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Natural occurrence of oviposition and adult emergence of the seed parasitoid wasp *Macrodasyceras hirsutum* Kamijo (Hymenoptera, Torymidae) on *Ilex latifolia* Thunberg in Japan

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ABSTRACT: *Macrodasyceras hirsutum* Kamijo (Hymenoptera: Torymidae) has been considered to be a specialized seed parasitoid wasp of *Ilex integra* Thunb. since the description as a new species. A previous study showed that they deposited eggs in seeds of the closely related tree *I. latifolia* when *I. integra* was experimentally absent. However, natural occurrence of oviposition into *I. latifolia* or successful emergence from *I. latifolia* as adults had not been investigated. Thus, to determine whether *M. hirsutum* utilizes *I. latifolia* seeds in the fields, we dissected 536 *I. integra* seeds and 501 *I. latifolia* seeds. *Macrodasyceras hirsutum* larvae were found in 152/307 fertilized seeds of *I. integra*, while found in 16/285 fertilized seeds of *I. latifolia*. Additionally, 12 male and 13 female *M. hirsutum* adults emerged from *I. latifolia* berries collected from field. The present study confirmed natural occurrence of oviposition by *M. hirsutum* females in *I. latifolia* seeds and the emergence of adults from berries.

KEY WORDS: *Ilex integra*, *Ilex latifolia*, *Macrodasyceras hirsutum*, seed parasitoid wasp, Torymidae.

INTRODUCTION

Macrodasyceras hirsutum Kamijo (Hymenoptera: Torymidae) has been considered to be a specialized seed parasitoid wasp of an ornamental tree *Ilex integra* Thunb. (Aquifoliaceae) since the description as a new species by Kamijo (1981). Small red berries of *I. integra* surrounded with dark green leaves are attractive to people in winter. However this wasp may reduce the ornamental value because the larvae within seeds keep the berries green (Takagi *et al*., 2012).

Macrodasyceras hirsutum is partially bivoltine; the adults of the overwintered generation emerge between May and June and some insects of the first generation emerge as adults in August (Takagi *et al*., 2010). Female adults of the overwintered generation selectively lay one to five eggs in each fertilized seed from late May to mid-June (Takagi *et al*., 2010; Takagi and Togashi, 2013a). The larvae only eat the seeds and never damage the berry flesh (Kamijo, 1981; Takagi *et al*., 2010, 2012). Only one larva develops in each seed (Takagi *et al*., 2012). The larvae overwinter in seeds within berries attached to twigs (Takagi *et al*., 2010, 2012).

Of 25 *Ilex* species distributed in Japan (Yao *et al*., 2020), *Ilex latifolia* Thunb. (Aquifoliaceae) is also planted in gardens and is known as a symbol tree of the post office in Japan. Jian *et al*. (2017), Park *et al*. (2019) and Yao *et al*. (2020) have revealed that *I. latifolia*, *I. integra* and 11 other *Ilex* species constitute a clade termed *Aquifolium* section in the molecular phylogenetic tree. *Ilex latifolia* and *I. integra* are distributed widely and sympatrically in Japan, although the northern limit is different between them, i.e., Miyagi Prefecture for *I. integra* and Shizuoka Prefecture for *I. latifolia* (Miyawaki *et al*., 1983; Katsuta *et al*., 1998). Unlike the two *Ilex* species, the remaining 11 species show restricted ranges; *I. matanoana* Makino, *I. mertensii* Maxim., *I. percoriacea* Tuyama and *I. beecheyi* (Loes.) Makino are endemic to the Bonin Islands and *I. maximowicziana* Loes., *I. dimorphophylla* Koidz., *I. warburgii* Loes and *I. liukiuensis* Loes are endemic to the Ryukyu islands (Yao *et al*., 2020). *Ilex rugosa* F. Schmidt is distributed in high-altitude areas, *I. leucoclada* (Maxim.) Makino in the heavy-snow region, and *I. buergeri* Miq. in the warm areas of western Japan (Satake *et al*., 1993).

Ilex latifolia and *I. integra* are extremely similar in phenology, morphology and reproduction biology. For example, they are dioecious, bird-dispersed, broad-leaved evergreen tree species (Miyawaki *et al*., 1983). The flower buds are completed by autumn in the leaf axils of the current-year twigs. The flowers open from late March to mid-April in the following year (Katsuta *et al*., 1998). The pistillate flowers of the two species are characterized by four dysfunctional, small stamens and one large superior ovary with three to five cavities, each

Table 1. Larval occurrence of the seed parasitoid ***Macrodasyceras hirsutum*** in ***Ilex integra*** and ***I. latifolia*** seeds.

Tree species	*I. integra*			*I. latifolia*		
Study site	Tokyo	Chiba	Osaka	Tokyo	Chiba	Osaka
Time when berries were sampled	July 2015	July 2015	July 2016	July 2015	July 2015	July 2016
No. of trees where berries were sampled	3	4	1	2	2	1
No. of berries dissected	60	60	15	50	50	25
No. of seeds dissected	239	237	60	201	200	100
No. of fertilized seeds (F)	99	152	56	131	81	73
No. of seeds with *M. hirsutum* larvae (I)	44	77	31	7	0	9
% of infested seeds (I / F)	44.4	50.7	55.4	5.3	0.0	12.3

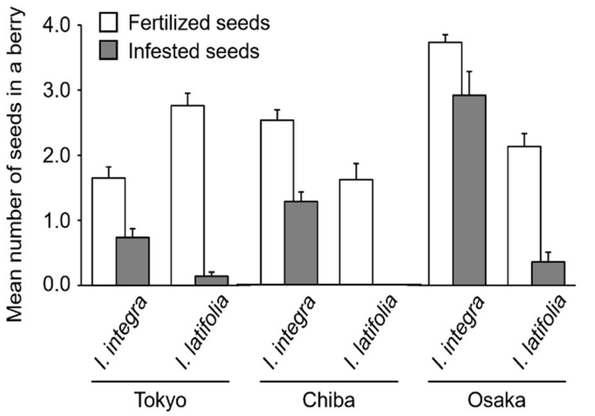

Fig. 1. Mean numbers of fertilized and ***Macrodasyceras hirsutum***-infested seeds in ***Ilex integra*** and ***I. latifolia*** berries sampled in Tokyo, Chiba, and Osaka. The numbers of fertilized seeds include the numbers of parasitized seeds because the parasitoid wasps deposit their eggs only into fertilized seeds. Bars = *SE*.

of which contains one ovule enclosed within the endocarp (Katsuta *et al*., 1998; Takagi *et al*., 2010). Immediately after flowering, each ovary of pistillate flower starts to develop into a spherical berry irrespective of pollination (Takagi *et al*., 2010). Both the berries and seeds are smaller in *I. latifolia* compared to *I. integra* (Katsuta *et al*., 1998). Individual *I. latifolia* and *I. integra* trees exhibit marked, yearly fluctuations in berry production (Katsuta *et al*., 1998; Takagi and Togashi, 2012). In addition, female *I. integra* trees can change their sex and the change of sex is synchronous in individual trees (Takagi and Togashi, 2012), whereas the sex change is unknown in *I. latifolia*.

Phenological and morphological similarities in berry development and phylogenetic relatedness between *I. integra* and *I. latifolia* imply the attack of *I. latiflora* berries by *M. hirsutum*. Actually, enclosure experiments with *I. latifolia* berries alone showed the deposition of the eggs into the seeds and the development into fully grown larvae in the seeds (Takagi and Togashi, 2013b). Thus, to determine whether *M. hirsutum* utilizes *I. latifolia* seeds in the fields, this study investigated: (1) natural occurrence of oviposition into *I. latifolia* seeds by *M. hirsutum* and (2) the successful emergence of *M. hirsutum* as adults from *I. latifolia* berries.

MATERIALS AND METHODS

Three areas of central Japan were selected to investigate the oviposition and development of *M. hirsutum* on the two *Ilex* species. The first was in urban areas of Nishi-Tokyo City, Chofu City and Bunkyo-ku, Tokyo Prefecture, which included the University of Tokyo Tanashi Forest in Nishi-Tokyo City and the campus of the University of Tokyo at Hongo, Bunkyo-ku. The second was in a hilly, rural area covered with forests in Kimitsu and Kamogawa Cities, Chiba Prefecture, which included the University of Tokyo Chiba Forest, where coniferous plantations, natural mixed forests of broad-leaved evergreen and coniferous trees and secondary broad-leaved forests covered the hills. The third was in an urban area of Osaka City, Osaka Prefecture, which included the Nagai Botanical Garden.

To determine the natural occurrence of oviposition by *M. hirsutum* into *I. latifolia*, 25 berries were randomly collected from each of four trees in the Tokyo and Chiba study areas in mid-July 2015 (Table 1). In the Osaka study area, 25 berries were randomly collected from a tree in mid-July 2016. As for *I. integra*, to confirm the oviposition by *M. hirsutum* in the areas, 15 berries were randomly collected from each of two trees in Nishi-Tokyo City and 30 berries from a tree in Hongo campus of the University of Tokyo in mid-July 2015 (Table 1). In the Chiba study area, 15 berries were randomly collected from each of four trees in mid-July 2015. In the Osaka study area, 15 *I. integra* berries were randomly collected from a tree in mid-July 2016. The berries on twigs were kept in the dark at 5°C before examination. The berries were dissected under a microscope to record the number of fertilized seeds in each berry and the presence or absence of wasp larvae in each seed.

To compare the proportion of infested seeds with *M. hirsutum* between *I. integra* and *I. latifolia* fertilized seeds, we conducted Fisher's exact test separately for each study site. The 5 % significance level was adjusted with a Bonferroni correction ($\alpha = 0.017$). We also used a generalized linear mixed model (GLMM) with binomial distribution linked with logit to compare the proportion of fertilized seeds that were infested with parasitoid wasps between *I. integra* and *I. latifolia*. The response variable in the model was the number of wasp-infested seeds out of the fertilized seeds in each berry.

Explanatory variables were tree species as fixed effect and study sites, within which trees were nested, as random effect. *Ilex integra* was used as reference. *P* value was calculated by Wald test. Computation was implemented using R 3.3.2 software (R Core Team, 2016) with package "lme4" (Bates *et al*., 2015).

To confirm the completion of wasp development in *I. latifolia* seeds in the field, two twigs bearing berries were collected from an *I. latifolia* tree in Hongo campus of the University of Tokyo on 13 May, 2013. The twigs were then placed in plastic bags and kept in the dark at 25°C to capture the wasps.

RESULTS AND DISCUSSION

The seed dissection showed that *M. hirsutum* larvae were present in 152/307 fertilized seeds within 135 *I. integra* berries and in 16/285 fertilized seeds within 125 *I. latifolia* berries when the berries were sampled during mid-July in the three study areas (Table 1) (Fig. 1). The proportion of larva-infested seeds was significantly higher in *I. integra* fertilized seeds than in *I. latifolia* ones (Fisher's exact test, $P < 0.001$ for all sites) (Table 1). GLMM also showed that the proportion of larva-infested seeds was significantly higher in *I. integra* than in *I. latifolia* (coefficient ± SE = -3.28 ± 1.38, $z = -2.38$, $P = 0.017$).

Enclosure experiments with *I. latifolia* berries alone showed the deposition of eggs into the seeds (Takagi and Togashi, 2013b). However, natural occurrence of oviposition into *I. latifolia* seeds by *M. hirsutum* had been unclear. The present study confirmed natural occurrence of oviposition by *M. hirsutum* females in *I. latifolia* seeds. To the best of our knowledge, this is a first record of natural occurrence of oviposition by *M. hirsutum*.

Twelve male and 13 female *M. hirsutum* adults emerged from *I. latifolia* berries that were collected from a tree in Hongo campus of the University of Tokyo in 2013. Takagi and Togashi (2013b) reported their development into mature larvae in the seeds. The present study also confirmed the emergence of adults from *I. latifolia* berries, indicating that *M. hirsutum* completes the life cycle on *I. latifolia*.

Ilex latifolia is the closely related species to *I. integra* (Yao *et al*., 2020), and their phenology and morphology are similar (Miyawaki *et al*., 1983; Katsuta *et al*., 1998). It is suggested that these similarity and phylogenetic relatedness between the two *Ilex* species allow the attack of *I. latifolia* berries by *M. hirsutum* and completion of the life cycle on *I. latifolia*.

Macrodasyceras hirsutum larvae prevent *I. integra* berries from turning red in autumn – winter (Takagi *et al*., 2012). Close relatedness between the two *Ilex* species makes us predict that the response of *I. latifolia* to wasp parasitism is similar to that of *I. integra*. If the parasitism by *M. hirsutum* also prevents *I. latifolia* berries from changing their color from green to red, it would reduce the ornamental value. The relationship between the wasp parasitism and berry color in *I. latifolia* remains to be determined.

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