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The scale and parasitoid community on native hemlocks in Japan

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HIGHLIGHTS

- Eighteen parasitoid species attack six scale species on hemlock in Japan.
- Eleven parasitoid species attack *F. externa* in Japan, and six of these are exclusive.
- Only *E. citrina* attacks all six scale species on hemlock in Japan.
- No evidence of cryptic species of *E. citrina* attacking scales on hemlock in Japan.

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ABSTRACT

Elongate hemlock scale (EHS), *Fiorinia externa* (Hemiptera: Diaspididae), is an invasive species in the United States of Japanese origin that was first detected in New York in 1908. In the United States, EHS attacks eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*), and has established in most states where these trees occur. Elongate hemlock scale density can be several orders of magnitude higher in the United States than in its native Japan despite the presence of the parasitoid *Encarsia citrina* (Hymenoptera: Aphelinidae) in both locations. We postulated three possible reasons for this: (1) cryptic diversity of *E. citrina* attacking EHS exists in Japan, but not in the United States, (2) additional parasitoid species attack EHS in Japan at significant rates, or (3) alternate armored scale hosts on hemlock in Japan enhance rates of parasitism of EHS by *E. citrina* or other parasitoid species. We surveyed 88 sites (or subsites) throughout Japan for all armored scales on hemlock to explore the scale community and the genetic diversity of the associated parasitoids, as well as the relationship between scale community species richness and percent parasitism of EHS. Six armored scale species were found on hemlock in Japan. Using two gene loci (28S-D2 and COI), 18 identified or putative species of parasitoids were reared from these six armored scale species. Only five of the 18 parasitoid species were found in more than one scale host species. Eleven of the 18 parasitoid species were found in EHS, and six of these were exclusive to EHS. *Encarsia citrina* was recovered from all six armored scale species, and there was no evidence for cryptic species of *E. citrina* or that it differed genetically from populations of this species found in the eastern United States. There was no relationship between the number of armored scale species present in a given location and percent parasitism of EHS by all parasitoid species. Our phylogenetic analysis corroborated the morphological identification of the parasitoid species, including those newly described, published in an earlier study. The results from this study suggest that the high densities and low parasitism rates of EHS in the United States may be due to a depauperate scale-parasitoid community compared to Japan. In particular, this study identified ten new parasitoid species attacking EHS in Japan, which suggests strong parasitoid pressure on EHS populations there. Despite the diverse parasitoid community associated with EHS, parasitism rates were often low. This may be indicative of natural variation in spatial/temporal host-parasitoid population dynamics, scale suppression from plant-mediated factors, or a combination of both. The hemlock scale parasitoids found in this study provide many potential biological control candidates should management of EHS or other hemlock scales become necessary in the future.

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1. Introduction

Elongate hemlock scale (*Fiorinia externa* Ferris) (EHS) (Hemiptera: Diaspididae) is a non-indigenous pest of hemlocks (*Tsuga* spp.) in the eastern United States. Originating from Japan (Takagi, 1963; Watanabe and Uchida, 1967), EHS was first detected in the United States in 1908 on Long Island, New York (Ferris, 1942). Since that time, EHS has spread throughout most of the range of eastern and Carolina hemlocks. Between 1998 and 2005, EHS spread further north in New England and increased in density (Orwig et al., 2002; Preisser et al., 2008a). This spread, together with experimental evidence (Preisser et al., 2008b), suggests that EHS is evolving greater cold tolerance and may continue to move north. This evolution poses a threat to eastern hemlock forests in areas previously protected from infestation by cold winter temperatures.

EHS feeds on the fluid within hemlock needles. Several scales feeding on the same needle can cause needle death. Heavy infestations result in the loss of a large proportion of a tree's foliage, and sustained defoliation can cause tree mortality. In North America, EHS density varies from 21 to 420 scales per 100 needles in Connecticut (McClure and Fergione, 1977; McClure, 1978) and as high as 400 scales per 100 needles in North Carolina and Tennessee (Lambdin et al., 2005). In Japan, density of elongate hemlock scale is low, ranging from 0.0 to 0.15 scales per 100 needles (McClure, 1986). These low densities are associated with attack by the parasitoid *Encarsia citrina* Crawford (Hymenoptera: Aphelinidae) (Watanabe and Uchida, 1967; McClure, 1986). Parasitism rates by this wasp of over 90% that prevent EHS from reaching injurious levels were reported by McClure (1986) in Japan (Kyoto, 34°55′–35°05′ latitude, 80–150 m elevation). One additional parasitoid of EHS in Japan, *Arrhenophagus albitibiae* Gerault (Hymenoptera: Encyrtidae), was reported, but only accounted for 11.6% of all parasitized scales (McClure, 1986). *Encarsia citrina* is a generalist parasitoid that also occurs in the United States, attacking various armored scales (Diaspididae) (Krombein and Hurd, 1979), including 2nd instar female EHS. Parasitism of EHS by *E. citrina* in the United States varies widely from near zero to greater than 90% (McClure, 1981; Lambdin et al., 2005; Abell and Van Driesche, 2012).

In this study we use data from surveys of the community of hemlock scales and parasitoids found in Japan to examine three possible reasons why *E. citrina* fails to suppress the density of EHS in the United States to levels comparable to those in Japan. First, we considered the possibility that, due to the presence of cryptic species, the entities described as *E. citrina* in Japan and the United States might not be identical. Many studies have found cases where what was once thought to be a generalist parasitoid species was in fact a complex of cryptic species each with more specific host preferences (e.g., Heraty et al., 2007; Smith et al., 2008; Desneux et al., 2009; Zhu and Fang, 2009). Second, there might be species of parasitoids attacking EHS in Japan that are not present in the United States; this is plausible since previous surveys in Japan to find EHS parasitoids were confined to the Kyoto area (McClure, 1986). Finally, there may be other species of armored scales on hemlock in Japan that act as alternate hosts for *E. citrina*, collectively supporting a larger *E. citrina* population better able to suppress EHS. To address these hypotheses, the objectives of this study were to (1) conduct surveys throughout Japan to discover new parasitoid species that attack EHS, (2) document other species of armored scales that occur on hemlocks in Japan, namely the two native species *Tsuga diversifolia* [M.] Masters and *Tsuga sieboldii* Carrière and two introduced species, the North American *Tsuga canadensis* [L.] Carrière and the Chinese species *Tsuga chinesis* [Franch.] Pritzel ex Diels, (3) explore the parasitoid community attacking these armored scales using phylogenetic analysis, and (4) determine if the presence of other scale species on hemlock affected parasitism of elongate hemlock scale.

2. Materials and methods

2.1. Specimen collection

A survey of diaspidid scales on *T. diversifolia*, *T. sieboldii*, *T. chinensis*, and *T. canadensis* throughout the main island of Japan (Honshu) was conducted in May of 2009. Surveys of *T. diversifolia* and *T. sieboldii* were primarily done in natural areas, while *T. chinensis* and *T. canadensis* were confined to botanical gardens. Eighty-eight geographically distinct sites (or subsites, see Table 1) from N 34°03.540′ to N 40°38.167′ latitude, and E 135°10.386′ to E 140°52.527′ longitude, and ranging in altitude from 30 to 2324 m were surveyed. Forty-six sites had *T. sieboldii*, 37 sites had *T. diversifolia*, four sites had *T. canadensis,* and one had *T. chinensis* (Fig. 1). Sites were chosen based on the knowledge of local guides and foresters. The number of scales collected and the time spent collecting was variable depending on the number of hemlock trees present at a site and limitations set by foresters and land managers on the amount of material that could be taken. Trees were searched by visually scanning the undersides of needles for the presence of scales around the entire circumference of each tree when possible for all or most branches within arm's reach, or using a pole pruner when needed. Small branches and sometimes individual needles with scales were cut off and placed inside a sealable plastic bag (Zip-Loc). Once a scale species was collected, subsequent sampling effort was focused on discovery of other scale species so as to maximize the number of scale species collected. Therefore, the number of each scale species collected was not representative of the population at each site and density could not be estimated. Within 48 h, individual hemlock needles bearing scales of any species were placed in gelatin capsules, and checked every two days during the survey for newly emerged parasitoids. Upon emergence of a parasitoid, the scales on the needle were checked for an emergence hole to confirm the species of scale that had been parasitized, and the parasitoid was placed in 100% ethanol. At the conclusion of the survey, all parasitoids reared were shipped to the University of Massachusetts for later molecular analysis. Scales that did not produce parasitoids were shipped live to the USDA-FS quarantine facility in Ansonia, Connecticut, where they were held for further observation. Scales were monitored for five more weeks in quarantine for additional parasitoids. After five weeks, all scales were placed in 100% ethanol and taken to the University of Massachusetts where they were counted, identified, and dissected to look for evidence of parasitoid pupae or larvae in hosts. Armored scales collected in Japan were identified to species by one of us (BBN). The total number of scales collected and the total percent parasitism (all parasitoid species combined) was calculated for each scale species by collection site and *Tsuga* species (Table 1). Twenty-four additional parasitoid specimens collected from scales on hemlock in Japan by Suzanne Lyon in 2006 were also included in the phylogenetic analyses of this study.

Encarsia citrina from EHS on *T. canadensis* in the eastern United States included in the analyses in this study were collected in separate surveys, whose methods are described in Abell and Van Driesche (2012).

2.2. DNA extraction, amplification, sequencing, and alignment

To obtain sequences from collected parasitoids for phylogenetic analyses, DNA was extracted from whole adults or pupae dissected from scales using the DNeasy Blood and Tissue Kit (Qiagen,

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