

# The Role of Arboreta in Studying the Evolution of Host Resistance to the Hemlock Woolly Adelgid

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The hemlock woolly adelgid, *Adelges tsugae*, is an introduced pest of hemlock which is, unfortunately, all too familiar to many readers of *Arnoldia*. Adelgids are a small family of sucking insects, related to aphids, which feed only on conifers<sup>1</sup>. Because they are so small and typically not very common, most adelgids usually go completely unnoticed by all but a handful of entomologists that specialize on them. This can change dramatically when an adelgid species is transported outside of its native range into an ecosystem that is not adapted to keeping it in check. In the United States and Canada, this was first experienced with the balsam woolly adelgid, which killed millions of fir trees (genus *Abies*) in first half of the 20th century and continues to severely threaten these ecosystems. We are now seeing similarly devastating effects by the hemlock woolly adelgid (HWA) on eastern hemlock, *Tsuga canadensis*, and Carolina hemlock, *T. caroliniana*<sup>2</sup>.

In this article, we will take a worldwide look at the relationship between the adelgid and its various hemlock hosts. While most of our research was done with plants growing in their native habitats, we also made extensive use of cultivated hemlocks growing in various botanical gardens around the world, including the Arnold Arboretum. The living collections and herbaria at these institutions have proved to be an invaluable resource for us in developing an evolutionary context for understanding hemlock resistance to HWA. In addition, the records and herbarium specimens from expeditions sponsored by the Arnold Arboretum—from the time of E. H. Wilson and Joseph Rock through the Sino-American Botanical Expedition of 1980—were invaluable in helping us to pinpoint where to look for hemlock specimens in southwestern China.

Our collaborative research on HWA began in 1999. Nathan had just received his master's degree in entomology from the University of Wisconsin and Mike needed someone to do a field evaluation of a tiny lady beetle (*Scymnus sinuanodulus*) that had been collected three years earlier in China, and had just been released from quarantine for biological control of the adelgid. Going to China to look for biological controls for HWA had been something of a gamble. The adelgid had never been collected from mainland Asia, only from Japan and Taiwan. But the fact that China was home to three of the nine species of *Tsuga* as well as several



Overwintering hemlock woolly adelgid nymphs settled on eastern hemlock, *Tsuga canadensis*.

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closely related genera (*Nothotsuga*, *Keteleeria*, and *Pseudolarix*) suggested that hemlock had a long evolutionary history in the region. Such a time span would have provided ample opportunity for stable tri-trophic relationships to have evolved between the host (*Tsuga*), its herbivores (HWA), and the predators of the herbivores. This hunch has proved correct, as more than sixty species of lady beetles have been collected from the hemlocks in China since the early 1990s, with twenty-five of them being new to science<sup>3</sup>.

In 2001, Mike artificially infested every hemlock species at Arnold Arboretum and at the Morris Arboretum in Philadelphia with HWA. These tests confirmed Dr. Peter Del Tredici's observation that Chinese hemlock (*T. chinensis*) growing in the Arboretum were immune to HWA<sup>4</sup>. This seemed odd, because in China we found this hemlock species to be infested by HWA, sometimes with very dense populations. We wondered if there were genetic and behavioral differences among the world's geographic populations of HWA and where the HWA introduced to the eastern U.S. originated. In the fall of that year, Nathan began to address these questions as part of his Ph.D. thesis for the Department of Ecology and Evolutionary Biology at Yale University.

**How Did HWA Get Here?**

The origin of HWA in North America has been the subject of considerable speculation. Most people have assumed that it arrived from Asia early in the 20th century, first on the west coast and then migrated to the east coast. By doing some detective work with museum specimens and modern molecular technology, we were able to separate fact from fiction. During the 19th and early 20th centuries, exotic hemlock nursery stock and bonsai purchased from Japanese nurseries usually arrived in the United States through ports on the West Coast. Around this same time, China was opening up as a new frontier for plant exploration, and live plants collected by the Arnold Arboretum as well as the United States Department of Agriculture were typically sent to San Francisco and then shipped east by rail. A particularly noteworthy example of this is a seedling of Chinese



A page from the 1914 Yokohama Nursery catalog showing the availability of live plants of *Tsuga sieboldii* and *T. diversifolia* for import to the United States from Japan (from the Archives of the Arnold Arboretum).

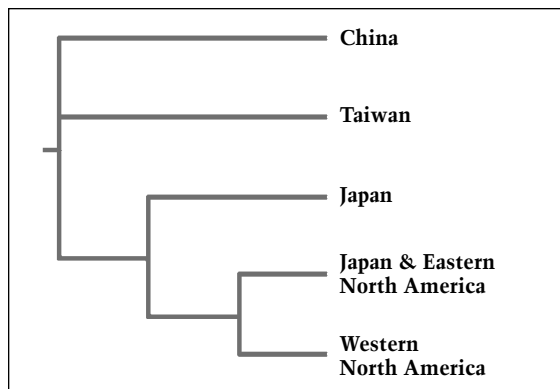
hemlock collected by E. H. Wilson in Hubei Province in 1910 that is still alive and well at the Arboretum. That imports like these had the potential to bring HWA with them to the U. S. was reinforced during a recent visit to the U. S. National Arboretum where we noticed that an herbarium specimen of *T. dumosa* collected in 1932 by Joseph F. Rock in southwestern China had the distinctive remains of HWA still attached to it.

At the U. S. National Collection of Insects in Beltsville, Maryland, we found a specimen collected in 1907 in South Bend, Washington that was not identified as HWA until 60 years later.

The first published account of an adelgid causing damage to North American hemlocks is from 1916 in Vancouver, British Columbia<sup>5</sup> and the formal description of HWA as a new species was based on insects collected in 1922 from Oregon and California<sup>6</sup>. In contrast, the first report of HWA in the eastern United States was not until 1951, from eastern hemlocks growing in Maymont Park in Richmond, Virginia. This 100-acre municipal park had formerly been part of the estate of Major James and Sallie Dooley (see <http://www.maymont.org>). Mrs. Dooley was an avid horticulturalist who collected plants from around the world. In 1911, with the help of the master Japanese gardener known simply as Muto, she created a traditional Japanese-style garden that was in vogue at the time. While we cannot be certain that HWA arrived on the east coast on nursery stock ordered by the Dooleys from Japan, its slow spread from a small area to several states is typical of introductions of non-native species.

Based on all of the circumstantial evidence, it seemed reasonable to assume that HWA had arrived on the west coast from Asia early in the 20th century. But we were not satisfied with this speculation and decided to look into the matter more deeply. Between 2002 and 2004, we collected samples from the mountains of Yunnan, Sichuan, Shaanxi, and Hubei provinces in China and throughout Honshu Island in Japan. Several collaborators sent us additional samples from eastern and western North America to include in our study.

When we compared DNA sequences from HWA collected in the different locations we found an exact match between HWA in eastern North America and HWA in southern Japan<sup>7,8</sup>. On the east coast, there was only a fraction of the natural variation found in Japan, which is characteristic of a recently introduced species. We also found that DNA sequences from HWA on the west coast do not match HWA from either the east coast or Asia, and that there was much more genetic variation in HWA on the west coast than on the east coast. These results suggest that HWA from western North America is a separate endemic lineage that has been diversifying there for thousands, or even millions of years. And finally, we were able to



*Phylogenetic relationships among geographic lineages of the hemlock woolly adelgid inferred using mitochondrial and nuclear DNA sequence data. Adelgids from China and Taiwan are different enough that they may be different species from the one that was introduced to eastern North America from Japan. There is a second lineage in Japan that is not the source of the introduction, and hemlock woolly adelgids in western North America are a separate lineage that appears to be native, not introduced as some have assumed (Figure based on the results of Havill et al. 2006, and Havill et al. 2007).*

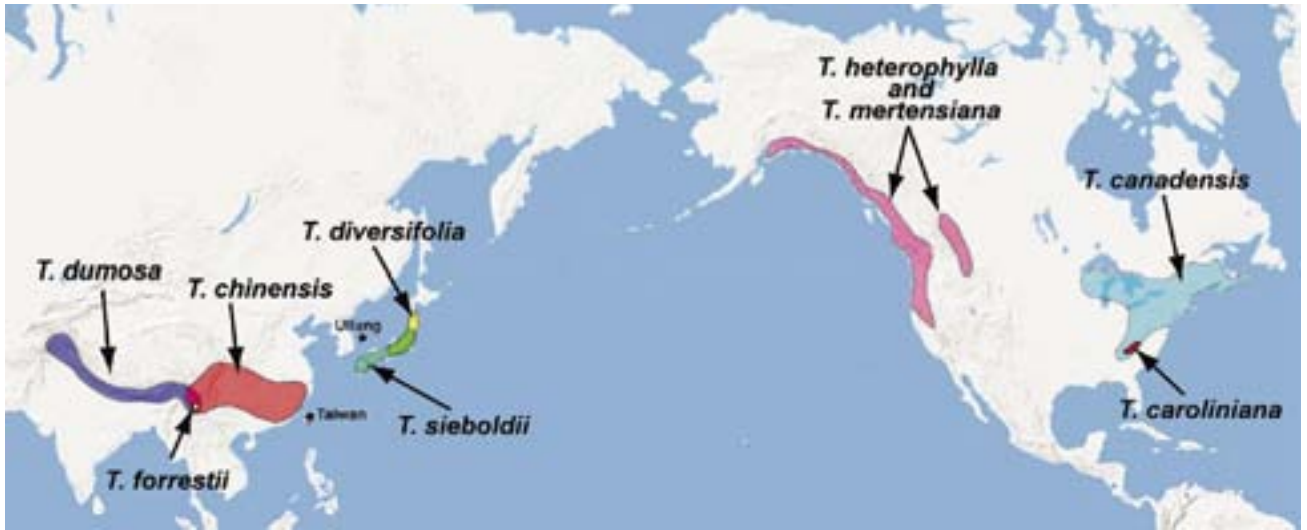
show that HWA in China is genetically divergent from HWA in Japan and North America and should probably be considered an entirely separate species.

### Hemlock Biogeography

In conjunction with this research on HWA genetics, we have also been exploring the evolutionary relationships among hemlock species around the world. Both of these studies were supported by grants from the USDA Forest Service, the Yale Institute for Biospheric Studies, and the Arnold Arboretum's Deland Endowment. We have also enjoyed the invaluable collaboration of colleagues in China and Japan, including Guoyue Yu, Li Li, Jianhua Zhou, and Shigehiko Shiyake.

Most plant taxonomists recognize nine species of hemlock worldwide<sup>9</sup>. There are four species in North America and five in Asia. There are no hemlocks native to Europe but the fossil record tells us that hemlock was once widespread on that continent but went extinct somewhere around one million years ago because of climate change and repeated glaciations<sup>10</sup>. There are two species of hemlock in eastern North America. The eastern hemlock, *T. canadensis*, is widely distributed from southern Canada to the Great





Map showing the worldwide distribution of the genus *Tsuga* (Reprinted from Havill et al., in press).

Lakes and New England down through the Appalachians into Georgia. The other species in the east is the Carolina Hemlock, *T. caroliniana*, which is native to the Blue Ridge Mountains from Virginia to Georgia. In western North America, there are also two species—western hemlock, *T. heterophylla*, usually found at low elevations, and the mountain hemlock, *T. mertensiana*, which grows at high elevations. There is a similar pattern in Japan, with *T. sieboldii* occurring mostly in the south and at low elevations, and *T. diversifolia* mostly in the north and at high elevations. There are three other hemlock species in Asia—*T. chinensis* has several described varieties and is widely distributed in China; *T. dumosa*, occurs in a narrow band from southwestern China along the Himalayas to Nepal; and *T. forrestii*, overlaps with the two other species in Yunnan and Sichuan provinces in southwestern China.

Our research has given us the pleasure of observing hemlocks growing in a variety of natural habitats in China and Japan. In both countries, hemlock occurs where it is cool and wet in the summer, such as the fog belt of high mountains. They are in the transition zone between deciduous hardwoods and boreal conifers and are often a climax species in diverse forests. The hemlocks may rise above the canopy, often with broad, domed, or flat crowns which is very different from the conical or pyramidal crowns of the North American species. The understory of a Chinese hemlock forest not



Dr. Nathan Havill standing next to a large *T. forrestii* in Lijiang, Yunnan Province, China.



*Tsuga chinensis* var. *tchekiangensis* growing on Mount Maoer in Guangxi, China.



*Tsuga sieboldii* in the background with fir in the foreground growing on Mount Tsurugi, Shikouku, Japan.



only contains *Rhododendrons* and other genera of plants commonly found in the forests where eastern hemlocks grow, but also has species of camellia, bamboo, peony, primrose, and other Asian plants which we only find here in cultivated landscapes.

In southwestern China, the range of hemlock and the panda overlap, and ancient hollow conifers are used as maternity dens by the panda. *Tsuga* is a Japanese word meaning “mother tree” and is the highlight of several national parks in Japan. Standing in a hemlock stand in east Asia, the opening lines of the poem *Evangeline* by Henry Wadsworth Longfellow comes to mind:

*This is the forest primeval. The murmuring pines and the hemlocks,  
Bearded with moss, and in garments green, indistinct in the twilight,*

### Modern Taxonomy Reveals Ancient Relationships

With the help of colleagues at Yale University, the University of Maine, the Academy of Natural Sciences in Philadelphia, and the University of Memphis, we used DNA sequences to reconstruct the evolutionary relationships and biogeographic history of hemlock, in part to see what this could tell us about how to manage HWA. We assembled multiple samples of each hemlock species, either collected by us in the field or from the living collections at Arnold Arboretum, the U.S. National Arboretum, Hangzhou Botanical Garden in China, and the Royal Botanic Garden in Edinburgh.

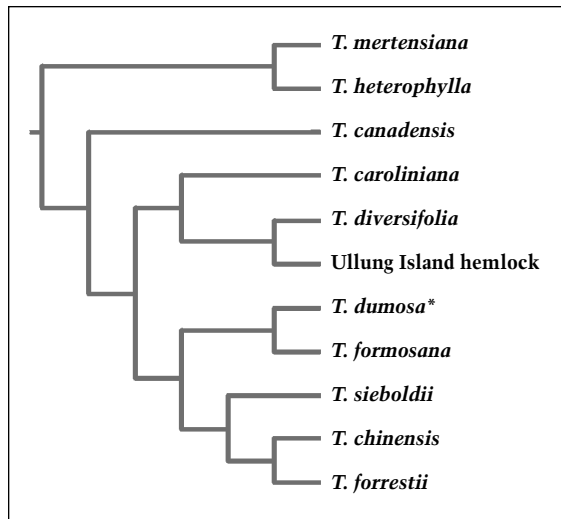
As with the HWA, some of the relationships among the hemlocks were a surprise to us<sup>11</sup>. One interesting result of this study was that the two hemlock species in eastern North America are not closely related to each other. *Tsuga caroliniana* is more closely related to the Japanese species *T. diversifolia* than to *T. canadensis*. Despite this close affinity, *T. caroliniana* is susceptible to HWA damage, while *T. diversifolia* is resistant.



Moss covered *T. dumosa* trunk in Laojun Shan in Yunnan, China.



*Tsuga chinensis* with hanging lichens growing near Danba in Sichuan, China.



Phylogenetic relationships among *Tsuga* species inferred using chloroplast DNA sequence data. Analysis using the nuclear ITS region agreed with this except that *T. dumosa* was sister to the rest of the Asian species plus *T. caroliniana*. This discordance may have resulted from an ancient hybrid origin of *T. dumosa* (Figure modified from Havill et al., in press).

Since the two species in eastern North America have different ancestries, their susceptibility to HWA probably arose independently in each species. Perhaps this resulted from living in a region where there are only a few inconsequential sucking insects that specialize on hemlock and where there was more selective pressure from chewing insects. Many studies have shown that plants have different defensive reactions to sucking versus chewing insects. Before HWA was introduced, the major pest of hemlock was a defoliator, the hemlock looper caterpillar. Recent chemotaxonomic studies of hemlock species and cultivars growing at the National Arboretum, Morris Arboretum, and Longwood Gardens suggest that the two hemlocks in eastern North America have adapted their terpenoid chemistry to provide protection against chewing insects, which seems to have made them vulnerable to non-native sucking pests such as HWA and the elongate hemlock scale<sup>12, 13</sup>. Out of thirteen cultivars of *T. canadensis* examined, the two with white-tipped foliage, 'Albo-spica' and 'Snowflake' grouped closer to the Asian species than to the "wild" *T. canadensis*. Careful testing is still needed to examine whether

these cultivars are more resistant to HWA and more susceptible to native chewing pests such as hemlock looper caterpillars.

Another surprising and very exciting discovery from the *Tsuga* phylogeny project involves two hemlocks growing at Arnold Arboretum (AA #1251-83). These trees were grown from seed collected on Ullung Island, South Korea in 1982 by an expedition from the Chollipo Arboretum. Ullung is a small, isolated volcanic island in the Sea of Japan—equidistant between Korea and Japan—that hosts many endemic plant species. Based on morphological characteristics, the hemlocks on Ullung Island have always been identified as *T. sieboldii*, the low-elevation Japanese species. DNA sequences from the trees growing in the Arboretum, however, consistently grouped, not with *T. sieboldii*, but with *T. diversifolia*, the other Japanese species that grows at higher elevations. To confirm this unexpected result, we obtained a fresh sample of Ullung hemlock from Dr. Nam Sook Lee at Ewha Womans University in Seoul. This sample, like those from the Arnold, independently verified that the Ullung hemlocks are closely related to, but distinct from, *T. diversifolia* rather than *T. sieboldii* as previously thought. A detailed study comparing the morphology of Ullung Island hemlock with *T. diversifolia* still needs to be done to decide whether it should be considered a new species.

### Adelgid Resistant Hemlocks

Previously, it was reported that *T. chinensis* and *T. diversifolia* had high resistance to HWA. Researchers at the National Arboretum have been able to produce viable hybrid crosses between *T. chinensis* and *T. caroliniana*<sup>14</sup>. These hybrids have been established in a field trial to evaluate their HWA resistance and growth characteristics. Recent expeditions to China have resulted in collection of hemlock seed from five provinces and more than 20 accessions are growing in experimental nurseries at the National, Morris, and Arnold Arboreta. It seems that the cultivation of *T. chinensis* and its hybrids may be an option available to gardeners in the foreseeable future.

Without the resources and expertise at the Arnold Arboretum, the U.S. National Arboretum, Morris Arboreum, Longwood Gardens,



A hemlock from Ullung Island, Korea growing at the Arnold Arboretum (AA #1251-83B). At 24 years of age, the tree was 8 meters tall by 6 meters wide. Ullung hemlocks have traditionally been identified as *T. sieboldii* based on morphology, but DNA analyses show that it is closely related to *T. diversifolia* and may be a new species (Photograph by P. Del Tredici, December 2007).

Chollipo Arboretum, Hangzhou Botanical Garden, and the Royal Botanical Garden at Edinburgh, this research would not have been possible. By highlighting the vital contributions that botanical gardens have made to the development of ways to control this devastating pest, hopefully we have reinforced the need for their continued commitment to research.

#### Endnotes:

- <sup>1</sup> Havill, N. P. and R. G. Foottit. 2007. Biology and evolution of Adelgidae. *Annual Review of Entomology* 52: 325–349.
- <sup>2</sup> Ward, J. S., M. E. Montgomery, C. A. S.-J. Cheah, B. Onken, and R. S. Cowles. 2004. *Eastern Hemlock Forests: Guidelines to Minimize the Impacts of Hemlock Woolly Adelgid*. USDA Forest Service, Morgantown, WV.
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*Adelges tsugae* Annand (Homoptera: Adelgidae). The Coleopterists Bulletin 54: 154–199.

- <sup>4</sup> Del Tredici, P. and A. Kitajima. 2004. Introduction and cultivation of Chinese hemlock (*Tsuga chinensis*) and its resistance to hemlock woolly adelgid (*Adelges tsugae*). *Journal of Arboriculture* 30: 282–286.
- <sup>5</sup> Chrystal, R. N. 1916. The forest insect problem in Stanley Park. *Proceedings of the Entomological Society of British Columbia* 9: 63–66.
- <sup>6</sup> Annand, P. N. 1924. A new species of *Adelges* (Hemiptera, Phylloxeridae). *Pan-Pacific Entomologist* 1: 79–82.
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- <sup>8</sup> Havill, N. P., R. G. Foottit, and C. D. von Dohlen. 2007. Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. *Molecular Phylogenetics and Evolution* 44: 357–370.
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- <sup>10</sup> LePage, B. A. 2003. A new species of *Tsuga* (Pinaceae) from the middle Eocene of Axel Heiberg Island, Canada, and an assessment of the evolution and biogeographical history of the genus. *Botanical Journal of the Linnean Society* 141: 257–296.
- <sup>11</sup> Havill, N. P., C. S. Campbell, T. F. Vining, B. A. LePage, R. J. Bayer, and M. J. Donoghue. In press. Phylogeny and biogeography of *Tsuga* (Pinaceae) inferred from nuclear ribosomal ITS and chloroplast DNA sequence data. *Systematic Botany*.
- <sup>12</sup> Lagalante, A. F. and M. E. Montgomery. 2003. Analysis of terpenoids from hemlock (*Tsuga*) species by solid-phase microextraction/gas chromatography/ion-trap mass spectrometry. *Journal of Agricultural and Food Chemistry* 51: 2115–2120.
- <sup>13</sup> Lagalante, A. F., M. E. Montgomery, F. C. Calvosa, and M. N. Mirzabeigi. In press. Characterization of terpenoid volatiles from cultivars of eastern hemlock (*Tsuga canadensis*). *Journal of Agricultural and Food Chemistry*.
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