Clonality in the endangered *Ambrosia pumila* (Nutt.) Gray (Asteraceae) inferred from RAPD markers; implications for conservation and management

Mitchell McGlaughlin and Elizabeth Friar

Rancho Santa Ana Botanic Garden / Department of Botany, Claremont Graduate University, 1500 N. College Ave., Claremont, CA, 91711

Abstract

Clonal plants have the ability to spread and survive over long periods of time by vegetative growth. For endangered species, the occurrence of clonality can have significant impacts on levels of genetic diversity, population structure, recruitment, and the implementation of appropriate conservation strategies. In the present study, we examine levels of clonal diversity in three populations of *Ambrosia pumila* (Nutt.) Gray (Asteraceae), an endangered clonal species from southern California. *Ambrosia pumila* is a perennial herbaceous species spreading from a rhizome like root, frequently found in dense patches of several hundred stems in a few square meters. The primary habitat for this species is upper terraces of rivers and drainages in areas that have been heavily impacted by anthropogenic disturbances and changing flood regimes. RAPD markers were employed to document the number and distribution of clones within multiple 0.5m^2 plots from each population. Thirty-one multi-locus genotypes were identified from the 201 stems sampled. The spatial distribution of clones was limited with no genotypes

shared between plots or populations. Mean clone size was estimated at 6.48 ramets per genet. Mean values of genotypic diversity (D=0.93) and evenness (E=0.93) were higher than the average for clonal plant species. Mean genetic similarity was 0.64 among all genets and ranged from 0.78 – 0.94 within populations. Significant divergence among populations was documented, with limited evidence of gene flow. Estimates of the number and distribution of genets within populations will significantly aid the conservation of this species.

Introduction

Clonality complicates many questions for plant biologists studying evolution, land managers protecting clonal populations, and our general understanding of species diversity. Clonal plants are classified at two levels of genetic organization, genets, which include all parts of a genetic individual, and ramets, which represent all stems that make up a genet (Avise, 1994; Harper, 1977). A ramet can be physically independent from the genet to which it belongs. Distinguishing unique genetic clones within a natural population is complicated by the interplay between genets and ramets, and cannot be conclusively elucidated without extensive genetic fingerprinting.

Clonality in rare and endangered plants makes the conservation and management of these species difficult (Spies and Wolf, 1997; Sydes and Peakall, 1998; Xie et al., 2001, Young et al., 2002). First, without genetic data, land managers do not know how many unique genets are represented within or among populations. A large population or an entire species may contain several unique genets (Chung and Epperson, 2000; Kreher et al., 2000) or in the extreme case very few genetic individuals (Kennington et al., 1996;

Sydes and Peakall, 1998; reviewed in Widen et al., 1994). Thus, traditional census procedures have the potential to significantly overestimate the number of individuals in a clonal population. Second, clonal diversity within populations can have impacts on levels of inbreeding, genetic drift, and the ability to react to changing ecological conditions (Barrett and Kohn, 1991; Spies and Wolf, 1997; Young et al., 2002). If a clonal species is self incompatible, a low level of genet diversity can limit the role of sexual reproduction and dispersal by seed (Eriksson, 1993). Third, the spatial distribution of genets can also impact the reproductive output of species. Since animal mediated pollen movement is frequently between neighbors (Spies and Wolf, 1997), if nearest neighbors are from a single genet, outcrossing will fail to create novel recombinant genotypes.

Ambrosia pumila (Nutt.) Gray is a rhizomatous, clonal, perennial species growing in several populations in San Diego and Riverside Counties, California (CNDDB, 2003). In 2002, *A. pumila* was federally listed as endangered (USFWS, 2002). Populations of this species are primarily found on upper river terraces and drainages, areas that are threatened by the construction and maintenance of highways, residential and commercial development, development of recreational facilities, and changing flood regimes. Recent monitoring efforts by the City of San Diego have focused on counting the number of aboveground stems to assess the size of populations (USFWS, 2002). However, these counting efforts may seriously overestimate the diversity of populations, as all aboveground stems may be offshoots of a single genet. The reproductive biology of this species is relatively unknown, however, the genus has both self-fertile and self-incompatible species (Payne, 1976). Thus, a population made up of a single genetic individual or even several closely related individuals could be incapable of setting seed if

it was self incompatible. Additionally, limited germination tests of field-collected seeds have not produced any viable seedlings (USFWS, 2002). It is apparent that census counts of aboveground stems without some information about levels of clonal diversity do not adequately address the potential threats to these populations.

The purpose of this study was to collect genetic data from several populations of *A. pumila* and estimate the number of genets within populations and levels of genetic diversity. Random Amplified Polymorphic DNA (RAPD) markers were employed to identify clones and undertake a fine scale examination of the spatial structure of clones. Three central questions were addressed: 1)What is the spatial distribution of clones within and among sampling plots in a given population? 2) Does this species have common genotypes found among populations across the species range? 3) Do measures of genetic and clonal diversity vary among populations?

Methods

Population Sampling

Ambrosia pumila (Nutt.) Gray (Asteraceae), the San Diego Ambrosia, is an herbaceous perennial species capable of spread by vegetative growth from an underground rhizome like root. Over time, the interconnection of underground roots can disintegrate leaving independent ramets. Aerial stems are 5 to 50cm tall, sprouting from the root in early spring and deteriorating in late summer. During the fall and winter there may not be any visible evidence of the plant above ground. This species is believed to be wind pollinated and bears separate male and female flower heads. *Ambrosia pumila* occurs most frequently on terraces of rivers and drainages. Plants have also been found in

grasslands, openings in costal sage scrub, adjacent to vernal pools, and disturbed sites such as dirt roads and fire breaks (Keck 1959; Payne, 1993; USFWS, 2002). Occurrences of this species have been documented from southern Riverside County, throughout San Diego County, and in north central Baja California, Mexico, from Colonet south to Lake Chapala (Figure 1).

During July 2003, leaf tissue was collected from three populations throughout the known range of *A. pumila* (Figure 1). Population size estimates of the sampled populations ranged from tens of thousands of above ground stems to a few hundred (USFWS, 2002). A plot sampling design was used, where all individuals within a $0.5m^2$ area were sampled. At least two $0.5m^2$ plots were sampled at each population. Plots within a population were separated from 5-500m depending on population size and dispersion. In addition, 2 voucher specimens were collected from each population. All leaf tissue was stored on ice until it could be frozen in a –80˚C freezer.

In the Sweetwater River, drainage plants were sampled from the San Diego National Wildlife Refuge (32°44'20N, 116°55'10W), hereafter abbreviated as population SD (Figure 1). At this population, 24, 11, 13, and 25 stems were sampled from four plots, for a total of 73. This population is found on a wide, gradually sloping river terrace dominated by grasses and some shrubs. At the south end of the site there is a grouping of plants covering ~0.1 hectares (ha) separated by ~400m from a larger northern grouping covering ~1.4ha (USFWS, 2002). Plot SD-A was sampled from the southern section, and Plots SD-B, SD-C, and SD-D were sampled from the northern section of the population (Figure 1). The northern section of the population is estimated to include tens of

thousands of stems, probably more than 50,000, and the southern section of the population is estimated to contain 1,000 stems.

In the San Diego River drainage, plants were sampled from Mission Trails Regional Park (32°50'19N, 117°02'29W), hereafter abbreviated as population MT (Figure 1). At this population, 33, 10, and 29 stems were sampled from three plots for a total of 72. This population is located on a river terrace and small drainage south of Mission Dam, continuing up a hillside dominated by grasses and non-native herbaceous species. This site covered 0.1ha and contained an estimated 10,000 stems. Plot MT-A was located at the bottom of a dense area of *A. pumila* stems, and a transect was run uphill through the center of the population, with the three plots evenly spaced. Other patches have been identified within 1.5km of this site that were not sampled.

In southwestern Riverside County, plants were sampled along Nichols Road near Alberhill (33°42'26N, 117°21'39W), hereafter abbreviated as population NR (Figure 1). At this population, 29 and 27 stems were sampled from two plots for a total of 56. This is one of the most northern known population of this species, and excluding two nearby occurrences, is separated from the rest of the rest of the range by ~40 km. This site is located along a dirt road in a grassland area, with an adjacent intermittent stream. This population covers about 0.05ha and contains fewer than 1000 stems. This population is threatened by a planned of expansion of Nichols Road.

Genetic Sampling

DNA was extracted from leaf tissue using a DNeasy Plant Mini Kit (QIAGEN Inc, Valencia, California). Approximately ten milligrams of tissue was used for each

extraction. DNA was quantified by gel electrophoresis with reference samples of lambda phage DNA of known concentrations. The Random Amplified Poylmorphic DNA (RAPD) technique was employed to genotype each sampled plant. Twenty RAPD primers from the University of British Columbia Biotechnology Laboratory were screened to identify primers that successfully amplified and provided polymorphic bands. Samples from two plots in each of the three populations were used in the initial screening procedure. Three primers that gave clear, polymorphic banding patterns were used for all subsequent analyses. These primers were 322 (GCCGCTACTA), 437 (AGTCCGCTGC), and 457 (CGACGCCCTG), which exhibited 15, 20, and 19 polymorphic bands, respectively. The size of the DNA fragments which were scored ranged from 425 bp to 1380 bp for primer 322, 580 bp to 1335 bp for primer 437, and 475 bp to 1315 bp for primer 457.

PCR reactions were first optimized using a MasterAmp PCR Optimization Kit (Epicentre, Madison, Wisconsin). PCR amplifications were performed in 17 µl reactions: 8.5 µl Epicenter 2X Optimization Premix buffer B, 4.5 µl ddH_2O, 1 µl BSA (10mg/ml), 1 µl oligonucleotide primer (15 µM), 1 µl template DNA (10ng/µl), and 0.1 µl (1 unit) Promega *Taq* Polymerase in storage buffer B (Promega, Madison, Wisconsin). For each PCR run, a single master mix was made for all samples and contained all of the PCR ingredients except for the template DNA. The PCR cycle began with an initial denaturation for 1 minute at 94˚C, followed by 44 cycles of 1 minute at 94˚C, 1 minute at 39˚C, and 2 minutes at 72˚C. The samples were given a final extension of 7 minutes at 72˚C, after which the samples were held at 4˚C.

PCR products were visualized on 2% high-resolution agarose gels (Agarose I, Amresco, Solon, Ohio) in 0.5X TBE buffer. Ethidium bromide was added directly to the agarose gel for a final concentration of 0.4 μg/ml of gel. Gels were run at 35miliamps for 12-14 hours. The banding phenotypes on each gel were visualized using a Stratagene Eagle Eye II digital imaging system (Stratagene, LaJolla, California). Gels were scored using the Eagle Sight Software version 3.2 (Stratagene, LaJolla, California). All bands were scored as present or absent.

Analyses

Each band position was treated as a separate locus, with band presence assumed to be dominant to band absence. All individuals sharing the same multilocus RAPD genotype were assumed to represent the same genet. Following the method of Sydes & Peakall (1998), the probability of obtaining a particular genotype was calculated as the product of the single locus genotype probabilities:

$$P_{gen} = \prod p_i$$

where p_i denotes the frequency of band presence or absence across all loci from all populations, for each observed genotype. The percentage of polymorphic loci (%P), was calculated for each genotype (frequency of band presence) and population.

Four descriptive statistics were employed to quantify genotype diversity at the population and plot level following the methods of (Ellstrand and Roose , 1987; McClintock and Waterway, 1993; Xie et al., 2001)

1) Mean clone size (N/G), where N is the number of sampled individuals and G is the number of genets resolved.

2) Proportion of genotypes detected (PD = G/N).

3) Simpson's diversity index (D) corrected for finite sample size (Pielou, 1969): $D = 1-[\sum n_i(n_i-1)/N(N-1)]$, where n_i is the number of samples of genotype i and N is the total number of samples, was used to as an additional measure of genotype diversity. D values range from 0 to 1, with a value of 0 in populations of only one genotype and a value of 1 in a population where each individual sampled has a unique genotype.

4) Fager's (1972) measure of evenness (E) was calculated as: $E = (D_{obs} - D_{min})/(D_{max} - D_{min})$, where $D_{min} = [(G - 1)(2N - G)]/[N(N - 1)]$ and $D_{max} = [N(G - 1)]/[G(N - 1)]$. Like Simpson's D, Fager's E ranges from 0 to 1, with a value of 0 in populations of only one genotype and 1 in a population where each individual sampled has a unique genotype.

A one tailed t-test was used to test differences between each population for the statistics used to quantify genotypic diversity.

Mean gene diversity (or expected heterozygosity, H_e) was calculated for each population according to Lynch and Milligan (1994), assuming Hardy-Weinberg equilibrium. The number of private alleles was calculated for each population. A similarity index (S_{xy}, Lynch 1990), which quantifies the fraction of shared bands, was also calculated for all pairs of plants within and among populations as:

$$S_{xy} = (2n_{xy})/(n_x + n_y)$$

where n_{xy} is the number of common bands between individual *x* and *y*, and n_x and n_y are the total number of bands for individual *x* and *y*, respectively. A matrix of dissimilarity ($1-S_{xy}$) was constructed for all pair-wise comparisons of genotypes. The software package PHYLIP (Felsenstein, 2004) was used to construct an unrooted UPGMA phenogram.

Results

A total of 201 individual stems were sampled from nine plots across three populations. Using three RAPD primers, 54 polymorphic loci were resolved. Thirty-one unique genotypes were identified across the three sampled populations. All genotypes were restricted to a single plot, and no genotypes were shared between populations (Figure 2). The probability of a given RAPD genotype arising by random was significantly low (assuming $P \geq 0.05$) and ranged from $1.8x10^{-5}$ to $8.1x10^{-11}$ (Table 1). This supports our assumption that each observed genotype represents a unique genet. About one third of all genotypes identified (29%) were represent by only one stem.

The mean clone size (N/G) across populations was 6.48 ramets per genet (Table 2). Mean clone size calculated by plot ranged from 2.20 to 29 ramets per genet (Table 2). The mean proportion of genotypes detected (PD) for all populations was 0.15 and ranged from 0.27 at SD to 0.07 at MT. At the plot level, proportion of genotypes detected ranged from 0.45 to 0.03 at SD-C and MT-C, respectively. Simpson's diversity index (D) was 0.93 for all populations, and ranged from 0.00 to 0.81 at the plot level. Fager's measure of genotypic evenness was 0.93 for all populations combined, and ranged from 0.00 to 0.76 at the plot level (Table 2).

Heterozygosity (H_E) was 0.33 for all populations combined and ranged from 0.04 at NR to 0.19 at SD (Table 3). Populations specific bands were found for each population with 8, 4, and 4 identified for SD, MT, and NR, respectively. The mean similarity index was 0.64 for all populations and ranged from 0.78 to 0.94 at populations SD and NR, respectively (Table 3). The level of genetic diversity appeared to be associated with population size.

The UPGMA phenogram resolved four major groupings of similar genotypes (Figure 4). Plot SD-A did not group with the other plots from population SD, but was more similar to plots from population MT. Genets within plots were more similar to each other than to other plots. Analyzing the dissimilarity data with the neighbor joining algorithm produced identical groupings.

Discussion

Population Level Processes

Levels of clonal diversity varied widely across the range of *Ambrosia pumila*. Multiple genets were identified in each population, however, two of the three plots sampled at the MT population were represented by a single genet unique to each plot. Of the three populations sampled, SD was by far the most diverse with 19 genets identified among four plots, the highest mean proportion of genotypes detected, the lowest mean clone size, and the highest observed D and E values (Table 2). Levels of clonal diversity seemed to be associated with population size. Population SD was the largest population sampled with an estimated 50,000 stems spread over a 1.5ha area. Despite its moderate size, 10,000 individuals spread over 0.1ha, population MT was found to have low levels of clone diversity with only six genets identified among the three plots containing 72 stems, and the lowest observed values of proportion of genotypes detected, and D and E values (Table 2). However, due to its low levels of genet diversity and the high number of stems sampled, population MT had the highest mean clone size of 14.4 ramets per genet. Population NR was the smallest population sampled, with 1,000 individuals covering 0.05 ha, and is the most northern known population of this species (Figure 1). Population

NR had moderate levels of mean clone size, clone diversity, and D and E values, compared to the other two sampled populations (Table 2). A single dominant genet was identified in eight of the nine plots sampled, indicating that clonal spread is occurring at each sampled population (Figure 2).

In the present study, genet size was found to be small. No shared genets were found among plots indicating that clones of *A. pumila* have limited spatial distributions. Additionally, the lack of shared genets among populations indicates that there are no regional genotypes that are present across large areas of the range. Mean clone size was estimated at 6.48 ramets per genet but this value varied widely across plots and populations (2.2 – 29.0). The high within plot genet diversity, especially at population SD, suggests that this species is capable of reproducing sexually, but it is difficult to differentiate between current and historic mating. Due to the clonal nature of this species, rare mating events can lead to the establishment of new genets that are maintained indefinitely through vegetative spread. The lower clone diversity at populations MT and NR can be attributed to lower population sizes, and all measures of genetic and clone diversity are consistent with this finding.

Levels of genetic diversity within populations and the level of divergence among populations can impact the evolutionary potential of a species. The level of genetic diversity in all populations was lower than the average for RAPD markers over all plant species, $H_{pop} = 0.22$, compiled by Nybom (2004; Table 3). Of the three populations sampled, MT and NR had very low levels of genetic diversity. The high values of within population similarity further confirm that most populations contained limited amounts of genetic diversity (Table 3). Despite the lack of diversity within populations, populations

were highly diverged from one another and had low mean similarity values (Table 3). The among population similarity value of 0.64 is similar to values documented for a clonal species of *Vaccinium* with a mixed mating system (Albert et al., 2003). The UPGMA phenogram indicates high similarity within plots, and substantial divergence between populations (Figure 4). Surprisingly, the four plots sampled at SD do not form a single closely related grouping. The fact that plot SD-1 is outside the main SD grouping and is separated by480m from the other SD plots demonstrates how localized sexual reproduction is. Although the level of genetic diversity contained in populations is low, the populations are significantly diverged and have unique genetic components.

Evolutionary Implications

The amount and distribution of clonal diversity has been shown to vary greatly among species and populations (Ellstrand and Roose, 1987; Widen et al., 1994). With the application of DNA techniques such as RAPDs, AFLPs, and microsatellites a growing number of researchers are identifying species with extensive clonal diversity (Albert et al., 2003; Hamelin and Reusch, 2003; Kreher et al., 2000; Persson and Gustavsson, 2001; Suyama et al., 2000). However, species with extremely low levels of clone diversity such as *Haloragodendron lucasii* with only seven known genets continue to be documented (Sydes and Peakall, 1998). In the current study, *Ambrosia pumila* is shown to have moderate to high levels of clone diversity within and among populations. Our measures of genotypic diversity (D = 0.93), the evenness value (E = 0.93), and the proportion of genotypes detected (PD = 0.18) for *A. pumila* were all higher than the clonal plant average complied by Ellstrand and Roose (1987; D = 0.62, E = 0.68, PD = 0.17). When

the same measures of geneotypic diversity and evenness are applied individually to the sampled populations we see that populations MT and NR are much less diverse than the SD population, containing less than half of the total number of genets. This finding suggests that different populations have unique evolutionary histories that are impacting their current levels of clonal diversity.

There are three major mechanisms that could impact the high level of clonal variation found in *A. pumila*. First, the current levels of clonal variation could be explained if populations originated from a genetically diverse set of founders, and that diversity has been maintained over time by clonal growth (Eriksson, 1993). The arrival of new immigrants to a population could also increase the level of clonal variation (Barrett and Kohn, 1991). This mechanism is likely to negatively impact the diversity contained within younger populations or populations that are geographically isolated. The relatively low level of clonal diversity seen in the NR population could be attributed to a lack of dispersal from more diverse populations in the southern part of the species range. Second, clonal diversity could be created and maintained by sexual reproduction and seedling recruitment (Eriksson, 1997). Due to the longevity of genets, relatively few successful matings can produce new genets that are maintained in the populations for long periods of time. Given the spatial distribution of clones within plots, and the high levels of diversity, especially in the SD population, it is likely that sexual reproduction is responsible for some of the variation. Young et al. (2002) documented that the maintenance of high levels of clone diversity in *Rutidosis leiolepis*, a species the exhibits sporophytic self-incompatibility, that could be attributed to sexual reproduction in most populations. Finally, due to the long life span of clonal plants, some levels of genetic

variation can be attributed to somatic mutations (Fernando and Cass, 1996; Klekowski, 1997). Of the three mechanisms presented, *A. pumila* seems to be most impacted by dispersal among populations, breeding within populations, and the maintenance of genets by vegetative spread.

Conservation Implications

The research presented here indicates that *A. pumila* is a genetically diverse clonal species with considerable clonal structure within and among populations. The SD population is by far the most diverse and warrants conservation priority. The NR population is unique due to its low levels of genetic diversity and its high levels of genetic similarity, suggesting that it has undergone a genetic bottleneck, or was founded by a limit number of genets. Genets were found to be very localized, indicating that most populations should contain several unique clones. The level of divergence between populations suggests that a large proportion of the genetic diversity of this species is contained among populations. The divergence between plot SD-1 in the southern portion of population SD clearly illustrates how divergence can occur even at distances of less than a kilometer. This leads to the conclusion that every occurrence of this species is likely to contain unique genetic material.

From the genetic data, it is clear that limited recruitment is occurring, and that the level of sexual reproduction varies by population. The allocation of resources between sexual and asexual reproduction is likely to be impacted by the habitat, resource availability, pollinator availability, and potentially shared self-incompatibility alleles (Alpert and Stuefer, 1997; Eriksson, 1997). The lack of genet diversity and the presence

of two large genets at the MT population indicate that it has a reduced frequency of seedling recruitment. The MT population differs from the other two populations by occurring on a hillside and having a higher density of non-native species. Without more information about the mating system of *A. pumila*, it is difficult to reach conclusions about what factors are having the greatest effect of genet diversity.

On average, a genet was found to contain a minimum of 6.5 ramets, but most likely several more. Since our sampling focused on $0.5m^2$ plots, it is likely that our sampling did not capture all of the ramets of a given genet. However, by sampling multiple plots within a population, we feel that our estimate of ramets per genet will be useful for land managers. When conducting censuses, we would recommend a conservative estimate that unique genets represent one order of magnitude less than the total number of stems growing in a population. From this we would estimate that the SD population contains approximately 5,000 genets, the MT population 1,000 genets, and the NR population 100 genets. It follows, that due to localized nature of genets, the best sampling strategy for seed collections or transplants for population relocation, is to sample widely across existing populations to obtain the maximum number of genets.

Unlike some clonal plant species that contain very low levels of genet diversity, the outlook for *A. pumila* is encouraging. The lack of genets shared across populations is particularly important because this indicates that clones could have unique, adaptive genotypes in different populations. The divergence among populations leads us to strongly recommend protecting as many populations across the species range as possible. Additional research with this species should focus on all aspects of the breeding system and on determining the age and longevity of genets and ramets.

Acknowledgements

We thank Jennifer Cruse-Sander and Hester Bell for comments on the manuscript, Gary Wallace for advice on population localities and the basic biology of *A. pumila*, John Barone and Paul Seeley (Mission Trails Regional Park), Val Urban (US Fish and Wildlife Service), and Debbie Waldecker (CalTrans) for field and permitting assistance. This research was funded by contract from the California Department of Fish and Game (#S0250014) and Rancho Santa Ana Botanic Garden. Collections were made under Federal Fish and Wildlife Permit TE009018-1 and Fish and Wildlife Service Special Use Permit 11720-03007.

Literature Cited

Albert, T., Raspe, O., and A.-L. Jacquemart. 2003. Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. *International Journal of Plant Science* 164: 649-655.

Alpert, P., and J.F. Stuefer. 1997. Division of labour in clonal plants. In *The Ecology and Evolution of Clonal Plants*. (eds. de Kroon, H., and J. van Groenendael). pp. 137-153. Backhuys Publishers, The Netherlands.

Avise, J.C. 1994. Molecular markers, natural history, and evolution. Chapman & Hall, New York.

Barrett, S.C.H., and J.R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: Implication for conservation. In: *Genetics and conservation of rare plants* (eds. Falk, D.A., and K.E. Holsinger), pp. 3-30. Oxford University Press, New York.

CNDDB (California Natural Diversity Database). 2003. Version 3.03, (11/03/2003). California Department of Fish and Game.

Chung, M.G., and B.K. Epperson. 2000. Clonal and spatial genetic structure in *Eurya emarginata* (Theaceae). *Heredity* 84: 170-177.

Ellstrand, N.C., and M.L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* 74: 123-131.

Eriksson, O. 1993. Dynamic of genets in clonal plants. *Trends in Ecology and Evolution* 8: 313-316.

Eriksson, O. 1997. Clonal life histories and the evolution of seed recruitment. In *The Ecology and Evolution of Clonal Plants*. (eds. de Kroon, H., and J. van Groenendael). pp. 211-226. Backhuys Publishers, The Netherlands.

Fager, E.W. 1972. Diversity: a sample study. *American Naturalist* 106: 293-310.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package) version 3.6. *Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.*

Harper, J.L. 1977. The population biology of plants. Academic Press, New York.

Hammerli, A., and T.B.H. Reusch. 2003. Genetic neighborhood of clone structures in eelgrass meadows quantified by spatial autocorrelation of microsatellite markers. *Heredity* 91: 448-455.

Keck, D.D. 1959. Compositae pp. 1073-1227. In *A California Flora* (eds. Munz, P.A., and D.D. Keck). University of California Press, Berkeley, California.

Kennington, W.J., Waycott, M., and S.H. James. 1996. DNA fingerprinting supports notions of clonality in a rare mallee, *Eucalyptus argutifolia*. *Molecular Ecology* 5: 693-696.

Klekowski, E.J. 1997. Somatic mutation theory of clonality. In *The Ecology and Evolution of Clonal Plants*. (eds. de Kroon, H., and J. van Groenendael). pp. 227-241. Backhuys Publishers, The Netherlands.

Kreher, S.A., Fore, S.A., and B.S. Collins. 2000. Genetic variation within and among patches of a clonal species, *Vaccinium stamineum* L. *Molecular Ecology* 9: 1247-1252.

Lynch, M., and B.G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3: 91-99.

Lynch, M. 1990. The similarity index and DNA fingerprinting. *Molecular Biology and Evolution* 7: 478-484.

McClintock, K.A., and M.J. Waterway. 1993. Patterns of allozyme variation and clonal diversity in *Carex lasiocarpa* and *C. pellita* (Cyperaceae). *American Journal of Botany* 80: 1251-1263.

Payne, W. 1976. Biochemistry and species problems in *Ambrosia* (Asteraceae-Ambrosieae). *Plant Systematics and Evolution* 125: 169-178.

Payne, W.W. 1993. *Ambrosia* pp. 193-194. In *The Jepson Manual: Higher Plants of California*. (ed. Hickman, J.C.). University of California Press, Berkeley, California.

Persson, H.A., and B.A. Gustavsson. 2001. The extent of clonality and genetic diversity in lingonberry (*Vaxxinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis.

Pielou, E.C. 1969. An introduction to mathematical ecology. Wiley-Interscience, New York, New York, USA.

Spies, S.D., and P.G. Wolf. 1997. Clonal structure and patterns of allozyme diversity in the rare endemic *Cycladenia humillis* var. *jonesii* (Apocynaceae). American Journal of Botany 84: 401-409.

Suyama, Y., Obayashi, K., and I. Hayashi. 2000. Clonal structure in a dwarf bamboo (*Sasa senanensis*) population inferred from amplified fragement length polymorphism (AFLP) fingerprints. *Molecular Ecology* 9: 901-906.

Sydes, M.A., and R. Peakall. 1998. Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragaceaea) revealed by allozymes and RAPDs. *Molecular Ecology* 7: 87-93.

USFWS (U.S. Fish and Wildlife Service). 2002. Endangered and threatened wildlife and plants; determination of endangered status for *Ambrosia pumila* (San Diego Ambrosia) from southern California. Federal Register 67(127): 44372-44382.

Young, A.G., Hill, J.H., Murray, B.G., and R. Peakall. 2002. Breeding system, genetic diversity and clonal structure in the sub-alpine forb *Rutidosis leiolepis* F. Muell. (Asteraceae). *Biological Conservation* 106: 71-78.

Widen, B., Cronberg, N., and M. Widen. 1994. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. *Folia Geobotanica et Phytotaxonomica* 29: 245-263.

Xie, Z.-W., Lu, Y.-Q., Ge, S., Hong, D.-Y., and F.-Z. Li. 2001. Clonality in wild rice (*Oryza rufipogon,* Poaceae) and its implications for conservation management. *American Journal of Botany* 88: 1058-1064.

Table 1: Descriptive statistics for observed multilocus RAPD genotypes, including number of ramets sampled, number of polymorphic loci, number of clone specific bands, and the probability of genotypes arising independently.

Genotype	Plot	Number of ramets sampled	Number of polymorphic bands (% total)	Private Alleles	Probability of genotype (P_{gen})
SD-A	SD-1	1	26 (48)	1	$1.4x10^{-7}$
SD-B	SD-1	4	26 (48)	0	$6.3x10^{-6}$
SD-C	SD-1	14	25 (46)	0	$2.8x10^{-5}$
SD-D	SD-1	5	26 (48)	0	$3.2x10^{-6}$
SD-E	SD-2	4	29 (54)	0	$8.1x10^{-11}$
SD-F	SD-2	2	27 (50)	0	$3.2x10^{-9}$
SD-G	SD-2	1	28 (52)	0	$3.2x10^{-9}$
SD-H	SD-2	1	28 (52)	0	$3.6x10^{-10}$
SD-I	SD-2	3	27 (50)	0	$6.4x10^{-8}$
SD-J	SD-3	9	26 (48)	0	$5.6x10^{-7}$
SD-K	SD-3	3	26 (48)	0	$2.5x10^{-6}$
SD-L	SD-3	1	26 (48)	0	$2.9x10^{-7}$
SD-M	SD-4	10	25 (46)	0	$3.3x10^{-7}$
SD-N	SD-4	6	24 (44)	0	$1.5x10^{-6}$
SD-O	SD-4	1	26 (48)	0	$3.8x10^{-8}$
SD-P	SD-4	1	27 (50)	0	$1.4x10^{-8}$
SD-Q	SD-4	1	25 (46)	0	$1.7x10^{7}$
SD-R	SD-4	3	26 (48)	1	$5.0x10^{-9}$
SD-S	SD-4	3	26 (48)	0	$3.8x10^{-8}$
MT-A	MT-1	27	28 (52)	0	$2.1x10^{-6}$
MT-B	MT-1	5	29 (54)	0	$4.8x10^{-7}$
MT-C	MT-1	1	29 (54)	0	$2.5x10^{-7}$
MT-D	MT-2	10	28 (52)	1	$1.3x10^{-7}$
MT-E	MT-3	29	27 (50)	0	$1.5x10^{-6}$
NR-A	NR-1	1	26 (48)	0	$3.5x10^{-7}$
NR-B	NR-1	21	25 (46)	0	$1.8x10^{-5}$
NR-C	NR-1	2	26 (48)	0	$1.8x10^{-6}$
NR-D	NR-1	5	26 (48)	0	$3.9x10^{-6}$
NR-E	NR-2	20	23 (43)	0	$7.1x10^{-5}$
NR-F	NR-2	3	24 (44)	0	$1.4x10^{-6}$
NR-G	NR-2	4	24 (44)	0	$8.1x10^{-6}$

Table 2: Genotypic diversity measures for each sampled population and plot.

Population	Plot	N[a]	G[b]	N/G[c]	PD[d]	D[e]	E[f]
SD		**73**	**19**	**3.65**	**0.27**	**0.92**	**0.91**
	SD-1	24	4	6.00	0.17	0.61	0.69
	SD-2	11	5	2.20	0.45	0.81	0.76
	SD-3	13	3	4.33	0.23	0.60	0.46
	SD-4	25	7	3.57	0.28	0.78	0.76
MT		**72**	**5**	**14.4**	**0.07**	**0.69**	**0.83**
	MT-1	33	3	11	0.09	0.33	0.37
	MT-2	10	1	10	0.10	0.00	0.00
	MT-3	29	1	29	0.03	0.00	0.00
NR		**56**	**7**	**8.00**	**0.13**	**0.77**	**0.85**
	NR-1	29	4	4.83	0.21	0.63	0.58
	NR-2	27	3	9.00	0.11	0.43	0.53
All Populations		**201**	**31**	**6.48**	**0.15**	**0.93**	**0.93**

a. Sample size.
b. The number of genotypes detected.
c. The mean clone size.
d. Proportion of genotypes detected (G/N).
e, f. $D = 1-[\sum n_i(n_i-1)/N(N-1)]$ and $E = (D_{obs} – D_{min})/(D_{max} – D_{min})$, where $D_{min} = [(G – 1)(2N – G)]/[N(N – 1)]$ and $D_{max} = [N(G – 1)]/[G(N – 1)]$, n_i is the number of samples of genotype i, and N is the total number of samples, G is the number of genotypes.

Table 3: Genetic diversity and similarity measures for each sampled population.

Population	# stems	# genets	%P	He	Private Alleles	S_{xy}
SD	73	19	57.41	0.19	8	0.78
MT	72	5	35.19	0.10	4	0.87
NR	56	7	14.81	0.04	4	0.94
All Populations	201	31	98.15	0.33		0.64

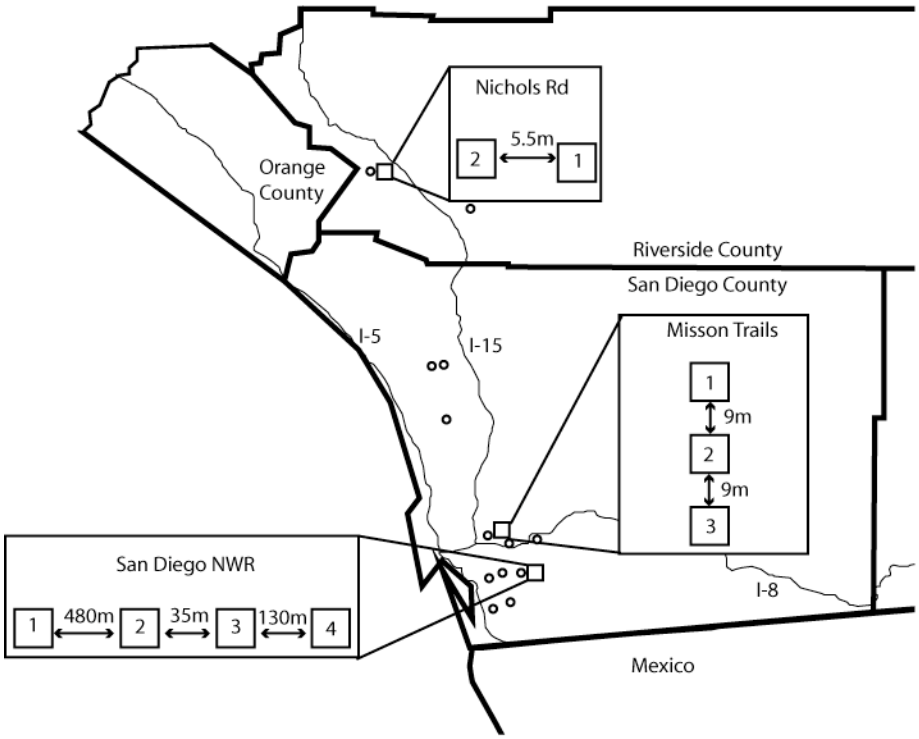

Figure 1: Map of the species range of *Ambrosia pumila*. The sampled populations are shown with squares and inset maps of the plot sampling schemes. Extant populations that were not sampled are indicated with circles. This species is known to occur in Mexico, but the exact location of populations in not well document so they are not shown here.

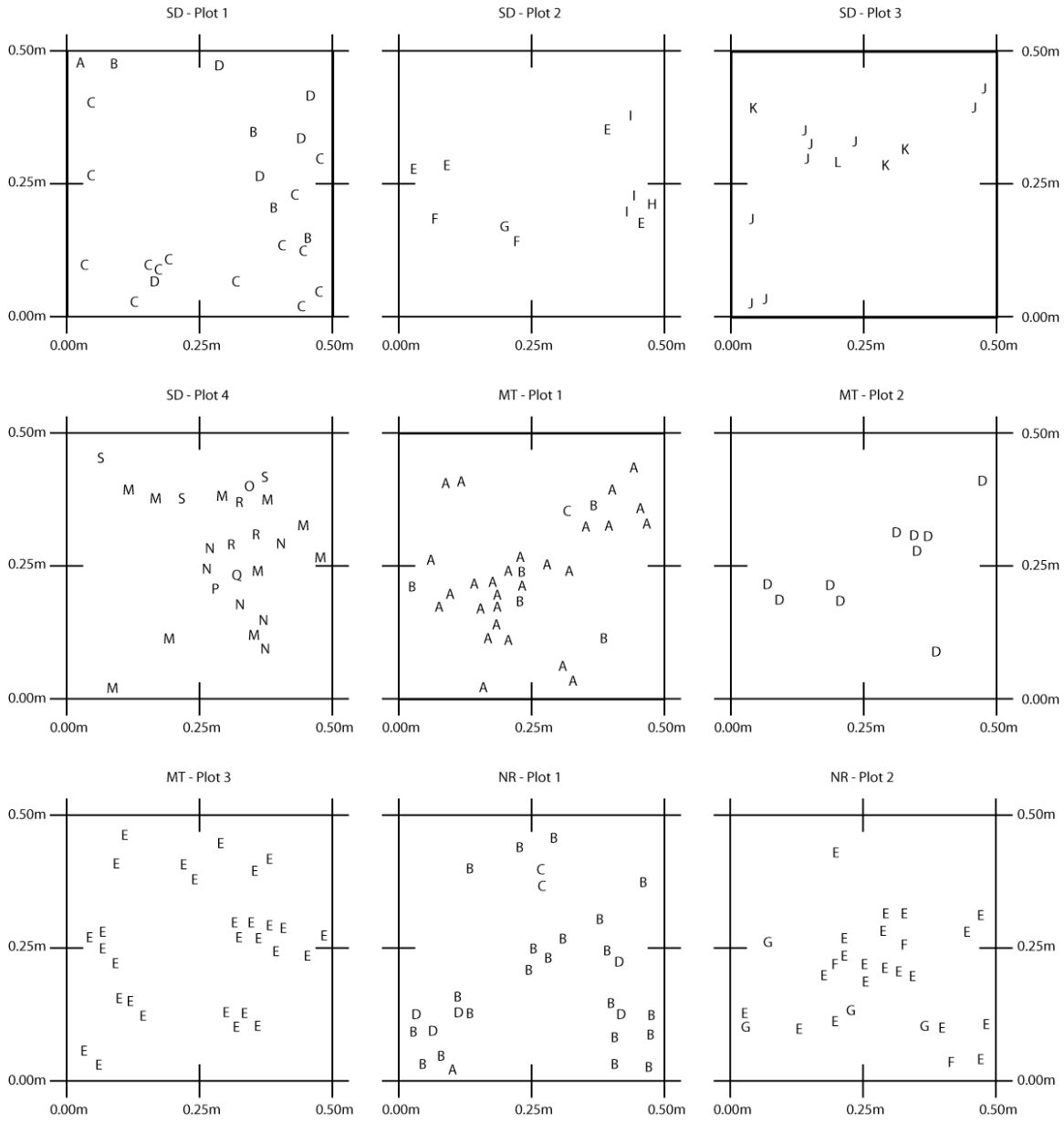

Figure 2: Plot maps for each of the 9 sampled plots. Each letter represents a sampled stem, matching letters within a plot indicate a shared genotype and hence membership to the same genet. No genotypes were shared between plot. Letters assigning genotype start with A in each population.

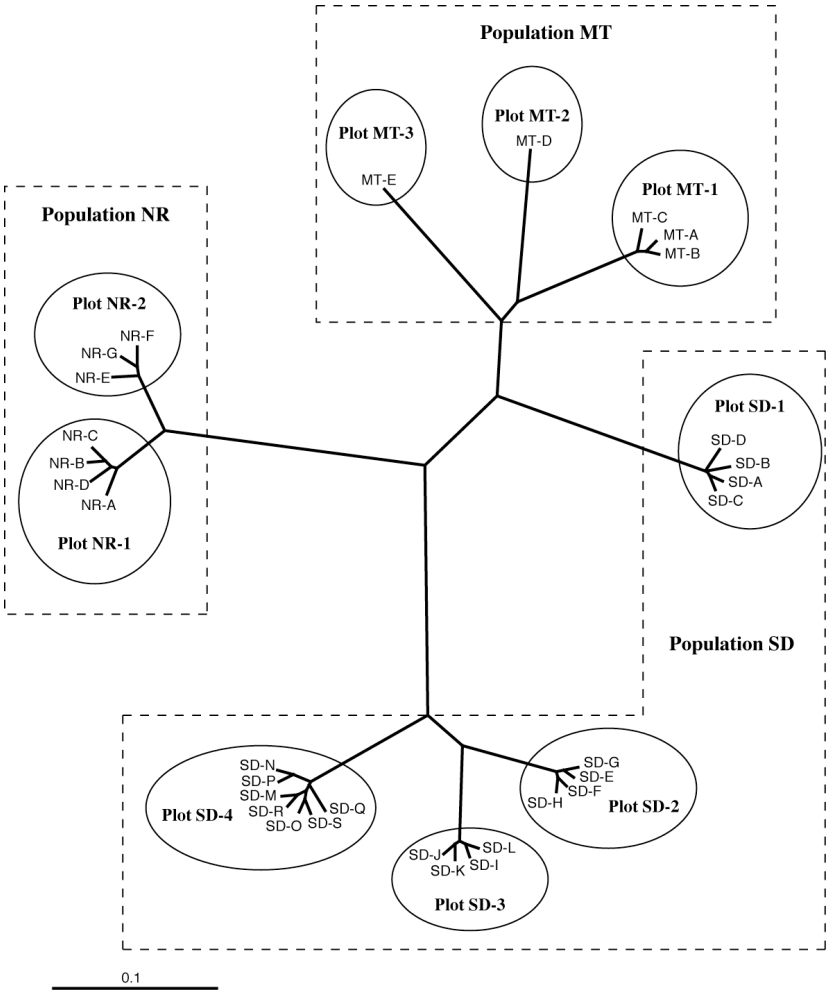

Figure 3: UPGMA phenogram based on dissimilarity values for each observed genotype. Genotypes found in a plot are grouped in solid ovals. Plots within a population are grouped by dashed lines.