THE POPULATION STRUCTURE OF *ARMILLARIA OSTOYAE* IN THE CENTRAL AND SOUTHERN INTERIOR OF BRITISH COLUMBIA

by

JEREMY RICHARD DETTMAN

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Department of Forest Sciences

The University of British Columbia
Vancouver, Canada

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ABSTRACT

The population structure of *Armillaria ostoyae* (Romagn.) Herink and *Armillaria sinapina* Bérubé & Dessureault was studied at two sites in the Interior of British Columbia using Random Amplified Polymorphic DNA (RAPD) analysis and somatic incompatibility testing. The first study site contained a small number of relatively large genets of *Armillaria ostoyae*. Each distinct infection center was occupied by a single genet of *A. ostoyae*, one of which displayed signs of a somatic mutation. *Armillaria sinapina* was also sampled from the first site but its population structure was considerably different from that of *A. ostoyae*. The second study site contained high densities of relatively small genets of *A. ostoyae*. It was estimated that over 70 % of the genets in this population occupied a domain less than one hectare in area. The distribution of genets could not be predicted by the distribution of mortality. The results of somatic incompatibility testing used to differentiate between genets corresponded with the results of RAPD analysis, with only one discrepancy. For both sites, regression analysis indicated no significant linear relationship between geographic distance and genetic similarity of genets.

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Chapter I:
Introduction

Background Information

The basidiomycete fungus *Armillaria ostoyae* (Romagn.) Herink is a common root pathogen of conifers in southern British Columbia. It is especially prevalent in the moist subzones of the Interior regions of the province, particularly within the Interior Cedar Hemlock (ICH) zone (Morrison and Mallett, 1996; Morrison et al, 1992). Timber and volume losses caused by decreased productivity, predisposition to windthrow, and tree/seedling mortality are quite significant. In B.C. alone, it is estimated that over 5 million cubic meters per year are lost to various root diseases, with *A. ostoyae* accounting for up to half of the total losses.

A. ostoyae is an obligate wood inhabitant, obtaining all of its nutrients from the degradation of woody substrates. Although it is quite pathogenic to its hosts, *A. ostoyae* is a weak competitor and usually cannot infect roots which are already colonized by other decay fungi. When an uninfected root comes in contact with the root of an infected tree, *A. ostoyae* is transferred by direct mycelial growth to the new host. If the roots are not in direct contact the fungus may form highly differentiated root-like aggregations of hyphae, called rhizomorphs, which ramify through the soil. These structures have a melanized outer rind which protects the fungus from deleterious agents in the soil and allows for growth into an otherwise unfavorable environment. When a rhizomorph contacts the root of a new host, the bark is penetrated and infection occurs. After the root is colonized, the pathogen advances up towards the root collar by mycelial fans growing through and decaying the phloem and cambial layers. This mode of infection generally allows *Armillaria* to avoid competition from other decay fungi, and thus colonize the root quite freely. The pathogen also advances via rhizomorphs which grow along the root surface and initiate new infections proximal to the original point of entry. When the mycelium reaches the root collar the tree is girdled, the remaining roots are colonized, and tree death follows.

During the late summer and early autumn, enormous numbers of basidiospores may be produced from basidiocarps (mushrooms) which occur at base of infected trees. Basidiospores are believed to initiate new infections through stumps or large wounds on susceptible hosts. After successful colonization of the first host, the domain occupied by the fungal mycelium generally expands radially outwards in all directions, creating a more or less spherical infection center. The area covered by an infection center may range in size from a single tree to tens of hectares (Kile, 1983, 1986; Korhonen, 1978; Legrand et al, 1996; Rishbeth 1978, 1991; Rizzo et al, 1995; Smith et al, 1992, 1994; Ullrich and Anderson, 1978; Worrall, 1994). Genetic recombination is not believed to occur during vegetative growth, therefore, the entire center is expected to be composed of genetically identical mycelia all derived from the same initial infection. The population structure of *Armillaria* is considered to be "clonal": vegetative growth predominates and sexual reproduction via basidiospores occurs at a relatively low frequency (Redfern and Filip, 1991).

Inoculum Potential vs. Host Vigor

The epidemiology of *A. ostoyae* can be best understood when the concepts of inoculum potential and host vigor are considered (Redfern and Filip, 1991; Wargo and Harrington, 1991). Inoculum potential is the energy available to the fungus at the point of infection (i.e. where the rhizomorph or mycelium contacts the root). The amount of inoculum potential is dependent upon the volume of substrate occupied, the quality of the substrate, and the distance the point of infection is from the inoculum source. For example, a large colonized dead root system provides an abundance of high quality woody material which *A. ostoyae* can degrade and obtain nutrients from, thereby increasing the inoculum potential. On the other hand, the inoculum potential of the fungus decreases as the point of infection moves farther and farther away from the inoculum source.

Host vigor refers to the overall health of the tree and is a function of several factors such as tree age, adaptation to site, stress levels, and crown position. The success of each possible infection event is determined by the balance between the inoculum potential of the fungus and the host's vigor. When inoculum potential is high and host vigor is low, infection can occur easily, allowing for the rapid spread of the pathogen from host to host. When inoculum potential is low and host vigor is high, *A. ostoyae*

cannot overcome the tree's resistance mechanisms and the pathogen cannot infect new hosts. If a tree has been successfully colonized, the moribund or dead root system may become an additional source of inoculum from which the pathogen can spread. Thus, as more and more hosts are infected, the inoculum potential steadily increases. When *A. ostoyae* is successfully spreading through the stand from host to host, the situation is referred to as the "active" state. When *A. ostoyae* has low inoculum potential and cannot infect new hosts, it is restricted to surviving within old inoculum sources or dormant lesions on the roots of previously infected trees. In this situation, *A. ostoyae* is prevented from spreading through the forest and remains generally harmless to the host. This is referred to as the "quiescent" state.

Conditions that stimulate *Armillaria* into an active state are well documented (Wargo and Harrington, 1991). They usually involve some form of disturbance which diminishes the vigor of the hosts, such as an insect defoliation or other environmental stressors. The harvesting of timber is often associated with the activation of *Armillaria*. The presently accepted hypothesis: After a tree has been felled, the fresh root system becomes a high quality food base which may be easily colonized by the pathogen, thereby increasing the inoculum potential. On the other hand, as the woody substrate is degraded the quality and amount of the inoculum decreases, as does the inoculum potential. Therefore, to remain active the pathogen must acquire new food bases at a greater rate than it degrades its present food bases. The regenerating seedlings within the center are easily infected, but the small volume of the under-developed root systems represents an insignificant increase in inoculum potential. As the surviving regeneration ages, its vigor and resistance both increase. If the inoculum potential decreases and the host vigor increases enough to prevent further infection, quiescence may eventually be attained. If conditions which are unfavorable to the host develop, *A. ostoyae* may once again revert back to an active state. Once activated in a well-stocked stand of susceptible host species, it may be many decades before it returns to a quiescent state.

Disease Prediction

In the coastal regions of British Columbia, the vigor of the mature trees is often great enough to resist infection by *A. ostoyae*. Host mortality is uncommon after age 25 (Morrison et al, 1989) and is usually restricted to young, small, suppressed trees. Thus,

the pathogen generally remains quiescent in most mature coastal stands. In the Interior Cedar Hemlock (ICH) zone, inoculum potential and host vigor are balanced in a very delicate equilibrium. Even small disturbances that only slightly reduce the vigor of the hosts can alter this equilibrium, allowing *A. ostoyae* to become active.

After a stand within the ICH has been harvested, the subsequent rotation may experience significant levels of infection and mortality (Morrison et al, 1989, 1992, 1996), but this varies considerably from site to site. In some sites, the pathogen may actively infect the majority of the regenerating hosts and cause high levels of mortality. In other sites, *A. ostoyae* may remain relatively quiescent and only cause minimal amounts of damage to the plantation. As with all other pathosystems, the characteristics of the host, pathogen, and environment all interact to create the resulting behavior. The areas demonstrating different *A. ostoyae* behavior are often ecologically similar and immediately adjacent to each other, with no apparent discrepancies in the environmental conditions. Also, the trees exhibit no physiological differences between sites, and presumably the genetic components of individual trees from both sites would be similar. Therefore, the behavior of *A. ostoyae* displayed in a particular site may be dependent upon the characteristics of the individual genets which occupy that site. Recent studies have indicated that certain genets may be extremely pathogenic while others may be only weakly pathogenic (Mugala et al, 1989; Omdal et al, 1995; Shaw and Loopstra, 1988). If this is the case, then *Armillaria*'s population structure and the factors which influence it would become very important for disease prediction. Information regarding the number, size, and distribution of genets in a particular site would be crucial for predicting damage in the near future. This information may allude to the epidemiology of *A. ostoyae* and the processes which have shaped the present day population structure, and allow for better disease prediction in the distant future.

The most common silvicultural treatment for areas known to be heavily infected with *A. ostoyae* is stump removal, of which the economic and ecological costs are quite substantial. If distinctions could be made between strongly and weakly pathogenic genets, silvicultural treatments may be adjusted accordingly. Strongly pathogenic genets would require severe treatment while weakly pathogenic genets may be left unmanaged, thus increasing the efficiency of our silvicultural practices. Similarly, the size and

of the genets in a site may affect the treatment regime. If a single genet is larger than the minimum silvicultural treatment unit, it may be treated separately and the characteristics of that individual genet should be taken into account. If several small genets occupy a site, then all genets would receive treatment of the same intensity, and the characteristics of individual genets would be irrelevant.

Objectives

Part I:

Presently, the clonal population structure of *Armillaria* root disease in the Interior of B.C. is simply assumed to exist. To date, no studies have determined if infection centers in this region are, as expected, occupied by single genetically homogeneous fungal individuals. It is unknown whether individual genets maintain their genetic integrity through time or not. Genetic variation within infection centers may be introduced by successive basidiospore infections or somatic mutations during vegetative growth. The ideal location to test for the existence of *A. ostoyae*'s clonal population structure is where the distribution of infection centers is quite simple. As one approaches the northern range boundary (approximately 52° N) of *A. ostoyae* in the central Interior, its prevalence gradually decreases. Near Williams Lake in the Sub-Boreal Spruce (SBS) zone, one finds distinct and isolated infection centers which are surrounded by large tracts of asymptomatic forest. Therefore, samples may be collected with a great deal of confidence that each patch of infected forest represents an individual infection center.

Objective of Part I: To verify the existence of *Armillaria* root disease's clonal population structure in the central Interior of British Columbia.

Part II:

In the ICH zone within the southern Interior, the distribution of infection and mortality is quite complex in comparison to the region studied in the central Interior in Part I. *A. ostoyae* is not found in distinct infection centers, rather mortality is distributed somewhat evenly throughout the forest region, with asymptomatic trees interspersed with symptomatic trees. The population structure of *A. ostoyae* is this area is yet to be

described. The distribution of mortality may represent numerous small genets with contacting or overlapping boundaries that have grown into each other over time, or it may represent a few large fragmented genets which occupy enormous areas. To understand the basic features of this pathogen that could lead to differences in disease behavior, the population structure must first be determined.

Objective of Part II: To describe the population structure of *Armillaria* root disease in the southern Interior of British Columbia.

Chapter II:

The Population Structure of *Armillaria ostoyae* and *Armillaria sinapina* in the Central Interior of British Columbia

INTRODUCTION

Armillaria root disease is the most damaging forest disease agent found within the central and southern Interior of British Columbia (Morrison and Mallett, 1996; Morrison et al, 1992). It negatively affects commercial timber production by causing tree mortality, reduction of tree growth, and predisposition to windthrow. Although six species of this basidiomycete genus occur in B.C. (Anderson et al, 1980; Anderson and Ullrich, 1979; Morrison et al, 1985; White et al, 1998), only two are found commonly in the Interior regions of the province: *Armillaria ostoyae* (Romagn.) Herink (North American Biological Species I, or NABS I) and *Armillaria sinapina* Bérubé & Dessureault (NABS V). *Armillaria ostoyae* is highly pathogenic to the majority of tree species and can infect and kill vigorously growing hosts of all ages. On the other hand, *Armillaria sinapina* is considered weakly pathogenic on deciduous trees and only saprophytic on coniferous trees (Allen et al, 1996; Morrison et al, 1985, 1992). Both species commonly occur on the same site (Cruickshank et al, 1997) but are not easily distinguished by field observations alone. Since *A. ostoyae* is most responsible for the losses in commercial timber production, forest pathologists are concerned mainly with this species.

All species of *Armillaria* are spread through the forest predominantly via subterranean vegetative growth along the root systems of susceptible hosts (Kile et al, 1991), resulting in patches of mortality referred to as infection centers. Each infection center is assumed to be occupied by a single mycelial individual (i.e. a genetically homogeneous genet) which has colonized the root systems of the included trees. A few studies (Kile, 1983; Rishbeth, 1978; Shaw and Roth, 1976) have verified that distinct infection centers are commonly occupied by single genets. The majority of studies that investigated the distribution of genets across the landscape have collected isolates from a

complex infected area where the delineation of separate infection centers was not possible. The ideal study site for the investigation of the genetic components of single infection centers would contain centers which displayed typical and obvious symptoms (Morrison et al, 1992), were sparsely distributed over the landscape, and were surrounded by mature forest which displayed no symptoms of infection.

Although several studies have produced indirect evidence of dispersal and infection via *Armillaria* basidiospores (Kile, 1983; Rishbeth, 1978, 1985, 1988; Smith et al, 1994; Worrall, 1994), it is still considered a rare event in nature. Despite the presumed low frequency of this phenomenon, it could become significant when the time scale of the entire forest is considered. Long distance dispersal via basidiospores is theoretically necessary to explain the present distribution of the disease and the genetic variation found within populations. By investigating the genetic relationships and spatial distribution of genets across the landscape, the extent to which *Armillaria* utilizes vegetative growth and/or dispersal via basidiospores can be estimated.

The population structure of various *Armillaria* species throughout the world have been studied by several authors. Distinguishing between individual *Armillaria* genets has been accomplished using several techniques: (1) somatic incompatibility or intra-specific antagonism (Adams, 1974; Guillaumin et al, 1996; Kile, 1983, 1986; Klein-Gebbinck et al, 1991; Korhonen, 1978; Legrand et al, 1996; Rishbeth, 1978, 1988, 1991; Rizzo et al, 1995; Rizzo and Harrington, 1993; Shaw and Roth, 1976; Smith et al, 1994; Worrall, 1994), (2) mating-type alleles (Anderson et al, 1979; Guillaumin et al, 1996; Kile, 1983; Korhonen, 1978; Smith et al, 1990, 1992; Ullrich and Anderson, 1978), (3) isoenzymes (Guillaumin et al, 1996; Morrison et al, 1989; Rizzo and Harrington, 1993), (4) nuclear or mitochondrial DNA restriction fragment length polymorphisms (Guillaumin et al, 1996; Rizzo et al, 1995; Schulze et al, 1997; Smith et al, 1990, 1992, 1994), and (5) random amplified polymorphic DNA analysis (Guillaumin et al, 1996; Schulze et al 1997; Smith et al, 1992). Although somatic incompatibility is the most commonly used technique, it has certain drawbacks: the complex genetic basis of somatic incompatibility reactions in basidiomycete fungi remains largely unknown. Also, it is possible that inbred genets with distinct genotypes may not be distinguished due to the shared incompatibility alleles (Korhonen, 1978; Kile, 1983). Comparative studies have indicated that different methods

of genet identification generally give the same delineation of genets (Smith et al, 1990, 1992), but with some slight differences (Guillaumin et al, 1996; Kile, 1983; Korhonen, 1978; Rizzo et al, 1995; Rizzo and Harrington, 1993; Smith et al, 1994). Recently it has been suggested that techniques using molecular markers are superior when attempting to distinguish between closely related fungal individuals (Jacobson et al, 1993).

The objectives of this study were: 1) to identify which *Armillaria* species were responsible for the observed mortality in the central Interior of B.C.; 2) to determine if distinct *Armillaria* infection centers were occupied by single or multiple genets; 3) to determine the relative importance of vegetative spread vs. sexual reproduction; 4) to compare the traditional technique of somatic incompatibility with the molecular technique of random amplified polymorphic DNA (RAPD) analysis in terms of distinguishing genets.

MATERIALS AND METHODS

Sampling

Since one objective of this study was to determine if individual infection centers were occupied by single, genetically homogenous fungal mycelia, delineation of infection centers and their respective boundaries was critical. Therefore, samples for this study were collected from the Gavin Lake Block (52°27'N, 121°49'W) of the University of British Columbia's Alex Fraser Research Forest which is approximately 50 km northeast of Williams Lake, British Columbia. The study area was located within the mh subzone of the Sub-boreal Spruce (SBS) biogeoclimatic zone (Meidinger and Pojar, 1991). This block was chosen as the study site because most root disease centers in the area displayed typical stand level symptoms and were surrounded by forest which displayed no above-ground symptoms, so the delineation of infection center boundaries was not difficult. Using forest cover maps and aerial photographs, sites which were suspected of root disease infection were chosen for inspection. During June and July 1997, nine infection centers (Centers A - I) that were caused by *Armillaria* root disease were discovered within the research forest (see Table 1 and Figure 1).

Table 1: Description of the nine sampled *Armillaria* infection centers.

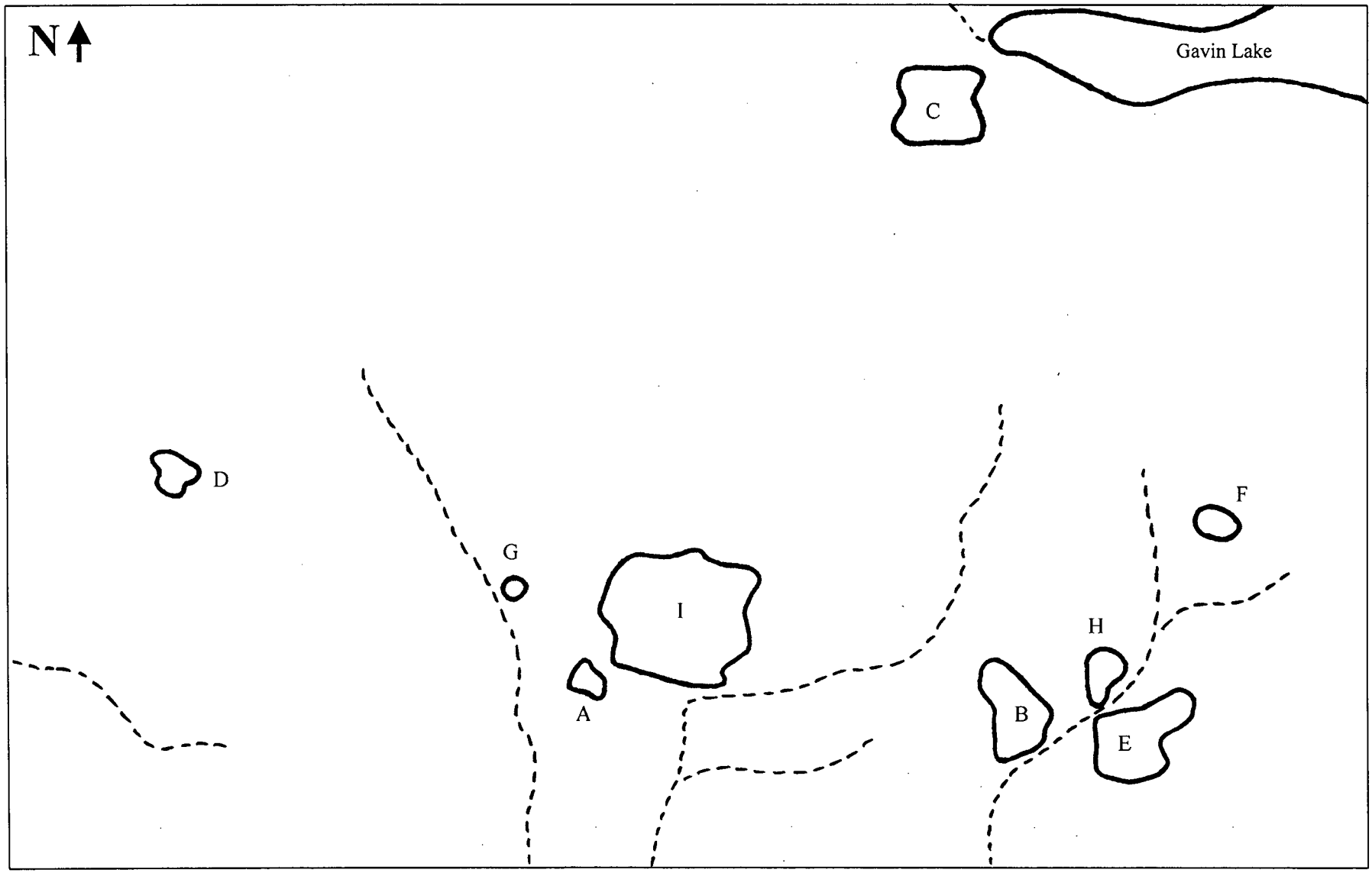
Center	Elev. (m)	Host Comp.*	Stand Age (yrs)	Size (ha)	Comments
A	965	Df, some Pl	100 – 120	0.50	shelterwood cut in 1992.
B	1060	Df, some Pl, Ba	100 – 120	3.80	partially bounded S by marsh, and E by stream. SW facing slope.
C	1025	Df, some Pl, Sp	120 - 140	7.43	very moist at N portion. N facing slope.
D	960	Df, some Pl, At	130 –150	1.81	SW facing slope.
E	1050	Pl, Df, Ba, some At	12	5.41	clearcut in 1985, planted 1986. Partially bounded NW and SE by streams.
F	1175	Df, Pl, At	100 - 120	0.69	SW facing slope.
G	1000	Df, some Pl	80 - 100	0.20	SW facing slope. Infected trees scattered around center.
H	1070	Df, Pl, Ba	100 - 120	1.08	S facing slope.
I	1000	Df, some Pl	100 – 120	15.83	SW facing slope. W end clearcut in 1992, planted in 1993. Bounded S by stream.

* Listed in descending order of abundance. Abbreviations: At = *Populus tremuloides* Michx., Ba = *Abies lasiocarpa* (Hook.) Nutt., Df = *Pseudotsuga mensiesii* (Mirb.) Franco, Pl = *Pinus contorta* Dougl. var.*latifolia* Engelm., Sp = *Picea glauca* (Moench) Voss X *Picea engelmannii* Parry.

After the infection centers were explored and mapped, 20 cm long root segments from 15 colonized conifers were collected from along the perimeter of each infection center (Figures 4 and 8), resulting in a total of 135 samples. Samples were collected from along the perimeter for two reasons. First, hosts located along the advancing front of the infection center have usually been infected quite recently. Isolates sampled from recently colonized hosts result in more successful culture isolation than samples collected from older decayed hosts. In addition to viability, perimeter samples are representative of the mycelium which occupies the inner regions of the center. Previous studies by other authors (Rishbeth, 1978; Shaw and Roth, 1976) have demonstrated that samples collected along the radius of an infection center all have the same genotype.

Any somatic mutation that occurred in the past would most easily be discovered by sampling along the perimeter. As the mycelium expands radially through the forest (much like a fungal culture growing on agar medium), the newly synthesized fungal cells along the perimeter of the infection center should have the same genotype as their precursor cells. If a mutation was to occur, it would be propagated radially outwards by vegetative growth and this genetic change would be expressed in sectors.

Figure 1: Map of Gavin Lake Block showing locations of nine sampled *Armillaria* infection centers. Letters indicate infection centers and dashed lines represent streams. Scale 1:20,000



In order to isolate the mycelium most likely to have caused the host's symptoms or mortality, all cultures were isolated from mycelial fans beneath the bark of the root. Collections were made from infected roots of juvenile regeneration, mature overstory trees, and occasionally from stumps. None of the cultures were isolated from rhizomorphs, fruiting bodies, or decaying wood for the following reasons. Legrand et al (1996) and Rizzo and Harrington (1993) discovered that different species of *Armillaria* occupied different niches, causing certain species to be sampled more commonly from rhizomorphs than others. Likewise, species (or genets) which produce an abundance of fruiting bodies would be over-represented if basidiocarps were sampled. Within 12 hrs from the time of sampling, a segment of a mycelial fan was aseptically removed from the root and placed on 2% malt extract agar medium. Cultures were incubated in the dark at room temperature (20-23 °C) and subcultured as necessary. Several isolates became contaminated and were lost; the remaining number of viable fungal cultures collected from each infection center is shown in Table 2.

DNA Extraction

In preparation for DNA extraction, all isolates were transferred to 2% malt extract broth and allowed to grow in the dark at room temperate for 2-3 weeks. Three different DNA extraction protocols (Flynn and Niehaus, 1997; Möller et al, 1992; modified version of Doyle and Dickson, 1987) were tested on ten random isolates which were sampled from the first infection center, Center A. When these three protocols were compared for yield, purity, and quality of resulting DNA, the modified version of Doyle and Dickson (1987, see Appendix A) was the most effective. This protocol was then used for the extraction of DNA from the remaining isolates.

Biological Species Identification

A PCR-based species identification method (Anderson and Stasovski, 1992; Harrington and Wingfield, 1995; White et al., 1998) was used to determine which species of *Armillaria* each isolate belonged to. The procedure involved the amplification of the intergenic spacer regions (IGS-1 and IGS-2) located between the various ribosomal RNA genes, and the restriction enzyme digestion of the resulting products. Final PCR reaction concentrations for IGS-1 amplification were as follows: 4.0 mM $MgCl_2$, 200 μM dNTPs,

0.5 μM of each primer (LR12R and O-1), 1.5 units *Taq* polymerase (Life Technologies, Grand Island, NY), 1X PCR Buffer (supplied with enzyme), and 10 ng DNA template with a final volume of 25 μl per reaction tube. After all reagents were aliquoted, two drops of light mineral oil were added to the surface of the mixture in each reaction tube to prevent evaporation. The thermal cycler (Perkin Elmer 480) conditions were: initial soak at 95°C for 2 min; then 35 cycles of 90°C for 30 sec, 60°C for 40 sec, 72°C for 2 min; a final elongation of 72°C for 10 min; then a final soak at 4°C. For the restriction enzyme digestion, two units of Alu I (Life Technologies) were added directly to the 25 μl of PCR products and incubated at 37°C for 2 hrs. Note that Alu I buffer was not necessary for successful digestion. The resulting fragments were separated on 2% TBE agarose gels at 4.75 V/cm, stained with ethidium bromide, and photographed under ultraviolet illumination. Further amplification of the IGS-2 region was not necessary for species identification.

Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD analysis is a PCR-based procedure which amplifies random segments of genomic DNA using a single decamer primer of arbitrary nucleotide sequence (Williams et al, 1990; Welsh and McClelland, 1990). Only three studies have applied RAPD analysis to the genus *Armillaria* (Smith et al, 1992; Guillaumin et al, 1996; Schulze et al, 1997). In these studies, a total of 13 different primer sequences were used for genetic analysis of various *Armillaria* species: 10 which were used on European *A. ostoyae* isolates and 3 which were used on a different species of North American *Armillaria*. The sequences of the 13 RAPD primers (Life Technologies) used in this study are listed in Tables 3 and 5. Final RAPD-PCR reaction concentrations for RAPD analysis were as follows: 2.0 mM $MgCl_2$, 100 μM dNTPs, 0.2 μM primer, 2.0 units *Taq* polymerase (Life Technologies), 1X PCR Buffer (supplied with enzyme), and 10 ng DNA template with a final volume of 25 μl per reaction tube. After all reagents were aliquoted, 2 drops of light mineral oil were added to each reaction tube to prevent evaporation. The thermal cycler (Perkin Elmer 480) conditions: initial soak at 94°C for 5 min; then 35 cycles of 94°C for 1min, 40°C for 30 sec, 72°C for 30 sec; then a final soak at 4°C. The amplification products were separated on 0.7% agarose / 0.4% Synergel (Diversified Biotech, Boston, MA) TBE

gel at 4.75 V/cm, stained with ethidium bromide, and photographed under ultraviolet illumination.

To ascertain the consistency of banding patterns, each PCR reaction was run at least twice. Amplification products may be dependent upon specific conditions of the PCR reaction, so to avoid artifactual variation (Ellsworth et al, 1993) the protocol was never modified. Only bands that were clearly resolved and consistent across *all* amplifications were recorded, and a binomial data matrix was compiled using the presence (1) or absence (0) of particular bands. The molecular weight of each band (= marker) was estimated by comparing gel migration distances with those of the 100 bp and λ/HindIII DNA ladders (Life Technologies). Data was stored and analyzed using the *RAPD*ISTANCE Package: Version 1.04 for the Analysis of Patterns of RAPD Fragments (Armstrong et al, 1994). Similarity matrices were calculated using Dice's similarity coefficient:

$S = 2a/(2a + b + c)$

where a is the number of RAPD markers shared by the two paired isolates, and b and c are the number of markers present only in one of the paired isolates, respectively. Monomorphic markers were not included in similarity coefficient calculations. Cluster analysis was performed using the UPGMA (Unweighted Pairwise Group Method with Arithmetical mean) option of the NEIGHBOR program of PHYLIP version 3.5 (Felsenstein, 1993), and the resulting dendrograms were viewed using TREEVIEW (Page, 1996).

Somatic Incompatibility Tests

Somatic incompatibility tests were performed on unamended 2% malt extract agar medium. Pairs of 2 mm agar cubes colonized by mycelium were placed approximately 4 mm apart from each other and incubated in the dark at room temperature. The cultures initially grew radially outwards in a concentric fashion until their margins contacted. If the two cultures were confluent, this indicated that both isolates belonged to the same somatic incompatibility group. If a gap remained between the cultures and the reaction zone consisted of sparse to absent mycelia, this indicated an antagonistic reaction between genetically distinct isolates (i.e. mutual inhibition). The reaction zone between the two cultures was inspected every seven days until a clear and obvious compatibility

or incompatibility reaction was observed. Any pairing which gave ambiguous or intermediate results was repeated. If an obvious reaction was not observed after four repetitions, results were considered "ambiguous".

Ramets vs. Genets

In the past, many terms have been used to describe the individual units of fungal populations (Anderson and Kohn, 1995; Rayner, 1991). In this study, the term "genet" is defined as all the mycelium produced by vegetative means following an initial sexual mating event. Hence, all of the isolates sampled from a single genet usually have the same genotype. Occasionally, genetic variability may be introduced into a fungal mitotic cell lineage; for example, if mitotic recombination or a somatic mutation occurs, isolates sampled from the same genet may posses different genotypes. The definition of a genet does not denote spatial contiguity of all the isolates, so a single genet may be divided into several non-contiguous patches called "ramets". In the following discussion, the terms "genet" and "ramet" are used according to the above definitions.

RESULTS

Biological Species Identification

Amplification and restriction enzyme digestion resulted in products similar in size to those reported by White et al (1998). Only two species of *Armillaria* have been reported from the region in which the study site was located (Morrison et al, 1985; White et al, 1998): *A. ostoyae* (NABS I) and *A. sinapina* (NABS V). These two species were successfully distinguished by comparing the IGS-1/Alu I restriction fragment patterns (Figure 2), so further amplification of the IGS-2 region was not required.

The details of the IGS-1/Alu I restriction fragment patterns encountered during species identification are summarized in Table 2. Twenty-five (19.5 %) of the isolates were identified as *A. sinapina*. Four of the five *A. sinapina* restriction fragment patterns described by White et al (1998) appeared in this population, with Pattern 3 being the most common. The remaining 103 samples were identified as *A. ostoyae*, with all isolates in this study producing the same restriction fragment pattern: Pattern 1. The second rare *A. ostoyae* fragment pattern described by White et al. (1998) was not detected.

Figure 2: IGS-1/Alu I restriction fragment patterns for *A. ostoyae* and *A. sinapina*. Lanes 1 and 8, DNA ladder. Lanes 2 and 4, *A. ostoyae* pattern 1. Lane 3, *A. sinapina* pattern 1. Lane 5, *A. sinapina* pattern 5. Lanes 6 and 7, *A. sinapina* pattern 2.

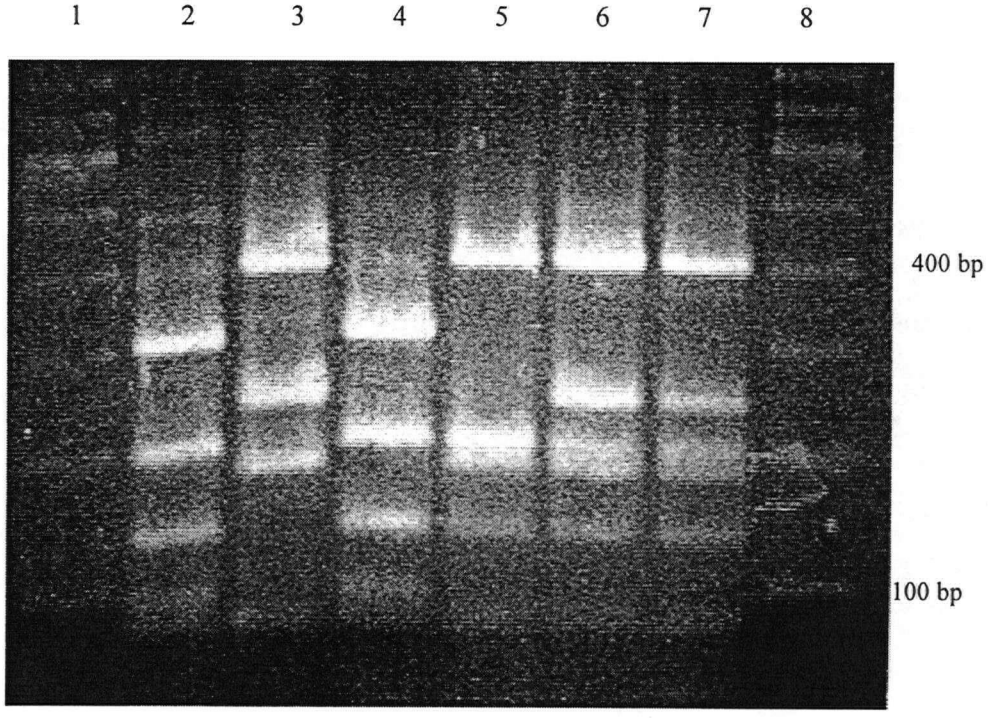


Table 2: Species, number of isolates, and frequency of IGS-1/Alu 1 restriction fragment patterns per center.

Center	Total no. of isolates	No. of *A. ostoyae* isolates	No. of *A. sinapina* isolates			
		all Pattern 1*	Pattern*			
			1	2	3	5
A	15	10		1	4	
B	15	15				
C	14	11	1	1		1
D	14	12		2		
E	14	14				
F	11	0	2	3	6	
G	14	14				
H	16	14		2		
I	15	13	1		1	
Total	128	103	4	9	11	1
			25			

*from White et al. (1998)

As shown in Table 2 and Figures 4 and 8, *A. ostoyae* occurred in eight of the nine infection centers with three of the centers (Centers B, E, G) being composed of only *A. ostoyae* isolates. Similarly, *A. sinapina* was recovered from six of the nine centers with one center (Center F) being composed of only that species. In the five infection centers that were occupied by both species, *A. ostoyae* was always sampled at least twice as frequently as *A. sinapina*. Clearly, *A. ostoyae* was the dominant species in the study site.

Primer Screening and Selection

The 13 primers were chosen specifically for their demonstrated ability to detect polymorphisms in *Armillaria* spp. Primers which failed to amplify or produced only monomorphic markers were originally screened out by the previous authors (Smith et al, 1992; Guillaumin et al, 1996; Schulze et al, 1997). These selected decamer primers have not been used on any *Armillaria* isolates from western North America, so it was necessary to screen the primers against some samples collected during this study. After the first set of samples were gathered from Center A, ten random isolates were used for preliminary screening. Products which were appropriately sized for the purpose of RAPD analysis were produced by all of the primers except one: primer JD09 gave no amplified products and was omitted from RAPD analysis. The remaining primers all produced scorable polymorphisms amongst the screening isolates. Therefore, 12 of the 13 primers were used to study the *Armillaria* isolates collected from the study site in the central Interior of British Columbia.

Armillaria ostoyae

A. ostoyae and *A. sinapina* are distinct intersterile biological species, so each species was analyzed independently from the other. Therefore, all data were species specific. For example, each particular RAPD marker was only compared within species, not across species; different species will have different markers. The results of the investigation into the population structure of *A. ostoyae* will be addressed first, and *A. sinapina* will be addressed later in this report. Since Center F was composed entirely of *A. sinapina*, it was omitted from the following *A. ostoyae* calculations.

Genotype Determination

The first objective was to determine how many different genotypes were represented by the 103 *A. ostoyae* isolates. The degree of confidence in genotype differentiation is proportional to the number of primers and polymorphic markers used to distinguish the genotypes. To attain an acceptable degree of confidence (see Appendix B), it was not necessary to use all 12 primers so only seven were chosen for this task (Table 3). Each of the seven primers was used in a RAPD-PCR reaction with all *A. ostoyae* isolates and the binomial (presence/absence) data were collected (Appendix C, Figures 3a and 3b). For *A. ostoyae*, a total of 41 polymorphic loci were detected, ranging in size from 415 to 2250 base pairs (bp). The average number of polymorphic markers produced per primer was 5.86, and the number of different banding patterns detected by each primer ranged from four to six. When the data from all seven primers were combined, a total of only seven distinct RAPD multilocus genotypes were detected among the 103 *A. ostoyae* isolates. The number of isolates of the same genotype ranged from one to 29, with a mean of 14.71 isolates per genotype (Table 4).

Table 3: Summary of RAPD analysis of 103 *A. ostoyae* isolates based on the first seven primers.

Primer*	No. of markers	No. of poly-morphic markers	No. of different banding patterns	Primer sequence (5'→3')
JD01	9	8	6	ACTTGAGGCG
JD02	9	5	4	ATGGATCCGC
JD03	9	8	6	CCGGCCTTCC
JD05	7	3	4	TGCCGAGCTG
JD06	7	4	5	AGTCAGCCAC
JD07	10	6	4	AATCGCGCTG
JD08	9	7	5	AGGGCTCTTG
Total	60	41		
Mean	8.57	5.86		

*Primer JD09 (GGTCCCTGAC) was omitted from RAPD analysis.

It was assumed that all isolates with the same genotype belonged to the same genet, but it is theoretically possible for the same genotype to arise, by chance alone, more than once in a randomly mating sexual population. To estimate the probabilities of identical RAPD genotypes arising independently, a method which multiplies individual single locus frequencies was utilized. The probability of the identical multilocus

Figure 3a: *A. ostoyae* RAPD-PCR products amplified by primer JD01. Lanes 1, 10, and 17, DNA ladder. Lanes 2 – 9 and 11 – 16, all *A. ostoyae* isolates collected from Center G (Genotype Ost-I).

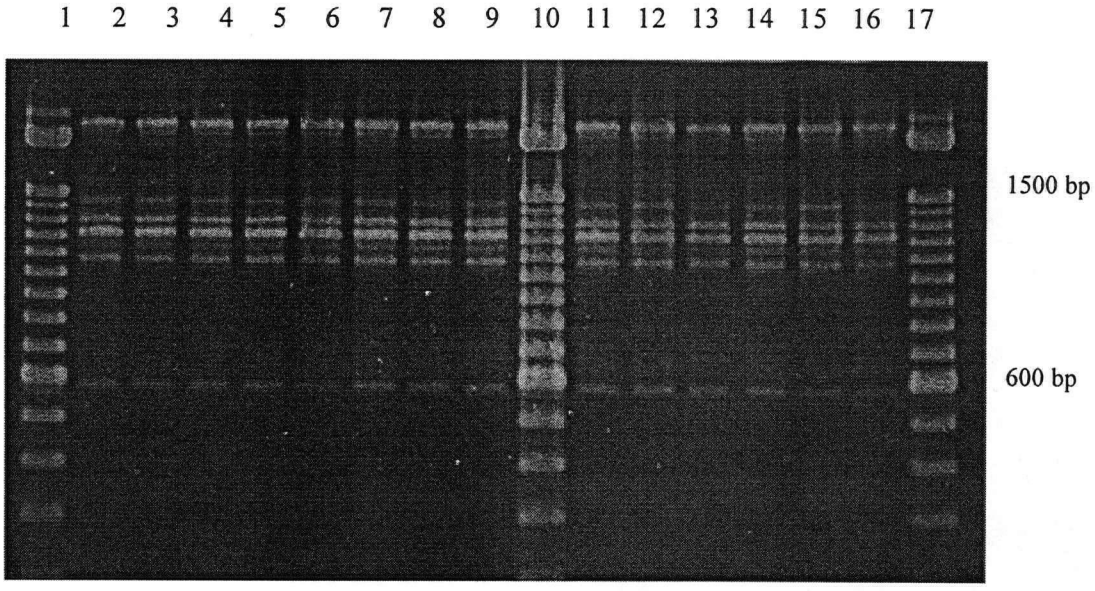


Figure 3b: *A. ostoyae* RAPD-PCR products amplified by primer JD01. Lanes 1 and 12, DNA ladder. Lanes 2 and 3, Genotype Ost-I. Lane 4, Genotype Ost-II. Lanes 5 – 7, Genotype Ost-III. Lane 8, Genotype Ost-IV. Lane 9, Genotype Ost-V. Lane 10, Genotype Ost-VI. Lane 11, Genotype Ost-VII.

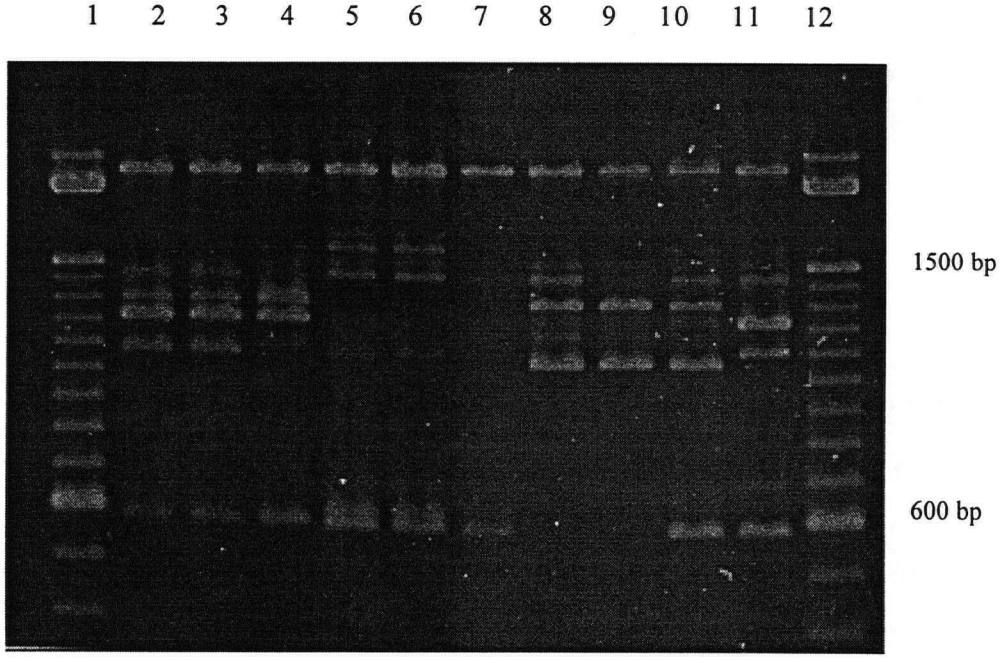


Table 4: The number and source of isolates belonging to the seven *A. ostoyae* genotypes.

Genotype	No. of isolates (*n*)	Source of isolates*
Ost-I	24	Center A (10) + Center G (14)
Ost-II	13	Center I
Ost-III	29	Center B(15) + Center H (14)
Ost-IV	1	Isolate E12 from Center E
Ost-V	13	Center E
Ost-VI	11	Center C
Ost-VII	12	Center D
Mean	14.71	

* Brackets indicate number of isolates from each center.

genotype occurring twice by chance alone is the product of all 41 single locus frequencies corresponding to that genotype. All calculated probabilities of particular genotypes arising independently multiple times were less than 8.96×10^{-81} (Appendix B). These extremely low probabilities verified that all isolates with the same RAPD genotype had arisen mitotically from an original source mycelium, and therefore were from the same genet.

Genetic Variability

To assess the genetic variability of the seven *A. ostoyae* genotypes, a commonly utilized band sharing coefficient (Dice's similarity coefficient) was calculated from the polymorphic RAPD markers produced by the first seven primers. The similarity coefficient values for the 21 pairwise genotype combinations ranged from a 0.333 to 0.950, with an overall average of 0.516 (Appendix C), which means that each genotype shares an average of 51.6% of its RAPD markers with the other genotypes. The confidence of the estimated genetic relatedness between genotypes increases as the number of RAPD markers used to calculate the similarity coefficients increases. For this reason, further RAPD analysis using the remaining five primers (Table 5) was conducted. To reduce the number of RAPD-PCR reactions, a single *A. ostoyae* isolate was chosen to represent each of the seven genotypes. An additional 31 polymorphic markers were produced which ranged in size from 350 to 2550 bp (Appendix D). The data from these five primers was combined with the data from the first seven primers, resulting in a total of 72 polymorphic markers with a mean of six per primer. Dice's similarity coefficients

were calculated for all pairwise genotype combinations based on the RAPD data produced from all 12 primers combined (Table 6).

Table 5: Summary of RAPD analysis of the seven *A. ostoyae* genotypes based on all 12 primers combined.

Primer	No. of bands	No. of poly-morphic bands	No. of different banding patterns	Sequence (5'→3')
First seven*	60	41	7	-
JD04	7	3	4	CAGGCCCTTC
JD10	7	5	6	CAAACGGGTG
JD11	14	10	6	GTGACGTAGG
JD12	10	9	6	GGGTAACGCC
JD13	6	4	5	GTGATCGCAG
Total	104	72		
Mean	8.67	6		

* see Table 3.

Table 6: Similarity matrix (using Dice's similarity coefficient) for the seven *A. ostoyae* genotypes based on all 12 primers combined.

Genotype	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
Ost-I	1.0						
Ost-II	0.754	1.0					
Ost-III	0.394	0.464	1.0				
Ost-IV	0.406	0.448	0.472	1.0			
Ost-V	0.448	0.486	0.533	0.959	1.0		
Ost-VI	0.400	0.508	0.441	0.455	0.522	1.0	
Ost-VII	0.633	0.603	0.529	0.485	0.522	0.484	1.0
Genotype Avg.	0.506	0.544	0.472	0.537	0.578	0.468	0.543
Overall Avg.	0.521						

The corresponding values for similarity coefficients based on the first seven primers vs. all 12 primers were quite similar, with no significant differences between genotypic similarity coefficient averages. This indicated that the second set of primers detected similar trends in genetic variability as the first set of primers did. Based on data from all 12 primers, the similarity coefficient values ranged from 0.394 to 0.959, with an overall average similarity coefficient of 0.521 (52.1%). Although the range of specific values was quite great, the ANOVA test did not reveal any significant differences (p>0.75) between the average similarity coefficients of the various genotypes.

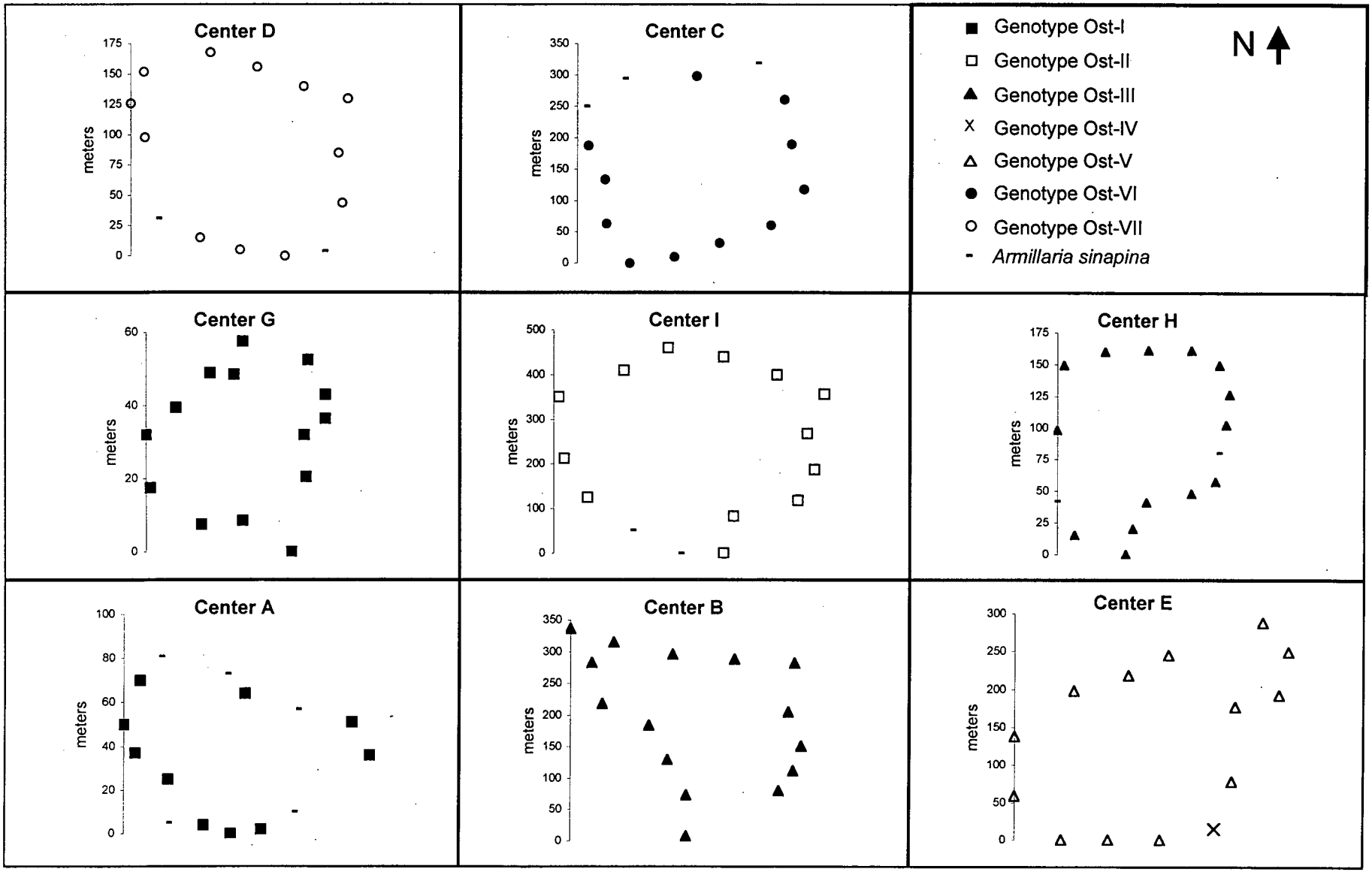
The abnormally high similarity coefficient (0.959) between Genotype Ost-IV and Ost-V suggested that one of these genotypes had possibly arisen via somatic mutation rather than sexual reproduction. Based on the distributions of Genotypes Ost-IV and Ost-V in relation to each other (Figure 4), Genotype Ost-IV was the likely mutant. The single isolate of Genotype Ost-IV (Isolate E12, see Table 4) was collected from Center E, the rest of which was occupied by isolates of Genotype Ost-V. The most likely explanation is that Isolate E12 was sampled from a mutated sector of the genet which occupied Center E. Another abnormally high similarity coefficient (0.754) was between Genotypes Ost-I and Ost-II. A similarity coefficient value of 0.750 was expected if the two genets had a parent-progeny relationship, and the distributions of Genotypes Ost-I and Ost-II in relation to each other did not exclude this possibility.

Distribution of Genotypes

A goal of this project was to determine if the eight distinct infection centers in the Gavin Lake Block were occupied by single or multiple genets of *A. ostoyae*. As shown in Figure 4, seven of the eight infection centers were internally genetically homogenous i.e. all isolates of *A. ostoyae* had the same genotype. The one exception was Center E, in which a single isolate displayed a different genotype than the rest of the isolates sampled from that center. As mentioned above, the data suggested that the unique genotype represented by Isolate E12 arose via somatic mutation rather than sexual reproduction. Since Genotype Ost-IV (Isolate E12) and Genotype Ost-V were both from the same mitotic cell lineage, they were both part of the same genet by definition. Therefore, Center E was occupied by two genotypes but only a single genet (Table 4), and the seven genotypes detected in the study area represented only six genets.

In two instances, the same genotype occurred in two different infection centers (Genotypes Ost-I and Ost-III). In both cases, the two infection centers which shared the same genotype were in close proximity to each other. For example, Centers B and H were both composed entirely of Genotype Ost-III and were only about 130 m apart from each other. This proximity of identical isolates presumably represented a large genet which had fragmented into two smaller non-contiguous ramets which now occupied discrete areas.

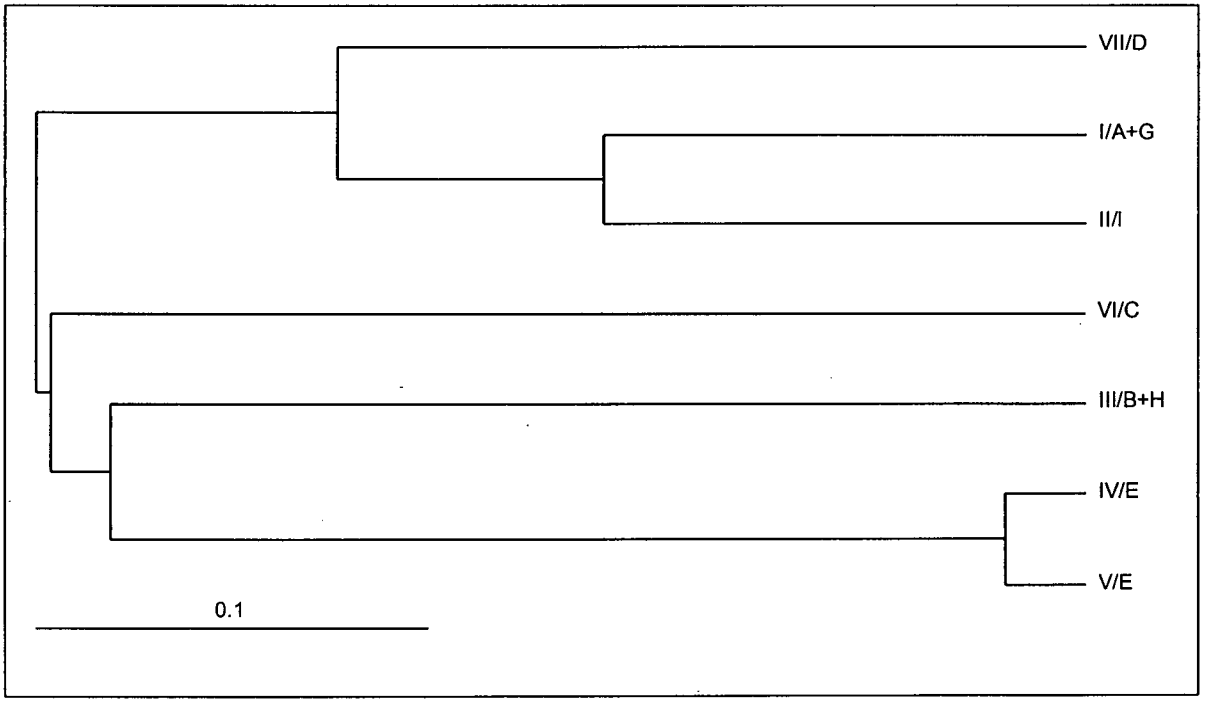
Figure 4: Distribution of isolates belonging to the seven *A. ostoyae* genotypes, along with distribution of *A. sinapina* isolates.



Based on the findings that *A. ostoyae* was the dominant and most pathogenic species in all eight infection centers, the area of each *A. ostoyae* genet was considered equivalent to the total area of the infection center it occupied (Table 1). If a genet had become fragmented into multiple ramets, then the areas of the ramets were summed to give the total area of the genet. The area occupied by the six *A. ostoyae* genets ranged from 0.7 ha to 15.83 ha, with a mean of 6.01 ha per genet.

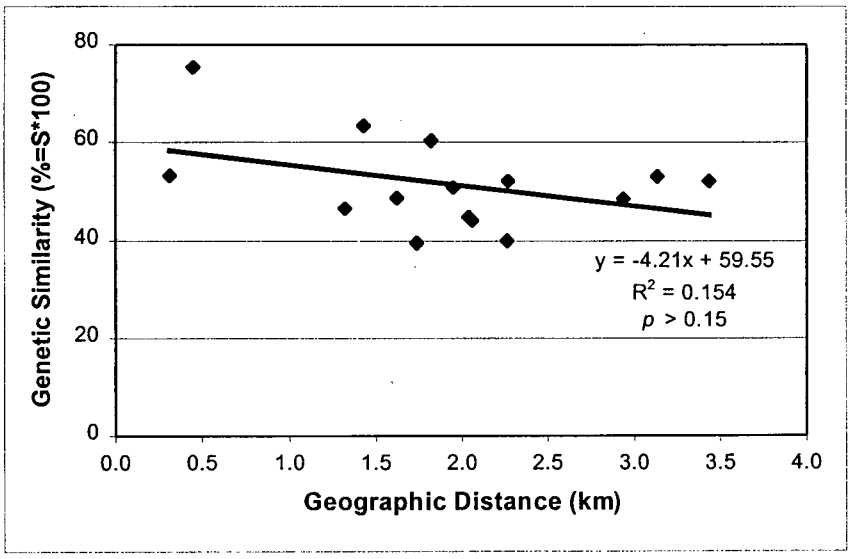
The relationship between geographic distance between genotypes and genetic similarity of genotypes was investigated. Based on the data from all 12 primers, cluster analysis was performed using the unweighted pairwise group method with arithmetical mean (UPGMA) and the resulting dendrogram appears as Figure 5.

Figure 5: UPGMA dendrogram for *A. ostoyae* produced from the similarity matrix in Table 6. Bar indicates phenetic distance (1 – S) and branch termini indicate genotypes/infection center.



The dendrogram shows that genotypes tended to cluster in a pattern which reflected their geographical spatial distribution over the landscape. Genotypes located in the eastern half of the study site were grouped together, as were the genotypes located in the western half of the study site. The first node in the dendrogram placed Genotypes Ost-I, Ost-II, and Ost-VII together in one cluster and Genotypes Ost-III, Ost-IV, Ost-V, and Ost-VI in the other (Figure 5). To determine if spatial proximity and genetic similarity were in fact related, linear regression analysis was performed using geographic distance vs. percent genetic similarity (S*100) between genotypes. (Figure 6). Since Genotype Ost-IV was a somatic mutation and therefore not independent, it was omitted from these calculations. To calculate geographic distances, the approximated midpoints of infection centers were used as the reference points for genotypes. Since some genotypes occurred in more than one infection center, the midpoint between all isolates belonging to that genotype was used as the reference point. For example, Genotype Ost-I was sampled from Centers A and G, so the midpoint between these two centers was the reference point. Although the dendrogram displayed an apparent relationship, regression analysis could detect no significant linear relationship ($P>0.15$) between geographic distance and genetic similarity.

Figure 6: Relationship between geographic distance and percent genetic similarity (S*100) for the six independent *A. ostoyae* genotypes.



Somatic Incompatibility Tests

Another objective of this study was to determine if RAPD analysis and somatic incompatibility testing differed in their abilities to distinguish *Armillaria* genets. First, somatic incompatibility tests were performed for all possible combinations of *A. ostoyae* isolates sampled from the same infection center. The results of within-center somatic incompatibility testing corresponded with the results of RAPD analysis, i.e. all isolates that displayed the same RAPD genotype were also somatically compatible. One anomaly was noticed from isolates within Center E: when Isolate E12 (Genotype Ost-IV) was paired with the 13 isolates of Genotype Ost-V, some reactions were too ambiguous to draw conclusions, even after several replications. Of those 13 reactions, six gave incompatible results while the other seven gave intermediate and therefore inconclusive results. Strangely, different isolates with Genotype Ost-V produced different degrees of incompatibility when paired with the Genotype Ost-IV isolate. Even though there was no noticeable differences in reaction conditions such as agar medium or incubation temperature, some reactions were more distinctive and apparently incompatible than others. Often multiple reactions were performed on the same agar plate, still resulting in different degrees of incompatibility for different isolates

Due to the large number of *A. ostoyae* samples collected, it was not feasible to run between-center somatic incompatibility tests using all isolates. Instead, a single isolate was chosen to represent each of the seven genotypes and all possible combinations were tested. The results were analogous to those of RAPD analysis: all isolates that displayed different RAPD genotypes were somatically incompatible (with the above exception). Note that when Genotype Ost-IV was paired with all genotypes other than Genotype Ost-V, clearly incompatible reactions were observed. The only pairing which produced intermediate reactions was Genotype Ost-IV vs. Ost-V. The ambiguous somatic incompatibility reactions between these two genotypes support the hypothesis that Genotype Ost-IV arose via somatic mutation from Genotype Ost-V.

Armillaria sinapina

Using the same procedures as mentioned above for *A. ostoyae*, the population structure of *A. sinapina* was investigated in the six infection centers where it was found. Since Centers B, E, and G were composed entirely of *A. ostoyae*, they were omitted from the following *A. sinapina* calculations.

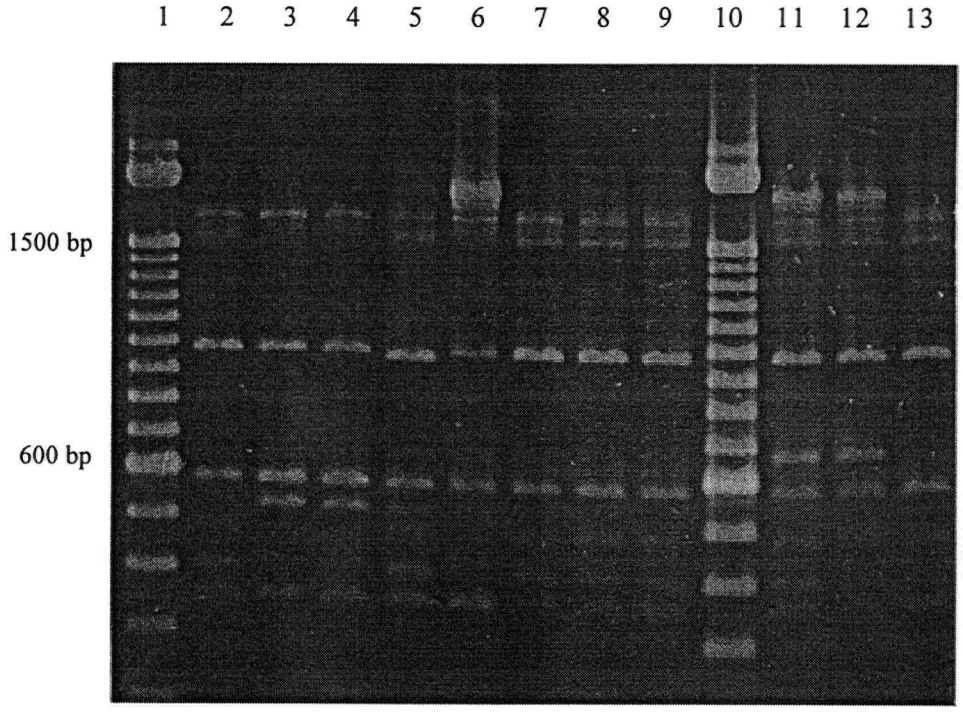
Genotype Determination and Genetic Variability

To determine how many different genotypes were represented by the 25 *A. sinapina* isolates, the first set of seven primers were applied (Table 7). Each primer was used in a RAPD-PCR reaction with all *A. sinapina* isolates and the binomial data were tabulated. (Appendix E, Figure 7). For *A. sinapina*, a total of 44 polymorphic markers were detected, ranging in size from 425 to 2100 bp. The mean number of polymorphic markers per primer was 6.29, and the number of different banding patterns detected by each primer ranged from five to ten. When the data from all seven primers were combined, a total of 18 distinct genotypes were detected among the 25 *A. sinapina* isolates. The number of isolates per genotype ranged from one to five, with 14 (77.8 %) of the genotypes being sampled only once (Figure 8). Somatic incompatibility tests for within-center combinations of *A. sinapina* isolates verified the genotypes delineated by RAPD analysis.

Table 7: Summary of RAPD analysis of 25 *A. sinapina* isolates based on seven primers.

Primer	No. of markers	No. of poly-morphic markers	No. of different banding patterns
JD01	6	5	7
JD02	6	5	8
JD03	8	6	5
JD05	7	7	8
JD06	7	7	10
JD07	8	7	10
JD08	7	7	10
Total	49	44	
Mean	7	6.29	

Figure 7: *A.sinapina* RAPD-PCR products amplified by primer JD01. Lanes 1 and 10, DNA ladder. Lanes 2 – 9 and 11 – 13, all *A. sinapina* isolates collected from Center F. Primer JD01 was able to distinguish between all five genets of *A. sinapina* found within the infection center.



RAPD analysis supported the species distinction based on the IGS-1/Alu I restriction fragment patterns: the RAPD banding patterns for the *A. sinapina* isolates were notably different from the banding patterns *A. ostoyae* isolates. For example, three markers were monomorphic across all isolates of *A. sinapina* but absent in *A. ostoyae*. Eleven markers were monomorphic in *A. ostoyae* but absent in *A. sinapina*, two of which were also monomorphic across all 52 *A. ostoyae* genotypes collected from the southern Interior of B.C. (see Chapter III).

The genetic variability of the 18 *A. sinapina* genotypes was assessed by calculating Dice's similarity coefficients based on the binomial data produced by the seven primers (Appendix E). Similarity coefficient values for 153 pairwise genotype combinations ranged from a minimum of 0.261 to a maximum of 0.774. No abnormally high similarity coefficients were noticed, so all genotypes were assumed to represent distinct genets. The overall average similarity coefficient for *A. sinapina* was 0.518 (51.8 %). This value is quite similar to that of *A. ostoyae* which, based on the first seven

primers, had an overall average similarity coefficient of 0.516. Although the same primers were used for both species, the calculations were based on completely different and often species-specific RAPD markers. In contrast to *A. ostoyae*, there were significant differences between the similarity coefficient averages of some of the 18 *A. sinapina* genotypes (Appendix E), but no trends were apparent.

Distribution of Genotypes

In regards to *A. sinapina,* the six sampled infection centers were internally genetically heterogeneous (Figure 8). All six centers were occupied by two to five distinct genotypes of *A. sinapina*, with a mean of three genotypes per center. There was a positive correlation between the number of *A. sinapina* isolates collected and the number of genotypes per center. For example, Center F had the greatest number of *A. sinapina* isolates collected from it and the greatest number of associated genotypes. Also, the same *A. sinapina* genotype was never collected from more than one infection center. Each genet was restricted to a single center and fragmentation into multiple ramets was not observed.

As shown in Figure 8, 14 (77.8 %) of the detected *A. sinapina* genotypes were represented by single isolates only. Centers A and F were the only infection centers in which multiple isolates of the same *A. sinapina* genotype were sampled. For the four genotypes that were sampled more than once, the maximum distance between two identical isolates ranged from 30 to 100 m, with an average of approximately 60 m. The maximum dimensions of genets represented by single isolates could be roughly estimated from the distances between sampling points along the perimeter of the centers. Hence, the average *maximum* genet length was approximately 85 m. This value likely over-estimates the actual sizes of genets because more *A. sinapina* genets would probably have been detected if the sampling intensity was increased. The positive correlation between the number of *A. sinapina* samples and the number of genotypes per center suggested that the average dimension of genets was less than the average distance of the sampling intervals. Clearly, the average size of the *A. sinapina* genets sampled from this study site was relatively small compared to the average size of the *A. ostoyae* genets.

Figure 8: Distribution of isolates belonging to the 18 *A. sinapina* genotypes, along with distribution of *A. ostoyae* isolates.
Like symbols (▲, ○, ●, ■) indicate isolates with the same genotype, except (X) indicates an *A. sinapina* isolate with a unique genotype (i.e. single isolate per genotype), and (-) indicates *A. ostoyae* isolates.

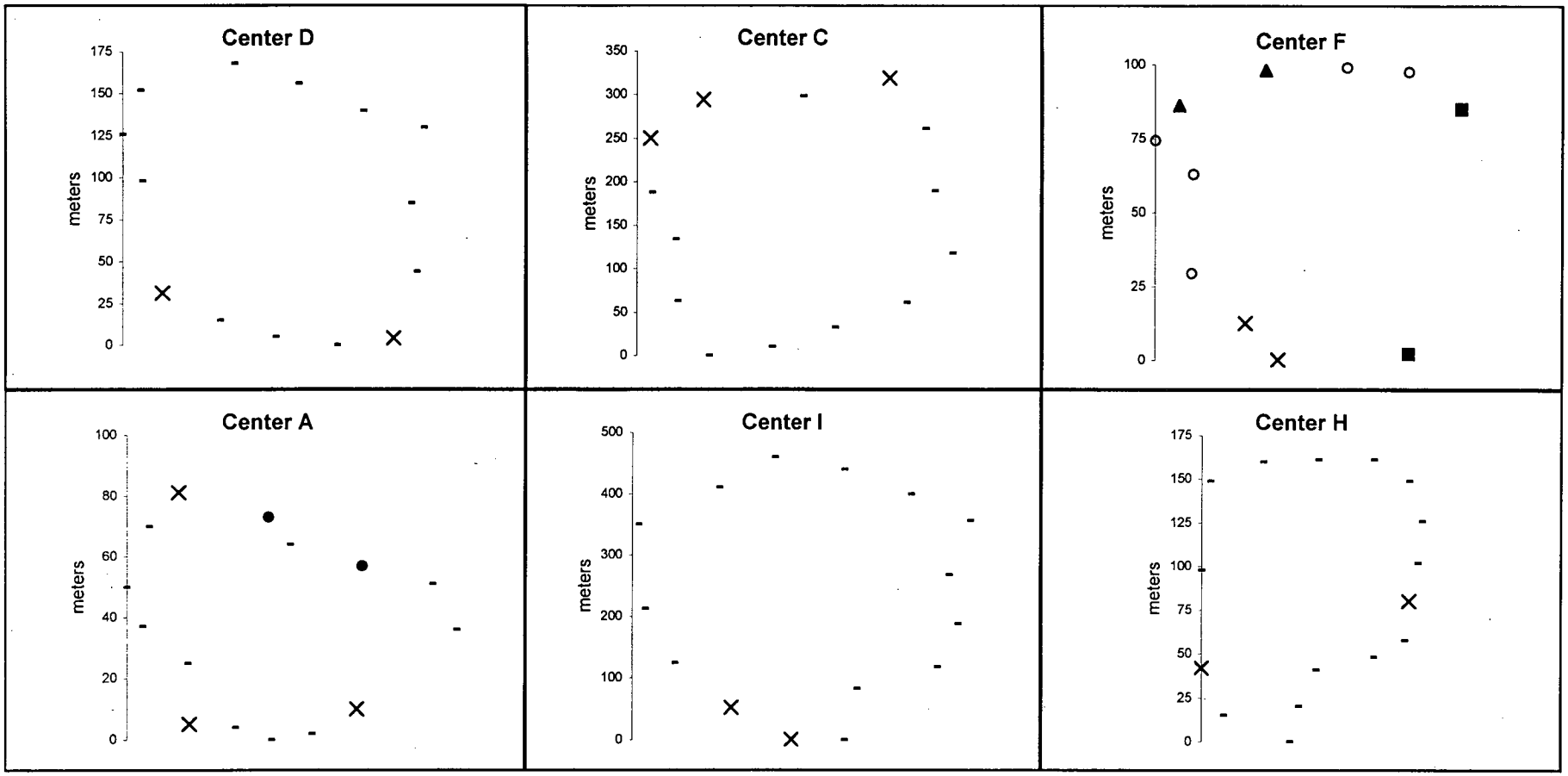
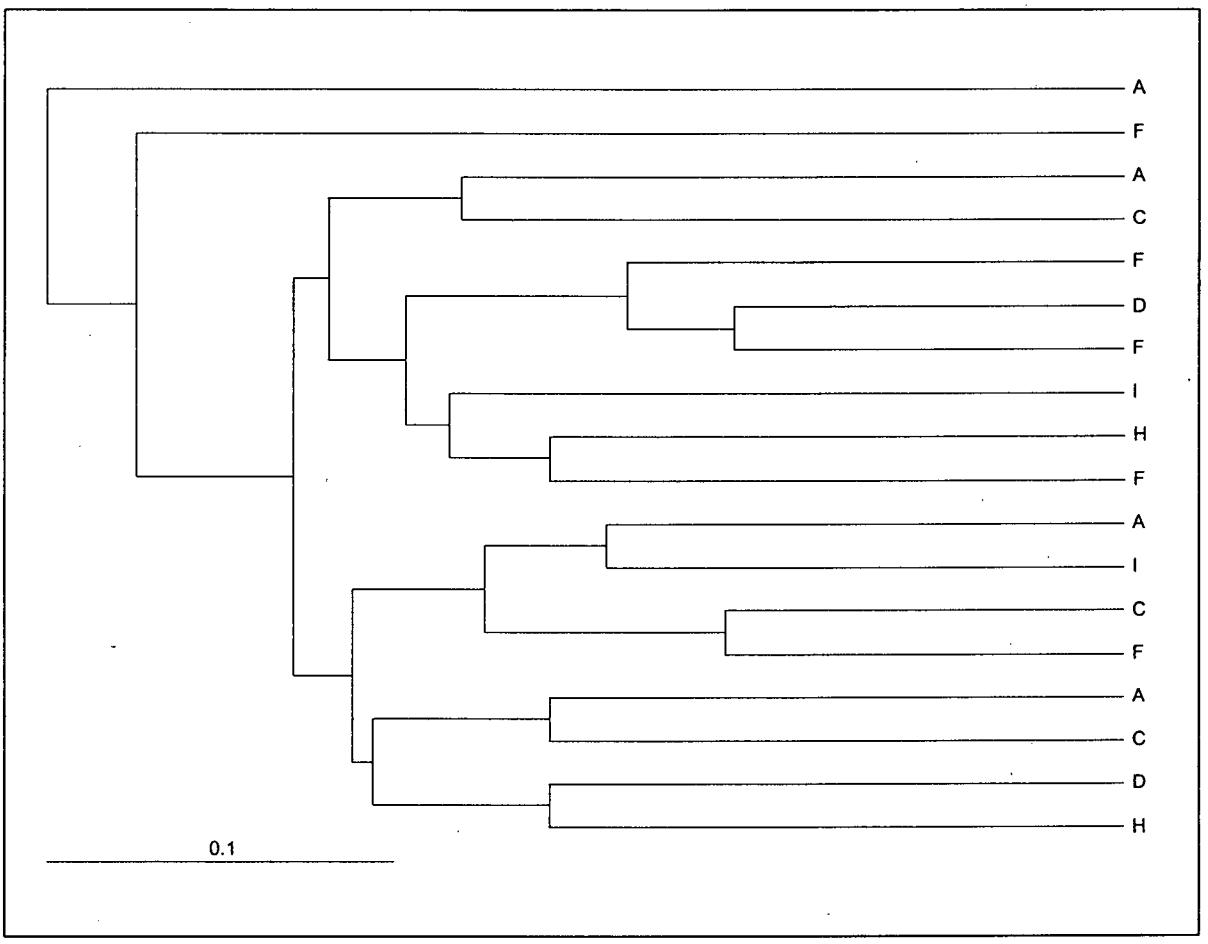
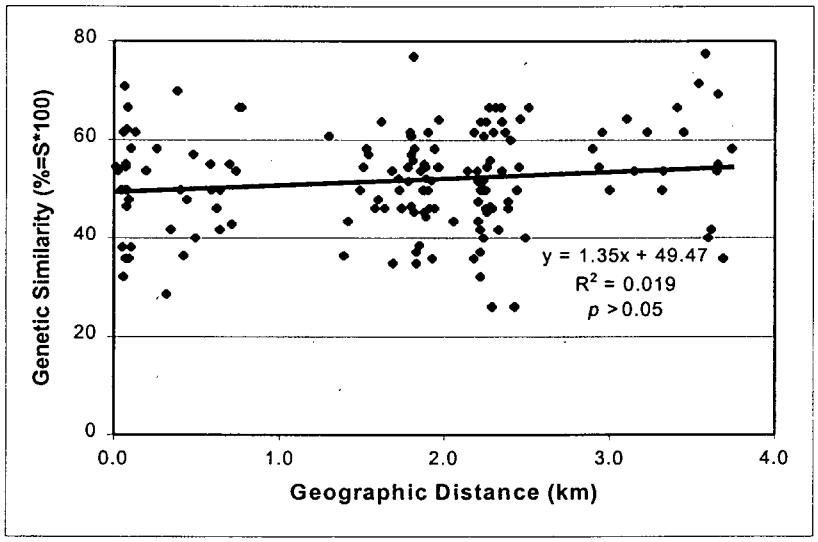


Figure 9: UPGMA dendrogram for the 18 *A. sinapina* genotypes. Bar indicates phenetic distance (1 – S) and branch termini indicate the center from which the genotype was sampled from.



Based on the RAPD data from the seven primers, cluster analysis was performed using the unweighted pairwise group method with arithmetical mean (UPGMA) and the resulting dendrogram appears as Figure 9. The arrangement of the above dendrogram suggested that the 18 *A. sinapina* genotypes were geographically distributed in a random fashion, i.e. they did not cluster in any discernible pattern. For instance, the five genotypes collected from Center F did not appear to aggregate at all, nor did those from Center A. Employing the same approach as for *A. ostoyae*, the results of linear regression analysis performed using geographic distance vs. percent genetic similarity (S*100) of genotypes is shown in Figure 10. Regression analysis indicated that there was no significant linear relationship between the two variables ($p>0.05$).

Figure 10: Relationship between geographic distance and percent genetic similarity (S*100) for the 18 *A. sinapina* genotypes.



DISCUSSION

Distribution of Genets

In regards to *A. ostoyae*, each infection center was occupied by a single genet, or ramet thereof. This genetic uniformity is consistent with the assertion that genets originate in a single mating event and spread exclusively by vegetative growth of mycelium and rhizomorphs. As the genet expands outwards through the forest along host root systems, an infection center develops. These results were similar to those of previous studies which investigated the genetic composition of distinct *Armillaria* infection centers. From Australia, Kile (1983) reported that 71% of infection centers were occupied by a single mycelium. Rishbeth (1978) and Shaw and Roth (1976) both reported single genets per infection center also, but the size of the sampled centers were notably smaller than those investigated here. Studies of the population structure of *Phellinus weirii*, a root-infecting fungus with a similar life cycle as *Armillaria*'s, produced similar results: the majority of infection centers were genetically uniform within (Bae et al, 1994; Dickman and Cook, 1988).

The results of the this study are also consistent with the assertion that the genus *Armillaria* does not reproduce via airborne asexual propagules. All isolates of the same genet were in relatively close proximity to each other. If the fungus was dispersed via asexual propagules then isolates of the same genet should be found scattered randomly throughout the forest. The extremely low probabilities of finding such a large number of identical isolates and the perfect spatial clustering of the isolates proved that *A. ostoyae* spreads predominantly by subterranean growth. When two infection centers shared the same genotype, they were always in close proximity to each other. In the past, a single large genet probably engulfed both centers and the area of forest between them. Through time, the middle portion of the genet perished, leaving two independent ramets separated by a tract asymptomatic forest. The fragmentation of a genet may be caused by several factors such as host unavailability, periods of unfavorable environmental conditions, or physical disruption of mycelial network. Since the areas of asymptomatic forest between infection centers was not examined for below-ground infection, it is possible that these regions are still occupied by inactive fungal mycelia which may become re-activated in the future. Thus after a period of independence, individual ramets of the same genet may coalesce to once again form a larger entity. The dissociation of genets into multiple discrete ramets separated by tracts of uninfected forest has been reported previously for *Armillaria* (Adams, 1974; Anderson et al, 1979; Kile, 1983; Shaw and Roth, 1976) and similarly for *Phellinus weirii* (Dickman and Cook, 1988).

Longevity and Genetic Stability

The longevity and genetic stability of genets over large time scales has been commonly reported (Anderson et al, 1979; Rishbeth, 1991; Shaw and Roth, 1976; Smith et al, 1992), with some genets reaching enormous sizes. Smith et al (1992) discovered an contiguous *A. bulbosa* genet with a minimum area of 15 hectares and considered it among the largest and oldest (~1500 yrs) living organisms. The largest *A. ostoyae* genet encountered in the present study was of similar magnitude: the *A. ostoyae* genet which was responsible for Center I was over 15 hectares in area. Average dimensions of contiguous *Armillaria* genets or ramets examined in other studies vary greatly: 50 m or less (Kile, 1986; Korhonen, 1978; Rishbeth 1978; Rizzo et al, 1995; Smith et al, 1994; Ullrich and Anderson, 1978; Worrall, 1994), 50 – 100 m (Legrand et al, 1996; Kile, 1983; Rishbeth,

1991), 100 m or greater (Smith et al, 1992). Average genet areas reported specifically for *A. ostoyae* ranged from 106 m^2 (Worrall, 1994) to 2029 m^2 (Legrand et al, 1996). The average area occupied by an *A. ostoyae* genet within the Gavin Lake study site was notably greater at 60100 m^2 (6.01 ha).

Assuming that, over time, each genet has expanded radially outwards from a single point of origin to attain its present size, the age of a genet can be estimated by the length of its radius divided by the annual rate of spread. The results of a previous study (van der Kamp, 1993) in the central Interior of B.C. indicated that *A. ostoyae* spread through the forest at a mean rate of 0.22 meters per year. The rate of spread of *A. ostoyae* was also measured in the southern Interior of B. C. (Peet et al, 1996) and in Oregon (Shaw and Roth, 1976); both obtained mean values of 1.0 meter per year. Since the study by van der Kamp (1993) was performed in the same area as the present study (Gavin Lake Block), 0.22 meters per year was the more appropriate value to use for genet age estimation. For the genet that occupied Center I, the average length of its radius was 250 m. In order for that genet to have attained its present dimensions, it must have been in existence for a minimum 1100 years. For both Genotypes Ost-I and Ost-III, half of the maximum distance between identical isolates was around 290 meters, i.e. over 1300 years of vegetative growth. Although these age estimates are quite crude, they illustrate the longevity of *A. ostoyae* genets in this region. In comparison, the average size of *A. sinapina* genets was considerably smaller. Since the rate of spread of *A. sinapina* in this region has not been determined, estimates of genet age could not be made.

A somatic mutation within an *Armillaria* genet has not been previously reported, which suggests that such an event is relatively rare. As a genet becomes larger and older, the chance of it experiencing a somatic mutation increases. Thus, the large sizes and old ages of *A. ostoyae* genets in this population made them possible candidates. However, out of 103 *A. ostoyae* samples collected, only one indicated that genetic variation within an *A. ostoyae* genet existed. Center E, which was approximately 5.41 ha in size, was occupied by two distinct genotypes (Genotypes Ost-IV and Ost-V). The high similarity between RAPD genotypes, the intermediate somatic incompatibility reactions, and the location of the samples in relation to each other indicated that Genotype Ost-IV had arisen by a somatic mutation from Genotype Ost-V. Genotypes Ost-IV and Ost-V were

distinguished from each other by only three of the 12 primers applied in this study. Their respective RAPD banding patterns differed by a total of three markers (one from each primer), and in all cases, the marker was absent in Genotype Ost-IV but present in Genotype Ost-V. It was assumed that these genetic differences originated during the period of time the genet existed in nature, not during the *in vitro* culturing of the individual isolates.

Establishment of New Genets

The initiation of new *Armillaria* genets via basidiospores has been investigated in field experiments by Kile (1983) and Rishbeth (1985). All inoculations of stumps with basidiospores by Kile (1983) failed to produce viable mycelia. Rishbeth (1985) performed similar inoculations and reported that occasionally the top 3 – 5 cm of the stumps became somewhat colonized. All attempts to infect large pruning wounds on trees failed, even near ground level. Although no studies to date have confidently demonstrated that basidiospores are capable of establishing new genets, long distance dispersal via basidiospores is theoretically necessary in order to explain the present distribution of the pathogen and the genetic variation found within populations. Every distinct genet represents a sexual mating event between two basidiospores and the subsequent infection of available substrate. Although the initiation of new genets occurs at an undetectably low frequency, the important role basidiospores play in the spread of this pathogen cannot be ignored.

In populations that reproduce sexually (or have in the recent past), individuals are expected to share approximately half of their RAPD markers resulting in similarity coefficient values of approximately 0.500. The average similarity coefficient between genotypes was 0.521 for *A. ostoyae* and 0.518 for *A. sinapina*. These intermediate levels of genetic similarity are consistent with genetic recombination resulting from sexual reproduction. Also, the spatial distribution of distinct genotypes reported in this study is further indirect evidence of independent infection via basidiospores. Such a large number of different genets separated by substantial distances can only by explained by basidiospore dispersal followed by successful substrate infection.

Population Structures of *Armillaria ostoyae* and *Armillaria sinapina*

This study demonstrated that the population structure of *A. sinapina* within the Gavin Lake study site was significantly different from that of *A. ostoyae*. When the distribution of species and genets were determined, some major differences between *A. sinapina* and *A. ostoyae* were apparent:

(1) *Size of genets*. For *A. sinapina*, the greatest distance between two isolates of the same genet was approximately 100 meters (in Center F), and 77.8 % of the genets were so small that only one isolate was recovered from them. The fact that the same *A. sinapina* genet was never sampled from more than one infection center was a reflection of small genet sizes also. For *A. ostoyae*, the greatest distance between two isolates of the same genet was considerably greater at 580 m. The genets of *A. ostoyae* were usually quite large (up to 15 hectares) and therefore sampled multiple times, occasionally from more than one infection center.

(2) *Number of genets*. A total of 103 *A. ostoyae* isolates were collected and only six genets were detected, while 18 genets were detected from only 25 *A. sinapina* samples. Therefore, only 19.5 % of the collected isolates were *A. sinapina*, but 75 % of the detected genets belonged to that species.

(3) *Distribution of genets*. All six centers that were occupied by *A. sinapina* contained a minimum of two *A. sinapina* genets. Multiple genets of *A. sinapina* within an infection center was presumably a result of successive infections via basidiospores. In contrast, each center that was occupied by *A. ostoyae* contained only a single genet of that species. When the two species were considered together, only Centers B, E, and G were occupied by single genets/ramets (Table 8). The mean number of individual *Armillaria* mycelia per infection center, regardless of species, was 2.89.

(4) *Genetic variability within study site*. When comparing the number of isolates per species to the number of genets per species, it was evident that *A. sinapina* displayed higher levels of genetic variability than *A. ostoyae* within the study site. Also, the IGS-1 rDNA region of *A. sinapina* was more variable than that of *A. ostoyae*. After restriction enzyme digestion, four *A. sinapina* fragment patterns were detected in this population while only one was detected for *A. ostoyae*. White et al (1998) reported a similar discrepancy in the variability of the IGS-1 region and proposed it indicated differences in

mating systems, prevalence of vegetative spread, population processes, or degree of ancestral polymorphism.

Table 8: Number of genets per species and total number of genets per infection center.

Center	No. of *A. ostoyae* genets/ ramets	No. of *A. sinapina* genets	Total no. of genets/ramets
A	1	4	5
B	1	0	1
C	1	3	4
D	1	2	3
E	1	0	1
F	0	5	5
G	1	0	1
H	1	2	3
I	1	2	3

Based on the results of this study, it appears that primary resource capture, which involves the new infection of available substrates via airborne basidiospores, occurs more frequently with *A. sinapina* than with *A. ostoyae*. The larger number of genets and the greater genetic variability of *A. sinapina* within infection centers suggests that dispersal via basidiospores plays a more important role in the epidemiology of *A. sinapina*. The greater variability in the IGS-1 region of *A. sinapina* may also indicate a higher frequency of sexual recombination. However, the extremely small sizes of *A. sinapina* genets suggests that this species has a limited ability to infect new hosts via root contact or rhizomorphs (known as secondary resource capture). In contrast, the small number of *A. ostoyae* genets encountered in the study site suggests that this species initiates new genets via basidiospore infection at a lower frequency than *A. sinapina*. Although new genet initiation via *A. ostoyae* basidiospores is rare, the occurrence of separate genets in distinct infection centers indicates that it happens often enough to propagate the species and maintain genetic variability within the population. The larger size of genets and genetic homogeneity within infection centers suggests that once established, *A. ostoyae* can spread quite aggressively and capture significant amounts of secondary resources.

Interactions Between Species

It is possible that interactions between the two *Armillaria* species in the Gavin Lake study site may have influenced their population structures. For instance, when both species were present in the same infection center, *A. ostoyae* was always the dominant species. This is consistent with *A. ostoyae* being more pathogenic to conifers than *A. sinapina* in the Pacific Northwest. On average, *A. ostoyae* was isolated four times as frequently as *A. sinapina*. As shown in Figures 4 and 8, *A. sinapina* dominated only when *A. ostoyae* was absent from the center completely (Center F). Of the four *A. sinapina* genets which were represented by multiple isolates, the three largest were all sampled from Center F. When *A. ostoyae* was present in a center, all *A. sinapina* genets except one were sampled only once. Perhaps the presence *A. ostoyae* inhibited the growth of *A. sinapina* genets, restricting the *A. sinapina* genets to relatively small sizes. It is unknown whether one species could displace the other from a root system, or whether the occurrence of *A. ostoyae* just prevents further colonization by *A. sinapina*. It is possible that the population structure of *A. sinapina* may be drastically different in regions where competing *Armillaria* species are absent.

When more than one species of *Armillaria* occurs on the same site, differences in average genet size between species have been reported (Legrand et al, 1996; Rishbeth, 1991; Smith et al, 1990). Conversely, Worrall (1994) investigated the population structure of four *Armillaria* in New York forests and found no significant differences between species in size or density of genets. As Legrand et al (1996) suggested, it is possible that two *Armillaria* species have evolved contrasting colonization strategies due to competition for the same woody substrates. Results of studies of the phylogeny of northern hemisphere species of *Armillaria* (Anderson and Stasovski, 1992; Miller et al, 1994) indicated that *A. ostoyae* and *A. sinapina* were amongst the most distantly related pairs of species. These findings are consistent with the genetic and apparent ecological differences observed between species in the present study.

Pathogenicity of *Armillaria sinapina*

The literature regarding *Armillaria* species in British Columbia (Allen et al, 1996; Morrison et al, 1985, 1992) states that *A. sinapina* is weakly pathogenic on deciduous trees and only saprophytic on coniferous trees. This species is usually incapable of

infecting healthy conifers, and it may colonize conifer root systems only after tree death (i.e. conifer stumps). In other regions of North America , *A. sinapina* has been associated mainly with deciduous substrates also (Blodgett and Worrall, 1992; Dumas, 1988; Harrington and Rizzo, 1993; Mallett, 1990; Mugala et al, 1989). However, Banik et al (1996) recently collected this species predominantly from coniferous substrates from the Olympic Peninsula in Washington. In the present study, samples were obtained from coniferous hosts only, and the large number of *A. sinapina* isolates recovered suggested that colonization of conifers by this species is not uncommon. Sampling from stumps did not increase the probability of isolating *A. sinapina* over *A. ostoyae*. A total of 16 isolates were collected from conifer stumps and only seven (43.8 %) were identified as *A. sinapina*. Therefore, only 28 % of *A. sinapina* isolates were collected from stumps, and the rest were sampled from coniferous trees. Several isolates (data not shown) were obtained from the roots of standing conifers which were clearly alive and only in the first stages of infection. Within Center F, *A. sinapina* was capable of colonizing and successfully infecting several coniferous trees. In fact, *A. sinapina* was the only species collected from Center F, so it was presumably responsible for the observed symptoms and mortality. These data indicate that *A. sinapina* in the central Interior of British Columbia may be more pathogenic to coniferous hosts than previously reported.

An unresolved problem remains: If basidiospore dispersal plays a major role in the spread of *A. sinapina*, then why were the *A. sinapina* genets clustered into infection centers at all? It is possible that successful initiation of *A. sinapina* genets has occurred throughout the entire Gavin Lake Block, but they remained undetected because they were not able to cause visible infection centers. Hence, the *A. sinapina* genets may have been detected simply because they were accidentally associated with an infection center already caused by *A. ostoyae*. Another possible explanation is that *A. sinapina* basidiospores can only infect stressed hosts with reduced vigor. Since infection centers caused by *A. ostoyae* create an abundance of such hosts, the probability of successful *A. sinapina* infection may be increased. However, neither of these explanations can account for Center F, the infection center which was occupied exclusively by five genets of *A. sinapina*.

Somatic Incompatibility vs. RAPD Analysis

The results of traditional somatic incompatibility reactions used to differentiate between genets corresponded quite well with the results of RAPD analysis. There was only one discrepancy between the two methods: RAPD analysis was superior when attempting to distinguish between isolates of Genotypes Ost-IV and Ost-V. Somatic incompatibility tests failed to give consistent and conclusive results, whereas RAPD analysis clearly indicated that these two genotypes were different from each other, albeit only slightly. When attempting to distinguish between isolates which differed significantly in genetic composition, both methods were equally consistent and dependable. When the isolates were relatively similar in genetic composition (i.e. somatic mutation), the DNA-based method was more successful at detecting these small differences.

These results are similar to those reported by other authors: Jacobson et al (1993) reported that somatically compatible isolates of *Suillus granulatus* were not necessarily genetically identical, and that RAPD analysis was more reliable and provided higher resolution of genets. Hansen et al (1993) studied the genetic control of somatic incompatibility in the fungal root pathogen *Heterobasidion annosum* (Fr.) Bref. and suggested that is was controlled by three to four multiallelic genes. They also observed that the intensity of the incompatibility reaction between two genets was proportional to the number of genes that had differing alleles. Consequently, the intensity of a somatic incompatibility reaction decreases as the relatedness of the individuals increases. These patterns have been noticed in other root-infecting basidiomycetes (Hansen, 1979; Stenlid, 1985) and other *Armillaria* species as well (Kile, 1983; Korhonen, 1978). When all combinations of diploid siblings were tested, Korhonen (1978) found that only 52% of the combinations gave incompatible results. As well, the parent diploids were compatible with some offspring and incompatible with others. Similarly, Kile (1983) reported that only 44% of pairings of inbred diploid siblings produced incompatible reactions. These analogous patterns of variable reaction intensity suggest that somatic incompatibility in all these genera is controlled in a similar fashion. Although it has limitations, somatic incompatibility remains a quick and efficient technique for genet differentiation.

Chapter III:

The Population Structure of *Armillaria ostoyae* in the Southern Interior of British Columbia

INTRODUCTION

The fungus *Armillaria ostoyae* (Romagn.) Herink causes significant losses to the commercial forest industry in British Columbia. Annual timber volume losses to *Armillaria* root disease have been estimated at two to three million cubic meters, with a large proportion of this damage occurring within the southern Interior regions of the province (Morrison and Mallett, 1996; Morrison et al, 1992). The disease causes tree mortality, diminished tree growth, and predisposition to windthrow, thereby reducing commercial timber production.

The Interior Cedar Hemlock (ICH) biogeoclimatic zone commonly experiences high levels of infection by *A. ostoyae.* Recent surveys (Morrison, unpublished) have demonstrated that up to 80 % of the root systems within a stand may be infected. In mature undisturbed stands, the host and fungus are usually in equilibrium: the hosts are growing vigorously enough to prevent the advance of the fungus, but not vigorously enough to eliminate the fungus from the root lesions entirely. Therefore, stands with high levels of infection may experience low levels of mortality. This delicate balance between host vigor and inoculum potential of the fungus can be upset by any disturbance which stresses host trees or causes mortality. Most forest management practices which involve the harvesting of timber (i.e. clearcutting, commercial thinning) may tip the scales in favor of *A. ostoyae* (Morrison et al, 1992). For instance, when an infected tree is felled during a clearcutting operation, the fungus may overcome the host's defenses and the remaining stump and root system is easily colonized. This woody tissue is degraded and utilized as a food base for the pathogen, allowing for increased inoculum levels. Any regenerating hosts whose root systems are in contact with the infected stump or associated rhizomorphs have a high probability of becoming infected.

In the southern Interior, mortality caused by *A. ostoyae* begins to appear within four to seven years after planting and may continue to stand maturity (Morrison and Mallett, 1996; Morrison et al, 1992). If sites with high levels of infection are harvested and planted with susceptible regeneration, the next rotation may experience up to 100 % infection and a devastatingly high levels of mortality. As Morrison and Mallett (1996) state, "The incidence of infected and killed trees is up to several times greater in disturbed than in undisturbed stands." As British Columbia's forests become more intensively managed for commercial timber production, the detrimental impact of *Armillaria* root disease will increase accordingly.

The population structure of *Armillaria* root disease in many regions of North America and Europe have been studied by several authors (Adams, 1974; Anderson et al, 1979; Kile, 1983, 1986; Klein-Gebbinck et al, 1991; Korhonen, 1978; Legrand et al, 1996; Rishbeth, 1978, 1991; Rizzo et al, 1995; Saville et al, 1996; Schulze et al, 1997; Shaw and Roth, 1976; Smith et al, 1990, 1992, 1994; Worrall, 1994). Distinguishing between individual *Armillaria* genets has been accomplished using several techniques: (1) somatic incompatibility, (2) mating-type alleles, (3) isozymes, (4) mtDNA RFLP's, and (5) RAPD's (see Chapter II, Introduction for summary). While studying *Armillaria* root disease in the central Interior for the first half of this thesis, two methods for distinguishing genets were employed: the traditional technique of somatic incompatibility and the molecular technique of Random Amplified Polymorphic DNA (RAPD) analysis. The results of these two techniques corresponded exactly, barring one exception: RAPD analysis was superior to somatic incompatibility in detecting slight genetic variation due to somatic mutation. This conclusion agrees with other comparative studies (Guillaumin et al, 1996; Jacobson et al, 1993; Rizzo et al, 1995) which suggest that techniques using molecular markers may be superior when attempting to distinguish closely related individuals.

The objective of this study was to investigate the population structure of *A. ostoyae* in selected plantations within the Interior Cedar Hemlock (ICH) biogeoclimatic zone of British Columbia's southern Interior. The goal was to determine the spatial distribution of genets throughout the sampled population, and to what extent the sexual and asexual components of *A. ostoyae*'s life cycle might affect this distribution. The

determination of patterns of genetic variability could indicate how important sexual reproduction and vegetative spread are to the epidemiology of *Armillaria* root disease. The present distribution of mortality within individual plantations in ICH does not suggest any obvious population structure. Unlike the Gavin Lake study site in the central Interior of B.C. (Chapter II), *Armillaria* root disease is not found in distinct infection centers surrounded by large areas of asymptomatic forest. Rather, mortality is distributed somewhat evenly throughout the forest region, with asymptomatic trees interspersed with symptomatic trees. The only apparent grouping of infected hosts is on a much smaller scale: patches of one to several adjacent symptomatic trees (called infection foci) may occur throughout the forest (van der Kamp, 1995). Since several different sized patches of mortality may be situated relatively close to each other, the delineation of individual infection foci is relatively arbitrary. It is possible that this pattern of infection represents many small *Armillaria* genets with contacting boundaries that have expanded into each other over time, or a few very large genets that have become fragmented into multiple ramets. Specifically, two questions regarding the population structure were posed: (1) What is the average size of a genet's domain? (2) Are individual infection foci occupied by single or multiple genets? To answer these questions, eight plantations which displayed typical symptoms and levels of infection by *Armillaria* root disease in the ICH zone of British Columbia's southern Interior were examined.

MATERIALS AND METHODS

Sampling

Samples for this study were collected within the Vernon Forest District in the Hidden Lake region (50°32'N, 118°49'W) which is situated approximately 25 km east of Enderby, British Columbia. The study area was located in the southern Interior of the province within the mw subzone of the Interior Cedar Hemlock (ICH) biogeoclimatic zone (Meidinger and Pojar, 1991). The distribution of mortality throughout plantations in this study site did not suggest any obvious population structure: observed mortality could not be allocated into putative infection centers. Since the pattern of infection was so random, the locations and boundaries of possible genets could not be predicted by inspection of

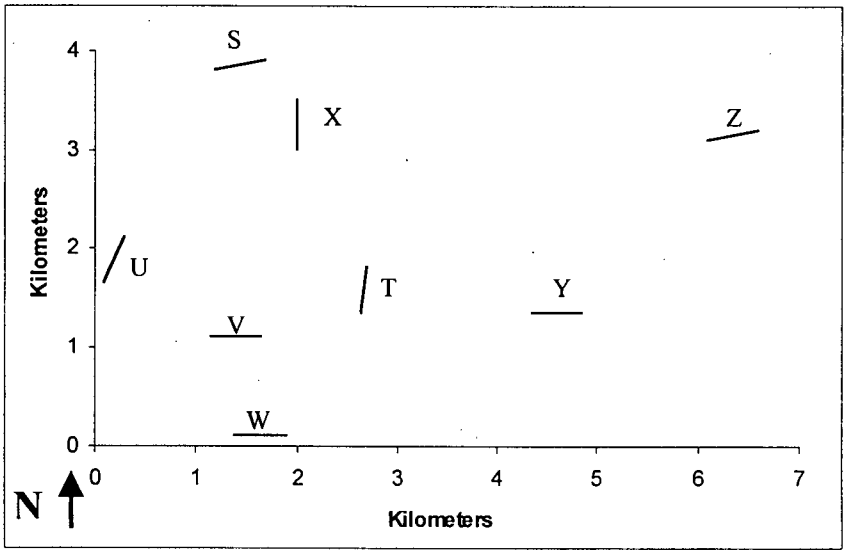
transects with systematically placed collection points was used to gather the samples for this study.

Eight conifer plantations within the study area which exhibited typical symptoms and levels of infection were chosen and a 500 m long transect was randomly laid through each of them (Transects S – Z, Figure 11). In order to avoid sampling the same genet in two different transects, the minimum distance between any two transects was 500 m. The plantations were initiated between 10 and 20 years ago and all were planted with *Pseudotsuga mensiesii* (Mirb.) Franco seedlings except one: Transect S was located in a *Picea engelmannii* Parry. plantation.

For Transects S and T, collection points were placed along the transect at 25 m intervals, and at 50 m intervals for all other transects. The closest symptomatic tree to each collection point was chosen for sampling, with a maximum perpendicular offset of 20 m (i.e. the sample could be no more than 20 m from the transect line). No limit was set for the maximum distance a host could be from the sample point along the transect. As long as the infected host was encountered before the adjacent sample points were, it could be sampled. Most plantations had a relatively high prevalence of infection, so a host displaying obvious symptoms was usually found every 15 to 20 m. Occasionally, an appropriate host could not be located and the sample point was omitted. Multiple-collection points were placed along the transects to investigate the composition of individual infection foci: when a focus with several recently infected hosts was encountered at a sample point, an additional three samples were collected. Therefore, multiple-collection points were not designated prior to sampling.

Infected root segments approximately 20 cm in length were gathered and within 12 hrs from the time of sampling, a segment of a mycelial fan was aseptically removed and placed on 2 % malt extract agar. In order to isolate the mycelium most likely to have caused the host's symptoms or mortality, all cultures were isolated from mycelial fans beneath the bark of the root. Cultures were incubated in the dark at room temperature (20-23 °C) and subcultured as necessary.

Figure 11: Diagram of Hidden Lake study site showing location and orientation of eight transects.



DNA Extraction

Isolates were prepared as described in Chapter II, and the protocol listed in Appendix A (modified version of Doyle and Dickson, 1987) was used for the extraction of DNA from the fungal isolates.

Biological Species Identification

The PCR-based species identification method (Anderson and Stasovski, 1992; Harrington and Wingfield, 1995; White et al., 1998) described in Chapter II was used to determine the species of each *Armillaria* isolate.

Random Amplified Polymorphic DNA (RAPD) Analysis

To delineate genets of *A. ostoyae* in the Hidden Lake study site, the molecular technique of RAPD analysis was used. Since 12 decamer primers utilized in Chapter II (JD01, JD02, JD03, JD04, JD05, JD06, JD07, JD08, JD10, JD11, JD12, JD13) were successful at detecting polymorphisms in *Armillaria* spp. isolates collected from the Gavin Lake study site, RAPD analysis was performed using these primers and the protocols described in Chapter II.

Ramets vs. Genets

When studying fungal population structures, using appropriate terms to describe the individual units of a fungal population is essential (Anderson and Kohn, 1995; Rayner, 1991). In this study, the term "genet" is defined as all the mycelium produced by vegetative means following an initial sexual mating event. Since a genet is a mitotic cell lineage, all isolates collected from the same genet should have the same genotype (in the absence of mitotic recombination or somatic mutation). Therefore, a genet is the *genetic* unit of a fungal population. The definition of a genet does not denote spatial contiguity of all the isolates: it is possible for a genet to be fragmented into several spatially distinct patches, referred to as "ramets". Since each ramet is an individual mycelium which occupies a discrete territory and can act independently from others, it is the *physical* unit of a fungal population. Hence, a spatially contiguous genet is composed of a single ramet, while a fragmented genet is composed of multiple ramets. In the following discussion, the terms "genet" and "ramet" are used according to the above definitions.

A limitation to sampling with linear transects as opposed to grids or quadrats is that measurements of individual ramet sizes can be collected in only one plane, therefore the true dimensions of ramets can never be determined. Also, not every infected host along the transect was sampled, so exact boundaries of genets could not be determined either. Along the length of the transect, it is possible for multiple isolates of the same genotype to be separated by isolates belonging to a different genotype. This non-contiguous distribution of isolates may represent either:

(A) Multiple discrete ramets. Each domain along the transect may be occupied by a separate individual mycelium, each a fragment of a previously larger genet.

(B) A single large genet. This genet may have other smaller genets established within its territory, or else the domains along the transect may represent four lobes of an extremely irregularly-shaped genet.

Depending on which assumption is taken, different calculations would follow. If the scenario A is assumed, then the areas of *ramet* domains could be estimated; if scenario B is assumed, then the areas of *genet* domains could be estimated.

Calculation of Ramet (or Genet) Domain Areas

The length of transect occupied by each ramet was calculated by the following manner: If two adjacent samples belonged to different ramets, the midpoint along the transect between the isolates was determined; this was calculated for all such pairs of isolates. The length of transect occupied by each ramet was the distance between the midpoints of itself and the two flanking ramets. For example, the distance occupied by the single ramet of Genet S-6 was measured from (A) the midpoint of Genet S-5 and the west sample of Genet S-6, to (B) the midpoint of east sample of Genet S-6 and west sample of Genet S-7 (Figure 14). An implicit assumption in these calculations was that the entire length of transect between two samples, from the same ramet or not, was occupied. For ramets which occur at the ends of transects, the domain boundary was an equal distance away from the terminal sample as was the midpoint between the last two samples on the transect. For example, the distance between the two eastern most samples of Genet S-7 was 30 m, so the domain boundary extended 15 m east of the terminal sample (Figure 14). In this study, the distance of transect occupied by a ramet was assumed to approximate the average length of that ramet. But when the term "length" is used, it does not imply that it is the largest dimension of a ramet or genet. Rather, "length" simply refers to the distance of transect which intersected the particular ramet or genet. Likewise, the term "width" simply describes the dimension of a ramet which is perpendicular to the ramet "length".

In areas such as the Hidden Lake study site where the distribution of genets is complex (i.e. small genets in high densities), the domains of ramets or genets are rarely spherical or symmetrical (Legrand et al, 1996; Rizzo et al, 1995; Smith et al, 1994; Worrall, 1994). In the present study, individual genets have likely had ample time to expand and confront each other, so unimpeded vegetative growth in all directions is not probable. Therefore, the lengths of ramets do not equal their widths. But if ramet lengths are collected for a large number of individuals in the population, the frequency distribution for different ramet lengths can be calculated. Since the transects were oriented randomly through the plantations, the ramet length frequency distribution should approximate the ramet width frequency distribution for the population. This assumption holds true unless the widths of ramets are dependent upon their lengths, i.e. longer ramets

have smaller widths. Based on these two distributions, the probable frequencies of various ramet length X ramet width combinations was then be determined for the population. The estimated area of a ramet's domain was therefore equivalent to the length multiplied by the width of the genet. Genet areas were calculated in a similar fashion.

RESULTS

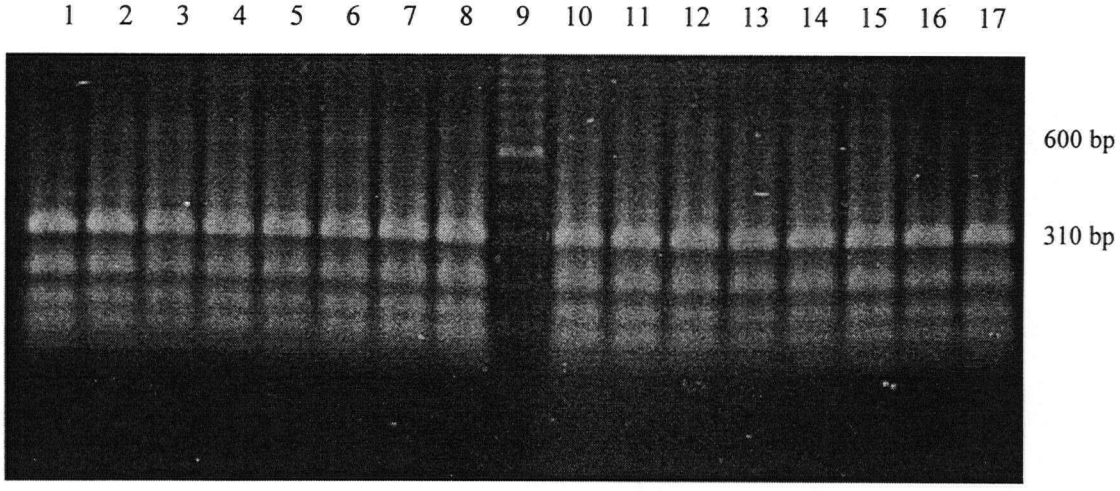
Sampling

While attempting to obtain pure cultures, several isolates became contaminated and were lost leaving a total of 122 viable fungal isolates. The number of remaining viable isolates collected from each transect ranged from 12 to 21, with an mean of 15.25 isolates per transect (Table 9).

Biological Species Identification

For all 122 isolates, amplification and restriction enzyme digestion resulted in products similar in size to those reported for *A. ostoyae* by White et al. (1998). The same *A. ostoyae* fragment pattern (Pattern 1) was produced by all isolates (Figure 12), and the second rare *A. ostoyae* fragment pattern described by White et al. (1998) was not detected.

Figure 12: IGS-1/Alu I restriction fragment patterns for *A. ostoyae*. Lanes 1 – 17: Genets S-1, S-2, T-1, T-5, U-2, U-3, V-1, V-2, DNA Ladder, Genets V-3, V-4, V-5, W-1, W-2, W-3, W-4, W-5.



Primer Screening

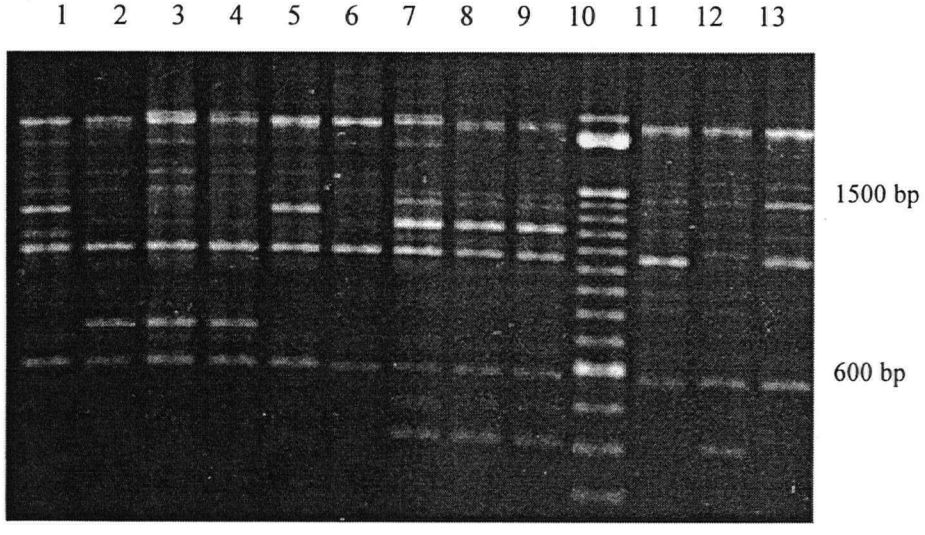
To verify that the 12 primers could be applied in RAPD analysis to *A. ostoyae* sampled from southern Interior, several isolates from various transects were used to screen for polymorphisms. Products of sufficient quality for RAPD analysis were produced by all primers except primer JD10, which yielded extremely faint and unscorable products. Primer JD11 yielded lower quality products when used with isolates from certain transects but overall, primer JD11 was still acceptable. Therefore, a total of 11 primers were used to study the *A. ostoyae* isolates collected from the plantations in the Hidden Lake study site.

Within-Transect Population Structure

Determination of Number of Genets Along Transects

The first goal was to determine how many different genets were represented by the *A. ostoyae* isolates sampled along each transect. As demonstrated in the Gavin Lake study site (Chapter II), RAPD analysis using seven primers yielded ample degrees of confidence in genet differentiation: the probabilities of the same RAPD genotype arising more than once by chance alone were negligible. Therefore, only six primers (JD01, JD02, JD03, JD05, JD06, and JD07) were chosen for within-transect genet determination in this portion of the project. For all isolates of each transect, the six primers were used

Figure 13: *A. ostoyae* RAPD-PCR products amplified by primer JD01. Lanes 1 – 13: Genets X-1, X-2, X-3, X-3, X-4, X-6, X-5, X-5, X-5, DNA Ladder, Genets X-6, X-7, X-6.



in RAPD-PCR reactions (Figure 13) and the data were collected in a binomial data matrix. Note that this portion of the study focused exclusively on genetic variation within transects, so isolates were compared to only those other isolates collected along the same transect. Genetic variation between isolates collected from different transects will be investigated in a following section of this thesis.

The results of within-transect genet determination are summarized in Table 9 and shown in Figure 14. Since each transect was occupied by different genets, the number of polymorphic markers produced by the six primers varied between transects. The average number of polymorphic markers used to delineate genets along a transect was 21.13 markers per transect. Consistently scorable markers ranged in size from 330 to 2200 bp (Appendix F), and the mean number of polymorphic markers produced per primer was only 3.52. Genets were named after their respective transects in the order they were encountered: the first from Transect S was named Genet S-1, the second was named Genet S-2, and so on. An average of 6.5 genets were detected per transect, with a cumulative total of 52 genets for all transects. Of these 52 genets, 22 (42.3 %) were represented by a single isolate only, which suggested the average genet size in this region may be relatively small. The maximum number of isolates per genet was eight, with an mean of only 2.35 isolates per genet.

Table 9: Number of isolates, markers, polymorphic markers, and genets per transect.

Transect	No. of isolates	No. of markers	No. of poly-morphic markers	No. of genets
S	21	39	30	7
T	20	23	13	7
U	13	44	29	4
V	13	34	22	6
W	14	37	25	8
X	12	30	14	7
Y	14	31	19	7
Z	15	33	17	6
Mean	15.25	33.88	21.13	6.5

Figure 14: Diagrams of eight transects showing the spatial distribution of the *A. ostoyae* genets.

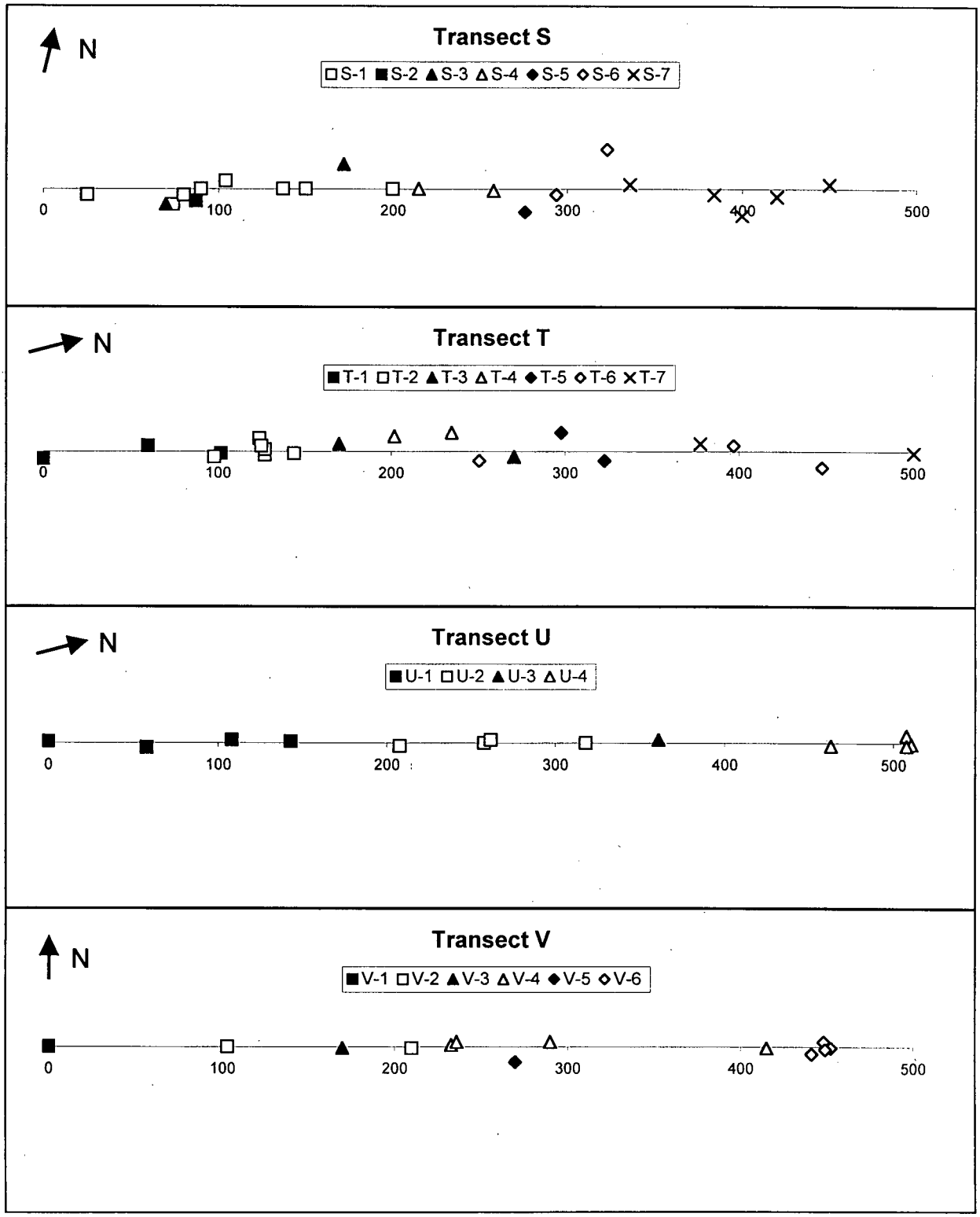
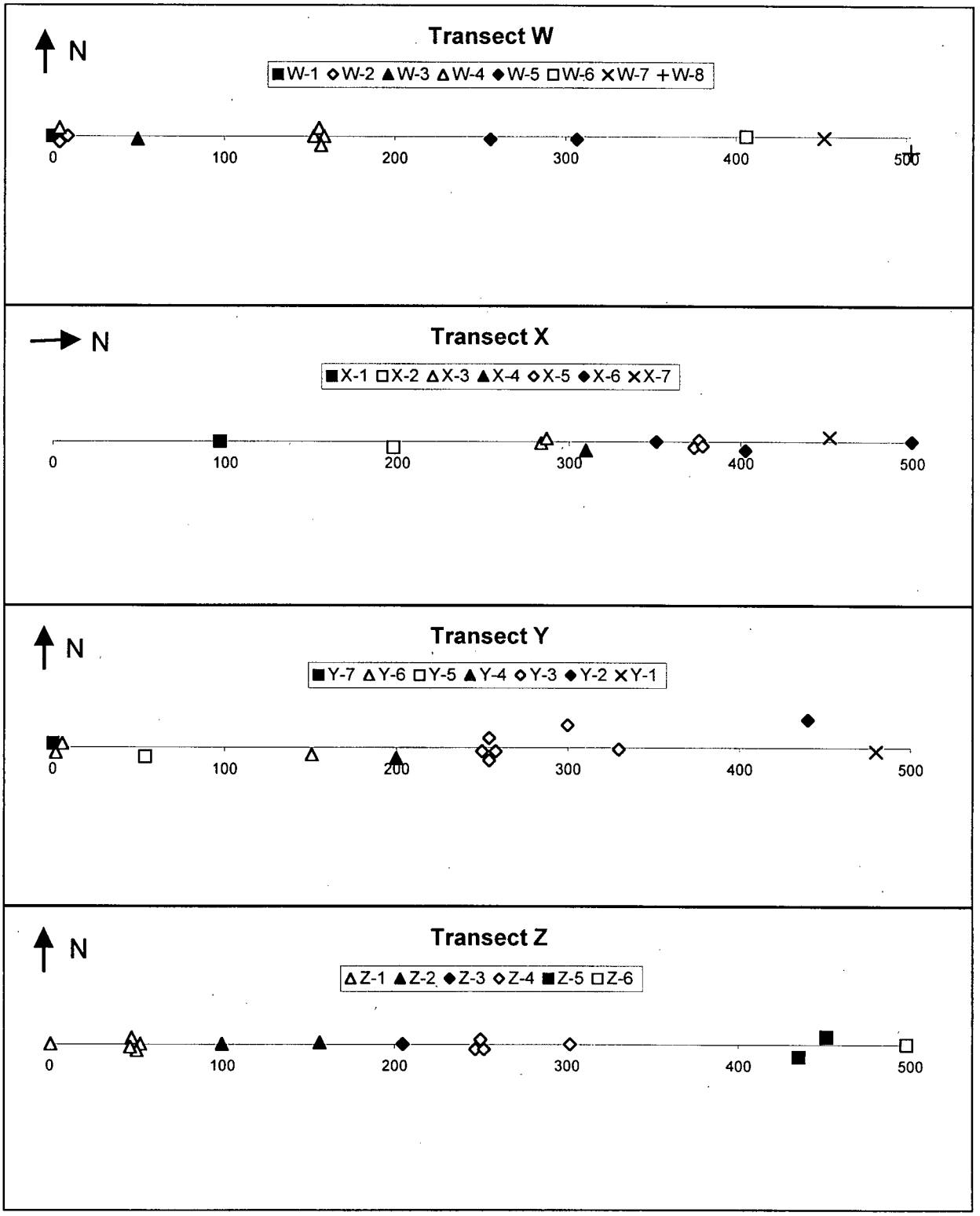


Figure 14 continued…



To estimate the probabilities of identical RAPD genotypes arising independently via random sexual recombination, the methods described in Appendix B were utilized. Calculated probability values for the 30 genets which were represented by multiple isolates ranged from 4.62×10^{-53} to 0.0015, so the probability of identical RAPD genotypes arising via random sexual recombination was negligible. The extremely low probabilities of finding several identical isolates and the spatial clustering of the isolates confirmed the assumption that isolates which displayed the same RAPD genotype belonged to the same genet and were derived via vegetative growth from a single initial mating event.

Of the 30 genets which were isolated multiple times, only 18 (60 %) occupied a contiguous domain along the transect. For example, the five isolates of Genet S-7 were contiguous for they were not separated by isolates from another genet and they occupied an uninterrupted segment of the transect. Conversely, the eight isolates of Genet S-1 were found divided into four different domains along the transect by isolates of other genets (Figure 14), and were therefore non-contiguous. As discussed earlier, this non-contiguous distribution of isolates may represent either: (A) multiple discrete ramets or (B) a single large genet. Depending on which scenario is assumed, different calculations would follow.

Areas of Ramet Domains

If scenario A is assumed, then non-contiguous isolates with the same genotype represented a genet which became fragmented into several smaller ramets and areas of *ramet* domains could be estimated. Therefore, each genetic unit of the population (genet) may be represented by several physical units (ramets). As the data in Table 10 indicates, 12 of the 52 (23 %) genets consisted of multiple ramets, with up to four ramets per genet.

The total number of *A. ostoyae* ramets detected along the eight transects was 67. Since a ramet is composed of an individual mycelium which may act independently from the other ramets, the following calculations considered each ramet as a separate entity which was weighted equally. Although a transect was 500 m in length, the actual cumulative length occupied by ramets was not necessarily 500 m. At the end of the transect, it was possible for the domain of the terminal ramet to extend beyond or fall

short of the 0 or 500 m mark. The mean cumulative length of occupied transect was 514.44 m per transect. The lengths of the 67 individual ramets ranged from 2.5 m to 204.5 m, with an overall mean of 61.43 m. The frequency distribution of ramets per length class is shown in Figure 15.

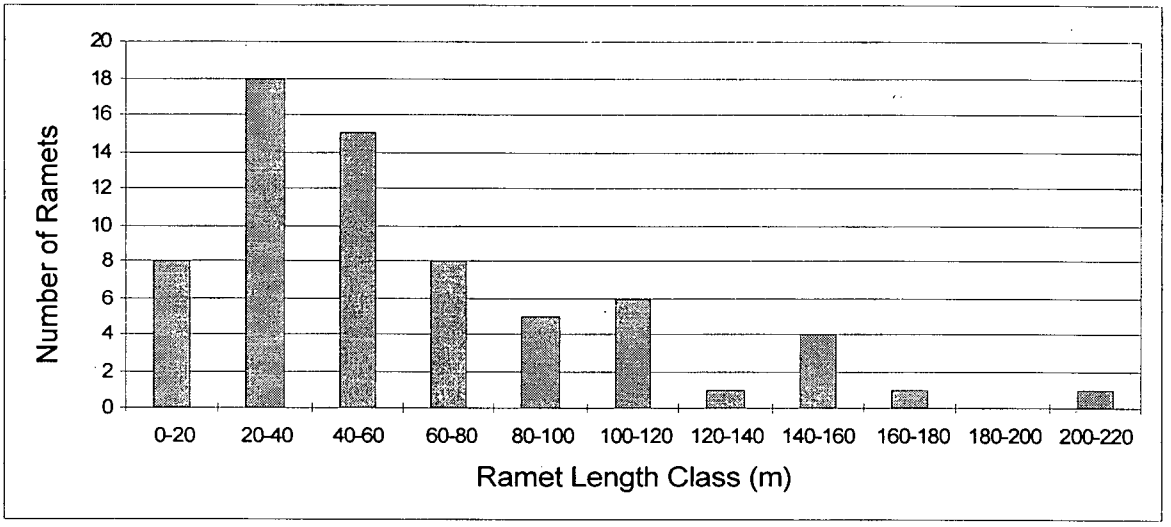
Table 10: Number of isolates and ramets per genet.

Genet	No. isolates per genet	No. ramets per genet	Genet	No. isolates per genet	No. ramets per genet
S-1	8	4	W-1	1	1
S-2	1	1	W-2	2	1
S-3	2	2	W-3	1	1
S-4	2	1	W-4	5	2
S-5	1	1	W-5	2	1
S-6	2	1	W-6	1	1
S-7	5	1	W-7	1	1
			W-8	1	1
T-1	3	2	X-1	1	1
T-2	6	2	X-2	1	1
T-3	2	2	X-3	2	1
T-4	2	1	X-4	1	1
T-5	2	1	X-5	3	1
T-6	3	2	X-6	3	3
T-7	2	2	X-7	1	1
U-1	4	1	Y-1	1	1
U-2	4	1	Y-2	1	1
U-3	1	1	Y-3	6	1
U-4	4	1	Y-4	1	1
			Y-5	1	1
			Y-6	3	2
			Y-7	1	1
V-1	1	1	Z-1	5	1
V-2	2	2	Z-2	2	1
V-3	1	1	Z-3	1	1
V-4	4	2	Z-4	4	1
V-5	1	1	Z-5	2	1
V-6	4	1	Z-6	1	1

Table 11: Number of ramets and average ramet length per transect.

Transect	No. of ramets	Average ramet length (m)
S	11	42.1
T	12	46.5
U	4	135.1
V	8	62.8
W	9	58.9
X	9	53.1
Y	8	62.7
Z	6	90.6
Overall Mean	8.38	61.43

Figure 15: Frequency distribution of the number of ramets within length classes.



To estimate ramet areas, the 67 ramet lengths were grouped into length classes with intervals of 50 m, and the cumulative frequency and the average ramet length was calculated for each class. As Table 12 shows, the majority of ramets were between 0 and 50 m in length and the cumulative frequency decreased with increased ramet length. The probable frequencies of all 25 possible ramet length X width combinations were calculated, and the areas of ramets of particular sizes were calculated using the average ramet lengths/widths of the respective length/width classes. For example (see Table 12),

Table 12: Summary of length/width classes used for ramet area calculations.

Ramet length/ width class (m)	Number of ramets	Frequency	Average ramet length/width (m)
0-50	34	0.51	28.3
50-100	20	0.30	71.1
100-150	10	0.15	120.5
150-200	2	0.03	161.0
200-250	1	0.01	204.5

a ramet with a length of 0 – 50 m and a width of 50 – 100 m would occur at a probable frequency of 0.15 (= 0.51 * 0.30), and the average area of such a ramet would be 2012.14 m^2 (= 28.32 m* 71.05 m). The resulting ramet areas were grouped into classes and the cumulative frequencies of each area class was converted into a percentage (Table 13).

Table 13: The percentages of ramets within various area classes.

Ramet area class (m^2)	Percentage of ramets
0-5000	74.23
5000-10000	19.34
10000-15000	4.90
15000-25000	1.33
25000-45000	0.20

Note that only the probable frequencies of particular ramet domain areas occurring within the population can be determined; domain areas of specific ramets cannot be calculated because measurements of individual ramets were only collected in one plane. Based upon the ramet area calculations employed in this study, 93.57 % of *A. ostoyae* ramets in this population occupied a domain less than 10,000 m^2 (1 hectare) in area, and the mean area occupied by a ramet was 3773.64 m^2. Thus, each hectare of plantation contained an average of 2.65 ramets of *A. ostoyae*.

Area of Genet Domains

If scenario B is assumed, then non-contiguous isolates with the same genotype represented a single unfragmented genet and areas of *genet* domains could be estimated. Therefore, each genetic unit of the population (genet) was represented by a single physical unit (ramet). Based on the distribution of isolates sampled in this study, the domains of different genets must overlap spatially. Some small genets may occupy a

territory within the territory of another (Genets S-2 within Genet S-1), or the edges of genets may just intermingle (Genet T-1 and T-2). Since the same region of transect may be occupied by multiple genets, that particular length of transect may be counted multiple times. The length of genets may be conservatively estimated by the maximum distance between two isolates of that genet, but since 42.3 % of the genets were sampled only once, this method was not applicable. Therefore, genet lengths were calculated using the same method as described for ramet lengths. Calculated lengths of the 52 genets ranged from 3 m to 231.5 m, with an overall mean of 94.37 m (Table 14). The frequency distribution of genets per length class is shown in Figure 16.

Table 14: Number of genets and average genet length per transect.

Transect	No. of genets	Average genet length (m)
S	7	86.6
T	7	125.7
U	4	135.1
V	6	97.1
W	8	78.4
X	7	79.0
Y	7	82.0
Z	6	90.6
Overall Mean	6.5	94.37

Figure 16: Frequency distribution of the number of genets within length classes.

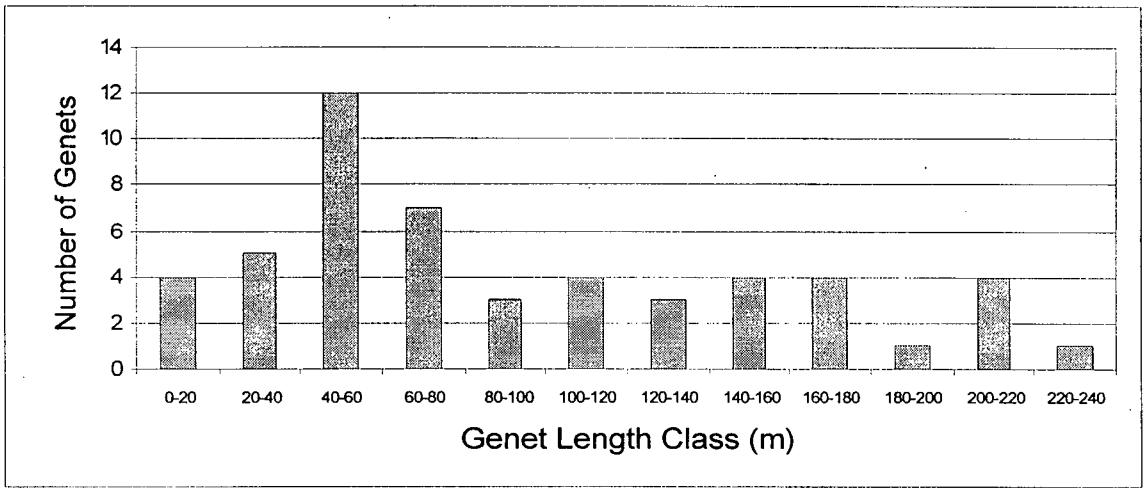


Table 15: Summary of length/width classes used for genet area calculations.

Genet length/ width class (m)	Number of genets	Frequency	Average genet length/width (m)
0-50	15	0.29	29.2
50-100	16	0.31	70.5
100-150	10	0.19	125.2
150-200	6	0.11	172.9
200-250	5	0.10	210.3

Table 16: The percentages of genets within various area classes.

Genet area class (m^2)	Percentage of genets
0-5000	46.63
5000-10000	24.04
10000-15000	13.02
15000-20000	3.70
20000-25000	4.44
25000-30000	5.03
30000-45000	3.14

The results of estimated genet area calculations are summarized in Tables 15 and 16. Again, note that only the probable frequencies of particular genet domain areas occurring within the population can be determined. Based on the above estimates, 70.67 % of *A. ostoyae* genets in this population occupied a domain less than 10,000 m^2 (1 hectare) in area, and the mean area of a genet's domain was 8905.70 m^2.

Genetic Variability of Genets Within Transects

The confidence of the estimated genetic relatedness between genets increases as the number of markers used to calculate the similarity coefficient increases. For this reason, further RAPD analysis using the remaining five primers (JD04, JD08, JD11, JD12, and JD13) was conducted using representative isolates of each genet (Appendix G). To assess the genetic variability of the *A. ostoyae* genets along each transect, Dice's similarity coefficients were calculated for pairwise genet combinations based on the RAPD data produced by all 11 primers combined. The similarity coefficient matrices for the transects are tabulated in Appendix H and are summarized in Table 17.

Figure 17: UPGMA dendrograms for the genets sampled from eight transects. Bar indicates phenetic distance (1 – S) and branch termini indicate genet.

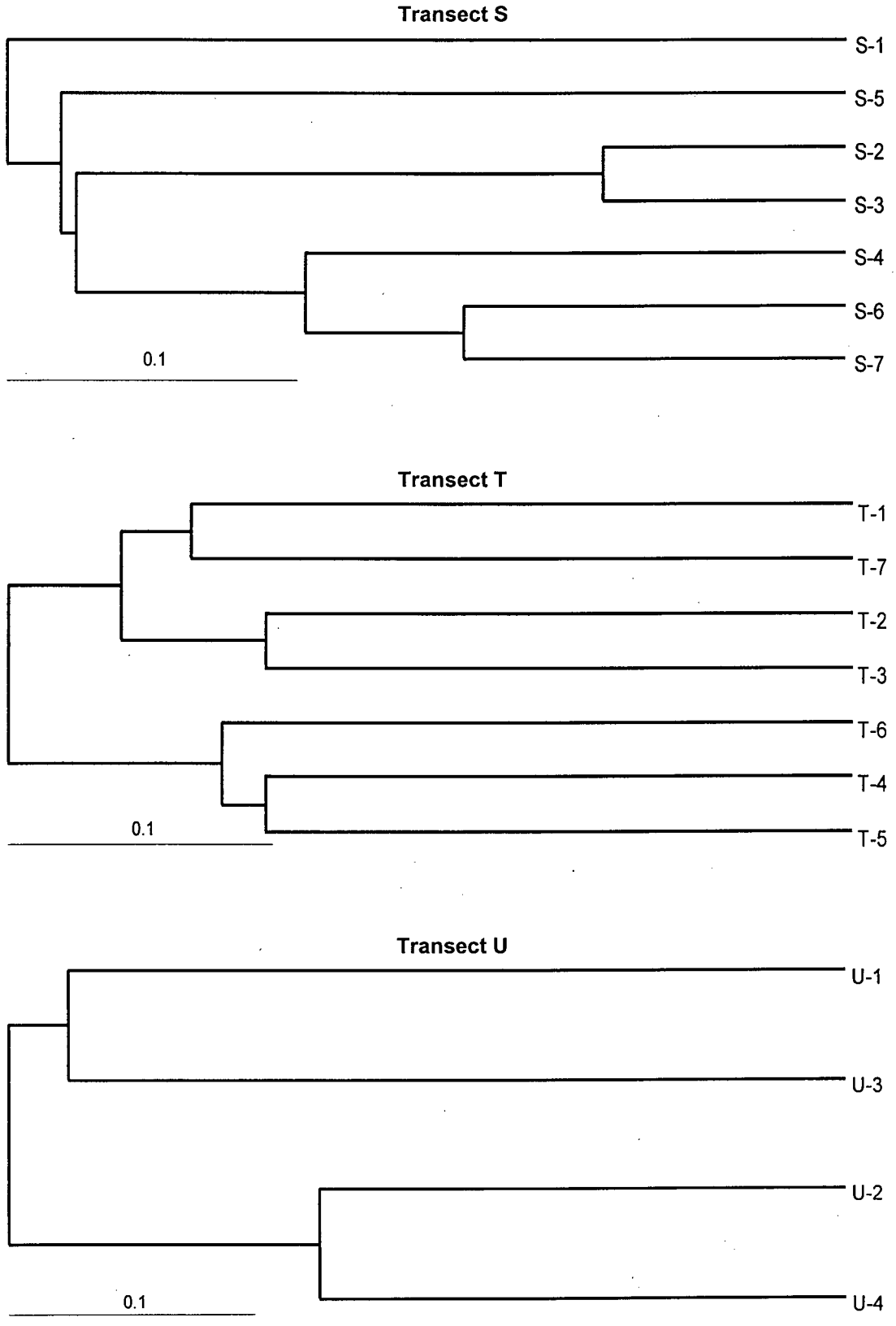


Figure 17 continued…

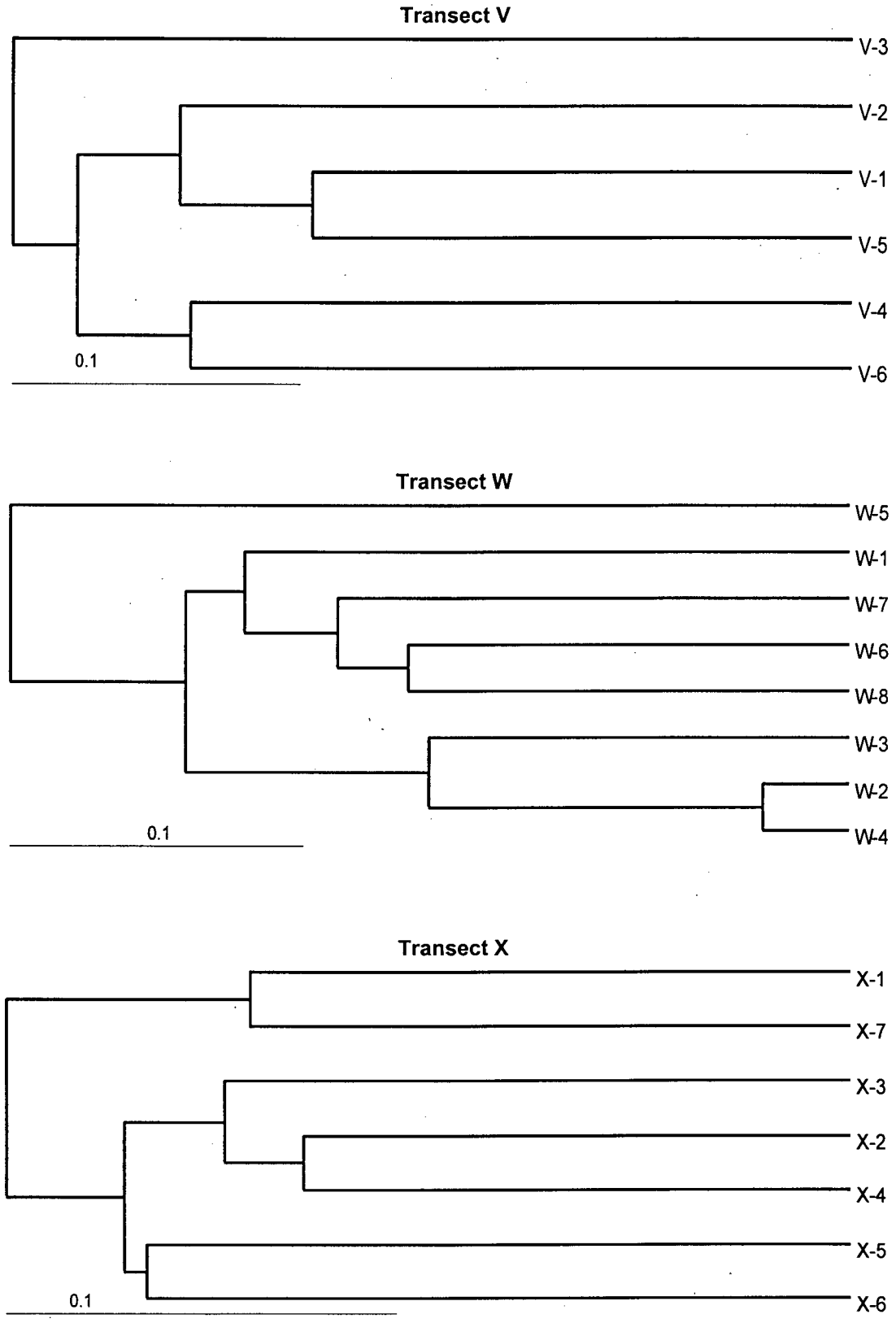


Figure 17 continued…

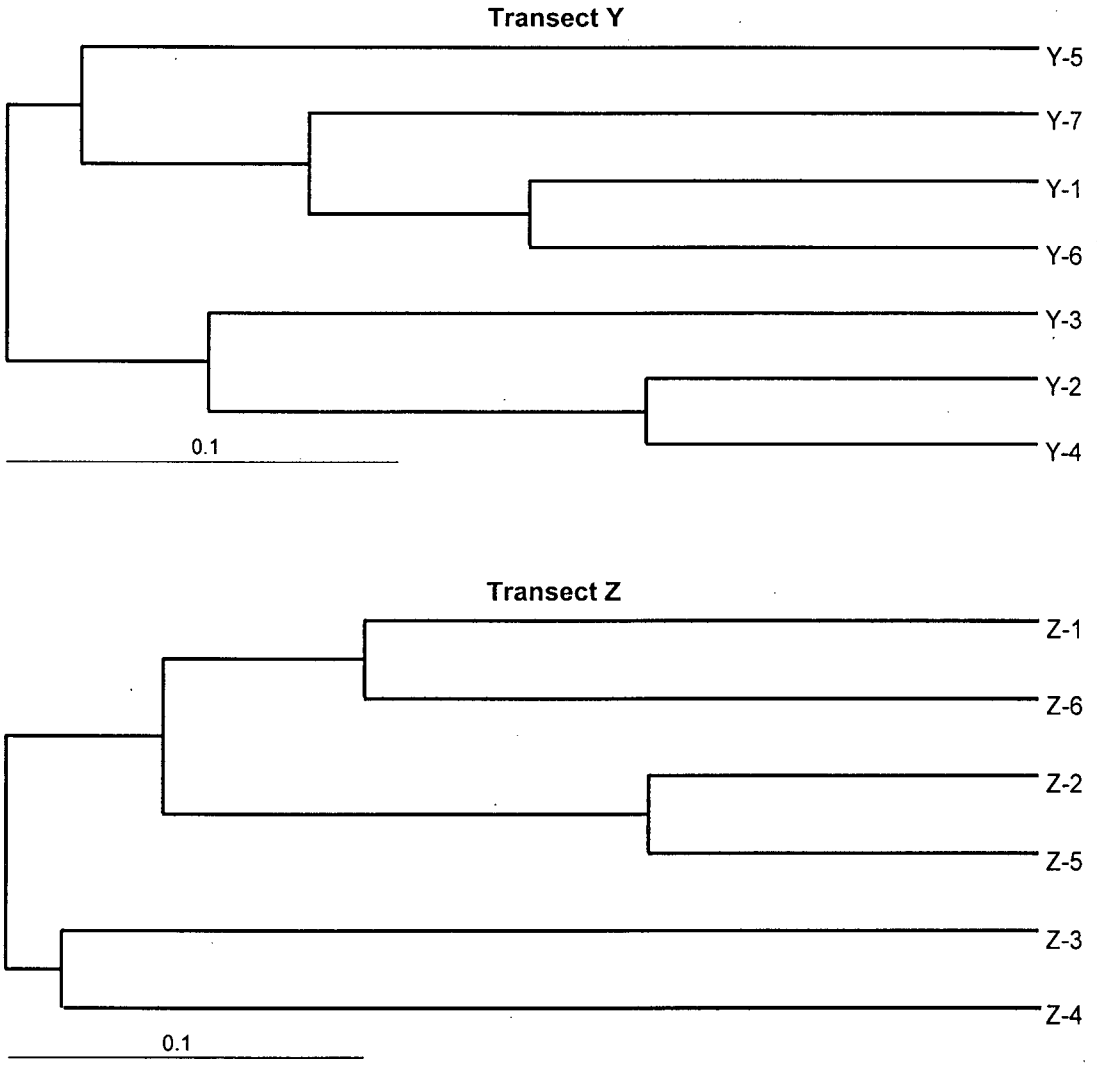


Table 17: Summary of within-transect genetic variability for eight transects using RAPD analysis with all 11 primers.

Transect	# Polymorphic Loci	Avg. Sim. Coefficient
S	55	0.499
T	25	0.418
U	46	0.370
V	36	0.473
W	33	0.561
X	26	0.612
Y	32	0.532
Z	39	0.483

When cluster analysis was performed using the unweighted pairwise group method with arithmetical mean (UPGMA), the results shown as dendrograms in Figure 17 did not display any general patterns of genet relatedness. For some transects, adjacent genets along the transect were somewhat related and clustered together in the dendrogram; for other transects they were not. Neighboring genets on the same transect were not necessarily closely related. To investigate this apparent random distribution of genets along the transects, linear regression analysis was performed for geographic distance vs. genetic similarity between genets. Geographic distance between genets was calculated using the midpoints of genet domains and comparisons were made only between genets from the same transect. For all eight transects, linear regression analysis indicated that there was no significant relationship between the two variables.

Individual Infection Foci

Multiple-collection points were distributed along the transects to investigate the genetic composition of individual infection foci. Each focus consisted of one to several adjacent hosts which displayed symptoms of infection or were dead. Although the average area occupied by an infection focus was much less than the average ramet or genet area, isolates collected from the same infection focus did not necessarily belong to the same genet. For example, four isolates were collected from an infection focus at the 5 m mark of Transect W (Figure 14) and three genets were detected among them. Conversely, the infection focus at the 155 m mark had four isolates collected from it, but they all were of the same genet. Therefore, spatial proximity of isolates suggested, but did not guarantee, they were sampled from the same genet. For all pairs of isolates which were sampled

within 10 m of each other, only 22.4 % them differed in genetic composition. In other words, two hosts which were situated 10 m or less apart had a 77.6 % chance of being infected by the same genet.

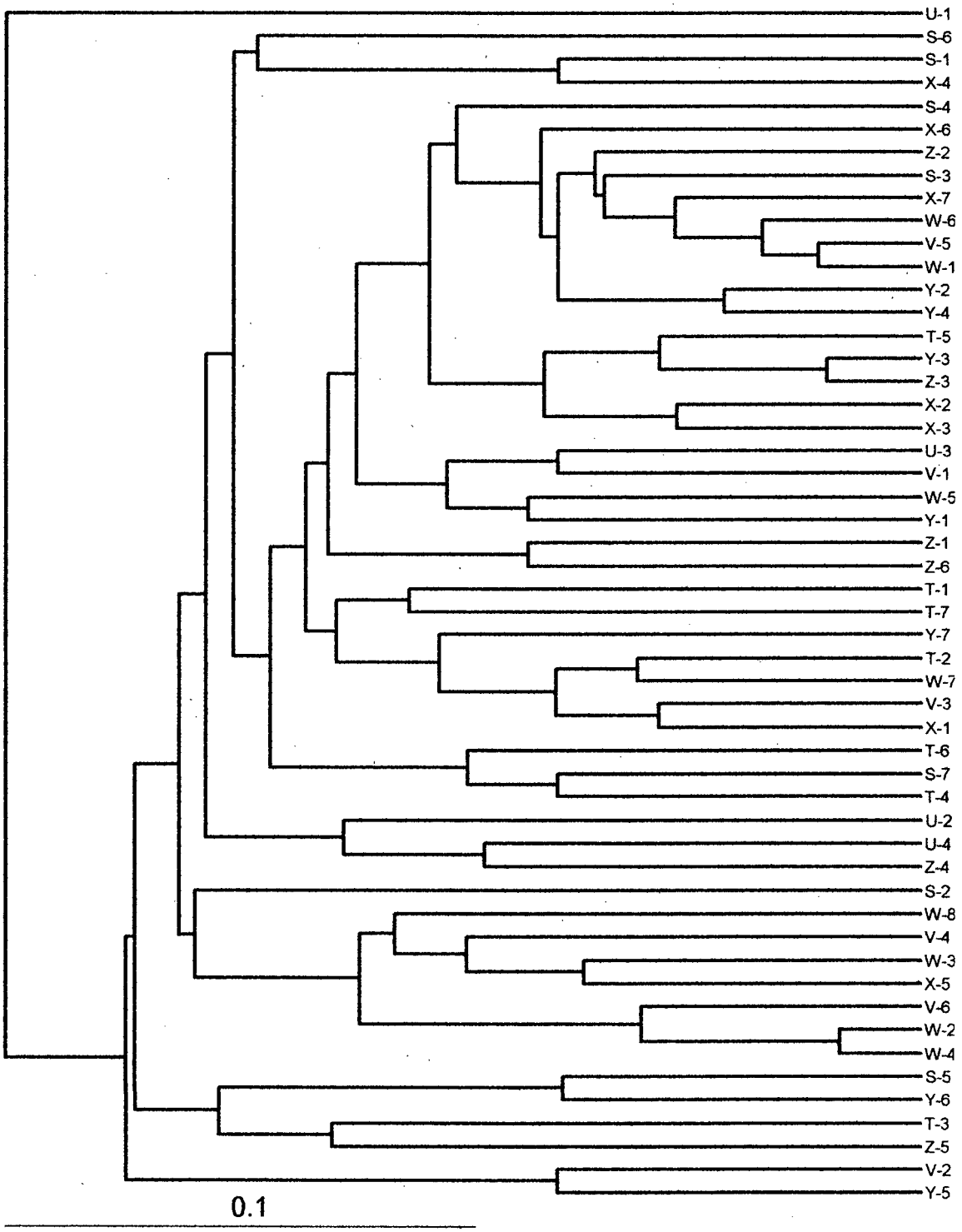
Between-Transect Population Structure

Genetic Variability of Genets Between Transects

To determine the genetic similarity of genets collected from the various transects within the Hidden Lake study site, RAPD analysis utilizing the above primers was repeated. A single isolate was chosen from each genet to represent it in the RAPD-PCR reactions. In the prior within-transect RAPD analysis, several markers which were consistently scorable within a particular transect were of insufficient quality in other transects. Only markers which were of adequate quality across *all* transects could be used for between-transect RAPD analysis. Primer JD11 was omitted for it yielded no scorable markers, and the remaining ten primers produced 46 markers in total, with 37 of them being polymorphic (Appendix I). These ten primers combined were successful at differentiating all 52 of the *A. ostoyae* genets detected during within-transect RAPD analysis. The distance between individual transects (500 m minimum) was great enough to prevent the same genet from being sampled from more than one transect.

A similarity matrix was calculated (Appendix J), cluster analysis was performed (UPGMA), and the resultant dendrogram is shown in Figure 18. The dendrogram displayed no apparent trends in genet distribution: genets did not cluster in any pattern associated with the geographical spatial distribution of the genets over the landscape. On a small scale, genets from the same transect did not cluster; on a larger scale, genets sampled from neighboring transects did not cluster either. It appeared that genets of *A. ostoyae* were randomly distributed throughout the Hidden Lake study site. This conclusion was supported further when linear regression analysis indicated that there was no significant relationship ($p>0.25$) between the average similarity coefficient between two transects and geographic distance between the two transect midpoints.

Figure 18: UPGMA dendrogram based on between-transect RAPD analysis showing genetic relationships of 52 genets. Bar indicates phenetic distance (1 – S) and branch termini indicate genet.



DISCUSSION

Population Structure

The results of this study demonstrated that the population structure of *A. ostoyae* in the Hidden Lake study site was quite complex. The most obvious characteristic was the relatively small sizes of ramets and genets. The average length of transect occupied by a ramet was 61.43 m and each hectare of plantation contained an average of 2.65 ramets. Of course, the average length of a genet in this area was somewhat larger at 94.37 m. It was estimated that 93.57 % of the ramets and 70.67 % of the genets within the sample site occupied domains less than one hectare in area. The greatest distance between two isolates of the same genet was 197 m (Genet T-6, Figure 14). Consistent with the conclusions from the Gavin Lake study site, the results of the this study indicate that *A. ostoyae* does not reproduce via airborne asexual propagules. The extremely low probabilities of finding several identical isolates and the spatial clustering of the isolates confirmed the assumption that isolates which displayed the same RAPD genotype belonged to the same genet and were derived via vegetative growth from a single initial mating event. The average size of genets in the Hidden Lake study site was quite small especially in comparison to the average size of genets in the Gavin Lake study site (Chapter II), but the average lengths of *Armillaria* genets examined in studies by other authors vary greatly: 50 m or less (Kile, 1986; Korhonen, 1978; Rishbeth 1978; Rizzo et al, 1995; Smith et al, 1994; Ullrich and Anderson 1978; Worrall, 1994), 50 – 100 m (Legrand et al, 1996; Kile, 1983; Rishbeth, 1991), 100 m or greater (Smith et al, 1992).

A second characteristic of the population structure in this region was the random distribution of genets in relation to infection foci. Single foci were not necessarily occupied by single mycelial individuals, and several foci were often occupied by the same genet. The distribution of genets within the plantations could not be predicted by the distribution of mortality. In the Gavin Lake study site, one could confidently assume that all infected hosts within an infection center of any magnitude were infected by the same genet. In the Hidden Lake study site, two hosts which were separated by a distance of 10 m or less only had a 77.6 % chance of being infected by the same genet. Klein-Gebbinck et al (1991) studied the distribution of *A. ostoyae* genets within plantations in Alberta and obtained results similar to those reported here.

The high density and small size of genets in this region caused the distribution of genets in relation to each other to be quite complex. Only 60 % of the genets which were isolated multiple times occupied an uninterrupted segment of the transect, and it was not uncommon for genets to have their isolates separated by isolates from another genet . In the Gavin Lake study site, individual genets did not interact with each other since they were always separated by significant distances of asymptomatic forest. In the Hidden Lake study site, two sampled hosts which were only one meter apart were infected by isolates belonging to different genets (Figure 14). The high levels of infection, high density of genets, and non-contiguous distribution of isolates strongly suggest that these genets have been expanding and interacting with each other for a great length of time, resulting in the complex mosaic of many small genets observed today.

Basidiospore Dispersal

In order to explain the large amounts of small scale genetic diversity and the high levels of infection, initiation of new genets via basidiospores must play, or have played in the past, an important role in the spread of *A. ostoyae* in the Hidden Lake study site. Based on the dynamics of basidiospore dispersal, two genets which occupy adjacent domains have higher probabilities of being related than two spatially separated genets. Also, if any form of genetic recombination was occurring between adjacent genets, those genets would share more RAPD markers and have high similarity coefficients. Since the eight transects were geographically separated by significant distances, the genets from the same transect would have a higher probability of being closely related than genets sampled from different transects. For instance, a genet sampled from Transect U was expected to be more closely related to other genets from Transect U than to genets from Transect V. Also, genets from neighboring transects would have a higher probability of being closely related than genets sampled from distant transects. The genets from Transect U were expected to be more closely related to the genets from Transect V than to those from Transect Z (Figure 11). When linear regression analysis was performed between genetic similarity and geographic distance for both within- and between-transect comparisons, no significant relationships were detected: genets of *A. ostoyae* were distributed randomly throughout the Hidden Lake study site.

In late summer and early autumn, each *A. ostoyae* genet has the potential to produce numerous basidiocarps which release an abundance of basidiospores. Due to basidiospore concentration gradients, the probability of basidiospores initiating infection decreases as the distance from their source increases. With large numbers of different genets concentrated into relatively small geographic areas, the concentration gradients from various basidiospore sources may overlap to a great extent. The atmosphere of the plantation could quickly become permeated with basidiospores from several local genets, to the extent that proximity to site may no longer affect the probability of initiating infection. This may possibly explain why there was no significant relationship between geographic distance and genetic similarity, and why the dendrogram (Figure 18) did not display any clustering of genets in a pattern which reflected their geographical spatial distribution. The atmosphere may be saturated with basidiospores from so many sources that the probability of initiating infection at a particular site is equal for several surrounding genets. This might explain why the genetic relations between established genets in the Hidden Lake study site were random, with no discernable patterns. Unfortunately, this speculative explanation cannot account for the random distribution of genets in the Gavin Lake study site. As discussed in Chapter II, the sampled genets represented only six basidiospore sources, most of which were separated from others by large tracts of asymptomatic forest.

Hidden Lake vs. Gavin Lake Study Site

The population structure of *A. ostoyae* in the Hidden Lake study site (southern Interior) was shown to be very different from that of *A. ostoyae* in the Gavin Lake study site (central Interior, Chapter II). In the Gavin Lake region, the study site was infected with a small number of relatively large genets which were sparsely distributed over the landscape. In the Hidden Lake region, the study site was infected with large numbers of relatively small genets occurring at high densities. These differences in population structure might have arisen for several *possible* reasons:

(1) The frequency of basidiospore-initiated infection is relatively low in both regions (as demonstrated in the Gavin Lake region), but *A. ostoyae* may have been present in the Hidden Lake region for a longer duration. If the pathogen spread slowly northward following the last glaciation event, it would have been re-established in the southern

Interior prior to the central Interior of B.C. New infections may have been occurring at the same frequency in both regions, but if the Hidden Lake region was exposed to the disease for a longer time, it would have accumulated many more infections. The increased levels of intra-specific competition for woody substrate may have caused the genets to occupy reduced domain sizes. Therefore, the complex population structure observed in the Hidden Lake study site may have developed simply because the pathogen was present in that area for a greater duration. In the course of time, the study site in the central Interior will continually be invaded by new genets and a more complex population structure may slowly develop.

(2) The frequency of fruiting, basidiospore-initiated infections, or resulting genet survival rates may be, or have been, substantially higher in the Hidden Lake study site than in the Gavin Lake study site. This would cause the plantations in the Hidden Lake region to develop higher levels of infection and to be completely colonized in a shorter period of time. The number of uninfected hosts decreases as the prevalence of infection increases, and this scarcity of available hosts would prevent established genets from expanding their domains uninhibited. The genets may have the potential to reach much larger proportions, but are restricted in size because the surrounding hosts have already been colonized by other competing genets.

(3) Differences in site disturbance history may have affected genet size and distribution in the two study sites. Sites occupied by small genets in high densities may have been associated with recent disturbance. In the Hidden Lake study site, all transects were laid through areas which had been clearcut and planted between 10 to 20 years ago. In the Gavin Lake study site, the majority of sampled infection centers were within stands which were 100 years or older in age. After a stand has been harvested, the remaining stumps act as infection courts for incoming *A. ostoyae* basidiospores. Since this abundance of available substrate increases the probability of successful genet initiation by basidiospores, the number of active genets on a particular site may increase rapidly after site disturbance. Similarly, genets which were previously restricted to dormant root lesions may now colonize the root systems of stumps and attempt to re-establish themselves. As time passes, the majority of genets may fail to acquire enough inoculum potential to continue spreading actively through the site, and either die or retreat to

isolated root lesions. Due to chance or selection for competitive advantages, relatively few genets may become securely established and continue to expand in size through time to stand maturity. In mature forest stands such as those in the Gavin Lake study site, the local community of *A. ostoyae* may have had ample time to stabilize and a small number of relatively large genets dominated the area. In recently disturbed sites such as those in the Hidden Lake study site, newly initiated genets along with reactivated genets may have created an initial community of relatively small genets in high densities. Therefore, the size and density of genets detected on a particular site may have been dependent upon the amount of time that has elapsed since the last site disturbance. Dahlberg and Stenlid (1990) studied the population structure of an ectomycorrhizal fungus and found that both increasing clonal size and decreasing clonal number was associated with increasing forest age and decreasing site disturbance. However, some of the results of the present did not support this hypothesis: In the Gavin Lake study site, one and a half of the sampled infection centers were located within a plantation, and one was within a shelterwood stand. These sites which were recently disturbed were all occupied by single large genets, not a complex mosaic of smaller genets.

Size and Distribution of Genets

There are several *possible* factors which may influence the size and distribution of genet territories: (1) Temporal: The genets which occupy larger domains may have been initiated via basidiospores before the genets which occupy smaller domains. In the absence of interactions with other genets, the size of a genet may simply be a reflection of its age: older genets have larger domains. (2) Physical: The organization of host root systems beneath the soil may dictate the size of a genet. Certain genets may have been restricted to a small area of forest because there was insufficient root contacts with surrounding trees to allow for mycelial or rhizomorph transfer. Genets which fortuitously became established in areas with extensive root closure could expand uninhibited and occupy larger domains. This factor is quite variable: different portions of a genet at different times may experience either condition. (3) Biological: More aggressively pathogenic genets may spread from host to host more quickly than others, thereby occupying larger domains and infecting more associated hosts. In the same period of time, other genets may remain confined to their present domains due to their

low levels of pathogenicity. Also, root closure may exist, but surrounding hosts may be unavailable for infection because the root systems are already colonized by another genet of *Armillaria* or another fungus. Therefore, intra- and inter-specific competition may restrict the size of genets by reducing the amount of available substrate. Although interactions between genets have not been directly studied in nature, mycelial antagonism at genet boundaries would presumably affect genet size and distribution more significantly as the local density of genets increases. It is likely that all three factors listed above have interacted to determine the territory occupied by individual genets in the Hidden Lake study site.

Experimental Error

There were some disadvantages to the transect sampling scheme applied in this study. First of all, the *actual* size and shape of domains occupied by genets was never truly known. Therefore, the above values regarding genet (or ramet) lengths and areas are all estimates based on certain assumptions. First, the distance of transect inclusive between two sampled isolates was considered fully occupied, even if the isolates belonged to different genets. This assumption is reasonable when the high levels of infection are considered. Morrison (unpublished) surveyed various plantations within the ICH and reported that 37 % of asymptomatic trees were actually infected by *Armillaria* root disease, and their distribution was not related to the distribution of visible infection foci. The second assumption is that the length of transect occupied by a genet was equal to the average dimension of that genet. Although this assumption may be false for individual genets, it becomes accurate when averaged across all genets combined.

It is possible that the sampling scheme employed in this study could, on average, overestimate the length of transect occupied by each genet. For example, as the distance between samples along the transect increases, the probability of detecting very small genets decreases. Also, what appeared to be one large genet may have turned out to be two separate smaller ramets if more intensive sampling was conducted. Assuming a set number of samples, a compromise exists between how accurate and how representative the results are. Every infected host encountered along the transect could be collected, but then only a few transects could be sampled. Thus, detailed information could be gathered on individual genets, but the results would be less representative due to the smaller

number of genets examined. Conversely, conclusions based on less detailed information from a larger number of genets would be more representative of the population. This study was designed to *estimate* the sizes and distribution of genets and ramets in the Hidden Lake population, so the chosen sampling intensity appeared sufficient. However, the large proportion (42.3 %) of genets that were sampled only once suggested that the sampling scheme was too coarse. Unfortunately, it is likely that several extremely small genets located between collection points remained undetected. Further studies which investigate the sizes of genets in heavily infected areas should reduce the collection point intervals.

If sampling intensity *did* influence observed genet or ramet lengths, then transects which yielded more isolates should have correspondingly smaller average genet or ramet lengths. Transects S and T had more samples collected from them (Table 9) because the sampling interval was less than that of the other six transects. However, the results of linear regression analysis indicated no significant relationship between the number of samples collected per transect and the average length of genets ($p>0.65$) or ramets ($p>0.25$) detected along those transects (Tables 12 and 15). Therefore, it was concluded that sampling intensity used in this study did not bias the calculated genet or ramet lengths.

Other sampling schemes such as plot or quadrat sampling have been used by various other authors (Legrand et al, 1996; Rizzo et al, 1995; Smith et al, 1990, 1994; Worrall, 1994) to study the distribution of *Armillaria* genets. With intensive sampling of quadrats, the exact size and shape of a few genets or ramets could be determined. Again, a compromise exists between how accurate and how representative the results are. Since this is a preliminary study on the population structure and generalizations were to be made, it was more appropriate to sample using transects.

In order to estimate the sizes of the fungal mycelia, non-contiguous isolates of the same genet were assumed to represent either (A) multiple ramets or (B) a single large genet. Most studies on the population structure of *Armillaria* and other root-inhabiting fungi have reported that spatial overlap of genets is quite rare (Bae et al, 1994; Dickman and Cook, 1989; Kile, 1983; Korhonen, 1978; Shaw and Roth, 1976; Smith et al, 1992, 1994; Stenlid, 1985; Worrall, 1994), which supported scenario A. But the arrangement

of isolates along the transect and the close proximity of different genets to each other supported scenario B. Both scenarios were equally plausible, but it was necessary to assume one scenario or the other for the purposes of area estimation. In actuality, the more appropriate assumption is a combination of both scenarios, thus the average size of an individual mycelium in the Hidden Lake study site was presumably greater than the estimated ramet sizes and less than the estimated genet sizes.

Management Implications

Recent studies have demonstrated that different genets of the same *Armillaria* species may possess different degrees of pathogenicity and virulence (Mugala et al, 1989; Omdal et al, 1995; Shaw and Loopstra, 1988). These discrepancies make disease prediction even more difficult for forest pathologists. Possibly, certain genets may spread quickly through the forest causing significant damage, while other genets remain dormant as harmless root lesions. When this type of variation is considered, the size and distribution of the genets throughout a particular stand become quite important. For instance, if a stand contains a single large genet which has been identified as extremely pathogenic and virulent, forest pathologists could predict significant losses within the infection center and the immediate periphery. Since the minimum silvicultural treatment unit is approximately one hectare, pathogenic genets which occupy a hectare or more could be treated individually. On the other hand, if a stand contains a complex mosaic of small genets with varying degrees of pathogenicity and virulence, predicting how the disease will develop through time would be quite difficult. Site treatment to prevent further infection could not focus on single pathogenic genets, so the entire area would undergo treatment of similar intensity.

Determining the population structure is essential to understanding the epidemiology of *Armillaria* root disease in a particular area. Once the key mechanisms of disease transmission are identified, management strategies which inhibit those specific mechanisms can be applied. For example, if infection via basidiospores (primary resource capture) is critical, then treatments which prevent colonization and infection of stumps should be employed. If transmission by mycelial fans and rhizomorphs (secondary resource capture) is critical, then treatments which reduce inoculum levels would be more appropriate. In the Gavin Lake study site, the small number of relatively

large genets indicated that *A. ostoyae* spreads predominantly by root to root mycelial transfer. Secondary resource capture also plays and important role in the Hidden Lake study site, but the high densities of relatively small genets suggested that basidiospore infection occurs more frequently in this area. For successful control and reduction of damage caused by *Armillaria* root disease, it may be necessary to apply different management strategies in each area. For more precise predictions of disease impact and more appropriate silvicultural prescriptions for infected forest stands in British Columbia, site-specific differences in the population structure of *A. ostoyae* should be addressed.

Summary

In summary, the genets of *A. ostoyae* investigated in the Hidden Lake study site occurred in high densities and occupied rather small domains. The distribution of genets in relation to one another was quite complex and isolates of the same genet were sometimes non-contiguous. It was estimated that approximately 70 % of the detected genets occupied a territory less then a hectare in area, with the average ramet being less than half the size of the average genet. Isolates collected from the same infection focus did not necessarily belong to the same genet, and genets were not restricted to single foci. There was not a significant relationship between geographic distance and genetic similarity so genets of *A. ostoyae* were randomly distributed throughout the study site.

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Appendices

Appendix A: Protocol for Fungal DNA Extraction
(modified version of Doyle and Dickson, 1987)

1) grind tissue in liquid nitrogen using pre-cooled mortar and pestle. Add appropriate amount of powder (30 - 60 mg) to lysis buffer (10 - 15 ml) which is pre-heated to 60 °C.

Lysis Buffer final concentrations:
- 100 mM Tris-HCl (pH 8.0)
- 1.4 M NaCl
- 20 mM EDTA
- 2 % B-mercaptoethanol
- 2 % CTAB

2) incubate at 60 - 64 °C for 30 - 45 min with gentle swirling every 10 min.
3) centrifuge at 3900 rpm for 5 min to pellet unwanted debris.
4) transfer supernatant to new tube and add an equal volume of chloroform-isoamyl alcohol (24:1). Mix gently for 2 min then centrifuge at 3900 rpm for 5 min.
5) transfer aqueous phase to new tube and add 2/3 volume of cold isopropanol. Centrifuge at 3900 rpm for 5 min to pellet the DNA.
6) pour off isopropanol, then rinse with 300 µl of wash buffer (75 % ethanol, 10 mM ammonium acetate) for 2 min.
7) with tweezers, remove pellet and resuspend DNA in 500 µl of distilled water.
8) add RNAse A to a final concentration of 10 µg/ml. Incubate at 37 °C for 30 min.
9) add Proteinase K to a final concentration of 10 µg/ml. Incubate at 37 °C for 30 min (or overnight).
10) add slightly less than an equal volume of phenol:chloroform (200 µl:200 µl). Wash for 2 min then centrifuge at 8000 rpm for 5 min.
11) transfer supernatant to new microtube, add slightly less than an equal volume of chloroform-isoamyl alcohol (400 µl), then wash for 2 min. Centrifuge at 10 000 rpm for 5 min.
12) transfer supernatant to new microtube and add NaCl to a final concentration of 0.15M (12 - 14 µl of 5M NaCl). Precipitate DNA with 2 volumes cold ethanol (800 µl) and mix gently.
13) centrifuge at 10 000 rpm for 5 min to pellet DNA.
14) remove supernatant, wash pellet with 150 µl cold 70 % ethanol, then air dry or dry DNA pellet in speedvac.
15) resuspend DNA in 300 µl of autoclaved water and store DNA in at -20 °C .

Appendix B: Probabilities of RAPD Genotype Occurrence

Assuming that a particular genotype has been discovered in the population, the probability of that identical genotype occurring again due to chance alone can be calculated by the formula:

$P_{gen} = \Pi p_i$

where p_i is the frequency of marker presence or absence observed for a single locus in the population. The probability of the identical multilocus genotype occurring *twice* by chance alone is the product of all 41 single locus frequencies corresponding to that genotype.

Probabilities of the seven Armillaria ostoyae *genotypes arising more than once independently.*

Genotype	No. of isolates (n)	P_{gen}*	P_{mult}**
Ost-I	24	4.38×10^{-10}	5.53×10^{-216}
Ost-II	13	2.43×10^{-10}	4.25×10^{-116}
Ost-III	29	2.17×10^{-15}	2.64×10^{-411}
Ost-IV	1	5.70×10^{-11}	-
Ost-V	13	2.05×10^{-9}	5.54×10^{-105}
Ost-VI	11	9.89×10^{-9}	8.96×10^{-81}
Ost-VII	12	1.52×10^{-9}	9.95×10^{-98}

* Probability of genotype being present twice by chance alone.
** Probability of genotype being present n times by chance alone $(P_{gen})^{n-1}$.

Similarly, the probability of (n-1) more replicates of the same genotype arising independently is:

$P_{mult} = (P_{gen})^{n-1}$

where n is the number of times the genotype was observed. As shown in the table above, calculated P_{mult} values for the genotypes were negligible.

Another formula (Tuskan et al, 1996) for calculating the probability that any two isolates share all markers by chance alone is:

$I = [S^2 + (1 - S)^2]^{n/S}$

where S is the average similarity coefficient (see Genetic Variability section) and n is the average number of markers per individual (n = 18.43, data not shown). For this population, $I = 1.86 \times 10^{-11}$ which was similar to the values of P_{gen} listed above.

Appendix C: Binomial Data and Similarity Matrix produced from the first seven primers for the seven *Armillaria ostoyae* genotypes.

Binomial Data: (1) indicates presence of marker, (0) indicates absence of marker

Primer	Molecular Weight (bp)	Genotype						
		Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD01	2200	1	1	1	1	1	1	1
JD01	1315	1	1	0	1	1	1	0
JD01	1225	1	1	0	0	0	0	1
JD01	1100	0	1	0	0	0	0	1
JD01	1085	1	0	1	0	0	0	0
JD01	1060	0	0	0	1	1	1	0
JD01	600	0	0	1	0	0	0	0
JD01	585	1	1	0	0	0	1	1
JD01	575	0	0	1	0	0	0	0
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD02	2200	0	0	1	1	1	1	0
JD02	1900	1	1	1	1	1	1	1
JD02	1775	0	0	1	0	0	0	0
JD02	1750	1	1	1	1	1	1	1
JD02	1725	1	1	1	1	1	1	1
JD02	1300	0	0	1	1	1	1	0
JD02	1270	0	0	0	0	0	0	1
JD02	1060	1	1	1	1	1	1	1
JD02	685	0	0	1	1	1	1	1
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD03	1975	1	1	1	0	1	1	1
JD03	1960	0	1	0	0	0	0	0
JD03	1900	0	0	1	1	1	1	1
JD03	1750	0	1	0	1	1	1	1
JD03	1400	0	0	1	1	1	0	0
JD03	1175	1	0	0	1	1	1	1
JD03	1110	0	1	1	0	0	0	0
JD03	1050	0	0	0	1	1	0	0
JD03	880	1	1	1	1	1	1	1
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD05	2250	0	0	0	1	1	0	0
JD05	1850	1	1	1	1	1	1	1
JD05	1700	1	1	1	1	1	1	1
JD05	1375	1	1	1	1	1	1	1
JD05	1180	0	0	1	1	1	0	1
JD05	1075	1	1	1	1	1	1	1
JD05	630	1	1	1	0	1	1	1

Primer	Molecular Weight (bp)	Genotype						
		Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD06	1800	1	1	1	1	1	1	1
JD06	1150	0	1	1	0	0	1	1
JD06	1015	1	1	1	1	1	0	1
JD06	1000	0	0	1	0	0	0	0
JD06	850	0	0	1	1	1	0	0
JD06	790	1	1	1	1	1	1	1
JD06	615	1	1	1	1	1	1	1
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD07	1825	0	0	1	0	0	0	0
JD07	1510	0	0	1	0	0	0	0
JD07	1490	1	1	1	1	1	1	1
JD07	1210	1	1	1	1	1	1	1
JD07	915	0	0	1	0	0	0	0
JD07	780	1	0	0	0	0	0	1
JD07	750	1	0	0	0	0	0	1
JD07	650	0	0	0	1	1	0	0
JD07	525	1	1	1	1	1	1	1
JD07	440	1	1	1	1	1	1	1
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD08	1650	1	1	1	0	0	0	1
JD08	1500	1	1	1	1	1	0	1
JD08	1425	1	1	1	0	0	1	1
JD08	1040	1	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1	1
JD08	760	0	0	0	1	1	0	0
JD08	560	1	1	0	1	1	1	1
JD08	525	1	1	0	1	1	0	0
JD08	415	1	1	0	0	0	0	0

Appendix C continued...

Similarity Matrix (Dice's Similarity Coefficients)

Genotype	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
Ost-I	1.0						
Ost-II	0.727	1.0					
Ost-III	0.359	0.400	1.0				
Ost-IV	0.343	0.333	0.429	1.0			
Ost-V	0.432	0.421	0.500	0.950	1.0		
Ost-VI	0.467	0.516	0.432	0.545	0.629	1.0	
Ost-VII	0.686	0.667	0.476	0.421	0.500	0.606	1.0
Gen. Avg.	0.502	0.510	0.433	0.504	0.572	0.533	0.559
Total Avg.	0.516, SD=0.150, SE=0.033						

Appendix D: Binomial Data produced from the last five primers for the seven *Armillaria ostoyae* genotypes.

Binomial Data: (1) indicates presence of marker, (0) indicates absence of marker

Primer	Molecular Weight (bp)	Genotype						
		Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD04	2100	1	1	1	1	1	1	1
JD04	1850	0	0	0	1	1	0	0
JD04	1650	1	1	1	1	1	1	1
JD04	1470	1	1	1	1	1	1	1
JD04	1300	0	0	1	0	0	0	1
JD04	1160	0	0	1	1	1	1	1
JD04	1090	1	1	1	1	1	1	1
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD10	2550	0	0	1	1	1	0	0
JD10	1610	1	1	0	1	1	1	0
JD10	1400	1	1	1	1	1	1	1
JD10	1310	1	1	1	1	1	1	1
JD10	1280	0	0	1	0	1	1	0
JD10	1100	1	1	0	1	1	0	1
JD10	980	0	0	0	0	0	1	1
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD11	2550	0	0	1	0	0	1	0
JD11	2500	1	1	1	1	1	1	1
JD11	2250	0	1	1	1	1	1	1
JD11	2175	0	0	0	1	1	1	0
JD11	2050	1	1	0	0	0	0	0
JD11	2000	1	1	1	1	1	1	1
JD11	1925	0	0	0	1	1	1	1
JD11	1850	1	1	1	0	0	1	0
JD11	1725	1	1	1	1	1	0	1
JD11	1625	0	0	0	0	0	1	0
JD11	1600	1	1	1	1	1	0	1
JD11	1000	1	1	1	1	1	1	1
JD11	525	1	1	1	1	1	1	1
JD11	350	0	0	1	0	0	0	0

Primer	Molecular Weight (bp)	Genotype						
		Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD12	1990	1	1	1	1	1	0	1
JD12	1825	1	1	1	0	0	1	1
JD12	1700	0	0	0	0	0	1	0
JD12	1500	1	0	0	1	1	0	0
JD12	1275	1	1	0	0	0	1	0
JD12	1150	1	1	1	1	1	1	1
JD12	1020	1	0	0	0	0	0	1
JD12	1000	0	1	1	1	1	1	0
JD12	590	0	0	0	1	1	0	0
JD12	375	0	1	0	0	0	1	0
Primer	**M.W.**	Ost-I	Ost-II	Ost-III	Ost-IV	Ost-V	Ost-VI	Ost-VII
JD13	1930	0	1	0	1	1	0	0
JD13	1900	1	1	0	0	0	1	0
JD13	1475	1	1	1	1	1	1	1
JD13	1275	1	1	1	1	1	0	1
JD13	930	1	1	1	1	1	1	1
JD13	750	0	0	0	0	0	1	0

Appendix E: Binomial Data and Similarity Matrix produced from the first seven primers for the eighteen *Armillaria sinapina* genotypes.

Binomial Data: (1) indicates presence of marker, (0) indicates absence of marker

	Molecular	Genotype																
Primer	Weight (bp)	A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD01	1875	1	1	0	1	0	1	1	1	0	0	0	0	1	1	1	0	0	1
JD01	1210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
JD01	990	1	0	1	0	1	0	1	1	0	1	1	0	1	1	0	0	1	1
JD01	960	0	1	0	1	0	1	0	0	0	0	0	1	0	0	1	1	0	0
JD01	680	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
JD01	590	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Primer	**M.W.**	A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD02	1800	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
JD02	1610	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD02	1550	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	1	1	0
JD02	1070	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0
JD02	1030	0	0	0	0	0	1	1	1	1	1	0	0	1	0	0	1	0	1
JD02	660	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primer	**M.W.**	A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD03	1520	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
JD03	1500	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
JD03	1480	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
JD03	1325	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD03	1050	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
JD03	890	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD03	850	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0
JD03	750	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1
Primer	**M.W.**	A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD05	2100	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
JD05	1070	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD05	1060	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD05	1050	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD05	980	0	1	0	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0
JD05	900	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
JD05	720	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1

Appendix E continued...

Primer	Molecular Weight (bp)	Genotype																	
		A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD06	1300	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
JD06	1200	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
JD06	1100	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
JD06	1000	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
JD06	975	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
JD06	600	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
JD06	425	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Primer	**M.W.**	A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD07	1590	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
JD07	1550	0	0	0	0	1	0	0	1	1	0	1	0	1	0	1	1	0	0
JD07	1480	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
JD07	800	1	0	1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0
JD07	670	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1
JD07	570	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
JD07	530	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
JD07	490	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Primer	**M.W.**	A01	A09	A12	A16	C01	C12	C13	D05	D07	F01	F02	F07	F08	F13	H02	H11	I05	I06
JD08	1900	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
JD08	1650	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
JD08	1300	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
JD08	1190	0	0	0	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0
JD08	900	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
JD08	680	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
JD08	510	1	0	0	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1

Appendix E continued...

Similarity Matrix (Dice's Similarity Coefficients)

Genotype	A01	A09	A12	A16	C01	C12	C13	D05	D07	H02	H11	I05	I06	F01	F02	F07	F08	F13
A01	1.0																	
A09	0.320	1.0																
A12	0.500	*0.381	1.0															
A16	0.500	*0.480	0.500	1.0														
C01	0.500	*0.400	0.667	0.643	1.0													
C12	0.417	*0.476	0.600	0.667	0.583	1.0												
C13	0.615	*0.261	0.545	0.462	0.538	0.545	1.0											
D05	*0.571	*0.480	0.583	0.500	0.643	0.583	0.615	1.0										
D07	0.462	*0.348	0.636	0.462	0.538	0.545	0.500	0.615	1.0									
H02	0.462	*0.522	0.545	0.615	0.615	0.636	0.500	0.615	0.500	1.0								
H11	0.385	*0.348	0.545	0.615	0.538	0.636	0.417	0.538	0.667	0.583	1.0							
I05	0.417	*0.286	0.700	0.500	0.667	0.500	0.455	0.500	0.545	0.364	0.545	1.0						
I06	0.480	*0.364	0.571	0.400	0.560	0.476	0.609	0.640	0.435	0.609	0.435	0.381	1.0					
F01	0.500	*0.320	0.667	0.500	0.500	0.500	0.538	0.714	0.538	0.538	0.462	0.500	0.560	1.0				
F02	0.615	*0.261	0.636	0.538	0.769	0.545	0.583	0.692	0.583	0.667	0.500	0.545	0.522	0.615	1.0			
F07	0.400	0.370	*0.462	0.467	*0.467	*0.462	0.357	*0.400	0.357	*0.429	0.500	0.462	0.370	*0.467	*0.357	1.0		
F08	0.516	*0.357	0.519	0.516	0.516	0.519	0.552	0.774	0.552	0.552	0.552	0.444	0.571	0.710	0.552	*0.545	1.0	
F13	0.538	*0.435	0.545	0.462	0.538	0.455	0.583	0.615	0.417	0.667	0.417	0.455	0.609	0.538	0.667	0.357	0.621	1.0
Gen Avg.	0.4822	0.3769	0.5649	0.5192	0.5696	0.538	0.5104	0.593	0.5118	0.5541	0.5108	0.4862	0.5054	0.5393	0.5676	0.4252	0.551	0.5246
Avg.	0.518																	

* indicates that the two average genotypic similarity coefficient's are significantly different as determined by a Tukey Test ($p < 0.05$)

Appendix F: Binomial Data produced from the first six primers for the *A. ostoyae* genets along ea transect.

Binomial Data: (1) indicates presence of marker, (0) indicates absence of marker

	Molecular	Genotypes from Transect S								Molecular	Genotypes from Transect T						
Primer	Weight (bp)	S-1	S-2	S-3	S-4	S-5	S-6	S-7	Primer	Weight (bp)	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD01	2150	1	1	1	1	1	1	1	JD01	2150	1	1	1	1	1	1	1
JD01	1360	0	1	1	0	0	0	1	JD01	1450	1	1	1	0	0	0	1
JD01	1300	1	0	0	0	1	0	0	JD01	1300	0	1	1	0	0	0	0
JD01	1220	0	1	1	0	1	0	0	JD01	1220	1	0	1	0	0	0	0
JD01	1120	1	1	1	0	0	0	1	JD01	1070	0	1	0	1	1	1	0
JD01	1070	0	0	0	0	1	0	0	JD01	590	1	1	1	1	1	1	1
JD01	840	0	1	1	0	0	1	0									
JD01	590	0	1	1	1	1	1	1									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD02	2200	1	1	1	0	1	1	1	JD02	2200	0	1	1	1	1	1	1
JD02	1750	1	1	1	1	1	1	1	JD02	1750	1	1	1	1	1	1	1
JD02	1060	1	1	1	1	1	1	1	JD02	1060	1	1	1	1	1	1	1
JD02	800	1	0	1	1	1	1	1									
JD02	690	0	1	1	1	1	1	1									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD03	2100	0	0	0	0	1	0	0	JD03	2000	1	1	1	0	0	1	0
JD03	2020	1	0	0	0	0	0	0	JD03	1750	0	0	0	0	0	1	1
JD03	2000	0	1	1	1	0	1	1	JD03	1100	1	1	0	1	0	0	0
JD03	1750	1	1	1	1	1	0	0	JD03	1060	0	0	0	0	0	1	0
JD03	1100	1	1	1	1	0	1	1	JD03	890	1	1	1	1	1	1	1
JD03	1060	0	0	0	0	1	0	0									
JD03	890	1	1	1	1	1	1	1									
JD03	840	0	1	1	0	0	0	0									
JD03	670	0	1	0	0	0	0	0									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD05	1625	0	0	0	1	1	1	1	JD05	620	1	1	1	1	1	1	1
JD05	1360	1	1	1	1	1	1	1									
JD05	1080	0	0	0	1	0	0	0									
JD05	1070	1	0	1	0	1	1	1									
JD05	960	0	1	0	0	0	0	0									
JD05	620	1	1	1	1	1	1	1									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD06	1990	0	0	0	0	1	0	0	JD06	1990	0	0	1	1	0	0	0
JD06	1400	1	0	0	0	0	0	0	JD06	1400	1	0	0	0	0	0	0
JD06	850	1	1	1	0	1	1	0	JD06	850	0	0	0	0	1	0	1
JD06	790	1	1	1	1	1	1	1	JD06	790	1	1	1	1	1	1	1
									JD06	610	1	1	1	1	1	1	1
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD07	1800	0	1	0	0	0	0	0	JD07	670	0	0	0	0	0	1	1
JD07	1510	0	1	1	0	0	0	0	JD07	520	1	1	1	1	1	1	1
JD07	990	0	1	0	0	0	0	0	JD07	440	1	1	1	1	1	1	1
JD07	520	1	1	1	1	1	1	1									
JD07	440	1	1	1	1	1	1	1									
JD07	400	0	1	0	0	0	0	0									
JD07	330	0	1	0	0	0	0	0									

Appendix F continued...

Primer	Molecular Weight (bp)	Genotypes from Transect U								Primer	Molecular Weight (bp)	Genotypes from Transect V						
		U-1	U-2	U-3	U-4							V-1	V-2	V-3	V-4	V-5	V-6	
JD01	2150	1	1	1	1					JD01	2150	1	1	1	1	1	1	
JD01	1450	1	1	1	0					JD01	1300	1	1	0	0	0	0	
JD01	1300	1	1	1	0					JD01	1290	0	0	0	1	0	1	
JD01	1220	1	1	0	0					JD01	1220	0	0	0	1	0	1	
JD01	1100	1	1	0	0					JD01	1070	1	1	1	1	0	1	
JD01	1070	0	0	0	1					JD01	590	1	1	1	1	1	1	
JD01	1040	0	0	1	0													
JD01	590	1	1	1	1													
JD01	410	1	0	0	0													
Primer	**M.W.**	U-1	U-2	U-3	U-4					**Primer**	**M.W.**	V-1	V-2	V-3	V-4	V-5	V-6	
JD02	2200	1	1	0	1					JD02	2200	0	0	0	0	1	0	
JD02	1750	1	1	1	1					JD02	1980	1	1	1	1	1	1	
JD02	1060	1	1	1	1					JD02	1750	1	1	1	1	1	1	
JD02	690	0	0	0	1					JD02	1060	1	1	1	1	1	1	
Primer	**M.W.**	U-1	U-2	U-3	U-4					**Primer**	**M.W.**	V-1	V-2	V-3	V-4	V-5	V-6	
JD03	2600	0	0	0	1					JD03	2150	1	1	1	1	1	0	
JD03	2000	0	0	1	0					JD03	1875	0	0	0	0	1	0	
JD03	1750	1	1	1	1					JD03	1750	1	1	1	1	1	1	
JD03	1400	1	0	1	0					JD03	1190	0	0	0	1	0	1	
JD03	1350	0	1	0	0					JD03	1100	0	1	1	0	1	0	
JD03	1100	0	1	0	1					JD03	890	1	1	1	1	1	1	
JD03	890	1	1	1	1					JD03	840	0	0	0	0	0	1	
JD03	780	0	0	1	0					JD03	510	0	0	1	1	0	0	
JD03	580	0	1	0	1													
JD03	480	1	0	0	0													
JD03	400	0	1	0	0													
Primer	**M.W.**	U-1	U-2	U-3	U-4					**Primer**	**M.W.**	V-1	V-2	V-3	V-4	V-5	V-6	
JD05	1850	0	1	0	1					JD05	1720	1	0	1	1	0	1	
JD05	1720	1	1	1	1					JD05	1460	1	0	1	1	1	1	
JD05	1380	1	1	1	1					JD05	1360	1	1	0	1	1	1	
JD05	1190	0	1	1	0					JD05	1200	1	1	0	0	0	1	
JD05	1070	1	1	1	1					JD05	1070	1	1	1	1	1	1	
JD05	620	1	1	1	1					JD05	620	1	1	1	1	1	1	
JD05	430	0	1	0	1													
Primer	**M.W.**	U-1	U-2	U-3	U-4					**Primer**	**M.W.**	V-1	V-2	V-3	V-4	V-5	V-6	
JD06	1850	1	1	1	1					JD06	1990	0	1	0	1	0	0	
JD06	1400	0	0	1	0					JD06	1040	0	1	0	1	0	1	
JD06	1140	1	1	0	1					JD06	1000	1	0	1	0	1	0	
JD06	1100	0	0	1	0					JD06	850	1	0	0	1	1	0	
JD06	1030	0	1	0	1					JD06	790	1	1	1	1	1	1	
JD06	850	0	1	1	1					JD06	610	1	1	1	1	1	881	
JD06	790	1	1	1	1													
JD06	610	1	1	1	1													
Primer	**M.W.**	U-1	U-2	U-3	U-4					**Primer**	**M.W.**	V-1	V-2	V-3	V-4	V-5	V-6	
JD07	1490	1	1	1	1					JD07	1800	0	0	0	0	0	1	
JD07	710	1	0	0	0					JD07	1390	0	1	0	0	0	1	
JD07	640	0	0	0	1					JD07	520	1	0	1	1	1	1	
JD07	520	0	1	1	1					JD07	440	1	1	1	1	1	1	
JD07	440	1	1	1	1													

Appendix F continued...

Primer	Molecular Weight (bp)	Genotypes from Transect W								Primer	Molecular Weight (bp)	Genotypes from Transect X						
		W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8			X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD01	2150	1	1	1	1	1	1	1	0	JD01	2150	1	1	1	1	1	1	1
JD01	1450	1	1	1	1	0	0	0	0	JD01	1390	0	0	0	0	1	0	0
JD01	1300	0	0	0	0	1	0	1	0	JD01	1300	1	0	0	1	0	0	0
JD01	1220	0	1	1	1	0	0	0	1	JD01	1220	0	0	0	0	1	0	0
JD01	1100	1	0	0	0	1	0	0	0	JD01	1100	1	0	0	0	0	0	1
JD01	1070	0	1	1	1	0	1	1	1	JD01	1070	1	1	1	1	1	1	0
JD01	590	1	1	1	1	1	1	1	1	JD01	710	0	1	1	0	0	0	0
										JD01	590	1	1	1	1	1	1	1
										JD01	410	0	0	0	0	1	0	1
Primer	**M.W.**	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	**Primer**	**M.W.**	X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD02	2200	1	1	1	1	0	0	1	1	JD02	2200	1	1	1	1	1	1	0
JD02	1980	1	1	1	1	1	1	1	1	JD02	1750	1	1	1	1	1	1	1
JD02	1750	1	1	1	1	1	1	0	1	JD02	1060	1	1	1	1	1	1	1
JD02	1060	1	1	1	1	1	1	1	1									
Primer	**M.W.**	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	**Primer**	**M.W.**	X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD03	3400	0	0	0	0	1	0	0	0	JD03	3200	1	1	1	1	1	1	1
JD03	3200	1	1	1	1	1	1	1	1	JD03	2000	1	1	1	1	1	1	1
JD03	3000	0	0	1	0	0	0	0	0	JD03	1750	1	0	0	1	1	1	1
JD03	1750	1	1	1	1	0	1	1	1	JD03	1100	1	1	1	1	1	1	1
JD03	1400	0	1	1	1	0	0	0	0	JD03	890	1	1	1	1	1	1	1
JD03	1100	1	0	0	0	1	1	0	0	JD03	780	0	0	0	0	0	1	0
JD03	1080	0	0	1	0	0	0	0	0	JD03	520	0	0	1	0	0	0	0
JD03	890	1	1	1	1	1	1	1	1									
JD03	840	0	1	0	1	0	0	0	0									
Primer	**M.W.**	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	**Primer**	**M.W.**	X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD05	1600	1	1	1	1	1	1	1	1	JD05	1720	1	0	1	1	1	1	1
JD05	1360	1	1	1	0	1	1	1	1	JD05	1380	1	1	1	1	1	1	1
JD05	1190	0	1	1	1	1	1	1	1	JD05	1070	1	1	1	1	1	1	1
JD05	1070	1	1	1	1	1	1	1	1	JD05	620	1	1	1	1	1	1	1
JD05	1000	1	0	0	0	0	0	1	0									
Primer	**M.W.**	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	**Primer**	**M.W.**	X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD06	1020	1	0	1	0	0	1	1	1	JD06	850	0	1	1	1	1	1	1
JD06	850	1	0	0	0	1	1	0	0	JD06	790	1	1	1	1	1	1	1
JD06	790	1	1	1	1	1	1	1	1									
Primer	**M.W.**	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	**Primer**	**M.W.**	X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD07	1800	0	1	1	1	0	0	0	0	JD07	1490	1	1	1	1	1	1	1
JD07	1550	0	0	1	0	0	0	0	0	JD07	1210	1	1	1	1	1	1	1
JD07	1490	1	1	1	1	1	1	1	1	JD07	910	0	0	1	0	0	1	0
JD07	1380	1	0	0	0	0	0	0	0	JD07	520	1	1	1	1	1	1	1
JD07	1210	1	1	1	1	1	1	1	1	JD07	440	1	1	1	1	1	1	1
JD07	810	1	0	0	0	0	0	0	0									
JD07	660	0	1	0	1	0	0	0	0									
JD07	520	1	1	1	1	1	1	1	1									
JD07	440	1	1	1	1	1	1	1	1									

Appendix F continued...

Primer	Molecular Weight (bp)	Genotypes from Transect Y						
		Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
JD01	2150	1	1	1	1	1	1	1
JD01	1450	0	1	1	1	0	0	0
JD01	1300	1	0	0	0	1	1	1
JD01	1220	0	0	0	1	1	0	0
JD01	1070	1	1	1	1	1	1	1
JD01	710	0	0	1	0	0	0	0
JD01	590	1	1	1	1	1	1	1
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
JD02	2200	0	0	1	0	0	1	1
JD02	1750	1	1	1	1	1	1	1
JD02	1060	1	1	1	1	1	1	1
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
JD03	2150	1	1	1	0	0	0	0
JD03	1750	1	1	0	1	1	1	1
JD03	1630	0	0	1	0	0	0	0
JD03	1100	1	1	1	1	1	0	1
JD03	1060	1	0	0	0	0	0	0
JD03	890	1	1	1	1	1	1	1
JD03	520	0	0	1	0	0	0	1
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
JD05	1070	1	1	1	1	1	1	1
JD05	620	1	1	1	1	1	1	1
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
JD06	1990	0	0	0	0	0	1	0
JD06	1260	0	1	1	1	0	0	0
JD06	1150	1	0	0	0	1	1	0
JD06	1020	1	1	0	1	0	1	1
JD06	850	1	1	1	1	0	1	1
JD06	790	1	1	1	1	1	1	1
JD06	610	1	1	1	1	1	1	1
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
JD07	1490	1	1	1	1	1	1	1
JD07	920	0	0	1	0	0	0	1
JD07	780	0	0	0	0	1	0	0
JD07	520	1	1	1	1	0	1	0
JD07	440	1	1	1	1	1	1	1

Primer	Molecular Weight (bp)	Genotypes from Transect Z					
		Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD01	2150	1	1	1	1	1	1
JD01	1450	1	1	1	1	1	1
JD01	1360	1	0	0	0	0	0
JD01	1220	0	0	0	0	1	0
JD01	1120	0	1	0	0	1	0
JD01	1070	1	0	1	1	0	1
JD01	1040	0	1	0	0	1	0
JD01	590	1	1	1	1	1	1
Primer	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD02	2200	1	1	1	1	0	1
JD02	1750	1	1	1	1	1	1
JD02	1270	1	1	0	0	1	0
JD02	1060	1	1	1	1	1	1
Primer	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD03	3000	1	0	0	0	0	0
JD03	2000	1	1	0	1	1	1
JD03	1875	1	1	1	0	1	1
JD03	1750	1	1	0	1	0	0
JD03	1100	0	1	1	1	0	0
JD03	890	1	1	1	1	1	1
JD03	840	0	0	0	0	1	0
Primer	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD05	1450	0	1	1	1	1	0
JD05	1380	1	1	1	1	1	1
JD05	1070	1	1	1	1	1	1
JD05	820	1	1	0	1	0	0
JD05	620	1	1	1	1	1	1
Primer	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD06	1050	0	0	1	0	0	0
JD06	860	1	1	1	1	1	1
JD06	790	1	1	1	1	1	1
JD06	610	1	1	1	1	1	1
Primer	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD07	2000	1	1	1	1	1	1
JD07	1490	1	1	1	1	1	1
JD07	900	1	0	0	1	1	1
JD07	520	1	1	1	1	1	1
JD07	440	1	1	1	1	1	1

Appendix G: Binomial Data produced from the next five primers for the *A. ostoyae* genets along e transect.

Binomial Data: (1) indicates presence of marker, (0) indicates absence of marker

Primer	Molecular Weight (bp)	Genotypes from Transect S							Primer	Molecular Weight (bp)	Genotypes from Transect T						
		S-1	S-2	S-3	S-4	S-5	S-6	S-7			T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD04	2100	1	1	1	1	1	1	1	JD04	2100	1	1	1	1	1	1	1
JD04	1980	0	0	0	0	1	0	0	JD04	1675	1	1	1	1	1	1	1
JD04	1750	1	0	0	0	0	0	0	JD04	1450	1	1	1	1	1	1	1
JD04	1675	1	1	1	1	1	1	1	JD04	1200	1	1	0	0	1	1	1
JD04	1600	0	0	0	1	0	0	0	JD04	1150	0	1	0	1	1	0	1
JD04	1450	1	1	1	1	1	0	1									
JD04	1340	0	0	0	0	0	1	0									
JD04	1200	1	1	1	0	1	1	1									
JD04	1150	0	0	0	1	0	1	1									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD08	2050	0	1	1	1	0	0	0	JD08	1725	0	0	0	1	0	1	0
JD08	1700	0	0	0	0	1	0	0	JD08	1430	1	1	1	0	1	1	1
JD08	1300	0	0	0	1	0	0	1	JD08	1140	1	1	1	1	1	1	1
JD08	1140	1	1	1	1	1	1	1	JD08	1030	1	1	1	1	1	1	1
JD08	1030	1	1	1	1	1	1	1	JD08	880	1	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1	1	JD08	740	1	0	0	1	0	1	1
JD08	740	0	0	0	0	1	0	1									
JD08	600	1	0	0	1	0	1	1									
JD08	510	1	1	1	0	1	0	0									
JD08	420	0	0	0	0	1	0	0									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD11	1170	1	0	0	0	0	1	0	JD11	1750	0	0	0	1	1	0	0
JD11	870	0	0	0	0	1	0	0									
JD11	430	1	0	0	1	1	1	1									
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD12	1010	1	1	1	1	1	1	1	JD12	1390	0	0	0	1	1	1	0
JD12	990	1	1	1	1	1	1	1	JD12	990	1	1	1	1	1	1	1
JD12	580	0	0	0	0	0	1	1	JD12	580	0	0	0	1	0	1	0
JD12	360	1	0	0	0	0	0	0	JD12	360	1	0	0	0	0	0	1
Primer	**M.W.**	S-1	S-2	S-3	S-4	S-5	S-6	S-7	**Primer**	**M.W.**	T-1	T-2	T-3	T-4	T-5	T-6	T-7
JD13	1490	1	1	1	1	1	1	1	JD13	1490	1	1	1	1	1	1	1
JD13	1250	0	1	1	0	0	0	0	JD13	1210	0	0	0	0	0	0	1
JD13	1200	0	0	0	0	0	0	1	JD13	1200	0	1	0	0	0	0	0
JD13	1180	0	1	1	0	0	0	0	JD13	930	1	1	1	1	1	1	1
JD13	1100	1	0	0	0	0	0	0	JD13	750	0	0	1	0	0	0	0
JD13	930	0	1	1	1	1	1	1									
JD13	920	1	0	0	0	0	0	0									

Primer	Molecular Weight (bp)	Genotypes from Transect U								Primer	Molecular Weight (bp)	Genotypes from Transect V						
		U-1	U-2	U-3	U-4							V-1	V-2	V-3	V-4	V-5	V-6	
JD04	2100	1	1	1	1					JD04	2100	1	1	1	1	1	1	
JD04	1980	1	1	0	0					JD04	1675	1	1	1	1	1	1	
JD04	1675	1	1	1	1					JD04	1450	1	1	1	1	1	1	
JD04	1470	0	1	0	0					JD04	1290	0	1	0	0	1	0	
JD04	1450	1	0	1	0					JD04	1200	1	1	1	1	1	1	
JD04	1340	0	0	0	1					JD04	1150	1	0	0	1	1	0	
JD04	1200	1	1	1	0													
JD04	1150	1	0	1	1													

Appendix G continued...

Primer	M.W.	U-1	U-2	U-3	U-4
JD08	1030	1	1	1	1
JD08	880	1	1	1	1
JD08	740	1	1	1	1
JD08	660	1	0	0	0
JD08	550	1	0	0	0
JD08	510	0	0	0	1
Primer	**M.W.**	**U-1**	**U-2**	**U-3**	**U-4**
JD11	1750	0	1	0	0
JD11	1160	1	1	0	1
JD11	520	1	0	0	0
Primer	**M.W.**	**U-1**	**U-2**	**U-3**	**U-4**
JD12	1875	0	1	1	1
JD12	990	1	0	1	0
JD12	580	0	0	1	0
Primer	**M.W.**	**U-1**	**U-2**	**U-3**	**U-4**
JD13	1490	1	1	1	1
JD13	1280	1	1	0	1
JD13	1200	0	0	1	0
JD13	930	1	1	1	1

Primer	M.W.	V-1	V-2	V-3	V-4	V-5	V-6
JD08	1430	1	1	1	1	1	1
JD08	1140	1	1	1	1	1	1
JD08	1030	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1
JD08	740	0	0	1	0	0	1
JD08	550	1	1	1	0	1	1
JD08	510	0	0	0	1	1	0
JD08	420	1	0	0	0	0	1
Primer	**M.W.**	**V-1**	**V-2**	**V-3**	**V-4**	**V-5**	**V-6**
JD11	980	1	1	0	0	1	1
JD11	920	0	0	0	1	0	0
JD11	590	0	0	0	0	1	0
Primer	**M.W.**	**V-1**	**V-2**	**V-3**	**V-4**	**V-5**	**V-6**
JD12	990	1	1	1	1	1	1
JD12	580	1	1	0	0	0	0
JD12	510	0	0	0	1	0	0
JD12	360	0	0	1	0	0	0
Primer	**M.W.**	**V-1**	**V-2**	**V-3**	**V-4**	**V-5**	**V-6**
JD13	1490	1	1	1	1	1	1
JD13	1280	1	1	0	1	1	1
JD13	1200	0	0	1	0	0	1
JD13	930	1	1	1	1	1	1

Primer	Molecular Weight (bp)	Genotypes from Transect W							
		W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8
JD04	2100	1	1	1	1	1	1	1	1
JD04	1675	1	1	1	1	1	1	1	1
JD04	1450	1	1	1	1	1	1	1	1
JD04	1340	0	0	1	0	0	0	0	0
JD04	1200	1	1	1	1	1	1	1	1
Primer	**M.W.**	**W-1**	**W-2**	**W-3**	**W-4**	**W-5**	**W-6**	**W-7**	**W-8**
JD08	1140	1	1	1	1	1	1	1	1
JD08	1030	1	1	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1	1	1
JD08	740	0	1	0	1	0	0	0	0
Primer	**M.W.**	**W-1**	**W-2**	**W-3**	**W-4**	**W-5**	**W-6**	**W-7**	**W-8**
JD11	920	0	1	0	1	0	0	0	0
Primer	**M.W.**	**W-1**	**W-2**	**W-3**	**W-4**	**W-5**	**W-6**	**W-7**	**W-8**
JD12	1390	1	1	1	1	0	1	1	1
JD12	1160	1	1	1	1	1	1	1	1
JD12	990	1	1	1	1	1	1	1	0
JD12	580	0	0	0	1	0	0	0	0
JD12	360	0	0	0	0	0	0	1	0
Primer	**M.W.**	**W-1**	**W-2**	**W-3**	**W-4**	**W-5**	**W-6**	**W-7**	**W-8**
JD13	1490	1	1	1	1	1	1	1	1
JD13	1200	0	0	0	0	0	0	1	0
JD13	930	1	1	1	1	1	1	1	1

Primer	Molecular Weight (bp)	Genotypes from Transect X						
		X-1	X-2	X-3	X-4	X-5	X-6	X-7
JD04	2100	1	1	1	1	1	1	1
JD04	1675	1	1	1	1	1	1	1
JD04	1450	1	1	1	1	1	0	1
JD04	1340	0	0	0	0	1	0	0
JD04	1200	1	1	0	1	1	1	1
Primer	**M.W.**	**X-1**	**X-2**	**X-3**	**X-4**	**X-5**	**X-6**	**X-7**
JD08	1140	1	1	1	1	1	0	1
JD08	1030	1	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1	1
JD08	740	1	1	0	0	0	0	0
JD08	600	0	1	0	1	0	0	0
JD08	550	1	0	0	0	0	0	1
Primer	**M.W.**	**X-1**	**X-2**	**X-3**	**X-4**	**X-5**	**X-6**	**X-7**
JD11	NO CONSISTENT BANDS							
Primer	**M.W.**	**X-1**	**X-2**	**X-3**	**X-4**	**X-5**	**X-6**	**X-7**
JD12	990	1	1	1	1	1	1	1
JD12	580	0	1	1	1	0	1	0
JD12	360	1	0	1	0	0	0	0
Primer	**M.W.**	**X-1**	**X-2**	**X-3**	**X-4**	**X-5**	**X-6**	**X-7**
JD13	1490	1	1	1	1	1	1	1
JD13	1190	1	0	1	1	0	0	1
JD13	1120	1	1	1	1	1	1	0
JD13	930	1	1	1	0	1	1	1

Appendix G continued...

Primer	Molecular Weight (bp)	Genotypes from Transect Y							Primer	Molecular Weight (bp)	Genotypes from Transect Z					
		Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7			Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD04	2100	1	1	1	1	1	1	1	JD04	2100	1	1	1	1	1	1
JD04	1860	1	1	1	0	1	1	1	JD04	1880	1	0	0	0	0	1
JD04	1770	0	0	1	0	0	0	0	JD04	1675	1	1	1	1	1	1
JD04	1675	1	1	1	1	1	1	1	JD04	1450	1	1	1	1	1	1
JD04	1450	1	0	1	1	1	1	1	JD04	1150	0	1	1	0	1	0
JD04	1300	0	0	0	1	1	0	0								
JD04	1200	1	1	0	1	1	1	1								
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	**Primer**	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD08	1030	1	1	1	1	1	1	1	JD08	1550	0	0	0	0	0	1
JD08	880	1	1	1	1	1	1	1	JD08	1430	1	1	0	1	1	0
JD08	740	1	0	0	0	0	1	0	JD08	1140	1	1	1	1	1	1
JD08	510	0	0	1	0	0	0	0	JD08	1030	1	1	1	1	1	1
JD08	420	0	1	1	1	1	1	0	JD08	880	1	1	1	1	1	1
									JD08	740	0	0	0	1	0	0
									JD08	600	0	0	0	1	0	0
									JD08	510	0	0	1	0	0	0
									JD08	420	0	1	1	0	1	0
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	**Primer**	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD11	NO CONSISTENT BANDS								JD11	1700	0	0	0	0	1	0
									JD11	1160	1	1	0	1	1	1
									JD11	590	0	0	0	1	0	0
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	**Primer**	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD12	1280	0	1	1	1	0	0	0	JD12	990	1	1	1	0	1	1
JD12	990	1	1	1	1	1	1	1	JD12	820	0	1	0	0	0	0
JD12	580	0	0	0	0	1	0	0	JD12	510	0	1	1	1	1	0
JD12	360	0	0	0	0	0	0	1								
Primer	**M.W.**	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	**Primer**	**M.W.**	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD13	1900	0	0	0	0	1	0	0	JD13	1490	1	1	1	1	1	1
JD13	1490	1	1	1	1	1	1	1	JD13	1250	1	1	0	0	1	0
JD13	930	1	1	1	1	1	1	1	JD13	1220	0	0	1	0	0	0
JD13	750	0	0	0	0	0	1	0	JD13	1200	0	0	0	1	0	0
									JD13	1190	0	0	0	0	0	1
									JD13	1160	1	1	0	0	1	0
									JD13	930	0	1	1	1	1	1
									JD13	750	1	1	0	0	1	1
									JD13	720	0	0	1	1	0	0

Appendix H: Similarity matrices (Dice's similarity coefficients) for the genets of each trans based on all 11 primers.

Genet	S-1	S-2	S-3	S-4	S-5	S-6	S-7
S-1	1.000						
S-2	0.348	1.000					
S-3	0.476	0.833	1.000				
S-4	0.333	0.381	0.474	1.000			
S-5	0.455	0.400	0.522	0.400	1.000		
S-6	0.474	0.409	0.550	0.588	0.476	1.000	
S-7	0.450	0.435	0.571	0.667	0.500	0.737	1.000

Genet	T-1	T-2	T-3	T-4	T-5	T-6	T-7
T-1	1.000						
T-2	0.526	1.000					
T-3	0.471	0.556	1.000				
T-4	0.211	0.400	0.222	1.000			
T-5	0.235	0.556	0.250	0.556	1.000		
T-6	0.381	0.455	0.300	0.545	0.500	1.000	
T-7	0.500	0.476	0.316	0.286	0.526	0.522	1.000

Genet	U-1	U-2	U-3	U-4
U-1	1.000			
U-2	0.465	1.000		
U-3	0.368	0.341	1.000	
U-4	0.256	0.571	0.216	1.000

Genet	V-1	V-2	V-3	V-4	V-5	V-6
V-1	1.000					
V-2	0.600	1.000				
V-3	0.500	0.308	1.000			
V-4	0.529	0.375	0.400	1.000		
V-5	0.625	0.467	0.429	0.471	1.000	
V-6	0.571	0.485	0.452	0.541	0.343	1.000

Genet	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8
W-1	1.000							
W-2	0.500	1.000						
W-3	0.545	0.743	1.000					
W-4	0.438	0.941	0.686	1.000				
W-5	0.560	0.370	0.357	0.296	1.000			
W-6	0.692	0.571	0.621	0.500	0.667	1.000		
W-7	0.571	0.533	0.581	0.467	0.435	0.667	1.000	
W-8	0.500	0.615	0.667	0.538	0.316	0.700	0.636	1.000

Genet	X-1	X-2	X-3	X-4	X-5	X-6	X-7
X-1	1.000						
X-2	0.593	1.000					
X-3	0.621	0.692	1.000				
X-4	0.714	0.720	0.667	1.000			
X-5	0.621	0.615	0.571	0.667	1.000		
X-6	0.538	0.609	0.640	0.667	0.640	1.000	
X-7	0.692	0.435	0.480	0.583	0.640	0.455	1.000

Appendix H continued...

Genet	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7
Y-1	1.000						
Y-2	0.640	1.000					
Y-3	0.400	0.621	1.000				
Y-4	0.538	0.800	0.533	1.000			
Y-5	0.538	0.400	0.267	0.538	1.000		
Y-6	0.741	0.538	0.387	0.519	0.519	1.000	
Y-7	0.640	0.500	0.483	0.480	0.480	0.615	1.000

Genet	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
Z-1	1.000					
Z-2	0.632	1.000				
Z-3	0.258	0.514	1.000			
Z-4	0.471	0.526	0.452	1.000		
Z-5	0.541	0.780	0.412	0.378	1.000	
Z-6	0.621	0.424	0.385	0.414	0.438	1.000

Appendix I: Binomial Data for all 52 *A. ostoyae* genets produced from 11 primers.

Binomial Data: (1) indicates presence of marker, (0) indicates absence of marker

	Molecuar	Genet																																																			
Primer	Weight (bp)	S-1	S-2	S-3	S-4	S-5	S-6	S-7	T-1	T-2	T-3	T-4	T-5	T-6	T-7	U-1	U-2	U-3	U-4	V-1	V-2	V-3	V-4	V-5	V-6	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	X-1	X-2	X-3	X-4	X-5	X-6	X-7	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD01	2150	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD01	1300	1	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1
JD01	1220	0	1	1	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0
JD01	1070	0	0	0	0	1	0	0	0	1	0	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1
JD01	710	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
JD01	590	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD01	410	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
JD02	2200	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	0	0	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1
JD02	1750	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
JD02	1070	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD03	2600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD03	1750	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0
JD03	1100	1	1	1	1	0	1	1	1	1	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	1	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	0
JD03	1060	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
JD03	890	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD03	840	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
JD03	670	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD05	960	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD06	1990	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
JD06	850	1	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
JD06	790	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD07	1800	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD07	780	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
JD07	520	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1
JD07	440	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD07	400	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD07	330	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD04	2100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD04	1980	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD04	1675	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD04	1450	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1
JD04	1340	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD04	710	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Appendix I continued...

Primer	Molecuar Weight (bp)	S-1	S-2	S-3	S-4	S-5	S-6	S-7	T-1	T-2	T-3	T-4	T-5	T-6	T-7	U-1	U-2	U-3	U-4	V-1	V-2	V-3	V-4	V-5	V-6
JD08	1030	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD08	740	0	0	0	0	1	0	1	1	0	0	1	0	1	1	1	1	1	1	0	0	1	0	0	1
JD08	660	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
JD08	600	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD08	420	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
JD12	990	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1
JD12	580	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	0
JD12	360	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
JD13	1490	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD13	1200	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1
JD13	930	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD13	750	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Primer	Molecuar Weight (bp)	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	X-1	X-2	X-3	X-4	X-5	X-6	X-7	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
JD08	1030	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD08	880	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD08	740	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0
JD08	660	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JD08	600	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
JD08	420	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	1	0
JD12	990	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
JD12	580	0	0	0	1	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
JD12	360	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
JD13	1490	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JD13	1200	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
JD13	930	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
JD13	750	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1

Appendix J: Similarity matrix (Dice's similarity coefficient) for 52 genets

Genet	S-1	S-2	S-3	S-4	S-5	S-6	S-7	T-1	T-2	T-3	T-4	T-5	T-6	T-7	U-1	U-2	U-3	U-4
S-1	1.000																	
S-2	0.600	1.000																
S-3	0.720	0.839	1.000															
S-4	0.727	0.643	0.783	1.000														
S-5	0.600	0.611	0.710	0.571	1.000													
S-6	0.667	0.600	0.720	0.727	0.533	1.000												
S-7	0.640	0.581	0.692	0.783	0.581	0.800	1.000											
T-1	0.609	0.621	0.750	0.762	0.621	0.609	0.750	1.000										
T-2	0.667	0.600	0.720	0.727	0.667	0.667	0.800	0.696	1.000									
T-3	0.583	0.600	0.720	0.636	0.733	0.583	0.640	0.696	0.750	1.000								
T-4	0.560	0.581	0.692	0.696	0.710	0.720	0.846	0.750	0.800	0.720	1.000							
T-5	0.636	0.643	0.783	0.700	0.714	0.727	0.696	0.667	0.818	0.727	0.783	1.000						
T-6	0.560	0.581	0.692	0.696	0.774	0.640	0.769	0.667	0.720	0.640	0.846	0.783	1.000					
T-7	0.750	0.667	0.800	0.727	0.733	0.667	0.720	0.783	0.667	0.667	0.720	0.818	0.800	1.000				
U-1	0.538	0.563	0.667	0.583	0.750	0.462	0.593	0.640	0.615	0.692	0.593	0.583	0.667	0.692	1.000			
U-2	0.640	0.645	0.769	0.609	0.774	0.640	0.615	0.667	0.640	0.640	0.615	0.609	0.615	0.720	0.741	1.000		
U-3	0.640	0.581	0.692	0.696	0.710	0.640	0.769	0.667	0.720	0.640	0.692	0.696	0.769	0.800	0.667	0.692	1.000	
U-4	0.560	0.581	0.692	0.609	0.645	0.720	0.615	0.583	0.640	0.480	0.692	0.696	0.692	0.720	0.519	0.769	0.615	1.000
V-1	0.640	0.581	0.692	0.696	0.774	0.640	0.615	0.583	0.720	0.640	0.692	0.783	0.769	0.720	0.593	0.615	0.846	0.615
V-2	0.583	0.533	0.640	0.727	0.667	0.583	0.640	0.609	0.750	0.667	0.800	0.636	0.720	0.583	0.615	0.560	0.720	0.560
V-3	0.640	0.581	0.692	0.783	0.645	0.560	0.769	0.833	0.800	0.560	0.769	0.696	0.769	0.800	0.593	0.615	0.769	0.692
V-4	0.583	0.667	0.800	0.727	0.800	0.583	0.560	0.696	0.667	0.750	0.720	0.818	0.720	0.750	0.615	0.640	0.720	0.640
V-5	0.750	0.733	0.880	0.818	0.733	0.750	0.720	0.696	0.833	0.667	0.800	0.909	0.800	0.833	0.615	0.720	0.720	0.800
V-6	0.444	0.667	0.714	0.640	0.727	0.444	0.643	0.692	0.667	0.593	0.643	0.640	0.714	0.667	0.621	0.571	0.714	0.571
W-1	0.783	0.759	0.917	0.857	0.690	0.783	0.750	0.727	0.783	0.696	0.750	0.857	0.750	0.870	0.640	0.750	0.750	0.750
W-2	0.538	0.750	0.815	0.667	0.750	0.538	0.667	0.720	0.692	0.692	0.741	0.750	0.815	0.769	0.714	0.667	0.667	0.667
W-3	0.560	0.710	0.769	0.696	0.710	0.640	0.615	0.667	0.720	0.720	0.692	0.783	0.769	0.720	0.667	0.615	0.615	0.692
W-4	0.519	0.727	0.786	0.640	0.727	0.593	0.714	0.692	0.667	0.667	0.786	0.720	0.857	0.741	0.690	0.643	0.714	0.643
W-5	0.727	0.643	0.783	0.800	0.643	0.727	0.696	0.762	0.818	0.727	0.696	0.800	0.609	0.727	0.583	0.696	0.783	0.609
W-6	0.696	0.690	0.833	0.857	0.690	0.696	0.667	0.727	0.783	0.609	0.750	0.857	0.750	0.783	0.560	0.667	0.750	0.750
W-7	0.720	0.581	0.692	0.696	0.710	0.560	0.692	0.667	0.880	0.720	0.692	0.783	0.769	0.800	0.667	0.615	0.769	0.615
W-8	0.476	0.593	0.727	0.632	0.667	0.476	0.545	0.600	0.667	0.667	0.636	0.737	0.727	0.667	0.609	0.636	0.545	0.636
X-1	0.769	0.625	0.741	0.750	0.750	0.615	0.741	0.800	0.846	0.692	0.815	0.750	0.815	0.846	0.714	0.741	0.741	0.741
X-2	0.667	0.606	0.714	0.720	0.667	0.815	0.857	0.692	0.741	0.593	0.857	0.800	0.786	0.741	0.552	0.643	0.714	0.714
X-3	0.692	0.625	0.741	0.667	0.625	0.769	0.741	0.720	0.769	0.615	0.815	0.833	0.741	0.769	0.500	0.593	0.667	0.667
X-4	0.846	0.625	0.741	0.750	0.688	0.769	0.741	0.560	0.769	0.615	0.741	0.750	0.741	0.692	0.571	0.667	0.741	0.667
X-5	0.667	0.727	0.857	0.720	0.727	0.741	0.643	0.692	0.741	0.667	0.714	0.800	0.714	0.741	0.690	0.714	0.643	0.786
X-6	0.667	0.667	0.800	0.727	0.667	0.833	0.720	0.609	0.750	0.583	0.800	0.818	0.800	0.750	0.538	0.720	0.720	0.800
X-7	0.696	0.690	0.833	0.857	0.621	0.696	0.667	0.727	0.696	0.609	0.667	0.762	0.667	0.783	0.640	0.667	0.750	0.667
Y-1	0.692	0.625	0.741	0.750	0.813	0.615	0.667	0.720	0.769	0.615	0.741	0.750	0.815	0.769	0.643	0.741	0.815	0.741
Y-2	0.609	0.621	0.750	0.762	0.690	0.696	0.583	0.636	0.696	0.522	0.667	0.762	0.667	0.696	0.480	0.667	0.667	0.750
Y-3	0.640	0.645	0.769	0.696	0.710	0.720	0.692	0.667	0.800	0.640	0.769	0.870	0.692	0.720	0.519	0.615	0.615	0.692
Y-4	0.640	0.710	0.846	0.783	0.774	0.640	0.615	0.750	0.720	0.640	0.692	0.783	0.692	0.720	0.593	0.692	0.692	0.692
Y-5	0.538	0.563	0.667	0.667	0.688	0.538	0.593	0.640	0.692	0.615	0.667	0.583	0.667	0.538	0.643	0.593	0.667	0.519
Y-6	0.593	0.545	0.643	0.560	0.848	0.519	0.571	0.538	0.667	0.741	0.714	0.720	0.714	0.741	0.621	0.643	0.714	0.643
Y-7	0.769	0.625	0.741	0.667	0.688	0.615	0.593	0.640	0.769	0.615	0.667	0.750	0.667	0.769	0.643	0.667	0.667	0.667
Z-1	0.696	0.621	0.750	0.667	0.690	0.609	0.583	0.545	0.696	0.696	0.667	0.857	0.750	0.783	0.560	0.583	0.667	0.667
Z-2	0.720	0.710	0.846	0.783	0.710	0.720	0.692	0.667	0.720	0.720	0.692	0.783	0.692	0.800	0.593	0.692	0.692	0.692
Z-3	0.667	0.667	0.800	0.727	0.733	0.750	0.720	0.696	0.833	0.667	0.800	0.909	0.720	0.750	0.538	0.640	0.640	0.720
Z-4	0.692	0.625	0.741	0.750	0.688	0.692	0.815	0.640	0.769	0.538	0.741	0.750	0.741	0.769	0.571	0.741	0.741	0.815
Z-5	0.500	0.667	0.800	0.636	0.667	0.583	0.560	0.696	0.583	0.750	0.560	0.727	0.560	0.667	0.538	0.560	0.640	0.480
Z-6	0.640	0.581	0.692	0.609	0.710	0.640	0.615	0.583	0.800	0.800	0.692	0.870	0.692	0.720	0.667	0.615	0.692	0.615

Appendix J continued...

V-1	V-2	V-3	V-4	V-5	V-6	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	X-1	X-2	X-3	X-4	X-5	X-6	X-7
1.000																				
0.800	1.000																			
0.692	0.720	1.000																		
0.800	0.750	0.720	1.000																	
0.800	0.750	0.800	0.833	1.000																
0.714	0.593	0.786	0.741	0.667	1.000															
0.750	0.696	0.750	0.783	0.957	0.615	1.000														
0.667	0.615	0.741	0.769	0.769	0.897	0.720	1.000													
0.692	0.640	0.692	0.800	0.800	0.786	0.750	0.889	1.000												
0.714	0.667	0.714	0.741	0.741	0.867	0.692	0.966	0.857	1.000											
0.783	0.727	0.696	0.727	0.818	0.560	0.857	0.583	0.609	0.560	1.000										
0.833	0.783	0.833	0.870	0.957	0.692	0.909	0.720	0.750	0.692	0.857	1.000									
0.769	0.720	0.846	0.720	0.800	0.714	0.750	0.741	0.769	0.714	0.696	0.750	1.000								
0.636	0.571	0.636	0.762	0.762	0.667	0.700	0.783	0.818	0.750	0.526	0.700	0.727	1.000							
0.741	0.769	0.889	0.692	0.846	0.690	0.800	0.786	0.741	0.759	0.750	0.800	0.889	0.696	1.000						
0.714	0.667	0.714	0.667	0.815	0.600	0.769	0.690	0.643	0.733	0.720	0.769	0.643	0.583	0.759	1.000					
0.741	0.692	0.741	0.692	0.846	0.552	0.800	0.643	0.667	0.690	0.750	0.800	0.741	0.609	0.786	0.897	1.000				
0.815	0.769	0.667	0.692	0.846	0.552	0.800	0.643	0.667	0.690	0.750	0.800	0.741	0.609	0.786	0.828	0.786	1.000			
0.714	0.667	0.714	0.815	0.889	0.667	0.846	0.759	0.857	0.733	0.720	0.846	0.714	0.750	0.759	0.733	0.759	0.759	1.000		
0.800	0.750	0.720	0.750	0.917	0.593	0.870	0.692	0.720	0.741	0.727	0.870	0.720	0.667	0.769	0.815	0.846	0.846	0.815	1.000	
0.750	0.696	0.750	0.783	0.870	0.615	0.909	0.640	0.667	0.615	0.857	0.909	0.667	0.600	0.720	0.692	0.720	0.720	0.846	0.783	1.000
0.815	0.769	0.815	0.769	0.846	0.690	0.800	0.714	0.667	0.690	0.833	0.880	0.741	0.609	0.857	0.759	0.714	0.786	0.759	0.769	0.800
0.833	0.696	0.750	0.783	0.870	0.692	0.818	0.640	0.667	0.615	0.762	0.909	0.667	0.600	0.720	0.692	0.720	0.720	0.769	0.870	0.818
0.769	0.640	0.692	0.720	0.880	0.643	0.833	0.667	0.692	0.643	0.783	0.833	0.692	0.636	0.741	0.857	0.889	0.741	0.786	0.800	0.750
0.846	0.720	0.769	0.880	0.880	0.786	0.833	0.741	0.769	0.714	0.783	0.917	0.692	0.727	0.741	0.714	0.741	0.741	0.857	0.800	0.833
0.815	0.846	0.667	0.692	0.692	0.690	0.640	0.643	0.667	0.690	0.667	0.720	0.667	0.609	0.714	0.621	0.643	0.714	0.690	0.692	0.640
0.786	0.667	0.643	0.741	0.741	0.667	0.692	0.690	0.643	0.667	0.640	0.692	0.714	0.583	0.759	0.667	0.621	0.690	0.667	0.667	0.615
0.741	0.769	0.741	0.692	0.846	0.552	0.800	0.643	0.667	0.621	0.750	0.800	0.815	0.609	0.857	0.690	0.786	0.786	0.759	0.769	0.720
0.750	0.609	0.667	0.783	0.870	0.615	0.818	0.720	0.750	0.692	0.667	0.818	0.750	0.700	0.720	0.692	0.720	0.800	0.769	0.783	0.727
0.769	0.640	0.692	0.720	0.880	0.643	0.917	0.667	0.692	0.643	0.783	0.833	0.692	0.636	0.741	0.714	0.741	0.741	0.786	0.800	0.833
0.800	0.667	0.720	0.750	0.917	0.667	0.870	0.692	0.720	0.667	0.818	0.870	0.720	0.667	0.769	0.815	0.846	0.769	0.815	0.833	0.783
0.667	0.615	0.815	0.692	0.846	0.690	0.800	0.714	0.667	0.690	0.667	0.800	0.741	0.696	0.786	0.828	0.714	0.786	0.759	0.769	0.720
0.720	0.500	0.560	0.750	0.667	0.741	0.696	0.692	0.640	0.667	0.727	0.696	0.560	0.571	0.538	0.593	0.615	0.538	0.667	0.583	0.696
0.769	0.640	0.615	0.720	0.800	0.571	0.750	0.667	0.692	0.643	0.783	0.750	0.769	0.636	0.741	0.714	0.741	0.741	0.786	0.720	0.750

Appendix J continued...

Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
1.000												
0.800	1.000											
0.741	0.833	1.000										
0.815	0.917	0.846	1.000									
0.714	0.720	0.667	0.815	1.000								
0.759	0.692	0.714	0.714	0.621	1.000							
0.786	0.720	0.741	0.741	0.714	0.690	1.000						
0.720	0.727	0.750	0.750	0.560	0.769	0.720	1.000					
0.741	0.833	0.846	0.846	0.667	0.786	0.741	0.833	1.000				
0.769	0.870	0.960	0.880	0.692	0.741	0.769	0.783	0.880	1.000			
0.786	0.720	0.741	0.741	0.571	0.690	0.714	0.720	0.741	0.769	1.000		
0.615	0.696	0.720	0.800	0.615	0.667	0.538	0.696	0.800	0.750	0.538	1.000	
0.741	0.667	0.769	0.692	0.593	0.786	0.741	0.833	0.769	0.800	0.667	0.720	1.000