



Pattern of flower size variation along an altitudinal gradient differs between *Impatiens textori* and *Impatiens noli-tangere*

Mitsuru Hattori, Yusuke Nagano, Yoshinori Shinohara & Takao Itino

To cite this article: Mitsuru Hattori, Yusuke Nagano, Yoshinori Shinohara & Takao Itino (2016) Pattern of flower size variation along an altitudinal gradient differs between *Impatiens textori* and *Impatiens noli-tangere*, *Journal of Plant Interactions*, 11:1, 152-157, DOI: [10.1080/17429145.2016.1226437](https://doi.org/10.1080/17429145.2016.1226437)

To link to this article: <http://dx.doi.org/10.1080/17429145.2016.1226437>



© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



[View supplementary material](#)



Published online: 26 Sep 2016.



[Submit your article to this journal](#)



Article views: 116



[View related articles](#)



[View Crossmark data](#)

RESEARCH ARTICLE

 OPEN ACCESS

Pattern of flower size variation along an altitudinal gradient differs between *Impatiens textori* and *Impatiens noli-tangere*

Mitsuru Hattori^{a*}, Yusuke Nagano^a, Yoshinori Shinohara^a and Takao Itino^{a,b}

^aDepartment of Biology, Faculty of Science, Shinshu University, Matsumoto, Nagano, Japan; ^bInstitute of Mountain Science, Shinshu University, Matsumoto, Nagano, Japan

ABSTRACT

Studies of factors influencing spatial variation in flower size offer insights into floral evolution. We investigated altitudinal variations in five flower dimensions of two native Japanese *Impatiens* species (*I. textori* and *I. noli-tangere*) and their interactions with their faunal visitors. These two species have similar floral traits, including flower shape, flowering time, and pollinator species; both species are pollinated mainly by *Bombus diversus*. In *I. textori*, all measured flower dimensions were negatively correlated with altitude. In contrast, in *I. noli-tangere*, no measured flower dimensions correlated with altitude. Thus, the altitudinal pattern of flower size variation differed between these congeneric co-habiting herbaceous plant species. The different patterns suggest that the factors (e.g. altitudinal variations of abiotic factors) that cause variation of flower size differ between these two *Impatiens* species even though focal species have similar floral traits (e.g. flower shape, flowering time, and pollinator species).

ARTICLE HISTORY

Received 7 April 2016
Accepted 16 August 2016

KEYWORDS

Altitudinal trait variation; bumblebees; floral characteristics; plant ecology; pollination

Introduction

Many recent studies have investigated factors (e.g. pollinator distribution, plant community composition) influencing the diversification and evolution of floral traits such as shape, color, and scent (Van der Niet et al. 2014). In many animal-pollinated plants, evidence has accumulated that floral diversification and adaptation occur in response to selection by pollinators (Schemske & Bradshaw 1999; Whittall & Hodges 2007; Pauw et al. 2009; Gómez et al. 2014; Nagano et al. 2014; Kuriya et al. 2015). Although many floral adaptations have undoubtedly arisen under selection pressure by pollinators, other selection agents also drive floral adaptation (reviewed by Strauss & Whittall 2006). Indeed, pollinator and non-pollinator agents often drive floral adaptation in different directions (Armbruster et al. 1997; Strauss & Whittall 2006). Therefore, to understand the relative importance of pollinator and non-pollinator agents and to elucidate how pollinators influence floral trait evolution, we should focus not on a single plant species but on multiple sympatric species (Itino & Hirao 2016). Furthermore, comparisons of related plant species may facilitate the detection of factors affecting floral trait variation (Whittall & Hodges 2007; Ollerton et al. 2009).


Mountain ecosystems are especially suitable for investigations of the relative importance of pollinator and non-pollinator agents in floral adaptation because steep environmental clines along an altitudinal gradient can strongly influence adaptive traits and often lead to adaptive diversification within a narrow geographic range (Byars et al. 2007; Milá et al. 2009). For example, clinal abiotic environmental changes along an altitudinal gradient (e.g. meteorological changes) cause variations in plant vegetative

size (Fabbro & Körner 2004; Anderson & Johnson 2009; Hautier et al. 2009). Moreover, in some bumblebee-pollinated species, altitudinal changes in biotic interactions have been shown to influence flower size indirectly (Dohzono & Suzuki 2010; Nagano et al. 2014; Kuriya et al. 2015). In this study, we investigated altitudinal variations in flower dimensions and flower visitors in two native Japanese *Impatiens* species that have broad altitudinal distributions.

Impatiens (Balsaminaceae) comprises over 900 species and is one of the largest plant genera in the world. *Impatiens* species, which are distributed mainly in tropical highlands and mountains in the Old World, and also in parts of temperate Asia and Europe (Song et al. 2003; Cai et al. 2008), have diverse reproductive characteristics and flower morphs (Yuan et al. 2004). Previous studies have investigated the relationship between flower morph and pollen transfer in *Impatiens* species (Wilson 1995). For example, Wilson (1995) showed that in *Impatiens pallida* and *Impatiens capensis*, both of which are pollinated by bumblebees, flower morph variations generally do not influence pollen transfer during the interaction with bumblebees. It is not known, however, whether this finding is applicable to other *Impatiens* species. In this study, we investigated altitudinal variations in the flower dimensions in *Impatiens textori* Miq. and *Impatiens noli-tangere* L. as well as altitudinal variations in their flower visitors. Both of these *Impatiens* species are annual herbs. They have similar vegetative and flower shapes (Tokuda et al. 2015) and produce both chasmogamous and cleistogamous flowers (Sugita 2001). In *I. noli-tangere*, however, the chasmogamous flowers are protandrous and always need pollen transfer from other flowers for fertilization, whereas in *I. textori* they are homogamous and do not always need

CONTACT Mitsuru Hattori  mtr.hattori@gmail.com

^{*}Present address: Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki, Nagasaki 852-8521, Japan

 Supplemental data for this article can be accessed at [doi:10.1080/17429145.2016.1226437](https://doi.org/10.1080/17429145.2016.1226437).

© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

pollen transfer (Sugita 2001; Hatcher 2003; Iwaizumi & Sakai 2004; Masuda et al. 2004). Therefore, although in central Japan these species are reported to be pollinated by the same pollinator, namely, *Bombus diversus* (Miyamoto 1961; Kato 1988; Tokuda et al. 2015), the importance of the pollinator to seed set may differ between them.

We hypothesized that altitudinal variations in flower dimensions would differ between these two species even if they were pollinated by the same pollinator species. To test this hypothesis, we measured floral dimensions and observed flower visitors in 11 populations of *I. textori* and 10 populations of *I. noli-tangere* distributed along an altitudinal gradient in central Japan.

Materials and methods

Plant materials

I. textori is distributed in moist to wet habitats (e.g. along streams and in marshes) in Japan, on the Korean Peninsula, and in northeastern China, and it has long-spurred magenta flowers (Figure S1). Although this species can produce chasmogamous and cleistogamous flowers, the frequency of cleistogamous flowers is very low (Sugita 2001). Iwaizumi & Sakai (2004) reported that the chasmogamous flower biomass in *I. textori* negatively correlates with the density of individuals, a finding that implies that flower biomass is influenced by resource competition (i.e. decreasing availability of resources). *I. noli-tangere* is distributed in moist habitats such as along streams across Europe, East Asia, and North America, and it has long-spurred yellow flowers (Figure S1). This species often produces more cleistogamous flowers than chasmogamous flowers (Masuda & Yahara 1994). The chasmogamous flowers of both species open between August and October in central Japan (Tokuda et al. 2015).

Study sites

We studied altitudinal changes in flower size and flower visitors in 11 *I. textori* populations (650–1350 m a.s.l.) and 10

I. noli-tangere populations (650–1480 m a.s.l.) in four mountain regions of central Japan in 2013 (Table 1). Each population was surveyed during its peak flowering season. The geographic distance between *I. textori* populations ranged from 600 m to 80 km, and that between *I. noli-tangere* populations ranged from 600 m to 45 km. The field study was approved by the Chushin District Forest Office (Forestry Agency) (25-16-60) and the Matsumoto Regional Office (Nagano Prefectural Government) (25Matsuchikan45-13, 25Matsuchikan45-14).

Variations in local flower visitors

In each of the 21 populations, we observed flower visitors for 1 h between 08:00 and 15:00 JST on a sunny day during that population's peak flowering season. We counted the number of individuals of each bumblebee, Anthophoridae, Lepidoptera (Macroglossinae), and Syrphidae species visiting each population.

Measurement of flower dimensions

From each population, we randomly picked out 11–20 individual plants and haphazardly selected one flower on each plant for morphological measurements. We measured five different flower dimensions on each flower (dimensions i–v, Figure 1). We measured flower dimensions (ii–v) with a digital caliper (precision, 0.01 mm), but for flower dimension (i), we first used a scanner (Scan 9900F, Canon Inc., Tokyo) to obtain a digital image of the flower and then we used Photo Measure software (Kenis Ltd, Osaka, Japan) to measure dimension (i) on the image. We defined the flower dimensions as follows: (i) the distance from the base of the stigma (or pistil) to the tip of the spur (i.e. lower sepal length + spur length); (ii) the distance from the base of the stigma (or pistil) to the bottom of the lower sepal entrance; (iii) the maximum lower sepal entrance width; (iv) the maximum lateral petal length; and (v) the maximum lateral petal width (Figure 1).

Table 1. Populations of *I. textori* and *I. noli-tangere* in which the altitudinal variation of flower dimensions and flower visitors were studied.

Species	Population ^a	Altitude (m)	Latitude (°N)	Longitude (°E)	No. of flowers for size measurements	Observed visitors ^b	
<i>I. textori</i>	Jn1	650	36.3130	137.8290	13	Bd: 3, Ma: 1	
	Uk1	780	38.2618	138.0003	15	Bd: 19	
	Jn2	850	36.2689	137.8329	12	Bd: 15	
	Af1	850	36.2704	138.5693	12	Bd: 10, Ma: 3, Ap: 2	
	Uk2	910	36.2616	138.0050	15	Bd: 6, An: 7	
	Jn3	965	36.2737	137.8302	13	Bd: 6	
	Uk3	1035	36.2600	138.0176	15	Bd: 8, Ma: 3	
	Af2	1150	36.2686	138.6025	13	Rh: 5, Sy: 2	
	Jn4	1250	36.3280	137.7844	20	–	
	Nk1	1335	36.0881	137.6477	14	Bd: 2, Bc: 5	
	Jn5	1350	36.3038	137.7571	13	Bh: 2	
	<i>I. noli-tangere</i>	Jn1	650	36.3130	137.8290	19	Bd: 15
		Uk1	780	36.2618	138.0003	12	Bd: 6
		Jn2	850	36.2689	137.8329	13	Bd: 13
		Jn3	965	36.2737	137.8302	12	Bd: 7
Jn4		1020	36.3052	137.7866	15	Bd: 6, Bc: 4	
Jn5		1180	36.2809	137.8281	15	Bd: 4, Bh: 1, Bhy: 1	
Nk1		1180	36.0824	137.6571	11	Bd: 1	
Nk2		1400	36.1480	137.6235	12	Bd: 3	
Uk2		1455	36.2501	138.0441	12	Bd: 8, Bc: 4	
Uk3		1480	36.2628	138.0982	15	Bd: 4, Bc: >20, Bu: 2	

Notes: The body size of the observed legitimate bumblebee pollinators decreases in the order Bc > Bd > Bu > Bh (Takamizawa 2005). Bc, *Bombus consobrinus*; Bd, *B. diversus*; Bh, *B. honshuensis*; Bhy, *B. hypocrita*; Bu, *B. ussurensis*; Ap, *Apis cerana*; An, Anthophoridae sp.; Ma, Macroglossinae sp.; Rh, *Rhingia* sp.; Syrphidae sp.; – no visitors observed.

^aThe surveyed populations were in the following mountain regions: Jn, Mt. Jyonen; Uk, Utsukushigahara; Af, Mt. Arafune; Nk, Mt. Norikura.

^bVisitors were observed for 1 h on a sunny day during the peak flowering season of each population.

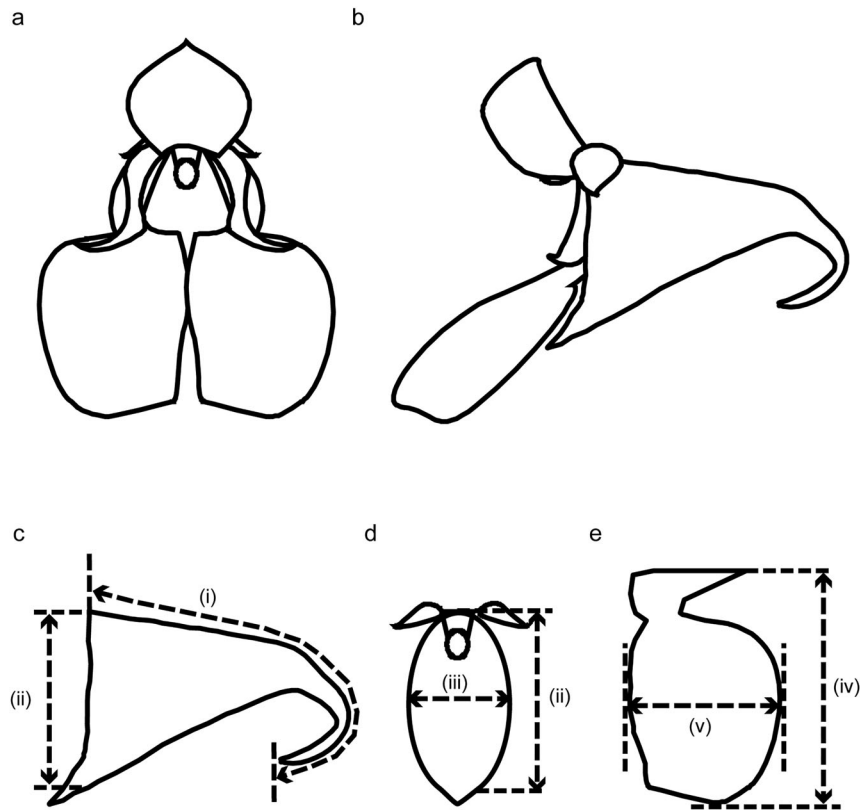


Figure 1. Sketches of a whole *Impatiens* flower and of its various parts showing the measured dimensions. (a) Whole flower (front view); (b) whole flower (side view); (c) corolla (side view); (d) corolla (front view); and (e) lateral petal. The dotted lines labeled (i)–(v) indicate the measured dimensions (see text for details).

When insects visit *I. textori* and *I. noli-tangere* flowers, they enter the corolla throat, crawl into the corolla, and insert their mouthpart into the innermost part of the spur (Figure S1). Pollen can be transferred from flower to flower if the flower's dimensions are such that the head and the dorsal thorax of visiting bumblebees, the presumptive pollinator, touch the anthers and stamens. Therefore, we consider flower dimensions (i–iii) to influence pollination efficiency. On the other hand, because lateral petals of the focal species are large and conspicuous in the wild, these traits are expected to play a role in attracting pollinators. Therefore, flower dimensions (iv) and (v) may influence the pollinator visitation rate.

Data analysis

We explored the variation in the five flower dimensions among populations by using one-way analysis of variance followed by pairwise comparisons with Tukey's HSD post hoc test. To examine whether flower size variation correlated with altitude, we calculated Kendall tau rank correlations between each of the five flower dimensions and population altitude. Significance levels for multiple tests were adjusted by using the sequential Bonferroni correction (Rice 1989). The statistical analyses were performed with the JMP version 12.0.1 software package (SAS Inc., Cary, NC, USA).

Results

In the study areas, *I. textori* flowers were visited primarily by *B. diversus*. However, most populations were visited not only by *B. diversus* but also by other insects (Table 1). The Nk1

population of *I. textori* was visited by *Bombus consobrinus*, which has the biggest body size among the observed flower visitors, but the Jn4 and Af2 populations of *I. textori* were not visited by any bumblebees during our observation period. Hoverflies and small bees, which have smaller body sizes than *B. diversus*, visited the Jn5, Uk3, Af1, and Af2 populations of *I. textori* (Table 1). Because the bodies of hoverflies and small bees do not touch the androecium and stigma when they visit *I. textori* flowers, however, they are not likely to be very effective pollinators.

I. noli-tangere flowers in the study areas were also visited primarily by *B. diversus*, but flowers in most populations were also visited by other insects (Table 1). In particular, the Jn4, Uk2, and Uk3 populations of *I. noli-tangere* were visited by *B. consobrinus*.

We found significant geographic variations in the flower dimensions of both species: [*I. textori*: (i) $F = 30.01$, $P < .001$; (ii) $F = 58.30$, $P < .001$; (iii) $F = 30.18$, $P < .001$; (iv) $F = 37.59$, $P < .001$; (v) $F = 34.25$, $P < .001$; *I. noli-tangere*: (i) $F = 7.44$, $P < .001$; (ii) $F = 12.8$, $P < .001$; (iii) $F = 2.46$, $P = .013$; (iv) $F = 7.92$, $P < .001$; (v) $F = 10.49$, $P < .001$; Table 2 and Figure 2]. The variations in flower dimensions (i–iii) were larger in *I. textori* than in *I. noli-tangere* (Figure 2). In particular, the variation in flower dimension (i) was within 5 mm in *I. noli-tangere*, whereas in *I. textori* it varied by more than 10 mm (Table 2 and Figure 2).

In *I. textori*, all flower dimensions gradually decreased along altitude: (i) $T = -0.27$, $P < .001$; (ii) $T = -0.30$, $P < .001$; (iii) $T = -0.33$, $P < .001$; (iv) $T = -0.30$, $P < .001$; and (v) $T = -0.39$, $P < .001$. In *I. noli-tangere*, however, no flower dimensions correlated with altitude: (i) $T = 0.004$, $P > .05$; (ii) $T = -0.09$, $P > .05$; (iii) $T = 0.03$, $P > .05$; (iv) $T = -0.08$, $P > .05$; and (v) $T = -0.058$, $P > .05$.

Table 2. Mean ± SE of each of five flower dimensions (see Figure 1) in the 11 *I. textori* populations and the 10 *I. noli-tangere* populations.

Species	Population	Dimension (i)	Dimension (ii)	Dimension (iii)	Dimension (iv)	Dimension (v)
<i>I. textori</i>	Jn1	33.02 ± 0.61 ^{cde}	10.34 ± 0.17 ^c	6.50 ± 0.13 ^{cd}	16.02 ± 0.75 ^{def}	9.31 ± 0.48 ^{de}
	Uk1	39.31 ± 0.39 ^{ab}	10.90 ± 0.16 ^{cd}	7.67 ± 0.14 ^a	21.52 ± 0.43 ^{ab}	12.56 ± 0.31 ^{ab}
	Jn2	38.79 ± 0.66 ^{ab}	12.43 ± 0.38 ^{ab}	7.36 ± 0.12 ^{ab}	20.87 ± 0.39 ^{ab}	12.25 ± 0.37 ^{abc}
	Af1	35.79 ± 0.87 ^{bc}	11.50 ± 0.27 ^{bcd}	6.81 ± 0.18 ^{bc}	18.01 ± 0.41 ^{cde}	10.76 ± 0.48 ^{cd}
	Uk2	41.03 ± 0.62 ^a	13.26 ± 0.27 ^a	7.51 ± 0.13 ^{ab}	22.85 ± 0.38 ^a	13.74 ± 0.34 ^a
	Jn3	38.66 ± 0.50 ^{ab}	12.00 ± 0.27 ^{bc}	7.17 ± 0.12 ^{abc}	19.97 ± 0.39 ^{bc}	10.60 ± 0.40 ^{cd}
	Uk3	34.33 ± 0.65 ^{cd}	9.07 ± 0.18 ^e	7.04 ± 0.17 ^{abc}	19.68 ± 0.53 ^{bc}	11.39 ± 0.38 ^{bc}
	Af2	30.24 ± 0.56 ^{ef}	9.06 ± 0.28 ^e	5.56 ± 0.20 ^{ef}	14.92 ± 0.64 ^{fg}	9.00 ± 0.43 ^{de}
	Jn4	28.11 ± 1.31 ^f	7.57 ± 0.22 ^f	4.97 ± 0.22 ^f	13.58 ± 0.31 ^g	7.00 ± 0.20 ^f
	Nk1	38.05 ± 0.65 ^{ab}	11.45 ± 0.11 ^{bcd}	6.88 ± 0.09 ^{bc}	18.22 ± 0.62 ^{cd}	8.00 ± 0.42 ^{ef}
	Jn5	31.18 ± 0.80 ^{def}	8.49 ± 0.32 ^{ef}	5.91 ± 0.18 ^{de}	15.74 ± 0.63 ^{efg}	8.52 ± 0.38 ^{ef}
<i>I. noli-tangere</i>	Jn1	31.91 ± 0.49 ^{bcdde}	9.95 ± 0.16 ^{cd}	7.46 ± 0.16 ^{ab}	22.78 ± 0.73 ^a	13.83 ± 0.62 ^{abc}
	Uk1	33.08 ± 0.57 ^{abc}	8.99 ± 0.18 ^{de}	7.67 ± 0.25 ^a	22.65 ± 0.67 ^{ab}	14.14 ± 0.50 ^{abc}
	Jn2	32.15 ± 0.30 ^{abcde}	11.47 ± 0.19 ^a	7.55 ± 0.16 ^{ab}	22.96 ± 0.63 ^a	14.40 ± 0.37 ^{ab}
	Jn3	30.56 ± 0.53 ^{cde}	11.01 ± 0.22 ^{ab}	7.39 ± 0.17 ^{ab}	19.63 ± 0.70 ^{bcd}	11.54 ± 0.68 ^{cde}
	Jn4	29.83 ± 0.47 ^e	10.26 ± 0.28 ^{bc}	7.17 ± 0.19 ^{ab}	17.80 ± 0.55 ^d	9.34 ± 0.37 ^e
	Jn5	34.41 ± 0.49 ^a	8.80 ± 0.14 ^e	6.71 ± 0.19 ^b	21.03 ± 0.73 ^{abc}	12.03 ± 0.67 ^{bcd}
	Nk1	32.01 ± 0.48 ^{abcde}	11.10 ± 0.29 ^{ab}	7.60 ± 0.25 ^{ab}	18.60 ± 0.64 ^{bcd}	11.11 ± 0.41 ^{de}
	Nk2	32.37 ± 0.62 ^{abcd}	10.10 ± 0.32 ^{bcd}	7.46 ± 0.25 ^{ab}	19.46 ± 0.64 ^{cd}	11.30 ± 0.53 ^{de}
	Uk2	33.87 ± 0.94 ^{ab}	9.95 ± 0.38 ^{bcd}	7.31 ± 0.21 ^{ab}	21.50 ± 0.75 ^{abc}	12.88 ± 0.60 ^{abcd}
	Uk3	30.54 ± 0.37 ^{de}	9.49 ± 0.20 ^{cde}	7.88 ± 0.19 ^a	23.09 ± 0.72 ^a	14.67 ± 0.53 ^a

Note: Lowercase letters indicate significant differences ($P < .05$) identified by Tukey's HSD post hoc pairwise comparisons.

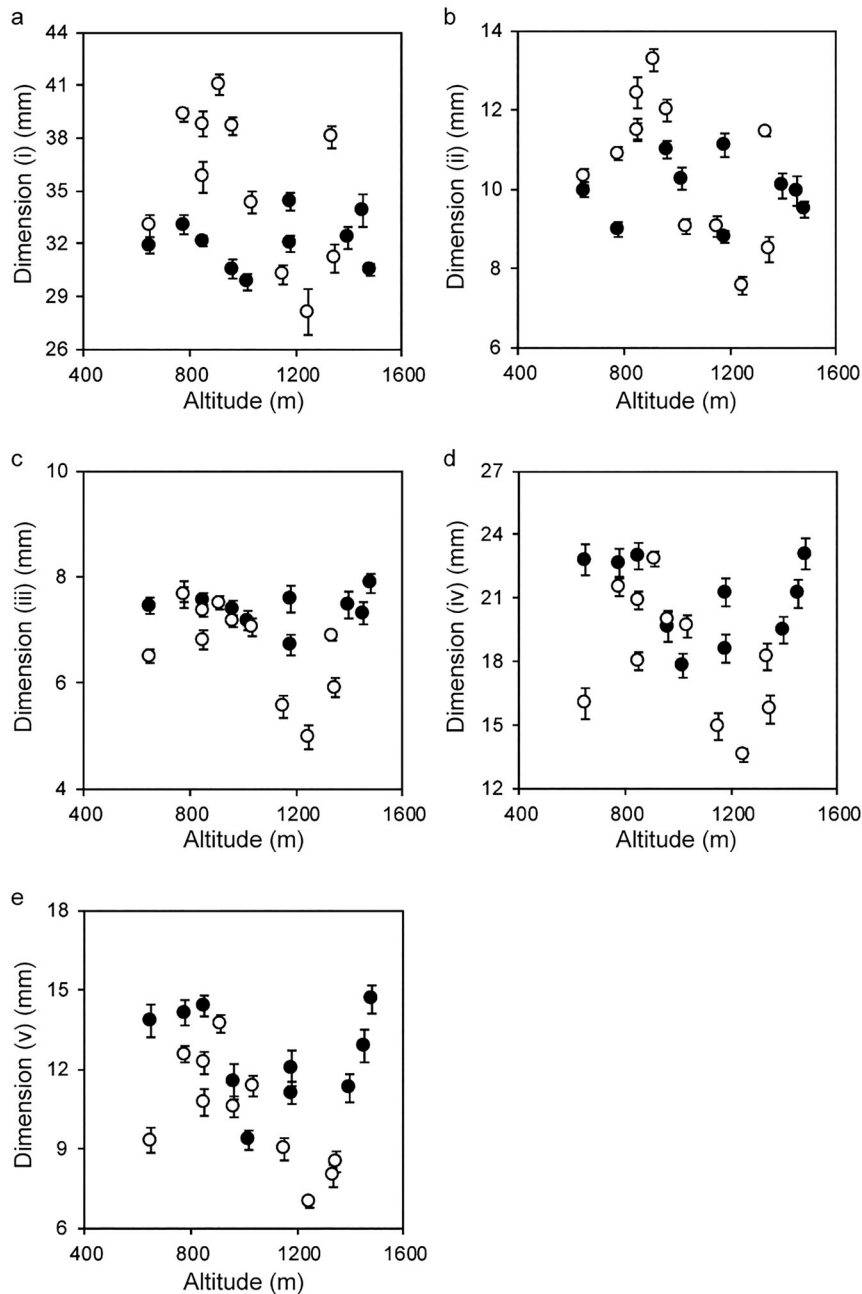


Figure 2. Altitudinal variation (mean ± SE) of each of the five measured flower dimensions (see Figure 1) in *I. textori* (white circles) and *I. noli-tangere* (black circles). Each symbol represents one population.

Discussion

Previous studies that have investigated flower size variations along an altitudinal gradient in mountainous environments have reported corresponding altitudinal variations in, for example, pollinator species composition and nectar robbing frequency (Hattori et al. 2014, 2015; Nagano et al. 2014; Kuriya et al. 2015; Zhao & Wang 2015). In this study, we showed that the pattern of flower size variation along an altitudinal gradient differed between two *Impatiens* species growing in similar habitats.

In *I. textori*, all measured flower dimensions correlated negatively with altitude (Figure 2), and the primary flower visitor was *B. diversus* (Table 1). Other studies (Miyamoto 1961; Kato 1988; Tokuda et al. 2015) have also reported that *I. textori* flowers are visited primarily by *B. diversus*. These findings suggest that the flower dimensions were more strongly affected by an abiotic environmental factor correlated with altitude (e.g. air temperature), or by an altitude-related pollen limitation (Cosacov et al. 2008), than by size-matching between flower and pollinator, because the pollinator species composition in central Japan seems to be similar, regardless of time or place. Various environmental factors such as air temperature and pollinator abundance change along an altitudinal gradient (Körner 1999; Nagano et al. 2014; Kuriya et al. 2015). These environmental factors can affect flower size variation along the gradient (Galen 2000; Knight et al. 2005; Parachnowitsch & Kessler 2010). For example, Galen (2000) and Carroll et al. (2001) reported negative correlations between flower size and resource availability as related to environmental stress and suggested that these negative correlations reflected the increased physiological cost of producing large flowers when resource availability is poor as a result of high environmental stress. Further study is needed to test how variations in flower dimensions might relate to variations in the abundance, diversity, and visit rate of pollinators.

In this study, *I. textori* flowers were smaller in higher altitude populations than in lower altitude populations (Figure 2). Iwaizumi and Sakai (2004) suggested that pollinators visit small flowers of *I. textori* less frequently than they visit large flowers. These facts suggest that the higher altitude populations of *I. textori* may suffer from pollen limitation. Pollen limitation can cause selection on various floral traits (e.g. flowering duration, flower size, and number of flowers) (Ashman et al. 2004; Parachnowitsch & Kessler 2010; Lázaro et al. 2015). Therefore, floral traits other than flower size may also differ between lower and higher altitude populations in *I. textori*. Further study is needed to determine whether pollen limitation is indeed stronger in higher altitude populations than in lower altitude populations of this species.

In *I. noli-tangere*, no measured flower dimensions were correlated with altitude (Figure 2). The primary flower visitor to *I. noli-tangere* was also *B. diversus*, as Tokuda et al. (2015) also reported (Table 1). The flower–pollinator size match often influences plant fitness more than other factors (Nagano et al. 2014; Kuriya et al. 2015). Therefore, these results suggest that flower dimensions were affected not by a clinal change in any abiotic environmental factor along altitude but by the size-match between the flowers and the *B. diversus* body size. Because in *I. noli-tangere*, the chasmogamous flowers are strongly protandrous and the pollens must be transferred from other flowers by insects

for fertilization (Hatcher 2003; Masuda et al. 2004), the production of flowers not size-matched to their primary pollinator would likely decrease the fitness of *I. noli-tangere*.

Conclusion

We showed that the altitudinal pattern of flower size variation differed between *I. textori* and *I. noli-tangere*, although these species have similar floral traits (i.e. the same flowering time, flower shape, and pollinator species) (Table 2 and Figure 2). Our result suggests that some factor that differs between them causes the different altitudinal pattern of flower size variation. To understand the factors that influence the altitudinal flower size variation in *Impatiens* species (e.g. interannual changes in pollinator species composition or altitudinal changes in plant reproductive systems), further study is needed.

Acknowledgements

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank the Chubu District Forest Office (Forestry Agency) and the Matsumoto Regional Office (Nagano Prefectural Government) for permission to work in the area.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the Global Environmental Research Fund (D-0904) of the Ministry of the Environment, Japan and by the Japanese Alps Inter-University Cooperative Project, Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References

- Anderson B, Johnson SD. 2009. Geographic covariation and local convergence of flower depth in a guild of fly-pollinated plants. *New Phytol.* 182:533–540.
- Armbruster WS, Howard JJ, Clausen TP, Debevec CEM, Loquvam JC, Matsuki M, Cerendolo B, Andel F. 1997. Do biochemical exaptations link evolution of plant defense and pollination systems? Historical hypotheses and experimental tests with *Dalechampia* vines. *Amer Nat.* 149:461–484.
- Ashman T-L, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG. 2004. Pollen limitation of plant reproduction: ecological and evolutionary cause and consequences. *Ecology.* 85:2408–2421.
- Byars SG, Papst W, Hoffmann AA. 2007. Local adaptation and cogeographical selection in the alpine plant *Poa hiemata*, along a narrow altitudinal gradient. *Evolution.* 61:2925–2941.
- Cai X-Z, Liu K-M, Tian S-Z. 2008. *Impatiens rupestris* (Balsaminaceae), a new species from Hunan, China. *Novon.* 18:9–11.
- Carroll AB, Pallardy SG, Galen C. 2001. Drought stress, plant water status, and floral trait expression in fireweed. *Epilobium angustifolium* (Onagraceae). *Amer J Bot.* 88:438–446.
- Cosacov A, Nattero J, Cocucci AA. 2008. Variation of pollinator assemblages and pollen limitation in a locally specialized system: the oil-producing *Nierembergia linariifolia* (Solanaceae). *Ann Bot.* 102:723–734.
- Dohzono I, Suzuki K. 2010. Morphological and genetic differentiation in *Isodon umbrosus* by altitudinal variation in bumblebee pollinator assemblages. *Plant Species Biol.* 25:20–29.
- Fabbro T, Körner C. 2004. Altitudinal differences in flower traits and reproductive allocation. *Flora.* 199:70–81.

- Galen C. 2000. High and dry: drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *Amer Nat.* 156:72–83.
- Gómez JM, Muñoz-Pajares AJ, Abdelaziz M, Lorite J, Perfectti F. 2014. Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Ann Bot.* 113:237–249.
- Hatcher PE. 2003. Biological flora of the British Isles: *Impatiens noli-tangere* L. *J Ecol.* 91:147–167.
- Hattori M, Nagano Y, Itino T. 2014. Geographic flower trait variation of *Auilegia buergeriana* Sieb. et Zucc. var. *buergeriana* on Mt. Norikura and the Utsukushigahara Highland, Japan. *Amer J Plant Sci.* 5:2686–2692.
- Hattori M, Nagano Y, Itino T. 2015. Geographic variation in flower size and flower-visitor composition of two bumblebee-pollinated, spring-flowering herbs, *Lamium album* L. var. *barbatum* (Lamiaceae) and *Meehania urticifolia* (Lamiaceae). *Amer J Plant Sci.* 6:737–745.
- Hautier Y, Randin CF, Stoecklin J, Guisan A. 2009. Changes in reproductive investment with altitude in an alpine plant. *J Plant Ecol.* 2:125–134.
- Itino T, Hirao AS. 2016. Structure and function of mountain ecosystems in Japan. Tokyo: Springer. Chapter 4, Plant genetic diversity and plant-pollinator interactions along altitudinal gradients; p. 63–88.
- Iwaizumi MG, Sakai S. 2004. Variation in flower biomass among nearby populations of *Impatiens textori* (Balsaminaceae): effects of population plant densities. *Can J Bot.* 82:563–572.
- Kato M. 1988. Bumblebee visits to *Impatiens* spp.: pattern and efficiency. *Oecologia.* 76:364–370.
- Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T-L. 2005. Pollen limitation of plant reproduction: pattern and process. *Annu Rev Ecol Evol.* 36:467–497.
- Körner C. 1999. Alpine plant life. Berlin: Springer.
- Kuriya S, Hattori M, Nagano Y, Itino T. 2015. Altitudinal flower size variation correlates with local pollinator size in a bumblebee-pollinated herb, *Prunella vulgaris* L. (Lamiaceae). *J Evol Biol.* 28:1761–1769.
- Lázaro A, Lundgren R, Totland Ø. 2015. Pollen limitation, species' floral traits and pollinator visitation: different relationships in contrasting communities. *Oikos.* 124:174–186.
- Masuda M, Yahara T. 1994. Reproductive ecology of a cleistogamous annual, *Impatiens noli-tangere* L., occurring under different environmental conditions. *Ecol Res.* 9:67–75.
- Masuda M, Yahara T, Maki M. 2004. Evolution of floral dimorphism in a cleistogamous annual, *Impatiens noli-tangere* L. occurring under different environmental conditions. *Ecol Res.* 19:571–580.
- Milá B, Wayne RK, Fitz P, Smith TB. 2009. Divergence with gene flow and fine-scale phylogeographical structure in the wedge-billed woodcreeper, *Glyphorhynchus spirurus*, a Neotropical rainforest bird. *Mol Ecol.* 18:2979–2995.
- Miyamoto S. 1961. Flower-visiting habits of bumblebees (Biological studies of Japanese bees XIX). *Jpn J Appl Entomol Zool.* 5:28–39.
- Nagano Y, Abe K, Kitazawa T, Hattori M, Hirao AS, Itino T. 2014. Changes in pollinator fauna affect altitudinal variation of floral size in a bumblebee-pollinated herb. *Ecol Evol.* 4:3395–3407.
- Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter CI, Rotenberry J. 2009. A global test of the pollination syndrome hypothesis. *Ann Bot.* 103:1471–1480.
- Parachnowitsch AL, Kessler A. 2010. Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytol.* 188:393–402.
- Pauw A, Stofberg J, Waterman RJ. 2009. Flies and flowers in Darwin's race. *Evolution.* 63:268–279.
- Rice WR. 1989. Analyzing tables of statistical tests. *Evolution.* 43:223–225.
- Schemske DW, Bradshaw HD. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc Natl Acad Sci.* 96:11910–11915.
- Song Y, Yuan Y-M, Küpfer P. 2003. Chromosomal evolution in Balsaminaceae, with cytological observations on 45 species from Southeast Asia. *Caryologia.* 56:463–481.
- Strauss SY, Whittall JB. 2006. Ecology and evolution of flowers. New York: Oxford University Press. Chapter 7, Non pollinator agents of selection on floral traits; p. 120–138.
- Sugita H. 2001. *Impatiens textori* and *I. nolitangere*: The world of plants (Herbaceous part in last volume). Tokyo: Newton press. p. 56–57.
- Takamizawa K. 2005. The Japanese social wasps and bees: Bombini. Nagano: Shinano Mainichi Shinbun. p. 178–248.
- Tokuda N, Hattori M, Abe K, Shinohara Y, Nagano Y, Itino T. 2015. Demonstration of pollinator-mediated competition between two native *Impatiens* species, *Impatiens noli-tangere* and *I. textori* (Balsaminaceae). *Ecol Evol.* 5:1271–1277.
- Van der Niet T, Peakall R, Johnson SD. 2014. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Ann Bot.* 113:199–211.
- Whittall JB, Hodges SA. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature.* 447:706–709.
- Wilson P. 1995. Selection for pollination success and the mechanical fit of *Impatiens* flowers around bumblebee bodies. *Biol J Linn Soc.* 55:355–383.
- Yuan Y-M, Song Y, Geuten K, Rahelivololona E, Wohlhauser S, Fischer E, Smets E, Küpfer P. 2004. Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. *Taxon.* 53:391–403.
- Zhao Z-G, Wang Y-K. 2015. Selection by pollinators on floral traits in generalized *Trollius ranunculoides* (Ranunculaceae) along altitudinal gradients. *PLoS ONE.* 10:e0118299.