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Differences in leafminer (*Phyllonorycter*, Gracillariidae, Lepidoptera) and aphid (*Tuberculatus*, Aphididae, Hemiptera) compositions among *Quercus dentata, Q. crispula, Q. serrata* and their hybrids

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Abstract Leafminer (*Phyllonorycter*, Gracillariidae, Lepidoptera) and aphid (*Tuberculatus*, Aphididae, Hemiptera) compositions were studied in three deciduous oak species, *Quercus dentata*, *Q. crispula* and *Q. serrata*, and their hybrids in Tomakomai Experimental Forest of Hokkaido University, Hokkaido, northern Japan. Identification of trees in this forest was done mainly on the basis of discriminant analysis on leaf morphology with reference to trees in pure *Q. dentata* and *Q. crispula* stands and a *Q. serrata* stand mixed with *Q. crispula*. The results suggested that hybridization occurred in all combinations (i.e., *Q. dentata*-*Q. crispula*, *Q. crispula*-*Q. serrata*, and *Q. serrata*-*Q. dentata*) and the frequency of hybrids was about 10 %. The composition of *Phyllonorycter* and *Tuberculatus* species differed between *Q. dentata* and *Q. crispula* or *Q. serrata*, but did not differ between *Q. crispula* and *Q. serrata*. Thus, *Q. dentata* would differ from *Q. crispula* and *Q. serrata* in chemical properties that determine herbivore host selection, survival and performance, possibly reflecting their eco-physiological differences or phylogenetic distances. The study insects were divided into three groups; species specialized to *Q. dentata* (three *Phyllonorycter* and one *Tuberculatus* species), those to *Q. crispula* and *Q. serrata* (six *Phyllonorycter* and two *Tuberculatus* species), and a species collected at least from *Q. dentata* and *Q. crispula* (one *Tuberculatus* species). Putative hybrid trees of *Q. dentata* and *Q. crispula* harbored both *Q. dentata*- and *Q. crispula*-specific insects.

Keywords Host specificity • Hybridization • Leaf morphology • Oak

Introduction

Herbivore insects are important components of forest ecosystems and sometimes cause serious damages to trees. In general, herbivore compositions differ in different tree species. One of processes leading to herbivore differentiation is coevolutionary interaction between trees and herbivores (Ehrlich and Raven 1964; Feeny 1975; Strong et al. 1984; Thompson 1994; Schoonhoven et al. 1998). If a tree population evolves novel defense mechanisms, herbivores may also evolve novel mechanisms to overcome the defenses, possibly at a cost of capacity to exploit trees of the original populations. Such process could lead to differentiation both in trees and herbivores. A number of factors would affect coevolutionary interactions of trees and herbivores. For example, Feeny (1975) considered that plant apparency affects the type of defense the plant evolves; i.e., large, highly abundant plants may evolve qualitative defense compounds such as tannins, whereas small, rarely found plants may evolve qualitative defense compounds such as alkaloids. Resource availability is also assumed to affect the evolution of defense mechanisms (Coley et al. 1985). Plants under favorable resource conditions may be able to survive high levels of damage, and then they may invest less to defense. In contrast, plants under limited resources may be unable to tolerate herbivory and then may evolve high levels of defense. This hypothesis further suggests that habitat shifts of plants (e.g., from favorable habitats to unfavorable ones) could alter their defense levels and then affect herbivore performance and compositions.

Hybridization of trees also affects the evolution and ecology of herbivore insects. For example, hybrid trees may serve as evolutionary bridges that allow herbivores to expand the host range (Floate and Whitham 1993). The importance of hybrids as

bridges depends, in part, on how extent introgression occurs between parental species and how hybrids respond to herbivores. For example, hybrids are often more susceptible to herbivores than parental tree species (Whitham 1989; Fritz et al. 1994), possibly due to hybrid breakdown of coadapted genes that determine resistance to herbivores (Sage et al. 1986), and they are also sometimes more resistant to herbivores than parental species or intermediate in resistance between parental trees (Boecklen and Spellenberg 1990; Aguilar and Boecklen 1992; Ishida et al. 2004). Thus, composition and abundance of herbivores on hybrid individuals are important information for understanding of herbivore speciation and host-shift. *Quercus* species often produce hybrids and have much contributed to the development of the hybrid-bridge and hybrid-as-sinks hypotheses (Boecklen and Spellenberg 1990; Aguilar and Boecklen 1992; Ishida et al. 2003, 2004; Tovar-Sánchez and Oyama 2006), as well as for the development of species concepts of plants (Anderson 1949; Burger 1975; Van Valen 1976; Rieseberg 1995; Arnold 1997).

Here, we studied compositions of *Phyllonorycter* (leafminers belonging to Gracillariidae, Lepidoptera) and *Tuberculatus* (aphids belonging to Aphididae, Hemiptera) species on three deciduous *Quercus* species, *Q. crispula* Blume, *Q. serrata* Murray and *Q. dentata* Thunberg, and their hybrids in Tomakomai Experimental Forest of Hokkaido University in northern Japan to understand the evolution of their associations. These *Quercus* species are predominant components of temperate forests from central to northern Japan and often produce hybrids (Ohba 1989; Hashizume et al. 1994; Matsuda 1996; Ishida et al. 2003; Kanno et al. 2004; Okaura et al. 2007; Matsumoto et al. 2009). Insects on these *Quercus* species have been studied to some extent, but information is still fragmentary (Yoshida 1985; Wada et al. 2000; Shibata et

al. 2001). Among a wide variety of insects on *Quercus* trees, the focal *Phyllonorycter* and *Tuberculatus* insects can be rather easily sampled and identified to species (Higuchi 1969; Quednau 1999; Fujihara et al. 2000).

In this study, we also estimate how often hybridization occurs between these *Quercus* species to understand its effects on their evolution, speciation and ecology. If hybridization is frequent, parental species could change in genetic structures, and, in extreme cases, they could fuse (Arnold and Hodges 1995; Jiggins and Mallet 2000). For these purposes, correct identification of hybrids is necessary. The identification of the present *Quercus* species and their hybrids have been done with morphological traits and DNA markers (Ohba 1989; Hashizume et al. 1994; Lee et al. 1997; Ishida et al. 2003; Matsumoto et al. 2009), as well as for European and American oaks and their hybrids (Kleinschmit et al. 1995; Bruschi et al. 2000; Tomlinson et al. 2000; Tovar-Sánchez and Oyama 2004; Himrane et al. 2004; González-Rodríguez et al. 2005). In this study, identification was done mainly on the basis of discriminant analysis on leaf morphology with reference to trees in pure *Q. dentata* and *Q. crispula* stands and a *Q. serrata* stand mixed with *Q. crispula*.

Materials and methods

Study sites and trees

The study was carried out in a cool-temperate forest in Tomakomai Experimental Forest (42°40' N, 141°36' E) of Hokkaido University in Hokkaido, northern Japan. This forest consists of ca. 50 tree species, many of which are deciduous broad-leaved trees

(Mishima et al. 1955, 1958; Igarashi 1978). *Quercus crispula* is dominant in this forest, whereas *Q. dentata* and *Q. serrata* are not abundant.

A total of 186 trees in Tomakomai Experimental Forest were examined. Among them, 36 (> 15 m in height) grew at scaffolding-system sites, and 150 (>1.5 m) did on transects that were set from the scaffolding systems. The scaffolding systems were constructed at three sites where two different *Quercus* species grew: i.e., two sites (A and B) with putative *Q. dentata* and *Q. crispula* and one site (C) with putative *Q. crispula* and *Q. serrata*; 15 *Quercus* trees grew at site A, 12 at site B, and 9 at site C. From each scaffolding system, two transects were set (one on the north-south direction and the other on the east-west direction), and 25 trees on each transect were examined. *Phyllonorycter* and *Tuberculatus* compositions were examined only for trees at scaffolding-system sites, since these insects could not be collected without scaffolding systems.

Reference trees were used to identify the trees in Tomakomai. Fifty *Q. dentata* reference trees were arbitrarily selected from its pure stand in Nakaotofuke (43°07' N, 143°05' E), and 50 *Q. crispula* trees were selected from each of its pure stands in Hamamasu (43°35' N, 141°28' E) and Toishiyama (43°00' N, 141°17' E). Leaf morphology of these *Q. dentata* and *Q. crispula* reference trees was already reported by Ishida et al. (2003). Reference trees of *Q. serrata* were obtained from a mixed stand of *Q. crispula* and *Q. serrata* in Aoba Park (43°00' N, 141°30' E), since no pure stand of *Q. serrata* was found in Hokkaido; 30 trees having *Q. serrata* characteristics were selected.

Collections of leaves, acorns and insects were carried out from 2005 to 2008 except for reference trees of *Q. dentata* and *Q. crispula*, which were examined in 1999 (Ishida et al. 2003).

Identification of trees

Ten undamaged shade leaves were collected from each tree in mid summer, and examined for areas, number of lobes, length/width proportion, LMA, number of stellate-hair clusters, length of stellate hairs, and the presence (or absence) of solitary and short hairs, according to Ishida et al. (2003). These traits co-varied little with each other (Ishida et al. 2003).

Reference trees were subjected to discriminant analysis using mean values of these eight traits, and discriminant formula obtained in this analysis was used to calculate canonical variate (CV) scores for trees in Tomakomai (Ishida et al. 2003). The analyses were performed with JMP ver. 6 (SAS institute, Cary, USA).

In addition to the above traits, petiole length was measured for trees except for *Q. dentata* and *Q. crispula* reference trees, since this trait is useful to discriminate *Q. serrata* from *Q. dentata* or *Q. crispula* (Huang et al. 1999). Ten leaves were examined for each tree. In addition, the length of scales on acorn caps was examined for trees at the scaffolding-system sites, if acorns were produced. This trait is useful to discriminate *Q. dentata* from *Q. crispula* or *Q. serrata* (Hashizume et al. 1994). Except for six trees that did not produce acorns during the study years, acorns with caps were collected (1-2 acorn(s) from trees of Nos. 32, 35 and 36; 5-11 from trees of Nos. 14, 16, 17, 18, 20, 25 and 29; 20 from the other trees), and a scale at the mid position of each cap was measured.

Phyllonorycter and *Tuberculatus* compositions

Leaves were collected from trees growing at the scaffolding-system sites in October, 2005, and stored outdoors until almost all of *Phyllonorycter* larvae grew to pupae. *Phyllonorycter* pupae were then collected from these leaves and identified to species by pupal exuviae (Fujihara et al. 2000).

Alate individuals of *Tuberculatus* were directly collected from trees at the scaffolding-system sites in August in 2005-2007, and identified to species by the number and position of chaetae on labrum and the number and size of spinal tubercle on pronotum (Higuchi 1969).

Table 1 lists the characteristics examined for trees at the study sites or populations; i.e., the scaffolding-system sites, transects from the scaffolding system, and reference populations of *Q. crispula, Q. dentata* and *Q. serrata*.

Results

Morphological analyses

Table 2 shows morphological data on reference trees. Area and leaf mass per area (LMA) were larger in *Q. dentata* followed by *Q. crispula*; the number of lobes and length/width proportion were smaller in *Q. dentata*; the density of stellate hairs was larger in *Q. dentata* followed by *Q. serrata*, while the hairs were longer in *Q. dentata* followed by *Q. crispula*; solitary hairs were observed more frequently in *Q. serrata* followed by *Q. crispula*, while short hairs showed an opposite tendency. Figure 1A shows the results of discriminant analysis on the reference trees. The three oak species,

Q. dentata, *Q. crispula* and *Q. serrata*, were clearly discriminated by the two CV scores; *Q. dentata* was discriminated from *Q. crispula* or *Q. serrata* by CV1, and trees having CV1 of <2 were differentiated into *Q. serrata* and *Q. crispula* by CV2.

With discriminant formula obtained by the above analysis, CV scores were calculated for 186 trees in Tomakomai Experimental Forest (for 36 trees at the scaffolding-system sites, CV scores are shown in Table 3, and morphological data are given in Table 4). CV scores of these trees varied continuously, but were almost confined within the ranges of scores for reference trees (Fig. 1B).

Length of scales of acorn caps and petioles of leaves in trees at the scaffolding-system sites were shown in Table 3. Scale length showed a strong correlation (r=0.86, P<0.001) with CV1, and showed a rather weak correlation (r=0.48, P=0.008) with CV2 when trees having CV1 of <2 were subjected to the analysis. Petioles were especially long in trees Nos. 33 and 36. However, petiole length did not show significant correlation with CV1 (r=0.32, P=0.14) or CV2 (r=0.32, P=0.15) among trees having CV1 of <2.

Phyllonorycter and *Tuberculatus* compositions

A total of 961 *Phyllonorycter* pupae belonging to nine species and 666 *Tuberculatus* individuals belonging to five species were collected from trees at the scaffolding-system sites (Table 5). Among them, *T. pilosus* (Takahashi) was recorded from Hokkaido for the first time.

The relative frequency of each insect species was calculated separately for *Phyllonorycter* and *Tuberculatus* in each of 35 trees (one tree without *Tuberculatus*

sample was excluded), and subjected to principle component analysis based on correlation matrix (Fig. 2). The first axis (PC1) explained 39.4 % of variation, PC2 did 14.3 %, PC 3 did 9.7 %, PC4 did 8.4 %, and PC5 did 6.9 %. PC1 showed a significant negative correlation (r=-0.84, P<0.001) with CV1, but other PC scores (PC2 - PC5) did not show significant correlation (r=-0.22 - 0.09, P=0.21 - 0.96) with CV1. For trees with CV1 of <2, all PC scores (PC1 - PC5) did not show significant correlation (r=-0.21 – 0.11, P=0.35 – 0.65) with CV2. These results suggested that the composition of these insects differed between *Q. dentata* and *Q. crispula* or *Q. serrata* but did not differ between *Q. crispula* and *Q. serrata*.

To evaluate host selection of each insect species, host selection index (*HSI*) was calculated by the following formula, $HSI=\Sigma(CV1_i \times n_i)/N$, where $CV1_i$ was CV1 of i tree at the scaffolding-system sites, n_i is the number of individuals of the focal insect species on i tree, and N is the total number of the focal species. Larger *HSI* indicates a stronger preference to trees with larger CV1 (i.e., *Q. dentata*). According to this index, the present insects can be classified into three groups, the first with *HSI* of 3.6 - 4.0 (*Phyllonorycter persimilis* Fujihara, Sato and Kumata, *P. leucocorona* (Kumata), *P. nigristella* (Kumata) and *Tuberculatus paiki* (Hille Ris Lambers)), the second with *HSI* of -1.4 - 0.5 (*P. matsudai* Kumata, *P. acutissimae* (Kumata), *P. similis* Kumata, *P. crenata* (Kumata), *P. pseudolautella* (Kumata), *P. mongolicae* (Kumata), *T. kashiwae* (Matsumura) and *T. yokoyamai* (Takahashi)), and the third with an intermediate score (1.8) of *HSI* (*T. japonicus* Higuchi). *Tuberculatus pilosus* was not examined for host selection since it was collected from only one tree.

Frequency of hybrids

To estimate the frequency of hybrids in Tomakomai Experimental Forest, *Quercus* trees were tentatively identified. A tree (No. 36 in Table 3) having a small CV1 score and a large CV2 score was assigned as *Q. serrata* (Fig. 1B). This tree also had long petioles, a characteristic of *Q. serrata* (Table 2). Among 11 trees having large CV1 scores (>3.7) and long acorn-cup scales (>5 mm), nine were assigned as *Q. dentata* (Fig. 1B), but two trees (Nos. 3 and 8) were assigned as hybrids between *Q. dentata* and *Q. crispula* since they harbored some *Phyllonorycter* and *Tuberculatus* species that are specific to *Q. crispula* and *Q. serrata* at rather high frequencies (Table 5). Among eight trees having intermediate CV1 scores (0.41<. >2.96), one (No. 18) was assigned as *Q. crispula* since it had short acorn-cap scales and scarcely harbored *Q. dentate*-specific insect species (Table 5). The others were assigned as hybrids between *Q. dentata* and *Q. crispula*. Most of them had relatively long acorn-cap scales (Table 3), and two (Nos. 14 and 16) harbored both *Q. dentata*-specific and *Q. crispula*(*Q. serrata*)-specific insects (Table 5).

Four trees having intermediate CV1 and CV2 scores between *Q. serrata* and *Q. dentata* were assigned as their hybrids (Fig. 1B). However, the length of petioles in these trees (4.4-5.7 mm) was included within the range of putative *Q. dentata* trees (4.1-7.0 mm), leaving a question on this identification.

Continuous variation was observed between *Q. crispula* and *Q. serrata*, and it is difficult to discriminate their hybrids from parental species. Here, eight trees having intermediate CV2 scores and/or petiole length were tentatively assigned as hybrids, and remaining trees were assigned as *Q. crispula* (Fig. 1B).

According to the above tentative identification, the frequency of hybrids was 33.3 % (12 out of 36 trees) at the scaffolding-system sites and 6.0 % (9 out of 150 trees) on the transects. When these data were pooled, the frequency was about 11.3 %.

Discussion

Trees from reference populations of *Q. dentata*, *Q. crispula* and *Q. serrata* were clearly discriminated from each other by canonical discriminant analysis using eight leaf traits (areas, number of lobes, length/width proportion, LMA, number of stellate-hair clusters, and length of stellate hairs and the presence (or absence) of solitary and short hairs); *Q. dentata* was discriminated from *Q. crispula* or *Q. serrata* by CV1, and trees having CV1 of <2 were differentiated into *Q. serrata* and *Q. crispula* by CV2.

The analysis on trees in Tomakomai Experimental Forest suggested that hybridization occurred in all combinations (i.e., *Q. dentata*-*Q. crispula*, *Q. crispula*-*Q. serrata*, and *Q. serrata*-*Q. dentata*), as has been reported in previous studies (Ohba 1989; Ishida et al. 2003; Kanno et al. 2004; Okaura et al. 2007; Matsumoto et al. 2009). However, the frequency of hybrids in this forest was, on average, not high (i.e., 11 % among all trees examined, and 6 % among trees on transects), suggesting that most *Quercus* trees in this forest retained morphological characteristics specific to the parental species. Also in other locations of Japan, most *Quercus* trees can be differentiated into either of the parental species (Ohba 1989; Ishida et al. 2003; Kanno et al. 2004; Okaura et al. 2007; Matsumoto et al. 2009). The maintenance of species distinctiveness despite frequent hybridization has also been reported for a number of hybridizing *Quercus* species pairs, e.g., *Q. grisea* – *Q. gambelii* (Howard et al. 1997), *Q.*

robur – *Q. petrea* (Muir et al. 2000), *Q. lobata* – *Q. douglasii* (Craft et al. 2002), *Q. crassifolia* – *Q. crassipes* (Tovar-Sánchez and Oyama 2004) and *Q. affinis* – *Q. laurina* (González-Rodríguez et al. 2005). Jiggins and Mallet (2000) assumed that distinctiveness of parental species is more likely maintained by their ecological divergence between parental species than genetic incompatibility; i.e., hybrid individuals (i.e., morphologically intermediate morphs) may be selected against, for example, due to stabilizing selection, although Williams et al. (2001) suggested that genetic incompatibility could cause reproductive isolation at least between *Q. grisea* and *Q. gambelii*. The Jiggins and Mallet's (2000) notion seems to agree with the present case, where no interspecific genetic incompatibility is observed (Kanazashi et al. 1997; Ubukata et al. 1999).

In the principle component analysis on *Phyllonorycter* (leafminers) and *Tuberculatus* (aphids) compositions, the first axis (PC1) showed a strong correlation with CV1, indicating that the compositions differed between *Q. dentata* and *Q. crispula* (or *Q. serrata*). On the other hand, none of PC scores was significantly correlated with CV2, suggesting that the compositions did not differ between *Q. crispula* and *Q. serrata*. According to the analysis on their host selection, the present insects were divided into three groups; species specialized to *Q. dentata* (three *Phyllonorycter* and one *Tuberculatus* species), those to *Q. crispula* and *Q. serrata* (six *Phyllonorycter* and two *Tuberculatus* species), and a species collected at least from *Q. dentata* and *Q. crispula* (one *Tuberculatus* species). The above results on *Phyllonorycter* species agreed well with the previous results (Sato 1991; Sato et al. 2002; Fujihara et al. 2000; Ishida et al. 2003). On the other hand, Higuchi (1969) and Quednau (1999) reported that *T. yokoyamai* and *T. kashiwae* occurred in the present three *Quercus* species whereas *T.*

paiki was rather specific to *Q. dentata*. In this study, however, the first two species were rarely found on *Q. dentata*. They were also rarely found in *Q. dentata* trees at Ishikari, 70 km north of Tomakomai (unpublished data). In addition to these leafminers and aphids, gall wasps differed between *Q. dentata* and *Q. crispula* or *Q. serrata*; i.e., *Andricus kashiwaphilus* Abe was specific to *Q. dentata* whereas *A. mukaigawae* (Mukaigawa) was associated with *Q. crispula* and *Q. serrata* (Abe 1986, 1988, 1998).

Thus, *Q. dentata* would differ from *Q. crispula* and *Q. serrata* in chemical properties which affect insect host selection, survival and performance. This differentiation pattern may reflect their phylogeny. *Quercus crispula* and *Q. serrata* are genetically more closely related with each other than to *Q. dentata* (Lee et al. 1997; Matsumoto et al. 2009), suggesting their phylogenetic closeness. *Quercus crispula* and *Q. serrata* may not have had enough time for chemical differentiation. Otherwise, the chemical difference may reflect the difference in their eco-physiological characteristics. *Quercus dentata* is a “sun” tree and frequently occurs in xeric environments, whereas *Q. crispula* and *Q. serrata* are rather shade tolerant (Ishida et al. 2004; Hattori et al. 2004; Matsuda 1996). Leaves of “sun” trees have thicker palisade parenchyma (i.e., larger LMA) and therefore have higher nitrogen concentration (Nabeshima et al. 2001; Hattori et al. 2004; Migita et al. 2007). Thus, *Q. dentata* leaves are nutritionally richer resources for herbivore insects, and therefore they must have higher capacity to protect themselves from herbivore attacks. In fact, *Q. dentata* leaves have higher concentrations of condensed tannins than *Q. crispula* leaves (Nabeshima et al. 2001; Kitamura et al. 2007). Such difference in defense capacity could have profound effects on their coevolutionary interactions with herbivores and

then lead to differentiation in herbivore composition (Strong et al. 1984; Schoonhoven et al. 1998).

Hybrids are often more susceptible to herbivores than parental tree species, a situation formulated as the “hybrids-as-sinks” hypothesis by Whitham (1989). In the present study, insect sampling was not quantitative, and therefore this hypothesis could not be tested. However, our previous study (Ishida et al. 2004) suggested that hybrids between *Q. dentata* and *Q. crispula* sustained intermediate herbivore densities in comparison with parental trees or similar densities with parental species, as well as hybrids of other *Quercus* species (Boecklen and Spellenberg 1990; Aguilar and Boecklen 1992; Tovar-Sánchez and Oyama 2006). Thus, the “hybrids-as-sinks” hypothesis has not been supported in *Quercus*.

On the other hand, morphological characteristics of the present hybrids showed continuous variation between the parental species, possibly suggesting that introgression has occurred through backcross. In addition, at least hybrids between *Q. dentata* and *Q. crispula* harbored both *Q. dentata*- and *Q. crispula*-specific herbivores. These results suggest that the present hybrids could act as “bridges” for host shift of pest insects (Floate and Whitham 1993; Tovar-Sánchez and Oyama 2006). However, it is not known whether the host shift truly occurred between the present oak species. Studies on genetic differentiation of herbivore populations on parental and hybrid trees may provide keys to address this issue.

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Table 1 Lists of characteristics examined for trees at the study sites or populations. Basic: Eight basic leaf traits (first eight traits in Table 2), Petiole: length of petioles, Acorn: length of scales on acorn cap, +: examined, -: not examined

Study sites or populations	No. of trees examined	Morphological traits			Insect composition
		Basic	Petiole	Acorn	
Scaffolding-system sites	36	+	+	+	+
Transects from scaffolding systems	150	+	+	-	-
Reference populations					
Q. crispula	100	+	-	-	-
Q. dentata	50	+	-	-	-
Q. serrata	30	+	+	-	-

Table 2 Leaf traits (mean ± SD) of reference populations of three *Quercus* species

Leaf traits	*Q. dentata*	*Q. crispula*	*Q. serrata*
Area (cm^2)	192.4±44.6	91.9±27.1	31.8±6.26
Number of lobes	16.7±1.64	23.1±2.23	22.2±2.20
Leaf mass per area (LMA: mg/cm^2)	6.24±0.53	4.15±0.55	4.72±1.00
Length/width proportion (L/W)	1.55±0.13	1.79±0.15	2.15±0.47
Density of stellate hairs (DS: $hairs/mm^2$)	3.63±0.91	0.23±0.29	23.0±7.67
Length of stellate hairs (LS:μm)	436.4±46.0	150.0±46.5	115.0±15.3
Individuals with solitary hairs (SO: %)	6	45	100
Individuals with short hairs (SH: %)	100	79	0
Petiole length (mm)	not measured	not measured	9.77±2.66

Table 3 Canonical variates 1 and 2 (CV1, CV2), length of scales on acorn caps (SL)and petioles (PL), and PC1 in principle component analysis on insect composition for trees at the scaffolding-system sites in Tomakomai. Tentative identification is also given (d-c: *Q. dentata*-*Q. crispula*, c-s: *Q. crispula*-*Q. serrata*)

Tree No.	CV1	CV2	SL (mm)	PL (mm)	PC1	Tentative Identification
1	6.420	2.305	7.7	6.4	-2.784	*Q. dentata*
2	5.682	2.383	11.0	4.1	-2.625	*Q. dentata*
3	5.383	0.661	-	4.3	-1.457	Hybrid (d-c)
4	5.213	1.314	5.0	6.0	-0.960	*Q. dentata*
5	4.984	1.929	8.3	4.6	-2.639	*Q. dentata*
6	4.829	2.530	5.4	4.5	-3.806	*Q. dentata*
7	4.491	2.546	9.4	4.3	-2.661	*Q. dentata*
8	4.395	0.848	-	4.1	-0.258	Hybrid (d-c)
9	4.268	2.202	7.9	6.2	-2.613	*Q. dentata*
10	4.143	2.835	10.4	7.3	-4.220	*Q. dentata*
11	3.770	1.779	-	5.0	-3.713	*Q. dentata*
12	2.960	1.025	4.0	5.6	-3.586	Hybrid (d-c)
13	2.877	0.318	4.4	4.6	-2.245	Hybrid (d-c)
14	2.004	0.515	2.0	3.9	-	Hybrid (d-c)
15	1.246	0.926	3.5	6.0	-3.056	Hybrid (d-c)
16	1.092	-0.238	3.3	4.9	1.360	Hybrid (d-c)
17	1.003	0.497	3.2	3.6	0.970	Hybrid (d-c)
18	0.415	-0.021	1.6	3.4	0.392	*Q. crispula*
19	-0.530	-0.274	1.9	1.2	1.140	*Q. crispula*
20	-0.603	0.539	-	3.7	1.838	*Q. crispula*
21	-0.755	-1.191	2.9	3.7	1.708	*Q. crispula*
22	-0.805	-1.104	-	4.7	2.309	*Q. crispula*
23	-0.830	-0.387	-	3.6	3.177	*Q. crispula*
24	-1.067	-1.439	1.6	5.7	1.502	*Q. crispula*
25	-1.075	-0.251	1.8	5.5	3.018	*Q. crispula*
26	-1.218	-1.033	2.2	2.8	1.171	*Q. crispula*

27	-1.313	-0.018	2.9	5.9	1.465	*Q. crispula*
28	-1.809	-0.106	2.9	3.8	2.028	*Q. crispula*
29	-1.822	-0.795	1.4	7.9	2.972	*Q. crispula*
30	-1.847	1.008	1.4	3.3	2.242	Hybrid (c-s)
31	-1.890	0.324	1.6	5.6	1.687	*Q. crispula*
32	-1.952	-1.507	1.3	4.8	1.319	*Q. crispula*
33	-2.405	0.536	1.2	10.0	1.213	Hybrid (c-s)
34	-2.419	2.366	1.1	4.5	1.311	Hybrid (c-s)
35	-3.407	1.058	1.3	8.2	2.864	Hybrid (c-s)
36	-4.272	5.684	1.0	17.5	0.937	*Q. serrata*

Table 4 Leaf traits of trees at the scaffolding-system sites in Tomakomai. See Table 2 for abbreviations

Tree No.	Area (cm^2)	No. of lobes	LMA (mg/cm^2)	L/W	DS	LS	SO	SH
1	165.5	13.7	7.08	1.5	4.2	428.0	0	1
2	165.7	14.6	8.05	1.3	6.4	328.0	0	1
3	233.5	17.6	5.60	1.2	1.7	339.6	0	1
4	206.8	14.6	8.20	1.4	5.2	174.4	0	1
5	141.1	14.9	6.64	1.4	3.7	421.0	1	1
6	148.1	14.9	7.56	1.7	7.7	330.3	0	1
7	109.1	16.7	8.12	1.7	8.1	374.5	0	1
8	122.1	14.9	5.58	1.7	1.6	362.9	0	1
9	100.8	14.0	8.04	1.6	7.0	279.1	0	1
10	121.6	14.9	7.00	1.5	8.6	346.6	0	1
11	138.9	15.2	5.46	1.6	5.6	337.3	0	1
12	140.3	14.7	4.67	1.5	4.1	255.9	0	1
13	105.8	17.6	8.15	1.7	4.6	155.8	0	1
14	179.0	19.5	5.45	1.4	1.5	246.6	1	0
15	127.7	19.7	6.28	1.7	8.2	165.1	0	1
16	106.7	19.0	5.80	1.5	3.2	181.4	1	1
17	58.7	18.0	7.38	1.6	6.7	114.0	0	1
18	121.5	19.1	5.62	1.4	1.6	144.2	1	0
19	74.7	19.4	5.35	1.5	1.4	141.9	1	0
20	53.4	17.8	6.01	1.7	7.3	104.7	1	1
21	86.3	21.7	4.85	1.9	2.9	137.2	1	1
22	51.6	23.0	6.79	2.1	0.8	144.2	1	0
23	94.3	19.4	4.64	1.4	1.4	118.6	1	0
24	86.4	24.9	6.48	1.9	0.8	116.3	1	0
25	46.5	18.9	5.09	1.8	5.1	118.6	1	1
26	59.9	20.4	5.20	1.5	0.1	100.8	1	0
27	89.5	22.4	6.01	1.7	4.6	109.3	1	0
28	54.9	20.9	5.16	1.8	3.8	123.3	1	0

29	40.8	23.9	6.93	2.1	3.4	100.0	1	0
30	61.1	22.0	6.32	1.7	8.0	121.0	1	0
31	73.4	21.9	5.33	1.5	5.6	116.3	1	0
32	67.0	23.4	4.79	1.8	0.2	112.4	1	0
33	47.8	21.0	5.20	2.1	6.6	116.7	1	0
34	62.4	23.8	7.43	1.5	17.2	88.4	1	1
35	53.9	24.3	5.00	1.9	9.9	136.1	1	0
36	39.6	19.6	5.68	2.2	24.5	138.9	1	0

Table 5 Number of *Phyllonorycter* and *Tuberculatus* individuals collected from trees at the scaffolding-system sites in Tomakomai. AC: *P. acutissimae,* SI: *P. similis*, CR: *P. cretata,* MO: *P. mongolicae, PS: P. pseudolautella,* MA: *P. matsudai,* PE: *P. persimilis*, LE: *P. leucocorona*, NI: *P. nigristella,* PA: *T. paiki*, YO: *T. yokoyamai,* KA: *T. kashiwae,* JA*: T. japonicus,* PI*: T. pilosus.* Host selection index (*HSI*) is also given (see text for explanation of *HIS*)

Tree	*Phyllonorycter*									*Tuberculatus*				
No.	AC	SI	CR	MO	PS	MA	PE	LE	NI	PA	YO	KA	JA	PI
1	1						17	4	4	21			1	
2	1						21	3	4	20	1	1		
3		1	2	2	1		18	9	2	1		7		
4	1						5	5		2				
5							17	9		21	1			
6		1					15	13	7	22				
7							7	4	8	16		1	1	
8	3	6		9	1		4	4	5			3		
9	1						11	8	3	20				
10							28	11	6	23	1		1	
11							27	5	9	15			1	
12	1	1					21	12	6	19		2		
13							15	8	3	6				
14	9	5	4	1			2	1	2					
15			1			1	24	6	7	16	2	1	2	
16	13	11	3	6			11		1	3	12	6	3	
17	3	1	10	3			1					1		
18	1	3		3	1		1				4	2		
19	3	7	4	1			1		3		7	14		
20		10		12	1		1				7	15		
21	2	8	3	12			3			1		21	1	
22	1		2	19	1	1	1				15	13	1	
23		8	6	3	14						4	31		
24	1	10		7						1	1	20		
25	4	5	8	13	4	1	1				6	16		

26	1	4		6							14	6		
27	2	6	3	9						2	11	7	1	
28	1	10		15							1	22		
29	2	17	4	7	3	1					12	10		
30	1	17	3	7		1			1	2	7	11		
31	6	13	2	19			2	3		11	8	5		
32	2	6	2	1			1				18	4		
33		8		4			3				9	14		
34	2	9	2	7							5	5		
35		18		4	6						2	22		3
36		8		1			5				16	5		
HSI	0.5	-1	-0	-1	-1	-1	3.6	4	3.7	3.84	-1.4	-1.1	1.81	-

Caption to Figure

Fig 1. Distribution of canonical variate scores (CV1 and CV2) in the discriminant analysis on eight leaf traits (see text for details). (A) Trees in reference populations; closed circles = *Quercus dentata* from Nakaotofuke, closed triangles = *Q. crispula* from Hamamasu and Toishiyama, closed squares= *Q. serrata* from Aoba Park. (B) Trees in Tomakomai Experimental Forest; closed circles = trees at the scaffolding-system sites, open circles = trees on the transects. Trees were tentatively identified based on the present results (see text and Table 3). Broken-line ovals: the distribution ranges of CV1 and CV2 in reference populations (see panel A)

Fig 2. Distribution of principal component scores (PC1 and PC2) in the analysis on *Phyllonorycter* and *Tuberculatus* compositions of 35 trees at the scaffolding-system sites. Trees were tentatively identified based on the present results (see text and Table 3)

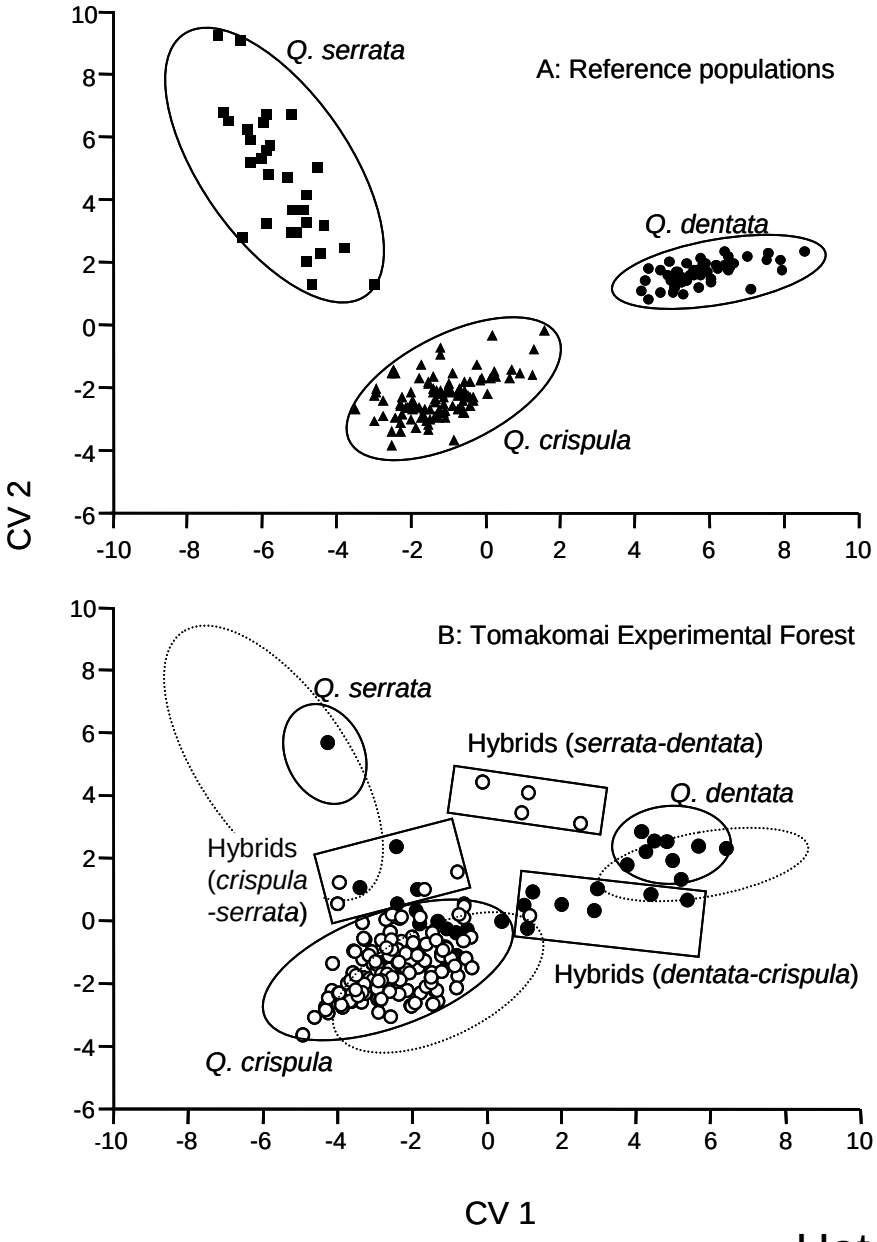
A: Reference populations
Q. serrata
Q. dentata
Q. crispula
B: Tomakomai Experimental Forest
Q. serrata
Hybrids (serrata-dentata)
Q. dentata
Hybrids (crispula -serrata)
Hybrids (dentata-crispula)
Q. crispula
CV 1
CV 2

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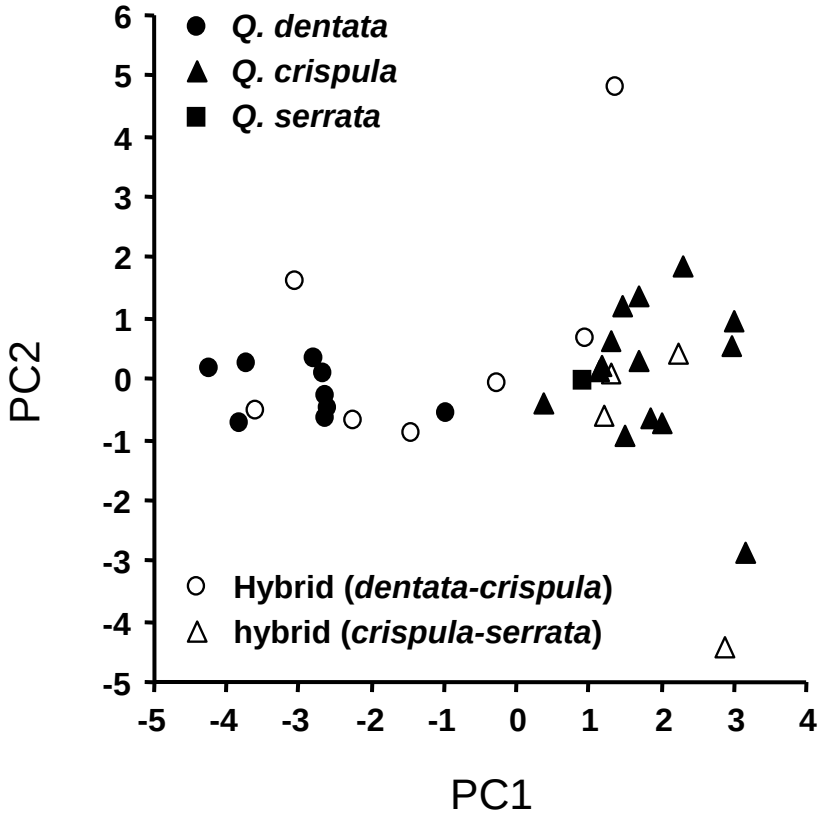
Fig. 2



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