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Findings of Agamic Generation Gall Caused by *Andricus moriokae* (Hymenoptera: Cynipidae) on *Quercus serrata* (Fagaceae)*

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Abstract. *Andricus moriokae* (Hymenoptera: Cynipidae) was originally described based on the bisexual generation alone and its agamic generation was not previously recognized. By caging bisexual generation females in a small pouch covering terminal buds of *Quercus serrata* (Fagaceae), we found the agamic generation galls of *A. moriokae* and determined the combination of the two generations. We also clarified the oviposition site by the bisexual generation females and described the shape, color, and size of agamic generation galls.

Key words: heterogonous annual life cycle, oak cynipid, oviposition site, bisexual generation, combination of generations.

Introduction

Haplodiploidy is a normal mode of reproduction in various species of Hymenoptera and thelytokous parthenogenesis is prevalent in the subfamily Cynipinae (Hymenoptera: Cynipidae). The most complex reproductive cycle is seen in many species of oak cynipids. They alternate a bisexual generation of males and females with a so-called agamic generation comprising only females although there are some exceptions (Askew, 1984; Wiebes-Rijks & Shorthouse, 1992; Yukawa & Masuda, 1996; Stone *et al.*, 2002).

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In Japan, 101 sorts of oak cynipid gall (including galls modified by inquilinous cynipids and those produced by thelytokous parthenogenetic cynipid species) are known to occur (Yukawa & Masuda, 1996). Among the 101 sorts of gall, the combinations of bisexual and agamic generations have been confirmed for 56 sorts (28 sets of combination) by rearing and oviposition experiments (Mukaigawa, 1914, 1920; Masuda, 1959, 1972; Yasumatsu & Masudà, 1955; Yano, 1973; Yukawa & Masuda, 1996). However, heterogony or life history has been left unclear until today for the remaining 45 sorts of the Japanese oak cynipid gall. Difficulties in combining the two generations are due chiefly to distinct differences between the two generations in the morphological features of female, the shape of gall, and galling site. Nevertheless, it is fundamentally necessary to clarify the combination of the generations as the first step toward future taxonomic, phylogenetic, and ecological studies of the oak cynipids.

Andricus moriokae Monzen (Hymenoptera: Cynipidae) is one of these species of which agamic generation has not been clarified, although its bisexual generation galls are known to occur in spring on the leaves of *Quercus serrata* Thunb. 'Konara' (Fagaceae) (Yukawa & Masuda, 1996). Recently we finally succeeded to combine the two generations of *A. moriokae* after several attempts of oviposition experiment. This paper intends to describe the oviposition site of bisexual generation females, the shape and position of agamic generation galls, and the annual life cycle of *A. moriokae*.

Materials and Methods

General information on A. moriokae

Andricus moriokae is distributed in Japan (except Hokkaido and the Ryukyus), the Korean Peninsula, and the Russian Far East (Yukawa & Masuda, 1996). During the bud burst in spring, bisexual generation larvae of *A. moriokae* make monothalamus, fleshy, and glassy drum-shaped galls that swell on both surfaces of the leaf of *Quercus mongolica* Fisch. 'Mongori-nara', *Q. mongolica* var. *grosseserrata* Rehd. et Wils. 'Mizu-nara' (= *Q. crispula* Blume), and *Q. serrata* (Fig. 1). Although *Q. mongolica* var. *grosseserrata* and *Q. crispula* have been synonymized with *Q. mongolica* (see Woods of the World: http://www.forestworld.com; Forestry Compendium, CAB International), we used the old names in this paper to distinguish the two known host trees that were previously separated into different species. The Japanese name of this gall is 'Nara-ha-taiko-tama-fushi' (Gall No. C-145 in Yukawa & Masuda, 1996).

Census field and trees

Field surveys were conducted from February 2001 to April 2002 in a secondary forest belonging to the Research Institute of Kyushu University Forests, Fukuoka

Prefecture, northern Kyushu, Japan. This forest consists mainly of deciduous oaks such as *Q. serrata* and *Quercus variabilis* Blume 'Abe-maki'. Two trees of *Q. serrata* (3 and 6 m in height, respectively; Qs-1 and Qs-2, hereafter) were selected as census trees for periodical field surveys and oviposition experiments.

Oviposition site of bisexual generation females

Five branches on Qs-1, each of which is about 30 cm in length from base to apex, were randomly selected on February 16, 2001 and covered with fine insect nets to prevent other cynipid species from galling and to avoid infestations by herbivores. On April 27, soon after bud burst on the branches, current leaves, developing acorns, and new terminal buds (overwintering buds later in season) on current shoots were covered individually with a small pouch made of gauze.

On April 1, 2001, bisexual generation galls of *A. moriokae* were first found on Qs-2. In order to avoid attacks by parasitoids, inquilines, and cecidophages, 256 bisexual generation galls were individually covered with a small pouch on the same day. These galls were monitored everyday until May 15 to obtain bisexual generation adults of *A. moriokae*. Forty-five adult males and 49 females of bisexual generation were obtained during the period from April 1 to May 15. As soon as they emerged from these galls, 45 pairs of male and female were put into a plastic container (7 cm in diameter, 8 cm in height), respectively, and the matings were confirmed by direct observation.

Forty-five mated females were divided into three groups (group A, B, and C), each containing 15 females. Females from group A were introduced individually into the aforementioned small pouch covering a current leaf. Similarly, those from group B and C were introduced into a small pouch covering a developing acorn and new terminal buds, respectively. Then, their oviposition behavior was observed intensively and the galling site was confirmed.

Agamic generation

On October 7, 2001, several months after the oviposition experiment, we found some galls produced by agamic generation larvae on the terminal buds (= overwintering buds) of Qs-1. Some of these galls were collected from the tree and dissected under a binocular microscope to examine the number of larvae and larval chambers per gall. At the same time, the color, shape, and size of these galls were recorded to examine whether or not these galls were already described in Yukawa & Masuda (1996).

In order to avoid attacks by parasitoids, inquilines, and cecidophages, the agamic generation galls that were gregariously produced on a bud were also covered with a small pouch immediately after these galls were first found on October 7, 2001. Some of the galls were dissected on December 16, 2001, January 16, February 16, and March

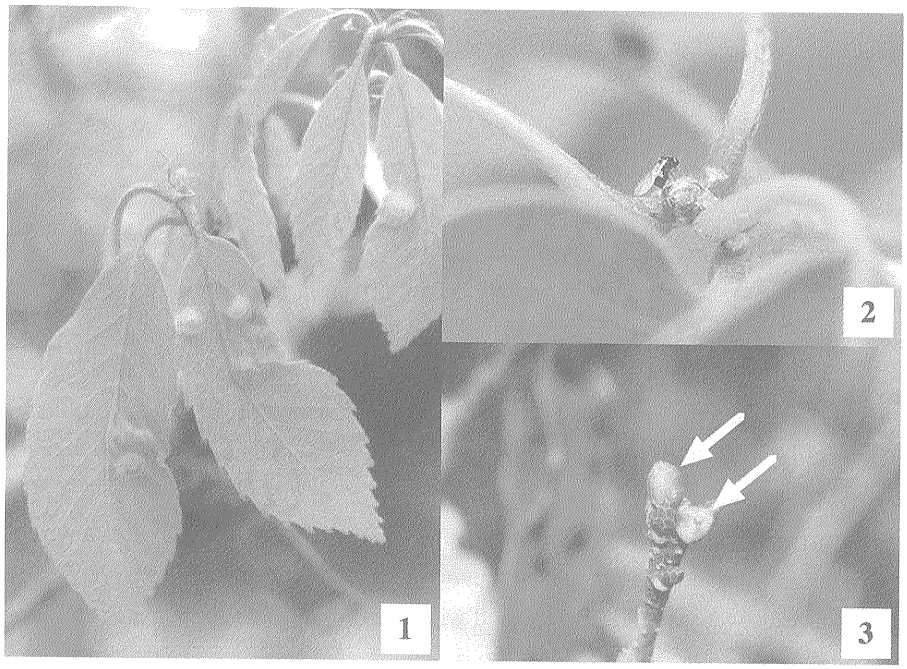

Fig. 1. Bisexual generation galls of *Andricus moriokae* on the leaves of *Quercus serrata.*

Fig. 2. A bisexual generation female of *Andricus moriokae* laying her eggs into a terminal bud of *Quercus serrata.*

Fig. 3. Agamic generation galls of *Andricus moriokae* on the terminal buds of *Quercus serrata.*

7, 2002 to determine the developmental stage of *A. moriokae.* Remaining galls were monitored once a week until April 10, 2002 and the emergences of agamic generation females from their galls were recorded.

Results

Oviposition site of bisexual generation females

Thirteen out of the 15 bisexual generation females belonging to group C were observed to have laid their eggs into newly produced terminal buds (overwintering buds) (Fig. 2). On October 7, 2001, 25 agamic generation galls of *A. moriokae* appeared on the overwintering buds (Fig. 3). In contrast, all the females of groups A and B did not lay their eggs either on current leaves or into developing acorns, respectively.

Agamic generation

The agamic generation gall was spindle-shaped and greenish brown, dark red, or

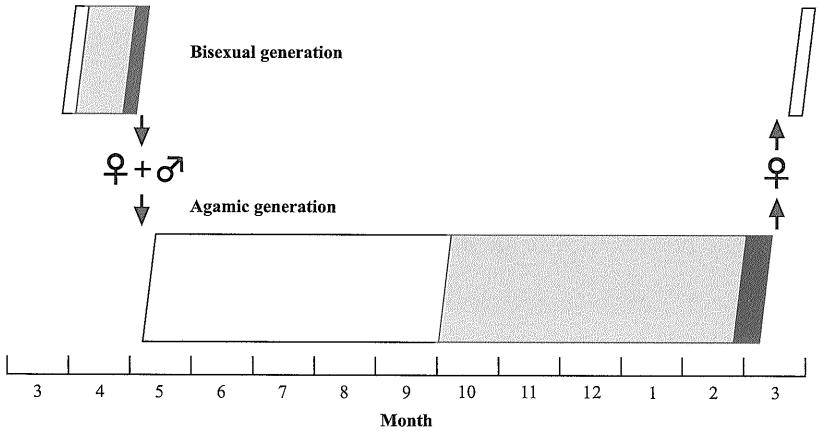

Fig. 4. Schematic representation of the annual life cycle of *Andricus moriokae*.

: egg stage : larval stage : pupal stage

blackish purple in color (Fig. 3). There was a small projection at the top of the gall (Fig. 3). The mean height of mature gall was 3.27±0.62 mm (mean±SD) (N = 17) and the mean diameter was 2.43±0.23 mm. This gall was monothalamus, containing a single agamic generation larva. Usually, the galls were produced gregariously on an overwintering bud.

The agamic generation gall of *A. moriokae* coincided well in appearance with that of an unidentified cynipid species that had been described under the Japanese name 'Nara-me-mure-togari-tama-fushi' (Gall No. C-133 in Yukawa & Masuda, 1996). Based on the similarity, we identified the agamic generation gall as C-133. This is the first record of this gall from Kyushu.

In the agamic generation, *A. moriokae* overwintered through December to February in the gall as a full-grown larva and pupated by March 7, 2002. Five females of the agamic generation emerged from the galls from March 12 to 22, 2002.

Conclusion

The findings of agamic generation revealed that *A. moriokae* has a heterogonous annual life cycle as seen in many other oak cynipids (Askew, 1984; Wiebes-Rijks & Shorthouse, 1992; Yukawa & Masuda, 1996; Stone *et al.*, 2002). In mid March, the agamic generation females lay their eggs into the overwintered buds of *Q. serrata* (Fig. 4). During the bud burst in early April, the bisexual generation larvae make swellings on both surfaces of host leaves (Fig. 4). The galls mature within 2 weeks. The larvae pupate in the galls in late April, and both males and females emerge in early May. After mating, the sexual generation females lay their eggs into the new terminal buds. The

agamic generation larvae produce galls on these buds in early October. They mature before winter, pupate in the galls by the following March, and adults emerge in mid March (Fig. 4).

As mentioned earlier, annual life cycle including the combination of generations is still unclear for many Japanese oak cynipids. In order to overcome the difficulties in combining the two generations, future DNA analysis may help to confirm suspected combinations and provide us with the full information of annual life cycle of respective species.

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