



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. *Theopsis* 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	
<i>Chrysantha</i>	Yellow	<i>C. longzhouensis</i>	3	40.90	1.21	cdh	39.60	1.25	ab	51.17	0.04	bgh	Inokuchi Tsubaki Kan
		<i>C. chrysantha</i> var. <i>phaeopubisperma</i>	3	48.80	5.63	bcdh	39.00	3.58	ab	57.61	0.07	ag	
		<i>C. quephongensis</i>	3	38.93	3.33	cdh	32.97	0.86	acd	44.63	0.06	fghi	
		<i>C. cucphuongensis</i>	3	36.67	3.66	dh	33.13	2.97	acd	39.26	0.00	gj	
<i>Theopsis</i>	White	<i>C. lutchuensis</i>	3	27.53	5.53	gh	14.20	1.22	gh	45.73	0.04	efghi	
		<i>C. transnokoensis</i>	3	41.03	1.76	cdh	20.70	0.75	eh	40.63	0.02	gj	
		<i>C. trichoclada</i>	3	23.00	1.14	h	11.53	1.12	h	24.93	0.10	jk	
		<i>C. fraterna</i>	3	32.87	1.35	fh	19.87	0.32	eh	56.53	0.02	agh	
<i>Longipedicellata</i>	White	<i>C. longicarpa</i>	3	33.73	2.31	efh	23.87	0.96	defg	63.00	0.04	abcdf	
<i>Oleifera</i>		<i>C. vietnamensis</i>	3	96.90	3.83	a	18.77	0.49	fh	17.00	0.02	k	
<i>Paracamellia</i>	White	<i>C. yuhsienensis</i>	3	96.73	3.50	a	13.53	0.61	gh	60.50	0.05	abcdf	
		<i>C. grijsii</i>	3	61.93	9.10	bcdf	11.87	0.78	h	38.11	0.06	gj	
<i>Camellia</i>	Red	<i>C. brevignyana</i>	3	64.60	8.49	bcd	29.70	1.92	abef	64.65	0.03	abcde	
		<i>C. lungshenensis</i>	3	61.27	7.83	bcdf	32.93	2.45	acd	72.19	0.03	a	
		<i>C. bailsinshanica</i>	3	62.40	1.74	bcdf	34.07	1.91	acd	70.48	0.01	ab	
		<i>C. brevicolumns</i>	3	66.30	7.48	bcd	36.50	2.65	ac	72.43	0.05	a	
		<i>C. kweichowensis</i>	3	60.10	0.36	bcdf	31.07	1.29	abe	50.06	0.02	cgh	
		<i>C. villosa</i>	3	61.43	6.70	bcdf	34.43	0.38	acd	46.95	0.00	dghi	
		<i>C. polyodonta</i>	3	63.23	7.78	bcde	26.23	1.27	cef	40.51	0.02	gj	
		<i>C. saluenensis</i>	3	59.87	12.32	bcdf	30.60	2.52	abe	67.38	0.05	abc	
		<i>C. lapidea</i>	3	53.43	5.45	bcdfg	33.17	3.76	acd	36.91	0.09	hj	
		<i>C. semiserrata</i>	3	54.33	9.76	bcdfg	40.60	5.21	a	53.91	0.03	agh	
		<i>C. pitardii</i> var. <i>pitardii</i>	3	68.17	3.32	ac	33.73	1.96	acd	66.43	0.09	abcd	
		<i>C. apolyodonta</i>	3	62.77	6.66	bcdf	26.07	1.70	cef	39.37	0.02	gj	
		<i>C. chekiangoleosa</i>	3	78.60	8.69	ab	28.33	2.24	bcef	37.91	0.04	gj	
		<i>C. japonica</i>	52	52.25	12.66	cdf	34.59	4.58	ab	62.50	0.07	abc	
<i>C. rusticana</i>	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

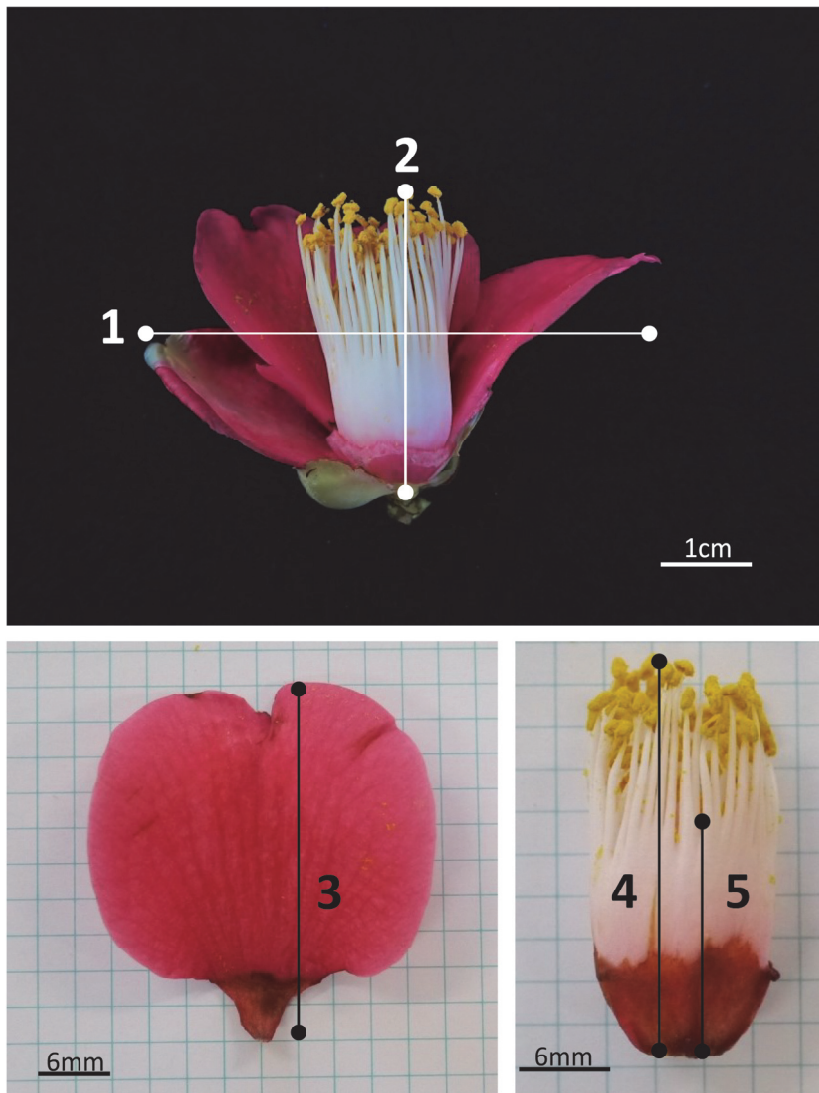


Fig. 1: Measurement of flower morphology

(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

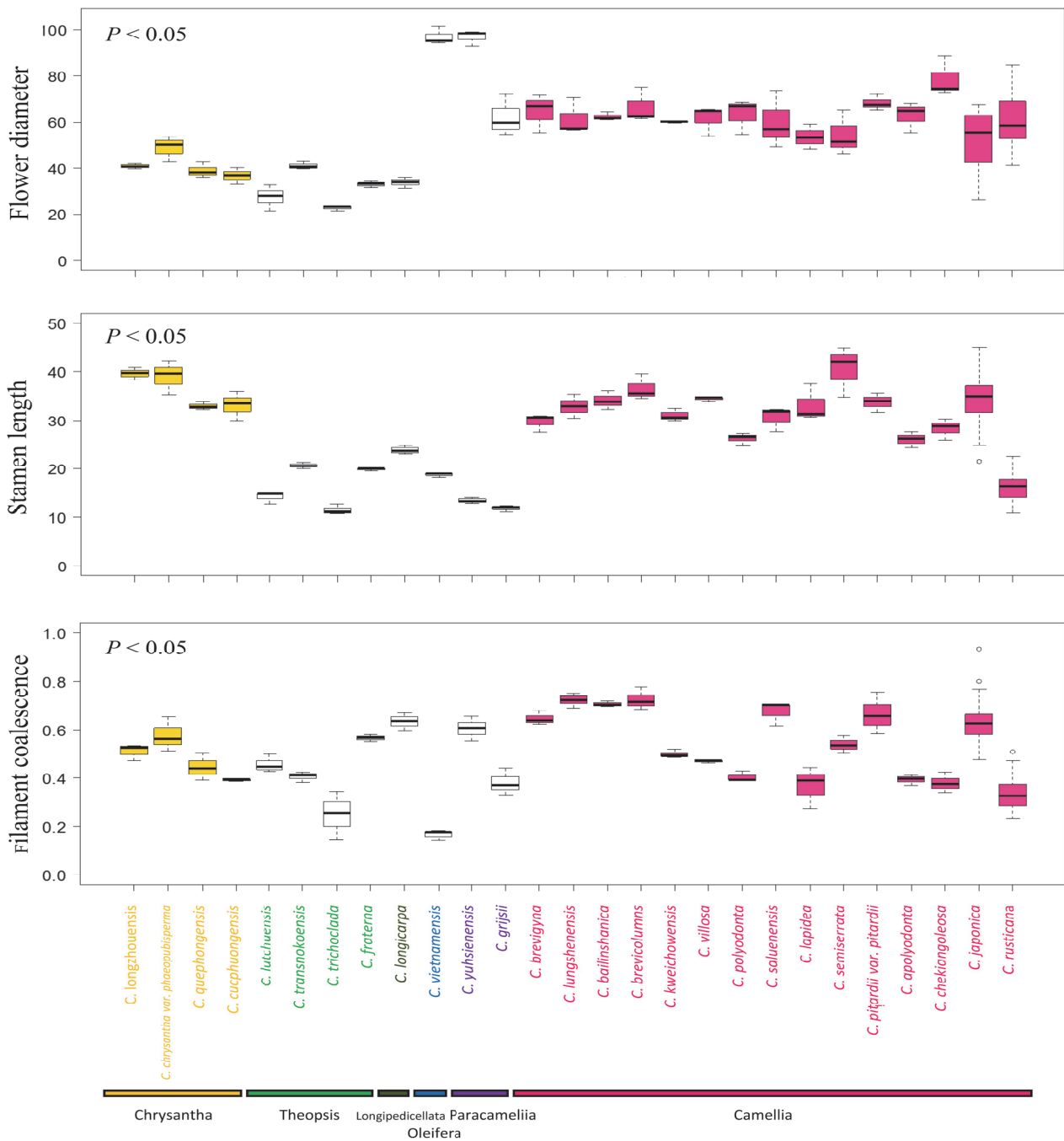


Fig. 2: Comparisons of flower diameter, stamen length and floral filament coalescence rates in the genus *Camellia*

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*

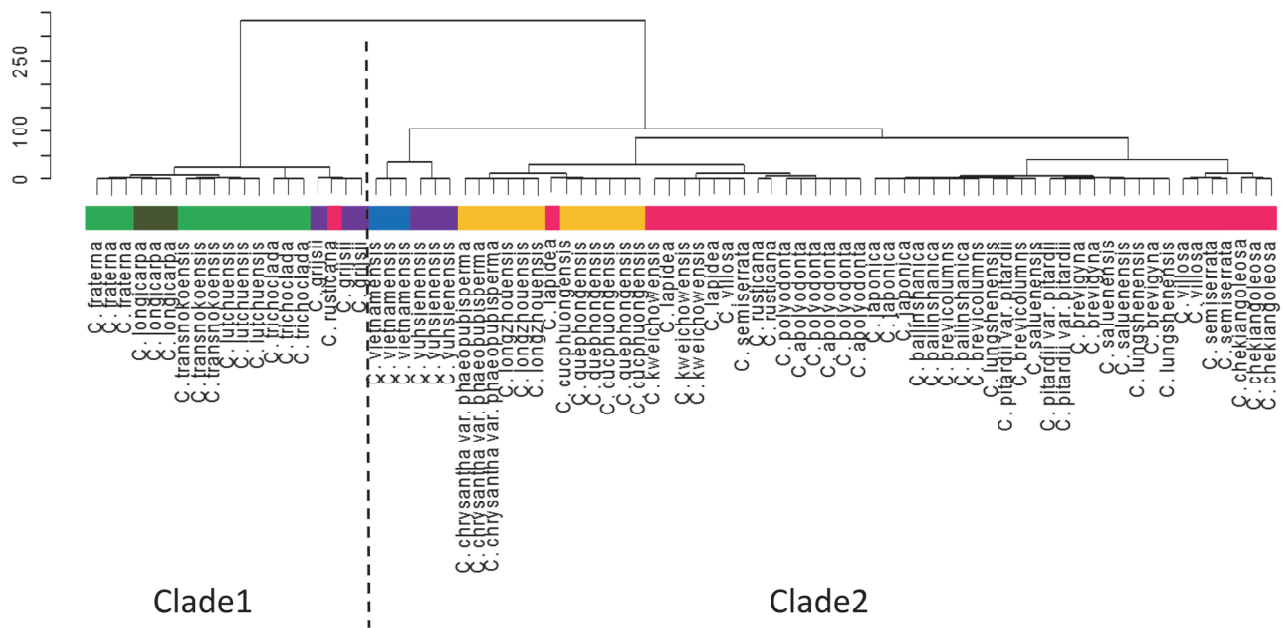


Fig. 3: Result of cluster analysis on flower morphology in the genus *Camellia*

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. brevistyla*. The clade consisting of *C. japonica* is consistent with the findings of Vijayan *et al.* (2009). *C. fluviatillis* and *C. brevistyla* have regions where their distribution overlaps with that of *C. japonica*. Additionally, it clarifies that *C. fluviatillis* and *C. brevistyla* 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. *Camelliopsis* 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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