Discrimination of hybrids between Quercus variabilis and Q. acutissima by using stellate hairs, and analysis of the hybridization zone in the Chubu District of central Japan

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Shozo Hiroki\textsuperscript{1} and Takahiro Kamiya\textsuperscript{1,2} : **Discrimination of hybrids between *Quercus variabilis* and *Q. acutissima* by using stellate hairs, and analysis of the hybridization zone in the Chubu District of central Japan**

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**Abstract**

To discriminate between *Quercus variabilis*, *Q. acutissima*, and their hybrids, we sampled 152 individuals in secondary forests in Japan, including a plantation of *Q. acutissima* in Nagoya City. We compared and identified the species and their hybrids on the basis of the density of stellate hairs on the undersurfaces of leaves. The density was high in *Q. variabilis*, zero in *Q. acutissima*, and low in their hybrids. We also studied the distribution patterns of the two species and found that *Q. variabilis* grows at lower altitudes in warmer regions than does *Q. acutissima*, and that a hybridization zone exists where the ranges of the two species overlap. We discuss differences in the distributions of the species on the basis of life-history features such as seed germination and seedling establishment. The results suggest that the main range of distribution of *Q. acutissima* corresponds to Kira’s Warm Temperate Deciduous Forest Zone, and that the unusual distribution pattern of *Q. acutissima* in warmer regions such as Kyushu may have resulted from planting of the species.

**Key words** : distribution, hybrid, *Quercus acutissima*, *Quercus variabilis*, stellate hairs.

**Introduction**

*Quercus* is a prolific genus of trees, and *Quercus* species frequently tend to hybridize (Stebbins et al. 1947; Palmer 1948; Stebbins 1950; Muller 1952). Recently, a number of studies on *Quercus* hybrids and hybridization zones have been published (Manos 1993; Dodd and Kashani 2003; Dodd and Afzal-Rafii 2004).

*Quercus variabilis* Blume and *Q. acutissima* Carruth. are closely related species within the *Cerris* section of the genus. Both species are widely distributed throughout Asia and grow in Japan, Korea, Taiwan, and China (Kitamura and Murata 1979; Menitsky 2005). Kitamura and Murata (1979) described hybrids between these species in Japan and northeastern China and named them *Q. acutissima × Q. variabilis*. They suggested that the hybrids are rare, and noted that Japan’s Shimane Prefecture is the main region where the hybrids occur. Sano (1990) compared the morphological characteristics of *Q. variabilis* and *Q. acutissima*, and reported the existence of hybrids of these species in Hiroshima Prefecture. Recently, in a preliminary survey in Iijima Town in Nagano Prefecture, central Japan, where the distributions of these species overlap, we found probable hybrids with characteristics intermediate between the two species. This finding suggested that hybridization between the two species may not be as rare as was formerly reported.

*Quercus variabilis* and *Q. acutissima*, along with *Q. serrata*, are major components of secondary forests in hilly regions of Japan. According to Horikawa’s species distribution map (Horikawa 1976), *Q. variabilis* is distributed mainly from central to western Japan and is absent from the warmest regions such as the southern part of the Kii Peninsula, southern Shikoku, and most of Kyushu. On the other hand, *Q. acutissima* occupies a wider range, being distributed from cooler regions such as the Kanto District and the southern Tohoku District to quite warm regions, including almost all of Kyushu (Horikawa 1972). Matsubara and Hiroki (1980) surveyed the distribution of *Q. vari-
Several Quercus trichome types might be hybrids. Spellenberg (1993) demonstrated that individuals with intermediate trichome types showed similar but not identical trichome types, and the presence of particular trichome types could be a clue to the existence of hybrids. Manos (1979) and Sano (1990) showed that similar patterns of trichomes occurred within the same Quercus section or series, and that the presence of particular trichome types might be hybrids. Spellenberg (1995) reported Q. basaseachicensis to be a hybrid between Q. rugosa and Q. depressipes on the basis of their trichomes. Whereas Q. rugosa had stellate hairs and large multicellular veriform hairs, Q. depressipes lacked stellate hairs and had minute veriform hairs, and Q. basaseachicensis had only medium-sized veriform hairs.

Here, we aimed to reveal the existence of hybrids between Q. variabilis and Q. acutissima based on the density of stellate hairs and to demonstrate the existence of a hybridization zone in Nagano Prefecture where the distribution ranges of the two species overlap. We did not use bark traits as a criterion for identifying hybrids, because the thickness of the cork layer varies continuously among hybrids between the two species (Sano 1990).

**Materials and methods**

We selected five sampling areas with developed secondary forests in Aichi Prefecture (the Higashiyama hills in Nagoya City) and Nagano Prefecture (Iida City, Iijima Town, Ina City, and Toyoshina Town; Figure 1). We selected one sampling site in each area except Toyoshina Town, where we selected three sites separated by more than 10 km. We added one more sampling site at Nagoya Castle Park (in Nagoya City), where Q. acutissima grows in an apparently artificial forest.

We selected 152 individuals of Q. variabilis and Q. acutissima, including intermediate types, from these sampling sites and sampled 3 to 8 leaves from each of them (Table 1). We tentatively sorted the 152 individuals into three types, the Q. variabilis type, the Q. acutissima type, and the intermediate type, based on the color of the undersurface of the leaf, which reflects the density of hairs.

Leaves with a low density of stellate hairs appear green or nearly green on their undersurfaces. Using a binocular microscope (Olympus, Tokyo, Japan) at ×29 magnification, we counted the number of stellate hairs on the undersurfaces of leaves of 106 individuals whose leaf undersurfaces appeared green or nearly green. To standardize our measurements, we chose the area of the lamina between the 9th and 10th veins from the base of the leaf, on the right-hand side.
Table 1. Sampling sites and number of samples

<table>
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<th>Locality of sampling site</th>
<th>Altitude (m)</th>
<th>Number of individuals</th>
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<tbody>
<tr>
<td>Aichi Prefecture</td>
<td></td>
<td></td>
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<tr>
<td>Nagoya City</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Higashiyama hills</td>
<td>50–65</td>
<td>20</td>
</tr>
<tr>
<td>Nagoya Castle Park</td>
<td>0</td>
<td>24</td>
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<tr>
<td>Nagano Prefecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iida City</td>
<td>590–620</td>
<td>21</td>
</tr>
<tr>
<td>Iijima Town</td>
<td>680–750</td>
<td>13</td>
</tr>
<tr>
<td>Ina City</td>
<td>800–1500</td>
<td>15</td>
</tr>
<tr>
<td>Toyoshina Town</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hikarujoyama</td>
<td>610–650</td>
<td>17</td>
</tr>
<tr>
<td>Toyoshina</td>
<td>640–650</td>
<td>26</td>
</tr>
<tr>
<td>Oshinoyama</td>
<td>545–570</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>152</td>
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of the leaf, as the measurement location. We measured the density of stellate hairs in 3 leaves per individual, and, to improve our estimates of variability, we measured 4 to 8 leaves per individual for the intermediate types obtained from Iijima Town.

We obtained a leaf with a white undersurface from an individual from the Higashiyama hills and photographed the surface with a camera (an automatic photographic apparatus from Olympus) attached to a microscope equipped with a 1-mm micrometer. We then counted the number of stellate hairs within an area of 0.75×1.07 mm using a hand-held counter. We performed this measurement three times for this single leaf of this particular individual.

We identified sampled individuals with high, zero, and intermediate degrees of stellate hair densities as *Q. variabilis*, *Q. acutissima*, and hybrids, respectively.

Furthermore, we analyzed the distribution pattern of the 152 individuals from Aichi Prefecture to Nagano Prefecture.

**Results**

The 152 individuals were tentatively classified into 46 individuals of the *Q. variabilis* type with white leaf undersurface, 103 of the *Q. acutissima* type with green undersurface, and 3 of the intermediate type with whitish green or almost green undersurface.

Of the 103 trees of the tentative *Q. acutissima* type, 16 had stellate hairs and were subsequently identified as hybrids (only 8 trees from Iijima Town are shown in Table 2). The rest of the individuals identified as *Q. acutissima* from Nagoya Castle Park and Nagano Prefecture had no stellate hairs. An individual identified as *Q. variabilis* from the Higashiyama hills had roughly 38,000 stellate hairs per square centimeter.

The other 19 individuals from the Higashiyama hills site identified as *Q. variabilis* had white leaf undersides and a seemingly thick cork layer in their bark. In Nagano Prefecture, 26 individuals also identified as *Q. variabilis* also had white leaf undersides.

Thus, we identified 46 trees as *Q. variabilis*, 87 as *Q. acutissima*, and 19 as hybrids.

The densities of stellate hairs varied greatly within an individual tree and among the 8 individuals identified as hybrids from Iijima Town (Table 3). Among those 8 individuals, 6 were tentatively judged to be *Q. acutissima* at first,
but had stellate hairs ranging from none to very few.

The distribution ranges of *Q. variabilis* and *Q. acutissima* overlapped a little in Nagano Prefecture (Fig. 2): *Q. variabilis* was abundant in the Higashiyama hills in Nagoya City (Aichi Prefecture) and in Iida City (Nagano Prefecture), but its numbers decreased markedly moving northward from Iida City (leftward on the chart). In contrast, numbers of *Q. acutissima* gradually increased moving northward from Iijima Town and at higher elevations. Hybrids occurred throughout the range where the two species coexisted.

The densities of stellate hairs in hybrids were lower where *Q. acutissima* was most abundant and higher where *Q. variabilis* was most abundant (n=19, Fig. 3). The hybrid from Iida, where *Q. variabilis* was abundant, had a mean density of about 600 stellate hairs per square centimeter.

**Discussion**

We identified 19 individuals as hybrids. Although *Q. acutissima* leaves are thought to have no stellate hairs, Sano (1990) reported the existence of 2 individuals with small amounts of stellate hairs in Hiroshima Prefecture and suggested that these might be hybrids. Thus, in future we need to study whether individuals with sparse stellate hairs fall within the normal range of variation for *Q. acutissima*, by studying its pure populations in the southern Tohoku District.

Our study showed that a zone of hybridization exists where the distribution ranges of *Q. variabilis* and *Q. acutissima* overlap between Iida and Toyoshina in Nagano Prefecture. We did not examine the bark traits of the populations in Nagano Prefecture, and we identified *Q. variabilis* solely by visual observation of the white leaf undersurface, which indicates a high density of stellate hairs. Thus, some hybrids may have...
erroneously been included in the populations that we identified as *Q. variabilis*. Sano (1990) described the existence of hybrids which had leaves densely covered with stellate hairs on their undersurfaces and the *Q. acutissima*-type bark. Additional investigation is necessary to reveal the variation in both bark thickness and density of stellate hairs in both species.

Among the hybrids, the density of stellate hairs was lower in areas where *Q. acutissima* was abundant and higher in areas where *Q. variabilis* was abundant. This suggests that backcrossing may have occurred between the hybrids and the parent species.

Our finding that *Q. variabilis* is distributed primarily in the warmer parts of the Chubu District is consistent with the conclusions of Matsubara and Hiroki (1980), who reported that *Q. variabilis* is abundant up to elevations from 400 to 500 m and that its presence at higher altitudes is limited to forest edges and sunny sites. Ishizuka et al. (1983) also reported that this species was present at sunny sites and in dry microhabitats below an elevation of 390 m near Yamagata in the southern part of the Tohoku District. The high densities of stellate hairs in *Q. variabilis* may be an adaptive characteristic for survival in dry, warm habitats; this hypothesis is supported by the development of a thick cork layer by this species. Dense leaf hairs are an adaptive characteristic for plants growing in dry habitats, because the hairs reduce moisture loss by creating a boundary layer of air around the leaf that reduces air motion and traps moisture (Ricklefs and Miller 2000). Similarly, the thick cork layer of *Q. variabilis* may be an adaptation to heat stress, considering the fact that *Q. suber*, which has thick cork layers, occurs in dry Mediterranean regions with low precipitation in the hot summer (More and White 2002).

The distribution of *Q. variabilis* seems to be strongly related to temperature. Matsubara and Hiroki (1980) estimated that the range of the species falls between 82 and 146 in the Warmth Index of Kira (1949): this index ranges from 85 to 180 for the warm temperate climate region where evergreen broad-leaved forests dominate. This, together with our present results, suggests that the main distribution of *Q. variabilis* corresponds closely to an area within the warm temperate climate region. In contrast, our results show that *Q. acutissima* is prevalent in such areas as Matsumoto, where the Warmth Index is about 91. *Quercus acutissima* is rarely found at elevations below 500 m in the Chubu District, except in plantations such as the one in Nagoya Castle Park. These facts suggest that the distribution of *Q. acutissima* corresponds to an area within the Warm Temperate Deciduous Forest Zone, as proposed by Kira (1949), where low winter temperatures restrict the growth of broad-leaved evergreen trees.

This difference in distribution between the two species can be explained by differences in their life histories. Hiroki and Matsubara (1982) compared the growth stages from seed to seedling in *Q. variabilis* and *Q. acutissima* and found differences in the patterns of seed germination and seedling growth. The seeds of *Q. variabilis* germinate in the year of seedfall so seedlings can establish before winter, whereas the seeds of *Q. acutissima* overwinter and do not germinate until the following spring, avoiding severe winter temperatures. These life-history features correspond well to the observed geographical distribution of each species: *Q. variabilis* in warmer regions and *Q. acutissima* in cooler regions.

Given these life-history traits, the distribution of *Q. acutissima* in warmer regions such as southern Kyushu is curious. Miyawaki (1981) regarded almost all of the *Q. acutissima* forests in Kyushu as plantations for the provision of logs used for growing *Lentinus edodes* (shiitake mushrooms). *Quercus acutissima* is also said to have been planted to support the production of wood charcoal (Matsubara and Hiroki 1980). The wood of *Q. acutissima* is much better for charcoal production than that of *Q. variabilis* (Kishimoto 1976), and ancient documents show that charcoal was produced from *Q. acutissima* in the Edo era in the upper reaches of the Ina River in the Hokketsu area of Hyogo and Osaka Prefectures (Hattori et al. 2005). Thus, the contribution of humans to the expansion of *Q. acutissima*'s distribution cannot be discounted.

Although acorns of *Q. variabilis* and *Q. acutissima* are also dispersed by animals such as rodents or jays (Hiroki and Matsubara 1982;
Chung and Chung 2002), the role of humans in the dispersal of large acorns also cannot be neglected. Hiroki and Ichino (1991) suggested that the distributional range of Castanopsis sieboldii expanded as a result of dispersal by humans, who used the nuts as food. It is also known that the distribution of Castanea sativa expanded rapidly around the Mediterranean about 3000 years ago by human interference (Fineschi et al. 2000).

Genetic analyses have shown the development of hybridization zones in areas where the distributions of Quercus species overlap in western North America (Howard et al. 1997; Dodd and Kashani 2003; Dodd and Afzal-Rafii 2004) and in Mexico (González-Rodríguez et al. 2004). We thus need to conduct more precise investigations into the formation of hybrids between Q. variabilis and Q. acutissima based not only on their morphological characteristics, but also on genetic analyses, to clarify the details of their hybridization, including such factors as the survival of hybrid seedlings.

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