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HYBRIDIZATION OF THE SAN FRANCISCO PEAKS' RARE ENDEMIC *PACKERA FRANCISCANA* WITH A LOWER-ELEVATION CONGENER: EVIDENCE FROM MORPHOMETRIC AND MOLECULAR DATA

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ABSTRACT—Morphometric and molecular data are presented that support the hypothesis that the rare endemic *Packera franciscana* (San Francisco Peaks groundsel) is hybridizing with another *Packera* species, *Packera werneriifolia*, which occurs at a lower elevation on the San Francisco Peaks near Flagstaff, Arizona. The morphological data show that most individuals from Snowslide Spring have an intermediate appearance or look more like *P. franciscana*, and that certain leaf traits show significant variation among the parental species and hybrids. Our genetic data show that hybrids share alleles with both parental species and suggest that hybridization may be more widespread than the single population at Snowslide Spring. Correlations between the morphology and genetics of these groups may be useful in providing managers with a tool for identifying hybrids without the expense of genetic analysis.

RESUMEN—Se presentan datos morfométricos y moleculares que apoyan la hipótesis de que la planta endémica rara *Packera franciscana* se hibrida con otra especie, *Packera werneriifolia*, que se encuentra a una elevación menor en los San Francisco Peaks cerca de Flagstaff, Arizona. Los datos morfológicos muestran que la mayoría de los individuos de Snowslide Spring tiene un aspecto intermedio o se parece más a *P. franciscana*, y que ciertas características de la hoja muestran una variación significativa entre las especies progenitoras y el híbrido. Nuestros datos genéticos indican que los híbridos comparten alelos con ambas especies parentales y sugiere que la hibridación puede ser más extendida que la sola población en Snowslide Spring. Las correlaciones entre la morfología y la genética de estos grupos pueden ser útiles para proporcionar a los administradores una herramienta para la identificación de los híbridos sin el gasto de análisis genético.

Packera is a New World genus composed of 75 taxa separated from the genus *Senecio* based on molecular, morphological, and palynological data. This genus was informally treated for over 100 years as the "aureoid *Senecio* complex" before officially being segregated. It is widely known that *Packera* species hybridize with other congeners when they co-occur (Barkley, 1988; Bain and Jensen, 1996; Bain and Golden, 2000; Trock, 2006).

San Francisco Peaks groundsel, *Packera franciscana* (Greene) W. A. Weber & Á. Löve, is a rare plant endemic to alpine tundra talus slopes of the San Francisco Peaks in Coconino County, Arizona. It is a short rhizomatous perennial, 3–7 cm tall, and easily separated from other species in Arizona by its often purplish, lyrate basal leaf blades, gradually reduced cauline leaves, and usually solitary heads (Trock, 2006). In 1983, the U.S. Fish and Wildlife Service listed the plant as threatened, and a 1987 recovery plan urged better management of the ecosystem as well as further monitoring of the taxon (USFWS, 1987).

In the late 1990s researchers located a population of *P. franciscana* with unusual leaf morphology (Fig. 1) in the Inner Basin near Snowslide Spring. A specimen from that locality collected in 1983 [Paul Boucher, without collection number, Deaver Herbarium (ASC)] had been originally identified as *P. franciscana* but was later annotated to *Packera werneriifolia* (A. Gray) W. A. Weber & Á. Löve by John Bain, a *Packera* specialist. The leaves are narrower and less lobed than those typical of *P. franciscana*. This unusual morphology may be the result of hybridization with another species of *Packera* or the result of natural variation within the species. If the leaf variation is the result of hybridization, the likely candidate is *P. werneriifolia*, a slightly lower-elevation species that also occurs in the San Francisco Peaks. *Packera werneriifolia* is widespread and occurs near or above timberline from the Rocky Mountains west to the Sierra Nevada (Trock, 2006). In the San Francisco Peaks, *P. werneriifolia*, also a rhizomatous perennial, occurs in openings in the *Picea-Abies* forests up to timberline. In general, this species is 7–15 cm tall, with 1 or 3–5 stems, each with 1–5 (sometimes as many as 8) heads. This species is morphologically variable throughout its range, but all populations within Arizona have leaves that are nearly linear with revolute margins (Trock, 2006). The phenology of the two species

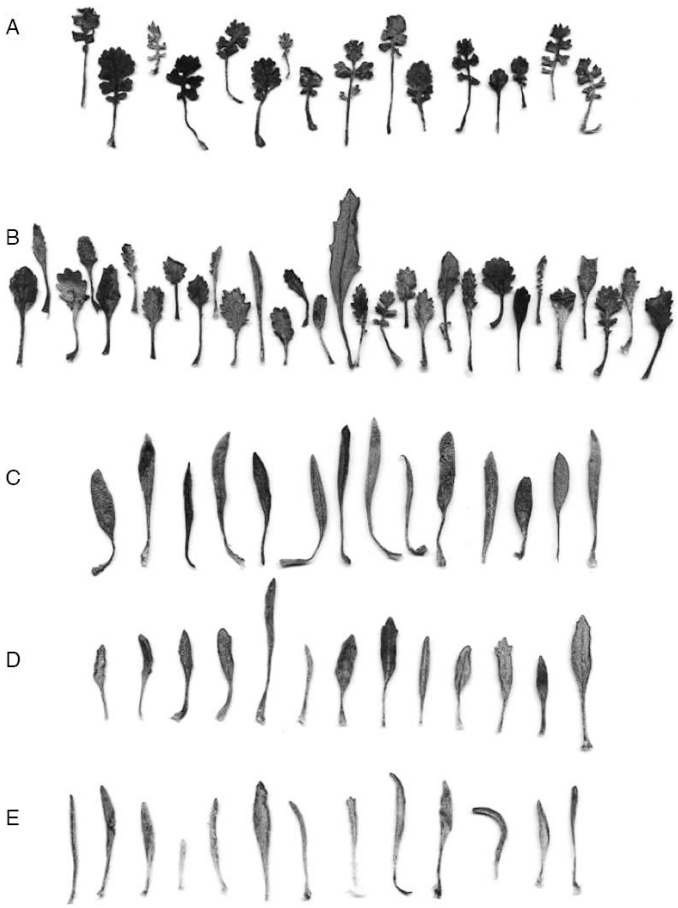

FIG. 1—Leaf morphology (measured June–August 2011) of parental species and hybrids representing all 86 individuals included in the molecular study. A) *Packera franciscana*, eastern slope of Agassiz Peak. B) Hybrid population at Snowslide Spring. C) *Packera werneriifolia,* Kendrick Peak. D) *Packera werneriifolia,* Inner Basin. E) *Packera werneriifolia,* Abineau Trail. Categorical character states for morphometric data set: 0, entire revolute margins; 0.25, teeth at tip; 0.5, shallow teeth to middle; 0.75, shallow teeth to base of blade; 1, deeply lobed.

in the San Francisco Peaks is also different. *Packera werneriifolia* generally flowers from the middle of June to the middle of July whereas *P. franciscana* flowers from the middle of August to October.

Hybridization can lead to the extinction of a parental species if the hybrids are fertile and their fitness does not decrease. In this case, genetic assimilation is possible due to total introgression between the hybrid and the parental species (Rhymer and Simberloff, 1996; Wolf et al., 2001). Because *P. franciscana* is a federally listed species, its ability to interbreed with sympatric species is critical to informing long-term management plans and monitoring efforts.

The purpose of this project was to gather evidence from morphological and molecular data as independent assessments to test the hypothesis that *P. franciscana* and *P. werneriifolia* are hybridizing in the Inner Basin of the San Francisco Peaks. It has become commonplace for studies of hybridization to include both morphological and molecular analyses. This combination of techniques serves to elucidate the extent of hybridization and introgression that may be occurring in a population (Guo et al., 2006; Field et al., 2009; Lindhardt et al., 2009). In addition, molecular tools can identify individuals of hybrid origin that may not be morphologically apparent.

MATERIALS AND METHODS—*Morphometrics*—We gathered a morphometric data set from 86 individuals using both vegetative and floral traits that have historically been used to delineate the two species (Trock, 2006). We measured nine quantitative traits: basal leaf length, basal leaf width, basal leaf length-width ratio, bract length, peduncle length, phyllary length and width, and ray length and width. We included only the leaf blades in the leaf length measurements and took width measurements from the widest part of the leaf. We treated leaf margins as categorical data and scored them in the following manner: 0, entire revolute margins; 0.25, teeth at tip; 0.5, shallow teeth to middle; 0.75, shallow teeth to base of blade; 1, deeply lobed. Between June and August 2011, we took three measurements from each individual and averaged them for each trait. Leaves from all 86 individuals are depicted in Fig. 1.

We sampled *P. werneriifolia* from three collection sites: Abineau Canyon, the Inner Basin, and Kendrick Peak. We collected *P. franciscana* samples from the eastern slope of Agassiz Peak along the Weatherford Trail. We collected the putative hybrid population near Snowslide Spring, which is found down slope from the *P. franciscana* population, and within a quarter mile of the populations of *P. werneriifolia* occurring in the Inner Basin. Specimen vouchers for each population were deposited at Northern Arizona University's Deaver Herbarium (ASC). *Packera franciscana* was photo-vouchered only.

We used principal components analysis (PCA) to examine possible morphological differences among the two parental species and the Snowslide Spring population and to visually explore patterns in the data (Fig. 2). In addition, we performed one-way analyses of variance (ANOVAs) on the principal components. This was because leaf traits and floral traits tended to load on the first and second principal components, respectively, and we wanted to check for significance between the three groups (two parental species and the Snowslide Spring population) and the morphological traits associated with each principal component. We performed analyses by using JMP Pro 10.0.0 (SAS Institute Inc., Cary, North Carolina). All principal components met the assumptions of ANOVA.

The PCA was run using the quantitative traits. We chose the appropriate number of principal components included in the analyses by using principal components with eigenvalues >1 (McGarigal et al., 2000). Once chosen, we performed the ANOVAs on the individual principal components.

Genetics—We collected fresh leaf tissue from each species and the hybrid population in the field and extracted the DNA using a Qiagen DNeasy Plant Kit according to the manufacturer's protocol with slight modifications (Qiagen, Valencia, California). The quality and quantity of DNA was checked using gel electrophoresis and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, Massachusetts). We used the amplified fragment length polymorphism

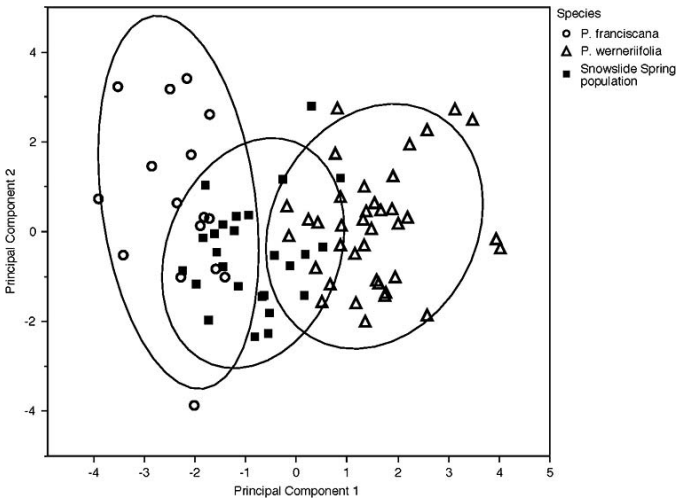

FIG. 2—Ordination of the principal components analysis including first and second principal components on the x and y axes respectively. Individuals of *Packera franciscana*, *Packera werneriifolia*, and the Snowslide Spring population are overlaid on the graph. Each genetic group is symbolized by a different shaped marker and overlaid with an ellipse encompassing 90% of the group's data points. We coded the Inner Basin population in this analysis as *P. werneriifolia* although it is clear from our analysis that some genetic admixture is also occurring in that population.

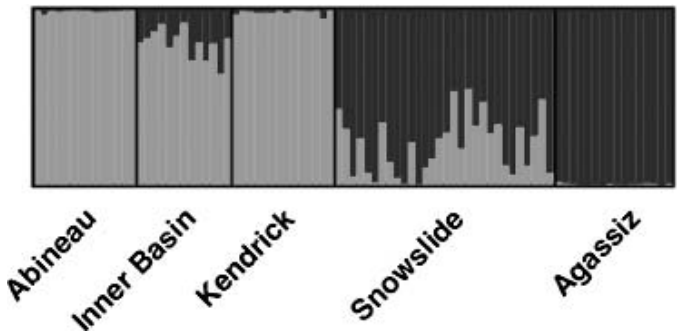

FIG. 3—Distruct graph. Visual representation of the entire hybrid index sorted according to collection site showing admixture of parental species in each of the 86 individuals in the study. *Packera franciscana* (Agassiz Peak), *Packera werneriifolia* (Abineau Canyon, Inner Basin, Kendrick Peak), hybrids (Snowslide Spring). *Packera franciscana*, dark gray; *P. werneriifolia*, light gray.

(AFLP) technique of Hersch-Green and Cronn (2009) to generate DNA profiles. For each individual, 15 ng of genomic DNA was digested by *Eco*RI and *Mse*I, with simultaneous ligation of corresponding adapters to the fragments. Primers complementary to the adaptor sequences plus one selective nucleotide (*Eco*RI + A and *Mse*I + C) were used for preselective amplification. For the preamplification process, we added a 1:5 dilution of the restriction-ligation product to the preamplification master mix, which consisted of 1× magnesium-free polymerase chain reaction buffer, 0.1 mg/mL bovine serum albumin, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 μM each of *Eco*RI + A and *Mse*I + C, and 1.25 units of *Taq* DNA polymerase.

For selective amplification, we tested 8 fluorescently labeled primer pairs containing the complement to the adaptor sequence plus 3 selective nucleotides on 10 individuals representing the 2 species and the Snowslide Spring population. We chose the four primer pairs that produced the largest number of fragments: *Eco*RI-ACA and *Mse*I-CAG; *Eco*RI-ACG and *Mse*I-CCG; *Eco*RI-ACT and *Mse*I-CAC; *Eco*RI-ACG and *Mse*I-CAC. The selective amplification reaction consisted of undiluted preamplification product 1× polymerase chain reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.375 μM *Eco*RI + 3 primer, 1.0 μM *Mse*I+3 primer, and 0.5 units *Taq* DNA polymerase.

The AFLP products were analyzed by using capillary electrophoresis on an ABI 3730XL (Applied Biosystems, Foster City, California). We mixed a 1:10 dilution of the AFLP product with formamide and GeneScan 600 LIZ size standard as per manufacturer's instructions. The AFLP fragments were evaluated using GeneMapper Software v.4.0 (Applied Biosystems, Foster City, California) and the profiles were analyzed with automated scoring by using a range of 100–600 base pairs and a peak height minimum of 1,000 for all primer combinations. To minimize scoring noise, we used only larger peaks for bin generation and hand-edited the bins for consistency and utility. Peaks present in the no-template controls were removed from the analysis.

Unique loci and loci with highly variable peak heights across samples were removed from the data set. Structure 2.3.4 (Pritchard et al., 2000) was used to estimate the number of interacting genetic groups (K) on the basis of the AFLP profiles. An admixture model with 10,000 burn-in and 1,000,000 iterations was used for each level of K from 1–10 and each level of K was replicated 10 times. We used Structure Harvester (Earl and von Holdt, 2012) to visualize likelihood values from the Structure output across all levels of K to detect which number of genetic groups best fit our data. CLUMPP (Jakobsson and Rosenberg, 2007) was used to pool all runs for the appropriate value of K, resulting in a hybrid index showing percentage of parental alleles present in each individual. We generated a graph (Fig. 3) from the hybrid index by using Distruct (Rosenberg, 2004). The Distruct graph (Fig. 3) is a graphical representation of the proportion of alleles from each of the parental species present in each individual.

Morphological and Genetic Correlation—We performed a linear regression combining the CLUMPP-derived hybrid index and the PCA-derived PC1 scores using JMP Pro 10.0.0 to assess the strength of correlation between the genetic admixture of individuals, particularly the Snowslide Spring population, and the suite of traits encompassed by the first principal component.

RESULTS—*Morphometrics*—The first three principal components of the PCA explain 49% (PC1), 31% (PC2), and 19% (PC3) of the total variation seen in our three groups (*P. franciscana*, *P. werneriifolia*, Snowslide Spring population) for a total of 99% of the variation. The first component largely represents original variation in leaf traits including leaf length:width ratio (88%), basal leaf width (54%) and length (53%), as well as peduncle length (58%). The second component mostly comprises variation in floral traits including ray length (63%),

phyllary length (50%), and ray width (31%). The third component mostly represents variation in phyllary width (71%). The first principal component distinguishes *P. franciscana* from *P. werneriifolia* with the Snowslide Spring population intermediate (Fig. 2). This pattern was corroborated by our statistical analysis. Although the second principal component encompasses some variation, it does not distinguish the groups from each other. The third principal component when mapped against both the first and second principal components does not lead to a visual separation of the three groups.

Results of the ANOVAs on the individual principal components corroborate the PCA results. The first principal component, which represented mostly variation in leaf traits, showed significance when analyzed against the three groups ($F_{2,78} = 122.2$, $P < 0.0001$; $R^2 = 0.76$). A Tukey's test showed the pairwise combinations of each parental species and the Snowslide Spring population to be significantly different ($P < 0.0001$). The second principal component, which represented mostly floral traits, also showed significance when analyzed by group ($F_{2,78} = 3.6$, $P = 0.0313$; $R^2 = 0.085$). A Tukey's test showed only *P. franciscana* and the Snowslide Spring population to be significantly different ($P = 0.0281$). The third principal component, when analyzed by group, was not significant ($F_{2,78} = 1.42$, $P = 0.248$; $R^2 = 0.035$). A Tukey's test showed no significant difference between any of the groups.

Genetics—Genetic analysis of 86 individuals from the five collection sites resulted in 321 polymorphic loci from four primer combinations. Structure Harvester recovered two distinct groups (K = 2). The population from Snowslide Spring (Fig. 3) shows various levels of admixture from the two parental species, with generally more alleles from *P. franciscana*. Almost all individuals of *P. werneriifolia* from the Inner Basin showed some admixture with *P. franciscana*.

Morphological and Genetic Correlation—The hybrid index, visually represented here by the Distruct graph (Fig. 3), is significantly correlated with the first principal component ($F_{1,79} = 143.4$, $P < 0.0001$). Additionally, 64% of the variation seen in the genetic makeup of each individual is due to the first principal component ($R^2 = 0.64$), which largely represents variation in leaf traits.

Discussion—Results of this study show there is hybridization occurring between *P. franciscana* and *P. werneriifolia* at Snowslide Spring in the San Francisco Peaks. PCA and ANOVAs performed on the individual principal components provide morphological evidence. When used together those traits comprising the majority of the first principal component, including leaf length to width ratio, basal leaf length and width, and peduncle length, might allow managers to distinguish the hybrids from the parental species. A management plan is beyond the scope of this paper, but there is potential for managers to use leaf morphology as a tool to distinguish hybrids from parental species.

The molecular evidence for hybridization can clearly be seen in the Distruct graph (Fig. 3). We used these analyses of the AFLP data to assess whether the individuals in the Snowslide Spring population were genetically identical to either parental species, or whether there was admixture of the parental species, creating a hybrid swarm. Admixture of the two parental species is occurring in the Snowslide Spring population, with *P. franciscana* alleles more highly represented. The Distruct graph also suggests that hybrids are fertile and capable of backcrossing in at least one direction with pure *P. franciscana*.

The molecular evidence also suggests that hybridization may be more widespread than was hypothesized at the beginning of this study, as seen in the admixture occurring in the Inner Basin population of *P. werneriifolia*. The Distruct graph (Fig. 3) shows that all individuals in this population are admixtures of the two parental species, with *P. franciscana* alleles present but less represented. Leaves of a few individuals from the Inner Basin population have two to three well-developed apical teeth (Fig. 1), a characteristic that is unusual in Arizona *P. werneriifolia*. Presence of apical teeth along with the quantitative characteristics noted above may help identify hybrids in the future. Mapping of high-elevation *P. werneriifolia* populations and surveys for additional hybrid populations should be a priority to assess whether hybridization is more widespread in the San Francisco Peaks or limited to the Inner Basin.

A more thorough population genetic analysis of *P. franciscana* is necessary to assess the extent of the hybrid zone on the San Francisco Peaks. Additional studies could investigate whether phenology or elevational distribution of one or both species is changing due to climatic shifts. If *P. werneriifolia* moves upslope as predicted by some models of climate change (Parmesan and Yohe, 2003; Thomas et al., 2004), then more hybrid populations would be likely in the future. In addition, because widespread species of *Packera* are known to hybridize where species distribution overlaps (Barkley, 1988), and because our small study included only two species from the area, future studies might include other sympatric and widespread *Packera,* including *Packera multilobata* and *Packera neomexicana,* to assess their involvement in this hybrid complex.

There are three possible evolutionary consequences of hybridization for the rare endemic *Packera* in the San Francisco Peaks. First, hybrid swarms may be ephemeral and have no long-lasting impact on the current parental species, thus maintaining the existing biodiversity. Second, hybridization may lead to the extinction of the rare endemic parent, *P. franciscana*, resulting in a destruction of biodiversity (Rhymer and Simberloff, 1996; Wolf et al., 2001). The third possibility is the creation of new diversity. Genetic diversity is directly related to the ability

of a species to respond to environmental changes. Because alpine endemics are particularly threatened by climate change due to limited range sizes and no escape route, increased genetic diversity may enable *P. franciscana* to persist, albeit in a genetically altered state.

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