

Doctoral thesis

Diverse adaptations to increase pollination success in
zoophilous plants

動物媒植物が送粉成功を高めるための多様な適応

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要旨

動物媒の被子植物は、送粉成功を高めるために、花形態や開花習性を進化させてきた。本論文は、(1) 不稔装飾花を伴う額咲き、(2) 早春の開花、(3) 花粉噴出という3つの現象に着目し、送粉者との関わりの中で植物が遂げてきた適応進化を解明することを目指した。

レンプクソウ科ガマズミ属 (*Viburnum*) とアジサイ科アジサイ属 (*Hydrangea*) には、不稔装飾花が周囲を取り囲んだ「額咲き」と呼ばれる花序を持つ種が多く見られる。9種のガマズミ属植物 (うち3種が額咲き種) と9種のアジサイ属植物 (うち8種が額咲き種) を対象に、日本列島の落葉樹林内のそれぞれの自生地において、装飾花を切除／付加する野外実験を行い、実験区と対照区それぞれにおける訪花昆虫群集を調査した。ほとんどの額咲き種において、装飾花の存在が昆虫の訪花頻度を高めていたが、非額咲き種では付加された装飾花の誘引効果は限定的であった。装飾花の誘引効果が特に高かったのはコハナバチ類、ハナカミキリ類、ハナアブ類であり、これらの昆虫の訪花頻度を高めるために、装飾花が進化したと考えられる。

早春はまだ寒い日が続いているが、そのような時期に開花するのがマンサク科のコウヤミズキ (*Corylopsis gotoana*) である。コウヤミズキの訪花昆虫を観察したところ、ビロウドツリアブ、ニホンミツバチの働き蜂、マルハナバチ類の女王、そして積翅目キシタカワゲラが訪花した。このカワゲラの雌は訪花して花粉を食し、送粉も果たしており、これはカワゲラ媒の世界初の確認となった。コウヤミズキの主要な送粉者は前三者であるが、幼虫が水生のカワゲラも代替送粉者として、陸上生態系が天候不順な年に送粉者の役割を果たしている可能性がある。

植物の中には、雄しべのバネ仕掛けによって花粉を噴出するものがあり、アワブキ科のミヤマハハソ (*Meliosma tenuis*) はその一例である。花の性表現と送粉様式を調査したところ、ミヤマハハソは自家和合性かつ雄性先熟で、コマルハナバチの雄蜂に特異的に訪花されていた。この雄蜂の訪花は花の花粉噴出を誘導し、腹部腹面はその花粉で覆われていた。

これらの植物の独自の花形態や開花習性は、それぞれの送粉昆虫との相互作用を通して、送粉成功を高めるために進化したと考えられた。

Summary

Diversification of angiosperms is attributed mainly to adoption of zoophily, because pollinator's behavior exhibiting a strong preference to specific flowers has canalized gene flow of the plants, and caused their speciation. Although zoophilous plants are believed to have coevolved with their pollinators, function of floral morphology and behavior of diverse groups of flowers are still little known. To explore adaptations of several groups of zoophilous angiosperms to attract pollinators and to increase pollination success, I focused on three unique aspects of flowering: (1) decorative sterile flowers in framed inflorescences, (2) flowering in the earliest spring, and (3) explosive pollen release.

Some plant species of Adoxaceae and Hydrangeaceae have 'framed' inflorescences, in which decorative sterile flowers are arranged around a dense cluster of small fertile flowers, while other members have inflorescences composed only of fertile flowers. It is intriguing why some species have framed inflorescences (FR species), but others in the same genus do not (n-FR species). The decorative flowers have been hypothesized to increase the attractiveness of the inflorescences, but few studies have tested the hypothesis. I explored the function of the decorative flowers to attract pollinators by conducting field manipulation experiments to remove/add decorative flowers to inflorescences in deciduous forests in the Japanese Archipelago.

In Chapter 2, I focused on pollination system of Adoxaceae. Insect visits to flowers were observed in nine *Viburnum* species, including three species having decorative flowers. *Viburnum* flowers were visited by diverse nectar/pollen-foraging insects, and the visitor assemblages varied among *Viburnum* species. Although both fertile and decorative sterile flowers of most *Viburnum* species were white and disciform, *V. urceolatum* was unique

in having pink tubular fertile flowers, which were visited exclusively by bumblebee workers. The manipulation experiments showed that the decorative flowers increased inflorescence visits by cerambycid beetles, syrphid flies and solitary bees only in FR species, and that decorative flowers in FR species additionally served as landing sites for these insects. After landing on decorative flowers, they walked to a cluster of fertile flowers and harvested nectar and pollen. The tendency to land on decorative flowers was considered an adaptation to avoid attack by crab spiders ambushing in clusters of fertile flowers. Fertile flowers tended to bloom more synchronously in n-FR species than in FR species, suggesting that decorative flowers retain the pollinator-attracting function for a longer period.

In Chapter 3, I focused on pollination system of Hydrangeaceae. Insect visits to flowers were observed in nine *Hydrangea* (sensu lato) species including eight FR and one n-FR species. The flowers were visited by diverse insects, and the insect visitor assemblages varied among plant species with different color and arrangement of decorative flowers. Especially, cerambycid beetles were correlated with white color of fertile/decorative flowers and sweet floral scents, and bumblebees were correlated with blue/purple color of fertile/decorative flowers and laterally projecting decorative flowers. Manipulation experiments revealed that the decorative flowers of most FR species increased inflorescence visits by various insect pollinator functional groups, but did not serve as landing sites. Although *H. sikokiana* is a FR species, the inflorescence had only a few small vestigial decorative flowers, and pollinator-attracting function of the decorative flowers was not detected. In *H. hirta*, the only n-FR species, added decorative flowers did not increase insect visits to inflorescences, suggesting that the

adoption of olfactory cues may have caused loss of decorative flowers.

These results on *Viburnum* and *Hydrangea* suggest that decorative flowers arranged around a corymbiform inflorescence have evolved to increase attractiveness to sight sensed, nectar/pollen-feeding small insects such as solitary bees, cerambycid beetles and syrphid flies. The high diversity of plant species having decorative flowers in the Japanese Archipelago as exemplified by *Viburnum* and *Hydrangea* is thought to be related with the high diversity and great abundance of solitary/eusocial halictid bees and anthophilous cerambycid beetles belonging to the genus *Pidonia*, which are also unique to the Archipelago. Each species of these plant genera often shares pollinator species and tended to bloom from April to August without overlapping flowering periods each other between congeneric plant species.

In the earliest spring in a temperate region, it is still cold and available pollinators are scarce, whereas flowers are easily visible because trees have not opened leaves, and competition for pollinators would be not strong. Even in this harsh season, some plants such as *Corylopsis* (Hamamelidaceae) start to bloom. In Chapter 4, I studied the pollination system of *C. gotoana* in a deciduous forest in Shiga Prefecture. The yellow pendent flowers were born on small racemes, and secreted nectar. I observed flower visitors of *C. gotoana*, and found that the flowers were mostly visited diurnally by bombyliid flies, Asian honeybee workers, bumblebee queens, solitary bees and one stonefly species, *Strophopteryx nohirae* (Taeniopterygidae: Plecoptera). The visits by stoneflies were unexpected because associations of terrestrial adult stoneflies with flowering plants have rarely been documented. Field observations and field experiments showed that the stonefly females feed on pollen and that the stoneflies dusted with pollen contribute to pollination of the flowers. This is the first report demonstrating that flowers are really pollinated by stoneflies. The stonefly

pollination is considered an option to secure pollination in the earliest spring in colder years, because the climatic regime is often different between terrestrial and aquatic habitats.

Zygomorphic flowers in several plant families have developed a special floral mechanism to release pollen explosively to attach pollen to remote long-tongued/billed pollinators. *Meliosma* (Sabiaceae) is a basal plant genus, that has superficially actinomorphic, but internally zygomorphic small dish-like flowers which release pollen explosively when certain flower visitors trigger the mechanism. To elucidate adaptive significance of the explosive pollen release in *Meliosma*, in Chapter 5, I studied the pollination and breeding systems of *M. tenuis* in a deciduous forest in Kyoto Prefecture. Artificial pollination experiments revealed that the plant was self-incompatible and protandrous, but automatic self-pollination never occurred. The explosive pollen release was triggered by slight tactile stimuli to anther filaments or staminodes in male-stage flowers. The flowers blooming from late May to early June were visited almost exclusively by drones of a bumblebee species, *Bombus ardens*, the emergence of which coincided with the flowering period of *M. tenuis*. The bumblebee drones could trigger the explosive pollen release mechanism, and pollen were attached on hairs on ventral side of abdomen and legs especially tarsi. The lack of bumblebee worker visits suggests that the explosive pollen release may discourage pollen-harvesting bumblebee workers. These results indicate that the bumblebee drones are the main pollinators of *M. tenuis*, and that the explosive pollen release enables the small flowers to attach pollen to the remote body parts of bumblebee drones, which visited exclusively *Meliosma* flowers for a long distance in search for newly emerging queens.

Thus, the special floral morphologies (e.g., arrangement of decorative sterile flowers) and flowering behaviors (e.g., flowering in the earliest spring and explosive pollen release) of several groups of zoophilous plants proved to

be adaptations to increase pollination success. Because zoophilous plants and their pollinators depend on each other, floral morphology and flowering behavior of the plants would have coevolved with visual/olfactory sensing system and foraging behavior of the pollinators. The coevolutionary approach will reveal how unique floral morphology and flowering behavior of zoophilous plants have evolved.

Chapter 1: General Introduction

Pollination mutualism

A mutualism is a symbiotic association between organisms of two different species in which each organism benefit from the activity of the other. In forest ecosystems there are four mutualisms: pollination mutualism, seed-dispersal mutualism, protection mutualism and mycorrhizal mutualism (Kato and Kawakita 2017). In the pollination mutualism a plant provides floral reward to an anthophilous animal visitor, and in turn, the visitor provides pollination service to the host plant. This mutualism has played an important role for shaping the great diversity of angiosperms, because nectar/pollen-foraging pollinators have evolved preference for specific flowers, and the foraging behavior of the pollinators has canalized gene flow of the host plants, thus causing speciation and diversification of zoophilous plants.

Among animal pollinators, winged insects have been the most important evolutionary partners of angiosperms. The five megadiverse insect order, Hemiptera, Coleoptera, Diptera, Hymenoptera and Lepidoptera, have diverged especially as herbivores along angiosperms since the cretaceous (Misof et al. 2014, Wiens et al. 2015), and members of the last four orders have diverged and played a prominent role as pollinators of angiosperms (Grimaldi and Engel 2005). Other insect orders also contain some anthophilous insects, some of which become pollinators, e.g., thysanopterans (Moog et al. 2002), orthopterans (Micheneau et al. 2010) and hemipterans (Ishida et al. 2009).

Diversity of pollination systems

In the pollination mutualism, floral reward and pollination service are exchanged between zoophilous plants and anthophilous animals. Because different groups of anthophilous animals potentially differ in sensory system and in energetic and trophic demand, the flowers need to send an appropriate signal and to provide proper floral reward to attract pollinator partners. Floral signals are generally visual and olfactory, but rarely tactile (Kevan and Lane 1985), thermal (Raguso 2004) and acoustic (von Helversen and von Helversen 1999). The most common floral reward is floral nectar because nectar is one of the most inexpensive chemicals for photosynthesizing plants. Pollen, floral oil, floral odor, floral tissue and even seed are also provided as reward in some plants. Thus, the floral morphology and floral reward correspond to specific groups of pollinators, and the correspondent relationship is called pollination syndrome. For example, bird-pollinated flowers tend to have red, odorless, tubular, deep flowers that secrete ample dilute nectar.

The characteristics to increase floral attractiveness involves in floral color, floral odor, flower shape, floral symmetry and arrangements of flowers. A mutational change of these floral characters may cause a switch of pollinator species, thus causing speciation (Okamoto et al. 2015). To help pollinator's access to floral reward and to secure pollination, floral guide in a corolla tube has evolved in some zygomorphic flowers pollinated by bees.

In addition to pollinator attraction, flowers have also evolved to avoid inbreeding. Self-incompatibility and sexual presentation such as dioecy, monoecy, dichogamy and herkogamy are common mechanisms to secure

Table 1-1. List of plant taxa having decorative flowers, with characteristics of decorative flowers and pollinators.

Clade	Order	Family	Genus	No. species	No. species with decorative flowers	Decorative flowers	Known pollinators	References on pollination
Monocot	Asparagales	Asparagaceae	<i>Bellevallia</i>	65	65	tubular sterile flowers are situated apically in vertical racem	bombyliid flies, bees	Dafni and Ivri 1981
			<i>Lachenalia</i>	115	115	tubular sterile flowers are situated apically in vertical racem	bird	Duncant et al 2004
			<i>Leopoldia</i>	12	12	tubular sterile flowers are situated apically in vertical racem	various bees	Morales et al. 2013; Canale et al. 2014
			<i>Muscari</i>	42	42	tubular sterile flowers are situated apically in vertical racem	bombyliid flies	Knoll 1921 †
Eudicot		Orchidaceae	<i>Oncidium</i>	324	> 10	small and degenerated sterile flowers are situated basically	<i>Trigona</i> bees	Parra-Tabla et al. 2000
						lilac brush-like flowers are situated basally in cylindrical spike	bees	Hopkins et al. 2010
	Fabales	Fabaceae	<i>Dichrostachys</i>	16	16			
	Cornales	Hydrangeaceae	<i>Hydrangea</i> (sensu lato)	73	ca. 69	large disciform flowers surrounding corymb	bees, flies, beetles	this study (Chater 3)
	Dipsacales	Adoxaceae	<i>Viburnum</i>	160	ca. 16	large disciform flowers surrounding corymb	bees, flies, beetles	Bell 1985; Jin et al. 2010; this study (Chapter 2)
	Asterales	Asteraceae ‡	<i>Aster</i>	214	> 200	ligular rays surrounding flat flower head	bees, flies	Proctor et al. 1996
			<i>Chrysanthemum</i>	43	43	ligular rays surrounding flat flower head	bees, flies	Brewer and Henstra 1974
			<i>Kalimeris</i>	14	14	ligular rays surrounding flat flower head	bees, flies	Shi et al 2009
			<i>Senecio</i>	1483	1483	ligular rays surrounding flat flower head	bees, butterflies	Anderson 1996
			<i>Madia</i>	11	11	ligular rays surrounding flat flower head	bees, flies	Celedón-Neghme et al. 2007
	Apiales	Apiaceae	<i>Anisosciadium</i>	3	3	lateral umbels with enlarged petals	—	
			<i>Coriandrum</i>	2	2	lateral umbels with enlarged petals	bees, syrphid flies	El-Berry et al. 1974
			<i>Daucus</i>	28	> 1	lateral umbels with enlarged petals	bees, flies, wasps	Lamborn and Ollerton 2000
			<i>Notopterygium</i>	6	2	lateral umbels with enlarged petals	—	
			<i>Pimpinella</i>	72	ca. 7	lateral umbels with enlarged petals	bees, syrphid flies	El-Berry et al. 1974

† As cited by Kevan and Baker (1983)

‡ Many other Asteraceae genera not noted in this table also share have decorative flowers.

outbreeding. Temporal changes of floral color observed in *Lonicera* and *Weigela* species (Caprifoliaceae) is also an adaptation to facilitate heterogamous pollination. Furthermore, floral morphology sometimes diverges among related plant species to avoid interspecific crossing and competition for pollinators.

Although zoophilous plants are believed to have coevolved with their pollinators, function of floral morphology and behavior of diverse groups of flowers are still little known. To explore adaptations of several groups of zoophilous angiosperms to attract

pollinators and to increase pollination success, I focused on three unique aspects of flowering: (1) decorative sterile flowers in framed inflorescences, (2) flowering in the earliest spring, and (3) explosive pollen release.

Decorative sterile flowers

Flowers are often born delicately arranged in an inflorescence, and the appearance of the inflorescence is critical to attract pollinators. For example, plants in Asteraceae have sunflower-like inflorescences called heads, which are composed of a cluster of small tubular flowers and lingular, usually

sterile, flowers surrounding the flower cluster. These decorative sterile flowers arranged in inflorescences are known in seven families of monocots and eudicots (Table 1-1), and have been believed to increase attractiveness of the inflorescences against pollinators (Darwin 1877, Bell 1985, Plitmann 1995, Anderson 1996, Jin et al. 2010, Morales et al. 2013, Meisel et al. 2014). Some recent studies confirmed that the decorative sterile flowers eventually increased pollinator visits to the inflorescences (Bell 1985, Jin et al. 2010).

Some plant species in Adoxaceae and Hydrangeaceae are known to have 'framed' inflorescences, in which a dense cluster of small fertile flowers is surrounded by large disciform decorative sterile flowers. While pollinator-attracting function of these decorative sterile flowers have been hypothesized, the functions have rarely been examined. Moreover, it is unknown why some species have framed inflorescences while other species of the same genus do not have framed inflorescences. The Japanese Archipelago is celebrated by the high diversity of plant species with framed inflorescences especially in *Viburnum* (Adoxaceae) and *Hydrangea* (Hydrangeaceae), giving us a rare chance to study the evolution of decorative sterile flowers.

Flowering in the earliest spring

In a temperate region, diverse plants bloom one after the other from the early spring to the fall, the flowering sequence is definite among years. Therefore, flowering time of each plant species is genetically determined, and each plant species tends to be pollinated by definite groups of pollinators every year. In the earliest spring, it is still cold, and available pollinators would be rare. Even in the harsh season, flowering is observed in some plant species of Hamamelidaceae genera, *Hamamelis* and *Corylopsis*, while the pollination systems of these plant species are almost unknown. My preliminary observations suggested that the

Corylopsis flowers were visited by stoneflies. Stoneflies (Plecoptera) are aquatic insects in their nymphal stages, and their associations with angiosperms are little documented even in the terrestrial adult stages. It is intriguing if the stoneflies contribute to pollination of the plants.

Explosive pollen release

Zoophilous plants generally have adaptations to attach pollen on proper position of pollinator bodies, and long protruded androecium has often evolved. In some zygomorphic tubular flowers, however, explosive pollen release aided by spring-loaded motion of stamens is known. In this pollination system, pollen is dusted to pollinators upon flower visit, instead of being innately presented. This mechanism has evolved several times in plants lineages with tubular/papilionaceous zygomorphic flowers (e.g., Fabaceae, Loranthaceae).

Meliosma is a genus belonging to Sabiaceae, an archaic plant family in Proteales, and is known to release pollen explosively. The flowers of *Meliosma* are small, superficially actinomorphic, but internally zygomorphic, contrasting with the abovementioned usual zygomorphic tubular flowers with explosive pollen release mechanism. Pollination of *Meliosma* species has not been observed, and it is unknown how the pollen release mechanism functions during flowering periods. To detect pollination system of *Meliosma*, I made extensive observation on pollinator visits to flowers and experiments to trigger the explosive pollen release mechanism.

Organization of the thesis

This study comprises six chapters. The first chapter presented here, Chapter 1, is the introduction. In the following two chapters (Chapters 2 and 3), I explore pollinator-attracting functions of sterile decorative flowers by conducting manipulation experiments removing/adding decorative flowers, and by evaluating visitation rates. In Chapter 2, studies

plants are nine *Viburnum* species (Adoxaceae) including three species with decorative flowers and six without them. In Chapter 3, studied plants are nine *Hydrangea* species (Hydrangeaceae) including eight species with decorative flowers and one without them. In Chapter 4, the pollination system of *Corylopsis gotoana* (Sabiaceae) was explored, especially focusing on contribution of stoneflies to pollination. In Chapter 5, I study the pollination and breeding systems of *Meliosma tenuis* (Sabiaceae), which explosively release pollen. Lastly, in Chapter 6, based on the results of the previous chapters, I discuss diverse adaptations of angiosperm flowers to attract pollinators and to avoid inbreeding.

Chapter 2: Pollinator-attracting and landing-site-providing functions of the decorative sterile flowers of *Viburnum* (Adoxaceae)

Introduction

The great majority of angiosperms are animal-pollinated (Ollerton et al. 2011, Abrol 2012), and their flowers are often designed to attract specific pollinators by intricate signaling strategies involving floral color, scent, and structure (Bawa and Beach 1981, Crepet 1984, Endress 2011, Van der Niet and Johnson 2012). Floral morphology, which refers to the size, color and shape, among other features, of reproductive and vegetative organs, plays a key role in the speciation of flowering plants through the attraction of pollinators. The arrangement of flowers with different morphologies (i.e. dimorphic flowers) is a strategy used by Asparagaceae (Morales et al. 2013), Orchidaceae (Meisel et al. 2014), Adoxaceae, Hydrangeaceae (Darwin 1877), Apiaceae, and Asteraceae (Plitmann 1995).

Darwin (1877) hypothesized that a unique floral arrangement known from some species of *Viburnum* (Adoxaceae) and *Hydrangea* (Hydrangeaceae) serves to attract pollinators. This floral arrangement, hereafter referred to as ‘framed inflorescence’, consists of inner and outer components: a central cluster of small, fertile flowers, surrounded by much larger, decorative sterile flowers. And similarly, Donoghue (1980) presumed that the decorative flowers of *Viburnum* species characterized by framed inflorescences would attract insect pollinators, and increase their fruit sets. Although *Viburnum* flowers are known to be visited by dipterans, hymenopterans and other insects (Donoghue 1980, Yumoto 1988, Inoue et al. 1990, Kato et al. 1990, Nebot and Mateu 1990, Englund 1993, Jin et al. 2010), few studies have investigated the pollinator attracting mechanism of decorative flowers and framed inflorescences. The efficacy of this mechanism has been established to some extent: removal of

decorative flowers has resulted in reduction of the fruit sets in *V. macrocephalum* and *V. lantanoides* (Bell (1985), although this manipulation did not significantly affect the fruit set in *V. opulum* (Krannitz and Maun (1991).

While at least some *Viburnum* species possess framed inflorescences that may attract pollinators (Bell 1985, Jin et al. 2010), the majority of *Viburnum* species studied by Clement and Donoghue (2011) do not possess decorative flowers. According to a phylogeny of *Viburnum* (Clement et al. (2014), decorative flowers may be a derived character that has independently evolved several times.

Here, I test two hypotheses using field experiments focusing on 9 of 16 Japanese *Viburnum* species with varying morphological and ecological traits. The first hypothesis is that the presence of decorative flowers increases the frequency of pollinator visits. Thus, clipping decorative flowers in species with inherently framed inflorescences (hereafter referred to as ‘FR species’) is expected to decrease the frequency of pollinator visits, while mounting decorative flowers on species with non-framed inflorescences (hereafter referred to as ‘n-FR species’) will increase the frequency of visits. The second hypothesis is that decorative flowers can serve as landing sites for flower-visiting insects. To test this hypothesis, I carried out field manipulation experiments in which decorative flowers were clipped in FR species and mounted on n-FR species. Furthermore, I compared morphological and ecological traits among these *Viburnum* species to elucidate the evolutionary significance of the framed inflorescence.

Materials and methods

Study sites and species

Viburnum is a genus of deciduous or evergreen shrubs and small trees. Around 160 *Viburnum* species are distributed in America, Europe and East Asia, mainly in Central America and Southeast Asia (Winkworth and Donoghue 2005). The inflorescences of *Viburnum* are usually terminal and compound corymbose or panicle, and the fertile flowers have five fused petals. The forms of fertile flowers in *Viburnum* can be classified into three types: (1) small bell-shaped flowers with a shallow corolla, (2) medium-sized funnel-shaped flowers, and (3) medium-sized tubular flowers.

In the Japanese archipelago, 4 of 16 *Viburnum* species are FR species. In FR species, the corolla of decorative flowers is larger and white or yellowish-white, and decorative flowers occasionally have remnants of stamens and styles (Donoghue 1980). Decorative flowers are found in some species that have bell-shaped fertile flowers, and bloom before and wilt after the fertile flowers.

I investigated the flower-visiting insects of nine *Viburnum* species (Table 2-1, Fig. 2-1): *V. dilatatum* Thunb., *V. erosum* Thunb., *V. furcatum* Blume ex Hook.f & Thomson., *V. opulus* L., *V. phlebotrichum* Siebold & Zucc., *V. plicatum* Thunberg, *V. sieboldii* Siebold & Zucc., *V. urceolatum* Siebold & Zucc. and *V. wrightii* Miq. Of these, three species were FR species (*V. furcatum*, *V. opulus* and *V. dilatatum*) (Figs. 2-1a-c) and six were n-FR species. *V. urceolatum* is unique in that the fertile flowers are tubular with red calyces (Fig. 2-1i).

Observation of flower visitors

Field experiments were carried out from May 2014 to August 2015 in the Honshu and Tohoku regions of Japan. The vegetation types and plant species studied at each site are listed in Table 2-2.

Table 2-1. Habitat, inflorescence type, features of fertile flowers, presence of decorative flowers, flowering period in Japan, distribution and date of observation of the nine *Viburnum* spp. See Table 2 for the study sites codes.

Viburnum species	Species code	Habit	Inflorescence type	Shape	Fertile flowers			Nectar segregation	Range of decorative flowers per inflorescence [no. in observed framed inflorescences †]	Flowering period in Japan	Distribution										Study site	Date of observation
					No. per inflorescence (mean ± SD, n=12)	In open % per inflorescence (mean ± SD, n=12)	FR species				Russia	China	Korea	Hokkaido	Honshu	Shikoku	Kyushu	Ryukyu	Taiwan			
FR species																						
<i>V. furcatum</i>	Vf	shrub	compound corymb	bell-shaped	68 ± 10	47.2 ± 7.1	–	7-9 [8]	April - May	+	+	+	+	+	+	+	+	+	+	S3	11 May 2014	
<i>V. opulus</i>	Vo	shrub	compound corymb	bell-shaped	94 ± 21	51.7 ± 7.9	d	10-14 [12]	May - July	+	+	+	+	+	+	+	+	+	+	S1	13 Jun 2015	
<i>V. plicatum</i>	Vpl	treelet	compound corymb	bell-shaped	49 ± 12	17.1 ± 7.2	–	4-6 [6]	May - June	+	+	+	+	+	+	+	+	+	+	S8	18 May 2014	
n-FR species																						
<i>V. dilatatum</i>	Vd	shrub	compound corymb	bell-shaped	409 ± 48	46.1 ± 23.2	d, n	– [4]	May	+	+	+	+	+	+	+	+	+	+	S9	21 May 2015	
<i>V. erosum</i>	Ve	shrub	compound corymb	bell-shaped	41 ± 5	88.8 ± 5.8	–	– [4]	April - May	+	+	+	+	+	+	+	+	+	+	S7	1 May 2015	
<i>V. wrightii</i>	Vw	shrub	compound corymb	bell-shaped	47 ± 9	76.2 ± 10.9	–	– [4]	May - July	+	+	+	+	+	+	+	+	+	+	S2	2 Jul 2014	
<i>V. sieboldii</i>	Vs	treelet	panicle	bell-shaped	117 ± 11	94.3 ± 2.3	d, n	– [4]	April - June	+	+	+	+	+	+	+	+	+	+	S6	23 May 2015	
<i>V. phlebotrichum</i>	Vph	shrub	pendent corymb	funnel-shaped	14 ± 2	99.0 ± 2.9	d	– [3]	April - June	+	+	+	+	+	+	+	+	+	+	S4	27 May 2015	
<i>V. urceolatum</i>	Vu	shrub	compound corymb	tubular	76 ± 5	21.5 ± 12.2	d	– [4]	June - July	+	+	+	+	+	+	+	+	+	+	S5	3 Jul 2015	
FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences; d, daytime; n, nighttime. † In species without framed inflorescences we mounted <i>V. plicatum</i> decorative flowers in treated inflorescences. Mounted decorative flowers in <i>V. phlebotrichum</i> were also pendant.																						

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences; d, daytime; n, nighttime. † In species without framed inflorescences we mounted *V. plicatum* decorative flowers in treated inflorescences. Mounted decorative flowers in *V. phlebotrichum* were also pendant.

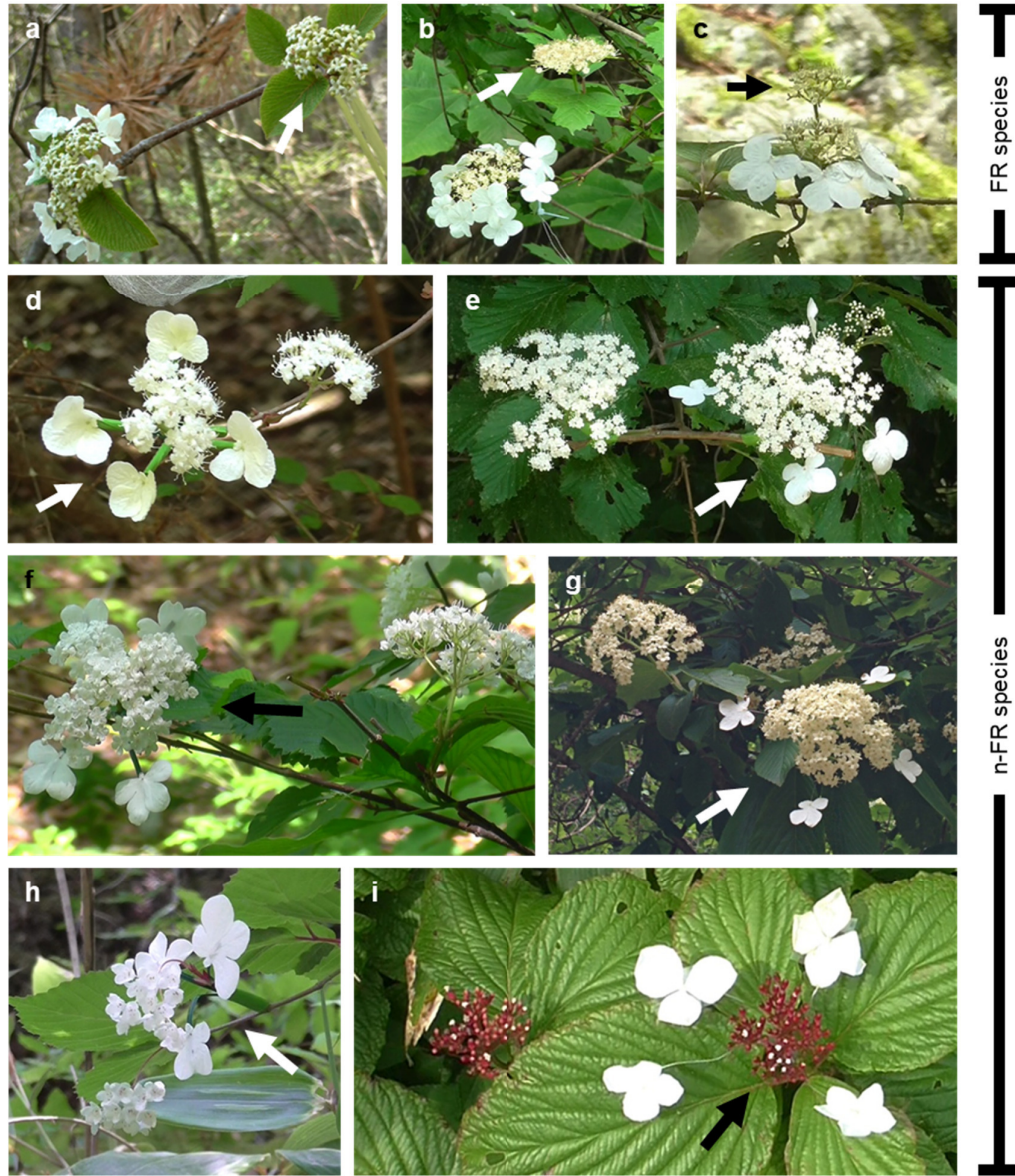


Fig 2-1. Intact and treated inflorescences of nine *Viburnum* species. (a) *V. furcatum*; (b) *V. opulus*; (c) *V. plicatum*; (d) *V. erosum*; (e) *V. dilatatum*; (f) *V. wrightii*; (g) *V. sieboldii*; (h) *V. phlebotrichum*; (i) *V. urceolatum*. Arrows: treated inflorescences. Decorative flowers of framed inflorescences (FR species) were clipped in treated inflorescences, while in non-framed inflorescences (n-FR species) decorative flowers of *V. plicatum* were mounted on treated inflorescences.

The insect visits to framed and non-framed inflorescences of nine *Viburnum* species were filmed with high-definition video cameras (Panasonic HC-V210M, Panasonic HC-V520M and Panasonic HC-V620M) fixed on tripods. To assess the pollinator-attracting function of decorative flowers in *Viburnum*, I compared flower visits to six pairs of framed inflorescences and non-framed inflorescences for each species. Two inflorescences with similar

forms (e.g. having a similar number of decorative flowers and a similar size of inflorescence) descending from the closest node were selected as a pair, and one of them was kept intact and the other one was treated. Treated inflorescences of FR species had the decorative flowers clipped, while treated inflorescences of n-FR species had *V. plicatum* decorative flowers mounted with thin wires to mimic natural framed inflorescences. For the treatment of

Table 2-2. List of study sites with their localities, coordinates, altitude, dominant vegetation and trees, and *Viburnum* species studied at each site

Site code	Locality	Latitude	Longitude	Altitude (m)	Vegetation	Dominant tree species	<i>Viburnum</i> species observed
S1	Iwaya, Higashidori-mura, Aomori Pref.	41°21'54" N	141°23'44" E	60	cool temperate coastal deciduous forest	<i>Quercus dentata</i>	<i>V. opulus</i>
S2	Shirakaba highland, Nagawa, Nagano Pref.	36° 8'9" N	138°14'17" E	1330	subalpine coniferous forest	<i>Larix kaempferi</i>	<i>V. wrightii</i>
S3	Kaida Highlands, Nagano Pref.	35°56'17" N	137°37'34" E	1140	cool temperate deciduous forest	<i>Quercus crispula</i>	<i>V. furcatum</i>
S4	Aburazaka, Ohno, Fukui Pref.	35°52'26" N	136°49'18" E	740	cool temperate deciduous forest	<i>Fagus crenata</i>	<i>V. phlebotrichum</i>
S5	Mt. Kanmuri, Ikeda, Fukui Pref.	35°47'19" N	136°23'49" E	1100	cool temperate deciduous forest	<i>Fagus crenata</i>	<i>V. urceolatum</i>
S6	Mt. Nabejiri, Taga, Shiga Pref.	35°14'25" N	136°21'26" E	670	temperate semi-evergreen forest	<i>Quercus salicina</i>	<i>V. sieboldii</i>
S7	Kurama, Sakyo, Kyoto Pref.	35° 7'59" N	135°46'40" E	460	temperate afforested coniferous forest	<i>Cryptomeria japonica</i>	<i>V. erosum</i>
S8	Kibune, Sakyo, Kyoto Pref.	35° 7'58" N	135°45'55" E	370	temperate rivarine deciduous forest	<i>Juglans mandshurica</i>	<i>V. plicatum</i>
S9	Iwakura, Sakyo, Kyoto Pref.	35° 5'27" N	135°47'11" E	160	warm temperate secondary deciduous forest	<i>Quercus serrata</i>	<i>V. dilatatum</i>

later-flowering species, I refrigerated *V. plicatum* inflorescences until the experiments to prevent the flowers from wilting. For each *Viburnum* species, I filmed insect visits to the six pairs of inflorescences for approximately 4 hours during the daytime on clear days.

I analyzed the films to obtain data on landing time (time [hh:mm:ss] when the visitor landed on the cluster of fertile flowers or on decorative flowers), departure time (time [hh:mm:ss] when the visitors departed from the inflorescence), and landing site (decorative flowers or fertile flowers) for each flower visitor. Insect visitors were netted and identified; most insects were identified at least to the family level, and the most predominant insects in Cerambycidae, Syrphidae and Apoidea were further identified to the species level. In addition to these diurnal observations, I directly observed nighttime insect visits to *V. wrightii*, *V. dilatatum*, *V. sieboldii* and *V. phlebotrichum* inflorescences.

I checked the video recordings and counted the numbers of unopened, opened and finished fertile flowers for each inflorescence. The percentage of open fertile flowers within an inflorescence is an indicator of flowering synchrony. To assess nectar production, I measured

the volume of floral nectar in bagged inflorescences with glass micropipettes (1 μ m) and the sugar concentration of the nectar with refractometers (Kikuchi 0–50% Brix and Kikuchi 45–80% Brix). I carried out additional nocturnal observations to assess nectar secretion for *V. wrightii*, *V. dilatatum*, *V. sieboldii*, and *V. phlebotrichum*.

Data analysis

For each *Viburnum* species, I compared insect visit rates (number of visits per inflorescence per hour) between framed and non-framed inflorescences (i.e. between intact and treated inflorescences) with a one-tailed, unpaired Mann-Whitney-Wilcoxon (MWW) test, using R software (ver. 3.3.2), at the 0.05 and 0.01 levels of significance. I also compared the visit rates of insect visitor orders and 17 insect functional groups between framed and non-framed inflorescences. The insect functional groups are as follows: Scarabaeidae, Cantharidae, Cerambycidae, other beetles, Empididae, Bombyliidae, Syrphidae, Muscomorpha, other flies, Andrenidae, Halictidae, Apid solitary bees, other bees, bumblebees, wasps, Macrolepidoptera and Others (i.e. Dermaptera, Hemiptera, Mecoptera). I excluded records of visitors that crawled or walked into the flowers (e.g., lepidopteran larvae, plant hoppers, ants).

Table 2-3. Visit rates of insect orders to framed and non-framed inflorescences of nine *Viburnum* species (N = 6).

<i>Viburnum</i> species	Inflorescence type	Visit rate (visits/inflorescence/h) $\pm$ SD				
		Coleoptera	Diptera	Hymenoptera	Lepidoptera	Others †
FR species						
<i>V. fuscum</i>	fr	—	4.7 $\pm$ 1.2	5.4 $\pm$ 5.6	0.1 $\pm$ 0.3	—
	n-fr	—	3.8 $\pm$ 1.8	3.7 $\pm$ 4.8	0	—
<i>V. opulus</i>	fr	7.7 $\pm$ 3.1 **	2.2 $\pm$ 1.1	1.0 $\pm$ 0.2 *	—	0
	n-fr	1.5 $\pm$ 0.8	1.3 $\pm$ 0.8	0.6 $\pm$ 0.2	—	0.1 $\pm$ 0.1
<i>V. plicatum</i>	fr	0.9 $\pm$ 0.7	2.2 $\pm$ 0.8	12.4 $\pm$ 1.9 **	—	—
	n-fr	0.8 $\pm$ 0.6	1.8 $\pm$ 0.5	5.0 $\pm$ 1.5	—	—
n-FR species						
<i>V. dilatatum</i>	fr	0.4 $\pm$ 0.5	0.2 $\pm$ 0.3	1.8 $\pm$ 0.5	—	0
	n-fr	0.3 $\pm$ 0.5	0.5 $\pm$ 0.4	1.6 $\pm$ 0.5	—	0 $\pm$ 0.1
<i>V. erosum</i>	fr	1.2 $\pm$ 1.0	3.9 $\pm$ 4.3	1.2 $\pm$ 1.3	—	—
	n-fr	0.5 $\pm$ 0.3	1.2 $\pm$ 0.8	0.6 $\pm$ 0.4	—	—
<i>V. wrightii</i>	fr	0.2 $\pm$ 0.3	1.8 $\pm$ 1.0	0.6 $\pm$ 0.8	—	—
	n-fr	0 $\pm$ 0.1	1.6 $\pm$ 0.9	0.3 $\pm$ 0.3	—	—
<i>V. sieboldii</i>	fr	0.1 $\pm$ 0.2	0.3 $\pm$ 0.3	2.7 $\pm$ 1.6	—	—
	n-fr	0.1 $\pm$ 0.2	0.2 $\pm$ 0.2	1.9 $\pm$ 1.1	—	—
<i>V. phlebotrichum</i>	fr	0.3 $\pm$ 0.6	0.4 $\pm$ 0.4	0.2 $\pm$ 0.3	—	0.1 $\pm$ 0.2
	n-fr	0.3 $\pm$ 0.8	0.1 $\pm$ 0.4	0.2 $\pm$ 0.2	—	0
<i>V. urceolatum</i>	fr	0.1 $\pm$ 0.2	1.6 $\pm$ 2.3 *	17.4 $\pm$ 11.5	0.1 $\pm$ 0.2	—
	n-fr	0	0.1 $\pm$ 0.2	29.8 $\pm$ 16.3 *	0	—

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences; fr, framed inflorescences; n-fr, non-framed inflorescences; SD, standard deviation. * $P < 0.05$, ** $P < 0.01$, One-tailed, unpaired Mann-Whitney test. † “Others” refers to visitors of the orders Dermaptera, Hemiptera and Mecoptera.

To detect whether flower visitors preferred to use decorative flowers as landing sites, I compared the rate of initial landing on decorative flowers and on fertile flowers for each *Viburnum* species with a one-tailed binomial test, considering similar chances to initially land on both flower types (1:1). I also compared the initial landing of insect visitor orders and main visitor groups.

Lastly, to compare insect visitor assemblages among intact inflorescences, and between framed and non-framed inflorescences of *Viburnum* species, I applied a non-metric multidimensional scaling (NMDS) analysis with a Bray-Curtis distance using the metaMDS function in R. In these analyses, the data comprised

the visit rates of the 17 insect functional groups on framed and non-framed inflorescences for nine *Viburnum* species. This analysis provided ordinates of the *Viburnum* inflorescences in a two-dimensional graphical representation according to their insect visitor assemblages.

Results

Floral Biology

The nine *Viburnum* species bloomed from spring to early summer (Table 2-1). Anthesis of the fertile flowers started in the morning, and the flower usually shed most of its pollen grains within a day. The main floral reward

of these *Viburnum* species was pollen. Pollen in buds was protected from pollinivorous insects by tightly enveloped tough calyces, and released from the anther only after the fertile flower opened and extended its stamens. Nectar was detected only in fertile flowers of *V. dilatatum*, *V. opulus*, *V. phlebotrichum* and *V. sieboldii*, with sugar concentrations of 48, 30,

19 and 28%, respectively. Nectar secretion was also observed in tubular flowers of *V. urceolatum*, but the sugar concentration could not be measured. Flowers of *V. sieboldii* and *V. dilatatum* also secreted nectar at nighttime.

The inflorescence size and floral display varied among *Viburnum* species. The num-

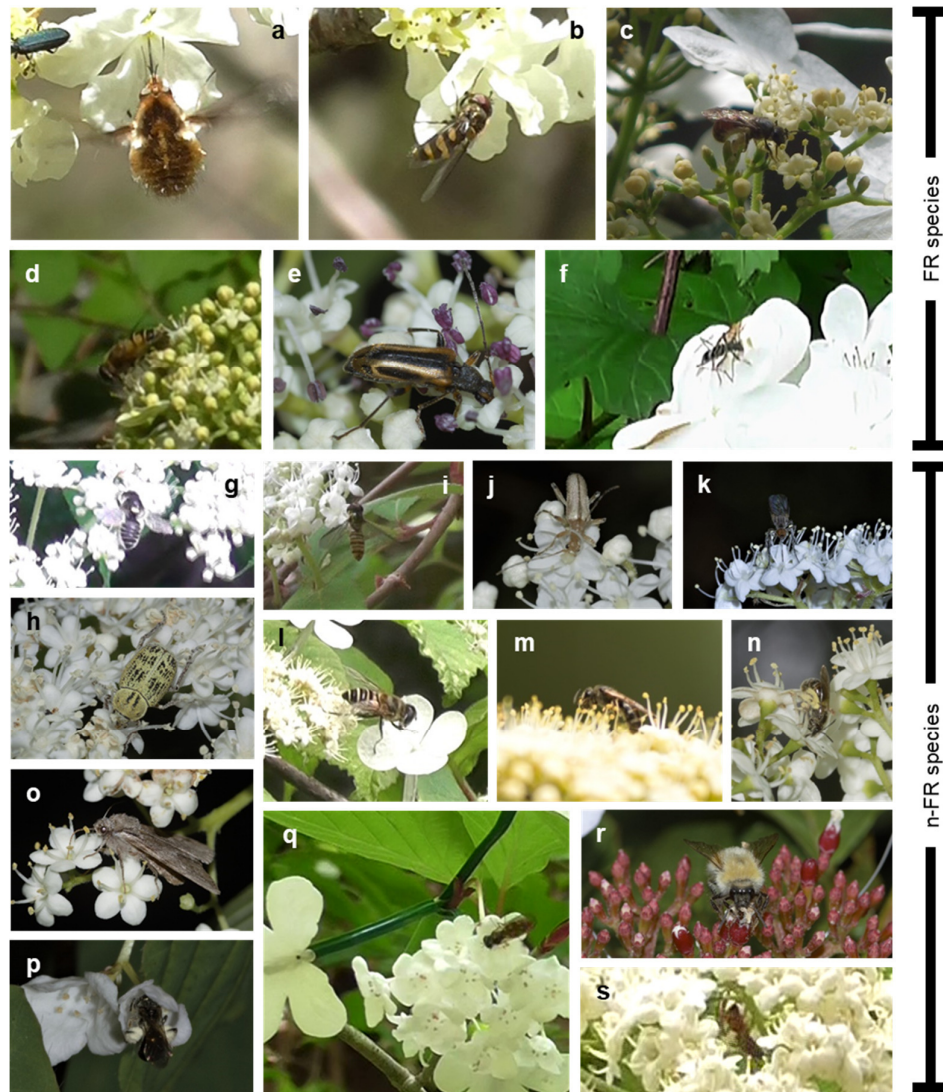


Fig 2-2. Insect visits to *Viburnum* flowers. *V. furcatum* visitors (a, b): (a) *Bombylius major* inserting its proboscis into a decorative flower; (b) *Parasyrphus* sp. landing on a decorative flower. *V. plicatum* visitors (c, d): (c) *Lasioglossum apristum* gathering pollen; (d) *Eristalis cerealis* feeding on pollen. *V. opulus* visitors (e, f): (e) *Pidonia* sp. feeding on nectar; (f) *Demonax transilis* landing on a decorative flower. *V. dilatatum* visitors (g, h): (g) *Megachile tsurugensis* gathering pollen; (h) *Ectinohoplia obducta* feeding on nectar at nighttime. *V. erosum* visitors (i-k): (i) *Episyrrhus balteatus* feeding on pollen; (j) *Pidonia aegrota* apparently feeding on nectar; (k) Empididae sp. 4 inserting its proboscis into a fertile flower. *V. wrightii* visitors (l, m): (l) *Eristalis cerealis* landing on a mounted decorative flower; (m) *Lasioglossum nipponicola* collecting pollen. *V. sieboldii* visitors (n, o): (n) *Lasioglossum apristum* feeding on nectar; (o) *Triaena intermedia* feeding on nectar. *V. phlebotrichum* visitors (p, q): (p) *Lasioglossum miyabei* feeding on nectar; (q) *Melanostoma* sp. feeding on pollen. *V. phlebotrichum* visitors: (r) *Bombus honshuensis* feeding on nectar. (s) Spider waiting for its prey in a cluster of fertile flowers on *V. sieboldii*.

ber of fertile flowers per inflorescence was largest in *V. dilatatum* and smallest in *V. phlebotrichum* (Table 2-1). Most fertile flowers in an inflorescence bloomed at the same time in *V. sieboldii* and *V. phlebotrichum*, whereas flowering among fertile flowers in an inflorescence was staggered to varying degrees in other *Viburnum* species. In FR species, the flowering of fertile flowers tended to be staggered, whereas it was more synchronous in n-FR species (except for *V. dilatatum* and *V. urceolatum*).

Flower-visiting insect fauna

A total of 120 insect species, belonging to seven orders and 35 families, visited the inflorescences of *Viburnum* spp. Ninety-one species visited intact inflorescences (supplementary table 2-1) and eighty-nine species visited treated inflorescences. The most species-rich order was Diptera (50%), followed by Hymenoptera (29.2%), Coleoptera (16.7%), Lepidoptera (1.7%), Hemiptera (0.8%), Dermaptera (0.8%) and Mecoptera (0.8%). Among dipterans, syrphid flies comprising 22 species were most dominant. In Hymenoptera, bees were dominant, comprising Apidae (11 species), Andrenidae (10 spp.), Halictidae (8 spp.), Colletidae (1 sp.) and Megachilidae (1 sp.). Coleopteran visitors were characterized by the dominance of Cerambycidae (14 species), Scarabaeidae (1 sp.), Cantharidae (2 spp.), and Elateridae (1 spp.). These beetles generally foraged for pollen, and some also ingested nectar. Insects observed to visit *Viburnum* inflorescences were generally diverse, belonging to three orders: Coleoptera, Hymenoptera and Diptera (Table 2-3, Figs. 2-2, 2-3). However, visits to *V. urceolatum* flowers were dominated by hymenopterans, particularly bumblebees (Table 2-4). Apart from *V. urceolatum*, the dominant visitors

Table 2-4. Visit rates of 17 visitor groups to framed and non-framed inflorescences of nine *Viburnum* species (N = 6)

Insect order	<i>V. furcatum</i>				<i>V. opulus</i>				<i>V. plicatum</i>				<i>V. dilatatum</i>				Visit rate (visits/inflorescence/h) ± SD				<i>V. erosum</i>				<i>V. wrightii</i>				<i>V. sieboldii</i>				<i>V. phlebotrichum</i>				<i>V. urceolatum</i>			
	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr								
Coleoptera	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
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Cerambycidae	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
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Other beetles	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
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Empididae	0 ± 0.1	0	0	0 ± 0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
	0.1 ± 0.2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
	4.5 ± 1.2	3.7 ± 1.9	1.0 ± 0.4	0.4 ± 0.3	0.9 ± 0.3	0.2 ± 0.3	0.2 ± 0.3	0.4 ± 0.4	3.5 ± 4.0 *	0.6 ± 0.5	1.1 ± 1.1	1.0 ± 0.9	0.1 ± 0.1	0	0.3 ± 0.4 *	0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2							
	0 ± 0.1	0.1 ± 0.1	1.2 ± 0.7	0.8 ± 0.7	1.3 ± 0.7	1.5 ± 0.4	0 ± 0.1	0 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.4 ± 0.7	0.3 ± 0.2	0.3 ± 0.4	0 ± 0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
Muscomorpha	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
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Hymenoptera	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
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Andrenidae	0.6 ± 0.5	0.2 ± 0.6	0.8 ± 0.2 *	0.6 ± 0.1	0.1 ± 0.2	0	0.5 ± 0.3	0.5 ± 0.5	0.8 ± 0.8	0.4 ± 0.4	0.1 ± 0.1	0	0.5 ± 0.5	0.5 ± 0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
	3.5 ± 4.0	2.4 ± 3.1	—	—	12.3 ± 2.0 **	5.0 ± 1.5	0.6 ± 0.5 *	0.1 ± 0.2	—	—	0.3 ± 0.5	0.3 ± 0.4	2.3 ± 1.3	1.4 ± 0.9	0.2 ± 0.3	0.2 ± 0.2	0	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2							
	0.9 ± 0.9	0.5 ± 0.6	0.1 ± 0.1	0	—	—	0.3 ± 0.4	0.5 ± 0.5	0.3 ± 0.5	0.2 ± 0.2	0.2 ± 0.4	0.1 ± 0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
	0 ± 0.1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
<i>Bombus</i>	0.3 ± 0.3	0.6 ± 0.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
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Other bees	0.1 ± 0.2	0	0.1 ± 0.1	0 ± 0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
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Lepidoptera	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—						
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Macrolepidoptera	0.1 ± 0.3	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—							
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fr, framed inflorescences; n-fr, non-framed inflorescences; SD, standard deviation. * P < 0.05, ** P < 0.01, one-tailed, unpaired Mann-Whitney test. † "Others" refers to visitors of the orders Dermaptera, Hemiptera and Mecoptera.

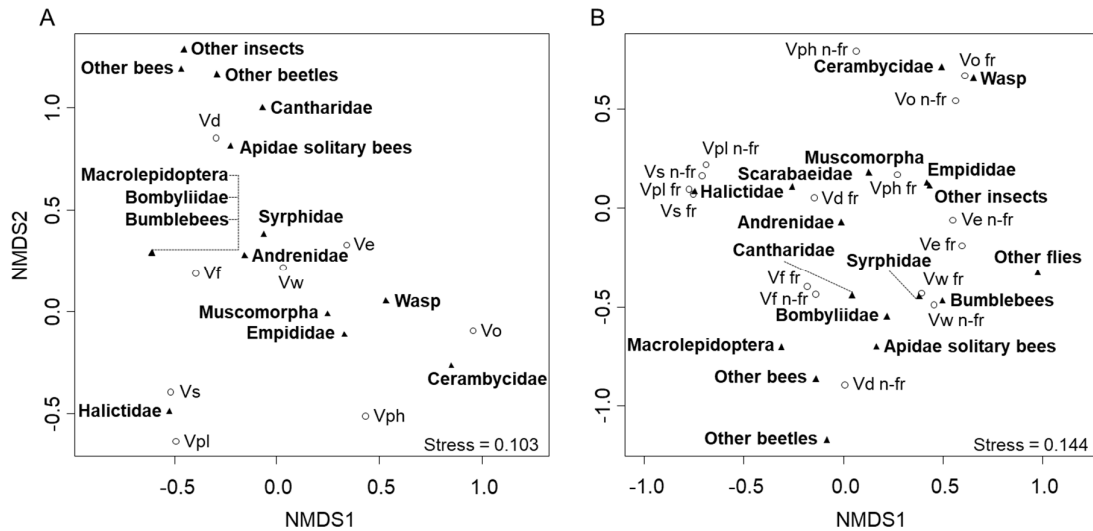


Fig 2-3. NMDS ordinations of inflorescences of *Viburnum* species according to their pollinator group assemblages. (A) Ordination of intact inflorescences. (B) ordination of framed and non-framed inflorescences. The ordinations were obtained by analyzing the visit rates for 17 visitor groups to framed and non-framed inflorescences of the studied *Viburnum* species, except for *V. urceolatum*, with NMDS, applying a Bray-Curtis distance. We omitted data of the framed and non-framed inflorescences of *V. urceolatum* in both analyses because they were outstandingly dominated by bumblebees, resulting in one-sided ordinations. The *Viburnum* inflorescences are represented by clear circles (○) and regular text, and the visitor groups by filled triangles (▲) and bold text. See Table 2-1 for *Viburnum* spp. codes. Abbreviations: fr = framed inflorescence; n-fr = non-framed inflorescence.

of other *Viburnum* species were cerambycid beetles, syrphid flies, and solitary bees, the proportions of which varied among *Viburnum* species (Table 2-4). In addition to these diurnal insects, nighttime visitors were observed for flowers of *V. dilatatum* and *V. sieboldii*. *V. dilatatum* was visited by nocturnal moths (Fig. 2-2o), and *V. sieboldii* was frequently visited by *Ectinohoplia obducta* (Scarabaeidae) (Fig. 2-2h).

The visitor assemblages were compared among intact inflorescences of *Viburnum* species, and between framed and non-framed inflorescences, using an NMDS analysis. The stress values were 0.10 and 0.14 for the intact inflorescences analysis and the framed and non-framed analysis, respectively. Both values were low enough to be considered useful in analyzing patterns (Clarke 1993), and in both analyses the ordination explained over 85% of the R^2 variance of the original matrix.

The ordination of the intact inflorescences (Fig. 2-3a) showed that the visitor assemblages were similar among some species and different among others. The assemblages of *V. opulus* and *V. phlebotrichum* had a predominance of cerambycid beetles, while the ones of *V. sieboldii* and *V. plicatum* had a predominance of halictid bees. The assemblages of *V. dilatatum*, *V. erosum*, *V. wrightii* and *V. furcatum* were similar to were similar to some extent, with a predominance syrphid flies. The ordination of framed and non-framed inflorescences (Fig. 2-3b) suggests that the assemblages of the same *Viburnum* species were largely similar, irrespective of the treatments of decorative flowers, and that the assemblages varied more interspecifically.

Groups for which visits were rarely recorded (Scarabaeidae, other beetles, Bombyliidae, other flies, wasps, Macrolepidoptera, Others) were dominant within the visitor assemblages of some *Viburnum* species. This is likely due to

the dominance being calculated based on the visit rate relative to other species.

After landing on inflorescences, insect visitors walked around on fertile flowers to ingest pollen or nectar. The time spent on an inflorescence varied among the visitor species (supplementary table 2-2). Some coleopterans and dipterans were frequently observed to copulate and rest in the inflorescences of *Viburnum* species, which is why some species of these taxa were recorded as spending a long time on inflorescences. In contrast to coleopterans and dipterans, most hymenopterans, especially bees, stayed on inflorescences for only a short time, and were not observed to copulate or rest in the inflorescences. Most insect visitors were dusted by pollen, especially around the mouthparts, the ventral side of the thorax and abdomen, and the legs. In general, bees accumulated more pollen than other visitors (supplementary table 2-2). Among coleopterans, cerambycid beetles, particularly *Pidonia* spp., accumulated moderate amounts of pollen. Among dipterans, *Bombylius major* accumulated large amounts of pollen, while some empidid flies and various syrphid flies accumulated moderate amounts of pollen. Among hymenopterans, only *Nomada* bees and wasps accumulated scarce amounts of pollen.

Although pollen was the main reward for the insect visitors to *Viburnum* flowers, I observed that some insects also collected nectar. Among hymenopterans, bumblebees frequently visited the tubular flowers of *V. urceolatum* to imbibe nectar (Fig. 2-2r), and solitary bees also

collected nectar from fertile flowers (Figs. 2-2n, 2-2p). Various cerambycid beetles also fed on nectar (Fig. 2-2e). *Pidonia aegrota* (Cerambycidae) was observed to feed on nectar from the fertile flowers of *V. erosum* (Fig. 2-2j). *Ectinohoplia obducta* (Scarabaeidae) fed on nectar and pollen from the fertile flowers of *V. dilatatum* (Fig. 2-2b). Empidid flies were observed to collect nectar from the fertile flowers of *Viburnum* species using their long proboscises (Fig. 2-2k). Additionally, *Bombylius major* (Fig. 2-2a) and *Pieris melete* (Pieridae) were observed to insert their long proboscises into the decorative flowers of *V. erosum* and *V. furcatum*, respectively, even though these flowers did not secrete nectar.

In addition to insects, thomisid spiders were observed in clusters of fertile flowers on *V. sieboldii* (Fig. 2-2s), *V. opulus* and *V. plicatum*.

Function of framed inflorescence

The numbers of insect visits to framed and non-framed inflorescences were compared for each *Viburnum* species. The visit rate for framed inflorescences was significantly higher than that for non-framed inflorescences in *V. plicatum* ($P < 0.01$), *V. opulus* ($P < 0.01$) and *V. erosum* ($P < 0.05$), but significantly lower in *V. urceolatum* (Fig. 2-4).

Next, I compared visit rates to framed and non-framed inflorescences among insect orders. Significantly higher visit rates to framed inflorescences were detected in coleopterans on

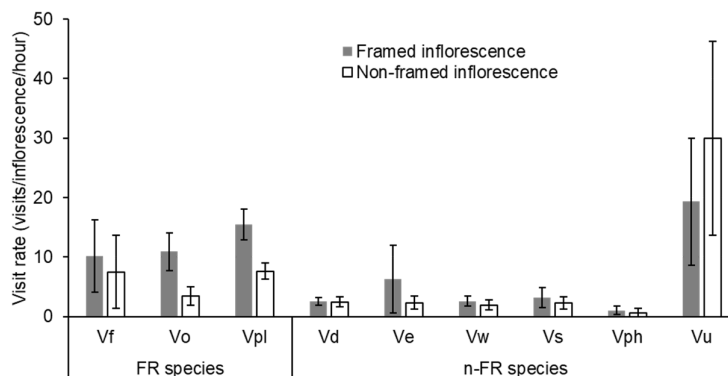


Fig. 2-4. Visit rates to framed and non-framed inflorescences. $N = 6$, error bars are $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, one-tailed, unpaired Mann-Whitney test. See Table 2-1 for *Viburnum* spp. codes. The framed inflorescences of most *Viburnum* species had higher visit rates, and this difference was significant for *V. opulus* and *V. plicatum*. Only *V. urceolatum* had higher visit rates to the non-framed inflorescences, but this difference was not significant. Abbreviations: FR species = framed species; n-FR species = non-framed species.

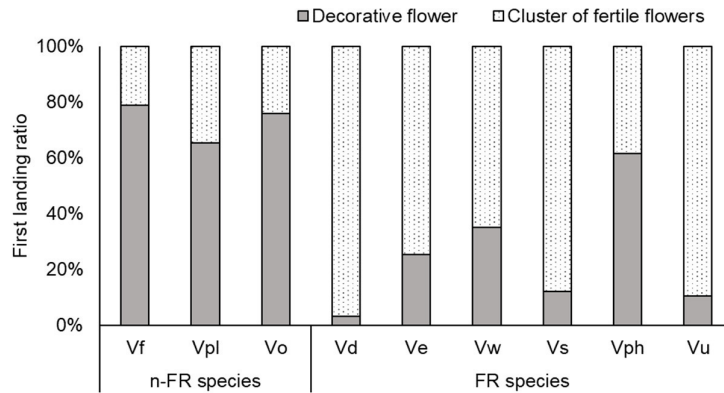


Fig. 2-5. Rates of initial landings on decorative flowers and fertile flowers in framed inflorescences of nine *Viburnum* species. N = 6, * P < 0.05, ** P < 0.01, one-tailed binominal test, p = 1/2. Only visitors of FR species showed preference to use decorative flowers as landing sites. See Table 3-1 for *Hydrangea* spp. codes. Abbreviations: FR species = framed species; n-FR species = non-framed species.

V. opulus flowers, dipterans on *V. urceolatum* flowers, and hymenopterans on *V. opulus*, *V. plicatum* and *V. urceolatum* flowers (Table 2-3). I made similar comparisons for 17 insect functional groups. Significantly higher visit rates to framed inflorescences were detected in cerambycid beetles on *V. opulus* and *V. dilatatum* flowers; in syrphid flies on *V. opulus*, *V. plicatum*, *V. erosum* and *V. phlebotrichum* flowers; in andrenid bees on *V. opulus* flowers; and in halictid bees on *V. plicatum* and *V. dilatatum* flowers (Table 2-4).

The flying insects attracted by the *Viburnum* inflorescences landed on the fertile or decorative flowers. By checking the video recordings, I observed the landing site of each insect visit. Insects landed on decorative flowers significantly more frequently in the three FR species (*V. furcatum*, *V. opulus* and *V. plicatum*). In contrast, insects landed significantly more frequently on fertile flowers than on mounted decorative flowers in four n-FR species: *V. erosum*, *V. dilatatum*, *V. sieboldii* and *V. urceolatum* (Fig. 2-5).

Next, I compared the rate at which insects landed first on decorative flowers among

insect orders. Among FR species, coleopterans, dipterans and hymenopterans showed preference for decorative flowers as landing sites, except for hymenopteran visitors of *V. opulus* (Table 2-5). Among n-FR species, only dipterans showed this preference in *V. urceolatum*. Among the flower visitor groups, cerambycid beetles, muscomorphan flies, and halictid bees preferred decorative flowers as landing sites in all FR species (Table 2-6, Figs. 2-2b, 2-2f).

Discussion

Although the flower-visitor assemblages varied among *Viburnum* species in the Japanese Archipelago, the flower-visitors comprised a diverse range of small insects including cerambycid beetles, syrphid flies, muscomorphan flies, andrenid bees and halictid bees. One exception was noted for *Viburnum urceolatum*, which was visited mainly by bumblebees (Table 2-4, supplementary table 2-1). The salient characteristic of these latter assemblages was the absence of honeybees, which suggests that the floral reward was not large or concentrated

Table 2-5. Rates of landing on decorative flowers for the orders, Coleoptera, Diptera and Hymenoptera in nine *Viburnum* species

Insect order	Landing ratio (%) ± SD †									
	FR species					n-FR species				
	<i>V. furcatum</i>	<i>V. plicatum</i>	<i>V. opulus</i>	<i>V. dilatatum</i>	<i>V. erosum</i>	<i>V. wrightii</i>	<i>V. sieboldii</i>	<i>V. phlebotrichum</i>	<i>V. urceolatum</i>	
Coleoptera	—	—	(6) 71.4% *	(6) 75.0% ** (4)	9.1%	(6) 12.9% (2)	40.0% (1)	50.0% (2)	50.0%	(2) 100%
Diptera	(6) 94.2% **	(6) 77.4% **	(6) 87.0% ** (4)	16.7%	(6) 31.8% (6)	33.3% (4)	62.5% (4)	60.0%	(5) 91.3% **	
Hymenoptera	(6) 66.2% **	(6) 62.7% **	(6) 56.5%	(6) 0.0%	(6) 15.6% (4)	38.5% (6)	4.7% (3)	100%	(6) 2.2%	

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences. * P < 0.05, ** P < 0.01, one-tailed binomial test, p = 1/2. † Numbers in parentheses represent the number of paired samples that were visited at least once by visitors of each insect pollinator order.

Table 2-6. Rates of landing on decorative flowers for 17 pollinator insect groups in nine *Viburnum* species

Insect order	Functional visitor group	First landing ratio (%) ± SD †																							
		FR species					n-FR species																		
		<i>V. furcatum</i>	<i>V. opulus</i>	<i>V. plicatum</i>	<i>V. dilatatum</i>	<i>V. erosum</i>	<i>V. wrightii</i>	<i>V. sieboldii</i>	<i>V. phlebotrichum</i>	<i>V. urceolatum</i>															
Coleoptera																									
	Scarabaeidae	—	—	—	—	(1)	0.0%	—	—	—	—	—	—	—	—										
	Cantharidae	—	—	(2)	100%	(0)	—	—	(1)	100%	—	—	—	—	—										
	Cerambycidae	—	—	(6)	71.4% *	(6)	75.0% **	(4)	10.0%	(6)	12.9%	(2)	40.0%	(1)	0.0%	(2)	50.0%	(2)	100%						
	Other beetles	—	—	—	—	(2)	50.0%	(0)	—	—	—	—	(1)	0.0%	—	—	—	—	—						
Diptera																									
	Bombyliidae	(1)	100%	—	—	—	—	—	(1)	0.0%	—	—	—	—	—	—	—	—	—						
	Empididae	(1)	100%	—	—	—	—	—	(3)	25.0%	(3)	25.0%	(1)	0.0%	(1)	100%	—	—	—						
	Muscomorpha	(1)	100%	(6)	81.3% **	(6)	86.2% **	(1)	0.0%	(5)	42.9%	(2)	66.7%	(3)	83.3%	—	—	(5)	90.9% **						
	Syrphidae	(6)	94.0% **	(6)	71.4% *	(6)	88.0% **	(3)	20.0%	(6)	30.9%	(5)	25.0%	(1)	0.0%	(3)	50.0%	(1)	100%						
	Other flies	—	—	—	—	—	—	—	(1)	100%	—	—	—	—	—	—	—	—	—						
Hymenoptera																									
	Bumblebees	(1)	100%	—	—	—	—	—	(1)	0.0%	—	—	—	—	—	—	—	(6)	2.2%						
	Andrenidae	(4)	50.0%	(1)	100%	(6)	52.6%	(5)	0.0%	(6)	4.5%	(2)	50.0%	(5)	10.0%	—	—	—	—						
	Halictidae	(4)	65.0% **	(6)	62.4% **	—	—	(6)	0.0%	—	(3)	28.6%	(6)	3.8%	(3)	100%	—	—	—						
	Apidae solitary bees	(6)	76.0% **	—	—	(2)	100%	(4)	0.0%	(3)	44.4%	(2)	50.0%	—	—	—	—	(1)	0.0%						
	Other bees	(3)	100% **	—	—	—	—	(5)	0.0%	—	—	—	—	—	—	—	—	—	—						
	Wasps	(1)	0.0%	—	—	(2)	50.0%	—	—	—	—	—	—	—	—	—	—	—	—						
Lepidoptera																									
	Macrolepidoptera	(1)	100%	—	—	—	—	—	—	—	—	—	—	—	—	—	—	(2)	100%						
Other insects ‡												—	—	—	—	—	—	—	—	—	—	(1)	0.0%	—	—

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences; dec, decorative flower; fer, fertile flower. * $P < 0.05$, ** $P < 0.01$, one-tailed binomial test, $p = 1/2$. $\dagger$ Numbers in parentheses represent the number of paired samples that were visited at least once by visitors of each insect pollinator group. $\ddagger$ "Others" refers to visitors of the orders Dermaptera, Hemiptera and Mecoptera.

enough for honeybees. The flower-visitor assemblages on each *Viburnum* species were largely similar between framed and non-framed inflorescences, regardless of whether the inflorescences were intact or treated (Fig. 2-3b).

Similar to previous observations made in the temperate forests of Kibune in Kyoto Prefecture, Japan (Inoue et al. 1990), pendent flowers of *V. phlebotrichum* were visited mainly by halictid bees and cerambycid beetles, and the low frequency of visits is likely due to the small size of the plant and its inflorescences. In contrast, the large inflorescences of *V. plicatum* at Kibune and Ashu in Kyoto Prefecture were more frequently visited by a wide range of cerambycid beetles and syrphid flies. The flowers of *V. furcatum* at Ashu were visited by syrphid flies and halictid bees, as was also observed in Yaku Island (Yumoto 1988). The high frequency of insect visits to the framed inflorescences of *V. furcatum* (Fig. 2-4) may be attributable to the conspicuousness of the decorative flowers on the bright forest floor, and to the rarity of concurrently blooming flowers in early spring, when canopy trees have not yet opened their leaves.

Compared with previous studies examining flower-visitor assemblages of *Viburnum*

flowers in Holarctic regions other than the Japanese Archipelago (Donoghue 1980, Nebot and Mateu 1990, Englund 1993, Jin et al. 2010), my data are characterized by a high frequency of visits by cerambycid beetles (Table 2-5), and in particular beetles of the genus *Pidonia* (supplementary table 2-1). The genus *Pidonia* has experienced an adaptive radiation in temperate forests in the Japanese Archipelago (Kuboki 1980). Adults of this genus are pollen-feeding visitors to various angiosperm flowers, such as the families Adoxaceae and Hydrangeaceae, both of which include many woody plant species with framed inflorescences. My data suggest that the diverse *Pidonia* beetles are important pollinators of the Japanese *Viburnum* species.

My observations of visits to framed and non-framed inflorescences suggest that removing the decorative flowers from inflorescences of FR species significantly decreased the attractiveness of the inflorescences (Fig. 2-4). This is consistent with previous studies by Bell (1985) and Jin et al. (2010). The greater visual attractiveness of framed inflorescences can be explained by one or a combination of the following visual cues: (1) enlarged inflorescence area, (2) accentuated contrast in the inflorescence (between the decorative and fertile flowers),

and (3) attractive silhouettes created by the decorative flowers (Thorpe and Horning 1983, Conner and Rush 1996, Goulson 2000, Spaethe et al. 2001, Makino et al. 2007). My data are consistent with these hypotheses, and suggest that flower-visits by solitary bees, syrphid flies and cerambycid beetles were facilitated by the presence of decorative flowers (Table 2-4). Thus, these small, pollen/nectar-foraging insects are thought to have been involved in the evolution of framed inflorescences.

Mounting decorative flowers on the inflorescences of n-FR species did not increase, or only slightly increased, their attractiveness (Fig. 2-4). For example, the intact, non-framed inflorescences of *V. urceolatum* were more frequently visited by bumblebees (their primary pollinator) than the treated, framed inflorescences. This result suggests that the mounted decorative flowers did not increase the attractiveness of the *V. urceolatum* inflorescences for bumblebees, and similar results were observed for other n-FR *Viburnum* species and their respective main pollinators. However, treated inflorescences were associated with increased attractiveness for cerambycid beetles, halictid bees, syrphid flies and other muscomorphan flies (Table 2-4). This suggests that the pollinator-attracting function of decorative flowers was less effective for visitor groups other than cerambycid beetles, syrphid flies, halictid bees and muscomorphan flies. Although the pollinator-attractive function of decorative flowers is associated with visual cues, it remains to be determined whether these flowers also employ olfactory cues to attract flower-visitors.

My observations of initial landing sites of flower-visitors on framed inflorescences suggest that the decorative flowers may serve as landing sites for visitors to FR *Viburnum* species, but not to n-FR species (Fig. 2-5). Results suggest that cerambycid beetles, syrphid flies and halictid bees are attracted by framed inflorescences, land on decorative flowers, and then walk to fertile flowers. On *Viburnum* inflorescences I observed that spiders, such as crab spiders (Thomisidae), ambushed flower-visiting

insects in fertile flower clusters, but not on decorative flowers. This is consistent with the observations of Lovell (1915). To reduce the risk of predation by spiders, some flower-visiting insects have evolved hesitation behaviors to assess the presence of spiders (Dukas 2001; Dukas and Morse 2003; Robertson & Maguire 2005; Yokoi & Fujisaki 2009). Because predators like crab spiders are usually absent on decorative flowers, and could be easily spotted if present, decorative flowers provide safe landing-sites. Thus, the observed tendency for cerambycid beetles and syrphid flies to land on decorative flowers may aid in avoiding spiders.

I hypothesize that another function of decorative flowers is to maintain the attractiveness of the inflorescence for a longer period, as suggested by the staggered flowering of fertile flowers within inflorescences in FR species (Table 2-1). In contrast, high levels of synchrony of flowering among fertile flowers within inflorescences was observed mostly in n-FR species. This flowering synchrony may enhance the pollinator-attracting function of the non-framed inflorescences, and reduce the cost of pollinivory by pollen-feeding flower-visitors at the cost of increased likelihood of geitonogamy. Thus, the attractive function of decorative flowers should be associated with the asynchronous flowering of fertile flowers within inflorescence in FR species.

My data suggest that the decorative flowers of FR *Viburnum* species increase inflorescence attractiveness for specific groups of insects: small, diurnal, pollen/nectar-feeding non-social insects, such as anthophilous cerambycid beetles, syrphid flies and solitary bees. My data also suggest that mounting decorative flowers on n-FR *Viburnum* species does not always increase inflorescence attractiveness, even though I observed that they were mainly visited by the above-mentioned groups of insects. Thus, the coexistence of FR and n-FR *Viburnum* species can be explained by the presence of multiple pollinator-attracting features.

Chapter 3: Pollinator-attracting functions of decorative sterile flowers of nine Japanese *Hydrangea* species (Hydrangeaceae)

Introduction

Insect pollination is by far the most common pollination system for the world's dominant terrestrial plant group, angiosperms (Grimaldi 1999; Hu et al. 2008; Abrol 2012), and it is widely hypothesized that insect pollinators drove the radiation of flowering plants (Crepet 1984; Dodd et al. 1999; Vamosi & Vamosi 2010; Willmer 2011). Some flowering plants use unique adaptations to attract and manipulate their pollinators to further increase their reproductive success (Kay & Sargent 2009). The use of dimorphic flowers within a single inflorescence is a particular adaptation observed in several families: Asparagaceae (Morales et al. 2013), Orchidaceae (Meisel et al. 2014), Adoxaceae, Hydrangeaceae (Darwin 1877), Apiaceae, Lamiaceae, and Asteraceae (Plitmann 1995). Few studies have evaluated the effects of dimorphic flowers in relation to pollination, though several have found positive results. In *Viburnum* spp. (Adoxaceae), Bell (1985) and Jin et al. (2010) showed that the presence of dimorphic flowers within a single inflorescence functioned to increase pollination success, while Krannitz & Maun (1991) reported neutral results. The pollinator-attracting functions of decorative flowers have also been observed in *Beaucarnea inermis* (Asparagaceae) (Morales et al. 2013) and *Leucanthemum vulgare* (Asteraceae) (Andersson 2008).

Hydrangea (Hydrangeaceae) is a genus of woody plants with large inflorescences composed of numerous small hermaphrodite flowers, and most species in the genus arrange several larger, showy, decorative flowers around a dense cluster of small fertile flowers.

The inflorescences with this floral arrangement will hereafter be referred as to 'framed inflorescences'. The decorative flowers are usually called 'sterile flowers' (McClintock 1957), while decorative flowers of at least some plants of *H. macrophylla* have male and female fertility (Uemachi et al. 2004). Darwin (1877) suggested that the decorative flowers of framed inflorescences of *Hydrangea* and *Viburnum* might increase their attractiveness to pollinators. This pollinator-attracting function has also been postulated by other authors (Donoghue 1980; Pilatowski 1982; Jacobs 2010), but the hypothesis has only been tested for *Viburnum* (Bell 1985; Krannitz & Maun 1991; Jin et al. 2010).

In Hydrangeaceae, 5 of 16 genera have species with dimorphic flowers in their inflorescences: *Cardiandra*, *Deinathe*, *Hydrangea*, *Platycrater*, and *Schizophragma* (Hufford 1997; Jacobs 2010). *Cardiandra*, *Hydrangea*, and *Schizophragma* contain species with framed inflorescences. Most *Hydrangea* species possess framed inflorescences (hereafter, 'FR species'), but a few species do not (hereafter, 'n-FR species'). Phylogenetic studies suggest that the loss of decorative flowers has only occurred independently four times, in *H. hirta*, *H. lingii*, *H. serratifolia* and *H. steyermarkii* (Hufford 1997; Jacobs 2010). Moreover, the morphology, color and arrangement of decorative flowers are diverse among the *Hydrangea* species. Considering this physiology, several intriguing questions have emerged: (1) Why do some *Hydrangea* species have framed inflorescences while others have non-framed inflorescences?; (2) What functions do decorative flowers have in local communities of anthophilous insects?;

and (3) What selection pressures have caused the evolution and diversification of decorative flowers?

To address these questions, I conducted field experiments in which decorative flowers were clipped in FR species, and mounted in n-FR species. I recorded the visits to these treated inflorescences, and to intact inflorescences. Next, I compared the visit frequencies and insect faunae types of flower visitors to framed and non-framed inflorescences (i.e. intact and treated inflorescences) among nine Japanese *Hydrangea* species comprising 8 FR and 1 n-FR species. Based on the data, I discuss the evolution and diversification of decorative flowers in Hydrangeaceae.

Material and methods

Study sites and species

Hydrangea species are broadly distributed in temperate regions of eastern Asia and eastern North America, and are scarcely present in the subtropical regions of Central and South America (McClintock 1957; Hu et al. 2008). The Japanese archipelago is characterized by high *Hydrangea* diversity (McClintock 1957; Hu et al. 2008), with 14 species (Hinkley 2003; Kawarada et al. 2010), most of which are shrubs. Most species of this genus display framed inflorescences: large, sterile, decorative flowers surround a cluster of numerous, small, fertile flowers. In some cultivar variations, the decorative flowers replace the fertile flowers. The fertile flowers are bisexual, have short pedicels, and are much more numerous than the decorative flowers. The decorative flowers of *Hydrangea* are usually sterile, though there are some rare cases of fertile decorative flowers in *H. macrophylla* (Uemachi et al. 2004).

Table 3-1. Features of the nine studied *Hydrangea* species, their decorative flowers and cluster of small flowers.

Hydrangea species	Species code	Habit	Type of inflorescence	Type of framing	Decorative flowers		Cluster of fertile flowers			Distribution ^d										
					No. per inflorescence ^b	Color	No. per inflorescence (mean ± SD) (N)	Average flowering synchrony in inflorescence (%)	Relative area in inflorescence (mean ± SD)	Color	Russia	Taiwan	China	Korea	Hokkaido	Honshu	Kyushu	Flowering period in Japan	Study site	Date of observation
FR species																				
<i>H. macrophylla</i>	Hm	shrub	compound corymb	complete	6–11 [8]	pale blue	170 ± 24 (6)	25.4	92.8 ± 1.3	blue					+			June - July	S5	7 Jun 2015
<i>H. serrata</i>	Hse	shrub	compound corymb	complete	4–10 [8]	blue	226 ± 30 (7)	53.9	55.5 ± 2.8	pale blue				+				June - Aug	S3	20 Jul 2014
<i>H. involucrata</i>	Hi	shrub	compound corymb	loose	6–17 [8]	white	246 ± 32 (7)	54.6	60.9 ± 1.3	pale purple					+			Aug - Sept	S4	3 Aug 2014
<i>H. petiolaris</i>	Hpe	vine	compound corymb	loose	4–11 [8]	white	420 ± 57 (8)	74.6	35.3 ± 2.0	white				+	+			June - July	S7	14 Jun 2015
<i>H. sikokiana</i>	Hsi	shrub	compound corymb	loose	4–10 [8]	white	676 ± 64 (6)	80.0	67.9 ± 4.2	white					+	+	+	July - Aug	S2	12 Jul 2014
<i>H. scandens</i>	Hsc	shrub	corymb	partial	2–5 [4]	white	32 ± 6 (10)	92.9	38.8 ± 2.4	white					+	+	+	May - June	S5	17 May 2015
<i>H. luteovenosa</i>	Hl	shrub	corymb	partial	2–4 [2]	white	11 ± 2 (12)	97.5	36.1 ± 2.5	white					+	+	+	June - July	S1	29 May 2015
<i>H. paniculata</i>	Hpa	treelet	compound panicle	complete ^a	6–16 [8–12]	white	332 ± 60 (7)	44.5	-	white				+	+	+	+	July - Aug	S4	31 Jul 2014
Non-FR species																				
<i>H. hirta</i>	Hh	shrub	compound corymb	not framed	0 [4]	purple	95 ± 6 (6)	97.8	25.4 ± 2.0	pale purple					+	+		June - July	S1	1–2 Jun 2014

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences. See Table 2 for the study sites codes. ^a Decorative flowers of *H. paniculata* complete framed the inflorescences when looked from the apical proportion of the panicle. ^b Range observed in the observed population of *Hydrangea* species. Number in brackets is the number of decorative flowers in observed framed inflorescences. For *H. hirta* we mounted purple decorative flowers from a *H. macrophylla* cultivar. ^c The number of fertile flowers was counted from some of the inflorescences which insect visits were recorded. ^d Distribution in Russia correspond to the Sakhalin island. Hokkaido, Honshu, Shikoku, Kyushu and Ryuku area the greater islands of the Japanese archipelago.

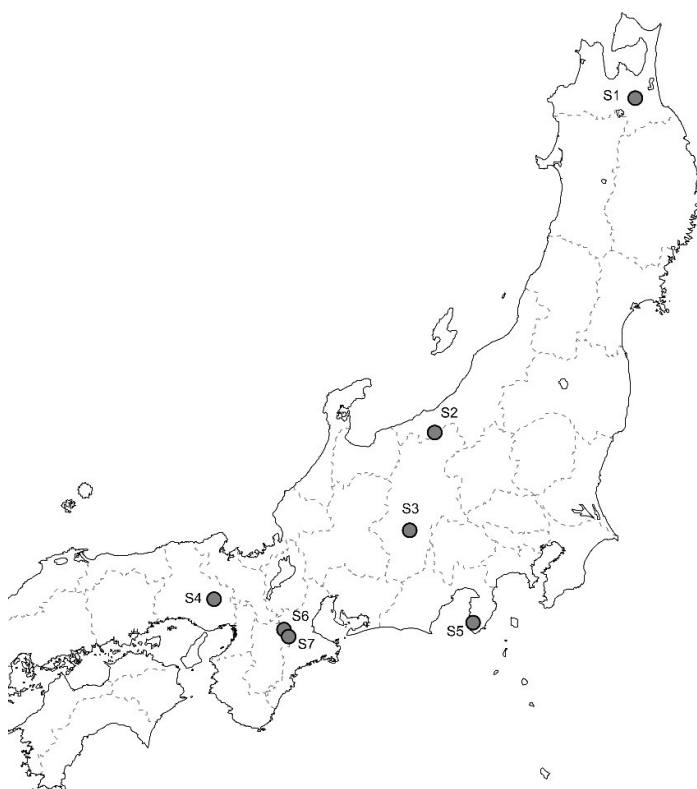


Fig. 3-1. Seven study sites for the *Hydrangea* species examined. The observation sites were all located in the Honshu region, Japan.

I observed and recorded insect visits to flowers of the following nine *Hydrangea* species: *H. hirta* (Thunberg) Siebold., *H. involucrata* Siebold., *H. luteovenosa* Koidz., *H. scandens* Seringe., *H. serrata* (Thunberg) Seringe., *H. sikokiana* Maxim., *H. paniculata* Sieb. and Zucc., *H. petiolaris* Sieb. and Zucc., and *H. macrophylla* (Thunb.) Ser. The observations and recordings were done in seven distinct locations on the Honshu island, Japan between 2014 and 2015 (Table 3-1, Fig. 3-1). Among the nine species, eight were FR species,

and *H. hirta* was a n-FR species (Table 3-2, Fig. 3-2). The decorative flowers had enlarged showy sepals surrounding a cluster of small fertile flowers forming a 'frame' (Fig. 3-2), but there are too few decorative flowers to form a complete frame in *H. scandens* and *H. luteovenosa* (Figs. 3-2f, 3-2g). The decorative flowers of most species point upwards, but those of *H. involucrata* are directed laterally (Fig. 3-2a). The decorative flowers of *H. sikokiana* are small and vestigial, and the sepal area of the decorative flowers occupy less than

Table 3-2. Study sites and their localities, coordinates, dominant vegetation and trees, and *Hydrangea* species studied at each site.

Site code	Observed period	Locality	Latitude	Longitude	Altitude (m)	Vegetation	Dominant tree species	Studied species
S1	14 Jun 2015	Towada, Horyo, Aomori Pref.	40°35'37" N	140°59'16" E	420	cool temperate, deciduous forest	<i>Fagus crenata</i> , <i>Acer mono</i>	<i>H. petiolaris</i>
S2	20 Jul 2014	Suginosawa, Myoko, Niigata Pref.	36°51'22" N	138° 7'27" E	1260	cool temperate, deciduous forest	<i>Fagus crenata</i> , <i>Cryptomeria japonica</i>	<i>H. serrata</i>
S3	31 Jul, 3 Aug 2014	Komagane Highlands, Nagano Pref.	35°44'18" N	137°53'15" E	910-980	cool temperate, deciduous forest	<i>Quercus crispula</i> , <i>Aesculus turbinata</i>	<i>H. involucrata</i> , <i>H. paniculata</i>
S4	2 Jun 2014, 29 May 2015	Kondacho Honjo, Sasayama, Hyogo Pref.	35° 0'49" N	135° 5'0" E	250-280	warm temperate, evergreen forest	<i>Quercus glauca</i> , <i>Quercus serrata</i>	<i>H. hirta</i> , <i>H. luteovenosa</i>
S5	7 Jun 2015	Matsuzaki, Kamo, Shizuoka Pref.	34°43'16" N	138°44'48" E	20	warm temperate, evergreen forest	<i>Castanopsis sieboldii</i> , <i>Camellia japonica</i>	<i>H. macrophylla</i>
S6	12 Jul 2014	Kooschidani, Nabari, Mie Pref.	34°34'9" N	136° 6'54" E	280	warm temperate, evergreen forest	<i>Quercus salicina</i> , <i>Quercus serrata</i>	<i>H. sikokiana</i>
S7	17 May 2015	Shorenji, Nabari city, Mie Pref.	34°33'3" N	136° 6'42" E	580	warm temperate, evergreen forest	<i>Quercus salicina</i> , <i>Abies firma</i>	<i>H. scandens</i>

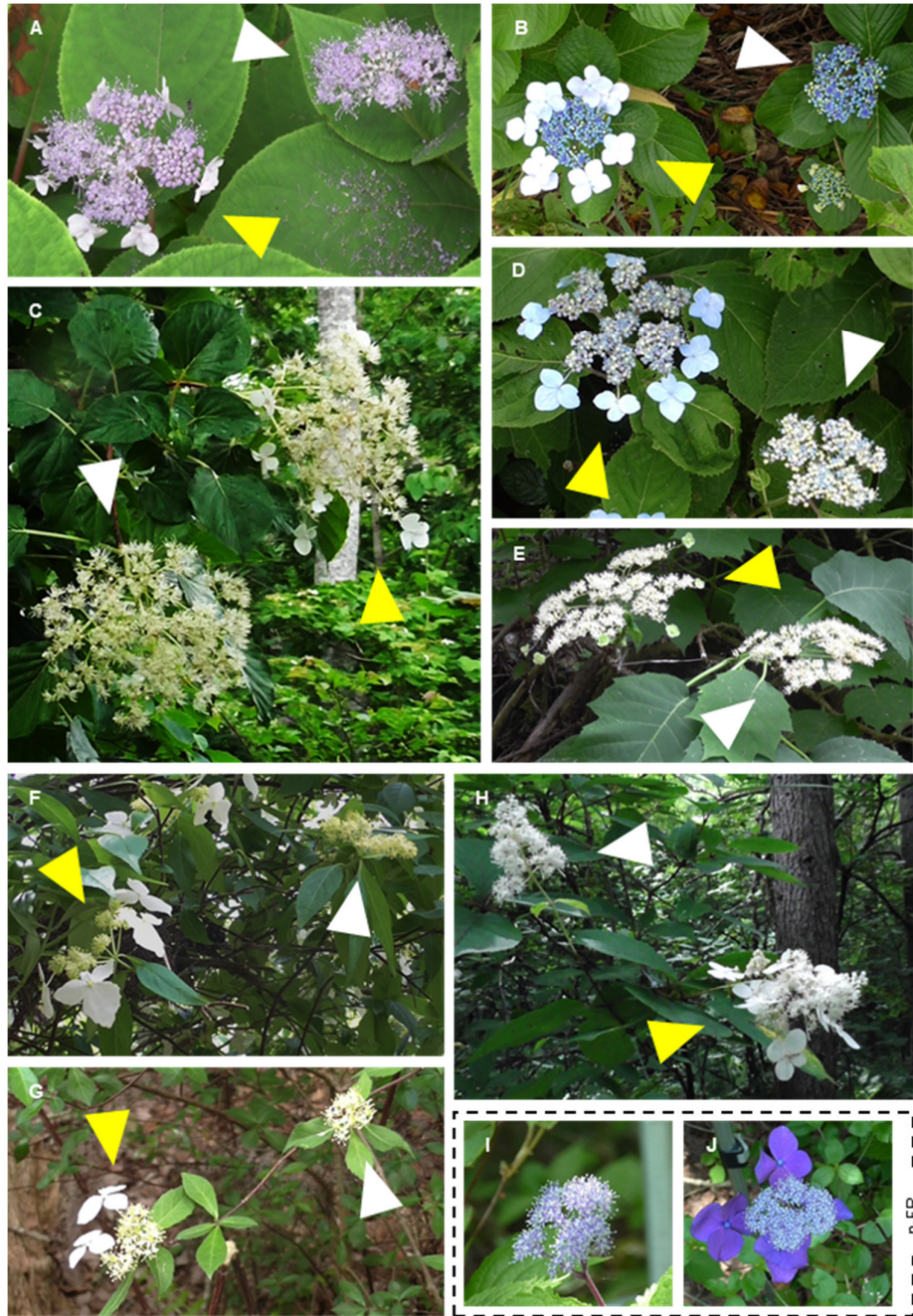


Fig. 3-2. Intact and treated inflorescences of nine *Hydrangea* species. (a) *H. involucrata*; (b) *H. macrophylla*; (c) *H. petiolaris*; (d) *H. serrata*; (e) *H. sikokiana*; (f) *H. scandens*; (g) *H. luteovenosa*; (h) *H. paniculata*; (i) intact inflorescence of *H. hirta*; and (j) treated inflorescence of *H. hirta*. In species with inherent sterile flowers: white arrows, treated inflorescences; yellow arrows, intact inflorescences. Decorative flowers were clipped in framed species and mounted in non-framed species. In *H. hirta*, we mounted decorative flowers of a purple *H. macrophylla* cultivar. Abbreviations: n-FR = non-framed species.

10% of the area of the whole inflorescence (Table 3-1, Fig. 3-2e). The inflorescences are all corymbs except in the case *H. paniculata*,

whose inflorescence is a panicle (Fig. 3-2h). See Table 3-2.

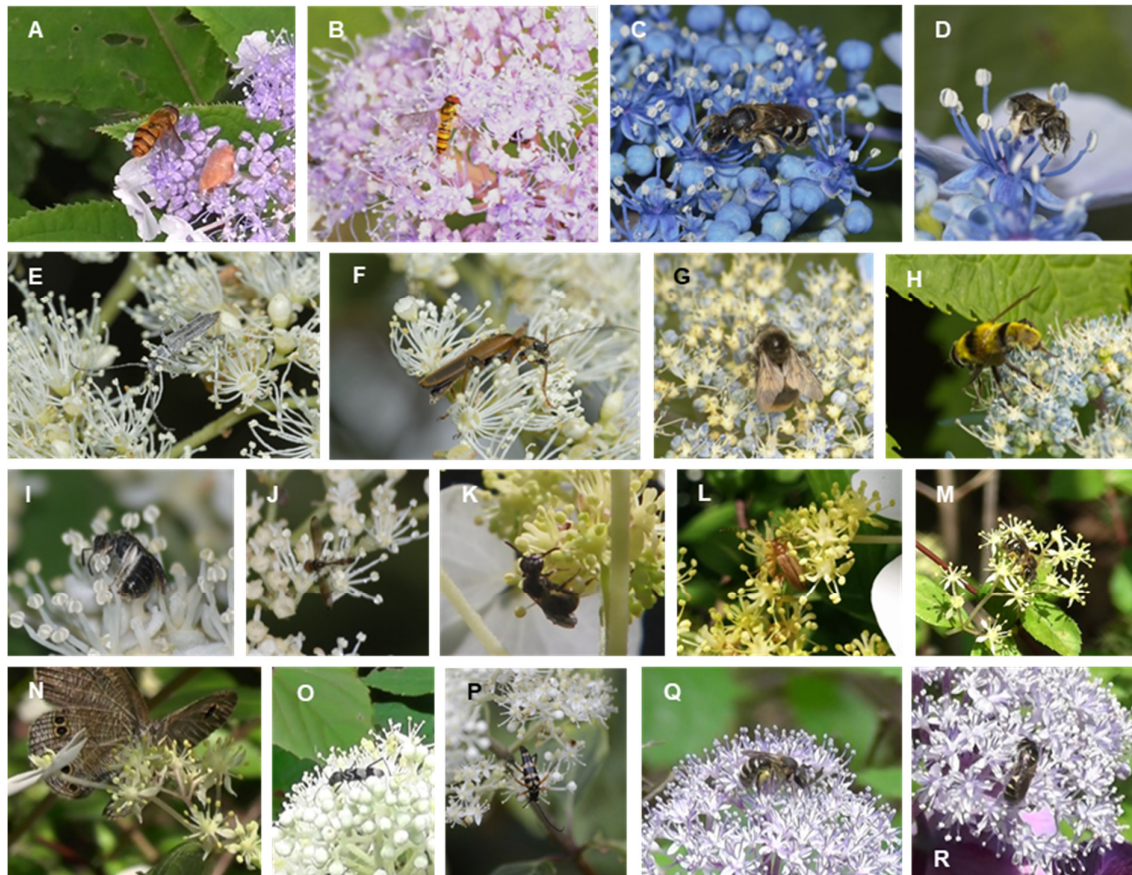


Fig. 3-3. Insect visits to flowers of nine *Hydrangea* species. *H. involucrata* visitors (a, b): (a) *Asarkina porcina* feeding on pollen; (b) *Episyrphus balteatus* feeding on pollen, *H. macrophylla* visitors (c, d): (c) *Lasioglossum harmandi* gathering pollen; (d) *Lasioglossum vulsum* gathering pollen, *H. petiolaris* visitors (e, f): (e) *Strangalomorpha tenuis aenescens* feeding on pollen; (f) *Lycocerus suturellus* feeding on pollen, *H. serrata* visitors (g, h): (g) *Bombus hypocrita* gathering pollen; (h) *Mallota dimorpha* feeding on nectar, *H. sikokiana* visitors (i, j): (i) *Lasioglossum allodelum* feeding on pollen; (j) *Allobaccha apicalis* feeding on pollen. *H. scandens* visitors (k, l): (k) *Lasioglossum vulsum* seemingly feeding on nectar; (l) *Pidonia aegrota* seemingly feeding on nectar, *H. luteovenosa* visitors (m, n): (m) *Lasioglossum nipponicola* seemingly feeding on nectar; (n) *Ypthima argus* seemingly feeding on nectar, *H. paniculata* visitors (o, p): (o) *Demonax transilis* waking in inflorescence; (p) *Judolia cometes* feeding on nectar. *H. hirta* visitors (q, r): (q) *Lasioglossum nipponicola* collecting pollen; and (r) *Ceratina japonica* collecting pollen.

Observation of flower visitors

I recorded insect visits to six pairs of inflorescences for each *Hydrangea* species, each pair with one framed and one non-framed inflorescence. These pairs had inflorescences of similar forms (e.g., size and number of decorative flowers), and were selected from the same plant individual when possible. If one individual plant did not have enough inflorescences, I used individuals close by. Within the inflorescence pair, one inflorescence

was kept intact, and the other was treated (i.e. decorative flowers were clipped in FR species, and mounted in n-FR species); for *H. hirta*, purple decorative flowers of an *H. macrophylla* cultivar were mounted. I recorded insect visits with video cameras (Panasonic HC-V210M, Panasonic HC-V520M and Panasonic HC-V620M) over the course of about 4 hours for each inflorescence pair.

I analyzed the recorded flower visits to obtain data on landing time (time at which the

visitor landed on the cluster of fertile flowers or on decorative flowers [hh: mm: ss]), departure time (time at which the visitors departed from the inflorescence [hh: mm: ss]), and landing site (decorative or fertile flowers) for each flower visitor. Insect visitors were netted nearby, and identified at least to family level.

To assess nectar production, I measured the volume of floral nectar in bagged inflorescences with glass micropipettes (0.5 µl), and the sugar concentration of the nectar with sugar refractometers (KIKUCHI, Tokyo, Japan).

Statistical analysis

To determine whether the presence of decorative flowers influenced the visit frequency of insect flower visitors, I compared the insect visitation rate (number of visits per inflorescence per hour) between the framed and non-framed inflorescences of the nine *Hydrangea* species with a one-tailed unpaired Mann-Whitney-Wilcoxon (MWW) U test, using R software (ver. 3.3.2; R Development Core Team), at the 0.05 and 0.01 levels of significance. I also compared the visitation rates of the insect orders and insect functional groups between framed and non-framed samples using a MWW U test with the same attributes and levels of significance. The 14 insect functional groups were: Cantharidae, Cerambycidae, Scarabaeidae, other beetles, Muscomorpha, Syrphidae, other flies, Andrenidae, Halictidae, bumblebees, other bees, wasps, Lepidoptera, and Dermaptera. I omitted data from insects that crawled or walked into the inflorescences (e.g., caterpillars, plant hoppers, ants, etc.).

To assess the tendency of flower visitors to land on decorative flowers, I compared the rate of landing on decorative flowers and on fertile flowers for all insect visitors of the framed inflorescences of each *Hydrangea* spp. using a one-tailed binomial test, considering similar chances to initially land on both flower types (1:1). I also compared the

landing tendency of the visitor orders of each *Hydrangea* spp. using a MWW test with the same attributes and levels of significance.

To compare the insect visitor assemblages and their influence among *Hydrangea* species, I clustered these species and applied two non-metric multidimensional scaling (NMDS) analyses based on the visitation rate proportions of the 14 functional pollinator groups. I clustered the *Hydrangea* species, according to the visitation rate proportions of the functional pollinator groups to the intact inflorescences, using a Bray-Curtis similarity index (also done in R). The number of optimal clusters was determined using the Silhouette method. I applied an NMDS analysis using a Bray-Curtis similarity index in R ('metaMDS' function) to compare the insect visitor assemblages among intact inflorescences, and between framed and non-framed inflorescences of the nine *Hydrangea* species. The data used in these analyses comprised the visitation rate proportions of the 14 insect functional pollinator groups on the framed and non-framed inflorescences of the nine *Hydrangea* species. The analyses provided ordinates of the *Hydrangea* inflorescences in a two-dimensional graphical representation according to their insect visitor assemblages.

Results

Flowering biology

The *Hydrangea* species bloom from spring to late summer (Table 3-2). Anthesis of fertile flowers starts in the morning, and the flowers usually shed most of their pollen grains within one or a few days. Among the studied species, *H. sikokiana* had the highest number of fertile flowers per inflorescence and *H. luteovenosa* had the lowest (Table 3-2). The flowering synchrony of fertile flowers varied among FR species, and peaked in *H. scandens*, *H. luteovenosa*, and *H. hirta* (Table 3-2).

The main floral reward from visiting *Hydrangea* spp. was pollen. Nectar was only detected in fertile flowers of *H. paniculata* and *H. petiolaris*, with sugar concentrations of 50.0% and 31.2%, respectively. The nectar-probing behavior of some insects on *H. scandens* and *H. luteovenosa* flowers suggests that these plant species also secrete nectar (Figs. 3-31-n). Inflorescences of *H. hirta*, *H. paniculata*, and *H. petiolaris* had sweet odors.

Insect visitors

By observing the inflorescences of nine *Hydrangea* species, a total of 166 insect species belonging to five orders and 40 families were recorded. The most species-rich order was Diptera (44.6%), followed by Coleoptera (24.1%), Hymenoptera (21.7%), Lepidoptera (8.4%), and Dermaptera (1.2%). Among the dipterans, syrphid flies were the most dominant,

comprising 41 species, followed by various muscomorphan families. Among the hymenopterans, bees were dominant, comprising Halictidae (16 spp.), Apidae (5 spp.), Andrenidae (4 spp.), and Megachilidae (1 sp.). Coleopteran visitors were characterized by a dominance of Cerambycidae (21 spp.), Scarabaeidae (10 spp.), and Cantharidae (3 spp.). These beetles usually feed on pollen, and sometimes nectar. Most lepidopteran visitors were macrolepidopterans, comprising Nymphalidae (5 spp.), Hesperidae (4 spp.), Lycaenidae (2 spp.), and Papilionidae (1 sp.).

Most insect visitors belonged to the orders Coleoptera, Hymenoptera, and Diptera, but the proportions of these orders varied among species (Table 3-3, Fig. 3-4b). Cluster analysis sorted the visitor assemblages into three clusters (Fig. 3-4a): cluster 'I' comprising *H. petiolaris* and *H. paniculata* was

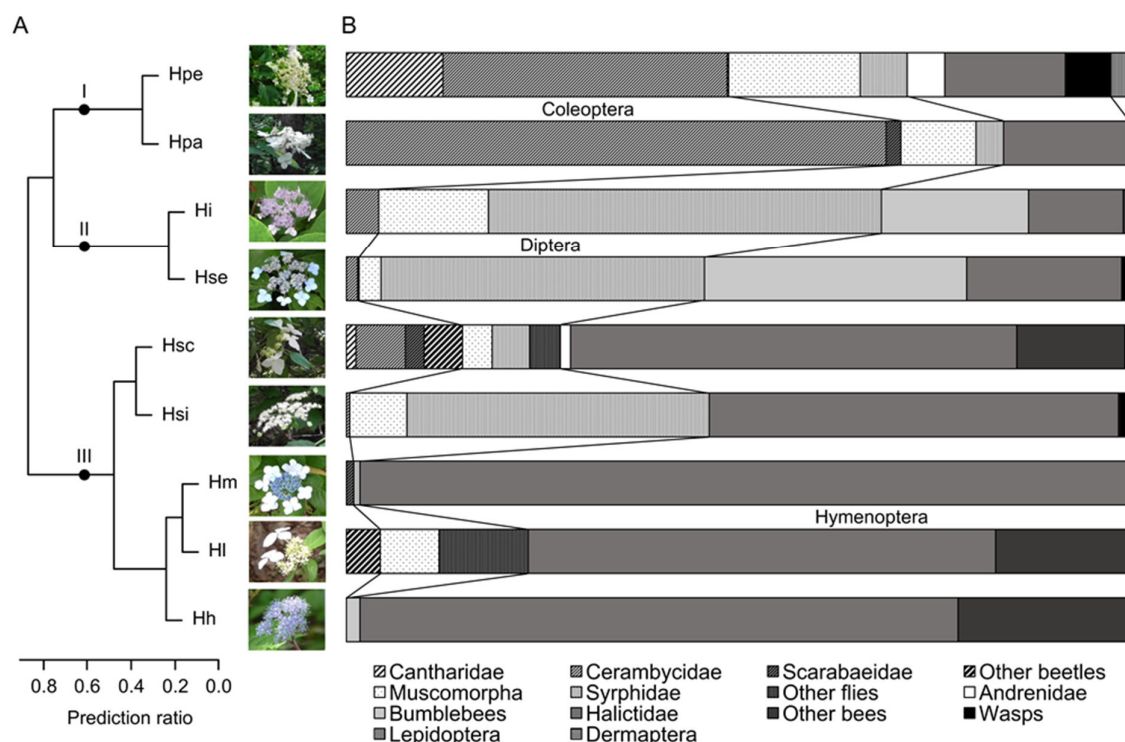


Fig. 3-4. Dendrogram and insect visitor spectra of *Hydrangea* species. (A) Dendrogram of nine *Hydrangea* spp. based on the visitation rate proportions of 14 insect visitor groups to intact inflorescences. (B) Insect visitor spectra of nine *Hydrangea* species based on the visitation rate proportions of 14 insect visitor groups to intact inflorescences. The visitor spectra of each *Hydrangea* species correspond to their respective position in the dendrogram. See Table 3-1 for *Hydrangea* spp. codes.

Table 3-3. Visit rates of insect orders to framed and non-framed inflorescences of nine *Hydrangea* species (N = 6).

inflorescenc		Visit rate (visits/inflorescence/h) $\pm$ SD				
<i>Hydrangea</i> species	e	Coleoptera	Diptera	Hymenoptera	Lepidoptera	Dermaptera
FR species						
<i>H. involucrata</i>	fr	0.3 $\pm$ 0.3	5.3 $\pm$ 3.7	2.6 $\pm$ 1.4	0.0 $\pm$ 0.1	—
	n-fr	0.4 $\pm$ 0.4	2.9 $\pm$ 1.7	1.9 $\pm$ 0.8	0.1 $\pm$ 0.1	—
<i>H. macrophylla</i>	fr	0.0 $\pm$ 0.1	0.0 $\pm$ 0.1	4.7 $\pm$ 2.1 *	—	—
	n-fr	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	2.0 $\pm$ 1.0	—	—
<i>H. petiolaris</i>	fr	9.5 $\pm$ 5.6	4.5 $\pm$ 3.8	5.1 $\pm$ 1.5	0.6 $\pm$ 0.8	—
	n-fr	5.5 $\pm$ 4.1	2.8 $\pm$ 1.7	4.0 $\pm$ 0.7	0.4 $\pm$ 0.5	—
<i>H. serrata</i>	fr	0.3 $\pm$ 0.3	8.5 $\pm$ 3.4 *	10.4 $\pm$ 2.4 *	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
	n-fr	0.1 $\pm$ 0.1	4.4 $\pm$ 1.3	6.2 $\pm$ 2.2	0.2 $\pm$ 0.2	0.0 $\pm$ 0.1
<i>H. sikokiana</i>	fr	0.1 $\pm$ 0.2	7.3 $\pm$ 3.2	8.7 $\pm$ 3.4	—	—
	n-fr	0.0 $\pm$ 0.1	6.9 $\pm$ 1.8	7.9 $\pm$ 3.5	—	—
<i>H. scandens</i>	fr	0.4 $\pm$ 0.5	0.4 $\pm$ 0.3	2.2 $\pm$ 1.8	0.0 $\pm$ 0.0	—
	n-fr	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	0.9 $\pm$ 0.9	0.0 $\pm$ 0.1	—
<i>H. luteovenosa</i>	fr	0.1 $\pm$ 0.3	0.5 $\pm$ 0.8	2.0 $\pm$ 1.0 *	—	—
	n-fr	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.6 $\pm$ 0.9	—	—
<i>H. paniculata</i>	fr	14.2 $\pm$ 9.8	2.6 $\pm$ 2.6	3.2 $\pm$ 4.4	0.1 $\pm$ 0.2	—
	n-fr	8.8 $\pm$ 6.4	2.2 $\pm$ 1.8	1.4 $\pm$ 1.4	0.0 $\pm$ 0.0	—
n-FR species						
<i>H. hirta</i>	fr	—	0.1 $\pm$ 0.1 *	5.7 $\pm$ 2.7	0.0 $\pm$ 0.1	—
	n-fr	—	0.0 $\pm$ 0.0	4.7 $\pm$ 4.3	0.0 $\pm$ 0.0	—

fr, framed inflorescences; n-fr, non-framed inflorescences; SD, standard deviation. * $P < 0.05$; ** $P < 0.01$. One-tailed, unpaired Mann-Whitney test.

characterized by a predominance of beetles, especially cerambycid beetles; cluster 'II' comprising *H. involucrata* and *H. serrata* was characterized by dominance of flies, particularly syrphid flies, and the occurrence of bumblebees; and cluster 'III' comprising *H. scandens*, *H. sikokiana*, *H. macrophylla*, *H.*

luteovenosa, and *H. hirta*, was characterized by the dominance of bees, particularly halictid bees.

The visitor assemblages were compared among intact inflorescences of *Hydrangea* species, and among framed and

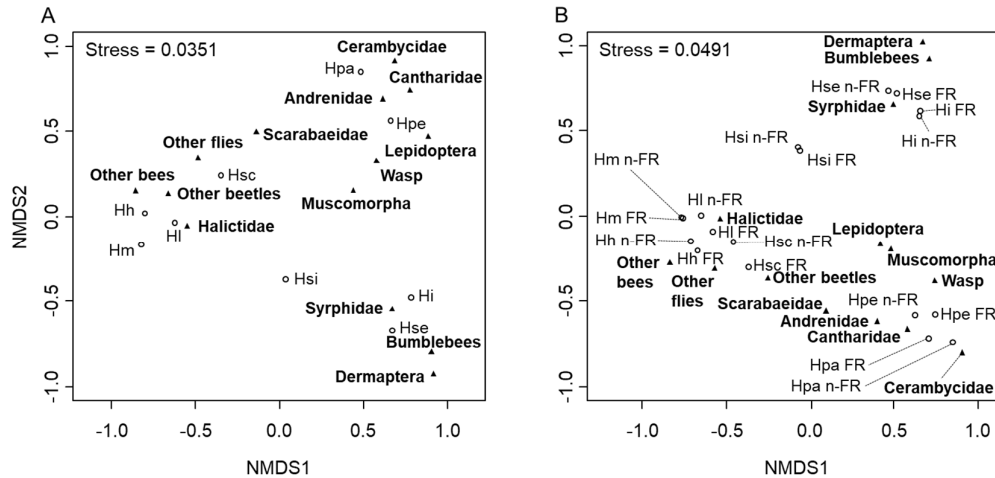


Fig. 3-5. Non-metric multidimensional scaling (NMDS) ordinations of flower visitor assemblages of nine *Hydrangea* species. (A) Ordination of visitation rate proportions of 14 visitor groups to intact inflorescences of nine *Hydrangea* species; the cluster was based on the Bray-Curtis similarity index. (B) Ordination of visitation rate proportions of 14 visitor groups to framed and non-framed inflorescences of nine *Hydrangea* species. The *Hydrangea* inflorescences are represented by filled triangles (▲) and bold text, 562 and the visitor groups by clear circles (○) and regular text. See Table 3-1 for *Viburnum* spp. codes. Abbreviations: FR = framed inflorescence; n-FR = non-framed inflorescence.

non-framed inflorescences, using NMDS analysis. The stress values of the intact inflorescences analysis, and of the framed and non-framed analysis, were less than 0.05, providing an excellent representation of the patterns (Clarke 1993) and explaining over 95% of the R^2 variance of the original matrix. The NMDS ordination of intact inflorescences (Fig. 3-5a) showed that the visitor assemblages differed among *Hydrangea* species. The first axis was positively correlated with the proportions of bumblebees, syrphid flies, and cerambycid beetles, and was negatively correlated with halictid bees; and the second axis was positively correlated with cerambycid beetles and negatively correlated with bumblebees and syrphid flies. The visitor assemblages of *H. involucrata* and *H. serrata* were dominated by bumblebees and syrphid flies, while those of *H. paniculate* and *H.*

petiolaris were dominated by visits from cerambycid beetles. The visitor assemblages of the remaining *Hydrangea* species were dominated by halictid bees. Visitor groups with generally low visitation rates were spread out in the ordination. The NMDS ordination including both framed and non-framed inflorescences (Fig. 3-5b) suggested that the visitor assemblages were largely similar between the two inflorescence types for each *Hydrangea* species.

Function of decorative flowers

To detect pollinator-attracting functions of decorative flowers, I compared the inflorescence-visitation rates between framed and non-framed inflorescences for nine *Hydrangea* species. In all FR species, the framed inflorescences had significantly higher visitation rates than the non-framed ones, with

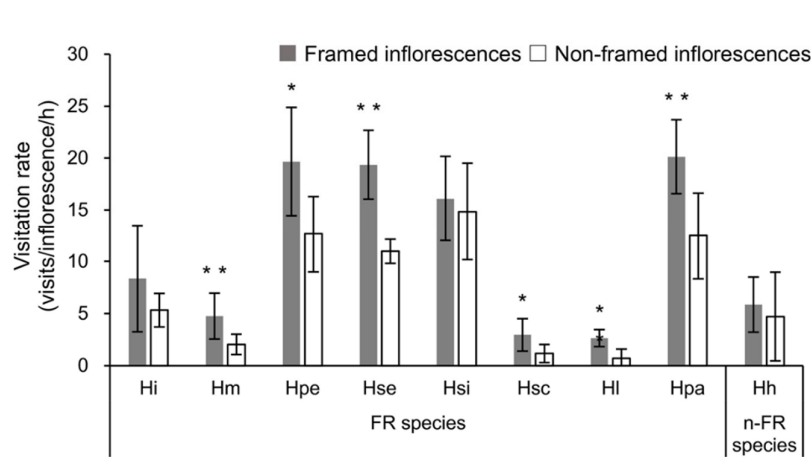


Fig. 3-6. Visitation rates to framed and non-framed inflorescences of nine *Hydrangea* species. $N = 6$, error bars are $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, one-tailed unpaired Mann-Whitney test. Most FR *Hydrangea* species had significantly higher visitation rates to the framed inflorescences, except *H. involucrata* and *H. sikokiana*, species with particular decorative flowers. *H. hirta*, the only n-FR species, showed no differences between visitation rates of framed and non-framed inflorescences. See Table 3-1 for *Hydrangea* spp. codes. Abbreviations: FR species = framed species; n-FR species = non-framed species.

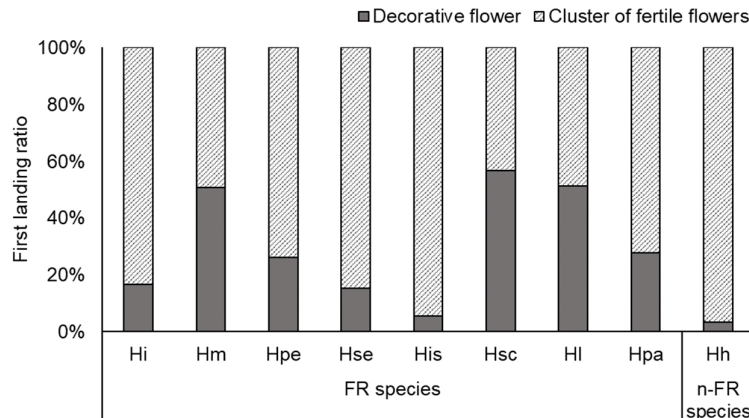


Fig. 3-7. Rates of initial landings on decorative flowers and fertile flowers in framed inflorescences of nine *Hydrangea* species. $N = 6$, * $P < 0.05$, ** $P < 0.01$, one-tailed binominal test, $p = 1/2$. No visitors of any studied species showed preference to use decorative flowers as landing sites. See Table 3-1 for *Hydrangea* spp. codes. Abbreviations: FR species = framed species; n-FR species = non-framed species.

the exceptions of *H. involucrata* and *H. sikokiana*. The only n-FR species, *H. hirta*, had a higher visitation rate to the framed inflorescences than the non-framed ones, but the difference was not significant (Fig. 3-6).

Next, I compared inflorescence-visitation rates between framed and non-framed inflorescences among insect orders and the 14 insect visitor groups. Most insect visitor orders had higher visitation rates for framed inflorescences than non-framed ones, significantly so in the dipterans on *H. serrata* and *H. hirta*, and in the hymenopterans on *H. serrata*, *H. luteovenosa*, and *H. macrophylla* (Table 3-3). Similar to the insect visitor orders, most insect visitor groups had higher visitation rates for the framed inflorescences, with significantly higher rates being seen for cantharid beetles on *H. petiolaris*, muscomorphan flies on *H. hirta*, *H. serrata*, and *H. scandens*, syrphid flies and bumblebees on *H. serrata*, halictid bees on *H. luteovenosa* and *H. macrophylla*, ‘other bees’ on *H. scandens*, and wasps on *H. petiolaris* (Table 3-4). Among the few visitor groups that visited the non-framed inflorescences more often, this trend was only significant for ‘other beetles’ on *H. petiolaris*.

To examine whether insect visitors tended to land first on decorative flowers in framed inflorescences, I checked the video recordings, and observed the initial landing spot for each flower visitor. In general, flower visitors of the studied *Hydrangea* species did not show preference to land in decorative flowers (Fig. 3-7).

Next, I analyzed the landing place for each insect order and each insect pollinator group. Coleopterans, dipterans and hymenopterans did not show preference to use decorative flowers as landing sites, except for dipteran flower visitors of *H. scandens* and *H. luteovenosa* (Table 3-5). Among insect

Table 3-4. Visit rates of 14 insect visitor groups to framed and non-framed inflorescences of nine *Hydrangea* species (N = 6).

Visitor group	Visit rate (visits/inflourescence/h) ± SD																	
	<i>H. involucrata</i>		<i>H. macrophylla</i>		<i>H. petiolaris</i>		<i>H. serrata</i>		<i>H. sikokiana</i>		<i>H. scandens</i>		<i>H. luteovenosa</i>		<i>H. paniculata</i>		<i>H. hirta</i> †	
	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr	fr	n-fr
Coleoptera																		
Cantharidae	—	—	—	—	2.4 ± 1.6 *	0.4 ± 0.8	—	—	—	—	0.0 ± 0.1	0.0 ± 0.1	—	—	—	—	—	—
Cerambycidae	0.3 ± 0.3	0.4 ± 0.4	—	—	7.1 ± 4.1	4.6 ± 3.3	0.3 ± 0.4	0.0 ± 0.1	0.1 ± 0.2	0.0 ± 0.1	0.2 ± 0.2	0.1 ± 0.2	—	—	13.8 ± 10.2	8.7 ± 6.6	—	—
Scarabaeidae	—	—	0.0 ± 0.1	0.0 ± 0.0	—	—	0.0 ± 0.1	0.0 ± 0.0	—	—	0.1 ± 0.1	0.0 ± 0.0	—	—	0.4 ± 0.5	0.1 ± 0.1	—	—
Other beetles	—	—	—	—	0.0 ± 0.1	0.5 ± 0.5 *	0.0 ± 0.0	0.0 ± 0.1	—	—	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	—	—	—	—
Diptera																		
Muscomorpha	1.2 ± 1.6	0.4 ± 0.3	—	—	3.3 ± 3.4	2.1 ± 1.6	0.6 ± 0.2 *	0.2 ± 0.2	1.2 ± 0.9	0.8 ± 0.7	0.1 ± 0.1 *	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.1	1.9 ± 2.4	1.7 ± 1.6	0.1 ± 0.1 *	0.0 ± 0.0
Syrphidae	4.2 ± 2.3	2.5 ± 1.5	0.0 ± 0.1	0.0 ± 0.0	1.2 ± 0.8	0.7 ± 0.5	7.9 ± 3.3 *	4.2 ± 1.1	6.2 ± 2.5	6.1 ± 1.6	0.1 ± 0.3	0.1 ± 0.1	—	—	0.7 ± 0.5	0.5 ± 0.4	—	—
Other flies	—	—	—	—	—	—	—	—	—	—	0.1 ± 0.2	0.0 ± 0.1	0.3 ± 0.7	0.0 ± 0.1	—	—	—	—
Hymenoptera																		
Andrenidae	—	—	—	—	0.9 ± 0.6	0.7 ± 0.6	—	—	—	—	0.0 ± 0.1	0.0 ± 0.1	—	—	—	—	—	—
Halictidae	1.0 ± 0.9	0.7 ± 0.6	4.7 ± 2.1 *	2.0 ± 1.0	3.0 ± 1.1	2.5 ± 1.1	3.8 ± 2.2	2.5 ± 1.5	8.4 ± 3.1	7.7 ± 3.5	1.7 ± 1.7	0.8 ± 0.8	1.6 ± 0.7 *	0.5 ± 0.7	3.2 ± 4.3	1.0 ± 0.8	4.0 ± 2.7	3.6 ± 3.3
Bumblebees	1.6 ± 0.7	1.1 ± 0.6	—	—	—	—	6.4 ± 2.5 *	3.7 ± 2.3	—	—	—	—	—	—	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.2
Other bees	0.0 ± 0.1	0.0 ± 0.0	—	—	0.0 ± 0.1	0.0 ± 0.0	—	—	0.0 ± 0.1	0.1 ± 0.1	0.4 ± 0.4 *	0.0 ± 0.0	0.5 ± 0.5	0.2 ± 0.2	—	—	1.6 ± 0.8	1.1 ± 1.1
Wasps	0.0 ± 0.1	0.1 ± 0.2	—	—	1.1 ± 0.3 *	0.8 ± 0.6	0.1 ± 0.1	0.0 ± 0.1	0.3 ± 0.6	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	—	—	0.0 ± 0.1	0.4 ± 0.8	—	—
Lepidoptera	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lepidoptera	0.0 ± 0.1	0.1 ± 0.1	—	—	0.6 ± 0.8	0.4 ± 0.5	0.1 ± 0.1	0.2 ± 0.2	—	—	0.0 ± 0.0	0.0 ± 0.1	—	—	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Dermoptera	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dermoptera	—	—	—	—	—	—	0.1 ± 0.1	0.0 ± 0.1	—	—	—	—	—	—	—	—	—	—

fr, framed inflourescences; n-fr, non-framed inflourescences; SD, standard deviation. * P < 0.05, ** P < 0.01, One-tailed, unpaired Mann-Whitney test. † Naturally non-framed species

fr, framed inflorescences; n-fr, non-framed inflorescences; SD, standard deviation. * P < 0.05, ** P < 0.01, One-tailed, unpaired Mann-Whitney test. † Naturally non-framed species

Table 3-6. Rates of landing on decorative flowers for 14 pollinator insect groups in nine *Hydrangea* species

Insect order	First landing ratio (%) $\pm$ SD †								
Visitor group	<i>H. involucrata</i>	<i>H. macrophylla</i>	<i>H. petiolaris</i>	<i>H. serrata</i>	<i>H. sikokiana</i>	<i>H. scandens</i>	<i>H. luteovenosa</i>	<i>H. paniculata</i>	<i>H. hirta</i> ‡
Coleoptera									
Cantharidae	—	—	(6) 34.5%	—	—	(1) 100%	—	—	—
Cerambycidae	(4) 33.3%	—	(6) 25.3%	(3) 85.7%	(1) 0.0%	(3) 60.0%	—	(6) 21.3%	—
Scarabaeidae	—	(1) 100%	—	(1) 0.0%	—	(2) 50.0%	—	(3) 0.0%	—
Other beetles	—	—	(1) 0.0%	(0) —	—	(2) 75.0%	(1) 66.7%	—	—
Diptera									
Muscomorpha	(4) 17.9%	—	(6) 33.3%	(6) 14.3%	(6) 9.4%	(3) 100%	(4) 80.0%	(4) 32.6%	(3) 33.3%
Syrphidae	(6) 20.8%	(1) 100%	(6) 35.7%	(6) 16.4%	(6) 7.1%	(2) 100%	—	(5) 17.6%	—
Other flies	—	—	—	—	—	(2) 100%	(1) 100% **	—	—
Hymenoptera									
Andrenidae	—	—	(6) 9.1%	—	—	(1) 100%	—	—	—
Halictidae	(6) 16.0%	(6) 50.0%	(6) 19.7%	(6) 18.8%	(6) 3.9%	(6) 45.7%	(6) 37.5%	(5) 57.9%	(6) 3.0%
Bumblebees	(6) 0.0%	—	—	(6) 7.9%	—	—	—	—	(1) 0.0%
Other bees	(1) 0.0%	—	(1) 0.0%	—	(1) 0.0%	(4) 45.5%	(4) 50.0%	—	(6) 0.0%
Wasps	(1) 100%	—	(6) 19.2%	(3) 33.3%	(2) 0.0%	(1) 100%	—	(1) 100%	—
Lepidoptera									
Lepidoptera	(1) 0.0%	—	(4) 14.3%	(3) 33.3%	—	—	—	(2) 33.3%	(1) 100%
Dermaptera									
Dermaptera	—	—	—	(2) 50.0%	—	—	—	—	—

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences. * $P < 0.05$, ** $P < 0.0$, one-tailed binomial test, $p = 1/2$. † Numbers in parentheses represent the number of paired samples that were visited at least once by visitors of each insect pollinator group. ‡ Only non-framed studied species.

pollinator groups, only the group “other flies” showed preference for the decorative flowers as landing sites (Table 3-6).

Discussion

The insect flower visitor assemblages of *Hydrangea* spp. were composed of diverse small pollen and/or nectar foraging insects. The main flower visitors, and likely main pollinators, were bees, syrphid flies, and cerambycid beetles, but the importance of these visitors varied among the *Hydrangea* species (Table 3-4, Figs. 3-4b, 3-5a, supplementary table 3-1). Most pollinators were mainly rewarded with pollen, and occasionally (in some species) with small amounts of nectar. The flower visitor assemblages were largely similar between framed and non-framed inflorescences, regardless of the species classification as FR or n-FR (Fig. 3-5b).

The flower visitor assemblage results were roughly similar to those of previous studies done in the same region in Japan (Kuboki 1980; Kato 1988; Inoue et al. 1990; Kato et al. 1990, 1993; Yamazaki & Kato 2003), and similar at in order level with observations

from other parts of the world (Robertson 1892; Smith-Ramírez et al. 2005). Nonetheless, a study conducted in the Oita Prefecture, in the Kyushu region of Japan, identified honeybees as the pollinator agents of *H. serrata* and *H. paniculata* (Yamazaki & Kato 2003), whereas I did not observe visiting honeybees for any of the plant species in this study. This inconsistency can be explained by the difference in habitats. My study sites were all forests, while the sites in Oita were natural grasslands.

Comparison of visitation rates between framed and non-framed inflorescences suggests that decorative flowers increase the attractiveness of *Hydrangea* species, except in species with particular decorative flowers (i.e. *H. involucrata* and *H. sikokiana*) and n-FR species (i.e. *H. hirta*). These results are consistent with previous studies of *Viburnum* spp. (Bell 1985; Jin et al. 2010; Chapter 2). Moreover, my results suggest that among the main visitor groups, insect-attracting functions of decorative flowers are more conspicuous to bees and syrphid flies than to cerambycid beetles. I attribute the higher visitation rates to framed inflorescences to visual cues and flower constancy. One or a few of the following visual

cues may be attractive to flower visitors: i) an enlarged inflorescence area created by the decorative flowers; ii) an emphasized contrast between decorative flowers and the cluster of fertile flowers, and/or the background (especially in colored species); and iii) a more attractive silhouette created by the decorative flowers. These visual features are appealing to bees (Prokopy & Owens 1983; Conner & Rush 1996; Goulson 2000; Spaethe et al. 2001; Makino et al. 2007; Willmer 2011), while their effects on dipterans and coleopterans are not well known. Flower constancy (i.e. fidelity to a plant species that previously provided a reward) is commonly found in social bees (Wilson & Stine 1996; Gegear & Lavery 2005) and solitary bees (Linsley & MacSwain 1958; Gross 1992), uncommonly found in syrphid flies (Goulson & Wright 1998), and rarely found in beetles (De Los Mozos Pascual & Medina Domingo 1991). I postulate that bees, and to a lesser degree flies, were mainly responsible for the selection pressure contributing to the appearance and diversity of decorative flowers in *Hydrangea*.

My observations of the landing of flower visitors shows that flower visitors of most *Hydrangea* FR species tend to land initially on the cluster of fertile flowers (Fig. 3-7). My results conflict with those of a previous study on *Viburnum* species (Chapter 2), where in various flower visitors, particularly cerambycid beetles, syrphid flies and halictid bees tended to land initially on decorative flowers. Although these decorative flowers do not function as landing sites, they are thought to contribute to maintenance of insect-attracting functions, through periods in which the inflorescences contain functional fertile flowers.

My results suggest that different combinations of floral characteristics created by decorative flowers increase species attractiveness to pollinators differently. Among these combinations, the arrangement of colored flowers and completely framed inflorescences seemed to be the most effective at increasing

attractiveness to pollinators; this was most notable in halictid bees in *H. macrophylla*, and in syrphid flies and bumblebees in *H. serrata*. This effectiveness is probably matched by the floral arrangement of *H. paniculata*, as the decorative flowers may attract various pollinators that glance at the inflorescence from various directions. Decorative flowers of species with partially and loosely framed inflorescences did not increase the attractiveness of their inflorescences, as was the case for completely framed species, regardless of flower coloration. Nonetheless, while few, the decorative flowers of *H. luteovenosa* and *H. scandens* (both partially framed species) served as landing sites more often than in other species (Fig. 3-7), likely due to their high relative area in the inflorescences, and greatly increase the inflorescence area (Table 3-1, Figs. 3-2f, 3-2g). Thus, they may increase attractiveness sufficiently to benefit the few fertile flowers found in the inflorescence.

Four of the nine studied *Hydrangea* species (*H. hirta*, *H. involucrata*, *H. macrophylla* and *H. serrata*) displayed blue- or purple-colored flowers (Fig. 3-2). These four species were visited predominantly by various bees and syrphid flies, and very rarely by beetles (Table 3-4, Fig. 3-4b). My results suggest that bees and syrphid flies are attracted by both blue/purple and white flowers, whereas beetles are attracted only by white flowers. Although the mechanism is still unknown, this differential attraction to flowers with different floral colors may result in reproductive isolation between species.

Among the studied *Hydrangea* species, only *H. hirta* was a non-FR species, and I did not detect increased insect-attracting capability when I attached decorative flowers (Fig. 3-6). The purple coloration of *H. hirta* flowers may be an adaptation designed to attract bees (Kevan & Baker 1983; Willmer 2011), and its high flowering synchrony may also increase the attractiveness of their inflorescences, as pointed out for n-FR *Viburnum* species in Chapter 2.

Phylogenetic analyses of *Hydrangea* showed that the loss of decorative flowers occurred independently four times (phylogenetically and geologically) in *Hydrangea* (i.e., *H. hirta*, *H. lingii* G. Hoo, *H. serratifolia* (Hook. and Arn.) F. Phil., and *H. steyermarkii* Standl) (Hufford 1997; Jacobs 2010). The loss of decorative flowers in *H. hirta* is peculiar, because most studied *Hydrangea* species that were visited by halictid bees had decorative flowers. At some point the purple-colored fertile flowers and/or a strong floral odor were adopted by *H. hirta*. Both visual and olfactory cues may suffice to attract pollinators and limit the use and evolution of decorative flowers.

Insect-attracting functions were not detected for the decorative flowers of *H. sikokiana* (Fig. 3-6). The fact that the visitor assembly of *H. sikokiana* was highly and similarly influenced by halictid bees and syrphid flies (Figs. 3-4b, 3-5a), two of the main visitor groups of the studied species, suggests that a selection pressure limiting the evolution of decorative flowers was not related to either pollinator group. Nevertheless, the large area of the cluster of fertile flowers of *H. sikokiana* might constitute a sufficiently attractive visual cue to limit the evolution of decorative flowers.

In the case of *H. involucrata*, the visitor-attracting function of the decorative flowers was not only limited by their lateral positioning, but also by the inflorescences not projecting from the bushes and being surrounded by leaves (Fig. 3-2a); this is in contrast to other colored FR species. The latter characteristics might have limited the use of decorative flowers and the insect-attracting capacity, which was compensated for by colored, fertile flowers.

Cerambycid beetles were dominant flower visitors in *H. paniculata* and *H. petiolaris*, and tended to more frequently visit framed inflorescences (Table 3-4). These *Hydrangea* species have been known to have sweet odors, and to be frequently visited by

cerambycid beetles (Kuboki 1980; Inoue et al. 1990; Kato et al. 1990, 1993). Because anthophilous cerambycid beetles are attracted by floral volatiles (Ikeda et al. 1993; Nakashima et al. 1994), they respond to both the visual and olfactory cues of *Hydrangea* flowers. Because pollen grains are attached to various body parts (Fig. 3-3e, supplementary table 3-2), these beetles are also important potential pollinators of Japanese *Hydrangea* spp. Although cerambycid beetles were the main visitors of *H. paniculata*, they were not as common in sympatric *H. involucrata*, which blooms during the same period (Tables 3-1, 3-2). The contrasting insect-visitor assemblages between these *Hydrangea* species suggest that their pollination syndromes are different. The most conspicuous differences in floral characteristics between the two species are the tepal color (white in the former, purple in the latter), direction of the decorative flowers (upward in the former, lateral in the latter), and their inflorescence type (compound-panicle in the former, compound-corymb in the latter). The postures of the decorative flowers correspond to that the tendency of cerambycid beetles to approach inflorescences from above, while bumblebees approach inflorescences with upward or horizontal movements (Kevan 1990).

My results suggest that decorative flowers generally increase the insect-attracting capabilities of inflorescences, and that the color, shape, and arrangement of decorative flowers vary among *Hydrangea* species, each of which is associated with different flower-visitor groups. Further observations of insect visits to artificially mounted decorative flowers, and phylogenetic comparisons of decorative flower morphology among diverse *Hydrangea* species, will reveal the evolution of each unique inflorescence.

Chapter 4: Pollination system of *Corylopsis gotoana* (Hamamelidaceae) and its stonefly (Plecoptera) co-pollinator

Introduction

Early angiosperm diversification is strongly attributed to reciprocal coevolution between entomophilous plants and anthophilous insects (Hu et al. 2008). The principal partners of entomophilous angiosperms consist of four orders of Neoptera: Coleoptera, Diptera, Hymenoptera and Lepidoptera (Crepet 1979). Pollination by other neopteran orders is less common, but thysanopterans (Moog et al. 2002), orthopterans (Micheneau et al. 2010) and hemipterans (Ishida et al. 2009) are documented facultative pollinators of some plants.

Plecoptera, a small neopteran order, is most well-known for its aquatic nymphs, which are a major component of the aquatic insect fauna in many stream ecosystems (Hynes 1976, Baumann 1979, DeWalt R. E. et al. 2015). The nymphs generally feed on submerged leaves, algae, and aquatic animals (Shapas and Hilsenhoff 1975, Sivec and Yule 2004), whereas adult feeding is mostly limited to species whose nymphs feed on detritus (DeWalt R. E. et al. 2015). Adults of some species have been reported to feed on specific vegetative and fungal matter (Newcomer 1918, Zwick 1990, Sweeney 1993). Although the behavior of adult stoneflies in the field is poorly understood, male and female adults of some arctoperlarian species display a remarkably advanced drumming behavior (Stewart and Zeigler 1984, Stewart 2001), during which the stoneflies beat or rub their abdomens upon certain materials to create vibrations to attract coupling mates (DeWalt R. E. et al. 2015). Associations

between adult stoneflies and plants appear to be rare. Previous studies have reported facultative flower visits of adult stoneflies (Willis and Burkill 1908, Porsch 1957, Thien et al. 1983, Inoue et al. 1990, Kato et al. 1990), yet their role in pollination remains unclear. In addition, two species of Taeniopterygidae have been documented as herbivorous pests of a *Prunus* sp. cultivar (Newcomer 1918).

Hamamelidaceae (Saxifragales) is a woody angiosperm family distributed in tropical and temperate regions of North and Central America, Asia, and some areas of Africa and Australia (Zhang and Lu 1995). This family displays various pollination forms, corresponding to a wide range of floral morphologies (Endress 1977), such as entomophily, ornithophily and anemophily; however, the pollination systems of most genera have not yet been studied.

The genus *Corylopsis* (Hamamelidaceae) occurs only in Asia, ranging from the Himalayas to Japan (Morley and Chao 1977, Zhiyun Z. and Endress 2003). In Japan, four species have been recorded in the regions of Kyushu, Shikoku and Honshu (Yamanaka 1986, Yamanaka et al. 2008) (Fig. 4-1). Based on floral morphology, Endress (1977, 1993) predicted that *Corylopsis* would be pollinated by generalist insects, such as dipterans and hymenopterans, and

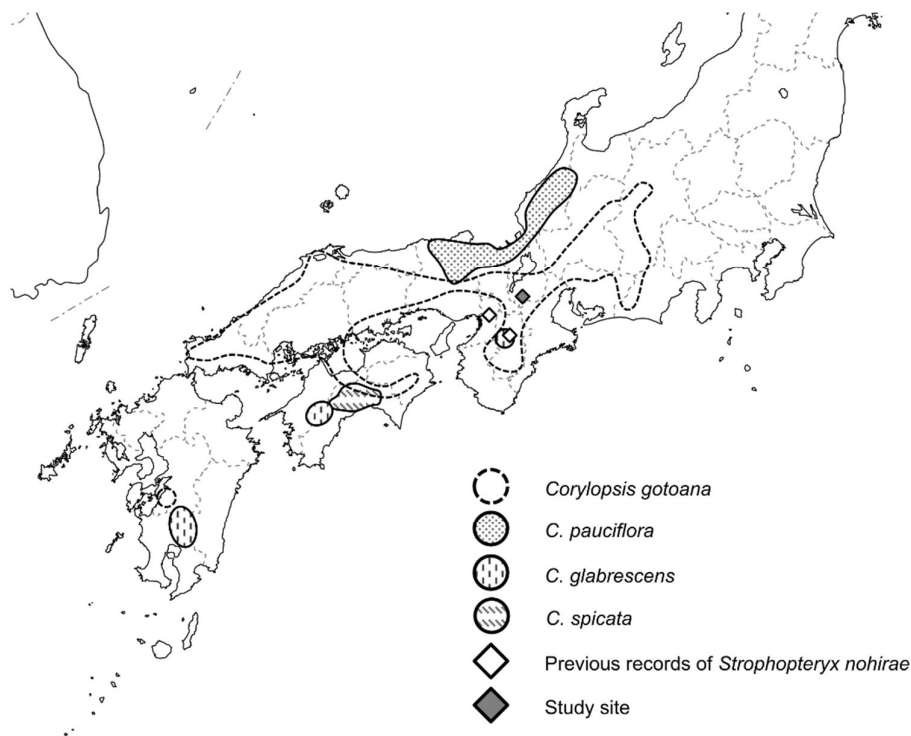


Fig. 4-1. Map of *Corylopsis* spp. distribution and *S. nohirae* reports of in Japan. The *Corylopsis* spp. distribution is based on the works of Yamanaka (1986) and Yamanaka (2008), the *S. nohirae* reports are from Okamoto (1922).

particularly by insects with long proboscises. Although *Apis mellifera* Linnaeus, 1758 (Apidae), *Melanostoma scalare* (Fabricius, 1794) (Syrphidae) and *Calliphora erythrocephala* Robineau-desvoidy, 1830 (Calliphoridae) have been reported as flower visitors of cultivars of *Corylopsis* spp. in Ireland (Morley and Chao 1977), little information exists regarding the pollination system of this genus in its native range.

In the present study, I report on the pollination system of *Corylopsis gotoana* Makino, based on field observations of flower-visiting insects in a temperate forest in Japan. My observations revealed that *C. gotoana* flowers were visited frequently by a stonefly species, *Strophopteryx nohirae* (Okamoto, 1922) (Taeniopterygidae), together with bees and flies. Hence, I further investigated the unique flower-visiting behavior of this stonefly and assessed its contribution to the pollination of *C. gotoana*.

Material and methods

Study species

Corylopsis gotoana is distributed in rocky terrain in the western part of Japan, primarily in western Honshu and Shikoku (Yamanaka 1986, Yamanaka et al. 2008) (Fig. 4-1). *Corylopsis gotoana* is a deciduous tree that grows to approximately 4.5 m tall, but smaller trees are common. Inflorescences are 3–6-cm-long racemes composed of 5–10 bisexual flowers. Each flower is composed of five petals, five sepals, five stamens and two pistils. The petals are yellow and ovate. The stamens and pistils are longer than the petals, and both are exerted from the corolla when matured. Most *Corylopsis* species show partial dichogamy: the stamens mature later than the pistils, which is probably a result of an autogamy-preventing mechanism (Morley and Chao 1977). In Japan, the flowers of *C. gotoana* bloom

between March and April. The study site was a deciduous broad-leaved secondary forest along the Tashiro River in Shiga Prefecture, Japan (34°55'5.70"N, 136°0'35.93"E). During the study period, eight woody species were in bloom at the study site: *Magnolia kobus* DC. (Magnoliaceae), *Camellia japonica* L. (Theaceae), *Eurya japonica* Thunb. (Pentaphylacaceae), *Stachyurus praecox* Siebold & Zucc. (Stachyuraceae), *Pachysandra terminalis* Siebold & Zucc. (Buxaceae), *Lindera umbellata* Thunb. (Lauraceae), *Cerasus jamasakura* (Siebold ex Koidz.) (Rosaceae) and *Salix integra* Thunb. (Salicaceae). Furthermore, various herbaceous plants, *Chrysosplenium grayanum* Maxim. (Saxifragaceae), *Cardamine scutata* Thunb. (Brassicaceae) and *Carex* spp. (Cyperaceae), were blooming in the riparian vegetation.

Observations of flower visitors

The flower visitors of *C. gotoana* were observed from March to April in 2014 and 2015, for 30 inflorescences in total. Daytime observations (08.00 – 18.00 hours) were conducted from 31 March to 3 April in 2014, and from 30 to 31 March and 2 to 3 April in 2015, on clear days only. Night-time observations (18.00 - 08.00 hours) were only conducted once, from the night of 3 April to the dawn of 4 April in 2014. Some flower visitors were captured for identification, and their pollen attachments were examined when possible. Insect flower visitors were identified to the species level but occasionally to order or family.

Assessment of floral rewards

The volume of floral nectar was measured using 0.5 µl glass micropipettes.

The sugar concentration of the nectar was determined using a refractometer (Kikuchi 0–50% Brix and Kikuchi 45–80% Brix).

Flower visitation and contribution to pollination of adult *S. nohirae*

To assess whether *S. nohirae* can pollinate *C. gotoana* when visiting its flowers, the quantity of pollen grains attached to flower-visiting individuals was counted and an introduction experiment was conducted in the field. Flower-visiting stoneflies were captured, and the attached pollen grains were counted for eight discrete body parts: antennae, head, mouthparts, thorax, abdomen, front legs, middle legs and hind legs. The stonefly introduction experiments were conducted on 27 March 2016 using 12 inflorescences with a total of 86 flowers. The flowers within an inflorescence were emasculated using fine tweezers, and each inflorescence was bagged with a fine-mesh nylon net to avoid pollination. For *Corylopsis* flowers whose pistils were matured, one female stonefly was introduced into each bagged inflorescence. The female individuals used for the experiments were those that had visited un-bagged flowers of *Corylopsis* and were thus dusted with a considerable amount of pollen. Twenty-four hours after the stonefly introduction, inflorescences were collected and fixed in 70% formalin-acetic acid-ethanol (FAA). Later, I stained the pollen tubes and counted pollen grains on the stigma and pollen tubes in the styles under UV irradiation, following the methods of Mori et al. (2006). In addition, the feces of *S. nohirae* were collected and examined for traces of pollen grains.

Results

Flowering and nectar secretion of *C. gotoana*

At the study site, the flowering period of *C. gotoana* lasted less than 2 weeks (between the end of March to the beginning of April). A single flower bloomed for approximately 4–5 days. *Corylopsis gotoana* showed partial dichogamy, as the pistils protruded and matured before the anthers. Nectar was secreted at the base of the petals. Of the 74 flowers observed from eight inflorescences, only 41 produced detectable

levels of nectar, with a mean volume of 0.22 mm³ (standard deviation [SD] = 0.18) and mean sugar concentration of 22.6% (SD = 8.7).

Flower visitors of *C. gotoana*

In total, 28 insect species of flower visitors from 14 families in four orders (Diptera, Hymenoptera, Lepidoptera and Plecoptera) were observed (Table 4-1). *Bombylius major* Linnaeus, 1758 (Bombyliidae), *Apis cerana* Fabricius, 1793 (Apidae) and queens of *Bombus ardens* Smith, 1879 (Apidae) were the main visitors of *C. gotoana* (Table 4-1). Thirteen



Fig. 4-2. Various flower visitors of *Corylopsis gotoana* obtaining nectar or pollen rewards. (a) *Bombylius major* feeding on nectar, (b) *Melanotoma* sp. feeding on nectar, (c) *Empididae* sp. feeding on nectar, (d) *Ceratina japonica* feeding on nectar, (e) *Bombus ardens* feeding on nectar, (f) *Andrena* (*Stenomelissa*) sp. feeding on nectar, (g) *Libythea celtis* feeding on nectar, (h) *Strophopteryx femoralis* feeding on pollen, (i) *Oraesia excavata* resting in an inflorescence. The primary rewards obtained by the flower visitors were pollen and nectar. *Strophopteryx femoralis* was usually observed feeding on pollen.

Table 4-1. List of insect visitors to *Corylopsis gotoana* flowers with their harvested floral reward, pollen attachment, and visit frequency.

Order	Superfamily/ Infraorder	Family	Species	Harvested		Visit frequency (visits/day to 30 inflorescences)	
				floral reward	Pollen attachment	2014	2015
Plecoptera	Euhognatha	Taeniopterygidae	<i>Strophopteryx nohirae</i>	p	+	5.5 ± 1.3	3.5 ± 1.7
Diptera	Culicomorpha	Chironomidae	Chironomidae sp.	n	+	0.3 ± 0.5	0 ± 0
		Bibionomorpha	Mycetophilidae	n, p	+	0.3 ± 0.5	0.5 ± 0.6
		Sciaridae	Sciaridae sp.	n, p	+	0.3 ± 0.5	0 ± 0
		Empidoidea	Empididae	n	+	0.8 ± 1.5	1 ± 0.8
	Tabanomorpha	Bombyliidae	<i>Bombylius major</i>	n	++	14.3 ± 4.9	10.8 ± 2.6
	Syrphoidea	Syrphidae	<i>Eristalis cerealis</i>	n, p	++	0.8 ± 1	1.5 ± 1.3
			<i>Eristalomyia tenax</i>	n, p	++	1.3 ± 1.5	1 ± 0.8
			<i>Episyrphus balteatus</i>	n, p	++	0.5 ± 0.6	0.5 ± 0.6
			<i>Melanostoma</i> sp.	n, p	++	0.8 ± 1	1.5 ± 0.6
			<i>Sphegina</i> sp. †	n, p	+	—	—
			<i>Cheilosia</i> sp. †	n, p	+	—	—
		Muscoidea	Anthomyiidae	n, p	++	0.5 ± 0.6	0.3 ± 0.5
			Tachinidae	n	+	0.8 ± 1	1 ± 0.8
	Hymenoptera	Apoidea	Andrenidae	n, p	+++	1 ± 0	0.5 ± 0.6
			<i>Andrena (Cnemidandrena)</i> sp.	n, p	+++	1.5 ± 0.6	0.5 ± 0.6
			<i>Andrena (Stenomelissa)</i> sp.	n, p	+++	0.3 ± 0.5	0.3 ± 0.5
			<i>Andrena (Micrandrena)</i> sp. 1	n, p	+++	0.5 ± 0.6	0.3 ± 0.5
			<i>Andrena (Micrandrena)</i> sp. 2	n, p	+++	0.5 ± 0.6	0.3 ± 0.5
		Halictidae	<i>Lasioglossum (Lasioglossum)</i> sp.	n, p	+++	1.8 ± 0.5	2 ± 1.4
			<i>Lasioglossum (Evyllaesus)</i> sp. 1	n, p	+++	0.5 ± 0.6	0.5 ± 1
			<i>Lasioglossum (Evyllaesus)</i> sp. 2	n, p	+++	1 ± 1.4	2 ± 0.8
		Apidae	<i>Apis cerana</i> [worker]	n, p	+++	9.3 ± 4.6	4.3 ± 1.7
			<i>Bombus ardens</i> [queen, rarely worker]	n, p	+++	2.8 ± 1	3.8 ± 0.5
			<i>Ceratina japonica</i>	n, p	+++	1.5 ± 1.3	2 ± 1.8
			<i>Xylocopa appendiculata</i> †	n, p	+++	—	—
			<i>Nomada</i> sp.	n	+	0 ± 0	0.3 ± 0.5
Lepidoptera	Papilionoidea	Nymphalidae	<i>Libythea lepita</i>	n	+	1.3 ± 0.5	2 ± 0.8
	Noctuoidea	Noctuidae	<i>Oraesia excavate</i> ‡	n	+	—	—

+, scarce; ++, moderate; +++ abundant; n, nectar; p, pollen. † Species observed outside the observation site. ‡ Species observed only during the night.

species of Diptera were observed; the most common fly visitors were one bombyliid fly species and six syrphid fly species. *Bombylius major*, the most common flower visitor of *C. gotoana*, harvested nectar secreted at the base of the petals using its long proboscis (Fig. 4-2a), and the hair on its head was covered by many pollen grains. Syrphid flies were less frequent visitors, but they did collect nectar and pollen (Fig. 4-2b) and pollen was attached to their bodies. Several of the rare fly species of flower visitors (three muscomorphans and two bibionomorphans, Fig. 4-2c; and one chironomid) also foraged for nectar. Twelve species of Hymenoptera were observed: 10 solitary bee species belonging to Andrenidae (four species), Halictidae (3 spp.) and Apidae (3 spp.) (Figs. 4-2d, 4-2f), and two eusocial Apidae species, *Apis cerana* and

Bombus ardens; these latter two hymenopterans foraged for nectar and pollen (Fig. 4-2e). Moreover, one species each of Lepidoptera and Plecoptera were observed during the daytime. A nymphalid butterfly, *Libythea lepita* Moore, 1857, consumed floral nectar with its proboscis, on which most of the pollen was attached. One taeniopterygid stonefly species, *S. nohirae*, foraged for pollen and mated on the plants but was not observed feeding on nectar.

Although most of the insects visited flowers during the daytime (Fig. 4-3), two flower visitors were observed during the night: a noctuid moth, *Oraesia excavate* (Butler, 1878), (Noctuidae) (Fig. 4-2i) and a fungus gnat (Mycetophilidae).

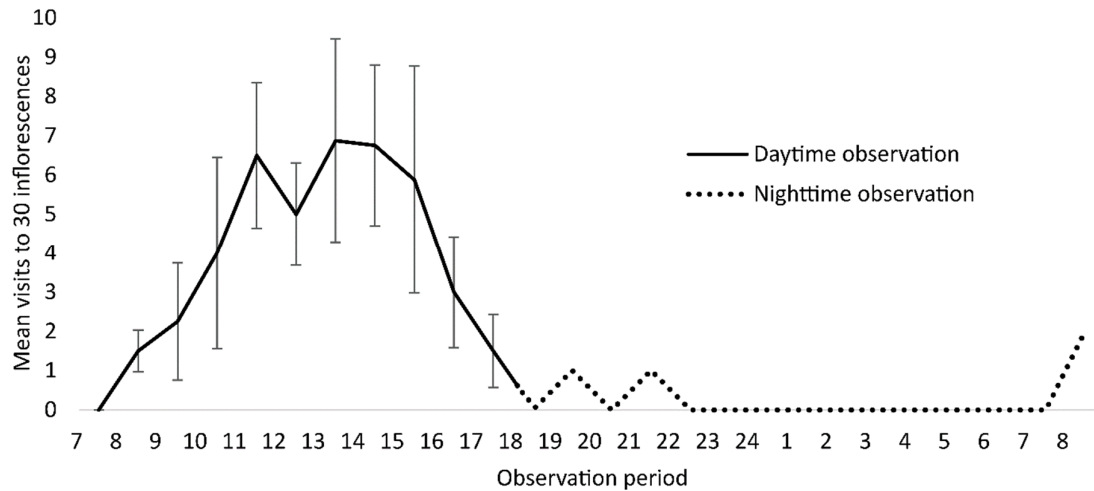


Fig. 4-3. Mean number of flower visits ($\pm$ standard deviation [SD]) to 30 inflorescences of *Corylopsis gotoana* during daytime and nighttime observations. Daytime observations (N = 8) in 2014–2015, and nighttime observations (N = 1) in 2014. Most visits were observed during the day, especially near midday.

Behavior of adult *S. nohirae*

Based on my observations, *S. nohirae* exclusively visited *C. gotoana* flowers, despite the close proximity of other woody or herbaceous plants in bloom. Moreover, examinations of the morphology of the pollen attached to the flower-visiting stoneflies indicated that it was solely composed of the pollen of *C. gotoana*. Female visits were more frequent than male visits, and behaviors clearly differed between the sexes: female stoneflies frequently ingested pollen

from mature and immature anthers (Figs. 4-2h, 4-4b) and rested on the inflorescences and branches (Fig. 4-4a), whereas male stoneflies seldom ingested pollen and actively walked on the branches. Mating was observed on the inflorescences and branches of flowering *Corylopsis* trees (Figs. 4-4c, 4-4d), but the drumming behavior was not observed. Pollen feeding by male and female flies was confirmed by fecal analysis.

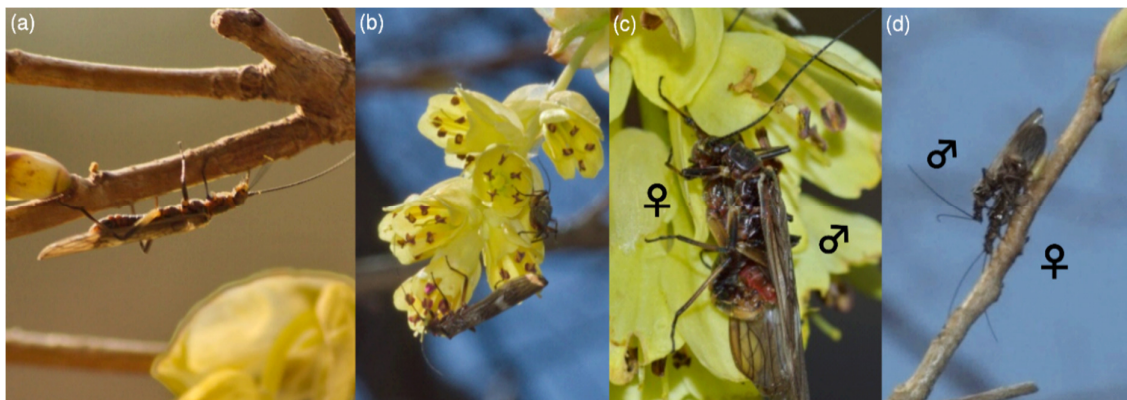


Fig. 4-4. *Strophopteryx femoralis* behavior in *Corylopsis gotoana*. (a) Female stonefly resting on a branch, (b) two female stoneflies foraging for pollen, (c) pair mating in an inflorescence, (d) pair mating on a branch. ♂, male stonefly; ♀, female stonefly. *Strophopteryx femoralis* were usually observed in *Corylopsis gotoana* trees. The female stoneflies were primarily observed resting or foraging for pollen. Stonefly mating was also observed.

***Strophopteryx nohirae* contribution to pollination**

Female stonefly samples harbored more attached pollen compared with male samples, and values were significantly higher for the antennae, head, mouthparts and thorax (Table 4-2). For both female and male samples, the thorax had the highest portion of attached pollen, whereas the antennae had the lowest (Table 4-2). In the field experiment, every inflorescence (N = 12) had at least one flower with either pollen grains or visible pollen tubes. Pollen grains were attached to the stigma in 89.1% of flowers, and pollen tubes were found in the pistils in 65.8% of flowers (N = 86) (Fig. 4-5).

Discussion

The flowers of *C. gotoana* were predominantly visited by generalist insects, especially dipterans and hymenopterans. These findings are consistent with the predictions of Endress (1977, 1993), as well as previous reports in Ireland for *C. gotoana* cultivars (Morley and Chao 1977). *Bombylius major*, *Apis cerana* and queens of *Bombus ardens* were frequent visitors with considerably high pollen attachment; thus, these species are the most probable main pollinators of *C. gotoana*, although their pollination efficiency remains to be evaluated. Similar to the observations of Morley and Chao (1977), dipteran and hymenopteran flower visitors, including the main visitors, primarily foraged for nectar. Nonetheless, some bees (e.g. *Apis cerana* and *Bombus ardens*) collected pollen as well.

My previous observations have indicated that *Bombylius major* is among the

most frequent and predominant visitors of *C. pauciflora* Siebold & Zucc., a congener native to Japan (A. A. Wong Sato, pers. obs., 2015). Moreover, the emergence of the bombyliid fly appears to be synchronous with the blooming of *Corylopsis* spp. in Japan. Taken together, a bombyliid pollination syndrome may be favored by *Corylopsis*, which flowers in early spring (March–April) when most insect pollinators are less active. However, the characteristics of the flowers (e.g. yellow color and morphology) of *Corylopsis* are not generally considered to be attractive to *Bombylius* spp. (Kastinger and Weber 2001).

In the present study, *S. nohirae* exhibited behaviors that have not been reported previously for Plecoptera; this stonefly consistently visited *C. gotoana* flowers, actively fed on its pollen and served as a co-pollinator. Although some stonefly species have been reported to occasionally visit flowers (Willis and Burkill 1908, Porsch 1957, Thien et al. 1983, Inoue et al. 1990, Kato et al. 1990), it remains unclear whether they function as pollinators. Thus, my observed interactions between *S. nohirae* and *C. gotoana* represent the first case of plecopteran pollination, as well as a rare case of plecopteran flower visitation for pollen foraging.

Table 4-2. Pollen grains attachment to different body parts of *Strophopteryx nohirae* males and females collected from *Corylopsis* flowers.

Body part	Pollen grains attachment (mean ± SD)	
	Females (n = 18)	Males (n = 4)
Antennae †	21 ± 21 **	4 ± 4
Head ‡	90 ± 56 **	31 ± 25
Mouthparts	92 ± 25 **	27 ± 16
Thorax	218 ± 166 *	103 ± 49
Abdomen	73 ± 27	68 ± 20
Fore legs ‡	88 ± 74	69 ± 32
Mid legs ‡	47 ± 42	61 ± 48
Hind legs ‡	100 ± 58	126 ± 93

SD, standard deviation; * P < 0.05, ** P < 0.01, One-tailed Welch corrected unpaired t-test. † Pollen grains of antennae, front legs, middle legs and hind legs were counted for pairs. ‡ Pollen grains of head do not include pollen of mouthparts and antennae.

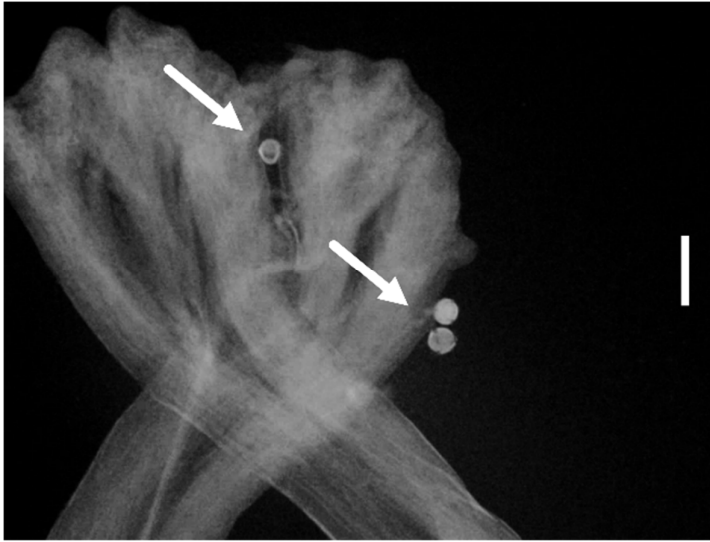


Fig. 4-5. Pistil of a flower used in the stonefly introduction experiment. White arrows indicate pollen grains in the stigma. Scale is 100 μm .

The lower visitation rate and lower volume of pollen attachment of *S. nohirae* suggest that this stonefly is a less valuable pollinator of *C. gotoana* compared with bombyliid flies and bees. Indeed, the field experiment demonstrated that the stonefly had a low pollination efficiency, probably because stoneflies are not well suited, morphologically or behaviorally, for pollination. Stoneflies lack structures for pollen attachment, and their ability to search for flowers is likely to be less developed than that of bombyliid flies and bees. Nevertheless, this stonefly may play an important role in the pollination of *C. gotoana* when the emergence of other pollinators is delayed or compromised.

My behavioral observations, and the presence of pollen grains on the mouthparts and in the feces of *S. nohirae*, suggest that adult feeding may be important to this stonefly, similar to other Nemouroidea species (Hynes 1942, Zwick 1990, Sweeney 1993, Tierno de Figueroa and Sánchez-Ortega 2000). Adult feeding may be especially important in female flies for reproductive purposes. For example, adult feeding is essential for ovary development in some other

species of Taeniopterygidae (Sweeney 1993). Notably, *S. nohirae* only visited *Corylopsis* flowers but did not visit concurrently blooming flowers of other woody and herbaceous plant species. Future research should compare the nutritive value of *Corylopsis* pollen with that of concurrently blooming plants. To the best of my knowledge, this report provides the first direct observation of pollen feeding in Plecoptera, although this has previously been inferred from gut content analyses (Zwick 1990, Tierno de Figueroa and Sánchez-Ortega 1999, Smith and Collier 2000, López-Rodríguez M. J. and I. 2007, Rúa and Tierno de Figueroa 2013).

In the present study, *Corylopsis* trees not only served as feeding sites, but also as mating sites for *S. nohirae*; thus, these trees are likely to be the preferred, or even the unique, breeding sites of this stonefly. The distinct behavioral differences between male and female *S. nohirae* in *C. gotoana* trees suggest that this stonefly engages in resource-based behaviors, during which the male partner intercepts the female partner at the foraging location to

copulate; similar behaviors have been reported in other Nemouroidea species (Zwick 1990). Although some species of Taeniopterygidae use drumming to attract mates (Stewart and Zeigler 1984, Stewart 2001), I did not observe this behavior in *S. nohirae*. Further observations of the behavior of *S. nohirae* will help to reveal how male and female stoneflies communicate and mate, as well as how mating behaviors affect the pollination process.

To conclude, my results showed that *S. nohirae* visited *C. gotoana*, fed on its pollen, and contributed to its pollination as co-pollinators. It is possible that in *S. nohirae* feeding on pollen is necessary for egg development, which may explain why the female stonefly fed on pollen more frequently than the male. For *Corylopsis* flowering in early spring, long winters may cause grave lagging of emergence of pollinators. Thus, Plecoptera pollination, although not very effective, may be an option to secure pollination in colder years, because the climatic regime is often different between terrestrial and aquatic habitats.

Chapter 5: Breeding system and bumblebee drone pollination of an explosively pollen releasing plant, *Meliosma tenuis* (Sabiaceae)

Introduction

Explosive pollination is an unusual mechanism in which pollen is violently released by movement of floral organs, mainly anthers and stamens. The explosive pollen release is usually triggered by slight tactile stimuli of flower visitors, and is considered to contribute to pollination attaching pollen to bodies of the visitors (Feehan 1985, Solomon Raju 1990, Solomon Raju and Subba Reddi 1995, Agostini et al. 2006). This mechanism, while rare, has been reported to assist cross pollination in 16 flowering plant families belonging to monocots and eudicots (Table 5-1, Elliot 1891, Vogel 1958, Meeuse 1961, Van Beusekom 1971, Harley 1971, Plitmann et al. 1973, Proctor and Yeo 1973, Brantjes and Vos 1981, Feehan 1985, Mosquin 1985, Solomon Raju 1990, Yeo 1993, Solomon Raju and Subba Reddi 1995, Atluri et al. 2004, Agostini et al. 2006, Pérez et al. 2006, Solomon Raju and Purnachandra Rao 2006, Alemán et al. 2014, Zúñiga 2015, Solomon Raju and Prasada Rao 2016, Montoya-Pfeiffer et al. 2016). Most of these explosively pollen releasing plants have tubular/papilionaceous zygomorphic flowers, and their main pollinators are birds and bees, which are dusted by pollen after visiting the flowers (Feehan 1985, Solomon Raju 1990, Solomon Raju and Subba Reddi 1995, Agostini et al. 2006) and in some cases, only insects with specific characteristics can trigger the mechanism (Meeuse 1961, Proctor and Yeo 1973, Solomon Raju and Subba Reddi 1995). Whereas plant species in Urticaceae, Cornaceae and Ericaceae have actinomorphic dish-shaped flowers, which are visited by bees and other insects, except in Urticaceae plants, where pollen is explosively released and dispersed by wind (Proctor and Yeo 1973,

Solomon Raju and Subba Reddi 1995, Montoya-Pfeiffer et al. 2016, Poinar Jr et al. 2016).

Among explosively pollen releasing plants, *Meliosma* in Sabiaceae (Proteales) is unique, because its flowers are minute dish-shaped and superficially actinomorphic, but internally zygomorphic. *Meliosma* is a paraphyletic genus (Zúñiga 2015), with about 50 species distributed in Southeast Asia, Central and South America (Guo and Brach 2007, Kubitzki 2007, De Craene and Wanntorp 2008). Even though the floral morphology of a few *Meliosma* species has been studied thoroughly, little is known about the pollination system of this genus, while flower visitors have been briefly reported in Japanese *Meliosma* species. (Inoue et al. 1990, Kato 2000).

To address the question why *Meliosma* plants have such peculiar floral morphology and behavior, I focused on a Japanese treelet species, *Meliosma tenuis*, which have explosively pollen-releasing flowers. In a temperate deciduous forest in central Japan, I made extensive observation of (1) flowering behavior, (2) insect flower visitors, (3) pollen attachment on bodies of the flower-visitors, and performed several controlled pollination treatments to determine the breeding system of the plant.

Materials and methods

Studied plant species and study site

Meliosma tenuis Maxim. is a small deciduous treelet of 2–3 m in height, growing in temperate deciduous forests. Inflorescences are terminal, peduncular panicles. Flowers are pentamerous, small (about 5 mm when opened), hermaphroditic. Five heteromorphic petals are

Table 5-1. Plant families and genera, in which explosive pollen release has been reported to assist cross pollination, with their floral symmetries and pollinators.

Clade	Subclade	Order	Family	Genus	flower shape	symmetry	pollinator	References
Monocot	Lilioid	Asparagales	Orchidaceae	<i>Listera</i> , <i>Neottia</i>	dish	zygomorphic	insect	Proctor and Yeo 1972; Raju 1995
			Musaceae	<i>Ravenala</i>	tubular	zygomorphic	bird	Proctor and Yeo 1972
Eudicot	basal eudicot	Ranunculales	Strelitziaceae	<i>Strelitzia</i>	tubular	zygomorphic	bird	Raju 1995
			Papaveraceae	<i>Corydalis</i> , <i>Fumaria</i>	tubular	zygomorphic	bee	Proctor and Yeo 1972; Raju 1995
			Sabiaceae	<i>Meliosma</i>	dish	actino/zygo †	insect	Van Beusekom 1971; De Zúñiga 2015
			Rhizophoraceae	<i>Bruguiera</i> , <i>Ceriops</i>	tubular	zygomorphic	bird, lepidoptera	Raju 1995
	Rosid	Malpighiales	Fabaceae	<i>Canavalia</i> ; <i>Cytisus</i> ;	papilionaceous	zygomorphic	bee, bird, bat	Scott-Elliott 1891; Vogel 1958;
				<i>Desmodium</i> ; <i>Harpalyce</i> ;				Meeuse 1961; Proctor and Yeo 1972;
				<i>Medicago</i> ; <i>Mucuna</i> ;				Yeo 1993; Raju 1995; Agostini and
	Rosales	Urticaceae		<i>Pongamia</i> ; <i>Psoralea</i> ;				Sazima 2006; Raju and Rao 2006;
				<i>Rhynchosia</i> ; <i>Spartinum</i>				Aleman 2013; Raju and Rao 2016
				<i>Boehmeria</i> ; <i>Pilea</i> ; <i>Urtica</i>	dish	actinomorphic	wind	Taylor 1942 ‡; Proctor and Yeo 1972;
Asterid	Malvales	Dipterocarpaceae		<i>Shorea</i>	dish	zygomorphic	wind	Raju 1995; Montoya-Pfeiffer et al. 2016
								Atturi et al. 2003
								Plitman 1973
								Feehan 1985; Raju 1995
	Myrtales	Onagraceae		<i>Lopezia</i>	tubular	zygomorphic	bird	
	Santalales	Loranthaceae		<i>Engleriana</i> ; <i>Erianthemum</i> ;	tubular	zygomorphic	bird	
	Cornales	Cornaceae		<i>Tapinanthus</i> ; <i>Vanwykia</i>	dish	actinomorphic	bee	
								Mosquin 1985
Asterid	Ericales	Ericaceae		<i>Cornus</i>	dish	actinomorphic	insect	
								Marie-Victorin 1942 ‡
	Lamiales	Lamiaceae		<i>Kalmia</i>	tubular	zygomorphic	bird, bee	
								Harley 1971; Brantjes and Vos 1981;
								Raju 1990; Raju 1995
								Proctor and Yeo 1972
								Pérez et al. 2006
Asterid	Solanales	Acanthaceae		<i>Odontonema</i>	tubular	zygomorphic	bird	
Asterid	Solanales	Solanaceae		<i>Schizanthus</i>	papilionaceous	zygomorphic	bee	

† Superficially actinomorphic, but internally zygomorphic. ‡ As cited by Solomon Raju and Subba Reddi (1995).

composed of three outer-petals, and two smaller inner-petals. Two fertile stamens (hereafter referred simply as to ‘stamens’) have flattened filaments attached to the inner-petals. Each stamen has two globular pollen sacs which open inward out, and three pouch-like staminodes. Ovary is superior and bicarpellate. The nectary disk surrounds the base of the ovary, and has various appendages which secrete nectar. The pistil is enclosed by the staminodes, and does not protrude through them. Fruits are round drupes with one stone. Unmatured fruits are green, and turn black when mature. *M. tenuis* is distributed along the Japanese Archipelago, in the Shikoku, Honshu and Kyushu regions, and flowers between June and July. Although *M. tenuis* has been recorded in China and Korea, it is not distributed in China (Guo and Brach 2007).

The study was conducted in a temperate deciduous forest of Kibune, Kyoto Prefecture, Japan (35° 8' N 135° 46' E, 370–480 m) during a monsoon season from June to mid-July in 2013 and 2017. During the research period, the following woody plant species were flowering: *Deutzia crenata* Siebold & Zuccarini (Hydrangeaceae), *Hydrangea serrata* (Thunb.) Ser. (Hydrangeaceae), *Alangium platanifolium* (Siebold & Zucc.) (Cornaceae), *Actinidia*

arguta (Siebold & Zucc.) Planch. ex Miq. (Actinidiaceae), *Neoshirakia japonica* (Siebold & Zucc.) Esser (Euphorbiaceae), among others and various flowering herbs.

Breeding system

To determinate the breeding system of *M. tenuis*, fruit-set was compared among the following six pollination treatments: automatic self-pollination (flowers intact and bagged) (Fig. 5-1a), natural pollination (control, flowers intact and unbagged) (Fig. 5-1b), restricted natural pollination (flowers intact, inflorescence covered with a metal net of 5*5 mm mesh size) (Fig. 5-1c), artificial self-pollination (flowers emasculated, hand-pollinated by its own pollen and bagged), artificial geitonogamous pollination (flowers emasculated, hand-pollinated by pollen from different flowers on the same plant and bagged), and artificial cross pollination (flowers emasculated, hand-pollinated by pollen from different plants and bagged). The three former treatments were applied in 2013, while the rest was applied in 2017.

The flowers of *M. tenuis* changed its sexual presentation during its flowering period. To monitor female function of flowers, fruit-set was compared between the following two

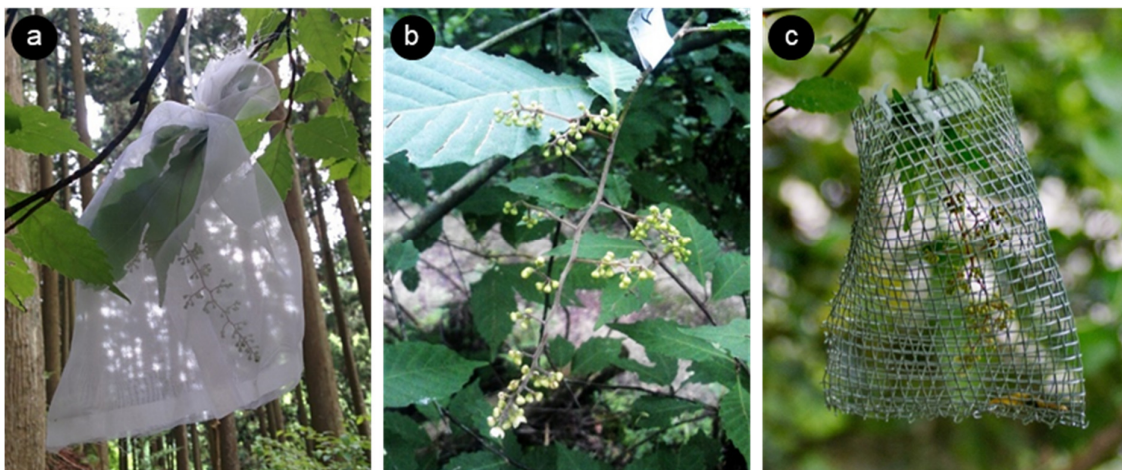


Fig. 5-1. Inflorescences of *Meliosma tenuis* used in pollination and breeding experiments. (a) Inflorescence used for the automatic self-pollination experiment bagged with nylon mesh; (b) Inflorescence used for the natural pollination experiment; (c) inflorescence used for the restricted natural-pollination experiment covered with a metal net of 5*5 mm mesh size. Inflorescences used for the hand pollination experiments were bagged as in the auto-self pollination experiment.



Fig. 5-2. Stages of *Meliosma tenuis* flower. (a) left: flower bud, right: beginning of flower opening; (b) male stage: flower with fully opened outer-petals, explosive mechanism triggered, and stamens revealed; (c) flower with fallen stamens and inner-petals; (d) female stage: flower with fallen staminodes and outer-petals, pistil revealed.

pollination sub-treatments applied to the hand-pollination treatments: pollination at male stage (pollen deposited in the apical portion of the staminodes (Fig. 5-2c)), and pollination at female stage (when the outer-petals fully opened, the outer-petals and staminodes were removed to reveal the pistils, and pollen directly deposited in the pistils (Fig. 5-2d)).

I applied a Duncan's multiple range test (MRT) to compare the fruit set among the multiple treatments, using the statistical software Infostat (2016 version, InfoStat Group), and one-tailed Mann–Whitney U-tests to detect difference of fruit set between pairs of treatments, using the statistical software R (v3.4.2., R Core Team).

Insect visits to flowers

I recorded all insect visits to 40 inflorescences of *M. tenuis*, during 8 – 17 h, on nine fine days in late June and early July in 2017 by direct observation and video recording. The data set of diurnal insect visits do not include visits by the visitors that crawled or walked to the inflorescences (e.g., lepidopteran larvae and ants).

To monitor secretion of nectar, I measured the volume of floral nectar of bagged flowers using 0.5 μ l glass micropipettes, and the sugar concentration of the nectar was determined using refractometers (Kikuchi, Tokyo, Japan).

Pollen attachment on bodies of flower visitors

I netted insect visitors from *Meliosma* inflorescences, others than the 40 inflorescences used for flower visits observations. Netted insect visitors were preserved as dried specimens. Pollen attachment of the specimens was checked by using an optic microscope and a scanning microscope in the following body parts: head, mouthparts, antennae, thorax, abdomen, hind legs, middle legs, and fore legs, and the general pollen attachment was categorized into 5 levels (none, scarce, little, considerable and abundant).

Trigger of explosive pollen release

To detect what stimuli on which floral parts trigger explosive pollen release, artificial stimulation experiments were conducted. Using a micro-pin, I picked at anthers, filaments, staminodes, inner petals and outer-petals of just opened flowers, and recorded whether explosive pollen release occurred.

Results

Flowering biology and explosive pollination mechanism

A single flower lasted for about four days. At opening, the three white outer-petals

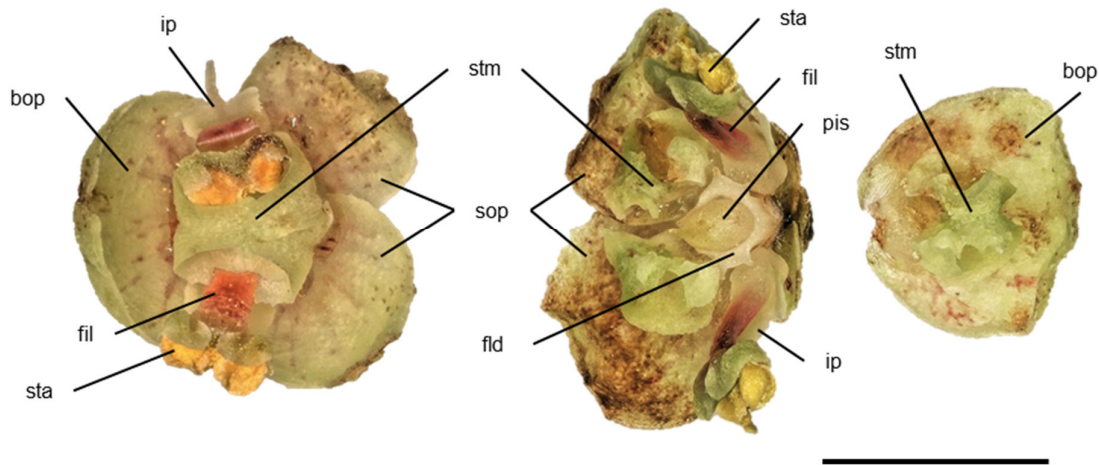


Fig. 5-3. Floral organs of *Meliosma tenuis*. Left: flower with one triggered stamen; right: flower with triggered stamens and one outer-petal separated. bop: big outer-petal; sop: small outer-petal; ip: inner-petal; stm: staminode; fil: filament; sta: stamen; fld: floral disk; pis: pistil. Scale is 5 mm.

slightly opened on the first day (Fig. 5-2a), and fully opened on the second day (Fig. 5-2b).

In a fully opened flower, the anthers of the two stamens are fixed downwards and inwards by a bend filament, and held by the three staminodes (Figs. 5-2a, 5-3). When the mechanism is triggered, the stress of the bending forces is released, and the filaments instantly return to their straight form, violently pulling the anthers outside and releasing pollen (Figs. 5-2b, 5-3). By the explosive release, pollen grains

were spewed out straightly up to about 4 cm distance from the flower, and the skewed pollen dust could be video-recorded. The explosive pollen release occurred altogether or separately for the two anthers in a flower.

The explosive pollen release was triggered by insect's touch to some specific parts in a flower. Artificial stimulating experiments revealed that the explosive pollen release was triggered almost consistently when the filaments and anthers were touched by micro-pins,

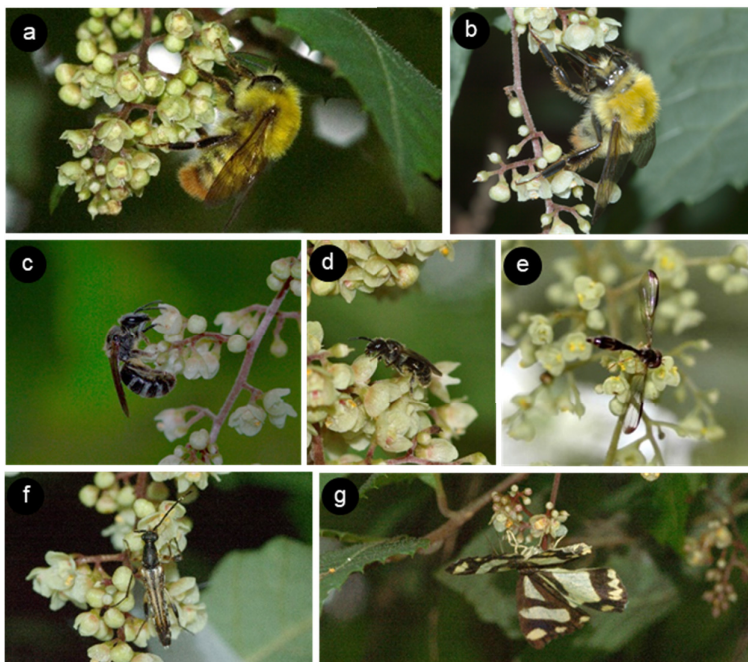


Fig. 5-4. Flower visitors of *Meliosma tenuis*. a, b: *Bombus ardens ardens* drone feeding on nectar; c: *Andrena (Andrena) aburana* (Andrenidae) feeding on nectar; d: *Lasioglossum (Evylaeus) allodalum* (Halictidae) feeding on nectar; e: *Baccha apicalis* (Sirphyidae) feeding on nectar; f: *Idiostrangalia contracta* (Cerambycidae) feeding on pollen; g: *Psychostrophia melanargia* (Epicopeiidae) feeding on nectar.

and occasionally when the staminodes were stimulated (Fig. 5-3).

When a flower accomplished explosive pollen release, the pistil was still enclosed under staminodes. About 12–24 hours after the explosive pollen release, stamens and inner-petals fell, and within about further 8 hours later, outer-petals and staminodes fell. The pistil emerged only after the staminodes fell. Therefore, the flowers are functionally protandrous: male stage before explosive pollen release, female stage after falling of staminodes, and a neutral stage (non-male and non-female) during the period after pollen release to the falling of the staminodes.

Inflorescences composed of 70–260 flowers develop basipetally (apical to basal), and similar among flower of a raceme (Fig. 5-1b). Flowering of an inflorescence lasted for about a week, and the flowering period varied among inflorescences with different number of flowers. Thus, a flowering inflorescence was composed of buds, male-stage flowers and female-stage flowers.

Nectar was produced by the nectar secreting glands of the floral disk (Fig. 5-3), and was found around the base of the outer-petals (Figs. 5-2b, 5-2c). The average nectar volume of bagged male-stage flower was 0.23 μ l ($\pm$

0.19 SD, standard deviation), and the average sugar concentration of the nectar was 33.1 % ($\pm$ 13.4 SD).

Breeding system

The fruit-set of *M. tenuis* varied among the six pollination treatments: automatic self-pollination, natural pollination, restricted natural-pollination, artificial self-pollination, artificial geitonogamous pollination, and artificial cross pollination (ANOVA, $F_{8, 58} = 16.71$, $P < 0.0001$). The fruit set was highest in the artificial cross pollination, followed by natural pollination (Table 5-2). The two treatments were classified in distinct groups with higher fruit sets from the other treatments by the MRT test ($df = 58$, $P < 0.05$) (Table 5-2).

Among treatments with intact flowers, fruit set of the natural pollination treatment was significantly greater than that of the restricted pollination treatment (U test, $Z = 2.47$, $P < 0.01$), suggesting that *M. tenuis* relies on large-sized (diameter > 5 mm) flower-visitors, i.e. bumblebees in this case.

In artificial cross pollination treatments, the flowers pollinated in female stage showed statistically higher fruit set than the flowers pollinated in male stage (U test, $Z = 3.18$, $P < 0.01$). The result supports the hypothesis that the

Table 5-2. Fruit sets of treated *Meliosma tenuis* flowers.

Treatments	N	Year	Mean fruit set (%) $\pm$ SD	Statistical group $\dagger$
artificial self-pollination (m)	6	2017	0.0 $\pm$ 0.0	A
artificial self-pollination (f)	6	2017	0.8 $\pm$ 1.3	A
artificial geitonogamous pollination (m)	7	2017	0.7 $\pm$ 1.3	A
artificial geitonogamous pollination (f)	6	2017	5.4 $\pm$ 3.1	A
artificial cross pollination (m)	7	2017	2.4 $\pm$ 2.5	A
artificial cross pollination (f)	8	2017	23.5 $\pm$ 8.7	C
natural pollination	9	2013	14.8 $\pm$ 10.1	B
restricted natural pollination	9	2013	3.4 $\pm$ 5.4	A
automatic self-pollination	9	2013	1.8 $\pm$ 2.8	A

SD, standard deviation; m, artificial pollination made on male-stage flower; f, artificial pollination made on female-stage flower. $\dagger$ Grouping by Duncan's multiple range test ($df = 58$, $P < 0.05$), common letters are not significantly different ($P > 0.05$).

flowers with pistils enclosed by the staminodes are functionally male-stages (Fig. 5-2b).

Among artificial pollination treatments of flowers in female stage, the fruit set of cross pollination treatment was significantly higher than that of self-pollination treatment (U test, $Z = 3.03$, $P < 0.01$) and geitonogamous pollination treatment (U test, $U = 3.03$, $P < 0.01$), suggesting that *M. tenuis* relies on allogamy, rather than autogamy and geitonogamy.

Insect visits to flowers

The flowers were diurnally visited by diverse insects. In total, 18 species belonging to 11 insect families in five orders, Diptera, Hemiptera, Hymenoptera and Lepidoptera, were observed to visit *M. tenuis* flowers during the observation period (Table 5-3). Nine hymenopteran species were observed, including three species of bumblebees, five species of solitary bees, and one species of parasitic wasp. *Bombus*

Table 5-3. Flower visitors of *Meliosma tenuis* and their visitation rates.

Insect flower-visitors	Average visitation rate (visits/40 inflorescences/10 h)	Total visit proportion (%)
Coleoptera		
Cerambycidae		
<i>Idiostrangalia contracta</i>	0.9 ± 1.1	1.8
Rhipiphoridae		
<i>Pelecotomoides</i> sp.	0.1 ± 0.3	0.2
Diptera		
Syrphidae		
<i>Baccha</i> spp.	1.8 ± 1.7	3.7
<i>Erystalis balteaus</i>	0.2 ± 0.4	0.5
<i>Melanostoma</i> sp.	0.2 ± 0.7	0.5
Mycetophilidae		
<i>Mycetophilidae</i> spp.	0.3 ± 0.7	0.7
Tipulidae		
<i>Tipulidae</i> sp.	0.1 ± 0.3	0.2
Hemiptera		
Miridae		
<i>Onomaus lautus</i>	0.3 ± 0.5	0.7
Hymenoptera		
Apidae		
<i>Bombus ardens ardens</i> (♂)	30.7 ± 9.9	63
<i>Bombus hypocrita hypocrita</i> (♀)	0.2 ± 0.7	0.5
<i>Bombus diversus diversus</i> (♀)	0.3 ± 0.5	0.7
<i>Ceratina flavipes</i>	0.6 ± 1.0	1.1
Colletidae		
<i>Hylaeus globulus</i>	1.6 ± 1.9	3.2
Andrenidae		
<i>Andrena (Andrena) aburana</i>	0.2 ± 0.4	0.5
Halictidae		
<i>Lasioglossum (Evylaeus) allodalum</i>	8.1 ± 4.9	16.7
<i>Lasioglossum (Evylaeus) hirashimae</i>	2.8 ± 2.0	5.7
Gasteruptiidae		
<i>Gasteruption</i> sp.	0.1 ± 0.3	0.2
Lepidoptera		
Epicopeiidae		
<i>Psychostrophia melanargia</i>	0.1 ± 0.3	0.2

♂, drones; ♀, workers .

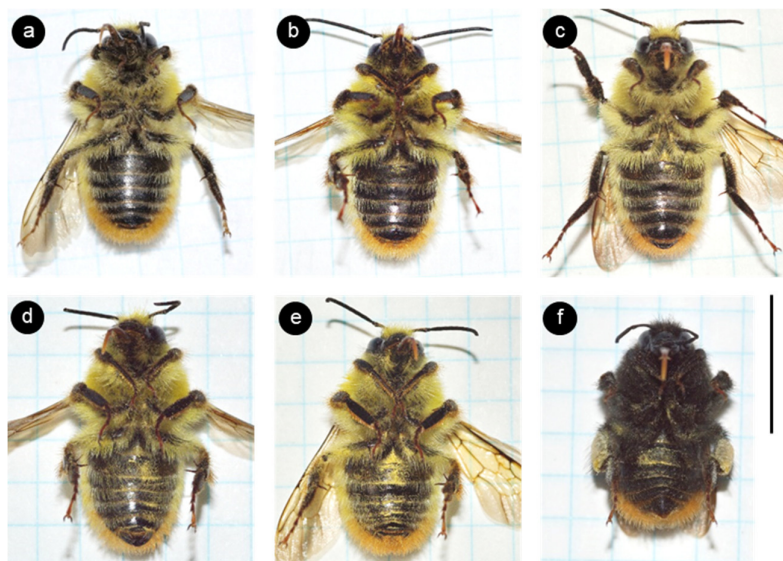


Fig. 5-5. *Meliosma tenuis* pollen attachment in several bumblebee visitors. *Bombus ardens ardens* drones with different levels of pollen attachment (a-e): (a) no visible clusters of pollen; (b) scarce pollen attachment; (c) little pollen attachment; (d) considerable pollen attachment; (e) abundant pollen attachment; (f) *Bombus hypocrita hypocrite* worker with *Meliosma tenuis* pollen in abdomen. Scale is 1 cm.

ardens drones (Figs. 5-4a, 5-4b) were the most common flower-visitors, and foraged for nectar; conspecific workers and queens were not observed. Workers of *Bombus diversus* Smith, 1869 and *B. hypocrita* Perez, 1905 were rarely observed, and also foraged for nectar. Five species of solitary bees belonging to Apidae (1 sp.), Colletidae (1 sp.), Andrenidae (1 sp.) and Halictidae (2 spp.), were also observed while they were rare. Among the solitary bees, the visitation frequency of *Lasioglossum (Evy-laeus) allodolum* Ebmer and Sakagami, 1985 (Halictidae) (Fig. 5-4d) was highest (Table 5-3). All these hymenopteran flower-visitors foraged for nectar (Fig. 5-4a-d).

Five dipteran species belonging to Syrphidae (3 spp.), Mycetophilidae (1 sp.) and Tipulidae (1 sp.) were observed on the flowers. The syrphid flies and mycetophilid flies were observed to feed on nectar (Fig. 5-4e). Coleopteran, lepidopteran and hemipteran visitors were rare visitors. The two coleopteran species, belonging to Cerambycidae (1 sp.) and Rhipiphoridae (1 sp.), fed on nectar inserting their mouth parts into the flowers. The cerambycid beetle visitor, *Idiostangalia contracta* (Bates, 1884), was observed once to ingest pollen grains remaining on the anthers of a triggered flower (Fig. 5-4f). *Psychostrophia melanargia* Butler, 1877 (Epicopeiidae), the only

lepidopteran visitor, fed on nectar using its long proboscis (Fig. 5-4g).

Bumblebee drones as pollinators

Drones of *B. ardens* were the main flower visitors, representing 63.0 % of the total visits, whereas conspecific workers and queens were not observed. The visitation rate (visits/40 inflorescences/10 h) of the drones was 30.7 ($\pm$ 9.9 SD).

The bumblebee drones landed on inflorescences softly and rarely harshly. Hesitation behavior was occasionally observed before landing, likely to assess the presence of nectar or to tag/examine drone's pheromone mark. The drones probed for nectar around the floral disk using their proboscises, more frequently from flowers that still had their outer-petals, in the male or neutral stages. Legs and proboscises of the drones were observed to trigger the explosive pollination mechanism.

Usually, pollen grains were found attached in dense clusters on hairs of ventral side of abdomen and on legs, especially tarsi (Figs. 5-5, 5-6a). From 76 netted *B. ardens* drones, 36 were dusked by pollen, while the rest were not (Fig. 5-5a). Amount of attached pollen grains varied among specimens (Figs. 5-5a-e). Although *B. hypocrita* workers were not observed to trigger the explosive pollination mechanism,

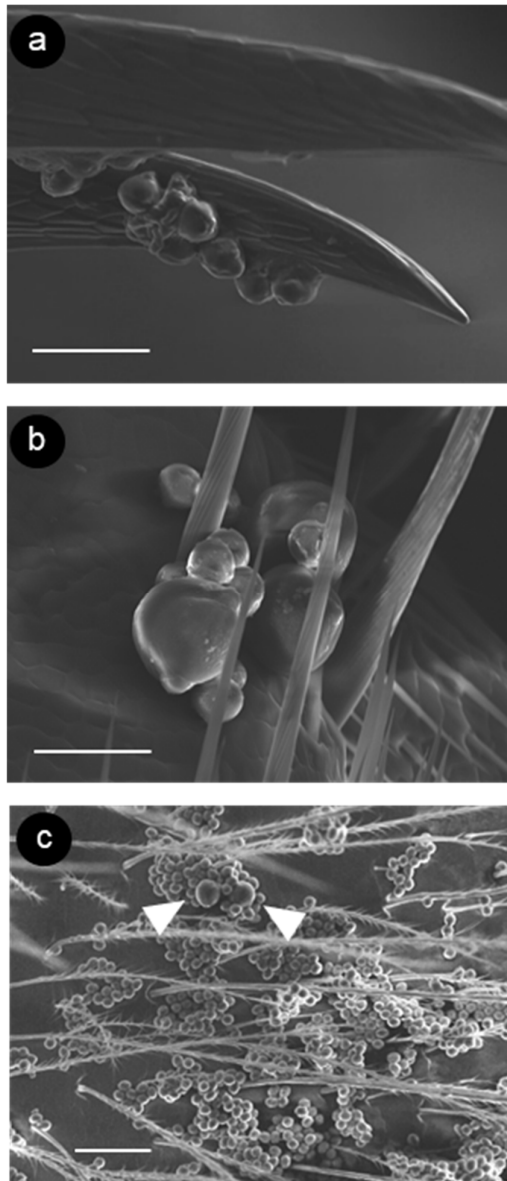


Fig. 5-6. Electron microscope pictures of pollen attachment in *Bombus ardens ardens* drones. (a) *Meliosma tenuis* pollen grains in pretarsus; (b) *Symplocos sawafutagi* (bigger) and *Meliosma tenuis* pollen grains (smaller) in pretarsus; (c) high concentration of *M. tenuis* pollen grains, and few *S. sawafutagi* pollen grains (white arrowhead). Scale is 100 μ m.

1 out of the 4 collected samples had *M. tenuis* pollen attachment, like the *B. ardens* drones (Fig. 5-5f). Almost all the pollen grains attached on *B. ardens* drones were identified as *M. tenuis*, and others were *Symplocos sawafutagi* Nagam. (Symplocaceae) (Fig. 5-6b, 5-6c).

Discussions

The flower of *M. tenuis* was self-compatible, but automatic self-pollination did not occur (Table 5-3), because the pistil was enclosed under staminodes when pollen was released explosively and until staminodes fell (Fig. 5-2). Thus, the flower was protandrous and allogamous (Table 5-3). In *Meliosma*, however, protogyny has been reported in a few species, e.g., *M. pinnata* (Roxb.) Maxim and, *M. veitchiorum* Hemsl. (Van Beusekom 1971). Although these results suggest that the explosive pollination in *Meliosma* is related with dichogamy, it is still unknown what selection pressure has favored protandry and protogyny.

Results of insect visits to the flowers and pollen attachment on visitor bodies suggest that *M. tenuis* is pollinated by bumblebee drones (Tables 5-2, 5-3). This is the first report on pollination system of Sabiaceae species, and simultaneously a rare report on pollination of explosively pollen-releasing, non-zygomorphic flowers. Since *Meliosma* flowers are very small and externally actinomorphic, and because the small flowers morphologically match small insects, it was unexpected that the flowers were pollinated by bumblebee drones. The bumblebee drone pollination system, however, was supported by the facts that only bumblebees triggered the explosive pollen release, and that the flowering season of *M. tenuis* during late May to early June coincides with the appearance of drones of *Bombus ardens*. In the study site, four bumblebee species inhabit, whereas the three other species (*B. diversus*, *B. hoshuen-sis* and *B. hypocrita*) reproduce in autumn.

Generally, bumblebee drones fly long distance in search for both new emerging queens and nectariferous flowers by following definite routes. Thus, bumblebee drones are potentially superior pollinators contributing to long-range pollen transfer, if they are available and abundant. The data of attached pollen on their bodies showed that the bumblebee drones have visited exclusively *Meliosma* flowers and inconsiderably *Symplocos sawafutagi*

(Symlocaceae) flowers. In contrast with bumblebee drones, bumblebee workers were rarely observed on *Meliosma* flowers, while workers of the other three bumblebee species were active. Result suggests that bumblebee workers have passed over the *Meliosma* flowers because the workers could not collect the explosively released pollen.

Transfer of *M. tenuis* pollen grains by bumblebee drones occurred while foraging for nectar, by contact of the legs, thorax, and/or abdomen with the pistil of the flowers. Transfer of pollen grains by the proboscis was rare, since the drones were observed to probe only from flowers with outer-petals, male and neutral stages. The dense attachment of pollen on hairs of ventral side of abdomen (Figs. 5-5, 5-6) is the exact result of explosive pollen release of *Meliosma* flowers, and the pollen would be transferred to pistils of flowers on distant trees.

Chapter 6: General Discussion

Zoophilous plants have developed diverse floral morphology and flowering behavior to attract pollinators and to avoid inbreeding along various direction of adaptations (Table 6-1). The excessive morphological diversity of zoophilous angiosperms is thus a product of adaptation to pollination by diverse specific pollinators. In this thesis, I examined three aspects of floral morphology and flowering behavior: (1) decorative sterile flowers in framed inflorescences, (2) flowering in the earliest spring, and (3) explosive pollen release.

(1) Decorative sterile flowers in framed inflorescences

Pollinator-attracting function of decorative flowers has been suggested by Darwin (1877), and few studies confirmed the function by field experiments. The manipulation experiments of clipping/mounting decorative flowers in Chapters 2 and 3 clearly showed that the main function of decorative flowers in *Viburnum* (Adoxaceae) and *Hydrangea* (Hydrangeaceae) species is to attract pollinators to framed inflorescences by visual cues. In general, pollinator-attracting function of decorative flowers is hypothesized to be caused by one or a combination of the following visual cues created by the decorative flowers: (1) wider inflorescence area, (2) accentuated inflorescence contrast (between decorative and fertile flowers, and between inflorescence and background), and (3) attractive inflorescence silhouettes (Thorp and Horning 1983, Conner and Rush 1996, Goulson 2000, Spaethe et al. 2001, Makino et al. 2007). Moreover, the decorative flowers also functioned as landing sites, notably for flower visitors of framed *Viburnum* species.

The general appearance of most framed inflorescences was largely similar between *Viburnum* and *Hydrangea*, irrespective of the difference of their phylogenetic clades. Furthermore, pollinator assemblages were also similar

between framed inflorescences of both the genera. These results suggest that the two genera of flowers have experienced convergent evolution of inflorescences and share similar pollinator assemblages.

My results on *Viburnum* and *Hydrangea* suggest that decorative flowers, mainly arranged around a corymbiform inflorescence have evolved to increase attractiveness to sight sensed, nectar/pollen-feeding small insects such as solitary bees, cerambycid beetles and syrphid flies. The high diversity of plant species having framed inflorescences in the Japanese Archipelago as exemplified by *Viburnum* and *Hydrangea* is thought to be related with the high diversity and great abundance of solitary/eusocial halictid bees and anthophilous cerambycid beetles belonging to the genus *Pidonia*.

(2) Flowering in the earliest spring

Because available pollinators such as bees are abundant from spring to early autumn, many zoophilous plants reasonably bloom in these seasons. And although flowering outside the boundaries of the previously mentioned seasons is thought would be an adaptation to avoid competition for pollinators, flowering in the earliest spring is unique, and it is intriguing how these plants reproduce.

The extensive observations on flowering and pollination of *Corylopsis gotoana* flowers suggested that the flowers were nectariferous and mainly pollinated by bombyliid flies, Asian honeybee workers and bumblebee queens. Bumblebee queens emerge from wintering underground in March. Asian honeybee colonies keep their activity in nests even in winter, and workers can fly on warmer days. Because the bombyliid flies are parasites of bumblebees, they go into action in early spring, i.e.,

Table 6-1. Adaptations of zoophilous plants to increase pollination success.

Target	Direction of adaptation	Attribute of adaptation	Adaptation	Example	Reference		
Pollinator attraction							
visual signal		floral color	non-green floral color stimulate pollinator's visual sense	diverse plants	Proctor et al. 1996; Willmer 2011		
		floral symmetry	zygomorphic flowers attract some bees and birds	Fabaceae, Lamiaceae, Orchidaceae; Ranunculaceae	Proctor et al. 1996; Cubas 2004		
		floral shape	specific floral shape attract some insects	diverse plants	Proctor et al. 1996		
		nectar guide	nectar guide lead bees coming deep into corolla tube	Balsaminaceae, Lamiaceae, Scrophlariaceae etc.	Proctor et al. 1996		
		decorative flowers	decorative flowers contribute to increase attractiveness of inflorescence	<i>Hydrangea</i> , <i>Viburnum</i>	this study (Chapters 2 and 3)		
olfactory signal		floral scents	floral volatiles attract osmatic pollinators	diverse plants	Proctor et al. 1996		
acourstic signal		reflection of ultrasound	special petal structure reflect ultrasound emitted by echolocating bats	<i>Mucuna</i> (Fabaceae)	von Helversen and von Helversen 1999		
tacticle signal			special type of cells in petals	<i>Antirrhinum</i> (Plantaginaceae)	Kevan and Lane 1985		
thermal signal			heat in floral chamber	<i>Chiloglottis</i> (Orchidaceae)	Raguso 2004		
timing of flowering	flowering at a season		adjust flowering season with the emergence time of specific pollinator	diverse plants	Proctor et al. 1996		
		flowering throughout a year	flowers support reproduction of the specific pollinators	<i>Ficus</i> (in part)	Kjellberg et al. 1987		
		flowering in early spring	utilize bees, beeflies and stoneflies	<i>Coryloppsis</i>	this study (Chapter 4)		
		flowering in winter	utilize Asian honeybees	<i>Cymbidium</i>	Tsuji and Kato 2010		
		staggered flowering	avoid interspecific competition for pollinators	<i>Hydrangea</i> , <i>Viburnum</i>	this study		
		flowering with rewarding plants	non-rewarding plants mimic rewarding plants	<i>Pogonia</i> (Orchidaceae)	Ushimaru and Nakata 2001		
		explosive pollen release	pollinator's tactile stimuli trigger explosive pollen release	Loranthaceae, Lamiaceae, Musaceae, <i>Meliosma</i>	this study (Chapter 5)		
		Floral reward	nectar	nectar is the most comonplace and chiepest sugar rewarr for anthophilous insects and birds	diverse plants	Proctor et al. 1996	
				pollen	pollen is protein reward for pollenivorous insects	diverse plants	Proctor et al. 1996
				floral oil	floral oil is harvested by oil-collecting bees belonging Mellitidae and Apidae	Malpighiaceae	Sigrist and Marlies 2004
perfume	perfume is harvedsted by euglossin bees			<i>Coryanthes</i> (Orchidaceae)	Gerlach and Schill 1991		
	resin	resin is harvested by euglossine bees	<i>Dalechampia</i> (Euphorbiaceae)	Armbruster and Webster 1979			
	floral tissue	brood site pollination	<i>Aristolochia</i> (Aristolochiaceae), <i>Macaranga</i> (Euphorbiaceae)	Sakai 2002; Ishida et al. 2009			
	seed	obligate pollination mutualism	<i>Ficus</i> (Moraceae), <i>Yucca</i> (Agavoideae), <i>Phyllanthaceae</i>	Kato and Kawakita 2017			
	mating site		<i>Alocasia</i> (Araceae)	Miyake and Yafuso 2003			
Outbreeding							
self-incompatibility			stigma inhibits germination of self-pollen, or style inhibitselongation of the pollen tube	diverse plants	Igic and Kohn 2006		
		dichogamy	protandry protogyny	anthers mature before stigma	<i>Meliosma</i> (Sabiaceae)	this study (Chapter 5) Griffin et al. 2000	
				stigma becomes receptable before maturation of anthers	<i>Aquilegia</i> (Ranunculaceae)		
		heterostily enantioistily		heteromorphism of styles and spistils	<i>Primula</i> (Primulaceae)	Boyd et al. 1990	
				gynoecium protrudes laterally, either to the right or to the left of the androecium	<i>Qualea</i> , <i>Ruizterania</i> (Vochysiaceae)	Rodríguez and Sanoja 2008	
herkogamy			spatial separation of the anthers and stigma	<i>Narcissus</i> (Amaryllidaceae)	Larrinaga, et al. 2009		
change of floral color			flower changes floral color during its floral period	<i>Weigela</i> (Caprifoliaceae)	Ida and Kudo 2003		

foraging floral nectar and seeking for bumblebee nests. These insects contributed to pollination of these flowers even in cold weather conditions, whereas their densities were very low.

The visits by stoneflies were unexpected because associations of terrestrial adult stoneflies with flowering plants have rarely been documented. Field observations and field experiments showed that the female stoneflies feed on pollen and that the stoneflies dusted with pollen contribute to pollination of the flowers. This is the first report demonstrating that flowers are certainly pollinated by stoneflies. The stonefly pollination is considered an option to secure pollination in the earliest spring in colder years, because the climatic regime is often different between terrestrial and aquatic habitats.

(3) Explosive pollen release

Zygomorphic flowers in several plant families have developed a special floral mechanism to release pollen explosively to attach pollen to remote long-tongued/billed pollinators. *Meliosma* (Sabiaceae) is a genus that has superficially actinomorphic, but internally zygomorphic small dish-like flowers which release pollen explosively when certain flower visitors trigger the mechanism. Artificial pollination experiments revealed that the plant was self-incompatible and protandrous, but automatic self-pollination never occurred. The combination of self-incompatibility and dichogamy has been documented in various self-incompatible angiosperm species (Griffin et al. 2000, Harder et al. 2000, Routley and Husband 2003), and is hypothesized to reduce pollen-pistil interference (Lloyd and Webb 1986, Bertin and Newman 1993). The reduction of pollen-pistil interference may be particularly beneficial to flowers of *M. tenuis* and other *Meliosma* species as these flowers do not expose pollen continuously.

The explosive pollen release was triggered by slight tactile stimuli to anther filaments or staminodes in male-stage flowers. The

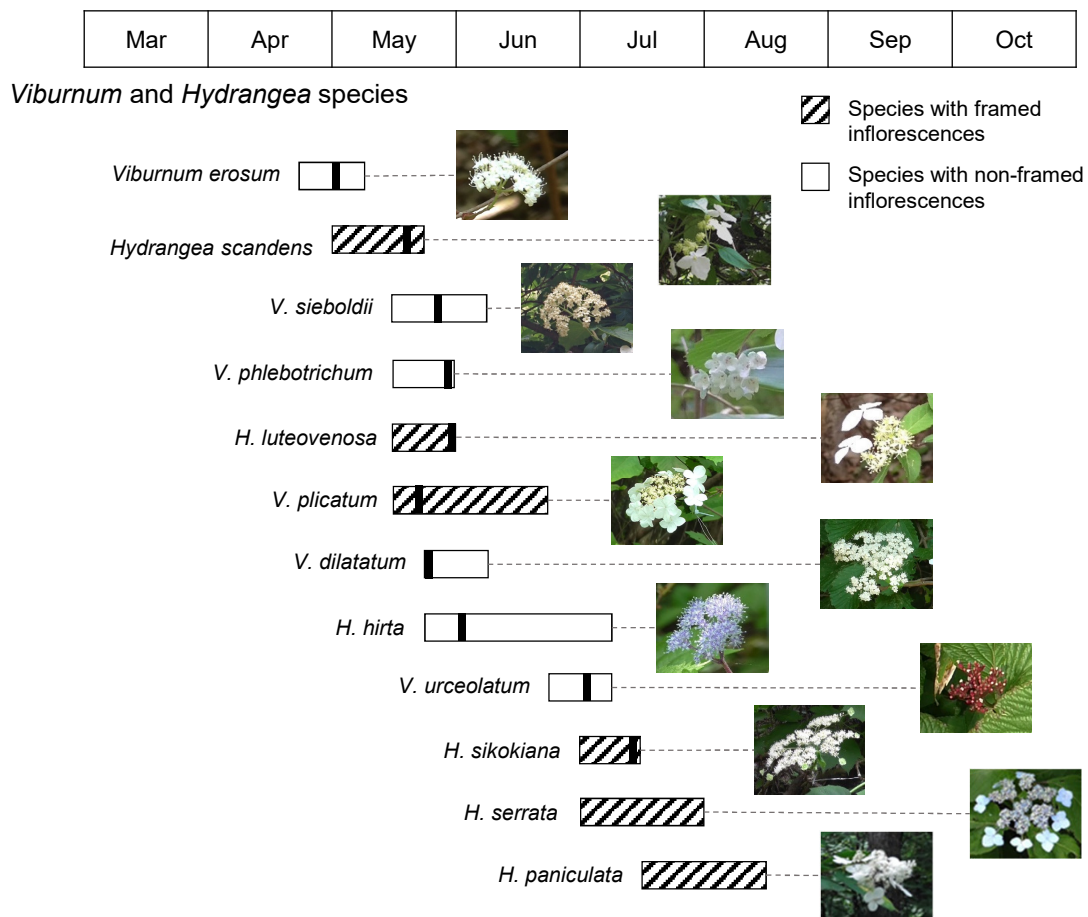
flowers blooming from late May to early June were visited almost exclusively by drones of a bumblebee species, *Bombus ardens*, the emergence of which coincided with the flowering period of *M. tenuis*. The bumblebee drones could trigger the explosive pollen release mechanism, and pollen were attached on hairs on ventral side of abdomen and legs especially tarsi. The lack of bumblebee worker visits suggests that the explosive pollen release may discourage pollen-harvesting bumblebee workers.

These results indicate that the bumblebee drones are the main pollinators of *M. tenuis*, and that the explosive pollen release enables the small flowers to attach pollen to the remote body parts of bumblebee drones, which visited exclusively *Meliosma* flowers for a long distance in search for newly emerging queens.

Morphological adaptations for pollination

Among the studied plant species, two morphological adaptations for pollination were studied. In Chapters 2 and 3, decorative sterile flowers: sterile flowers, found in some *Viburnum* and *Hydrangea* species, have been modified to become larger and more attractive to pollinators. These sterile flowers surround a cluster of fertile flowers, which remain small and offer pollen and nectar as rewards to their insect visitors. And in Chapter 5, explosive pollen release: the modifications of several flower organs of *M. tenuis* for explosive pollen release resulted in a specialized flower, pollinated almost solely by *B. ardens* drones.

As framed *Viburnum* and *Hydrangea* species flower in spring and summer (Fig. 6-1) and *M. tenuis* in summer, their floral morphological adaptations probably function to avoid competition for pollinators at flowering peak times (April - July). Additionally, these adaptations are similar to the solutions of Mosquin (1971) for flowering plants to avoid competition for pollinators flowering peak times: for framed *Viburnum* and *Hydrangea* species, the



Insect pollinator groups

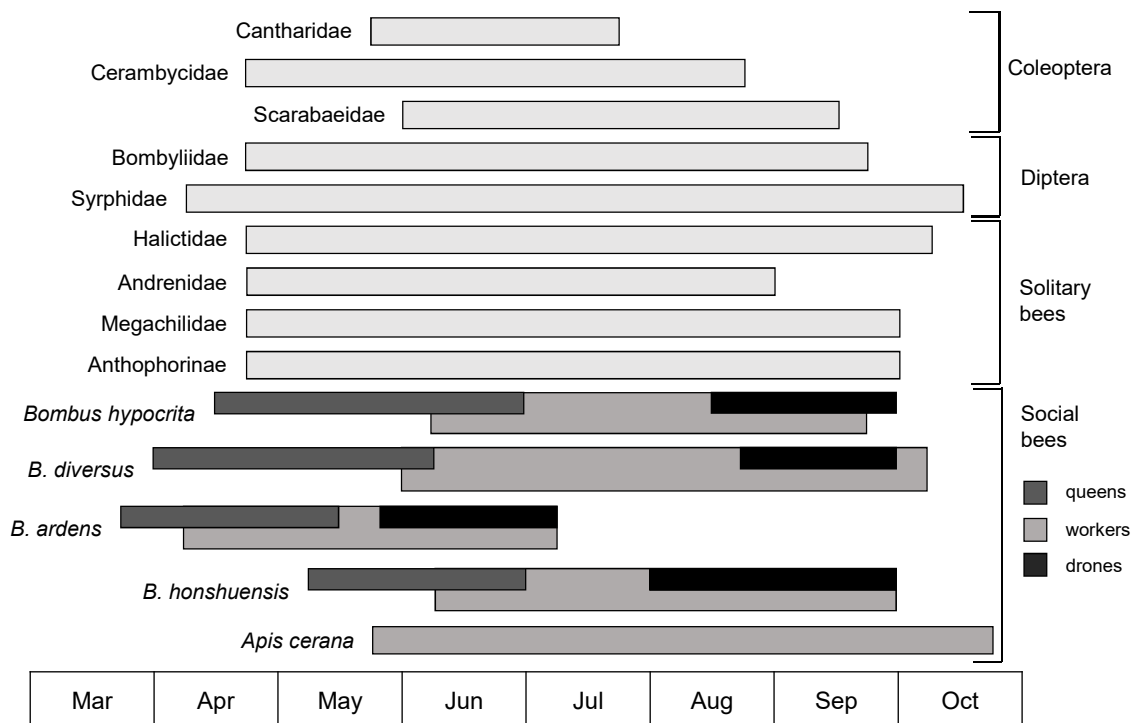


Fig. 6-1. Flowering phenology of *Viburnum* and *Hydrangea* with active periods of pollinators. Based on information of Inoue et al. (1990), Kato et al. (1990) and personal observations. Vertical bars in flowering phenology are my observation periods of each plant species around Kyoto prefecture.

development of a large visual target and readily accessible rewards, and for *M. tenuis*, the evolution of specialized flowers.

Shifts in flowering phenologies as adaptations for pollination

Shift in flowering phenologies are another adaptation to avoid competition for pollinator. Shifts in flowering phenologies are found in the early spring flowering *C. gotoana*, and in *Viburnum* and *Hydrangea* species.

Corylopsis species flowering in the very early spring is probably an adaptation to avoid competition for pollinators in the later warmer seasons. This shift in the flowering phenology of *C. gotoana* resulted in a pollination system that relies on early emerging and active insects, such as bumble queens, honeybee workers and bombyliid flies, and that additionally adopts an aquatic insect, *Strophopteryx nohirae* (Taeniopterygidae, Plecoptera) as a co-pollinator.

Viburnum and *Hydrangea* species show a different adaptation in their flowering phenologies: staggered flowering, where the blooming times of congeneric species do not totally overlap (Fig. 6-1). This adaptation likely occurred due to congeneric species sharing pollinators and blooming times, from April to August. The staggered flowering adaptation also improves efficiency of intraspecific pollination and minimizes interspecific hybridization.

Importance of Pidonid beetles (Cerambycidae) as pollinators of Japanese flora

Pidonia (Cerambycidae) is a genus of cerambycid beetles which is mainly distributed in eastern Asia. Beetles of this genus have greatly diversified in the Japanese archipelago (Kuboki & Shimamoto 1979; Kuboki 1980;

Kuboki 1987), where are mainly found in primary humid forests (Maeto et al. 2002; Ohsawa 2004). Even though these beetles have been documented to visit flowers of various families (Kuboki and Shimamoto 1979; Kuboki 1980; Inoue et al. 1990; Kato et al. 1990; Yamazaki & Kato 2003), including flowers of various *Viburnum* and *Hydrangea* species, few studies have focused on the pollination potential of these beetles.

In Chapters 2 and 3, I recorded cerambycid beetles, including various pidonid beetles, to visit *Viburnum* and *Hydrangea* species (supplementary Tables 2-1, 3-1). These beetles showed preference to visit framed inflorescences (Table 2-4) and used decorative flowers as landing sites in framed *Viburnum* species (Table 2-6). The predilection of inflorescences with decorative flowers, and the use of these flowers as landing sites, suggest that these beetles are even related to the high diversity of *Viburnum* and *Hydrangea* framed species in the Japanese Archipelago. My findings show that pidonid beetles are important pollinators of part of the indigenous Japanese flora. Nonetheless, to further understand the importance of pidonid beetles as pollinators, more extensive study about the ecology and natural history of these beetles is needed.

Acknowledgments

Firstly, I would like to express my most sincere gratitude to my advisor, Prof. M. Kato, who patiently and continuously guided me through the years I have been on his care. The wealth of knowledge and experience that I have gained from his guidance has greatly improved my scientific abilities and stimulated my enthusiasm for study and self-improvement. His teachings and appreciation for nature will surely stay with me.

Thanks are also due to Dr. K. Kenji and Dr. Y. Imada for fruitful discussion and helpful insights on my research. I also thank Dr. A. Kawakita, Dr. H. Toju and Dr. K. Kadowaki for sharing their knowledge and valuable technical knowledge with me, and Dr. Y. Nakase and Ms. Y. Yamane for using their valuable time to lead me to plant populations used in this study.

I also express my gratitude to all the members of Kato lab. and Itioka lab. for their support throughout my doctoral research and stay in the Kato lab.

Also, I thank the Japanese Ministry of Education, Culture, Sports, Science and Technology for its student scholarship, which made my studies at the Kyoto University possible.

Last but not the least, I would like to thank to all my family, especially to Lucho and Eiko, my parents, and Aisa, my sister, for always supporting and believing in me.

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Supplemental Material

Supplementary Table 2-1. Visit rates of all visitor species to intact inflorescences of the *Viburnum* species (N = 6).

Visitor species	Visit rate (visits/inflorescence/h) $\pm$ SD to intact inflorescences								
	FR species			n-FR species					
	Vf	Vo	Vpl	Vd	Ve	Vw	Vs	Vph	Vu
Coleoptera									
Cantharidae									
<i>Lycocerus suturellus</i>	—	0 $\pm$ 0.1	—	0.1 $\pm$ 0.2	—	—	—	—	—
<i>Themus episcopalis</i>	—	0 $\pm$ 0.1	—	—	—	—	—	—	—
Cerambycidae									
<i>Demonax transilis</i>	—	4 $\pm$ 1.2	0.9 $\pm$ 0.7	0 $\pm$ 0.1	0.4 $\pm$ 0.2	—	0.1 $\pm$ 0.1	—	—
<i>Gaurotes doris</i>	—	0.4 $\pm$ 0.5	—	—	—	—	—	—	—
<i>Parastrangalis lesnei</i>	—	2.3 $\pm$ 1.8	—	—	—	—	—	—	—
<i>Pidonia (Cryptopidonia) sp.</i>	—	0 $\pm$ 0.1	—	—	—	—	—	—	—
<i>Pidonia (Pidonia) signifera</i>	—	0.2 $\pm$ 0.3	—	—	—	—	—	—	—
<i>Pidonia (Pidonia) sp.</i>	—	—	—	—	—	—	—	0.2 $\pm$ 0.6	—
<i>Pidonia aegrota</i>	—	0.6 $\pm$ 0.4	—	—	0 $\pm$ 0.1	0 $\pm$ 0.1	—	—	—
<i>Pidonia testacea</i>	—	—	—	—	0 $\pm$ 0.1	—	—	0.1 $\pm$ 0.2	—
<i>Strangalomorpha tenuis</i>	—	—	—	—	—	—	0 $\pm$ 0.1	—	—
Elateridae									
<i>Dolerosomus gracilis</i>	—	—	—	0.2 $\pm$ 0.5	—	—	—	—	—
Stenotrachelidae									
<i>Cephaloön pallens</i>	—	0.1 $\pm$ 0.1	—	—	—	—	—	—	—
Diptera									
Acroceridae									
<i>Oligoneura sp.</i>	—	0.3 $\pm$ 0.3	—	—	—	—	—	—	—
Anthomyiidae									
<i>Anthomyiidae sp. 2</i>	—	—	—	—	0.1 $\pm$ 0.2	—	—	—	—
Bombyliidae									
<i>Bombylius major</i>	0.1 $\pm$ 0.2	—	—	—	—	—	—	—	—
Calliphoridae									
<i>Calliphoridae sp. 1</i>	—	—	—	—	—	0.1 $\pm$ 0.1	—	—	—
<i>Calliphoridae sp. 3</i>	—	0 $\pm$ 0.1	—	—	—	—	—	—	—
<i>Lucilia sp.</i>	—	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2	—	—	—	—	—	—
<i>Stomorphina obsoleta</i>	—	—	0.1 $\pm$ 0.2	—	—	—	—	—	—
Empididae									
<i>Empididae sp. 1</i>	0 $\pm$ 0.1	—	—	—	—	—	—	—	—
<i>Empididae sp. 2</i>	—	—	—	—	—	0.3 $\pm$ 0.4	—	—	—
<i>Empididae sp. 3</i>	—	—	—	—	0.3 $\pm$ 0.4	—	—	—	—
<i>Empididae sp. 4</i>	—	—	—	—	0.1 $\pm$ 0.1	—	—	—	—
<i>Empididae sp. 5</i>	—	—	—	—	—	—	0.1 $\pm$ 0.1	—	—
<i>Empididae sp. 6</i>	—	—	—	—	—	—	—	0.1 $\pm$ 0.4	—
Fanniidae									
<i>Fanniidae sp.</i>	0 $\pm$ 0.1	—	—	—	—	—	—	—	—
Lauxaniidae									
<i>Lauxaniidae sp. 1</i>	—	—	—	—	0 $\pm$ 0.1	—	—	—	—
<i>Lauxaniidae sp. 2</i>	—	—	—	—	—	—	0 $\pm$ 0.1	—	—
Muscidae									
<i>Muscidae sp. 1</i>	—	—	0.4 $\pm$ 0.3	—	—	—	—	—	—
<i>Muscidae sp. 2</i>	—	—	—	—	—	0.2 $\pm$ 0.2	—	—	—
<i>Muscidae sp. 3</i>	—	—	—	0 $\pm$ 0.1	—	—	—	—	—
<i>Muscidae sp. 4</i>	—	0.7 $\pm$ 0.6	—	—	—	—	—	—	—
Rhagionidae									
<i>Rhagionidae sp.</i>	—	0 $\pm$ 0.1	—	—	—	—	—	—	—
Sarcophagidae									
<i>Sarcophaga sp.</i>	—	—	—	—	0 $\pm$ 0.1	—	—	—	—
<i>Sarcophagidae sp. 1</i>	—	—	0.6 $\pm$ 0.6	—	—	—	—	—	—
Scathophagidae									
<i>Scathophagidae sp.</i>	—	0 $\pm$ 0.1	—	—	—	—	—	—	—
Syrphidae									
<i>Baccha sp.</i>	—	—	—	—	—	0.4 $\pm$ 0.5	—	—	—
<i>Cheilosia omogensis</i>	—	—	0.7 $\pm$ 0.2	—	—	—	—	—	—
<i>Epistrophe sp. 2</i>	—	—	—	0 $\pm$ 0.1	—	—	—	—	—
<i>Cheilosia sp. 1</i>	0 $\pm$ 0.1	—	—	—	—	—	—	—	—
<i>Cheilosia sp. 2</i>	—	—	—	—	—	0.1 $\pm$ 0.1	—	—	—
<i>Cheilosia sp. 3</i>	—	0.8 $\pm$ 0.5	—	—	—	—	—	—	—
<i>Dasyisyrphus bilineatus</i>	—	—	—	—	0 $\pm$ 0.1	0.2 $\pm$ 0.3	—	—	—

(continued)

Supplementary Table 2-1. continued

<i>Epistrophe</i> sp. 1	—	—	—	—	—	0.1 ± 0.1	—	—	—
<i>Episyrphus balteatus</i>	—	0 ± 0.1	—	0.4 ± 0.5	0.1 ± 0.2	0.1 ± 0.1	—	—	—
<i>Eristalis cerealis</i>	—	—	0.1 ± 0.1	—	—	0.1 ± 0.1	—	—	—
<i>Eristalis tenax</i>	—	0 ± 0.1	—	—	—	—	—	—	—
<i>Eupeodes</i> sp.	0.5 ± 0.5	—	—	—	—	—	—	—	—
<i>Mallota yakushimana</i>	—	—	—	0 ± 0.1	—	—	—	—	—
<i>Parasyrphus</i> sp.	4 ± 1.5	—	—	—	—	—	—	—	—
<i>Sp.haerophoria</i> sp. 1	—	—	—	—	—	0.1 ± 0.3	—	—	—
<i>Sp.haerophoria</i> sp. 2	—	0.1 ± 0.1	—	—	—	—	—	—	—
<i>Sp.hegina</i> sp. 1	—	—	—	—	0.4 ± 0.4	—	—	—	—
<i>Sp.hegina</i> sp. 2	—	0.1 ± 0.3	—	—	—	—	—	—	0.1 ± 0.1
Tachinidae									
<i>Tachinidae</i> sp. 2	—	—	0.1 ± 0.1	—	—	—	—	—	—
<i>Tachinidae</i> sp. 4	—	—	—	—	—	—	—	—	0.1 ± 0.2
Hymenoptera									
Andrenidae									
<i>Andrena (Calomelissa)</i> sp.	—	—	—	0 ± 0.1	—	—	—	—	—
<i>Andrena (Euandrena) luridiloma</i>	—	—	—	—	0.4 ± 0.4	—	—	—	—
<i>Andrena (Hoplendrena) akitsushimae</i>	—	—	0.1 ± 0.2	—	—	—	—	—	—
<i>Andrena (Micandrena) minutula</i>	—	—	—	—	—	—	0.5 ± 0.2	—	—
<i>Andrena (Micandrena) falsificissima</i>	0.2 ± 0.2	—	—	—	—	—	—	—	—
<i>Andrena (Micandrena) subopaka</i>	—	0.8 ± 0.2	—	—	—	—	—	—	—
<i>Andrena (Simandrena) nippon</i>	0.3 ± 0.4	—	—	—	—	—	—	—	—
<i>Andrena (Simandrena) opacifovea</i>	—	—	—	0.4 ± 0.4	—	—	—	—	—
Apidae									
<i>Bombus ardens</i>	—	—	—	—	—	—	—	—	0.1 ± 0.1
<i>Bombus diversus</i>	—	—	—	—	—	—	—	—	0.2 ± 0.2
<i>Bombus honshuensis</i>	—	—	—	—	—	—	—	—	28.7 ± 15.9
<i>Bombus hypocrita</i>	0 ± 0.1	—	—	—	—	—	—	—	—
<i>Ceratina esakii</i>	0.6 ± 0.5	—	—	—	—	0 ± 0.1	—	—	—
<i>Ceratina japonica</i>	—	—	—	—	—	—	—	—	0.7 ± 1
<i>Nomada fukuina</i>	0.2 ± 0.2	—	—	—	—	—	—	—	—
<i>Nomada hakonensis</i>	0 ± 0.1	—	—	—	—	—	—	—	—
<i>Nomada nipponica</i>	0.1 ± 0.3	—	—	—	—	—	—	—	—
<i>Nomada</i> sp.	—	0.1 ± 0.1	—	—	—	—	—	—	—
<i>Eucera nipponensis</i>	—	—	—	0.5 ± 0.5	—	—	—	—	—
Argidae									
<i>Argidae</i> sp.	—	0 ± 0.1	—	—	—	—	—	—	—
Colletidae									
<i>Hylaeus niger</i>	0.2 ± 0.3	—	—	—	—	—	—	—	—
Halictidae									
<i>Lasioglossum (Evylaeus) hoffmanni</i>	3.5 ± 4	—	9.1 ± 5.3	—	—	—	—	—	—
<i>Lasioglossum (Evylaeus) miyabei</i>	—	—	—	—	—	—	—	0.2 ± 0.2	—
<i>Lasioglossum (Hemihalictus) pumilum</i>	—	—	—	—	—	—	—	—	0.2 ± 0.4
<i>Lasioglossum (Lasioglossum) apristum</i>	—	—	3.3 ± 4.7	—	—	—	0.1 ± 0.2	—	—
<i>Lasioglossum (Lasioglossum) exiliceps</i>	—	—	—	0.1 ± 0.2	—	—	—	—	—
<i>Lasioglossum (Lasioglossum) nipponicola</i>	—	—	—	—	—	0.3 ± 0.4	—	—	—
<i>Lasioglossum (Lasioglossum) proximum</i>	—	—	—	—	—	—	1.3 ± 0.7	—	—
Ichneumonoidae									
<i>Ichneumonoidae</i> sp.	—	0 ± 0.1	—	—	—	—	—	—	—
Megachilidae									
<i>Megachile tsurugensis</i>	—	—	—	0.5 ± 0.8	—	—	—	—	—
Sphecidae									
<i>Sp.hex</i> sp.	0 ± 0.1	—	—	—	—	—	—	—	—
Vespidae									
<i>Polistes snellei</i>	0 ± 0.1	—	—	—	—	—	—	—	—
Lepidoptera									
Pieridae									
<i>Pieris melete</i>	0.1 ± 0.3	—	—	—	—	—	—	—	—
Mecoptera									
Panorpidae									
<i>Panorpida</i> sp.	—	—	—	0 ± 0.1	—	—	—	—	—

FR species, species with inherently framed inflorescences; n-FR species, species without framed inflorescences; SD, standard deviation. See Table 2-1 for *Viburnum* spp. codes.

Supplementary Table 2-2. Pollen accumulation and visit duration on intact inflorescences for all visitors to the *Viburnum* species.

Visitor species	Pollen attachment	Stay time in intact inflorescences	
		<i>Viburnum</i> spp.	Stay time $\pm$ SD (sec)
Coleoptera			
Cantharidae			
<i>Lycocerus suturellus</i>	+	Vo	185 $\pm$ 0
		Vd	884 $\pm$ 260
<i>Themus episcopalis</i>	+	Vo	28 $\pm$ 0
Cerambycidae			
<i>Demonax transilis</i>	++	Vo	65 $\pm$ 91
		Vpl	15 $\pm$ 22
		Vd	380 $\pm$ 0
		Ve	60 $\pm$ 84
		Vs	0 $\pm$ 0
<i>Gaurotes doris</i>	+	Vo	85 $\pm$ 75
<i>Parastrangalis lesnei</i>	+	Vo	99 $\pm$ 143
<i>Pidonia (Cryptopidonia) sp.</i>	++	Vo	391 $\pm$ 0
<i>Pidonia (Pidonia) signifera</i>	++	Vo	59 $\pm$ 55
<i>Pidonia (Pidonia) sp.</i>	++	Vph	28 $\pm$ 14
<i>Pidonia aegrota</i>	++	Vo	174 $\pm$ 259
		Ve	20 $\pm$ 0
		Vw	1167 $\pm$ 0
<i>Pidonia testacea</i>	++	Ve	4 $\pm$ 0
		Vph	62 $\pm$ 0
<i>Strangalomorpha tenuis</i>	+	Vs	0 $\pm$ 0
Elateridae			
<i>Dolerosomus gracilis</i>	+	Vd	96 $\pm$ 100
Stenotrachelidae			
<i>Cephaloon pallens</i>	+	Vo	69 $\pm$ 78
Diptera			
Acroceridae			
<i>Oligoneura sp.</i>	+	Vo	13 $\pm$ 15
Anthomyiidae			
<i>Anthomyiidae sp. 2</i>	+	Ve	129 $\pm$ 136
Bombyliidae			
<i>Bombylius major</i>	+++	Vf	3 $\pm$ 0
Calliphoridae			
<i>Calliphoridae sp. 1</i>	+	Vw	43 $\pm$ 24
<i>Calliphoridae sp. 3</i>	+	Vo	199 $\pm$ 0
<i>Lucilia sp.</i>	+	Vo	16 $\pm$ 13
		Vpl	69 $\pm$ 83
<i>Stomorphina obsoleta</i>	+	Vpl	68 $\pm$ 58
Empididae			
<i>Empididae sp. 1</i>	+	Vf	1 $\pm$ 0
<i>Empididae sp. 2</i>	++	Vw	84 $\pm$ 73
<i>Empididae sp. 3</i>	+	Ve	191 $\pm$ 131
<i>Empididae sp. 4</i>	++	Ve	233 $\pm$ 304
<i>Empididae sp. 5</i>	+	Vs	0 $\pm$ 0
<i>Empididae sp. 6</i>	+	Vph	117 $\pm$ 69
Fanniidae			
<i>Fanniidae sp.</i>	+	Vf	11 $\pm$ 0
Lauxaniidae			
<i>Lauxaniidae sp. 1</i>	+	Ve	27 $\pm$ 0
<i>Lauxaniidae sp. 2</i>	+	Vs	0 $\pm$ 0

(continued)

Supplementary Table 2-2. continued

Muscidae			
<i>Muscidae</i> sp. 1	+	Vpl	80 ± 77
<i>Muscidae</i> sp. 2	+	Vw	29 ± 15
<i>Muscidae</i> sp. 3	+	Vd	13 ± 0
<i>Muscidae</i> sp. 4	+	Vo	159 ± 230
Rhagionidae			
<i>Rhagionidae</i> sp.	+	Vo	27 ± 0
Sarcophagidae			
<i>Sarcophaga</i> sp.	+	Ve	25 ± 0
<i>Sarcophagidae</i> sp. 1	+	Vpl	29 ± 26
Scathophagidae			
<i>Scathophagidae</i> sp.	+	Vo	34 ± 0
Syrphidae			
<i>Baccha</i> sp.	++	Vw	42 ± 29
<i>Cheilosia omogensis</i>	++	Vpl	30 ± 26
<i>Cheilosia</i> sp. 1	++	Vf	16 ± 0
<i>Cheilosia</i> sp. 2	++	Vw	176 ± 38
<i>Cheilosia</i> sp. 3	++	Vo	59 ± 97
<i>Dasyrphus bilineatus</i>	+	Ve	0 ± 0
		Vw	18 ± 23
<i>Epistrophe</i> sp. 1	+	Vw	17 ± 23
<i>Epistrophe</i> sp. 2	+	Vd	35 ± 0
<i>Episyrphus balteatus</i>	++	Vo	8 ± 0
		Vd	103 ± 127
		Ve	10 ± 9
		Vw	167 ± 124
<i>Eristalis cerealis</i>	++	Vpl	29 ± 14
		Vw	25 ± 23
<i>Eristalis tenax</i>	++	Vo	5 ± 0
<i>Eupeodes</i> sp.	++	Vf	115 ± 208
<i>Mallota yakushimana</i>	++	Vd	57 ± 0
<i>Parasyrphus</i> sp.	++	Vf	43 ± 141
<i>Sphaerophoria</i> sp. 1	++	Vw	83 ± 72
<i>Sphaerophoria</i> sp. 2	++	Vo	8 ± 5
<i>Sphegina</i> sp. 1	++	Ve	187 ± 231
<i>Sphegina</i> sp. 2	++	Vo	28 ± 24
		Vu	34 ± 0
Tachinidae			
<i>Tachinidae</i> sp. 2	+	Vpl	46 ± 63
<i>Tachinidae</i> sp. 4	+	Vu	14 ± 0
Hymenoptera			
Andrenidae			
<i>Andrena (Calomelissa)</i> sp.	+++	Vd	73 ± 0
<i>Andrena (Euandrena) luridiloma</i>	+++	Ve	24 ± 19
<i>Andrena (Hoplandrena) akitsushimae</i>	+++	Vpl	13 ± 5
<i>Andrena (Micandrena) minutula</i>	+++	Vs	1 ± 0
<i>Andrena (Micandrena) falsificissima</i>	+++	Vf	48 ± 71
<i>Andrena (Micandrena) subopaka</i>	+++	Vo	117 ± 130
<i>Andrena (Simandrena) nippon</i>	+++	Vf	21 ± 20
<i>Andrena (Simandrena) opacifovea</i>	+++	Vd	11 ± 8

(continued)

Supplementary Table 2-2. continued

Apidae			
<i>Bombus ardens</i>	+++	Vu	31 ± 0
<i>Bombus diversus</i>	+++	Vu	7 ± 8
<i>Bombus honshuensis</i>	+++	Vu	10 ± 7
<i>Bombus hypocrita</i>	+++	Vf	1 ± 0
<i>Ceratina esakii</i>	+++	Vf	24 ± 14
		Vw	11 ± 0
<i>Ceratina japonica</i>	++	Ve	41 ± 48
		Vu	54 ± 98
<i>Nomada fukuina</i>	+	Vf	35 ± 27
<i>Nomada hakonensis</i>	+	Vf	11 ± 0
<i>Nomada nipponica</i>	+	Vf	53 ± 7
<i>Nomada</i> sp.	+	Vo	12 ± 4
<i>Eucera nipponensis</i>	+++	Vd	19 ± 17
Argidae			
<i>Argidae</i> sp.	+	Vo	9 ± 0
Colletidae			
<i>Hylaeus niger</i>	+++	Vf	29 ± 34
Halictidae			
<i>Lasioglossum (Evyllaes) hoffmanni</i>	+++	Vf	22 ± 30
		Vpl	19 ± 23
<i>Lasioglossum (Evyllaes) miyabei</i>	+++	Vph	7 ± 2
<i>Lasioglossum (Hemihalictus) pumilum</i>	+++	Vu	18 ± 8
<i>Lasioglossum (Lasioglossum) apristum</i>	+++	Vpl	18 ± 21
		Vs	0 ± 0
<i>Lasioglossum (Lasioglossum) exiliceps</i>	+++	Vd	62 ± 105
<i>Lasioglossum (Lasioglossum) nipponicola</i>	+++	Vw	33 ± 43
<i>Lasioglossum (Lasioglossum) proximatum</i>	+++	Vs	1 ± 1
Ichneumonoidae			
<i>Ichneumonoidae</i> sp.	+	Vo	0 ± 0
Megachilidae			
<i>Megachile tsurugensis</i>	+++	Vd	5 ± 4
Sphecidae			
<i>Sphex</i> sp.	+	Vf	15 ± 0
Vespidae			
<i>Polistes snellei</i>	+	Vf	51 ± 0
Lepidoptera			
Pieridae			
<i>Pieris melete</i>	+	Vf	4 ± 4
Mecoptera			
Panorpidae			
<i>Panorpida</i> sp.	+	Vd	60 ± 0

Pollen attachment: +++, abundant; ++, moderate; +, mediocre. See Table 2-1 for *Viburnum* spp. codes.

Supplementary Table 3-1. Visit rates of all visitor species to intact inflorescences of the *Hydrangea* species (N = 6).

Visitor species	Visitation rate (visits/inflorescence/h) $\pm$ SD to intact inflorescences								
	Hi	Hm	Hpe	Hse	Hsi	Hsc	HI	Hpa	Hh †
Coleoptera									
Cantharidae									
<i>Lycocerus suturellus</i>	—	—	1.6 $\pm$ 0.7	—	—	—	—	—	—
<i>Podabrus ishiharai</i>	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Themus episcopalis</i>	—	—	0.8 $\pm$ 1.1	—	—	—	—	—	—
Cerambycidae									
<i>Aredolpona succedanea</i>	0.0 $\pm$ 0.1	—	—	—	—	—	—	0.0 $\pm$ 0.1	—
<i>Cyrtoclytus caproides</i>	—	—	0.0 $\pm$ 0.1	—	—	—	—	—	—
<i>Demonax transilis</i>	—	—	0.1 $\pm$ 0.2	—	—	—	—	0.2 $\pm$ 0.3	—
<i>Gaurotes doris</i>	—	—	0.2 $\pm$ 0.4	—	—	—	—	—	—
<i>Idiostrangalia hakonensis</i>	—	—	—	—	—	—	—	1.6 $\pm$ 1.6	—
<i>Idiostrangalia</i> sp. 2	—	—	—	—	0.1 $\pm$ 0.2	—	—	—	—
<i>Judolia cometes</i>	—	—	—	—	—	—	—	1.3 $\pm$ 1.6	—
<i>Leptostrangalia lesnei</i>	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Leptura ochraceofasciata</i>	0.3 $\pm$ 0.3	—	—	0.0 $\pm$ 0.1	—	—	—	6.1 $\pm$ 5.5	—
<i>Nakanea vicaria</i>	—	—	—	—	—	—	—	0.5 $\pm$ 0.9	—
<i>Parastrangalis nymphula</i>	—	—	—	—	—	0.0 $\pm$ 0.1	—	2.4 $\pm$ 2.8	—
<i>Parastrangalis</i> sp. 1	—	—	—	0.0 $\pm$ 0.1	—	—	—	—	—
<i>Parastrangalis</i> sp. 2	—	—	—	—	—	—	—	0.1 $\pm$ 0.2	—
<i>Pidonia (Pidonia) signifera</i>	—	—	0.0 $\pm$ 0.1	—	—	—	—	—	—
<i>Pidonia (Pidonia)</i> sp.	—	—	—	0.2 $\pm$ 0.3	—	—	—	—	—
<i>Pidonia aegrota</i>	—	—	0.3 $\pm$ 0.4	—	—	0.1 $\pm$ 0.2	—	0.8 $\pm$ 1.0	—
<i>Stenocorus caeruleipennis</i>	—	—	—	0.0 $\pm$ 0.1	—	—	—	—	—
<i>Strangalia koyaensis</i>	—	—	—	—	—	—	—	0.6 $\pm$ 0.4	—
<i>Strangalomorpha tenuis aenescens</i>	—	—	6.4 $\pm$ 3.9	—	—	—	—	—	—
Mordellidae									
<i>Mordellidae</i> sp.	—	—	—	—	—	—	0.1 $\pm$ 0.3	—	—
Scarabaeidae									
<i>Anomala orientalis</i>	—	—	—	—	—	—	—	0.0 $\pm$ 0.1	—
<i>Anomala rufocuprea</i>	—	—	—	—	—	—	—	0.1 $\pm$ 0.2	—
<i>Anomala</i> sp. 1	—	—	—	—	—	—	—	0.0 $\pm$ 0.1	—
<i>Eucetonia roelofsi</i>	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Gametis jucunda</i>	—	0.0 $\pm$ 0.1	—	—	—	—	—	—	—
<i>Lasiotrichius succinctus</i>	—	—	—	0.0 $\pm$ 0.1	—	—	—	0.2 $\pm$ 0.4	—
<i>Nipponovaigus</i> sp.	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Popillia japonica</i>	—	—	—	—	—	—	—	0.0 $\pm$ 0.1	—
Scraptiidae									
<i>Scraptiidae</i> sp.	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
Stenotrachelidae									
<i>Cephaloon pallens</i>	—	—	0.0 $\pm$ 0.1	—	—	—	—	—	—
Tenebrionidae									
<i>Tenebrionidae</i> sp.	—	—	—	—	—	0.1 $\pm$ 0.2	—	—	—
Dermaptera									
<i>Dermaptera</i> sp. 1	—	—	—	0.0 $\pm$ 0.1	—	—	—	—	—
<i>Dermaptera</i> sp. 2	—	—	—	0.0 $\pm$ 0.1	—	—	—	—	—
Diptera									
Asilidae									
<i>Asilidae</i> sp. 1	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Asilidae</i> sp. 2	—	—	—	—	—	—	0.0 $\pm$ 0.1	—	—
<i>Choerades komurai</i>	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
Calliphoridae									
<i>Calliphoridae</i> sp. 1	0.0 $\pm$ 0.1	—	—	—	—	—	—	0.0 $\pm$ 0.1	—
<i>Calliphoridae</i> sp. 2	—	—	—	—	—	—	—	0.2 $\pm$ 0.4	—
<i>Calliphoridae</i> sp. 3	—	—	0.0 $\pm$ 0.1	—	—	—	—	—	—
<i>Stomorphina obsoleta</i>	1.1 $\pm$ 1.6	—	—	0.2 $\pm$ 0.4	—	—	—	1.5 $\pm$ 2.0	—
Drosophilidae									
<i>Drosophilidae</i> sp.	—	—	0.0 $\pm$ 0.1	—	—	—	—	—	—
Empididae									
<i>Empididae</i> sp. 1	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Empididae</i> sp. 2	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Empididae</i> sp. 3	—	—	—	—	—	—	0.3 $\pm$ 0.7	—	—
Lauxaniidae									
<i>Lauxaniidae</i> sp.	—	—	—	—	—	—	0.2 $\pm$ 0.2	—	—
Muscidae									
<i>Musca</i> sp.	—	—	1.3 $\pm$ 1.7	—	—	—	—	—	—
<i>Muscidae</i> sp. 1	—	—	—	—	—	—	—	0.2 $\pm$ 0.5	—
<i>Muscidae</i> sp. 2	—	—	—	—	—	0.0 $\pm$ 0.1	—	—	—
<i>Muscidae</i> sp. 3	—	—	0.0 $\pm$ 0.1	—	—	—	—	—	—
<i>Neomya</i> sp.	—	—	—	—	0.6 $\pm$ 0.4	—	—	—	—

(continued)

Supplementary Table 3-1. continued

Sarcophagidae									
<i>Sarcophagidae</i> sp. 2	—	—	—	0.1 ± 0.1	—	—	—	—	—
<i>Sarcophagidae</i> sp. 3	—	—	—	0.1 ± 0.1	—	—	—	—	—
Scathophagidae									
<i>Scathophagidae</i> sp.	—	—	1.9 ± 2.3	—	—	—	—	—	—
Syrphidae									
<i>Allobaccha apicalis</i>	—	—	—	—	0.7 ± 0.7	—	—	—	—
<i>Asarkina porcina</i>	0.7 ± 1.0	—	—	—	—	—	—	—	—
<i>Baccha</i> sp.	—	—	—	—	3.0 ± 2.6	—	—	—	—
<i>Betasyrphus</i> sp. 1	—	—	—	0.7 ± 0.7	—	—	—	—	—
<i>Betasyrphus</i> sp. 2	0.8 ± 1.2	—	—	—	—	—	—	—	—
<i>Chalcosyrphus</i> sp.	—	—	—	—	—	0.0 ± 0.1	—	—	—
<i>Cheilosia</i> sp. 1	—	—	—	—	0.4 ± 0.6	—	—	—	—
<i>Cheilosia</i> sp. 2	—	—	—	1.0 ± 0.8	—	—	—	—	—
<i>Cheilosia</i> sp. 3	—	—	—	—	—	—	0.0 ± 0.1	—	—
<i>Cheilosia</i> sp. 4	—	—	—	—	—	0.0 ± 0.1	—	—	—
<i>Cheilosia</i> sp. 5	—	0.0 ± 0.1	—	—	—	—	—	—	—
<i>Cheilosia</i> sp. 6	—	—	0.0 ± 0.1	—	—	—	—	—	—
<i>Chrysotoxum grande</i>	0.3 ± 0.4	—	—	—	—	—	—	—	—
<i>Dasysyrphus bilineatus</i>	0.0 ± 0.1	—	—	—	—	—	—	—	—
<i>Episyrphus balteatus</i>	0.1 ± 0.1	—	0.5 ± 0.6	0.3 ± 0.3	—	0.0 ± 0.1	—	—	—
<i>Eristalis</i> sp.	—	—	—	1.6 ± 1.0	—	—	—	—	—
<i>Eristalis tenax</i>	—	—	0.1 ± 0.2	—	—	—	—	—	—
<i>Eupeodes bucculatus</i>	0.3 ± 0.4	—	—	—	—	—	—	—	—
<i>Mallota dimorpha</i>	0.1 ± 0.1	—	—	0.4 ± 0.2	—	—	—	0.1 ± 0.2	—
<i>Mallota yakushimana</i>	—	—	—	0.2 ± 0.2	—	—	—	0.1 ± 0.1	—
<i>Milesia undulata</i>	0.3 ± 0.4	—	—	—	—	—	—	—	—
<i>Paragus</i> sp.	—	—	—	0.2 ± 0.3	—	—	—	—	—
<i>Pterallastes unicolor</i>	—	—	—	0.1 ± 0.1	—	—	—	—	—
<i>Sphaerophoria</i> sp. 1	—	—	—	—	2.0 ± 1.4	—	—	—	—
<i>Sphaerophoria</i> sp. 2	—	—	—	0.4 ± 0.6	—	—	—	—	—
<i>Sphaerophoria</i> sp. 3	—	—	—	0.5 ± 0.7	—	—	—	—	—
<i>Sphaerophoria</i> sp. 4	1.2 ± 1.7	—	—	—	—	—	—	—	—
<i>Sphaerophoria</i> sp. 5	—	—	0.1 ± 0.1	—	—	—	—	—	—
<i>Sphegina</i> sp. 1	—	—	—	—	—	—	—	0.0 ± 0.1	—
<i>Sphegina</i> sp. 2	0.0 ± 0.1	—	—	—	—	—	—	—	—
<i>Sphegina</i> sp. 3	—	—	0.4 ± 0.4	—	—	—	—	—	—
<i>Syrphus</i> sp. 1	—	—	—	2.4 ± 1.7	—	—	—	—	—
<i>Syrphus</i> sp. 2	—	—	—	—	—	—	—	0.2 ± 0.4	—
<i>Temnostoma apiform</i>	—	—	—	0.0 ± 0.1	—	—	—	—	—
<i>Temnostoma nitobei</i>	0.4 ± 0.4	—	—	—	—	—	—	—	—
<i>Temnostoma vespiforme</i>	—	—	—	0.0 ± 0.1	—	—	—	—	—
<i>Volucella thompsoni</i>	—	—	—	—	—	—	—	0.1 ± 0.1	—
<i>Xylota</i> sp. 1	—	—	—	0.3 ± 0.4	—	—	—	—	—
Tachinidae									
<i>Cylindromyia</i> sp.	—	—	—	—	0.5 ± 0.5	—	—	—	—
<i>Ectophasia rotundiventris</i>	—	—	—	0.2 ± 0.2	—	—	—	—	—
<i>Tachinidae</i> sp. 1	—	—	—	—	0.0 ± 0.1	—	—	—	—
<i>Tachinidae</i> sp. 2	—	—	—	—	0.0 ± 0.1	—	—	—	—
Tipulidae									
<i>Tipulidae</i> sp.	—	—	—	—	—	0.1 ± 0.2	—	—	—
Hymenoptera									
Andrenidae									
<i>Andrena (Andrena) lapponica</i>	—	—	0.4 ± 0.6	—	—	—	—	—	—
<i>Andrena (Calomelissa) prostomias</i>	—	—	—	—	—	0.0 ± 0.1	—	—	—
<i>Andrena (Chemidandrena) denticulata</i>	—	—	0.0 ± 0.1	—	—	—	—	—	—
<i>Andrena (Micrandrena) subopaka</i>	—	—	0.5 ± 0.3	—	—	—	—	—	—
Apidae									
<i>Bombus ardens</i>	—	—	—	—	—	—	—	—	0.1 ± 0.2
<i>Bombus hypocrite</i>	1.6 ± 0.7	—	—	6.4 ± 2.5	—	—	—	—	—
<i>Ceratina japonica</i>	0.0 ± 0.1	—	—	—	—	0.4 ± 0.5	0.5 ± 0.5	—	1.1 ± 1.1
<i>Nomada</i> sp. 1	—	—	—	—	—	0.0 ± 0.1	—	—	—
<i>Nomada</i> sp. 2	—	—	0.0 ± 0.1	—	—	—	—	—	—
Argidae									
<i>Argidae</i> sp.	—	—	—	0.0 ± 0.1	—	—	—	—	—
Braconidae									
<i>Braconidae</i> sp.	—	—	—	—	0.3 ± 0.6	—	—	—	—

(continued)

Supplementary Table 3-1. continued

Halictidae									
<i>Lasioglossum (Evylaeus) allodatum</i>	—	—	2.7 ± 1.3	—	7.9 ± 3.0	—	—	—	—
<i>Lasioglossum (Evylaeus) apristum</i>	—	—	0.3 ± 0.5	—	—	—	—	—	—
<i>Lasioglossum (Evylaeus) caliginosum</i>	—	—	—	—	—	—	1.5 ± 0.7	—	3.5 ± 3.3
<i>Lasioglossum (Evylaeus) duplex</i>	—	3.6 ± 1.8	—	—	—	—	—	—	—
<i>Lasioglossum (Evylaeus) pallilomum</i>	0.9 ± 0.9	—	—	—	—	—	—	2.9 ± 4.2	—
<i>Lasioglossum (Evylaeus) sibilicatum</i>	—	—	—	3.4 ± 2.1	—	—	—	—	—
<i>Lasioglossum (Evylaeus) vulsum</i>	—	0.4 ± 0.3	—	—	0.5 ± 0.8	1.6 ± 1.7	—	—	—
<i>Lasioglossum (Lasioglossum) exiliceps</i>	—	—	—	—	—	—	—	0.3 ± 0.4	—
<i>Lasioglossum (Lasioglossum) harmandi</i>	—	0.7 ± 1.0	—	0.3 ± 0.2	—	—	—	—	—
<i>Lasioglossum (Lasioglossum) leviventre</i>	—	—	—	0.1 ± 0.1	—	—	—	—	—
<i>Lasioglossum (Lasioglossum) nipponicola</i>	—	—	—	—	—	—	0.1 ± 0.1	—	0.0 ± 0.1
<i>Lasioglossum (Lasioglossum) occidentis</i>	0.1 ± 0.2	—	—	—	—	—	—	—	—
<i>Lasioglossum (Lasioglossum) proximatum</i>	—	—	—	—	—	0.1 ± 0.2	—	—	—
Ichneumonidae									
<i>Picardiella tarsalis</i>	—	—	—	—	—	0.0 ± 0.1	—	—	—
Megachilidae									
<i>Megachile subalbata</i>	—	—	—	—	0.0 ± 0.1	—	—	—	—
Pompilidae									
<i>Anoplius</i> sp.	0.0 ± 0.1	—	—	—	—	—	—	—	—
Sphecidae									
<i>Sphecidae</i> sp.	—	—	—	0.1 ± 0.1	—	—	—	—	—
Tenthredinidae									
<i>Tenthredo nigropicta</i>	—	—	1.1 ± 0.3	—	—	—	—	—	—
Vespidae									
<i>Eumenidae</i> sp.	—	—	—	—	—	—	—	0.0 ± 0.1	—
<i>Polistes</i> sp.	—	—	—	—	0.0 ± 0.1	—	—	—	—
Lepidoptera									
Adelidae									
<i>Nemophora aurifera</i>	—	—	0.1 ± 0.2	—	—	—	—	—	—
Hesperiidae									
<i>Daimio tethys</i>	—	—	0.4 ± 0.6	—	—	—	—	—	—
<i>Notocrypta</i> sp.	—	—	—	—	—	—	—	0.0 ± 0.1	—
Lycaenidae									
<i>Rapala arata</i>	—	—	—	0.0 ± 0.1	—	—	—	—	—
Nymphalidae									
<i>Damora sagana</i>	—	—	—	—	—	—	—	0.1 ± 0.2	—
<i>Inachis io</i>	—	—	—	0.0 ± 0.1	—	—	—	—	—
<i>Minois dryas</i>	0.0 ± 0.1	—	—	—	—	—	—	—	—
<i>Speyeria aglaja</i>	—	—	—	0.0 ± 0.1	—	—	—	—	—
Papilionidae									
<i>Parnassius stubbendorffii</i>	—	—	0.0 ± 0.1	—	—	—	—	—	—

SD, standard deviation. † only studied species without framed inflorescences. See Table 3-1 for *Hydrangea* spp. codes.

Supplementary Table 3-2. Pollen accumulation and visit duration on intact inflorescences for all visitors to the *Viburnum* species.

Visitor species	Pollen attachment	Stay time in intact inflorescences	
		<i>Hydrangea</i> spp.	Stay time $\pm$ SD (sec)
Coleoptera			
Cantharidae			
<i>Lycocerus suturellus</i>	+	Hpe	169 $\pm$ 226
<i>Podabrus ishiharai</i>	+	Hsc	234 $\pm$ 0
<i>Themus episcopalis</i>	+	Hpe	80 $\pm$ 144
Cerambycidae			
<i>Aredolpona succedanea</i>	+	Hpa	35 $\pm$ 0
		Hi	35 $\pm$ 0
<i>Cyrtoclytus caproides</i>	+	Hpe	760 $\pm$ 0
<i>Demonax transilis</i>	++	Hpa	1006 $\pm$ 1020
		Hpe	33 $\pm$ 5
<i>Gaurotes doris</i>	+	Hpe	833 $\pm$ 940
<i>Idiostrangalia hakonensis</i>	+	Hpa	206 $\pm$ 352
<i>Idiostrangalia</i> sp. 2	+	Hsi	105 $\pm$ 35
<i>Judolia cometes</i>	+	Hpa	421 $\pm$ 1380
<i>Leptostrangalia lesnei</i>	+	Hsc	6 $\pm$ 0
<i>Leptura ochraceofasciata</i>	+	Hse	695 $\pm$ 0
		Hpa	570 $\pm$ 2096
		Hi	70 $\pm$ 65
<i>Nakanea vicaria</i>	+	Hpa	17 $\pm$ 19
<i>Parastrangalis nymphula</i>	+	Hpa	314 $\pm$ 793
		Hsc	2327 $\pm$ 0
<i>Parastrangalis</i> sp. 1	+	Hse	450 $\pm$ 0
<i>Parastrangalis</i> sp. 2	+	Hpa	65 $\pm$ 22
<i>Pidonia (Pidonia) signifera</i>	+	Hpe	10 $\pm$ 0
<i>Pidonia (Pidonia)</i> sp.	+	Hse	31 $\pm$ 26
<i>Pidonia aegrota</i>	+	Hpa	491 $\pm$ 448
		Hsc	821 $\pm$ 1294
		Hpe	187 $\pm$ 311
<i>Stenocorus caeruleipennis</i>	+	Hse	0 $\pm$ 0
<i>Strangalia koyaensis</i>	+	Hpa	73 $\pm$ 132
<i>Strangalomorpha tenuis aenescens</i>	++	Hpe	180 $\pm$ 433
Mordellidae			
<i>Mordellidae</i> sp.	+	Hl	943 $\pm$ 1413
Scarabaeidae			
<i>Anomala daimiana</i>	+	Hpa	—
<i>Anomala orientalis</i>	+	Hpa	1 $\pm$ 0
<i>Anomala rufocuprea</i>	+	Hpa	277 $\pm$ 298
<i>Anomala</i> sp. 1	+	Hpa	10 $\pm$ 0
<i>Eucetonia roelofsi</i>	++	Hsc	55 $\pm$ 0
<i>Gametis jucunda</i>	++	Hm	98 $\pm$ 0
<i>Lasiotrichius succinctus</i>	++	Hse	380 $\pm$ 0
		Hpa	7 $\pm$ 6
<i>Nipponovaigus</i> sp.	++	Hsc	444 $\pm$ 0
<i>Popillia japonica</i>	+	Hpa	94 $\pm$ 0
Scraptiidae			
<i>Scraptiidae</i> sp.	+	Hsc	45 $\pm$ 0
Stenotrachelidae			
<i>Cephaloon pallens</i>	+	Hpe	195 $\pm$ 0
Tenebrionidae			
<i>Tenebrionidae</i>	+	Hsc	614 $\pm$ 263

(continued)

Supplementary Table 3-2. continued

Dermaptera			
<i>Dermaptera</i> sp. 1	+	Hse	35 ± 0
<i>Dermaptera</i> sp. 2	+	Hse	144 ± 0
Diptera			
Asilidae			
<i>Asilidae</i> sp. 1	+	Hsc	7 ± 0
<i>Asilidae</i> sp. 2	+	Hi	14 ± 0
<i>Choerades komurai</i>	+	Hsc	216 ± 0
Calliphoridae			
<i>Calliphoridae</i> sp. 1	+	Hpa	37 ± 0
		Hi	19 ± 0
<i>Calliphoridae</i> sp. 2	+	Hpa	547 ± 722
<i>Calliphoridae</i> sp. 3	+	Hpe	645 ± 0
<i>Stomorphina obsoleta</i>	+	Hse	136 ± 121
		Hpa	872 ± 693
		Hi	1441 ± 1773
Drosophilidae			
<i>Drosophilidae</i> sp.	+	Hpe	6 ± 0
Empididae			
<i>Empididae</i> sp. 1	+	Hsc	52 ± 0
<i>Empididae</i> sp. 2	+	Hsc	15 ± 0
<i>Empididae</i> sp. 3	++	Hi	295 ± 321
Lauxaniidae			
<i>Lauxaniidae</i> sp.	+	Hi	93 ± 83
Muscidae			
<i>Musca</i> sp.	+	Hpe	93 ± 125
<i>Muscidae</i> sp. 1	+	Hpa	23 ± 38
<i>Muscidae</i> sp. 2	+	Hsc	24 ± 0
<i>Muscidae</i> sp. 3	+	Hpe	107 ± 0
<i>Neomya</i> sp.	+	Hsi	17 ± 15
Sarcophagidae			
<i>Sarcophagidae</i> sp. 2	+	Hse	131 ± 66
<i>Sarcophagidae</i> sp. 3	+	Hse	95 ± 131
Scathophagidae			
<i>Scathophagidae</i> sp.	+	Hpe	167 ± 225
Syrphidae			
<i>Allobaccha apicalis</i>	++	Hsi	27 ± 35
<i>Asarkina porcina</i>	++	Hi	216 ± 567
<i>Baccha</i> sp.	++	Hsi	42 ± 63
<i>Betasyrphus</i> sp. 1	++	Hse	23 ± 43
<i>Betasyrphus</i> sp. 2	++	Hi	39 ± 38
<i>Chalcosyrphus</i> sp.	++	Hsc	37 ± 0
<i>Cheilosia</i> sp. 1	++	Hsi	29 ± 23
<i>Cheilosia</i> sp. 2	++	Hse	123 ± 173
<i>Cheilosia</i> sp. 3	++	Hpa	82 ± 0
<i>Cheilosia</i> sp. 4	++	Hsc	6 ± 0
<i>Cheilosia</i> sp. 5	++	Hm	16 ± 0
<i>Cheilosia</i> sp. 6	++	Hpe	69 ± 0
<i>Chrysotoxum grande</i>	+	Hi	12 ± 10
<i>Dasyrphus bilineatus</i>	++	Hi	1 ± 0
<i>Episyrphus balteatus</i>	++	Hse	42 ± 82
		Hi	25 ± 11
		Hsc	0 ± 0
		Hpe	55 ± 76
<i>Eristalis</i> sp.	++	Hse	111 ± 238
<i>Eristalis tenax</i>	++	Hpe	26 ± 1

(continued)

Supplementary Table 3-2. continued

<i>Eupeodes bucculatus</i>	++	Hi	17 ± 22
<i>Mallota dimorpha</i>	++	Hse	34 ± 41
		Hpa	12 ± 7
		Hi	9 ± 6
<i>Mallota yakushimana</i>	++	Hse	28 ± 19
		Hpa	58 ± 63
<i>Milesia undulata</i>	+	Hi	20 ± 35
<i>Paragus</i> sp.	++	Hse	333 ± 519
<i>Pterallastes unicolor</i>	++	Hse	26 ± 11
<i>Sphaerophoria</i> sp. 1	+	Hsi	31 ± 49
<i>Sphaerophoria</i> sp. 2	++	Hse	176 ± 200
<i>Sphaerophoria</i> sp. 3	++	Hse	58 ± 80
<i>Sphaerophoria</i> sp. 4	++	Hi	147 ± 358
<i>Sphaerophoria</i> sp. 5	++	Hpe	37 ± 22
<i>Sphegina</i> sp. 1	++	Hpa	27 ± 0
<i>Sphegina</i> sp. 2	++	Hi	22 ± 0
<i>Sphegina</i> sp. 3	++	Hpe	67 ± 80
<i>Syrphus</i> sp. 1	++	Hse	47 ± 74
<i>Syrphus</i> sp. 2	++	Hpa	33 ± 64
<i>Temnostoma apiform</i>	++	Hse	5 ± 0
<i>Temnostoma nitobei</i>	++	Hi	46 ± 53
<i>Temnostoma vespiforme</i>	+	Hse	46 ± 0
<i>Volucella thompsoni</i>	++	Hpa	22 ± 11
<i>Xylota</i> sp. 1	++	Hse	19 ± 17
Tachinidae			
<i>Cylindromyia</i> sp.	+	Hsi	28 ± 53
<i>Ectophasia rotundiventris</i>	+	Hse	31 ± 34
<i>Tachinidae</i> sp. 1	+	Hsi	32 ± 0
<i>Tachinidae</i> sp. 2	+	Hsi	36 ± 0
Tipulidae			
<i>Tipulidae</i> sp.	+	Hsc	943 ± 1324
Hymenoptera			
Andrenidae			
<i>Andrena (Andrena) lapponica</i>	+++	Hpe	152 ± 222
<i>Andrena (Calomelissa) prostomias</i>	+++	Hsc	25 ± 0
<i>Andrena (Cnemidandrena) denticulata</i>	+++	Hpe	392 ± 0
<i>Andrena (Micrandrena) subopaka</i>	+++	Hpe	83 ± 82
Apidae			
<i>Bombus ardens</i>	+++	Hh	4 ± 1
<i>Bombus hypocrita</i>	+++	Hse	20 ± 141
		Hi	9 ± 9
<i>Ceratina japonica</i>	+++	Hh	75 ± 93
		Hi	23 ± 0
		Hsc	53 ± 68
		Hl	15 ± 16
<i>Nomada</i> sp. 1	++	Hsc	83 ± 0
<i>Nomada</i> sp. 2	++	Hpe	26 ± 0
Argidae			
<i>Argidae</i> sp.	+	Hse	13 ± 0
Braconidae			
<i>Braconidae</i> sp.	+	Hsi	30 ± 9
Halictidae			
<i>Lasioglossum (Evylaeus) allodatum</i>	+++	Hsi	59 ± 148
		Hpe	70 ± 109
<i>Lasioglossum (Evylaeus) apristum</i>	+++	Hpe	76 ± 79

(continued)

Supplementary Table 3-2. continued

<i>Lasioglossum (Evylaeus) caliginosum</i>	+++	Hh	86 ± 97
		Hl	17 ± 22
<i>Lasioglossum (Evylaeus) duplex</i>	+++	Hm	67 ± 103
<i>Lasioglossum (Evylaeus) pallilomum</i>	+++	Hpa	53 ± 211
		Hi	166 ± 188
<i>Lasioglossum (Evylaeus) sibiliacum</i>	+++	Hse	67 ± 80
<i>Lasioglossum (Evylaeus) vulsum</i>	+++	Hsi	16 ± 17
		Hsc	69 ± 90
		Hm	66 ± 82
<i>Lasioglossum (Lasioglossum) exiliceps</i>	+++	Hpa	18 ± 15
<i>Lasioglossum (Lasioglossum) harmandi</i>	+++	Hse	33 ± 41
		Hm	52 ± 85
<i>Lasioglossum (Lasioglossum) leviventre</i>	+++	Hse	7 ± 9
<i>Lasioglossum (Lasioglossum) nipponicola</i>	+++	Hh	7 ± 0
		Hl	13 ± 13
<i>Lasioglossum (Lasioglossum) occidens</i>	+++	Hi	21 ± 7
<i>Lasioglossum (Lasioglossum) proximatum</i>	+++	Hsc	20 ± 18
Ichneumonidae			
<i>Picardiella tarsalis</i>	+	Hsc	3 ± 0
Megachilidae			
<i>Megachile subalbata</i>	+++	Hsi	12 ± 0
Pompilidae			
<i>Anoplius</i> sp.	+	Hi	8 ± 0
Sphecidae			
<i>Sphecidae</i> sp.	+	Hse	5 ± 4
Tenthredinidae			
<i>Tenthredo nigropicta</i>	++	Hpe	110 ± 287
Vespidae			
<i>Eumenidae</i> sp.	+	Hpa	11 ± 0
<i>Polistes</i> sp.	+	Hsi	2 ± 0
Lepidoptera			
Adelidae			
<i>Nemophora aurifera</i>	++	Hpe	179 ± 27
Hesperiidae			
<i>Daimio tethys</i>	+	Hpe	56 ± 50
<i>Hesperiidae</i> sp.	+	Hh	—
<i>Notocrypta</i> sp.	+	Hpa	16 ± 0
Lycaenidae			
<i>Rapala arata</i>	+	Hse	7 ± 0
Nymphalidae			
<i>Damora sagana</i>	++	Hpa	6 ± 7
<i>Inachis io</i>	+	Hse	5 ± 0
<i>Minois dryas</i>	+	Hi	11 ± 0
<i>Speyeria aglaja</i>	+	Hse	5 ± 0
Papilionidae			
<i>Parnassius stubbendorffi</i>	++	Hpe	0 ± 0

Pollen attachment: +++, abundant; ++, moderate; +, mediocre. See Table 3-1 for *Hydrangea* spp. codes.