A biosystematic study of the rare plant *Paronychia virginica* Spreng. (Caryophyllaceae) employing morphometric and allozyme analyses

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(ABSTRACT)

Paronychia virginica Spreng. (Caryophyllaceae) is a perennial evergreen herb of exposed, relatively xeric habitats. Approximately 10 mid-Appalachian populations remain in Virginia, West Virginia, and Maryland and are disjunct from populations located primarily in Texas, Oklahoma, and Arkansas. A study was conducted to test the hypothesis that eastern and western populations differ significantly and, therefore, represent at least two distinct taxa. Statistical analyses of 8 qualitative and 24 quantitative morphological characters indicated very highly significant ($P \leq 0.001$) variation between eastern and western populations of *P. virginica.* Characters differing most significantly included sepal pubescence, awn length, awn pubescence, awn curvature, length-width ratio of leaves, and shape of leaf apices. Starch gel electrophoresis was performed and six enzyme systems/nine loci (EST-2, EST-3, LAP, MDH-1, MDH-2, PGI, PGM-1, PGM-2, and SKDH) were identified as being consistently scorable and informative. Although gene flow between populations of *P. virginica* was shown to be restricted (mean $F_{ST} = 0.353$), populations are maintaining relatively high levels of genetic diversity. Genetic variability was quantified for each population and mean values for number of alleles per locus (A), percent loci polymorphic (P), and expected heterozygosity (H_{EXP}) were found to be 1.95, 47.22%, and 0.204, respectively, exceeding those values reported for seed plants, widespread species, and endemic species. Hierarchical F statistics suggest higher levels of genetic variability within individual populations than among populations, regardless of geographic location. All statistically significant ($P \leq 0.05$) deviations from Hardy-Weinberg equilibrium indicated a deficiency in heterozygotes at the respective loci. Considering results from both the morphometric and allozyme analyses, the current author suggests recognizing two distinct subspecies, *P. virginica* subsp. *virginica* in the eastern U.S. and *P. virginica* subsp. *scoparia* in the south-central U.S. Conservation efforts should be focused on the maintenance of existing populations in both eastern and western regions of the U.S. in order to preserve the genetic and evolutionary potential of these taxa.

Dedicated to Mr. Michael McDonough,
who taught me to notice and to question the obvious.

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Table of Contents

List of Figures

List of Tables

Introduction

At the 1981 Botanical Congress in Sydney, Australia, Peter Raven indicated that as much as 25% of living organisms on earth may become extinct by the beginning of the twenty-first century (Bennett, 1984). The diversity of plant life in the Commonwealth of Virginia is not immune to such threats of significant reduction. Porter (1979) included approximately 350 species in a list of rare and endangered vascular plants of Virginia. By 1991, the number had increased to some 600, including those species suspected to be rare or endangered and being monitored at that time by the Virginia Division of Natural Heritage (Porter and Wieboldt, 1991).

There are a variety of possible reasons for such a sharp increase in the reported numbers of rare vascular plants in Virginia and other geographic and political regions. One explanation may be that government agencies have established more rigorous methods of surveying plant populations. Consistent surveying of landscapes may result in an increased number of citations of rare species. If this were the only reason the reported number has increased, we would surely have reason to celebrate the increased efforts of botanists rather than to mourn the possible loss of more plant species. Unfortunately, the number of rare, threatened, and endangered species has probably increased more because of habitat destruction than because of more numerous botanical forays. The opening of mines and quarries, the presence of hikers clambering around on rocks or hillsides, and even collection by over-zealous botanists have created circumstances that are detrimental to many plant species (Porter and Wieboldt, 1991).

Paronychia virginica Spreng. is an example of a rare and fascinating member of the central Appalachian flora. The following paper discusses *P. virginica,* focusing primarily on morphometric and allozyme variation within and among populations of the species over its entire geographic range. The introduction will discuss the higher taxa to which *P. virginica* belongs as well as objectives and hypotheses of the present study. A description of the materials and methods used to investigate the taxon will be followed by a report of results and a detailed discussion of the significance and implications of this study to the taxonomy and conservation of *P. virginica.*

Description of higher taxa

Paronychia virginica belongs to the family Caryophyllaceae. This diverse family consists of two to several subfamilies (Thomson, 1942; Tutin *et al.*, 1964; Shaw, 1980) or four tribes (Fernald, 1950; a treatment of the family as it occurs in the northeastern United States), depending on the classification scheme used by the author. Caryophyllaceae is known primarily for its weedy (*Silene, Cerastium*, and *Stellaria*) and ornamental (*Dianthus, Gypsohila,* and *Arenaria*) representatives. Also referred to as the pink family because of the "pinked" (notched) appearance of the petals of various members, Caryophyllaceae is represented by approximately 70 genera and 1,750 species (Zomlefer, 1994).

The subfamily Paronychioideae has been separated from the Caryophyllaceae by a number of authors and raised to familial rank and referred to as the Illecebraceae (Thomson, 1942). Neither chromosome number nor pollen morphology follow the subfamily lines clearly, and anatomical similarities between Paronychioideae and other

subfamilies seem to support retention of one taxonomic family (Thomson, 1942). Indeed, an examination of more recent classification schemes of angiosperm taxa reveals the inclusion of members of the so-called Illecebraceae within the family Caryophyllaceae (Takhtajan, 1980; Thorne, 1983; Cronquist, 1988).

The Caryophyllaceae were placed close to families of the order Centrospermae (more recently referred to as Caryophyllales) by Bentham and Hooker (1862) and have been accepted in that position by most authors since that time. Although members of Caryophyllaceae possess anthocyanins rather than betalains (a floral pigment common to 9 of the families within Caryphyllales), morphological and anatomical features (particularly P-type sieve-tube plastids) support the notion of a common evolutionary history between this family and other members of Caryophylles (Chang and Mabry, 1973; Rodman *et al.*, 1984).

Numerous studies have been conducted to examine the relationships among members of the order Centrospermae. Chang and Mabry (1973) suggest that the eleven families of Caryophyllales (Centrospermae) were derived from a common ancestor that diverged into two evolutionary lines prior to the origin of the present floral pigments. Rodman *et al.* (1984) dispute this notion suggesting instead that members of Caryophyllaceae merely retained the ancestral condition (anthocyanin production) while other members of the order made use of another biochemical pathway and thus of another biochemical (betalain). Both anthocyanins, flavonoid pigments formed by the condensation of 3 malonyl-CoA units with *p*-courmaryl-CoA, and betalains, products of the condensation of dopa (3,4-dihydroxyphenylalanine) with a second amino acid, occur

in flowers and fruits providing color to attract potential dispersers of pollen and seeds. Members of the Caryophyllaceae are generally considered to be derived from the Phytolaccaeae that represent a transitory group between the apetalous and polypetalous families of the Centrospermae (Thomson, 1942). However, Downie and Palmer (1994) report that forcing Caryophyllaceae and Molluginaceae, the only other anthocyanin-producing family in the order, together at the base of the Caryophyllales involved adding five steps to the most parsimonious cladograms produced in their study. Instead, Downie and Palmer (1994) indicate that, through analyses of a highly conserved inverted repeat region of the chloroplast genome in conjunction with cladistic methods, the Caryophyllaceae nest with other families of the order in concensus trees. Caryophyllaceae seems to belong in the order Caryophyllales (Centrospermae), although this will probably remain a matter of debate among plant systematists.

Morphological diversity within the Caryophyllaceae has made the delimitation of genera somewhat ambiguous (Zomlefer, 1994). Although phenotypically diverse, members of Caryophyllaceae are generally herbaceous in habit and either annual or perennial. Stems branch dichotomously and typically have swollen nodes at the point of attachment of simple, opposite, and narrow leaves. Stipules are generally absent; however, the genus *Paronychia* is noted for the presence of papery white stipules. Gleason and Cronquist (1991) support this observation by making note of the presence of hyaline or scarious stipules among representatives of the subfamily Paronychioideae.

Within the Caryophyllaceae, floral morphology varies widely and may differ even within the same species. Inflorescences are generally cymose, although flowers may be

solitary. Shaw (1980) describes the inflorescence as “caryophyllaceous,” noting that it is a unique type of helicoid cyme. Gleason and Cronquist (1991) describe the cymes as being “dichasial,” that is, each axis produces two opposite or subopposite lateral axes. Flowers may have a corolla of notched petals or the petals may be extremely reduced or absent. Sepals are generally present and may be petal-like in appearance. The androecium consists of 5 or 10 or occasionally 1 to 4 stamens with distinct filaments that are occasionally adnate to the petals or sepals. Anthers are basifixed and dehisce longitudinally. Nectaries can often be found at the bases of the filaments. The gynoecium consists of a 2- to 5-carpellate 1-locular ovary, though this may vary, with free-central placentation. Styles may number 2 to 5, as is true with stigmas. Fruit type among members of the Caryophyllaceae is generally a capsule or utricle bearing seeds that may have an ornamented seed coat. An anatomical feature that is characteristic of representatives of the family is the presence of P-type plastids.

The reproductive biology of many members of Caryophyllaceae is understood. Bees, flies, and butterflies are attracted to nectar produced at the bases of the stamens. Shaw (1980) discusses several adaptations of various members of the family to pollination by bees and Lepidopterans. Such adaptations include elongated floral tubes, red and white coloration of petals and sepals, the occurrence of night flowering, and the emission of sweet scents only after dark. Flowers are commonly protandrous, with the androecium maturing prior to the gynoecium; however, self-pollination occurs in several species (Zomlefer, 1994). Dispersal of seeds is facilitated by either wind or animals,

which act to shake seeds loose from the capsule or utricle. Seeds tend to be light and readily dispersed after being knocked free of the fruit (Shaw, 1980).

Caryophyllaceae is a relatively cosmopolitan family. Although representatives occur primarily in north temperate regions, a few species occur in south temperate areas, montane tropics, and the Arctic. Zomlefer (1994) notes that the Mediterranean region hosts a particularly diverse assemblage of members of Caryophyllaceae.

Paronychia virginica belongs to the subfamily Paronychioideae, tribe Paronychieae, subtribe Paronychiinae, genus *Paronychia,* subgenus *Paronychia,* section *Paronychia,* and subsection *Chartaceifoliae*. M.N. Chaudhri (1968) provides a comprehensive discussion and delineation of representatives of Paronychiinae. In brief, taxa included in Paronychiinae are distinguished from other members of the Caryophyllaceae by possessing stipulate leaves and perigynous flowers. The genus *Paronychia* (Tournef.) Miller, Gardn. Dict., abridged ed. 4, vol. 3 (1754) includes 109 species divided into three subgenera (*Paronychia, Siphonicia,* and *Anoplonychia*) and is distributed worldwide (Core, 1941; Chaudhri, 1968). Although most abundant in North America, the Mediterranean region, and the South American countries of Argentina and Chile, *Paronychia* may also occur in Europe, Asia, Africa, and has been found most recently in Mexico (Chaudhri 1968; Turner, 1995 a,b). Rare in the tropics, the genus flourishes primarily in temperate and warm regions. Of the 21 North American species of *Paronychia,* 10 are perennial and 11 are annual.

The reproductive biology of this entire subtribe is not fully understood; however, Chaudhri (1968) suggests that the species are, in general, self-pollinated. He also notes

the prevalence of protandrous flowers in which stamens cling to the stigma(s) when the flower opens and the style extends upward. There are a few examples of pollination by ants in *Herniaria.* Dispersal of fruits is generally by wind, often carrying entire inflorescences away from the plant, scattering seeds as they travel. The current author observed a similar phenomenon at the Potts Creek, Virginia, collection site in late November 1996. Several *P. virginica* plants were seen with complete, dried cymes broken off and lying at the bases of the plants. The large percentage of endemism within the genus *Paronychia* (90%; Chaudhri, 1968) may be caused, in part, by the lack of long-distance dispersal (i.e., several hundred meters or kilometers per dispersal event) in the genus. Few, if any, studies have been performed to study the reproductive and pollination biology and mechanisms for dispersal used by members of *Paronychia.* The only reference made to any sort of greenhouse or garden study performed on *P. virginica* found by the author was a single statement made by Chaudhri (1968) regarding habit variation observed while "growing the plants from seed in a garden." Chaudhri did not elaborate.

Paronychia is the largest of the three subgenera within the genus. This subgenus includes 57 species and is subsequently divided into two sections determined by the presence (sect. *Paronychia*) or absence (sect. *Nyachia*) of an apical cusp or awn on the abaxial surfaces of the hooded sepals. Section *Paronychia* consists of five subsections (*Fasciculatae, Polygonoideae, Anychia, Chartaceifoliae*, and *Paronychia*), of which *Paronychia virginica* belongs to *Chartaceifoliae*. Subsections are characterized by the presence of a short versus long and distinct awn at the apices of the sepals, axillary

fascicled leaves rather than leaf pairs being separated by distinctly discernible internodes, flowers occurring in lateral pseudo-axillary clusters or in lax terminal cymes, purplish plant color or no purplish plant color, and the presence or absence of linear and subchartaceous (slightly papery in texture and achlorophyllous) leaves. Members of subsection *Chartaceifoliae* are characterized by linear and subchartaceous or somewhat rigid leaves. Representatives of *Paronychia* possess flowers that occur in terminal repeatedly-forked, lax, or compact cymes and are subtended by foliar bracts or leaves, much reduced in size. The perigynous zone (hypanthium) is generally well developed. Sepals include long awns that are setaceous (bristle-like) or rigid. The reader is again referred to Chaudhri (1968) for a full treatise of the subtribe Paronychiinae and its subsequent divisions.

A review of morphological features shared by members of the genus *Paronychia* is appropriate because of the morphometric portion of the present study. Chaudhri's (1968) description of the general morphology of *Paronychia* is particularly informative and will be summarized in the following paragraphs. The plants belonging to this genus are most often small, low herbs that form compact mats. Although generally perennial, as in the case of *P. virginica*, there are annual species as well. The perennial species have a long, sparsely-branched taproot, while annual species generally have a long, slender tap root with numerous, short, lateral rootlets.

Vegetative morphology differs between perennial and annual species. Stems in the perennial species rise from a woody caudex and are usually branched from the base. Depending on the species, the plant's growth habit is either upright or prostrate with the

flowering cymes generally always erect and ascending from the vegetative structure. Annual species have no woody tissue and are rather delicate-looking, erect or sub-erect plants. Although Chaudhri (1968) reports that *P. virginica* is one of the tallest species, reaching a height of 50 centimeters, plants observed by the current author were never over 20 centimeters in height. Leaves are opposite, generally sessile in perennial species, small and narrow, and elliptical-oblong to obovate-oblanceolate in shape. The midrib is usually quite distinct, with lateral veins extending out to form a reticulate or netted pattern of venation. Perhaps one of the more diagnostic characters of *Paronychia* is the presence of silvery-scarious stipules (resembling tiny slivers of tissue paper) occurring in pairs at the base of each pair of leaves. The stipules may be deeply split lengthwise, often appearing as two individual structures.

Floral characteristics resemble those of other members of the Caryophyllaceae. Individual flowers are extremely inconspicuous (1.5-3 mm and rarely up to 7 mm in diameter); however, in the species that have flowers in cymes, one may observe a dense cluster of tiny flowers that appear to be yellow in color (the color coming from sepals or stamens, not petals). The flowers are arranged in helicoid-like cymes, as mentioned above in the description of the family Caryophyllaceae. The cymes of *P. virginica* are condensed into large, pedunculate, corymbose clusters terminating the more-or-less erect flowering shoots. Individual flowers are usually subtended by silvery-scarious bracts, which are often quite prominent. In some species, the bracts are actually enlarged stipules. The flowers of *Paronychia virginica* are subtended by two leaf-like or foliaceous bracts, shorter and more rigid than leaves occurring along the stem, and two

papery stipules. Chaudhri (1968) suggests that such foliaceous bracts may represent a primitive stage in the development of larger more scarious floral bracts. The sepals form the most conspicuous part of the flower, as the petals are highly reduced or absent. Occurring in fives and of equal length, the sepals are often linear-oblong to linear-lanceolate in shape and have a small hood-like structure at the apex. The abaxial apex of the hood is often prolonged into a slender awn with a whitish membranous margin. The hood-like structures, when well-developed, serve as pockets for holding the anthers (Chaudhri, 1968). *Paronychia virginica* possesses the most rigid or stout form of awn, which has a thickened base and may be glabrous or pubescent. The hoods accompanying stout, rigid awns are generally stiff and thickened, while membranous hoods accompany slender awns. The stamens are situated opposite the sepals and are usually five in number. The filaments are opposite and attached to the base of each sepal, forming a perigynous ring or hypanthium. The anthers are orange-yellow in color and usually mature earlier than the stigmas, although the anthers are often found clasping the latter, perhaps to ensure autogamy (Chaudhri, 1968). Two fused carpels comprise the pistil. The ovary, always one-loculed and one-ovuled, is often ovoid to subglobose and narrowed upwards into the style. The ovary may be entirely glabrous or slightly papillose. The style is often quite reduced and may be short and thick with free or sessile stigmas, although this varies depending on the species. The ovule is solitary and basal, erect or attached to the carpel wall by the funiculus. The fruit is an utricle, ovoid to almost globose, often enclosed by sepals. The pericarp may be membranous and smooth

or papillose and somewhat warty. The seed is ovoid-subglobose to nearly ellipsoidal or occasionally reniform in shape, with a smooth dark-brown testa.

The majority of the taxonomic studies on species of *Paronychia* has been limited to botanical surveys and morphological descriptions. Only a limited amount of information is available regarding the karyology of members of the genus. Chaudhri (1968) reports a basic number of x = 7, 8, 9 (and 6 or 12). Diploid chromosome numbers include 2n = 14, 28, 32, 36, and 42, which varied within and among the six species examined. More recent publications (Goldblatt 1981, 1984, 1988; Goldblatt and Johnson 1990, 1991,1994, 1996) report chromosome numbers as n = 9, 14, 18, and 27 while 2n = 14, 18, 28, 32, and 64. Most species for which chomosome numbers were reported occur in the Mediterranean region with no mention of *P. virginica* . With the exception of Hartman (1974), no studies have been done on the chemical and molecular composition of the members of the genus. Such studies would surely prove interesting and may help to resolve some unanswered questions within and among members of *Paronychia.* Further comments on this topic will be presented in the Discussion section of this paper.

Species of interest

Paronychia virginica (Figure 1) is a member of Virginia's native flora that is currently listed as "extremely rare" (S1) in the Commonwealth (Ludwig, 1994). Such a listing at the state level indicates that the species occurs at five or fewer localities. Commonly known as the yellow nailwort, Virginia nailwort, or whitlow-wort, the species is currently divided into two varieties: *P. virginica* var. *virginica* and *P. virginica* var. *parksii* (Cory) Chaudhri. *Paronychia virginica* var. *virginica,* occurs in the eastern

United States and Missouri, Texas, Arkansas, Oklahoma, and Alabama, while *P. virginica* var. *parksii* occurs only in central Texas (Chaudhri, 1968)(Figure 2). Contrary to the Greek translation of the word paronychia--"whitlow" or "felon," a disease of the nails and the plants with whitish scaly parts supposed to cure it--there is no real economic significance of the genus or species in question.

As is usually the case with species that have received little attention, there has been, and continues to be, some confusion regarding the taxonomic treatment of *P. virginica.* Core (1940) writes that Linnaeus first described the plant in 1767 under the name *Achyranthes dichotoma*. Willdenow (1797) refers to the same plant (he gives the same locality as did Linnaeus) but does not use the name, *Illecebrum dichotomum*, attributed to him by Nuttall (1818). De Candolle (1804) described an entirely different plant under the name *Paronychia dichotoma*. De Candolle (1828) reduced his *Paronychia dichotoma* and *Illecebrum dichotomum* to synonymy under *Herniaria dichotoma.* In the latter publication, De Candolle recognizes a *Paronychia dichotoma* Nutt., which is Linnaeus's plant from Harpers Ferry, West Virginia (the same locality is given). De Candolle adopted Nuttall's binomial, *Paronychia dichotoma*, for his plant because it had been used as a synonym for his *Herniaria dichotoma* (even though it was perhaps not the same plant; De Candolle gave no locality for the plant he originally named *P. dichotoma*). Unfortunately, De Candolle violated the current rules of nomenclature, and the name *Paronychia dichotoma*, widely used by many botanists for over a century, had to be regarded as invalid. Sprengel (1825) recognized the discrepency and proposed the new name *Paronychia virginica* to be used to refer to Linnaeus's original specimen collected at

Harpers Ferry. Small's (1897) use of the binomial *P. dichotoma* indicates botanists' overlooking or ignoring Sprengel's action. The taxonomy of *P. virginica* is given in Appendix A.

Nomenclatural confusion has plagued sub-taxa within this species as well. Small (1897) recognized *P. dichotoma* (L.) Nutt., referring to Nuttall's erroneously-named entity as well as a second, similar species occurring in the western United States, which he named *P. scoparia* Small. *Paronychia scoparia* differed from *P. virginica* in having a more robust habit, strictly erect and few-flowered cymes, minutely pubescent foliage, larger calyces, more strongly abaxially ribbed sepals, and longer cusps or awns on the sepals. Core (1941) also recognized the species *P. virginica* but considered Small's *P. scoparia* to be a synonym of *P. virginica.* Cory (1944) acknowledged the differences noted by Small (1897), but disagreed with the justification of specific separtation and instead recognized *P. scoparia* as a western variety of the eastern *P. virginica.* After conferring with Core, Cory (1944) named a new species, *P. parksii* Cory, which had been discovered in central Texas by H.B. Parks. Differences between *P. virginica* and *P. parksii* noted by Cory include the following: the latter grows farther west and at somewhat higher elevations, is 1.5 to 2 times taller than the former, has strictly erect stems versus prostrate stems, possesses longer sepals, and tends to be reddish in color when mature versus the yellowish color of mature *P. virginica* plants. The most recent treatment by Chaudhri (1968) recognizes *P. scoparia* as a synonym of *P. virginica* and names *P. parksii* as a central Texas variety of *P. virginica.* Chaudhri recognizes the following differences between *P. virginica* var. *virginica* and *P. virginica* var. *parksii*: the

latter has slightly longer sepals and slightly longer cusps or awns attached to the sepals than the former, is scabrous and spreading versus glabrous and erect as in *P. virginica* var. *virginica*, and occurs only in cental Texas. Correll and Johnston (1970) follow Cory (1941) [cited as Cory (1943)] in Correll and Johnston (1970). This appears to be a typographical error because Volume 26 of the American Midland Naturalist, in which Cory's article appears, was published in 1941, not 1943. The present author examined Volume 43 and could find no articles written by Cory on *Paronychia*. Correll and Johnston also comment on Cory's (1941) treatment, by stating that *P. parksii* "appears to be little more than a habit variation of *P. virginica* var. *scoparia*" yet they continue to recognize *P. parksii* as a distinct species "until further study" suggests otherwise (Correll and Johnston, 1970, p. 628). Correll and Johnston (1970) continued to follow Cory's (1941) treatment, perhaps because they were not aware of Chaudhri's 1968 treatise of the subtribe Paronychiinae, as the book is a relatively obscure publication, not available in many university libraries or herbaria. In his treatment of the Texas species of *Paronychia,* Turner (1983) agrees with Chaudhri (1968) and Correll and Johnston (1970) that the differences noted between *P. parksii* and *P. virginica* var. *scoparia* represent ecotypes of the same taxon, not warrenting taxonomic status.

Although *P. virginica* var. *virginica* is found in only a handful of locations in Virginia, West Virginia, and Maryland combined, the species as a whole is not considered to be in danger of extinction, because it is presumed to be relatively abundant in the mid to south-central parts of the United States. The Commonwealth of Virginia does rank its variety as "S1" or "extremely rare" indicating that there are five or fewer locations at

which the plant has been found (Porter and Wieboldt, 1991). Such ranking, however, does not afford the plant, or its habitat, any state or federal protection. The official status of *P. virginica* is G4T1Q/S1. A global status of four (G4) indicates that the genus is quite common worldwide. The letter "T" indicates that the number following it applies to a subspecies of the species in question. In the case of *P virginica,* one of the varieties is "very rare" (1). [Note also that Rawinski and Cassin (1986) indicate that *P. virginica* var. *parksii* is listed as T1 in Texas.] The letter "Q" indicates that the taxonomic status of the species of interest is questionable. The rank of "S1" was discussed previously. The species has also been given a federal rank of "Category 2," suggesting that information does exist to support the proposal of protection for the species; however, conclusive data regarding the biological vulnerability of the plant is needed before official protection can be given (Porter and Wieboldt, 1991; Ludwig, 1994).

Little is known about the life history of *P. virginica.* The plant is an evergreen, perennial herb that flowers from mid-summer (July) through mid-autumn (November) depending on environmental conditions and geographic location of the population (W. Rohrer, personal observation). Its niche in Virginia (three sites) and West Virginia (one site) tends to be rock outcrops and bluffs of a variety of substrates, either metamorphic or sedimentary in nature. Maryland sites (two) and a single West Virginia site occur on metamorphic slate along edges of the Shenandoah and Potomac Rivers. As one travels farther west into Arkansas and Texas (and presumably Missouri, Oklahoma, and Alabama, though no specimens were found in these states by the current author), *P. virginica* is found in rocky soils on limestone hills and outcrops. All sites are relatively

xeric, particularly on the surface, and fully exposed to sunlight. Cliff faces are generally southern or southwestern in their exposure and competition is kept minimal by the presence of thin soil and by periodic flooding of riverine sites. Although there is some concern that severe flooding may eliminate riparian populations of this rare plant, it is thought that perhaps flood-scouring along river banks acts as fire does in grassy plains--to remove debris and create openness, thus enabling shade-intolerant species to thrive (Rawinski and Cassin, 1986).

Objectives and Hypotheses

The questions remaining about the taxonomic status of *P. virginica* are the focus of the current paper. The primary objective of the project was to clarify the systematics of *P. virginica.* The second objective was to compare the use of two types of information, morphometric and allozyme, for determining variation within and among populations. The final objective was to provide useful information for determining the vulnerability of eastern populations to extinction. A null hypothesis was formed and can be stated as follows: eastern and western populations of *P. virginica* do not differ significantly and, therefore, represent one taxon. An alternate hypothesis was formed as well: eastern and western populations of *P. virginica* differ significantly and, therefore, represent at least two distinct taxa. The remainder of this paper will inform the reader of materials and methods used to investigate *P. virginica,* communicate results of both morphometric and allozyme studies, and provide a discussion as to the significance of the current author's findings as they relate to the taxonomy, nomenclature, and conservation of this species.

Materials and Methods

Morphometric analysis

In order to determine the amount of morphological variation between eastern and western populations of *Paronychia virginica*, a total of 105 individuals representing 12 populations was examined using a binocular stereoscope equipped with a micrometer. Individual samples served as Operational Taxonomic Units (OTUs) and were examined for phenotypic similarity and morphological variation using hierarchical cluster analysis, analysis of variance (ANOVA and MANOVA), and principal components analyses.

In order to adequately evaluate the entire geographic range of *P. virginica*, samples were collected from the eastern United States, northern Texas, and central Texas. A total of five populations in Virginia, West Virginia, and Maryland and a total of seven populations in Texas were sampled (See Table 1). One to several vegetative and flowering stems were taken from four to ten random individuals. The number of samples taken from each site was determined by the number of plants flowering in the population at the time of collection. Ten individuals was the desired sample size; however, numbers lower than ten indicate that fewer than ten flowering plants could be found in the population or, in the case of the rare eastern populations, the author believed collecting more than ten samples would be detrimental to the population (Table 1). Specimens were placed into sealable plastic bags and labeled to indicate collection site. Samples were transported on ice and placed into a refrigerator soon after collection. Specimens collected in Texas were mailed to T. F. Wieboldt at the Massey Herbarium, Virginia Tech,

Blacksburg, Virginia, for refrigeration. All specimens were pressed and dried as soon after collection as possible and stored for later examination.

In order to study the populations numerically, a total of 56 qualitative and quantitative traits was examined for use in a phenetic analysis. A data matrix was constructed using 32 (24 quantitative traits and 8 qualitative traits) of the original 56 characters (Table 2). Twenty-four traits were discarded because of excessive variability of the character among all OTUs, lack of variability of the character among all OTUs, and/or inability to consistently and accurately measure and score the character among all OTUs. Several quantitative characters were combined and expressed as ratios to minimize variation among environmentally-influenced characters (Watson and Estes, 1990).

Statistical analyses were performed to provide estimates of phenotypic similarity and variation between eastern and western populations. The data were standardized prior to all analyses. Agglomerative hierarchical clustering analysis was performed using MINITAB (Release 11.1 for Windows, 1996) to produced a hierarchical, non-overlapping dendrogram illustrating the associations among the OTUs. Analysis of variance (ANOVA) was performed for all characters and subsequently for quantitative characters only. Multivariate analysis of variance (MANOVA) was also performed for all characters and subsequently for quantitative characters only. The MANOVA was performed primarily to reveal statistically significant morphological gaps or differences among populations of *P. virginica* examined (Negron-Ortiz and Hickey, 1996). Principal components analysis (PCA) was used to construct two-dimensional models of

morphological variation and to examine any underlying patterns of geographical variation (Watson and Estes, 1990; Elisens and Nelson, 1993). A correlation matrix of the characters was constructed to calculate eigenvectors for the first three principal components, and the OTUs were projected onto these components (Watson and Estes, 1990). The PCA was performed twice, once for all characters and a second time using only quantitative characters.

Allozyme analysis

To determine the amount of genetic variation within and among populations of *P. virginica*, allozymes from a total of 403 individuals from 16 populations were examined using horizontal starch gel electrophoresis. Each population served as a separate Operational Taxonomic Unit (OTU) and was treated as a distinct entity in the statistical analysis using BIOSYS-1 (Swofford and Selander, 1981).

In order to adequately evaluate the entire geographic range of *P. virginica*, samples were collected from the eastern United States, Arkansas, northern Texas, and central Texas. A total of seven populations in Virginia, West Virginia, and Maryland, one population in Arkansas, and a total of eight populations in Texas were sampled (Table 1). (Sites sampled for the allozyme analysis differ slightly from those sampled for the morphometric analysis. A number of factors contributed to this discrepancy including flood/hurricane damage to populations after the initial collecting trip.) One to several vegetative stems were collected from three to forty-five random individuals from each population. The number of samples collected was determined by the number of plants in the population. Twenty individuals per population was the desired sample size;

however, sample sizes lower than twenty indicate that either fewer than twenty plants could be found in the population or the author believed collecting more than twenty samples would be detrimental to the population (see footnote "b" in Table 1). Specimens were placed into sealable plastic bags and sprayed with water to maintain moisture during storage. The bags were slightly inflated prior to sealing to avoid rotting of the moist specimen. Each bag was tagged with a label indicating site of collection. All samples were stored on ice immediately after collection and placed in a refrigerator as soon after collection as possible. Samples collected in Arkansas and Texas were mailed to T. F. Wieboldt at the Massey Herbarium, Virginia Tech, for refrigeration.

Horizontal starch gel electrophoresis of various enzyme systems (allozyme analysis) was performed at Mountain Lake Biological Station, Pembroke, Virginia, and Texas Tech University, Lubbock, Texas. Extraction of enzymes was performed by grinding 5-10 leaves per sample in "Microbuffer" as modified by Werth (1985) in the presence of 5 g PVP (polyvinyl pyrrolidone) and 5 drops ß-mercaptoethanol in ceramic spot plates using small test tubes as pestles. Sand was added to each well to aid in breaking down the relatively tough leaf tissue. Both the microbuffer and spot plates containing leaf samples were kept on ice throughout the extraction process. Excess microbuffer could be stored at -20°C for up to three days with no apparent negative effects. Extracts were stored in the spot plates at -60°C for up to five weeks before electrophoresis with no detrimental effects when compared to extracts prepared and used immediately prior to electrophoresis.

Samples were fractionated in 12% starch (potato) gels. Four types of gels were prepared for use in three types of electrophoretic buffers. Preparation of gels followed Werth (1985). All gels were prepared to a final volume of 400 ml and poured into 350 ml capacity gel molds. Gels were allowed to cool and then covered with plastic wrap to prevent evaporation and stored at 4°C until use.

In order to proceed with the loading of samples into gels, extracts were thawed on ice and allowed to absorb onto wicks cut from chromatography paper. The wicks were loaded into vertically-sliced gels. Twenty to twenty-seven samples plus a bromophenol blue standard were loaded per gel. A sample of *Paronychia argyrocoma* (Michx.) Nutt. was included on each gel to illustrate the banding pattern of a distinctly different species within the same genus. The bromophenol blue standard was included to allow for monitoring of the progress of migration during electrophoresis. Thirteen enzyme systems were assayed in various combinations using four buffer systems (Table 3). Lithium hydroxide (LiOH) was used to resolve leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), glutamic oxaloacetic transaminase (GOT), phosphoglucumutase (PGM), triose phosphate isomerase (TPI), hexokinase (HK), and esterase (EST). Morpholine (Morph) was employed to resolve malate dehydrogenase (MDH), shikimic dehygrogenase (SKDH), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6P), aldolase (ALD), and acid phosphatase (ACPH). EST, PGM, PGI, GOT, TPI, and LAP were resolved on System #6 (#6). MDH, ACPH, IDH, SKDH, and 6P were resolved on histidine citrate (HC). Enzyme activity staining protocols and agarose overlays followed Werth (1985). Each gel was photographed using

100 speed Kroger brand film and a light box. Banding patterns were also sketched on paper and scored when possible. Six enzyme systems encompassing nine loci (EST-2, EST-3, LAP, MDH-1, MDH-2, PGI, PGM-1, PGM-2, SKDH) produced consistently clear and scorable bands (see Table 4 for a complete allele roster). A statistical analysis was completed using BIOSYS-1 (Swofford and Selander, 1981).

Results

Morphometric analysis

In order to determine the amount of morphological variation between eastern and western populations of *Paronychia virginica*, a morphometric analysis was completed. Visual observation of specimens indicated an ability to predict region of origin for most samples. Specimens of *P. virginica* collected from eastern populations tended to have less pubescence on stems, leaves, sepals, and awns than specimens collected from western localities. Eastern populations also tended to possess more apicular leaf apices rather than the prominant, mucronate leaf apices of western specimens. The third distinct difference between eastern and western specimens was the occurrence of slight to no back-curvature of abaxial awns on samples collected in the eastern United States. Specimens from the western U.S. generally exhibited distinctly back-curved awns. Statistical significance of these and all other phenotypic characters examined was determined by performing various statistical analyses as described below.

The dendrogram produced by hierarchical cluster analysis has two major clusters. Cluster I (Figure 3) is composed primarily of OTUs (individual specimens of *P. virginica*) representing eastern populations. Several exceptions to this separation occur. Individuals 80 through 89 represent a western population from northern Texas (TX 121) that cluster together. Individual 81, also from TX 121, occurs in cluster I but farther to the right of the dendrogram. Other exceptions include individual 65, also from northern Texas (FM 1220), and individual 105, from central Texas (WRP). Cluster II (Figure 4) consists exclusively of representatives from the west. No real trends in separation occur

within each cluster with the exception of, in cluster I, representatives of the eastern population from Cave Mountain, West Virginia (26-35), clustering together at the far right of the diagram. In cluster II, OTUs from the Windmill Run Park, Texas population (96-105) cluster together at the far right of the dendrogram. The remainder of the OTUs are randomly dispersed, with representatives from each population mingling together within each of the major clusters. Populations within each geographic area show no clear separation amongst themselves, with the exceptions noted above.

The statistical significance of morphological differences between eastern and western populations of *P. virginica* was determined by performing one-way analysis of variance (ANOVA) between eastern and western OTUs. An ANOVA was performed using all morphological characters, determining whether or not the separation of eastern and western OTUs was statistically significant. F statistics determined if the separation between the two geographic groups was quantitatively and statistically more significant than variation within the two groups. Statistical significance ($P \leq 0.05$) in variation between eastern and western representatives occurred for 19 of the 32 characters examined. Characters 17, 27, and 28 (see Table 2 for a list of characters with associated numbers) were determined to be significantly different ($P \leq 0.05$) between eastern and western OTUs while characters 1, 2, 8, 14, 20, 26, and 32 showed highly significant ($P \leq 0.01$) variation. The remaining characters that indicated statistically-significant morphological differences between eastern and western populations (4, 5, 9, 10, 11, 12, 19, 21, and 22) indicate a very high level of significance ($P \leq 0.001$)(Table 5). A

multivariate analysis of variance (MANOVA) of the same data set produced similar results. The variation between eastern and western OTUs, when considering all characters together, was very highly significant ($P \leq 0.001$) using four different statistical tests (Table 6). When only quantitative characters were used with the ANOVA (Table 7), this was also the case for MANOVA (Table 8).

The principal components plots (Figures 5-10) confirmed the ability to distinguish between eastern and western populations of *P. virginica* on the basis of morphological characters. A principal components analysis using individuals (OTUs) for which all 32 variables were present (i.e., no missing data) produced two-dimensional figures indicating definite separation among populations of the plant (Figures 5-7). An eigenanalysis of the correlation matrix indicated that 20.2% of the total variation distributed across all specimens examined can be explained by principal component one (PC 1). This suggests that PC 1, which explains the greatest variation among populations, is an "east/west" factor. In other words, there is measurable and statistical separation between eastern and western populations of *P. virginica.* Characters that caused the greatest separation between points on the principal components plots (Figures 5-7) are characters 2, 4, 9, 10, 11, 12, 19, 20, 21, and 22--length of internodes on reproductive stems, vegetative stem pubescence, shape of leaf apex, vegetative leaf pubescence, length-width ratio of leaves on vegetative stems, length-width ratio of leaves on reproductive stems, sepal pubescence, awn length, awn pubescence, and awn curvature, respectively.

An additional principal components analysis was conducted using only quantitative characters (Figures 8-10). Separation between eastern and western samples, as plotted on the two-dimensional representation of the principal component analysis, was not as distinct, suggesting that both qualitative and quantitative characters are important in distinguishing between eastern and western populations of *P. virginica.* Eigenanalysis of the correlation matrix indicated that 17.6% of the variation existing among all specimens examined is accounted for by PC 1. Characters 2, 11, 12, 17, 20, 23, 26, 28, and 31--internode length of reproductive stems, length-width ratio of leaves on vegetatives stems, length-width ratio of leaves on reproductive stems, sepal length, awn length, sepal:awn length ratio, filament length, style length, and length of the fork of the style, respectively--explained most of the separation between graphed points.

Allozyme analysis

In order to determine the genetic variation within and among eastern and western populations of *P. virginica,* an allozyme analysis was performed using starch gel electrophoresis. Results were obtained following electrophoresis and scoring of visible bands. Of the thirteen enzyme systems evaluated, six (EST, LAP, MDH, PGI, PGM, SKDH) could be consistently resolved and scored (Figures 11-13). The remaining seven enzyme systems (ACPH, ALD, GOT, HK, IDH, 6P, TPI) were not interpretable because of complex banding patterns (Figure 13). The six scorable enzyme systems were coded by nine loci (EST-2, EST-3, LAP, MDH-1, MDH-2, PGI, PGM-1, PGM-2, SKDH). BIOSYS-1 (Swofford and Selander, 1981) output reported results for genetic variability, conformance to Hardy-Weinberg equilibrium, and divergence among

populations as determined by F statistics (Wright, 1965, 1969) and pairwise values for Nei's Genetic Identity I (Nei, 1978) and Rogers's Genetic Similarity S (Rogers, 1972).

Forty alleles were identified among the nine interpretable loci. Allele frequencies were tallied and are reported in Table 9. Three loci (EST-3, MDH-1, PGI) were monomorphic for all populations. The remaining six loci were polymorphic, with several instances of rare or novel (frequency < 0.05) alleles. Allele SKDH-D occurred only in population one (Ford Hollow, Virginia). Population four (Harpers Ferry, West Virginia) contained the rare allele LAP-A. A novel allele (MDH-2E) occurred in population six (Paw Paw Tunnel, Maryland). The population at Magazine Mountain, Arkansas, (population eight) exhibited the rare allele PGM2-B. Population eleven (National Guard Base, Texas) contains the rare allele SKDH-A. Allele MDH-2B was rare and occurred only in population twelve (TX 121, Texas). Population thirteen (Bee Cave, Texas) exhibited four rare alleles (EST-2D, EST-2F, EST-2G, LAP-K) while population fourteen (Wimberley, Texas) consisted of two rare alleles (EST-2A and LAP-G). Several alleles occurred more frequently in eastern (LAP-B, PGM-1E) or western (EST-2B, EST-2E, LAP-F, LAP-H, LAP-I, LAP-J, PGM-2A, PGM-2D, SKDH-B) populations. Seven alleles (EST-2C, LAP-D, MDH-2A, MDH-2C, PGM-1B, PGM-2C, SKDH-C) were common in all populations. Many of the polymorphic loci were only weakly polymorphic or were monomorphic in various populations.

Genetic variability was quantified for each population by computing values for three indices of polymorphism: mean number of alleles per locus (A), percent polymorphic loci (P; 0.95 criterion), and mean expected heterozygosity (H_{exp}).

Population-level values for *P. virginica* (means: A = 1.95, P = 47.22%, H_{exp} = 0.204, Table 10) exceeded mean values for seed plants (A = 1.53, P = 34.2%, H_{exp} = 0.113; Hamrick and Godt, 1996). Population level values also exceeded those for widespread species (A = 1.72, P = 43.0%, H_{exp} = 0.159) and greatly exceeded those for endemic species (A = 1.39, P = 26.3%, H_{exp} = 0.063).

Genotype proportions were compared to those expected under Hardy-Weinberg equilibrium by computing the fixation index F (also referred to as the inbreeding coefficient) for each polymorphic locus in each population. Statistical difference of the value F from 0 was evaluated using the chi-square test (Table 11). After pooling allele frequencies for those loci whose distributions may have been too skewed to carry out the chi-square test, eighteen loci showed non-conformance to Hardy-Weinberg proportions ($P \leq 0.05$) such that the computed value of F was significantly different from 0. In all cases, statistically-significant deviations from Hardy-Weinberg expectations indicated a deficiency of heterozygotes (i.e., F values were greater than 0).

In order to evaluate population substructure, hierarchical F_{XY} analysis was carried out using BIOSYS-1. The designated hierarchy consisted of total population sample, regions (east and west), states (Virginia, West Virginia, Maryland, Arkansas, northern Texas, central Texas), and localities (each population as designated in Table 1). In the hierarchical analysis (Table 12), the variance among localities with respect to region and among localities with respect to the total population sample were large, F_{XY} = 0.354 and F_{XY} = 0.333, respectively. A major component of each of these values is explained by the variation occurring among localities with respect to state (F_{XY} = 0.248). These

results support the notion that more variation occurs among populations (localities) regardless of geographic location (i.e., eastern populations differ from each other as much as they differ from western populations and vice versa).

Genetic divergence among populations was quantified by computing F_{ST} values (Wright, 1965, 1978) and pairwise values for Nei's Genetic Identity I (Nei, 1978) and Rogers Genetic Similarity S (Rogers, 1972). Values of F_{ST} ranged from 0.169 (EST-2) to 0.461 (PGM-1), with a mean value of F_{ST} = 0.353 (Table 13). All values were tested for statistical difference from 0 using contingency chi-square analysis and were determined to be very highly significant ($P \leq 0.001$)(Table 14). It is generally agreed that F_{ST} values greater than 0.2 indicate low rates of gene flow among populations (C. Werth, personal communication). Four out of six polymorphic loci in *P. virginica* have F_{ST} values greater than 0.2 (Table 13) indicating that gene flow between populations of this species is relatively restricted, even among populations in the same state or geographic region.

The matrix of pairwise values (Table 15) indicates that measures of genetic similarity (S) (mean = 0.778, range 0.641 to 0.913) are considerably higher than measures of genetic identity (I) (mean = 0.145, range 0.004 to 0.349). Nei's (1978) unbiased genetic identity is a measure of genetic distance, thus low values for I indicate little variation among populations of *P. virginica.* This is supported by the relatively high values of genetic similarity. Results suggest that populations of *P. virginica,* as determined by allozyme analysis, are similar genetically and cannot be differentiated using the above statistical analysis.

A phenogram was constructed using unweighted pair-group method using an arithmetic average (UPGMA)(Figure 14). Populations were clustered on the basis of their pairwise values for S. Two major clusters show somewhat of a geographic trend (east and west) but there are exceptions. Two eastern populations (Harpers Ferry and Bear Island) cluster with the western populations. The two subclusters within the major cluster containing predominantly western populations show northern Texas (and Arkansas) and central Texas populations clustering together in the phenogram. Overall, there seems to be some correlation between clusters and geographic location, however, separation within the entire cluster diagram is relatively weak and should be interpreted with caution.

Discussion

Distribution

Patterns of plant distribution have interested botanists for centuries. The study of geography, geology, and ecology as distinct disciplines has revealed a more intimate association between the three areas. Plants may occur primarily in one geographic location (endemic), scattered throughout many geographic locations (continuous distribution), or primarily in one geographic location with isolated populations occurring in another separate location (disjunct distribution). *Paronychia virginica* is an example of a plant whose distribution is disjunct. Found primarily in the western United States (Texas, Oklahoma, Arkansas, Missouri, and Alabama), populations of *P. virginica* can be found in the mid-Appalachians (Virginia, West Virginia, and Maryland)(Figure 2). Such a curious distribution has led to taxonomic and nomenclatural confusion (Small, 1897; Core, 1941; Cory, 1944; Chaudhri, 1968) and to studies such as the present one. Eastern and western populations traditionally have been recognized as distinct taxonomic entities; however, taxonomic rank has been disputed.

Included in the following paragraphs are several hypotheses that have been proposed to explain the origin of disjunct plant populations in North America. The discussion focuses on ideas regarding east-west disjuncts that occur in the continental United States. A synopsis of geologic events is presented followed by a description of the hypotheses and their pertinence to *P. virginica.*

At glacial maximum, the Wisconsin ice sheet covered half of the North American continent. The ice sheet extended from the Canadian Rockies across the northern plains

and southward to central Illinois, northern Pennsylvania, and southern New England (Hutton, 1989). South of the enormous field of glaciers there existed a belt of tundra, particularly wide in the east, extending several hundred kilometers south into the high elevations of the Appalachians (Hutton, 1989). What effect the Wisconsin ice sheet, its advancement and its retreat, had on vegetation south of its borders is not entirely understood. Braun (1955) suggests that the vegetation south of the ice border was essentially as it is today. Deevey (1949), in contrast, believes that substantial changes have taken place during and since the Pleistocene glaciation. Whitehead (1973) puts forth the hypothesis that modern plant communities have been established within the last several thousand years and are continuously changing. Perhaps the effects of the northern ice sheets were even farther-reaching than previously thought and could help to explain the curious distributions of many taxa held in common between the eastern and western regions of North America. For an excellent discussion of glacial and post-glacial events of northern North America, the reader is referred to Pielou (1991).

The first of several hypotheses regarding the origin of disjunct plant populations to be presented is dependent on refugia and "nunataks." Populations peripheral to the main area of existence are said to have survived glaciation by retreating to more hospitable areas free of ice (Fernald, 1925). Such areas were perhaps no more than small islands of unglaciated earth surrounded by a sea of ice. Though they must have been hostile places on which to live, refugia or nunataks offered more hospitable environments to inhabitants than did the glaciers. Taxa that survived glacial events, particularly the Wisconsin-period glaciation, on refugia may have remained in the area after the glaciers receded. Minimal

dispersion resulted in isolated disjuncts from the main population. Keener (1983), however, suggests that the southern Appalachians, which are home to many disjunct species, were not the lush glacial refugia as previously thought. The Appalachians were perhaps more suitable for colonization than more northern areas, but probably not to the extent that the number of disjuncts in the area would suggest. Other supposed refugia include the Ozarks and Orange Island, Florida (Braun, 1955). Evidence in support of the idea of glacial refugia in the Appalachians lies in the similarity in habitats occupied by western species and their eastern disjunct relatives (and vice versa). The encroaching glacial ice sheets of the Wisconsin geologic period may have forced some central and western populations to "seek out" (not actively, of course) environments equally hospitable to their place of origin. Keener (1983) has suggested that if refugia had been occupied during glaciation, they would have been in the southwestern United States, not the southern Appalachians, and that the inhabitants subsequently (i.e., after the retreat of the ice sheets) migrated north and east to barrens and glades of the Appalachians. Only after such migration did the populations become isolated from the "parental" population.

A second hypothesis used to explain the occurrence of disjunct plant populations purports that there were originally single, gap-free ranges of species across North America (Pielou, 1991). Various events may have occurred in parts of the range that resulted in fragmentation of the original, expansive population. One such event could have been the advancement of the Wisconsin ice sheets. This supposes that ice sheets destroyed large tracts of vegetated areas and that survivors took refuge in ice-free areas as discussed previously in the "refugia hypothesis." Inferred by this hypothesis is the

existence of modern vegetation prior to the Wisconsin glaciation. Braun's (1955) explanation for fragmentation of the original range of a taxon is as follows: changes in environmental conditions allowed other, "foreign" species to invade and crowd out or surround already-established species. As a result, various colonies of plants became isolated, scattered throughout the original range. The fragmentation of a continuous range may be brought about by topographic, and/or climatic change or by changes in continental configuration (Braun, 1955). The persistence of the newly-disjunct populations may depend on suitability of the newly-modified habitat, reproductive strategies, and maintenance of minimal competition.

Combining ideas from both the refugia and fragmentation hypotheses is a hypothesis that explains disjunct populations in terms of the migration and recession over time and space by community types. Following such recessions, remnant plant populations remain behind. Such remaining populations would have migrated with the original population but perhaps found suitable habitat in which to remain as the bulk of the group figuratively retreated out of the area. One of the most intriguing explanations for the existence of the large number of east-west disjunct taxa is the theory of the "Prairie Peninsula" (Transeau, 1935). During the post-Wisconsin Xerothermic Period (also called the Hypsithermal), vegetation common to the prairies of North America extended eastward into areas of favorable climate and openings left by retreating spruce-larch forests (Takhtajan, 1986; Hutton, 1989). Although the peninsula extended only as far east as the modern-day city of Cleveland, Ohio, this theory has been used to explain the existence of mainly western species in West Virginia and Virginia. Perhaps some

elements of the prairie flora migrated into the highlands of the Appalachian states and remained there in areas similar in climate and exposure to the western prairies. Evidence lies in the similar habitats occupied by many disjunct species in the east and in the west. Habitats in the east, such as exposed bluffs, barrens, and glades, are very similar to the plains and prairies of the central and western regions of North America. The most isolated of such "prairie remnants" occur on bluffs, hill tops, cliff edges, sandstone outcrops, sand plains and dunes, eroding limestone outcrops, limestone with underground streams, ponds, bogs, and marl marshes (Transeau, 1935). Such habitats are found throughout the prairies of interior North America and scattered throughout eastern regions.

The prairies began to retreat westward by about six thousand years ago, four thousand years after the initiation of eastward advancement. Left behind were scattered patches with various combinations of prairie species. Braun (1955) refers to barrens (including shale) as "prairie inclusions," and I would suggest that the term is also suitable for open glades such as the limestone glades of West Virginia (Bartgis, 1993). The communities found in central and western prairies and in eastern glades and barrens are strikingly similar and do suggest some sort of historical association between the geographic regions. Perhaps the flora of the prairie peninsula invaded a newly-suitable habitat following the retreat of spruce-larch forests and initiated the succession of a new forest of shrubs and trees (Hutton, 1989). As the canopy closed above the initial invaders, obligate heliophytes, such as *Paronychia virginica*, were left to survive in areas

with minimal competition such as shale barrens, limestone and dolomite cliffs, open limestone glades, and flood-scoured river banks.

The final hypothesis of origination of disjunct plant populations to be discussed is that of long-distance dispersal. Perhaps a feasible explanation for pteridophytes with light, airborne spores, the idea of long-distance (several hundred or thousand kilometers) dispersal of fruits, seeds, and/or propagules of flowering plants does not seem to hold much credence. Exceptions may be noted, for instance members of the Orchidaceae and some capsule-bearing members of the Caryophyllaceae in which small seeds are dispersed after wind or an animal frees the seed from the capsule (*P. virginica* bears a utricle, not a capsule). There are, of course, examples of birds and other animals carrying propagules long distances as well. At least one interesting, if not peculiar, hypothesis evolved from the idea of long-distance dispersal. Harvill (1983) and Harvill *et al.* (1992) put forth the idea of a southwest to northeast distribution of plants as the result of prevailing winds and the Coriolis Force. According to Harvill (1983), during most of the "seed-dispersal season" (May through November) the winds are predominantly southern to southwestern 97% of the time. This suggests that airborne materials, including seeds and spores, are carried in a northern and northeasterly direction. In conjunction with the idea of prevailing winds playing a major role in the dispersal of southwestern species to more northeastern stations is the suggestion that the Coriolis Force helps to direct northward moving particles slightly to the east. Instead of winds, and suspended objects therein, moving directly northward, the Coriolis Force acts to move the air a bit to the east because the earth is moving more slowly at middle latitudes. Harvill *et al.* (1992) suggest

that such migrations started about 16,500 years ago and were indeed due to the Coriolis Force assisted by prevailing winds. Although there are no doubt other plausible explanations for the existence of east-west disjuncts in North America, the three presented seem to be most feasible.

The use of morphometric and genetic information may be useful in determining the historical distributions of plants; however, it is difficult to determine the geographic origin and direction of migration of populations of *P. virginica* using the results of the present study. The cluster diagram based on allozyme data (Figure 14) shows weak separation among populations but perhaps not enough to support a hypothesis regarding direction of migration of the species. The existence of populations of *P. virginica* in Alabama, a mid-point between eastern and western populations (Figure 2), suggests some historical connection between the currently disjunct populations; however, direction of migration or place of origin cannot be determined. Although the data generated for the current study do not clearly support one theory on the origin of disjunct plant populations, I have come to believe that the theory of the Prairie Peninsula offers the best explanation for the disjunction in this taxon. The eastward expansion and subsequent westward retreat are evidenced by the similarity in habitats occupied by the eastern and western populations of *P. virginica.* Long-distance dispersal does not seem to be a plausible explanation for the distribution of this plant because of its limited means of dispersing seeds.

Taxonomic status

For plant systematists, the notion of taxonomic categories is paramount to any project. Most, if not all, such categories are arbitrary and are the construct of the human mind (Levin, 1979). Categorical organization is necessary if a common ground is to be established to facilitate discussion of specific entities. In biological hierarchical systems of taxonomy and nomenclature, the species is the keystone. All other ranks are built from this, the least inclusive of the taxonomic categories. Mayr (1969) suggests two levels of difficulty in determining the taxonomic affiliation of natural populations: 1) the delimitation of the taxon (whatever it may be) and 2) ranking the taxon in the proper category (i.e., species, genus, family). The focus of this project has been the taxonomic rank of species (and subsequently subspecies and variety).

Perhaps one of the most difficult taxonomic categories to determine, species must be clearly identified as it is the concept with which we have the most frequent and direct contact in the field, laboratory, and/or herbarium. However, because of a lack of experimental research, the species is, according to Grant (1976b, p. 319), ". . . one of the most poorly understood of all basic units of biological organization." Unfortunately, as stated appropriately by Harrison (1991), the study of speciation has never emerged as a coherent discipline. Instead it falls at the interface between population biology and systematic biology and does not fit comfortably into either domain. I believe that taxonomic confusion comes about as the result of catching a glimpse of speciation as it is happening. As observers, we are arriving "on the scene" in the middle of the action, so to speak, in which case determining whether or not populations have diverged enough to be

named as separate species or subspecies is difficult. In addition, not all organisms are at the same "stage" in speciation, therefore rendering useless one catch-all species concept. Indeed, Templeton (1994) contends that a species concept can be evaluated only in terms of a particular goal or purpose. Jonsell (1984) indicates that botanists have been aware of the difficulty of defining species for most of history. Stemming from this difficulty have been a variety of ideas and concepts proposing criteria for deciding when a group of organisms merits the rank of species. Perhaps the most familiar and most rigorously-defined concept of species is the biological species concept (BSC), whose best-known advocate is zoologist Ernst Mayr (1963, 1969). This notion of species has also been called the isolation concept and is well known to scientists and non-scientists alike.

The biological concept of species emerged in the mid-nineteenth century. Over the course of approximately 150 years, much thought was put into clarifying the idea of defining species on purely biological (versus typological) terms (Mayr, 1969). By defining a species as groups of interbreeding natural populations that are reproductively isolated from other such groups, a concept based on the biology of the organism, not just its physical characteristics, was put into place. Mayr expanded upon the concept and defined species as "groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups" (Mayr, 1963). The use of the word "potentially" in the definition is obviously ambiguous and later was removed by Mayr (1969).

The BSC has been attractive to biologists for a variety of reasons. Perhaps most appealing is the idea of having to use only one characteristic to determine the rank of a

taxon. If one can determine that two or more individuals can interbreed and produce viable offspring, then, according to the BSC, one can determine the taxonomic rank of the individuals in question. (Another ambiguous word in the definition of a biological species is "viable." Must the offspring be able to further reproduce or just be able to function adequately and survive in the environment?) Another appealing aspect to the BSC is its relative ease of understanding. The BSC also conforms to many current hypotheses of speciation that stress the importance of reproductive isolating mechanisms (Jonsell, 1984). Finally, and perhaps most significantly, the BSC does tend to be useful in Kingdom Animalia, with which most people are familiar. Because much emphasis is placed on the study and understanding of animal (primarily vertebrate) anatomy, physiology, and behavior in introductory level biology courses, the BSC is generally the only species concept introduced and discussed (if species concepts are discussed at all).

The BSC is indeed useful when discussing sexually-reproducing, interbreeding individuals for which reproductive habits are known. The problem lies in the fact that not all organisms are sexually reproducing or interbreeding or even understood to the extent that we can determine this information. Many examples of asexual or apomictic plants occur. Not every plant species is sexual and not every plant population consists of individuals linked by mating bonds (Grant, 1976a).

Ehrlich and Raven (1969, p. 1230) state that, "There is an abundance of inferential evidence indicating that, at least in many cases, gene flow is of little or no importance in maintaining many of the phenetic units we call 'species'." They cite examples of asexual organisms that are kept similar not by gene flow, but as a result of similar selection

regimes. Endler (1989) states that speciation and reproductive isolation are evolutionarily independent. Environmental and ecological, not reproductive, mechanisms seem to be the modes of speciation for some if not most organisms. According to Dobzhansky *et al.* (1977), the production of sterile hybrids as a criterion by which to distinguish between species is far from the truth and should not be the sole determinant for the rank of species. Where there is no interbreeding, the BSC cannot be applied and another more plausible concept of species is required.

As implied previously, plant populations tend to exhibit a broad spectrum of breeding systems. Some are promiscuous outcrossers, swapping pollen with a wide range of closely-related species to form hybrid swarms (e.g., *Quercus* spp.). Other plant populations are strictly uniparental or clonal in their reproduction (e.g., *Populus tremuloides*). There are also intermediate, non-obligate self-pollinators, that can be sexual or apomictic, depending on environmental conditions. *Paronychia virginica* exhibits similar problems in following the BSC because it seems as though mating occurs between nearest neighbors, which are quite often close relatives, thus simulating self-pollination in some cases. Selfing may also occur within *P. virginica* as evidenced by the anthers clasping the style/stigma in many of the specimens examined for the morphometric portion of the present study. Thus, *P. virginica* seems to rely on more than one method of reproduction (please note that this is the opinion of the current author and that only greenhouse or common garden studies can shed additional light on this subject). The BSC does not help one to delimit any taxon that exhibits such a variety of mating regimes.

Sibling species also present a problem to the systematist when delimiting species using the BSC, particularly when discussing nomenclature (Heywood, 1963). Sibling species are those groups of organisms that are reproductively isolated from one another yet appear, morphologically, to be almost if not completely identical. Only extensive fertility experiments would allow one to determine that the two are distinct biological species. Because of the necessity to perfom such lengthy studies, the BSC is not practical for use in the field.

Perhaps the most challenging problems for the BSC to resolve are those dealing with geographically isolated populations of organisms, such as *P. virginica.* Keeton and Gould (1986) suggest that with increased geographic distance between two populations there is a decreased chance of gene flow and an increased chance of more marked differences between the populations; however, the two populations could remain interfertile, producing viable progeny. Morphological and isozymic results of the current study support the notion of increased differentiation and decreased gene flow, respectively. Whether or not eastern and western populations of *P. virginica* have remained interfertile has yet to be determined. By definition, a biological species can interbreed successfully with another of its kind. If two populations are disjunct geographically, occupy different ecological niches, are morphologically distinct, and can produce viable offspring when artificially crossed in the laboratory or greenhouse, are they the same species? According to the BSC, they are. But the obvious question is, would the two populations have ever come into contact in nature? If so, would they have produced so-called viable offspring? In many cases there is no good test for determining

whether or not two allopatric populations belong to the same or different species according to the BSC (Keeton and Gould, 1986). Sokal and Crovello (1970) also raise the question of whether laboratory tests of interbreeding should be considered as evidence when contrasted with field observations. I believe that once an organism is taken from its natural environment and placed into an artificial setting such as a laboratory or greenhouse, the organism may perform differently. Dobzhansky *et al.* (1977) support this notion and warn their readers to beware of crossability in the laboratory or greenhouse, because organisms may have other barriers in the field that keep them isolated. Most botanists agree that the BSC is lacking in its ability to be applied to many members of the plant kingdom. *Paronychia virginica* is no exception. The lack of information regarding the mating system employed by this taxon prevents one from making an accurate statement regarding the effectiveness of the BSC in this case. Results from both the allozyme and morphometric studies do support some sort of taxonomic division within the taxon; however, the line between species and subspecies or variety is fine and to be considered carefully before changing the taxonomic treatment as it now stands.

Of particular interest in the case of *P. virginica,* and other relatively rare plants, are 1) the use of the BSC in defining species in legal documents and 2) the delineation of infraspecific taxa, namely subspecies and variety. A state that defines a species as populations of interbreeding, genetically-cohesive units will neglect to protect rare organisms whose breeding system is anything other than sexual and outcrossing (Standley, 1992). Statutes governing the protection of wild plants or animals may include

subspecies and variety within the taxonomic rank of species, or they may not. Historically, the term "subspecies" has been used primarily in zoology and the term "variety" in botany. While subspecies remains the infraspecific taxonomic rank of choice among zoologists, contemporary botanists use both terms. Variety was originally intended to indicate any marked variation that occurred within a species, regardless of the geographic location of populations (Standley, 1992). Subspecies, on the other hand, generally refers to variation caused by geographic separation of populations. Variety is a taxonomic category below subspecies. In his treatment of the subtribe Paronychiinae, Chaudhri (1968) differentiates between the ranks of subspecies and variety as follows: the term subspecies is used to describe any group of individuals differing from the main population in more than one character and, in addition, occupying a rather well-defined area, while the term variety is used to refer to variants differing from the main population in one morphological character, regardless of distributional pattern. It is this definition of subspecies and variety that the current author will follow when deciding on the most appropriate taxonomic treatment of *P. virginica.*

Unfortunately, the seemingly trivial discussion of the difference between subspecies and variety must be of concern to conservation biologists. The rank of variety often lacks explicit legal protection under endangered species statutes (see Standley, 1992). Many laws, federal and state, only offer protection to infraspecific taxa at the rank of subspecies, neglecting to recognize rare "varieties" of plants (Standley, 1992). The terms variety and subspecies often have been used interchangeably (generating more confusion) and, for this reason, Standley (1992) suggests that taxonomists concerned with

rare plant conservation should adopt the usage of the rank of subspecies in place of variety to ensure that legal protection is not denied because of semantics.

Regarding the most appropriate taxonomic treatment for *P. virginica,* morphometric and allozyme data lead to somewhat different conclusions. While morphological data support the notion of clear taxonomic distinction between eastern and western populations, allozyme data illustrate less distinct geographic separation and suggest instead considerable variation among all populations, regardless of geographic region. Hillis (1987) reminds us that systematists have always had to deal with discordance among data sets even if two or more data sets include only morphological data. If differences in results are to be expected when using similar types of input data, then certainly studies that make use of two or more types of information (e.g., morphological and isozymic, isozymic and molecular) can be expected to yield different conclusions based on the results obtained. Hillis (1987) also suggests that the difficulty in correlating results from a morphological analysis with those of a molecular analysis may arise because of varying species concepts used to explain each type of data. For example, a systematist conducting a study using primarily morphological characters will tend to define species as those groups of individuals that are morphologically distinct from one another, regardless of variation at the molecular level. On the other hand, a systematist working with cpDNA sequences may define species as those groups of individuals that exhibit some minimal amount of genetic divergence, regardless of morphological similarities and/or differences. Linhart and Grant (1996) indicate that there are good reasons to predict a difference in patterns of character variation in naturally-

occurring populations. One reason is that different characters, whether morphological or isozymic, may be either single-gene or polygenic characters. A second reason given is that characters may be either neutrally-responsive or selectively responsive to the environment. A neutrally responsive character is one that is not affected by the processes of natural selection. As predicted by Hillis (1987) and Linhart and Grant (1996), morphological and allozymic results suggest different taxonomic treatments for *P. virginica.*

The high number of morphological characters exhibiting statistically-significant variation between individuals from eastern and western populations (Tables 5 and 7) supports the work of previous investigators who relied exclusively on phenotypic traits in determining taxonomic classification of *P. virginica.* In their systematic study of taxa within the genus *Ernodea,* Negron-Ortiz and Hickey (1996) use the following definition of a morphological species: a morphological species is a group of organisms with morphological discontinuities separating it from other groups; the attainment of morphological differentiation may not always be correlated with ecological or reproductive isolation or genetic differences. According to the results of the morphometric portion of the current study and considering the above definition of a morphological species, I believe that *P. virginica* represents two easily-distinguishable *morphological species*. That is, a botanist in the field would be able to differentiate between *P. virginica* (in the eastern United States) and *P. scoparia* (resurrecting Small's taxonomic treatment), using more than one character. With phenotypic characters such as pubescence, shape of leaf apex, and curvature of awns, one can distinguish between

eastern and western specimens. Although variable according to environmental conditions and age of plant (Chaudhri, 1968), pubescence is, in my opinion, the most reliable character for differentiating between eastern and western populations of *P. virginica.* Also from the evidence collected in this study, I do not support the notion that *P. parksii* is either a distinct species or a distinct subspecies from *P. virginica* when considering morphological evidence alone, thus disputing Chaudhri's (1968) treatment of the taxa.

As indicated previously, the results of the allozyme study lead to somewhat different conclusions regarding the taxonomic treatment of *P. virginica.* Hierarchical F statistics (Table 12) suggest that there is very little variance between regions (east and west) when compared to that of the total population sample (F_{XY} = -0.033). Most of the variation occurs among localities as compared to region (F_{XY} = 0.354), among localities as compared to total population sample (F_{XY} = 0.333), and among localities as compared to state (F_{XY} = 0.248). Because there is such discrimination between populations, regardless of geographic region, one may assume that there is very little, if any, gene flow among populations of *P. virginica.* Each population seems to be maintaining high levels of genetic diversity without the help of incoming migrants or long-distance dispersal. Contrary to popular belief and supported by the results of the current study, small, isolated plant populations are not necessarily genetically depauperate (Hamrick and Godt, 1996).

In addition to functioning as seemingly separate units, several populations of *P. virginica* exhibit a deficiency of heterozygotes at various loci. Several explanations may be offered to explain heterozygote deficiency. Karron (1991) suggests that there is some

support to the notion that plants with restricted distributions have higher levels of self-fertilization than more widespread species. Selfing populations do tend to have lower frequencies of heterozygotes; however, if *P. virginica* was indeed an autogamous plant, one would expect all allozyme loci to be deficient in heterozygotes. This is not the case (Table 11). Various alleles conform to Hardy-Weinberg equilibrium while others are not, suggesting that a mechanism other than selfing is driving the occurrence of heterozygote deficiency.

A second explanation for the deficiency in heterozygotes in some but not all populations of *P. virginica* deals with gel-scoring inaccuracies. Because of the inconsistency in conformance to Hardy-Weinberg equilibrium, it was suggested that perhaps the deficiency of heterozygotes was an artifact of improper scoring of allozymes (C. Werth, personal communication). MDH-2 proved to be the most consistently interpretable of the loci examined (Figure 11). Close examination of allele frequencies within this locus and across populations revealed the same results, heterozygote deficiency in some, not all, populations at the MDH-2 locus. This lends credibility to the scoring of gels and suggests that the reported heterozygote deficiencies and inconsistent conformance to Hardy-Weinberg equilibrium are "real" and not artifacts resulting from improper scoring.

A final explanation could be that the sample size for several populations was not adequate. Sample size, however, should not affect conformance to Hardy-Weinberg equilibrium (C. Werth, personal communication) and this seems to be the case in the present study. Two populations in particular were extremely limited in the number of

individuals examined (Table 1). One, Johnsons Creek, a Virginia population, consisted of only six samples because there were only six plants belonging to the population. The second small population sample was obtained along Farm Road 1220 in northern Texas. Sample size was limited in this case because only three plants could be found. By examining the information in Table 10, one might notice that, while the small population from Johnson Creek lacked allelic diversity (it was monomorphic), the small population from north Texas exhibits tremendous diversity for having sampled only three individuals. It should be mentioned that plants were collected for allozyme analysis prior to flowering and that finding *P. virginica* in the midst of a drought along a north Texas highway was like trying to find the proverbial needle in a haystack. Plants examined during the summer of 1996 were extremely reduced in size because of a lack of rain. This suggests that perhaps the three samples located in the north Texas populations were merely the only representatives that could be found and collected. In other words, the population sampled along Farm Road 1220 probably had many more members that went undetected, thus uncollected, by the present author.

The most plausible explanation for the deficiency of heterozygotes in several populations of *P. virginica* may have to do with population substructuring. Such sub-structuring, also known as the Wahlund effect or force (Hartl, 1988), may cause an uneven sampling of genotypes by the investigator because of uneven distribution of alleles within the population. The patchiness in allele occurence seems to be an acceptable explanation for the lack of heterozygotes in several of the sampled populations of *P. virginica.*

The mating system of *P. virginica* can perhaps be speculated upon after examining the results of the present study. The fact that each population seems to be acting as a "closed" system, that is few migrants come into the populations and few propagules are dispersed far from the population, suggests that breeding among close relatives may be occurring. Such a mating system, although not entirely self-fertilizing, would surely affect the ratio of heterozygotes to homozygotes in the population, although not to the extreme degree of an entirely autogamous population. *Paronychia virginica* seems to be a facultatively-selfing organism. The anthers were often found to be clasping the style and stigma in specimens examined for use in the morphometric component of this project. Such strategic positioning of reproductive organs was also noted by Chaudhri (1968). The present author believes that a combination of mating with close relatives and occasional selfing, when few neighbors are available for pollen exchange, is the cause of the excessive occurrence of homozygotes. Karron (1991) supports this conclusion by stating that certain aspects of the biology of rare plants predisposes them to self-fertilization and random or mixed mating regimes. The author suggests that small population size (few available mates) and limited pollination services may result in selfing or mating with close relatives, thus decreasing the frequency of heterozygotes.

Determining taxonomic treatment for *P. virginica* using allozyme evidence was not as straightforward as it was when using morphometric evidence. When considered in conjunction with morphological evidence, allozyme data support the delimitation of several sub-taxa; however, Baum and Shaw (1995, p. 301) state it most appropriately: "Few (if any) real objects have absolute boundaries if they are observed carefully

enough." As taxonomic classifications are dynamic and forever changing, the present author makes the following suggestions regarding the most logical taxonomic treatment for *P. virginica* at the present time, realizing that the next individual to study this plant may reach entirely different conclusions.

An avid "splitter" would be tempted to identify each population as a distinct entity unto itself, and may be justified in doing so. This is not practical for many reasons, the least of which is the nomenclatural havoc it would create. Therefore, I would suggest that, used in combination with the morphological evidence, allozymes suggest the delineation of two subspecies, *P. virginica* subsp. *virginica* occurring in the eastern United States, and *P. virginica* subsp. *scoparia* occurring in Arkansas and Texas (and presumably Missouri, Oklahoma, and Alabama). (See Appendix B for the synonomy of these taxa.) I use the rank subspecies because I believe the differentiation between the entities is substantial enough to warrant such a position. I would also suggest that the morphological differences found among populations of this species is more indicative of genomic variation than of ecological or environmental variation. The cluster diagram constructed using allozyme data (Figure 14) hinted at geographic variation within "quasi-neutral" (those not affected by environment) characters, such as proteins and DNA, but only subsequent analysis will support or deny this notion.

Key to sub-taxa of *Paronychia virginica* Spreng.

A. Sepals and awns glabrous; awns having slight to no back-curvature; leaves glabrous with apiculate apices.....1. subsp. *virginica*

A. Sepals and awns pubescent; awns obviously back-curved; leaves pubescent with mucronate apices.....2. subsp. *scoparia*

Description of sub-taxa

1. *P. virginica* Spreng. subsp. *virginica*

A perennial herb; vegetative and reproductive stems branched from the base of the plant; extending up from a woody caudex. Stems may be erect and upright or spreading and prostrate. Plants 10-30 cm in diameter and 15-25 cm in height. Stems and leaves glabrous or minutely pubescent; pubescence tends to be isolated at nodes along vegetative stems. Leaves linear and sessile, apicular at apices. Flowering cymes branch in a pseudo-dichotomous manner. Sepals glabrous; 3.0-4.9 mm (avg. 3.8 mm) long, including awns. Awns attached to abaxial surfaces of sepals, glabrous, with little or no back-curvature; 0.2-1.1 mm (avg. 0.6 mm) long. Stipules white and papery, often split down center to give appearance of two stipules per leaf or floral bract.

2. *P. virginica* subsp. *scoparia* (Small) Rohrer

A perennial herb; vegetative and reproductive stems branched from the base of the plant; extending up from a woody caudex. Stems may be erect and upright or spreading and prostrate. Plants 10-30 cm in diameter and 15-25 cm in height. Stems and leaves moderately to densely pubescent. Leaves linear and sessile, mucronate or spine-like at apices. Flowering cymes branch in a pseudo-dichotomous manner. Sepals densely

pubescent; 3.3-5.4 mm (avg. 4.2 mm) long, including awns. Awns attached to abaxial surfaces of sepals, densely pubescent, moderately to extremely back-curvature; 0.4-1.5 mm (avg. 0.8 mm) long. Stipules white and papery, often split down center to give appearance of two stipules per leaf or floral bract.

Conservation

Suggestion for the conservation of *Paronychia virginica* is no different than for any of our endangered species--protect habitat. This is not limited to populations of *P. virginica* in the eastern United States, although that is where it is most urgently needed. Millar and Libby (1991) suggest conserving adequate genetic diversity of widespread species before they become imperiled. Allozyme results indicate that rare, eastern populations seem to be as genetically diverse as the more abundant western populations, implying that conservation of all populations of *P. virginica* is required to maintain adequate genetic diversity in the species. Karron (1991) supports this notion by emphasizing the importance of conserving genetic material from as many populations as possible, recognizing that there are novel alleles in even the smallest population. Table 9 shows that there are indeed rare alleles in many of the sampled populations of *P. virginica.* It is imperitive in cases such as that of *P. virginica,* where populations are genetically distinct and isolated entities, to preserve as much of the natural germplasm as is possible. Hamrick *et al.* (1991) suggest that conservation efforts should be toward preserving genetic variability and evolutionary potential of a species. It is not enough to save the species if the habit upon which it depends is being irreparably destroyed and,

vice versa, it is not enough to save habitat if the populations dwindle and become genetically deficient.

The Future

Much has been learned regarding the morphological and genetic variation among populations of *P. virginica,* yet this project represents only a fraction of what can still be gained from studying this plant. I would suggest that chromosome counts be attempted to investigate the ploidy level of this particular species. Such information may be helpful in interpreting allozyme banding patterns, predicting breeding system, and determining historical distribution. A greenhouse study would also be particularly helpful in determining the breeding system used by this plant, as well as requirements for seed germination. Particularly interesting would be testing the biological species concept by evaluating interfertility between plants from different geographic locations. In the field, pollination and dispersal studies would be most helpful in determining the mechanisms used by this plant to ensure maintained genetic diversity. The use of ultraviolet radiation may provide information regarding the use of floral markings to guide insects to nectar. Also in the field, assessing the available seed bank in areas surrounding populations may indicate whether or not recruitment, either natural or human-induced, is possible. Finally, a determination of the most desirable population density, not size, may help in determining the most appropriate size of protected area for the species (Huenneke *et al.*, 1992).

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Appendix A

Taxonomic and nomenclatural history of *Paronychia virginica* as presented by Chaudhri (1968).

P. virginica Sprengel, Syst. 1:822 (1825)
Type: See below under *Achyranthes dichotoma* L.

Syn.: *Achyranthes dichotoma* L., Mant. 51 (1767).
Type: "Habitat in Virginia", Herb. Linn. 287/11 (LINN).

Illecebrum dichotomum Willd., Sp. Pl. ed. 5, 1:1196 (1797).

Paronychia dichotoma (L.) Nutt., Gen. N. Amer. 1:159 (1818), non DC. in Poiret, Encycl. 5:25 (1804).

Plottzia dichotoma Arnott apud Torr. & Gray, Fl. N. Amer. 171 (1838).

Paronychia scoparia Small in Bull. Torr. Bot. Club 24:335 (1897).
Type: Oklahoma: Indian Territory, between Fort Cobb and Fort Arbuckle 1868, *Ed. Palmer 27* (holotype, NY).

Appendix B

Synonomy of sub-taxa of *Paronychia virginica* Sprengel.

1. *Paronychia virginica* Spengel subsp. *virginica*, Syst. 1:822 (1825)
   Type: See below under *Achyranthes dichotoma* L.

   Synonyms: *Achyranthes dichotoma* L., Mant. 51 (1767).
   Type: "Habitat in Virginia", Herb. Linn. 287/11 (LINN).

   Illecebrum dichotomum Willd., Sp. Pl. ed. 5, 1:1196 (1797).

   Paronychia dichotoma (L.) Nutt., Gen. N. Amer. 1:159 (1818), non DC. in Poiret, Encycl. 5:25 (1804).

   Plottzia dichotoma Arnott apud Torr. & Gray, Fl. N. Amer.171 (1838).

2. *Paronychia virginica* subsp. *scoparia* (Small) Rohrer, **comb. nov**.
   Basionym: *Paronychia scoparia* Small in Bull. Torr. Bot. Club 24:335 (1897).
   Type: Oklahoma: Indian Territory, between Fort Cobb and Fort Arbuckle 1868, *Ed. Palmer* 27 (NY, holotype).

   Synonyms: *P. scoparia* var. *scoparia* (Small) Cory, Rhodora 46:280 (1944).

   P. parksii Cory, loc. cit. 46:280 (1944).

   P. virginica var. *parksii* (Cory) Chaudhri, A revision of the Paronychiinae, p. 140 (1968).

Figure 1. Photograph of *Paronychia virginica* Spreng. Plants are generally 10-30 cm in diameter and 15-30 cm in height. Photo taken by T.F. Wieboldt at the Cave Mountain, West Virginia, collection site.

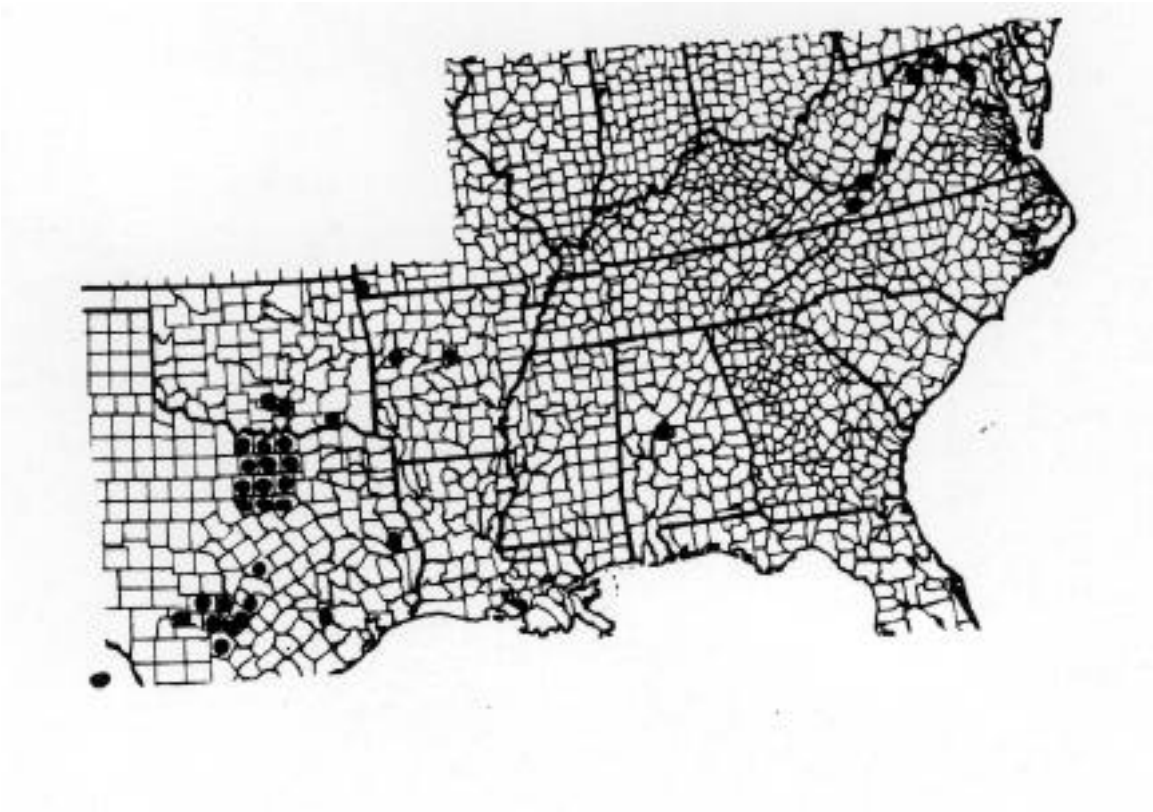

Figure 2. Range map of *Paronychia virginica* Spreng. Dots represent counties in which *P. virginica* has been collected.

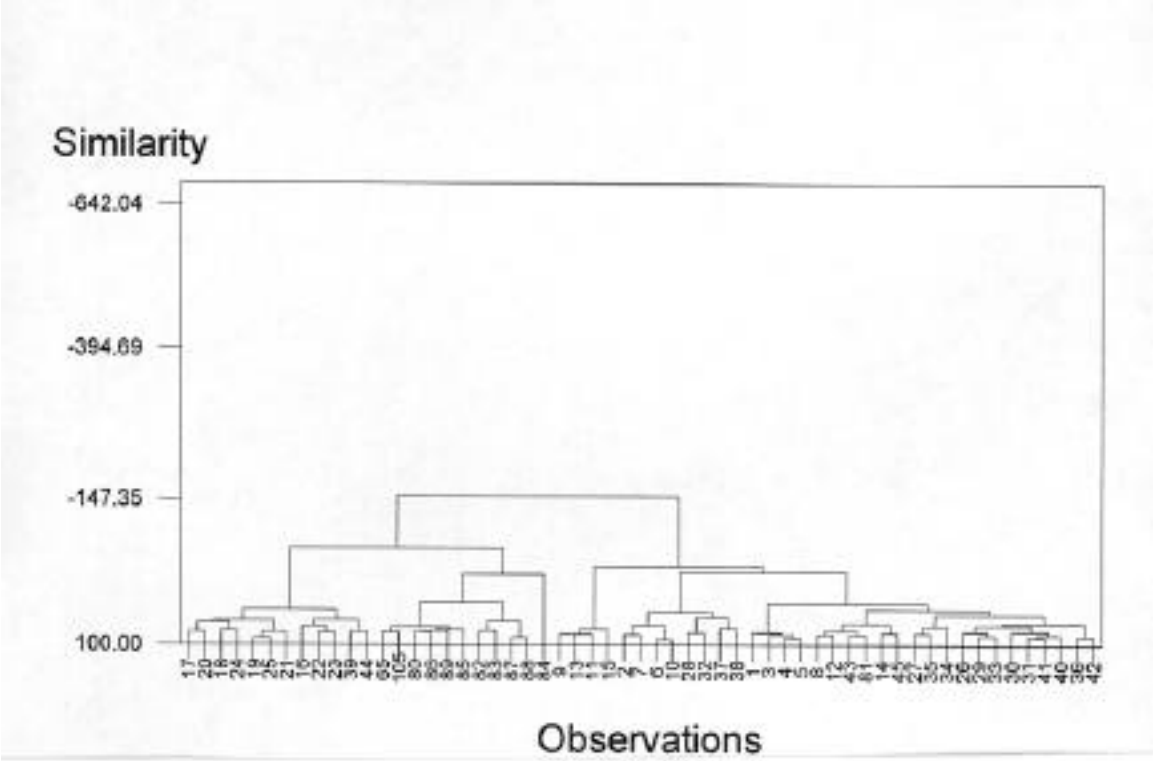

Figure 3. Cluster I of the hierarchical cluster analysis performed on morphological characters and character ratios. Numbers, when included in the cluster, indicate individual specimens as follows: 1-5, Bear Island, MD; 6-15, Paw Paw Tunnel, MD; 16-25, Cave Mountain, WV; 26-35, Ford Hollow, VA; 36-45, Potts Creek, VA; 46-55, Bee Cave, TX; 56-65, Farm Road 1220, TX; 66-69, Mount Bonnell, TX; 70-79, Peden Road, TX; 80-89, Texas Route 121, TX; 90-95, Wimberley, TX; and 96-105, Windmill Run Park, TX.

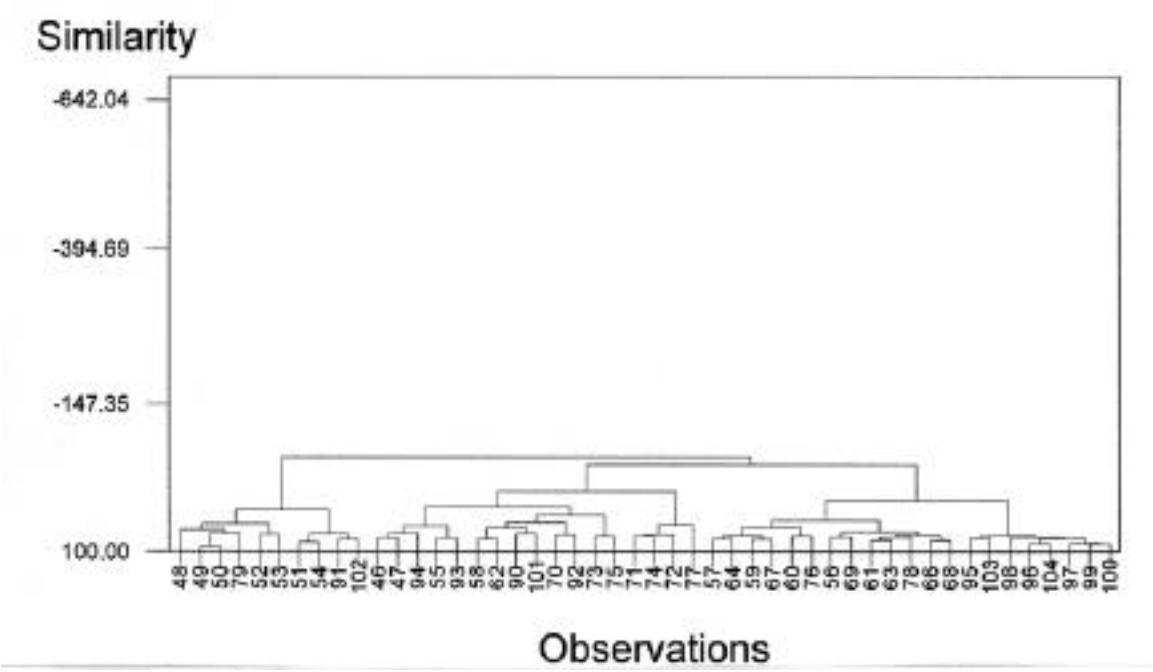

Figure 4. Cluster II of the hierarchical cluster analysis performed on morphological characters and character ratios. Numbers, when included in the cluster, indicate individual specimens as follows: 1-5, Bear Island, MD; 6-15, Paw Paw Tunnel, MD; 16-25, Cave Mountain, WV; 26-35, Ford Hollow, VA; 36-45, Potts Creek, VA; 46-55, Bee Cave, TX;56-65, Farm Road 1220, TX; 66-69, Mount Bonnell, TX; 70-79, Peden Road, TX; 80-89, Texas Route 121, TX; 90-95, Wimberley, TX; and 96-105, Windmill Run Park, TX.

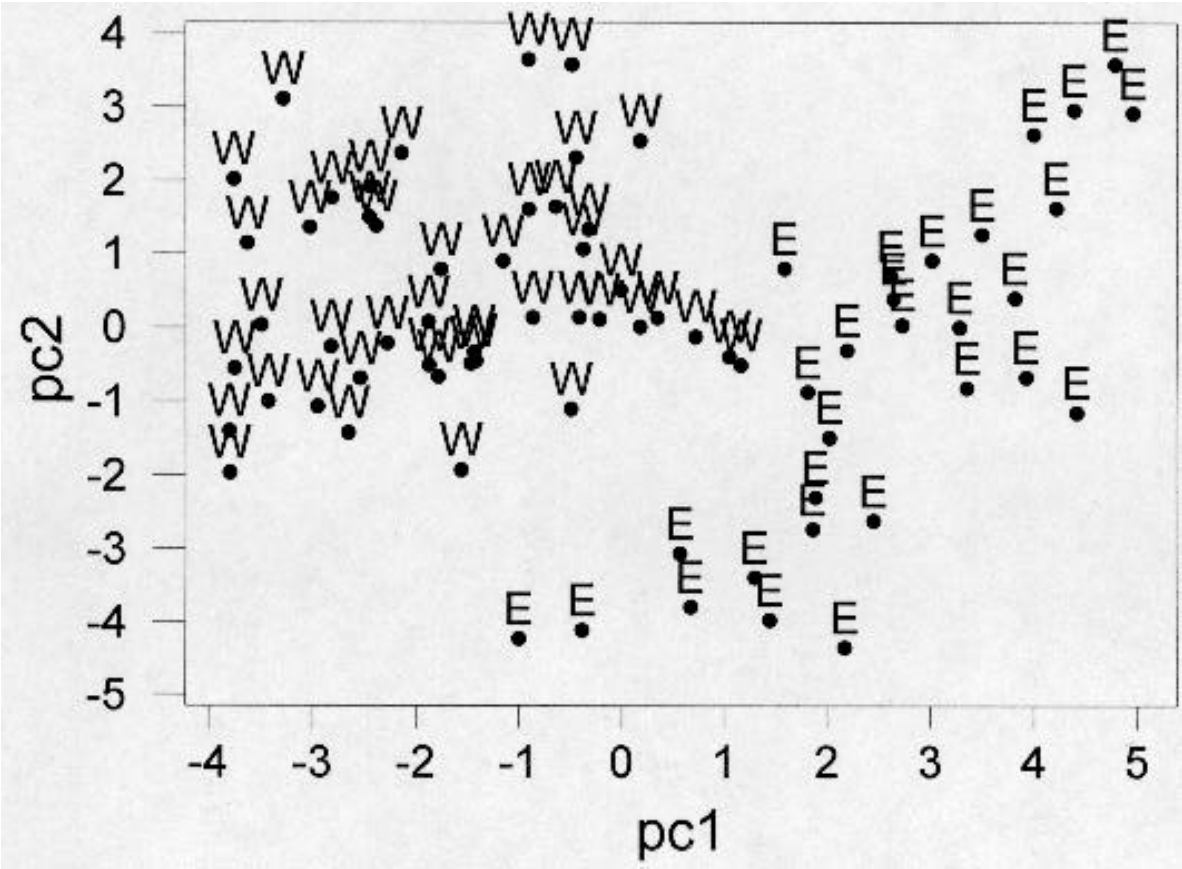

Figure 5. Two-dimensional representation of the principal components analysis of individual specimens (OTUs) for which data were available for all 32 morphological characters and character ratios. Comparison of principal components one and two. E indicates OUTs from eastern populations and W indicates OTUs from Western populations.

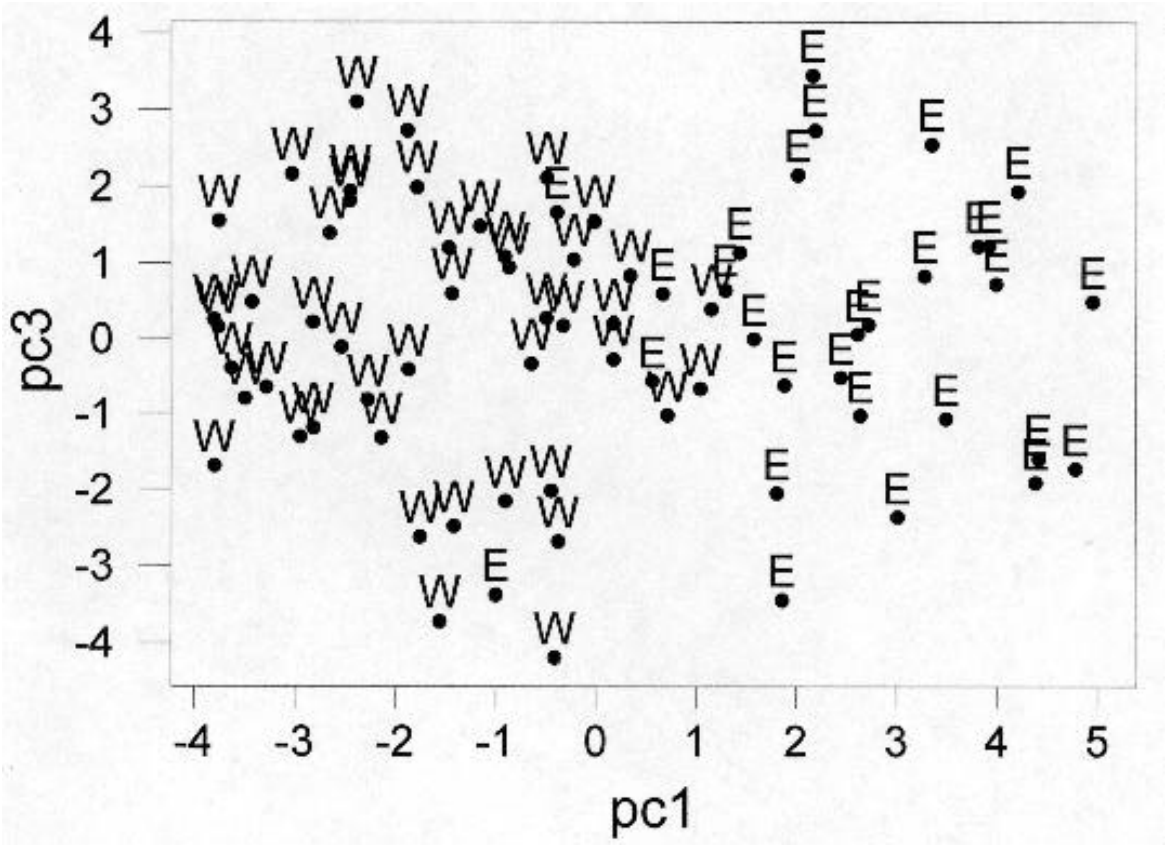

Figure 6. Two-dimensional representation of the principal components analysis of individual specimens (OTUs) for which data were available for all 32 morphological characters and character ratios. Comparison of principal components one and three. E indicates OTUs from eastern populations and W indicates OTUs from western populations.

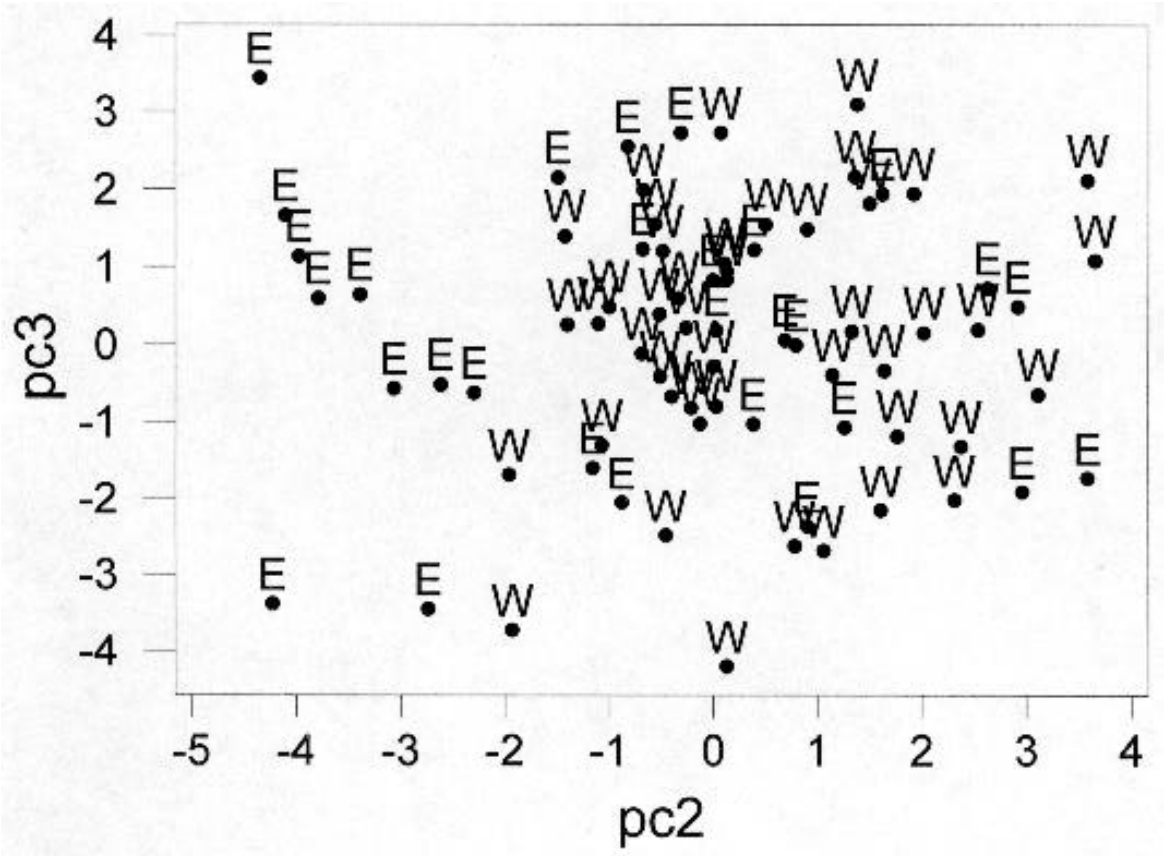

Figure 7. Two-dimensional representation of the principal components analysis of individual specimens (OTUs) for which data were available for all 32 morphological characters and character ratios. Comparison of principal components two and three. E indicates OTUs from eastern populations and W indicates OTUs from western populations.

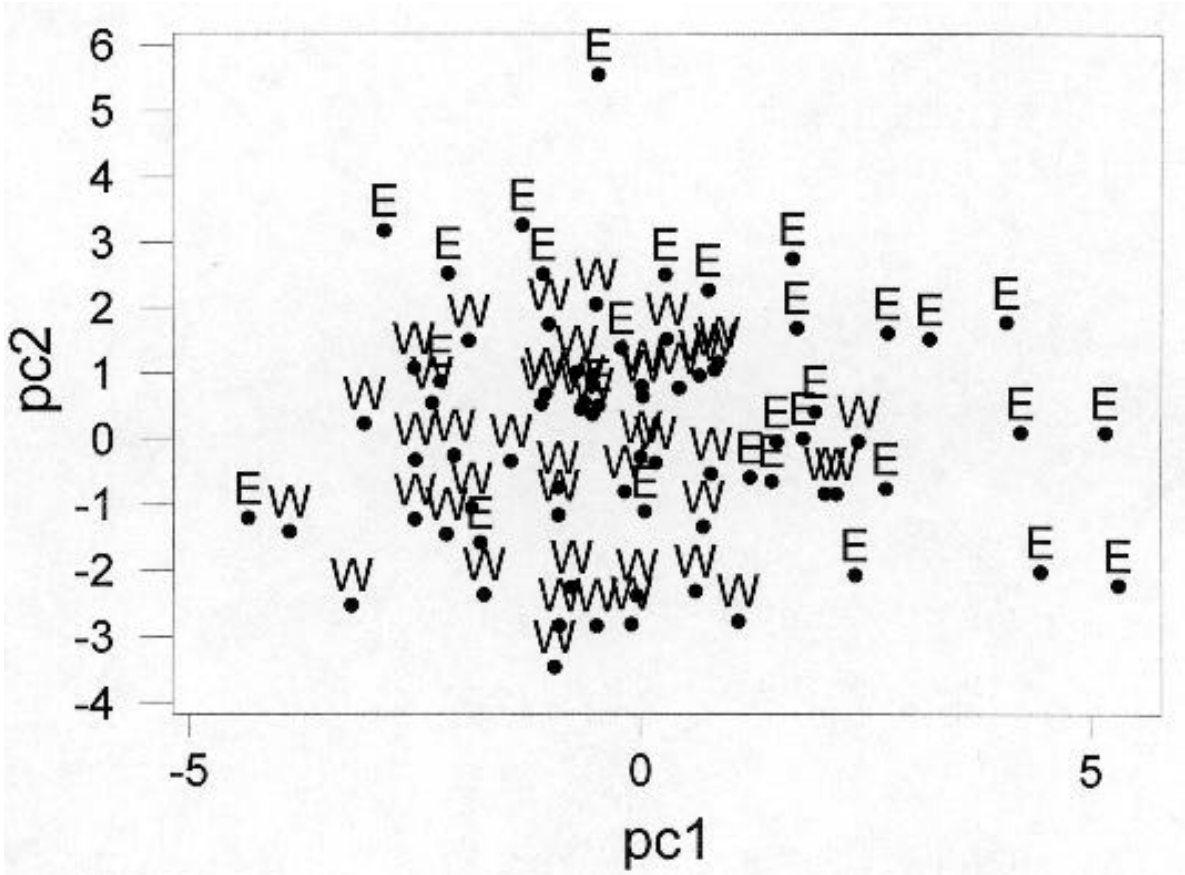

Figure 8. Two-dimensional representation of the principal components analysis of individual specimens (OTUs) for which data were available for all quantitative morphological characters and character ratios. Comparison of principal components one and two. E indicates OTUs from eastern populations and W indicates OTUs from western populations.

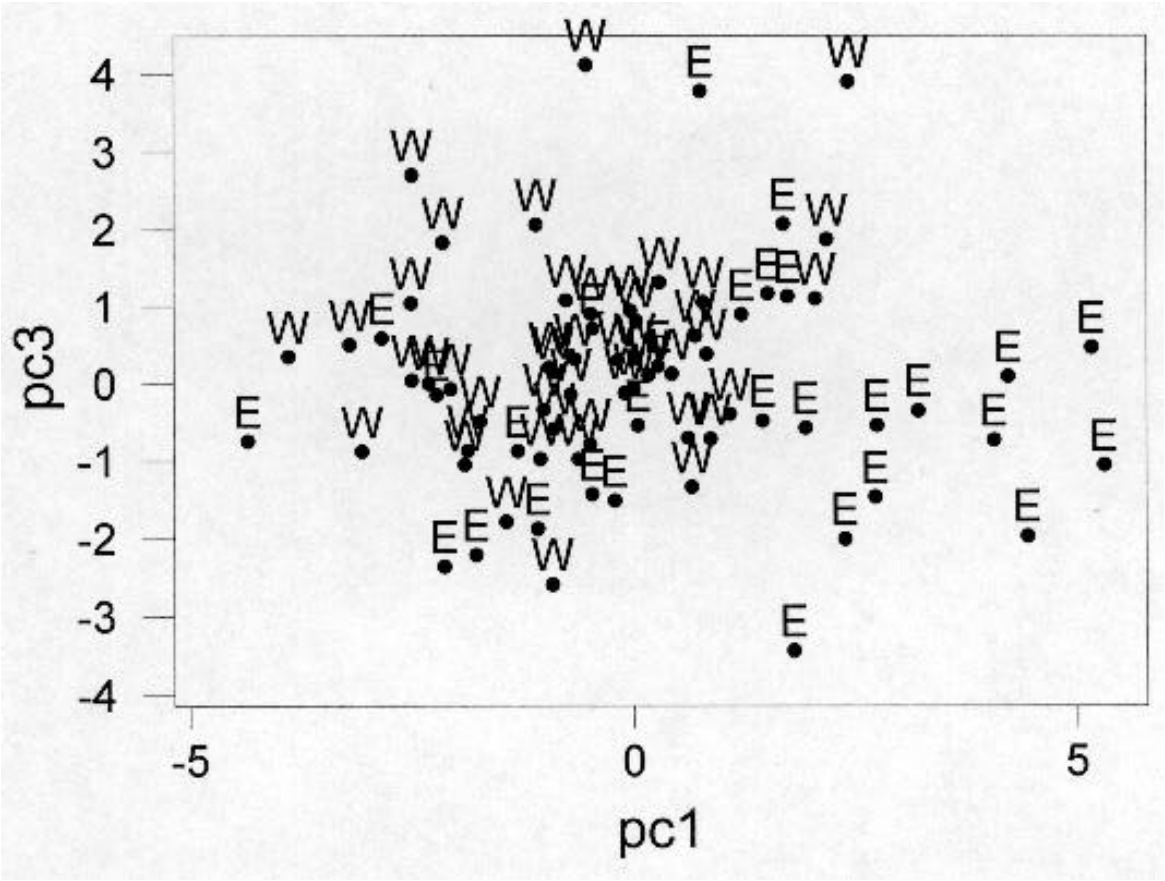

Figure 9. Two-dimensional representation of the principal components analysis of individual specimens (OTUs) for which data were available for all quantitative morphological characters and character ratios. Comparison of principal components one and three. E indicates OTUs from eastern populations and W indicates OTUs from western populations.

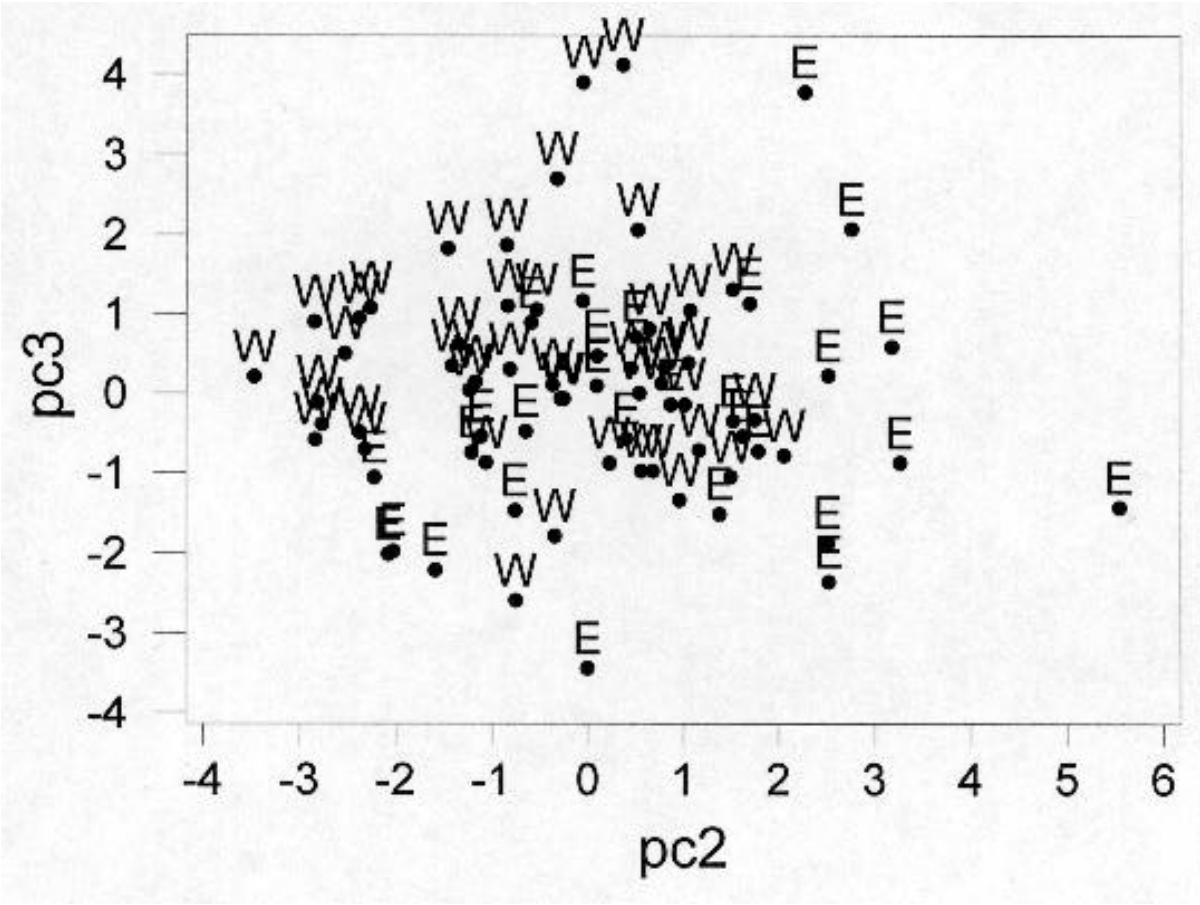

Figure 10. Two-dimensional representation of the principal components analysis of individual specimens (OTUs) for which data were available for all quantitative morphological characters and character ratios. Comparison of principal components two and three. E indicates OTUs from eastern populations and W indicates OTUs from western populations.

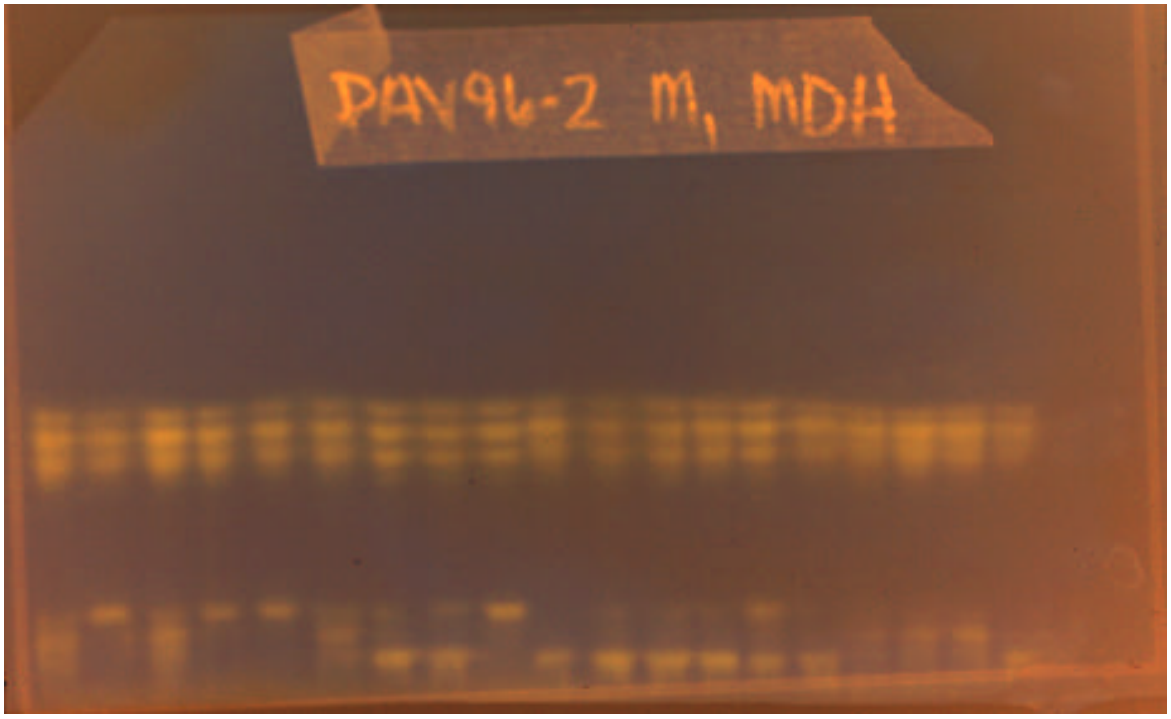

Figure 11. Photograph of an electrophoretic gel on which the enzyme malate dehydrogenase (MDH) was run using a morpholine buffer system. Notice the presence of two loci, MDH-1 (monomorphic and towards top of photo) and MDH-2 (polymorphic and towards bottom of photo).

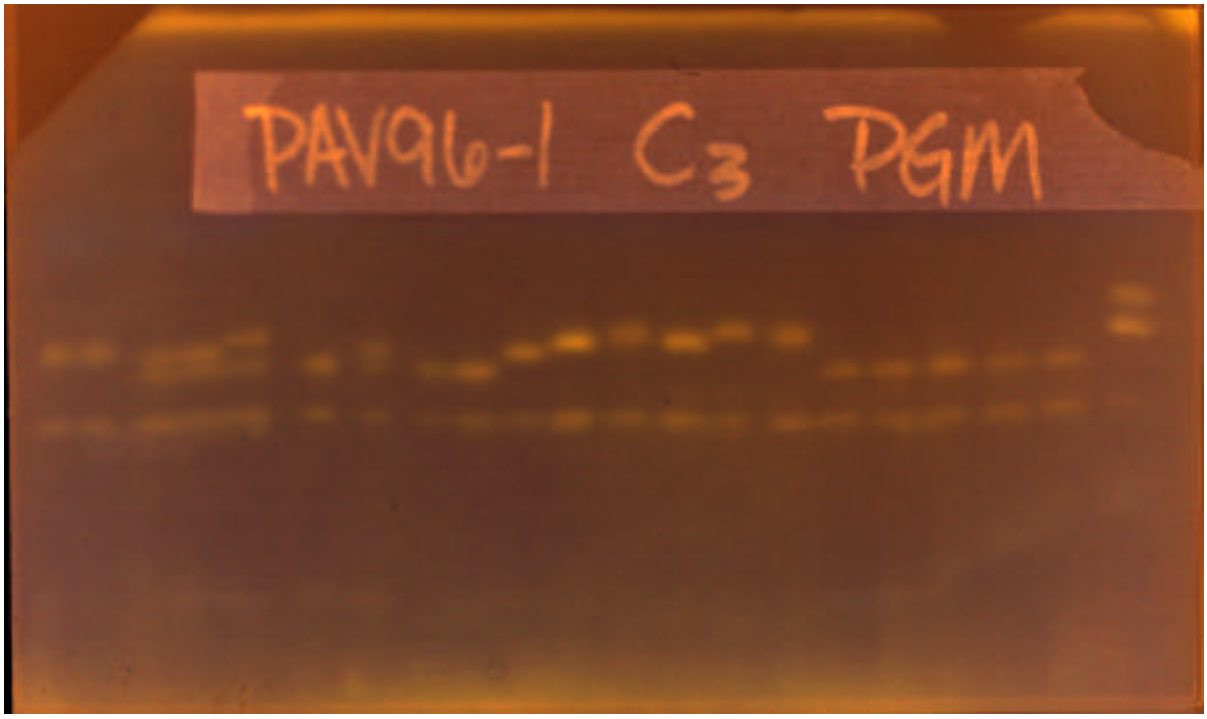

Figure 12. Photograph of an electrophoretic gel on which the enzyme phosphoglucomutase (PGM) was run using a system #6 buffer system. Notice the presence of two loci, PGM-1 (towards top of photo) and PGM-2 (towards bottom of photo).

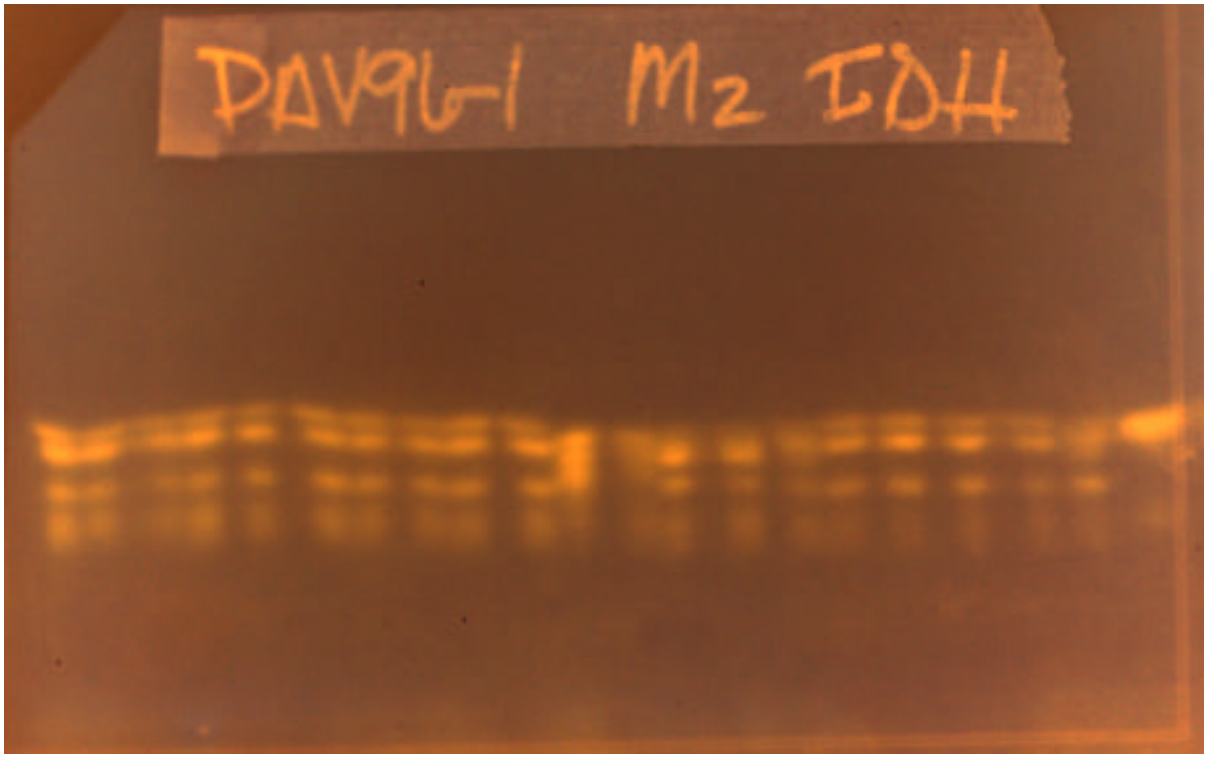

Figure 13. Photograph of an electrophoretic gel on which the enzyme isocitrate dehydrogenase (IDH) was run using a morpholine buffer system. IDH represents an enzyme with a complex banding pattern that prevented reliable scoring and interpretation.

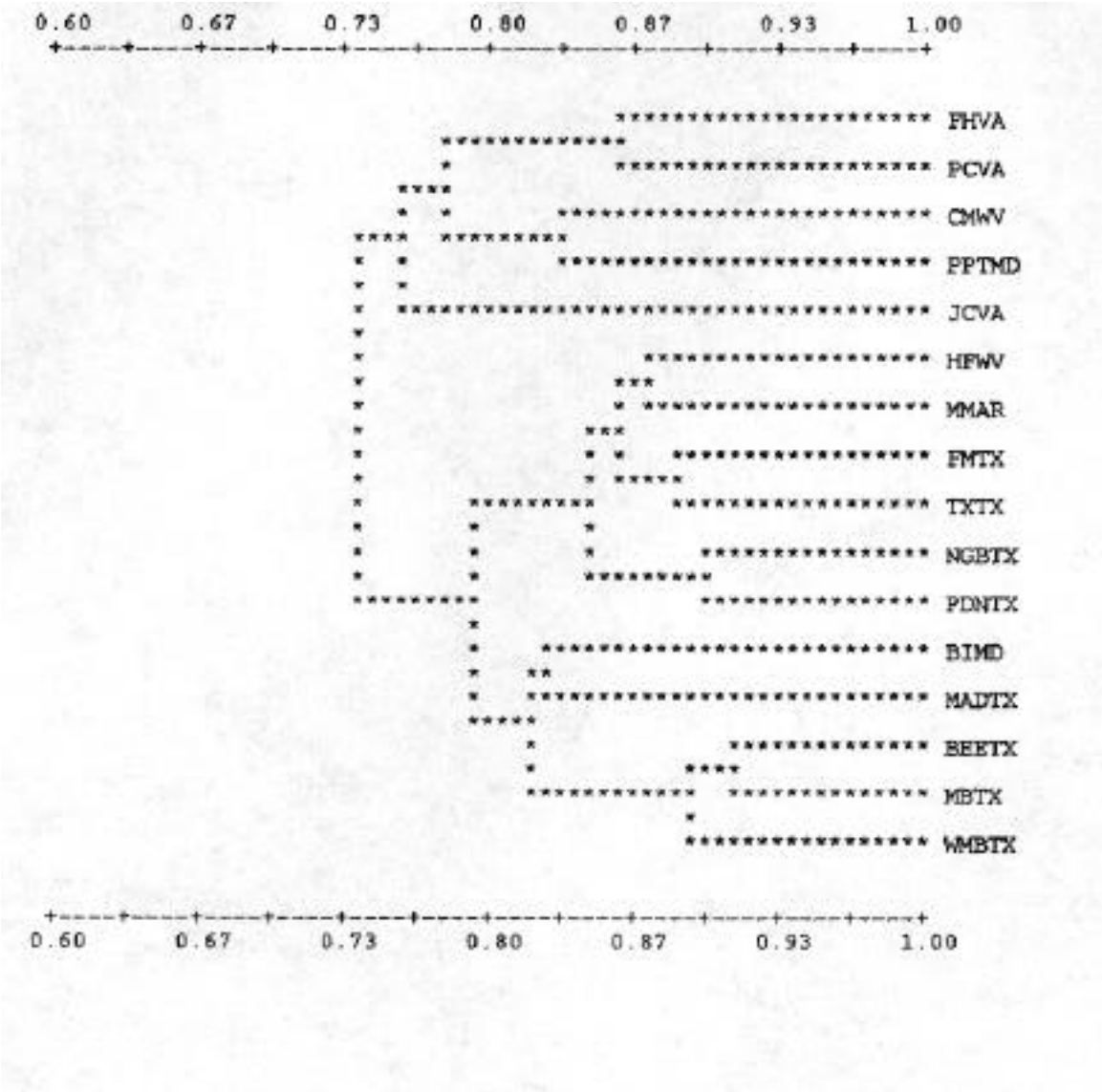

Figure 14. Cluster analysis of allozyme data using unweighted pair group method. Populations (OTUs) were clustered according to their pairwise values for Rogers (1972) Genetic Similarity S.

Table 1. Information on sites from which samples of *Paronychia virginica* Spreng. were obtained

Name of site	Population no./ Name abbreviation	Location	No. of plants in population	No. of plants examined (Morphometric study)[a]	No. Plants Examined (Allozyme study)[b]
Bear Island	7/BI	Montgomery Co., MD	22-25	5	12
Paw Paw Tunnel	6/PPT	Allegany Co., MD	75-100	10	20
Harpers Ferry	4/HF	Jefferson Co., WV	500+	0	38
Cave Mountain	5/CM	Grant Co., WV	1000+	10	18
Ford Hollow	1/FH	Alleghany Co., VA	75	10	44
Johnsons Creek	3/JC	Alleghany Co., VA	10	0	6
Potts Creek	2/PC	Alleghany Co., VA	35	10	20
Magazine Mountain	8/MM	Logan Co., AR	100+	0	33
Bee Cave	13/BEE	Travis Co., TX	50	10	45
Farm Road 1220	9/FM1220	Tarrant Co., TX	3/20[c]	10	3
Madrone	10/MAD	Travis Co., TX	28	0	11
Mount Bonnell	15/MB	Travis Co., TX	50/10[c]	4	31
National Guard Base	11/NGB	Wise Co., TX	14	0	14
Peden Road	16/PDN	Tarrant Co., TX	200+	10	44
Texas Route 121	12/TX121	Collin Co., TX	300+	10	36
Wimberley	14/WMB	Hays Co., TX	22/10[c]	6	28
Windmill Run Park	na/WRP	Travis Co., TX	75	10	0
TOTAL	---	---	---	105	403

[a] Desired sample size was ten individuals per population. Sample sizes lower than ten indicate that either fewer than ten plants were flowering at the time of collection or that the population was not sampled (a value of zero).

[b] Desired sample size was twenty individuals per population. Sample sizes lower than twenty indicate that either few than twenty plants could be located at the site, plants became unusable after collection (i.e., rotting), or that the population was not sampled (a value of zero).

[c] The first number indicates the number of plants observed at the site on the first collecting trip. Only vegetative material for use in the molecular analysis was collected at that time. The second number indicates the number of plants observed to be flowering during the second collecting trip; however, not all plants may have been flowering at that time.

Table 2. Characters used in the morphometric analysis of *Paronychia virginica* Spreng. Numbers used to refer to individual characters in the text are indicated in the first column. N = quantitative character; L = qualitative character. V = vegetative characters; F = floral character.

No.	N/L	V/F	Description of Character
1	N	V	Average length of intrnodes on vegetative stem (avg. of 5th and 10th) (mm)
2	N	V	Average length of internodes on reproductive stem (avg of 5th and 10th) (mm)
3	N	V	Ratio of average lengths of internodes (repro:veg)
4	L	V	Degree of pubescence on vegetative stem 0 (pubescence absent)-5 (fully pubescent)
5	L	V	Degree of pubescence on reproductive stem 0 (pubescence absent)-5 (fully pubescent)
6	N	V	Length of longest trichome present on reproductive stem (mm)
7	N	V	Width of vegetative stem at center of internode mid-way down stem (mm)
8	N	V	Width of reproductive stem at center of internode mid-way down stem (mm)
9	L	V	Shape of leaf (on vegetative stem) apex 0-apiculate; 1-apiculate to mucronate; 2-mucronate
10	L	V	Degree of pubescence on vegetative leaf; 0 pubescence absent-5 (fully pubescent)
11	N	V	Length-width ratio of longest leaf on vegetative stem
12	N	V	Length-width ratio of longest leaf on reproductive stem
13	N	V	Length-width ratio of random stipule from apical clump of leaves on vegetative stem
14	N	V	Length-width ratio of stipule occurring at base of first dichotomous branch of inflorescence
15	N	V	Length ratio of vegetative leaf:stipule
16	N	V	Length ratio of leaves occurring on vegetative stems:reproductive stems
17	N	F	Length of sepal including awn (mm)
18	N	F	Width of sepal (mm)
19	L	F	Sepal pubescence 0-no pubescence; 1-sparsely pubescent; 2-moderately pubscent; 3-extremely pubescent
20	N	F	Length of awn (mm)
21	L	F	Awn pubescence 0-no pubescence; 1-sparsely pubescent; 2-moderately pubescent; 3-extremely pubescent
22	L	F	Awn curvature 0-no backcurve; 1-slightly/moderately backcurved; 2-extremely backcurved
23	N	F	Length ratio of total sepal:awn
24	N	F	Length-width ratio of petal
25	N	F	Anther length (mm)
26	N	F	Filament length without anther (mm)
27	N	F	Length ratio of sepal (without awn):filament
28	N	F	Length of style from attachment to ovary to tip of stigma (mm)
29	N	F	Length ratio of style:filament
30	L	F	Presence/absence of dichotomously forked style 0-absent; 1-present
31	N	F	Length of fork of style (mm)
32	N	F	Length ratio of style:fork

Table 3. Summary of enzymes assayed in Paronychia virginica (after Pasteur et al., 1988 and Kephart, 1990).

Abbreviation of Enzyme	Name of Enzyme	Buffer System(s) Used to Resolve Enzyme Loci[a]	Structure	Localization	Presumed Function/Types of Reactions Involved In
ACPH	Acid phosphatase	Morph, HC	Monomeric or dimeric	Varies	Hydrolyzes phosphate esters
ALD	Aldolase	Morph	Tetrameric	Cytosol, plastid	Glycolosis, Calvin cycle
EST[b]	Esterase	LiOH, #6[c]	Monomeric, sometimes dimeric, many loci	Cytosol	Hydrolyzes ester linkages, in vitro
GOT	Glutamic oxaloacetic transaminase	LiOH, #6	Dimeric	Cytosol, plastid, mitochondrion, microbody	Metabolizes amino acids
HK	Hexokinase	LiOH	Monomeric	Cytosol, plastid, mitochondrion	Glycolosis
IDH	Isocitrate dehydrogenase	Morph, HC	Dimeric	Cytosol, plastd	Citric acid cycle
LAP[b]	Leucine aminopeptidase	LiOH[c], #6	Monomeric	Cytosol	Catabolyzes proteins
MDH[b]	Malate dehydrogenase	Morph[c], HC	Dimeric	Cytosol, mitochondrion, microbody	Citric acid cycle, photorespiration, C4 photosynthesis
6P	6-Phosphogluconate dehydrogenase	Morph, HC	Dimeric	Cytosol, plastid	Pentose phosphate pathway
PGI[b]	Phosphoglucoisomerase	LiOH[c], #6	Dimeric	Cytosol, plastid	Glycolysis, starch synthesis
PGM[b]	Phosphoglucomutase	LiOH, #6[c]	Monomeric, frequent sub-bands	Cytosol, Plastid	Conversion of starches to sugars
SKDH[b]	Shikimic dehydrogenase	Morph[c], HC	Monomeric	Cytosol, plastid	Synthesis of aromatic compounds
TPI	Triose phosphate isomerase	LiOH, #6	Dimeric	Cytosol, plastid	Glycolysis, Calvin cycle

[a] *Buffer systems are abbreviated as follows: Lithium hydroxide (LiOH), System #6 (#6), Morpholine (Morph), Histidine citrate (HC).*

[b] *Enzymes from which consistently scorable and interpretable bands were produced. All data in the current paper are derived from these enzymes.*

[c] *Buffer systems used to resolved the consistently informative enzyme loci.*

Table 11. Values of the fixation index (inbreeding coefficient) F. Conformance to Hardy-Weinberg equilibrium was tested using chi-square analysis and is indicated by asterisks: --- - no significant deviation from Hardy-Weinberg equilibrium; ns - not significant; * - P ≤ 0.05; ** - P ≤ 0.01; * - P ≤ 0.001.**

Population	EST-2	LAP	MDH-2	PGM-1	PGM-2	SKDH
FH	-0.011ns	0.343**	0.166ns	0.307**	---	-0.030ns
PC	0.608ns	0.893ns	0.560ns	0.647**	-0.818ns	---
JC	---	---	---	---	---	---
HF	---	0.114ns	-0.233ns	0.374ns	---	-0.152ns
CM	-0.076ns	-0.006ns	-0.029ns	0.364*	---	---
PPT	---	-0.111ns	0.327ns	-0.081ns	---	---
BI	0.011ns	0.517ns	-0.023ns	0.111ns	---	---
MM	0.371*	0.538***	0.147ns	---	0.224ns	-0.245ns
FM1220	-0.500ns	0.333ns	1.000ns	-0.200ns	---	---
MAD	0.417ns	-0.119ns	-0.158ns	0.868***	0.614ns	---
NGB	-0.167ns	---	0.152ns	---	-0.077ns	-0.200ns
TX121	0.409*	0.144ns	-0.162ns	0.719***	0.219ns	-0.014ns
BEE	0.459***	0.271ns	0.451ns	1.000ns	0.480**	---
WMB	0.576***	0.482**	0.780ns	1.000ns	0.280ns	0.012ns
MB	0.792***	0.118*	-0.033ns	1.000ns	0.026ns	0.333*
PDN	0.327*	0.198ns	-0.110ns	---	0.344ns	0.336***

Table 15. Matrix of pairwise values for Rogers's (1972) Genetic Similarity S (above diagonal) and Nei's (1978) Genetic Identity I (below diagonal).

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 FH	*****	0.863	0.734	0.827	0.774	0.794	0.786	0.763	0.779	0.729	0.783	0.792	0.781	0.741	0.737	0.768
2 PC	0.052	*****	0.754	0.799	0.796	0.756	0.740	0.767	0.790	0.688	0.765	0.756	0.737	0.679	0.693	0.797
3 JC	0.198	0.140	*****	0.728	0.741	0.801	0.769	0.670	0.754	0.731	0.820	0.753	0.764	0.704	0.725	0.735
4 HF	0.096	0.116	0.246	*****	0.791	0.761	0.842	0.876	0.858	0.737	0.779	0.853	0.801	0.759	0.744	0.823
5 CM	0.138	0.098	0.216	0.125	*****	0.834	0.754	0.785	0.789	0.695	0.694	0.776	0.703	0.653	0.674	0.756
6 PPT	0.138	0.147	0.188	0.198	0.085	*****	0.743	0.685	0.722	0.679	0.674	0.709	0.683	0.641	0.627	0.679
7 BI	0.117	0.197	0.192	0.072	0.200	0.215	*****	0.766	0.804	0.823	0.800	0.869	0.852	0.810	0.787	0.757
8 MM	0.137	0.158	0.305	0.038	0.141	0.286	0.152	*****	0.877	0.716	0.827	0.858	0.802	0.754	0.786	0.891
9 FM1220	0.109	0.125	0.174	0.030	0.118	0.232	0.092	0.007	*****	0.776	0.838	0.887	0.822	0.776	0.816	0.888
10 MAD	0.192	0.242	0.224	0.189	0.280	0.300	0.106	0.236	0.141	*****	0.748	0.801	0.820	0.833	0.805	0.718
11 NGB	0.129	0.161	0.135	0.134	0.250	0.326	0.145	0.102	0.032	0.202	*****	0.853	0.856	0.776	0.811	0.897
12 TX121	0.126	0.161	0.170	0.051	0.162	0.260	0.041	0.053	0.009	0.129	0.049	*****	0.881	0.810	0.843	0.863
13 BEE	0.112	0.192	0.156	0.113	0.247	0.280	0.052	0.126	0.057	0.089	0.051	0.034	*****	0.900	0.913	0.832
14 WMB	0.159	0.257	0.228	0.160	0.315	0.332	0.081	0.178	0.108	0.060	0.131	0.085	0.021	*****	0.887	0.758
15 MB	0.151	0.233	0.190	0.172	0.289	0.349	0.090	0.144	0.071	0.102	0.067	0.053	0.019	0.030	*****	0.798
16 PDN	0.130	0.139	0.206	0.083	0.161	0.295	0.165	0.035	0.004	0.228	0.027	0.042	0.083	0.153	0.101	*****

Table 4. Roster of alleles scored for in the allozyme analysis of *Paronychia virginica* Spreng.

Locus	Allele Number	Allele Letter	Population(s) of Occurrence
Est-2	1+	A	WMB
	1	B	PC,NGB, MM,TX,BEE, WMB,BI,CM
	2	C	FH,PC,JC,HF,FM,MAD,NGB,MM,TX,BEE, WMB,BI,CM,PPT
	3+	D	BEE
	3	E	FH,FM,MAD,MM,TX,BEE,WMB,BI, CM
	4+	F	BEE
	4	G	BEE
Est-3	2	A	BEE,BI,CM,FH,FM,HF,JC,MAD,MB, MM,NGB,PC,PDN,TX,WMB,PPT
Lap	1+	A	HF
	1	B	PPT,BI,CM,FH,PC,HF,BEE,MB,PDN
	2+	C	MM,PDN
	2	D	PPT,BI,CM,FH,PC,JC,HF,FM,NGB, MM,TX,BEE,WMB,MB,PDN
	3+	E	CM,MM,BEE,MB,PDN
	3	F	BI,CM,FH,HF,FM,MAD,MM,TX,BEE, WMB,MB,PDN
	3-	G	WMB
	4+	H	FM,MAD,BEE,WMB,MB,PDN
	4	I	MAD,TX,BEE,WMB,MB
	5	J	TX,WMB,MB
	6	K	BEE
Mdh-1	1	A	BEE,BI,CM,FH,FM,HF,JC,MAD,MB,MM, NGB,PC,PDN,TX,WMB,PPT
Mdh-2	1	A	FH,PC,JC,HF,FM,MAD,NGB,MM,TX,BEE, WMB,BI,CM
	2+	B	TX
	2	C	FH,PC,HF,FM,MAD,TX,BEE,WMB,BI, CM,PPT
	3	D	NGB,MM,PPT
	4	E	PPT
Pgi	2	A	BEE,BI,CM,FH,FM,HF,JC,MAD,MB, MM,NGB,PC,PDN,TX,WMB,PPT
Pgm-1	1	A	FH,HF,FM,MAD,TX,BI,CM,PPT
	2	B	FH,PC,HF,FM,MAD,NGB,MM,TX, BEE,WMB,BI,CM
	3+	C	HF
	3	D	FH,PC,HF,MAD,TX
	4	E	FH,PC,JC,MAD,CM,PPT
Pgm-2	1	A	MAD,NGB,MM,TX,BEE,WMB
	2+	B	MM
	2	C	FH,PC,JC,HF,FM,MAD,NGB,MM TX, BEE,WMB,BI,CM
	3	D	TX,BEE,WMB
Skdh	1+	A	NGB
	1	B	BEE,WMB
	2	C	FH,PC,JC,HF,FM,MAD,NGB,MM,TX, BEE,WMB,MB,BI,CM
	3+	D	FH
	3	E	FH,PC,HF,NGB,MM,TX,WMB,MB

Table 5. Results of the ANOVA for all characters used in the morphometric study of *Paronychia virginica*. Characters are listed in descending order according to respective F values. See Table 2 for a complete list of characters and their associated number. ns - not significant; * - P £ 0.05; ** - P £ 0.01; *** - P £ 0.001

Character No.	F value	P value
19	261.95	0.000***
21	93.75	0.000***
10	67.97	0.000***
4	57.92	0.000***
11	39.21	0.000***
22	36.67	0.000***
12	30.37	0.000***
5	19.82	0.000***
9	13.45	0.000***
14	10.41	0.002**
2	10.27	0.002**
8	8.38	0.005**
1	7.82	0.007**
32	7.69	0.007**
26	7.56	0.008**
20	7.08	0.010**
17	5.70	0.020*
28	5.34	0.024*
27	4.05	0.048*
18	2.85	0.095ns
13	2.43	0.123ns
25	2.43	0.123ns
16	1.97	0.165ns
7	1.24	0.269ns
15	0.78	0.380ns
6	0.27	0.604ns
3	0.23	0.635ns
29	0.08	0.783ns
31	0.04	0.836ns
23	0.00	0.977ns
24	0.00	0.982ns
30	NA■	NA■

■ Character 30 (presence or absence of a dichotomously forked style) was not used in the ANOVA because a score of "0" or absent, automatically invalidated characters 31 and 32, decreasing the number of characters upon which to base the analysis of variance.

Table 6. Results of the MANOVA for all characters used in the morphometric study of *Paronychia virginica*. ns - not significant; * - P ≤ 0.05; ** - P ≤ 0.01; * - P ≤ 0.001.**

Criterion	Test Statistic	F value	P value
Wilk's[a]	0.07908	16.153	0.000***
Lawley-Hotelling[a]	11.64546	16.153	0.000***
Pillai's[a]	0.92092	16.153	0.000***
Roy's[a]	11.64546		

[a] See Johnson and Wichern (1992).

Table 7. Results of the ANOVA for only quantitative characters used in the morphometric study of *Paronychia virginica.* Characters are listed in descending order according to their respective F values. See Table 2 for a complete list of characters and their associated number. ns - not significant; * - P ≤ 0.05; ** - P ≤ 0.01; * - P ≤ 0.001.**

Character No.	F value	P value
11	39.20	0.000***
12	30.37	0.000***
14	10.41	0.002**
2	10.27	0.002**
8	8.38	0.005**
1	7.82	0.007**
32	7.69	0.007**
26	7.56	0.008**
20	7.08	0.010**
17	5.70	0.020*
28	5.34	0.024*
27	4.05	0.048*
18	2.85	0.095ns
13	2.43	0.123ns
25	2.43	0.123ns
16	1.97	0.165ns
7	1.24	0.269ns
15	0.78	0.380ns
6	0.27	0.604ns
3	0.23	0.635ns
29	0.08	0.783ns
31	0.04	0.836ns
23	0.00	0.977ns
24	0.00	0.982ns

Table 8. Results of the MANOVA for only quantitative characters used in the morphometric study of *Paronychia virginica*. ns - not significant; * - P ≤ 0.05; ** - P ≤ 0.01; * - P ≤ 0.001.**

Criterion	Test Statistic	F value	P value
Wilk's[a]	0.22746	7.076	0.000***
Lawley-Hotelling[a]	3.39642	7.076	0.000***
Pillai's[a]	0.77254	7.076	0.000***
Roy's[a]	3.39642		

[a] See Johnson and Wichern (1992).

Table 9. Allele frequencies in populations 1 through 16.

	Population								
Locus	1	2	3	4	5	6	7	8	9
Est2									
(N)	44	20	6	38	17	20	11	33	3
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.150	0.000	0.000	0.412	0.000	0.182	0.121	0.000
C	0.989	0.850	1.000	1.000	0.529	1.000	0.773	0.682	0.667
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.011	0.000	0.000	0.000	0.059	0.000	0.045	0.197	0.333
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Est3									
(N)	44	20	6	38	18	20	12	33	3
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lap									
(N)	44	20	6	38	18	20	12	33	3
A	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000
B	0.750	0.375	0.000	0.118	0.639	0.900	0.292	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000
D	0.239	0.625	1.000	0.263	0.306	0.100	0.083	0.394	0.667
E	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.182	0.000
F	0.011	0.000	0.000	0.566	0.028	0.000	0.625	0.394	0.167
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh1									
(N)	44	20	6	38	18	20	12	33	3
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh2									
(N)	44	20	6	37	18	18	8	33	3
A	0.568	0.350	1.000	0.189	0.028	0.000	0.813	0.121	0.333
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.432	0.650	0.000	0.811	0.972	0.278	0.188	0.879	0.667
D	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.389	0.000	0.000	0.000
Pgi									
(N)	44	20	6	38	18	20	12	33	3
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm1									
(N)	44	20	6	38	18	20	12	33	3
A	0.091	0.000	0.000	0.303	0.028	0.075	0.625	0.000	0.167
B	0.500	0.075	0.000	0.553	0.194	0.000	0.375	1.000	0.833
C	0.000	0.000	0.000	0.026	0.056	0.000	0.000	0.000	0.000
D	0.239	0.450	0.000	0.118	0.000	0.000	0.000	0.000	0.000
E	0.170	0.475	1.000	0.000	0.722	0.925	0.000	0.000	0.000
Pgm2									
(N)	44	20	6	38	18	20	12	33	3
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.061	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.894	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skdh									
(N)	44	20	6	38	18	20	12	33	3
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.489	0.450	1.000	0.868	1.000	1.000	1.000	0.803	1.000
D	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.455	0.550	0.000	0.132	0.000	0.000	0.000	0.197	0.000

Table 9 continued.

	Population						
Locus	10	11	12	13	14	15	16
Est2							
(N)	7	7	36	45	27	31	37
A	0.000	0.000	0.000	0.000	0.019	0.000	0.000
B	0.000	0.143	0.292	0.156	0.019	0.129	0.257
C	0.571	0.857	0.625	0.778	0.704	0.468	0.730
D	0.000	0.000	0.000	0.011	0.000	0.000	0.000
E	0.429	0.000	0.083	0.033	0.259	0.403	0.014
F	0.000	0.000	0.000	0.011	0.000	0.000	0.000
G	0.000	0.000	0.000	0.011	0.000	0.000	0.000
Est3							
(N)	11	14	36	45	28	31	44
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lap							
(N)	11	14	36	44	27	31	40
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.057	0.000	0.097	0.012
C	0.000	0.000	0.000	0.000	0.000	0.000	0.063
D	0.000	1.000	0.486	0.318	0.037	0.403	0.825
E	0.000	0.000	0.000	0.034	0.000	0.081	0.025
F	0.045	0.000	0.458	0.182	0.167	0.177	0.037
G	0.000	0.000	0.000	0.000	0.019	0.000	0.000
H	0.091	0.000	0.000	0.239	0.389	0.113	0.037
I	0.864	0.000	0.014	0.159	0.315	0.081	0.000
J	0.000	0.000	0.042	0.000	0.074	0.048	0.000
K	0.000	0.000	0.000	0.011	0.000	0.000	0.000
Mdh1							
(N)	11	14	36	45	28	31	44
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh2							
(N)	11	14	36	45	28	31	44
A	0.864	0.786	0.597	0.911	0.911	0.968	0.352
B	0.000	0.000	0.028	0.000	0.000	0.000	0.000
C	0.136	0.214	0.375	0.089	0.089	0.032	0.636
D	0.000	0.000	0.000	0.000	0.000	0.000	0.011
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgi							
(N)	11	14	36	45	28	31	44
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm1							
(N)	11	14	36	23	16	19	9
A	0.364	0.000	0.250	0.261	0.375	0.211	0.000
B	0.273	1.000	0.736	0.739	0.625	0.789	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.318	0.000	0.014	0.000	0.000	0.000	0.000
E	0.045	0.000	0.000	0.000	0.000	0.000	0.000
Pgm2							
(N)	11	14	36	35	27	19	44
A	0.136	0.071	0.083	0.171	0.370	0.316	0.193
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.864	0.929	0.903	0.800	0.537	0.684	0.807
D	0.000	0.000	0.014	0.029	0.093	0.000	0.000
Skdh							
(N)	11	14	36	45	20	31	44
A	0.000	0.143	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.211	0.150	0.242	0.023
C	1.000	0.786	0.986	0.789	0.750	0.710	0.841
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.071	0.014	0.000	0.100	0.048	0.136

Table 10. Estimates of genetic variability at 9 loci in 16 populations of *Paronychia virginica* (standard errors in parentheses).

Population	Mean sample size per Locus N	Mean no. of alleles per locus A	Percentage of loci polymorphic[a] P	Mean heterozygosity: Direct-count H_{obs}	Mean heterozygosity: HdyWbg expected[b] H_{exp}
1. FH	44.0 (0.0)	2.0 (0.4)	44.4	0.189 (0.078)	0.236 (0.095)
2. PC	20.0 (0.0)	1.7 (0.2)	55.6	0.161 (0.096)	0.255 (0.085)
3. JC	6.0 (0.0)	1.0 (0.0)	0.0	0.000 (0.000)	0.000 (0.000)
4. HF	37.9 (0.1)	1.9 (0.4)	44.4	0.171 (0.071)	0.193 (0.086)
5. CM	17.9 (0.1)	2.0 (0.4)	33.3	0.158 (0.079)	0.175 (0.084)
6. PPT	19.8 (0.2)	1.4 (0.2)	33.3	0.088 (0.051)	0.112 (0.075)
7. BI	11.4 (0.4)	1.7 (0.3)	44.4	0.156 (0.063)	0.193 (0.079)
8. MM	33.0 (0.0)	2.0 (0.4)	55.6	0.148 (0.052)	0.210 (0.081)
9. FM1220	3.0 (0.0)	1.6 (0.2)	44.4	0.148 (0.081)	0.222 (0.091)
10. MAD	10.6 (0.4)	1.9 (0.4)	55.6	0.113 (0.043)	0.222 (0.087)
11. NGB	13.2 (0.8)	1.6 (0.2)	44.4	0.127 (0.056)	0.125 (0.054)
12. TX121	36.0 (0.0)	2.3 (0.4)	55.6	0.182 (0.074)	0.245 (0.084)
13. BEE	41.3 (2.5)	2.8 (0.7)	66.7	0.151 (0.065)	0.266 (0.086)
14. WMB	25.4 (1.5)	2.6 (0.6)	66.7	0.155 (0.063)	0.313 (0.092)
15. MB	28.3 (1.8)	2.4 (0.6)	55.6	0.179 (0.080)	0.298 (0.098)
16. PDN	38.9 (3.8)	2.3 (0.6)	55.6	0.159 (0.060)	0.199 (0.066)
AMONG POPULATION MEANS	24.2	1.95	47.22	0.143	0.204

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[b] Unbiased estimate (see Nei, 1978).

Table 12. Hierarchical analysis--variance components and F-statistics combined across loci.

Comparison X	Comparison Y	Variance component	F XY
LOCALITY	- STATE	0.60334	0.248
LOCALITY	- REGION	1.00410	0.354
LOCALITY	- TOTAL	0.91425	0.333
STATE	- REGION	0.40077	0.141
STATE	- TOTAL	0.31091	0.113
REGION	- TOTAL	-0.08985	-0.033

Table 13. F-statistics for 6 polymorphic loci in Paronychia virginica Spreng. Values of F(ST) were tested for statistical difference from 0 using contingency chi square analysis (Table 14). Significant values are indicated by asterisks: * - P ≤ 0.05; ** - P ≤ 0.01; * - P ≤ 0.001**

Locus	F(IS)	F(IT)	F(ST)
Est2	0.278	0.400	0.169***
Lap	0.299	0.574	0.392***
Mdh2	0.198	0.544	0.432***
Pgm1	0.555	0.760	0.461***
Pgm2	0.274	0.401	0.175***
Skdh	-0.106	0.149	0.231***
Mean	0.276	0.532	0.353***

Table 14. Contingency chi-square analysis at 6 polymorphic loci in 16 populations of Paronychia virgnica.

Locus	No. of alleles	Chi-square	D.F.	P
Est2	7	282.880	90	0.00000
Lap	11	1282.278	150	0.00000
Mdh2	5	878.405	60	0.00000
Pgm1	5	695.295	60	0.00000
Pgm2	4	191.804	45	0.00000
Skdh	5	459.009	60	0.00000
Totals		3789.671	465	0.00000

Vita

Wendy Lynn Rohrer grew up in the small farming community of Quarryville, Pennsylvania. Family vacations to the mountains of Lycoming and Tioga Counties, Pennsylvania, and Keene, New Hampshire, made a lasting impression and helped to determine the direction Wendy would choose to move in academically. Not until the second semester of a pre-medicine program at Wilson College, however, did she realize that her potential could be more fully realized and that her life could be more her own with a career in botany. After graduating from Wilson College with a B.S. in Biology in May of 1993, Wendy moved to Durango, Colorado, where she was employed as an instructor of laboratory chemistry at Fort Lewis College. Volunteering as naturalist and interpreter for both the San Juan National Forest Association and the Colorado Division of Wildlife enabled Wendy to remain active in the field of biology. Following her return to Pennsylvania, Wendy accepted a position teaching chemistry and organic chemistry at her Alma Mater, Wilson College. A volunteer position working with bluebirds at King's Gap Environmental Education and Training Center, Carlisle, Pennsylvania, once again offered her a chance to be in the field. After a two year break from life as a student, Wendy chose to attend Virginia Tech to pursue a Masters degree in Biology, focusing on Plant Systematics and Evolution. Wendy now plans to return to her roots and pursue a career in agriculture and plant breeding.