

## CLONE STRUCTURE IN FOUR *SOLIDAGO ALTISSIMA* (ASTERACEAE) POPULATIONS: RHIZOME CONNECTIONS WITHIN GENOTYPES<sup>1</sup>

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### ABSTRACT

We studied clone structure and degree of genotypic mixing of *Solidago altissima* L. (Asteraceae) clones in four old fields near Ithaca, New York. The fields differed in time from agricultural abandonment and were approximately 1, 5, 20, and 35 years old. In the three older fields, three 0.75 m<sup>2</sup> plots were excavated intact and rhizome connections among ramets were mapped. In the youngest field 30 ramets were dug up singly. The genotype of all ramets was determined using electrophoresis of four polymorphic enzyme systems. Fields differed in the number and dispersion of genotypes within plots, and the degree of connection among ramets in the same clone. The one-year-old field was composed of single ramet genotypes which had probably established from seed the previous year. The five-year-old field contained many small contiguous clones of *S. altissima* with highly interconnected ramets. In the oldest two fields clones were highly intermixed and ramets of the same genotype were not extensively interconnected. These results demonstrate that clones of *S. altissima* display considerable phenotypic variability between fields and patterns of clone development may differ. The causes of this variability remain to be identified. We suggest that either selection for different genotypes or changing habitat conditions during succession may lead to changes in clone form.

ECOLOGISTS HAVE BEGUN to focus increasing attention on the biology of clonal species (Cook, 1983; Jackson, Buss, and Cook, 1985; Harper, Rosen, and White, 1986). Many species of plants form clones through the underground growth of structures such as rhizomes and root buds that render the study of clonal biology in the field difficult and time-consuming. In addition, the physical connections between parent and daughter ramets often decay, thereby obscuring the genetic relatedness of ramets within a clone. Consequently we know relatively little about the physical and genetic structure of most natural populations of clonal plant species.

Three elements of structure may affect the functioning and interaction of clones in the field. First, the relative position of ramets will

influence the outcome of interactions with neighboring plants (Hartnett and Bazzaz, 1983; Goldberg and Werner, 1983; Bulow-Olsen, Sackville Hamilton, and Hutchings, 1984; Schmidt, 1986). Second, the degree of physiological connection among ramets will determine how much resource sharing between ramets can potentially occur. Such sharing may minimize differences in the performance of individual ramets and cause the clone to function as a single physiological individual (Pitelka and Ashmun, 1985). Finally, if plant genotype affects competitive ability, insect and disease resistance, or the probability of outcrossing, then the genetic identity of ramets will determine how neighboring clones interact with each other and other species (e.g., Kinsman, 1982).

Although studies of the overall genetic diversity of clonal plant populations have been conducted (Silander, 1985; Ellstrand and Roose, 1987), they usually do not examine the genotypic structure on a scale small enough to assess the potential for interactions among genotypes. For many species we do not know whether clones form discrete spatial units or whether ramets from different clones grow intermixed. Although a number of studies have measured the overall size of natural clones (Cook, 1983, for references), very few studies have mapped genotypes at a scale that permits definition of potential ecological interactions (Edwards, 1984; Reinartz and Popp, 1987).

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TABLE 1. Characteristics of the four fields used in the study. Values of density, height, and species number are mean values ( $\pm$ SE) of the three 0.75-m<sup>2</sup> plots in each site (14 plots at Varna)

Site	<i>Solidago altissima</i>		Field age (yr) (approx.)	Field size (ha) (approx.)	Mean species no. per 1/8 m <sup>2</sup>
	Ramet density per m <sup>2</sup>	Ramet height (cm)			
Varna	2.6 $\pm$ 1.0	48.7 $\pm$ 11.2	1	1.0	5.0 $\pm$ 0.20
Jacksonville	61.7 $\pm$ 2.6	103.7 $\pm$ 2.4	5	13.0	1.3 $\pm$ 0.29
Whipple	40.7 $\pm$ 4.4	72.0 $\pm$ 14.9	20	1.0	9.7 $\pm$ 0.63
Lydell	50.0 $\pm$ 5.9	83.0 $\pm$ 24.0	35	1.3	6.2 $\pm$ 0.63

Yet genotype strongly affects the performance of individual ramets. Studies of many plant species have shown that genotypes vary in a variety of fitness components, such as reproductive behavior, competitive ability, and susceptibility to herbivores and pathogens (e.g., Antonovics, 1984; Maddox and Root, 1987). Since genotypes may consist of multiple ramets that are physiologically integrated, a knowledge of the clonal structure is critical to understanding the biology of a clonal species and its role in small scale community dynamics.

Species of the genus *Solidago* (Asteraceae), particularly *S. canadensis* L. and its hexaploid relative *S. altissima* L., form a dominant component of many old field communities in eastern North America (Werner and Bradbury, 1980; Marks, 1983; Melville and Morton, 1983). Although much of the work done on these species concerns the biology of individual ramets, it is well known that *Solidago* produces multiple ramets from underground rhizomes (Smith and Palmer, 1976; Werner and Bradbury, 1980; Melville and Morton, 1983) that create clones. These clones have been reported to form discrete, expanding clumps of ramets of a single genotype with little intergenet mixing (Smith and Palmer, 1976; Armstrong, 1983; Hartnett and Bazzaz, 1985). Rhizome connections are thought to persist for a number of years resulting in physiologically integrated clones, although this has not been explicitly studied. Experimental manipulations such as shading and rhizome severing have suggested that ramets within clones share resources (Hartnett and Bazzaz, 1983), and that expanding clones integrate the locally adverse effects of neighbors (Hartnett and Bazzaz, 1985).

These experimental manipulations involve neither the mapping of clones in the field, nor the identification of ramet genotype. Consequently we do not know whether clonal expansion always occurs in a regular, uniform fashion leading to discrete, contiguous clones, or whether natural populations consist of a diversity of highly intermixed clones. The phenotypic appearance of individual ramets varies

among fields and this variation may be due to the pattern of growth underground. Our study has employed mapping and identification of genotypes in four different aged populations of *Solidago altissima* growing in abandoned agricultural fields in Ithaca, New York. Three questions were investigated: 1) How connected are ramets within *S. altissima* clones? 2) Do genets form discrete clones expanding at the periphery, or are ramets of different genetic identity intermixed? 3) Do these aspects of clone structure vary within and among fields?

**METHODS**—Four old field populations of *Solidago altissima* were chosen in central New York. These fields were 4–20 km apart and differed in age, successional stage, and historical treatment (Table 1). The work described below was labor intensive. Thus, two fields (Whipple and Lydell) were sampled in August and September 1984; Varna and Jacksonville were sampled in August and September 1985.

Varna was planted annually in corn until August 1984. By August 1985 a variety of annuals and perennials, including *Solidago altissima*, had colonized the field. Jacksonville was last planted annually in corn in approximately 1980. This large field (ca. 13 ha, Table 1) had a virtual monoculture of *S. altissima* in 1985. Whipple Farm was used for hay production until approximately 1965 (Root and Kareiva, 1984) and now consists primarily of various *Solidago* species, small herbaceous perennials (e.g., *Fragaria*, *Hieracium*, *Aster*) and grasses (particularly *Poa pratense* and *Phleum pratense*). Lydell was abandoned before 1960 and its agricultural use is not known. Lydell is dominated by small shrubs (*Rubus*, *Viburnum*) and trees (e.g., *Pinus strobus*) which interrupt patches of herbaceous old field species. Composition of herbaceous species is similar to Whipple.

In each of the three older fields, three circular plots (ca. 0.75 m<sup>2</sup>) were randomly located (i.e., no attempt was made to identify goldenrod clones or patches in advance). Each ramet of *S. altissima* was tagged, its height recorded,

and its upper, healthy leaves collected for electrophoresis. We used a 10 dm<sup>2</sup> grid and a plumb line to map the exact location of each ramet. The entire plot was carefully excavated to a depth of 20 cm, preserving rhizome connections, and placed on a table in the field. The soil was removed by hand. The locations of rhizome connections and old stem stubs were mapped.

Varna was sampled differently because of the low density of goldenrod (ca. 3 ramets/m<sup>2</sup>). Fourteen 0.75 m<sup>2</sup> circular plots were located at random to include a total of 30 ramets. The relative positions of all ramets within a plot were mapped and leaves were collected for electrophoresis. Each ramet was excavated to determine the number of rhizomes growing from it.

After each plot was excavated four 0.125 m<sup>2</sup> plots were located one meter north, east, south, and west of the plot. The total number of species growing in this 0.5 m<sup>2</sup> area was determined. This measure of local species richness is reported in Table 1.

*Electrophoresis*—After collection leaves were stored at -80 C until analysis (within 16 weeks). Approximately 3 cm<sup>2</sup> of frozen leaf material was ground with 0.5 ml of extraction buffer. The liquid extract was absorbed on filter paper wicks and placed on the gel. The extraction buffer consisted of 0.25% mercaptoethanol, 0.25 M dibasic sodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>), and 0.25 M monobasic sodium phosphate (NaH<sub>2</sub>PO<sub>4</sub>).

Starch gels were prepared as in Huenneke (1985). Gels were given a 15-min prerun at 75 mA, the wicks were removed, and the gels were run for an additional 2 hr at less than 75 mA covered with an ice pack.

After completion the gel was sliced horizontally and stained for each enzyme used. Of 29 enzyme systems initially used, 10 had readable activity. Six of these were monomorphic and four were polymorphic (TPI, ADH, LAP, AAT). These four systems were used to distinguish genotypes. TPI gels had two loci and all systems had a number of alleles. Our systems appeared to contain enough variability to distinguish all genotypes we encountered at Jacksonville, Whipple, and Lydell. For Varna plants the bands for some individuals were difficult to read and thus we could confidently distinguish only 20 genotypes in 30 plants. However, all plants at Varna were single ramets, presumably established from seed during the preceding year.

Several individuals from five known clones growing naturally and transplanted to a com-

mon garden were sampled repeatedly over the summer of 1984. For each clone the banding patterns were identical over the two sites throughout the summer. We therefore assume that differences in banding patterns consistently and accurately reflect genotypic differences.

*Analysis*—A *G* test of independent spatial pattern of genotypes was conducted for each plot (Pielou, 1977) under the hypothesis that genotypes were distributed at random with respect to other genotypes within that plot. The test only analyzes distribution within the plot and suggests nothing about the relative distribution of genotypes at larger scales.

The probability that a nearest neighbor was the same genotype or connected was calculated in the following way. Each plot was divided into 10 cm<sup>2</sup> sections. One ramet was randomly chosen from each section and the characteristics of its nearest neighbor (whether it was the same genotype and whether it was connected) recorded. The probability that a ramet's nearest neighbor was the same genotype was the number of ramet-neighbor pairs that were the same genotype divided by the total number of ramet-neighbor pairs.

A group of interconnected ramets is treated as a potential physiological unit. Connectedness is the total number of ramets divided by the number of physiological units. It is the mean number of ramets per physiological unit.

We have included a clone map of one arbitrarily chosen plot from Jacksonville (Fig. 1), Whipple (Fig. 2), and Lydell (Fig. 3). Copies of all nine maps are available from the authors on request.

**RESULTS**—The pattern of clonal interconnection and genotype dispersion differed among fields. The Varna *S. altissima* population (1 year old) was sparse ( $2.6 \pm 1.0$  ramets/m<sup>2</sup>) and existed as single unconnected ramets (Table 2). Each ramet was clearly only one year old (there were no shoot stubs from previous years) and was vigorous and producing new rhizomes. Each of the 20 electrophoretically readable ramets was a different genotype.

The Jacksonville *S. altissima* populations (5 years old) formed a dense continuous stand. Although apparently distinct clones could be visually distinguished only at the field's periphery, electrophoresis showed that the field consisted of many small, densely packed clones (Fig. 1). The mean number of genotypes among three 0.75 m<sup>2</sup> plots was  $9.3 \pm 2.6$  (Table 2).

The probability that a ramet was connected to its nearest neighbor, and thus of the same

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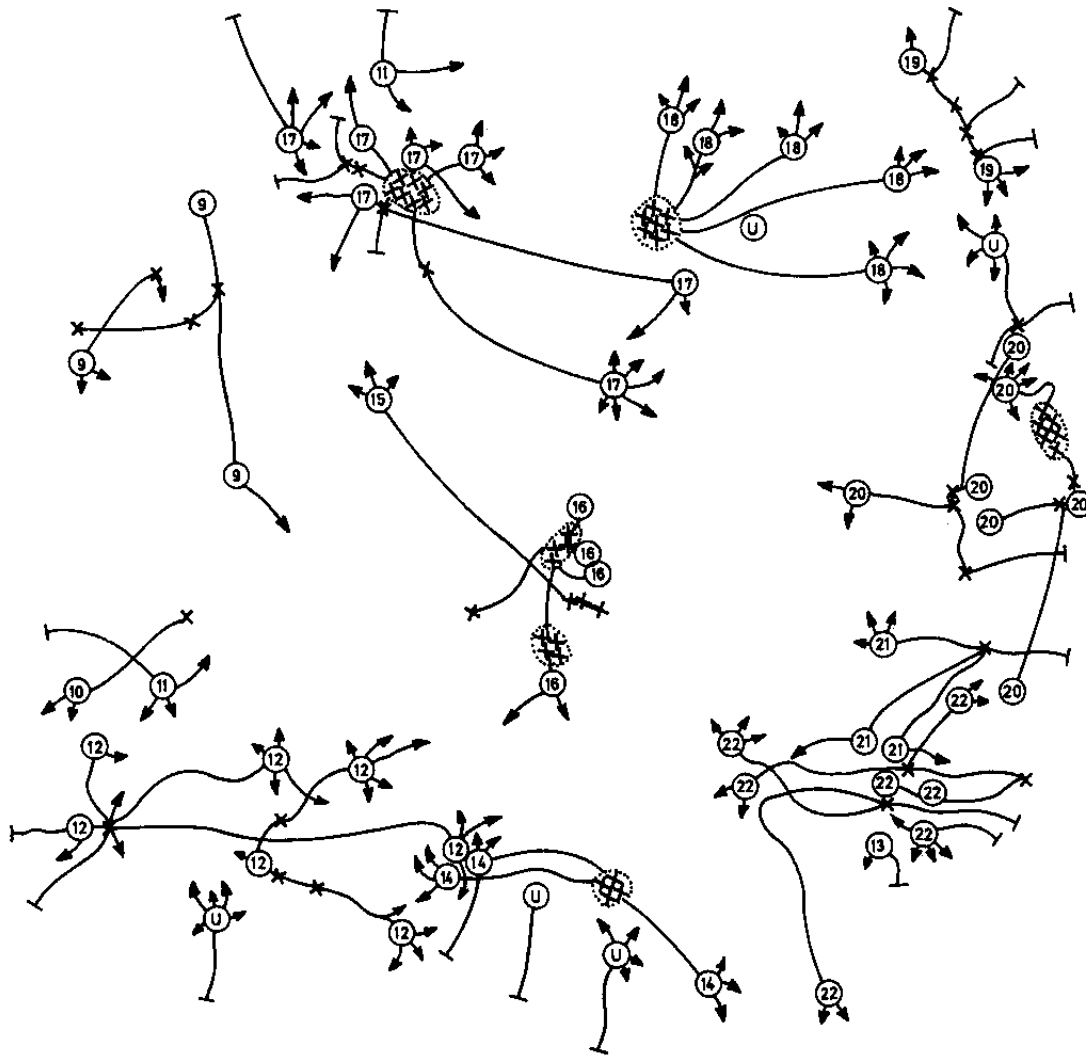


Fig. 1. Map of underground connections among genotypes at Jacksonville 3 in 1985. Circles denote current living ramets. Numbers within circles denote genotypes (different numbers denote different electrophoretic genotypes); U means that the genotype was not identified. X denotes a stump from a previous year's ramet. Lines are rhizome connections: T endings are rotten or cut rhizomes and arrowed endings are actively growing rhizome tips. All maps are the same scale and represent an area of 0.75 m<sup>2</sup>.

genotype, was highest in this field (Table 2). Many genets had a tangled, woody, center with many dead shoot stubs from previous years. Rhizomes were relatively short (Table 3) and emerged from these masses to terminate in living ramets (Fig. 1). A *G* test of random relative distribution suggests that genotypes in each of the three Jacksonville plots tended to be clumped in space (Table 2). Ramets at Jacksonville were highly connected; potential physiological units contained a mean of  $3.9 \pm 0.6$  ramets (Table 2), the highest of any of the fields.

The Whipple Farm population (20 years old) apparently contained somewhat fewer genotypes (mean number of genotypes in plots:  $3.3 \pm 0.9$ , Table 2). Rhizome growth seemed to have little directionality and rhizomes grew longer distances than those at Jacksonville (Fig. 2, Table 3). Consequently, ramets that were connected to one another usually were not each other's nearest neighbors. The *G* test suggests that genotypes were randomly arrayed in each plot (Table 2). Few ramets were connected; most were connected only to previous year's

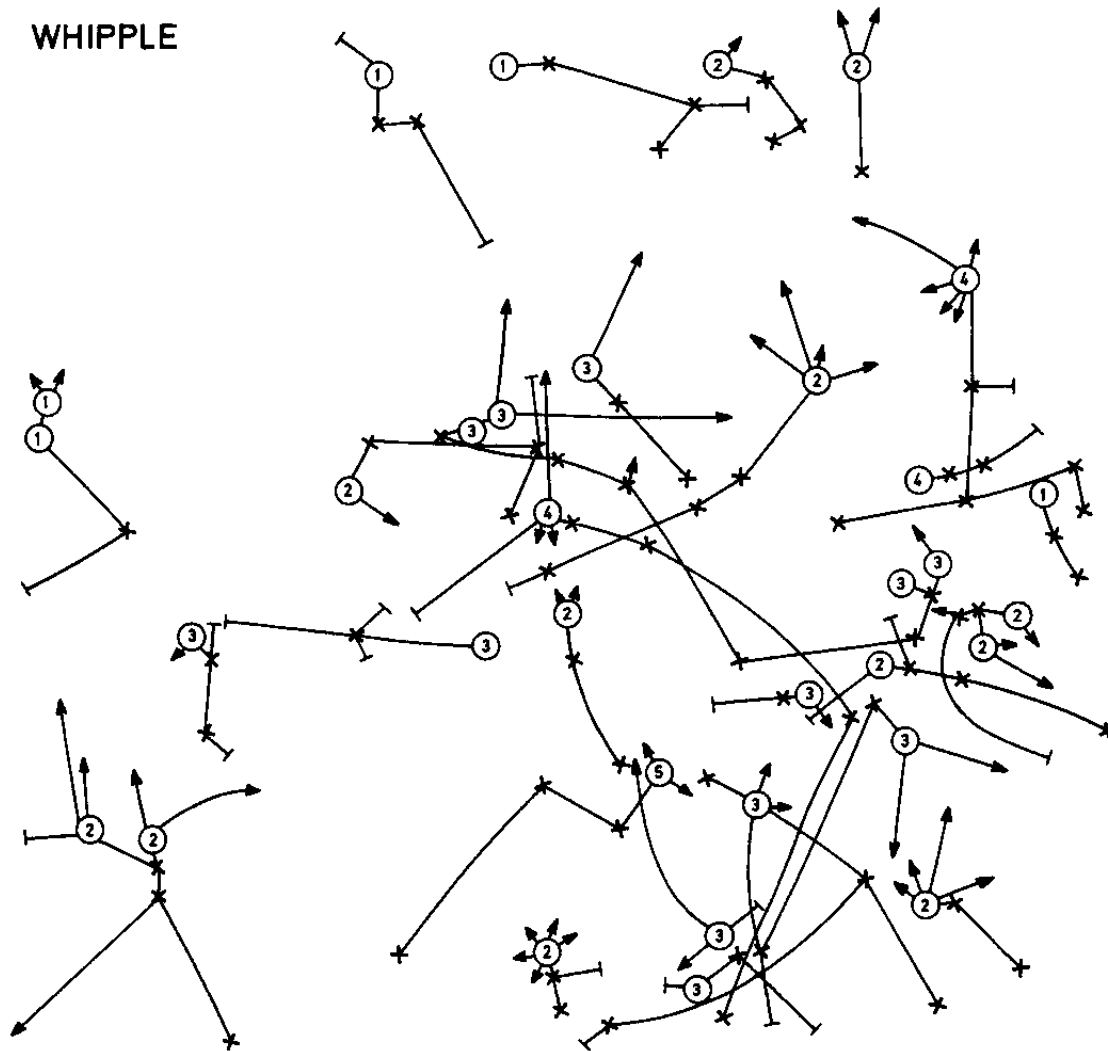


Fig. 2. Map of underground connections among genotypes at Whipple 1 in 1984. Symbols are the same as for Fig. 1.

stem stubs (connectedness =  $1.4 \pm 0.2$ ; Table 2). The connectedness was the lowest of the three older fields.

Clone structure at the Lydell population (35 years old) was similar to that at Whipple, although one plot (Lydell 1, Table 2) contained just one genotype. Genotypes were randomly distributed in one of the other two plots (Table 2). Connectedness, the mean number of ramets in a potential physiological unit, was low in all three plots ( $2.1 \pm 0.4$ , Table 2). Even in the monotypic plot (Lydell 1) the clone did not form a single integrated unit. In fact, a ramet at Lydell 1 had the lowest probability of being connected to its nearest neighbor (0.08). In general, the probability that ramets were connected to their neighbors was lowest at Lydell,

and rhizomes were very long (Table 3), suggesting that clones were becoming less compact and more widely distributed. Rhizomes were found to be significantly longer at Whipple and Lydell ( $F = 6.10$ ,  $P < 0.05$ ) using nested analysis of variance (Table 3). There were no differences in the number of rhizomes per ramet among fields (Table 3).

**DISCUSSION**—Our study examined the form and genotypic composition of sampled ramets of *S. altissima* in natural populations in four different fields in central New York. By combining genotype identification through electrophoresis with mapping of the physical connections among ramets, we were able to examine variation in the form of clones and