

Morphological characteristics of leaves and trichomes in reciprocal hybrids of *Quercus dentata* and *Q. serrata*

Jeong-Ho Lee*

Korea Forest Seed & Variety Center, Chungju 380-941, Korea

ABSTRACT: The morphological characteristics of leaves, trichomes, and acorns in 8- to 11-year-old reciprocal hybrids of *Quercus dentata* and *Q. serrata* were investigated. The reciprocal hybrids had obovate to obovate-elliptic leaves that were smaller than those of *Q. dentata* but similar to *Q. serrata* in their leaf size, obtuse leaf apex, narrow-tipped leaf base, and shape of small to intermediate-sized serrations. The ray length of stellate hairs was intermediate between those of the parents, and the hairs showed dense growth. Long single hairs are present among the stellate hairs on the abaxial side of the leaves. Therefore, the hybrids of *Q. dentata* and *Q. serrata* could be identified by the size and shape of leaves, shape of serrations, and type of leaf trichomes.

Keywords: Interspecific hybrid, leaf trichome, *Quercus dentata*, *Quercus serrata*.

Introduction

Hybridization and introgression are common phenomena in many plants and important processes in evolution. Interspecific gene flow has received much attention, not only as a potential source of genetic variation in plant populations, but also for its implications in the speciation process (Anderson, 1949; Stebbins, 1969; Rieseberg and Brunfeldt, 1992; Avise, 1994).

Since Darwin (1859), the genus *Quercus* has attracted the attention of evolutionists for its very poor reproductive barriers between species (Rushton, 1993). Many oak species grow in mixed stands over large sympatric zones. In those mixed populations, hybridization appears to be a common phenomenon (Rushton, 1993). However, with the exception of a small proportion, most oak species remain discernible even in conditions of sympatry and hybridization.

In mixed stands of *Q. dentata*, *Q. serrata*, and *Q. mongolica* var. \times *crispula*, investigation of leaf and acorn types revealed high frequencies of individuals with intermediate characteristics (Somego et al., 1986; Toyoda et al., 1986; Miyazaki, 1988, 1989; Okada et al., 1994, 1995). Hashizume et al. (1994a) conducted artificial crossing studies of *Quercus* spp. and carried out an analysis to prove whether individuals with intermediate characteristics were natural hybrids.

Artificial crossings of *Q. serrata*, *Q. dentata*, and *Q. aliena* of the section *Prinus* can produce interspecific hybrids (Orita et al., 1991; Hashizume et al., 1994a). However, *Quercus* spp.

generally have low fructification percentages since many immature seeds drop after pollination (Suzuki et al., 1992; Hashizume et al., 1994a).

In this study, the leaves, trichomes, and acorns of 16 individuals produced by artificial hybridization between *Q. dentata* and *Q. serrata* were investigated if the interspecific hybrids can be identified by those characteristics.

Materials and Methods

1. Materials

From 1991 to 1994, interspecific hybrids were obtained by artificial crossing of *Q. dentata* and *Q. serrata* that had been planted in the Hiruzen experimental forest of Tottori University. Table 1 provides data on the trees. The mother tree and the pollen trees used for artificial crossing were individuals of *Q. dentata* No. 2 and *Q. serrata* No. 9 aged 24 years in 2003. The species of each individual was determined by morphological features such as leaf form, serration, trichome, acorn, and bark. The diameter at breast height (DBH) of the 24-year-old mother and pollen trees were 15 cm and 18 cm, respectively. The interspecific hybrids studied were between 10 and 13 years old in 2003 and had stem base diameter of 0.9 to 2.7 cm and heights of 0.6 to 1.6 m. Sample leaves were picked from each individual in May 2003. The characteristics of *Q. dentata* were: Leaves are larger and obovate, from thick large stellate hair to a little large stellate hair, there is no long hair in *Q. dentata*. The characteristics of *Q. serrata*: Leaves are smaller and oblong, obovate-oblong, thick small stellate hair and a little long hair.

*Author for correspondence: mtmac@forest.go.kr

Table 1. Information for the samples studied.

Parents and F ₁ Hybrids	No. of trees investigated	Age (years)	DBH (cm)	Height (m)
<i>Q. dentata</i> (No. 2)	1	24	18	11
<i>Q. serrata</i> (No. 9)	1	24	15	7
<i>Q. dentata</i> (No. 2) × <i>Q. serrata</i> (No. 9) F ₁	6	10, 12, 13	(1.4~2.7)*	0.8~1.6
<i>Q. serrata</i> (No. 9) × <i>Q. dentata</i> (No. 2) F ₁	5	10, 11	(0.9~1.9)	0.6~1.0

*A diameter of a stem was measured at stem base

2. Methods

1) Artificial crossing

• Inspection of flow blooming : It is marked 5 branches with buds respectively, observed and recorded flower blooming on a daily basis.

• Pollen collection : Before a male flower blooms, the flower is picked dried on a piece of paper and then selected pollen by using mesh with 0.1 mm density.

• Storage of pollen : After drying selected pollen inside for one day, you may put the pollen into 3 cm wide vial and then put into desiccator with silica Gel and store it at 2 or below zero 2 Celsius degree.

• Germination test of pollen : A culture ground is made with a portion of the following density(1% of agar to 20% of sugar), pouring the liquid culture into a 2 cm wide and 4 cm height bottle. It is spread the pollen and plug the bottle by using a cork. Germination rates are inspected after cultivating it at 25°C within 24 to 28 hours.

• Artificial crossing

- To mating bag : The branch with many female flowers is selected and then cover the branch with a crossing mating bag. The mating bag (20 cm wide and 40 cm height) after removing male flowers. The mating bag is covered right before a flow blooms. As there are difference in the time of flowering depending on species, *Q. serrata* is pollinated from April 18th to April 28th, from May 6th to 9th. for *Q. dentata*.

- Controlled pollination : We pollinated on stigma by using a small brush. We conducted inter species hybrid of an controlled pollination before the flower of parents tree blooms. We pollinated *Q. serrata* from April 23th to May 3rd, from May 9th to 14th for *Q. dentata*.

• Inspection on Acorn & Collection of the Seed : Before uncovering the envelope, the number of acorn is counted and then the screen is covered in the early of September to prevent the falling of acorn afterwards. Mature acorns are gathered from the end of September to the early of October.

2) Measurement of leaf characteristics

Ten normal leaves from each individual were copied on a photocopy machine. Blade length (L), blade width (W), petiole length (T), leaf area, and serration were measured from the

leaf copy. Using these measurements, the blade form ratio (L/W), petiole length ratio (T/L × 100%), and serration density (number of serrations/10 cm²) were calculated. The types of serration, leaf apex, and leaf base were also studied. Raw data matrices were analyzed by the principal component analysis (PCA) function of the SAS program.

2) Measurement of trichomes

Thirty mature leaves were incised at their centers. They were then attached to a slide glass, and images were taken with a microscope. Using these images, the density of stellate hairs (number of hairs/cm²), the number and length of rays, and the density and length of long hairs were measured. The number of stellate hairs and the length of long hairs were measured on 20 leaves of each sample tree. In each leaf, 50 to 100 stellate hairs were measured, and the length of the longest stellate hair was determined.

Results

1. Leaf characteristics of interspecific hybrids

Figs. 1 and 2 present comparisons of the leaf characteristics. The leaves of *Q. dentata* were larger in size and obovate to oblong-obovate in shape, with round leaf apices and auriculate

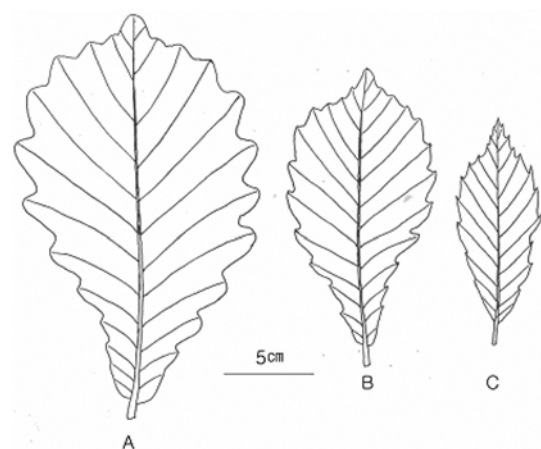


Fig. 1. Comparison of leaf morphology in *Q. dentata*, *Q. serrata*, and *Q. dentata* × *Q. serrata* (A: mother tree of *Q. dentata* No. 2; B: *Q. dentata* × *Q. serrata* F₁; C: pollen tree of *Q. serrata* No. 9).

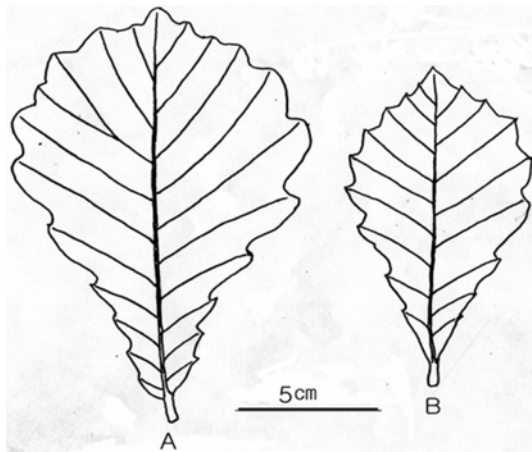


Fig. 2. Comparison of two types in *Q. serrata* × *Q. dentata* (A: *Q. dentata* type; B: *Q. fabri* type)

leaf bases. The serration was crenate and large, and the apex was round (Fig. 1-A). The leaves of *Q. serrata* were smaller in size and oblong to obovate-oblong. The leaf apex was sharp, and the leaf base was obtuse. The serration was small and sharp (Fig. 1-C). The leaves of F₁ hybrids of *Q. dentata* × *Q. serrata* were obovate to oblong-obovate, and many leaves were smaller than *Q. dentata* leaves but similar in size to *Q. serrata* leaves. The leaf apex was obtuse, and the leaf base was cuneate. The splitting of the leaf margin was deeper than in *Q. dentata*, and the serrations were small to medium in size with sharp ends. Many leaves had serratus margins (Fig. 1-B).

The leaves of *Q. serrata* × *Q. dentata* F₁ were smaller than those of *Q. dentata*, but many were similar in size to *Q. serrata* leaves. The leaves were obovate in shape, with an obtuse or round leaf apex and cuneate leaf base. The leaf margin had deep splitting, and the serrations were small with sharp ends (Fig. 2-B). Interestingly, individual intermediate-sized serrations with round ends were similar to those of *Q. dentata*.

Table 2 lists the variations in leaf characteristics in the artificial hybrids and their parents. The leaf blade of the interspecific hybrids was smaller than that of the mother tree (*Q. dentata*). However, many leaves had intermediate sizes

compared to those of their parents. Fig. 3 depicts the relationship of the leaf area and the form ratio. The interspecific hybrids had smaller leaves than *Q. dentata*, but many leaves were similar in size to those of *Q. serrata*, particularly for the family of *Q. serrata* × *Q. dentata* with the exception of one individual. The interspecific hybrid of *Q. dentata* and *Q. serrata* had a leaf form ratio intermediate to that of the parents.

The petiole length of the interspecific hybrids was shorter than that of *Q. serrata*, while the petiole length percentage was similar to that of *Q. dentata* (Table 2, Fig. 4). In both the *Q.*

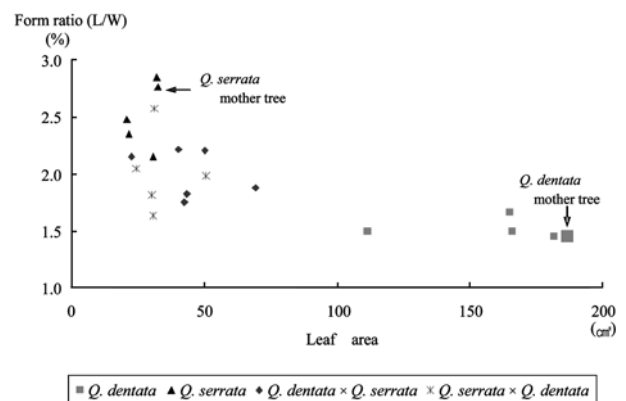


Fig. 3. Comparison of leaf size and form ratio in parents and interspecific hybrids.

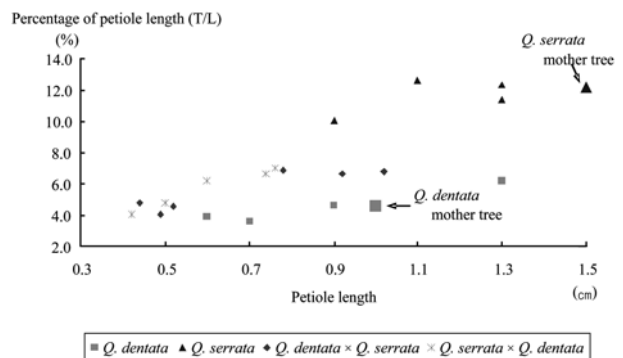


Fig. 4. Comparison of petiole length and petiole length percentage in parents and interspecific hybrids.

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

Parents and F ₁ Hybrids	Blade length L (cm)	Blade width W (cm)	Leaf area (cm ²)	Petiole length (cm)	No. of serrations	Blade form ratio (L/W)	Petiole length percentage (%)	Density of serrations (number/10 cm ²)	Serration pattern	Leaf apex
<i>Q. dentata</i> (No. 2)	21.1 ± 1.90	14.6 ± 1.98	186.3 ± 21.1	1.0 ± 0.24	8.6 ± 1.12	1.47 ± 0.14	4.6 ± 0.75	4.1 ± 0.7	big, repand	round
<i>Q. serrata</i> (No. 9)	11.9 ± 2.12	4.4 ± 0.74	32.7 ± 8.09	1.5 ± 0.43	11.0 ± 0.18	2.77 ± 0.35	12.2 ± 1.21	9.3 ± 1.30	small, serrate	aristulate
<i>Q. dentata</i> × <i>Q. serrata</i> F ₁	12.2 ± 2.58	6.2 ± 1.11	44.9 ± 15.5	0.7 ± 2.15	9.0 ± 0.90	2.01 ± 0.19	5.6 ± 1.11	7.6 ± 1.89	small to medium, subserrate	acute
<i>Q. serrata</i> × <i>Q. dentata</i> F ₁	10.4 ± 1.51	5.4 ± 1.12	33.6 ± 8.56	0.6 ± 0.11	9.2 ± 0.52	2.01 ± 0.19	5.8 ± 1.14	9.0 ± 1.35	small to medium, subserrate, lobed	acute to round

dentata × *Q. serrata* and *Q. serrata* × *Q. dentata* families, the number of serrations was between that of the parents (Fig. 5). However, in most individuals, the number of serrations was similar to that of *Q. dentata* (Fig. 5). As a result of principal component analysis (PCA), *Q. dentata* × *Q. serrata* F₁ and *Q. serrata* × *Q. dentata* F₁ are closer than their parents (Fig. 7).

2. Leaf trichomes of interspecific hybrids

Table 3 provides a comparison of the trichomes, and Fig. 6 depicts variations in the ray length of stellate hairs. Interspecific hybrids had dense growth of long stellate hairs. However, long

stellate hairs were not observed in the *Q. dentata* mother tree. The ray length of the stellate hairs ranged from 250 to 650 μm (average 425 μm); most stellate hairs were 350–450 μm in length.

While the *Q. serrata* mother tree had denser coverage of small stellate hairs, long hairs were also present. The average length of stellate hair was 107 μm, with most stellate hairs less than 120 μm in length. In the artificial hybrid of *Q. dentata* and *Q. serrata*, there was thick coverage of intermediate-sized stellate hairs, and long hairs were also present. The ray lengths of the stellate hairs ranged from 115–370 μm (average 179 μm), with most ray lengths between those of *Q. dentata* and *Q. serrata*.

The average number of rays of the stellate hairs was 3.6 in *Q. dentata* and 4.5 in *Q. serrata*. This result reveals slight differences between the species. The density of the stellate hairs was lowest in *Q. dentata* and highest in *Q. serrata*.

Therefore, major differences among the leaf trichomes of *Q. dentata*, *Q. serrata*, and their hybrids were found in the ray length of stellate hairs and in the existence of simple hairs. Stellate hairs had an average ray length of 198 μm in the artificial hybrid of *Q. dentata* × *Q. serrata* and 224 μm in the family of *Q. dentata* × *Q. serrata*.

In the two hybrid families, the average ray length of the stellate hairs was approximately 200 μm (Fig. 6). Lengths of

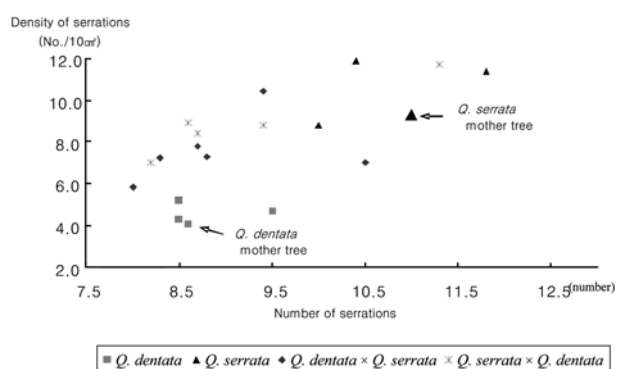


Fig. 5. Comparison of number of serrations and density of serrations in parents and interspecific hybrids.

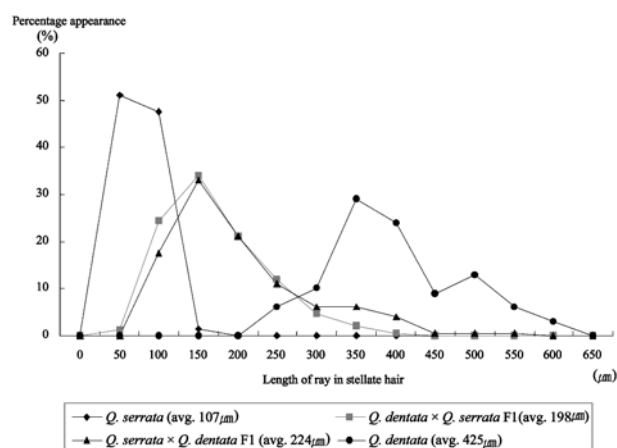


Fig. 6. Variations in ray length of stellate hairs of *Q. dentata*, *Q. serrata*, and their interspecific hybrids.

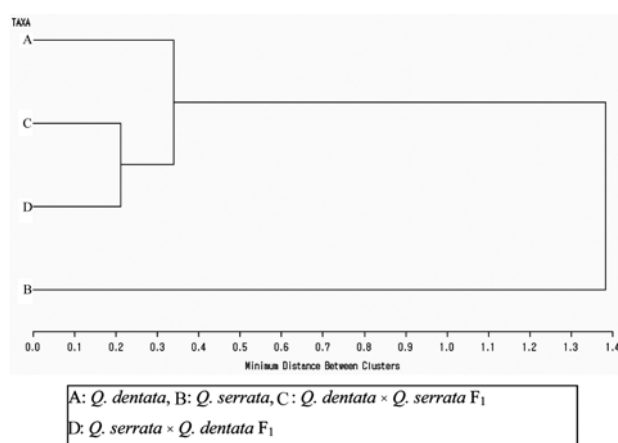


Fig. 7. Dendrogram of 110 individuals of *Quercus* spp. for the first and third principal components.

Table 3. Comparison of trichomes in interspecific hybrids and parents.

F ₁ Parents and Hybrids	Stellate hair			Long hair	
	Ray length (μm)	Number of rays	Density (number/cm ²)	Length (μm)	Density (number/cm ²)
<i>Q. dentata</i> (No. 2)	425 ± 17.2	3.6 ± 0.2	480 ± 48.6	-	-
<i>Q. serrata</i> (No. 9)	107 ± 14.5	4.5 ± 0.2	7054 ± 728.5	288 ± 19.8	350 ± 27.8
<i>Q. dentata</i> × <i>Q. serrata</i> F ₁	198 ± 7.7	2.9 ± 0.3	2330 ± 234.1	548 ± 67.74	231 ± 64.5
<i>Q. serrata</i> × <i>Q. dentata</i> F ₁	224 ± 31.2	2.7 ± 0.5	1611 ± 369.3	474 ± 22.43	411 ± 134.5

smallest stellate hairs were below 150 μm , and those of longest stellate hairs were below 300 μm ; these two types of stellate hairs appeared in a mixed pattern.

The stellate hairs of the interspecific hybrids of *Q. dentata* and *Q. serrata* had an average of 2.7-2.9 rays; long single hairs similar to those observed in *Q. serrata* were found in the interspecific hybrid of a mixed forest.

On the basis of the principal component analysis, individuals were grouped in four groups by the main components (1 and 3) making high contributions: *Q. dentata*, *Q. serrata*, *Q. dentata* \times *Q. serrata* F_1 , and *Q. serrata* \times *Q. dentata* F_1 (Fig. 7).

Discussion

Hardin (1975) observed that gene exchange occurs, or at least has the potential to occur, among nearly all species of the subgenus *Quercus* in eastern North America. Whittemore and Schaal (1991) also reported that sympatric oak species remain distinct despite considerable introgression, suggesting that the concept of total genetic isolation between species as an explanation for species distinctness clearly does not apply for *Quercus*. Hybrids of *Quercus* spp. were easily found to grow in forest stands with a mixed population of *Q. dentata*, *Q. serrata*, and *Q. mongolica* var. *crispula*, and studies of leaf and nut characteristics revealed a high frequency of intermediate-type individuals of those species in such stands (Miyazaki, 1988, 1989; Okada et al., 1994, 1996).

Hashizume et al. (1994b) compared and examined leaves and acorns sampled from a forest stand of *Quercus* spp. in the Hiruzen experimental forest of Tottori University. They measured the leaf blade, leaf width, form ratio, serration, petiole percentage length, acorn ratio, cupular diameter, length, width, and form ratio of the cupule scale of the *Q. fabri* type. The values were intermediate compared to those of *Q. serrata* and *Q. dentata*, and thus *Q. fabri* was presumed to be a natural hybrid.

In the present study, many leaves of the *Q. dentata* and *Q. serrata* artificial hybrids were obovate to obovate-oblong in shape, with sizes smaller than those of *Q. dentata* but similar to those of *Q. serrata*. The leaf apex was obtuse, slightly sharp, and not round as observed in *Q. dentata*. However, the cuneate leaf base resembled that of *Q. dentata*. Serration was smaller than those of *Q. dentata* but larger than those of *Q. serrata*. The margin was sharp, and many leaves had a serrate margin similar to those of *Q. serrata*. Therefore, the leaves of the hybrids had characteristics of both *Q. dentata* and *Q. serrata*.

Compared with *Q. fabri* individuals (Hashizume et al., 1994b) from the Hiruzen district, *Q. dentata* and *Q. serrata* artificial hybrids in this study had smaller leaves. The form ratio (L/W)

of the leaves of the interspecific hybrids was intermediate to that of the parents in the families of *Q. dentata* \times *Q. serrata* and *Q. serrata* \times *Q. dentata*. However, the leaf size of the hybrid family was smaller than that of the *Q. dentata* father, and the petiole was smaller than that of *Q. dentata* and *Q. serrata*. This difference can be explained by the age of the studied artificial hybrids, which were immature seedlings under 7 years of age, while the parent trees were 18 years old. The leaf size, petiole length, and serration number of *Q. serrata*, *Q. dentata*, and *Q. aliena* have been reported to differ according to tree age, with these values increasing with tree age and differing among the species (Lee 1997).

According to the *Q. serrata* subgenus key (Satake et al., 1989), *Q. dentata* possesses a large leaf blade, and stellate hairs are present on the reverse side of the leaf. *Quercus serrata* has a small leaf blade, and the reverse side of the leaf has sericeous stellate hair. Leaf trichomes have been reported to be a distinguishing factor for the *Q. serrata* species (Kurata, 1949; Kim et al., 1992; Lee, 1997).

Lee (1997) reported that seedlings of the density and ray number in stellate hair had lower stellate hair density and ray number than *Q. serrata*, *Q. dentata* which are mature trees. However, the density and ray number of *Q. serrata*, *Q. dentata*, and other *Quercus* species were found to differ by tree age. The density and ray number increased with the age of the tree, while ray length barely changed with tree age.

Thus to identify a seedling of *Q. serrata*, it is preferable not to use the density and number of rays but the lengths of rays. The trichomes present on the reverse side of the leaf were considerably different in the *Q. dentata* and *Q. serrata* hybrid. In a majority of the artificial hybrids of *Q. dentata* and *Q. serrata*, the nut characteristics were not clear because these hybrids had not yet produced acorns.

Literature Cited

- Anderson, E. 1949. Introgressive Hybridization. Wiley, New York.
- Awise, J. C. 1994. Molecular Markers, Natural History, and Evolution. Chapman and Hall, New York.
- Darwin, C. 1859. On the Origin of Species by Means of Natural Selection, 1st ed. Murray, London.
- Hardin, J. W. 1975. Hybridization and introgression in *Quercus alba*. J. Arnold Arbor. 56: 336-363.
- Hashizume, H., Z. Suo, J. H. Lee and F. Yamamoto. 1994a. Fundamental studies on the breeding of *Quercus* species (I): Flowering, pollination and seed bearing by artificial pollination. Trans. Jpn. For. Soc. 105: 321-324 (in Japanese).
- Hashizume, H., Z. Suo, J. H. Lee, S. Okada and F. Yamamoto.

- 1994b. Fundamental studies on the breeding of *Quercus* species (II) Characters of leaves and fruits in natural hybrids of *Q. dentata*, *Q. serrata*, and *Q. mongolica* var. *grosseserrata*. Trans. Jpn. For. Soc. 105: 325-328 (in Japanese).
- Kim, M. H., H. S. Song and C. S. Kim. 1992. Morphological types and seasonal loss of the trichomes of some *Quercus* species in Korea. Korean J. Pl. Taxon. 22: 13-21.
- Kurata, M. 1949. Trees for Byproducts. Asakura Publish. Ltd., Tokyo, Japan. Pp.114-116.
- Lee, J. H. 1997. Morphological, ecological, and molecular biological studies on the identification of *Quercus* spp. and their interspecific hybrids. Doctoral thesis. Tottori Univ. Tottori, Japan.
- Miyazaki, Y. 1988. Studies of introgression and natural hybridization of oaks in Hokkaido. For. Tree. Breed. Hokkaido. Japan. 31: 5-8.
- Miyazaki, Y. 1989. Ecological genetic studies of *Quercus crispula* in Hokkaido. For. Tree. Breed. 153: 1-5.
- Okada, S., Y. Nakagawa and H. Hashizume. 1994. Hybridization of deciduous *Quercus* species in Chugoku Mountain (I): a classification of leaf hair type by scanning-electron microscopy. Trans. Kansai Branch. Jpn. For. Soc. 3: 133-136.
- Okada, S., R. Yamamoto, H. Hashizume and F. Yamamoto. 1996. Variation of leaf morphological traits in *Quercus acutissima* and *Q. variabilis*. Trans. Kansai Branch Jpn. For. Soc. 5: 73-76.
- Orita, H., K. Koono, K. Okuyama and S. Eiga. 1991. Genetic characters of leaf forms in *Quercus*. Trans. Hokkaido Branch Jpn. For. Soc. 39: 44-46.
- Rieseberg, L. H. and J. F. Brunsfeld. 1992. Molecular Evidence and Plant introgression. In Molecular Systematics of Plants. Soltis, P. S., D. E. Soltis and J. D. Doyle (eds.), Chapman and Hall, New York. Pp. 151-176.
- Rushton, B. S. 1993. Natural hybridization within the *Quercus* L. Ann. Sci. For. 50: 73-90.
- Satake, Y., H. Hara, S. Watari and T. Tominari. 1989. Woody Flowers of Japan, Trees I. Heibonsha Co., Ltd., Tokyo, Japan. Pp. 70-72.
- Somego, M., T. Toyoda and T. Okawara. 1986. Variation of pollen in the genus *Quercus* spp. of forests of Mt. Haruna. Trans. Kanto Branch. Jpn. For. Soc. 38: 99-100.
- Stebbins, G. L. 1969. The significance of hybridization for plant taxonomy and evolution. Taxon 18: 26-35.
- Suzuki, T., H. Miguchi and T. Kamitani. 1992. Reproductive behavior of Konara (*Quercus serrata*) in coastal Japanese red pine (*Pinus densiflora*) stand. J. Jpn. For. Soc. 74: 342-345.
- Toyoda, T., M. Somego and T. Okawara. 1986. Variety of pollen in the genus *Quercus* spp. of forests of Mt. Zinba. Trans. Kanto Branch Jpn. For. Soc. 38: 101-102.
- Whittemore, A. T. and B. A. Schaal. 1991. Interspecific gene flow in sympatric oaks. Proc. Natl. Acad. Sci. USA 88: 2540-2544.