Chapter 21: The Introduction of *Laricobius nigrinus* as a Biological Control Agent for the Hemlock Woolly Adelgid: Is There a Threat to the Native Congener, *L. rubidus*?

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BACKGROUND

Laricobius Rosenhauer is one of four genera in the family Derondontidae (Coleoptera) that occupies the temperate regions of the Northern Hemisphere (Lawrence 1989). Members of this genus are only known to prey on adelgids (Hemiptera: Adelgidae) (Lawrence and Hlavac 1979, Lawrence 1989). There are three species native to North America: *L. nigrinus* and *L. laticollis* are native to western North America, and *L. rubidus* is native to eastern North America. *Laricobius nigrinus* is being used in the eastern United States as a biological control of the hemlock woolly adelgid. Previously, *L. erichsonii* was introduced to both coasts of North America from Europe for control of the balsam woolly adelgid (summarized in Montgomery et al. 2011). This species was reported to have established, but its most recent recorded recovery was in 1978 (Schooley et al. 1984). A molecular study of the relationships among the four *Laricobius* species reported in North America, plus *L. kangdingensis* and *L. osakensis* from Asia, showed that, surprisingly, the two species from western North America were not the most closely related (Montgomery et al. 2011). Instead, it was found that *L. nigrinus* is very closely related to the eastern species, *L. rubidus*. In fact, the genetic distance between these species (using a portion of the mitochondrial COI gene) was only slightly higher than within each species (Davis et al. 2011). This suggests that they diverged very recently and may not be reproductively isolated. Recent work (described in more detail below) has shown that *L. nigrinus* and *L. rubidus* are in fact interbreeding at sites in the eastern U.S. where *L. nigrinus* was released. It is not yet known if this will enhance or hinder hemlock woolly adelgid (HWA) biological control.

ACKNOWLEDGING RISK

It is important to weigh the benefits and risks when making decisions for natural resource management (Loomans and van Lenteren 2005). Benefits of hemlock woolly adelgid are nil, while the risks and costs are great. Loss of hemlock timber and pulpwood (Burns and Honkala 1990, Ward et al. 2004) and residential property values (Holmes et al. 2006) can be tabulated, while calculating costs associated with intangible environmental and aesthetic benefits are much more difficult (Anders 1977, McConnachie et al. 2003). Left uncontrolled, HWA has the potential to cause hemlock mortality within all 25 forest cover types of which it is a component (Burns and Honkala 1990, Orwig et al. 2002). Loss of hemlock alters eco-hydrological systems (Ford and Vose 2007) and accelerates growth of invasive plants (Eschtruth and Battles 2008). It can also negatively impact temperature-sensitive streams (Snyder et al. 2002, Ross et al. 2003) and habitat for numerous wildlife species (Yamasaki et al. 1999, Onken and Souto 2000, Lishawa et al. 2007).

Some of the risk associated with a biological control agent can be evaluated by laboratory experiments prior to its introduction, yet it is recognized that environmental variability and other sources of uncertainty are cause for continued post-release assessment (Louda et al. 2003, Hopper et al. 2006). An unexpected risk that was recently discovered in association with the release of *L. nigrinus* is its ability to hybridize with a native species, *L. rubidus*. In this report, we summarize what is currently known about interbreeding between *L. nigrinus* and *L. rubidus*, and we discuss research directions to evaluate the implications for biological control of HWA.

Laricobius nigrinus Fender

Laricobius nigrinus Fender is a small (2-3 mm), black beetle native to western North America (Fender 1945, Zilahi-Balogh et al. 2006) where it has been found to be a widespread and abundant natural enemy of HWA, at both low and high densities of the pest (Kohler et al. 2008).

Both adults and larvae feed on *A. tsugae* eggs, nymphs, and adults. Eggs of *L. nigrinus* are laid in late winter and early spring. Larvae develop through four instars, feeding on HWA progrediens eggs, and drop to the forest floor to pupate. Adults diapause during summer in the soil and emerge in fall to feed on HWA sistens nymphs in the fall and winter (Zilahi-Balogh et al. 2003). The life cycles of *L. nigrinus* and HWA are highly synchronized (Zilahi-Balogh et al. 2003).

Laricobius nigrinus was imported into the eastern United States from Victoria, British Columbia for further evaluation and was determined to be host-specific in the laboratory (Zilahi-Balogh et al. 2002). Federal and State approval for environmental release of *L. nigrinus* was granted in 2000. Laboratory mass-rearing methods were developed for *L. nigrinus* and adults are currently being reared in a number of laboratories (Lamb et al. 2005). Free releases of *L. nigrinus* began in 2003. As of 2009, *L. nigrinus* adults were released in 15 eastern states, spanning USDA plant hardiness zones 5a to 7a (Roberts et al. 2010). It was found to establish in 13/22 (59%) of initial release sites (Mausel et al. 2010). The probability of establishment was greater at sites with higher minimum annual temperatures and where more beetles were released. Additional *L. nigrinus* from Idaho has been released in several New England states in an attempt to establish a more cold-hardy strain in the north.

Laricobius rubidus

Laricobius rubidus is the only species of *Laricobius* native to eastern North America (Clark and Brown 1960; Lawrence 1989). Its known distribution extends from the District of Columbia, north to New Brunswick, west to Minnesota, and south to North Carolina (Brown 1944, Raske and Hodson 1964, Lawrence 1989, Wallace and Hain 2000). Its primary host is the pine bark adelgid (PBA), *Pineus strobi* Hartig (Clark and Brown 1960). *Laricobius rubidus* has also been found to occasionally feed on the balsam woolly adelgid, *Adelges piceae* Ratz. (Lawrence and Hlavac 1979) and has been collected from eastern hemlock infested with HWA throughout its introduced range (Montgomery and Lyon 1996, Wallace and Hain 2000, Mausel et al. 2008). Laboratory studies have shown that it can reproduce and complete development on HWA, but has an ovipositional preference for pine bark adelgid (PBA) (Zilahi-Balogh et al. 2005).

The life cycle of *L. rubidus* is well synchronized with that of PBA (Clark & Brown 1960). Adults are active between late March and early June with peak activity between mid-April to mid-May (Clark and Brown 1960, Zilahi-Balogh et al. 2005). Four instars are present late April through early June (Clark and Brown 1960), migrating to the soil to pupate by late June (Zilahi-Balogh et al. 2005). Emerging adults undergo an aestival diapause, becoming active in October through early November (Zilahi-Balogh et al. 2005). In Virginia, *L. rubidus* adults have been observed migrating from the branches to the duff where they are thought to undergo a hibernal diapause, but adults associated with HWA have also been found to be active in the winter (Zilahi-Balogh et al. 2005, Mausel et al. 2008).

POTENTIAL FOR INTERBREEDING

Adult *L. nigrinus* can be distinguished morphologically from *L. rubidus*. *Laricobius nigrinus* (Fig. 1) has unicolorous (black) elytra, the distance across the posterior of the pronotum is greater than across the anterior, and the apices of the lateral parameres of the male genitalia are narrowly acute. In contrast, *L. rubidus* (Fig. 2) has bicolor (red and black) elytra, the distances across the posterior and anterior of the pronotum are subequal, and the apices of the lateral parameres are truncate (Montgomery et al. 2011, Leschen 2011). The immature life stages are morphologically indistinguishable.

Several observations prompted questions about the potential of *L. nigrinus* and *L. rubidus* to interbreed: 1) both species are routinely recovered from HWA-infested hemlock trees in the eastern U.S. at sites where *L. nigrinus* was released; 2) molecular analysis of the genus *Laricobius* found, surprisingly, that *L. nigrinus* and *L. rubidus* are very closely related suggesting that they are recently diverged species that may have the ability to produce viable offspring (Klein et al. 2010, Montgomery et al. 2011); 3) members of the two species were observed copulating with each other on HWA infested hemlock at the Virginia Tech field insectary, which neighbors a white pine stand infested with pine bark adelgid (Mausel et al. 2008); and 4) morphological and molecular species identification were found to be in conflict for two beetles collected from a *L. nigrinus* release site in Maryland, suggesting that these individuals could be of hybrid origin.

This prompted the development of microsatellite markers that could be used to distinguish *L. nigrinus* and *L. rubidus* from their hybrids (Klein et al. 2010). This method exposed a trend of an increasing proportion of hybrids recovered at *L. nigrinus* release sites in Pennsylvania, North Carolina, and Tennessee between 2007 and 2009 (Havill et al. 2010). It was also used to identify *Laricobius* adults collected from HWA-infested hemlock the Virginia Tech field insectary where PBA-infested white pine grows in close proximity (N. Havill, unpublished data). Data from six microsatellite loci analyzed with the software NEWHYBRIDS (Anderson & Thompson 2002) were used to classify beetles. In 2008 we collected 27 *L. nigrinus*, 15 *L. rubidus*, and 13 hybrids. In 2010 we collected 87 *L. nigrinus*, 4 *L. rubidus*, and 8 hybrids, and in 2011 we collected 87 *L. nigrinus*, 7 *L. rubidus*, and 9 hybrids. Further confirmation that these species can interbreed was shown in a 2009 laboratory study where three interspecific pairs produced viable offspring (T. Dellinger, unpublished data).

Figure 1. *Laricobius nigrinus* (photo by Gina Davis).

Figure 2. *Laricobius rubidus* (photo by Gina Davis).

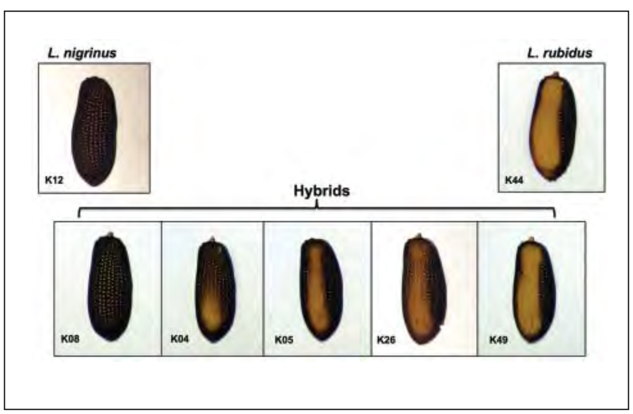
Field collected beetles that were identified as having mixed parentage had morphological characters that resembled either parent species or were intermediate—i.e., they had black or bicolored elytra, and the parameres of the male genitalia were either accute, truncate, or intermediate (Fig. 3). It is therefore not possible to use morphology to distinguish beetles of mixed parentage from the parent species.

The ecological niches occupied by *Laricobius* species and their offspring may affect the geographic distribution and extent of interbreeding. Laboratory host range studies show that *L. nigrinus* prefers HWA on hemlock (Zilahi-Balogh et al. 2002), and *L. rubidus* prefers PBA on white pine (Zilahi-Balogh et al. 2005). It may therefore be more likely for the two species to encounter each other in areas where hemlock and white pine co-occur than in areas with only one host species is present. The extent to which *Laricobius* adults migrate between stands would also affect the rate of interbreeding. *Laricobius nigrinus* was found to be common within 300 m of the original release trees by the fourth generation (G. Davis, unpublished data). Other observations suggest that *L. nigrinus* can disperse greater distances. For example, McDonald (2010) recovered *L. nigrinus* from at least 1.6 km from the release area, five years post-release. Preliminary data suggest that the geographic overlap of hemlock and white pine may indeed affect the rate and incidence of interbreeding between the species. Recovery of *L. rubidus* on hemlock was lower where eastern white pine was sparse or absent from stands in which *L. nigrinus* was released (G. Davis, unpublished data). In addition, we collected *Laricobius* from white pine at the Virginia Tech field insectary in 2011, and all 47 were classified as pure *L. rubidus* (Havill, unpublished data). Additional samples from hemlock and white pine in *L. nigrinus* release sites, as well as laboratory choice tests with hybrid beetles will help to further predict the importance of ecological factors in determining the outcome of interbreeding.

POSSIBLE HYBRIDIZATION SCENARIOS

Introductions of nonnative species can have large impacts on the genetics of native species through hybridization and introgression (i.e. gene flow) (Mooney and Cleland 2001, Mallet 2007). Hybridization between *L. rubidus* and *L. nigrinus* could have several outcomes, including:

- Hybrid Incompatibility
  - Sterility of hybrids
  - Outbreeding depression
  - Reinforcement of premating isolation
- Hybrid vigor
  - Speciation
  - Genetic assimilation



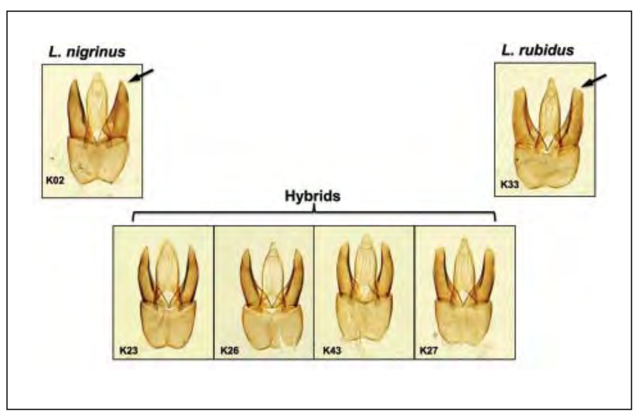


Figure 3. Examples of elytra (left) and slide-mounted male genitalia (right) of *L. nigrinus*, *L. rubidus* and their hybrids. Arrows point to the lateral parameres that are acute in *L. nigrinus* and truncate in *L. rubidus*. Hybrids can resemble either parent species or can be intermediate.

Hybrid Incompatibility

Reproductive isolation between populations can result in the accumulation of genetic incompatibilities over time. This could make reproductive isolation permanent, even if the cause of isolation were removed (Palmer and Feldman 2009). Hybridization produces recombinant genotypes that have not previously been subjected to selection. These genotypes will typically be less well adapted than those of their parents, resulting in selection against hybrids (Burke and Arnold 2001).

Sterility

Selection against hybrids is often exhibited as sterility or inviability (Haldane 1922, Mallet 2007). The production of sterile or inviable offspring would result in a decrease in fitness of the parental species due to an overall decrease in reproductive output. Although *Laricobius* hybrid sterility is a possibility, there is evidence of F_2 hybrid individuals and backcrosses in the field (N. Havill, unpublished data), suggesting that at least some of the F_1 hybrids are fertile.

Outbreeding depression

Outbreeding depression is a reduction in hybrid fitness, possibly due to the hybrid offspring being less well adapted to environmental conditions than the parental species (Klug and Cummings 2003). Outbreeding could result in lower reproductive potential (Arnold 1997). The reduction in reproductive output may occur as a result of a decrease in the number of offspring produced or as a result of lower levels of fertility or vigor among the hybrid progeny (Arnold 1997). We do not know whether *Laricobius* hybrids are less fit than their parents. Ongoing laboratory and field studies are explicitly testing this.

Reinforcement of pre-mating isolation

Hybridization can lead to an increase in reproductive isolation between parent species when mating barriers evolve due to selection against unfit hybrids (Mallet 2007). If reinforcement is occurring as *L. nigrinus* is released into sites where *L. rubidus* is present, over time we will see a decrease in hybridization and eventually a termination of hybridization as reinforcement becomes more powerful. This would also allow the parent species to remain genetically intact. Assessment of whether this is occurring will require long-term monitoring of the frequency of interbreeding.

Hybrid Vigor/Heterosis

Hybrids are often assumed to be less fit than their parents, but this is not always the case (Arnold 1997). A review by Arnold and Hodges (1995) found that hybrids were not uniformly less fit than parental genotypes.

Speciation

Unique adaptations might arise from combining divergent genomes (Arnold 1997, Mooney and Cleland 2001, Mallet 2007). The increased genetic variability that results from crossing divergent genotypes can result in offspring that are better adapted to changed and changing environments, allowing them to occupy a habitat that was not available to the parents (Arnold 1997, Mooney and Cleland 2001). If hybrids tend to assemble in habitats different than the parents by means of seasonality, drift in small populations, or change in host preference, then gene flow between hybrids and parents will be reduced, and hybrid speciation (the origin of a new species) can occur (Mooney and Cleland 2001, Mallet 2007). Adelgids on hemlocks in the eastern U.S. is a new niche that was created when HWA was introduced from Japan. *Laricobius* hybrids could be better adapted to this niche than their parents if they receive a preference for hemlock woolly adelgid from their *L. nigrinus* parents, and hardiness in eastern climates from *L. rubidus*. This possibility is being evaluated in laboratory and field studies.

Genetic assimilation

Open niches are not the only possible habitats for hybrids to invade (Arnold and Hodges 1995). If hybrids have an equivalent or higher fitness than the parents in their own habitat, the hybrids may replace the "pure" parental species due to competition (Arnold 1997, Mallet 2007). For example, if hybrids were to show greater feeding efficiencies than those of the parental species, this could result in a greater reproductive capacity of hybrids and the displacement of the parental species locally (Grant and Grant 1996).

HYBRIDIZATION IN OTHER CLASSICAL BIOLOGICAL CONTROL PROGRAMS

There are very few examples in the literature of introduced biocontrol agents interbreeding with native species. We are aware of just three systems in which this was investigated in the laboratory, one of which also tracked hybridization in the field. Naka et al. (2005, 2006) found that a *Chrysoperla carnea* (Chrysopidae) introduced from Germany was able to produce fertile F_1, F_2, and backcrossed offspring with native Japanese *C. nipponensis* in the laboratory, but concluded that they were unlikely to hybridize extensively in the field because hybrid fertility was low, and the parent species have different courtship songs. Davies et al. (2009) used DNA sequence data to confirm that introduced *Diadegma semiclausum* (Ichneumonidae) can hybridize with native Japanese *D. fenestrale* in the lab, and encouraged field studies to follow up. Finally, Moriya et al. (1992) showed that an introduced parasitoid of chestnut gall wasps, *Torymus sinensis* (Torymidae), from China can hybridize with a native Japanese species, *T. beneficus*. The native species has an early-spring and a late-spring strain. Using field-collected wasps from a single chestnut orchard, Yara et al. (2010) found that the early-spring strain was displaced by the introduced species without evidence of hybridization, while the late-spring strain showed increasing frequency of hybrids over time. The effects on pest control were not evaluated.

CONCLUSIONS

Hybridization between *L. nigrinus* and *L. rubidus* has been confirmed in several *L. nigrinus* release sites where eastern white pine and eastern hemlock co-occur. We know hybrids are feeding on HWA and are capable of reproducing but there is no indication as yet whether hybridization will negatively or positively affect the HWA biocontrol program. Laboratory tests are underway to assess the feeding preferences and fitness of hybrids relative to the parent species. Both species readily feed and reproduce on hemlock woolly adelgid although laboratory studies indicate *L. nigrinus* is not able to successfully reproduce on pine bark adelgid. Laboratory studies have also shown each predator species to have a preference for one or the other adelgids when presented with a choice. Based on preliminary results of genotyping more than 1700 specimens collected from across the landscape where *L. nigrinus* has been released, the rate of hybridization has thus far been shown to be approximately 7 percent. These results and the known differences in host preference suggest that species separation is likely to be maintained with infrequent gene flow between the two species. We will continue to monitor this unusual hybridization event as it plays out over time.

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