Biological Control 63 (2012) 359–369

Contents lists available at SciVerse ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control

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HIGHLIGHTS

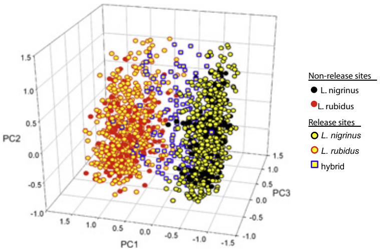
- We report hybridization between *Laricobius nigrinus* and *Laricobius rubidus*.
- Six microsatellite markers plus mitochondrial COI haplotypes were used.
- Introgression was widespread and asymmetrical towards *L. nigrinus*.
- The outcome could be a mosaic of genetic introgression across the landscape.
- Evaluating hybridization between biocontrol agents and native species is important.

ARTICLE INFO

Article history:
Received 25 June 2012
Accepted 8 August 2012
Available online 17 August 2012

Keywords:
Hemlock woolly adelgid
Adelges tsugae
Laricobius nigrinus
Laricobius rubidus
Landscape genetics
Non-target effects

GRAPHICAL ABSTRACT



ABSTRACT

Hybridization between introduced biological control agents and native species has the potential to impact native biodiversity and pest control efforts. This study reports progress towards predicting the outcome of hybridization between two beetle species, the introduced *Laricobius nigrinus* Fender and the native *L. rubidus* LeConte. *L. nigrinus* is a predator from western North America introduced to hemlock stands in the eastern United States as a biological control of the hemlock woolly adelgid [*Adelges tsugae* Annand (Hemiptera: Adelgidae)]. *Laricobius rubidus* is a closely related eastern species that also feeds on *A. tsugae* but prefers pine adelgids (*Pineus strobi* Hartig) on white pine (*Pinus strobus* L.). Six microsatellite markers plus mitochondrial COI haplotypes were used to examine genetic structure of these two *Laricobius* species across North America. In their native ranges, major geographic features have impacted gene flow: the intermountain region in the West, and the Appalachian Mountains in the East. Analysis of 1229 individuals from adelgid-infested hemlock trees in release sites in the eastern United States found widespread hybridization with asymmetrical introgression towards *L. nigrinus* on hemlock. The ultimate outcome of hybridization could therefore be a complex mosaic of genetic introgression across the landscape, depending on the distribution of hemlock and pine. This study confirms the importance of evaluating the potential for introduced biological control agents to hybridize with their native relatives. This system also provides an excellent opportunity to improve our understanding of emerging hybrid zones by tracking its progress over time.

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1049-9644/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.biocontrol.2012.08.001

1. Introduction

Hybridization is widespread in nature with complex ecological and evolutionary dynamics (Dobzhansky, 1940; Hewitt, 1988; Harrison, 1993; Burke and Arnold, 2001). Factors that influence whether hybridizing species ultimately merge or remain distinct include the permeability of reproductive barriers and the strength of selection against hybrids. Additional complexity is introduced when fitness is differentially impacted by environmental factors. Together, this makes it very difficult to predict the outcome of any particular hybridization event.

Accidental or deliberate introduction of non-native species that leads to hybridization with native species is a conservation concern (Rhymer and Simberloff, 1996; Allendorf et al., 2001; Mooney and Cleland, 2001; Seehausen et al., 2008; Ellstrand et al., 2010). Hybridization between introduced biological control agents and native species has also been discussed in this context because of potential impacts on native biodiversity or on the success of pest control (Hopper et al., 2006; van Lenteren et al., 2006).

We are aware of just three cases of hybridization between a classical biological control agent and a native species. *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is used for biological control of greenhouse pests. Naka et al. (2005, 2006) were able to produce laboratory crosses between *C. carnea* introduced from Germany, and native Japanese *C. nipponensis* (Okamoto). In this case, hybridization was not expected to threaten the integrity of the native species because hybrid fertility was low, and each parent species has a different courtship song. The second example involves *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae), a parasitoid used to control diamondback moth, which was shown to hybridize with native Japanese *D. fenestrale* (Holmgren) in the lab (Davies et al., 2009). Field evaluations have not been reported. Finally, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), introduced from China, was found to hybridize with native Japanese *T. beneficus* Yasumatsu et Kamijo in the lab (Moriya et al., 1992) and in the field (Toda et al., 2000; Yara et al., 2000). The native *Torymus* species has separate early-spring, and late-spring strains. The introduced species displaced the early-spring strain, without evidence of hybridization, while an increasing proportion of hybrids with the late-spring strain was detected over time (Yara et al., 2010). Release of *T. sinensis* resulted in successful control of the pest species, but the specific effects of hybridization on biological control were not evaluated.

Based on these few examples, it is not possible to predict the impact of hybridization on a particular biological control program. This requires specific information about the extent of interbreeding and the relative fitness of hybrids versus parents in the ecological context in which they are likely to be found. Hybrids can be less fit as expressed by outbreeding depression or hybrid sterility (Dobzhansky, 1940), which could maintain species integrity but impair control efforts if hybrids have less of an impact on the pest than their parents. Conversely, hybridization can be a source of novel genetic variation, introducing advantageous alleles or allele combinations that can contribute to adaptive evolution (Lewontin and Birch, 1966). This could enhance establishment and efficacy of the biological control agents but disrupt the genetic integrity of the native species with cascading effects on its own predator–prey dynamics.

The hemlock woolly adelgid, *Adelges tsugae* Annand, was introduced from Japan to the eastern United States (Havill et al., 2006) some time before its discovery in Virginia in the early 1950s. It has become a serious pest of eastern hemlock, *Tsuga canadensis* L. (Carrière) and Carolina hemlock, *T. caroliniana* Engleman. In western North America there is a native lineage of *A. tsugae* (Havill et al., 2007) that has a suite of predators that may be instrumental in regulating its populations (Kohler et al., 2008). Western North America has therefore been considered a promising source of biological control agents for introduction to the eastern United States.

One species, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), has been released since 2003 throughout the introduced range of *A. tsugae* in the eastern United States (Mausel et al., 2010). It has become established at many of the release sites and has been shown to reduce adelgid densities in the field, but the recovery of hemlock forest health has not been documented to date (Mausel et al., 2008, 2011b). *Laricobius* species are known to feed only on Adelgidae (Leschen, 2011). In laboratory tests, *L. nigrinus* preferred feeding and ovipositing on *A. tsugae* settled on *T. canadensis* over the native eastern pine bark adelgid, *Pineus strobi* (Hartig) settled on *Pinus strobus* L., and *L. nigrinus* completed development only on *A. tsugae* (Zilahi-Balogh et al., 2002). In western North America, *L. nigrinus* has been collected from *Tsuga heterophylla* (Raf.) Sarg., *Pseudotsuga menziesii* (Mirb.) Franco, *Picea engelmannii* Parry ex Engelm., *Pinus monticola* Douglas ex D. Don (Mausel et al., 2011b), and *Larix occidentalis* Nutt. (this study).

Laricobius rubidus LeConte is the only member of the genus that is endemic to eastern North America (Leschen, 2011). It is most commonly associated with *Pineus strobi* on *Pinus strobus* (Clark and Brown, 1960), but has also been reported feeding on three non-native adelgids: *A. tsugae* on *T. canadensis* (Montgomery and Lyon, 1996; Wallace and Hain, 2000; Mausel et al., 2008), *Adelges piceae* Ratz., on *Abies* spp. (Clark and Brown, 1960), and *Pineus pini* (Macquart) on *Pinus sylvestris* L. (this study). In laboratory tests, *L. rubidus* developed and survived equally well when reared on *A. tsugae* or *P. strobi*, but preferred to oviposit on *P. strobi* (Zilahi-Balogh et al., 2005). The two *Laricobius* species can be distinguished as adults by the color of their elytra and by differences in the shape of male genitalia (Leschen, 2011; Montgomery et al., 2011). Larvae of the two species cannot be distinguished morphologically.

Davis et al. (2011) evaluated genetic diversity within and differentiation between *L. nigrinus* and *L. rubidus* using a section of the cytochrome c oxidase subunit I (COI) mitochondrial gene commonly used for DNA barcoding. They found low sequence divergence between the two species. A phylogeny of the genus *Laricobius* reconstructed by Montgomery et al. (2011) confirmed that *L. nigrinus* and *L. rubidus* are recently diverged sister species. These results, plus observations of *L. nigrinus* and *L. rubidus* mating in the field (Mausel et al., 2008), drew attention to the possibility of hybridization between the two species.

The purpose of this study is to make progress towards predicting the impacts of hybridization between *L. nigrinus* and *L. rubidus* on biological control of *A. tsugae* and on native biodiversity in eastern North America. Microsatellite markers were evaluated for their ability to distinguish each species and their hybrids and were used to test for population structure within each species. Microsatellites and mitochondrial COI DNA sequence variation were then used to evaluate the incidence of hybridization in the field using samples collected on *T. canadensis* infested with *A. tsugae* at sites in the eastern United States where *L. nigrinus* was released. Adult beetle morphology was also examined to determine whether there are characters that can be used to distinguish parent species from hybrids.

2. Methods

2.1. Sample collection and data acquisition

Beetles were collected between 2006 and 2011 from 57 sites in the United States and one site in Canada (Fig. 1; Supplementary Table 1). Some of these specimens were also analyzed in Davis