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# Morphological Characteristics of Leaves, Trichomes and acorns in artificial hybrids of *Quercus aliena* × *Q. mongolica* var. *crispula* and *Q. serrata* × *Q. mongolica* var. *crispula* hybrids

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**Abstract -** Morphological characteristics of leaves, trichomes and acorns were investigated in 6-year-old artificial hybrids of *Q. aliena* × *Q. mongolica* var. *crispula* and *Q. serrata* × *Q. mongolica* var. *crispula*. Leaf shapes of *Q. aliena* × *Q. mongolica* var. *crispula* $F_1$ were obovate and resembled to that of *Q. aliena*. But several characters including the size of leaf and petiole and the shape of leaf base resembled to those of *Q. mongolica* var. *crispula*. In $F_1$ hybrids, small stellate hairs distributed sparsely on the abaxial surface and their lay length was intermediate between both parents. There were no big differences on characters of nuts and cupules between both parents and $F_1$ hybrids. Leaf shapes of *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ were obovate-elliptic, and the leaf shape and leaf base and the length of petiole resembled to those of *Q. mongolica* var. *crispula*, but leaf size and serration resembled to those of *Q. serrata*. The number of serration in a leaf was intermediate between both parents. Small stellate hairs distributed sparsely and large single hairs were mixed on the reverse side of leaves. there were no big differences on the number and size of stellate hairs between $F_1$ hybrid and *Q. serrata*. It is able to distinguish $F_1$ hybrids from both parents by the size leaf size and shapes, leaf base and serration, petiole length and trichome type in the leaf.

**Key words -** *trichome type*, *interspecific hybrid. Quercus aliena, Q. mongolica* var. *crispula*, *Q. serrata*

## Introduction

Natural hybrids of *Q. serrata* and *Q. mongolica* var. *crispula* is named as *Quercus* × *serratoides* Uyeki var. *mizukonara* Kitam., distributed throughout Honshu and Kyushu in Japan, and there is a place that *Q. serrata* upon *Q. mongolica* var. *crispula* are distributed(Kitamura and Murata, 1979). There is *Q.* × *alieno-crispula* H.Ohba regarded as natural hybrids of *Quercus aliena* and *Q. mongolica* var. *crispula*, and this is distributed in Honshu (Satake et al, 1989). Okada et al.(1994) tried to classify species by type of leaf trichome on the abaxial surface in mixed forest of *Quercus* species in the Tottori University Forests Agriculture in Hiruzen district. They reported that there were, in addition to 3 species of trees such as *Q. mongolica* var. *crispula*, *Q. serrata*, and *Quercus dentata*, *Quercus* × *serratoides*, *Q. anguste-lepidota* and *Q. takatorensis* regarded as hybrids of these by 8~9% respectively in this stand. It is easy to classify *Q. mongolica* var. *crispula*, *Q. serrata*, and *Quercus dentata* by the size and form of leaves, the form of serration, and the length of petiole, but it is difficult to decide what species hybrids are from only by the form of leaves and serration in case of intermediate individual regarded as hybrids of these species. However, if this is investigated by adding leaf trichome type on the abaxial surface and the character of cupules to the form of leaves, it is possible to decide what species are hybridized. Authors hybridized *Quercus* species artificially to clarify whether intermediate individual growing naturally in the *Quercus* forest in the Tottori University Forests Agriculture in Hiruzen district was natural hybrids or not(1994).

It is expected to report the results of investigating morphological characteristics of individual leaves, leaf trichome type, and acorns fructified in *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ and *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ in 2001 from among artificial hybrids of *Quercus aliena* × *Q. mongolica* var. *crispula* and *Q. serrata* × *Q. mongolica* var. *crispula* that had been cultivated since they were hybridized in 1994.

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Table 1. Information charateristics of investigated sample

| Parents and $F_1$ Hybrids | No. of investigated tree | Age(year) | D.B.H(cm) | Height(m) |
|---|---|---|---|---|
| *Q. aliena* | 1 | 20 | 12 | 10 |
| *Q. serrata* | 1 | 20 | 16 | 8 |
| *Q. mogoica* var. *crispula* | 1 | 20 | 10 | 8 |
| *Q. aliena* × *Q. mogolica* var. *crispula* $F_1$ | 6 | 6 | (2.6~5.0) | 1.4~3.2 |
| *Q. serrata* × *Q. mogolica* var. *crispula* $F_1$ | 4 | 6 | (3.1~3.5) | 1.6~2.6 |

## Materials and Methods

### Materials

Artificial hybrids test was conducted for 4 years from 1991~1994 with *Quercus dentata*, *Q. aliena*, and *Q. serrata* planted in test place of department of agriculture at Tottori University to make interspecific hybrids, and the result is shown in Table 1. A seed tree and a pollen tree used for artificial hybrids are *Quercus aliena* 5, *Q. serrata* 9, and *Q. mongolica* var. *crispula* 1, and they were 20-year-old at that moment(14 years old at the time of artificial hybrids). An external form, namely the form of leaves, serration, leaf trichome type, nut, and cupule shows the characteristics of each of seed tree and pollen tree well. Combination of *Quercus aliena* × *Q. mongolica* var. *crispula* and *Q. serrata* × *Q. mongolica* var. *crispula* was used in interspecific hybrids. However, inverse hybrids were not carried out because *Q. mongolica* var. *crispula* from among 3 species had too fast flowering time. Although more than 250 female flowers were hybridized with combination of 2 seed trees, the rate of fruit-bearing was low, and also there was a damage by harmful insects even after seeding. According to this, 6 individuals from *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$, and 4 individuals from *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ were cultivated respectively. The diameter of root of 6 year-old individuals was 2.6-5.0cm, and its height was 1.4-3.2m. Leaves and fruits were gathered on September and October respectively to investigate the characteristics.

### Methods

#### Measurement of the characteristics of leaf

The author gathered 10 normally grown leaves each from one individual, and then copied them by copy machine. Blade length (L), maximum leaf width (W), petiole length (T), leaf area and the number of serration of this copy were measured to calculate the aspect ratio (L/W), the petiole rate (T/L × 100, %), and the density of serration (the number of serration per 10 cm of leaf length). Also the form of serration, leaf apices, and leaf base were recorded.

#### Measurement of leaf trichomes type

The author cut the center part of 30 ripe leaves, put on slide glass by turning the underside of leaf upward, and took a picture with microscope. The author measured the number of stellate hairs, the length of strands(20~50 strands each), and the length of large single hairs(10 strands each) with the image data.

* The number of stellate hairs and the length of large single hairs were measured on 20 strands of 1 individual. The longest strand of stellate hairs from among one image data was measured, and 50~100 strands each from one individual were measured.*

That is to say, the longest strand among stellate hairs was measured.

#### Measurement of fruits

The author measured the length projected externally with the height and the diameter of nut and cupule, the diameter of hilum, the length of involucre bract, and margin of involucre, and calculated the aspect ratio(height/diameter) and diameter ratio of hilum(diameter of hilum/diameter of nut). Namely, the length of involucre bract and the longest acorn scale of rementum on the center of cupule were measured.

## Results

### Leaf characteristics of interspecific hybrids

The leaf shapes of both parents and interspecific hybrids is shown in Fig. 1 and 2. Leaves of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ are obovate, leaf apex is obtuse, and the leaf base is auriculate. The form of lamina resembles to that of

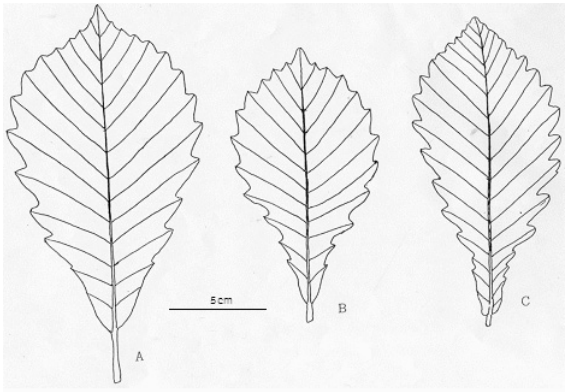


Fig. 1. Comparison of leaf morphology in parents and *Q. aliena* × *Q. mongolica* var. *crispula* $F_1$. ( A; mother tree , B; *Q. aliena* × *Q. mongolica* var. *crispula* $F_1$, C; Pollen tree )

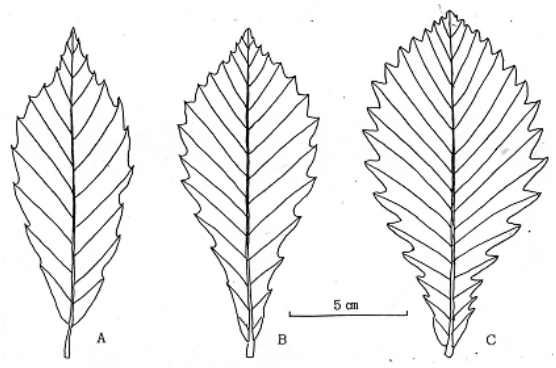


Fig. 2. Comparison of leaf morphology in parents and *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$. ( A; mother tree , B; *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$, C; Pollen tree )

Table 2. Variation of leaf characteristics in interspecific hybrids and parents

| Parents and $F_1$ Hybrids | No. of trees investigated | Blade length (cm) | Blade width (cm) | Leaf area ($cm^2$) | Petiole length (cm) | No. of serrations | Blade ratio (L/W) | Petiole length percentage (%) | Density of serrations (no./10 $cm^2$) | Type of serration | Leaf apex |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Q. aliena* No.5 | 1 | 19.8±2.31 | 10.2±1.27 | 121.7±13.5 | 2.9±0.39 | 9.8±0.35 | 1.94±0.08 | 14.6±1.06 | 4.9±0.14 | medium, lobed | acute |
| *Q. serrata* No.9 | 1 | 11.9±2.12 | 4.3±0.74 | 32.7±8.09 | 1.5±0.43 | 11.0±0.18 | 2.77±0.35 | 12.2±1.21 | 9.3±0.70 | small, serrate | aristulate |
| *Q. mogolica* var. *cripula* No.1 | 1 | 13.5±1.27 | 5.7±0.07 | 45.5±4.31 | 0.3±0.14 | 13.5±1.98 | 2.37±0.22 | 2.2±1.14 | 10.0±1.01 | medium, lobed | acute, acuminate |
| *Q. aliena* × *Q. mogolica* var. *crispula* $F_1$ | 6 | 12.3±1.72 | 6.4±0.80 | 45.3±6.07 | 0.6±0.23 | 11.1±1.17 | 1.92±0.15 | 5.0±1.65 | 9.1±1.21 | medium, lobed | obtuse |
| *Q. serrata* × *Q. mogolica* var. *crispula* $F_1$ | 4 | 10.2±0.31 | 4.7±0.06 | 27.7±2.25 | 0.6±0.10 | 12.4±0.35 | 2.19±0.13 | 6.2±1.16 | 12.2±0.33 | small to medium, serrate | subserrate, obtuse |

*Quercus aliena*, but the form of leaf base is similar to *Q. mongolica* var. *crispula*. Serration is weak triangle phased, dug part was low, and the head was obtuse.

Leaf of *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ is obovate-oblong, the apex is acute, the base is auriculate, petiole is short, the form of leaf resembles to that of *Q. mongolica* var. *crispula* rather than that of *Q. serrata*. Serration is triangle phased, bigger than *Q. serrata*, and the head has tendency to be sharp spot phased.

The result of measuring the characteristics of leaves of both parents and interspecific hybrids is shown in Table 2, and the correlation between each characteristic is shown in Fig. 3, 4, and 5. The leaf length of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ was average 12 cm, and the leaf area was similar to *Q. mongolica* var. *crispula*. Its leaf width was wider than that of *Q. mongolica* var. *crispula*, and the numerical value of the aspect ratio of leaf was almost similar to *Quercus aliena* (Fig. 3). The leaf length of *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ was about 10 cm, the leaf area was smaller than that of *Q. mongolica* var. *crispula*, and the size of leaf was similar to *Q. serrata*. However, the leaf width was wider than that of *Q. serrata*, and the numerical value of the aspect ratio of leaf was close to *Q. mongolica* var. *crispula*(Fig. 3).

Petiole of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ and *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ was shorter than that of *Q. serrata* and *Q. aliena*, and the numerical value of the petiole rate was also low(Fig. 4). The number of serration of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ was intermediate between both parents, but *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ had a great width of transition between

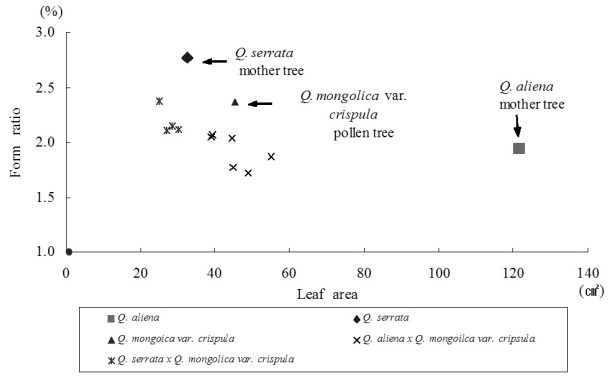


Fig. 3. Comparison of leaf size and form ratio in parents and interspecific hybrids.

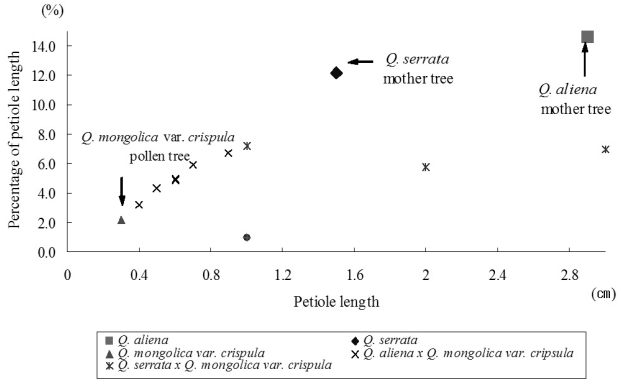


Fig. 4. Comparison of petiole length and petiole length percentage in parents and interspecific hybrids.

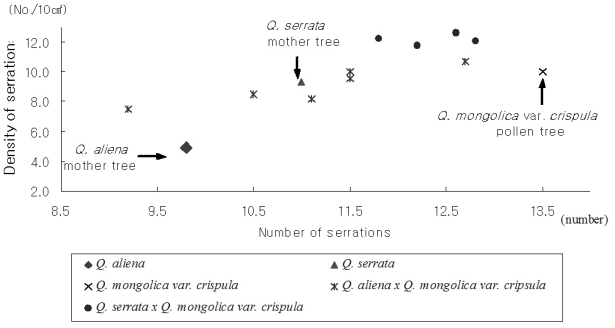


Fig. 5. Comparison of number of serrations and density of serrations in parents and interspecific hybrids.

individuals, so there were an individual intermediate between both parents and others close to a seed tree or pollen tree (Fig. 5).

As the result of the serration density, *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ was higher than both parents, and *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ had many individuals close to *Q. mongolica* var. *crispula*(Fig. 5).

In characteristics of leaf of interspecific hybrids of 2 combinations, the length and shape leaf of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ resemble to *Q. aliena*, but the size, the form of leaf base, and the length of petiole resemble to *Q. mongolica* var. *crispula*. The form of leaf and the length of petiole of *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ resembled to *Q. mongolica* var. *crispula*, but the size of leaf was as big as *Q. serrata*, and the number of serration was intermediate between both parents. Namely, there are leaves that resemble to a seed tree, leaves that resemble to pollen tree, and leaves intermediate between both parents in the characteristics of leaves of interspecific hybrids, the size of leaf had tendency to resemble to one of parent that had smaller size of leaf, and the length of petiole had tendency to resemble to one of parent that had shorter length of petiole.

### Leaf trichomes of interspecific hybrids

The comparison of leaf trichome type between both parents and interspecific hybrids is shown in Table 3. Small stellate hairs which have many strands get thick in *Q. aliena*, but there are no large single hairs. Small stellate hairs get thick in *Q. serrata*, and small number of large single hairs are distributed. There is almost no leaf trichome type or only a few small stellate hairs in *Q. mongolica* var. *crispula*. Three species of tree can be distinguished by the density, the number of strands, and the strand length of stellate hairs, and the existence of large single hairs.

There was only a few small stellate hairs in *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$, but there were no large single hairs. The density of stellate hairs was considerably less than Quercus aliena by 2,000 strands/$cm^2$ or less, but the number of strands were average 6.3, and the large number of them were intermediate between *Quercus aliena* and *Q. mongolica* var. *crispula*. Small stellate hairs and large single hairs existed together in *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$. The density of stellate hairs was much less than *Q. serrata* by average 665 strands/$cm^2$, and close to the numerical value of *Q. mongolica* var. *crispula*. The number of strands was average 4.4, and the length of strand was average 113 μm, *Q. serrata*, so there was a big difference from *Q. mongolica* var. *crispula*.

The transition of the number of strands of stellate hairs of Quercus aliena × *Q. mongolica* var. *crispula* $F_1$ was compared with both parents (Fig. 6). *Quercus aliena* 5, a seed tree, had

Table 3. Comparison of trichomes in interspecific hybrids and parents

| Parents and $F_1$ Hybrids | No. of investigated tree | Stellate hair | | | Long hair | |
|---|---|---|---|---|---|---|
| | | Length of ray (μm) | Number of ray | Density (no./cm$^2$) | Length (μm) | Density (no./cm$^2$) |
| *Q. aliena* | 1 | 160±8.35 | 8.9±1.0 | 9,121±526.3 | - | - |
| *Q. serrata* | 1 | 107±11.5 | 4.5±0.7 | 7,054±728.5 | 350±12.4 | 288±10.5 |
| *Q. mogolica* var. 00000 | 1 | 129±13.2 | 4.2±1.0 | 280±11.4 | - | - |
| *Q. aliena* × *Q. mogolica* var. *crispula* $F_1$ | 6 | 91±9.82 | 6.3±1.38 | 695±16.3 | - | - |
| *Q. serrata* × *Q. mogolica* var. *crispula* $F_1$ | 4 | 113±11.31 | 4.4±0.9 | 665±15.7 | 420±13.1 | 509±11.5 |

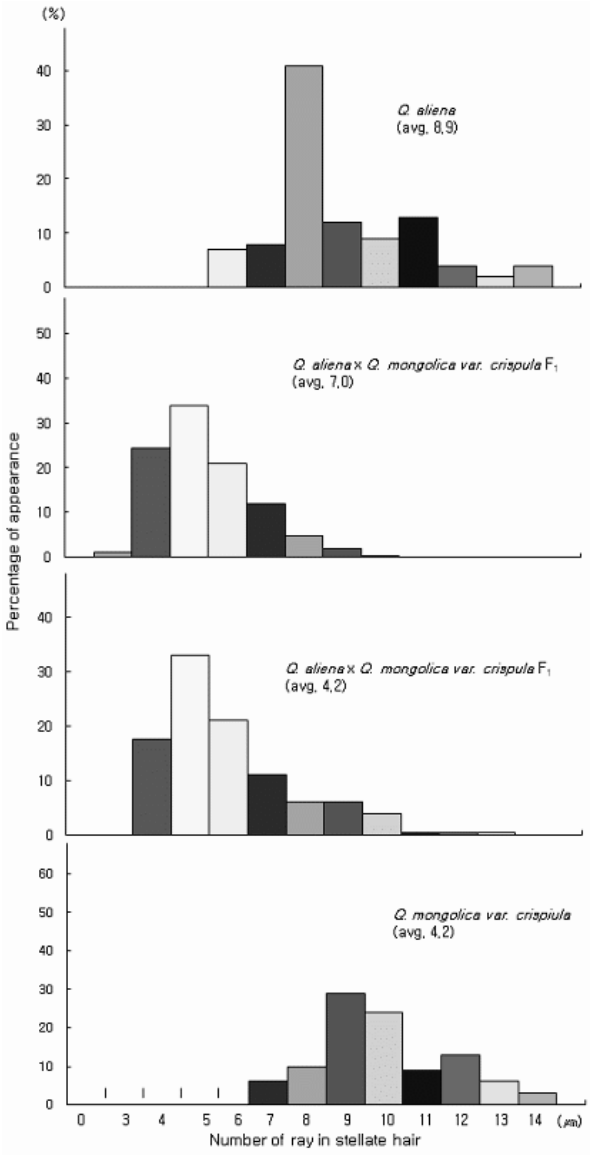


Fig. 6. Variation of length of ray in stellate hair of *Q. dentata*, *Q. serrata* and their interspecific hybrids

8.9 strands in average of stellate hairs, the number of strands of stellate hairs was between 6 and 14, an individual having 8 strands was the most. *Q. mongolica* var. *crispula*, a pollen tree, had 3~7 strands of stellate hairs, and an individual having 4 strands was the most. An individual having 7~8 strands in average was the most in 4 individuals from among 6 individuals of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$. However the number of strands of stellate hairs was average 4.9 in other 2 individuals, and an individual having 4 strands was the most. As the result of investigating the number of strands of stellate hairs in hybrids, there were a type close to *Quercus aliena*, and a type close to *Q. mongolica* var. *crispula*.

The characteristics of fruits of interspecific hybrids

The characteristics of fruits and cupules of both parents and *Q. aliena* × *Q. mogolica* var *crispula* $F_1$ are shown in Fig. 7.

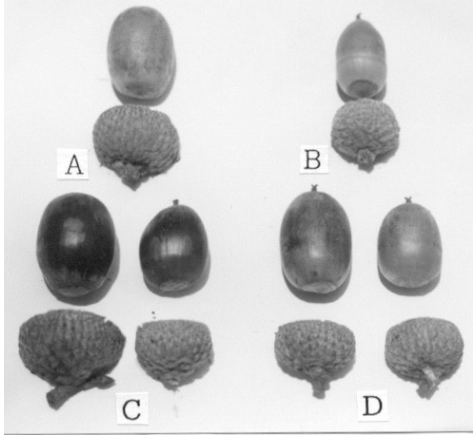


Fig.7. Morphology of nut and cupule in partens and *Q. aliena* × *Q. mongolica* var. *cripula* $F_1$. (A: mother tree, B: pollen tree, C, D: Q. aliena × *Q. mongolica* var. *crispula* $F_1$)

The fruits of *Quercus aliena* 5, a seed tree, was rectangular, and the aspect ratio was 1.31, but the fruits of *Q. mongolica* var. *crispula* 1, a pollen tree, was oval, and the aspect ratio was 1.39. Fruits of $F_1$ were egg shaped~oval. Cupules of both parents and $F_1$ were bowl shaped, the height and the diameter of $F_1$ were little bit smaller than *Quercus aliena*. The diameter of calyx of nut base was smaller than that of both parents, and the length of involucre was shorter than that of both parents, and almost not came out externally from margin of cupules. The back of involucre was came out like a wen, and fat in case of *Q. mongolica* var. *crispula*, but was not shown in case of *Quercus aliena* and $F_1$. There was no difference on the characteristics of fruits and cupules between *Quercus aliena* and *Q. mongolica* var. *crispula*, and also there was no characteristics to characterize the hybrids of both.

## Discussion

Kitamura and Murata et al.(1979) called hybrids of *Q. mongolica* var. *crispula* and *Q. serrata* as Q. × *serratoides* Uyeki var. *mizukonara* Kitam, and found it at the place where *Q. serrata* and *Q. mongolica* var. *crispula* were distributed together. Sawatake et al. (1989) described that hybrids of both existed at the place where *Q. serrata* and *Q. mongolica* var. *crispula* were distributed together, (Mizukonara) *Q. crispuloserrata* (sugimoto) M. Kikuchi; called as *Q. serratoides* Uyeki var. *crispuloserrata* Sugimoto, and there is only a few of stellate hairs or nothing in young leaves. Through this article, it is impossible to distinguish which one from *Q. mongolica* var. *crispula* and *Q. serrata* is a seed tree or a pollen tree of *Q.* × *serratoides* Uyeki var. mizukonara kitam. As examined the flowering time of *Quercus* species in Quercus species forest (550-650 m above sea level) of the Tottori University Forests Agriculture in Hiruzen district where both species were distributed sparsely by Lee et al.(1996), for the flowering of male flower, *Q. mongolica* var. *crispula* was the fastest, and *Q. serrata* and Q. dentata were followed in order, but an individul with slow flowering time from among *Q. mongolica* var. *crispula* and an individual with fast flowering time from among *Q. serrata* and Q. *dentata* were duplicated. Accordingly in case of *Quercus* species, a female flower is bloomed earlier than a male flower by a couple of days, so *Q.* × *serratoides* Uyeki var. *mizukonara* Kitam has the higher possibility that a pollen of *Q. mongolica* var. *crispula* is pollunated on a female flower of *Q. serrata*.

Leaf of *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$ is obovate-oblong, wider than that of *Q. serrata*, and also the base is auriculate, the petiole is short, and the form of leaf resembles to *Q. mongolica* var. *crispula*. However the serration is smaller than that of *Q. mongolica* var. *crispula*, and similar to *Q. serrata*, for example the head is sharp spot-phased. That is to say, the characteristics of both parents were mixed. There are small number of stellate hairs almost same as *Q. serrata* in mesophill on the abaxial surface, and there are large single hairs together upon it. Small stellate hairs get thick, and large single hairs get sparsely in *Q. serrata*, but there are few of stellate hairs, and almost no large single hairs in *Q. mongolica* var. *crispula*. As seen that small stellate hairs are distributed sparsely in *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$, it is considered that it received the characteristics of *Q. mongolica* var. *crispula*, and as seen that there are large single hairs, it is considered that it received the characteristics of *Q. serrata*.

*Quercus* × *alienocrispula* H. Ohba(Satake et al., 1989); *Q. paucilepis* Uyeki var. *naramizugashiwa* Kitam. et T. Horik is considered as hybrids of *Q. mongolica* var. *crispula* and *Q. aliena*, and it is seldom found in Honshu (Kinki district). The flowering time of *Q. aliena* in Tottory city is almost same as that of *Q. serrata*, but later than that *Q. mongolica* var. *crispula* by couple of days(Hasizume et al., 1994). The flowering of *Quercus* spp. is protogynous, a female flower started to bloom earlier than a male flower by couple of days, so *Q. naramizugashiwa* has high possibility that a pollen of *Q. mongolica* var. *crispula* can be pollinated on *Quercus aliena*. Leaf of *Quercus aliena* × *Q. mongolica* var. *crispula* $F_1$ is obovate, wider than that of *Q. mongolica* var. *crispula*, and the form of leaf resembles to *Quercus aliena*. However the size of leaf is similar to *Q. mongolica* var. *crispula*, the base of the leaf length is auriculate, petiole is short, and these characteristics are similar to *Q. mongolica* var. *crispula*. Leaf trichome type on the underside of leaves have small stellate hairs distributed sparsely, presents the characteristics close to *Q. mongolica* var. *crispula*, but *Quercus aliena* type of individual having the large number of strands of small stellate hairs(more than 8 strands) get sparsely depending on individuals, and the density and form of stellate hairs presents mutual character of both

parents. Leaf trichome type is an important standard to classify species of *Quercus spp*.. As the result that authors selected 10 individuals each from each species of tree in natural forest to examine, for the density of stellate hairs, *Q. serrata* was 8,141, *Q. mongolica* var. *crispula* was 101, and Quercus aliena was 10,071(strands/$cm^2$) respectively. For the number of strands of stellate hairs, *Q. serrata* was 4.6, *Q. mongolica* var. *crispula* was 4.9, and Quercus aliena was 78.2(strands) respectively. For the strand length of stellate hairs, *Q. serrata* was 114, *Q. mongolica* var. *crispula* was 127, and *Quercus aliena* was 158 (μm). There are almost no or only a few of stellate hairs in *Q. mongolica* var. *crispula*, and stellate hairs get thick in *Quercus aliena*, the number of strands are great, so they can be distinguished each other. *Q. serrata* is classified because small stellate hairs and large single hairs get thick. Small stellate hairs are distributed get sparsely in *Q. aliena* × *Q. mongolica* var. *crispula* $F_1$, so the density is similar to *Q. mongolica* var. *crispula*, there is an individual having strands more than 8 that *Q. aliena* type of stellate hairs get sparsely, and this individual is considered as hybrids when being decided as leaf trichome type. There are sparsely small stellate hairs having very few strands in *Q. serrata* × *Q. mongolica* var. *crispula* $F_1$, and large single hairs get sparsely, so it can be distinguished from *Q. aliena* and *Q. serrata*.

It is difficult to decide the species with the size and the form of acorn and cupules, because there is a big difference between individuals even in same species. The shape of cupule rementum is genetically stable characteristics, and can be used as the base of classifying species(Kadomathu, 1992). However involucre of *Q. aliena*, *Q. serrata*, and *Q. mongolica* var. *crispula* is pressured, and there is no difference on the length, so it is not enough to make it as the base of classifying interspecific hybrids of these 3 species of tree. It is reported that the length of involucre of $F_1$ presented the intermediate form of both parents in interspecific hybrids of *Q. dentata* involucre, *Q. mongolica* var. *crispula* × *Q. dentata* $F_1$ (Ubukata, 1996). It is necessary to synthesize the characteristics of leaf, leaf trichome type, and rementum of cupules to decide the hybrids of *Quercus* species.

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