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Iridoid and secoiridoid glycosides in a hybrid complex of bush honeysuckles (*Lonicera spp.*, Caprifolicaceae): Implications for evolutionary ecology and invasion biology

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ABSTRACT

Interspecific hybridization among non-native plant species can generate genotypes that are more reproductively successful in the introduced habitat than either parent. One important mechanism that may serve as a stimulus for the evolution of invasiveness in hybrids is increased variation in secondary metabolite chemistry, but still very little is known about patterns of chemical trait introgression in plant hybrid zones. This study examined the occurrence of iridoid and secoiridoid glycosides (IGs), an important group of plant defense compounds, in three species of honeysuckle, *Lonicera morrowii* A. Gray, *Lonicera tatarica* L., and their hybrid *Lonicera* × *bella* Zabel. (Caprifoliaceae), all of which are considered invasive in various parts of North America. Hybrid genotypes had a diversity of IGs inherited from both parent species, as well as one component not detected in either parent. All three species were similar in that overall concentrations of IGs were significantly higher in fruits than in leaves, and several compounds that were major components of fruits were never found in leaves. However, specific patterns of quantitative distribution among leaves, unripe fruits, and ripe fruits differed among the three species, with a relatively higher allocation to fruits in the hybrid species than for either parent. These patterns likely have important consequences for plant interactions with antagonistic herbivores and pathogens as well as mutualistic seed dispersers, and thus the potential invasiveness of hybrid and parental species in their introduced range. Methods established here for quantitative analysis of IGs will allow for the exploration of many compelling research questions related to the evolutionary ecology and invasion biology of these and other related species in the genus *Lonicera*.

1. Introduction

Hybridization between plant species has been implicated as an important mechanism that can underlie the evolution of invasiveness (Ellstrand and Schierenbeck, 2000; Schierenbeck and Ellstrand, 2009). A large source pool of genetic variation in hybrid genotypes provides increased raw material on which natural selection can act, potentially leading to evolutionary novelty in life history, morphology, phenology, or secondary metabolite chemistry that can make some hybrid populations better adapted to new environments (e.g. Geiger et al., 2011; Oberprieler et al., 2010; Schweitzer et al., 2002). However, despite the importance of interspecific hybridization in invasion biology, plant evolution, and the structuring of ecological communities (Barton, 2001; Hegarty and Hiscock, 2005; Martinsen et al., 2001; Schierenbeck and Ellstrand, 2009; Whitham et al., 1999), there are still many unanswered questions about patterns of trait introgression in hybrids, particularly for secondary metabolite chemistry (Orians, 2000). Hybrids can differ chemically from the parental species both qualitatively and quantitatively—they may have chemical compounds typical of one or both parents, fail to express certain compounds produced by parents, or have novel compounds not typical of either parent (Cheng et al., 2011; Orians, 2000; Orians and Fritz, 1995; Rehill et al., 2006; Rieseberg and Ellstrand, 1993). Because plant chemistry has important consequences for species interactions and, therefore, the reproductive success of plants (Coley and Barone, 2001; Eisner and Meinwald, 1995), a better understanding of the chemical variation among hybrids may provide important insights into why certain hybrids become invasive in introduced ranges while others never establish viable populations (Ellstrand and Schierenbeck, 2000; Fritz, 1999; Fritz et al., 1999; Strauss, 1994; Whitney et al., 2006).

Exotic bush honeysuckles (*Lonicera spp.*, Caprifoliaceae) are some of the most problematic invasive species in the eastern and mid-western United States (Nyboer, 1992; Webster et al., 2006). Most species fruit in abundance and are thought to be dispersed primarily by birds (Bartuszevige and Gorchov, 2006; Ingold and Craycraft, 1983), although white-tailed deer may also be important

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as dispersers (Vellend, 2002; Whitehead, personal observation). Their introduction and spread have led to altered plant communities and reduced native plant diversity in many areas (e.g. Collier et al., 2002; Woods, 1993), which may be due to competitive (Gorchov and Trisel, 2003) or allelopathic effects (Cipollini et al., 2008). High densities of honeysuckle shrubs can also have cascading effects in ecosystems, including alteration in resource availability for birds (Bartuszevige and Gorchov, 2006; Ingold and Craycraft, 1983), declines in amphibian communities due to high levels of allelochemicals produced by the plants (Watling et al., 2011), and even increased disease risk for humans through indirect effects on deer populations that serve as reservoirs for parasites and pathogens (Allan et al., 2010). Some of the most invasive species include *Lonicera tatarica* L., *Lonicera morrowii* A. Gray, and their hybrid progeny *Lonicera* × *bella* Zabel., which form hybrid swarms throughout much of the introduced range (Barnes and Cottam, 1974; Nyboer, 1992; Webster et al., 2006). The hybrid species appears to be more successful in North America than either parent, as evidenced by the wide variety of habitats that the hybrid inhabits, its higher abundance relative to the parent species, and the high frequency of hybrid individuals that exhibit morphological traits intermediate to the parents (Barnes and Cottam, 1974; Whitehead, personal observation).

The *L.* × *bella* hybrid complex provides an intriguing system for phytochemical research. A comparison of secondary metabolites produced in parental and hybrid species would add an important new component to a growing literature on the chemical consequences of hybridization and establish analytical methods that will allow researchers to address many questions related to the evolutionary ecology and invasion biology of these species. The phytochemistry of *Lonicera* has been previously investigated due to the importance of various species in traditional pharmacopeias, and the genus contains at least two classes of secondary compounds with known ecological and economic importance: iridoid and secoiridoid glycosides (IGs) and phenolics (Chen et al., 2007; Cipollini et al., 2008; Ikeshiro et al., 1992; Li et al., 2003; Song et al., 2006; Svobodova et al., 2008; Wang et al., 2003; Zadernowski et al., 2005). This study focuses on IGs, which are an important class of plant defensive compounds found in over 50 plant families (Bowers, 1991), but have not been previously investigated in the context of plant hybridization. Souzu and Mitsuhashi (1969, 1970) and Ikeshiro et al. (1992) have provided initial descriptions of six IGs in fruits and leaves of *L. morrowii*, and there is one report of secologanin (**6**) (Fig. 1) in *L. tatarica* (Hermanslokkerbol and Verpoorte, 1987). However, to our knowledge, there is no information of the role of IGs in the ecology and evolution of these species, and there are no studies that have described the occurrence of IGs in the hybrid species, *L.* × *bella*.

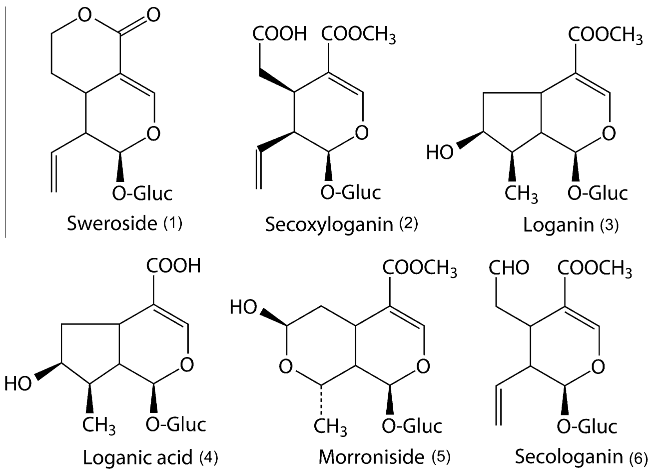

Fig. 1. Structures of major iridoid and secoiridoid glycosides from *Lonicera* × *bella*, *L. morrowii*, and *L. tatarica*.

IGs play an important ecological role in plant defense against both herbivores and pathogens (Bowers, 1991; Marak et al., 2002a, b). Their composition and concentration can vary considerably between species, among individual plants and plant organs, and throughout plant development (Jamieson and Bowers, 2010; Penuelas et al., 2006; Quintero and Bowers, 2011a). This spatial and temporal variation has direct implications for plant antagonists as well as cascading effects that can influence interactions with higher trophic levels (Dyer and Bowers, 1996; Jamieson and Bowers, 2010; Lampert et al., 2011; Lindstedt et al., 2010; Penuelas et al., 2006; Quintero and Bowers, 2011b; Reudler et al., 2011). Although past work on the ecological role of IGs has focused primarily on leaves; these compounds have also been found in the fruits of various species (Ikeshiro et al., 1992; Makarevich et al., 2009; Ono et al., 2005). Fruit secondary compounds may function to defend fruits against antagonists (e.g. insect seed predators and fungal pathogens) and/or to regulate the foraging and feeding behaviors of vertebrate seed dispersers (Cipollini and Levey, 1997a; Herrera, 1982; Levey et al., 2007; Tewksbury et al., 2008); however IGs have not been examined in this context. The potential role of IGs in fruit/frugivore interactions is of particular interest in the context of invasion biology, because the spread of an invasive species can be greatly accelerated by an effective dispersal mechanism in the novel habitat (Gosper et al., 2005; Higgins and Richardson, 1999; Richardson et al., 2000).

The objectives of this study were to: (1) provide a detailed method for extraction and quantification of IGs in *Lonicera* species that will be useful to ecologists and evolutionary biologists; (2) describe the occurrence of IGs in the hybrid honeysuckle *L.* × *bella* and its parental species *L. tatarica* and *L. morrowii*; and (3) compare the composition and concentration of IGs among leaves, unripe fruits, and ripe fruits in these three species. Results are discussed in the context of their potential implications for the evolution of chemical traits in hybrid genotypes, species interactions with herbivores and seed dispersers, and invasion biology.

2. Results and discussion

Leaf and fruit samples of *L. morrowii*, *L. tatarica*, and *L.* × *bella* were obtained from the living collections at the Arnold Arboretum of Harvard University (Cambridge, MA, USA). *L. morrowii* was originally wild-collected from the Honshu Provenance in Japan in 1984, *L. tatarica* was wild-collected from Tajikistan in 1978, and the hybrid species, *L.* × *bella*, is of cultivated origin and was received at the arboretum in 1919 from Boston, MA (BG-BASE, 2011). In addition, samples were collected from three wild populations of *L.* × *bella* growing near Boulder, CO, USA. The identification and quantification of IGs was carried out using gas chromatography with mass spectrometry detection (GC–MS).

Six major IG components (on average representing 89.1% of the estimated total IGs) were identified by comparison to authentic reference standards (Table 1). One other presumably related major component (Unknown G, 10.0% of estimated total IGs) and six minor components (totaling <1% of estimated total IGs) were also detected and provisionally characterized as IGs based on characteristic fragmentation patterns in mass spectra as described in detail in Inouye et al. (1976) and Popov and Handjieva (1983). Although there is no spectral peak associated with the molecular ion for silylated iridoids, several peaks associated with the aglycone portion of the molecule are very informative, and, in combination with peaks originating from the sugar moiety, served as a means for positive identification of previously characterized IGs.

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