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# Development and verification of SNP arrays to monitor hybridization between two host-associated strains of knotweed psyllid, *Aphalara itadori*

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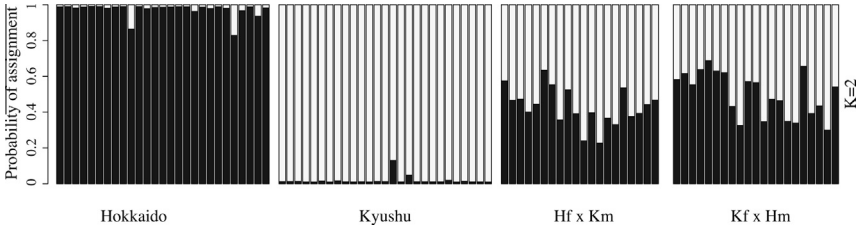
## HIGHLIGHTS

- Hybrid crosses established between two strains of the psyllid *Aphalara itadori*.
- We developed two SNP arrays to identify pure and hybrid individuals.
- A broader SNP array successfully identified all individuals.
- A reduced array failed to accurately identify most hybrid individuals.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Three species of invasive knotweeds (*Fallopia japonica*, *Fallopia sachalinensis*, and *Fallopia* × *bohemica*) cause extensive damage to riparian and roadside habitats in North America. Currently, two strains of the psyllid *Aphalara itadori* are being evaluated for introduction into the United States and Canada for the biological control of these knotweeds following the introduction of *A. itadori* into the United Kingdom. If approved and released, hybridization between individuals from these two strains is likely and understanding whether barriers to hybridization exist could have an important impact on the sustainability of this biological control program. Here we developed two single nucleotide polymorphism (SNP) arrays and examined their utility for identifying individuals of known pure strains (Hokkaido and Kyushu) and hybrid origins. Using an array of 141 SNPs we correctly identified all individuals to pure and hybrid classes, whereas using a smaller array of 29 SNPs we were able to correctly identify pure line individuals, but not hybrids.



## 1. Introduction

Hybridization in nature was long considered a relatively rare event with both pre- and/or post-zygotic barriers preventing the transmission of genomic material between species (Kirkpatrick, 2000; Lukhtanov et al., 2005; Orr and Presgraves, 2000; Presgraves, 2002). These barriers may, in part, be the result of incompatibilities among nuclear or cytoplasmic loci (Dobzhansky, 1936; Hurst and Pomiankowski, 1991) and/or incompatibilities between cytoplasmic loci and either nuclear loci or sex chromosomes (Ellison et al., 2008; Hurst and Pomiankowski, 1991). Yet, increasingly it is being recognized that hybridization between closely related organisms occurs in a broad range of taxonomic groups (Allendorf et al., 2001; Harrison and Larson, 2014) and that it has important consequences for evolution and speciation (Mallet, 2005). These hybridization events can lead to shifts

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in host-preference (Feder et al., 2003) and host-associated speciation events (Schwarz et al., 2005); furthermore, the rate at which hybridization events occur has undoubtedly been increased through anthropogenic activities that accidentally facilitate encounters between allopatric lineages (Allendorf et al., 2001).

Among insect taxa, hybridization appears to be a common occurrence (Schwenk et al., 2008). Given the extensive use of insect species as natural enemies in classical biological control programs (Hoddle, 2004; Van Driesche et al., 2010), hybridization has also likely played a frequent role in the sustainability of biological control services. Historically, many programs were established by collecting individuals of a selected natural enemy species from different localities and/or hosts within its region of origin. These populations have been referred to as strains, biotypes, and/or ecotypes (Heraty, 2009). This practice is much less common today, however, due to the recognition that each strain needs to be tested separately for its host specificity (Hopper et al., 2005). From an evolutionary perspective, strains likely represent an early point in the process of speciation (i.e., before genetic isolation [Dres and Mallet, 2002]) and can be influenced by factors such as gene-flow, genetic diversity, and phenotypic plasticity (Ruiz-Montoya and Nunez-Farfan, 2013). How frequently these strains hybridize, or what effect hybridization has on the sustainability of biological control services is unclear as relatively few studies have examined the effects of post-introduction hybridization. Those studies that have examined the potential effects of hybridization between strains have found evidence of both reduced (e.g. Messing and AliNiazee, 1988; Hoffmann et al., 2002) and increased (e.g., Szűcs et al., 2012) fitness for hybrids relative to their parents.

One system that provides a valuable model for testing the potential effects of hybridization on the host specialization and performance of a natural enemy in a classical biological control setting is the proposed program for the biological control of invasive knotweeds (*Fallopia* spp. [Caryophyllales: Polygonaceae]) in North America. These invasive weeds include Japanese (*Fallopia japonica* [Houttuyn] Ronse Decraene), giant (*Fallopia sachalinensis* Schmidt ex Maxim.), and Bohemian knotweed (*Fallopia* × *bohemica* [Chrtek & Chrtková] JP Bailey), and have been introduced to North America and Europe on several occasions beginning in the 1840s (Beerling et al., 1994). Invasive species of knotweed occur along riverbanks and roadways and in wetlands and disturbed areas where they invade riparian communities (Maerz et al., 2005; Siemens and Blossey, 2007) and reduce native plant and insect species diversity (Gerber et al., 2008; Murrell et al., 2011). The psyllid *Aphalara itadori* Shinji (Hemiptera: Aphalaridae) was recently released in Europe for the biological control of knotweeds (Djeddour and Shaw, 2010; Shaw et al., 2009), and has also been proposed for introduction into the United States and Canada. Two strains of *A. itadori* that differ in their fitness among *Fallopia* species have been proposed for introduction (Grevstad et al., 2013) due to regional differences in the abundance of each species of knotweed in North America (Gaskin et al., 2014). One of these strains was recently released in the UK for the Japanese knotweed biological control program there (Shaw et al., 2009), and originates from the Japanese island of Kyushu. This strain performs best on Japanese and Bohemian knotweeds, while the other strain originates from the Japanese island of Hokkaido and performs best on giant knotweed (Grevstad et al., 2013). Due to overlapping distributions of the invasive species of knotweed in some parts of the United States and Canada, the potential for hybridization between the released strains of psyllid is expected to be high. As there are no known morphological differences between the two psyllid strains, neutral molecular markers are needed to be able to distinguish them post introduction and to detect the incidence of hybridization.

One potential barrier to hybridization between these strains may be the presence of reproductive manipulating endosymbionts. Psyllids are known to harbor a diverse assemblage of endosymbionts, including the maternally transmitted primary endosymbiont *Candidatus* Carsonella ruddii (Gammaproteobacteria) that provides essential amino acids absent from their host's diet (Baumann, 2005; Moran and Bennett, 2014), and a range of secondary endosymbionts that have coevolved with *C. ruddii* and its insect hosts to provide additional metabolic functions (Chrudimský et al., 2012; Sloan and Moran, 2012). Some secondary endosymbionts are also known to cause sex-ratio distortion (Bordenstein et al., 2001; Ferrari and Vavre, 2011; Turelli, 1994), including species of *Wolbachia* (Rickettsiaceae), *Cardinium* (Bacteroidaceae), *Rickettsia* (Rickettsiaceae), *Spiroplasma* (Spiroplasmataceae), and *Arsenophonus* (Enterobacteriaceae) that are commonly found in insects (Engelstaedter and Hurst, 2009; Hilgenboecker et al., 2008). In the context of classical biological control programs, the presence of endosymbionts that either provide essential functions to their hosts or engage in reproductive manipulation may have important consequences for the establishment and impact of introduced natural enemies through the creation of barriers to geneflow (Cheyppe-Buchmann et al., 2011) and/or through influencing patterns of host specialization (Branca et al., 2011).

Therefore, the objectives of this study were (1) to develop neutral molecular markers for the two *A. itadori* strains, (2) to test the ability of these markers to successfully differentiate pure and hybrid individuals of *A. itadori*, and (3) to catalog the diversity of endosymbionts present in populations of both strains.

## 2. Materials and methods

### 2.1. Laboratory colonies

The insects in this study were sampled from laboratory populations maintained at the Insect Quarantine Facility at Oregon State University (OSU) and the Insect Microbial Containment Facility at Agriculture and Agri-Food Canada, Lethbridge (AAFC). The Hokkaido strain was originally field collected by RSB and FSG from giant knotweed on the Island of Hokkaido, Japan in July of 2007. The Kyushu strain was originally collected from Japanese knotweed on the Island of Kyushu in 2004 and was maintained in quarantine in the United Kingdom until a portion of the colony was transferred to OSU and AAFC in 2010. This strain was released into the United Kingdom in 2010 (Shaw et al., 2009; Djeddour and Shaw, 2010). Hybrid colonies were established at AAFC by placing individual females and males from respective strains together for 24 h to ensure mating occurred before moving them into rearing cages with either giant, Bohemian, or Japanese knotweed. All rearing of parent and hybrid lines at AAFC were conducted using the following controls. Colonies were established in separate and isolated rearing rooms, with only one parent colony being accessed per quarantine visit and one-way movement from parent colonies to hybrid colonies. Re-suiting was conducted for subsequent quarantine visits. For the Hokkaido female (H♀) × Kyushu male (K♂) hybrids, colonies were established in 2011 by rearing individuals for at least 25 generations before adults were placed in 95% ethanol, while for the Kyushu female (K♀) × Hokkaido male (H♂) hybrids, colonies were established in 2014 by rearing individuals for between 4 and 5 generations before adults were placed in 95% ethanol.

### 2.2. DNA extractions for identification of A. itadori strains

DNA was extracted from individuals of each strain of *A. itadori* from colonies maintained at OSU and AAFC, as well as from