Patterns of occurrence of hybrids of Castanopsis cuspidata and C. sieboldii in the IBP Minamata Special Research Area , Kumamoto Prefecture , Japan

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journal or publication title	植物地理・分類研究 = The journal of phytogeography and toxonomy
volume	51
number	1
page range	63-67
year	2003-06-25
URL	http://hdl.handle.net/2297/48538

Journal of Phytogeography and Taxonomy **51** : 63-67, 2003

Satoshi Kobayashi and Shozo Hiroki : **Patterns of occurrence of hybrids of *Castanopsis cuspidata* and *C. sieboldii* in the IBP Minamata Special Research Area, Kumamoto Prefecture, Japan**

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Castanopsis cuspidata (Thunb.) Schottky and *C. sieboldii* (Makino) Hatus. ex T. Yamaz. et Mashiba are dominant components of the evergreen broad-leaved forests of southwestern Japan, including parts of Honshu, Kyushu and Shikoku but excluding the Ryukyu Islands (Hattori and Nakanishi 1983). Although these 2 *Castanopsis* species are both climax species, it is very difficult to distinguish them because of the existence of an intermediate type (hybrid). *Castanopsis cuspidata* and *C. sieboldii* were regarded to be varieties in the same species until it was decided that *C. sieboldii* was a variety of *C. cuspidata* (Nakai 1939 ; Yamanaka 1966). However, Yamazaki and Mashiba (1987 a, b) classified the 2 trees as separate species on the basis of differences in nut shape and leaf epidermis. *Castanopsis cuspidata* has a single-layered leaf epidermis and bears small, globular nuts, whereas *C. sieboldii* has a 2-layered leaf epidermis and bears large, oblong nuts (Yamazaki and Mashiba 1987 a).

A type of nut or wood morphology that is intermediate between the 2 species is recognized (Kobayashi and Sugawa 1959 ; Yamanaka 1966 ; Yamazaki and Mashiba 1987 a). On the basis of its wood anatomy, Kobayashi and Sugawa (1959) suggested that the intermediate type was a hybrid. However, Yamanaka (1966) considered that the intermediate type was merely an intraspecific variation. By comparing its nut shape with the typical nut shape of each species, Hiroki and Ichino (1991) showed that individuals with the intermediate type of nut that were growing around temples and shrines in the Chubu District were hybrids of the 2 species. However, it is difficult to identify the hybrids by nut morphology alone, because the nut shapes of the 2 species are variable and can overlap with each other. Kobayashi et al. (1998) showed that hybrids have a chimeric structure of both 1 and 2 epidermal layers within a leaf. These morphological differences among *C. cuspidata, C. sieboldii* and their hybrid can be confirmed by genetic differences in nuclear species-specific markers (paper in preparation by the authors). Therefore, the morphology of the leaf epidermis is a useful key characteristic for the identification of *C. cuspidata, C. sieboldii* and their hybrid.

Hiroki and Ichino (1991) reported that hybrids occurred in forests around the temples and shrines of the Mikawa District of Aichi Prefecture and suggested that this hybridization was caused by the planting of *C. sieboldii* in the *C. cuspidata* distribution area. In 2001 we suggested that, in the coastal areas of the Chubu District, hybrids may be growing in natural forests because *C. sieboldii* coexists with *C. cuspidata* in these areas, although this hybrid origin is questionable because of uncertainty about the origin of the distribution of *C. sieboldii* (Hiroki and Kobayashi 2001).

To conduct a thorough examination of hybridization in natural forests, we chose the *Castanopsis* population in the Minamata Special Research Area of the International Biological Program (IBP), Kyushu. Although Tagawa (1973, 1979) reported that only *C. cuspidata* is present in this area, we had received information that *C. sieboldii* also exists there (personal communication by Tagawa and Okubo in 1999). We eventually

Table 1. The number of individuals of *Castanopsis cuspidata*, cuspidata, *C. sieboldii* and hybrids found in the investigated area

	Castanopsis cuspidata	*Castanopsis siboldii*	Hybrid	total
No. of individuals	147	105	64	316

found that both *C. cuspidata* and *C. sieboldii* are distributed in the area (Omura et al. 1978). Therefore, we expected hybrids to be present too.

We also intended to investigate the distribution of the 2 species to see whether the hybrid population occurred only in the region where the 2 species overlapped.

Study site and methods

In October 1999, we sampled leaves of *Castanopsis* from 316 individuals from the foothills to the hilltops of the IBP Minamata Special Research Area (about 12 ha) (lat 32°30′ N, long 131°35′ E, 400–642 m a.s.l.) at Minamata in Kumamoto Prefecture, where mature forests remain in their natural state. In this area *Castanopsis* dominates the tree layer, accompanying *Cyclobalanopsis gilva*, *C. salicina* and *Machilus japonica*, with *Eurya japonica* in the shrub layer (Omura et al. 1978).

The sampled leaves were transversely cross-sectioned (30-μm thickness) with a plant microtome at the widest point of the leaf. The leaf epidermis was observed under a microscope (10×20 and 10×40). Individuals with a leaf epidermis of 1 layer, 2 layers, and both 1 and 2 layers were identified as *C. cuspidata*, *C. sieboldii* and the hybrid, respectively. All individuals sampled were recorded on a topographic map (1 : 5000).

Results and discussion

From our examination of the leaf epidermis, we identified 147 plants as *C. cuspidata*, 105 as *C. sieboldii* and 64 as hybrids (Table 1). Individuals with a chimeric structure of both 1 and 2 epidermal layers within a leaf were identified as

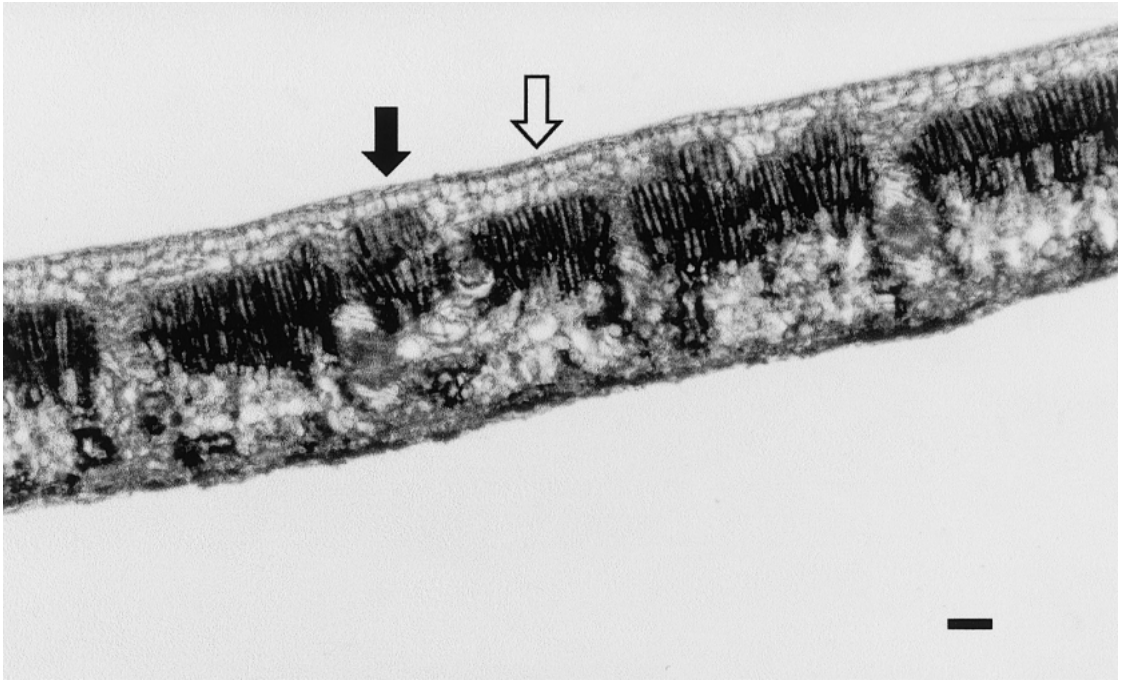

Fig. 1. Leaf epidermis of a hybrid between *Castanopsis cuspidata* and *C. sieboldii*. Black arrow indicates 1–layered leaf epidermis ; white arrow points to 2–layered leaf epidermis. Bar = 20 μm.

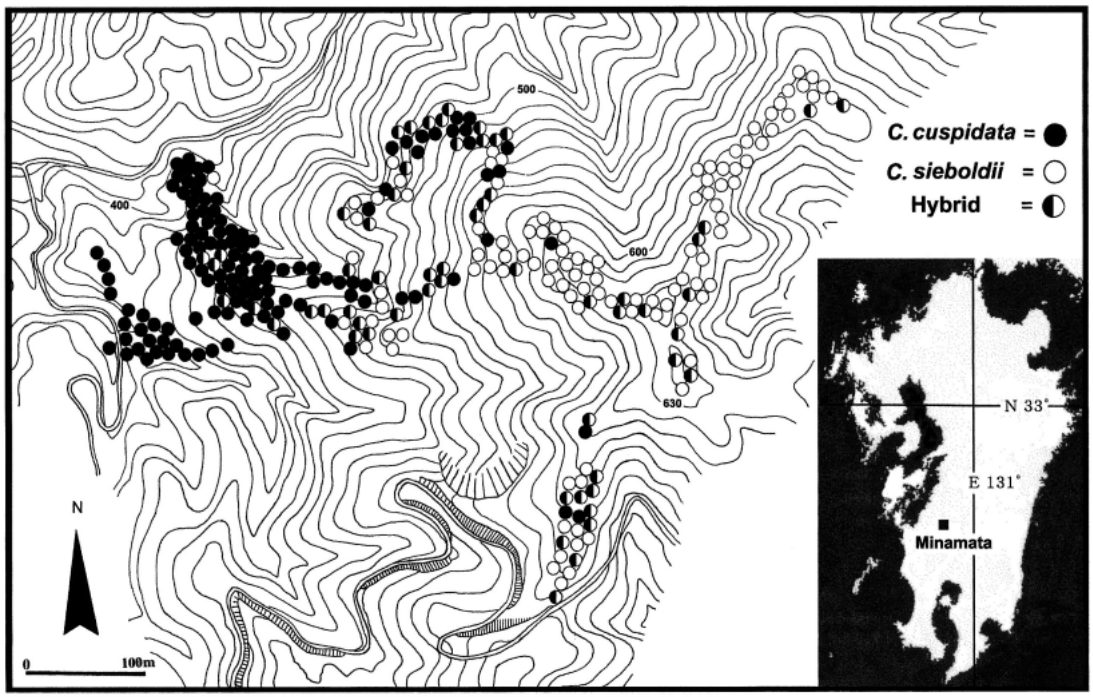

Fig. 2. Distribution of *Castanopsis cuspidata*, *C. sieboldii* and their hybrids in the IBP Minamata Special Research Area.

hybrids (Fig. 1). These results confirm the existence of *C. sieboldii* in the Minamata Special Research Area, as already recorded by Omura et al. (1978), although Tagawa (1973, 1979) referred only to the presence of *C. cuspidata*. Our results also show that hybrids occur in the inland areas of Kyushu where the 2 *Castanopsis* species coexist. Coexistence of *C. cuspidata* and *C. sieboldii* is common in southern Kyushu (Sako and Saida 1990), so one would expect hybridization of the 2 species to occur widely.

Although we demonstrated that hybrids occur in the natural forests of Kyushu, hybrids are largely due to human influence in inland Chubu. This is because *C. sieboldii* is not naturally distributed in inland Chubu (Inami 1966), and planted *C. sieboldii* are needed for hybrid formation (Hiroki and Ichino 1991). Yamada and Nishimura (2000) reported the coexistence of *C. cuspidata* and *C. sieboldii* in the forests around temples and shrines in and around Okayama Prefecture and referred to the existence of an intermediate type of leaf epidermis. We presume that also these hybrids originate through human influence for the same reason as described above.

In the Minamata Special Research Area, many *C. cuspidata* individuals were distributed on the lower slopes or in the valleys, and their distribution was sparser at higher altitudes (Fig. 2). In contrast, *C. sieboldii* occurred mainly in higher areas, such as on hilltops and ridges. These results are consistent with the reports of Yamanaka (1966, 1975) on Shikoku, in which *C. sieboldii* was found to be distributed inland at higher altitudes than *C. cuspidata*. In the Minamata Special Research Area, only *C. sieboldii* is distributed on the hilltops and ridges. We assume that *C. sieboldii* is more tolerant to drought stress or immature soils than *C. cuspidata*. Hiroki and Ichino (1998) suggested that *C. sieboldii* may be able to establish itself more successfully than *C. cuspidata* in such stressful habitats as hilltops and ridges because larger nuts give more rapid early growth.

Hybrid occurrence is not confined to the zone where *C. cuspidata* and *C. sieboldii* meet. This suggests a long history of hybridization of the 2 species.

Acknowledgments

We thank Dr. H. Tagawa (President of Kagoshima Prefectural College, Kagoshima) and Dr. K. Okubo (Shinshu University, Matsumoto) for providing us with information about the distribution of *Castanopsis* species in Minamata. We would also like to thank the late Mr. M. Nishioka (formerly Lecturer at Hyogo College of Medicine, Nishinomiya City) for acting as our guide in the IBP Minamata Special Research Area.

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(Received November 29, 2001; accepted January 27, 2003)

小林悟志・広木詔三：熊本県水俣市（IBP 調査地域内）におけるツブラジイとスダジイの雑種個体群の分布調査

天然林として知られる熊本県水俣市の IBP の調査区域内のシイ個体群は，ツブラジイのみの分布とされていたが，スダジイが分布しているとの情報を得た。本研究では，天然林においてツブラジイとスダジイの交雑種がどの程度存在しているかを明らかにするため，IBP 調査地域内の標高の低い山麓から標高の高い尾根にかけて，任意に 316 個体分の葉を採集した。葉の表皮組織の判別による結果，ツブラジイ（147 個体）は，標高の低い山麓部に多く存在し，スダジイ（105 個体）は，山頂や尾根部に多く存在し，雑種個体（64 個体）は，山の中腹から尾根部にかけて分布していることが明らかになった。

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