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Preface to the 20th International Symposium on Integrated Field Science "Biodiversity and Phylogeography"

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Biodiversity is becoming more important in biology and agriculture and is a common issue for humankind. One of its research fields is phylogeography, which attempts to elucidate the historical process of the geographical distribution of modern individuals, populations, and species based on the analysis of genetic genealogy patterns. In this international symposium, four invited talks by up-and-coming researchers and 12 posters with short oral presentations by young students were held under the broad theme of "Biodiversity and Phylogeography." The purpose of this symposium was not only to share the latest research information on the topic but also to provide the "diverse" participants with valuable presentation and discussion opportunities on the international stage, which can be utilized in the future.

I sincerely hope that this international symposium will contribute to the understanding and conservation of global biodiversity, the development of phylogeography, and the future of the participants.

Symposium mini review

A Comprehensive Comparison of Flower Morphology in the Genus *Camellia*, with a Focus on the Section *Camellia*

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Abstract

This study compared floral traits between the bird-pollinated sect. *Camellia* and other sections within the genus *Camellia* to investigate the relationship between floral traits and shifts in pollinators. In the cluster analysis of floral traits, bird-pollinated species in sect. *Camellia* (red camellias) and yellow camellias were found to form the same clade. These traits are possible adaptations for bird pollination. Yellow camellias are smaller in size than flowers in sect. *Camellia*. While sect. *Camellia* mainly relies on Japanese white-eyes, which suck nectar by hooking their feet on the petals for pollination, yellow camellias have adapted to hovering bird pollinators, which require a different set of characteristics. Interestingly, *C. rusticana*, which is the only insect-pollinated species within sect. *Camellia*, and sect. *Theopsis*, which includes insect-pollinated white flowers, were included in the same clade. Our results revealed a high level of uniformity in floral traits among species within sect. *Camellia*. A comparison of these findings with those of previous molecular phylogenetic studies revealed that the rapid diversification of sect. *Camellia* may not be attributed to a common ancestor. Instead, it may be attributed to different lineages influenced by environmental factors. To gain a more comprehensive understanding, it is essential to conduct a thorough quantitative analysis of the morphological traits within the *Camellia* genus. This analysis should also involve a reassessment of their relationship with molecular phylogeny, as molecular data can provide valuable insights into the evolutionary history and relationships among *Camellia* species.

Introduction

The family Theaceae is known to comprise approximately 28 genera and 600 species that are mainly distributed in moist temperate regions worldwide (Iwatsuki, 2006). Among them, the genus *Camellia* is distributed in East and Southeast Asia and consists of approximately 125 species (Ming and Zhang, 1995). Sect. *Camellia*, which is the focus of this study, is characterized by large red flowers that bloom in winter. Various traits, such as shape, size, color, reward to pollinators (e.g., amount and quality of nectar), and timing of flowering, are important characteristics of these species that attract pollinators and are specialized according to the pollination syndrome (Faegri and van der Pijl, 1979). The bird pollination

syndrome involves the presence of large flowers, red or orange colors, and abundant and thin nectar. The characteristic red petals in sect. *Camellia* can be considered a bird pollination syndrome trait.

Early classifications of *Camellia* were primarily based on morphological differences in leaves, flowers, and fruits (e.g., Sealy, 1958; Chang, 1998; Ming, 1999; Ming, 2007). Recently, these classifications have been revised using molecular phylogenetic analyses (e.g., Rao *et al*., 2018; Zhang *et al*., 2019; Cheng *et al*., 2022; Wu *et al*., 2022; Yan *et al*., 2021). According to these analyses, the ancestral sections in the subgenus *Camellia* are sect. *Furfuracea*, sect. *Thea*, sect. *Glaberrima*, and sect. *Longissima*; they are characterized by white petals and a floral fragrance, which are adaptations

to insect pollinators. The results of molecular phylogenetic studies suggest that the major clades of *Camellia* radiated in the late Miocene along with the formation of subtropical evergreen broad-leaved forests in East Asia (Shengyuan *et al.*, 2023; Zan *et al.*, 2023). The polymorphism of flower color and size within the genus *Camellia* is thus thought to be a result of adaptation to pollinators (pollinator shifts). Moreover, it is predicted that the ancestral sect. *Camellia* with insect pollination traits differentiated into sect. *Camellia* with bird pollination traits. However, the relationship between molecular phylogeny and floral traits is not always straightforward, as some ancestral *Camellia* sections, such as sect. *Corallina* and sect. *Brachyandra*, have yellow or pink petals and are pollinated by bees or sunbirds (Sun *et al.*, 2017). Therefore, the relationship between molecular phylogeny and floral traits is more complex than considered previously. While some studies have attempted to examine the relationship between morphology and molecular phylogeny (Zan *et al.*, 2023), more quantitative assessments and comparisons between floral traits and pollination strategies are required.

The complex relationship between molecular phylogeny and floral traits is exemplified in two species of sect. *Camellia* that are distributed in Japan. *C. japonica* and *C. rusticana*, which belong to sect. *Camellia*, have characteristic large red petals, suggesting that they are bird-pollinated species. Although *C. japonica* is a bird-pollinated species (e.g., Yumoto, 1988; Abe *et al.*, 2008; Abe *et al.*, 2011), a different pollination system involving insects has been reported in *C. rusticana* (Ishizawa, 1988). The floral traits of *C. rusticana*, except for flower color, are characteristic of insect pollination (e.g., Ishizawa, 2005; Abe and Miura *et al.*, 2020).

Therefore, in this study, we comprehensively compared floral traits, with a focus on sect. *Camellia* which exhibits bird pollination traits. We also compared white-flowered species with insect pollination traits and yellow-flowered species with bird pollination traits. Our study assessed the systematic position of sect. *Camellia* and its relationship with pollinator shifts based on floral traits and their relationship with existing molecular phylogenies.

Materials and Method

A total of 27 *Camellia* species, primarily belonging to sect. *Camellia*, were used in this study. Of these, 25 species were collected from the greenhouse of Inokuchi Tsubaki Kan in Nanto City, Toyama Prefecture. In addition, data from two species, *C. japonica* and *C. rusticana*, which grow naturally in Niigata Prefecture and were used in the study of Abe and Miura *et al.* (2020), were included in our study (Table 1). The breakdown was as follows: 15 species from sect. *Camellia*, two species from sect. *Paracamellia*, one species from sect. *Oleifera*, one species from the sect. *longipedicellata*, four species from sect. *Theopsis*, and four yellow species from sect. *Chrysantha*. Of these, 15 species had red flowers (sect. *Camellia*), eight had white flowers (sect. *Paracamellia*, sect. *Oleifera*, sect. *Longipedicellata*, and sect. *Theopsis*), and four had yellow flowers (sect. *Chrysantha*) (Table 1). To assess the differences in flower morphology of *Camellia* species, quantitative measurements of the flower diameter, corolla length, petal length, maximum stamen length, and stamen attachment rate (length of stamen attachment/maximum stamen length) were performed (Fig. 1). Corolla and petal lengths were excluded from the measurement items because of large differences between flowers. Measurements were performed by disassembling the flowers into as many parts as possible. For the measurements, three flowers were collected from each species at the greenhouse of Inokuchi Tsubaki Kan from January to March 2018. For *C. japonica* and *C. rusticana*, data for one flower out of 52 and 59 individuals, respectively, were used from the study of Abe and Miura *et al.* (2020). One-way ANOVA was performed for each measurement item related to flower diameter, stamen length, and stamen attachment rate, and significant differences in the mean values among groups were calculated using Tukey's method. Furthermore, the obtained measurement values were standardized, and cluster analysis was performed using the squared Euclidean distance and Ward's method. R version 3.2.3 (R Core Team 2015) was used for statistical analysis.

Results

Although no significant trend was noted in the measured values among species (Table 1), some trends were observed depending on flower color (Figs. 2–3). The flower diameter tended to be larger in sect. *Camellia* and smaller in sect. *Chrysantha*, indicating a high uniformity within the sections. However, in species with white flowers, the flower diameter of sect. *Paracamellia* and sect. *Oleifera* was larger, while that of sect. *Theopsi*s and sect. *Longipedicellata* was smaller (P < 0.05, Tukey's test; Fig. 2, Table 1). In particular, *C. vietnamensis* belonging to sect. *Oleifera* and *C. yuhsienensis* belonging to sect. *Paracamellia* had a significantly larger flower diameter. Regarding stamen length, sect. *Camellia* and sect. *Chrysantha* tended to have longer stamens. In contrast, all species with white flowers tended to have shorter stamens. Furthermore, in sect. *Camellia*, only *C. rusticana* had a significantly shorter stamen length (P < 0.05, Tukey's test; Fig. 2, Table 1). No trends were observed among sections in terms of the anther filament cohesion rate, while variation was noted among species (Fig. 2, Table 1).

In the cluster analysis, the five sections and 27 species analyzed in this study were divided into two large clades (Fig. 3). Clade 1 consisted of *C. grijsii* in sect. *Paracamellia*, sect. *longipedicellata* and sect. *Theopsis*, as well as *C. rusticana* belonging to sect. *Camellia*. Clade 2 consisted of sect. *Camellia*, sect. *Chrysantha*, sect. *Oleifera*, and *C. yuhsienensis* belonging to sect. *Paracamellia*. The sections within clade 2 were further divided into two subclades. Sect. *Camellia* and sect. *Chrysantha* were found to be similar in terms of flower morphology.

Discussion

Diversification of floral traits

As shown in the results, the floral traits of the bird-pollinated species, namely sect. *Camellia* (red camellias) and yellow camellias, were found to belong to the same clade. Between red- and yellow-flowered species, although the flower size tended to be larger in sect. *Camellia*, the stamens were longer with a higher degree of cohesion between filaments and a structure that made it easy to accumulate nectar. A comparison of flower size and stamen length indicated that

Table 1. Comparative results of floral morphology of the genus *Camellia*

Section	Petal color	Species	No. of samples	length of flower diameter (mm)			stamen length (mm)			Rate of filament coalescence (%)			Location and Data ref.
				Mean	±SD	SD	Mean	±SD	SD	Mean	±SD	SD	
Chrysantha	Yellow	*C. longzhouensis*	3	40.90	1.21	cdh	39.60	1.25	ab	51.17	0.04	bgh	Inokuchi Tsubaki Kan
		C. chrysantha var. phaeopubisperma	3	48.80	5.63	bcdh	39.00	3.58	ab	57.61	0.07	ag	
		C. quephongensis	3	38.93	3.33	cdh	32.97	0.86	acd	44.63	0.06	fghi	
		C. cucphuongensis	3	36.67	3.66	dh	33.13	2.97	acd	39.26	0.00	gj	
Theopsis	White	*C. lutchuensis*	3	27.53	5.53	gh	14.20	1.22	gh	45.73	0.04	efghi	
		C. transnokoensis	3	41.03	1.76	cdh	20.70	0.75	eh	40.63	0.02	gj	
		C. trichoclada	3	23.00	1.14	h	11.53	1.12	h	24.93	0.10	jk	
		C. fraterna	3	32.87	1.35	fh	19.87	0.32	eh	56.53	0.02	agh	
Longipedicellata		*C. longicarpa*	3	33.73	2.31	efh	23.87	0.96	defg	63.00	0.04	abcdf	
Oleifera		*C. vietnamensis*	3	96.90	3.83	a	18·77	0.49	fh	17.00	0.02	k	
Paracamellia		*C. yuhsienensis*	3	96.73	3.50	a	13.53	0.61	gh	60.50	0.05	abcdf	
		C. grijsii	3	61.93	9.10	bcdf	11.87	0.78	h	38.11	0.06	gj	
Camellia	Red	*C. brevigyna*	3	64.60	8.49	bcd	29.70	1.92	abef	64.65	0.03	abcde	
		C. lungshenensis	3	61.27	7.83	bcdf	32.93	2.45	acd	72.19	0.03	a	
		C. bailinshanica	3	62.40	1.74	bcdf	34.07	1.91	acd	70.48	0.01	ab	
		C. brevicolumns	3	66.30	7.48	bcd	36.50	2.65	ac	72.43	0.05	a	
		C. kweichowensis	3	60.10	0.36	bcdf	31.07	1.29	abe	50.06	0.02	cgh	
		C. villosa	3	61.43	6.70	bcdf	34.43	0.38	acd	46.95	0.00	dghi	
		C. polyodonta	3	63.23	7.78	bcde	26.23	1.27	cef	40.51	0.02	gj	
		C. saluenensis	3	59.87	12.32	bcdf	30.60	2.52	abe	67.38	0.05	abc	
		C. lapidea	3	53.43	5.45	bcdfg	33.17	3.76	acd	36.91	0.09	hj	
		C. semiserrata	3	54.33	9.76	bcdfg	40.60	5.21	a	53.91	0.03	agh	
		C. pitardii var. pitardii	3	68.17	3.32	ac	33.73	1.96	acd	66.43	0.09	abed	
		C. apolyodonta	3	62.77	6.66	bcdf	26.07	1.70	cef	39.37	0.02	gj	
		C. chekiangoleosa	3	78.60	8.69	ab	28.33	2.24	bcef	37.91	0.04	gj	
		C. japonica	52	52.25	12.66	cdf	34.59	4.58	ab	62.50	0.07	abc	Abe and Miura et al. (2019)
		C. rusticana	59	60.48	10.98	bc	15.97	2.71	gh	33.86	0.07	ij	

±SD indicates standard deviations. One-way ANOVA was performed for each measurement item, and significant differences (SD) in the mean values among groups were calculated using Tukey's method ($P < 0.05$). Non-capital letters indicate statistical (in)significance in a concise way: if treatment groups share the same non-capital letter, then the differences between the groups are not statistically significant.

sect. *Camellia* had a larger and more homogeneous flower size. These traits can be predicted to be adaptations to bird pollination, as hypothesized. The stamen length may also be an adaptation to pollination by larger birds rather than insects. In addition, *C. rusticana* belonging to sect. *Camellia* and species belonging to sect. *Theopsis* with white flowers, known as insect-pollinated species, were included in the same clade. Assessment of the characteristics of yellow camellias revealed that the flower size was smaller than that of sect. *Camellia* (red camellias) but larger than that of white camellias. Moreover, the stamen was as long as that in sect. *Camellia*. According to Sun *et al.* (2017), *C. petelotii*, which belongs to sect. *Chrysantha* (yellow camellias) with yellow petals, is pollinated by small birds called sunbirds (Nectariniidae). Although sunbirds are slightly smaller than the Japanese white-eye (the main pollinator of *C. japonica*), they hover to suck nectar instead of perching on branches or petals. While sect. *Camellia* (red camellias) is mainly pollinated by the Japanese white-eye (*Zosterops japonicus*) and brown-eared bulbul (*Hypsipetes amaurotis*) that perch on branches or petals to suck nectar, yellow camellias are pollinated by hovering birds and do not require a large flower size. However, the stamen has likely lengthened to facilitate pollination by birds. Regarding the homogeneity of sect. *Camellia*, it is suggested to have diverged relatively quickly from the ancestral lineage of white-flowered species. This will be discussed in the following section.

Relationship between molecular phylogeny and floral morphology

In this section, we discuss that the homogeneity of floral traits in sect. *Camellia* is caused by rapid evolution resulting from pollinator shifts for birds, based on the results of previous molecular phylogenetic analyses. Moreover, we discuss whether the ancestral insect-pollinated *C. rusticana* is phylogenetically ancestral or a new species.

As per previous studies on the molecular phylogeny of the genus *Camellia*, the diversification of sect. *Camellia* occurred in the following order in ancient times: the origin of the genus *Camellia* dates back to 39.5 million years ago, with rapid diversification of the sections having occurred around 20–30 million years ago (Zhao *et al.*, 2022). Other studies have reported that diversification occurred around
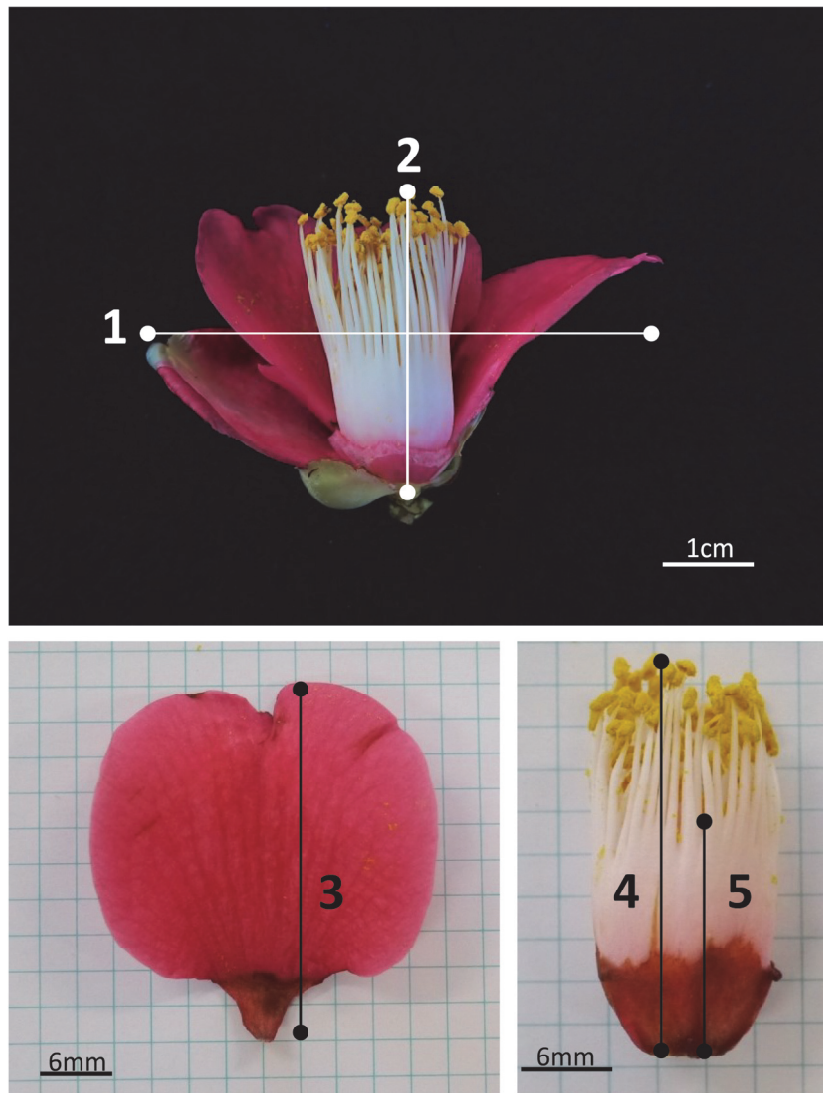
Fig. 1: Measurement of flower morphology
(1) length of flower diameter, (2) height from petal base to corolla, (3) petal length, (4) stamen length, and (5)/(4) Rate of filament coalescence
(2) and (3) were not used for analysis due to significant variations within individuals. The photographs are *Camellia japonica*.

25 million years ago (Cheng *et al*., 2022); radiation of major clades occurred during the late Miocene around 23–19 million years ago, followed by the occurrence of a species burst between 10 and 5 million years ago (Zan *et al*., 2023); diversification occurred around 13 million years ago (Zhang *et al*., 2022); diversification occurred during the middle to late Miocene (around 13.70–12.42 million years ago) in the genus *Camellia* (Shengyuan *et al*., 2023); diversification of sections occurred around 12 million years ago (Zhang *et al*., 2014); and diversification of sect. *Camellia* and sect. *Oleifera* and related sections in this study peaked around 6–7 million years ago in the late Miocene after section diversification began around 10.54 million years ago (Wu *et al*., 2022). Although the origin and diversification of the sections occurred from 6 to 30 million years ago and in the Miocene epoch, respectively, they generally fall within the Miocene epoch.

When summarizing previous studies on the molecular phylogeny of sect. *Camellia* and its closely related sections, the divergence of sect. *Paracamellia* is estimated to have occurred 19 million years ago (Zhao *et al*., 2022) and that of

C. oleifera belonging to sect. *Oleifera* is estimated to have occurred 5.88 million years ago (Wu *et al*., 2022). Moreover, in the study of Shengyuan *et al*. (2023), assessments using a molecular phylogenetic tree based on nuclear genes suggested that sect. *Oleifera* should be merged with sect. *Paracamellia* and that sect. *Paracamellia* branched off after *C. japonica*. These previous studies revealed that sect. *Camellia* did not undergo rapid speciation at a particular time compared to other sections. Instead, it underwent speciation during the late Miocene, similar to other species. Therefore, the floral uniformity of sect. *Camellia* is considered to be a convergent trait specialized for bird pollination. Personal communication with Abe has confirmed that not only *C. japonica* but also other species within the sect. *Camellia* are primarily visited by white-eyes and bulbuls. In this study, it was shown that despite being closely related to sect. *Camellia* in terms of molecular phylogeny, sect. *Paracamellia* was classified into a separate clade morphologically and is likely to be grouped with insect-pollinated species (Fig. 3) Thus, it can be concluded that species with insect pollination traits are not necessarily
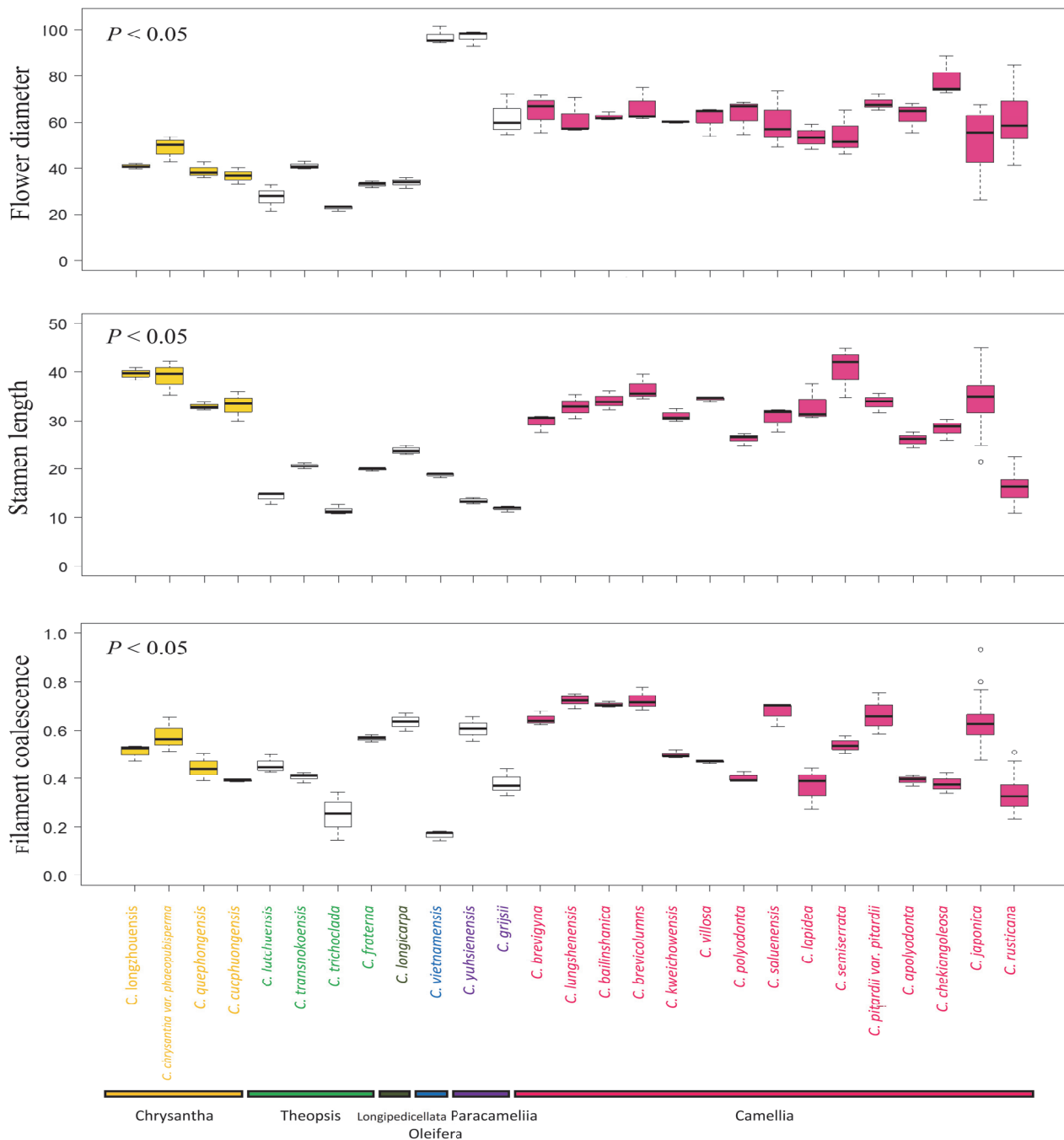
Fig. 2: Comparisons of flower diameter, stamen length and floral filament coalescence rates in the genus *Camellia*
The thick line inside the box represents the median. The bottom of the box corresponds to the first quartile, while the top of the box represents the third quartile. The whiskers extend to the farthest values within a distance of 1.5 times the interquartile range from the lower or upper quartile. Any data points outside this range are depicted as individual points on the graph and are considered potential outliers. The color of the box indicates floral color, and the color of the species name is categorized by section. The color scheme for each section is as follows: red for sect. *Camellia*, purple for sect. *Paracamellia*, blue for sect. *Oleifera*, green for sect. *Longipedicellata* and sect. *Theopsis*, and yellow for sect. *Chrysantha*.

ancestral in terms of molecular phylogeny. *C. chrysantha*, which belongs to sect. *Chrysantha* with yellow petals and is a bird-pollinated species, is classified in the subgenus *Thea* and is considered to be in a more ancestral position than the subgenus *Camellia*, which includes sect. *Camellia*, because of having diverged from the subgenus *Camellia* in the early to middle Miocene (Wu *et al*., 2022; Zhao *et al*., 2022). Similar to the subgenus *Camellia*, the diversification of yellow camellias with bird pollination traits occurred during the Miocene period; therefore, the pollination syndrome is considered to

have evolved independently rather than following the order of molecular phylogenetic evolution.

Despite its belonging to sect. *Camellia*, little molecular phylogenetic research has been conducted on *C. rusticana*, which is morphologically classified as a member of sect. *Theopsis* within the subgenus *Metacamellia* in this study. However, Vijayan *et al*. (2009) used nrITS sequences of 112 *Camellia* species and found that *C. chekiangoleosa* occupies a more ancestral position compared to *C. japonica* and *C. rusticana*, and is separated into two clades: the *C. rusticana*

Fig. 3: Result of cluster analysis on flower morphology in the genus Camellia
Flower diameter, stamen length, and rate of filament coalescence were measured for each of three individuals, and the obtained measurements were standardized for cluster analysis using the Euclidean square distance and Ward's method. The color scheme for each section is as follows: red for sect. *Camellia*, purple for sect. *Paracamellia*, blue for sect. *Oleifera*, green for sect. *Longipedicellata* and sect. *Theopsis*, and yellow for sect. *Chrysantha*.

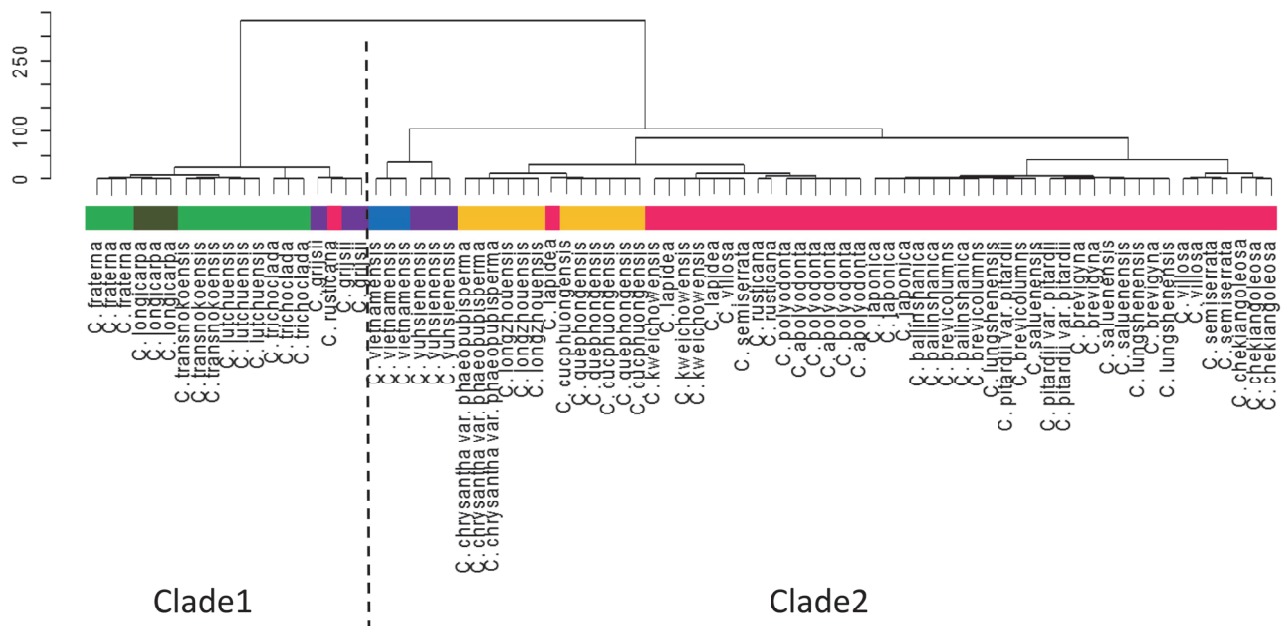clade (including *C. azalea* and *C. edithae*) and the *C. japonica* clade (including *C. fluviatilis* and *C. brevistyla*). According to molecular phylogenetic research examining *C. chekiangoleosa* and *C. japonica*, Zhao *et al.* (2022) suggested that *C. japonica* and *C. chekiangoleosa* are sister species, while Cheng *et al.* (2022) suggested that *C. azalea* belonging to sect. *Camellia* similar to *C. chekiangoleosa* forms a sister group and that *C. japonica* diverged from that clade 15 million years ago. In the study of Shengyuan *et al.* (2023), phylogenetic trees based on nuclear genes demonstrated that sect. *Oleifera* should be merged into sect. *Paracamellia* and that *C. japonica* is phylogenetically related to *C. chekiangoleosa*, which is ancestral (albeit with short branches). Yan *et al.* (2021) reported similar results. Based on chloroplast genome sequences, Wu *et al.* (2022) reported that *C. chekiangoleosa* diverged from *C. sasanqua* in sect. *Paracamellia*, following which *C. chekiangoleosa* split into two sister species: *C. japonica* and *C. oleifera*. Moreover, Rao *et al.* (2018) suggested that *C. chekiangoleosa* is ancestral and that the *C. japonica* clade (including *C. fluviatilis* and *C. brevistyla*) diverged from it. Based on these findings, it is highly likely that *C. chekiangoleosa* is ancestral among the three species and that it is related to *C. japonica*. *C. chekiangoleosa* has a lower stamen cohesion rate (Table 1) and can be considered more ancestral than *C. japonica*. However, *C. rusticana*, which has more insect pollination traits, was derived later than *C. chekiangoleosa*.

In summary, based on the results of our research on floral traits, the uniformity of traits in sect. *Camellia* is considered to have undergone rapid diversification in recent years. However, according to previous studies on molecular phylogeny, other sections also showed species diversification in the late Neogene period, similar to sect. *Camellia*. Thus, the results of molecular phylogenetic analyses have provided no clear indication regarding the uniformity of floral traits in sect.

Camellia.

How did the evolution of floral traits occur?

In this study, no correlation was noted between floral characteristics and molecular phylogeny. Rao *et al.* (2018) evaluated the environmental factors and evolutionary dynamics (speciation times and diversification rates) that form the diversity patterns of 145 species in the family Theaceae, consisting of the genus *Camellia*, based on chloroplast and nuclear ITS regions. They reported that the overall species diversity was significantly correlated with environmental variables, particularly soil pH. At the section level, the species diversity was correlated with the phylogenetic structure. Despite being in different sections, many species that are geographically (and environmentally) close to each other can form a clade together based on molecular phylogeny, indicating that environmental factors and patterns of species diversification are likely related. For example, studies focusing on species related to *C. japonica* revealed that *C. oleifera* in sect. *Oleifera*, which has a wide distribution in mainland China and widely cultivated, forms the same clade as *C. japonica* with a bootstrap value of 50 (while other species have a high independence of almost 100) (Zhang *et al.*, 2019; Wu *et al.*, 2022). According to the results of Rao *et al.* (2018), *C. chekiangoleosa* differentiated first, becoming an ancestral lineage. This was followed by the emergence of two clades: one consisting of *C. azalea* and *C. edithae* and the other consisting of *C. japonica*, *C. fluviatillis*, and *C. bravistyla*. The clade consisting of *C. japonica* is consistent with the findings of Vijayan *et al.* (2009). *C. fluviatillis* and *C. bravistyla* have regions where their distribution overlaps with that of *C. japonica*. Additionally, it clarifies that *C. fluviatillis* and *C. bravistyla* belong to sect. *Paracamellia*, while *C. japonica* belongs to a different section. According to the findings of Cheng *et al.* (2022), the clade that includes

C. japonica has a subclade consisting of *C. sasanqua* from sect. *Oleifera*, which is distributed in Japan and diverged from *C. japonica* around 13-14 million years ago. This is followed by a shorter branch of *C. fluviatillis*, as well as the subsequent differentiation of *C. cordifolia* (distributed in Taiwan, Guangdong, and Jiangxi) from sect. *Camelliopis* and *C. oleifera* from sect. *Oleifera*. Thus, the derivatives after *C. japonica* do not belong to sect. *Camellia*. There are other examples within the *C. japonica* clade that do not fall under sect. *Camellia*. Shengyuan *et al.* (2023) also demonstrated that sect. *Paracamellia* branched off later than *C. japonica*. Furthermore, it is difficult to clearly distinguish between sect. *Oleifera* and sect. *Paracamellia* (Wu *et al.*, 2022; Shengyuan *et al.*, 2023), as mentioned above. Based on molecular phylogenetic results, Yan *et al.* (2021) reported that the closest sister species to *C. japonica* is *C. lutchuensis*, classified under sect. *Theopsis*; it exclusively grows in the Ryukyu Islands and exhibits significant morphological differences. As mentioned above, in cases where species from different sections form the same clade as *C. japonica* or are derived from it, the species are often geographically close or overlapping. When species not only belong to a different section from *C. japonica* but also exhibit completely different morphologies, the influence of overlapping distribution and hybridization under wild conditions can be considered. Moreover, for species like *C. oleifera*, which have been widely cultivated for oil production or used as ornamental varieties, human influences should be considered along with geographical factors. Thus, when considering the correlation between environmental factors and molecular phylogeny, careful consideration of the influence of hybridization is necessary.

We considered speciation by a pollination shift to birds as an environmental factor in sect. *Camellia*. The genus *Camellia* is widely distributed across the temperate forests of mainland Asia, ranging from northern regions, such as Honshu in Japan, to southern regions, such as Southeast Asia. The center of diversity for this genus lies in southern China, which is also considered the core of the Sino-Japanese Floristic Region (SJFR). During the early to middle Miocene, East Asia experienced an increase in temperature and precipitation. The warm and humid climate, along with the intensified summer monsoon system in Asia (Sun and Wang, 2005), facilitated the widespread distribution of the genus *Camellia* (which consists of warm-temperate evergreen broad-leaved trees) throughout the SJFR (Yang *et al.*, 2016). These geological and climatic events likely played a significant role in influencing the speciation and diversification of the genus *Camellia*. The genus *Camellia* is known for its characteristic of flowering primarily during the winter season. Within this genus, sect. *Camellia* consists of species that are geographically distributed to the north. The diversification of sect. *Camellia* occurred during the Miocene, which was the warmest period from the Neogene to the present, characterized by generally warm climates and gradual cooling thereafter. During this cooling trend, species in the northern regions that were geographically distributed adapted from insect pollination, which is limited by the low presence of insects during winter, to bird pollination, which is facilitated by endothermic animals capable of winter activity. This advantageous trait, possibly resulting from the spread of beneficial mutations within the population, could

have led to the evolution of sect. *Camellia*.

In summary, species belonging to the genus *Camellia* with bird pollination traits are not believed to have rapidly diversified from a common ancestor. Instead, they may have originated from different lineages because of environmental conditions, such as climate. As this is currently a matter of speculation, it is necessary to comprehensively and quantitatively organize the morphological traits of species in the genus *Camellia* and reevaluate their relationship with molecular phylogeny.

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Proceedings of
the 20th International Symposium on Integrated Field Science
"Biodiversity and Phylogeography"

November 23, 2022
Graduate School of Agricultural Science, Tohoku University,
Sendai, Japan

The 20th International Symposium on Integrated Field Science
"Biodiversity and Phylogeography"

Registration
   10:10–
Opening remarks
   10:30–10:40   Organizer: **Yoshihisa Suyama** (Tohoku University, Japan)

Invited Presentations
   10:40–11:10   **Harue Abe** (Niigata University, Japan)
                  Evolution of the genus *Camellia* based on the biological interaction and the
                  historical background

   11:10–11:40   **Gustavo Maruyama Mori** (São Paulo State University (UNESP), Brazil)
                  Like a mangrove out of water: dispersal limitation and adaptive responses of
                  dominant coastal trees to freshwater limitation

   11:40–12:00   Q & A

   12:00–13:00   Lunch

   13:00–13:30   **Yuji Isagi** (Kyoto University, Japan)
                  Tailor-made biological conservation of endangered plant species with genomic
                  information

   13:30–14:00   **Alison Kim Shan Wee** (University of Nottingham Malaysia Campus, Malaysia)
                  Mangroves in a changing world: biogeography and ecological genomics of
                  Southeast Asian mangroves

   14:00–14:10   Q & A

   14:10–14:20   Tea Break

Poster Preview
   14:20–14:35

Poster Presentations
   14:35–15:50

Closing Remarks
   15:50–16:00   **Yoshihisa Suyama** (Tohoku University, Japan)

The 20th International Symposium on Integrated Field Science
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Organizer: Yoshihisa Suyama (Field Science Center, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan)

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Evolution of the Genus *Camellia*
Based on the Biological Interaction and the Historical Background

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Yunguang SHEN[7], Yoshihisa SUYAMA[8] and Zhonglang WANG[7]

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The genus *Camellia* (Theaceae) is particularly prevalent in East and Southeast Asia (82-280 species). According to the Flora of China, there are 13 species in the sect. *Camellia*, of which 12 are found in China (11 being endemic), and only two species, *C. japonica* and *C. rusticana*, are found in Japanese islands. Since these two species are distributed at the northern limit of the genus *Camellia*, it is thought to have come from the continent and have speciated in Japan. Comparing the morphology, most species of *Camellia* have separated flower filaments, but the sect. *Camellia* species except for *C. rusticana* and *C. chekiangoleosa* have connected filaments, which indicates the bird-pollinated flower characteristics to keep a large amount of nectar. *C. japonica* blooms in the winter when insects are absent and must depend on bird pollination whereas other *Camellias* depend on both pollination, insects and birds. Therefore *C. japonica* might be the most adaptable species for bird pollination. On the other hand, *C. rusticana* has ancestral traits with not-connected filaments in the sect. *Camellia*. We hypothesized that the pollinator shift from insects to birds may have caused speciation from the ancestral genus *Camellia* to the sect. *Camellia*. Later, *C. rusticana* might get back to insect pollination to fit the snowy environment in Japan. Hence, this study aims to examine the speciation of Japanese *Camellias* based on pollinator shift by comparing the floral morphologies and the genetic differentiation of the genus *Camellia*. Next, we examined the demographic history of *C. japonica*, which is widely distributed in the Japanese archipelago, the Korean peninsula, and the coastal areas of mainland China and Taiwan. Through the above examinations, we discuss the evolution of the genus *Camellia* in Japan based on biological interaction and the historical background.

We compared their morphologies of leaf hypodermis, flower form, petal color, filament color, and filament color in the twenty populations and estimate quantitatively the differentiation. In the results, PCA analysis clearly distinguished between these species, and both petal and filament colors were also statistically different between these species. The floral traits of *C. japonica* such as filament connection rates, nectar, and sugar contents are one of the most adapted characteristics for bird pollination. The eight cpSSR and the genome-wide SNPs by MIG-Seq revealed largely congruent results indicating that the genetic structures are divided into 3; southern and northern *C. japonica*, and *C. rusticana*. The relationship between the three species including the related species in China was further inferred from the phylogeny generated by RAxML using SNP data. It was found that *C. chekiangoleosa* is the ancestral species and that *C. rusticana* and *C. japonica* are more related. However, demographic analysis, discussed below, reveals that the divergence date between *C. rusticana* and *C. japonica* is also older. The intraspecific genetic structure of *C. japonica* was investigated using ADMIXTURE 1.3.0, and the populations were divided into (1) mainly northern and (2) mainly southern populations in the Japanese archipelago, (3) Chinese and Korean populations, and (4) Okinawa and Taiwanese populations. Demographic analysis using DIYABC was carried out on the four populations in which hybrid individuals were excluded by ADMIXTURE predicted that the northern Japanese populations firstly differentiated from the southern Japanese populations, and then the Okinawa and Taiwan populations differentiated. Later, the Chinese and Korean populations returned to the mainland (the continent) from the southern Japanese populations. This result is consistent with the results of ENM. The optimum distribution of *C. rusticana* during the last glacial period was almost the same as that of the present distribution on the Sea of Japan, suggesting that *C. rusticana* is a relict species in Japan since before the glacial period. The northern population is the ancestral group as a relict in Honshu Japan. The results of this study could provide not only the evolutionary history of the species but also the population priorities that should serve as the focus for future management and conservation of these species.

Photo. White-eye feeding on camellia nectar

Invited Talk 2

Like a Mangrove out of Water:
Dispersal Limitation and Adaptive Responses of Dominant Coastal Trees to Freshwater Limitation

Gustavo Maruyama MORI

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As climate changes, freshwater unavailability due to reducing precipitation, lower air humidity, and rising temperatures may increase mass tree mortality not only in arid regions, but also in tropical and subtropical environments like mangroves forests. These tree communities inhabit the habitats between land and sea and are composed by few broadly distributed plants whose populations face high environmental heterogeneity, from arid to rainy/wet sites, and from subtropical to equatorial regions. Thus, mangroves provide suitable models to unveil the mechanisms underlying trees adaptation to freshwater limitation. First, this presentation will be focused on how geography and oceanography shape the dispersal of mangroves along the Brazilian coast. As intraspecific gene flow is limited in this region, local adaptation is expected to take place as one observes contrasting environments, at different geographic scales. Then, I will explore recent findings on the adaptive responses of two black mangroves species (*Avicennia*) across the Brazilian coast to drought tolerance or response to hydric stress. Evidence of natural selection has also been observed at much smaller geographic scale. There are signs of natural selection in *A. germinans* in response to abrupt limitation in access to soil freshwater, after a road was constructed in the mid-70s. To conclude, there will be a brief discussion on how these findings may support mangrove trees conservation and its associated challenges.

Tailor-made Biological Conservation of Endangered Plant Species with Genomic Information

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The multifaceted importance of biodiversity is increasingly recognized, but the situation in various ecosystems is crucial and many endangered species are recognized. Based on the "Conservation of Species Act", the Japanese government has designated the species with the highest conservation priority as Domestic Rare Wild Fauna and Flora and is trying to conserve them, and the number of the designated species is scheduled to be as many as 700 in the near future. However, limited resources available for the conservation of biodiversity require the development of rational and effective methods for the conservation of many endangered species. How can we appropriately preserve such a large number of endangered species with limited conservation resources? Until now, the conservation status of endangered species has been assessed primarily by the number of individuals surviving, but it is difficult to prioritize species for conservation based only on their population size. We explored ways to appropriately conserve a large number of endangered species with three criteria: (I) genetic diversity of remnant populations, the most common value for assessing the status of endangered species, (II) conservation value based on the phylogenetic uniqueness of the taxon, and (III) conservation difficulty estimated from the status of the genome. We evaluated the uniqueness of populations based on genetic diversity and phylogenetic perspectives through contracted genome sequencing on individuals growing in Japan and overseas of rare species. Furthermore, by comprehensively analyzing the expressed genes by RNA-seq, we found genetic indicators characteristic of endangered species in terms of genetic diversity, deleterious mutation accumulation, and duplicated gene rate, thus estimating the vulnerability of species and local populations and their ability to adapt to the environment.

As a result of detailed genome-level analysis of endangered plant species growing in the Ogasawara and Ryukyu islands, which are designated World Natural Heritage sites, we found significant differences between species in phylogenetic uniqueness, amount of deleterious mutation, and ability to adapt to a variety of environments. By combining these evaluation axes I, II and III described above based on the different types of genomic information, we categorized the conservation status of endangered species and developed a tailor-made conservation strategy by which conservation resources can be allocated more effectively and rationally according to the species' unique conservation status (**Figure**).
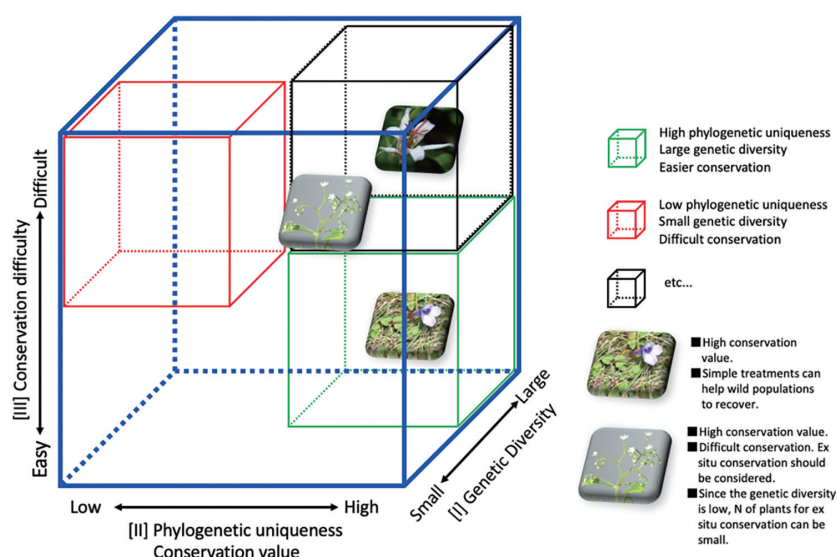

Figure. Categorization of critically endangered species based on [I] genetic diversity, [II] phylogenetic uniqueness and [III] conservation difficulty

Mangroves in a Changing World:
Biogeography and Ecological Genomics of
Southeast Asian Mangroves

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Mangroves are one of the most threatened ecosystems in the world. Understanding the drivers and limitations of gene flow, phylogeography, genetic adaptation and tools for natural resource management is crucial to effectively address the threats, conserve the long-term evolutionary potential, and ensure the sustainable management of mangroves. The first part of this talk summarizes key research findings on the biogeography of major mangrove tree species in Southeast Asia and the greater Indo-West Pacific region. In essence, propagule dispersal capabilities, land barriers and ocean currents are drivers of gene flow and underscores the importance of long-distance dispersal in connecting fragmented mangrove populations. The second part of the presentation describes our work on abiotic stress response in mangroves, especially at the species range limits. Current understanding of the molecular mechanism underlying stress adaptation points toward diverging strategies in stress response, even among closely related species. These studies will be important in estimating the adaptive potential of mangroves under climate change. The third part of this talk focuses on recent proof-of-concept studies on the application of environmental DNA (eDNA) as a biomonitoring tool in mangroves. Findings showed that fish communities change across the salinity gradient, and that biological invasion is a threat to the mangrove ecosystem. Collectively, these studies highlight the genetic uniqueness in Southeast Asian mangroves, the urgency of their conservation and how recent technological advances can contribute to this cause.

Poster 1

Effects of Cultivation Methods on Paddy Rice Growth Observed by UAV-mounted Multispectral Camera

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Remote sensing technology is effective for monitoring paddy rice growth and yield estimation. Vegetation indices (VIs) obtained from remote sensing data are related to rice grain protein content, which is an indicator of rice taste and has been used to estimate the best harvesting time. It is observed that the red-edge band spectrum is sensitive to chlorophyll and nitrogen content, the reflectance from the plants changes rapidly in this spectral region, and the VI obtained using the red-edge band is less saturated than that obtained using the red band. In this study, considering that different cultivation methods affect rice growth, we compared the VI based on the red-edge band with two other VIs based on red and green bands to observe the differences in the growth of paddy rice in conventional and organic farming paddy plots. We captured images using an unmanned aerial vehicle (UAV) mounted multispectral camera, on August 5, August 22, and September 13, 2022, in a paddy field located at the Kawatabi Field Science Center of Tohoku University in Osaki City, Miyagi Prefecture, Japan. The drone DJI Inspire 2 was equipped with a RedEdge-MX Dual camera, which can observe in 10 bands. As a result, the mean values of all three VIs showed a decreasing tendency with increasing growth period. Growth difference between conventional and organic cultivation methods were more pronounced for the VI obtained using the red-edge band compared with the VIs obtained using green and red bands. The difference between the two cultivation methods was apparent before the heading date, and it became difficult to discern thereafter. The values of the VIs in conventional farming were larger than those in organic farming.

Phylogeography of Infraspecific Differentiation of *Hydrangea serrata* (Hydrangeaceae), a Widespread Shrub Species in the Japanese Archipelago

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Hydrangea serrata (Thunb.) Ser. is a deciduous shrub species, distributed throughout Japan and in a part of East Asia. This species comprises of six varieties, var. *serrata*, var. *acuminata* (Siebold et Zucc.) H. Ohba et S. Akiyama, var. *yesoensis* (Koidz.) H. Ohba, var. *minamitanii* H. Ohba, var. *australis* T. Yamaz., and var. *angustata* (Franch. et Sav.) H. Ohba (Ohba and Akiyama, 2016); since their distributions are allopatric in Japan and composes sect. *Macrophyllae* together with *H. macrophylla* (De Smet et al., 2015), *H. serrata* will be a useful material to understand allopatric speciation in the plants distributed widely in the Japanese Archipelago. Although several phylogenetic studies of *H. serrata* have been previously conducted, the phylogenetic relationships among these varieties remain unclear because of limited information by genetic markers used and incomplete taxon samplings (Uemachi et al., 2014; Hirota et al., 2022). Therefore, more comprehensive phylogenetic analyses are required to elucidate the phylogenetic relationships and the history of diversification within sect. *Macrophyllae*, particularly at infraspecific level of *H. serrata*.

In this study, we reconstructed an infraspecific phylogeny of *H. serrata* and estimated divergence time of sect. *Macrophyllae*, including *H. serrata*, from the sister species, based on genome-wide SNPs. Additionally, we reconstructed the ancestral geographic areas to investigate phylogeographic history of *H. serrata*.

The phylogenetic trees reconstructed in this study showed the monophyly of most varieties of *H. serrata*. The divergence time between sect. *Macrophyllae* species and the outgroups was estimated to be in the Pleistocene period. The ancestral biogeographic history of *H. serrata* suggested some evidence of biogeographic isolations at various scales in the Japanese Archipelago.
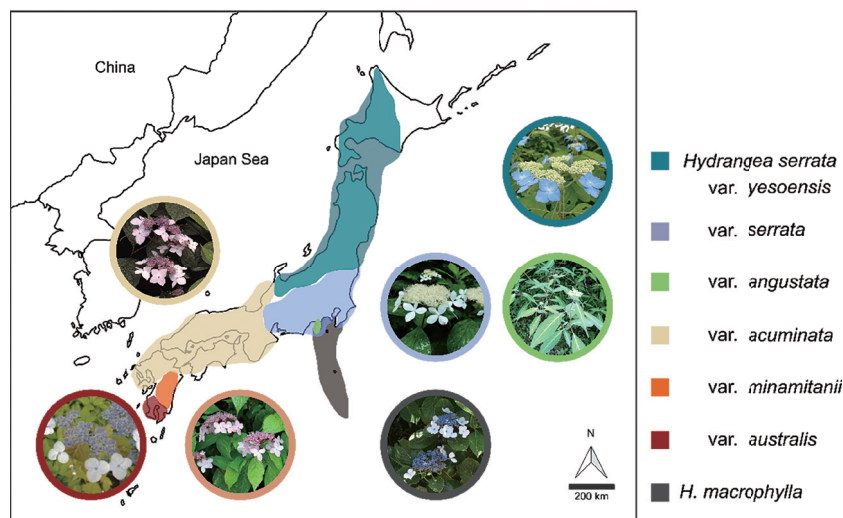

Figure. Distributions of varieties of *Hydrangea serrata* and *H. macrophylla* in the Japanese Archipelago

Poster 3

Comparison of Vegetation Indices and Spectral Reflectance Observed by Two Types of UAV-mounted Multispectral Camera

Ryohei YOKOYAMA[1], Chinatsu YONEZAWA[2], Mizuhiko NISHIDA[2], Ryosuke TAJIMA[2], Muxiye[2], Koharu OKADA[1], Kouki TAKAMURA[3], Kaori AMAYA[3] and Ken ICHIKAWA[3]

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Remote sensing in agriculture using multispectral cameras has increased in availability due to their advantages, such as high flexibility, ease of operation, and high spatial resolution. Several multispectral cameras with UAVs have been developed and utilized. The observed value can be converted to spectral reflectance and vegetation indices are calculated. However, the accuracy of the observed reflectance should be confirmed for vegetation monitoring. In this study, we compared images obtained by the two types of multispectral cameras mounted on UAVs, RedEdge MX-dual with Inspire-2 and P4 Multispectral (P4M) for paddy rice fields in the Kawatabi Field Science Center. We obtained spectral reflectance and calculated vegetation indices such as Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI), and Red Edge Normalized Difference Vegetation Index (RENDVI). On August 5th, 2022, and September 13th, 2022, we observed target area almost simultaneously by two cameras. Because of the battery duration, the region was separated into two portions and monitored individually. Radiometric calibration was performed for the orthomosaic images by RedEdge MX-dual using a reflectance panel. P4M has a sunlight sensor, and it is possible to obtain vegetation indices without calibration, however, spectral reflectance is uncertain. Therefore, radiometric calibration was performed for the observation on September 13. For the comparison of RedEdge MX-dual and P4M, the average of vegetation indices and spectral reflectance were computed on each of the agricultural parcels for paddy rice. The results show that the spectral reflectance and RENDVI obtained by P4M without calibration were inconsistent with those of RedEdge MX-dual. The differences were clearly visible, especially in the spectral reflectance. NDVI and GNDVI obtained by P4M without calibration on September 13 corresponded to those obtained by the RedEdge MX-dual, however, RENDVI was smaller than that of the RedEdge MX-dual. After the calibration, RENDVI and spectral reflectance, except for blue and red edge, acquired by the first flight of P4M on September 13 almost corresponded to those obtained by RedEdge MX-dual. Blue was larger than that by RedEdge MX-dual and red edge was smaller than that by RedEdge MX-dual. RENDVI and spectral reflectance were discordant with those of the RedEdge MX-dual on the second flight on September 13. Blue, green, and RENDVI were larger than these by RedEdge MX-dual. Red, red edge, and near infrared were smaller than these by RedEdge MX-dual. These results show that the UAV multispectral remote sensing technology is instructive for precision agriculture; however, calibration and evaluation are necessary to obtain accurate observation results.

Poster 4

Morphological and Genetic Data Do Not Support the Intraspecific Taxonomic Classification of *Pimenta pseudocaryophyllus*

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Pimenta pseudocaryophyllus Landrum (Myrtaceae) is an important plant for traditional communities in southwestern Brazil, used both medicinally and for cultural. Due to the predatory exploitation, it may be at risk of vulnerability requiring. However, little is known about this species, including whether the name *P. pseudocaryophyllus* corresponds to a species or a species complex. This plant is widely distributed in South America, occupying different biomes but the distribution records are concentrated in the Atlantic Forest and the Cerrado. It presents three taxonomic varieties defined based on vegetative morphology, especially on the shape of the leaves. *P. pseudocaryophyllus* var. *hoehnei* (DC.) Landrum is in the coastal region of southern to southeastern Brazil, mainly in herbaceous, shrubby, and arboreal coastal sandy habitats (*restingas*); *P. pseudocaryophyllus* var. *pseudocaryophyllus* (Gomes) Landrum, is distributed in southeastern Brazil mainly in the highlands of ombrophilous dense forests, and *P. pseudocaryophyllus* var. *fulvescens* (Burret) Landrum that occurs in the Cerrado, with rare exceptions in the Bolivian Chaco. However, in the field and in collections records, a great variation in the shape of the leaves is observed. This study sought to analyze whether there is a congruence between genetics and morphological aspects of representatives of these taxonomic varieties. We sampled 153 *P. pseudocaryophyllus* individuals across four sites and genotyped them using a set of 11 polymorphic microsatellites. We quantified the genetic diversity of each sample and described their population genetic structure. Complementarily, we used a geometric morphometry of leaves from a subset of the total samples. We considered the leaf closest to the site's average and normalized them using General Procrustes Analysis and performed a Principal Component Analysis (PCA) to describe how taxonomic varieties are organized considering the leaves' shapes and sizes. The PCA of the morphology showed individuals comprised groups according to sampling site and variety. Conversely, the population genetic structure was not congruent with the observed morphological variation. Despite forming clearly separated clusters. We observed that varieties *pseudocaryophyllus* and *fulvescens* grouped together, whereas *hoehnei* was clearly differentiated. This preliminary study suggests that, because of the absence of correspondence between leaf morphology and genetics, leaf shape is likely not a reliable character for the intraspecies taxonomy.

Phylogeography of *Juniperus* Species (Cupressaceae) with a Disjunct Distribution in the Island Areas of Japan

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A coastal conifer, *Juniperus taxifolia* var. *lutchuensis* (Cupressaceae) is distributed in the Ryukyu Islands, Izu Islands, and a part of the Izu peninsula. This species is treated as a variety of *J. taxifolia* var. *taxifolia* which is endemic to the Bonin (Ogasawara) Islands. Since these three Island groups are separated by more than 1,000 km away, it is interesting where this species originated and how it expanded its distribution. On the other hand, *J. conferta* is distributed in Hokkaido, Honshu, and Kyushu Islands. This species grows in a coastal environment like *J. taxifolia* var. *lutchuensis* and the two taxa are distinguished by the traits of their leaves. In areas where distributions of these two species overlap, morphologically intermediate individuals have been reported; however, their phylogenetic relationships are unclear.

In this study, we collected 267 samples of 7 *Juniperus* species including 27 populations of *J. taxifolia* and *J. conferta* from Japan and Taiwan Island in order to reveal the phylogeographic relationship among these three taxa. We extracted DNA from leaves by the CTAB method and obtained genome-wide SNPs by the MIG-seq analysis. Based on MIG-seq data, we conducted phylogenetic and population genetic analyses.

The result of the phylogenetic analysis indicated that *J. taxifolia* var. *lutchuensis* didn't form a monophyletic clade with var. *taxifolia* but with *J. conferta*. Moreover, *J. taxifolia* var. *taxifolia* was monophyletic with *J. formosana* which is endemic to Taiwan Island and southeast of mainland China. The results of the population genetic analyses indicated that *J. taxifolia* var. *lutchuensis* from Izu Islands is genetically different from the Ryukyu Islands population and is closely related to *J. conferta* from Honshu Island. These results suggest that *J. taxifolia* var. *taxifolia* originated from Taiwan or mainland China and that *J. taxifolia* var. *lutchuensis* has the same origin as *J. conferta* with some genetic differentiation by expanding its distribution along the coast.

Photo. *Juniperus taxifolia* var. *lutchuensis* in Amami Oshima Island

Decay Types and Fungal Communities of Norway Spruce Dead Wood in Europe

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Fungi are the major decomposers of dead wood in forest ecosystems. Differences in fungal community structure affect the lignin decomposition of dead wood through differences in ligninolytic enzyme profiles of the fungal species in the community. Such differences in decomposition patterns are expected to affect carbon stocks in forests. Therefore, it is important to understand the driving factors and functions of wood-inhabiting fungi (WIF) communities in dead wood, and how they may respond to environmental changes. In this study, we investigated the relationship between fungal community composition and environmental variables in dead wood of Norway spruce (*Picea abies*) across the latitudinal gradient in Europe.

We sampled 374 dead Norway spruce logs from forests in six sites in five European countries along the latitudinal gradient. DNA metabarcoding was used to examine the fungal community structure of each log, and the functions of the fungal species that compose the community were estimated by the FUNGuild database. In this study, we also quantitatively evaluated the decay type of dead woods by using the Dilute Alkali Solubility (DAS) method, which is a method for quantifying and evaluating the chemical changes associated with the decomposition and alteration of lignocellulose in dead woods. The value of DAS (%) tends to be higher in the brown rot decay type, in which lignin remains with little modification.

DNA metabarcoding identified 1408 OTUs, of which 18 OTUs were brown rot fungi. The structure of the fungal community varies across a climatic gradient, DAS (%), an index of brown rot, was significantly correlated with mean annual temperature. The frequency of occurrence of brown rot fungi was positively correlated with DAS and annual mean temperature. These results suggested that the frequency of brown rot may increase with temperature as some species of brown rot fungi, which are sensitive to the site environment, become more dominant in a warmer temperature.
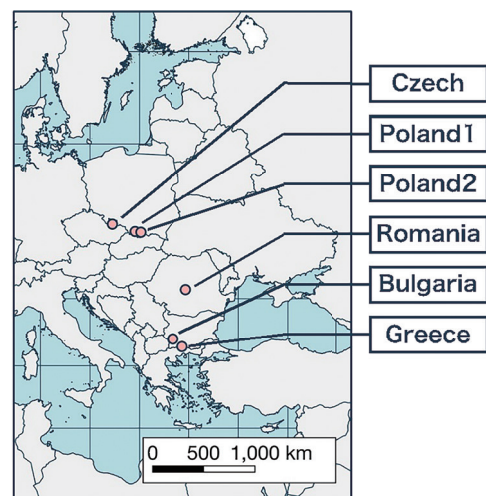

Photo. Norway spruce forest

Figure. Study sites in Europe (6 sites)

Population Genetic Structure of a Bamboo Taxon, *Pleioblastus* Sect. *Pleioblastus*, in the Ryukyu Islands Formed by Geohistory and Human Activity

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In island ecosystems, past geohistorical events, such as the formation of land bridges and trenches, have strongly influenced the distribution of organisms that constitute the ecosystem. However, some plant species commonly used in human life may be influenced by significant anthropogenic effects on their distribution and population genetic structure. In this study, we focused on a bamboo taxon, *Pleioblastus* Sect. *Pleioblastus* (*P. hindsii* sensu Nakai, *P. linearis*, *P. gramineus* and *P. gozadakensis*), which have been used in human life in the Ryukyu Islands. A total of 187 ramets from 11 islands were collected for population genetic analysis using the MIG-seq methods. First, the clone identification analysis revealed that several identical genets were found tens of kilometers apart on the same island, and even from different islands separated by the ocean. Such long-distance dispersal could not be expected under natural conditions, suggesting that their distribution is due to human transplantation and propagation. On the other hand, the results of population genetic structure analysis excluding these widely distributed genets indicated that distinct local genetic clusters are formed in the islands as observed in other organisms. These results clearly demonstrate not only geological history, but also human activities have a strong impact on the distribution and formation of population genetic structure in human-related plant species.

Left Photo. A high-density population of *P. linearis* at Cape Miyako, Amami Oshima Island which is considered to be a natural distribution.
Right Photo. *P. linearis* growing on the roadside at Amami Oshima Island which is considered to be a planted population.

Effects of the Resource Distance on the Decisions of Mycelial Behavior

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Cord-forming basidiomycetes develop large mycelial networks and play a major role in organic matter decomposition and nutrient cycling in forest ecosystems. Fungal mycelia alter their foraging and migrating behavior flexibly in response to the presence of outer resource and their nutritional status. Previous studies showed that, when a mycelium extending from a wood block (inoculum) finds a new piece of wood (bait), the mycelium makes a decision whether or not to move from the inoculum to the bait depending on the bait size and the timing of bait placement. For example, relatively larger baits tend to induce mycelial migration from inoculum wood to bait wood more frequently than smaller ones. This suggests that mycelia may decide to move out based on the size of the wood resources and their own nutritional status. However, the influence of other factors, such as distance from the inoculum to the bait, on the decision to move out is not clear. We hypothesized that, if the distance from the inoculum is longer, mycelia might move to the baits even with smaller size, because maintaining the hyphal connection between the inoculum and the bait at a longer distance might be energetically more expensive than that in a shorter distance. Therefore, this study examined the effects of distance from the inoculum to the bait and the size of the bait on the decision-making of mycelial migration.

A beech wood block (0.5 cm^3), incubated with a basidiomycete *Phanerochate veltina* for 1 month on 2% malt extract agar medium for colonization, was put onto a corner of a tray (24 cm × 24 cm) filled with soil ca. 5 mm depth (Fig. 1). When mycelium grew at least 15 cm from the inoculum, a bait wood block was placed 1 cm or 15 cm away from the inoculum and incubated at 20˚C in the darkness for 50 days. We prepared two sizes of bait wood blocks (4 cm^3 and 8 cm^3). After incubation, inoculum wood blocks were retrieved from the tray, surface cleaned, transferred to new soil trays, and incubated at 20˚C in the darkness for 8 days to check the re-growth of mycelium and its direction. Trays were photographed every 3 days during the experiment. We measured hyphal coverage by ImageJ. Inoculums and baits were dried at 70˚C and weighed before and after incubation to check their weight loss during incubation. The dry weight of 40 inoculums at the time of bait placement was estimated by creating a regression line from the dry weight and mycelial area of 73 inoculums removed at that time.
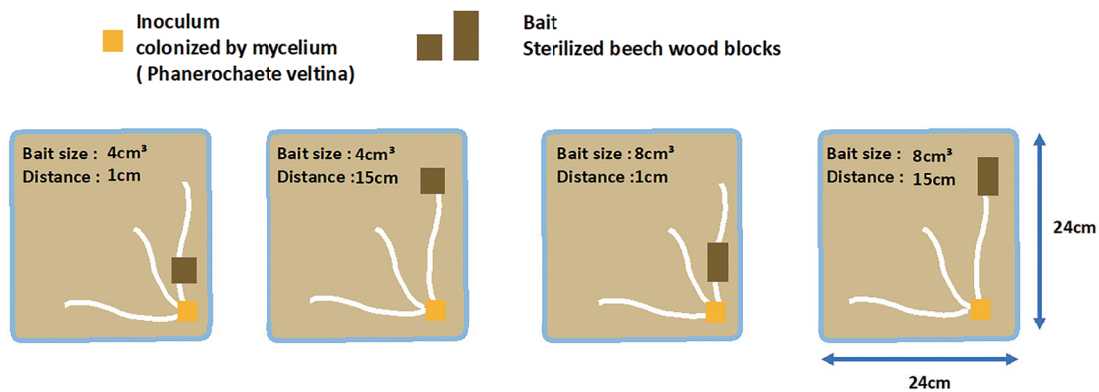

Figure. Experimental design of microcosm

Development of
Plant Environmental DNA Analysis Method for Forest Soil

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Environmental DNA analysis is a useful method for identifying species that live in a place. In our laboratory, we are developing technology with the aim of applying this method to intraspecific diversity analysis. As a preliminary step of this study, we examined forest soil samples to confirm whether the plant DNA detected in the soil matches the composition of the surrounding plant species. Soil samples were collected from an artificial forest of *Cryptomeria japonica* and DNA was extracted using a commercially available soil DNA extraction kit. Multiple plant DNA barcode regions were amplified from the extracted DNA, and sequence data were obtained by next-generation DNA sequencing. In addition, plant samples growing in the vicinity of the sampling site were also collected, and sequence data were obtained by the same method after DNA extraction. As a result of analyzing the sequence data detected from the soil sample, the sequences were six species of woody plants including *C. japonica* and seven species of herbaceous plants growing in the vicinity. From these results, it was confirmed that plant environment DNA analysis of forest soil is possible, but it was also found that there is room for improvement in DNA extraction library construction methods. In the next step, we will conduct a similar analysis of forest soils with different vegetation to confirm the validity of the acquired data. And then we plan to develop it into an intraspecific level diversity analysis method after improving the basic technology by referring to the DNA extraction and library construction methods of ancient soil DNA conducted in previous studies.

Photo 1. Soil and plant samples were collected in an artificial forest of *Cryptomeria japonica*

Photo 2. Soil samples were collected from *ca.* 5 cm depth

Poster 10

Effects of Oak Wilt Disease on
Fungal Community Composition and Wood Decomposition
in Dead *Quercus serrata* Trunks

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In Japan, mass attacks of an ambrosia beetle *Platypus quercivorus*, vectoring a pathogenic fungus *Raffaelea quercivora*, cause oak wilt outbreaks in recent decades. Since *P. quercivorus* inoculates symbiotic fungi into wood tissue, it can alter the fungal community composition within the dead wood. A change in the fungal community composition may alter the decomposition function of the community, as well as the physical and chemical properties of the dead wood. The difference in properties of the dead wood may impact surrounding biological communities. Therefore, understanding the effects of oak wilt disease on a dead wood fungal community is important for predicting wood decomposition and forest biodiversity after the dieback. In this study, we used DNA metabarcoding to compare the fungal communities between *Q. serrata* trunks killed by oak wilt and healthy trunks that felled artificially at three sites across latitude in Japan. A total of 1200 samples from 5-time points during the 2016 autumn – 2018 spring were subjected to amplicon sequencing of the fungal internal transcribed spacer 1 region and 1128 fungal operational taxonomic units (OTUs) were detected. Fungal community composition differed significantly between *Q. serrata* trunks killed by oak wilt and healthy-cut trunks (Fig. 1), and diversity indices showed that trunks killed by oak wilt were significantly lower than that of healthy-cut trunks (Fig 2). The Fungal Trait database was used to estimate the function of each OTU and revealed a dominance of wood-decomposing fungi, mainly white rot fungi, which have the ability to decompose wood. The generalized linear mixed model (GLMM) showed that the OTU richness and DNA copy number of white rot fungi were significantly affected by the sampling period, mean air temperature, precipitation, and oak wilt infestation, and that the OTU richness and DNA copy number of white rot fungi increased with oak wilt. During the ca. 1.5-year experiment, wood density decreased by an average of 9.2%. Structural equation modeling (SEM) showed that oak wilt and environmental variables significantly affected the fungal community, but the fungal community didn't significantly affect wood decomposition (Fig 3). Rather, trunk moisture and mean air temperature strongly affected decomposition. This study focused on the initial decomposition of the trunks during ca. 1.5 years after the start of the experiment. It is important to conduct further research over a longer time period to evaluate the effects of oak wilt and associated fungal communities on trunk decomposition.
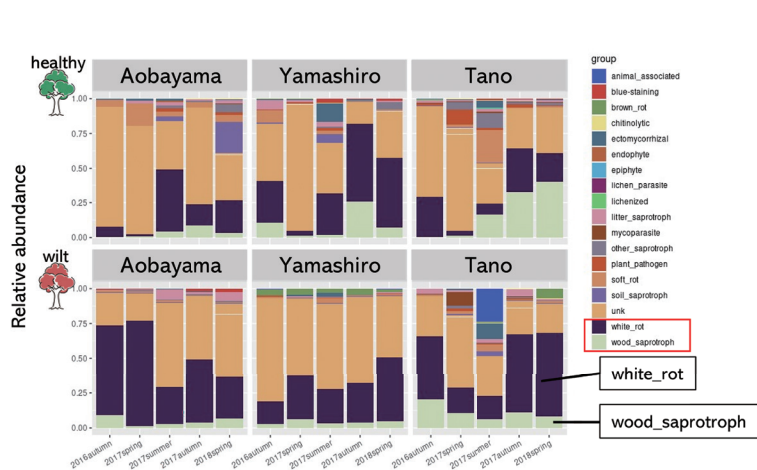

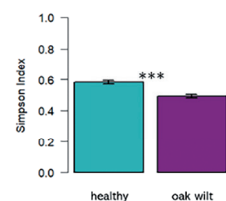**Figure 1.** Relative abundance of fungi (functional group)
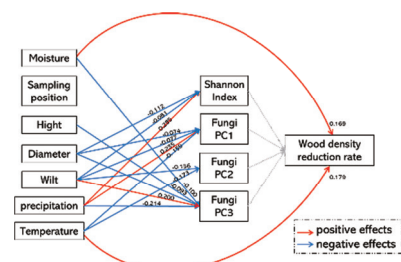

Figure 2. Diversity index

Figure 3. SEM

Poster 11

Phylogenetic Origin of
Cryptomeria japonica on Kinkasan Island in Japan

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Sugi（杉）– *Cryptomeria japonica* is widely planted throughout Japan accounting for about 44 % of the total area of planted forests but its natural populations with its original local lineage are limited. Natural forests and local tree lineages are important for regional biodiversity and future breeding resources. Kinkasan in Miyagi prefecture is known as an island where the natural forest has been protected; therefore, we thought that it is a candidate for an unknown natural population of the species. Therefore, we wanted to figure out if the population on the island has a surviving local lineage of this region or not.

We collected 24 samples from the island including individuals from natural habitats as well as planted populations. We also used reference samples (83 samples from 17 natural populations in Japan and 28 samples from local populations near Kinkasan) to compare the genetic structure. Genome-wide SNP data was collected with the MIG-seq approach and genetic analysis was conducted by using Stacks (ver. 2.60), RAxML (ver.8.2.12), and ADMIXTURE (ver. 1.3.0).

The results showed that the eight trees from the natural habitats were supposed to be originated from a local lineage related to the local populations near Kinkasan. Their values of genetic diversity and inbreeding coefficient are almost the same levels as other natural populations. In addition, the lineage of the Pacific Ocean side was found in planted populations and partial mixing of the Sea of Japan side lineage was also observed in some individuals which may indicate genetic disturbance in the natural genetic structure.

The trees with local lineage discovered in this study are important for the conservation of the local genetic resources and diversity. It is important to consider the conservation of local genetic diversity in upcoming afforestation and restoration projects.

Photos 1 and 2. Over hundred years old trees of *Cryptomeria japonica* on Kinkasan island

Molecular Phylogenetic Analyses of
Geographical and Ecological Speciation of *Anaphalis margaritacea*

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Anaphalis margaritacea (Asteraceae) is a perennial species distributed widely from Asia to North America. In Japan, *A. margaritacea* consists of three varieties; var. *margaritacea* distributed mainly in the mountain areas in eastern Japan, var. *angustifolia* distributed in mountain areas in western Japan, var. *yedoensis* distributed in riverbanks covered with pebbles throughout Japan. These varieties are considered to have been derived via different speciation patterns such as geographical and ecological speciations. They seem to need taxonomic reexamination based on the molecular analysis because morphological intermediates between these varieties have been sometimes reported. In this study, we collected these three varieties from all over Japan and examined the genetic differentiation among the three varieties using the genome-wide SNPs by MIG-seq.

Analyses based on the SNPs showed that the populations of *A. margaritacea* var. *angustifolia* and var. *yedoensis* were monophyletic, respectively and these lineages were contained with *A. margaritacea* var. margaritacea, suggesting that they are of a single origin from *A. margaritacea* var. *margaritacea.* Population genetics analyses showed that the *A. margaritacea* complex comprised three clades as a whole: "*A. margaritacea* var. *margaritacea* in Hokkaido" clade, "*A. margaritacea* var. *margaritacea* in Honshu" clade, and "*A. margaritacea* var. *yedoensis* in Hokkaido" clade. *Anaphalis margaritacea* var. *angustifolia* was included in "var. *margaritacea* in Honshu" clade. On the other hand, it is revealed that *A. margaritacea* var. *yedoensis* in Honshu have a admixture of the two clades of *A. margaritacea* var. *margaritacea* in Honshu and *A. margaritacea* var. *yedoensis* in Hokkaido. Based on these results, we will discuss differentiation processes among the three varieties of *A. margaritacea.*

List of scientific papers in 2022 published by field science group in Graduate School of Agricultural Science, Tohoku University

The Forest-Andisols Group

Cardoni, S., Piredda, R., Denk, T., Grimm, G.W., Papageorgiou, A.C., Schulze, E-D., Scoppola, A., Shanjani, P.S., Suyama, Y., Tomaru, N., Worth, J.R.P. and Simeone, M.C. (2022) 5S-IGS rDNA in wind-pollinated trees (*Fagus* L.) encapsulates 55 million years of reticulate evolution and hybrid origins of modern species. *Plant J.*, **109**(4): 909-926.

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Fukasawa, Y. (2022) Mushroom thinking. *Seibutsukougaku*, **100**: 251. (in Japanese)

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Kusuma, Y.W.C., Matsuo, A., Suyama, Y., Wanke, S. and Isagi, Y. (2022) Conservation genetics of three *Rafflesia* species in Java Island, Indonesia using SNP markers obtained from MIG-seq. *Conserv. Genet.*, **23**: 1039-1052.

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References are listed after the main text in alphabetical order under the first author's name: Last name(s), followed by initials of first and middle names; year of publication (in parentheses); title of article; title of periodical (abbreviated according to Index Medicus and italicized); number of volume (bold); and the first and last pages. Publications by the same author(s) in the same year should be listed as 2007a, 2007b, etc. In a reference with more than fifteen authors, give the first fifteen authors and then use et al.

When a book is referred to, the reference should include the author's name; year of publication (in parentheses); title of book (capitalized and italic); edition (if any); publisher; place of publication; and page(s).

Note the following examples.

1) A journal article:

Momota, K., Takagi, R., Sasaki, A., Tamai, S. and Ikeda, M. (2021) The effect of birds and cows on marine ecosystem in Miyagi Prefecture. *J. Integr. Field Sci.*, **6**: 109-115.

2) A chapter in an edited book:

Aragaki, Y. and Ikeda, M. (2021) The effect of courtship dance of fish on marine biodiversity in Onagawa Bay. In *Fish, Cattle and Biodiversity, 2nd ed*., edited by Ogura, S. and Fukasawa, M. Tohoku University Press, Sendai, pp. 108-150.

3) A book:

Ogura, S. and Fukasawa, M. (2021) *Fish, Cattle and Biodiversity, 2nd ed*., Tohoku University Press, Sendai.

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