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# The Population Biology and Demography of *Cimicifuga rubifolia* Kearney and the Genetic Relationships Among North American *Cimicifuga* Species

Rebecca Ann Cook
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To the Graduate Council:

I am submitting herewith a dissertation written by Rebecca Ann Cook entitled "The Population Biology and Demography of *Cimicifuga rubifolia* Kearney and the Genetic Relationships Among North American *Cimicifuga* Species." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.

Edward E. C. Clebsch, Major Professor

We have read this dissertation and recommend its acceptance:

Murray Evans, Cliff Amundsen, Gary McCracken

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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# THE POPULATION BIOLOGY AND DEMOGRAPHY OF *CIMICIFUGA RUBIFOLIA* KEARNEY AND THE GENETIC RELATIONSHIPS AMONG NORTH AMERICAN *CIMICIFUGA* SPECIES

A Dissertation

Presented for the

Doctor of Philosophy Degree

The University of Tennessee, Knoxville

Rebecca Ann Cook

August 1993

## DEDICATION

"Knowledge" puffs up, but love builds up. If any one imagines that he knows something, he does not yet know as he ought to know.

1 Corinthians 8:1-2

This dissertation is dedicated to my parents

Thomas Cory Cook

and

Betty Snodgrass Cook

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ABSTRACT

In this beginning study of the population biology of Cimicifuga species, the life history and demography of the long-lived herbaceous perennial, Cimicifuga rubifolia Kearney, were investigated, the genetic structure of some of its populations was studied, and an investigation of the genetic relationships among the North American species was begun.

The life history and demography were monitored in two populations, one of approximately 1400 individuals (1987-1990) and the second of about 400 individuals (1988-1990). A model of leaf area was used to determine the leaf area (photosynthetic size) of individuals and this was followed during the study. Relationships between the leaf area of individuals and flowering, fruit set, mortality, dormancy and size change were investigated. The population size structures were considered using size class transition matrices. Population genetic structure from throughout the range of Cimicifuga rubifolia was assayed using starch gel electrophoresis, and the genetic relationships of the North American Cimicifuga species were studied using electrophoretic methods.

Leaf area was positively related to ability to flower and set seed while mortality and dormancy were negatively related to leaf area. Reproduction was primarily sexual, with asexual reproduction by rhizome fragmentation occurring rarely. Several plant responses thought to be related to the low precipitation amounts during 1987 and 1988 were noted. The mean size of the plants in both populations increased by approximately 30 percent during the study and the size at which the

probability of flowering reached 50 percent varied. Size structure based on size transition probabilities, was not constant. Plant dormancy was frequent and the number of seedlings noted yearly varied widely.

Seven loci were assayed electrophoretically in C. rubifolia. Accumulated gene differences per locus, as measured by genetic distance among populations was insubstantial but, genetic divergence among the populations is indicated by large $F_{ST}$ values (.197-.468). This appears to be due to reproductive isolation of populations, indicated by high total fixation indices. Gene flow within populations seems to be limited. The ten loci assayed in 6 North American species of Cimicifuga showed the mean genetic identity from pairwise comparisons of the species to be .543. Mean $G_{ST}$ values ranged from .086 to .503 and seem to be related, in part, to varying breeding systems among the species.

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# CHAPTER 1

# INTRODUCTION

Population biology considers changes in a population and the relationship of these changes to the life cycle of the species, the genetic structure of the population and the interaction of the species with environmental and physiological factors. Changes in the number of individuals in a population and how the changes are related to the various life stages of the plant are considered in demographic studies. Life history studies consider how various characters such as age or size specific survival, fecundity, and mortality influence the dynamics of the population. As life history strategies are the result of selection factors, they are also indicators of the evolutionary fitness of the population. The study of the population genetics of a species considers both how the life history strategy has affected the genetic structure of the population and the evolutionary potential of the population. To even begin to understand the population biology of a species, all of these different aspects of the population's dynamics must be studied (Davey and Smith; Silverton 1987).

This study is a preliminary investigation of the population biology of Cimicifuga rubifolia Kearney. The demography, life history, and genetic structure of the species are considered. Cimicifuga rubifolia was chosen as a study subject for several reasons. Primary among them is that little is known about the species. Although Kearney described C. rubifolia in 1897, it was not generally accepted as a species until after the work of Ramsey (1965). Cimicifuga rubifolia is

also of interest as a woodland herbaceous perennial. Most population biology studies of herbaceous plants have focused on annual and biennial species. Most of the long-lived perennials that have been studied have either lacked clonal growth or have had vegetative reproductive structures that are located above ground (Bierzychudek 1982a; Erikkson 1989). Even in terms of population genetics, comparatively few studies have been on long-lived herbaceous perennials. In an extensive review of the literature, Hamrick and Godt (1990) compared the available allozyme information from 653 studies which included 449 species and 165 genera. Only four of those taxa were long-lived herbaceous perennials. This study will, therefore, contribute information to an area of plant population biology that has been relatively neglected.

There is additional interest in Cimicifuga since it includes some species that are considered rare. Three of the North American species are candidates for threatened status on the Federal Register of Endangered and Threatened Species (Ayensu and DeFilips 1978). Before a species can be placed on the Endangered and Threatened Species list, a large amount of information about it is needed, including basic information about its population biology. This study contributes information needed in the evaluation of the status of C. rubifolia. The methods developed, particularly the size classification system and its use in a population projection model, should also be applicable to the other candidate species, C. arizonica and C. laciniata.

RANGE AND DESCRIPTION

The genus Cimicifuga contains 12 species. These occur in the northern temperate zones of Europe, Asia, and North America. Six species are found in North America. Cimicifuga rubifolia Kearn., C. racemosa (L.) Nutt., and C. americana Michx. occur in eastern North America. Three, C. arizonica Wats., C. elata Nutt., and C. laciniata Wats., are found in western North America. The species are distinguishable in the field by a number of characteristics including terminal leaflet shape, number of leaflets, fruit morphology, pistil number, and petiole morphology (Ramsey 1965, 1988).

Cimicifuga rubifolia occurs mainly in the Ridge and Valley region of Tennessee and southern Virginia, and scattered populations also occur in northwest Tennessee, southern Illinois, western Kentucky, southern Indiana, and northern Alabama (Pellmyr 1986a). Cimicifuga racemosa ranges from southern Ontario to central Georgia, west to Arkansas and north to northern Ohio. It is generally restricted to elevations below 1500 m. Cimicifuga americana is found at higher elevations (274-1950 m) in the Appalachian Mountains from southern Pennsylvania to northern Georgia. Although C. rubifolia and C. americana are not known to occur together, C. racemosa is commonly found with both species (Ramsey 1965).

Cimicifuga laciniata is known from a limited number of sites in the Cascade Mountains of the Pacific Northwest. Originally known from only two sites, the number of known sites has increased with the logging of old growth forests (E. Alverson, personal communication). It is typically found on very steep slopes between elevations of 950-1100 m. Cimicifuga elata is also endemic to the Pacific Northwest. Its original

range was from southern British Columbia to northwest Oregon, although no extant populations are known in Canada (Pellmyr 1986a). Cimicifuga arizonica is known only to occur in canyon bottoms in two counties in Arizona (Pellmyr 1985a; Ramsey 1988).

Cimicifuga rubifolia is typically found on the lower slopes of north-facing bluffs, very often on clay soils which are those formed over limestone or calcareous shale. While most populations are found on slopes above rivers or streams they are typically found above the high water level. It is associated with Tilia heterophylla, Acer saccharum, Fraxinus americana, Lindera benzoin, Parthenocissus quinquefolia, Toxicodendron radicans, Impatiens pallida, and Polymnia canadensis (Ramsey 1965).

Cimicifuga rubifolia is an herbaceous perennial. Stems arise from a thick, horizontal rhizome that may be 10 cm in length. Stem heights range from 3-22 dm. The ternate or biternate leaves have 3-17 leaflets. The deeply cordate base of the terminal leaflet contributed to its previous classification as C. cordifolia Pursh. or C. racemosa (L.) Nutt. var. cordifolia Pursh. The inflorescence is a simple panicle of racemes. The white apetalous flowers are numerous, with 1-2 pistils and many stamens (Ramsey 1987). Flowering occurs in late summer. It is self-infertile and relies on insects for cross-pollination (Pellmyr 1986a). Fruits are follicles, containing an average of 8-9 seeds (Ramsey 1987). Although the rhizome may branch and have active apices with leaves located on different portions, there has been no previous documentation of vegetative reproduction from rhizome fragmentation (Ramsey, personal communication).

PURPOSE

The purpose of this study is to answer basic questions about the population biology of C. rubifolia and its relationships with the other North American species of Cimicifuga. The questions to be answered can be stated as follows: 1) Can the size of an individual, as evidenced by photosynthetic area, be used to estimate life history characters such as mortality, dormancy, and fecundity? 2) In the populations studied, what is the size structure of the population and do these populations appear to be currently stable? 3) Does asexual reproduction by rhizome fragmentation occur and, if so, is asexual or sexual reproduction more common? 4) Is there apparent genetic variability in C. rubifolia and how is it distributed within and between populations? 5) How is genetic structure of C. rubifolia and the other North American species of Cimicifuga affected by their differing mating systems? 6) What is the genetic relationship of C. rubifolia to the other North American species of Cimicifuga?

# CHAPTER 2

## PLANT SIZE AND ITS RELATIONSHIP TO LIFE HISTORY CHARACTERS IN TWO POPULATIONS OF *CIMICIFUGA RUBIFOLIA*

### INTRODUCTION

Life history and demographic studies are concerned with the dynamics of populations. Life history studies, directed toward the organisms' life cycles, link the ecological role of plants with population dynamics. Life history characters include age and size specific survival, fecundity, and mortality. Closely related to life history studies are demographic studies which look at the changes in number of individuals or classes of individuals in a population over time (Cochran 1986; Venable 1984).

Although many forest herbs are perennials, life history and demographic studies of forest herbs have typically focused on annual or biennial plants. The herbaceous perennials that have been studied have usually lacked clonal reproduction (Bierzychudek 1982b). Those studies have indicated several trends in the life history and demography of herbaceous perennials. Plant size has been shown to often be more important in determining reproductive capability, both sexual and asexual, than chronological age. Eriksson (1988) found that the probability of stolon production and occurrence of flowering were both positively related to plant size in *Potentilla anserina*. Similarly Newell et al. (1981) found that both stolon production and the number of fruit produced per plant increased with plant size in *Viola*. In a study of 57 herbaceous species, Shipley and Dion (1992) found a weak but highly significant relationship between (ln) number of seeds and the

(ln) average weight of individuals. While there was significant correlation between leaf area and flower number in Arisaema, there was none between leaf area and either seed set or corm production (Bierzychudek 1982a). There is evidence to indicate that climatic variation can influence both occurrence of flowering and the number of seeds produced. In their long term study Inghe and Tamm (1988) were able to link variation in flowering in several species to specific climatic variables such as drought during a critical period in the previous year.

Several studies have shown mortality rates to be negatively related to plant size by several studies. Newell et al. (1981) recorded the highest mortality rates in the smallest plants with mortality rates being age independent. Similarly, Bierzychudek (1982a) reported that in Arisaema the smallest and youngest plants had a high mortality rate with no increase in mortality in the very old and very large. In Echinacea tennesseensis mortality is highest among juvenile plants but decreases with plant size (Drew 1991). Eriksson (1988) also found that in Potentilla anserina size of the ramets influenced mortality, although in years with poor environmental conditions the relationship was obscured.

Many of the herbaceous perennial populations studied have exhibited overall stability in total number of ramets despite a relatively high turnover in individual ramets. In other species, however, changes in the number of individuals from year to year seem to be common. Most of the species studied have similar size class distributions with the seeds and seedlings comprising the largest portion of the population and with the largest plants making up the smallest portion of the population.

Changes in the number of individuals may result from either seedling recruitment or from asexual reproduction (Barkham 1980a, 1980b; Bierzychudek 1982b; Tamm 1956a, 1956b).

Many earlier studies of herbaceous perennial species indicated that in species with both vegetative and sexual reproduction, recruitment was primarily through vegetative reproduction with limited production of new genets (Cook 1983; Lovett-Doust 1981; Newell et al. 1981; Sarukhan 1974; Sarukhan and Gadgil 1974; Sarukhan and Harper 1973; Solbrig 1981; Solbrig et al. 1980, 1988). However, these studies primarily considered plants whose asexual reproductive parts are above ground. In a review Eriksson (1989) found that although this group of plants does primarily use vegetative reproduction for recruitment this is not true for all potentially clonal herbs. He found that 40% of the species had repeated seedling recruitment. However, grassland species were found to reproduce sexually more often than woodland species. Plants with clonal growth above ground recruited more often by seedlings than did those with below ground structures. Similarly, Bierzychudek (1982b) found that while about half of the clonal species reviewed (13 of 24) did use asexual reproduction as the major method, the rest primarily used seedlings for recruitment. Additionally, Bierzychudek (1982a) found that the importance of asexual reproduction can vary between populations of a species.

There is growing evidence that a number of herbaceous perennials may undergo periods of dormancy and may remain underground for one or more growing seasons (Bierzychudek 1982a; Cochran 1986; Keeler 1991; Oostermeijer et al. 1992). The reported number of individuals that

undergo dormancy within a population is typically very small (<1%). The number of species capable of dormancy may be under-reported as many demographic studies have followed the relative numbers of individuals in plots rather than specific individuals.

This portion of the study will determine in the size, as measured by photosynthetic area, of an individual can be used to estimate such life history characters such as mortality, dormancy and fecundity in Cimicifuga rubifolia. It will also investigate whether C. rubifolia, an herbaceous perennial, undergoes asexual reproduction by rhizome fragmentation. The inflorescence is a simple panicle of racemes. Fruits are follicles, containing an average of 8-9 seeds (Ramsey 1987). Flowering occurs in late summer. It is not known to self-pollinate, relying on insects for cross-pollination (Pellmyr 1986a). Stems arise from a thick, horizontal rhizome that may be 10 cm in length. The rhizome may branch and have active apices with leaves located on different portions. Cimicifuga rubifolia is presumed to be capable of clonal reproduction even though prior to this study there was no direct documentation that this occurred naturally. Books on plant propagation list fragmentation of Cimicifuga rhizomes as a means of propagation (Plumridge 1976; Thompson 1989).

Cimicifuga rubifolia occurs in the Ridge and Valley and Cumberland Plateau regions of Tennessee and Virginia. Scattered populations also occur in southern Illinois, southern Indiana, northern Alabama, northwestern Tennessee, and western Kentucky (Pellmyr 1986a). It is typically found on the lower slopes of north-facing bluffs. The clay soils in which it grows are usually those formed over limestone or

calcareous shale. While most populations are on slopes adjacent to rivers and streams they are usually located above the apparent high water level (Ramsey 1965).

## MATERIALS AND METHODS

Two sites were used in this study. One is located in Roane County, Tennessee and is situated on the lower portion of the north-facing slope of Chestnut Ridge in the area adjacent to the Grassy Creek embayment on Watts Bar Reservoir on land owned by the Tennessee Valley Authority (TVA). The lowest part of the population is situated in the margin of the Grassy Creek floodplain although most of the population is situated well above the high water level due to the steepness of the slope. The other site is in Anderson County, Tennessee on the Oak Ridge National Laboratory Reservation. This site is located near the base of north-facing Bull Bluff and overlooks Melton Hill Reservoir. The lowest portion of the population is approximately 10 feet above the reservoir pool level. Elevation at both sites is approximately 800 feet. Aerial photographs from 1937 to 1982 show no signs of disturbance to the forest near the sites. These two sites represent two general types of C. rubifolia habitat (personal observation). Much of the population at Bull Bluff is located in loose rock of various sizes that shows some evidence of continuing movement. The slope at Grassy Creek is more stable. Other than occasional rock outcrops, little rock is exposed, however, in most areas there is only a thin layer of soil over underlying rocks.

In 1987, 274 C. rubifolia plants were marked and measured at the Grassy Creek site, using a ruler. An individual or plant was considered to be a rhizome and all of its leaves. Measurements included plant height as petiole length (cm), length and basal width of each leaflet (cm), leaf length (length of rachis to apical leaflet tip) (cm), and leaf width (distance from basal leaflet tip to opposite basal leaflet tip) (cm). The leaves of these plants were collected. The area ($cm^2$) was measured on a Licor Area Meter. Since destructive sampling was undesirable for the remainder of the study, a simple model of leaf area was constructed using the field area measurements compared to the Licor area measurements. This model was:

$$\text{Area} = 0.5LW$$

where L equals leaf length and W equals leaf width. Because both the Licor area and the model area are subject to error, Model I regression is not appropriate for analysis (Sokal and Rohlf 1981). Correlations and Model II regression were done.

In addition to the plants marked and measured for the model determination, an additional 1041 plants were marked at the Grassy Creek site in 1987. This gave a total of 1315 marked individuals. An individual plant was considered to be a single rhizome and all of its shoots. All plants were double marked with a numbered plastic pot label and wire stake flag. Proximity of the rhizomes to the soil surface made it possible to ascertain if a single rhizome had multiple ramets or shoots. Each shoot was also marked and labeled if multiple shoots were present. After the measurements necessary for the model were determined, only the measurements of leaf width and leaf length were

taken. In addition to these measurements the number of leaves and leaflets per individual and per shoot and the number of shoots per individual were recorded. The number of inflorescences per individual was also noted. Flower stalk height, number of flowers, and number of follicles that developed were recorded for all flowering individuals. Because of the large size of this population, only the central portion of the population was used. All plants located within this area were used in the study. The area was located so as to encompass a variety of possible microhabitats within the population.

In 1988, 1989 and 1990 the same measurements where recorded for the marked plants. The number and identity of any multiple shoots of a individual were also recorded. Multiple shoot rhizomes were checked annually to see if rhizome fragmentation, or asexual reproduction, had occurred. If the tags were located but no plant was found, the plant was listed as absent. If tags for a plant were not located, the plant was listed as missing and was not used in calculations. Seedlings were marked but were not measured to minimize possible damage. Instead, seedlings were assigned an arbitrary size based on the averaged measurements of seedlings from outside the study plot. Any other unmarked plants were listed as new and were marked and measured.

The study was begun at the Bull Bluff population in 1988 and was continued through 1990. The measurements and observations on the Bull Bluff populations were duplicates of those on the Grassy Creek plants. Because of the small population size, all 312 plants at this site were used in the study.

## RESULTS

### Leaf Area Relationships

The simple area model (0.5LW) is highly positively correlated to the area as measured by the Licor meter ($r^2$ = .9739). Therefore, leaf area will be assumed to be linearly related to the model (Figure 2.1). Model II regression shows the following relationship between the model area ($A_m$) and the measured area ($A_l$):

$$A_m = 1.141\ A_l + 43.844.$$

In cases where linear correlation is very high, both Model I and Model II regression give similar equations (Sokal and Rohlf 1981). Model I regression gives the following relationship between the model area and the measured area:

$$A_m = 1.161\ A_l + 37.802.$$

For the purposes of this study, the size ($cm^2$) of an individual plant was considered to be the sum of the areas of all its leaves. The frequencies of the sizes of all populations were plotted. Seedlings were excluded from these calculations due to the extreme variation in number of seedlings from year to year. In all cases, an inverse J-shaped curve was seen (Figure 2.2a). Base 10 log transformation of area was done to obtain a size distribution that approximated normal distribution (Figure 2.2b). The Shapiro-Wilk statistic, W, (SAS 1987) was used to test both populations in all years for normal distribution of log transformed leaf area. The null hypothesis that the samples were taken from a population with a normal distribution was accepted. Single Factor ANOVA (SAS 1987) showed the population size distribution was

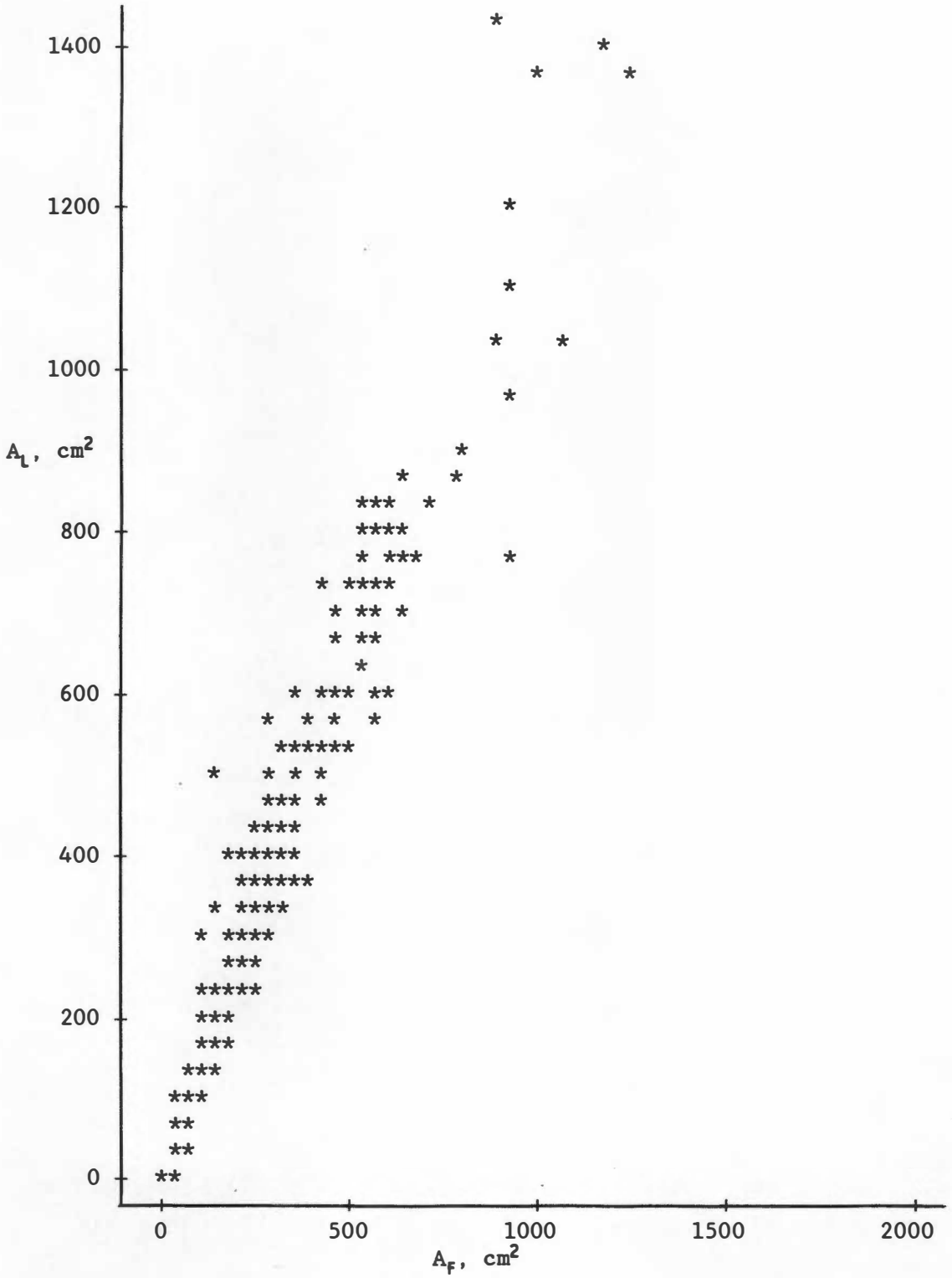
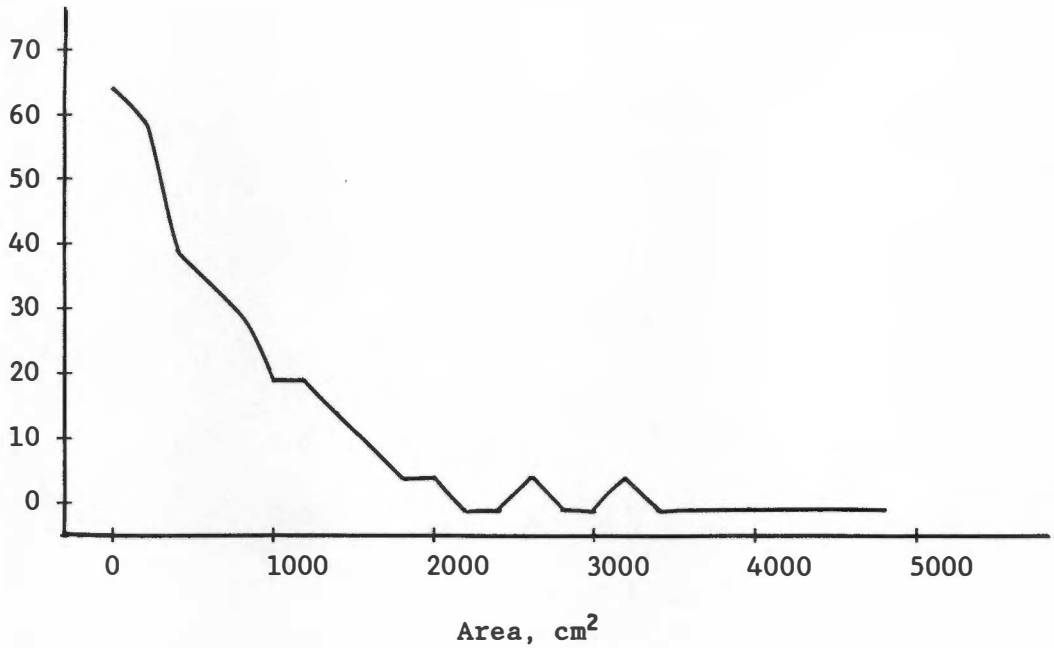



Figure 2.1 Leaf area of Cimicifuga rubifolia from the model ($A_F$ = .5 x length x width) versus leaf area from measurement ($A_L$).

a.




b.

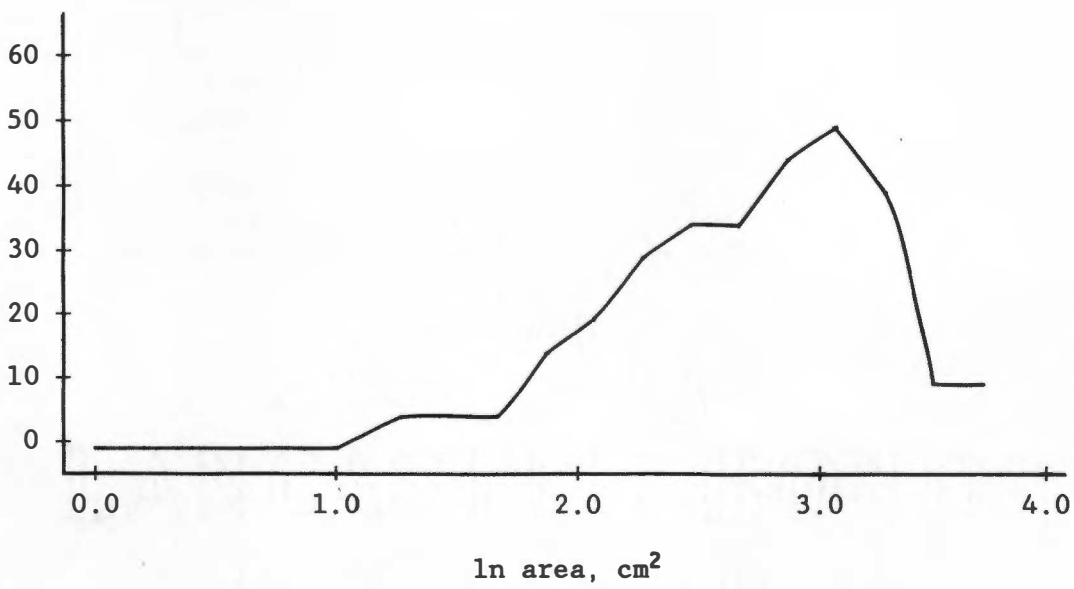



Figure 2.2 Leaf area distribution of individuals of Cimicifuga rubifolia at Bull Bluff in 1988.

significantly different from population to population and from year to year (Table 2.1).

It should be noted that the mean size of the individuals in the populations did not remain constant from year to year. An overall increase in size occurs yearly (Table 2.2). This is thought to be primarily related to changes in environmental conditions, particularly precipitation amounts (Table 2.3). While the mean size of the individual in both populations increased yearly, some individuals decreased in size. The size changes in the largest individuals (in the first year of the study) are shown in Table 2.4. Many of the largest 10 at Grassy Creek increased in size and remained among the largest in the population. Of those that decreased in size, the changes were relatively small. In contrast, most of the 10 largest at Bull Bluff decreased dramatically in size. The mean size of the Grassy Creek population increased by 62.7% from 1987 to 1988 while the mean size of the largest 10 increased by 44.4%. The mean size of the Bull Bluff

Table 2.1 Single Classification Analysis of Variance (SAS 1987) between populations for log (leaf area). Ho: the variances of the groups are equal.

| Classification | df/df[a] | F | Pr > F |
|---|---|---|---|
| Populations | 1/6339 | 144.16 | 0.0001[b] |
| Population and year | 6/6334 | 76.66 | 0.0001 |
| Grassy Creek by year | 4/6337 | 121.70 | 0.0001 |
| Bull Bluff by year | 3/1339 | 123.85 | 0.0001 |

[a] numerator degrees of freedom/ denominator degrees of freedom
[b] reject Ho at all significance levels

Table 2.2 Increase in mean size of individuals. The population mean for area, ($A_t$ in $cm^2$), shoot number, inflorescence number, leaf number, and leaflet number for both populations in all years. Seedlings are excluded.

| Site/Year | N | Area | Shoot | Inflorescence | Leaf | Leaflet |
|---|---|---|---|---|---|---|
| Bull Bluff | | | | | | |
| 1988 | 312 | 733.8 | 1.15 | 0.12 | 1.8 | 8.8 |
| 1989 | 326 | 768.8 | 1.17 | 0.27 | 2.0 | 10.1 |
| 1990 | 420 | 797.0 | 1.18 | 0.21 | 2.2 | 10.2 |
| Grassy Creek | | | | | | |
| 1987 | 1315 | 308.1 | 1.08 | 0.03 | 1.7 | 7.0 |
| 1988 | 1414 | 451.9 | 1.10 | 0.06 | 1.8 | 8.1 |
| 1989 | 1314 | 624.1 | 1.11 | 0.12 | 2.0 | 10.6 |
| 1990 | 1320 | 803.5 | 1.15 | 0.11 | 2.5 | 13.7 |

Table 2.3 Annual precipitation in Oak Ridge, Tennessee. Data is from the National Oceanic and Atmospheric Administration. Water equivalent in inches is given.

| Month | Year | | | | | Mean[a] |
|---|---|---|---|---|---|---|
| | 1986 | 1987 | 1988 | 1989 | 1990 | |
| January | 1.16 | 4.87 | 5.44 | 6.94 | 5.29 | 4.80 |
| February | 5.15 | 5.64 | 3.43 | 5.07 | 8.01 | 4.71 |
| March | 2.70 | 2.82 | 3.80 | 6.03 | 5.09 | 5.66 |
| April | 1.73 | 2.97 | 3.42 | 2.76 | 2.57 | 4.07 |
| May | 2.74 | 2.02 | 2.65 | 6.14 | 6.59 | 4.26 |
| June | 1.45 | 4.26 | 0.53 | 11.14 | 1.53 | 4.21 |
| July | 2.84 | 3.94 | 7.60 | 3.62 | 5.06 | 5.13 |
| August | 2.84 | 1.92 | 2.39 | 3.90 | 5.09 | 3.72 |
| September | 4.70 | 5.64 | 5.63 | 8.86 | 1.44 | 3.83 |
| October | 4.51 | 0.69 | 1.97 | 2.46 | 4.07 | 2.99 |
| November | 3.67 | 2.11 | 6.56 | 6.06 | 2.40 | 4.56 |
| December | 5.34 | 3.43 | 5.53 | 3.03 | 12.64 | 10.92 |
| Total | 38.83 | 40.31 | 48.95 | 66.01 | 59.78 | 58.86 |

[a] The mean is calculated from data collected from 1951 through April 1993.

Table 2.4 Fate of 10 largest individuals in the first year of the survey. ID is identification number of the plant, Area its area, $cm^2$, in that year, Shoot is the number of shoots, and Inflorescence is the number of inflorescences present that year.

| ID | Area | | | | Shoot | | | | Inflorescence | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 87 | 88 | 89 | 90 | 87 | 88 | 89 | 90 | 87 | 88 | 89 | 90 |
| **Bull Bluff** | | | | | | | | | | | | |
| 74 | | 2886 | 2469 | 1018 | | 1 | 1 | 2 | | 1 | 1 | 0 |
| 125 | | 3067 | 1159 | 177 | | 1 | 1 | 2 | | 0 | 0 | 0 |
| 119 | | 3206 | 2826 | 2108 | | 1 | 2 | 2 | | 1 | 2 | 1 |
| 80 | | 3212 | 2562 | 1608 | | 1 | 1 | 1 | | 1 | 1 | 1 |
| 70 | | 3340 | 3649 | 1872 | | 1 | 1 | 2 | | 1 | 1 | 0 |
| 91 | | 3460 | 2763 | 2806 | | 2 | 2 | 2 | | 2 | 1 | 2 |
| 156 | | 3893 | 4015 | 4313 | | 2 | 3 | 2 | | 0 | 1 | 2 |
| 85 | | 4109 | 4139 | 3153 | | 3 | 3 | 4 | | 1 | 1 | 1 |
| 88 | | 4638 | 5158 | 5158 | | 2 | 2 | 3 | | 0 | 2 | 2 |
| 95 | | 4809 | 5245 | 2665 | | 1 | 1 | 1 | | 1 | 1 | 1 |
| **Grassy Creek** | | | | | | | | | | | | |
| 150 | 2123 | 2623 | 2362 | 2279 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 95 | 2164 | 3390 | 5504 | 7144 | 2 | 2 | 2 | 2 | 0 | 1 | 1 | 1 |
| 30 | 2213 | 3102 | 3724 | 3881 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| 98 | 2455 | 5155 | 6796 | 5225 | 2 | 3 | 3 | 4 | 1 | 1 | 2 | 1 |
| 509 | 2691 | 4437 | 5019 | 2228 | 4 | 3 | 3 | 3 | 1 | 1 | 1 | 0 |
| 22 | 2845 | 4228 | 5756 | 5717 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 |
| 848 | 2943 | 3182 | 3410 | 3994 | 2 | 2 | 2 | 3 | 1 | 1 | 1 | 1 |
| 843 | 3977 | 4798 | 3947 | 4621 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 837 | 4186 | 6788 | 8997 | 9969 | 2 | 2 | 2 | 3 | 0 | 2 | 2 | 2 |
| 828 | 5285 | 7056 | 9032 | 10444 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |

population increased by 31.4% although the mean size of the largest 10 decreased by 32.0%.

In addition to the changes in the mean size of individuals in the population, the composition varied between the populations and between years. The greatest source of variation was the uneven production of seedlings from year to year (Table 2.5).

Shoot Dynamics

Individuals composed of a single shoots were significantly smaller ($p < 0.05$) than those with multiple shoots or ramets (Table 2.6). However, in only 2 cases was there a significant size difference between individuals with 2 shoots or those with more than 2 shoots (Table 2.7). It should be noted that the mean area of individuals with more than 2 shoots was consistently larger than the mean area of 2 shoot individuals.

Changes in the number of shoots from year to year does not always cause a significant size change (Table 2.8). In most cases there was no significant difference in the size of plants that increased shoot number and those that decreased in shoot number. In all but two instances (the BB 1989-90 increase and GC 1988-89 decrease) individuals increased in overall size regardless of whether shoot number increased or decreased.

It was thought that asexual reproduction would occur by the branching and splitting of multiple shoot rhizomes. In almost all cases the rhizomes of these individuals were close enough to the surface to observe without extensive excavation. While placement of some of the rhizomes suggests that this process does occur, no such asexual

Table 2.5 Population composition as seedlings, single shoot rhizomes, and multishoot rhizomes. Percentage of the total is given in parentheses.

| Population | Year | Seedling | Single shoot Rhizomes | Multishoot Rhizomes | Total |
|---|---|---|---|---|---|
| Bull Bluff | 1988 | 0 | 276 (88.5) | 36 (11.5) | 312 |
| Bull Bluff | 1989 | 164 (32.3) | 298 (58.8) | 45 (8.9) | 507 |
| Bull Bluff | 1990 | 523 (50.0) | 465 (44.4) | 59 (5.6) | 1047 |
| Grassy Creek | 1987 | 0 | 1219 (92.7) | 96 (7.3) | 1315 |
| Grassy Creek | 1988 | 17 (1.2) | 1331 (90.4) | 125 (8.5) | 1473 |
| Grassy Creek | 1989 | 4 (0.3) | 1357 (91.4) | 124 (8.4) | 1485 |
| Grassy Creek | 1990 | 1274 (46.1) | 1335 (48.4) | 152 (5.5) | 2761 |

Table 2.6 Comparison of the means of log area, A(t) in $cm^2$ of single shoot rhizomes versus mean of log area of multiple shoot rhizomes. One-tailed t-test of the hypothesis that single and multishoot genets have equal mean areas.

| Site/Year | Type | N | A(t) | s | t | Prob >\|t\| |
|---|---|---|---|---|---|---|
| BB 1988 | single shoot | 276 | 2.52 | 0.62 | -6.25 | 0.0001[a] |
| | multishoot | 36 | 3.03 | 0.44 | | |
| BB 1989 | single shoot | 281 | 2.53 | 0.61 | -6.94 | 0.0001 |
| | multishoot | 45 | 3.03 | 0.42 | | |
| BB 1990 | single shoot | 361 | 2.15 | 1.06 | -13.95 | 0.0001 |
| | multishoot | 59 | 3.16 | 0.32 | | |
| GC 1987 | single shoot | 1219 | 2.10 | 0.60 | -8.95 | 0.0001 |
| | multishoot | 96 | 2.62 | 0.54 | | |
| GC 1988 | single shoot | 1289 | 2.21 | 0.68 | -11.25 | 0.0001 |
| | multishoot | 125 | 2.78 | 0.53 | | |
| GC 1989 | single shoot | 1190 | 2.37 | 0.64 | -13.28 | 0.0001 |
| | multishoot | 124 | 2.99 | 0.47 | | |
| GC 1990 | single shoot | 1168 | 2.49 | 0.65 | -12.49 | 0.0001 |
| | multishoot | 152 | 3.04 | 0.49 | | |

[a] difference is highly significant

Table 2.7 Comparison of the mean of log area A(t), in $cm^2$, of individuals with 2 shoots versus mean of log area of individuals with more than 2 shoots (2+ shoots). One-tailed t-test of the hypothesis that individuals with 2 shoots and individuals with more than 2 shoots will have equal mean areas.

| Site/Year | Type | N | A(t) | s | t | Prob > \|t\| |
|---|---|---|---|---|---|---|
| BB 1988 | 2 shoots | 29 | 3.04 | 0.42 | 0.27 | 0.7928 |
| | 2+ shoots | 7 | 2.98 | 0.55 | | |
| BB 1989 | 2 shoots | 36 | 3.01 | 0.40 | -0.41 | 0.6891 |
| | 2+ shoots | 9 | 3.09 | 0.50 | | |
| BB 1990 | 2 shoots | 48 | 3.13 | 0.35 | -1.00 | 0.3352[a] |
| | 2+ shoots | 11 | 3.25 | 0.35 | | |
| GC 1987 | 2 shoots | 83 | 2.61 | 0.56 | -0.86 | 0.3983 |
| | 2+ shoots | 13 | 2.72 | 0.39 | | |
| GC 1988 | 2 shoots | 110 | 2.75 | 0.54 | -2.70 | 0.0126[a] |
| | 2+ shoots | 15 | 3.04 | 0.36 | | |
| GC 1989 | 2 shoots | 105 | 2.96 | 0.49 | -1.59 | 0.1221 |
| | 2+ shoots | 19 | 3.11 | 0.34 | | |
| GC 1990 | 2 shoots | 117 | 3.02 | 0.47 | -0.84 | 0.4065 |
| | 2+ shoots | 35 | 3.11 | 0.57 | | |

[a] difference is significant, for all others there is no significant difference

Table 2.8 Comparison of means of log Area, A(t) and A(t+1) in $cm^2$, of individuals that had changes in shoot number. Two-tailed t-test of the hypothesis that the mean areas of individuals that increased in shoot number (type = increased) and those that decreased in shoot number (type = decreased) are equal.

| Site/Year | Year | Type | N | A | s | t | Prob > \|t\| |
|---|---|---|---|---|---|---|---|
| BB 1988-89 | A(1988) | decreased | 15 | 2.76 | 0.49 | 0.14 | 0.8946 |
| | | increased | 8 | 2.79 | 0.70 | | |
| BB 1988-89 | A(1989) | decreased | 15 | 2.84 | 0.44 | 0.08 | 0.9359 |
| | | increased | 8 | 2.86 | 0.68 | | |
| BB 1989-90 | A(1989) | decreased | 7 | 2.78 | 0.44 | -2.05 | 0.0762 |
| | | increased | 25 | 3.13 | 0.31 | | |
| BB 1989-90 | A(1990) | decreased | 7 | 2.94 | 0.56 | 0.561 | 0.4324 |
| | | increased | 25 | 3.13 | 0.37 | | |
| GC 1987-88 | A(1987) | decreased | 9 | 2.76 | 0.48 | 1.34 | 0.2054 |
| | | increased | 39 | 2.53 | 0.46 | | |
| GC 1987-88 | A(1988) | decreased | 9 | 2.90 | 0.49 | 0.844 | 0.4154 |
| | | increased | 39 | 2.75 | 0.47 | | |
| GC 1988-89 | A(1988) | decreased | 27 | 2.46 | 0.61 | -2.83 | 0.0069[a] |
| | | increased | 29 | 2.87 | 0.44 | | |
| GC 1988-89 | A(1989) | decreased | 27 | 2.36 | 0.62 | -4.25 | 0.0001[a] |
| | | increased | 29 | 2.99 | 0.47 | | |
| GC 1989-90 | A(1989) | decreased | 19 | 2.97 | 0.11 | 0.769 | 0.4465 |
| | | increased | 61 | 2.88 | 0.55 | | |
| GC 1989-90 | A(1990) | decreased | 19 | 3.05 | 0.38 | 0.468 | 0.6421 |
| | | increased | 61 | 2.99 | 0.59 | | |

[a] difference is significant, for all others there is no significant difference

reproduction was observed during the study. However, two instances were noted in 1992 after the completion of the study, both in the Grassy Creek population.

Flowering

Only a relatively small portion of the plants were capable of blooming (determined by the presence of an inflorescence at some time during the growing season) in any given year. Of those individuals that did have inflorescences, approximately 20% did not produce follicles with seeds (Table 2.9). The percentage of flowering plants per year fluctuated and may be linked in part to climatic variability. However, seedling number also caused some variation. At Grassy Creek in 1990, the large number of seedlings present caused a drop in the percentage of flowering individuals (10.2 - 5.1%) even though the actual number of flowering individuals stayed about the same (134 - 132). A similar situation occurred at Bull Bluff between 1989 and 1990. In addition to a low percentage of flowering individuals in the population, a number of plants that had inflorescences did not ever bloom. In those cases the inflorescences were broken off by branch fall, were damaged by herbivores or died from undetermined causes. Of those that did bloom, a small portion did not develop follicles, sometimes due to the death of the inflorescence after blooming. In some cases information about inflorescence fate was missing. Those individuals were not included in further calculations.

Flowering was closely related to plant size. The mean size of flowering (one or more inflorescences) plants was significantly larger

Table 2.9 Portion of each population with one or more inflorescences and comparative fate of the inflorescences. Percentages are given in parentheses.

| Site/Year | Flowering Plants | Inflorescence Death | No Follicle Development | Follicle Development | Unknown Fate |
|---|---|---|---|---|---|
| BB 1988 | 36/312 (11.5) | 7 (19.4) | 1 (2.8) | 25 (69.4) | 3 (8.3) |
| BB 1989 | 82/490 (16.7) | 16 (20.3) | 1 (1.3) | 62 (78.5) | 3 (3.7) |
| BB 1990 | 82/943 (8.7) | 1 (1.2) | 1 (1.2) | 77 (93.9) | 3 (3.7) |
| GC 1987 | 38/1315 (2.9) | 9 (23.7) | 6 (15.8) | 22 (57.9) | 1 (2.6) |
| GC 1988 | 82/1432 (5.7) | 9 (11.0) | 2 (2.4) | 71 (86.6) | 0 |
| GC 1989 | 144/1318 (10.9) | 19 (13.2) | 2 (1.4) | 113 (78.5) | 10 (6.9) |
| GC 1990 | 139/2594 (5.4) | 20 (14.4) | 3 (2.2) | 109 (78.4) | 7 (5.0) |

($p < 0.0001$) than the mean size of those that had no inflorescence (Table 2.10). The mean size of plants with 2 or more inflorescences was also significantly larger than the mean size of those with only 1 inflorescence (Table 2.11). A weak but highly significant positive relationship was also seen between the number of ovaries and follicles (not all ovaries developed into seed-bearing follicles) that a plant produced and its size (Tables 2.12 and 2.13). The closest correlations were between plant size and ovary number and plant size and follicle number in the same years. Generally, there is also positive correlation between plant size and ovary number and follicle number in years t-1 and t+1.

The probability of flowering based on size, area in $cm^2$, was calculated using the probit procedure in SAS (1987) for both populations in all years. This procedure calculates the probability of a plant flowering based on its size in a given year. The model generated was defined by the equation:

$$p = \exp(u + yr + A*.0029)/1 + \exp(u + yr + A*.0029)$$

where p is the probability of flowering, u is the estimated mean or intercept, yr is the mean area for that year, A is the size in $cm^2$, and .0029 is the slope. Using this model, plots were made of the probability of flowering based on size. These plots were overlain on plots of relative frequency of plants flowering by size (Figures 2.3-2.9). A comparison of the plots by year, by site, and by year and site show that all are significantly different ($p < 0.05$) except for the years of 1988 and 1990 (Table 2.14). The equation was also used to

Table 2.10 Comparison of mean size as log area, A(t) in $cm^2$, of nonflowering plants versus mean size of flowering plants. One-tailed t-test of the hypothesis that the mean sizes are equal.

| Site/Year | Type | N | A(t) | s | t | Prob > \|t\| |
|---|---|---|---|---|---|---|
| BB 1988 | nonflowering | 276 | 2.486 | 0.612 | -15.38 | 0.0001 |
| | flowering | 36 | 3.262 | 0.210 | | |
| BB 1989 | nonflowering | 245 | 2.395 | 0.572 | -18.81 | 0.0001 |
| | flowering | 81 | 3.201 | 0.201 | | |
| BB 1990 | nonflowering | 338 | 2.054 | 1.027 | -20.87 | 0.0001 |
| | flowering | 82 | 3.292 | 0.181 | | |
| GC 1987 | nonflowering | 1277 | 2.101 | 0.593 | -31.94 | 0.0001 |
| | flowering | 38 | 3.200 | 0.184 | | |
| GC 1988 | nonflowering | 1332 | 2.195 | 0.653 | -40.25 | 0.0001 |
| | flowering | 82 | 3.299 | 0.188 | | |
| GC 1989 | nonflowering | 1171 | 2.323 | 0.612 | -37.30 | 0.0001 |
| | flowering | 143 | 3.289 | 0.224 | | |
| GC 1990 | nonflowering | 1186 | 2.458 | 0.631 | -34.41 | 0.0001 |
| | flowering | 134 | 3.352 | 0.213 | | |

Table 2.11 Comparison of mean size as log area, A(t) in $cm^2$, of plants with 1 inflorescence versus mean size of plants with 2 or more inflorescences. One-tailed t-test of the hypothesis that the mean sizes are equal.

| Site/Year | Type | N | A(t) | s | t | Prob > \|t\| |
|---|---|---|---|---|---|---|
| BB 1988 | 1 inflorescence | 34 | 3.245 | 0.209 | -3.64 | 0.0660 |
| | 2+ inflorescences | 2 | 3.485 | 0.077 | | |
| BB 1989 | 1 inflorescence | 75 | 3.179 | 0.145 | -4.72 | 0.0028 |
| | 2+ inflorescences | 6 | 3.477 | 0.145 | | |
| BB 1990 | 1 inflorescence | 75 | 3.266 | 0.163 | -7.04 | 0.0001 |
| | 2+ inflorescences | 7 | 3.574 | 0.105 | | |
| GC 1987 | 1 inflorescence | 37 | 3.186 | 0.164 | [a] | |
| | 2+ inflorescences | 1 | 3.723 | | | |
| GC 1988 | 1 inflorescence | 79 | 3.284 | 0.170 | -3.41 | 0.0733 |
| | 2+ inflorescences | 3 | 3.715 | 0.217 | | |
| GC 1989 | 1 inflorescence | 136 | 3.264 | 0.197 | -8.04 | 0.0001 |
| | 2+ inflorescence | 7 | 3.775 | 0.162 | | |
| GC 1990 | 1 inflorescence | 126 | 3.334 | 0.197 | -2.85 | 0.0231 |
| | 2+ inflorescences | 8 | 3.622 | 0.280 | | |

[a] test is invalid if done with n=1

Table 2.12 Correlation between plant size, area in $cm^2$, and the number of ovaries produced. The Pearson Correlation Coefficients are shown with the probability of getting a greater r shown below the coefficient.

| | Site/Year of Blooming | | | | | | |
|---|---|---|---|---|---|---|---|
| Year | BB88 | BB89 | BB90 | GC87 | GC88 | GC89 | GC90 |
| Year t | .305 | .406 | .519 | .539 | .663 | .686 | .405 |
| | .0847 | .0002 | .0001 | .0001 | .0001 | .0001 | .0001 |
| Year t-1 | | .011 | .159 | | .559 | .477 | .336 |
| | | .9270 | .1690 | | .0001 | .0001 | .0001 |
| Year t+1 | .419 | .381 | | .404 | .622 | .606 | |
| | .0170 | .0005 | | .0132 | .0001 | .0001 | |
| n | 33 | 79 | 79 | 37 | 82 | 134 | 132 |

Table 2.13 Correlation between plant size, area in $cm^2$, and the number of follicles that develop. The Pearson Correlation Coefficients are shown with the probability of getting a greater r shown below the coefficient.

| | Site/Year of Blooming | | | | | | |
|---|---|---|---|---|---|---|---|
| Year | BB88 | BB89 | BB90 | GC87 | GC88 | GC89 | GC90 |
| Year t | .375 | .428 | .509 | .480 | .685 | .633 | .314 |
| | .0313 | .0001 | .0001 | .0027 | .0001 | .0001 | .0001 |
| Year t-1 | | ..057 | .125 | | .564 | .438 | .246 |
| | | .0619 | .1690 | | .0001 | .0001 | .0001 |
| Year t+1 | .470 | .347 | | .396 | .620 | .564 | |
| | .0067 | .0017 | | .0152 | .0001 | .0001 | |
| n | 33 | 79 | 79 | 37 | 82 | 134 | 132 |

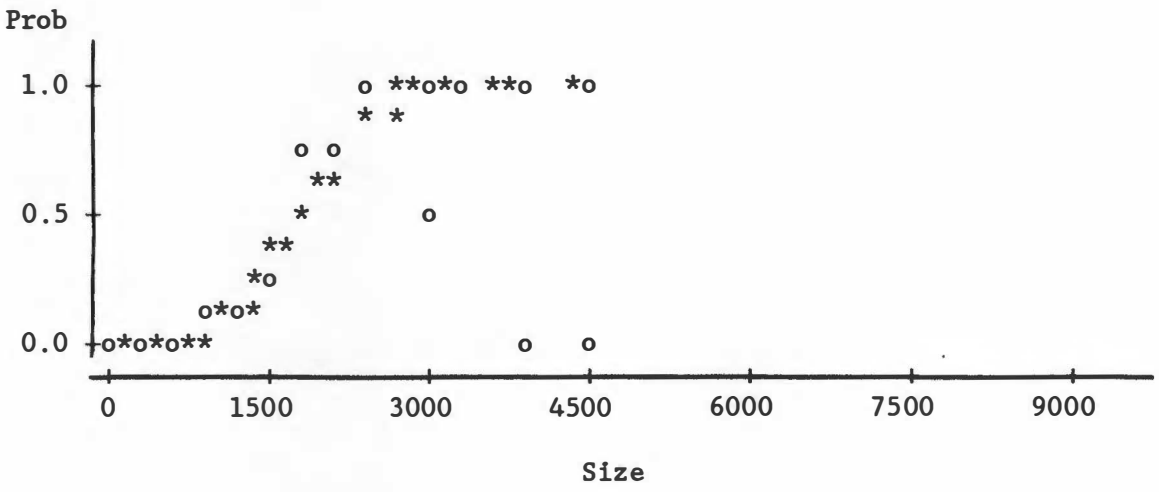



Figure 2.3 Plot of the probability of an individual at Bull Bluff in 1988 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is the relative probability.

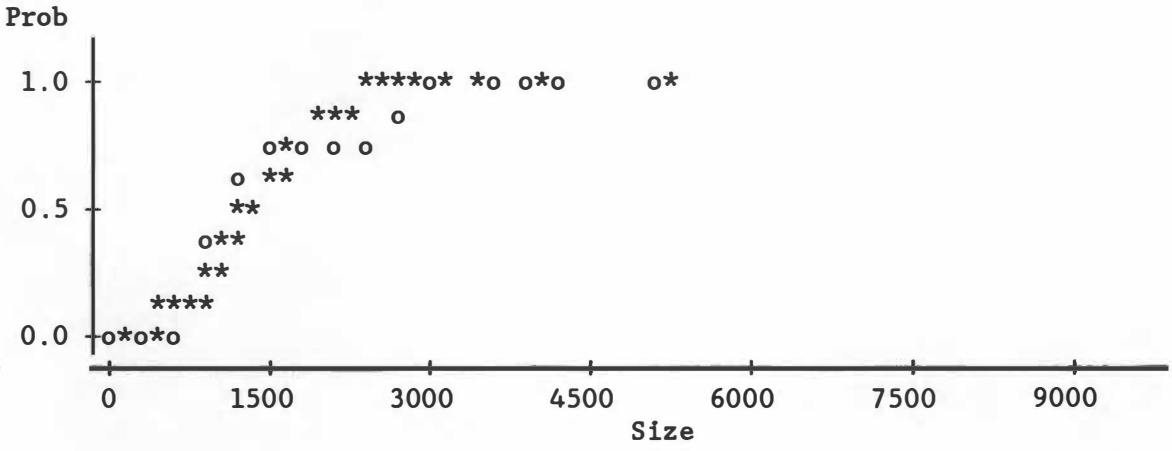



Figure 2.4 Plot of the probability of an individual at Bull Bluff in 1989 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is relative probability.

Bull Bluff 1990

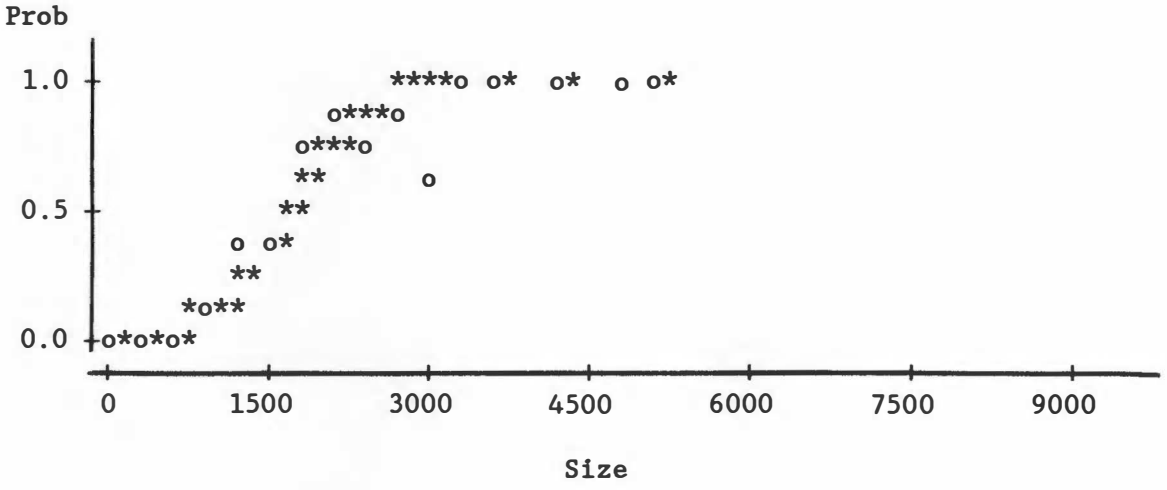



Figure 2.5 Plot of the probability of an individual at Bull Bluff in 1990 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is relative probability.

Grassy Creek 1987

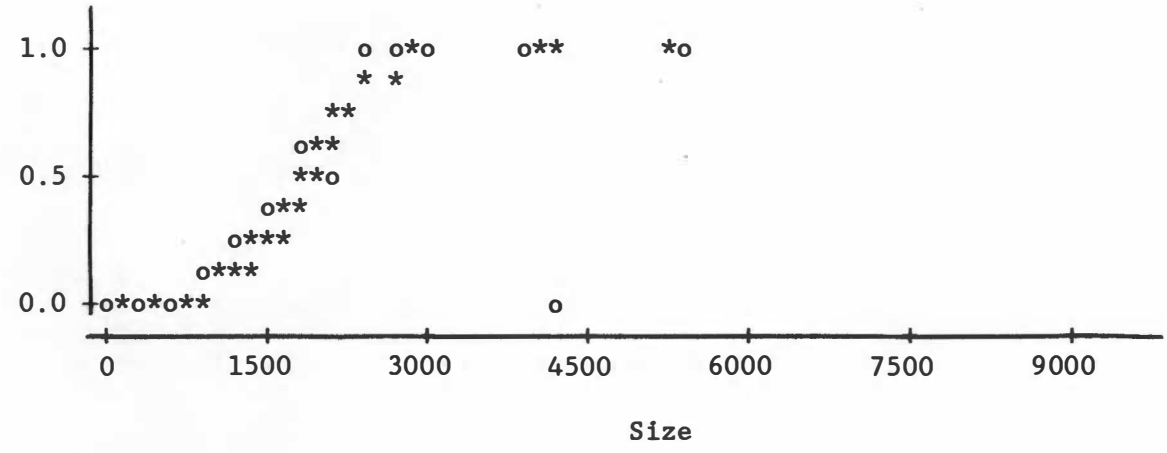



Figure 2.6 Plot of the probability of an individual at Grassy Creek in 1987 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is relative probability.

Grassy Creek 1988

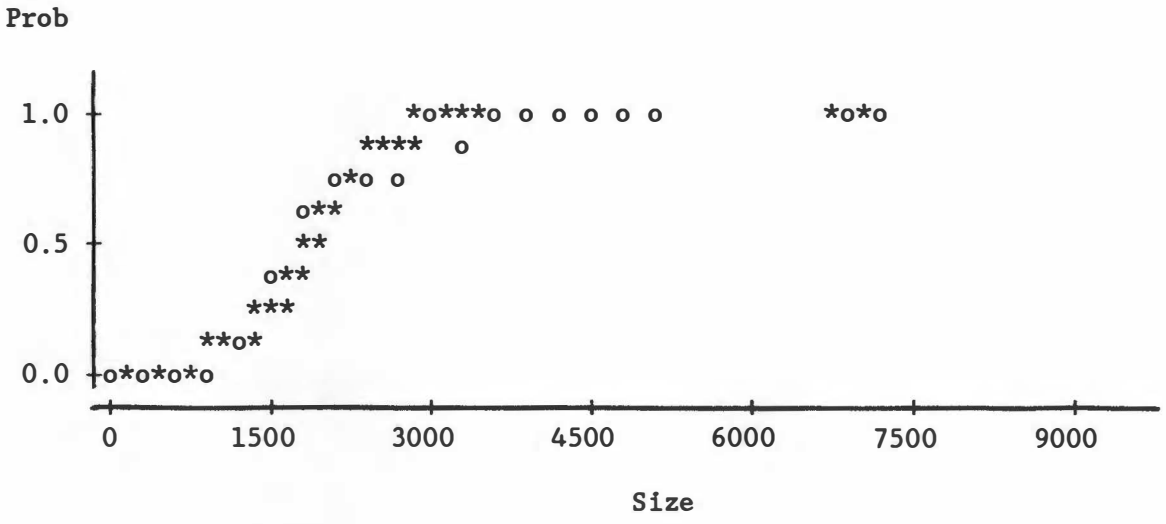



Figure 2.7 Plot of the probability of an individual at Grassy Creek in 1988 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is relative probability.

Grassy Creek 1989

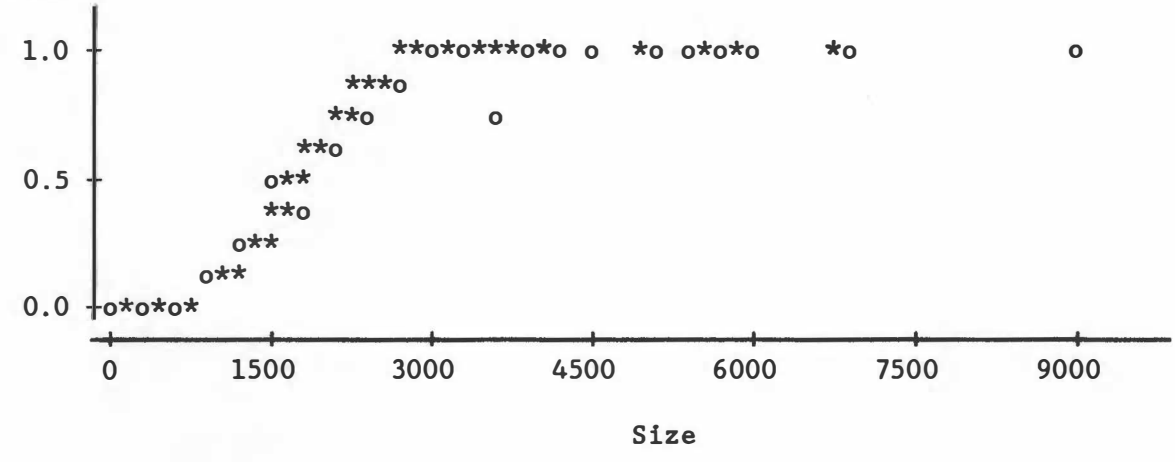



Figure 2.8 Plot of the probability of an individual at Grassy Creek in 1989 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is relative probability.

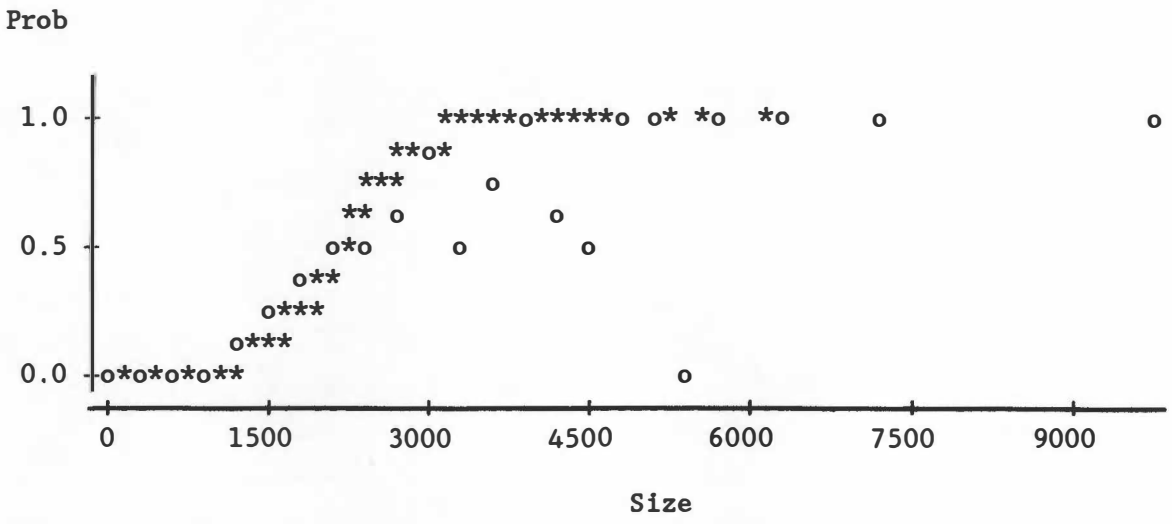



Figure 2.9 Plot of the probability of an individual at Grassy Creek in 1990 flowering based on its size, $cm^2$ (*) overlain on a plot of the relative frequency of plants flowering by size, $cm^2$ (o). Prob is relative probability.

Table 2.14 Comparison of the estimated intercepts from the flowering probability model. The Grassy Creek 1990 population was used as the expected for a Chi Square analysis. Significance was tested at the 0.05 level.

| Site/Year | Estimated Intercept | $X^2$ | df | $p > X^2$ |
|---|---|---|---|---|
| SITE/YEAR | | | | |
| Bull Bluff 1988 | 1.0844 | 5.45 | 1 | 0.0196 |
| Bull Bluff 1989 | 2.4782 | 43.27 | 1 | 0.0001 |
| Bull Bluff 1990 | 1.4526 | 14.93 | 1 | 0.0001 |
| Grassy Creek 1987 | 0.9224 | 5.11 | 1 | 0.0238 |
| Grassy Creek 1988 | 0.9043 | 6.84 | 1 | 0.0089 |
| Grassy Creek 1989 | 1.2558 | 16.10 | 1 | 0.0001 |
| SITE | | | | |
| Bull Bluff | 1.0478 | 21.68 | 1 | 0.0001 |
| YEAR | | | | |
| 1987 | 0.7618 | 3.86 | 1 | 0.0490 |
| 1988 | 0.4649 | 2.75[a] | 1 | 0.0973 |
| 1989 | 1.1619 | 21.77 | 1 | 0.0001 |
| MODEL | 0.0029 | 370.33 | 1 | 0.0001 |

[a] difference is not significant, all others are significantly different

estimate the size at which 50% probability of flowering is attained (Table 2.15).

Dormancy and Mortality

The majority of individuals in each population were present in all years of the survey (Table 2.16), although a large number of individuals were absent for one or more years of the study (29.2% at Bull Bluff and 21.9% at Grassy Creek). In many instances, an individual that was missing one year would be present in a later year. The appearance of previously unmarked nonseedling individuals (to be referred to as reemergents) in the fourth year of the study at Grassy Creek indicates that these plants remained dormant for at least three years. Because of this it was usually impossible to determine if missing individuals were dead or dormant without destructive sampling. Therefore, no distinction was made between dead and dormant individuals. In both populations, the group of plants that was present for all years of the study had the largest mean size and also included the largest individuals. It should be noted that, of the Grassy Creek plants that were missing in 1990, 37.5% were in an area that was flooded during 1989. Of the 10 largest plants that were missing 1990, 9 were in the flood area. Only the plants that were on the edges of the flooded areas have reappeared since the end of the study. In 1989 the plants had been measured prior to the flooding so presence and size were not affected that year.

A number of reemergents were observed each year in both populations (Table 2.17). These reemergents are thought to be

Table 2.15 Size, area as $cm^2$, at which the probability of flowering reaches 50 percent.

| Site | Year | A |
|---|---|---|
| Bull Bluff | 1988 | 1342 |
| Bull Bluff | 1989 | 1696 |
| Bull Bluff | 1990 | 1879 |
| Grassy Creek | 1987 | 1879 |
| Grassy Creek | 1988 | 1885 |
| Grassy Creek | 1989 | 1885 |
| Grassy Creek | 1990 | 2196 |

Table 2.16 Comparison of the size of plants that were present for all years of the study to the size of plants that were absent for portions of the study. The range of size and mean of size are in $cm^2$. Mean size is from the first year the group of plants was present.

| Years present | N | Range | Mean |
|---|---|---|---|
| **Bull Bluff** | | | |
| 88 89 90 | 287 | 2.61- 4809.26 | 758.89 |
| 89 90 | 28 | 3.25- 1112.76 | 172.18 |
| 88 89 | 7 | 1.36- 2005.83 | 439.87 |
| 88 | 9 | 0.90- 1626.48 | 427.01 |
| 88 90 | 8 | 5.25- 1528.27 | 474.11 |
| 89 | 3 | 1.26- 351.77 | 121.59 |
| 90 | 3 | 7.05- 208.56 | 74.65 |
| **Grassy Creek** | | | |
| 87 88 89 90 | 1151 | 1.80- 5285.30 | 336.05 |
| 88 89 90 | 91 | 1.44- 1324.30 | 96.16 |
| 87 88 90 | 43 | 2.30 441.61 | 69.48 |
| 87 88 89 | 40 | 1.68- 953.33 | 203.51 |
| 87 | 37 | 0.90- 863.50 | 81.87 |
| 87 88 | 33 | 1.32- 437.79 | 82.19 |
| 88 | 29 | 1.26- 200.10 | 24.03 |
| 88 90 | 12 | 2.00- 32.64 | 14.44 |
| 88 89 | 10 | 1.14- 113.68 | 32.23 |
| 89 90 | 8 | 2.43- 990.59 | 155.10 |
| 87 89 90 | 7 | 14.00- 598.26 | 169.34 |
| 90 | 6 | 1.56- 908.14 | 211.41 |
| 89 | 3 | 1.43- 42.55 | 15.56 |
| 87 90 | 2 | 31.63 95.24 | 63.43 |
| 87 89 | 2 | 2.81- 104.08 | 53.44 |

Table 2.17 Comparison of mean leaf area, log A(t), of nondormant versus mean leaf area of reemergent (dormant in the previous year) individuals. One-tailed t-test of the hypothesis that nondormant and reemergent plants have equal mean areas.

| Site/Year | Type | N | A(t) | s | t | Prob>\|t\| |
|---|---|---|---|---|---|---|
| BB 1989 | nondormant | 300 | 2.667 | 0.541 | 5.62 | 0.0001 |
| | reemergent | 25 | 1.840 | 0.719 | | |
| BB 1990 | nondormant | 401 | 2.378 | 0.989 | 9.01 | 0.0001 |
| | reemergent | 14 | 0.815 | 0.623 | | |
| GC 1988 | nondormant | 1261 | 2.369 | 0.592 | 16.22 | 0.0001 |
| | reemergent | 148 | 1.406 | 0.693 | | |
| GC 1989 | nondormant | 1304 | 2.467 | 0.646 | 5.13 | 0.0012 |
| | reemergent | 8 | 1.510 | 0.506 | | |
| GC 1990 | nondormant | 1316 | 2.553 | 0.654 | 2.22 | 0.1124[a] |
| | reemergent | 4 | 1.239 | 1.180 | | |

[a] difference is not significant, all others are significantly different

individuals that were dormant during the first survey year. Some of these may be individuals that died back early in the year before being censused. A small number may be individuals overlooked during the initial survey. In all cases the mean size of the reemergents was significantly smaller than that of plants that had not been dormant.

The mean size of plants that were present for all years of the study is significantly larger than that of plants that became dormant or died with the exception of plants that were missing from Bull Bluff in 1989 (Table 2.18).

Table 2.18 Comparison of the mean leaf area, log A(t-1), of dead and dormant individuals (absent) versus mean leaf area of individuals present all years of the study (present). One-tailed t-test of the hypothesis that dead and dormant individuals and individuals always present have equal mean area.

| Site/Year | Type | N | $A_{(t-1)}$ | s | t | Prob > \|t\| |
|---|---|---|---|---|---|---|
| BB 1989 | absent | 17 | 2.154 | 0.912 | 1.99 | 0.0629[a] |
| | present | 295 | 2.600 | 0.594 | | |
| BB 1990 | absent | 11 | 1.694 | 1.124 | 2.74 | 0.0206 |
| | present | 315 | 2.627 | 0.567 | | |
| GC 1988 | absent | 40 | 1.372 | 0.655 | 7.59 | 0.0001 |
| | present | 1267 | 2.167 | 0.593 | | |
| GC 1989 | absent | 112 | 1.387 | 0.719 | 13.70 | 0.0001 |
| | present | 1292 | 2.347 | 0.609 | | |
| GC 1990 | absent | 54 | 1.778 | 0.985 | 5.05 | 0.0001 |
| | present | 1257 | 2.461 | 0.615 | | |

[a] difference is not significant, all others are significantly different

## DISCUSSION

Size distributions in the populations of C. rubifolia studied follow the general pattern found in many herbaceous perennials with most individuals being in the smaller size classes. This held true even though there was an increase in the mean size of individuals during the study.

It was interesting that while there was an overall increase in size it did not hold true for all individuals. In the Bull Bluff population, the 10 largest individuals the first year of the study showed a progressive decline in size, number of ramets, flowering

occurrence and number of follicles produced. This trend was also seen in some of the larger individuals at Grassy Creek after the end of the study. This may be a result of sampling error or it may indicate that individuals reach a maximum size at which they will stay for a period of time before they begin a slow decline in size that eventually results in death. Another possibility is that individuals may undergo cyclic size changes with increases in size and fecundity followed by periods of smaller size and lowered fecundity. If older individuals do decrease in size it would mean that size is an inadequate determinant of age. A much longer study is needed to determine if it is a real trend or if the size decrease is reversible.

As in studies of most other herbaceous perennials, size (as photosynthetic area) proved to be an important determinant in the state of the plant. Generally it appears that a certain minimum size must be attained before a rhizome develops additional shoots. The fact that there were only two instances (at Bull Bluff in 1990 and Grassy Creek in 1988) where there was a significant difference between rhizomes with two shoots as opposed to those with more than two suggests that once this minimum size is attained the actual shoot number may be influenced by factors other than size. It was expected that size would increase as shoot number increased and decrease as shoot number decreased. This did hold true as individuals went from one to two ramets. However, among the multishooted rhizomes there were only two cases were the mean size of the plants decreased and one of these was with a ramet number increase (Table 2.6). In all other instances the mean size of multishoot rhizomes increased regardless of whether ramet number increased or

decreased. This suggests that the yearly size increase seen in most individuals during the period of the study masked other trends that might be notable during periods of more consistent precipitation conditions.

Because of the destructive sampling required, no attempt was made to determine what degree of physiological communication exists between different shoots on the same rhizome. There is evidence that rhizomes tend to be physiologically persistent (Pitelka and Ashman 1985). The behavior of ramets observed in the field suggest that this is also true for C. rubifolia rhizomes. In plants with multiple shoots or ramets, there would sometimes be alternation from year to year as to which of the ramets was largest or which had an inflorescence. In the two instances where rhizome fragmentation was noted, the connecting section of the rhizome had become visibly decayed. This finding indicates that asexual reproduction by rhizome fragmentation does occur but that it is a relatively slow process and will not be easily observed.

Size was also an important correlate to flowering in C. rubifolia. It appears as if there is a minimum size threshold that must be attained before a plant flowers. There was variation in the size at which the probability of flowering reached 50%. This indicates that there may be other factors involved such as amount of precipitation. However, it is also probably a reflection of the increase in both mean size of individuals and in numbers of individuals flowering.

The question still remains of whether size is linked to precipitation and flowering to size or if both are independently linked to precipitation. It is unknown whether flower primordia develop the

year of flowering or the year before as new shoot primordia develop. In the spring when the leaves have just finished expanding, some plants will have what appears (from field observation only) to be a very small flower stalk primordium that never develops. This implies the flower primordia develop as the leaf primordia develop. Even assuming formation of flower primodia is begun in the year prior to blooming, the presence of undeveloped primoridia would suggest that the maturation of primordia into infloresences is also linked to size the plant attains in year of flowering.

The weak but highly significant relationship between number of ovaries and follicles to size suggests that once an individual attains the size needed to support flowering, fluctuations in size beyond this is less important. The very low correlation coefficients found in the Bull Bluff population are probably due to the size decrease observed in several of the larger individuals where their size dropped below the apparent flowering size threshold.

The change in population size from year to year is typically for a number of species (Barkham 1980a, 1980b; Bierzychudek 1982a; Tamm 1956a, 1956b). The precipitation patterns for the time of the study may have resulted in a somewhat greater population size flux than might be expected between years that were more similar in precipitation. Much of the population size fluctuations were due both to variation in number of seedling recruits and the large number of reemergents and dormant individuals.

It is probable that part of the second year reemergents were those individuals that were missed the first year of the study or that had

died back before censusing. However, even if the reemergent plants from the second year of the survey and those plants that disappeared and did not reappear by the end of the survey are eliminated from the calculations, the percentage of the populations that were dormant for part of the study is still high (5% for Bull Bluff and 6.7% for Grassy Creek).

Most of the plants that experienced either dormancy or mortality were smaller plants. However, at both populations there were disturbances that resulted in the size independent death of a number of plants. In 1989 at Grassy Creek an area at the base of the slope was either underwater or had saturated soil for a large part of the growing season. Plants in this area have not reappeared and attempts to locate rhizomes have been unsuccessful. Four of the plants were of sufficient size to have flowered the previous year. The other known cause of mortality was tree fall disturbance from the uprooting of a tree at Bull Bluff. Some individuals were able to reroot and survived the displacement while a few others were never relocated and were presumed to be buried or lost in the reservoir.

The relationships between size as photosynthetic area, and life history characters of C. rubifolia were similar to those observed in other long-lived herbaceous perennials. Both mortality and dormancy were negatively correlated to the size of the individual. Flowering capability was highly positively correlated to plant size while the number of flowers produced was less strongly related to size. Asexual reproduction by rhizome fragmentation was shown to be a possible but very rare occurrence in the populations studied.

# CHAPTER 3

# DEMOGRAPHY AND SIZE CLASSIFICATION IN TWO POPULATIONS OF *CIMICIFUGA RUBIFOLIA*

## INTRODUCTION

Demography is the study of the number of individuals in a population and how it changes. The purpose then of a demographic study is to attempt to understand how the population will change over time. Changes in the population will be the result of both the pattern of births and deaths in a population as well as of the population structure (Sarukahn and Gadgil 1974; Silverton 1991). The structure of the population, as a size hierarchy, is important for reasons other than predicting the future size of the population. Such studies also provide information on the number of individuals that are contributing genes to future generations. If only a few large individuals in a population are reproducing, there will be an effect on both the ecology and the evolutionary potential of the populations (Heywood 1986; Weiner and Solbrig 1984).

Recruitment is an important factor in determining the dynamics of a population. Mode of reproduction, timing of reproduction, seed production, seed germination, and seedling survival influence the population dynamics. Within herbaceous perennials, a number of recruitment possibilities exist. In many species, asexual reproduction is most common with recruitment of new genets occurring only rarely. In other species, asexual reproduction either does not occur or occurs only rarely so recruitment is primarily through seed production. Other species may show variation between populations or between years as to

whether asexual reproduction or sexual reproduction is predominant. If the species is long-lived, recruitment by seedlings every few years may be sufficient to maintain the population at a stable level (Bierzychudek 1982b; Chapman et al. 1989; Inghe and Tamm 1988; Keeler 1991; Matlack 1987).

Because population size is influenced by the pattern of births and deaths, fertility and mortality rates within a population can be used to predict changes in size and structure of the population. Different types of models have been designed to make these predictions. One such model is the population projection matrix. Leslie (1945) designed a matrix model for species in which fecundity and survival depend on the age of the individual. In this model, individuals are grouped into age classes. The probabilities of survival, mortality and reproduction for each class are determined for a given time period. The model is then used to estimate the stable age distribution and size of the population under the given conditions (Meagher 1982; Vandermeer 1981).

In many species, age may not be the primary determinant of the physiological state of the individual. This is particularly true of herbaceous perennials where size has been found to have more impact on the physiological condition of the individual than its age (Bierzychudek 1982a; Kirkpatrick 1984; Sohn and Policansky 1977; Werner 1975; Werner and Caswell 1977). In addition, it may not be possible to determine the age of many herbaceous plant species, making age classification difficult (if not impossible) in natural populations. The Lefkovitch matrix model (Lefkovitch 1965) is a modification of the Leslie model that is based on either size or life history stages rather than age

classes. In cases where fecundity cannot be readily determined it is impossible to predict population dynamics using Leslie or Lefkovitch models. However, these models can be used to predict stable age or size classification distributions under these circumstances.

In many studies of herbaceous perennials, the classification for the model is based on some measurement of photosynthetic area although other morphological or physiological characters may be used (Bierzychudek 1982a; Cochran 1986; Meagher 1982; Werner and Caswell 1977). The underlying assumption is that most or all reproductive investment comes from current photosynthesis. This is supported by studies that have shown strong correlations between aboveground vegetative biomass, leaf number, or leaf area and some measure of reproductive effort such as seed number or seed size (Fone 1989; Harper 1977; Solbrig 1981; Weiner 1988). Leaf removal also has been shown to reduce reproductive effort and implies that current photosynthetic products rather than stored assimilates are used (Lubbers and Lechowicz 1989; McKone 1989; Spears and May 1988). There are a number of difficulties in using herbaceous perennials in this type of study. In species where above ground parts die back each winter, verification of individuals can be difficult. Additionally, there is frequently some change in position of above ground parts between growing seasons as underground portions of the plant grow. Also, unlike woody perennials, the size of individuals in some herbaceous perennials has been shown to be reversible between years (Bierzychudek 1982a; Cochran 1986). This has also be shown to be true for C. rubifolia (Chapter 2). If individuals are capable of undergoing dormancy, it may not be possible to

distinguish between dead and dormant individuals. The unit of study is usually chosen arbitrarily as the ramet as genets cannot be accurately determined without destructive sampling. In plants with rhizomes, a single rhizome may produce multiple shoots, making identification of individuals difficult (Bierzychudek 1982a; Cochran 1986; Oostermeijer et al. 1992; Tamm 1972a, 1972b; Willems 1982).

The main purpose of this portion of the study was to develop a size classification model for Cimicifuga rubifolia. This model will then be used to determine if the structure of the study populations are currently stable and to estimate their stable size structure. The recruitment capability of the populations, in terms of seed production, seed germination, and seedling survival, will also be investigated.

Cimicifuga rubifolia is a herbaceous perennial. Stems rise from thick, horizontal rhizomes that may be 10 cm in length. The rhizome may branch and have active apices with leaves located on different portions. The current study has shown that asexual reproduction in the populations studied occurs rarely and for the purposes of this part of the study will be ignored. The inflorescence is a simple panicle of racemes. Fruits are follicles, containing an average of 8-9 seeds (Ramsey 1987). Flowering occurs in late summer. Fruit is set constantly at about 45% throughout the flowering period (Pellmyr 1986a). Seeds of Cimicifuga racemosa have been shown to have epicotyl dormancy or are "two year seeds". The seeds will germinate and produce radicles if incubated at suitable temperatures. However, epicotyls do not emerge until after exposure to low temperatures. If seeds are subjected to low temperatures before germination, radicles do not emerge until

temperatures have increased and epicotyls will not emerge until exposure to a second period of cold stratification (Baskin and Baskin 1985).

Two sites were used in this study. The first is located in Roane County, Tennessee and is situated on the lower slopes of Chestnut Ridge adjacent to the Grassy Creek embayment on Watts Bar Reservoir on land owned by the Tennessee Valley Authority (TVA). The second site is in Anderson County, Tennessee on the Oak Ridge National Laboratory Reservation. This site is located near the base of Bull Bluff and overlooks Melton Hill Reservoir. Aerial photographs from 1937 to 1982 show no signs of disturbance to the sites.

## MATERIALS AND METHODS

In 1987, 1315 individuals were marked in the Grassy Creek population. As described in Chapter 2, a simple leaf area model was constructed using field area measurements as compared to Licor area measurements. The field area measurement was derived by multiplying the 0.5 by the leaf height, cm, and by leaf width, cm, with length being the length of the rachis to the terminal leaflet tip and width being the distance between the tips of the basal leaflets. Correlation between the field area measurement and the Licor area measurement was 0.974 and the field area measurements were considered adequate to use for size determinations. From 1987 to 1990, the marked individuals in the populations were measured for annual expression of size. Rhizomes producing multiple shoots were checked annually to see if rhizome fragmentation had occurred. If the tags were located but no emergent plant was found, the plant was listed as absent. If tags for a plant

were not located, the plant was listed as missing and was not used in calculations. Seedlings were marked but were not measured to minimize possible damage. Instead, they were assigned a standard size based on the measurement of seedlings from outside the study plots. Any other unmarked plants were listed as reemergents and were marked and measured. The size of the plant was considered to be the sum of the areas of its leaves as determined by the model given above. The number of inflorescences per rhizome was noted. The number of flowers and the number of follicles that developed were recorded for all flowering individuals. Because of the large size of the Grassy Creek population, only a portion of the population, from near its western edge to a gap in the central portion, was used. All plants located within this area were used in the study.

The study was expanded to the Bull Bluff population in 1988 and was continued until 1990. The same measurements and observations were taken and recorded at this population as at Grassy Creek. Because of the small population size, all plants at this site were used in the study.

For each year, each population was divided into five classes with equal numbers of individuals in each class. The sizes of the largest individuals of each of these classes were averaged. Those averages were used as the dividing size for a classification system where the number of individuals in each class was relatively evenly distributed for both populations in all years. Seedlings were not used in these determinations. Another class, absent, was added for those individuals that were not present in that year, due either to death or to dormancy.

In the fall of 1988, seeds were collected for a germination test. Seed sources were plants from outside the study plot at Grassy Creek and a second population a few miles away. A soil mixture of 2 parts by volume mineral soil: 2 parts compost: 1 part sand was used. Soil pH was checked to ensure it was in the range of soils in which C. rubifolia occurs (Ramsey 1965). Seeds were sown on top of the soil and covered by maple-oak leaf litter. Two sets, each with 4 replicates of 200 seeds, were used. One set was placed inside a greenhouse and the other set was placed outside in an adjacent sheltered walkway. Seeds were watered weekly, except when the soil was frozen. The experiment was continued until July 1991. Each spring, the number of seedlings was counted. Seedlings were counted after the cotyledons became visible.

## RESULTS

### Reproduction

No seedlings were observed in the set of seeds that was placed inside the greenhouse. A small percentage of seeds from the set outside the greenhouse germinated in 1989, after 1 winter (Table 3.1). No seeds were observed to have germinated in 1991. The highest percentage of germination was in 1990, after exposure to 2 cold periods. This indicates that C. rubifolia seeds most likely undergo the same epicotyl dormancy as does C. racemosa. Seedlings were marked after germination but were not removed from the flats. Two 1990 seedlings produced inflorescences in 1991.

Seed production varied greatly from year to year at both the Grassy Creek and Bull Bluff populations (Table 3.2). This variation is

Table 3.1 Results of the germination experiment for seeds of Cimicifuga rubifolia. Percentages are given in parentheses. N for each replicate is 200.

| Year | Replicate A | B | C | D |
|---|---|---|---|---|
| 1989 | 8 (4.0) | 2 (1.0) | 0 | 12 (6.0) |
| 1990 | 53 (26.5) | 86 (43.0) | 40 (20.0) | 51 (25.5) |
| 1991 | 0 | 0 | 0 | 0 |
| Total | 61 (30.5) | 88 (44.0) | 40 (20.0) | 63 (31.5) |

Table 3.2 Estimated seed production per year for C. rubifolia at both sites. I is the number of individuals that produced seeds, $X_f$ is the average number of seed-bearing follicles per plant, F is the total number of follicles for the population, S is the estimated number of seeds produced, G is the number of seedlings observed and G% is percent germination.

| Site | Year | I | $X_f$ | F | S | G | G% |
|---|---|---|---|---|---|---|---|
| Bull Bluff | 1988 | 25 | 54.7 | 1422 | 11376 | 164 | 1.9 |
| Bull Bluff | 1989 | 62 | 72.2 | 4546 | 36368 | | |
| Bull Bluff | 1990 | 77 | 78.4 | 6094 | 48752 | | |
| Grassy Creek | 1987 | 22 | 9.9 | 277 | 2216 | 4 | 0.2 |
| Grassy Creek | 1988 | 71 | 59.8 | 4363 | 34904 | 1274 | 4.9 |
| Grassy Creek | 1989 | 113 | 41.3 | 4750 | 228000 | | |
| Grassy Creek | 1990 | 109 | 49.8 | 5578 | 267744 | | |

linked primarily to variation in the number of flowering plants, although there are also differences in the average number of follicles produced per plant from year to year. Seed production was estimated by multiplying the number of seed-bearing follicles by 8, the average number of seeds per follicle (Ramsey 1987). Because of the 2 years apparently required for maximum seed germination, it was impossible under the time constraints of the study to get counts needed for percentage field germination estimates for each year's seed crop.

Very little information on the survivorship of seedlings is available due to the very low production of seedlings during most years of the study (Table 3.3). However, it should be noted that, because of the very large number of seeds produced in some years, even with low germination and seedling survival rates, a relatively large number of new individuals could be introduced into the population sporadically. For instance, if only 1% of the seedlings produced at Grassy Creek in 1990 survive to maturity, approximately 13 new plants will have been recruited. Additional observations made at both populations since the end of the study have shown that large numbers of seedlings were produced again in both 1991 and 1992.

Size Classifications

For the purposes of this study, an individual was considered to be a rhizome and all of the shoots coming off of that rhizome. It is possible that in the case of some of the larger plants, the shoots were not connected physiologically and were functioning as independent individuals. However, excavation of rhizomes at other sites showed no

Table 3.3 Fate of C. rubifolia seedlings. The year given is the year of germination. Survival rate, as a percentage, is given in parentheses under the number of survivors, N. A is the mean leaf area in $cm^2$ of the survivors in a given year and t is the year in which the seedling was first observed.

| Site | Year | N(t) | N(t+1) | A(t+1) | N(t+2) | A(t+2) |
|---|---|---|---|---|---|---|
| Bull Bluff | 1989 | 164 | 80 (48.7) | 27.8 | | |
| Grassy Creek | 1988 | 17 | 2 (11.2) | 2.8 | 1 (5.9) | 5.0 |
| Grassy Creek | 1989 | 4 | 1 (25.0) | 1.6 | | |

examples of this and it was decided that the effects on the model would be negligible. In addition, no asexual reproduction was observed during the time of the study and was therefore not considered in the model.

As was previously reported (Chapter 2), population size distribution was significantly different from population to population and from year to year. In the same report it was noted that the mean size of the individuals in the populations did not remain constant from year to year. An overall increase in size occurred yearly and is thought to be primarily related to changes in precipitation. A summary of population composition by size class is given in Table 3.4. The size classes are as follows: class 0, absent; class A, 0-78 $cm^2$; class B, 79-240 $cm^2$; class C, 241-539 $cm^2$; class D 540-1055 $cm^2$; and class E, greater than 1055 $cm^2$.

Table 3.4 Composition of C. rubifolia populations by size classification (in $cm^2$). The percentage of the population is given in parenthesis. Range of size in each class is given in the text.

| Site | Year | Class 0 | Class A | Class B | Class C | Class D | Class E | Total |
|---|---|---|---|---|---|---|---|---|
| Bull Bluff | | | | | | | | |
| | 1988 | 0 | 36 (11.6) | 64 (20.6) | 60 (19.3) | 75 (24.1) | 76 (24.4) | 311 |
| | 1989 | 17 (5.0) | 37 (10.8) | 66 (19.3) | 65 (19.0) | 73 (21.3) | 84 (24.6) | 342 |
| | 1990 | 104 (19.9) | 108 (20.7) | 50 (9.6) | 69 (13.2) | 63 (12.0) | 129 (24.7) | 523 |
| Grassy Creek | | | | | | | | |
| | 1987 | 0 | 429 (32.6) | 379 (28.8) | 282 (21.5) | 151 (11.5) | 73 (5.6) | 1314 |
| | 1988 | 40 (2.8) | 389 (26.9) | 344 (23.8) | 293 (20.3) | 217 (15.0) | 161 (11.1) | 1444 |
| | 1989 | 167 (11.3) | 256 (17.3) | 321 (21.7) | 254 (17.2) | 235 (15.9) | 247 (16.7) | 1480 |
| | 1990 | 167 (11.2) | 223 (15.0) | 246 (16.5) | 259 (17.4) | 230 (15.5) | 363 (24.4) | 1488 |

Because of the two years apparently required for seed germination, it was not possible to incorporate seeds and seedlings into the matrix model. Therefore, the model was only used to predict the stable size distributions of the populations and not the dynamics in terms of changes in numbers of individuals.

Transition probability matrices of the Lefkovitch type can be used to predict the stable size distribution of a population provided they are temporally constant (the probability of moving from size class i to size class j is the same each year). Tests of the one year transition matrices (Table 3.5) from both populations show that the transition probabilities are not constant through time ($X^2$ = 111.82, df = 30, $p < 0.001$; Anderson and Goodman 1957). The greatest deviation came from the comparison of the Grassy Creek 1987-1988 and Grassy Creek 1988-1989 matrices ($X^2$ = 513.08, df = 30, $p < 0.001$). Within these two matrices, the greatest deviation for the constancy assumption comes in the largest size class (Class E, $X^2$ = 122.38, df = 5, $p < 0.001$). However, the transition probabilities of both the smallest class (Class A, $X^2$ = 4.96, df = 5, $p > 0.001$) and the largest class (Class E, $X^2$ = 7.76, df = 5, $p > 0.001$) in the Bull Bluff population are constant through time even though the matrices as a whole do not hold constant.

The two year transition matrices for Grassy Creek were also tested to see if they were constant through time (Table 3.6). They also were not constant ($X^2$ = 302.02, df = 30, $p < 0.001$) with the greatest deviation being in the largest size class (Class E, $X^2$ = 161.00, $p < 0.001$) and the least deviation being in the second smallest size class (Class B, $X^2$ = 22.70, df = 5, $P < 0.001$).

Table 3.5 One-year transition probabilities for individuals based on 0.5 x leaf length x leaf width ($cm^2$). Entry $a_{ji}$ = (probability of rhizome size i in year t becoming size j in year t+1). Class 0 consists of those rhizomes with 0 leaf area. Class n represents rhizomes with n-1 < leaf area <= n. Column sample sizes are given in parentheses.

| Site | | Class A[a] | Class B | Class C | Class D | Class E | Class 0 |
|---|---|---|---|---|---|---|---|
| Bull Bluff | | | | 1988 | | | |
| | A[b] | 0.528 | 0.031 | 0.017 | 0.013 | 0.000 | 0.000 |
| | B | 0.250 | 0.609 | 0.117 | 0.027 | 0.000 | 0.000 |
| 1989 | C | 0.056 | 0.281 | 0.467 | 0.107 | 0.026 | 0.000 |
| | D | 0.000 | 0.016 | 0.317 | 0.547 | 0.158 | 0.000 |
| | E | 0.000 | 0.000 | 0.050 | 0.293 | 0.763 | 0.000 |
| | 0 | 0.167 | 0.063 | 0.030 | 0.013 | 0.053 | 0.000 |
| | | (36) | (64) | (60) | (75) | (76) | (0) |
| Bull Bluff | | | | 1989 | | | |
| | A | 0.324 | 0.030 | 0.000 | 0.000 | 0.000 | 0.176 |
| | B | 0.351 | 0.409 | 0.046 | 0.014 | 0.024 | 0.000 |
| 1990 | C | 0.054 | 0.500 | 0.292 | 0.110 | 0.048 | 0.176 |
| | D | 0.054 | 0.061 | 0.492 | 0.219 | 0.083 | 0.000 |
| | E | 0.000 | 0.000 | 0.138 | 0.644 | 0.833 | 0.118 |
| | 0 | 0.216 | 0.000 | 0.031 | 0.014 | 0.012 | 0.529 |
| | | (37) | (66) | (65) | (73) | (84) | (17) |
| Grassy Creek | | | | 1987 | | | |
| | A | 0.289 | 0.227 | 0.255 | 0.219 | 0.110 | 0.000 |
| | B | 0.256 | 0.306 | 0.202 | 0.152 | 0.178 | 0.000 |
| 1988 | C | 0.179 | 0.214 | 0.245 | 0.172 | 0.247 | 0.000 |
| | D | 0.140 | 0.145 | 0.188 | 0.219 | 0.055 | 0.000 |
| | E | 0.096 | 0.084 | 0.078 | 0.225 | 0.397 | 0.000 |
| | 0 | 0.040 | 0.024 | 0.032 | 0.013 | 0.014 | 0.000 |
| | | (429) | (379) | (282) | (151) | (73) | (0) |

Table 3.5 (cont.)

| Site | | Class A[a] | Class B | Class C | Class D | Class E | Class 0 |
|---|---|---|---|---|---|---|---|
| Grassy Creek | | | | 1988 | | | |
| | A | 0.285 | 0.160 | 0.143 | 0.088 | 0.037 | 0.225 |
| | B | 0.252 | 0.294 | 0.157 | 0.189 | 0.137 | 0.250 |
| 1989 | C | 0.123 | 0.215 | 0.236 | 0.161 | 0.118 | 0.150 |
| | D | 0.103 | 0.142 | 0.242 | 0.235 | 0.130 | 0.100 |
| | E | 0.090 | 0.096 | 0.154 | 0.230 | 0.478 | 0.100 |
| | 0 | 0.147 | 0.093 | 0.068 | 0.097 | 0.099 | 0.175 |
| | | (389) | (344) | (293) | (217) | (161) | (40) |
| Grassy Creek | | | | 1989 | | | |
| | A | 0.438 | 0.087 | 0.071 | 0.068 | 0.041 | 0.216 |
| | B | 0.246 | 0.340 | 0.079 | 0.111 | 0.041 | 0.108 |
| 1990 | C | 0.078 | 0.327 | 0.268 | 0.111 | 0.105 | 0.078 |
| | D | 0.031 | 0.084 | 0.350 | 0.268 | 0.117 | 0.078 |
| | E | 0.090 | 0.118 | 0.157 | 0.349 | 0.623 | 0.156 |
| | 0 | 0.117 | 0.044 | 0.075 | 0.094 | 0.073 | 0.365 |
| | | (256) | (321) | (254) | (235) | (247) | (167) |

[a] size, $cm^2$ in year t
[b] size, $cm^2$ in year t+1

Table 3.6 Two-year transition probabilities for individuals based on 0.5 x leaf length x leaf width ($cm^2$). Entry $a_{ij}$ = (probability of rhizome size i in year t becoming size j in year t+2). Class 0 consists of those rhizomes with 0 leaf area. Class n represents rhizomes with n-1 < leaf area <= n. Column sample sizes are given in parenthesis.

| Site | | Class A | Class B | Class C | Class D | Class E | Class 0 |
|---|---|---|---|---|---|---|---|
| Bull Bluff | | | | 1988 | | | |
| | A | 0.278 | 0.031 | 0.017 | 0.000 | 0.000 | 0.000 |
| | B | 0.278 | 0.297 | 0.017 | 0.040 | 0.026 | 0.000 |
| 1990 | C | 0.194 | 0.422 | 0.233 | 0.147 | 0.066 | 0.000 |
| | D | 0.028 | 0.141 | 0.383 | 0.200 | 0.092 | 0.000 |
| | E | 0.028 | 0.063 | 0.317 | 0.587 | 0.776 | 0.000 |
| | 0 | 0.194 | 0.047 | 0.033 | 0.027 | 0.039 | 0.000 |
| | | (36) | (64) | (60) | (75) | (76) | (0) |
| Grassy Creek | | | | 1987 | | | |
| | A | 0.186 | 0.140 | 0.120 | 0.093 | 0.055 | 0.000 |
| | B | 0.254 | 0.211 | 0.206 | 0.199 | 0.178 | 0.000 |
| 1989 | C | 0.196 | 0.201 | 0.156 | 0.152 | 0.164 | 0.000 |
| | D | 0.140 | 0.169 | 0.202 | 0.192 | 0.110 | 0.000 |
| | E | 0.140 | 0.153 | 0.181 | 0.245 | 0.425 | 0.000 |
| | 0 | 0.084 | 0.127 | 0.135 | 0.119 | 0.068 | 0.000 |
| | | (429) | (379) | (282) | (151) | (73) | (0) |
| Grassy Creek | | | | 1988 | | | |
| | A | 0.260 | 0.125 | 0.096 | 0.097 | 0.056 | 0.175 |
| | B | 0.201 | 0.241 | 0.133 | 0.092 | 0.112 | 0.125 |
| 1990 | C | 0.177 | 0.265 | 0.164 | 0.111 | 0.093 | 0.225 |
| | D | 0.103 | 0.151 | 0.225 | 0.207 | 0.112 | 0.200 |
| | E | 0.108 | 0.160 | 0.300 | 0.392 | 0.547 | 0.100 |
| | 0 | 0.152 | 0.058 | 0.082 | 0.101 | 0.081 | 0.175 |
| | | (389) | (344) | (293) | (217) | (161) | (40) |

Even though matrices were not temporally constant, stable size distributions were generated for comparative purposes (Tables 3.7 and 3.8). Each size distribution was qualitatively different from the others with the most differences occurring between the populations rather than within the populations.

## DISCUSSION

Experiments indicate that seeds of C. rubifolia may show the same epicotyl dormancy as described by Baskin and Baskin (1985) for C. racemosa. Cimicifuga racemosa seeds begin germination with radicle emergence the year they are produced with epicotyl emergence occurring after the first winter. However, C. racemosa seeds are produced much earlier in the growing season than are C. rubifolia seeds. In some instances C. rubifolia follicles do not open until after the first frost (personal observation). Because of this exposure to low temperatures before radicle emergence, dormancy is apparently initiated and two periods of stratification are required for C. rubifolia. The few C. rubifolia seeds that had epicotyl emergence after only 1 winter were possibly formed early enough in the growing season to begin radicle emergence before dormancy was initiated.

The experimental germination rates of C. rubifolia were comparable to those at the lower end of the rates reported by Baskin and Baskin (1985) for C. racemosa. It is possible that seed viability varies from year to year or that the conditions for the test were not comparable to optimal conditions for C. rubifolia.

Table 3.7 Comparison of observed size distribution with the stable size distributions (expressed as percentage of total population) associated with one year size transition matrices.

| Size Class | Bull Bluff | | | Grassy Creek | | | |
|---|---|---|---|---|---|---|---|
| | Average[a] | 88-89[b] | 89-90 | Average | 87-88 | 88-89 | 89-90 |
| A | .144 | .019 | .011 | .230 | .227 | .145 | .125 |
| B | .165 | .080 | .045 | .227 | .227 | .211 | .129 |
| C | .172 | .132 | .108 | .191 | .211 | .170 | .153 |
| D | .191 | .278 | .142 | .145 | .150 | .161 | .157 |
| E | .246 | .450 | .661 | .145 | .158 | .206 | .325 |
| 0 | .083 | .041 | .033 | .063 | .026 | .107 | .111 |

[a] average observed size distribution for that site, all years
[b] years of the transition matrix used for projection

Table 3.8 Comparison of observed size distribution with the stable size distributions (expressed as percentage of total population) associated with two year size transition matrices.

| Size Class | Bull Bluff | | Grassy Creek | | |
|---|---|---|---|---|---|
| | Average[a] | 88-90[b] | Average | 87-89 | 88-90 |
| A | .144 | .005 | .230 | .110 | .113 |
| B | .165 | .039 | .227 | .204 | .142 |
| C | .172 | .113 | .191 | .173 | .153 |
| D | .188 | .144 | .145 | .159 | .157 |
| E | .246 | .661 | .145 | .249 | .336 |
| 0 | .083 | .038 | .063 | .105 | .098 |

[a] average observed size distribution for that site, all years
[b] years of the transition matrix used for projection

The field germination rates observed are probably low for two reasons. The first is that this number does not take into account the number of seeds that may have produced radicles but did not survive for epicotyls to emerge. Because the first years of the study (1987 and 1988) were dry compared to long term precipitation means, a higher than average rate of mortality for germinating seeds might be expected.

Two things of note are not shown in the tables. The number of seedlings at Bull Bluff in 1989 (164) was substantially higher than those seen at Grassy Creek (4) during the same year. This indicates that there was a difference in the survival of 1987 seeds when comparing Bull Bluff and Grassy Creek. At Bull Bluff in both 1989 and 1990, the most seedlings seemed to be in areas where litter was thin or absent. Also the appearance of the very large number of seedlings at Grassy Creek in 1990 was preceded by a very hard rain that washed much of the litter off the slopes and left more soil exposed that was usually observed. As these were unanticipated events, no measure had been taken to monitor substrate effect on germination. Any future studies should investigate the effects of both variation in precipitation and in litter presence and depth on germination and seedling survival.

Under garden conditions only one year of growth was required for seedlings to obtain sufficient size to flower. This was not observed in the field. In most cases there was little difference in plant size in its first and second years in the field. Ed Alverson (personal communication) has made similar observations with C. elata plants grown from seed. However, this large size increase in plants from seed substantiates that the large changes in size seen in the field are

possible and are not errors resulting from plant misidentification. This has interesting implications as it is sometimes assumed that in long lived perennials a number of years may be required before plants are able to flower and contribute genetically to the population. Also there are relatively few individuals blooming during any time period so that the population genetics are influenced by only those individuals for relatively long periods of time. However, if new genets are able to begin flowering within one or two years after germination it is possible that there is a faster 'genetic turnover' in long lived herbaceous perennials than is sometimes assumed.

The fact that the matrices did not show temporal constance was not surprising given the increase in mean size of the individuals and the variation in precipitation during the study. It is possible that the size increase of individuals was related to the increased amounts of rainfall in 1989 and 1990. What was expected was for the highest probability to be for an individual to remain in the same size class with the next highest probability being to go to the next larger size class (excepting the largest class, E and class 0). While this was sometimes true (transitions for Bull Bluff 1988 to 1989) at other times there was a higher probability of individuals moving to the next larger class than of remaining in the same class. Generally speaking, at Bull Bluff from 1988 to 1989 plants had a higher probability of remaining in their class rather than moving to any other size class. However, from 1989 to 1990 there was a higher probability that they would move into the next larger class than that they would remain in the same class. Similarly at Grassy Creek from 1987 to 1988 and from 1988 to 1989 there

was a greater probability of plants remaining in the same size class than of moving to another class while between 1989 and 1990 there was a slightly higher probability of moving into the next larger class. Based on the assumption that the increased precipitation was an important size determinant, this implies a year lag in the effect of precipitation on size. This is supported by comparison of the two year matrices for Grassy Creek. Between 1987 and 1989 there is a slightly higher probability of increasing in size class; however, there is a much larger probability of moving up a size class between 1988 and 1990.

Even though the stable size distributions generated from the transition matrices could not be used to accurately predict the future size distributions, they do indicate that the dynamics of the two populations are different. The most obvious difference is the large number of Class E individuals predicted for Bull Bluff, 45 to 66.1%, as compared to the 15.8 to 32.5% projected for Grassy Creek. While the average observed distribution at Bull Bluff does contain more large individuals than smaller individuals, this very large projected increase within the largest class was unexpected. Although it is possible that this is a reasonably accurate projection, it is more probably an illustration of why projections should not be done when the matrices are not temporally constant. In contrast the projected distributions for Grassy Creek are not as obviously different from the average observed distribution. The comparison of the projected distributions to the observed average distributions suggests that the Grassy Creek population is more stable than the Bull Bluff population. This may be a reflection of the site differences with the Bull Bluff site being subjected more to

the movement of loose rocks and more frequent tree falls (two during the study) than is the Grassy Creek population. There may also be some effect of the change in slope moisture and light as well as a presumed eradication of the lower part of the population that resulted from the filling of the Melton Hill Reservoir during the mid 1960's.

The number of seeds and seedlings produced per year varied greatly in both of the populations studied. The production of very large numbers of seedlings, even sporadically, should be sufficient to maintain the populations. The population projection matrices produced were not temporally constant and the projected stable size structure of the populations differed from the observed structure. This is thought to be due, at least in part, to the variable precipitation amounts during the study.

# CHAPTER 4

# INVESTIGATION OF THE POPULATION GENETICS OF CIMICIFUGA RUBIFOLIA

## INTRODUCTION

The knowledge of the amount and distribution of genetic variation within a species is necessary to understand the evolutionary potential of the species. The distribution of this variation results from the interaction of a number of evolutionary factors such as selection, population size, and amount of gene flow within and between populations. Basic genetic information is also needed before conservation strategies can be made. Allozyme studies are a relatively quick and inexpensive way of obtaining this information (Hamrick 1989; Hamrick and Godt 1990). Allozymes are enzymes that are coded for by different alleles at the same locus. Because of relationship between DNA and protein, allozymes give information on changes in DNA. The major disadvantage in the use of allozyme analysis is that it only tests for structural genes coding for soluble proteins and enzymes (which may or may not be what selection is acting on). It also underestimates the number of mutational events that occur since only those resulting in an electrical charge difference are detectable (Ayala et al. 1974; Clegg 1990; Hartl 1980).

In their most recent review of 653 plant studies, Hamrick and Godt (1990) noted a number of trends in the distribution of genetic variation within different taxa with correlations to life history traits, ecological traits, and geographic range. Within species, an average of 50% of the loci are polymorphic and mean genetic diversity, as mean Hardy-Weinberg expected heterozygosity, was found to be 15%. Most of

the diversity was found within populations with only 22% of the total allozyme variation resulting from differences between populations. Endemic species typically had lower levels of heterozygosity than more widespread species. Species that are predominantly outcrossing exhibited more diversity as heterozygosity than plants with other breeding systems. Long-lived herbaceous perennials had a total mean heterozygosity of .205 with 39.6% of the loci being polymorphic. This was somewhat lower than that of long-lived woody perennials or of short-lived herbaceous perennials. However, as studies of the genetic structure of plant populations have tended to focus on temperate annuals, short-lived perennials, and coniferous trees, few studies are available on long-lived herbaceous perennials. This review included only 4 long-lived herbaceous perennial taxa (species and subspecies) with an average of 6 populations each.

Once allelic frequencies have been determined through allozyme analysis, a number of statistical tests can be done. Observed heterozygosity ($H_o$) can be determined and allelic frequencies used to calculate expected heterozygosity ($H_e$) per individual in a population. The Hardy-Weinberg model is usually used for these calculations. Deviations from the assumptions on which the model is based result in observed heterozygosities that are different from those expected. The model assumes the population is of large size with negligible gene flow, selection and mutation rates. Mating between individuals in the population is assumed to be random (Hartl 1980). The observed and expected heterozygosities can then be used in a number of other

statistics to estimate which of the model's assumptions are being violated.

The genotypic structure of populations at a single locus may be measured using a fixation index, F. This can be used as a measure of the reduction in heterozygosity due to inbreeding or as the probability that two alleles in an individual are identical by descent. Originally derived by Wright (1965), it has been corrected for small population size by Kirby (1975) where:

$$F=1-H_o/[2pq(1+1/2N-1)].$$

The denominator portion of the equation is equivalent to $H_e$ where p and q are allelic frequencies. Here N is the number of individuals in the population. In outbreeding populations there is a general excess of heterozygotes resulting in an F value that is negative in value. Positive F values may be the result of inbreeding (or consanguineous matings) or of pooling of subpopulations with differing allelic frequencies (Brown 1979; Husband and Barrett 1992; Patton and Feder 1981).

Different levels of population subdivisions may also be studied using Wright's hierarchical F-statistics (1965). The inbreeding coefficient, $F_{IS}$, considers the variation in observed from expected heterozygote frequency at the subpopulation level. The inbreeding coefficient may be expressed as:

$$F_{IS} = H_S - H_I / H_S$$

where $H_S$ is the expected heterozygosity of an individual in the subpopulation and $H_I$ is the observed heterozygosity of an individual in the subpopulation. $F_{IS}$ may range from -1.0 to +1.0 and indicates the

relative amount of inbreeding that occurs within subpopulations. Negative values, indicating an excess of heterozygotes, are typically observed in outbreeding populations while positive values are found in inbreeding populations. The fixation index, $F_{ST}$, considers the amount of differentiation between the subpopulations and as such is a measure of genetic drift or differentiation between the subpopulations. The fixation index is represented by the equation:

$$F_{ST} = H_T - H_S / H_T$$

where $H_T$ is the expected heterozygosity of an individual in the total population. $F_{ST}$ ranges from 0.0 to 1.0 with values of 0.0 indicating no differentiation between subpopulations. The overall inbreeding coefficient, $F_{IT}$, considers the combined effects of breeding system at the subpopulation level and genetic drift between subpopulations. The overall inbreeding coefficient may be expressed as:

$$F_{IT} = H_T - H_I / H_T.$$

If populations are subdivided into inbreeding subpopulations $F_{IT}$ will be positive. However, if the population is not subdivided and inbreeding is not significant $F_{IT}$ values will be negative (Hartl 1980; Silander 1984; Walker 1987).

As populations become isolated, either geographically or ecologically, there is an accumulation of genetic differences due to factors such as selection, genetic drift, or founder effect. Allozyme data can be used to estimate the accumulated number of gene substitutions per locus in the different populations. This calculation assumes a constant rate of gene substitutions with time. The relative degree of divergence between populations can be measured using either

Nei's genetic identity, I, or genetic distance, D (Nei 1978). These use electrophoretic data to estimate the number of gene substitutions per locus that have accumulated between populations. I is expressed as:

$$I = \frac{Jxy}{\sqrt{JxJy}}$$

where Jxy, Jx and Jy represent the arithmatic means, over all loci, of $\Sigma x_i y_i$, $\Sigma x_i^2$ and $\Sigma y_i^2$. Here $x_i$ and $y_i$ are the frequencies all alleles assayed in populations X and Y. Identity values range from 1.0 if the populations have not diverged to 0.0 if there are no alleles in common. Genetic distance considers the number of allelic differences per locus that have occurred since the populations became separated. D is expressed as:

$$D = \frac{-\ln Jxy}{\sqrt{JxJy}}$$

Values of 0.0 indicate no detectable divergence has occurred while values of 1.0 indicate total divergence of the populations (Avise and Smith 1977; Nei 1971, 1972; Walker 1987).

Cimicifuga rubifolia is a federal C2 candidate. These are taxa for which available information indicates they should be listed as either endangered or threatened but for which substantial data on its biological vulnerability is unavailable. It occurs primarily in the Ridge and Valley region of Tennessee and southwestern Virginia. Within this region, most populations have been found along four major river systems: the Tennessee, Clinch, Powell, and Holston. It is typically found on the lower slopes of north-facing bluffs that are adjacent to rivers or streams. While most populations are found close to rivers or streams, there are a few populations located away from waterways such as

those occurring in gaps near the top of Clinch Mountain. Populations also occur in the Cumberland Plateau of Tennessee, as well as in southern Illinois, southern Indiana, western Kentucky, and northern Alabama. Site characteristics for these populations are the same as for those in the main range of the species (Ramsey 1965).

Cimicifuga rubifolia Kearney is a long-lived herbaceous perennial in the Ranunculaceae. It is not know to self-pollinate, relying on insects for cross-pollination. A nectarless species, it appears to rely on other plant species, such as Impatiens pallida and Polymnia canadensis, to attract pollinators to the population (Pellmyr 1986a). In populations that have been studied, reproduction is almost exclusively sexual, although seedling production varies greatly between years (see chapter 2).

This portion of the study will use allozyme analysis to test whether reproduction is primarily sexual. In addition, this study will investigate the degree of genetic variability of C. rubifolia and how the variability is distributed within and between populations.

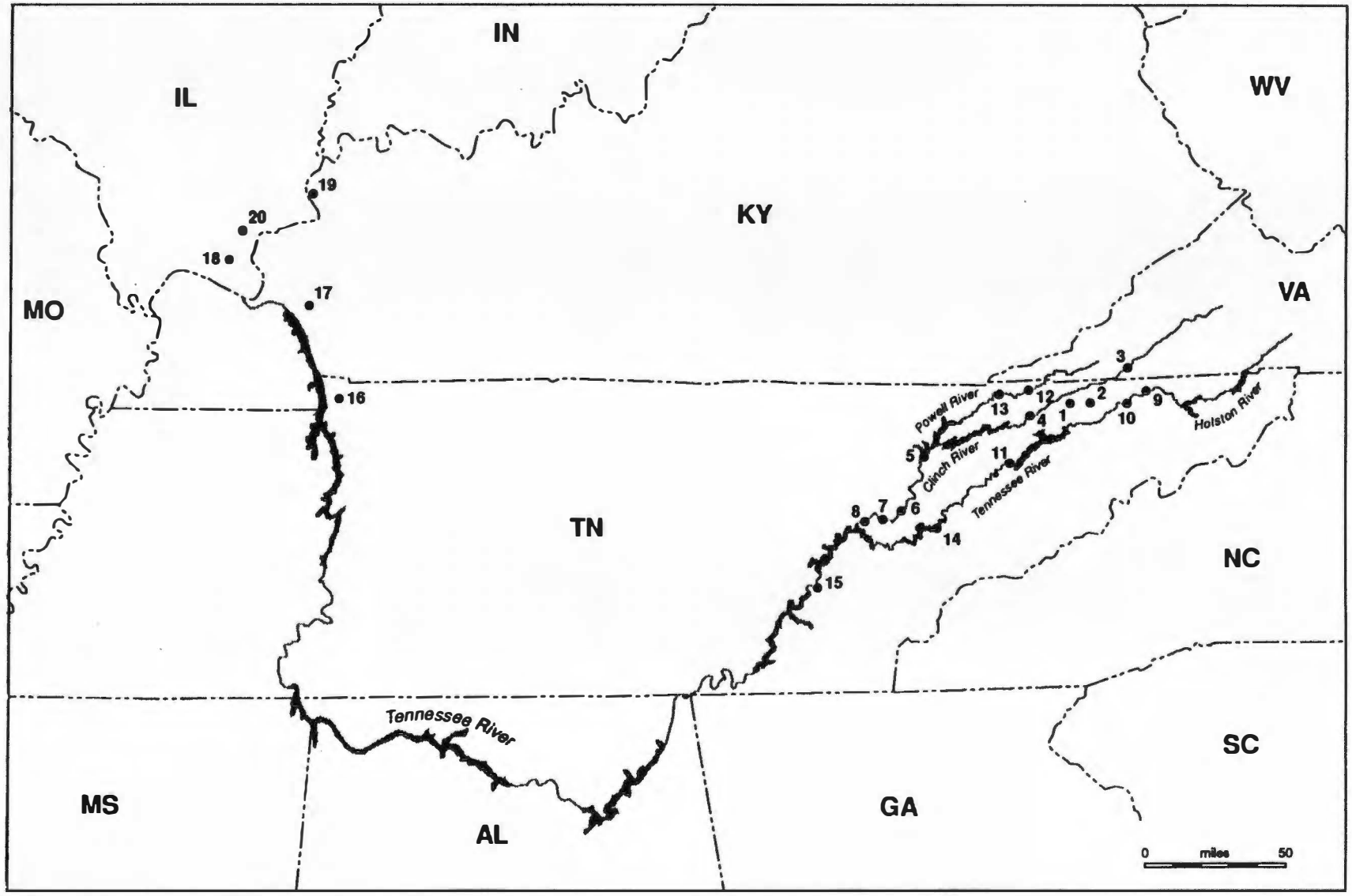
## MATERIALS AND METHODS

Populations were selected to encompass both the main range and disjunct populations of C. rubifolia. Within the main range of the Ridge and Valley Provence, populations along each of the major river systems were included (Figure 4.1 and Table 4.1). For each population included in the study, a list of associated species was made. This was not to provide quantitative vegetational analyses of the sites but rather to see if other rare plant species were consistently appearing

Table 4.1 Location of Cimicifuga rubifolia populations used for electrophoretic analysis. Sites are grouped by geographic location.

| Site | | | County | USGS 7.5' Quadrangle |
|---|---|---|---|---|
| Clinch Mountain | | | | |
| 1 | BWG | Big War Gap | Hawkins, TN | Lee Valley |
| 2 | LWG | Little War Gap | Hawkins ,TN | Kyles Ford |
| Clinch River | | | | |
| 3 | VIR | Virginia | Scott, VA | Duffield |
| 4 | PPG | Pawpaw Grove | Hancock, TN | Swan Island |
| 5 | NRB | Norris River Bluffs | Anderson, TN | Norris |
| 6 | BLB | Bull Bluff | Anderson, TN | Lovell |
| 7 | GRC | Grassy Creek | Roane, TN | Elverton |
| 8 | STB | Stowe Bluff | Roane, TN | Harriman/Bacon Gap |
| Holston River | | | | |
| 9 | KPQ | Kingsport Quarry | Sullivan, TN | Kingsport |
| 10 | CHB | Christain Bend | Hawkins, TN | Stony Point |
| 11 | MSR | Mill Springs Road | Jefferson, TN | Joppa |
| Powell River | | | | |
| 12 | WRG | Wallens Ridge | Hancock, TN | Coleman's Gap |
| 13 | PRB | Powell River Bridge | Claibourne, TN | Middlesboro South |
| Tennessee River | | | | |
| 14 | GEO | Georges Creek | Blount, TN | Louisville |
| 15 | EVF | Eaves Ferry | Meigs, TN | Decatur |
| Disjuncts | | | | |
| 16 | LBL | Bear Creek LBL | Stewart Co., TN | Thorpe |
| 17 | EDV | Eddyville | Lyon Co., KY | Eddyville |
| 18 | LOL | Lola | Livingston Co., KY | Lola |
| 19 | ANC | Antioch Church | Hardin Co., IL | Dekoven KY-IL |
| 20 | LSC | Lusk Creek | Pope Co., IL | Waltersburg |

Figure 4.1 Distribution of Cimicifuga rubifolia sites assayed. Numbers of the study sites are those given in Table 4.1.


IL
IN
KY
WV
VA
MO
TN
NC
SC
GA
AL
MS
Tennessee River
Powell River
Clinch River
Tennessee River
Holston River
1
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13
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16
17
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19
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0
miles
50

with C. rubifolia and as a check for the known and possible unknown pollinator-attractor species.

For the purpose of analyzing intrapopulation genetic architecture, from 8 to 10 sampling points were selected within populations and the seven plants nearest to each point were sampled. Points were selected so as to include all possible microhabitats. Field maps were made at the site with collection points located to check against any genetic pattern seen. Plants were checked to insure that each collection was from a different rhizome. Actual number of points and number of samples collected were dependent on the population size. Leaflets from each individual were collected and placed in labeled plastic bags. Bags were immediately placed in a cooler with ice. Samples were taken to the lab where each leaflet was cut into squares approximately 1 $cm^2$. These were placed in labeled individual plastic bags and stored at -80° C until needed. Voucher specimens from each population were deposited in the University of Tennessee, Knoxville Herbarium (TENN).

Samples from the freezer were placed in liquid nitrogen, and were ground immediately on a chilled grinding block in grinding buffer (Werth 1985). Enzymes were resolved on 12.5% starch gels utilizing two different buffer systems. A morpholine system (Clayton and Tretiak 1972) was used for the separation of Shikimic acid dehydrogenase (SKD), Isocitrate dehydrogenase (IDH), and Phosphoglucomutase (PGM). A histidine citrate buffer system (Soltis et al. 1983) was used for Phosphoglucoisomerase (PGI) and 6-Phosphoglucodehydrogenase (6-PGD). Staining procedures followed those of Werth (1985). Recipes for all solutions are included in Appendix A. After gels were stained, they

were fixed in an acetic acid: ethanol solution and were photographed. Loci were designated sequentially with the most anodally migrating isozyme designated as 1. Alleles were also designated sequentially with the most anodally migrating allele designated as A. Distances of the bands were measured from the origin.

All genetic variability tabulations, genetic distance, genetic identity, cluster analysis, and F-statistics were calculated using BIOSYS-1 (Swofford and Selander 1981). Goodness of fit between observed heterozygosities and those expected under Hardy-Weinberg equilibrium were tested using G-tests (Sokal and Rohlf 1981). In addition to the study of the genetic differences between the populations of C. rubifolia, analysis was done of the genetic variation and distribution within populations. Each sample point within a population was treated as an individual subdivision and heterozygosity for each subdivision was calculated. Cluster analysis using genetic identity and F-statistics were also done for each population. In addition, the number of genotypes in each population and within each subdivision was determined as a method of looking for evidence of asexual reproduction.

## RESULTS

Associated plant species did not show consistent presence of any other rare species with C. rubifolia. The plant lists complied are in Appendix B.

Of the seven loci tested, two (PGI-1 and 6PGD-1) were monomorphic. All other loci were polymorphic in two or more populations. For the purposes of this study, a locus is considered polymorphic if the

frequency of the most common allele is less than .95. Polymorphic loci are SKD, 2 alleles; IDH, 3 alleles; PGM, 3 alleles; PGI-2, 2 alleles; and 6PGD-2, 3 alleles. Allele frequencies determined for the seven loci used in this study are presented in Table 4.2.

The SKD locus is polymorphic in 6 of the 20 populations studied with the less common allele, E, present on all river systems but not in the disjunct populations or on Clinch Mountain. The IDH locus is polymorphic in 9 populations with monomorphic populations fixed at the D allele. The C allele was found in 10 populations while the F allele was present in only 3 populations. Only one population, Pawpaw Grove, had all three alleles. The PGM locus is polymorphic in 7 populations with monomorphic populations fixed at the B allele. Only one population, Bull Bluff, had all three alleles present. Eight populations were polymorphic for 6PGD-2. Monomorphic populations were fixed on the C allele. The second most common allele, D, was found in populations on the Clinch, Holston, and Powell Rivers and in the disjunct populations. The least common allele, B, was found only in 3 sites, Powell River Bridge, Wallens Ridge, and Norris River Bluffs. In two populations, Antioch Church and Kingsport Quarry, the most common allele was not C but the D allele. All populations in the main range were monomorphic at the PGI-2 locus with fixation on the C allele. A second allele, G, was present in 3 of the disjunct populations, Bear Creek LBL, Lusk Creek, and Eddyville.

The direct count (observed) heterozygosity is less than the Hardy-Weinberg expected heterozygosity in all but two of the polymorphic

Table 4.2 Allele frequencies for loci of Cimicifuga rubifolia populations assayed.

| | Population | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Locus | BWG | LWG | EVF | GEO | STB | PPG | VIR | NRB | BLB | GRC |
| SKD | | | | | | | | | | |
| B | 1.000 | 1.000 | .952 | 1.000 | .780 | .934 | .949 | .838 | 1.000 | 1.000 |
| E | .000 | .000 | .048 | .000 | .220 | .066 | .051 | .162 | .000 | .000 |
| IDH | | | | | | | | | | |
| C | .000 | .428 | .000 | .105 | .000 | .015 | .000 | .106 | .000 | .086 |
| D | 1.000 | .572 | 1.000 | .895 | 1.000 | .949 | .971 | .894 | 1.000 | .914 |
| F | .000 | .000 | .000 | .000 | .000 | .037 | .029 | .000 | .000 | .000 |
| PGM-2 | | | | | | | | | | |
| A | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .079 | .003 |
| B | .993 | .696 | 1.000 | 1.000 | .962 | .971 | 1.000 | .782 | .614 | .997 |
| C | .007 | .304 | .000 | .000 | .038 | .029 | .000 | .218 | .307 | .000 |
| 6PGD-2 | | | | | | | | | | |
| B | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .190 | .000 | .000 |
| C | 1.000 | 1.000 | 1.000 | 1.000 | .652 | 1.000 | .848 | .592 | .779 | .986 |
| D | .000 | .000 | .000 | .000 | .348 | .000 | .152 | .218 | .221 | .014 |
| 6PGD-1 | | | | | | | | | | |
| A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| PGI-2 | | | | | | | | | | |
| C | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| G | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| PGI-1 | | | | | | | | | | |
| B | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Table 4.2 (cont.)

| Locus | Population MSR | KPQ | CHB | PRB | WRG | LBL | LSC | ANC | LOL | EDV |
|---|---|---|---|---|---|---|---|---|---|---|
| **SKD** | | | | | | | | | | |
| B | .935 | 1.000 | .967 | .578 | .951 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| E | .065 | .000 | .033 | .422 | .049 | .000 | .000 | .000 | .000 | .000 |
| IDH | | | | | | | | | | |
| C | .000 | .078 | .175 | .000 | .000 | .058 | .101 | .045 | .000 | .000 |
| D | 1.000 | .922 | .825 | .991 | 1.000 | .942 | .899 | .955 | 1.000 | 1.000 |
| F | .000 | .000 | .000 | .009 | .000 | .000 | .000 | .000 | .000 | .000 |
| PGM-2 | | | | | | | | | | |
| A | .007 | .000 | .000 | .000 | .000 | .000 | .000 | .052 | .000 | .000 |
| B | .993 | 1.000 | .992 | .629 | .721 | .964 | .862 | .948 | 1.000 | 1.000 |
| C | .000 | .000 | .008 | .371 | .279 | .036 | .138 | .000 | .000 | .000 |
| 6PGD-1 | | | | | | | | | | |
| A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| 6PGD-2 | | | | | | | | | | |
| B | .000 | .000 | .000 | .207 | .074 | .000 | .000 | .000 | .000 | .000 |
| C | 1.000 | .141 | 1.000 | .310 | .697 | 1.000 | 1.000 | .231 | 1.000 | 1.000 |
| D | .000 | .859 | .000 | .483 | .230 | .000 | .000 | .769 | .000 | .000 |
| PGI-1 | | | | | | | | | | |
| B | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| PGI-2 | | | | | | | | | | |
| C | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | .978 | .580 | 1.000 | 1.000 | .714 |
| H | .000 | .000 | .000 | .000 | .000 | .022 | .420 | .000 | .000 | .286 |

populations (Table 4.3). The Big War Gap site had the same observed and expected frequencies but all assayed individuals in the population were genetically identical except for 1 heterozygous individual. The Eddyville population had a very slight heterozygote excess, .061 observed to .059 expected, but only 5 individuals that were homozygous for the rare allele were observed.

The Fixation Indices, F, indicate that many of the deviations from expected heterozygote proportions are significant (Table 4.4). F values show extreme variation, ranging from -.179 to 1.00. Negative values, indicating an excess of heterozygotes, were found most often for the PGM locus. F values for SKD and IDH loci were usually positive, indicating heterozygote deficits. Fixation indices of 1.00, signifying a total lack of heterozygotes in a polymorphic population, were found in three populations and for three different loci.

The F statistics for individual alleles (Tables 4.5 to 4.9) indicate a fairly high degree of both inbreeding within populations and divergence between populations, resulting in high total fixation indices. For SKD, IDH and PGM, most of the high $F_{IT}$'s are the result of large $F_{IS}$ values. The $F_{ST}$ value is much larger than $F_{IS}$ for 6PGD-2 and PGI-2. The presence of a rare allele at both of these loci that occurs in only a few populations accounts for much of the divergence between populations with respect to these loci. Additionally, 6PGD-2 allele D being more common in three of the populations, rather than the C allele, appears to contribute. The $F_{IS}$ for the PGI-2 is the only negative value present.

Table 4.3 Genetic variability at seven loci in populations of *Cimicifuga rubifolia*. Standard errors are in parentheses.

| Population | Mean sample size per Locus | Mean no. of alleles per locus | Percentage of loci polymorphic[a] | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | Direct-count | HdyWbg expected[b] |
| BWG | 68 | 1.1 ( .1) | 14.3 | .002 ( .002) | .002 ( .002) |
| LWG | 69 | 1.3 ( .2) | 28.6 | .072 ( .047) | .131 ( .085) |
| BLB | 70 | 1.4 ( .3) | 28.6 | .098 ( .063) | .125 ( .083) |
| GRC | 145 | 1.4 ( .2) | 42.9 | .004 ( .003) | .027 ( .022 |
| STB | 66 | 1.4 ( .2) | 42.9 | .095 ( .063) | .125 ( .073) |
| VIR | 69 | 1.4 ( .2) | 42.9 | .058 ( .042) | .059 ( .036) |
| PPG | 68 | 1.6 ( .3) | 42.9 | .038 ( .020) | .040 ( .020) |
| NRB | 71 | 1.7 ( .3) | 57.1 | .141 ( .063) | .197 ( .082) |
| PRB | 58 | 1.7 ( .3) | 57.1 | .172 ( .088) | .230 ( .108) |
| WRG | 61 | 1.6 ( .3) | 42.9 | .059 ( .038) | .137 ( .078) |
| GEO | 62 | 1.1 ( .1) | 14.3 | .002 ( .002) | .027 ( .027) |
| EVF | 62 | 1.1 ( .1) | 14.3 | .009 ( .009) | .013 ( .013) |
| CHB | 60 | 1.4 ( .2) | 42.9 | .033 ( .025) | .053 ( .041) |
| KPQ | 64 | 1.3 ( .2) | 28.6 | .031 ( .023) | .056 ( .037) |

Table 4.3 (cont.)

| Population | Mean sample size per Locus | Mean no. of alleles per locus | Percentage of loci polymorphic[a] | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | Direct-count | HdyWbg expected[b] |
| MSR | 69 | 1.3 ( .2) | 28.6 | .004 ( .003) | .020 ( .017) |
| LBL | 69 | 1.4 ( .2) | 42.9 | .021 ( .011) | .032 ( .017) |
| LSC | 69 | 1.4 ( .2) | 42.9 | .122 ( .076) | .131 ( .071) |
| ANC | 67 | 1.4 ( .2) | 42.9 | .047 ( .036) | .078 ( .050) |
| EDV | 70 | 1.1 ( .1) | 14.3 | .061 ( .061) | .059 ( .059) |
| LOL | 79 | 1.0 ( .0) | .0 | .000 ( .000) | .000 ( .000) |

[a] A locus is consider polymorphic if the frequency of the most common allele does not exceed 0.95.
[b] Unbiased estimate (Nei 1978).

Table 4.4 Fixation indices (F) for polymorphic Cimicifuga rubifolia populations. When more than 3 alleles were present, the least common alleles were pooled.

| Population | Loci | | | | |
|---|---|---|---|---|---|
| | SKD | IDH | PGM | 6PGD-2 | PGI-2 |
| Big War Gap | | | -.007 | | |
| Little War Gap | | .437[b] | .452[b] | | |
| Eaves Ferry | .299 | | | | |
| Georges Creek | | .914[b] | | | |
| Stowe Bluff | .602[b] | | -.039 | -.001 | |
| Virginia | .248 | .485b | | -.179 | |
| Pawpaw Grove | -.071 | .225 | -.030 | | |
| Bull Bluff | | | .371[b] | -.036 | |
| Grassy Creek | | .869[b] | -.003[b] | 1.000[b] | |
| Norris River Bluffs | .326[a] | .478[b] | .216[b] | .229[b] | |
| Powell River Bridge | .187 | -.009 | .520[b] | .094 | |
| Wallens Ridge | 1.000[b] | .511[b] | | .533[b] | |
| Christain Bend | .483[b] | .365[b] | -.008 | | |
| Kingsport Quarry | | .566[b] | | .354[b] | |
| Mill Spring Road | .881[b] | | -.007 | | |
| LBL Bear Creek | | .735[b] | -.038 | | -.022 |
| Lusk Creek | | .205 | .329[a] | | .130 |
| Antioch Church | | 1.00[b] | .246 | .287[b] | |
| Eddyville | | | | | -.050 |

[a] $p < 0.05$
[b] $p < 0.001$ if rejected here, rejected at all levels

Table 4.5 F-Statistics calculated for individual alleles of SKD in populations of Cimicifuga rubifolia.

| Allele | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| B | .390 | .510 | .197 |
| E | .390 | .510 | .197 |
| Mean | .390 | .510 | .197 |

Table 4.6 F-Statistics calculated for individual alleles of IDH in populations of Cimicifuga rubifolia.

| Allele | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| C | .554 | .631 | .172 |
| D | .526 | .601 | .158 |
| F | .170 | .192 | .027 |
| Mean | .526 | .602 | .160 |

Table 4.7 F-Statistics calculated for individual alleles of PGM in populations of Cimicifuga rubifolia.

| Allele | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| A | .261 | .302 | .057 |
| B | .369 | .500 | .208 |
| C | .371 | .499 | .203 |
| Mean | .365 | .491 | .199 |

Table 4.8 F-Statistics calculated for individual alleles of 6PGD-2 in populations of Cimicifuga rubifolia.

| Allele | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| B | .276 | .391 | .159 |
| C | .206 | .608 | .507 |
| D | .118 | .539 | .477 |
| Mean | .176 | .562 | .468 |

Table 4.9 F-Statistics calculated for individual alleles of PGI-2 in populations of Cimicifuga rubifolia.

| Allele | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| C | -.090 | .271 | .331 |
| G | -.090 | .271 | .331 |
| Mean | -.090 | .271 | .331 |

The mean F-statistics for all loci in all populations of C. rubifolia studied (Table 4.10) show that the high total fixation index, $F_{IT}$, is the result of both a high positive $F_{IS}$ and $F_{ST}$ values. This indicates there is both a high level of inbreeding within populations and genetic divergence among populations. The deficit of heterozygotes in all but two of the polymorphic populations indicates that the populations are inbred or they are subdivided in smaller breeding groups with differing allele frequencies between the groups or both.

Several genetic similarity and distance measures were employed for all pairwise comparisons of the populations. The matrix of genetic identities and genetic distance are shown in Table 4.11. Unbiased genetic identity values, I, range from .865 to 1.00. Genetic distance values range, D, range from 0 to .161. Both UPGMA cluster analysis and Wagner procedure were used to produce phenograms from several I and D matrices. Figure 4.2 shows the cluster analysis of genetic identity, I. All branches occur at I values of greater than .90. Two major cluster

Table 4.10 Summary of F-statistics at all loci in populations of Cimicifuga rubifolia.

| Locus | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| SKD | .390 | .510 | .197 |
| IDH | .526 | .602 | .160 |
| PGM | .365 | .491 | .199 |
| 6PGD-2 | .176 | .562 | .468 |
| PGI-2 | -.090 | .271 | .331 |
| Mean | .301 | .520 | .313 |

Table 4.11 Matrix of genetic similarity and distance coefficients calculated for populations of Cimicifuga rubifolia. Above diagonal: Nei (1978) unbiased genetic identity: Below diagonal: Nei (1978) unbiased genetic distance

| Site | BWG | LWG | EVF | STB | GEO | VIR | NRB | PPG | CHB | KPQ |
|---|---|---|---|---|---|---|---|---|---|---|
| BWG | **** | .961 | 1.000 | .977 | .999 | .997 | .974 | .999 | .996 | .891 |
| LWG | .039 | **** | .960 | .932 | .972 | .956 | .957 | .965 | .978 | .851 |
| EVF | .000 | .041 | **** | .979 | .998 | .997 | .975 | 1.000 | .996 | .890 |
| STB | .024 | .071 | .021 | **** | .974 | .991 | .989 | .979 | .972 | .952 |
| GEO | .001 | .029 | .002 | .027 | **** | .995 | .973 | .999 | 1.000 | .890 |
| VIR | .003 | .045 | .003 | .010 | .005 | **** | .984 | .997 | .993 | .924 |
| NRB | .027 | .044 | .026 | .011 | .027 | .016 | **** | .976 | .973 | .939 |
| PPG | .001 | .036 | .000 | .021 | .001 | .003 | .024 | **** | .997 | .888 |
| CHB | .004 | .023 | .004 | .028 | .000 | .007 | .027 | .003 | **** | .887 |
| KPQ | .115 | .161 | .117 | .050 | .116 | .080 | .063 | .118 | .120 | **** |
| MSR | .001 | .041 | .000 | .021 | .002 | .003 | .025 | .000 | .004 | .117 |
| PRB | .108 | .134 | .103 | .039 | .114 | .080 | .030 | .101 | .115 | .067 |
| WRG | .020 | .043 | .021 | .015 | .024 | .013 | .005 | .020 | .027 | .070 |
| LBL | .000 | .031 | .001 | .025 | .000 | .004 | .025 | .001 | .002 | .117 |
| LSC | .029 | .051 | .030 | .061 | .029 | .035 | .057 | .030 | .030 | .158 |
| ANC | .092 | .138 | .093 | .036 | .094 | .061 | .048 | .095 | .097 | .001 |
| EDV | .011 | .056 | .012 | .039 | .013 | .016 | .045 | .013 | .017 | .133 |
| LOL | .000 | .040 | .000 | .024 | .001 | .003 | .027 | .001 | .004 | .115 |
| BLB | .023 | .039 | .025 | .026 | .027 | .020 | .014 | .024 | .030 | .087 |
| GRC | .001 | .031 | .001 | .025 | .000 | .004 | .026 | .001 | .001 | .112 |

Table 4.11 (cont.)

| Site | MSR | PRB | WRG | LBL | LSC | ANC | EDV | LOL | BLB | GRC |
|---|---|---|---|---|---|---|---|---|---|---|
| BWG | .999 | .898 | .980 | 1.000 | .972 | .912 | .989 | 1.000 | .977 | .999 |
| LWG | .959 | .875 | .958 | .970 | .950 | .871 | .945 | .961 | .962 | .970 |
| EVF | 1.000 | .902 | .979 | .999 | .970 | .911 | .988 | 1.000 | .975 | .999 |
| STB | .980 | .962 | .985 | .975 | .941 | .965 | .962 | .977 | .974 | .976 |
| GEO | .998 | .892 | .977 | 1.000 | .972 | .911 | .987 | .999 | .973 | 1.000 |
| VIR | .997 | .923 | .987 | .996 | .965 | .941 | .984 | .997 | .980 | .996 |
| NRB | .975 | .970 | .995 | .975 | .945 | .953 | .956 | .973 | .986 | .975 |
| PPG | 1.000 | .904 | .980 | .999 | .971 | .910 | .987 | .999 | .977 | .999 |
| CHB | .996 | .892 | .974 | .998 | .970 | .907 | .983 | .996 | .970 | .999 |
| KPQ | .889 | .935 | .932 | .890 | .854 | .999 | .875 | .891 | .917 | .894 |
| MSR | **** | .904 | .979 | .999 | .970 | .911 | .988 | .999 | .975 | .998 |
| PRB | .101 | **** | .957 | .897 | .865 | .944 | .877 | .897 | .943 | .896 |
| WRG | .021 | .044 | **** | .981 | .954 | .949 | .964 | .979 | .998 | .978 |
| LBL | .001 | .109 | .020 | **** | .975 | .911 | .989 | 1.000 | .978 | 1.000 |
| LSC | .030 | .145 | .047 | .025 | **** | .876 | .994 | .971 | .955 | .972 |
| ANC | .093 | .058 | .052 | .093 | .132 | **** | .897 | .913 | .937 | .914 |
| EDV | .012 | .132 | .036 | .011 | .006 | .109 | **** | .989 | .961 | .987 |
| LOL | .001 | .109 | .021 | .000 | .029 | .092 | .011 | **** | .976 | .999 |
| BLB | .025 | .059 | .002 | .022 | .047 | .065 | .040 | .024 | **** | .975 |
| GRC | .002 | .110 | .022 | .000 | .029 | .090 | .013 | .001 | .025 | **** |

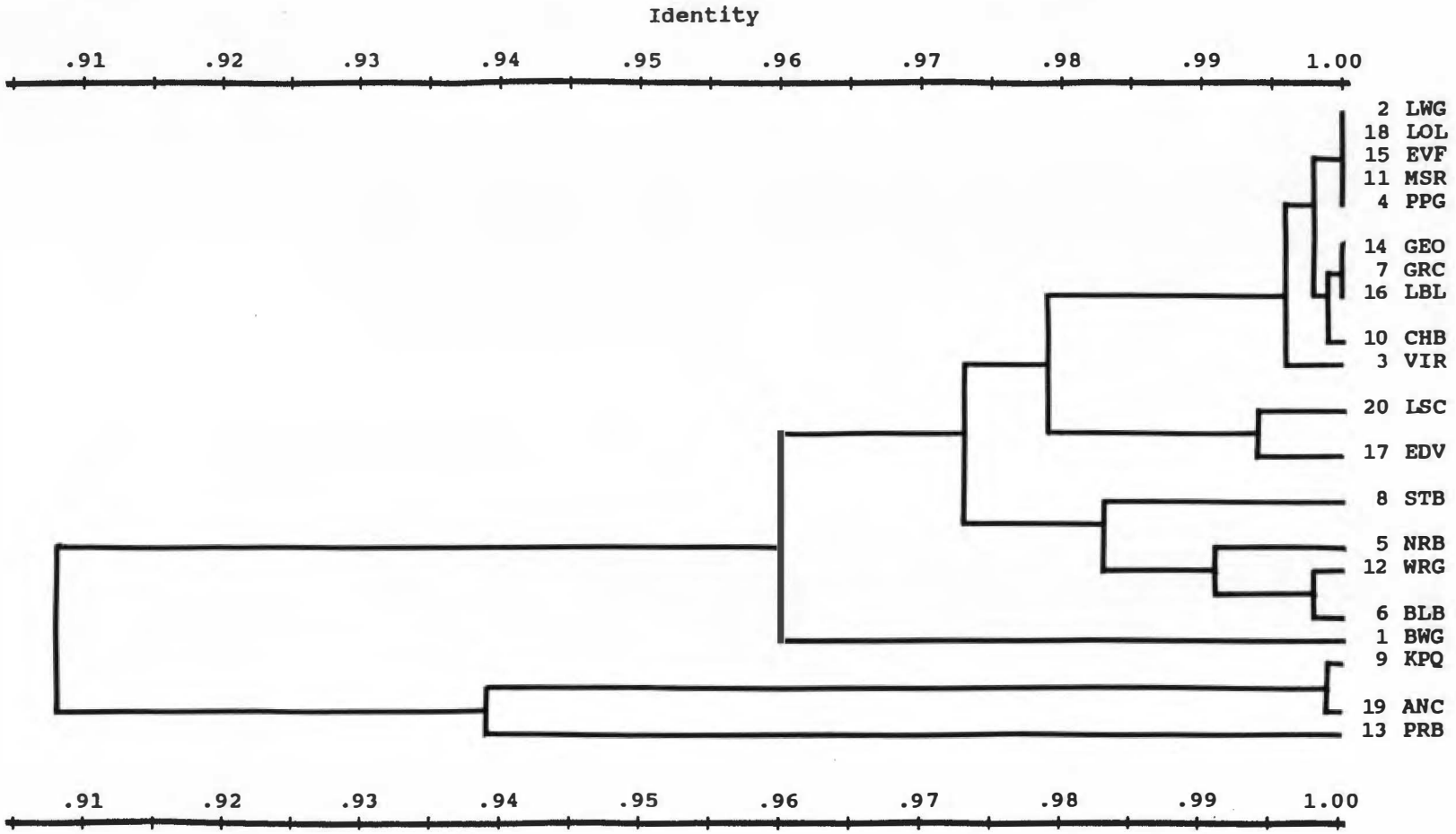


**Figure 4.2** Cluster analysis of populations of *Cimicifuga rubifolia* using unweighted pair-group method with arithmetic averaging based on Nei's (1978) unbiased identity.

were defined with Kingsport Quarry, Antioch Church and Powell River Bridge populations being separated from all other populations. There was no definable pattern of clustering based on location (river system), geographic proximity, or apparent disturbance. Other types of cluster analysis such as Wagner trees or Prevost and Roger's similarity index, produced similar results with only slight shifts in the arrangement of the populations.

Intrapopulation Genetic Architecture

Analysis of individual populations using F-statistics (Table 4.12) indicate there is a great deal of variation in the genetic architecture within the populations. Mean $F_{IT}$'s range from -.060 to .914. The mean $F_{IS}$ values also vary greatly, ranging from -.241 to .898. Less variation is seen in the mean $F_{ST}$ values which run from .065 to .315.

Using each sampling point as a subdivision, genetic distance and genetic identity also were calculated within each population. Unweighted pair group analysis was done for each to see if any pattern could be seen in the grouping of the sampling points (Figure 4.3). There was no apparent grouping of subdivisions based on possible microhabitats such as slope position, moisture conditions, or sunlight availability. In several populations, many of the subdivisions were monomorphic for all loci and the major clusters were based on monomorphic versus polymorphic genotypes. In the population shown in Figure 4.3, groups A, B, C, F, and G were all monomorphic for the common alleles. The other major clustering factor was geographic proximity, with the subdivisions closest together geographically being grouped

Table 4.12 Mean F-statistics calculated for individual polymorphic populations of *Cimicifuga rubifolia* throughout its range.

| Population | Mean F(IS) | Mean F(IT) | Mean F(ST) |
|---|---|---|---|
| Georges Creek | .898 | .914 | .161 |
| Grassy Creek | .811 | .861 | .263 |
| Mill Springs Road | .747 | .774 | .105 |
| Wallens Ridge | .490 | .571 | .160 |
| Big War Gap | .261 | .440 | .243 |
| Kingsport Quarry | .285 | .420 | .190 |
| Antioch Church | .126 | .402 | .315 |
| Christian Bend | .250 | .388 | .185 |
| Bear Creek LBL | .226 | .342 | .150 |
| Eaves Ferry | .190 | .290 | .123 |
| Norris River Bluffs | .139 | .278 | .161 |
| Powell River Bridge | .042 | .258 | .226 |
| Stowe Bluff | .168 | .238 | .083 |
| Bull Bluff | .046 | .218 | .180 |
| Pawpaw Grove | -.193 | .069 | .220 |
| Lusk Creek | -.160 | .056 | .187 |
| Virginia | -.056 | .024 | .076 |
| Little War Gap | -.077 | -.007 | .065 |
| Eddyville | -.241 | -.060 | .146 |

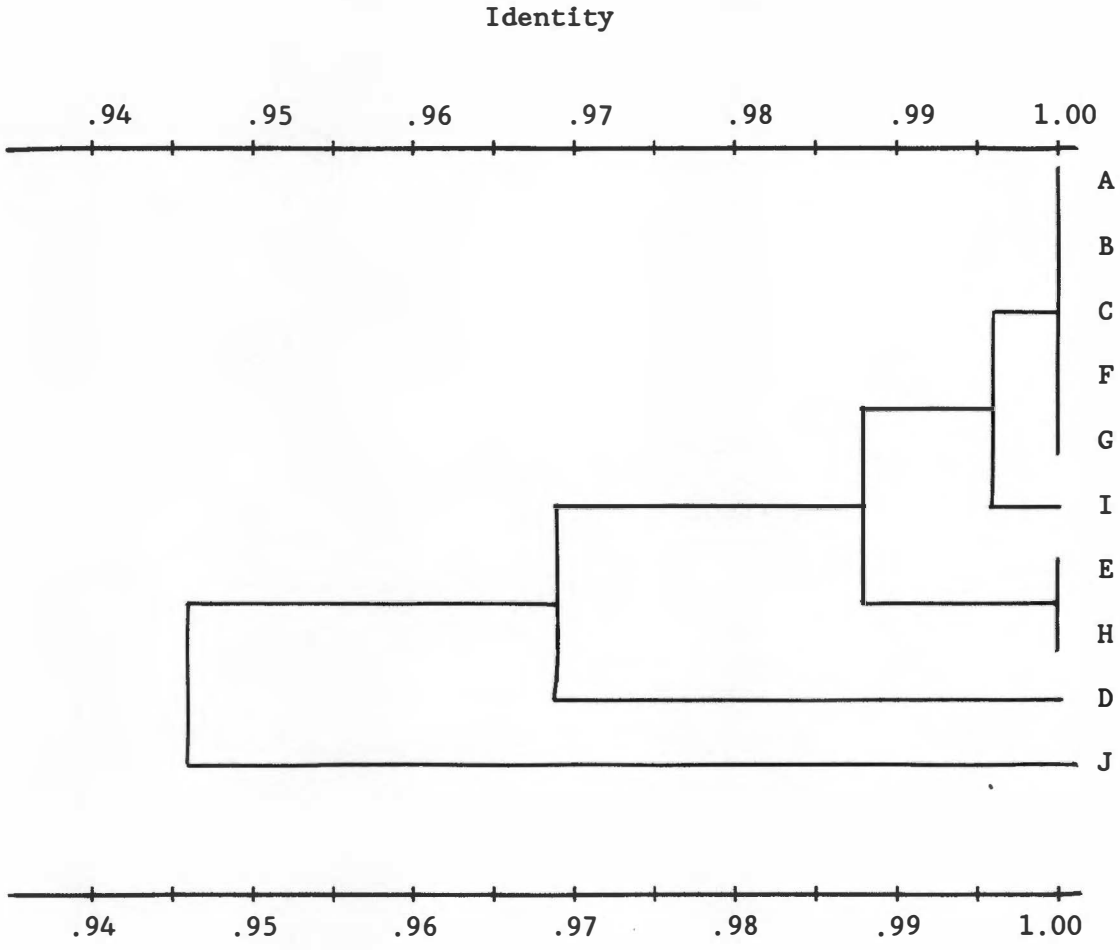


Figure 4.3 Cluster analysis of the Bull Bluff Population using unweighted pair group method. Similarity coefficient used is Nei's (1978) unbiased genetic identity.

together (Figure 4.4). The number of genotypes per sampling point was calculated as a check for possible asexual reproduction. In most of the populations there was insufficient variation to make any accurate determination (Table 4.13). In these populations, the most frequent genotype was consistently that in which all loci were fixed for the most common allele with only one or two individuals per subdivision showing any variation. Occasionally a subdivision would have 3 or 4 individuals that were heterozygous for a particular locus while the rest of the individuals were homozygous at all loci. The distribution of genotypes suggests that sexual reproduction is the predominant form of reproduction if not the only form in most populations. A number of different genotypes were observed in these populations; however, identical genotypes were not clustered as would be expected if cloning was occurring. That is particularly true for the Norris River Bluffs population.

## DISCUSSION

Because of the proximity of most populations to streams, one possible route for gene flow between populations could be along river systems. Based on this assumption, one would expect populations along the same river system to be more similar to each other than to those along other rivers. This was not supported by the cluster analysis done with genetic identity, probably because of the overall similarity of all populations. However, the distribution of 6PGD-2 allele A does suggest this may occur. The only populations in which the A allele was found were the two Powell River populations and the Norris River Bluffs

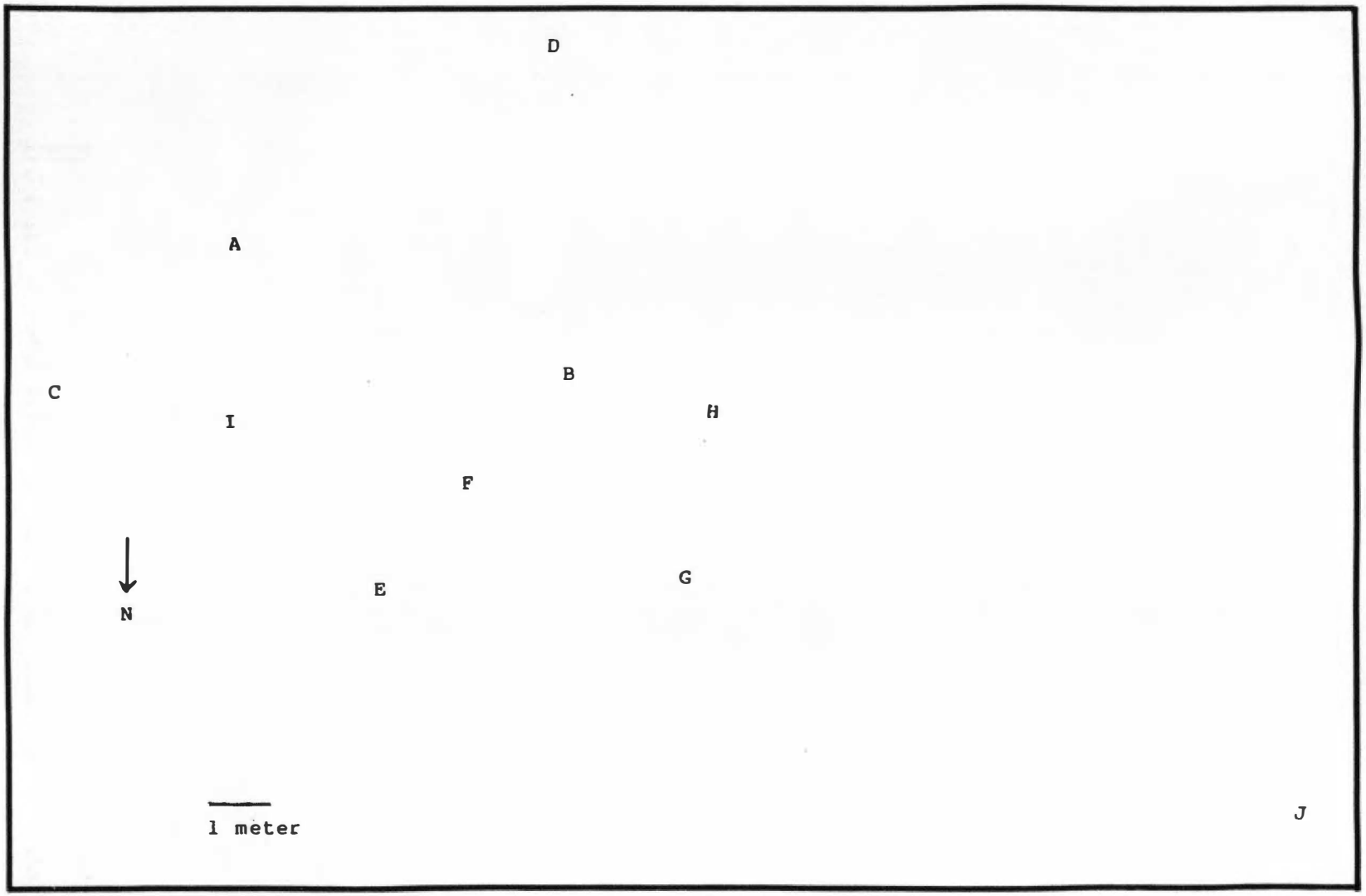


Figure 4.4 Map of sampling points at the Bull Bluff population.

Table 4.13 Genotypes at each sampling point in *Cimicifuga rubifolia* populations. The upper number is the number of genotypes observed at that point and the lower number is the number of individuals assayed from that point.

| Population | Subdivision | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K |
| Norris River Bluffs | 5 | 9 | 6 | 7 | 7 | 5 | 4 | 6 | 3 | | |
| | 8 | 9 | 6 | 8 | 8 | 7 | 8 | 8 | 8 | | |
| Powell River Bridge | 3 | 2 | 3 | 4 | 3 | 4 | 5 | 5 | 3 | | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | | |
| Wallens Ridge | 3 | 2 | 3 | 3 | 3 | 4 | 5 | 4 | 4 | | |
| | 7 | 7 | 7 | 6 | 7 | 7 | 7 | 7 | 7 | | |
| Georges Creek | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 1 | | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | | |
| Eaves Ferry | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | | |
| | 6 | 7 | 7 | 7 | 8 | 7 | 7 | 7 | 7 | | |
| Virginia | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| Stowe Bluff | 5 | 3 | 5 | 5 | 3 | 4 | 3 | 3 | 4 | 5 | |
| | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| Kingsport Quarry | 3 | 7 | 7 | 2 | 2 | 1 | 2 | 3 | 2 | | |
| | 12 | 7 | 7 | 4 | 8 | 6 | 6 | 10 | 2 | | |
| Mill Springs Road | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 2 | 1 | 1 | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| Little War Gap | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | | |
| Big War Gap | 2 | 3 | 5 | 4 | 5 | 4 | 5 | 5 | 5 | 2 | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| Bear Creek LBL | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | | |
| | 7 | 7 | 7 | 6 | 8 | 7 | 7 | 7 | 8 | | |
| Lusk Creek | 3 | 5 | 3 | 4 | 4 | 3 | 4 | 5 | 2 | 3 | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| Antioch Church | 1 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 2 |
| | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 7 | 7 | 7 | 7 |

Table 4.13 (cont.)

| Population | Subdivision | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K |
| Eddyville | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | |
| | 7 | 7 | 7 | 7 | 7 | 6 | 7 | 7 | 6 | 10 | |
| Bull Bluff | 3 | 3 | 4 | 6 | 4 | 4 | 3 | 5 | 5 | 4 | |
| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 8 | 8 | 8 | |
| Grassy Creek | 2 | 1 | 3 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 |
| | 7 | 7 | 7 | 7 | 7 | 2 | 7 | 7 | 8 | 7 | 8 |
| Grassy Creek (cont.) | 1 | 1 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | |
| | 7 | 7 | 8 | 7 | 7 | 9 | 7 | 7 | 8 | 7 | |

population. The latter population is located on the Clinch River just below the point at which the Clinch and Powell Rivers join. This suggests the allele originated in a population along the Powell River and has somehow been carried to populations downstream. The presence of the rare allele G of PGI-2 only in disjunct populations of Kentucky, Illinois, and western Tennessee was also interesting. It is probable that the G allele arose after these populations became isolated from the populations in the main range of C. rubifolia.

The mean number of alleles per locus within populations of C. rubifolia (1.3) is less than the average reported by Hamrick and Godt (1990) for other dicots and long-lived herbaceous perennials (1.44). It is also lower than those of animal-pollinated (1.54) and exclusively sexually reproducing species (1.53) although it is comparable to that given for other endemic species (1.39). The mean percentage of

polymorphic loci per population of C. rubifolia (33.6%) is more comparable to those of other animal-pollinated (35.9%) and exclusively sexually reproducing (34.9%) species. It is higher than that reported for endemic species (26.3%) but is lower than other long-lived herbaceous perennials (39.3%). It should be noted that the Hamrick and Godt study was based on data that had a mean population sample size of 12.7 and where an average of 16.5 loci per species were assayed.

Most of the variance in the genetic distance values is due to the presence of rare alleles that are found in only a few populations and to what is probably genetic drift at the 6PGD-2 allele in two populations (Kingsport Quarry and Antioch Church). The uniformly high values of I and low values of D show that overall, there has been little accumulation of gene differences among populations of C. rubifolia at the loci studied. The mean genetic identity of .971 for C. rubifolia is comparable to those found (above .90) for other conspecific populations of flowering plants (Crawford 1990).

High total fixation indices, $F_{IT}$'s, were observed at all loci in populations of C. rubifolia. For three of the loci; SKD, IDH, and PGM, most of the $F_{IT}$ value is comprised of the $F_{IS}$ value with divergence among populations contributing less to the total fixation. At the other two polymorphic loci, 6PGD-2 and PGI-2, the presence of rare alleles in only a few populations increased the degree of divergence between the populations and thus their $F_{ST}$ value contributes to their relatively large $F_{IT}$ values. Combined, C. rubifolia has a high degree of total fixation due both to allelic frequency differences within the populations and the divergence among populations. Wright (1978)

suggested that $F_{ST}$'s of .25 and greater indicate a very great degree of divergence. Westerbergh and Saura (1992) refer to the $F_{IT}$ of .216 in Silene dioica as being relatively high, indicating divergence of allele frequencies among populations. Cimicifuga rubifolia, with a mean $F_{IT}$ of .520 and a mean $F_{ST}$ of .313, then shows relatively high divergence with respect to allelic frequencies.

When considering the F-statistics for individuals populations, it becomes evident why the species level F-statistics indicated a high level of divergence. The mean $F_{IT}$'s range from -.060 to .914. Consideration of factors such as apparent disturbance and population size revealed no particular patterns for which populations have high total fixation indices and which do not. Georges Creek, which has the highest $F_{IT}$, is a very small population and is highly inbreed, as indicated by the $F_{IS}$. However, the Grassy Creek population, with the next highest $F_{IT}$, was one of the largest seen with in excess of 3000 individuals. This population is comprised of several large patches with pollinators observed moving within each patch, yet its $F_{IT}$ has a larger contribution from the $F_{IS}$ than $F_{ST}$ value. The Lusk Creek population was also a very large, patchy population but has a very low $F_{IT}$. Disturbance also does not appear to be a direct factor. The most disturbed sites appear to be Kingsport Quarry (in the talus of the quarry), Lola (by a planted field), Wallens Ridge (on the upper and lower banks of a road), and Mill Springs Road (also on a road bank). Other sites were in protected natural areas where there is no evidence of any type of human disturbance. These sites include Bear Creek LBL, Lusk Creek, Norris River Bluff, and Bull Bluff.

Another factor to be considered is the presence or absence of the known pollinator-attractor species, Impatiens pallida and Polymnia canadensis (Pellmyr 1986a). Neither of these species were present at Georges Creek, Grassy Creek, Antioch Church, Bear Creek LBL or Eaves Ferry. All of these populations have mean $F_{IS}$ values of .190 or greater. Several other populations were located in or near large populations of one or both of those species. These populations, which include Norris River Bluffs, Pawpaw Grove, and Little War Gap, all have mean $F_{IS}$ values less than .139. This suggests that the presence of the I. pallida and P. canadensis does influence gene flow within C. rubifolia populations. This cannot be the only contributing factor as the F values for the Eaves Ferry and Norris River Bluffs populations are very similar.

The number of large positive mean $F_{IS}$ values is a reflection of the heterozygote deficits in many of the populations. These are probably the result of two factors. The first is inbreeding within the populations. Even though C. rubifolia is not know to self-pollinate (Pellmyr 1986a), there is probably a high level of mating between closely related individuals. While many of the populations are small in total number of individuals the effective population size will be much smaller as only 5 to 20% of the population may bloom in a year (see Chapter 2). The second probable contributor to the heterozygote deficit is statistical pooling of small, breeding populations with differing allelic frequencies into a single population (or the Wahlund effect). In most of the populations, the distribution of C. rubifolia is patchy. While the patches are often easily within the flight range of

bumblebees, the primary pollinators (Pellmyr 1986), it is possible that the pollinators do not fly directly to another patch. If Pellmyr's hypotheses that C. rubifolia relies on other species such as Polymnia canadensis and Impatiens pallida to attract pollinators is correct, it is more probable that the pollinators will move to those species after leaving C. rubifolia patches, thus isolating those patches. This is also supported by personal observation. Additionally, seed dispersal appears to be through gravity and results in clumps of sibling plants in close proximity to each other. The pattern of seedling emergence supports this idea. Seedlings are frequently observed in groups at distances from a large plant that is about the length of a flower stalk. These factors should result in the production of groups of plants with gene frequencies differing from other parts of the population. This seems to fit the computer model tested by Turner et al. (1982) in which pollination of self-incompatible plants by their nearest neighbor resulted in increased homozygosity in patches and increased divergence between patches.

There is no conclusive evidence, from this genetic analysis, that asexual reproduction by rhizome fragmentation occurs. In many populations there is insufficient variability to determine any type of genotype patterns. However, in some of the populations or in subdivisions with variation, the relative number of genotypes present indicates that reproduction is almost (if not completely) exclusively sexual. There were a few subdivisions (1 at Wallens Ridge, 1 at Virginia, and 1 at Little War Gap) where small groups of heterozygous genotypes were found. However, they could be the result of either

asexual reproduction or clusters of siblings. In most populations, individuals are located far enough apart and were separated by rocks indicating that asexual reproduction by rhizome fragmentation was highly unlikely.

Predictions about the genetic diversity and architecture of C. rubifolia can be made based on life history and ecological traits. Taken separately, some of the results of this study seem to contradict what would be expected. As an animal pollinated plant, a high level of heterozygosity would be predicted, yet many populations, and the species as a whole, show a large degree of homozygosity. A large degree of genetic divergence between the widespread and isolated populations of C. rubifolia would be expected. This is supported by the large $F_{ST}$ values seen. Much of the genetic variation within the species appears to be due to some genetic drift between populations and the presence of rare alleles in a few populations. Genetic architecture within populations shows a great deal of variation but seems to primarily be the result of limited gene flow (with respect to both pollen and seed dispersal) within the populations and the varying number of alleles in different populations.

# CHAPTER 5

## STUDY OF THE BREEDING SYSTEMS AND GENETIC RELATIONSHIPS AMONG THE NORTH AMERICAN SPECIES OF CIMICIFUGA

### INTRODUCTION

In 1957 Hunter and Markert first described the separation of enzymes by starch gel electrophoresis. Since that time allozymes have become commonly used to describe the genetic structure of individuals, populations, species, or even higher taxonomic categories. Allozymes are considered to be a quick and easy way to evaluate genetic differences because, as proteins, their structure is determined by the DNA sequence. Since they exhibit Mendelian inheritance and are codominantly expressed, interpretation of data is relatively simple. The major disadvantage of allozyme analysis is that they only measure the diversity of soluble, enzyme coding genes and will not reveal differences in allozymes if the change in the protein structure did not result in electrophoretic mobility (Weeden and Wendel 1989).

There have been a number of studies on the relationship between genetic diversity and plant breeding systems. In those studies several trends were noted. Typically, species with restricted gene movement exhibit greater genetic differentiation between populations than those with widely dispersed pollen and seeds. Plants that are predominantly self-pollinating have more diversity among populations than within populations, while the opposite is more common for obligate out-breeders (Brown et al. 1989; Crawford 1989; Marshall and Brown 1975; Rossi et al. 1992). Many of the cited studies have shown large variation in genetic structure between populations of the same species. Inbreeding species

have frequently exhibited higher mean heterozygosity than expected while heterozygote deficits (with respect to Hardy-Weinberg expected) are common in many outbreeding plants. In self-compatible, insect pollinated species, a high level of variation in out-crossing rates, both within and between populations, has been noted (Brown et al. 1989; Scacchi et al. 1991). There is also some debate about the effectiveness of genetic analysis in the study of mating or breeding systems. Most studies take into account such factors as asexual reproduction, self-pollination, obligate out-breeding, and mixed pollination systems but neglect factors such as sibling matings, pollen distribution patterns, limited pollen flow, and pattern of pollen flow (Hamrick 1989).

A number of different statistics are used to study the relationship between genetic diversity, genetic structure, and plant breeding systems. Among the simpler statistics are the comparison of values such as mean heterozygosity and mean number of polymorphic loci. Other genetic diversity statistics are based on the total genetic diversity ($H_T$) and mean diversity within populations ($H_S$). These may be used for either hierarchical F-statistics (Wright 1965) or for Nei's (1973) $G_{ST}$ diversity statistics.

In most cases, Wright's $F_{ST}$ (described in chapter 4) and Nei's $G_{ST}$ are the same (Hartl 1980). $G_{ST}$ is the proportion of the genetic diversity due to variability among the populations where:

$$G_{ST} = D_{ST} / H_T.$$

The variation among populations, $D_{ST}$, is the difference in the total genetic diversity and the mean diversity within populations or:

$$D_{ST} = H_T - H_S$$

$F_{ST}$ considers the amount of differentiation between the populations and as such is a measure of genetic drift between the populations. The inbreeding coefficient, $F_{IS}$, considers the departure of observed from expected heterozygote frequency at the population level. The overall inbreeding coefficient, $F_{IT}$, considers the combined effects of breeding system at the population level and genetic drift between populations.

Hamrick and Godt (1990) reviewed plant allozyme literature and compared genetic diversity of taxa divided into various categories including life form, geographic range, and breeding system. They found that the more widespread the species is the higher the level of its diversity. Predominantly outcrossed species had higher levels of genetic diversity than self-pollinated species or those with mixed-mating systems, but information on only 4 taxa of long-lived herbaceous perennials was included in the review.

Allozymes have also been used to study the relationships between congeneric species. Many of those studies have focused on particular types of postulated relationships such as sister species or progenitor-offspring species (Crawford and Smith 1982; Riesberg and Soltis 1987). Most of these studies have looked for the presence of alleles, or even loci, unique to a species. Additionally, the species are compared using a variety of similarity and distance statistics such as Nei's (1972) genetic identity and genetic distance. As species become isolated there is an accumulation of genetic differences due to factors such as mutation, selection, and genetic drift. Allozyme data are used to estimate the accumulated number of gene substitutions per locus in the different species. The relative degree of divergence between species

can be measured using either genetic distance, D, or genetic identity, I. Identity values range from 1.0 (if the populations have not diverged) to 0.0 if there are no alleles in common (Avise and Smith 1977; Nei 1971, 1972; Walker 1987). Gottlieb (1977) calculated the mean genetic identity between congeneric plant species to be 0.67. Updates of Gottlieb's estimate have not produced any significant differences (Crawford 1989).

The aim of this study was to determine the effect of different breeding systems on the distribution of genetic variability, both among and within populations of the six North American species of Cimicifuga. Additionally, the relationships among the species in terms of relative amount of divergence will be considered.

Three species of Cimicifuga are found in eastern North America. Cimicifuga rubifolia Kearney is found primarily in the Ridge and Valley Province in eastern Tennessee and southwestern Virginia, with disjunct populations in northern Alabama, southern Illinois, southern Indiana, western Kentucky, and northwestern Tennessee. It is typically found on steep, north-facing limestone or calcareous slopes above the rivers. Cimicifuga americana Michx. is found from east-central Pennsylvania southward to northwestern South Carolina and north central Georgia, primarily at elevations from 274 to 1950 m. Cimicifuga racemosa (L.) Nutt. has the widest range of the North American species. It extends from southeastern Ontario southward to South Carolina and westward to northern Arkansas (Ramsey 1965). Cimicifuga racemosa occurs with both C. rubifolia and C. americana, although no hybrid or suspected hybrid individuals have been reported. This is most likely due to differences

in blooming periods. Cimicifuga racemosa blooms during June and July while both C. rubifolia and C. americana bloom from August to October. Cimicifuga rubifolia and C. americana are not known to be sympatric.

The range of Cimicifuga elata Nutt. extends from southern British Columbia to southwestern Oregon where it is found primarily in the Coast Range in a variety of habitats (Ramsey 1965). Cimicifuga laciniata Wats. is known only from about 10 populations in the Cascade Mountains in Oregon and Washington (Alverson personal communication). Cimicifuga arizonica Wats. is endemic to Coconino and Gila Counties of Arizona where it is found in deep shade with moist soils. Only 6 sites of C. arizonica are known (Phillips et al. 1982). None of the western species are known to occur together.

Morphologically the group has been split into three sets of sister species with C. rubifolia and C. elata, C. americana and C. laciniata, and C. racemosa and C. arizonica comprising the sets (Ramsey 1965). On the other hand, Pellmyr's (1985a, 1985b, 1986a, 1986b) study of the pollination ecology of the genus revealed some intriguing differences among the species. Two of the species, C. americana and C. laciniata, are nectariferous while the other 4 are nectarless. Cimicifuga racemosa is primarily pollinated by tachinid flies but all others are primarily pollinated by various species of bumblebees. Two of the species, C. elata and C. arizonica, are capable of self-pollination but none of the others are known to self-pollinate.

## MATERIALS AND METHODS

Populations for sampling were selected from throughout the ranges of all species (Table 5.1 and Figures 5.1, 5.2 and 5.3). Because sampling was done by a number of individuals, there was no consistent collection pattern. All plants were checked to insure that each collection was from a different rhizome. Number of samples collected was dependent on the population size. Among the eastern species, leaflets from each individual were collected and placed in labeled plastic bags. Bags were immediately placed in a cooler with ice. Samples were taken to the lab where each leaflet was cut into squares approximately 1 $cm^2$. These were placed in labeled individual plastic bags and stored at -80° C until needed. Leaflets from the western species were placed in labeled plastic bags with moist paper towels and mailed. Procedures following their receipt was the same as those previously described. Voucher specimens were deposited in the University of Tennessee, Knoxville Herbarium. Electrophoretic procedures were those described in Chapter 4. Recipes for all solutions are included in Appendix A.

Loci were designated sequentially with the most anodally migrating isozyme designated as 1. Alleles were also designated sequentially with the most anodally migrating allele designated as A. Distances of the bands were measured from the origin.

All genetic variability tabulations, genetic distance, genetic identity, cluster analysis, and F-statistics were calculated using BIOSYS-1 (Swofford and Selander 1981). Hierarchical F-statistics were used with hierarchical categories being species and population.

Table 5.1 Sites of Cimicifuga populations assayed for electrophoretic study. N is number of individuals assayed.

| Designation of Population | N | County and State |
|---|---|---|
| C. americana | | |
| Ashe | 19 | Ashe Co., North Carolina |
| Aurora | 18 | Preston Co., West Virginia |
| Carter 1 | 18 | Carter Co., Tennessee |
| Carter 2 | 20 | Carter Co., Tennessee |
| Haywood | 20 | Haywood Co., North Carolina |
| Monroe[a] | 3 | Monroe Co., Tennessee |
| Rockbridge 1 | 19 | Rockbridge Co., Virginia |
| Rockbridge 2 | 22 | Rockbridge Co., Virginia |
| Tucker | 15 | Tucker Co., West Virginia |
| Unicoi | 4 | Unicoi Co., Tennessee |
| C. arizonica | | |
| Oak Creek[b] | 9 | Coconino Co., Arizona |
| Workman Creek[c] | 29 | Gila Co., Arizona |
| C. elata[d] | | |
| Angels Rest | 24 | Multnomah Co., Oregon |
| Battleground | 18 | Clark Co., Washington |
| Beacon Rock | 18 | Skamania Co., Washington |
| Beacon Day | 18 | Skamania Co., Washington |
| Fox Hollow | 8 | Lane Co., Oregon |
| Lewis and Clark | 32 | Lewis Co., Washington |
| Pike | 18 | Yamhill Co., Oregon |
| Pilot Butte | 55 | Douglas Co., Oregon |
| Spencer Butte | 14 | Lane Co., Oregon |
| Sulphur Springs | 18 | Benton Co., Oregon |
| Yampo | 18 | Yamhill Co., Oregon |
| C. laciniata[d] | | |
| Eagle Creek | 18 | Clackamas Co., Oregon |
| Lost Lake | 18 | Hood River Co., Oregon |
| Puny Creek | 30 | Skamania Co., Washington |
| Wahtum Lake | 18 | Hood River Co., Oregon |

Table 5.1 (cont.)

| Designation of Population | N | County and State |
|---|---|---|
| *C. racemosa* | | |
| Aurora | 20 | Preston Co., West Virginia |
| Bull Bluff | 22 | Anderson Co., Tennessee |
| Rockbridge 3 | 20 | Rockbridge County, Virginia |
| Cattaraugus[e] | 14 | Cattaraugus Co., New York |
| Christain Bend | 20 | Hawkins Co., Tennessee |
| Eaves Ferry | 20 | Meigs Co., Tennessee |
| Grassy Creek | 20 | Roane Co., Tennessee |
| Haywood | 23 | Haywood Co., North Carolina |
| Kingsport Quarry | 20 | Sullivan Co., Tennessee |
| LBL Bear Creek | 20 | Stewart Co., Tennessee |
| Little War Gap | 15 | Hawkins Co., Tennessee |
| Mill Springs Rd | 20 | Jefferson Co., Tennessee |
| Pawpaw | 20 | Hancock Co., Tennessee |
| Preston | 9 | Preston Co., West Virginia |
| UT Woodlot | 23 | Knox Co., Tennessee |
| *C. rubifolia* | | |
| Antioch Church | 19 | Hardin Co., Illinois |
| Big War Gap | 18 | Hawkins Co., Tennessee |
| Bull Bluff | 19 | Anderson Co., Tennessee |
| Christain Bend | 18 | Hawkins Co,, Tennessee |
| Eaves Ferry | 18 | Meigs Co., Tennessee |
| Eddyville | 18 | Lyon Co., Kentucky |
| Georges Creek | 18 | Blount Co., Tennessee |
| Grassy Creek | 19 | Roane Co., Tennessee |
| Kingsport Quarry | 18 | Sullivan Co., Tennessee |
| LBL Bear Creek | 19 | Stewart Co., Tennessee |
| Little War Gap | 19 | Hawkins Co., Tennessee |
| Lola | 18 | Livingston Co., Kentucky |
| Lusk Creek | 18 | Pope Co., Illinois |
| Mill Springs Road | 19 | Jefferson Co., Tennessee |
| Norris River Bluffs | 18 | Anderson Co., Tennessee |
| Pawpaw Grove | 19 | Hancock Co., Tennessee |
| Powell River Bridge | 19 | Claibourne Co., Tennessee |
| Stowe Bluff | 18 | Roane Co., Tennessee |
| Virginia | 19 | Scott Co., Virginia |
| Wallens Ridge | 18 | Hancock Co., Tennessee |

[a] collection made by E.E.C. Clebsch
[b] collection made by G. Goodwin
[c] collection made by M. Ross
[d] collections made by E. Alverson
[e] collection made by A.M. Evans

Figure 5.1 Distribution of Cimicifuga rubifolia and C. americana. Circles designate C.rubifolia; and triangles C. americana. Modified from Ramsey 1965.

Figure 5.2 Distribution of Cimicifuga racemosa. Modified from Ramsey 1965.

Figure 5.3 Distribution of the Western North American Species of Cimicifuga. Circles designate C. elata; triangles, C. laciniata; and squares, C. arizonica. Modified from Ramsey 1965.

RESULTS

Ten loci were assayed. One locus, PGM-1, could not be consistently scored in C. rubifolia and was therefore used only in intraspecific analysis of the other species and not in interspecific comparisons. There also appeared to be additional bands present in C. racemosa for PGI-2 or PGI-3. These bands were also inconsistent and the gels were scored conservatively for the bands that were always present. Two of the loci were unique to single species. PGM-3 was present only in C. americana and PGI-3 was present only in C. racemosa. Allelic frequencies are given in Table 5.2. Of the 36 alleles detected 25 (69%) were unique to a particular species. Most of the loci had at least one allele that was common to most species. PGM-2 B and 6PGD-2 C were both present in all species while 6PGD-1 A was in 5 species and SKD E was in 4 species. For each locus there were also alleles that were unique to a particular species. A unique allele of PGI-2 was present in each species. The eastern species had more unique alleles than the western species. Cimicifuga americana had a total of 7 unique alleles, C. racemosa and C. rubifolia each had 6, C. arizonica had 4, and C. elata and C. laciniata had only 3. If total number of alleles present in a species is calculated, C. rubifolia has the most with 15 alleles, C. americana has 14, C. racemosa has 13, C. elata has 10, C. laciniata has 9 and C. arizonica has 8.

These differences in number of loci and alleles were also seen in terms of the percentages of polymorphic loci (Table 5.3). The two populations of C. arizonica sampled showed no variation while only one locus (PGI-2) in C. laciniata was polymorphic. Cimicifuga elata

Table 5.2 Summary of allele frequency data for all loci assayed in six species of *Cimicifuga*.

| Locus/allele | AMER[a] | ARIZ | ELAT | LACI | RACE | RUBI |
|---|---|---|---|---|---|---|
| SKD | | | | | | |
| A | | | | | .185 | |
| B | | | | | | .948 |
| C | .747 | | | | | |
| D | | | | | .815 | |
| E | | 1.000 | 1.000 | 1.000 | | .052 |
| F | .253 | | | | | |
| IDH | | | | | | |
| A | | | | 1.000 | | |
| B | .995 | 1.000 | | | .977 | |
| C | | | | | | .045 |
| D | | | 1.000 | | | .952 |
| E | .005 | | | | | |
| F | | | | | .023 | .003 |
| PGM-1 | | | | | | |
| A | 1.000 | | | 1.000 | | |
| B | | | .930 | | 1.000 | n.s.[b] |
| C | | | .070 | | | |
| D | | 1.000 | | | | |
| PGM-2 | | | | | | |
| A | | | .041 | | | .014 |
| B | 1.000 | 1.000 | .959 | 1.000 | .987 | .894 |
| C | | | | | | .092 |
| D | | | | | .013 | |
| PGM-3 | | | | | | |
| A | .147 | | | | | |
| B | .853 | | | | | |
| 6PGD-1 | | | | | | |
| A | 1.000 | | 1.000 | 1.000 | 1.000 | 1.000 |
| B | | 1.000 | | | | |
| 6PGD-2 | | | | | | |
| A | .037 | | | | | |
| B | | | | | | .020 |
| C | .963 | 1.000 | 1.000 | 1.000 | .986 | .813 |
| D | | | | | | .167 |
| E | | | | | .014 | |

Table 5.2 (cont.)

| Locus/allele | AMER | ARIZ | ELAT | LACI | RACE | RUBI |
|---|---|---|---|---|---|---|
| PGI-1 | | | | | | |
| A | | | | 1.000 | | |
| B | 1.000 | | 1.000 | | 1.000 | 1.000 |
| C | | 1.000 | | | | |
| PGI-2 | | | | | | |
| A | | | | .340 | | |
| B | | 1.000 | | | | |
| C | | | | | | .970 |
| D | | | 1.000 | | | |
| E | .111 | | | .660 | | |
| F | .889 | | | | | |
| G | | | | | 1.000 | .030 |
| PGI-3 | | | | | | |
| A | | | | | 1.000 | |

[a] AMER is C. americana, ARIZ is C. arizonica, ELAT is C. elata, LACI is C. laciniata, RACE is C. racemosa, and RUBI is C. rubifolia.

[b] n.s. present but not consistently scorable

Table 5.3 Indices of heterozygosity in the six North American species of Cimicifuga. Standard deviation (s) is in parenthesis.

| Species | Mean no. of alleles locus | Mean Percentage of loci polymorphic[a] | Mean heterozygosity | |
|---|---|---|---|---|
| | | | Direct-count | HdyWbg expected[b] |
| C. americana | 1.2 (6.5) | 22.2 (.15) | .049 (.033) | .052 (.030) |
| C. arizonica | 1.0 (0.0) | .0 (0.0) | .000 (.000) | .000 (.000) |
| C. laciniata | 1.1 (0.0) | 12.5 (0.0) | .058 (.016) | .050 (.050) |
| C. elata | 1.1 (0.1) | 11.4 (8.8) | .022 (.022) | .025 (.022) |
| C. racemosa | 1.1 (0.1) | 14.1 (9.8) | .024 (.023) | .137 (.028) |
| C. rubifolia | 1.3 (0.2) | 22.9 (15.5) | .050 (.050) | .107 (.065) |

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed .95

[b] Unbiased estimate (Nei 1978)

exhibited more polymorphisms with some populations having 25% of the loci polymorphic. Cimicifuga rubifolia had the highest level of polymorphism with populations averaging 22.9% polymorphic loci. Although the number of individuals assayed per population varied, this appears to have only had limited effects on the data. Only 3 of the 11 populations of C. elata were monomorphic at all loci, including the population with the most individuals assayed (Pilot Knob, n=55). In C. laciniata, the rare allele was present in all populations assayed. The smallest populations of C. americana assayed (Unicoi, n=4, and Monroe, n=3) were monomorphic.

There was a great deal of variation in the mean F-statistics of the species (Table 5.4). Cimicifuga arizonica is not included because it exhibited no polymorphisms. Mean $F_{IS}$ values ranged from -.187 in C. laciniata to .335 in C. racemosa. Mean $F_{IT}$ values were comparable in C. americana, C. rubifolia, and C. racemosa with values of .521, .520 and .456 respectively. Cimicifuga laciniata had the lowest $F_{IT}$ with a value

Table 5.4 Summary of Mean F-statistics at all loci for all polymorphic species

| Species | F(IS) | F(IT) | F(ST) |
|---|---|---|---|
| C. Americana | .007 | .521 | .517 |
| C. elata | .078 | .154 | .083 |
| C. laciniata | -.187 | -.033 | .130 |
| C. racemosa | .335 | .456 | .182 |
| C. rubifolia | .301 | .520 | .313 |

of -.033. Mean $F_{ST}$ values also ranged widely, from .083 in C. elata to .517 in C. americana.

The mean F-statistics were very interesting in terms of the different breeding systems in Cimicifuga. Only C. elata and C. arizonica are known to be capable of self-fertilization, while all of the other species are thought to not self-pollinate. In outbreeding populations, $F_{IS}$ is expected to be negative or close to 0, yet two of the species which do not self-pollinate, C. racemosa and C. rubifolia, have a much higher $F_{IS}$ than does the self-pollinating C. elata. Two of the species, C. americana and C. rubifolia, show a higher degree of genetic divergence between populations than do the other species. This high level of divergence between C. americana and between C. rubifolia populations is probably related to the degree of isolation of most populations. The lower levels of divergence between both C. elata and C. laciniata populations are more likely due to the low number of alleles per locus in these species than any gene flow between populations. Because of the low number of loci analyzed in this study, these results should only be considered as preliminary and not truly indicative of the levels of diversity in all of the species.

Table 5.5 also shows the degree of allozyme variation at the population levels for the species, but in terms of $G_{ST}$ values. Hamrick and Godt (1990) found that typically species that are self-pollinating have higher $G_{ST}$'s than do species with mixed pollination systems or those that are obligate out-crossers. The $G_{ST}$ value for C. americana is much more typical for self-pollinators than it is for a self-incompatible species. In contrast, the $G_{ST}$ value for C. elata is much

Table 5.5 Levels of allozyme variation at the population level for the North American species of Cimicifuga.

| Species | $G_{ST}$ | $D_{ST}$ | $H_T$ | $H_S$ |
|---|---|---|---|---|
| C. americana | .503 | .091 | .181 | .090 |
| C. elata | .086 | .009 | .105 | .096 |
| C. laciniata | .129 | .058 | .449 | .391 |
| C. racemosa | .180 | .018 | .100 | .082 |
| C. rubifolia | .314 | .049 | .156 | .107 |

lower than those typical for plants using a mixed mating system, or even for many obligate outbreeding species. This suggests that even though C. elata can self-pollinate, it is primarily out-crossed.

Several genetic similarity and distance measures were employed for all pairwise comparisons both within and between the species. The results of the Nei's unbiased genetic identity (Nei 1978) tests are shown in Table 5.6. Within species, C. arizonica and C. elata show the least divergence of populations, with average identities of 1.000. Cimicifuga americana populations show the least similarity with an average I value of .942. Between the species, C. arizonica is consistently the least similar to all of the other species with average identity values ranging from .433 to .568. The two most similar species were C. rubifolia and C. elata which had pairwise identity of .763. A

Table 5.6 Mean values for Nei's (1978) genetic identity.

| | Identity | Range |
|---|---|---|
| **Within species** | | |
| *C. americana* | .942 | .757-1.000 |
| *C. arizonica* | 1.000 | 1.000-1.000 |
| *C. elata* | 1.000 | .999-1.000 |
| *C. laciniata* | .992 | .981-1.000 |
| *C. racemosa* | .992 | .960-1.000 |
| *C. rubifolia* | .971 | .888-1.000 |
| **Between species** | | |
| *C. americana* x | | |
| *C. arizonica* | .454 | .435- .470 |
| *C. elata* | .566 | .519- .584 |
| *C. laciniata* | .473 | .452- .516 |
| *C. racemosa* | .573 | .535- .601 |
| *C. rubifolia* | .553 | .470- .599 |
| *C. arizonica* x | | |
| *C. elata* | .554 | .549- .556 |
| *C. laciniata* | .568 | .565- .572 |
| *C. racemosa* | .447 | .421- .458 |
| *C. rubifolia* | .433 | .357- .456 |
| *C. elata* x | | |
| *C. laciniata* | .631 | .372- .636 |
| *C. racemosa* | .561 | .548- .572 |
| *C. rubifolia* | .763 | .683- .793 |
| *C. laciniata* x | | |
| *C. racemosa* | .460 | .447- .471 |
| *C. rubifolia* | .560 | .478- .586 |
| *C. racemosa* x | | |
| *C. rubifolia* | .552 | .470- .637 |

number of procedures such as UPGMA clusters analysis (Sneath and Sokal 1973) and Wagner procedure (Farris 1972) were used to produce phenograms from several I and D matrices. All produced similar phenograms in terms of the relationships of the species. Figure 5.3 shows the cluster analysis of unbiased genetic identity, I. Cimicifuga arizonica branches off from the rest of the species with an I of approximately .470. The rest of the species are divided into two clusters with C. racemosa and C. americana in one cluster. The other cluster contains C. rubifolia and C. elata with C. laciniata as an outlying group.

## DISCUSSION

In their review, Hamrick and Godt (1990) found that long-lived herbaceous perennials had a mean $G_{ST}$ value of .213. The mean $G_{ST}$ value, .202, for the Cimicifuga species studied was relatively close, given that Hamrick and Godt's information was based on studies of only 4 taxa.

Both the hierarchical F-statistics and $G_{ST}$ values indicate that there are differences in the distribution of genetic variation in the species. In C. americana most of the variation is found between populations while in C. rubifolia the variation is distributed relatively evenly between and within populations. In contrast, C. racemosa has most of its variation within the populations rather than between them. These values are not particularly close to those that would be expected based on comparison to those reported by Hamrick and Godt (1990) for taxa with similar breeding systems. They found that self-pollinated species typically have high $G_{ST}$ values (.510). Plants with a mixed breeding system of self and animal pollination had

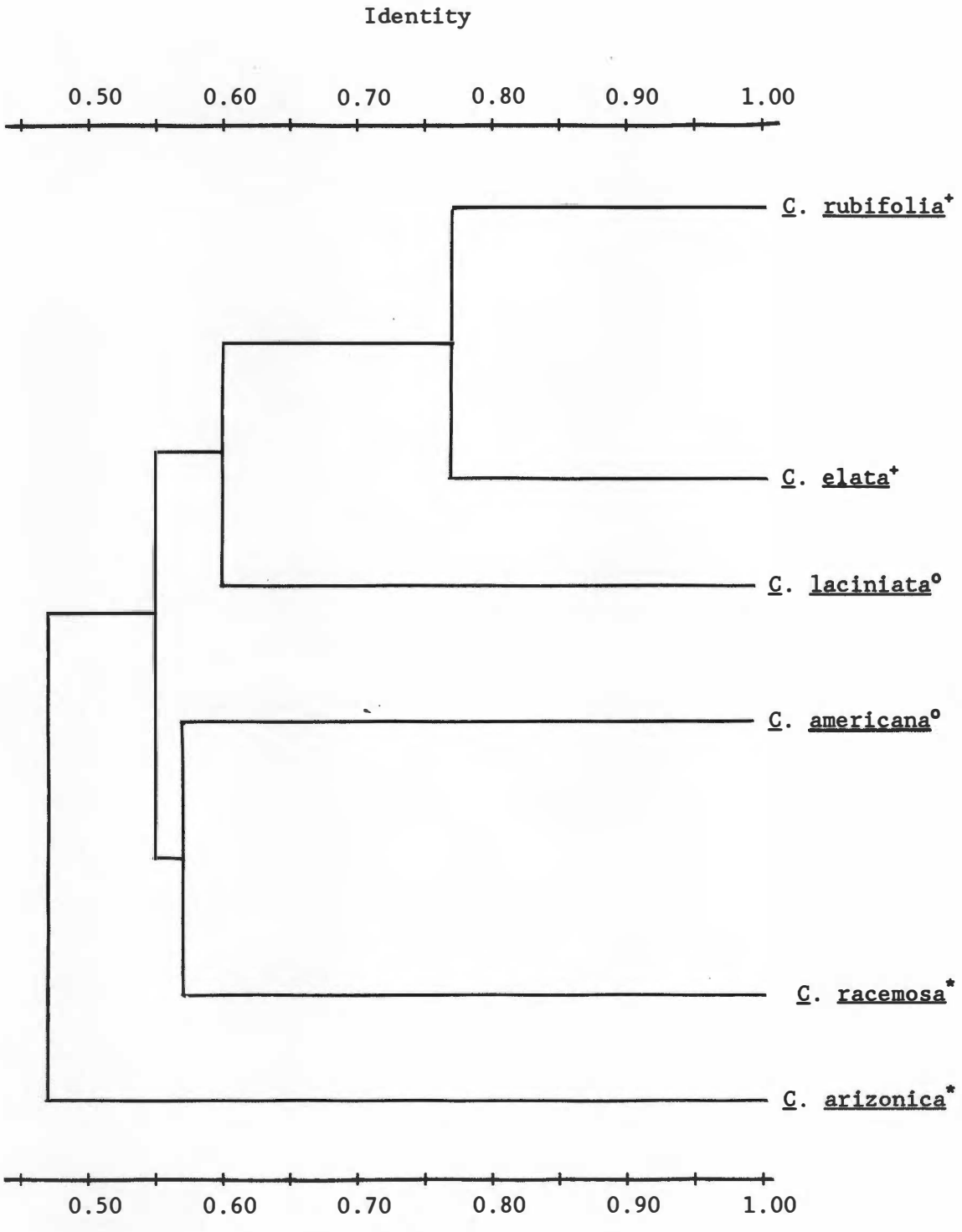


Figure 5.4 Cluster analysis of populations of North American Cimicifuga species using Unweighted Pair-Group Method with arithmatic averaging based on Nei's (1978) Unbiased Genetic Identity. Morphologically similar species are denoted by symbol.

intermediate $G_{ST}$ values (.216) and those species that were solely animal pollinated had $G_{ST}$'s that were slightly lower (.197). In contrast, C. americana had a mean $G_{ST}$ very close to that typical of self-pollinated species. C. racemosa and C. laciniata had values close to those reported for other outcrossing-animal pollinated species. The other species that does not self-pollinate, C. rubifolia, had a mean $G_{ST}$ substantially higher than that expected for plants with animal pollination systems. C. elata had a mean $G_{ST}$ value much lower than those reported for outcrossing animal pollinated plants.

There are a number of factors that could be affecting the distribution of genetic variation. One is the low number of polymorphic loci, particularly in C. laciniata and C. elata. As only 1 or 2 polymorphic loci, respectively, were observed in these species, little genetic variation, either among or within populations, can be expected. Because of this, little can be concluded about breeding systems from these data, but the number of heterozygous individuals also suggests they are predominantly outcrossing. It should be noted that there is an assumption being made that in these species, as in C. rubifolia, asexual reproduction is rare.

The higher than expected mean $G_{ST}$ values for the other species are probably affected by combinations of small population size and pollination between relatives. As mentioned in the previous chapter, it appears that C. rubifolia populations are subdivided into inbreeding subpopulations, which can have the same effect in terms of genetic structure as self-pollination. It is possible that the high mean $G_{ST}$ value of C. americana is a result of a high rate of pollinations between

relatives. Because of the small size of many of the populations assayed, this is highly probable. Only C. racemosa, which typically occurs in larger populations and apparently had a more reliable pollinator, is close to the mean $G_{ST}$ expected for an outcrossing, animal pollinated species.

The number of alleles and loci that were unique for each species indicates that all of these species have been reproductively isolated for a relatively long time. This is supported by the mean genetic identity of .543 (s=.085) for all pairwise species comparisons, which is lower than that reported by Gottlieb (1977) for congeneric plant species.

Only one pair of the morphologically similar species, C. elata and C. rubifolia, were clustered together. This also suggests that the species have been isolated for a long time. This study indicates that C. arizonica is the least similar to all of the other species assayed. Given the habitat differences of C. arizonica, it is probable that some of the divergence is due to selection pressures.

From this study it can be concluded that the six North American species of Cimicifuga have been reproductively isolated for a relatively long period of time. This is supported by the number of unique alleles present in each species as well as the relatively low genetic identity values between species. However, the data about the breeding systems of the Cimicifuga species is inconclusive. For three of the species, C. arizonica, C. elata, and C.laciniata, the lack or low levels of polymorphisms made analysis impossible or suspect. The data obtained from C. elata and C. laciniata does suggest that they are probably

predominantly out-crossing despite their ability to self-fertilize. Cimicifuga racemosa, which does not self-pollinate, shows a distribution of genetic variation comparable to that of other reported for other animal pollinated species. Cimicifuga rubifolia values are more similar to those of plants with mixed pollination systems and is probably a result of inbreeding among close relatives.

# CHAPTER 6

# SUMMARY AND CONCLUSIONS

## SUMMARY

### Population biology of *Cimicifuga rubifolia*

Plant size, based on photosynthetic area, proved to be an important correlate to life history characters. Presence of an inflorescence was strongly correlated to plant size. Reproductive capacity, as number of ovaries or follicles produced, was also positively related to size. Both dormancy and mortality were negatively correlated to size, although noted causes of mortality were size independent. The data suggest that once *Cimicifuga rubifolia* attains a certain size (as photosynthetic area), changes in size above this become less important in terms of reproductive capacity and change in the number of shoots per rhizome. This study also suggested that plant size is strongly influenced by the amount of precipitation.

The size structure of the populations studied was typical of that found in other long-lived perennials, but the structure based on size classification was not statistically stable. This is apparently another influence of the variability of precipitation during the study. The increasing mean size of the individual is reflected in the relatively high probabilities found for individuals to move into a larger size class.

The primary mode of reproduction in *C. rubifolia* was found to be sexual reproduction. Asexual reproduction by rhizome fragmentation was shown to be possible but rare in the 2 populations studied. The

electrophoretic analysis of other populations supported this conclusion, although in some populations low levels of polymorphisms made analysis difficult.

The study did not reveal a substantial amount of accumulated gene differences per locus as measured by genetic distance among populations of C. rubifolia. However a large amount of genetic divergence among the populations is indicated by the large $F_{ST}$ values. This is probably due to the reproductive isolation of the populations, indicated by the generally high total fixation indices, $F_{IT}$. In addition to being isolated from other populations, many populations appear to be subdivided into small, inbreeding subpopulations. F-statistics for individual populations indicate the intrapopulational genetic architecture varies greatly between populations. A number of possible influencing factors, such as disturbance, pollinator abundance, and population size, were considered as possible determinates of the distribution of genetic variability within populations.

## Genetic Relationships and Breeding Systems of the North American *Cimicifuga* Species

There is a relatively high level of genetic divergence, as measured by genetic identity, among the North American species of *Cimicifuga*, indicating the species have been isolated for a long period of time. C. rubifolia was the most similar to C. elata, the western species that most closely resembles it morphologically. Such a relationship did not hold up for the other pairs of morphologically similar species.

The data about the breeding systems of the Cimicifuga species is inconclusive. The lack of detected polymorphism in C. arizonica made analysis of its breeding system impossible. The low levels of polymorphisms in C. elata and C. laciniata limit the effectiveness of their analysis but data indicates both are predominantly out-crossing species, despite the self-fertility of C. elata. In contrast, the $G_{ST}$ values for both C. americana and C. rubifolia indicate that a high level of inbreeding occurs in both species, even though neither self-pollinate.

## CONCLUSIONS

In general, the life history and demography of Cimicifuga rubifolia were found to be very similar to those reported for other long-lived herbaceous perennials. However, there were a number of specific aspects that were unexpected or unusual. While it is not uncommon for perennial herbs to be able to undergo dormancy, the portion of individuals that were dormant for some portion of the study was larger than anticipated. Also, the length of time that some of the plants were dormant, at least 3 years, was unexpected. It is probable that both the high dormancy levels and length of dormancy were results of the prolonged dry period prior to and during the first years of the study.

The size plasticity of individuals and the relationship of this plasticity to precipitation also proved interesting. While herbaceous perennials are known to be capable of either increasing or decreasing in size from year to year, the variability in C. rubifolia seems to be

unusually great, with the size of the largest individuals doubling during the course of the study. The change from year to year of the size at which a plant has a 50% probability of flowering was also very unusual. Most population projection models are based on the assumption that relationships between size and life history characters are constant. This is apparently not true for C. rubifolia as there is variation in the size at which 50% probability of flowering is attained. This type of variability suggests a weakness in many of the current population models.

This study indicates that asexual reproduction is a rare occurrence in the populations studied. Based on general trends noted in the literature, it was initially thought that reproduction in C. rubifolia would be predominantly sexual but asexual reproduction by rhizome fragmentation would occur frequently. In retrospect, asexual reproduction by rhizome fragmentation would probably not be a particularly effective means of reproduction given the habitat of C. rubifolia. One of the habitat characteristics of C. rubifolia is that it occurs on rocky slopes. Frequently, individual rhizomes are separated by rocks. This would make reproduction by underground mechanisms inefficient, since such substrate barriers would effectively prevent the separation of propagules.

Geographically and reproductively isolated populations of C. rubifolia was indicated by the high $F_{ST}$ values. These differences between populations may be the result of genetic drift, founder effect or selection factors. Some alleles were found in only a few populations. The presence of a unique allele in the Powell River

drainage suggests that there is at least limited gene flow along river systems. Limited gene flow within the populations has resulted in their subdivision into inbreeding groups. The high level of inbreeding and subsequent subdivision of the populations indicated by allozyme analysis is most likely due to limited gene flow within the populations.

This study on the population biology of C. rubifolia should only be considered as a preliminary investigation. Although the questions initially asked were answered, a number of new questions were raised. Among them is that of what factor or factors are controlling the distribution of genetic variability within populations of C. rubifolia. An investigation of the cause or causes of limited gene flow within the populations (pollinator availability, pollen viability, fertility among closely related plants) should be made. Another important line of study that should be considered is the exact relationship between plant size, flowering and precipitation. This study was done during a time period with unusual precipitation patterns. While this made interpretation of some of the data difficult, it also revealed some very interesting relationships between the species and its environment.

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# APPENDICES

# APPENDIX A

## ALLOZYME EXTRACTION BUFFER, ELECTRODE BUFFERS AND STAIN RECIPES

Extraction Buffer
from Werth 1985

| | |
|---|---|
| 0.2 M Tris HCL pH 8.0 | 100 ml |
| 0.5% Sodium (meta) Bisulfite | 0.5 g |
| 0.05% EDTA, Tetrasodium salt | 0.05 g |
| 0.01 M Magnesium Chloride | 1 ml of 1M solution |

adjust pH to 7.5
2-Mercaptoethanol (0.1%) added just prior to grinding.

Electrode and Gel Buffers

Morpholine
from Clayton and Tretiak 1972

Electrode:

| | |
|---|---|
| 0.4 M Citric Acid, monohydrate | 8.4 g/l |
| N-3(3-Aminopropyl)-Morpholine | |
| add until pH reaches 8.0 | |

Gel: 1:19 dilution of electrode buffer

Soltis # 1
from Soltis et al. 1983

Electrode:

| | |
|---|---|
| 0.4M Citric Acid, | |
| trisodium salt dihydrate | 117.64 g/l |

adjust pH to 7.0 with 1.0 M HCl

Gel:

| | |
|---|---|
| 0.02 M Histidine-HCl, | |
| monohydrate | 4.19 g/l |

adjust pH to 7.0 with 1.0 M NaOH

Stain Recipes
modified by Werth from Werth 1985

All solutions were prepared ahead of time and frozen in 5 ml aliqouts. Noted ingredients were added to the 5 ml aliqouts just prior to use.

| IDH (Isocitrate dehydrogenase) | |
|---|---|
| 0.2 M Tris HCL pH 7.0 | 85 ml |
| 1M $MgCl_2$ | 10 ml |
| Isocitric acid, trisodium salt | 0.5 g |
| NADP | 0.05 g |
| 1% MTT | 5 ml |
| 1% PMS | 2 drops to 5 mls just prior to assay |

| PGI (Phosphoglucose isomerase) | |
|---|---|
| 0.2M Tris HCl pH 8.0 | 85 ml |
| 1M $MgCl_2$ | 10 ml |
| Fructose-6-Phospahte | 0.2 g |
| NADP | 0.05 g |
| 1% MTT | 5 ml |
| 1%PMS | 2 drops to 5 mls just prior to assay |
| G-6-PDH | 10 units to 5 mls just prior to assay |

| PGM (Phosphoglucomutase) | |
|---|---|
| 0.2M Tris HCl pH 8.0 | 85 ml |
| 1M $MgCl_2$ | 10 ml |
| Glucose-1-Phosphate, (Sigma G-1259) | 0.5 g |
| NADP | 0.05 g |
| 1% MTT | 5 ml |
| 1% PMS | 2 drops to 5 mls just prior to assay |
| G-6-PDH | 10 units to 5 mls just prior to assay |

| 6-PGDH (6-Phosphogluconate dehydrogenase) | |
|---|---|
| 0.2M Tris HCl pH 8.0 | 85 ml |
| 1M $MgCl_2$ | 10 ml |
| 6-Phosphogluconic Acid, barium salt | 0.1 g |
| NADP | 0.05 g |
| 1% MTT | 5 ml |
| 1% PMS | 2 drops to 5 mls just prior to assay |

| SKD (Shikimate dehydrogenase) | |
|---|---|
| 0.2M Tris HCl pH 8.0 | 95 ml |
| Shikimic Acid | 0.1 g |
| NADP | 0.05 g |
| 1% MTT | 5 ml |
| 1% PMS | 2 drops to 5 mls just prior to assay |

# APPENDIX B

## SPECIES LIST FOR ELECTROPHORETIC FIELD STUDY SITES

Plant lists were compiled after site observation and the identification of some collections. The primary source used for identification and nomenclature was Radford et al. (1973) although others were also used. The sources include:

Gleason, H.A. 1952. The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. Lancaster Press, Inc., Lancaster, Penna.

Radford, A.E., H.E. Ahles, and C.R. Bell. 1973. Manual of the Vascular Flora of the Carolinas. The University of North Carolina Press, Chapel Hill. Third edition.

Steyermark, J.A. 1977. Flora of Missouri. The Iowa State University Press, Ames, Iowa. Fifth edition.

Wofford, B.A. 1989. Guide to the Vascular Plants of the Blue Ridge. The University of Georgia Press, Athens.

## DISJUNCT POPULATIONS

### ANTIOCH CHURCH, ILLINOIS (ANC)

*Acer saccharum*
*Actaea pachypoda*
*Adiantum pedatum*
*Arisaema triphyllum*
*Asarum canadense*
*Astilbe biternata*
*Calycarpon lyonii*
*Campanula americana*
*Carya cordiformis*
*Carya ovata*
*Carya tomentosa*
*Chelone lyonii*
*Cimicifuga rubifolia*
*Cornus florida*
*Cystopteris protrusa*
*Dioscorea villosa*
*Fagus grandifolia*
*Fraxinus americana*
*Hybanthus concolor*
*Hydrangea arborescens*
*Ipomoea hederaecea*
*Jeffersonia diphylla*
*Lindera benzoin*
*Liquidambar styraciflua*
*Liriodendron tulipifera*
*Parthenocissus quinquefolia*
*Phytolacca americana*
*Platanus occidentalis*
*Polemonium reptans*
*Polygonatum biflorum*
*Quercus alba*
*Quercus muhlenbergii*
*Quercus prinoides*
*Sanguinaria canadensis*
*Staphylea trifolia*
*Toxicodendron radicans*
*Trillium sp.*
*Ulmus rubra*
*Urtica dioica*
*Viola canadensis*
*Vitus sp.*

EDDYVILLE, KT (EDV)

*Acer saccharum*
*Actaea pachypoda*
*Aesculus glabra*
*Arisaema triphyllum*
*Asarum canadense*
*Botrychium virginianum*
*Carya ovata*
*Caulophyllum thalictroides*
*Celtis occidentalis*
*Cimicifuga rubifolia*
*Cystopteris protrusa*
*Dioscorea villosa*
*Fagus grandifolia*
*Fraxinus americana*
*Hydrangea arborescens*
*Impatiens capensis*
*Lindera benzoin*
*Liriodendron tulipifera*
*Parthenocissus quinquefolia*
*Platanus occidentalis*
*Polemonium reptans*
*Polygonatum biflorum*
*Polystichum acrostichoides*
*Quercus montana*
*Quercus muhlenbergii*
*Quercus prinoides*
*Quercus velutina*
*Sanicula canadensis*
*Staphylea trifolia*
*Toxicodendron radicans*
*Trillium sp.*
*Ulmus rubra*
*Urtica dioica*
*Viola canadensis*
*Vitus aestivalis*

BEAR CREEK LBL, TN (LBL)

*Acer saccharum*
*Aesculus glabra*
*Aesculus flava*
*Arisaema triphyllum*
*Asarum canadense*
*Asimina triloba*
*Asplenium rhizophyllum*
*Aster divaricatus*
*Brachyelytrum erectum*
*Cacalia muhlengergii*
*Campanula americana*
*Carex oligocarpa*
*Carex platyphylla*
*Carpinus caroliniana*
*Carya cordiformis*
*Carya ovata*
*Caulophyllum thalictroides*
*Cimicifuga rubifolia*
*Celtis sp.*
*Cornus florida*
*Fagus grandifolia*
*Fraxinus americana*
*Geum canadense*
*Hydrophyllum canadense*
*Juglans nigra*
*Lindera benzoin*
*Microstegium vimineum*
*Osmorhiza claytonii*
*Pachysandra procumbens*
*Parthenocissus quinquefolia*
*Platanus occidentalis*
*Podophyllum peltatum*
*Polygonum virginianum*
*Polypodium virginianum*
*Quercus alba*
*Quercus rubra*
*Sanguinaria canadensis*
*Sassafras albidum*
*Smilax tamnoides*
*Stellaria pubera*
*Toxicodendron radicans*
*Ulmus rubra*
*Urtica dioica*
*Viola canadensis*
*Viola sp.*

LOLA, KT (LOL)

*Acer saccharum*
*Actaea pachypoda*
*Arisaema triphyllum*
*Arundinaria gigantea*
*Asarum canadense*
*Asimina triloba*
*Campanula americana*
*Carya ovata*
*Celtis occidentalis*
*Cimicifuga rubifolia*
*Dicentra sp.*
*Dioscorea villosa*
*Eupatorium rugosum*
*Geum canadense*
*Gymnocladus dioicus*
*Hybanthus concolor*
*Impatiens pallida*
*Juglans nigra*
*Ligustrum vulgare*
*Lindera benzoin*
*Menispermum canadenses*
*Parthenocissus quinquefolia*
*Phytolacca americana*
*Quercus velutina*
*Setaria faberi*
*Sorghum halepense*
*Staphylea trifolia*
*Toxicodendron radicans*
*Trillium sp.*
*Uvularia perfoliata*
*Woodsia obtusa*

LUSK CREEK, IL (LSC)

*Acer saccharum*
*Adiantum pedatum*
*Aesculus glabra*
*Aesculus flava*
*Ambrosia trifida*
*Arisaema triphyllum*
*Asarum canadense*
*Asimina triloba*
*Botrychium virginianum*
*Cacalia muhlengergii*
*Campanula americana*
*Carex sp.*
*Carpinus caroliniana*
*Celtis occidentalis*
*Cimicifuga rubifolia*
*Cornus florida*
*Cystopteris protrusa*
*Diarrhena americana*
*Festuca obtusa*
*Fraxinus americana*
*Galium asprellum*
*Geum canadense*
*Impatiens pallida*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Menispermum canadenses*
*Nyssa sylvatica*
*Parthenocissus quinquefolia*
*Phytolacca americana*
*Platanus occidentalis*
*Polemonium reptans*
*Polygonum virginianum*
*Polypodium virginianum*
*Polystichum acrostichoides*
*Prunus serotina*
*Quercus alba*
*Quercus montana*
*Quercus muhlenbergii*
*Quercus rubra*
*Quercus velutina*
*Sanguinaria canadensis*
*Staphylea trifolia*
*Stylophorum diphyllum*
*Thelypteris hexagonoptera*
*Tilia sp.*
*Toxicodendron radicans*
*Tradescantia subaspera*
*Trillium sp.*
*Ulmus rubra*
*Urtica dioica*
*Viola canadensis*
*Viola sororia*

## CLINCH MOUNTAIN POPULATIONS

### BIG WAR GAP, TN (BWG)

*Acer saccharum*
*Aesculus flava*
*Asimina triloba*
*Astilbe biternata*
*Botrychium virginianum*
*Cacalia muhlengergii*
*Caulophyllum thalictroides*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Collinsonia canadensis*
*Cornus florida*
*Cystopteris protrusa*
*Delphinium tricorne*
*Dicentra sp.*
*Disporum lanuginosum*
*Dryopteris marginalis*
*Dryopteris goldiana*
*Erythonium americanum*
*Fraxinus americana*
*Galium triflorum*
*Geranium maculatum*
*Hydrophyllum canadense*
*Impatiens pallida*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Magnolia acuminata*
*Osmorhiza claytonii*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Phlox sp.*
*Platanus occidentalis*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polystichum acrostichoides*
*Quercus rubra*
*Sanguinaria canadensis*
*Sassafras albidum*
*Sedum ternatum*
*Thalictrum thalictroides*
*Tilia heterophylla*
*Toxicodendron radicans*
*Trillium grandiflorum*
*Ulmus rubra*
*Uvularia perfoliata*
*Viburnum acerifolium*
*Viola sororia*

### LITTLE WAR GAP, TN (LWG)

*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Arisaema triphyllum*
*Asarum canadense*
*Aster divaricatus*
*Astilbe biternata*
*Athyrium asplenioides*
*Campanula americana*
*Caulophyllum thalictroides*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cornus florida*
*Cystopteris protrusa*
*Dentaria diphylla*
*Disporum lanuginosum*
*Dryopteris marginalis*
*Dryopteris goldiana*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Magnolia acuminata*
*Osmorhiza claytonii*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Phlox divaricata*
*Pilea pumila*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polymnia canadensis*
*Polystichum acrostichoides*
*Quercus rubra*
*Sanguinaria canadensis*
*Sanicula gregaria*
*Scrophularia marlandica*
*Sedum ternatum*

LITTLE WAR GAP, TN (cont.)

*Erythonium americanum*
*Eupatorium rugosum*
*Fagus grandifolia*
*Geranium maculatum*
*Heuchera villosa*
*Homalosorus pycnocarpos*
*Hybanthus concolor*
*Hydrangea arborescens*
*Hydrophyllum virginianum*
*Impatiens pallida*
*Smilacina racemosa*
*Stellaria pubera*
*Tilia heterophylla*
*Toxicodendron radicans*
*Trillium erectum*
*Trillium grandiflorum*
*Urtica dioica*
*Viola canadensis*
*Viola sororia*

HOLSTON RIVER POPULATIONS

MILL SPRINGS ROAD, TN (MSR)

*Acer negundo*
*Acer rubrum*
*Acer saccharum*
*Adiantum pedatum*
*Arisaema triphyllum*
*Asarum canadense*
*Asimina triloba*
*Asplenium rhizophyllum*
*Aster divaricatus*
*Astilbe biternata*
*Carpinus caroliniana*
*Carya tomentosa*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Conopholis americana*
*Cornus florida*
*Cystopteris bulbifera*
*Dentaria diphylla*
*Dioscorea villosa*
*Dryopteris marginalis*
*Fagus grandifolia*
*Fraxinus americana*
*Galium triflorum*
*Hamamelis virginiana*
*Heuchera villosa*
*Hexastylis arifolia*
*Hydrangea arborescens*
*Impatiens pallida*
*Lindera benzoin*
*Liriodendron tulipifera*
*Lonicera japonica*
*Magnolia acuminata*
*Osmorhiza claytonii*
*Parthenocissus quinquefolia*
*Phlox sp.*
*Platanus occidentalis*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polygonum virginianum*
*Polymnia canadensis*
*Polystichum acrostichoides*
*Quercus muhlenbergii*
*Quercus rubra*
*Robinia pseudoacacia*
*Sanguinaria canadensis*
*Sassafras albidum*
*Sedum ternatum*
*Smilacina racemosa*
*Staphylea trifolia*
*Thalictrum dioicum*
*Thalictrum thalictroides*
*Thaspium barbinode*
*Tiarella cordifolia*
*Tilia heterophylla*
*Toxicodendron radicans*
*Ulmus rubra*
*Viburnum prunifolium*

KINGSPORT QUARRY, TN (KPQ)

*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Amphicarpaea bracteata*
*Arisaema triphyllum*
*Aristolochia macrophylla*
*Asarum canadense*
*Asimina triloba*
*Astilbe biternata*
*Carpinus caroliniana*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Dryopteris marginalis*
*Fraxinus americana*
*Galium triflorum*
*Heuchera villosa*
*Hydrangea arborescens*
*Impatiens pallida*
*Lindera benzoin*
*Lonicera dioica*
*Parthenocissus quinquefolia*
*Platanus occidentalis*
*Polygonatum biflorum*
*Polymnia canadensis*
*Quercus muhlenbergii*
*Sedum ternatum*
*Solidago flexicaulis*
*Staphylea trifolia*
*Thuja occidentalis*
*Tilia heterophylla*
*Toxicodendron radicans*
*Tsuga canadensis*

CHRISTIAN BEND, TN (CHB)

*Acer negundo*
*Acer saccharinum*
*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Alliaria petiolata*
*Anemone virginiana*
*Arisaema triphyllum*
*Asarum canadense*
*Asimina triloba*
*Asplenium platyneuron*
*Astilbe biternata*
*Bignonia capreolata*
*Botrychium virginianum*
*Caulophyllum thalictroides*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cornus florida*
*Cystopteris bulbifera*
*Dentaria diphylla*
*Dicentra sp.*
*Dioscorea villosa*
*Diphylleia cymosa*
*Dryopteris marginalis*
*Elymus villosus*
*Fagus grandifolia*
*Fraxinus americana*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Lonicera japonica*
*Magnolia macrophylla*
*Morus rubra*
*Osmorhiza claytonii*
*Panicum sp.*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Platanus occidentalis*
*Podophyllum peltatum*
*Polygonatum pubescens*
*Polymnia canadensis*
*Polystichum acrostichoides*
*Quercus coccinea*
*Quercus muhlenbergii*
*Quercus rubra*
*Quercus velutina*
*Robinia pseudoacacia*
*Sanguinaria canadensis*
*Smilacina racemosa*
*Solidago flexicaulis*
*Staphylea trifolia*
*Stellaria pubera*
*Stylophorum diphyllum*
*Tilia heterophylla*
*Toxicodendron radicans*

CHRISTIAN BEND, TN (cont.)

*Hamamelis virginiana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hybanthus concolor*
*Hydrangea arborescens*
*Hydrophyllum canadense*
*Impatiens pallida*
*Jeffersonia diphylla*
*Trillium grandiflorum*
*Ulmus rubra*
*Urtica dioica*
*Uvularia perfoliata*
*Viola canadensis*
*Viola sp.*
*Vitus sp.*

CLINCH RIVER POPULATIONS

BULL BLUFF, TN (BLB)

*Acer saccharum*
*Aesculus flava*
*Arisaema triphyllum*
*Asarum canadense*
*Asimina triloba*
*Asplenium platyneuron*
*Asplenium rhizophyllum*
*Aster divaricatus*
*Astilbe biternata*
*Bignonia capreolata*
*Carpinus caroliniana*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cystopteris bulbifera*
*Dentaria diphylla*
*Dioscorea villosa*
*Dryopteris marginalis*
*Erythonium americanum*
*Fagus grandifolia*
*Fraxinus quadrangulata*
*Hepatica acutiloba*
*Heuchera villosa*
*Jeffersonia diphylla*
*Juglans nigra*
*Lindera benzoin*
*Magnolia acuminata*
*Magnolia macrophylla*
*Panax quinquefolius*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Polygonatum biflorum*
*Polymnia canadensis*
*Polypodium polypodioides*
*Quercus muhlenbergii*
*Quercus rubra*
*Sanicula canadensis*
*Sanicula gregaria*
*Sedum ternatum*
*Smilacina racemosa*
*Solidago flexicaulis*
*Staphylea trifolia*
*Thalictrum thalictroides*
*Tiarella cordifolia*
*Tilia heterophylla*
*Tipularia discolor*
*Toxicodendron radicans*
*Trillium erectum*

GRASSY CREEK, TN (GRC)

*Acer negundo*
*Acer saccharum*
*Actaea pachypoda*
*Aesculus flava*
*Arisaema dracontium*
*Arisaema triphyllum*
*Asarum canadense*
*Asplenium platyneuron*
*Aster divaricatus*
*Botrychium virginianum*
*Carex platyphylla*
*Carpinus caroliniana*
*Caulophyllum thalictroides*
*Celtis laevigata*
*Cercis canadensis*
*Chelone lyonii*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Claytonia virginica*
*Cornus florida*
*Cystopteris bulbifera*
*Delphinium tricorne*
*Dicentra cucullaria*
*Dryopteris marginalis*
*Erythonium americanum*
*Eupatorium rugosum*
*Fagus grandifolia*
*Geum canadense*
*Heliopsis helianthoides*
*Hepatica acutiloba*
*Hepatica americana*
*Heuchera villosa*
*Hydrophyllum canadense*
*Impatiens capensis*
*Juglans nigra*
*Juniperus virginiana*
*Lindera benzoin*
*Liriodendron tulipifera*
*Magnolia acuminata*
*Magnolia macrophylla*
*Mitella diphylla*
*Panax quinquefolius*
*Parthenocissus quinquefolia*
*Phlox divaricata*
*Phryma leptostachya*
*Pilea pumila*
*Podophyllum peltatum*
*Polemonium reptans*
*Polystichum acrostichoides*
*Quercus muhlenbergii*
*Sanguinaria canadensis*
*Sanicula trifoliata*
*Sedum ternatum*
*Solidago flexicaulis*
*Stellaria pubera*
*Thalictrum thalictroides*
*Tiarella cordifolia*
*Tilia heterophylla*
*Toxicodendron radicans*
*Trillium erectum*
*Trillium luteum*
*Urtica dioica*
*Viola canadensis*
*Viola sororia*
*Vitus aestivalis*

NORRIS RIVER BLUFFS, TN (NRB)

*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Arisaema triphyllum*
*Asarum canadense*
*Asplenium rhizophyllum*
*Astilbe biternata*
*Carpinus caroliniana*
*Carya cordiformis*
*Caulophyllum thalictroides*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Claytonia virginica*
*Meehania cordata*
*Mitella diphylla*
*Monarda clinopodia*
*Osmorhiza claytonii*
*Ostrya virginiana*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Phlox divaricata*
*Polygonatum biflorum*
*Polymnia canadensis*
*Polypodium virginianum*
*Polystichum acrostichoides*
*Quercus muhlenbergii*

NORRIS RIVER BLUFFS, TN (cont.)

*Cornus alternifolia*
*Cystopteris bulbifera*
*Dentaria diphylla*
*Desmodium glutinosum*
*Dryopteris marginalis*
*Equisetum hyemale*
*Erythonium americanum*
*Fagus grandifolia*
*Fraxinus americana*
*Fraxinus quadrangulata*
*Galium triflorum*
*Hamamelis virginiana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hydrangea arborescens*
*Hydrophyllum canadense*
*Impatiens pallida*
*Liriodendron tulipifera*
*Magnolia acuminata*
*Ribes cynosbati*
*Sanguinaria canadensis*
*Sanicula trifoliata*
*Sedum ternatum*
*Smilacina racemosa*
*Stellaria pubera*
*Stylophorum diphyllum*
*Taraxacum officinale*
*Thalictrum thalictroides*
*Tiarella cordifolia*
*Tilia heterophylla*
*Toxicodendron radicans*
*Trillium erectum*
*Trillium luteum*
*Viburnum acerifolium*
*Viburnum rufidulum*
*Viola sororia*
*Vitus cinerea var. bailyana*

STOWE BLUFF, TN (STB)

*Acer negundo*
*Acer rubrum*
*Acer saccharum*
*Actaea pachypoda*
*Adiantum pedatum*
*Aesculus flava*
*Arisaema triphyllum*
*Asimina triloba*
*Asplenium platyneuron*
*Asplenium rhizophyllum*
*Aster cordifolius*
*Aster divaricatus*
*Bignonia capreolata*
*Botrychium virginianum*
*Campanula americana*
*Celtis occidentalis*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cornus florida*
*Cystopteris bulbifera*
*Cystopteris protrusa*
*Diervilla sessilifolia v. sessilifolia*
*Dioscorea villosa*
*Fagus grandifolia*
*Fraxinus americana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hydrangea arborescens*
*Liriodendron tulipifera*
*Lonicera japonica*
*Ostrya virginiana*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Polygonatum biflorum*
*Polygonum virginianum*
*Polymnia canadensis*
*Prunus americana*
*Quercus muhlenbergii*
*Quercus palustris*
*Sassafras albidum*
*Saxifraga caroliniana*
*Sedum ternatum*
*Senecio obovatus*
*Smilacina racemosa*
*Solidago flexicaulis*
*Staphylea trifolia*
*Thaspium barbinode*
*Tilia heterophylla*
*Toxicodendron radicans*
*Ulmus rubra*
*Viburnum acerifolium*
*Viola canadensis*
*Vitus vulpina*

PAWPAW GROVE, TN (PPG)

*Acer negundo*
*Acer nigrum*
*Acer rubrum*
*Acer saccharum*
*Aesculus flava*
*Ailanthus altissima*
*Aquilegia canadensis*
*Arisaema triphyllum*
*Aristolochia macrophylla*
*Asarum canadense*
*Asimina triloba*
*Asplenium platyneuron*
*Asplenium resiliens*
*Asplenium rhizophyllum*
*Bignonia capreolata*
*Campanula americana*
*Carpinus caroliniana*
*Carya glabra var. glabra*
*Carya sp.*
*Caulophyllum thalictroides*
*Celtis occidentalis*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cornus alternifolia*
*Cornus florida*
*Cystopteris bulbifera*
*Dentaria diphylla*
*Dioscorea villosa*
*Diphylleia cymosa*
*Dryopteris marginalis*
*Fraxinus americana*
*Fraxinus quadrangulata*
*Galium triflorum*
*Geum canadense*
*Hamamelis virginiana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hexastylis arifolia*
*Hydrangea arborescens*
*Hydrophyllum virginianum*
*Hystrix patula*
*Impatiens capensis*
*Impatiens pallida*
*Jeffersonia diphylla*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Magnolia acuminata*
*Mitella diphylla*
*Morus rubra*
*Osmorhiza claytonii*
*Ostrya virginiana*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Phlox divaricata*
*Platanus occidentalis*
*Poa sylvestris*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polymnia canadensis*
*Polystichum acrostichoides*
*Quercus muhlenbergii*
*Ribes sp.*
*Sanguinaria canadensis*
*Sedum ternatum*
*Smilacina racemosa*
*Smilax walteri*
*Staphylea trifolia*
*Thaspium barbinode*
*Tilia heterophylla*
*Toxicodendron radicans*
*Ulmus rubra*
*Urtica dioica*
*Uvularia perfoliata*
*Viburnum sp.*
*Vitus sp.*

VIRGINIA (VIR)

*Acer nigrum*
*Acer saccharum*
*Aesculus flava*
*Arisaema triphyllum*
*Aristolochia macrophylla*
*Aruncus dioicus*
*Asarum canadense*
*Asplenium rhizophyllum*
*Astilbe biternata*
*Bignonia capreolata*
*Botrychium virginianum*
*Caulophyllum thalictroides*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cornus alternifolia*
*Cornus florida*
*Cystopteris bulbifera*
*Delphinium tricorne*
*Dentaria diphylla*
*Dicentra sp.*
*Disporum lanuginosum*
*Dryopteris marginalis*
*Erythonium americanum*
*Fagus grandifolia*
*Fraxinus americana*
*Geranium maculatum*
*Hamamelis virginiana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hydrangea arborescens*
*Impatiens pallida*
*Jeffersonia diphylla*
*Lindera benzoin*
*Magnolia acuminata*
*Mitella diphylla*
*Osmorhiza claytonii*
*Ostrya virginiana*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Phlox divaricata*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polystichum acrostichoides*
*Quercus muhlenbergii*
*Quercus velutina*
*Sanguinaria canadensis*
*Sedum ternatum*
*Senecio obovatus*
*Smilacina racemosa*
*Smilax tamnoides*
*Staphylea trifolia*
*Stellaria pubera*
*Stylophorum diphyllum*
*Thalictrum thalictroides*
*Tiarella cordifolia*
*Tilia heterophylla*
*Toxicodendron radicans*
*Trillium erectum*
*Trillium grandiflorum*
*Ulmus rubra*
*Urtica dioica*
*Uvularia grandiflora*
*Viola canadensis*
*Vitus cinerea var. bailyana*

TENNESSEE RIVER POPULATIONS

GEORGES CREEK, TN (GEO)

*Acer saccharum*
*Aesculus flava*
*Asimina triloba*
*Bignonia capreolata*
*Carya ovata*
*Cimicifuga rubifolia*
*Cystopteris bulbifera*
*Dioscorea villosa*
*Dryopteris marginalis*
*Fraxinus americana*
*Juglans nigra*
*Parthenocissus quinquefolia*
*Polygonatum biflorum*
*Quercus muhlenbergii*
*Quercus velutina*
*Sanguinaria canadensis*
*Smilacina racemosa*
*Smilax tamnoides*
*Tilia heterophylla*
*Toxicodendron radicans*

EAVES FERRY, TN (EVF)

*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Anemone sp.*
*Arisaema triphyllum*
*Asimina triloba*
*Asplenium rhizophyllum*
*Aster divaricatus*
*Bignonia capreolata*
*Carpinus caroliniana*
*Carya glabra var. glabra*
*Carya ovata*
*Caulophyllum thalictroides*
*Celtis occidentalis*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Collinsonia verticillata*
*Conopholis americana*
*Cornus florida*
*Cystopteris protrusa*
*Dioscorea villosa*
*Dryopteris marginalis*
*Erythonium americanum*
*Fagus grandifolia*
*Fraxinus americana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hydrangea arborescens*
*Lindera benzoin*
*Liriodendron tulipifera*
*Lonicera japonica*
*Ostrya virginiana*
*Panax quinquefolius*
*Parthenocissus quinquefolia*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polypodium virginianum*
*Polystichum acrostichoides*
*Quercus muhlenbergii*
*Quercus rubra*
*Sassafras albidum*
*Sedum ternatum*
*Smilacina racemosa*
*Tiarella cordifolia*
*Tilia heterophylla*
*Toxicodendron radicans*
*Trillium luteum*
*Ulmus rubra*
*Vitus sp.*

POWELL RIVER POPULATIONS

WALLENS RIDGE, TN (WRD)

*Acer negundo*
*Acer nigrum*
*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Aristolochia macrophylla*
*Arundinaria gigantea*
*Asarum canadense*
*Aster cordifolius*
*Aster divaricatus*
*Astilbe biternata*
*Bromus racemosus*
*Campanula americana*
*Campsis radicans*
*Carpinus caroliniana*
*Carya ovata*
*Impatiens pallida*
*Jeffersonia diphylla*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Magnolia acuminata*
*Magnolia tripetala*
*Mitella diphylla*
*Parthenocissus quinquefolia*
*Phacelia bipinnatifida*
*Phlox divaricata*
*Platanus occidentalis*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Prunus sp.*
*Pueraria lobata*

WALLENS RIDGE, TN (cont.)

*Celastres scandens*
*Cercis canadensis*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Collinsonia canadensis*
*Cystopteris protrusa*
*Delphinium tricorne*
*Fagus grandifolia*
*Fraxinus americana*
*Galium triflorum*
*Glecoma hederacea*
*Heliopsis helianthoides*
*Hepatica acutiloba*
*Hydrangea arborescens*
*Hydrophyllum virginianum*
*Impatiens capensis*
*Quercus muhlenbergii*
*Quercus velutina*
*Sanguinaria canadensis*
*Sanicula smallii*
*Sedum ternatum*
*Senecio obovatus*
*Smilax glauca*
*Solidago flexicaulis*
*Taraxacum officinale*
*Thalictrum thalictroides*
*Tilia heterophylla*
*Toxicodendron radicans*
*Ulmus rubra*
*Urtica dioica*
*Uvularia perfoliata*

POWELL RIVER BRIDGE, TN (PRB)

*Acer negundo*
*Acer saccharum*
*Adiantum pedatum*
*Aesculus flava*
*Anemone virginiana*
*Aquilegia canadensis*
*Arisaema triphyllum*
*Aristolochia macrophylla*
*Aruncus dioicus*
*Asarum canadense*
*Asimina triloba*
*Asplenium rhizophyllum*
*Aster sp.*
*Astilbe biternata*
*Bignonia capreolata*
*Brachyelytrum erectum*
*Carex pedunculata?*
*Carpinus caroliniana*
*Carya ovata*
*Caulophyllum thalictroides*
*Cercis canadensis*
*Chamaelirium luteum*
*Cimicifuga racemosa*
*Cimicifuga rubifolia*
*Cornus florida*
*Cystopteris bulbifera*
*Dentaria diphylla*
*Dioscorea villosa*
*Disporum lanuginosum*
*Hydrophyllum virginianum*
*Impatiens pallida*
*Jeffersonia diphylla*
*Juglans nigra*
*Lindera benzoin*
*Liriodendron tulipifera*
*Magnolia macrophylla*
*Mitella diphylla*
*Monarda clinopodia*
*Osmorhiza claytonii*
*Parthenocissus quinquefolia*
*Phlox divaricata*
*Podophyllum peltatum*
*Polygonatum biflorum*
*Polystichum acrostichoides*
*Pyrularia pubera*
*Quercus muhlenbergii*
*Quercus velutina*
*Rhododendron maximum*
*Sanguinaria canadensis*
*Sanicula gregaria*
*Sedum ternatum*
*Senecio obovatus*
*Smilacina racemosa*
*Solidago flexicaulis*
*Staphylea trifolia*
*Stellaria pubera*
*Thalictrum dioicum*
*Thalictrum thalictroides*

POWELL RIVER BRIDGE, TN (cont.)

*Dodecatheon meadia*
*Dryopteris marginalis*
*Erythonium americanum*
*Fagus grandifolia*
*Fraxinus americana*
*Geranium maculatum*
*Hamamelis virginiana*
*Hepatica acutiloba*
*Heuchera villosa*
*Hydrangea arborescens*
*Hydrophyllum canadense*
*Thaspium trifoliatum var. trifoliatum*
*Tiarella cordifolia*
*Tilia americana*
*Toxicodendron radicans*
*Trillium erectum*
*Trillium grandiflorum*
*Uvularia grandiflora*
*Uvularia perfoliata*
*Viburnum rufidulum*
*Viola canadensis*
*Waldsteinia fragarioides*

# VITA

Rebecca Ann Cook was born in Pocahontas, AR September 21, 1958. She attended Alma Spike Elementary School in that town and graduated from Pocahontas High School in 1976. In the fall of that year she entered Hendrix College in Conway, AR and received a Bachelor of Arts degree with distinction in biology in 1980. After working as a seed analyst for the Arkansas State Plant Board for a year she began study for a Master's degree in Environmental Science at Rice University in Houston, TX. After completion of the requirements for the degree in 1983, she was awarded the degree in 1984. Between 1983 and 1986 she did analytical work for CORE, Inc, in Tulsa Ok and worked as an intern in the Sunship Earth Program at Camp Wyman in Eureka, Mo. In the fall of 1986 she began a graduate program at the University of Tennessee, Knoxville. From 1986 to 1991 she served as a Graduate Teaching Assistant for the Botany Department. She was an adjunct instructor in general biology at Pellissippi State Technical Community College from 1991 to 1992. In the fall of 1992 she accepted a position as general biology instructor at the University of Tennessee, Knoxville. In addition, she has worked as a rare plant and wetlands consultant for Oak Ridge National Laboratory since 1991. She was awarded the degree of Doctor of Philosophy with a major in Botany in August of 1993.