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## **(106) Genetic diversity of the pinewood nematode, *Bursaphelenchus xylophilus* after 100 years of invasion in Japan**

Akiba M, Kanzaki N, and Sahashi N

*Forestry and Forest Products research Institute, Matsunosato 1, Tsukuba, Ibaraki, 305-8687, Japan*

*Email: akiban@ffpri.affrc.go.jp*

The pinewood nematode *Bursaphelenchus xylophilus* is a pathogen that causes pine wilt disease. The nematode is thought to have been introduced into southwestern Japan (Nagasaki Prefecture in 1905 and Hyogo Prefecture in 1921) from North America followed by dispersion throughout Japan, except to Hokkaido, the northernmost island. However, details on the origins of the invading nematodes and the routes of dispersal in Japan are unknown. We collected 223 nematode isolates from dead trees in affected areas covering almost all damaged areas in Japan and analyzed their nuclear ribosomal DNA (ITS1-5.8SRNA-ITS2) and mitochondrial DNA (cytochrome oxidase subunit I [COI]) sequences. Three SNPs in the ITS1 region, 2 SNPs and 2 indels of two bases in the ITS2 region, and 6 haplotypes (R1–R6) were detected in the nuclear DNA sequence data. Five percent of isolates showed heterogeneity of two haplotypes. Haplotype diversity was  $0.625 \pm 0.019$  and nucleotide diversity was  $0.0016 \pm 0.0001$  among all isolates. R1 and R2 were the dominant haplotypes (38.2% and 47.2%, respectively) and only these two haplotypes occurred in northeastern Japan, where disease expanded after the 1970s. In southwestern Japan where the history of pine wilt disease is older, four other haplotypes (R3–6) were detected. Twenty-eight variable sites were detected within the 658-bp mitochondrial DNA sequence and the isolates separated into 11 haplotypes (4 haplotype groups). Haplotype diversity was  $0.596 \pm 0.032$  and nucleotide diversity was  $0.0109 \pm 0.0005$ . The dominant haplotypes in all areas of Japan were C1a (60.1%) and C4a (18.8%). The C3 haplotype was detected only on Okinawa Island, possibly due to the founder effect of introduced nematodes. Differences in both nuclear and mitochondrial DNA in each haplotype may indicate different geographic origins of *B. xylophilus*, and therefore, multiple invasions of nematodes from different native locations. The higher diversity of haplotypes in southwestern Japan may reflect multiple invasions in that region followed by expansion of several haplotypes to northeastern Japan. The ITS haplotype pattern did not correspond to that of COI. Considering the different inheritance

modes of nuclear and mitochondrial DNA, hybridization between nematodes of different origins must have occurred after introduction to Japan. Comparison of Japanese haplotypes with sequences registered in public DNA databases showed that the R3 and R4 ITS haplotypes and some COI haplotypes were unique. Almost all *B. xylophilus* isolates from Portugal were consistent with the R2 ITS haplotype and the C1a COI haplotype, which were dominant haplotypes in Japan. The diversity of *B. xylophilus* in Japan was higher than in Portugal where nematode invasion occurred in 1999. This study shows that multiple invasions are likely to have occurred in Japan, but the origins of the invading nematodes are unknown. To address this gap in our knowledge, extensive sampling of nematodes in native locations (USA and Canada) and more detailed analysis using molecular markers with high resolution such as SSRs or SNPs are necessary.