Biological Journal of the Linnean Society, 2020, **131**, 12–25. With 3 figures.

Florivory defence: are phenolic compounds distributed non-randomly within perianths?

SAYA NAKANO[1], MICHIO OGURO[2,*], TOMOYUKI ITAGAKI[1], and SATOKI SAKAI[1]

[1]*Graduate School of Life Sciences, Tohoku University, Sendai 980-8578, Japan*
[2]*Forestry and Forest Products Research Institute, Tsukuba 305-8687, Japan*

Received 30 March 2020; revised 31 May 2020; accepted for publication 3 June 2020

Plants might allocate chemical defences unequally within attractive units of flowers including petals, sepals, and bracts because of variations in the probability of florivory. Based on optimal defence theory, which predicts that plants allocate higher chemical defences to tissues with higher probabilities of herbivore attack, we predicted that distal parts and sepals would have higher chemical defence allocations than proximal parts and petals. To test this prediction, we compared total phenolics and condensed tannins concentrations as well as presence of florivory within attractive units of ten angiosperm species. In agreement with the prediction, the overall results showed that the distal parts had higher total phenolics and condensed tannins than the proximal parts. On the other hand, contrary to the prediction, petals and sepals showed no tissue-specific variations. Florivory was more severe on the distal parts than the proximal parts, although statistical support for the variation was slightly weak, while the variations in presence of florivory between petals and sepals differed between the distal and proximal parts. These results may support the prediction of the optimal defence theory because distal parts of attractive units had higher presence of florivory and concentration of chemical defences.

ADDITIONAL KEYWORDS: bracts – condensed tannins – florivory – floral herbivory – perianths – petals – sepals – total phenolics.

INTRODUCTION

Although most studies of plant–herbivore interactions have been focused on herbivory of leaves, other parts of plants such as flowers are also attacked by herbivores. Several studies have shown that florivory (herbivory on flowers) can have detrimental effects on plant reproduction in both direct and indirect ways (see the review by McCall & Irwin, 2006). The direct effects result from consumption of petals, stamens, stigmas or ovaries that cause reduction in probability of buds to mature (Krupnick & Weis, 1998), viable pollen (Kirk, 1987) or seed set (McCall, 2008). Indirect damage alters floral visual appearance (Mothershead & Marquis, 2000; McCall, 2008; Tsuji & Ohgushi, 2018) or scent (Lucas-Barbosa *et al.*, 2016), causing declines in pollinator visitation (but see Malo *et al.*, 2001; Soper Gorden & Adler, 2016). This reduction in reproduction can decrease population growth rates of plants (Louda & Potvin, 1995) and affect floral evolution (Ashman & Penet, 2007; Sandring *et al.*, 2007).

These direct and indirect effects of florivory have been mitigated by various defence mechanisms developed by flowering plants (see the review by McCall & Irwin, 2006). For example, flowers of some species contain phenolics (Tsuji & Sota, 2010; Alhakmani *et al.*, 2013; Oguro & Sakai, 2014), alkaloids (Adler, 2000; Alves *et al.*, 2007), and glucosinolates (Strauss *et al.*, 2004). In some species, secondary metabolites in flowers such as anthocyanins (Johnson *et al.*, 2008), nicotine (Kessler *et al.*, 2008) and furanocoumarin (Zangerl & Berenbaum, 1993) were shown to act as floral defences.

Even within a flower, plants can allocate chemical defences unevenly in different functional units such as petals, stamens and pistils (Detzel & Wink, 1993; Bravo & Copaja, 2002; Frolich *et al.*, 2006). The optimal defence theory (ODT) predicts that plants allocate more defences to tissues with higher probabilities of herbivory and/or higher fitness values (McKey, 1974; Stamp, 2003). The probability of attack by florivores may vary among the functional units of a flower. For example, anthers and perianths are attacked more often than ovaries in *Iris gracilipes* (Onodera *et al.*, 2014). Also, sepals and petals are more consumed than

*Corresponding author. E-mail: mog@ffpri.affrc.go.jp

stamens and carpels in two species of Brassicaceae (Abdalsamee & Müller, 2015). In these species, concentrations of secondary metabolites are also different among the functional units yet congruence of the distributions with the ODT is different among the species (Onodera *et al.*, 2014; Abdalsamee & Müller, 2015).

However, relative allocation of chemical defences can also differ within a functional unit because the value and/or probability of attack can be different within the functional unit. Attractive units of flowers, consisting of petals, sepals and/or bracts, would be good materials for elucidating defence allocation within a functional unit for the following reasons. First, attractive units enclose gametes and may protect them from direct effects of florivory before anthesis while they are also used for attraction of pollinators and consequently mediate indirect effects on reproduction. Therefore, these multiple relationships with florivory could cause different selection on chemical defences in different parts of attractive units of flowers. Second, probabilities of florivory attack seem to be different for different parts of attractive units. For example, distal parts of attractive units are attacked more often than proximal parts in many species (McCall, 2008: fig.1; Oguro & Sakai, 2015: fig.1; Thompson & Johnson, 2016: fig. 2B; Wakabayashi *et al.*, 2018: fig. 1; S.N. and M.O., pers. obs.) yet this pattern has not been measured quantitatively. Also, sepals are exposed to the external environment longer than the other parts of flowers during their lives (from bud formation to blooming), and hence the sepals may suffer florivory more often. In fact, attacks by insect larvae occur more often in the bud stage than in the open flower stage in *I. gracilipes* (Oguro & Sakai, 2009), and the sepals are damaged more severely than the petals in this species (M.O., pers. obs.). Yet, despite the possibility that variations in probability of florivory could have caused part-dependent allocation of chemical defences, little is known about relative allocation of chemical defences within attractive units of flowers. As far as we know, chemical variations between petals and sepals have only been studied in *Acanthus mollis* (Bravo & Copaja, 2002) and *Phalaenopsis* hybrids (Frolich *et al.*, 2006).

In this study, we examined relative allocation of chemical defences and presence of florivory within attractive units of flowers in ten angiosperm species in north-east Japan. Although many plants are known to produce specific chemical compounds that contribute to defence against herbivory, such as nicotine in tobacco (Steppuhn *et al.*, 2004) and glucosinolates in the Brassicales (Rask *et al.*, 2000), we focused on phenolic compounds for the measure of chemical defences because they are ubiquitous putative defence compounds found in plants (Appel, 1993) and could be examined in many species by measuring total phenolics and condensed tannins. Phenolic compounds are thought to act as plant defences against herbivory and to be negatively related to the intensity of herbivory (Pearse & Hipp, 2009; Kurokawa *et al.*, 2010; Fan *et al.*, 2016), though some researchers doubt their defence functions (Bernays *et al.*, 1981; Hemming & Lindroth, 1995; Close & McArthur, 2002). Based on the observed variations in the intensity of florivory and the optimal defence theory, we predicted that both the concentrations of phenolic compounds and the presence of florivory would be higher in sepals than in petals, and higher in distal parts than proximal parts of sepals, petals and bracts.

MATERIAL AND METHODS

Study species

We selected ten perennial species (Table 1) whose flowers are mono-coloured or have a uniform pattern along their attractive units, based on visual inspection. We selected these species to obtain sufficient samples for chemical measurements and to minimize the effects of colour pattern on distributions of chemical compounds throughout the attractive units. One exceptional species (*Houttuynia cordata*) having no perianth but white showy petaloid bracts (Tucker, 1981) was also used. In Japan, this species propagates by underground stems and by parthenogenesis (Shibata & Miyake, 1908; Mihara, 1960). Hence, although pollinator attraction might not be necessary, *H. cordata* still maintains showy petaloid bracts, and before opening, these cover the spikes of flowers consisting of pistils and stamens. In addition, because *H. cordata* bracts are sometimes severely damaged (S.N., pers. obs.), we included them in this study, and treated their showy bracts in the same way as the petals and sepals of other species. For species belonging to the Asteraceae (*Aster ageratoides*, *Aster microcephalus* and *Helianthus tuberosus*), we used ray flowers because dissecting small tubular flowers was impractical. *Aquilegia buergeriana* has two floral phenotypes (purple and yellow) and we measured the chemical defences and florivory separately for each phenotype.

Study sites

We collected samples from twelve locations in Miyagi, Iwate and Aomori prefectures in northern Japan (Table 1). Aobayama, Aramaki and Kawauchi are suburbs of Sendai city, Miyagi Prefecture, where we sampled flowers growing in natural vegetation facing paved or unpaved roads. Kagitoriyama and Izumigatake are mountainous regions in Miyagi Prefecture, where we collected samples growing in deciduous-coniferous

Table 1. Detailed information about the species and sampling sites. Numbers of flowers and sample groups for each sampling site are also shown

Family	Species	Size of one flower or flower head (cm)	Segmentation	Site name	Location	Numbers of flowers used for chemical traits measurements	Numbers of sample groups used for chemical traits measurements	Numbers of flowers used for floriovry measurement
Asparagaceae	*Hosta sieboldii* (Paxton) J.W.Ingram var. *sieboldii* f. *spathulata* (Miq.) W.G.Schmid	4–5	Distal/proximal	Aramaki	38°15'27.7"N 140°50'32.4"E	50	10	25
				Izumigatake	38°23'20.5"N 140°43'09.7"E	50	10	50
Asphodelaceae	*Hemerocallis fulva* L. var. *kwanso* Regel	10	Distal/proximal of petal/sepal	Aobayama	38°15'31.9"N 140°51'21.0"E	15	15	30
Asteraceae	*Aster ageratoides* Turcz. var. *ageratoi*des	1.5–2	Distal/proximal	Kagitoriyama	38°14'24.4"N 140°49'50.1"E	100	5	50
Asteraceae	*Aster microcephalus* (Miq.) Franch. et Sav. var. ovatus (Franch. et Sav.) Soejima et Mot.Ito	2.5	Distal/proximal	Kawauchi	38°15'37.6"N 140°50'57.4"E	60	4	50
				Tsuta	40°35'52.4"N 140°57'16.4"E	90	6	50
Asteraceae	*Helianthus tuberosus* L.	5–10	Distal/proximal	Koeji	38°14'45.8"N 140°52'49.3"E	15	15	50
				Nakanose	38°15'38.4"N 140°51'30.4"E	10	10	0
Liliaceae	*Cardiocrinum cordatum* (Thunb.) Makino	12–18	Distal/proximal of petal/sepal	Aobayama	38°15'17.7"N 140°51'23.4"E	30	10	50
Liliaceae	*Lilium auratum* Lindl.	20–25	Distal/proximal of petal/sepal	Aramaki	38°15'30.8"N 140°50'34.4"E	10	10	25
				Kagitoriyama	38°14'27.2"N 140°49'30.6"E	5	5	25
Ranunculaceae	*Aconitum japonicum* Thunb. subsp. *subcuneatum* (Nakai) Kadota	3.5–4.5	Distal/proximal of petal/sepal†	Tsuta	40°35'52.4"N 140°57'16.4"E	100	10	50
				Tashiro	40°41'59.5"N 140°55'10.9"E	150	15	200

Table 1 Continued

Family	Species	Size of one flower or flower head (cm)	Segmentation	Site name	Location	Numbers of flowers used for chemical traits measurements	Numbers of sample groups used for chemical traits measurements	Numbers of flowers used for floriovry measurement
Ranunculaceae	*Aquilegia buergeriana* Siebold et Zucc. var. *oxysepala* (Trautv. et C.A.Mey.) Kitam. (Purple sepal)	3–3.5	Distal/ proximal of petal/pepal	Hayasaka	39°50'58.2"N 141°30'29.9"E	30	5	50
				Sodeyama	40°01'42.3"N 141°32'28.8"E	30	5	50
				Hakkoda	40°38'54.5"N 140°51'07.8"E	30	5	50
	Aquilegia buergeriana Siebold et Zucc. var. *oxysepala* (Trautv. et C.A.Mey.) Kitam. (Yellow sepal)			Hayasaka	39°50'58.2"N 141°30'29.9"E	18	3	0
				Sodeyama	40°01'42.3"N 141°32'28.8"E	30	5	50
Saururaceae	*Houttuynia cordata* Thunb.	(bracts)	Distal/ proximal	Aobayama	38°15'33.0"N 140°51'20.9"E	50	5	50
		3–4		Aramaki	38°15'31.6"N 140°50'31.2"E	150	15	150
				Kawauchi	38°15'27.6"N 140°50'49.1"E	50	5	50

†Because petals of *A. japonicum* are too small for separate measurements of chemical traits for distal and proximal parts, chemical traits of entire petals were measured for this species.

mixed forests. Koeji and Nakanose are also suburbs of Sendai City, where we collected samples growing in natural vegetation along the Hirose River. Tsuta, Tashiro and Hakkoda are mountainous regions in Aomori Prefecture, where we sampled flowers growing in damp vegetation in Tsuta, in dry vegetation in Tashiro, and in natural vegetation in the Botanical Garden of Tohoku University in Hakkoda. Hayasaka and Sodeyama are plateau grassland areas in Iwate Prefecture, where we sampled flowers growing in natural vegetation.

Sample collection

We sampled non-damaged flowers without apparent colour changes caused by aging for chemical measurement during June to September 2016. For the chemical trait measurements, a minimum amount of dry mass was required. Although measuring the chemical traits for each individual would be ideal, for many species it was not possible to obtain sufficiently large samples from single individuals. Therefore, to keep some natural variation in the chemical traits among individuals and simultaneously fulfil the minimum requirement for the chemical measurements, we made sample groups consisting of several individuals. Because the number of flowers needed for one measurement depended on the flower size, we collected a suitable number of flowers for each group for each species (Table 1). In each location, we randomly selected patches of several individuals so that we could treat flowers collected from one patch as one sample group. We collected flowers for each sample group from individuals growing close to each other. All procedures after collection of flowers were applied to each sample group so that flowers from different groups were never mixed together. We cut flowers at their pedicels or peduncles and returned them to the laboratory as soon as possible.

In the laboratory, we separated attractive units, i.e. petal, sepal and bract samples into several parts using scissors or razors to compare the variations in chemical concentrations among them. Because of the diversity of floral morphology, we couldn't apply a single segmentation method to all species. Therefore, we applied simple anatomical segmentations for most species but applied specific segmentation methods for some species. Detailed segmentation methods for each species are shown in the Supporting Information (Appendix S1; Fig. S1; Table S1) and the list of segmentation methods of attractive units of flowers for each species and assignment of categories [i.e. position (distal or proximal) and part (petal or sepal)] for each segmented attractive unit is shown in Supporting Information (Table S2).

During sample dissection, we removed any pollen attached to floral samples by brushing gently. For the species having spurs (*A. japonicum* and *A. buergeriana*), we also removed nectar from them. We dried the separated samples with silica gel and kept them at room temperature in the silica gel until they were used for the chemical traits measurement.

Chemical trait measurement

Before the chemical traits measurement, we ground dried samples of a segmented group into powder using Micro Smash™ MS-100R (Tomy Digital Biology Co., Ltd., Tokyo, Japan). Following the Folin-Ciocalteu method (Julkunen-Tiitto, 1985), we measured total phenolics concentration using tannic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as the standard. We followed the butanol-hydrochloric acid-iron assay to measure condensed tannin concentration using commercially available cyanidin chloride as the standard (Makkar *et al.*, 1999). The concentrations of total phenolics and condensed tannins were calculated from the absorbance values obtained using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). To control for possible effects of variations in conditions among the measurement batches (i.e. sets of samples measured simultaneously), we measured all flower parts from the same sample group in one measurement batch and measured only one sample group in one batch for each species so that the fluctuation among measurement batches similarly affects all flower parts.

In addition, our preliminary measurements showed that measurements with different sample weights resulted in slightly different measured values even for the same sample (Supporting Information, Fig. S2). To reduce the effects of sample weight variation, we equalized the sample weight of each part to 15 mg, the standard weight of a powdered sample needed for the measurements, when possible. However, because some separated parts of some sample groups lacked the required weight, we equalized the sample weight of each part to the minimal weight among the parts in the same sample group. Sample weights for sample groups for each species are shown in Supporting Information (Table S3). Because the dry weights were too light to equalize, we omitted the distal part of *H. sieboldii* in three sample groups. Also, because the weights of distal and proximal samples of lateral sepals in eight sample groups of *A. japonicum* were less than 5 mg, the chemical traits were measured using mixed samples of distal and proximal parts to avoid inaccurate measurement due to the measurement limit of the spectrometer. These measurements were omitted from the analyses comparing distal and proximal parts (see *Statistical analyses* section below).

Measurements for presence of florivory

It was impractical to precisely measure the amount of florivory for each part of attractive units for species in the Asteraceae because they have many small ray flowers and in the Ranunculaceae because they have complex floral structures. To apply the same method to all species and all parts of attractive units, we measured florivory as presence/absence. During the sample collections for the chemical measurements, we measured presence of florivory for randomly selected flowers. We recorded signs (i.e. missing parts on petals, sepals and bracts) of florivory damage for proximal and distal parts of petals/sepals/bracts of each sampled flower in a binary manner. After the measurements, non-damaged flowers without apparent colour changes due to aging were included in the chemical measurement samples. The numbers of flowers examined are shown in Table 1.

Statistical analysis

All analyses were done using R v.3.6.0 (R Core Team, 2019) unless otherwise noted. Because attractive parts of flowers measured in this study consist of analogous tissues (petals, sepals and bracts), we did not include phylogenetic information in the analyses described below. We did not use a model selection procedure with Akaike's information criteria (AIC) because the models had only up to three fixed effects (Table 2, 3) and the purpose of such procedure is improving the predictive ability of the models (Hastie *et al.,* 2008), which was not our purpose (Tables 2, 3 in Results).

Chemical traits

Variations in total phenolics and condensed tannins between distal and proximal parts of the petals/sepals/bracts in all species were tested using generalized linear mixed models (GLMMs) with the glmmADMB package v.0.8.3.3, which estimates parameters by maximum likelihood, using the Laplace approximation via AD Model Builder software (Fournier *et al.*, 2012; Skaug *et al.*, 2016). For these analyses, the concentrations of total phenolics and condensed tannins were used for the response variables and the positions of the attractive units (distal or proximal) were used as the explanatory variables of the models. For the error distribution of the models, a gamma distribution with the log link function was used because the distributions of the chemical traits were closer to gamma distribution than Gaussian distribution (Supporting Information,

Table 2. Results of the GLMMs analysing variations in total phenolics and condensed tannins between the positions (distal and proximal) and the parts (petal and sepal)

Response		df	*F*	*P*
(A) Total phenolics	Position (proximal/distal)	1	5.63	0.018*
	Residuals	706		
(B) Condensed tannins	Position (proximal/distal)	1	5.48	0.019*
	Residuals	706		
(C) Total phenolics	Part (sepal/petal)	1	2.76	0.097†
	Residuals	510		
(D) Condensed tannins	Part (sepal/petal)	1	0.99	0.319
	Residuals	510		

*P <0.05; †P <0.1.

Table 3. Results of the GLMMs analysing variations in presence of florivory between the positions (distal and proximal) and the parts (petal and sepal)

Response		df	χ^2	*P*
(A) Presence of florivory	Position (proximal/distal)	1	3.72	0.054†
(B) Presence of florivory	Position (proximal/distal)	1	0.00	0.988
	Part (sepal/petal)	1	0.57	0.450
	Position × Part	1	5.88	0.015*

*P <0.05; †P <0.1.

Appendix S2, Table S4). Because the values of the chemical traits as well as the variations between distal and proximal parts could be different among species, we included species as random slope and random intercept terms (Zuur *et al.*, 2009) in the models so that we could obtain a general trend for all species as well as estimated variations for each species from one model. Because the two colour morphs in *A. buergeriana* had different chemical trait values and the variation was even larger than variations between distal and proximal parts or between sepals and petals in other species (Supporting Information, Table S2), we treated them as separate "species" for the random effect. Also, to control statistical dependence of the samples collected in the same location and in the same sample group, nested random effects (the sample group is nested within the location) were included as random intercepts in the models. Finally, to control statistical dependence of samples measured in the same measurement batches, batch number was included as a random intercept in the models.

Variations in the chemical traits between petals and sepals were also tested by GLMMs. At first, we tried preliminary analyses by GLMMs with similar settings as the models described above using the four species for which we measured the chemical traits for distal and proximal parts of petals and sepals (i.e. *H. fulva, C. cordatum*, *L. auratum* and both phenotypes of *A. buergeriana*). In the models, the position (distal/proximal), part (petal/sepal) and their interaction were used as explanatory variables. However, the preliminary analyses did not find significant interactions between the position and part terms (Supporting Information, Table S5). Therefore, we compared petals and sepals by including both proximal and distal samples because by omitting the position and interaction terms from the model, we could include *A. japonicum* in the analyses. Omission of these terms caused the main effect of part to become significant, likely due to increased sample size (see *Results* section and Table 2). For the models testing the chemical traits between petals and sepals, we used the same response variables, error distribution, link function and random factors as the models described above but used part of flower (petal or sepal) as an explanatory variable. The *P* values of the models testing variations in the chemical traits among floral parts and positions were obtained by the likelihood ratio test with the *F* statistic using the *car* package (Fox & Weisberg, 2019) in R.

Florivory

Presence of florivory in all species was compared between proximal and distal parts by a GLMM. The response variable of the model was presence of florivory on each position of petals/sepals/bracts and the explanatory variable of the model was position (proximal or distal). For the error distribution of the model, a binomial distribution with the logit link function was used. For the same reason as in the models analysing the chemical traits, species was included as a random slope and a random intercept in the model. The sampling location and flower ID nested within sampling location were also included as random intercepts in the model.

Presence of florivory was also compared between petals and sepals using the species having both petals and attractive sepals (*A. japonicum*, *H. fulva, C. cordatum*, *L. auratum* and both phenotypes of *A. buergeriana*) by a GLMM. For the explanatory variables of the model, position (proximal or distal), part (petal or sepal) and their interaction were used. For the response variable, error distribution, link function and random factors, the settings were the same as the model testing variation in florivory between distal and proximal parts. The *P* values of the models were obtained using methods similar to those used with the models for the chemical traits, except that the χ^2 statistic was used for the calculation of *P*.

RESULTS

Chemical traits

Although there were large variations in total phenolics and condensed tannins among the species (Figs 1-2), considering all the species together, the distal parts of the attractive units had 30% higher total phenolics (Fig. 1A) and 15% higher condensed tannins (Fig. 2A) concentrations than the proximal parts: results of the GLMMs showed significant variations in the total phenolics and condensed tannins between distal and proximal parts (Table 2A, B). This was also true for most of the individual species, but the magnitude of the variations were species-specific and total phenolics in petals of *H. tuberosus* showed an opposite trend (Fig. 1A). On the other hand, while sepals appeared to have 60% and 27% higher concentrations of total phenolics (Fig. 1B) and condensed tannins (Fig. 2B) than petals, respectively, these variations were not significant (Table 2C, D).

Florivory

The distal parts of attractive units had 203% higher probability of presence of florivory than the proximal parts as we expected. However, the analysis provided slightly weak statistical support for this idea ($P = 0.054$) when all species were considered

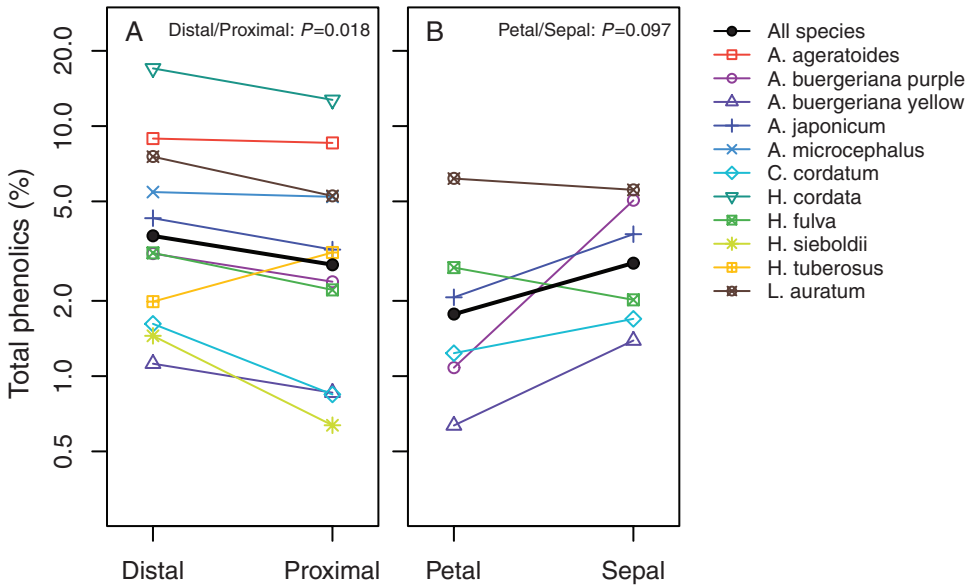

Figure 1. Estimated variations in the total phenolics concentration (A) between the distal and proximal parts of the petals/sepals/bracts in all ten species and (B) between the petals and sepals in five species. The thick black lines represent estimated variations for all species and the thin lines represent those for individual species.

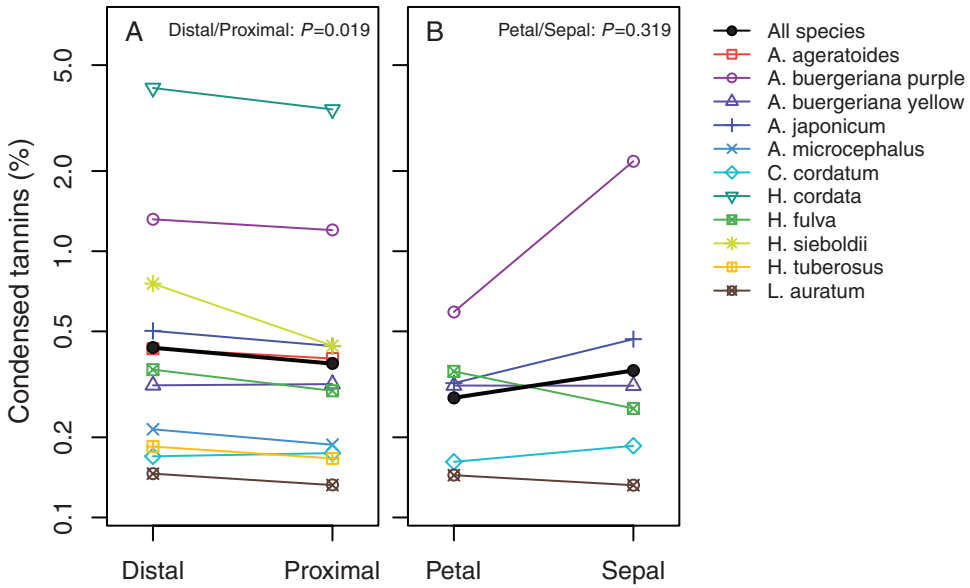

Figure 2. Estimated variations in the condensed tannins concentration (A) between the distal and proximal parts of the petals/sepals/bracts in ten species and (B) between the petals and sepals in five species. The thick black lines represent estimated variations for all species and the thin lines represent those for individual species.

together (Fig. 3A; Table 3A). For most of the individual species, the distal parts of attractive units had a higher presence of florivory than the proximal parts; however, both phenotypes of *A. buergeriana* showed opposite trends (Fig. 3A). The variations in florivory between the petals and sepals were also different between the distal and proximal parts: the result of the GLMM using the five species having petals and attractive sepals showed significant interaction between the position (distal or proximal) and part (petal or sepal) (Fig. 3B; Table 3B). The model showed that the sepals had a 73% higher presence of florivory

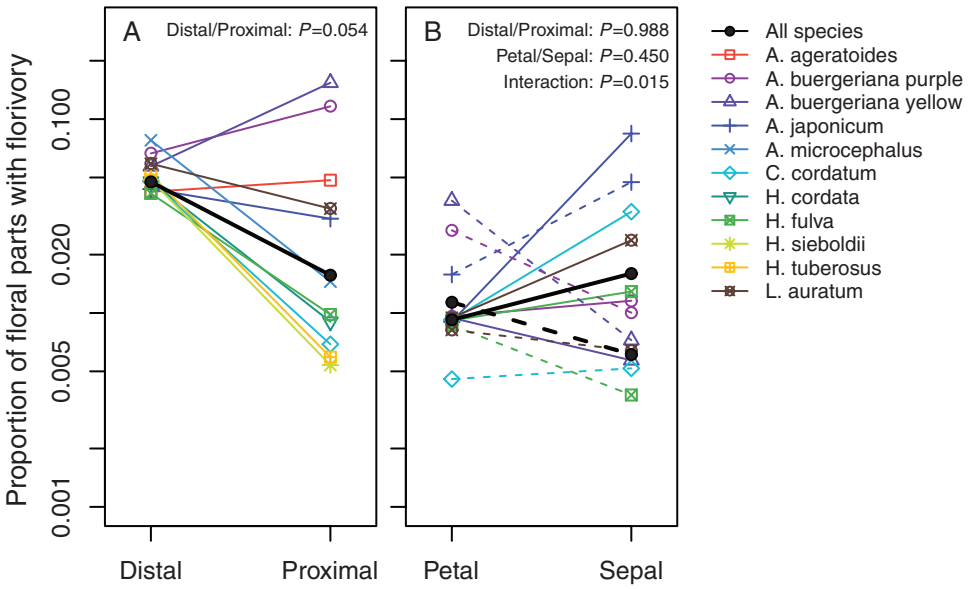

Figure 3. Estimated variations in the proportion of attacked floral parts (A) between the distal and proximal parts of the petals/sepals/bracts in ten species and (B) between the distal and proximal parts of the petals and sepals in five species. The thick black lines represent estimated variations for all species and the thin lines represent those for individual species. The solid and dashed lines in panel (B) represent estimated variations for the distal and proximal parts, respectively. Note that because the values plotted in the two panels were derived from different models with different datasets, the mean values for the distal and proximal parts are different for each panel.

than the petals in the distal parts whereas the petals had a 86% higher presence of florivory than the sepals in the proximal parts (Fig. 3B). When looking for the estimated variations for each species, most species showed higher or similar presence of florivory on the distal and proximal parts of sepals; however, *H. fulva* and both phenotypes of *A. buergeriana* showed much higher presence of florivory in the proximal parts of the petals (Fig. 3B).

DISCUSSION

Variations in the phenolic compounds and florivory between distal and proximal parts of attractive units

Although part-dependent presence of florivory within attractive units has been observed in many species (McCall, 2008; Oguro & Sakai, 2015; Thompson & Johnson, 2016; Wakabayashi *et al.*, 2018), uneven distribution of florivory and chemical defences within attractive units has not been measured quantitatively. This is the first study measuring part-dependent florivory and allocation of chemical defences within attractive units of flowers, as far as we know. The results showed that, in general, the distal parts of attractive units had higher total phenolics and condensed tannins concentrations than the proximal parts (Figs 1A, 2A). Also, the distal parts tended to suffer more florivory: nine out of eleven comparisons showed distal parts having more presence of florivory (Fig. 3A). However, the *P* value of the main effect was slightly higher than 0.05 (Table 3A), though it is possible that the analysis had weak statistical power due to the small number of flowers used for the measurement (Table 1). Also, the correlations between relative allocation of the chemical defences and variations in the presence of florivory within attractive units were weak (Supporting Information, Appendix S3). Therefore, the observed variations in total phenolics and condensed tannins might be in part explained by the prediction of the ODT [i.e. plants allocate more defences to the parts with higher probability of attack (McKey, 1974; Stamp, 2003)], but statistical support for the prediction was slightly weak.

This weak support for the prediction of the ODT might be explained by one of the following reasons. First, although we measured phenolic compounds as a determinant of florivory, other chemical properties of attractive units could also affect preferences of insects. For example, plants or parts of plants having higher water and nitrogen (Coley, 1987; Johnson *et al.*, 2014) and lower phosphorus (Oguro & Sakai, 2015) tended to suffer herbivory. Onodera *et al.* (2014) found that the concentrations of nitrogen and phosphorus differed among the anther, ovary and perianth and showed their

possible relationships with florivory. Therefore, part-dependent distribution of other chemical properties within attractive units could modify the relationships between the chemical traits we measured and florivory.

Second, spatiotemporal variation of florivory could weaken the relationship between relative allocation of the chemical defences and variations in presence of florivory within attractive units. It is known that intensity of florivory is different among sites and years (Oguro & Sakai, 2009; Kawagoe & Kudoh, 2010). We measured florivory in one location in 1 year for each species. This design might make it difficult for us to detect the relationship between chemical defences and florivory due to the spatiotemporal variation of florivory. In addition, the large variation in the chemical traits and florivory among the species (Figs 2-3) might weaken our ability to detect the relationship between chemical defences and florivory owing to the small number of species used in the present study.

Although the ODT assumes that plant defences reduce herbivory (Stamp, 2003), whether florivores can respond to part-dependent allocation of plant defences within attractive units is unknown because feeding site selection by herbivores including florivores might be affected by visual and olfactory cues of flowers. To the best of our knowledge, no studies have investigated the relative importance of chemical defences and visual and olfactory cues, but because feeding behaviour of herbivores would have evolved to maximize their fitness (Pyke *et al.*, 1977), their preferences for feeding sites based on visual and/or olfactory cues would relate to the nutritional value of plant tissues. However, little is known about the effect of within-plant distribution of chemical defences on herbivore foraging (Kohler *et al.*, 2015) or about the clues herbivores use for feeding site selection. What is known so far indicates herbivores can respond to within-plant allocation of defences. The best-known cases are the many specialist herbivores of the Brassicales: they select floral tissues having higher nutrition and glucosinolates using gravity and/or visual rather than olfactory cues (Smallegange *et al.*, 2007; Bandeili & Muller, 2010; Abdalsamee & Müller, 2015; Tsuji *et al.*, 2018). Moreover, Shroff *et al.* (2008) showed that herbivores can even respond to spatial variations in chemical defences within leaves: generalist lepidopteran larvae *Helicoverpa armigera* avoided the midvein and periphery of *Arabidopsis thaliana* leaves containing high glucosinolate concentrations and fed almost exclusively on the inner lamina. The results from these studies suggest that florivores may be able to respond to part-dependent chemical defences if they affect their performance.

Although we explained allocation patterns of the phenolic compounds by probability of attack, the ODT predicts that the values of plant parts also affect the allocation pattern of defences (McKey, 1974; Rhoades, 1979; Stamp, 2003). Therefore, although no previous studies elucidated the relationship between defence allocation and values of tissues within flowers, variations in values within attractive units could lead to variations in defence allocation within them. In fact, Morinaga and Sakai (2006) showed that different parts of the perianth have different values for pollinator attraction and fitness in *I. gracilipes* by the experimental manipulation of perianth size. Future studies investigating values of parts as well as intensity of florivory on different parts of attractive units would help our understanding of evolution of chemical defences within attractive units.

Variations in the phenolic compounds and florivory between the petals and sepals

Contrary to our prediction, there were no significant variations in total phenolics (Fig. 1B) and condensed tannins (Fig. 2B) concentrations between petals and sepals. These unclear patterns might be partly due to the complex pattern of presence of florivory between the petals and sepals: although we expected sepals to have a higher presence of florivory, this was only observed for the distal parts and a weak opposite pattern was observed in the proximal parts in all the species studied (Fig. 2B). Also, the variations in presence of florivory among the distal and proximal parts of petals and sepals differed among the species (Fig. 2B). This species-specific and position-dependent pattern of florivory on the petals and sepals might have imposed different selection pressures on the relative allocation of phenolic compounds between petals and sepals.

Species-specific morphology of attractive units might play some role in determining the observed presence of florivory among the petals and sepals. The higher presence of florivory on the petals than the sepals was most remarkable for the proximal parts of both phenotypes of *A. buergeriana* (Fig. 3B). In *A. buergeriana*, the proximal parts of petals have spurs and signs of florivory on the petals were mostly observed on spurs. Therefore, we suspect these signs might include floral damage caused by nectar robbers. This is because *A. buergeriana* is mainly pollinated by bumblebees (Misaki *et al.*, 2018), which act as nectar robbers for many plant species (Irwin, 2003; Mayer *et al.*, 2014; Soper Gorden & Adler, 2016; Ye *et al.*, 2017) and species having nectar spurs likely experience nectar robbing (Irwin & Maloof, 2002). On the other hand, among the species studied, *A. japonicum* also has spurs but their spurs were not attacked by florivores or nectar robbers in our observations (Fig. 3B). The variation in the pattern of florivory on spurs between the

two species might be caused by their morphological differences in arrangement of floral parts: the spurs of *A. buergeriana* are exposed to the outside of the flowers whereas those of *A. japonicum* are covered by sepals and invisible from the outside of the flowers. Although interactions between florivores and nectar robbers have not been well studied, Soper Gorden and Adler (2016) showed that floral condensed tannins reduce visitation of nectar robbers and floral anthocyanins (phenolic compounds) affect both florivores and nectar robbers in *Impatiens capensis*. Therefore, not only florivores but also nectar robbers might affect evolution of floral defence allocations. This species-specific morphology and its relationship with florivores and nectar robbers might have contributed to the inconsistent pattern of defence allocation in petals and sepals.

Limitations of the current study

Although we regarded the concentration of total phenolics and tannins as measures of a plant's defence against florivory, phenolic compounds are known to have other functions in plants such as pollinator attraction (Harborne & Williams, 2000) and UV damage protection (Stapleton & Walbot, 1994; Rice-Evans *et al.*, 1997; Harborne & Williams, 2000). Furthermore, there are several examples of the same phenolic compounds having multiple roles. Floral anthocyanin may act as a floral colour and a florivory defence simultaneously (Johnson *et al.*, 2008). Phenolics acting in UV resistance also act as defences against herbivory (Grant-Petersson & Renwick, 1996; Rousseaux *et al.*, 2004). Therefore, we think these biotic/abiotic factors other than florivory might affect evolution of phenolic compounds in flowers.

In this study, we selected species by human-visible colour of flowers. However, some pigments including phenolics are not visible to the human eye (Eisner *et al.*, 1969). In the Asteraceae, this invisible colour pattern on petals is known to result from the distribution of UV-absorbing phenolic compounds (flavonols) (Schlangen *et al.*, 2009). In the species used in this study, *H. tuberosus* (Asteraceae; Supporting Information, Fig. S3) and *H. fulva* (Hirota *et al.*, 2019) are known to have invisible an colour pattern on proximal parts of attractive units. Such invisible colour might have some effects on the measured chemical trait values, yet the effect would not be so simple because *H. tuberosus* had higher total phenolics on the proximal parts whereas *H. fulva* showed opposite pattern (Fig. 1).

Although we measured the chemical traits and florivory of open flowers, flowers can be attacked by florivores before flower opening (Oguro & Sakai, 2009, 2015). Our previous study (Oguro & Sakai, 2014) showed that concentrations of total phenolics did not differ between buds and flowers in 34 Asteraceae species; however, relative allocation of chemical defences within attractive units may differ depending on floral development. To understand the role of chemical defences on plant-florivore interactions in more detail, further studies examining relative allocation of chemical defences and occurrence of florivory on several developmental stages of flowers would be helpful.

CONCLUSION

We found consistent uneven distribution patterns of phenolic compounds and florivory within attractive units among species: in agreement with our prediction, the distal parts generally had higher levels of chemical defences and presence of florivory than the proximal parts in the ten species studied, although the statistical support for the variation in florivory was slightly weak. Among the functional units in flowers, attractive units are unique because they can have several functions such as protecting the inner reproductive tissues and advertising to pollinators. Also, the main function can differ between the bud and flowering stages. Moreover, phenolics can serve as defensive compounds, pigments and UV protectants in plants (Harborne & Williams, 2000). Although in this study we examined only florivores as potential selection agents affecting distribution of phenolics, other factors such as pollinator attraction and UV protection could act as selection pressures causing the observed distribution. Thus, attractive units may acquire elaborate mechanisms to maintain their functions, balancing several effects of phenolics in the biotic and abiotic environments in which they live. Considering all such roles of phenolics in each part of attractive units may help us to reveal the more exact functions of phenolics and to understand the evolution of chemical defence allocation within attractive units.

ACKNOWLEDGEMENTS

We thank H. Sasaki, J. Mochizuki, K. Hoshi, M. Ito and Y. Aoyagi for their help during the field work, H. Kurokawa, M. Kawabe, Y. Matsumoto and J. Aoki for their advice during the chemical measurements, T. Fukuhara for permitting us to use the pictures of *H. tuberosus*, two anonymous reviewers for their helpful comments, and J. Glazebrook for carefully proofreading the manuscript. We also appreciate the valuable suggestions of our colleagues, particularly

T. Nakashizuka, K. Hikosaka, M. Aiba, R. Oguchi and K. Wakabayashi, throughout this study.

REFERENCES

Abdalsamee MK, **Müller C. 2015.** Uncovering different parameters influencing florivory in a specialist herbivore. *Ecological Entomology* **40:** 258–268.

Adler LS. 2000. Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *American Naturalist* **156:** 92–99.

Alhakmani F, **Kumar S**, **Khan SA. 2013.** Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pacific Journal of Tropical Biomedicine* **3:** 623–627.

Alves MN, **Sartoratto A**, **Trigo JR. 2007.** Scopolamine in *Brugmansia suaveolens* (Solanaceae): defense, allocation, costs, and induced response. *Journal of Chemical Ecology* **33:** 297–309.

Appel HM. 1993. Phenolics in ecological interactions: the importance of oxidation. *Journal of Chemical Ecology* **19:** 1521–1552.

Ashman T-L, **Penet L. 2007.** Direct and indirect effects of a sex-biased antagonist on male and female fertility: consequences for reproductive trait evolution in a gender-dimorphic plant. *The American Naturalist* **169:** 595–608.

Bandeili B, **Muller C. 2010.** Folivory versus florivory-adaptiveness of flower feeding. *Naturwissenschaften* **97:** 79–88.

Bernays EA, **Chamberlain DJ**, **Leather EM. 1981.** Tolerance of acridids to ingested condensed tannin. *Journal of Chemical Ecology* **7:** 247–256.

Bravo HR, **Copaja SV. 2002.** Contents and morphological distribution of 2,4-dihydroxy-1,4-benzoxazin-3-one and 2-benzoxazolinone in *Acanthus mollis* in relation to protection from larvae of *Pseudaletia impuncta*. *Annals of Applied Biology* **140:** 129–132.

Close DC, **McArthur C. 2002.** Rethinking the role of many plant phenolics – protection from photodamage not herbivores? *Oikos* **99:** 166–172.

Coley PD. 1987. Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. *New Phytologist* **106:** 251–263.

Detzel A, **Wink M. 1993.** Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology* **4:** 8–18.

Eisner T, **Silberglied RE**, **Aneshansley D**, **Carrel JE**, **Howland HC. 1969.** Ultraviolet video-viewing: the television camera as an insect eye. *Science* **166:** 1172.

Fan X, **Fan B**, **Wang Y**, **Yang W. 2016.** Anthocyanin accumulation enhanced in *Lc*-transgenic cotton under light and increased resistance to bollworm. *Plant Biotechnology Reports* **10:** 1–11.

Fournier DA, **Skaug HJ**, **Ancheta J**, **Ianelli J**, **Magnusson A**, **Maunder MN**, **Nielsen A**, **Sibert J. 2012.** AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* **27:** 233–249.

Fox J, **Weisberg S. 2019.** *An R companion to applied regression*. Thousand Oaks: Sage.

Frolich C, **Hartmann T**, **Ober D. 2006.** Tissue distribution and biosynthesis of 1,2-saturated pyrrolizidine alkaloids in *Phalaenopsis* hybrids (Orchidaceae). *Phytochemistry* **67:** 1493–1502.

Grant-Petersson J, **Renwick JAA. 1996.** Effects of ultraviolet-B exposure of *Arabidopsis thaliana* on herbivory by two crucifer-feeding insects (Lepidoptera). *Environmental Entomology* **25:** 135–142.

Harborne JB, **Williams CA. 2000.** Advances in flavonoid research since 1992. *Phytochemistry* **55:** 481–504.

Hastie T, **Tibshirani R**, **Friedman J. 2008.** Model assessment and selection. In: Hastie T, Tibshirani R and Friedman J, eds. *The elements of statistical learning data mining, inference, and prediction*. 2nd ed. New York: Springer-Verlag, 219–260. Available at:https://www.springer.com/jp/book/9780387848570.

Hemming JDC, **Lindroth RL. 1995.** Intraspecific variation in aspen phytochemistry - effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* **103:** 79–88.

Hirota SK, **Miki N**, **Yasumoto AA**, **Yahara T. 2019.** UV bullseye contrast of *Hemerocallis* flowers attracts hawkmoths but not swallowtail butterflies. *Ecology and Evolution* **9:** 52–64.

Irwin RE. 2003. Impact of nectar robbing on estimates of pollen flow: conceptual predictions and empirical outcomes. *Ecology* **84:** 485–495.

Irwin RE, **Maloof JE. 2002.** Variation in nectar robbing over time, space, and species. *Oecologia* **133:** 525–533.

Johnson E, **Berhow M**, **Dowd P. 2008.** Colored and white sectors from star-patterned petunia flowers display differential resistance to corn earworm and cabbage looper larvae. *Journal of Chemical Ecology* **34:** 757–765.

Johnson MTJ, **Ives AR**, **Ahern J**, **Salminen JP. 2014.** Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist* **203:** 267–279.

Julkunen-Tiitto R. 1985. Phenolic constituents in the leaves of northern willows - methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* **33:** 213–217.

Kawagoe T, **Kudoh H. 2010.** Escape from floral herbivory by early flowering in *Arabidopsis halleri* subsp. *gemmifera*. *Oecologia* **164:** 713–720.

Kessler D, **Gase K**, **Baldwin IT. 2008.** Field experiments with transformed plants reveal the sense of floral scents. *Science* **321:** 1200–1202.

Kirk WDJ. 1987. How much pollen can thrips destroy? *Ecological Entomology* **12:** 31–40.

Kohler A, **Maag D**, **Veyrat N**, **Glauser G**, **Wolfender J-L**, **Turlings TCJ**, **Erb M. 2015.** Within-plant distribution of 1,4-benzoxazin-3-ones contributes to herbivore niche differentiation in maize. *Plant Cell and Environment* **38:** 1081–1093.

Krupnick GA, **Weis AE. 1998.** Floral herbivore effect on the sex expression of an andromonoecious plant, *Isomeris arborea* (Capparaceae). *Plant Ecology* **134:** 151–162.

Kurokawa H, **Peltzer DA**, **Wardle DA. 2010.** Plant traits, leaf palatability and litter decomposability for co-occurring woody species differing in invasion status and nitrogen fixation ability. *Functional Ecology* **24:** 513–523.

Louda SM, **Potvin MA. 1995.** Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. *Ecology* **76:** 229–245.

Lucas-Barbosa D, **Sun P**, **Hakman A**, **van Beek TA**, **van Loon JJA**, **Dicke M**, **Koricheva J. 2016.** Visual and odour cues: plant responses to pollination and herbivory affect the behaviour of flower visitors. *Functional Ecology* **30:** 431–441.

Makkar HPS, **Gamble G**, **Becker K. 1999.** Limitation of the butanol-hydrochloric acid-iron assay for bound condensed tannins. *Food Chemistry* **66:** 129–133.

Malo JE, **Leirana-Alcocer J**, **Parra-Tabla V. 2001.** Population fragmentation, florivory, and the effects of flower morphology alterations on the pollination success of *Myrmecophila tibicinis* (Orchidaceae). *Biotropica* **33:** 529–534.

Mayer C, **Dehon C**, **Gauthier A-L**, **Naveau O**, **Rigo C**, **Jacquemart A-L. 2014.** Nectar robbing improves male reproductive success of the endangered *Aconitum napellus* ssp. *lusitanicum*. *Evolutionary Ecology* **28:** 669–685.

McCall AC. 2008. Florivory affects pollinator visitation and female fitness in *Nemophila menziesii*. *Oecologia* **155:** 729–737.

McCall AC, **Irwin RE. 2006.** Florivory: the intersection of pollination and herbivory. *Ecology Letters* **9:** 1351–1365.

McKey D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* **108:** 305–320.

Mihara T. 1960. On the reduction division of *Houttuynia cordata* Thunb. *Journal of Plant Research* **73:** 498–498.

Misaki A, **Itagaki T**, **Matsubara Y**, **Sakai S. 2018.** Intraflower variation in nectar secretion: secretion patterns and pollinator behavior in male- and female-phase flowers. *American Journal of Botany* **105:** 842–850.

Morinaga S-I, **Sakai S. 2006.** Functional differentiation in pollination processes between the outer and inner perianths in *Iris gracilipes* (Iridaceae). *Canadian Journal of Botany* **84:** 164–171.

Mothershead K, **Marquis RJ. 2000.** Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* **81:** 30–40.

Oguro M, **Sakai S. 2009.** Floral herbivory at different stages of flower development changes reproduction in *Iris gracilipes* (Iridaceae). *Plant Ecology* **202:** 221–234.

Oguro M, **Sakai S. 2014.** Difference in defense strategy in flower heads and leaves of Asteraceae: multiple-species approach. *Oecologia* **174:** 227–239.

Oguro M, **Sakai S. 2015.** Relation between flower head traits and florivory in Asteraceae: a phylogenetically controlled approach. *American Journal of Botany* **102:** 407–416.

Onodera H, **Oguro M**, **Sakai S. 2014.** Effects of nutrient contents and defense compounds on herbivory in reproductive organs and leaves of *Iris gracilipes*. *Plant Ecology* **215:** 1025–1035.

Pearse IS, **Hipp AL. 2009.** Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 18097–18102.

Pyke GH, **Pulliam HR**, **Charnov EL. 1977.** Optimal foraging: a selective review of theory and tests. *The Quarterly Review of Biology* **52:** 137–154.

R Core Team. **2019.** *R: a language and environment for statistical computing*, *3.6.0th edn*. Vienna: R Foundation for Statistical Computing.

Rask L, **Andréasson E**, **Ekbom B**, **Eriksson S**, **Pontoppidan B**, **Meijer J. 2000.** Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Molecular Biology* **42:** 93–114.

Rhoades DF. 1979. Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH, eds. *Herbivores: their interaction with secondary plant metabolites*. New York: Academic Press, 1–55.

Rice-Evans C, **Miller N**, **Paganga G. 1997.** Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2:** 152–159.

Rousseaux MC, **Julkunen-Tiitto R**, **Searles PS**, **Scopel AL**, **Aphalo PJ**, **Ballaré CL. 2004.** Solar UV-B radiation affects leaf quality and insect herbivory in the southern beech tree *Nothofagus antarctica*. *Oecologia* **138:** 505–512.

Sandring S, **Riihimäki MA**, **Savolainen O**, **Ågren J. 2007.** Selection on flowering time and floral display in an alpine and a lowland population of *Arabidopsis lyrata*. *Journal of Evolutionary Biology* **20:** 558–567.

Schlangen K, **Miosic S**, **Castro A**, **Freudmann K**, **Luczkiewicz M**, **Vitzthum F**, **Schwab W**, **Gamsjager S**, **Musso M**, **Halbwirth H. 2009.** Formation of UV-honey guides in *Rudbeckia hirta*. *Phytochemistry* **70:** 889–898.

Shibata K, **Miyake K. 1908.** Ueber Parthenogenesis bei *Houttuynia cordata*. *Journal of Plant Research* **22:** 141–144.

Shroff R, **Vergara F**, **Muck A**, **Svatos A**, **Gershenzon J. 2008.** Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. *Proceedings of the National Academy of Sciences* **105:** 6196–6201.

Skaug H, **Fournier D**, **Bolker B**, **Magnusson A**, **Nielsen A. 2016.** *Generalized linear mixed models using 'AD Model Builder'*. R package version 0.8.3.3.

Smallegange RC, **van Loon JJA**, **Blatt SE**, **Harvey JA**, **Agerbirk N**, **Dicke M. 2007.** Flower vs. leaf feeding by *Pieris brassicae*: glucosinolate-rich flower tissues are preferred and sustain higher growth rate. *Journal of Chemical Ecology* **33:** 1831–1844.

Soper Gorden NL, **Adler LS. 2016.** Florivory shapes both leaf and floral interactions. *Ecosphere* **7:** e01326.

Stamp N. 2003. Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* **78:** 23–55.

Stapleton AE, **Walbot V. 1994.** Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. *Plant Physiology* **105:** 881–889.

Steppuhn A, **Gase K**, **Krock B**, **Halitschke R**, **Baldwin IT. 2004.** Nicotine's defensive function in nature. *PLoS Biology* **2:** e217.

Strauss SY, **Irwin RE**, **Lambrix VM. 2004.** Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. *Journal of Ecology* **92:** 132–141.

Thompson KA, **Johnson MT. 2016.** Antiherbivore defenses alter natural selection on plant reproductive traits. *Evolution* **70:** 796–810.

Tsuji J, **Logan T**, **Russo A. 2018.** A hierarchy of cues directs the foraging of *Pieris rapae* (Lepidoptera: Pieridae) larvae. *Environmental Entomology* **47:** 1485–1492.

Tsuji K, **Ohgushi T. 2018.** Florivory indirectly decreases the plant reproductive output through changes in pollinator attraction. *Ecology and Evolution* **8:** 2993–3001.

Tsuji K, **Sota T. 2010.** Sexual differences in flower defense and correlated male-biased florivory in a plant–florivore system. *Oikos* **119:** 1848–1853.

Tucker SC. 1981. Inflorescence and floral development in *Houttuynia cordata* (Saururaceae). *American Journal of Botany* **68:** 1017–1032.

Wakabayashi K, **Oguro M**, **Itagaki T**, **Sakai S. 2018.** Floral-induced and constitutive defense against florivory: a comparison of chemical traits in 12 herb species. *Plant Ecology* **219:** 985–997.

Ye ZM, **Jin XF**, **Wang QF**, **Yang CF**, **Inouye DW. 2017.** Pollinators shift to nectar robbers when florivory occurs, with effects on reproductive success in *Iris bulleyana* (Iridaceae). *Plant Biology* **19:** 760–766.

Zangerl AR, **Berenbaum MR. 1993.** Plant chemistry, insect adaptations to plant chemistry, and host plant utilization patterns. *Ecology* **74:** 47–54.

Zuur AF, **Ieno EN**, **Walker NJ**, **Saveliev AA**, **Smith GM. 2009.** Mixed effects modelling for nested data. In: Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM, eds. *Mixed effects models and extensions in ecology with R*. New York: Springer-Verlag, 101–142.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Detailed segmentation methods of attractive units for each species.
Appendix S2. Checking the error distribution of the chemical traits.
Appendix S3. Inter-specific relationship between variations in florivory and the chemical defences.
Fig. S1. Diagrams of the segmentation patterns of flowers.
Fig. S2. Relationship between measured sample weight and concentration of total phenolics in *Lilium auratum* shown by the preliminary measurements.
Fig. S3. Normal and ultraviolet photographs of *H. tuberosus*.
Table S1. Differences in results of the GLMMs testing variations in the chemical traits between the distal and proximal parts of attractive units.
Table S2. List of sample segmentation methods for each species.
Table S3. The distribution of the measured dry weights of sample groups for each species used for chemical measurements.
Table S4. Results of the Shapiro-Wilk normality test and Kolmogorov-Smirnov test checking congruence of the chemical trait values of each species for normal and gamma distributions.
Table S5. Results of the GLMMs testing differences in total phenolics and condensed tannins contents between floral parts (petal/sepal) and positions (distal/proximal) including an interaction term.