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Flower visitor fauna of the narrow endemic lily ***Lilium rubellum*** Baker in a lowland habitat in Yamagata, northern Japan

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Abstract

Floral visitor fauna of the narrow endemic lily *Lilium rubellum* was examined in a lowland habitat in Kaminoyama City, Yamagata Prefecture, northern Japan. Flowers of *L. rubellum* bloomed from early to late June. During 23 h of observing floral visitors, 64 insects were detected on *L. rubellum* flowers. Although coleopteran insects were most frequently found on *L. rubellum* flowers, they did not seem to be effective pollinators because of their body size. Bees were less frequently observed than coleopteran insects, but all individuals contacted sexual organs in *L. rubellum* flowers. Syrphid flies were seen less frequently, but they were also considered effective pollinators. From our observations, *L. rubellum* is a bee-pollinated species of the genus *Lilium*. Bee pollination has also been recorded in a species of *Lilium* sect. *Archelirion*, *L. japonicum* var. *abeanum*. The floral characteristics of *L. japonicum* var. *abeanum* (e.g., pinkish color, relatively small and tubular corolla, and lateral insertion of anthers into the corolla) were similar to those of *L. rubellum* and the character combination may be related to the bee-pollination mode in *Lilium*.

Keywords : bee pollination, *Bombus*, *Ceratina*, flower visitor fauna, *Lilium japonicum* var. *abeanum*, *Lilium rubellum*, nitidulid beetle, syrphid fly

Introduction

In angiosperms, entomophily is the dominant mode of pollination, and various types of entomophilous flowers have evolved through interactions

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with diverse arrays of insects (Endress 1994; Proctor et al. 1996; Armbruster 2006). When considering floral evolution in angiosperms, it is essential to take relationships with insect pollinators into account. Surveys of flower visitor fauna are the first step in studying plant–insect pollinator interactions and floral evolution mediated by insect pollinators.

Lilium L. is a large monocot genus of the Northern Hemisphere that includes about 120 spp., many of which are widely cultivated as ornamental plants (Shimizu 1987; McRae 1998). Highly diverse and conspicuous floral features within the genus demonstrate adaptations to various arrays of pollinating insects. Of the 15 *Lilium* species in Japan, about half are endemic (Shimizu 1987), and these species also show conspicuous diversity in their floral features.

Among Japanese lilies, species of sect. *Archelirion* are the most important because most of the species are endemic to Japan and show highly divergent floral characteristics. Some have quite large, widely opened, pale-colored flowers with strong scents. These species are pollinated by lepidopteran insects, moths and butterflies. For example, *L. auratum* Lindl., which is endemic to central and northern Honshu, is pollinated during the day by butterflies such as large *Papilio* species and nocturnally by hawkmoths (Hayashi and Kawano 2007; Morinaga et al. 2009). *Lilium japonicum* Thunb. is also known as a lepidopteran-pollinated species and is pollinated by nocturnal hawkmoths (Chiba and Shimizu 2004; Yokota and Yahara 2012). Some species in the section, however, have relatively small and funnel-shaped flowers and are thought to rely on a different pollination mode from other large-flowered species. One of these species, *L. japonicum* Thunb. var. *abeanum* (Honda) Kitam., a serpentine variety of *L. japonicum* that is found in Tokushima Prefecture, Shikoku district, is known to be bee-pollinated (Yokota and Yahara 2012).

Another small-flowered species of sect. *Archelirion* is *L. rubellum* Baker (Fig. 1). This species is a narrow endemic to the side of northern Japan that faces the Japan Sea. It only occurs in Yamagata, western Miyagi and Fukushima, and northern Niigata (Shimizu 1987). Flower color, shape, and size are quite similar to *L. japonicum* var. *abeanum* and it is thought to be bee-pollinated. However, no published observations of pollinators of this species exist. Because sect. *Archelirion* is mostly endemic to Japan, the evolution of species in the section is important when considering the speciation processes of plants in the Japanese archipelago.

In this study, we observed floral visitors of *L. rubellum* that occurred in a lowland habitat in Yamagata Prefecture. We also recorded flowering phenology of the population. Lowland populations of *L. rubellum* are critically endangered and the results of this study are also important for conservation purposes.

Fig. 1. A flower of *Lilium rubellum* in the study population of Kaminoyama, Yamagata. Note that pinkish color, tubular corolla, and lateral insertion of anthers into the corolla.

Materials and methods

Study site

Observations of flowering phenology and flower visitors were conducted in a population of *L. rubellum* in Togo, Kaminoyama-shi, Yamagata Prefecture. Detailed information about the study site can be provided by the authors upon request. *Lilium rubellum* occurred on an east-facing open slope that was sparsely covered with *Miscanthus sinensis*.

Observations

Observations were conducted in 2- to 5-day intervals from June 6 to 29, 2010. The population included 90 flowering *L. rubellum* individuals. Each individual was labeled when flower opening was observed. Labeled individuals were continuously observed until all flowers died. We also recorded when stigmas in opened flowers received pollen grains.

Flower visitors were recorded when they landed on flowers or were found on flowers. Visitors were collected as often as possible for exact identification. Before collection, we recorded whether a visitor's body touched the anthers or stigmas of *L. rubellum*. Collected visitors were also examined for the presence of *L. rubellum* pollen grains using a stereomicroscope.

Results

Flowering phenology

Flowers of five individuals opened on June 6, and the peak flowering in the population occurred on June 17 (62 flowering individuals). All flowers were finished by June 29. The longevity of individual flowers was 5–10 days and flowering duration in this population was approximately 1 month (Fig. 2).

Flower visitors

During the study period, observations of flower visitors were conducted on 8 days and the total observation time was 23 h. In total, 64 flower visitors were recorded during the study period (Table 1). Among the visitors, coleopteran insects were observed most frequently (45.3% of total visits) and a nitidulid beetle (*Carpophilus chalybeus*) represented about one-third of all visitors (20 individuals). The second-most frequent visitor group was bees (25.0% of total visits). Small bee genera, especially *Ceratina*, accounted for more than half of all bee records (2 species, 9 individuals in total). Syrphid flies were less frequent visitors of *L. rubellum* (9.4%).

The proportion of flower visitors bearing pollen grains of *L. rubellum* on their bodies differed among groups. All bee visitors had pollen grains on their bodies, suggesting that they touched the reproductive organs of *L. rubellum* when they visited flowers. All syrphid flies were also observed bearing pollen grains of *L. rubellum*. In contrast, only 13.7% of coleopteran insects had pollen grains of *L. rubellum*. *Carpophilus chalybeus* in particular was so small that it did not make contact with either anthers or stigmas when visiting flowers. Other flower visitors were considered nectar thieves (ants), predators of flower visitors (spiders), or accidental visitors (grasshoppers).

Discussion

Our results indicated that bees were the most effective flower visitors for the pollination of *L. rubellum*. This pattern was also observed in *L. japonicum* var. *abeanum* (Yokota and Yahara 2012). Floral traits such as pinkish color, relatively small and tubular corolla, and lateral insertion of anthers into the corolla are suitable for bee pollination (Yokota and Yahara 2012; Fig. 1). Bee-pollination modes were also found in lilies in North America (*L. parvum* Kellogg, *L. maritimum* Kellogg), although these species were also visited by hummingbirds (Skinner 1988). To attract hummingbird visitors, flower color in these species is red or reddish orange, but other floral traits are similar to those of *L. rubellum* or *L. japonicum* var. *abeanum*, including having laterally opening flowers, small tubular

Table 1. Flowering phenology of *Lilium rubellum* in a study population.

Individual ID	Observation date 6	8	10	12	15	17	18	22	24	29
1					1	1	1	×		
2					1	1	1	×		
3					1	1	2	1	×	
4						1	2	1	×	
5		2	2	1	×					
6		1	1	2	×					
7					2	2	2	×		
8		1	1	1	×					
9					1	1	1	1	×	
10	1	1	1	1	×					
11				1	1	1	1	×		
12		1	1	1	1	1	×			
13					1	1	1	×		
14			1	1	1	1	×			
15	1	1	1	1	×					
16				1	1	1	×			
17	1	1	1	1	1	×				
18		1	1	2	2	2	×			
19		1	1	1	1	×				
20			2	3	3	1	×			
21			1	1	×					
22	1	1	1	1	×					
23	1	1	1	×						
24			1	3	3	1	×			
25			1	1	1	1	×			
26			1	1	1	1	×			
27			1	1	1	1	×			
28				1	1	1	1	×		
29					1	1	1	1	×	
30				1	1	1	1	×		
31			1	1	1	1	×			
32				1	1	1	1	×		
33			1	1	1	1	×			
34			1	1	1	1	1	×		
35				1	1	1	1	×		
36					1	1	1	×		
37			1	1	1	×				
38			1	1	1	1	1	×		
39				1	×					
40					1	1	1	×		
41				1	×					
42					1	1	1	×		
43					1	1	1	×		
44				1	1	1	1	×		
45					1	2	2	1	×	
46		1	1	2	2	×				
47					1	1	1	×		
48				1	2	2	×			
49				1	1	1	×			
50				1	1	1	×			
51					4	5	5	×		
52				1	1	1	×			
53			1	1	1	1	1	×		
54			1	1	×					
55				1	1	×				
56		1	1	3	3	×				
57						1	1	1	1	×
58					1	1	1	1	×	
59					2	2	2	×		
60					1	1	1	×		
61					1	1	2	×		
62						1	1	1	1	×
63				1	1	1	1	×		
64					2	2	2	×		
65					1	1	1	×		
66							1	2	1	×
67						1	1	1	1	×
68					1	1	1	×		
69					1	1	1	×		
70						1	1	1	1	×
71					1	1	1	1	×	
72							2	2	1	×
73						1	1	×		
74								2	2	×
75						1	1	1	1	×
76								1	1	×
77					1	3	3	×		
78						1	1	1	×	
79					3	3	1	×		
80					1	1	1	×		
81					1	1	2	×		
82					2	2	1	×		
83					1	×				
84							1	1	×	
85					×					
86						1	1	1	×	
87								1	1	×
88								1	2	×
89								1	1	×
90								1	1	×
total indivisuals	5	13	27	41	60	62	50	23	13	0
total flowers	5	14	29	50	78	77	65	26	15	0

Numbers indicate flowers on individuals. Gray areas indicate flowering dulation of individuals.
Crosses indicate date that flowering was finished.

Table 2. Floral visitors observed on flowers of *Lilium rubellum*.

Class	Order	Suborder	Family	Genus	Species	No. of visits	with pollen(%)
Insecta	Hymenoptera		Apidae	*Bombus*	*Bombus diversus diversus* Smith	2	100
				Ceratina	*Ceratina flavipes* Smith	8	100
					Ceratina esakii Yasumatsu et Hirashima	1	100
			Halictidae	*Halictus*		5	100
			Formicidae			4	0
	Diptera	Brachycera	Syrphidae	*Mesembrius*	*Mesembrius flavipes* Matsumura	1	100
				Syritta		1	100
				Episyrphus		3	100
				Blera	*Blera kyotoensis* (Shiraki)	1	100
		Nematocera				2	0
	Coleoptera		Oedemeridae	*Oedemeronia*	*Oedemeronia lucidicollis* (Motschulsky)	9	33
			Nitidulidae	*Carpophilus*	*Carpophilus chalybeus* Murray	20	5
	Orthoptera	Caelifera	Acrididae			2	50
Arachnida	Araneae		Thomisidae			5	0

corollas, and lateral insertion of anthers. Although additional observations of pollinators need to be accumulated before inferring trends in floral morphology in relation to pollinator fauna, the correlation between bee pollination and the above suite of floral characters provides a good working hypothesis for the evolution of pollination syndromes in *Lilium*.

Phylogenetic relationships among species of sect. *Archelirion* have not been fully resolved. Analyses based on ITS sequences have produced very poorly resolved relationships, although the section forms a monophyletic group, except for Chinese *L. brownii* (Dubouzet and Shinoda 1999; Nishikawa et al. 1999; Nishikawa et al. 2001). In contrast, analyses based on chloroplast DNA sequences have shown that the sister species of *L. rubellum* is *L. auratum*, and *L. japonicum* is the most closely related to *L. auratum* var. *platyphyllum*, an insular variety of *L. auratum* that is distributed in the Izu Islands (Nishikawa et al. 2002). If these relationships are correct, although the evolutionary direction has not been determined, either bee pollination or lepidopteran pollination has evolved independently within sect. *Archelirion*. To confirm these evolutionary patterns, more precise phylogenetic analyses must be conducted.

The flowering period of *L. rubellum* in the lowland habitat was earlier than the period of any other species of *Lilium* in northern Japan. Early flowering may be one of the reasons for the evolution of bee pollination in this species. In northern Japan, although the life histories of hawkmoths are not obvious, the appearance of the second brood of large butterflies, such as *Papilio* spp., occurs later than the flowering season of *L. rubellum* (Fukuda et al. 1982). The scarcity of large lepidopteran insects may be an important selective pressure driving *L. rubellum* to evolve characters that are suited to bee pollination. Observations of pollinators in various populations with different environmental conditions may provide empirical estimates of selections on floral characters by pollinators. *Lilium rubellum* is also distributed in high-altitude habitats, and the insect fauna in these areas differs from that of the lowland habitats observed during this study. Extensive comparisons should be made between pollinators in the current and other populations.

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