A Conservation Assessment of *Packera millefolium*, a Southern Appalachian Endemic

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Masters of Science in the Curriculum of Ecology.

Chapel Hill
2006

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ABSTRACT

Andrea Elizabeth Gramling: A Conservation Assessment of *Packera millefolium*, a Southern
Appalachian Endemic
(Under the direction of Robert K. Peet)

Packera millefolium is a rare species endemic to rock outcrops in the southern

Appalachians thought to hybridize with its more common congener, *P. anonyma*.

Morphological analyses and cross-pollination experiments were used to verify the existence

of the hybrid *Packera ×memmingeri*. Preliminary results show that introgression between *P.

millefolium* and *P. anonyma* is probable at some locations. Information on population size

and the presence or absence of hybrids was compiled for populations of *P. millefolium* from

historical records and on-site surveys. An analysis of this data shows that hybrids occur at

approximately 40% of the populations in North and South Carolina. The data also suggests

that anthropogenic disturbance of the landscape surrounding once isolated populations of *P.

millefolium* might have broken down the ecological barriers that historically prevented gene

flow between *P. millefolium* and *P. anonyma.* The management implications for *P.

millefolium* due to hybridization with *P. anonyma* are discussed.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

INTRODUCTION

Packera millefolium (Torr. & Gray) W.A. Weber & A. Löve has been described as "an enigmatic taxon; rare in occurrence, restricted in distribution, and with no obvious relatives in its general range" (Barkley 1968). *P. millefolium* is "one of the rarest and most highly endemic of native ragworts, "is confined to a small area of the Southern Appalachians, and exhibits "finely cut leaves," "free-flowering character," and "brilliant color" (Alexander 1937). It faces several threats to its continued persistence including loss of habitat due to development and loss of genetic identity due to introgression with its more common congener *Packera anonyma* (Wood) W.A. Weber & A. Löve (Kral 1983; Uttal 1984; NatureServe 2006).

The hybridization between *P. millefolium* and *P. anonyma* to form *Senecio memmingeri* Britton ex Small (here after referred to as *Packera ×memmingeri*) and the potential introgression between these species may have serious conservation implications for *Packera millefolium* due to possible "genetic swamping" (Uttal 1984). The extent of hybridization is unknown and confounded by intergrading species boundaries (Barkley 1988).

In the following assessment the threats to the continued persistence of this rare species will be evaluated by: 1) verifying the hybrid origin of *Packera ×memmingeri* via morphological data and cross-pollination experiments; 2) comparing historical occurrences of *P. millefolium* with present-day occurrences to gain a perspective on *P. millefolium*'s persistence in the region; and 3) describing some theoretical and practical concerns that may influence future conservation/restoration practices with this species.

I: Does hybridization occur between *Packera millefolium* and *Packera anonyma*?

Introduction

Packera: A history of hybridization and introgression

The genus *Packera* was separated from the super-genus *Senecio* in 1976 by A. Löve and D. Löve, and incorporates the informal "Aureoid" assemblage of *Senecio*. *Packera* is made distinct from the rest of the genus *Senecio* by sharing some or all of the following characters: perennial herbs arising from creeping rootstocks or a caudex; basal leaves well developed, cauline leaves progressively reduced upward; leaf margins without callose denticles; roots fibrous, thin, and branching; haploid chromosome numbers 22 or 23, or numbers derived there from (Freeman and Barkley 1995). *Packera* includes about 60 species, which range from Mexico to the Arctic and into eastern Siberia, but the majority of them are found in North America (Freeman And Barkley 1995). Recent molecular evidence strongly supports the separation of *Packera* from other North American members of *Senecio* s.l. (Bain and Golden 2000). In addition, it shows that the closest out groups to *Packera* (*Senecio jacobaea* and *Pericallis*) are Old World taxa (Bain and Golden 2000).

The separation of *Packera* from *Senecio sensu lato* does not make the relationships within the genus any clearer. The members of *Packera* have "achieved some notoriety as being difficult to define and distinguish from one another" (Yates et al. 1999). Delimitation of species within *Packera* is made difficult by widespread hybridization and introgression within the group (Freeman and Barkley 1995). Apparently, the combination of a "pliable" cytological structure (they are functioning as diploids, but are historically polyploid), obligate outbreeding, and good seed dispersal allows *Packera* species to hybridize and

introgress easily when their ranges overlap (Barkley 1988). It has even been suggested that the complex is behaving like a single highly variable biological species (Bain and Jansen 1996). Unusually high levels of intrapopulation cpDNA polymorphism within many species and populations of *Packera* support the idea that introgression is occurring today or has occurred in the past (Bain and Golden 2000).

Study Species

Packera millefolium ranges from the southern tip of Virginia, through North and South Carolina and into Georgia (Ogle 1991; NatureServe 2006). Plants are restricted to the sandy soils that form on and around granite or limestone outcrops, usually in full sun, in cracks, or in small depressions (NatureServe 2006). It is believed that *P. millefolium* is a disjunct that resulted from an eastward migration of a western progenitor, possibly during the last glaciation (Barkley 1988). *P. millefolium* is presently listed as critically imperiled in Georgia and Virginia and imperiled in North and South Carolina (NatureServe 2006).

Packera millefolium is best identified by its tri-pinnate basal leaves. Leaf segments are typically 1-3 mm wide contributing to the lacey texture of the leaves and suggesting the common names "thousand leaf" or "yarrow leaf" groundsel (Figure 1). *P. millefolium* blooms from late April to early June.

Packera anonyma is a common species that ranges from Pennsylvania to Florida and west into Louisiana. It can tolerate a wide range of environmental conditions, is associated with a variety of community types, and can be termed "weedy." Its range overlaps that of *P. millefolium*, and at some sites the two occur sympatrically.

Packera anonyma is distinguishable from *P. millefolium* by its basal leaves. *P. anonyma*'s basal leaves are petiolate with oblanceolate blades cuneate at their base (Figure 1). The leaves are also serrate. *P. anonyma* blooms from May to early June (Radford 1968).

Figure 1: Basal leaves of *Packera millefolium* and *Packera anonyma*

Note the tri-pinnate leaf with narrow leaf segments of *P. millefolium* (A) and the entire leaf of *P. anonyma* (B).

History of the putative hybrid, *Packera ×memmingeri*

The literature describing both *Packera millefolium* and *P. anonyma* indicates that identification of these two species in the field (or on herbarium sheets) is complicated. For example, Barkley (1968) notes that some collections of *P. millefolium* exhibit the *P. anonyma*-like characters of a permanent dense tomentum on the lower stems and conspicuously small and numerous heads. In addition, several collections of *P. anonyma* from rocky habitats within the range of *P. millefolium* exhibit leaves more dissected than those of plants from more general habitats (Uttal 1984). There is at least a superficial resemblance between the two species that may make it difficult to distinguish them in the field (Massey 1980, Massey et al. 1983).

To complicate matters, there is a putative hybrid between the two species, *Packera ×memmingeri*. It has been suggested that like many other species of *Packera*, hybridization and introgression occur when both parents are present in the same range. The hybrid, *Packera ×memmingeri* was first collected in 1887 by Mr. E. R. Memminger in Henderson County, N.C. and recognized as a species in 1898 (Britton, Small). It has also been considered an aberrant form or variety of *P. millefolium* (Britton 1892, Alexander 1937), a variety of *P. anonyma* (Radford 1968), or simply included within a broad concept of *P. millefolium* (Kartesz 1999). The synonymy for *P. millefolium* (Table 1) reflects the taxonomic confusion created by the hybrid.

Based on morphological evidence from herbarium specimens (primarily the difference in leaf segment widths), Uttal (1984) concluded that *Packera ×memmingeri* is indeed an interspecific hybrid. In addition, agencies and property owners like the U.S. Forest Service, the Heritage Program Network, and the Nature Conservancy all recognize the existence of the hybrid (personal communications). However, there are currently no published studies that have looked in more detail at the morphological differences between the three taxa or made an attempt to experimentally test the hybrid origin of *Packera ×memmingeri*. Because of *Packera millefolium'*s status as a threatened plant throughout its range, the issue of

hybridization between the taxa is of some interest for the future management and protection of this species. In an attempt to better understand the extent of hybridization and introgression between *P. millefolium* and *P. anonyma*, morphological analyses and cross-pollination experiments were performed between the summers of 2000 and 2004.

Table 1: Synonymy for *Packera millefolium*

Synonym	Author
1. *Senecio millefolium*	Torrey and Gray (1843)
2. *Senecio millefolium* var. *memmingeri*	(T. & G.) Britton (1892)
3. *Senecio memmingeri*	Britton (1898)
4. *Packera millefolium*	(T. & G.) W.A. Weber & A. Love (1981)
5. *Packera ×memmingeri*	(unpublished combination)

(adapted from Barkley 1978; Weber and Love 1981)

Methods

Morphology

Hybridization may be detected by measuring morphological characters, with hybrids showing intermediate characters to either parent (Wilson 1992). Basal leaf and flower samples from all three taxa were collected on or near outcrops where *Packera millefolium* occurs in N.C. and S.C. Morphological analyses of these samples were used to document hybridization between *P. millefolium* and *P. anonyma*. Several morphological characters for basal leaf and flower samples were observed or measured using a caliper including: total leaf length, length of petiole, length of leaf blade, width of leaf blade at widest point, degree of dissection of leaves, width of widest leaf division, flower ligule length, and flower involucre

width. These characters were chosen because prior morphological work with *P. anonyma* and *P. ×memmingeri* indicate that they are useful diagnostic characters (Chapman and Jones 1971; Uttal 1984).

Leaf and flower samples were collected at each study site (for site locations see Appendix One). Plants were identified in the field based largely on the characteristics of their basal leaves. Plants were considered to be of hybrid origin if the basal leaves were largely bi-pinnate with wide (greater than 3 mm) leaf segments (Figure 2). Care was taken to obtain leaf samples from a variety of microhabitats across the site from both flowering and non-flowering individuals. Basal leaves were collected and then pressed for analysis in the lab. Flower samples were taken from flowering individuals after counting the number of flower heads per inflorescence. Two flower heads from each plant were collected and placed in a cooler for pollen analysis and two more were preserved in an 80% ethanol solution for morphological analysis.

Slides were prepared for pollen fertility analysis by rubbing a flower head onto each slide and then staining the pollen with 1% aniline blue in lactophenol. One hundred pollen grains were counted on each slide and scored as fertile (dark blue) or infertile (light blue/clear).

Seed germinability

Seed germinability tests were conducted in the fall of 2001 using *P. millefolium* and *P. anonyma* seeds collected from the field. F1 hybrid seeds were collected from a pilot cross-pollination study conducted in the field (*P. millefolium* as the maternal plant and *P. anonyma* as the pollen donor). Seeds were rinsed in a weak bleach and water solution to sterilize them. Filter paper was placed in Petri dishes and moistened with distilled water and 100 seeds were placed in each dish and sealed with parafilm. Five replicates were made for each of the three taxa. Petri dishes were placed in a growth chamber set to 18°C with 12 hours of light and 12

Figure 2: Varied basal leaf morphology of *Packera millefolium* and *Packera anonyma* hybrids

hours of dark per day. Petri dishes were checked daily and seeds started germinating within 10-15 days. Small spots of mold developed in most

Cross-Pollination experiment

A cross-pollination experiment was performed to determine the extent to which gene flow is possible between *P. anonyma* and *P. millefolium*. An experimental garden was created at the Highlands Biological Station in Highlands, N.C. during the summer of 2001 in order to conduct the cross-pollination experiment. An experimental garden was used for the labor-intensive hybridization experiments for two reasons: 1) *P. millefolium* sites are often hard to access and are widely separated; and 2) it is unlikely that large populations of all three taxa will be found at the same site. The 3m x 5m garden is a raised bed filled with granite-derived sand. About 50 individuals of *P. millefolium* and *P. anonyma* and three F1 Hybrids (seeds were collected from a small field cross-pollination experiment in the summer of 2000) were propagated from seed and planted in the garden.

In early May the emerging inflorescences were bagged prior to flowering to exclude insects. The cross-pollination bags were perforated plastic to allow some air-flow and to prevent problems experienced with bridal-veil mesh bags used during the summer of 2003 (bridal-veil mesh allowed small pollinators and fertile seeds through the mesh and became heavy enough to weigh down the inflorescence when it rained). Each inflorescence was staked using a wooden dowel to keep the plastic bags from weighing down the inflorescence. All inflorescences remained bagged after being crossed to ensure 100% recovery of all fruits. Crosses were conducted in the following combinations: 1) *P. millefolium* X *P. millefolium*; 2) *P. anonyma* X *P. anonyma*; 3) *P. millefolium* X *P. anonyma*; 4) *P. millefolium* X the putative

hybrid; 5) *P. anonyma* X the putative hybrid; and 6) the putative hybrid X the putative

hybrid.

Chapman and Jones (1971) suggested that *P. anonyma* was largely self-sterile. To

confirm this fact and to determine if *P. millefolium* was also self-sterile, inflorescences of

both species were bagged prior to flowering and the dried flower heads were collected at the

end of the season. No fertile seeds were found and it was concluded that both species were

nonautogamous. Artificial crosses were made by rubbing flower heads together at least once

daily from late May until early June (Chapman and Jones 1971). Mature achenes were

collected from June 3rd until June 16th. After being collected the percentage of fertile

achenes per cross were counted to estimate percentage fruit-set per cross.

Results

Leaf Morphology

Morphological and ecological data are presented in Table 2. The putative natural hybrids

were intermediate in total leaf length, leaf blade length, leaf blade width, and number of

flower heads per plant when compared with the parents. In general, basal leaves of *P.*

millefolium were longer and wider than those of *P. anonyma* or the hybrid. This is reflected

by the ratio of leaf length to leaf width (*P millefolium*-4.5; hybrid-5.4; *P. anonyma*-10.6).

The natural hybrids had a larger average segment width (5.7 mm) than either parent (*P.*

millefolium-2.0 mm; *P. anonyma*-3.9 mm). Most *P. anonyma* leaves sampled did not have

segments at all. Occasionally, basal leaves of *P. anonyma* had "wings" off of the petiole

below the leaf blade. These wings were measured to obtain values of leaf segment width for

P. anonyma.

There was no significant difference in % seed germination between the three taxa. The %

pollen fertility was significantly different with *P. anonyma* having the highest pollen fertility

(91%). Percent pollen fertility was calculated for flowers collected from hybrid plants in the

field as well as from F1 individuals in the experimental garden. The F1 individuals in the

garden show a significantly lower % pollen fertility (66%) than hybrid individuals collected

in the field (86%).

Cross-pollination Experiments

The results of the cross-pollination experiment are shown in Table 3. All of the artificial

crosses had a lower % of fertile seed set than the % fertile seed set from *P. millefolium* that

was naturally pollinated in the field (71%). Of the artificial crosses, the average % fertile

seed set was highest when *P. anonyma* was crossed with pollen from *P. millefolium* (69%).

When *P. millefolium* was crossed with pollen from *P. anonyma*, the % fertile seed set was

much lower (37%). The F1 hybrid was a more successful pollen donor than seed producer

(52% fertile seeds when donating pollen to *P. millefolium* and 42% when donating to *P.

anonyma*; 40% when crossed with *P. millefolium* pollen and 23% fertile seed set when

crossed with *P. anonyma* pollen).

Table 2: A comparison of *P. millefolium, P. anonyma,* and their natural hybrids

Mean and standard deviation are given. An analysis of variance was conducted for each characteristic.

Characteristic	*P. millefolium*	Hybrids	*P. anonyma*	ANOVA, p< 0.05
Total leaf length, mm	213.4, +/- 62.0	206.7, +/- 52.5	182.3, +/- 47.9	*
Petiole length, mm	97.3, +/- 36.1	98.7, +/- 32.7	96.0, +/- 33.7	
Blade length, mm	116.5, +/- 37.4	108.0, +/- 30.9	86.3, +/- 25.2	*
Blade width, mm	50.7, +/- 19.7	39.6, +/- 10.4	19.0, +/- 11.5	*
Ratio of leaf length to leaf width	4.5, +/- 1.1	5.4, +/- 1.2	10.6, +/- 3.4	*
Segment width, mm	2.0, +/- 0.8	5.7, +/- 2.3	3.9, +/- 2.3	*
Flower Heads per plant	34, +/- 20	37, +/- 17	61, +/- 37	*
% Seed germination	54, +/- 15	60, +/- 9	50, +/- 10	
% Pollen Fertility	87, +/- 16	86, +/- 15 F1 only: 66, +/- 21	91, +/- 9	*

Table 3: Percent fertile seed set for cross-pollination experiments.

Results were analyzed using a one way ANOVA. Differences in % fertile seed set between crosses were found to be significant, $p < 0.05$. M= *P. millefolium*, A= *P. anonyma*, F1=F1 hybrid, p=pollen donor.

Cross	% Fertile Seeds
M x Mp	57%, +/- 21
M x Ap	37%, +/- 18
M x F1p	51%, +/- 20
A x Ap	47%, +/- 29
A x Mp	69%, +/- 18
A x F1p	42%, +/- 29
F1 x Ap	23%, +/-16
F1 x Mp	40%, +/- 21
M seed set from field	71%, +/- 21

Discussion

The morphological analyses support the hybrid origin of *Packera ×memmingeri* and possibly a variety of backcross or later generation hybrids. The morphological data show that there are plants with leaf characteristics intermediate to both *P. millefolium* and *P. anonyma*. The most useful characteristic for identifying hybrids in the field proved to be the width of the leaf segments--hybrids have bi-to tri-pinnate basal leaves with wide leaf segments (>3mm), *P. millefolium* has bi-to tri-pinnate basal leaves with very thin leaf segments (<3mm), and *P. anonyma* typically does not have any segmentation of its basal leaves, although it may have "wings" at the base of the leaf blade. Leaf segment width is especially useful because it applies to the wide range of hybrid leaf morphologies observed in nature (Figure 2).

The cross-pollination experiments also support the existence of hybridization between *P. millefolium* and *P. anonyma*. Lamont et al. (2003) note that for natural interspecific hybridization to occur, four conditions must be met by the parent species. They must be in close physical proximity; have overlapping flowering times; have some overlap in pollinators; and have some degree of pollen compatibility. *P. millefolium* and *P. anonyma* are known to co-occur at some locations and occur in close proximity at others, they have overlapping flowering times (flowering times naturally overlapped in the common garden), and they both have a variety of generalist pollinators (personal observation). The cross-pollination experiment shows that *P. millefolium* and *P. anonyma* meet the fourth requirement for naturally occurring hybridization: there is some degree of pollen compatibility between the species. Even though some crosses were more successful than others, all combinations of crosses produced some fertile seeds.

The rate and direction of naturally occurring interspecific pollen exchange is unknown. Hybrid plants, however, are almost always associated with populations of *P. millefolium* and not with closely situated populations of *P. anonyma*. Thus, it is surprising that the cross-pollination experiments indicated that *P. anonyma* appears to be a better maternal parent than *P. millefolium* when the two are crossed. Similar results were found when Chapman and Jones (1971) conducted a cross-pollination experiment between *P. anonyma* and *P. tomentosa,* a species commonly found on piedmont granite outcrops. They also found that *P. anonyma* was a better maternal parent. The larger numbers of hybrid individuals found in populations of *P. millefolium* may be explained by the larger overall global population size of *P. anonyma*, because a numerically smaller population will produce a higher percentage of hybrid seed than a numerically larger population (Levin et al. 1996).

Along with the variable morphology of the hybrids found in the field, the cross-pollination experiments indicate that introgression between *P. millefolium* and *P. anonyma* is a strong possibility. The results of the cross-pollination experiment show that while F1 hybrids may not have high fertility as a maternal parent when crossed with either *P. millefolium* or *P. anonyma*, both *P. millefolium* and *P. anonyma* have a relatively high seed set when they are crossed with F1 pollen, despite the relatively low F1 pollen fertility. The production of fertile seed from all back cross combinations could provide many avenues for gene flow between the taxa.

In addition, the opportunity for backcrossing and introgression in a natural setting may be higher than indicated by this study. One reason for this is that the percent seed set in this study may be lower than that in natural populations. It was observed that the seed set of *P. millefolium* in a naturally occurring population that included hybrids and nearby individuals

of *P. anonyma* was higher than any seed set observed in the experimental crosses. If seed set is higher in naturally occurring populations, then there may be a higher percentage of hybrid or backcross seeds entering the seed pool each year. Finally, there is evidence of contact and hybridization between *P. anonyma* and *P. millefolium* as long ago as the late 1800's when specimens labeled *Senecio memmingeri* and resembling hybrid individuals were collected. Given the long period of contact between the parent species and hybrids at some locations and assuming that introgression between these species is possible, there has been ample opportunity for backcrossing and introgression to occur.

Further experimentation is necessary to better quantify the extent of introgression between the species and to identify possible barriers to gene flow, including hybrid breakdown. It is possible that F2 individuals would have poor germination, little to no survivorship, or abnormal meiosis as found in other *Packera* hybrids (Chapman and Jones 1971). Thus, seeds collected from back crosses should be germinated, propagated and then used in further cross-pollination experiments. Because seeds that appeared fertile were collected from these backcrosses and the germination experiment shows that F1 seeds that appear fertile can be germinated with as much success as fertile seeds of *P. millefolium* and *P. anonyma* in a lab setting, it is possible that some of the F2 seeds that appear fertile may germinate. Even if it turns out that there is low fertility or viability of early-generation hybrids, other instances of natural hybridization indicate that it is still possible for extensive gene flow to occur (Arnold et al. 1999).

II: Historical Range and Continued Persistence of *Packera millefolium*

Introduction

Rarity is an emergent trait of a species and its environment that has both a spatial and temporal component (Kunin 1997). A rare species is one that can be characterized (on a population scale) by low abundances, restricted (local) distribution, and/or low local population densities (Rabinowitz 1981, Gaston 1994; Orians 1997). *P. millefolium* is thought to be rare because it is limited to a specific habitat type: granite or limestone outcrops found in the Southern Appalachians between 1500-4000 feet in elevation. Although its geographical range is limited, some populations of *P. millefolium* are very large, with thousands or hundreds of thousands of individual plants (NatureServe Explorer 2006).

Rare species, like *Packera millefolium,* are likely to be at a higher risk for extinction than more common species (Gaston 1994). It is important to note, however, that the state of rarity describes a population only at a particular time and not all rare species are equally likely to go extinct (Harper 1981). For example, in the future a rare species may decline in numbers or range (to the point of extinction), expand to become more common, or persist at the same level of rarity. Therefore, from a conservation and management standpoint, it is important to assess the stability of the populations of rare species over time. One way to assess the stability of a rare species is to monitor its populations over time.

Conservation and management organizations like the state Natural Heritage programs, NatureServe and The Nature Conservancy have kept records of historical occurrences of many rare or endangered plants like *P. millefolium*. These records can be used as a baseline for monitoring changes in populations of *P. millefolium*. Revisiting these locations 5-20 years later and observing any major changes in the location or the population may help identify threats to the continued persistence of *P. millefolium* in the region. In order to assess

possible threats to *P. millefolium* and to gain a better understanding of its population stability in the region, historical populations of *P. millefolium* were visited between the summers of 2001 and 2004.

Methods

Historical Locations

A list of historical locations for *Packera millefolium* in North and South Carolina was compiled using Heritage Program rare species occurrence records for North and South Carolina, herbarium records, and documents from The Nature Conservancy, NatureServe, the U.S. Forest Service, and the Highlands Biological Station (Appendix Two). Several new localities recorded in South Carolina by Patrick McMillan were also added. Twenty one of these sites in North and South Carolina were re-visited between the summers of 2001 and 2004 (Appendix One). A population estimate, the presence or absence of *P. anonyma*, the hybrid, reproductive individuals, and seedlings were recorded for each site. Observations were compared to historical records and site localities matched when possible. Data for populations in Virginia were obtained from the Virginia Natural Heritage Program (Wilson and Tuberville 2003).

The total number of known occurrences of *Packera millefolium* throughout its range depends on how populations are defined. The patchy nature of the outcrop habitats where *P. millefolium* is found leads to a naturally patchy distribution of plants and populations. It is not uncommon to find large numbers of *Packera millefolium* on outcrops that are in fairly close proximity, but separated by forest and other habitat patches unsuitable for *P. millefolium*. Historic records may name one locality that encompasses several populations spread out on nearby, but separate outcrops. For the purposes of this study, a population was defined as any group of *Packera millefolium* individuals separated from another group by 100 meters or more of habitat unsuitable (forested) for *Packera millefolium.*

<u>Results</u>

Rare plant occurrence records and herbarium specimens indicate that there were 28 historic locations documented in North Carolina and 17 historic locations in South Carolina. At the beginning of this study, records indicate that at least four historic locations were believed to be extirpated: a population on/near the peak of Whiteside Mountain in Jackson County, NC; Flat Rock in Hendersonville, NC; Horse Creek Waterfall in Polk County, NC; and Paris Mountain in Greenville County, SC (Massey et al. 1980). Between the summers of 2001-2004, ten historic locations were revisited in North Carolina and 11 in South Carolina. A total of 32 populations were visited in North and South Carolina as some historical locations accounted for more than one population. *Packera millefolium* was searched for, but not found at two other localities and these populations may also be extirpated (Chimneytop Mountain and Shelton Pisgah Mountain in Jackson County, NC). The current estimate for the number of extant populations derived from this study is 26 populations in NC (although there are possibly 5 extirpated localities in NC, some historic localities accounted for more than one population) and 29 populations in SC (with some historical locations having many populations). There is also one population in Rabun County, Georgia (Massey et al. 1980; NatureServe 2006) and a total of 11 populations in Virginia (Wilson and Tuberville 2003). Combined there are 67 known populations of *Packera millefolium* throughout its range, depending on how populations are grouped. This number is slightly higher than the 40-51 occurrences of *P. millefolium* estimated by NatureServe (NatureServe 2006).

NatureServe (2006) gives a scale for the viability of *P. millefolium* populations. Their ranking system lists populations with >200 rosettes as having excellent viability; those with 101-200 rosettes as having good viability; those with 51-100 rosettes as having fair viability; and those with just 1-50 rosettes as having poor viability. Using this system to analyze populations with population estimates in North and South Carolina there are 14 populations that would be ranked as having excellent viability, 6 as good, 4 as fair, and 17 as poor.

Another way to determine the viability of populations would be to compare population estimates from historical records to population estimates made during the course of this study. Of the eight historical locations that had historical population estimates and also were revisited, only two populations were estimated to have a smaller population than was recorded historically. In both cases, large numbers of hybrids and plants more closely resembling *P. anonyma* were found. These sites could be examples of populations of *P. millefolium* that have been genetically swamped by large nearby populations of *P. anonyma*.

Of the 55 populations recorded for North and South Carolina, hybrid plants had been observed at 23 of those. At five sites, the presence of hybrids has been noted in historical records, but no estimate of the percentage of the population represented by hybrid individuals had been made. Hybrids were estimated to represent between 1% and 5% of individuals at two populations; between 5% and 25% of individuals at 5 populations; between 25% and 50% of individuals at 5 populations; and between 50% and 100% at 6 populations. Of the populations where hybrids were estimated to represent between 50% and 100% of individuals, 2 populations were estimated to be made up of 75% or more hybrid individuals and in one of these no pure *P. millefolium* individuals were observed.

At 17 of the 23 sites with hybrids in North and South Carolina, some evidence of anthropogenic disturbance was observed (or noted in a historic record) on or near the outcrop. The disturbance ranged from a seemingly major onsite disturbance, like a road being built to the top of the outcrop and trails running through the populations of *P. millefolium* as on Glassy Mountain in Pickens County, SC to a more general disturbance of the landscape surrounding an outcrop, like clearing land for agriculture or housing developments. In much of the open landscape along the escarpment between North and South Carolina (along SC Highway 11) and in the open fields and roadways around the Highlands Plateau, large populations of *P. anonyma* were ubiquitous. Populations of *P. anonyma* were observed near (separated laterally by trees or other unsuitable habitat at the same elevation or vertically with the population of *P. anonyma* visible on cleared land below

the *P. millefolium* outcrop) but not on some outcrops with *P. millefolium* where hybrids were also observed

Discussion

It is estimated that there are over a million individuals of *Packera millefolium* spread throughout its range and that the global long-term trend for the persistence of *P. millefolium* is "relatively stable" (NatureServe 2006). The results of this study partially support this evaluation, but also raise some interesting questions about the continued persistence of *P. millefolium* in the region.

There are only 4 definite populations (perhaps 6) out of more than 50 populations of *P. millefolium* that have been extirpated over 150 years of documented population occurrences. Where records exist, 75% of populations have maintained a similar population size over time. In addition, there are 14 populations in North and South Carolina that have excellent viability. Six of those are very large populations with 1000+ individuals. All of these statistics are positive indicators that support the idea that the continued persistence of *P. millefolium* in the region is likely.

On the other hand, 17 populations in North and South Carolina are very small (less than 50 individuals) and considered to have poor viability. There are another 15 populations with no population estimates and very little overall information about population health. Most disturbing is that nearly half of all populations in North and South Carolina have some hybrid presence.

Hybridization may have many detrimental affects on a species including changes in genetic diversity, population dynamics, and interspecific interactions (Arnold et al. 1999). There are two main negative consequences of hybridization for a rare species: outbreeding depression and genetic assimilation (Ellstrand and Elam 1993). Outbreeding depression manifests itself when hybrid offspring are sterile or have reduced vigor, whereas genetic

assimilation is a greater risk when hybrid offspring are vigorous and fertile (Ellstrand and Elam 1993).

There is some evidence from the cross-pollination experiment and observations of populations with high percentages of hybrid individuals that suggests genetic assimilation is the main concern for the outcome of hybridization between *P. millefolium* and *P. anonyma*. It has been suggested that small populations are more likely to decline over time due to genetic assimilation than larger ones (Ellstrand and Elam 1993; Levin et al. 1996). Therefore, the populations of *P. millefolium* most susceptible to genetic assimilation are those with small population sizes that may come into contact with a much larger population of *P. anonyma*. These populations are already considered to have poor viability and introgression with *P. anonyma* may increase the time-frame and likelihood of their extirpation.

Another risk factor for the continued persistence of *P. millefolium* in the region may be loss of habitat and deterioration of that habitat (NatureServe 2006). Kral (1983) cites the development of rocky areas with views and Massey et al. (1980) site trampling by hikers and wildlife as threats to some populations. Turner et al. (2003) have shown in landscape analyses that building density in forested habitats of the southern Appalachians increased substantially between 1950 and 1990. Because *P. millefolium* is considered to be intrinsically threatened by its limited availability of specific habitat (NatureServe 2006), any loss of habitat may be detrimental. Many populations of *P. millefolium* are so remote or steep that it is unlikely that future development will lead to a direct loss of habitat. The larger threat may be the interaction between anthropogenic disturbance of the landscape and hybridization.

Hybrids have long been thought to be associated with disturbance (Anderson 1948). Human disturbance can break down ecological and geographic barriers between species allowing previously allopatric species to come into contact and hybridize (Riesberg and Wendel 1993; Rhymer and Simberloff 1996; Wendt et al. 2001; Parsons and Hermanutz

2006). In addition, disturbance may aid in the establishment and survival of hybrids, thereby providing unique habitats for their unique genotypes or by reducing suitable habitat for the parental species (Anderson 1948; Riesberg and Wendel 1993; Riesberg and Gerber 1995; Levin et al. 1996; Lamont et al. 2003).

Although the data from this study is observational in nature, it does suggest that over 70% of the sites with hybrids were in some way connected to anthropogenic disturbance. Even if the landscape immediately surrounding an outcrop and population of *P. millefolium* is not disturbed, the disturbance of the landscape in the surrounding areas may provide suitable habitat for *P. anonyma*. If the ecological barrier between these populations is small enough, then it is possible for the wind dispersed seeds or pollen of *P. anonyma* to traverse those barriers at a rate that may be evolutionarily significant, especially if the population of *P. anonyma* is very large compared to the population size of *P. millefolium* (Ellstrand 1992). Several populations of *P. millefolium* with a hybrid presence are still surrounded by strips of forest, but these thin barriers are surrounded by roads and fields that contain large populations of *P. anonyma* (personal observation). Kreyer et al. (2003) show that some bumblebees may cross forest borders of up to 600 meters to reach floral resources. It is likely that the fragmentation of habitat in the landscape surrounding once isolated populations of *P. millefolium* is allowing some gene flow between these species once isolated from each other by much larger distances.

III: Management Implications

<u>**Introduction**</u>

Packera millefolium is listed as imperiled or critically imperiled throughout its range (NatureServe 2006). Any management strategy developed to protect *P. millefolium* will be complicated by two main factors. First of all, managers must decide whether or not *P. millefolium's* hybridization and possible genetic assimilation with *P. anonyma* should be considered a natural part of the evolutionary process not to be interfered with or as a major threat to the continued persistence of *P. millefolium* that should be actively managed and prevented. Secondly, managers are faced with making management decisions based on incomplete data concerning the population sizes and general health of many populations and without information regarding the overall genetic variation within and between populations of *P. millefolium*. The following discussion will address these concerns and offer suggestions for the continued management and preservation of *P. millefolium*

<u>**Discussion**</u>

Hybridization and Management Decisions

Hybridization has been recognized as both an evolutionarily constructive process (Arnold 1992, Arnold et al. 1999) and as a process that may lead to the extinction of populations and species (Levin et al. 1996, Rhymer and Simberloff 1996, Wolf et al. 2001). This dual role for hybridization leaves conservation biologists and managers with a conundrum. When should they view the process of hybridization as a positive evolutionary process and when should they regard it as a negative process that may endanger rare species?

The case could be made that the hybridization between *P. millefolium* and *P. anonyma* is a natural process. Both species naturally occur in the southeastern United States and have formed natural hybrids in several naturally occurring populations. There are other examples of species within the genus *Packera* hybridizing historically or in the present day (Chapman and Jones 1971; Freeman and Barkley 1995). Why shouldn't we consider the hybridization between *P. millefolium* and *P. anonyma* as just another example of the ability of two *Packera* species to hybridize? There are two lines of reasoning that may help mangers to answer this question and decide if hybridization is a natural and positive process or an anthropogenic and negative process.

Allendorf et al. (2001) categorize hybridization into natural and anthropogenic categories to help guide management decisions. In this framework, natural hybridization is viewed as hybridization that results in a new species, a stable hybrid zone, or when populations contain alleles from other taxa, but hybridization is not ongoing or increasing the frequency of alleles in the population. In each of these three cases, Allendorf et al. (2001) conclude that hybridization should be considered as a part of the natural evolutionary process. Other authors agree that hybridization may have an evolutionarily constructive outcome when the hybrid offspring have become stabilized (it no longer crosses with either parent), are taxonomically distinct from either parent, and if they thrive in habitats unique to either parent (Riesberg 1991, Rhymer and Simberloff 1996). In this situation, the hybrid offspring would be considered a new species and would add to the species diversity of an area, rather than detract from it.

Allendorf et al.'s (2001) second category includes any instance of hybridization caused by human activities. These anthropogenic hybridization events may have negative impacts on rare species including wasted reproductive effort and the eventual loss of the rare parental species due to introgression. The implication of this category is that most cases where a rare species is at risk due to hybridization with a more abundant species can be attributed to

25

human activities (species introductions, fragmentation, and habitat modification) that allowed otherwise allopatric species to come into contact (Allendorf et al. 2001).

Other authors do not make a distinction between natural or anthropogenic causes of hybridization as a rationale for taking management action, but focus instead on the outcome of the hybridization. Ellstrand and Elam (1993) suggest that managers should act swiftly when populations are at a high risk for interspecific gene flow where the outcome is likely to be genetic assimilation or outbreeding depression. Rhymer and Simberloff (1996) suggest that management actions should be taken when hybridization results in outbreeding depression or when introgression between rare and widespread congeners is so severe that extinction of the rare species is likely. Others agree that management action should be taken when a rare species is threatened by hybridization with a more widespread congener (Soltis and Gitzendanner 1999; Wolf et al. 2001).

When subjected to Allendorf's anthropogenic vs. natural test the hybridization between *P. millefolium* and *P. anonyma* falls into the anthropogenic category. Not only does the hybridization between *P. millefolium* and *P. anonyma* appear to be ongoing and not forming a stable hybrid zone or new species, *P. millefolium* and *P. anonyma* should not be considered to be naturally co-occurring at most localities. *P. millefolium* is restricted to granite outcrops and it is unlikely that it was historically found sympatrically with *P. anonyma*. It is more likely that human disturbance of the forested areas around outcrops allowed the weedy *P. anonyma* to increase its range and establish in areas where the two species could come into contact. In addition, the hybrid is only found on sites where *P. millefolium* previously occurred alone. The hybrid, therefore, is not increasing diversity by filling a new niche, but could be out competing *P. millefolium* and replacing it.

Even if the hybrid is not out competing *P. millefolium*, the proportion of hybrids in a population will increase progressively, especially if the hybrids are fertile and mate with themselves and parental individuals, because all offspring of hybrids will be hybrids and the end result would still be a decrease in the parental population of *P. millefolium* (Huxel 1999,

Allendorf et al. 2001). The *P. millefolium* populations that contain over 50% hybrid individuals are indicators that the result of the hybridization between *P. millefolium* and *P. anonyma* is likely to be genetic assimilation. In addition, *P. millefolium* is at a higher risk of genetic assimilation because it is numerically inferior to the widespread *P. anonyma* (Ellstrand and Elam 1993; Levin et al. 1996). Thus, based on what we currently know, the hybridization between these two species may result in genetic assimilation of *P. millefolium* if left unmanaged. Therefore, this case meets the "results test" of when to take management action to prevent hybridization when possible.

Suggested Management Strategies

Although the outcome of hybridization in any one population may be genetic assimilation, the extinction of *Packera millefolium* via genetic assimilation is unlikely unless all of its populations are invaded by *Packera anonyma* (Levin et al. 1996). Management strategies, therefore, should focus on diminishing the effects of hybridization where it is occurring and preventing contact between the two species where there is no current evidence of hybridization.

In general, the conservation value of a hybridized population increases as the number of pure populations decreases (Allendorf et al. 2001). As long as there is a fairly large number of large, pure, and isolated populations of *P. millefolium* remaining, the best management strategy for the continued persistence of *P. millefolium* in the region may be to improve and protect the habitat surrounding pure populations of *P. millefolium* (Wolf et al. 2001). Road construction or other habitat disturbances should be minimized to decrease the risk of contact between the two *Packera* species (Parsons and Hermanutz 2006). One complication of this conservation strategy is that the populations of *P. millefolium* are found on lands with a variety of ownerships across four states. Luckily, many of the populations are currently on lands which afford some legal protection or are in relatively inaccessible habitats (Massey et

al. 1980). Populations are found on land owned or protected by the United States Forest Service, the South Carolina State Park system, the South Carolina Heritage Preserve System, The Nature Conservancy, as well as on several privately owned tracts. It may be difficult to coordinate an effort between these agencies and surrounding land owners to minimize habitat disturbance, especially as the pressure to develop land in the southern Appalachians increases. If the incidence of hybridization among populations increases and the number of pure populations decline, it may become important to manage hybridized populations.

At locations where hybridization is ongoing, there are two possible management strategies that could be used: eliminate the less desired species and hybrid individuals from a site, and/or transplant pure individuals from the rare population to a remote location where hybridization is not occurring (Riesberg 1991). Even if removal and rescue efforts are feasible, these efforts should be taken with caution because there are several complicating factors.

First of all, the estimates of the proportion of hybrid individuals in a population of *P. millefolium* made in this study were based solely on morphological evidence and morphological estimates may underestimate the true proportion of hybrids in natural populations (Riesberg and Linder 1999; Wolf et al. 2001). Therefore, "pure" parental individuals identified on morphological evidence may in fact contain some proportion of alleles from the more common congener. Before any removal of hybrids or transfer of parental individuals, it may be prudent to do a more thorough genetic study of the population.

If a genetic study of the population is not feasible, Allendorf et al. (2001) only recommend rescuing pure parental individuals from populations that remain largely pure with a few F1 hybrids. Transferring a large number of individuals would involve finding a remote location with suitable habitat that may be hard to access and monitor in the future. If you could find such a location, the survival rates in the new population may be low and repeated attempts to establish new populations would continue to deplete the source population (Riesberg 1991). If a new smaller "founder" population was established, it could

28

be at an increased risk of inbreeding depression and there is no guarantee that the new population would survive.

Given the inherent risks of rescuing individuals and establishing new populations, it may be more reasonable to remove all hybrid individuals and individuals of *P. anonyma* from a population. If the population size was significantly diminished after removal of all suspected hybrid individuals, it might be possible to restock the population with plants grown from seeds taken from the population to be restored (Godt et al. 1996). This study has shown that growing large numbers of *P. millefolium* from seed is possible. Increasing the size of a population could prevent a further loss of genetic diversity and buffer the population against extinction and demographic stochasticity (Godt et al. 1996).

At the same time, *P. anonyma* is widespread and ubiquitous along roadsides and disturbed areas. In some situations, the hybridization between *P. anonyma* and *P. millefolium* may be facilitated by pollen transfer across forest boundaries and there is no practical way to remove thousands of *P. anonyma* individuals to prevent this type of gene flow. One example of this situation would be the hybrids found on Pinnacle Mountain in Table Rock Park, Greenville County, SC. There are hybrids on the outcrop, but the source of the *P. anonyma* pollen is likely the large population of *P. anonyma* along Highway 11 below. In other instances, removal of *P. anonyma* and hybrids from an outcrop might be possible, but the process would likely have to be repeated over time because there is continued disturbance in the landscape that would allow for the re-establishment of *P. anonyma* over time. Glassy Mountain in Pickens County, South Carolina, for example, has large populations of *P. anonyma* and hybrid individuals that could be removed from the outcrop habitat while maintaining a large population of pure *P. millefolium*. Because a road leads directly to the top of the mountain, this road is frequently mown to prevent woody encroachment, and there are large source populations of *P. anonyma* in the agricultural land and roadsides surrounding the mountain, it is likely that *P. anonyma* could re-establish itself over time.

Because each hybridized population may have unique problems and characteristics and because resources are limited, populations should be evaluated on their potential for conservation before any management action is taken at the population level. The manager should look at each population individually to assess the level of hybridization, the location of the closest population of *P. anonyma,* whether or not any isolation between the population of *P. millefolium* and *P. anonyma* could be achieved, and the number of pure individuals of *P. millefolium* remaining. Pure populations, isolated populations, large populations, and populations that have the fewest number of hybrids may be the easiest to manage in terms of preventing hybridization or diminishing the effects of current hybridization. Populations that consist predominantly of hybridized individuals may be salvageable if all hybrids are eradicated; the number of *P. millefolium* individuals is increased by restocking the population with pure individuals; and it is likely that future hybridization could be prevented. It may be best not to waste resources trying to restore populations that consist predominantly of hybridized individuals where it is unlikely that the continued gene flow with *P. anonyma* could be prevented even after the eradication of hybrid individuals.

Research as a Management Strategy

Although this study begins to shed light on the process of hybridization and potential introgression between *Packera millefolium* and *Packera anonyma,* it also raises many new questions and highlights the lack of some basic information that would greatly facilitate making scientifically sound management decisions. Any management plan would not be complete without an emphasis on continued research and monitoring. There should be two major priorities for research and monitoring: gathering basic population information on those populations that have not been resurveyed for several years (including population estimates and the proportion of hybrids in each population) and gathering genetic data on

populations to asses the genetic diversity within populations, between populations, and to better characterize the introgression and gene flow between *P. millefolium* and *P. anonyma.*

The value of removing hybrid individuals from populations, restocking populations with pure *P. millefolium* individuals, or rescuing pure *P. millefolium* individuals from hybridized populations largely depends on the number, health, and isolation of the remaining pure populations. There are at least 15 populations with little or no population data and 5 populations known to have hybrid individuals, but without an estimate of the percentage of the population made up of hybrids. More detailed information about these populations and others not visited in this study could alter the perceived need for management action at the population level.

One way to improve the available data on the potential introgression between *Packera millefolium* and *P. anonyma* is to use molecular markers to determine the actual frequencies of the hybrid and parental groups in each population (Wolf et al. 2001). Maximum likelihood methods may also be employed to estimate the frequencies of different hybrid classes in a population (Nason and Ellstrand 1993). If a much higher proportion of individuals in a population is of hybrid origin than suspected by morphological data alone, there may be a more pressing need to actively manage populations. Conversely, molecular data could indicate that very little backcrossing is occurring and that there is less of a threat of genetic assimilation than previously imagined.

In general, even without the question of introgression, the importance of understanding the genetic diversity within a species to guide development of effective management strategies has been recognized (Hamrick et al. 1991). Genetic information can be used to assess overall genetic diversity within or between populations, gene flow between populations, effective population size, and population viability (Deyoung and Honeycutt 2005). The patchy and isolated nature of *P. millefolium* populations may cause the genetic diversity within this species to be spatially structured (Escudero et al. 2003).

The spatial structure of *P. millefolium* throughout the landscape is complex. At the smallest scale, *P. millefolium* may occupy a variety of microsites on an outcrop. Several populations may occur on different outcrops separated by only a few hundred meters on one mountain. These populations may then be clustered a few miles apart within a mountain range. At the largest scale, populations are spread out across several states and may be separated by hundreds of miles. If this spatial structuring does affect the genetic diversity and gene flow within and between populations, then designing effective management strategies may depend on understanding these patterns.

For example, if each population is highly adapted to its local environment and genetically distinct from other populations and a manager wants to restore *P. millefolium* at a location where all hybrid individuals have been eradicated, then care should be taken when transplanting individuals that they come from a source population that is as closely genetically and environmentally similar to the original population as possible (Pegtel 1998). Any transplant or reintroduction efforts should consider the adaptive variation within a population to help ensure survivorship and fecundity at the new location (Huenneke 1991).

Understanding gene flow between populations may also aid management decisions. Gene flow by pollen could increase the effective size of a population to include several smaller populations at a local scale (Ellstrand 1992). This could be important because it could decrease the threat of inbreeding depression. In the case of *P. millefolium*, gene flow between populations may also be important when considering which, if any, populations should be managed to diminish the effects of hybridization. For example, consider three populations that are found within a mile or two of each other. One population is near a developed area and hybrids represent 75 % of this population. The other two populations contain only a few hybrids and are more isolated from the developed area and other populations of *P. anonyma*. Managers might decide that the population with the highest proportion of hybrids is not salvageable and focus their efforts on removing the few hybrids in the two more isolated populations. However, if genetic tests reveal significant gene flow

between the two more isolated populations and the population that contains a majority of hybrids, the strategy would be open to question. Even if managers are successful at removing the few hybrids from the more isolated populations, the problem can be expected to recur over time if gene flow with the first predominately hybrid population continued.

These hypothetical examples show that understanding *P. millefolium's* genetic diversity and the spatial structure of that diversity may provide managers with useful information that can be used to make practical management decisions.

CONCLUSION

The results of this study indicate that *Packera millefolium's* continued persistence in the southern Appalachians may be threatened by its hybridization with *Packera anonyma* to form *Packera ×memmingeri.* Not only do these two species appear to hybridize with ease, initial data indicate that introgression and genetic assimilation are possible. On the other hand, the isolated and patchy distribution of *P. millefolium* populations may serve to help maintain ecological barriers between the two species, despite increased human disturbance in the Southern Appalachians.

Future research should address the need for updated population information, for a more detailed molecular investigation of the hybridization between these two species, and for data on the genetic variation within and between populations of *P. millefolium*. Until more information is gathered, the most pressing management concern is to protect populations of *P. millefolium* that remain "pure" and to preserve the forested habitat around these populations.

Site Name	County, State	Latitude	Longitude	Hybrid Present?
Bald Rock	Greenville Co., SC	35.08277	-82.6203	YES
Cedar Mountain	Greenville Co., SC	35.0802	-82.25533	YES
Caesar's Head	Greenville Co., SC	35.1005	-82.59254	NO
Eva Russell Chandler Heritage Preserve	Greenville Co., SC			
Outcrop 1		35.08205	-82.60477	YES
Outcrop 2		35.08225	-82.60315	YES
Outcrop 3 (Small, below main outcrops)		35.0769	-82.6036	YES
Persimmon Ridge	Greenville Co., SC	35.07608	-82.60338	NO
Wattacoo Creek	Greenville Co., SC	35.0888	-82.57912	NO
Wildcat Wayside Park	Greenville Co., SC	35.0775	-82.58458	NO
Cedar Rock Mountain	Pickens Co., SC	34.9646	-82.79922	YES
Drawbar Cliffs	Pickens Co., SC			
Outcrop 1		35.02982	-82.74437	NO
Outcrop 2		35.02875	-82.74465	NO
Glassy Mountain	Pickens Co., SC	34.89988	-82.66022	YES
Long Ridge	Pickens Co., SC			
Outcrop 1 (with Petroglyphs)		35.02074	-82.74554	YES
Outcrop 2		35.02737	-82.741	NO
Outcrop 3		35.02643	-82.74145	NO
Outcrop 4		35.02587	-82.74242	NO

Site Name	County, State	Latitude	Longitude	Hybrid Present?
Table Rock, Pinnacle Mountain	Pickens Co., SC	35.02907	-82.73422	YES
Table Rock	Pickens Co., SC			
Outcrop 1 (summit)		35.05077	-82.70114	NO
Outcrop 2 (below summit)		35.05005	-82.70374	NO
Outcrop 3 (Governor's Rock)		35.04757	-82.71442	NO
Outcrop 4 (near CCC Shelter)		35.04268	-82.71443	NO
Snake Den Rock	Buncombe Co., NC	35.75458	-82.35548	YES
Big Sheep Ridge Cliff	Jackson Co., NC	35.13452	-83.09765	YES
Rock Mountain	Jackson Co. NC			
Outcrop 1		35.11525	-83.08225	YES
Outcrop 2 (small)		35.11033	-83.07103	NO
Outcrop 3		35.10988	-83.07	NO
Terrapin Mountain	Jackson Co. NC	35.05732	-83.08357	NO
Black Rock, Macon	Macon Co., NC	35.05139	-83.14861	NO
McDowell Mountain	Macon Co., NC	35.02972	-83.37083	YES
Pinnacle Mountain	Macon Co., NC	35.09265	-83.34427	YES
Slick Rock	Macon Co., NC	35.02502	-83.14827	YES
Chimney Rock Park	Rutherford Co., NC	35.42498	-82.24731	YES

APPENDIX TWO: List of historical occurrence records of *Packera millefolium* not visited in this study

Site Name	County, State	Latitude	Longitude	Hybrid Present?
Laurel Creek	Greenville Co., SC	35.07861	-82.72361	No
Old Indian Mountain	Greenville Co., SC	35.13944	-82.3825	No
Paris Mountain*	Greenville Co., SC	34.945	-82.42111	No
Plumley Mountain	Greenville Co., SC	35.1625	-82.39861	No
Round Mountain 1	Greenville Co., SC	35.16361	-82.30833	No
Round Mountain 2	Greenville Co., SC	35.16139	-82.30722	No
The Stool	Pickens Co., SC	35.05111	-82.69333	No
Cedar Cliff Knobb	Buncombe Co., NC	35.77667	-82.39361	No
Craggy Scenic Area	Buncombe Co., NC	35.72639	-82.37639	No
Ivy Knob	Buncombe Co., NC	35.80222	-82.37972	Yes
Locust Ridge Outcrop	Buncombe Co., NC	35.74444	-82.39333	Yes
Flat Rock*	Henderson Co., NC			
Glassy Mountain	Henderson Co., NC	35.26667	-82.45972	No
Chatooga River Gorge	Jackson Co., NC	35.04306	-83.12167	No
Chimney Top*	Jackson Co., NC	35.10278	-83.06833	No
Dismal River Cliffs	Jackson Co., NC	35.23444	-83.04028	Yes
Little Terrapin Mountain	Jackson Co., NC	35.08504	-83.08831	No

Site Name	County, State	Latitude	Longitude	Hybrid Present?
Shelton Pisgah Mountain	Jackson Co., NC	35.17278	-82.99056	No
Whiteside Mountain	Jackson Co., NC	35.07778	-83.14861	Yes
Cedar Cliff	Macon Co., NC	35.04111	-83.4	Yes
Double Top	Macon Co., NC	35.02727	-83.42297	No
Buck Mountain	Polk Co., NC	35.22722	-82.29583	No
Miller Mountain	Polk Co., NC	35.25556	-82.25556	No
Tryon Peak	Polk Co., NC	35.26667	-82.24444	Yes
Long Mountain	Rutherford Co., NC	35.53309	-82.0408	Yes

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