZAKI, 1987).

The aims of this investigation were to determine if the morphological differences between *Ilex rugosa* specimens from eight Japanese mountains could segregate the specimens into the varieties accepted by YAMAZAKI (1987; 1989), and whether populations of *I. rugosa* differ morphologically based on mountain of origin, altitude, or plant gender (male or female).

Material and methods

Mountains studied

Fieldwork was conducted in the autumn of 1999 on eight Japanese mountains on Hokkaido and Honshu (Fig. 3 and Tab. 1). The climate in the investigated areas is described in Tab. 2.

Field methods

On each mountain we registered the occurrence of *Ilex rugosa*, working down from the timberline. The altitude and position of the first observed *I. rugosa* was measured by altimeter and Global Positioning System (GPS) and recorded. Each 100 or 200 m lower in altitude we described the morphology of five plants per cluster and collected cuttings for further study. As *I. rugosa* normally grows beneath conifers, distribution varied between the mountains depending on altitude (Tab. 1). We recorded the distribution of *I. rugosa* in the accessible area of each mountain, ignoring aspect. The growth habit, leaf colour, and rooting ability of lateral shoots of each accession were

Tab. 1: Mountains investigated, peak altitude (m above sea level = m a.s.l.), collecting altitude (m a.s.l.), collecting area, districts, and prefectures of the eight investigated Japanese mountains. The numbers of the mountains relate to Fig. 3. The positions of the collecting areas were determined using the global positioning system (GPS).

Mountain	Mountain peak (m a.s.l.)	Collecting altitude (m a.s.l.)	GPS coordinates	District	Prefecture
1. Mt. Furano	1950	1600, 1400, 1200, 1000, 800	43°21'661"— 43°23'447"N 142°36'090"—142°37'754"E	Hokkaido	Kamikawa
2. Mt. Dairoku	1460	1460, 1260, 1060, 830, 600	43°18'592"— 43°20'377"N 142°35'490"—142°37916"E	Hokkaido	Kamikawa
3. Mt. Oodake	1584,6	1500, 1400, 1300	40°39'646"— 40°39'909"N 140°52'317"—140°52'674"E	Tohoku	Aomori
4. Mt. Nishidaiten	1981,8	1980, 1880, 1780, 1680, 1580	37°44'022"— 37°44'111"N 140°06'943"—140°07'874"E	Tohoku	Fukushima
5. Mt. Motoshirane	2156	2124, 2053, 1983	36°37'426"— 36°37'766"N 138°31'961"—138°32'333"E	Kanto	Gunma
6. Mt. Norikura	3026	2200, 2100, 2000, 1900	36°07'224"— 36°07'324"N 137°34'559"—137°35'852"E	Chubu	Nagano
7. Mt. Haku	2702	2200, 2100, 2050, 2030, 1880, 1780, 1500	36°07'697"— 36°08'583"N 136°43'890"—136°46'692"E	Chubu	Ishikawa
8. Mt. Fuji	3776	No *Ilex rugosa* plants found		Chubu	Shizuoka

described. All woody plant species within a radius of 2.5 m of the investigated plant were recorded (Tab. 3). The shading caused by surrounding plants of other species was also observed.

Laboratory registrations

The internode length for each cutting was registered. For each plant, the length and width of the fully developed leaves of the 10 uppermost leaves were measured using an electronic digital calliper. The cuttings were sent to Aarslev, Denmark, where they were rooted and grown in a greenhouse to determine the sex of the plants. After two years growth in the greenhouse, the plants were planted outside in a field at Aarslev in a fertile clay soil (11 % clay) to observe growth in the Danish climate. The internode length at each cutting and the length and width of the fully developed leaves of the 10 uppermost leaves were measured in the surviving plants after two years' growth.

Statistical analysis

The measured variables (length, width, colour, and internode length of leaves, growth habit of plant, and rooting ability of lateral shoots) were analysed using mixed linear normal models, with mountain, sex (categorical), altitude, shading caused by surrounding plants of other species (continuous covariate) as fixed effects. The repeated measurements arising from our sampling in clusters from different altitudes were modelled by introducing sampling cluster as a random effect in the model. For some variables – length and width of leaves, and internode length – a log transformation was necessary to validate the normal distribution assumption. Residual variance was estimated for each mountain for those variables in which variance homogeneity resulted in an obviously inferior model.
The initial model included interactions between the fixed effects, and a stepwise model reduction was performed using a 0.05 level of significance as a criterion for exclusion. The similarity of the plants within a sampling cluster was expressed as the intra-class correlation

Tab. 2: Climatic features of the eight investigated Japanese mountains. Data obtained from isotherm maps (Tatehira, 1991; Nitta, 1993). The numbers of the mountains relate to Fig. 3.

Mountain	Annual amount precipitation (mm)	Annual max. depth of snow cover (cm)	Annual mean temp. (°C)	Monthly min. temp. (°C)	Monthly max. temp. (°C)	Lowest monthly mean temp. (°C)	Highest monthly mean temp. (°C)
1. Mt. Furano	800—1000	100—150	3—6	Jan, -15,3	Aug, 25,9	Jan, -9,4	Aug, 20,8
2. Mt. Dairoku	800—1000	100—150	3—6	Jan, -15,3	Aug, 25,9	Jan, -9,4	Aug, 20,8
3. Mt. Oodake	1200—1400	100—150	3—6	Jan, -10,4	Aug, 21,5	Jan, -8,1	Aug, 18,6
4. Mt. Nishidaiten	1600—2000	50—100	6—9	Jan, -9,2	Aug, 25,5	Jan, -2,8	Aug, 22,2
5. Mt. Motoshirane	1600—2000	20—50	6—9	Jan, -7,4	Aug, 23,4	Jan, -4,6	Aug, 19,4
6. Mt. Norikura	1600—2000	50—100	9—12	Jan, -9,1	Aug, 26,2	Jan, -4,0	Aug, 22,0
7. Mt. Haku	2400—2800	150—200	9—12	Jan, -4,7	Aug, 28,7	Jan, -5,4	Aug, 23,3
8. Mt. Fuji	2000—2400	20—50	6—9	Jan, -9,5	Aug, 24,9	Jan, -2,9	Aug, 21,6

Tab. 3: Woody plant species found in the overstorey of *Ilex rugosa* in the collecting area of the seven mountains in Japan where *I. rugosa* was found. The numbers of the mountains relate to Fig. 3.

Mountain 1.	**Mt. Furano**	**Mt. Furano**	**Mt. Furano**	**Mt. Furano**	**Mt. Furano**
Collecting altitude (m a.s.l.)	1600	1400	1200	1000	800
Species found in the overstorey of *Ilex rugosa*	*Pinus pumila* Regel	*Pinus pumila* Regel *Pinus thunbergii* Parl. *Sasa kurilensis* (Rupr.) Makino et Shibata	*Picea glehnii* Mast. *Sasa kurilensis* (Rupr.) Makino et Shibata	*Picea glehnii* Mast. *Sasa kurilensis* (Rupr.) Makino et Shibata *Sorbus commixta* Hedl. Makino et Shibata	*Abies sachalinensis* Fr. Schm. *Betula ermanii* Cham. *Picea glehnii* Mast. *Sasa kurilensis* (Rupr.) *Vaccinium smallii* A. Gray

Mountain 2.	**Mt. Dairoku**	**Mt. Dairoku**	**Mt. Dairoku**	**Mt. Dairoku**	**Mt. Dairoku**
Collecting altitude (m a.s.l.)	1460	1260	1060	830	600
Species found in the overstorey of *Ilex rugosa*	*Picea glehnii* Mast. *Pinus pumila* Regel *Sasa kurilensis* (Rupr.) Makino et Shibata *Vaccinium vitis-idaea* L.	*Abies sachalinensis* Fr. Schm. *Menziesia pentandra* Maxim. *Picea glehnii* Mast. *Pinus pumila* Regel *Sasa kurilensis* (Rupr.) Makino et Shibata	*Abies sachalinensis* Fr. Schm. *Menziesia pentandra* Maxim. *Picea glehnii* Mast.	*Abies sachalinensis* Fr. Schm. *Picea jezoénsis* Carr. *Sasa kurilensis* (Rupr.) Makino et Shibata *Sorbus commixta* Hedl.	*Abies sachalinensis* Fr. Schm. *Picea jezoénsis* Carr. *Sasa kurilensis* (Rupr.) Makino et Shibata

Mountain 3.	**Mt. Oodake**	**Mt. Oodake**	**Mt. Oodake**
Collecting altitude (m a.s.l.)	1500	1400	1300
Species found in the overstorey of *Ilex rugosa*	*Pinus pumila* Regel *Rhododendron brachycarpum* D. Don *Sorbus commixta* Hedl. *Vaccinium sp.* L.	*Abies mariesii* Mast. *Acer tschonoskii* Maxim. *Leucothoe grayana* Maxim. *Menziesia multiflora* Maxim. *Rhododendron brachycarpum* D. Don *Sorbus commixta* Hedl.	*Abies mariesii* Mast. *Acer tschonoskii* Maxim. *Sasa sp.* *Sorbus commixta* Hedl.

Mountain 4.	**Mt. Nishidaiten**	**Mt. Nishidaiten**	**Mt. Nishidaiten**	**Mt. Nishidaiten**	**Mt. Nishidaiten**
Collecting altitude (m a.s.l.)	1980	1880	1780	1680	1580
Species found in the overstorey of *Ilex rugosa*	*Abies mariesii* Mast. *Sasa sp.* *Vaccinium uliginosum* L.	*Abies mariesii* Mast. *Oplopanax japonicus* Nakai *Sasa sp.*	*Abies mariesii* Mast. *Oplopanax japonicus* Nakai *Sasa sp.* *Viburnum furcatum* Blume	*Abies mariesii* Mast. *Oplopanax japonicus* Nakai *Sasa sp.* *Sorbus commixta* Hedl. *Virburnum furcatum* Blume	*Abies mariesii* Mast. *Sasa sp.* *Viburnum furcatum* Blume

Mountain 5.	**Mt. Motoshirane**	**Mt. Motoshirane**	**Mt. Motoshirane**
Collecting altitude (m a.s.l.)	2124	2053	1983
Species found in the overstorey of *Ilex rugosa*	*Abies veitchii* Lindl. *Pinus pumila* Regel *Prunus nipponica* Matsum. *Sasa sp.* *Vaccinium uliginosum* L.	*Abies veitchii* Lindl. *Menziesia pentandra* Maxim. *Prunus nipponica* Matsum. *Sasa sp.* *Sorbus commixta* Hedl. *Vaccinium smallii* A. Gray *Viburnum furcatum* Blume	*Abies veitchii* Lindl. *Acer tschonoskii* Maxim. *Alnus hirsuta* Turcz. *Betula ermanii* Cham. *Menziesia pentandra* Maxim. *Sorbus commixta* Hedl. *Tsuga diversifolia* Mast. *Vaccinium smallii* A. Gray *Viburnum furcatum* Blume

Mountain 6.	**Mt. Norikura**	**Mt. Norikura**	**Mt. Norikura**	**Mt. Norikura**
Collecting altitude (m a.s.l.)	2200	2100	2000	1900
Species found in the overstorey of *Ilex rugosa*	*Abies veitchii* Lindl. *Acer tschonoskii* Maxim. *Rhododendron albrechtii* Maxim. *Sorbus commixta* Hedl. *Vaccinium ovalifolium* J.E. Smith *Viburnum furcatum* Blume	*Abies veitchii* Lindl. *Acer tschonoskii* Maxim. *Euonymus macropterus* Rupr. *Menziesia pentandra* Maxim. *Sorbus commixta* Hedl. *Vaccinium ovalifolium* J.E. Smith *Viburnum furcatum* Blume	*Abies veitchii* Lindl. *Acer tschonoskii* Maxim. *Menziesia pentandra* Maxim. *Sasa senanensis* (Franch. et Savat.) Rehd. *Sorbus commixta* Hedl. *Tsuga diversifolia* Mast.	*Abies veitchii* Lindl. *Sasa sp.* *Sorbus commixta* Hedl. *Tsuga diversifolia* Mast.

Mountain 7.	**Mt. Haku**	**Mt. Haku**	**Mt. Haku**	**Mt. Haku**	**Mt. Haku**
Collecting altitude (m a.s.l.)	2200	2100	2050	2030	1880
Species found in the overstorey of *Ilex rugosa*	*Abies mariesii* Mast. *Betula ermanii* Cham. *Oplopanax japonicus* Nakai *Sasa palmata* (Marliac) Nakai *Sorbus commixta* Hedl.	*Abies mariesii* Mast. *Pinus pumila* Regel *Sasa cernua* Makino *Sorbus matsumurana* (Makino) Koehne*Vaccinium smallii* A. Gray	*Abies mariesii* Mast. *Sasa cernua* Makino	*Abies mariesii* Mast. *Sasa palmata* (Marliac) Nakai *Viburnum furcatum* Blume	*Sasa palmata* (Marliac) Nakai

Mountain 7.	**Mt. Haku**	**Mt. Haku**
Collecting altitude (m a.s.l.)	1780	1500
Species found in the overstorey of *Ilex rugosa*	*Abies mariesii* Mast. *Acer tschonoskii* Maxim. *Betula ermanii* Cham. *Menziesia pentandra* Maxim. *Sasa palmata* (Marliac) Nakai *Viburnum furcatum* Blume	*Acer micranthum* Sieb. et Zucc. *Clethra barbinervis* Sieb. et Zucc. *Hydrangea paniculata* Siebold *Ilex sugerokii* Maxim. *Rhododendron brachycarpum* D. Don

coefficient, i.e., the variance within a cluster as a proportion of the total variance (within cluster + residual variance). The calculations were made using the MIXED procedure of SAS for Windows 8.02 (SAS INSTITUTE, 1999).

For each plant, the leaf length, leaf width, and internode length were measured in Japan. After four years growth in Denmark, the same plants were measured again if they had survived. The measurements were analysed pairwise using the GLM procedure of SAS for Windows 8.02 (SAS INSTITUTE, 1999), comparing Japanese measurements against Danish measurements for each surviving plant.

Results

I. rugosa was found on seven of the eight investigated mountains. *I. rugosa* was not observed on the south slope of Mt. Fuji, even though it had been reported in this area ten years earlier (KONTA, 1999, personal communication).

I. rugosa was found growing most often beneath conifers, such as *Abies mariesii* Mast., *Abies sachalinensis* Fr. Schm., *Abies veitchii* Lindl., *Picea glehnii* Mast., *Picea jezoënsis* Carr., or *Pinus pumila* (Pallas) Regel, but also in deciduous forest or beneath *Sasa* spp., as shown in Tab. 3.

The intra-class correlation of the plants growing at a single sampling site was similar for the different variables and ranged between 0.2 and 0.4.

Morphological diversity related to locality

Internode length varied significantly among mountains, with mean values lying between 0.16 and 0.23 (Tab. 4). The shading caused by surrounding plants of other species (data not shown) had no influence on internode length. Analysis found no significant difference in the length or width of leaves between mountains. Mean leaf length varied between 2.25 and 2.54 cm, and width varied between 0.76 and 0.89 cm. The growth habit, rooting ability of lateral shoots, and leaf colour (statistical data not shown) did not vary significantly between mountains.

Tab. 4: Internode length (cm) for plants of *I. rugosa* collected at the seven mountains in Japan. Internode length followed by different letters is significantly different at 5 % significance level. N = number of observations.

Mountain		Internode length (cm)	
Furano	(N=104)	0.17 ± 0.02	cd
Dairoku	(N=135)	0.23 ± 0.03	a
Oodake	(N=70)	0.21 ± 0.03	abc
Nishidaiten	(N=113)	0.16 ± 0.02	d
Motoshirane	(N=53)	0.16 ± 0.03	cd
Norikura	(N=69)	0.18 ± 0.03	bcd
Haku	(N=153)	0.21 ± 0.03	ab

Morphological diversity related to altitude

Leaf width decreased significantly (at 5 % significance level) with increasing altitude. Statistical analysis showed an intercept of 1.023 ± 0.11 (standard error) and a slope of -0.00013 ± 0.000055 (standard error) per km, meaning that the width decreased 12 % ± 10 % as the altitude increased 1 km (retransformed from log data). Fig. 4 shows the predicted values of leaf width relative to altitude. Leaf length, however, was not influenced by altitude. The growth habit, rooting ability of lateral shoots, and leaf colour were not significantly affected by altitude.

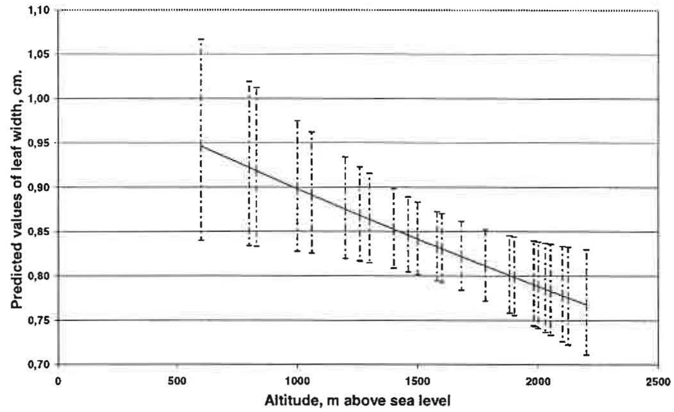

Fig. 4: Predicted values of leaf width (cm) of *I. rugosa* depending on the altitude where they were collected. The slope is significant at a 5 % level. Error bars are 95 % confidence intervals.

Morphological diversity related to sex

The standard deviation of the internode length differed significantly between male and female, meaning that internode length being more variable in male (0.4291 ± 0.0645) than in female plants (0.3373 ± 0.0459). The other morphological characteristics studied did not vary significantly between male and female plants.

Growth habit, rooting ability of lateral shoots, and leaf colour

Growth habit, rooting ability of lateral shoots and leaf colour of the plants found on each mountain appear in Tab. 5. Fig. 5 displays the different growth habits registered in this investigation. Most plants displayed decumbent to shrubby growth, with few to a moderate number of lateral shoots producing roots. Only a minority of plants had many lateral shoots producing roots.

Few plants had long twigs: one plant had twigs over 2 m long, but twigs were generally under 0.5 m in length. The investigated plants mostly had dark-coloured leaves. Shading caused by surrounding plants did not have a statistically significant influence on leaf colour. Different soil types could possibly affect leaf colour and growth habit, but soil type was not considered in this study.

The results from the statistical analysis of the abovementioned different investigated variables measured in Japan are gathered and shown in Tab. 6.

Japanese versus Danish measurements of leaves

The leaves measured on plants in Japan were significantly smaller than leaves measured on the same plants in Denmark (Tab. 7). The internode length did not change significantly. Considering the different mountains where the plants were collected, pairwise measurements of the plants in Japan and those in Denmark revealed significantly larger leaves in Denmark after four years growth (Fig. 6).

Considering the altitudes at which the plants were collected, only a few plants from different altitudes were significantly alike, e.g. leaf

Tab. 5: Description of growth habit, rooting ability of lateral shoots, and leaf colour of *Ilex rugosa* growing on seven Japanese mountains. The numbers (in %) represent plants investigated on each mountain. N = number of specimens.

Growth habit		**Procumbent**	**Decumbent**	**Shrubby**	**Upright**
Mt. Furano	(N=25)	12	36	44	8
Mt. Dairoku	(N=25)	8	20	56	16
Mt. Oodake	(N=15)	27	13	53	7
Mt. Nishidaiten	(N=25)	20	16	36	28
Mt. Motoshirane	(N=15)	27	40	33	0
Mt. Norikura	(N=20)	40	25	20	15
Mt. Haku	(N=35)	14	29	37	20
Means of mountains		21	26	40	13
Lateral shoots forming roots		**None**	**Few**	**Medium**	**Many**
Mt. Furano	(N=25)	0	56	28	16
Mt. Dairoku	(N=25)	8	68	16	8
Mt. Oodake	(N=15)	0	67	20	13
Mt. Nishidaiten	(N=25)	0	56	36	8
Mt. Motoshirane	(N=15)	0	100	0	0
Mt. Norikura	(N=20)	0	75	25	0
Mt. Haku	(N=35)	0	69	31	0
Means of mountains		1	70	22	7
Leaf colour		**Light-coloured green**	**Intermediate colour**	**Dark-coloured green**	**Very dark-coloured green**
Mt. Furano	(N=25)	0	48	52	0
Mt. Dairoku	(N=25)	0	8	88	4
Mt. Oodake	(N=15)	0	33	67	0
Mt. Nishidaiten	(N=25)	0	0	92	8
Mt. Motoshirane	(N=15)	7	20	73	0
Mt. Norikura	(N=20)	0	25	50	25
Mt. Haku	(N=35)	3	40	51	6
Means of mountains		1	25	68	6

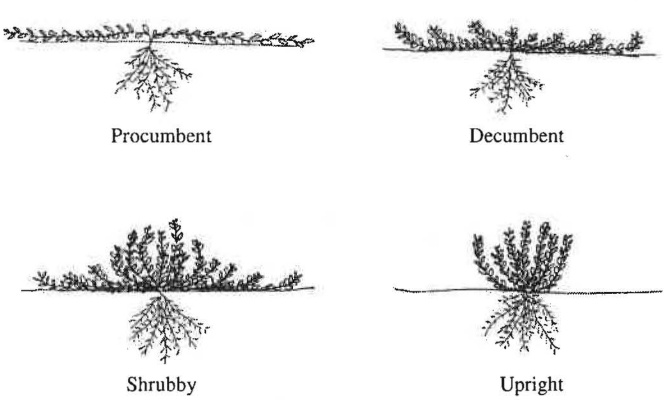

Fig. 5: Different growth habits of *I. rugosa* recorded in this investigation.

length at 800 m a.s.l. (Fig. 7). The significance of this is that most plants had significantly larger leaves after growth in Denmark. Internode length did not display the same pattern; internode length in Japan was not significant different from internode length in Denmark.

Tab. 6: The statistical results in the investigation of collected plant material of *Ilex rugosa* from Japan. NS = non-significance. * * * = 5 % significance level.

	Mountains	**Altitude**	**Sex**
Internode length	* * *	NS	NS
Standard deviation of internode length	–	–	* * *
Leaf length	NS	NS	NS
Leaf width	NS	* * *	NS
Growth habit	NS	NS	NS
Rooting ability	NS	NS	NS
Leaf colour	NS	NS	NS

Danish measurements of leaves

The plants growing in Denmark were analysed individually. After four years growth, significant differences were found in leaf length, leaf width, and internode length between plants native from different Japanese mountains and between plants native from different altitudes (Tab. 8).

Tab. 7: Mean values for leaf length, leaf width and internode length (cm) measured in Denmark and Japan, respectively. Values followed by different letters are significantly different at 5 % significance level. Each variable was analysed individual.

	Leaf length (cm)	Leaf width (cm)	Internode length (cm)
Denmark	3.0 a	1.1 a	0.21 a
Japan	2.5 b	0.8 b	0.21 a

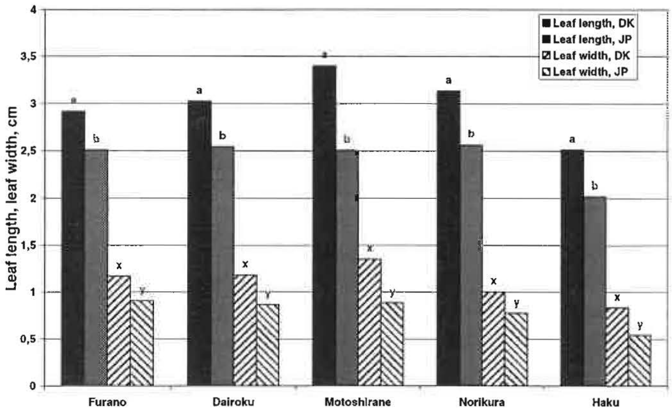

Fig. 6: Leaf length and leaf width (cm) of plants native to Japanese mountains measured in Japan and Denmark, respectively. Values followed by different letters are significantly different at 5 % significance level. Each variable was analysed individual.

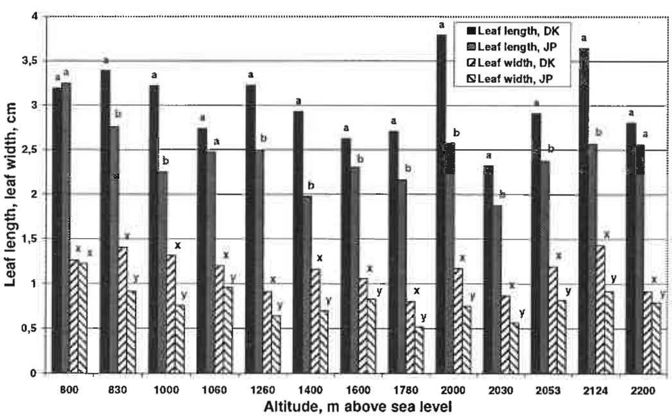

Fig. 7: Leaf length and leaf width (cm) of plants native to different altitudes at Japanese mountains measured in Japan and Denmark, respectively. Values followed by different letters are significantly different at 5 % significance level. Each variable was analysed individual.

Tab. 8: Mean values for leaf length, leaf width and internode length (cm) measured in Denmark after four years growth. Values followed by different letters are significantly different at 5 % significance level. Each variable was analysed individual.

Native mountain	Leaf length (cm)	Leaf width (cm)	Internode length (cm)
Furano	2.9 b	1.2 b	0.23 a
Dairoku	3.0 b	1.2 b	0.23 a
Motoshirane	3.4 a	1.4 a	0.16 b
Norikura	3.1 ab	1.0 c	0.17 b
Haku	2.5 c	0.8 d	0.22 a
Native altitudes (m a.s.l.)	**Leaf length (cm)**	**Leaf width (cm)**	**Internode length (cm)**
800	3.2 cf	1.3 bc	0.22 bf
830	3.4 bc	1.4 a	0.20 cdefg
1000	3.2 ce	1.3 ab	0.30 a
1060	2.7 gh	1.2 bd	0.23 bd
1260	3.2 cdg	0.9 g	0.25 b
1400	2.9 defg	1.2 cdef	0.20 cdefg
1600	2.6 h	1.1 f	0.23 bc
1780	2.7 gh	0.8 g	0.22 be
2000	3.8 a	1.2 bf	0.20 cdefg
2030	2.3 i	0.9 g	0.22 bg
2053	2.9 defh	1.2 def	0.16 h
2124	3.6 ab	1.4 a	0.16 h
2200	2.8 gh	0.9 g	0.16 h

Discussion

YAMAZAKI (1987; 1989) recognized three varieties of *I. rugosa*, mainly based on leaf size. He described *I. rugosa* var. *rugosa* ('Maruba-tsuru-tsuge'), distribution in Hokkaido, south of Chishima Island, and Sakhalin, with leaf length 2.5-4 cm and leaf width 1-2 cm, *I. rugosa* var. *hondoensis* ('Tsuru-tsuge'), distribution in Tohoku, Kanto and Chubu, with leaf length 1.5-4 cm and leaf width 0.5-1.3 cm, and *I. rugosa* var. *stenophylla* ('Hosoba-tsuru-tsuge'), distribution in Southern part of Chubu, Kinki and Shikoku (also found North of Chubu district to the South of Tohoku district), with leaf length 2.5-5 cm and leaf width 0.3-0.7 cm.

The present statistical analysis of leaf length and width did not support this segregation. The mean leaf lengths of all the investigated plants growing in the areas where the three described varieties grow (Tab. 9) were 2.5, 2.5, and 2.4 cm, respectively, and the mean widths were 0.8, 0.9, and 0.9 cm, respectively. These means are very much alike even though the plants are from all three of the described areas. The variation found in leaf length and width appears in Tab. 9. Our investigation included all fully developed leaves from the tip of the cutting to 10 leaves down, regardless of size. This procedure influenced the values obtained for mean leaf length and width, as not only the largest leaves were chosen and measured. YAMAZAKI (1987; 1989) gives no information on the position of the leaves used to establish

Tab. 9: Variation in leaf size (cm) of *I. rugosa* examined in Japan in this study.

Distribution districts	Leaf length (cm)	Leaf width (cm)
Hokkaido, south of Chishima Island and Sakhalin	0.4-4.8	0.1-1.8
Tohoku, Kanto and Chubu	0.7-4.3	0.2-1.5
Southern part of Chubu, Kinki and Shikoku (also found North of Chubu district to the South of Tohoku district)	0.6-4.2	0.2-1.3

the three varieties, making comparison with the present investigation difficult.

YAMAZAKI (1987) describes individuals of *I. rugosa* var. *rugosa* from Hokkaido as having smaller leaves, similar to plants from Honshu. The described variation in leaf size may result from differences in the habitats of plants growing on different mountains (YAMAZAKI, 1987). However, the individual specimens from Hokkaido, the southern Chishima Islands, and Sakhalin Island had larger leaves than those from other locations (YAMAZAKI, 1987). YAMAZAKI (1987; 1989) describes hybridisations between varieties of *I. rugosa,* raising the possibility that growth habits alone may not influence leaf size. This possibility could explain why this investigation did not obtain a segregation in terms of leaf size (Tab. 9) similar to that described by YAMAZAKI.

Internode length differed significantly between mountains. Latitude seems to be of no importance to internode length; the mountains in Tab. 4 are listed according to latitude, from Hokkaido to Honshu (numbers 1-7 in Fig. 3 and Tab. 1). Likewise, precipitation (Tab. 2) does not seem to be responsible for the variation in internode length. The annual precipitation on Mt. Dairoku is 800-1000 mm and that on Mt. Haku 2400-2800 mm, but the internode length of *I. rugosa* plants from these two mountains is not significantly different. Furthermore, Mt. Furano and Mt. Dairoku both have annual precipitation in the range of 800-1000 mm, and plants originating from one mountain or the other differ significantly from each other.

By way of comparison, CREMER-BACH and BACH (1994) found that the leaf size and number of nodes in the *Medicago* species decreased with increasing altitude and precipitation in Morocco. Whether the decreasing number of nodes is due to altitude or precipitation is unknown, as genetic differences or soil type may also influence the results.

Leaf width of *I. rugosa* did not vary significantly among the mountains, but did decrease significantly with increasing altitude. In the descriptions of *I. rugosa* varieties, YAMAZAKI (1987; 1989) provided no information on altitude.

Decreasing leaf size with increasing altitude has also been found in *Fagus* (BIALOBRZESKA et al., 1988), *Metrosideros polymorpha* (GEESKE et al., 1994; CORDELL et al., 1998; CORDELL et al., 1999), and in various broad-leaved evergreen trees (TANG and OHSAWA, 1999). This investigation tried to determine if the morphological differences between *Ilex rugosa* specimens from eight Japanese mountains could segregate the specimens into the varieties accepted by YAMAZAKI (1987; 1989). That was not supported by this investigation.

A comparison of different taxonomic studies of the species *I. rugosa* shows that the taxonomic descriptions have changed in course of time. SCHMIDT described the species *I. rugosa* in 1868 (SCHMIDT, 1868), in 1936 some varieties of *I. rugosa* were described (IWATSUKI et al., 1999), which later on were described as forma (GALLE, 1997) and finally these forma-descriptions were not recognized at all in IWATSUKI et al. (1999).

This investigation found no morphological differences in terms of leaf size between the sexes of the plants. Plants of *I. rugosa* displayed various growth habits in the investigated areas, but we found no relationship between growth habit and geographical location. Internode length was found to differ significantly with geographic location, but neither latitude nor precipitation could explain this.

I. rugosa plants growing in Denmark for four years showed significantly larger leaves than those measured in Japan. Significant differences were found in leaf size and internode length for different Japanese native locations and for different Japanese native altitudes. This fact could indicate that the genotypes of the plants in Japan were suppressed by environmental factors, as the phenotypes grown under fixed climatic conditions in Denmark changed.

Further study would likely uncover additional aspects of this fascinating plant.

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References

BAUERS, B.M., 1986: *Ilex rugosa* F. Schmidt. Holly Society J. 4, 37.

BIALOBRZESKA, M., TRUCHANOWICZ, J., ZARZYCKI, K., 1988: Shape and size of leaves and fruits of four European and Japanese *Fagus* species. Veröff. Geobot. Inst. ETH, 98, 373-385. Stiftung Rübel, Zürich.

CORDELL, S., GOLDSTEIN, G., MUELLER-DOMBOIS, D., WEBB, D., VITOUSEK, P.M., 1998: Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. Oecologia 113, 188-196.

CORDELL, S., GOLDSTEIN, G., MEINZER, F.C., HANDLEY, L.L., 1999: Allocation of nitrogen and carbon in leaves of *Metrosideros polymorpha* regulates carboxylation capacity and $\delta^{13}C$ along an altitudinal gradient. Functional Ecology 13, 811-818.

CREMER-BACH, M., BACH, M., 1994: Morphologische und agronomische Merkmale autochthoner annueller *Medicago*-Arten in Marokko. J. Agronomy Crop Science 172, 81-89.

GALLE, F.C., 1997: *Hollies*. The genus *Ilex*. Timber Press, Portland, Oregon.

GEESKE, J., APLET, G., VITOUSEK, P.M., 1994: Leaf morphology along environmental gradients in Hawaiian *Metrosideros polymorpha*. Biotropica 26, 17-22.

HUXLEY, A., 1999: Dictionary of Gardening 2, D to K. The New Royal Horticultural Society. Macmillan Reference Ltd., London.

IWATSUKI, K., BOUFFORD, D.E., OHBA, H., 1999: Flora of Japan, Volume IIc. Angiospermae, Dicotyledoneae, Archichlamydeae(c). Kodansha Ltd., Tokyo, Japan.

KITAMURA, S., OKAMOTO, S., 1972: Coloured illustrations of trees and shrubs of Japan. Hoikusha Publishing Co., Ltd. Osaka, Japan.

NITTA, T., 1993: Climatic Atlas of Japan 1961-1990. Japan Meteorological Agency, Tokyo.

OHWI, J., 1965: Flora of Japan. Smithsoniana Institution, Washington, D.C.

OHWI, J., 1984: Flora of Japan. Smithsoniana Institution, Washington, D.C.

SAS INSTITUTE, 1999: SAS Systems for Windows 8.02. SAS Institute, Inc., Cary, NC, USA.

SCHMIDT, FR., 1868: Reisen im Amur-Lande und auf der Insel Sachalin, im auftrage der Kaiserlich-Russischen Geographischen Gesellschaft ausgefuehrt. Mémoires de l'Académie Impériale des Sciences de St.-Pétersbourg, VII[E] Série. Tome XII, No 2. Commissionnaires de l'Académie Impériale des sciences, St.-Pétersbourg.

TANG, C.Q., OHSAWA, M., 1999: Altitudinal distribution of evergreen broad-leaved trees and their leaf-size pattern on a humid subtropical mountain, Mt. Emei, Sichuan, China. Plant Ecology 145, 221-233.

TATEHIRA, R., 1991: Climatic Table of Japan 1961-1990. Vol. 1. Japan Meteorological Agency, Tokyo.

YAMAZAKI, T., 1987: On *Ilex rugosa* Fr. Schm. The J. Japanese Botany 62, 144. (In Japanese, with English abstract).

YAMAZAKI, T., 1989: *Ilex rugosa* Fr. Schm. In: Satake, Y., Hara, H., Watari, S., Tominari, T., (eds.), Wild Flowers of Japan, Woody Plants, 31. Heibonsha Ltd., Publishers, Tokyo (In Japanese).

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