

Latitudinal gradient in leaf defense traits of woody plants along Japanese archipelago

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General Introduction

It is estimated that over the twenty million species of organisms are living on our planet, and all of these organisms adapted to their own living environment, namely niche (Hatchinson 1957). Not only the abiotic factors but biotic interaction plays a key role in the maintenance of biodiversity. Animal-plant interactions are one of the most important topic in community ecology (e.g. Morin 1999). Plants and herbivore insects have accounted for about half of the entire diversity on the earth (Strong et al., 1984). Plant-herbivore interactions are extremely complex, which should lead the tremendous diversity of both plants and herbivores (e.g. Gutierrez et al., 1984; Hay et al., 1989). Although the interaction between these two components, namely co-speciation, should account for this diversification, most of the studies so far, tend to explain this interaction only from one side of them. Plants have interacted with insect herbivores for several hundred million years, which should lead to complex defense systems against various herbivores (Fürstenberg-Hägg et al., 2013). This interaction between plants and herbivores has long proposed the opportunity for studying the mechanism of the creation and maintenance of biological diversity because of its universality and generality (Strong et al. 1984; Ali and Agrawal 2012).

It is believed that the evolution of plant defense traits followed by counter-adaptations in herbivores could lead to bursts of adaptive radiation of both components (Ehrlich and Raven 1969). Understanding the coevolution of plant and insect species and macroevolution of adaptive traits has inspired biologists for some decades, yet has been challenging to study even present days (Schluter, 2000). Plant defense strategy is very important when studying the macroevolution of herbivore insects by directly affecting the distribution and the diversity of insect herbivore assemblage (Agrawal 2006). To examine this phenomenon, the latitudinal gradient in the intensity of animal-plant interactions should be examined. Many studies have

examined the “low latitude high defense” (LLHD) hypothesis (Bolser and Hay 1996), which posits that plant species distributed at lower latitudes will show higher degrees of defense (Dobzhansky 1950, Coley and Aide 1991, Schmitt et al. 1995, Marquis et al. 2012). Although these studies have been conducted with the aim of confirming a latitudinal gradient in defense strength (see review by Moles et al. 2011), few have found the support for the hypothesis. For example, Moles et al. (2011) recently showed that both chemical (tannins and phenols, flavonoids, alkaloids, resins/oils and other), and physical defense (physical mechanical strength, extrafloral nectarines and other) traits, across a wide range of latitude, and found conflicting trends in response to predictions based on the LLHD hypothesis. They suggested the sampling bias, the mixed functional types of woody plants and variations of data analysis is the biggest problem for these complex result.

In this thesis, we sampled the woody plants leaf all around Japanese archipelago with the main spot of Okinawa, Amami Island, Kyusyu, Mie, Kansai, middle range of Japan, Titibu, Yamanasi, Obihoro, Furano Etc. We collected total number of 736 broad-leaved tree species with 267 evergreen and 469 deciduous tree species. This is the most complete data for the traits analysis now for Japanese tree species (1225 species in total, Satake et al. 1989).

One of the major reasons for this complexity might be the variation of "functional types" of woody plant species (e.g., Reich et al 1997, Chapin et al 1996). Although many classifications of the functional type are possible, broad-leaved woody evergreen species with leaf life-span >1 year and broad-leaved woody deciduous species with leaf life-span < 1 year occupied consistently different positions in their trends in leaf defense traits regardless of ecosystem or biome (Reich et al 1997). Thus, the separate examination of the latitudinal gradients in leaf defense traits between evergreen and deciduous tree species might be efficient way to clarify the trends.

To understand the defense strategies among plant species, many previous studies

compared the leaf quality, which should affect the performance of herbivores. Variety of leaf traits both chemical and physical ones were measured and served as the proxy for the leaf quality (see Moles et al 2011, 2013). On the other hand, herbivorous insects evolved variety of counter adaptation to these defense mechanisms (Agrawal and Fishbein 2006). These make it quite difficult to fairly evaluated the leaf availability for the herbivores. Therefore, in this study, I examined the leaf availability by examining the performance of generalist herbivore, Eri silk-moth (*Samia cynthia ricini*). Eri silk-moth is a generalist herbivore and completely lose the preference among plant species. The larvae are highly polyphagous, but natural host-plants include Castor (*Ricinus*), *Ailanthus* tree, Cassava (*Manihot*), Kesseru (*Heteropanax*), or *Plumeria*. The larvae of Eri silk-moth eventually eat any kind of plant leaves unless the leaves are too hard or hairy (Hirayama & Konno, 2007). For this feature, Eri silk-moth larvae have been used in bioassays and analyses to evaluate the defense activities and defense levels of plants against herbivorous insects (Konno *et al.*, 2006).

In chapter one, I analyzed comprehensive data on the functional leaf traits of woody plant species around Japanese archipelago, and check the latitudinal trends in these leaf traits. In chapter two, I carried out the raring experiments using the larvae of the Eri silk-moth to objectively evaluate the "leaf availability" of broad-leaved tree species for the herbivorous insects. Then the latitudinal trend of this "leaf availability" was checked to examine the “low latitude high defense” (LLHD) hypothesis. With these examinations, we have separately analyzed the evergreen broadleaved tree species and deciduous broadleaved tree species.

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Chapter 1

A paradox of latitudinal leaf defense strategies in deciduous and evergreen broadleaved trees

Abstract

The classical “low latitude–high defense” hypothesis is seldom supported by empirical evidence. In this context, we tested latitudinal patterns in the leaf defense traits of deciduous broadleaved (DB) and evergreen broadleaved (EGB) tree species, which are expected to affect herbivore diversity. We examined the co-occurrence of leaf defense traits (tannin and phenol content, leaf mechanical strength, leaf dry matter content, leaf mass per area, and leaf thickness) in 736 broadleaved tree species and their correlations with species geographical range in East Asian island flora. We discovered contrasting latitudinal defense strategy gradients in DB and EGB tree species. DB species employed chemical defenses (increasing tannin and phenol content) at higher latitudes and physical defenses (softer and thinner leaves) at lower latitudes, whereas EGB tree species exhibited opposite latitudinal defense patterns. The “low latitude high defense” hypothesis included a paradoxical aspect in chemical and physical defense traits across broadleaved tree species. To reconcile paradoxical defense strategies along the latitudinal gradient, we conclude that interactive correlations among leaf traits are controlled by leaf longevity, which differs between DB and EGB tree species.

Keywords: chemical defense, phenol, physical defense, plant–animal interactions, tannin.

Introduction

Biological interactions among organisms are believed widely to intensify at lower latitudes (Lewinsohn and Roslin 2008), leading to the development of latitudinal diversity gradient (LDG) hypotheses to explain large-scale biodiversity patterns (Dobzhansky 1950; MacArthur 1972; Pennings and Silliman 2005). As plants and herbivores comprise at least 40% of global terrestrial biodiversity (Price 2002), evaluation of the consequences of plant–herbivore interaction on the LDG should attract much interest (Marquis et al. 2012). Several studies have examined the “low latitude high defense” (LLHD) hypothesis (Bolser and Hay 1996) which posits that plant species distributed at lower latitudes will show higher degrees of defense (Schmitt et al. 1995, Dobzhansky 1950; Coley and Aide 1991). Although these studies have been conducted with the aim of confirming a latitudinal gradient in defense strength (see review by Moles et al. 2011), few have found support for the hypothesis.

Moles et al. (2011) performed a meta-analysis of defense trait data, both chemical (tannins and phenols, flavonoids, alkaloids etc.) and physical (physical strength, extra floral nectarines etc.) defense traits, across a wide range of latitude, and found conflicting trends in response to predictions based on the LLHD hypothesis, with increasing and decreasing gradients, as well as nonsignificant trends, in plant defense traits across latitudes. One pitfall of this study is the large bias caused by variation in various plant functional types among individual studies (Sitch et al. 2003), which reduced the statistical power to detect or identify specific latitudinal gradients in the targeted trait variables. For the broadleaved (BL) tree species, several functional types (or groups), e.g., trees or shrubs, N fixers or not, are recognized (Wright et al. 2004, Kattge et al. 2011). Among them, the contrast between deciduous broadleaved (DB) and evergreen broadleaved (EGB) tree species should be the most evident (Wright et al. 2004). For example, the results of Pringle, E. G. et al (2011)

shows that evergreen and deciduous trees have a distinguished leaf traits and syndromes in seasonally dry tropical forest. Also, Kikuzawa et al. (2013) showed clear contrast between DB and EGB tree species in leaf longevity along latitude. Thus, the separation of these functional types, namely DB vs. EGB, might clarify the conflicting trends in latitudinal gradient in plant defense traits. On the other hand, many empirical studies were based on trait data for particular plant taxa in phylogenetically narrow ranges (see supplemental tables in Moles et al. 2011; Anstett et al. 2016; see also Moles et al. 2013), which may make the detection of latitudinal gradients in defense strategies difficult.

As the defense strategies of each plant species, particular set of multiple defense traits were observed. Agrawal and Fishbein (2006) proposed the defense syndrome concept, in which several sets or combinations of defense traits are selected convergent as a syndrome. For example, they found three defense strategies in *Asclepias* plants: high physical and chemical defense with high nutrition, tolerance/escape, and low nutritional quality. Therefore, multiple functional traits should be examined simultaneously to explore plant defense strategies relevant to herbivores (Levin and York 1978). Thus, a comprehensive dataset including the entire species assemblage in the focal region for multiple sets of functional leaf traits should help us to rigorously examine the LLHD hypothesis (see also Anstett et al. 2016).

To test the LLHD hypothesis, we compiled a dataset of plant defense traits by thoroughly sampling BL tree species across the East Asian continental archipelago from the Hokkaido to Iriomote Islands (Maeshiro et al. 2014; Kusumoto et al. 2015; Shiono et al. 2015). In the present study, we first examined trait co-occurrence with respect to the chemical and mechanical defense strategies of each plant species using ordination. We then explored the latitudinal gradients of multiples of leaf defense trait of DB and EGB tree species by path analysis. Our goal is to clarify which leaf traits show the latitudinal gradients,

and how are the directions of the gradient both for DB and EGB tree species. Although the dominant theory is the LLHD (Dobzhansky 1950; Coley & Aide 1991), we expect that much complex trends will be observed depends on the plant functional types as well as the leaf defense traits examined.

Materials and Methods

Plant functional traits and latitudinal species distribution

We examined 267 EGB and 469 DB tree species, a total of 736 BL tree species (see Table S2). We focused on leaf traits that might function as defense against herbivores: tannin and phenol contents (%), leaf mechanical strength (g/cm^2), leaf dry matter content (LDMC, %), leaf mass per area (LMA, g/cm^2), and leaf thickness (μm). To build a dataset of leaf defense traits, we collected five replicate trees over the distributional range of each species across the East Asian islands, including the Ryukyu Islands. The latitudinal range of samplings was from 24°N to 45°N . Four shoots with leaves were collected from each five-tree, and average values of each tree individual were calculated (Shiono et al. 2015). The all leaves we collected were mature leaves generally from sun exploded side. LDMC and leaf thickness were measured following the protocols of Cornelissen et al. (2003). Leaf mechanical strength was measured using a “penetrometer” (Feeny 1970). Although Aranwela et al. (1999) showed the bias of using different area of fracture surface of punching rod (see also Onoda et al. 2013), in the present study, all the mechanical strength of leaves was measured by the standardized penetrometer made by MM. The diameter of steel punching rod was 3mm, thus the area of fracture surface was 7.07 mm^2 and punch perimeter was 9.42 mm. A proanthocyanidin assay was performed to determine tannin concentrations, using a

commercially available quebracho powder as the standard (Julkunen-Titto 1985). The Folin-Ciocalteu method was used to determine the total phenol content (Waterman and Mole 1994) with a tannic acid (Wako Co. Ltd., Tokyo, Japan) serving as the standard. The distribution data for woody plant species were collected from botanical literature on the flora of Japan. Data collection methods are described in detail in Kubota et al. (2015).

Statistical analysis

The relationships between leaf defense traits and the distributional range of each plant species were examined for BL tree species as a whole, and separately for DB and EGB tree species. The average values of five replicates for each tree species were used for the following analyses. Although intraspecific variations in leaf defense traits, and even intraspecific gradients in these traits along latitude, were reported (Moles et al. 2013), the shortages of within species replicates prevent us to examine these trends. To assess multivariate relationships among leaf defense traits, we performed principal component analysis (PCA), which deals with collinearity among multiple leaf traits (Pearse and Hipp 2012). Then, we developed full SEM model a priori based on the latitudinal effects on all the leaf defense traits and possible correlations among the traits (see Fig. 1-1). The fit of SEM was assessed by a χ^2 goodness-of-fit test of the model, the root mean square error of approximation (RMSEA) and the comparative fit index (CFI). A satisfactory model fit was indicated by: (1) a non-significant χ^2 goodness-of-fit test ($\alpha = 0.05$), (2) CFI > 0.9, and (3) lower 90% confidence intervals (CIs) of RMSEA < 0.05 (Blackburn et al 2016, Zhang et al 2013). Based on species distributional data, we analyzed the relationships between latitude (1° intervals) and the trait values of species distributed at the focal latitudes. The integer values of latitude at the lower limits were given for the all analysis. All variables used in path analyses were standardized (Legendre and Legendre 1998) by subtracting the mean and dividing by the

standard deviation.

All analyses were conducted in the R 3.3.2 statistical platform (R Development Core Team 2016). The SEMs were calculated with the R package *lavaan* 0.5–16 (Rosseel, 2012), and the packages *stats* and *MASS* (R Development Core Team 2016) were used for PCA and other analyses.

Results

Relationships among functional leaf traits

Among the BL tree species overall, the PCA results showed that 40% of the total variance was explained by the first axis and 30% was explained by the second axis. In a separate PCA of DB species, 40% of the total variance was explained by the first axis, with high LMA values and high LDMC values. The second axis, which distinguished species with high mechanical defense trait values from those with high tannin and phenol content values, explained 24% of the variation. The PCA of EGB species showed that 38% of the total variance was explained by the first axis, which separated species with low LMA values from those with high leaf mechanical strength (Fig. S1, Table 1-1). The second axis, which ordinated species with high chemical defense trait values and those with low mechanical defense trait values, explained 29% of the variation. The vectors for chemical and mechanical defense traits were at right angles to each other for DB and EGB tree species, indicating the separator of physical and chemical defense traits.

Path analysis

The effects of latitude on leaf traits contrasted markedly between DB and EGB species

(Fig.1-1, 1-2). Positive latitudinal effects on tannin and phenol content were detected in DB species, whereas a negative effect on tannin and no effect on phenol was detected in EGB species. We also observed contrasting latitudinal effects on leaf mechanical strength and thickness; these effects were positive for EGB species and negative for DB species. Effects on LDMC were positive in DB and EGB species.

Upon removal of the effects of latitude on individual leaf traits, the interactive correlations among leaf traits were fairly similar in DB and EGB species (Fig. 1-1). These trends were consistent even when the phylogenetic bias was removed using phylogenetic independent contrasts (PICs; Table S1; Felsenstein 1985).

Discussion

We found significant latitudinal gradients in leaf defense traits in both EGB and DB tree species. However, EGB and DB tree species demonstrated contrasting latitudinal gradients in mechanical and chemical defense traits. Among the mechanical defense traits, EGB species showed increasing trends in leaf mechanical strength and thickness along the latitudinal gradient, with tougher and thicker leaves occurring at higher latitudes. DB species showed the opposite trend, with softer and thinner leaves occurring at higher latitudes. Although Onoda et al. (2012) also observed the positive trends in leaf mechanical strength along latitude for the woody species, the present analysis on EGB and DB tree species showed contrasting trends between them. Among the chemical defense traits, EGB species exhibited a decreasing trend along the latitudinal gradient in tannin content, whereas DB species showed increasing trends with latitude in tannin and phenol content. These results indicate greater chemical defenses at lower latitudes in EGB species and greater physical defenses at

lower latitudes in DB species. Thus, the LLHD hypothesis should accommodate a paradoxical aspect in the latitudinal gradients in chemical and physical defense traits across DB and EGB tree species.

One possible explanation for these trends involves trade-offs between leaf defense traits along latitudinal gradients, which would limit total costs of defense against herbivores and might lead to contrasting patterns in latitudinal trends in chemical and mechanical defense traits (Eichenberg *et al.* 2015). In the present study, many pairs of leaf defense traits showed significant positive correlations in path analyses (Fig. 1-1), PCA (Fig. S1) and PIC (Table S1); these correlations were detected more frequently than in Moles *et al.* (2013) who observed them in only three of 45 pairwise comparisons. Moles *et al.* (2013) argued that this low incidence of significant correlations in pairwise comparisons might partly be explained by the bias in the measurement of defense traits, but not the allocation of resources that might drive trade-offs, in the majority of included studies. However, the present results show much clearer trends in correlations among leaf defense traits, likely due to the separate analyses of DB and EGB tree species and the comprehensive dataset of leaf defense traits obtained by thorough sampling (see Discussion in Moles *et al.* 2013).

One possible explanation for the contrasting trends in physical and chemical defense traits shown in both DB and EGB tree species is "trade-off" between these traits. Classical examinations on defense strategies considered defenses as singleton strategies, and assumed the trade-offs among different antiherbivore strategies (Steward and Keeler 1988, Herms and Mattson 1992). However, in the present study, we can observe the independent trends between physical and chemical leaf defense traits in PCA (Fig. S1, Table 1-1). These suggested that the contrasting trends of these leaf traits along the latitude were not trade-off but independent responses between physical and chemical leaf defense traits. These paradoxical trends in defense strategies observed in DB and EGB tree species could be

explained by trends in leaf longevity along temperature gradients. Kikuzawa et al. (2013) found decreasing trends in leaf longevity among EGB tree species along a temperature gradient, and opposing trends in DB tree species (Fig.1-3). They also detected a positive correlation between leaf longevity and leaf mass per area, which is generally correlated positively with leaf thickness, a surrogate for physical defense. Thus, EGB-specific higher physical defense (or DB-specific lower physical defense) at higher latitudes likely is a by-product of or reflects a correlation with the leaf longevity gradient along the temperature gradient (Fig. 1-3). Latitudinal gradients in leaf defense traits could be driven not only by herbivory, but also by abiotic conditions, e.g., soil fertility or UV radiations (Moles et al. 2011). Although this study examined multiple defense traits simultaneously across a wide range of species, broader and more consistent measurements of plant functional traits may be needed to obtain a better understanding of plant defense strategies.

Furthermore, the correlative patterns among leaf defense traits were consistent between DB and EGB tree species when the covariate effect of latitude was removed by path analysis (Fig. 1-1). These results suggest the existence of a fixed core structure in multiple defense traits. LMA showed consistent positive correlations with other leaf defense traits, suggesting that it has a defensive function or just correlate with them. Leaf economics spectrum studies indicated the existence of exploitative plant species, with fast growth associated with thinner leaves and lower LMA (Wright *et al.* 2004). Thus, species with lower LMA may abandon this trait to defend themselves from herbivores, and grow faster to escape herbivory. Conversely, LDMC showed consistent positive correlations with other leaf defense traits, suggesting that higher LDMC is a surrogate for a higher defense strategy in these species. Many previous studies have shown that LDMC is a surrogate for a variety of leaf properties (e.g., Shipley et al. 2006); higher LDMC is correlated with lower water content (Niinemets 2001) and lower soil fertility (Rusch et al. 2009). Positive correlations of

LDMC with plant mechanical strength and resistance to biotic agents have also been observed (Chave et al. 2009, Preston et al. 2006). These showed the common core structures in defense traits of DB and EGB tree species and also specific responses to environmental gradients between these tree species.

Finally, many recent researches have improved kinds of factors that affect the plant defense trait level. Intraspecific plant traits suggested that varied by the climate change (Chiho Kamiyama et al., 2014). They suggested intraspecific variation also made a large contribution. To avoid this variation, in the present study, we collected the leaf sampling as well as possible in the distribution range, then took the average value for subsequent analysis. We did not consider about the other impact index of environment for plant defensive traits in this research because of data form. For example, lightning, solid environment (Read J. et al., 2016) and etc.

Based on the examination of a comprehensive dataset of BL from East Asian flora, we discovered complex latitudinal trends in leaf defense traits involving higher chemical defense at lower latitudes for EGB species and higher physical defense at lower latitudes for DB species. In a meta-analysis derived from various empirical studies of leaf traits across functional types (e.g., tree, shrub, and herbaceous species) and in different regions, Moles et al. (2011) reported no such trend. Our evaluation of LLHD hypothesis that was characterized by a paradoxical aspect in chemical and physical defense traits suggests the importance of more detailed exploration of separate plant functional groups to test this hypothesis.

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Table 1. The results of Principal Component Analysis on broad-leaved (BL), evergreen (EGB) and deciduous (DB) tree species. The explanatory powers and the cumulative contributions (%) of each factor were shown.

	BL			EGB			DB		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
	(40)	(70)	(84)	(38)	(67)	(84)	(40)	(64)	(79)
tannin	0.09	-0.58	-0.42	-0.15	0.57	-0.34	0.34	-0.46	-0.45
phenol	0.15	-0.59	-0.30	-0.24	0.54	-0.37	0.45	-0.37	-0.32
strength	0.51	0.19	0.08	-0.48	-0.30	0.17	0.33	0.35	0.29
LDMC	0.35	-0.39	0.68	-0.35	0.37	0.67	0.51	-0.15	0.49
thickness	0.46	0.34	-0.51	-0.42	-0.40	-0.51	0.16	0.63	-0.60
LMA	0.61	0.04	0.06	-0.62	-0.05	0.06	0.53	0.34	0.08

Figure Captions

Figure 1-1. Results of a structural equation model (SEM) depicting hypothesized causal relationships among leaf defense traits and the effects of latitude on them. Solid lines indicate the positive effects or interactions. While the negative effects or interactions are indicated by broken lines. The dashed lines show the non-significant paths. The deciduous (DB) and evergreen (EGB) tree species were separately examined. Standardized coefficients are provided for each path with significant ($P > 0.05$) effect. For DB; Degree of freedom = 1, P -value (Chi-square) = 0.150 (indicating close model-data fit). For EGB; Degree of freedom = 2, P -value (Chi-square) = 0.249 (again indicating close model-data fit).

Figure 1-2. The distribution of leaf defense traits for deciduous and evergreen broad-leaved tree species. The signs (+, -) after the trait codes show the direction of latitudinal trends in the path analysis (Fig. 1-1), respectively.

Figure 1-3. Schematic relationships of latitudinal gradients of leaf longevity, chemical defense, and physical defense between deciduous (DB) and evergreen (EGB) broad leaved trees species. The pattern shown in the panel for leaf longevity was derived from the Kikuzawa et al. (2013).

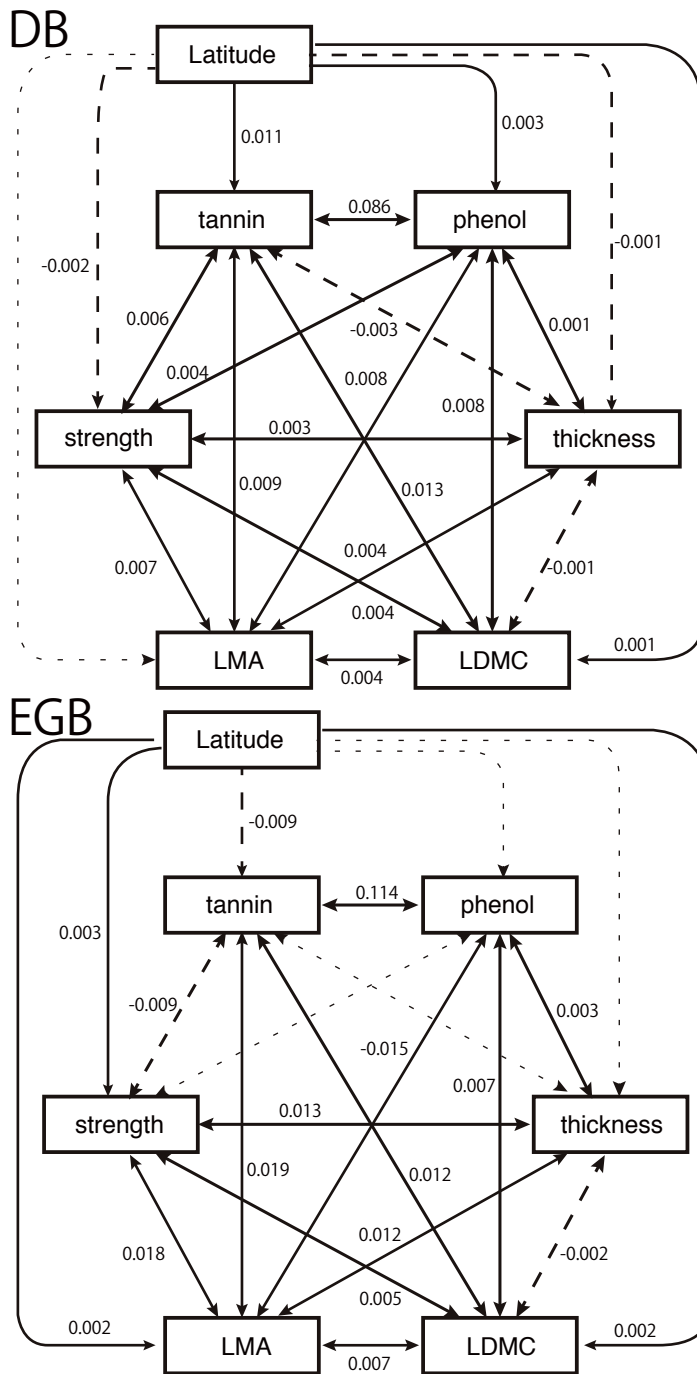


Figure 1-1

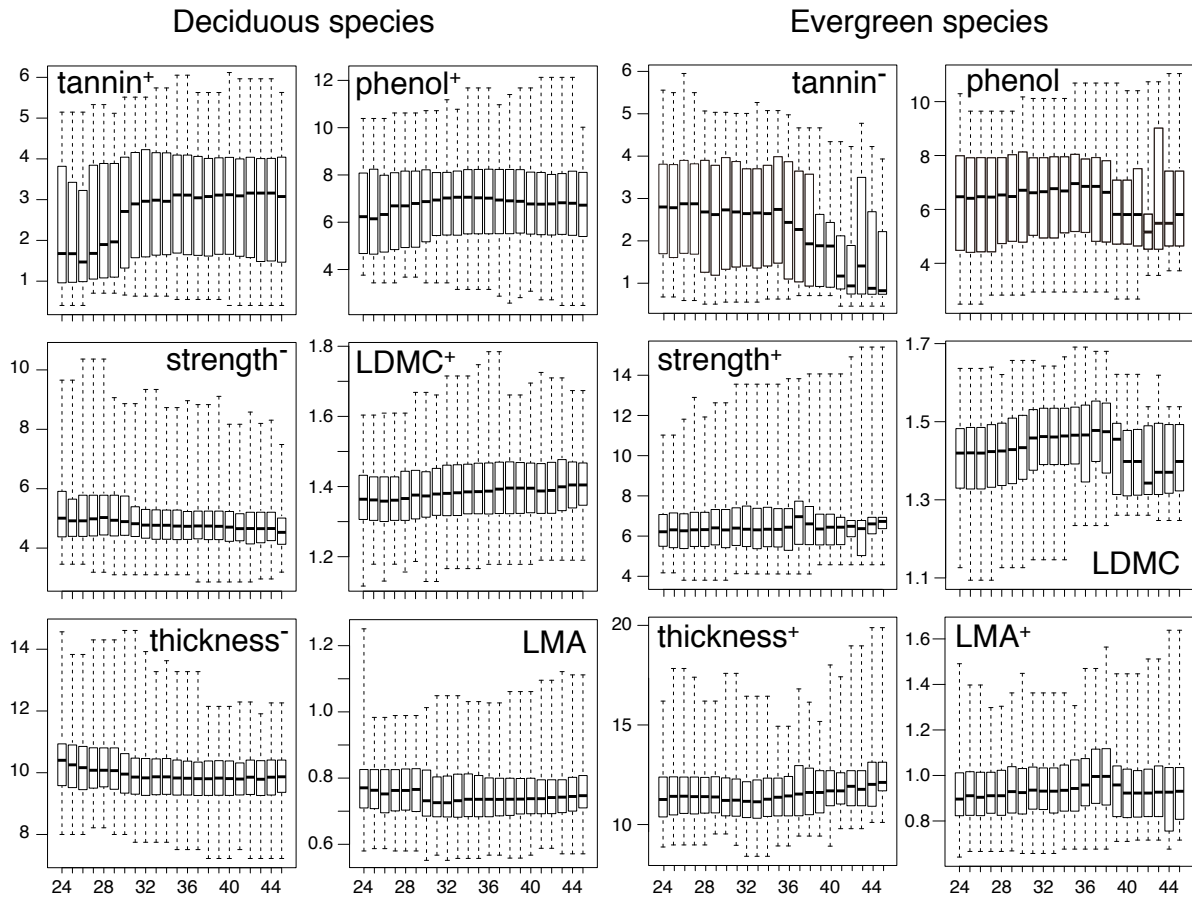


Figure 1-2

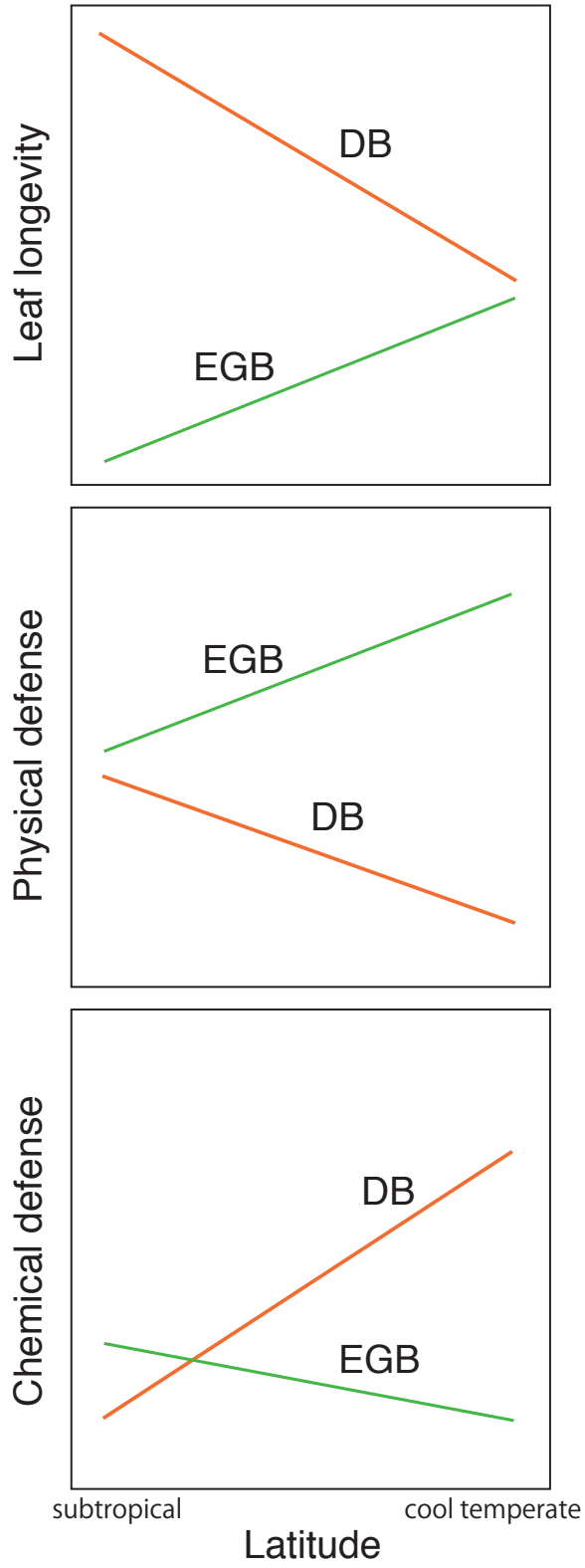


Figure 1-3

Chapter 2

The functional evaluation of leaf availability of Japanese trees using Eri silkmoth (*Samia cynthia ricini*, Lepidoptera, Saturniidae)

Abstract

To evaluate the leaf availability of broad-leaved tree species, I performed rearing experiment using Eri silkmoth (*Samia cynthia ricini*) on 310 tree species. Eri silkmoth was utilized because of their lack in host plant preference. The preliminary analysis revealed the affectivity of this moth larvae as the "objective index" of leaf availability. The performance was limited by both physical (leaf mechanical strength) and chemical (phenol content) leaf defense traits, although variety of specific chemical compounds was not measured in the present study. Contrasting effects of leaf mechanical strength were observed between evergreen and deciduous broad-leaved species. Whereas negative effect of mechanical strength was observed in evergreen species, unexpected positive relationship with leaf mechanical strength was detected in deciduous trees. Furthermore, the effect of leaf mechanical strength was fully excluded by treatment with artificial diet, which suggested the effect of potential factors correlate with leaf mechanical strength even the leaves were grained to powder. The effect of phenol content on larval performance was detected only in deciduous tree species. When the latitudinal trends in larval performance were examined, the increasing trend in larval performance was observed for deciduous tree species, but not in evergreen tree species. These results suggested that the strength of leaf defense in general is higher in higher latitude for the deciduous tree species, but for the evergreen tree species such gradient was not detected.

Introduction

Plants and herbivores comprise at least 40% of global terrestrial biodiversity (Price, 2002). It is believed that the evolution of plant defense traits followed by counter-adaptations in herbivores could lead to bursts of adaptive radiation of both components (Ehrlich and Raven 1969). After the seminal paper by Ehrlich and Raven (1969), despite the understanding of the macroevolution of leaf defense traits be attracted much interests for nearly fifty years, it has still been a big challenge for the ecologists (Moles et al. 2013). One of the key questions on the plant defenses is the examination on latitudinal diversity gradient (LDG) hypothesis, which explains large-scale biodiversity patterns (Dobzhansky 1950; MacArthur 1972; Pennings and Silliman 2005). This hypothesis argues that why the biological interactions among organisms are intensifying at lower latitudes (Lewinsohn and Roslin 2008). Although there are several studies confirmed the higher defense levels in tropical plant species (Rasmann and Agrawal 2011), the counter examples which suggested the opposite trends are also presented. The potential reason for this confusion must be a lack of objective measures of the strength of leaf defenses against herbivores.

Plants defend themselves by various ways, such as leaf mechanical strength, trichome and spine as physical defenses and secondary metabolites, such as tannin, phenol, and alkaloid, etc. as chemical defenses (War et al. 2012). The chemical defenses can be further classified into quantitative and qualitative chemical defenses (Rhoades 1979, Coley et al. 1985). Qualitative defenses are defined as toxins that interferes a specific-metabolism often by blocking several biochemical reactions (Theis et al., 2003). Thus, qualitative chemicals are generally not dosage dependent. On the other hand, quantitative chemicals are digestibility reducers that make plant cell walls indigestible to animals, and thus equally effective against both the specialist and generalist herbivores. Woody plant species tend to

produce the more amounts of quantitative chemicals such as tannin, because quantitative defenses are the most cost-effective in long-lived leaves with high synthetic costs (Coley et al., 1985; Coley, 1988). Of course, this does not rule out the possibility that woody plants possess the qualitative defenses, such as Aglycone in *Nerium* (Franz 1989), or Urushiols in *Toxicodendron* (Wheeler et al. 2014). Plant defenses are composed of multiple traits (Sánchez-Sánchez and Morquecho-Contreras, 2017), which might be organized into co-adapted trait complexes (Dobzhansky 1970). Agrawal & Fishbein (2006) proposed the concept of defense syndrome by examining the 24 species of milkweeds (*Asclepias* spp.) in a field experiment. They considered this trend as a consequence of shared evolutionary ancestry or because of adaptive convergence.

Although many defense traits had been repeatedly measured, and the effectiveness of these traits as for the defense were evaluated (e.g., Moles et al. 2013), however, for the fair evaluation of the availability of plants leaf, the availability of the leaf for the herbivores should be objectively measured. Therefore, in this study, I set an "objective index" of leaf availability for each plant species against the insect herbivores. Here, I use the larvae of the Eri silkmoth (*Samia cynthia ricini*) to evaluate the leaf availability. Eri silk-moth is a generalist herbivore and completely lose the preference among plant species. The larvae are highly polyphagous, but natural host-plants are castor (*Ricinus*) and *Ailanthus* tree. The larvae of Eri silkmoth eventually eat any kind of plant leaves unless the leaves are too hard or hairy, and also eat artificial diets containing extracts from various plants, then subsequently show the symptoms such as death from poisoning and growth inhibition in response to the respective plant (Hirayama & Konno, 2007). For this feature, Eri silk-moth larvae have been used in bioassays and analyses to evaluate the defense activities and defense levels of plants against herbivorous insects (Fukui et al., 2002; Konno *et al.*, 2004; Konno *et al.*, 2006).

The aim of present study is to elucidate the factor explaining the leaf availability of

woody tree species all over the Japanese archipelago, and also evaluate the latitudinal gradient observed in leaf availability. I sampled the tree species as many as possible to analyze the effects of the leaf defense traits on the larval growth of highly polyphagous herbivore. Many past studies show that some of plant defensive traits shows a significant phylogenetic signal among species (e.g. Agrawal et al., 2009). My previous research also gives the evidence that most of the defensive trait I will use in this study shows a significant phylogenetic signal (Saihanna et al. in submit.). However, the study to related herbivore growth rate and host plant phylogeny is rare. Thus, my first aim is trying to make clean this question: Is there a phylogenetic signal in the growth of Eri silk-moth larvae what the relationship between the defensive traits and the growth of Eri silk-moth larvae?

Materials & Methods

Leaf Trait Data

I sampled mature leaves from 310 species, which are native to Japan for raring experiment. For the dataset of leaf defense traits, I collected five replicates over the distributional range of each species across the East Asian islands. Four shoots with leaves were collected from each tree; average values were used in analysis (see Shiono et al. 2015 for detail). Phylogenetic relationship among tree species examined in the present study, was estimated by Phylocom (Webb et al. 2008). I selected 4 leaf traits as the physical leaf defense traits: leaf mechanical strength, leaf thickness, leaf dries mass content (LDMC) and leaf mass per area. Additionally, we collected the C: N ratio as nutritional trait. For the chemical defense traits, I used the contents of condensed tannin and total phenolic, which are well known as the defense for the herbivores (Feeny 1970).

Raring experiment

The Eri silkmoth was used for the experiment. I performed two types of raring experiment as follow. Fresh leaf (FL) treatment; I collected the fresh mature leaves for 190 broad leaved tree species. 10 silkmoth larvae were applied to one leaf for 48 hours in condition of 25°C, appropriate humidity, for 16 hours illumination, 8 hours darkroom. This was replicated five times for each tree species. Artificial food (AF) treatment; the mature leaves of 310 species (including the 190-species for FL) of broad leaved tree species were sampled, and dried at room temperature over silica gel. Dried leaves were pulverized and made into the artificial diet. For the artificial food, dried leaf powder of each tree species was mixed with the powder of a ready-made diet material, Insecta F-II (Nihon-Nosan-Kogyo Co., Tsukuba, Japan). The proportion of Insecta, leaf powder, and water is 1:1:6. Insecta F-II (Nosan Corporation Life-Tech Department, Japan) is a common artificial food for herbivore insect larvae which is without the leaf component. The main component of the Insecta F-II is chlorella powder, defatted soybeans, starch, sugar, cellulose, modeling agent, citric acid, vitamins, minerals, preservative, and antibiotics. This paste was utilized for the raring experiment. Again, 10 silkmoth larvae were applied to the paste for 48 hours. The AF treatment was set for the purpose of removing the effect of leaf mechanical strength on the larval performance in FL treatment. After 48 hours, I measured the average body mass (nearest to 0.1 mg) as an index of the growth on the leaf. For the growth data, zero values were added for the dead individuals. I also calculated the survival rate for each treatment. Furthermore, the decreased difference of fresh leaf and artificial food were also measured (nearest to 0.1 mg) as an index of feeding mass of the larvae.

Evaluation of the performance of Eri silkmoth as an "objective index"

Although Eri silkmoth is highly polyphagous, they still have several tree species as "host plant". If there is any preference of the moth related to the closeness from the original hosts, the performance of this moth couldn't be evaluated as "objective". The larval performances on each experimental tree species were compared with the phylogenetic distance from the natural host plants of Eri silkmoth, namely *Ricinus* and *Ailanthus* trees, respectively.

Although *Ricinus* and *Ailanthus* are not native to Japan, rearing experiments were also carried out for these two tree species. The residual of larval growth on target tree species from these two tree species, respectively, were regressed by phylogenetic distance (Generalized linear model; GLM) from these two species (Phylocom; Webb et al. 2008).

Statistical Analyses

To assess multivariate relationships among leaf defense traits, I performed principal component analysis (PCA), which deals with collinearity among multiple leaf traits (Pearse and Hipp 2012). Then I choose the representative leaf defense traits which shows independent trends each other. These variables were served for linear model (LM) to examine the factors that explain the differences in the larval performances among tree species. First, the full model with examining the all the variables was prepared, and the best model with lowest AIC value was selected by stepAIC function in the MASS library implemented in R.

I further applied GLM to examine relationships between the latitudinal distributions of plant species and the leaf availability evaluated by larval performance of silkmoth. Based on species distributional data of tree species, I analyzed the relationships between latitude (1° intervals) and the values of larval performance on the focal tree species.

Results

Performance of Eri silkmoth

There was no sign of the effect of the phylogenetic distance from original host plant species on the larval performance of Eri silkmoth when fed on the wide range of plant species (Table 2-1).

Effects of leaf traits on larval performance of Eri silkmoth

When the effects of leaf defense traits were examined, the GLM selected the both chemical and physical defense traits. For the evergreen species, the larval growth was negatively affected by leaf mechanical strength and phenol contents (Table 2-2). Interestingly this effect was observed even on the larvae fed on artificial diets. For the deciduous tree species, the larval growth was enhanced when fed on tougher leaves contrary to the expectation (Table 2-2). The effects of LDMC and CN were generally observed on the larval growth. The leaf consumptions were also affected by LDMC and CN in general (Table 2-2). The negative effect of phenol contents was observed on the leaf consumption for deciduous trees.

I picked up the lower 5% of the species, which grow worst among all the tree species (Table 2-4). There are 18 plant species on which none of larvae show any growth. The larvae were fatal on 13 plant species. These included 16 of evergreen species, and 6 of deciduous species.

Latitudinal gradient of leaf availability

When the larval growth of Eri silkmoth was regressed with the distribution range of plant species used for the rearing experiment, I observed clear increasing trends in deciduous tree species (Table 2-3, Fig. 2-1). On the other hand, there was no trend for the evergreen tree species (Table 2-3, Fig. S2). The amount of leaf consumption also showed similar trend with

those for larval growth of Eri silkworm. These suggested that the leaf availability for herbivores is higher in high latitude area for the deciduous tree species, but show no trends for evergreen tree species.

Discussion

None of the effect of the distance from original host plants was observed in larval performance of Eri silkworm (Table 2-1), which showed the affectivity of this moth as the "objective index" of leaf availability of broad-leaved tree species in general.

For EGB species, leaf mechanical strength showed negative effects on larval growth, which is straight forward and reasonable result. However, for the DB species, this relationship was opposite, and positive effect of leaf mechanical strength was found on the larval growth. This result suggests that the leaf mechanical strength functions as a defense in EGB species, but not in the DB species. It is expected that the most of the leaves of DB trees are sufficiently soft for the larvae, and the leaf mechanical strength shows the usefulness of the leaves through the thickness of them. In the previous chapter, I showed the differential trends of leaf mechanical strength along the latitudinal gradient between DB and EGD tree species (Fig. 1-2), and I examined this trend in relation with the variation of leaf life spans (see Kikuzawa et.al, 2013). Although many previous studies utilized the leaf mechanical strength as the index of physical defense of the plants (Feeny 1970, Murakami and Wada 1997 etc.), the present result suggested the malfunction of leaf mechanical strength as the leaf defense trait at least for the deciduous tree species. As Moreira et al. (2017) suggested that leaf mechanical strength is the fundamental and structurally important for EGB oak species, the function of leaf mechanical strength might be quite diverse, and careful examination is

needed to examine the strength of leaf defense in general.

The interesting point of the effect of leaf mechanical strength on larval growth is that even the effect of mechanical strength was removed by feeding the artificial diet, the effect of the mechanical strength on larval growth was detected in EGD species (Table 2-2). In the case of deciduous tree species, the effect of leaf mechanical strength be excluded as expect. This suggests that leaf mechanical strength of EGD species correlates with several defense traits other than physical ones. Although I did not find any defensive trait which have a correlation with leaf mechanical strength, there is an evidence that the mechanical strength has a positive correlation with total phenolic content (Read et al. 2009).

The effect of phenol contents was also confirmed, although only a weak effect was detected in the raring experiment. Phenols, including the tannin were repeatedly reported as one of the major agent of leaf defense as "quantitative" defense (Feeny 1976). However, in this study, the effect of tannin was never detected. Because phenols are the biosynthetic material for the tannins (e.g., Herms and Mattson 1992), it is expected the interaction among phenol and tannin with the other chemicals such as lignin. The present results might be show that the effect of qualitative defense such as tannin or phenols is weak but significant for the larval performance.

When the outlier of the plant species in the result of raring experiments, I can find several "extreme" species in their defense strategies. The most of outliers possessed very hard leaves. For the DB species, they were *Celtis boninensis*, *Castanea crenata*, *Carpinus laxiflora*, and *Carpinus tschonoskii*, which all show crispy or very hard leaves. Only the *Cerasus incisa* has soft leaves but the larvae did not grow up well. *Garcinia subelliptica* and *Maesa montana* showed lower survival and also growth. The leaf of *Garcinia* species is known to contain toxic chemicals, garcinia-cambodia (hydroxycitric acid) (Heymsfield et al 1998). However, I have no toxicity information about *Maesa montana*. I also find that many

of the zero-growth plant species are medicinal plant, such as *Stachyurus praecox* is one of the medicinal plant with high tannic acid content.

Because plant defense strategies are complicated, it is hard to make clear the whole mechanism of how plant defense traits interact and the availability for the herbivores is determined. In our result, we found that the leaf mechanical strength what usually known as a physical defense trait, actually not defense in DB if the leaf is not hard to the certainly strength (Fig. 2-2). But we cannot say that leaf mechanical strength is work as a defense trait in EGB but not in DB. Agrawal and Fishbein (2006) proposed the so-called defense syndrome hypothesis that includes several defense categories focused on two-dimensional array, edibility/digestibility and toxicity/barrier axes. However, these studies examined the defense strategy only from the view from plant side, namely leaf defense trait. On the other hand, the present study considered the leaf availability from the point of view from herbivores, which should be worth to be taken in future challenges.

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Table 2-1. The results of GLM on the variation of larval performance among plant species examined.

	Comparison with <i>A. altissima</i> Δ AIC	Comparison with <i>R. communis</i> Δ AIC
DB	-1.6	-1.9
EGB	0.2	-1.5

Table 2-2. The explanatory variables, which was selected as the best model under the criteria of AIC. The plus and minus at the parenthesis shows the sign of the coefficients for each explanatory variable.

	EGB	DB
Growth FL	strength-, phenol-	LDMC+, strength+
Growth AF	strength-, LDMC+, cn+	cn+, LDMC+
LC FL	LDMC+, cn-	phenol-
LC AF	LDMC+, cn-	LDMC+, cn+

LC:leaf consumption; FL: fresh leaf; AF: artificial feed

Table 2-3. Results of regression of larval growth on latitude by GLM.

	EGB FL		DB FL	
	Δ AIC	coeff.	Δ AIC	coeff.
Growth	-1.0	-	5.0	0.013
Leaf consumption	-1.6	-	26.1	0.005

Table 2-4. A list of plant species on which less-growth (1) or fatal (1) (lower 5% of species) performance of Eri silkmoth were observed when fed on fresh leaves. EGB shows broad-leaved evergreen species, and DB shows broad-leaved deciduous species.

plant species	DB/EGB	fatal	zero growth
<i>Carpinus laxiflora</i>	DB		1
<i>Carpinus tschonoskii</i>	DB		1
<i>Castanea crenata</i>	DB		1
<i>Celtis boninensis</i>	DB		1
<i>Cerasus incisa</i>	DB		1
<i>Ficus benguetensis</i>	DB		
<i>Ardisia sieboldii</i>	EGB	1	1
<i>Camellia japonica</i>	EGB		1
<i>Camellia lutchuensis</i>	EGB	1	1
<i>Camellia sasanqua</i>	EGB		1
<i>Cleyera japonica</i>	EGB	1	1
<i>Garcinia multiflora</i>	EGB	1	1
<i>Garcinia subelliptica</i>	EGB	1	1
<i>Maesa montana</i>	EGB	1	1
<i>Melicope triphylla</i>	EGB	1	1
<i>Sarcandra glabra</i>	EGB	1	1
<i>Symplocos prunifolia</i>	EGB	1	
<i>Symplocos stellaris</i>	EGB	1	1
<i>Tarenna gracilipes</i>	EGB	1	1
<i>Wikstroemia retusa</i>	EGB	1	1

Figure Captions

Figure 2-1. The distribution of leaf consumption and larval growth along the latitudinal range in the present study, when the larvae were fed on deciduous tree species (DB).

Figure 2-2. Schematic representation of ecological function of leaf strength on larval performance.

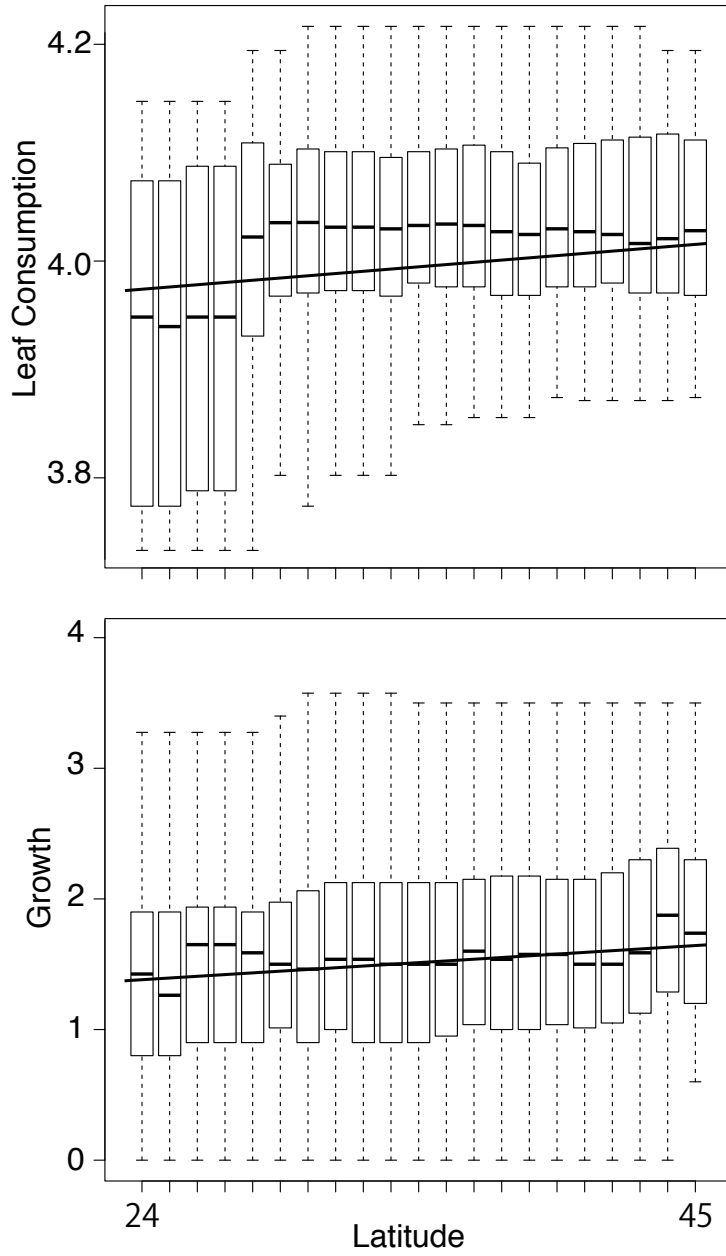


Fig. 2-1

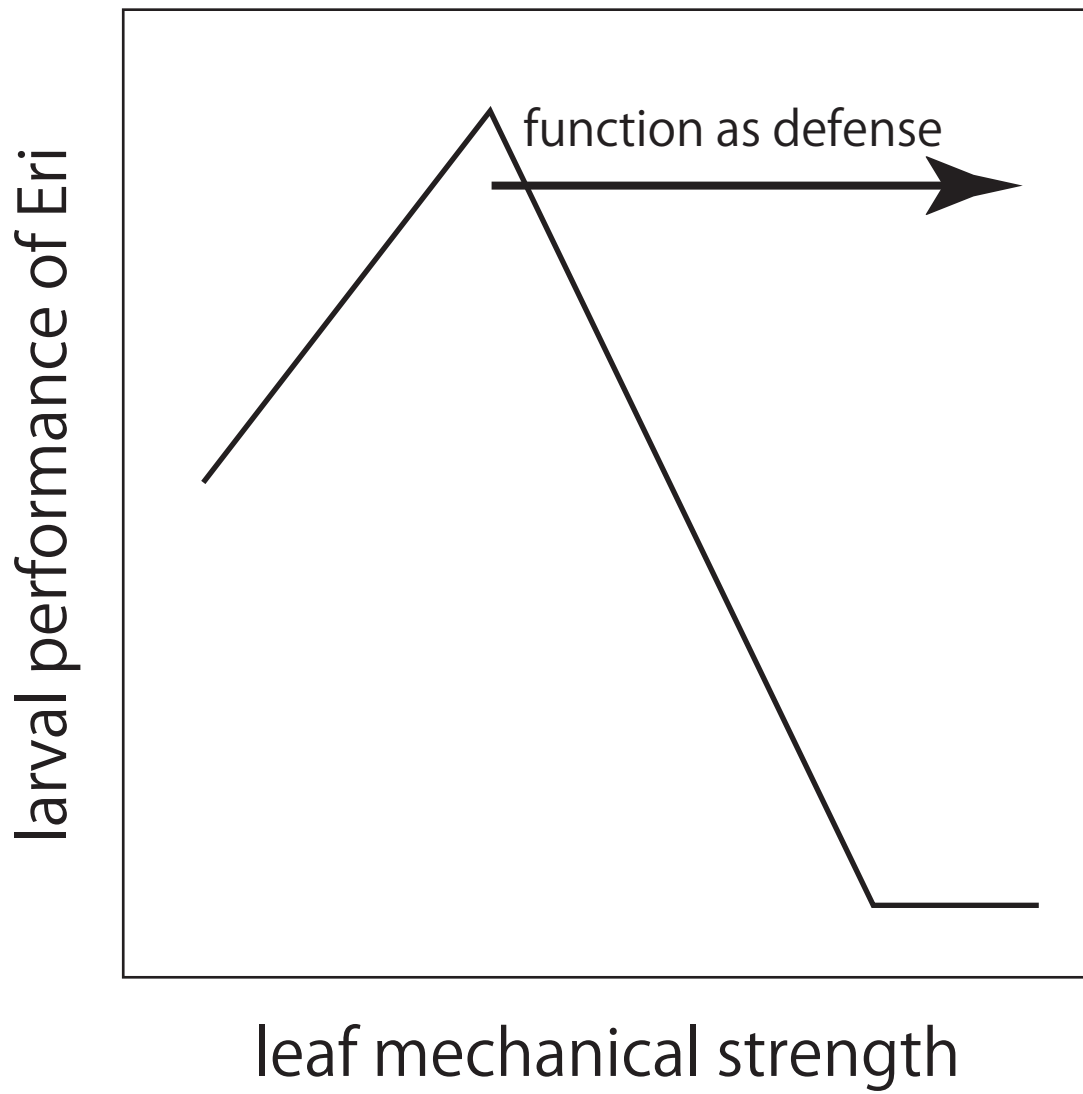


Fig. 2-2

General Discussion

In this study, 60% (736 species) of broad-leaved tree flora recorded in Japanese archipelago (1225 species in total, Satake et al. 1989) was sampled and multiple functional leaf traits were measured (Chapter 1). These are the biggest and the most thorough examination of geographic trends and the functional effects of leaf defense traits along temperate to subtropical gradient through Japan (see also Moles et al. 2011). Furthermore, large-scale rearing experiments using the Eri silkmoth revealed the function of each leaf defense trait on the performance of herbivorous insects in general (Chapter 2).

Clear latitudinal gradients in leaf defense traits were observed when the EGB and DB tree species were separately examined. For the EGB species, increasing trends in leaf mechanical strength and thickness along latitude were observed, hence the higher the latitude the tougher and thicker the leaves. The larval growth of Eri silkmoth showed the significant negative correlation with leaf mechanical strength in evergreen species as expected. On the other hand, the DB species showed the opposite trends in leaf mechanical strength and thickness along latitude; the higher the latitude the softer and thinner the leaves. Then the leaf mechanical strength showed a "positive" correlation with larval growth. These results suggest the importance of the separate analyses among functional types like EGB and DB tree species (Kikuzawa et al, 2013). In EGB tree species, the leaf mechanical strength functions as the defense for the herbivore, but in DB tree species, the leaves are sufficiently soft for the herbivore at least for Eri silkmoth, and the leaf mechanical strength represented the "usefulness" of the leaves with the leaf thickness. Although this is a very common way of thinking, but the function of leaf mechanical strength for plants are not simply for the defense from the herbivores (Moreira et al, 2017). The leaf mechanical strength should facilitate the structural strength of the leaves and also the resistance to desiccation (Brenes Arguedas et al.

2013), which should also change along the latitude. When the factors regulating the variation in the strength of interactions between plants and herbivore are examined, e.g., the examination of low latitude high defense, the causal relationships of each leaf traits and the multiple functions should be inspected.

Several studies showed that mean annual temperature (MAT) had contrasting effects on the leaf lifespan of evergreen and deciduous tree species, respectively; leaf lifespan increases with increasing MAT for deciduous species, while leaf lifespan decreases with increasing MAT for evergreen species (see Wright et al. 2005, Kikuzawa et al. 2013). Furthermore, it is obvious that leaf mechanical strength positively associated with leaf lifespan both in evergreen and deciduous tree species (Reich et al. 1991). Although I did not measure the leaf lifespan in the present study, many other leaf traits should potentially affect the observed latitudinal trends in leaf defense traits. We also found that larvae growth increasing with C: N ratio in deciduous tree species (Table 2-2). Since it is known that plant will investment in alkaloids defense substances when N increased, at low latitudes may include other defenses such as alkaloids. About the evergreen tree species, the results in chapter 1 came to the conclusion that low latitude has higher chemical defense and at higher latitudes, there is became a higher physical defense (Fig. 1-2, Fig. 1-3). Also, at the chapter 2, we found that in raring experiment both chemistry and physical defense both prevent larvae growth in evergreen species (Table 2-2). Perhaps for the evergreen species there is a trade-offs between chemical defense and physical defense so that total defense did not change along the latitude, so it seems that the growth of the larvae did not show a latitude trend. We think the defense strategy choice will be different depending on the latitude. However, since evergreen tree species are more widely distributed at low latitudes and we collected less samples at higher latitudes, is likely to affect the results. Still, all these strongly imply that the leaf defense strategy itself should be evolved under the multiple factors affecting the variety

of function of leaves. It is hard to clarify the causal mechanisms how the latitudinal gradient in defense.

In this thesis, I simply use only one species of generalist herbivore, Eri silkmoth in a rearing experiment. Nevertheless, I can draw a lot of useful results and general trends. For example, the dual function of leaf mechanical strength on the larval performance could be represented by this assay (Fig. 2-3). Furthermore, the results on several species showed unexpected low value of larval performance, e.g., *Maesa Montana*, on which none of specific defense mechanisms are reported yet. This result might be informative to seek the novel defense mechanism employed by plants. I could appeal that the common garden experiment using the diverse kinds of herbivore including specialist herbivore might have effective tools to evaluate the leaf quality for variety of herbivore species.

Although there are many studies about the plant-herbivore interaction, the results are very different. These mixed results mainly due to the inconsistency of the research methods and the scattered scope of the research object (Moles et al. 2011). I advocate that it is necessary to using a unified approach to evaluate the leaf availability and to create a unified global database to facilitate the integration of research results across all regions and plant/herbivore species. The integration of data from these single-region or species research results to the global unified dataset should be required to the general understandings of the leaf defense traits.

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Supporting Information

Plant Phylogeny

For the broadleaved tree species, phylogenetic trees were derived from Phylocom (Webb et al. 2008) which is the stand-alone version of plant lines creating software Phylomatic (Davies et al. 2004). A phylogenetic tree of the broadleaved tree species was constructed from an APGIII consensus tree (R20120829.new) choosing the maximally resolved seed plant tree option. The tree was resolved at the genus level and any unresolved genera were placed as basal polytomies within their families. Branch lengths in the phylogenetic tree were adjusted using the application bladp in phylocom (Webb et al. 2008).

Table S1. The results of correlation analysis using PIC among plant traits. The correlation coefficients were shown with the asterisks showing the significant correlation with a 5% level. R packages “ape” (Paradis et al. 2004) and “picante” (Kembel et al. 2010) were used the analysis.

	tannin	phenol	strength	LDMC	thickness
<hr/>					
BL					
phenol	0.53*				
strength	-	-			
LDMC	0.23*	0.31*	0.26*		
thickness	-0.14*	-0.09*	0.52*	-0.11*	
LMA	-	0.12*	0.61*	0.51*	0.63*
<hr/>					
DB					
phenol	0.54*				
strength	0.09*	0.1*			
LDMC	0.29*	0.36*	0.32*		
thickness	-	-	0.15*	-0.15*	
LMA	0.13*	0.34*	0.43*	0.66*	0.47*
<hr/>					
EGB					
phenol	0.53*				
strength	-	-			
LDMC	0.3*	0.29*	0.27*		
thickness	-	-	0.48*	-	
LMA	0.22*	0.23*	0.55*	0.54*	0.62*
<hr/>					

Table S2. A list of deciduous broad-leaved tree species used in this thesis. All the species used in the analysis in chapter 1 were listed. “1” shows the species used in rare experiments in chapter2, AF for artificial food, and FL for fresh leaf experiments, respectively.

Species	Family	AF	FL
<i>Abelia serrata</i>	Caprifoliaceae		
<i>Abelia spathulata</i>	Caprifoliaceae	1	
<i>Abelia tetrasepala</i>	Caprifoliaceae		
<i>Acer amoenum</i>	Sapindaceae	1	
<i>Acer argutum</i>	Sapindaceae	1	1
<i>Acer australe</i>	Sapindaceae		
<i>Acer capillipes</i>	Sapindaceae	1	1
<i>Acer carpinifolium</i>	Sapindaceae	1	1
<i>Acer cissifolium</i>	Sapindaceae	1	1
<i>Acer crataegifolium</i>	Sapindaceae	1	1
<i>Acer diabolicum</i>	Sapindaceae	1	
<i>Acer distylum</i>	Sapindaceae	1	1
<i>Acer ginnala</i>	Sapindaceae	1	
<i>Acer insulare</i>	Sapindaceae		
<i>Acer japonicum</i>	Sapindaceae	1	
<i>Acer maximowiczianum</i>	Sapindaceae		
<i>Acer micranthum</i>	Sapindaceae		
<i>Acer miyabei</i>	Sapindaceae	1	1
<i>Acer morifolium</i>	Sapindaceae		
<i>Acer nipponicum</i>	Sapindaceae		
<i>Acer palmatum</i>	Sapindaceae	1	
<i>Acer pictum</i>	Sapindaceae	1	
<i>Acer pycnanthum</i>	Sapindaceae		
<i>Acer rufinerve</i>	Sapindaceae	1	1
<i>Acer shirasawanum</i>	Sapindaceae	1	1
<i>Acer sieboldianum</i>	Sapindaceae	1	1
<i>Acer tenuifolium</i>	Sapindaceae		
<i>Acer tschonoskii</i>	Sapindaceae	1	1
<i>Acer ukurunduense</i>	Sapindaceae		
<i>Aesculus turbinata</i>	Sapindaceae	1	1
<i>Alangium platanifolium</i>	Cornaceae		
<i>Alangium premnifolium</i>	Alangiaceae		
<i>Albizia julibrissin</i>	Fabaceae	1	
<i>Albizia kalkora</i>	Fabaceae		
<i>Albizia retusa</i>	Fabaceae		

<i>Alchornea liukiensis</i>	Euphorbiaceae		
<i>Alnus fauriei</i>	Betulaceae		
<i>Alnus firma</i>	Betulaceae		
<i>Alnus hirsuta</i>	Betulaceae	1	
<i>Alnus inokumae</i>	Betulaceae		
<i>Alnus japonica</i>	Betulaceae	1	1
<i>Alnus matsumurae</i>	Betulaceae		
<i>Alnus pendula</i>	Betulaceae	1	1
<i>Alnus serrulatoides</i>	Betulaceae		
<i>Alnus sieboldiana</i>	Betulaceae		
<i>Alnus trabeculosa</i>	Betulaceae		
<i>Alnus viridis</i>	Betulaceae		
<i>Amelanchier asiatica</i>	Rosaceae		
<i>Aphananthe aspera</i>	Ulmaceae		
<i>Aralia bipinnata</i>	Araliaceae		
<i>Aralia elata</i>	Araliaceae	1	
<i>Aria alnifolia</i>	Rosaceae	1	
<i>Aria japonica</i>	Rosaceae		
<i>Berberis amurensis</i>	Euphorbiaceae	1	
<i>Berberis sieboldii</i>	Berberidaceae		
<i>Berberis thunbergii</i>	Berberidaceae	1	
<i>Berberis tschonoskyana</i>	Berberidaceae		
<i>Berchemia lineata</i>	Rhamnaceae		
<i>Berchemia longiracemosa</i>	Rhamnaceae		
<i>Berchemiella berchemiifolia</i>	Rhamnaceae		
<i>Betula apoiensis</i>	Fagaceae		
<i>Betula chichibuensis</i>	Betulaceae		
<i>Betula corylifolia</i>	Betulaceae		
<i>Betula ermanii</i>	Betulaceae	1	
<i>Betula globispica</i>	Betulaceae		
<i>Betula grossa</i>	Betulaceae	1	1
<i>Betula maximowicziana</i>	Betulaceae	1	
<i>Betula ovalifolia</i>	Betulaceae		
<i>Betula platyphylla</i>	Betulaceae	1	
<i>Betula schmidtii</i>	Betulaceae		
<i>Boehmeria nivea</i>	Urticaceae		
<i>Boehmeria spicata</i>	Urticaceae	1	
<i>Broussonetia kazinoki</i>	Moraceae	1	1
<i>Broussonetia papyrifera</i>	Moraceae	1	
<i>Buckleya lanceolata</i>	Santalaceae		
<i>Buddleja curviflora</i>	Scrophulariaceae		
<i>Buddleja davidii</i>	Scrophulariaceae		
<i>Buddleja japonica</i>	Scrophulariaceae		

<i>Caesalpinia decapetala</i>	Fabaceae		
<i>Callicarpa dichotoma</i>	Lamiaceae		
<i>Callicarpa formosana</i>	Lamiaceae		
<i>Callicarpa japonica</i>	Lamiaceae	1	1
<i>Callicarpa kochiana</i>	Lamiaceae		
<i>Callicarpa longissima</i>	Lamiaceae		
<i>Callicarpa mollis</i>	Lamiaceae		
<i>Callicarpa oshimensis</i>	Lamiaceae	1	
<i>Carpinus cordata</i>	Betulaceae	1	
<i>Carpinus japonica</i>	Betulaceae		
<i>Carpinus laxiflora</i>	Betulaceae	1	1
<i>Carpinus tschonoskii</i>	Betulaceae	1	1
<i>Carpinus turczaninovii</i>	Betulaceae		
<i>Castanea crenata</i>	Fagaceae	1	1
<i>Celtis boninensis</i>	Ulmaceae	1	1
<i>Celtis jessoensis</i>	Ulmaceae		
<i>Celtis sinensis</i>	Ulmaceae	1	
<i>Cerasus apetala</i>	Rosaceae		
<i>Cerasus incisa</i>	Rosaceae	1	1
<i>Cerasus jamasakura</i>	Rosaceae		
<i>Cerasus leveilleana</i>	Rosaceae		
<i>Cerasus maximowiczii</i>	Rosaceae	1	
<i>Cerasus nipponica</i>	Rosaceae	1	
<i>Cerasus sargentii</i>	Rosaceae	1	1
<i>Cerasus spachiana</i>	Rosaceae	1	1
<i>Cerasus speciosa</i>	Rosaceae		
<i>Cercidiphyllum japonicum</i>	Cercidiphyllaceae	1	1
<i>Cercidiphyllum magnificum</i>	Cercidiphyllaceae		
<i>Chengiopanax sciadophylloides</i>	Araliaceae	1	
<i>Chionanthus retusus</i>	Oleaceae		
<i>Choerospondias axillaris</i>	Anacardiaceae		
<i>Cladothamnus bracteatus</i>	Ericaceae		
<i>Cladrastis platycarpa</i>	Fabaceae	1	
<i>Cladrastis shikokiana</i>	Ericaceae		
<i>Clerodendrum trichotomum</i>	Ericaceae	1	
<i>Clethra barbinervis</i>	Clethraceae	1	1
<i>Codariocalyx microphyllus</i>	Fabaceae		
<i>Cordia dichotoma</i>	Boraginaceae		
<i>Coriaria japonica</i>	Coriariaceae	1	1
<i>Cornus controversa</i>	Cornaceae	1	
<i>Cornus kousa</i>	Cornaceae	1	
<i>Cornus macrophylla</i>	Cornaceae	1	
<i>Corylopsis glabrescens</i>	Hamamelidaceae		

<i>Corylopsis pauciflora</i>	Hamamelidaceae		
<i>Corylopsis spicata</i>	Hamamelidaceae		
<i>Corylus heterophylla</i>	Betulaceae		
<i>Corylus sieboldiana</i>	Betulaceae	1	1
<i>Crataegus chlorosarca</i>	Rosaceae	1	1
<i>Crataegus jozana</i>	Rosaceae		
<i>Crateva formosensis</i>	Rosaceae	1	1
<i>Daphne jezoensis</i>	Thymelaeaceae	1	
<i>Daphne pseudomezereum</i>	Thymelaeaceae	1	
<i>Debregeasia orientalis</i>	Urticaceae		
<i>Desmodium heterocarpon</i>	Fabaceae		
<i>Deutzia crenata</i>	Saxifragaceae	1	
<i>Deutzia floribunda</i>	Saxifragaceae		
<i>Deutzia gracilis</i>	Saxifragaceae		
<i>Deutzia maximowicziana</i>	Saxifragaceae		
<i>Deutzia naseana</i>	Saxifragaceae		
<i>Deutzia scabra</i>	Saxifragaceae	1	
<i>Deutzia uniflora</i>	Saxifragaceae		
<i>Deutzia yaeyamensis</i>	Saxifragaceae		
<i>Deutzia zentaroana</i>	Saxifragaceae		
<i>Diospyros japonica</i>	Ebenaceae	1	
<i>Diospyros oldhamii</i>	Ebenaceae		
<i>Diplomorpha ganpi</i>	Thymelaeaceae		
<i>Diplomorpha pauciflora</i>	Thymelaeaceae		
<i>Diplomorpha phymatoglossa</i>	Thymelaeaceae		
<i>Diplomorpha sikokiana</i>	Thymelaeaceae		
<i>Diplomorpha trichotoma</i>	Thymelaeaceae		
<i>Disanthus cercidifolius</i>	Hamamelidaceae	1	1
<i>Edgeworthia chrysantha</i>	Thymelaeaceae		
<i>Ehretia acuminata</i>	Boraginaceae		
<i>Ehretia dicksonii</i>	Boragiceae		
<i>Elaeagnus montana</i>	Elaeagnaceae		
<i>Elaeagnus multiflora</i>	Elaeagnaceae	1	
<i>Elaeagnus thunbergii</i>	Elaeagnaceae		
<i>Elaeagnus umbellata</i>	Elaeagnaceae		
<i>Eleutherococcus divaricatus</i>	Araliaceae		
<i>Eleutherococcus senticosus</i>	Araliaceae		
<i>Eleutherococcus spinosus</i>	Araliaceae	1	
<i>Elliottia paniculata</i>	Ericaceae		
<i>Enkianthus campanulatus</i>	Ericaceae	1	
<i>Enkianthus cernuus</i>	Ericaceae	1	1
<i>Enkianthus perulatus</i>	Ericaceae		
<i>Enkianthus sikokianus</i>	Ericaceae		

<i>Enkianthus subsessilis</i>	Lauraceae		
<i>Eubotryoides grayana</i>	Ericaceae		
<i>Euonymus alatus</i>	Celastraceae	1	1
<i>Euonymus macropterus</i>	Celastraceae	1	1
<i>Euonymus melananthus</i>	Celastraceae		
<i>Euonymus oxyphyllus</i>	Celastraceae	1	1
<i>Euonymus planipes</i>	Celastraceae		
<i>Euonymus sieboldianus</i>	Celastraceae		
<i>Euonymus tricarpus</i>	Celastraceae		
<i>Euptelea polyandra</i>	Eupteleaceae	1	1
<i>Euscaphis japonica</i>	Staphyleaceae	1	1
<i>Fagus crenata</i>	Fagaceae	1	1
<i>Fagus japonica</i>	Fagaceae		
<i>Ficus erecta</i>	Moraceae	1	
<i>Firmiana simplex</i>	Malvaceae		
<i>Flueggea suffruticosa</i>	Euphorbiaceae		
<i>Forsythia japonica</i>	Oleaceae		
<i>Forsythia togashii</i>	Oleaceae		
<i>Frangula crenata</i>	Rhamnaceae	1	
<i>Fraxinus apertisquamifera</i>	Oleaceae	1	1
<i>Fraxinus insularis</i>	Oleaceae		
<i>Fraxinus japonica</i>	Oleaceae		
<i>Fraxinus lanuginosa</i>	Oleaceae	1	1
<i>Fraxinus longicuspis</i>	Oleaceae		
<i>Fraxinus mandshurica</i>	Oleaceae	1	
<i>Fraxinus platypoda</i>	Oleaceae		
<i>Fraxinus sieboldiana</i>	Oleaceae	1	1
<i>Gamblea innovans</i>	Araliaceae		
<i>Glochidion obovatum</i>	Euphorbiaceae	1	1
<i>Hamamelis japonica</i>	Hamamelidaceae		
<i>Helwingia japonica</i>	Helwingiaceae	1	
<i>Hibiscus makinoi</i>	Malvaceae		
<i>Hovenia dulcis</i>	Rhamnaceae	1	
<i>Hovenia trichocarpa</i>	Rhamnaceae		
<i>Hydrangea chinensis</i>	Saxifragaceae		
<i>Hydrangea hirta</i>	Saxifragaceae		
<i>Hydrangea involucrata</i>	Saxifragaceae	1	
<i>Hydrangea kawagoeana</i>	Saxifragaceae		
<i>Hydrangea liukiensis</i>	Ericaceae		
<i>Hydrangea luteovenosa</i>	Saxifragaceae		
<i>Hydrangea paniculata</i>	Saxifragaceae	1	
<i>Hydrangea scandens</i>	Saxifragaceae		
<i>Hydrangea serrata</i>	Saxifragaceae	1	1

<i>Hydrangea sikokiana</i>	Saxifragaceae		
<i>Idesia polycarpa</i>	Salicaceae	1	1
<i>Ilex geniculata</i>	Ericaceae		
<i>Ilex macrocarpa</i>	Aquifoliaceae		
<i>Ilex macropoda</i>	Aquifoliaceae	1	1
<i>Ilex micrococca</i>	Aquifoliaceae		
<i>Ilex nipponica</i>	Aquifoliaceae		
<i>Ilex serrata</i>	Aquifoliaceae		
<i>Indigofera decora</i>	Fabaceae	1	
<i>Indigofera pseudotinctoria</i>	Fabaceae		
<i>Itea japonica</i>	Saxifragaceae		
<i>Juglans mandshurica</i>	Juglandaceae	1	
<i>Kalopanax septemlobus</i>	Araliaceae	1	1
<i>Kerria japonica</i>	Rosaceae	1	
<i>Koelreuteria paniculata</i>	Sapindaceae		
<i>Lagerstroemia subcostata</i>	Lythraceae	1	
<i>Ledum palustre</i>	Ericaceae		
<i>Lespedeza bicolor</i>	Fabaceae	1	1
<i>Lespedeza buergeri</i>	Fabaceae	1	1
<i>Lespedeza cyrtobotrya</i>	Fabaceae	1	
<i>Lespedeza thunbergii</i>	Fabaceae		
<i>Lespedeza virgata</i>	Fabaceae		
<i>Ligustrum obtusifolium</i>	Oleaceae	1	
<i>Ligustrum tschonoskii</i>	Oleaceae	1	1
<i>Lindera glauca</i>	Lauraceae		
<i>Lindera lancea</i>	Lauraceae	1	
<i>Lindera obtusiloba</i>	Lauraceae	1	1
<i>Lindera sericea</i>	Lauraceae		
<i>Lindera triloba</i>	Lauraceae	1	1
<i>Lindera umbellata</i>	Lauraceae	1	1
<i>Liriodendron tulipifera</i>	Magnoliaceae	1	1
<i>Litsea cubeba</i>	Lauraceae	1	
<i>Lonicera alpigena</i>	Caprifoliaceae		
<i>Lonicera caerulea</i>	Caprifoliaceae	1	1
<i>Lonicera chамissoi</i>	Caprifoliaceae		
<i>Lonicera chrysantha</i>	Caprifoliaceae		
<i>Lonicera demissa</i>	Caprifoliaceae		
<i>Lonicera gracilipes</i>	Caprifoliaceae	1	1
<i>Lonicera maackii</i>	Caprifoliaceae	1	1
<i>Lonicera ramosissima</i>	Caprifoliaceae		
<i>Lonicera sachalinensis</i>	Caprifoliaceae		
<i>Lonicera strophiphora</i>	Caprifoliaceae		
<i>Lonicera vidalii</i>	Caprifoliaceae	1	1

<i>Lycium chinense</i>	Solanaceae		
<i>Lyonia ovalifolia</i>	Ericaceae	1	1
<i>Maackia amurensis</i>	Fabaceae	1	1
<i>Macrodiervilla middendorffiana</i>	Caprifoliaceae		
<i>Magnolia kobus</i>	Magnoliaceae	1	1
<i>Magnolia obovata</i>	Magnoliaceae	1	
<i>Magnolia salicifolia</i>	Magnoliaceae	1	
<i>Magnolia sieboldii</i>	Magnoliaceae		
<i>Magnolia stellata</i>	Magnoliaceae		
<i>Mallotus japonicus</i>	Euphorbiaceae	1	
<i>Mallotus paniculatus</i>	Euphorbiaceae		
<i>Malus baccata</i>	Rosaceae		
<i>Malus spontanea</i>	Rosaceae	1	
<i>Malus toringo</i>	Rosaceae	1	1
<i>Malus tschonoskii</i>	Rosaceae	1	
<i>Margaritaria indica</i>	Euphorbiaceae	1	1
<i>Melanolepis multiglandulosa</i>	Euphorbiaceae	1	1
<i>Melia azedarach</i>	Meliaceae	1	
<i>Meliosma arnottiana</i>	Sabiaceae		
<i>Meliosma myriantha</i>	Sabiaceae		
<i>Meliosma tenuis</i>	Sabiaceae		
<i>Menziesia multiflora</i>	Ericaceae		
<i>Menziesia pentandra</i>	Ericaceae		
<i>Menziesia purpurea</i>	Ericaceae		
<i>Morus australis</i>	Moraceae	1	1
<i>Myrica gale</i>	Myricaceae		
<i>Neillia incisa</i>	Rosaceae	1	1
<i>Neillia tanakae</i>	Rosaceae	1	
<i>Neoshirakia japonica</i>	Euphorbiaceae	1	1
<i>Ohwia caudata</i>	Fabaceae		
<i>Oreocnide frutescens</i>	Urticaceae		
<i>Orixa japonica</i>	Rutaceae	1	
<i>Ormocarpum cochinchinense</i>	Fabaceae		
<i>Ostrya japonica</i>	Betulaceae	1	
<i>Padus avium</i>	Rosaceae		
<i>Padus buergeriana</i>	Rosaceae	1	1
<i>Padus grayana</i>	Rosaceae	1	1
<i>Padus ssiori</i>	Rosaceae	1	
<i>Paliurus ramosissimus</i>	Rhamnaceae		
<i>Paulownia tomentosa</i>	Scrophulariaceae	1	
<i>Pertya scandens</i>	Asteraceae	1	1
<i>Phellodendron amurense</i>	Rutaceae		
<i>Philadelphus satsumi</i>	Saxifragaceae	1	1

<i>Phyllanthus flexuosus</i>	Euphorbiaceae	1	
<i>Picrasma quassioides</i>	Simaroubaceae		
<i>Platycarya strobilacea</i>	Juglandaceae		
<i>Platycrater arguta</i>	Saxifragaceae		
<i>Populus suaveolens</i>	Salicaceae	1	
<i>Populus tremula</i>	Salicaceae	1	
<i>Pourthiaea villosa</i>	Rosaceae	1	1
<i>Premna microphylla</i>	Lamiaceae		
<i>Pterocarya rhoifolia</i>	Juglandaceae		
<i>Pterostyrax corymbosa</i>	Styracaceae		
<i>Pterostyrax hispida</i>	Styracaceae	1	1
<i>Pyrus calleryana</i>	Rosaceae		
<i>Pyrus pyrifolia</i>	Rosaceae		
<i>Pyrus ussuriensis</i>	Rosaceae	1	1
<i>Quercus acutissima</i>	Fagaceae	1	1
<i>Quercus aliena</i>	Fagaceae		
<i>Quercus crispula</i>	Fagaceae	1	
<i>Quercus dentata</i>	Fagaceae	1	
<i>Quercus serrata</i>	Fagaceae	1	
<i>Quercus variabilis</i>	Ericaceae		
<i>Rhamnella franguloides</i>	Rhamnaceae	1	
<i>Rhamnus costata</i>	Rhamnaceae		
<i>Rhamnus davurica</i>	Rhamnaceae		
<i>Rhamnus japonica</i>	Rhamnaceae		
<i>Rhamnus liukiuensis</i>	Rhamnaceae		
<i>Rhamnus yoshinoi</i>	Rhamnaceae		
<i>Rhododendron albrechtii</i>	Ericaceae	1	
<i>Rhododendron amagianum</i>	Fagaceae		
<i>Rhododendron amakusaense</i>	Ericaceae		
<i>Rhododendron dauricum</i>	Ericaceae		
<i>Rhododendron dilatatum</i>	Ericaceae	1	1
<i>Rhododendron hyugaense</i>	Ericaceae		
<i>Rhododendron kaempferi</i>	Ericaceae	1	1
<i>Rhododendron kiusianum</i>	Ericaceae	1	
<i>Rhododendron kiyosumense</i>	Rosaceae		
<i>Rhododendron lagopus</i>	Ericaceae		
<i>Rhododendron mayebarae</i>	Ericaceae		
<i>Rhododendron molle</i>	Ericaceae	1	1
<i>Rhododendron mucronulatum</i>	Ericaceae		
<i>Rhododendron nipponicum</i>	Ericaceae		
<i>Rhododendron nudipes</i>	Ericaceae		
<i>Rhododendron osuzuyamense</i>	Ericaceae		
<i>Rhododendron pentaphyllum</i>	Ericaceae		

<i>Rhododendron quinquefolium</i>	Ericaceae		
<i>Rhododendron reticulatum</i>	Ericaceae		
<i>Rhododendron sanctum</i>	Ericaceae		
<i>Rhododendron semibarbatum</i>	Ericaceae		
<i>Rhododendron wadanum</i>	Ericaceae		
<i>Rhododendron weyrichii</i>	Ericaceae		
<i>Rhodotypos scandens</i>	Rosaceae		
<i>Rhus javanica</i>	Anacardiaceae	1	
<i>Ribes fasciculatum</i>	Saxifragaceae		
<i>Ribes japonicum</i>	Saxifragaceae		
<i>Ribes latifolium</i>	Saxifragaceae		
<i>Ribes maximowiczianum</i>	Saxifragaceae		
<i>Ribes sachalinense</i>	Saxifragaceae		
<i>Ribes sinanense</i>	Saxifragaceae	1	1
<i>Robinia pseudoacacia</i>	Fabaceae	1	1
<i>Rosa acicularis</i>	Rosaceae		
<i>Rosa fujisanensis</i>	Rosaceae		
<i>Rosa hirtula</i>	Rosaceae		
<i>Rosa luciae</i>	Rosaceae		
<i>Rosa multiflora</i>	Rosaceae	1	
<i>Rosa onoei</i>	Rosaceae		
<i>Rosa paniculigera</i>	Rosaceae	1	1
<i>Rosa rugosa</i>	Rosaceae		
<i>Rosa sambucina</i>	Rosaceae		
<i>Rubus crataegifolius</i>	Rosaceae	1	1
<i>Rubus croceacanthus</i>	Rosaceae	1	1
<i>Rubus grayanus</i>	Rosaceae	1	1
<i>Rubus idaeus</i>	Rosaceae		
<i>Rubus mesogaeus</i>	Rosaceae		
<i>Rubus microphyllus</i>	Rosaceae		
<i>Rubus palmatus</i>	Rosaceae	1	1
<i>Rubus parvifolius</i>	Rosaceae		
<i>Rubus subcrataegifolius</i>	Rosaceae		
<i>Rubus trifidus</i>	Rosaceae		
<i>Salix arbutifolia</i>	Salicaceae		
<i>Salix caprea</i>	Salicaceae	1	1
<i>Salix cardiophylla</i>	Salicaceae		
<i>Salix dolichostyla</i>	Salicaceae		
<i>Salix eriocarpa</i>	Salicaceae		
<i>Salix futura</i>	Salicaceae		
<i>Salix gracilistyla</i>	Salicaceae		
<i>Salix hukaoana</i>	Fagaceae		
<i>Salix integra</i>	Salicaceae	1	1

<i>Salix japonica</i>	Salicaceae		
<i>Salix miyabeana</i>	Salicaceae		
<i>Salix pierotii</i>	Salicaceae		
<i>Salix reinii</i>	Salicaceae		
<i>Salix rorida</i>	Salicaceae		
<i>Salix schwerinii</i>	Salicaceae		
<i>Salix sieboldiana</i>	Salicaceae		
<i>Salix taraiensis</i>	Salicaceae		
<i>Salix triandra</i>	Salicaceae		
<i>Salix udensis</i>	Salicaceae	1	1
<i>Salix vulpina</i>	Salicaceae		
<i>Salix warburgii</i>	Salicaceae	1	1
<i>Salix yoshinoi</i>	Salicaceae		
<i>Sambucus racemosa</i>	Caprifoliaceae	1	
<i>Sapindus mukorossi</i>	Sapindaceae	1	
<i>Schoepfia jasminodora</i>	Olacaceae	1	
<i>Sinoadina racemosa</i>	Rubiaceae		
<i>Sorbaria sorbifolia</i>	Rosaceae		
<i>Sorbus commixta</i>	Rosaceae		
<i>Sorbus gracilis</i>	Rosaceae		
<i>Sorbus matsumurana</i>	Rosaceae		
<i>Sorbus sambucifolia</i>	Rosaceae		
<i>Spiraea betulifolia</i>	Rosaceae		
<i>Spiraea chamaedryfolia</i>	Rosaceae		
<i>Spiraea dasyantha</i>	Rosaceae		
<i>Spiraea japonica</i>	Rosaceae		
<i>Spiraea media</i>	Rosaceae		
<i>Spiraea miyabei</i>	Rosaceae		
<i>Spiraea nipponica</i>	Rosaceae		
<i>Spiraea salicifolia</i>	Rosaceae	1	
<i>Spiraea thunbergii</i>	Rosaceae	1	1
<i>Stachyurus praecox</i>	Stachyuraceae	1	1
<i>Staphylea bumalda</i>	Staphyleaceae	1	
<i>Stewartia monadelphica</i>	Theaceae		
<i>Stewartia pseudocamellia</i>	Theaceae		
<i>Stewartia serrata</i>	Theaceae		
<i>Styrax japonica</i>	Styracaceae	1	
<i>Styrax obassia</i>	Styracaceae	1	1
<i>Styrax shiraiana</i>	Styracaceae	1	1
<i>Symplocos coreana</i>	Symplocaceae		
<i>Symplocos konishii</i>	Symplocaceae		
<i>Symplocos paniculata</i>	Symplocaceae		
<i>Symplocos sawafutagi</i>	Symplocaceae	1	1

<i>Syringa reticulata</i>	Oleaceae	1	
<i>Terminalia catappa</i>	Combretaceae		
<i>Tetradium glabrifolium</i>	Rutaceae	1	1
<i>Tilia japonica</i>	Malvaceae	1	
<i>Tilia kiusiana</i>	Malvaceae		
<i>Tilia mandshurica</i>	Malvaceae		
<i>Tilia maximowicziana</i>	Malvaceae	1	
<i>Toxicodendron succedaneum</i>	Anacardiaceae	1	
<i>Toxicodendron sylvestri</i>	Anacardiaceae		
<i>Toxicodendron trichocarpum</i>	Anacardiaceae	1	1
<i>Ulmus davidiana</i>	Ulmaceae	1	
<i>Ulmus laciniata</i>	Ulmaceae		
<i>Ulmus parvifolia</i>	Ulmaceae	1	1
<i>Uraria crinita</i>	Fabaceae		
<i>Urena lobata</i>	Malvaceae		
<i>Vaccinium hirtum</i>	Ericaceae	1	1
<i>Vaccinium japonicum</i>	Ericaceae		
<i>Vaccinium oldhamii</i>	Ericaceae		
<i>Vaccinium ovalifolium</i>	Ericaceae		
<i>Vaccinium smallii</i>	Ericaceae	1	1
<i>Vaccinium yakushimense</i>	Ericaceae		
<i>Vernicia cordata</i>	Celastraceae		
<i>Viburnum carlesii</i>	Caprifoliaceae		
<i>Viburnum dilatatum</i>	Caprifoliaceae	1	
<i>Viburnum erosum</i>	Caprifoliaceae		
<i>Viburnum furcatum</i>	Caprifoliaceae	1	1
<i>Viburnum opulus</i>	Caprifoliaceae	1	
<i>Viburnum phlebotrichum</i>	Caprifoliaceae		
<i>Viburnum plicatum</i>	Caprifoliaceae	1	
<i>Viburnum sieboldii</i>	Caprifoliaceae	1	
<i>Viburnum tashiroi</i>	Caprifoliaceae		
<i>Viburnum urceolatum</i>	Caprifoliaceae		
<i>Viburnum wrightii</i>	Caprifoliaceae	1	
<i>Vitex rotundifolia</i>	Lamiaceae	1	1
<i>Vitex trifolia</i>	Lamiaceae		
<i>Volkameria inermis</i>	Lamiaceae		
<i>Weigela coraeensis</i>	Celastraceae		
<i>Weigela decora</i>	Caprifoliaceae		
<i>Weigela floribunda</i>	Caprifoliaceae		
<i>Weigela hortensis</i>	Caprifoliaceae		
<i>Weigela japonica</i>	Caprifoliaceae		
<i>Weigela maximowiczii</i>	Caprifoliaceae		
<i>Zanthoxylum ailanthoides</i>	Rutaceae		

<i>Zanthoxylum piperitum</i>	Rutaceae	1	
<i>Zanthoxylum schinifolium</i>	Rutaceae	1	1
<i>Zanthoxylum yakumontanum</i>	Rutaceae		
<i>Zelkova serrata</i>	Ulmaceae	1	1

Table S3 A list of evergreen broad-leaved tree species used in this thesis. All the species used in the analysis in chapter 1 were listed. “1” shows the species used in rare experiments in chapter2, AF for artificial food, and FL for fresh leaf experiments, respectively.

species	family	AF	FL
<i>Acer oblongum</i>	Sapindaceae		
<i>Actinodaphne acuminata</i>	Lauraceae	1	1
<i>Adina pilulifera</i>	Rubiaceae		
<i>Adinandra ryukyuensis</i>	Fagaceae	1	1
<i>Adinandra yaeyamensis</i>	Theaceae		
<i>Aidia canthioides</i>	Rubiaceae	1	1
<i>Aidia cochinchinensis</i>	Rubiaceae		
<i>Allophylus timoriensis</i>	Sapindaceae		
<i>Antidesma japonicum</i>	Euphorbiaceae	1	1
<i>Antidesma pentandrum</i>	Euphorbiaceae		
<i>Archidendron lucidum</i>	Fabaceae		
<i>Ardisia crenata</i>	Primulaceae		
<i>Ardisia crispa</i>	Primulaceae		
<i>Ardisia quinquegona</i>	Primulaceae	1	
<i>Ardisia sieboldii</i>	Primulaceae	1	1
<i>Aucuba japonica</i>	Aucubaceae	1	1
<i>Avicennia marina</i>	Acanthaceae		
<i>Barringtonia asiatica</i>	Lecythidaceae		
<i>Barringtonia racemosa</i>	Lecythidaceae	1	1
<i>Beilschmiedia erythrophloia</i>	Lauraceae		
<i>Bischofia javanica</i>	Euphorbiaceae	1	1
<i>Blastus cochinchinensis</i>	Melastomataceae		
<i>Boehmeria densiflora</i>	Urticaceae		
<i>Bredia okinawensis</i>	Melastomataceae		
<i>Bredia yaeyamensis</i>	Melastomataceae		
<i>Breynia vitis-idaea</i>	Euphorbiaceae		
<i>Bridelia insulata</i>	Euphorbiaceae		
<i>Bruguiera gymnorhiza</i>	Rhizophoraceae		
<i>Buxus liukiensis</i>	Buxaceae	1	1
<i>Camellia japonica</i>	Theaceae	1	1
<i>Camellia lutchuensis</i>	Theaceae	1	1
<i>Camellia sasanqua</i>	Theaceae	1	1
<i>Castanopsis cuspidata</i>	Fagaceae	1	
<i>Castanopsis sieboldii</i>	Fagaceae	1	
<i>Cerbera manghas</i>	Apocynaceae	1	1

<i>Cinnamomum camphora</i>	Lauraceae	1	1
<i>Cinnamomum daphnoides</i>	Lauraceae		
<i>Cinnamomum doederleinii</i>	Lauraceae	1	1
<i>Cinnamomum sieboldii</i>	Lauraceae	1	1
<i>Cinnamomum tenuifolium</i>	Lauraceae	1	1
<i>Citrus depressa</i>	Rutaceae	1	1
<i>Citrus tachibana</i>	Rutaceae		
<i>Cleyera japonica</i>	Theaceae	1	1
<i>Cocculus laurifolius</i>	Menispermaceae		
<i>Crossostephium chinense</i>	Asteraceae		
<i>Croton cascarilloides</i>	Euphorbiaceae		
<i>Damnacanthus biflorus</i>	Rubiaceae	1	1
<i>Damnacanthus macrophyllus</i>	Rubiaceae		
<i>Daphne kiusiana</i>	Thymelaeaceae		
<i>Daphne miyabeana</i>	Thymelaeaceae		
<i>Daphniphyllum teijsmannii</i>	Daphniphyllaceae	1	1
<i>Dendrolobium umbellatum</i>	Fabaceae		
<i>Dendropanax trifidus</i>	Araliaceae	1	1
<i>Diospyros egbert-walkeri</i>	Ebenaceae	1	1
<i>Diospyros eriantha</i>	Ebenaceae		
<i>Diospyros maritima</i>	Ebenaceae	1	1
<i>Diospyros morrisiana</i>	Ebenaceae	1	1
<i>Diplospora dubia</i>	Rubiaceae	1	1
<i>Discocleidion ulmifolium</i>	Euphorbiaceae		
<i>Distylium racemosum</i>	Hamamelidaceae	1	1
<i>Dodonaea viscosa</i>	Sapindaceae		
<i>Ehretia microphylla</i>	Ericaceae	1	1
<i>Elaeagnus macrophylla</i>	Elaeagnaceae		
<i>Elaeagnus pungens</i>	Elaeagnaceae		
<i>Elaeocarpus japonicus</i>	Elaeocarpaceae	1	
<i>Elaeocarpus multiflorus</i>	Elaeocarpaceae		
<i>Elaeocarpus zollingeri</i>	Elaeocarpaceae	1	
<i>Eriobotrya japonica</i>	Lauraceae		
<i>Euchresta japonica</i>	Fabaceae		
<i>Euonymus carnosus</i>	Celastraceae		
<i>Euonymus chibae</i>	Celastraceae		
<i>Euonymus japonicus</i>	Celastraceae	1	
<i>Euonymus lutchuensis</i>	Celastraceae		
<i>Euonymus tashiroi</i>	Celastraceae		
<i>Euonymus trichocarpus</i>	Celastraceae		
<i>Eurya emarginata</i>	Theaceae	1	1
<i>Eurya japonica</i>	Theaceae	1	
<i>Eurya osimensis</i>	Theaceae		

<i>Eurya sakishimensis</i>	Theaceae		
<i>Eurya yaeyamensis</i>	Theaceae		
<i>Excoecaria agallocha</i>	Euphorbiaceae		
<i>Fatsia japonica</i>	Araliaceae	1	1
<i>Ficus ampelas</i>	Moraceae	1	1
<i>Ficus benguetensis</i>	Moraceae	1	1
<i>Ficus caulocarpa</i>	Moraceae		
<i>Ficus irisana</i>	Moraceae		
<i>Ficus microcarpa</i>	Moraceae	1	1
<i>Ficus septica</i>	Moraceae	1	1
<i>Ficus superba</i>	Moraceae	1	1
<i>Ficus variegata</i>	Moraceae		
<i>Ficus virgata</i>	Moraceae	1	1
<i>Flemingia macrophylla</i>	Fabaceae		
<i>Fraxinus griffithii</i>	Oleaceae	1	1
<i>Garcinia subelliptica</i>	Ericaceae	1	1
<i>Gardenia jasminoides</i>	Rubiaceae	1	
<i>Glochidion acuminatum</i>	Euphorbiaceae		
<i>Glochidion rubrum</i>	Euphorbiaceae		
<i>Glochidion zeylanicum</i>	Euphorbiaceae	1	1
<i>Guettarda speciosa</i>	Rubiaceae		
<i>Gymnosporia diversifolia</i>	Celastraceae	1	1
<i>Helicia cochinchinensis</i>	Proteaceae		
<i>Heliotropium foertherianum</i>	Boraginaceae	1	1
<i>Heritiera littoralis</i>	Malvaceae		
<i>Hernandia nymphaeifolia</i>	Hernandiaceae	1	
<i>Hibiscus tiliaceus</i>	Malvaceae		
<i>Ilex buergeri</i>	Aquifoliaceae		
<i>Ilex chinensis</i>	Aquifoliaceae	1	1
<i>Ilex crenata</i>	Aquifoliaceae		
<i>Ilex dimorphophylla</i>	Aquifoliaceae		
<i>Ilex goshiensis</i>	Aquifoliaceae	1	1
<i>Ilex integra</i>	Aquifoliaceae	1	
<i>Ilex latifolia</i>	Aquifoliaceae		
<i>Ilex leucoclada</i>	Aquifoliaceae		
<i>Ilex liukuensis</i>	Aquifoliaceae	1	1
<i>Ilex maximowicziana</i>	Aquifoliaceae	1	1
<i>Ilex pedunculosa</i>	Aquifoliaceae		
<i>Ilex rotunda</i>	Aquifoliaceae	1	
<i>Ilex sugerokii</i>	Aquifoliaceae		
<i>Ilex warburgii</i>	Aquifoliaceae	1	1
<i>Illicium anisatum</i>	Schisandraceae	1	
<i>Indigofera zollingeriana</i>	Fabaceae		

<i>Intsia bijuga</i>	Fabaceae		
<i>Itea oldhamii</i>	Saxifragaceae		
<i>Kandelia obovata</i>	Rhizophoraceae		
<i>Lasianthus attenuatus</i>	Rubiaceae		
<i>Lasianthus curtisii</i>	Rubiaceae		
<i>Lasianthus fordii</i>	Rubiaceae		
<i>Lasianthus hirsutus</i>	Rubiaceae		
<i>Lasianthus hispidulus</i>	Rubiaceae		
<i>Lasianthus japonicus</i>	Rubiaceae		
<i>Lasianthus verticillatus</i>	Rubiaceae		
<i>Laurocerasus spinulosa</i>	Rosaceae		
<i>Laurocerasus zippeliana</i>	Myrtaceae		
<i>Leucothoe keiskei</i>	Ericaceae		
<i>Ligustrum liukuense</i>	Oleaceae	1	1
<i>Ligustrum ovalifolium</i>	Oleaceae		
<i>Lindera erythrocarpa</i>	Lauraceae		
<i>Lithocarpus edulis</i>	Fagaceae	1	1
<i>Lithocarpus glaber</i>	Fagaceae		
<i>Litsea coreana</i>	Lauraceae	1	
<i>Litsea japonica</i>	Lauraceae	1	1
<i>Lonicera morrowii</i>	Caprifoliaceae		
<i>Loropetalum chinense</i>	Hamamelidaceae		
<i>Lumnitzera racemosa</i>	Combretaceae		
<i>Lycium sandwicense</i>	Solanaceae		
<i>Macaranga tanarius</i>	Euphorbiaceae	1	1
<i>Machilus japonica</i>	Lauraceae	1	
<i>Machilus thunbergii</i>	Lauraceae	1	1
<i>Maclura cochinchinensis</i>	Moraceae		
<i>Maesa japonica</i>	Primulaceae		
<i>Maesa montana</i>	Primulaceae	1	1
<i>Magnolia compressa</i>	Magnoliaceae		
<i>Mallotus philippensis</i>	Euphorbiaceae		
<i>Melastoma candidum</i>	Melastomataceae	1	
<i>Melicope triphylla</i>	Rutaceae	1	1
<i>Meliosma rigida</i>	Sabiaceae	1	1
<i>Meliosma squamulata</i>	Sabiaceae	1	1
<i>Microtropis japonica</i>	Celastraceae	1	1
<i>Millettia pinnata</i>	Fabaceae		
<i>Morella rubra</i>	Myricaceae	1	1
<i>Morinda citrifolia</i>	Rubiaceae		
<i>Murraya paniculata</i>	Rutaceae	1	
<i>Myoporum bontioides</i>	Scrophulariaceae		
<i>Myrsine seguinii</i>	Myrsinaceae	1	1

<i>Nandina domestica</i>	Berberidaceae	1	
<i>Neolitsea aciculata</i>	Lauraceae	1	1
<i>Neolitsea sericea</i>	Lauraceae	1	
<i>Nothapodytes nimmonianus</i>	Icacinaceae		
<i>Oreocnide pedunculata</i>	Urticaceae	1	
<i>Osmanthus heterophyllus</i>	Oleaceae	1	1
<i>Osmanthus insularis</i>	Oleaceae		
<i>Osmanthus marginatus</i>	Oleaceae	1	1
<i>Osmanthus rigidus</i>	Oleaceae		
<i>Osteomeles anthyllidifolia</i>	Rosaceae		
<i>Photinia glabra</i>	Rosaceae		
<i>Photinia serratifolia</i>	Rosaceae		
<i>Photinia wrightiana</i>	Rosaceae		
<i>Pieris japonica</i>	Ericaceae	1	1
<i>Pipturus arborescens</i>	Urticaceae		
<i>Pisonia umbellifera</i>	Nyctaginaceae		
<i>Pittosporum tobira</i>	Pittosporaceae	1	1
<i>Planchonella obovata</i>	Sapotaceae	1	
<i>Premna serratifolia</i>	Verbenaceae		
<i>Psychotria manillensis</i>	Rubiaceae		
<i>Psychotria rubra</i>	Rubiaceae	1	1
<i>Pterocarpus vidalianus</i>	Fabaceae	1	1
<i>Putranjiva matsumurae</i>	Euphorbiaceae		
<i>Pyrenaria virgata</i>	Theaceae	1	1
<i>Quercus acuta</i>	Fagaceae		
<i>Quercus gilva</i>	Fagaceae		
<i>Quercus glauca</i>	Fagaceae	1	
<i>Quercus hondae</i>	Fagaceae	1	
<i>Quercus miyagii</i>	Fagaceae	1	1
<i>Quercus myrsinifolia</i>	Fagaceae		
<i>Quercus phillyreoides</i>	Fagaceae		
<i>Quercus salicina</i>	Fagaceae		
<i>Quercus sessilifolia</i>	Fagaceae	1	
<i>Rhaphiolepis indica</i>	Rosaceae	1	1
<i>Rhizophora mucronata</i>	Rhizophoraceae		
<i>Rhododendron amanoi</i>	Ericaceae		
<i>Rhododendron aureum</i>	Ericaceae		
<i>Rhododendron brachycarpum</i>	Ericaceae		
<i>Rhododendron degronianum</i>	Rosaceae		
<i>Rhododendron indicum</i>	Ericaceae		
<i>Rhododendron japonoheptamerum</i>	Ericaceae		
<i>Rhododendron keiskei</i>	Ericaceae	1	

<i>Rhododendron komiyamae</i>	Ericaceae		
<i>Rhododendron lapponicum</i>	Ericaceae		
<i>Rhododendron latoucheae</i>	Ericaceae		
<i>Rhododendron macrosepalum</i>	Ericaceae		
<i>Rhododendron makinoi</i>	Ericaceae		
<i>Rhododendron ripense</i>	Ericaceae		
<i>Rhododendron scabrum</i>	Ericaceae	1	1
<i>Rhododendron simsii</i>	Ericaceae		
<i>Rhododendron tashiroi</i>	Ericaceae	1	1
<i>Rhododendron tosaense</i>	Ericaceae		
<i>Rhododendron uwaense</i>	Ericaceae		
<i>Rhododendron yakuinsulare</i>	Ericaceae		
<i>Rhododendron yakushimanum</i>	Ericaceae		
<i>Rhodomyrtus tomentosa</i>	Myrtaceae		
<i>Rosa bracteata</i>	Rosaceae		
<i>Rubus nesiotes</i>	Rosaceae		
<i>Rubus pectinellus</i>	Rosaceae		
<i>Rubus sieboldii</i>	Rosaceae	1	
<i>Rubus swinhoei</i>	Rosaceae		
<i>Sarcandra glabra</i>	Chloranthaceae	1	1
<i>Scaevola taccada</i>	Goodeniaceae	1	1
<i>Schefflera heptaphylla</i>	Fagaceae	1	
<i>Schima wallichii</i>	Theaceae	1	1
<i>Scolopia oldhamii</i>	Salicaceae		
<i>Skimmia japonica</i>	Rutaceae	1	1
<i>Solanum erianthum</i>	Solanaceae		
<i>Solanum macaonense</i>	Solanaceae		
<i>Sophora tomentosa</i>	Fabaceae		
<i>Symplocos cochinchinensis</i>	Symplocaceae		
<i>Symplocos formosana</i>	Symplocaceae	1	
<i>Symplocos glauca</i>	Symplocaceae		
<i>Symplocos kuroki</i>	Symplocaceae	1	1
<i>Symplocos lancifolia</i>	Symplocaceae	1	
<i>Symplocos liukuensis</i>	Symplocaceae		
<i>Symplocos myrtacea</i>	Symplocaceae		
<i>Symplocos nakaharae</i>	Symplocaceae		
<i>Symplocos okinawensis</i>	Symplocaceae		
<i>Symplocos prunifolia</i>	Symplocaceae	1	1
<i>Symplocos sonoharae</i>	Symplocaceae		
<i>Symplocos stellaris</i>	Symplocaceae	1	1
<i>Symplocos tanakae</i>	Lauraceae		
<i>Symplocos theophrastifolia</i>	Symplocaceae		
<i>Syzygium buxifolium</i>	Myrtaceae	1	1

<i>Syzygium jambos</i>	Myrtaceae		
<i>Tarenna gracilipes</i>	Rubiaceae	1	1
<i>Ternstroemia gymnanthera</i>	Lauraceae	1	
<i>Thespesia populnea</i>	Malvaceae		
<i>Trema orientalis</i>	Ulmaceae	1	
<i>Trochodendron aralioides</i>	Trochodendraceae		
<i>Turpinia ternata</i>	Staphyleaceae	1	1
<i>Vaccinium bracteatum</i>	Ericaceae		
<i>Vaccinium wrightii</i>	Ericaceae	1	1
<i>Viburnum japonicum</i>	Caprifoliaceae	1	1
<i>Viburnum odoratissimum</i>	Caprifoliaceae	1	1
<i>Viburnum suspensum</i>	Caprifoliaceae	1	
<i>Vitex quinata</i>	Lamiaceae		
<i>Wendlandia formosana</i>	Rubiaceae	1	1
<i>Wikstroemia retusa</i>	Thymelaeaceae	1	1
<i>Xylosma congesta</i>	Salicaceae		
<i>Zanthoxylum armatum</i>	Rutaceae	1	
<i>Zanthoxylum beecheyanum</i>	Rutaceae		

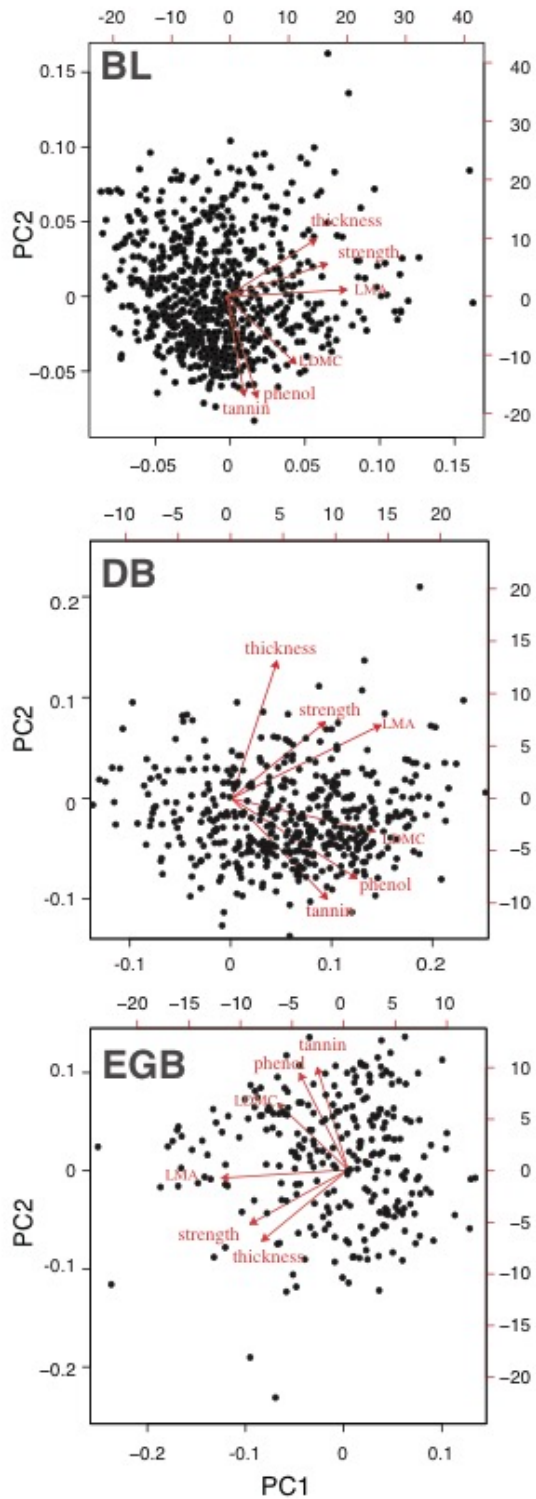


Figure S1. Principal Component analyses (PCAs) of leaf defense traits of the tree species in Eastern Asia continental archipelago. The broad-leaved tree species (BL), deciduous (DB) and evergreen (EGB) tree species were separately examined. Biplots of species coordinates (●) arrayed on the first two axes of the PCAs.

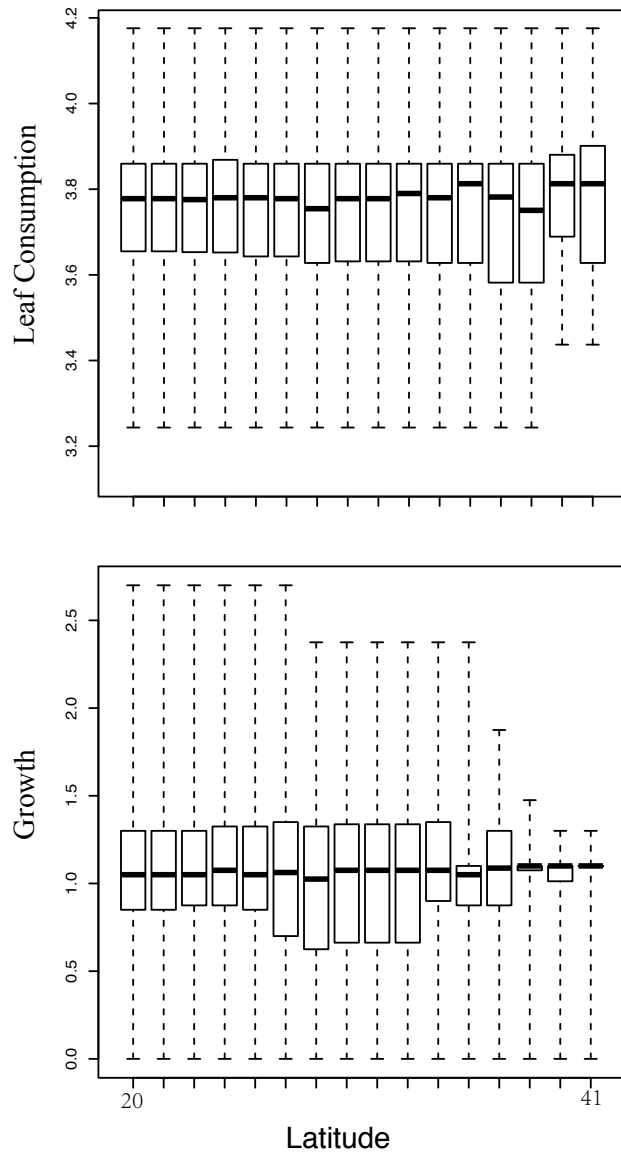


Figure S2. The distribution of leaf consumption and larval growth along the latitudinal range, when the larvae were fed on evergreen tree species (EGB).

Acknowledgements

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