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ABSTRACT—We used data on alleles of seven polymorphic microsatellite loci in 142 individuals of the star cactus *Astrophytum asterias* from five subpopulations to estimate genetic parameters. Four of the subpopulations had high levels of heterozygosity and allelic diversity compatible with efficient outcrossing, and low F_{ST}-values that suggested high rates of gametic gene flow effected by winged-insect pollinators. The fifth subpopulation that was small and occupied the smallest area had low levels of heterozygosity and allelic diversity, which can be caused by small populations, inbreeding, geographic isolation, and founder effect. Our research indicated that all subpopulations, except one, were genetically suitable sources of propagules for reintroduction or for augmentation of other populations.

RESUMEN—Utilizamos datos de los alelos de siete loci polimórficos de microsatélites en 142 individuos del cactus *Astrophytum asterias* provenientes de cinco subpoblaciones para estimar los parámetros genéticos. Cuatro de las subpoblaciones mostraron altos niveles de heterozigosidad y diversidad alélica, siendo compatibles con cruzamiento exógeno eficiente, y bajos valores de F_{ST}, los cuales sugirieron flujo gamético de genes intermediados por insectos voladores como polinizadores. La quinta subpoblación, que era pequeña y ocupaba el área más pequeña, mostró bajos niveles de heterozigosidad y diversidad alélica, lo que puede ser consecuencia de un pequeño tamaño poblacional, endogamia, aislamiento geográfico y el efecto de fundadores. Nuestra investigación indicó que todas las subpoblaciones, menos una, eran genéticamente apropiadas para servir como fuentes para la reintroducción de la especie o el aumento de otras poblaciones.

The star cactus (*Astrophytum asterias*) is an endangered species that, except for a small area of ca. 100 km^2 in Starr County, Texas, is endemic to northeastern Mexico (United States Fish and Wildlife Service, 1993; Martínez Ávalos et al., 2004). In Texas, the species has a generally sparse and patchy distribution with several dense stands covering small areas across the fragmented remains of Tamaulipan-thornscrub habitat (Poole et al., 2007).

Important anthropogenic pressures on populations of *A. asterias* include illicit collecting, the agricultural practice of root-plowing, and urban development (Clover, 1932; Strong and Williamson, 2007; Terry et al., 2007). In addition to these threats, natural threats that typically are fatal to the cactus include disease caused by the pathogen *Phytophora infestans*, infestation by a herbivorous cerambicid beetle (*Moneilema armatum*), and consumption of the aerial portion of the stem by vertebrate herbivores (Martínez Ávalos et al., 2007; G. K. Janssen et al., in litt.). These pressures would be expected to give rise to small populations, fragmentation of habitat, and genetic effects, such as decreased heterozygosity associated with inbreeding, fixation of alleles, and loss of alleles. All of these are manifestations of decreased genetic diversity, which may precipitate loss of fitness and decreased evolutionary potential (Moritz, 1994).

Herein, we report a study of seven microsatellite markers identified from genomic DNA of *A. asterias* from Starr County, Texas. We undertook the study to determine the degree of genetic structure within and among five subpopulations and to indicate which, if any, of these presumed subpopulations may constitute genetically distinct populations. We evaluated the breeding system of *A. asterias* based on genetic data, as well as gene flow in the form of gametic migration, and we examined genetic evidence for a recent reduction in size of populations. We also assessed suitability of the subpopulations as sources of propagules for reintroduction of *A. asterias* into suitable habitat within the historical range of the species,

or for augmentation of demographically inviable or genetically impaired populations.

MATERIALS AND METHODS—We sampled 142 individuals of *A. asterias* from five locations that covered the known range of the species in the United States as of April 2007. To avoid risk of mortality from wounding of stem tissues, we collected only tepals for subsequent extraction of DNA. The number of individuals sampled was the number of individuals in flower on the day sampling took place at a given site. Distribution of the 142 individuals among the five study sites (denoted SE, SSW, SW, N and NE) was as follows: SE, 84; SSW, 14; SW, 14; N, 22; NE, 8. Due to poaching of this endangered species, we are not disclosing exact locations of study sites.

Following methods described by Terry et al. (2006), we placed tepals into vials containing desiccant pellets (t.h.e. Desiccant, EMD Chemicals, Cincinnati, Ohio) and stored them at ambient temperature until DNA was extracted. We stored solutions of DNA at −20°C until needed, we developed seven microsatellite loci from genomic DNA from one individual of *A. asterias* from SE, and we amplified microsatellite loci by PCR and performed fragment-size determinations by capillary electrophoresis.

We estimated population-genetic parameters from microsatellite data using Genepop (Raymond and Rousset, 1995) and GenAlEx (Peakall and Smouse, 2006). In partitioning input data for analyses using Genepop and GenAlEx, we treated clusters of individuals from the five study sites as five discrete populations. However, for all other purposes we employed Wright's (1965, 1978) terminology, whereby our five geographically defined clusters are denoted as subpopulations comprising a single all-inclusive population. We analyzed structure of populations using the Bayesian method of STRUCTURE (Pritchard et al., 2000), wherein we generated 10 replicates for each assumed value of *K* (number of differentiated populations), with a burn-in of 50,000 and 100,000 iterations. We assumed admixture and independent frequencies of alleles for *K* = 1–5.

We used Genepop to test for linkage disequilibrium between members of each pair of loci by the method of Garnier-Gere and Dillmann (1992) for the purpose of identifying any linked loci that should be eliminated from subsequent analyses. We used the Markov chain method of Guo and Thompson (1992) to obtain multilocus values of *P* to test for Hardy-Weinberg equilibrium for each subpopulation. We tested genotypic data for increased heterozygosity and reduced heterozygosity, in proportions expected in a randomly breeding population, using the scoring method of Rousset and Raymond (1995) in Genepop. We assessed allelic differentiation within and among subpopulations using the method of Weir and Cockerham (1984) to estimate Wright's (1965) allele frequency-based *F*-coefficients F_{IS}, F_{ST}, and F_{IT}, where F_{IS} measures genetic differentiation among individuals within subpopulations, F_{ST} measures differentiation among subpopulations, and F_{IT} measures differentiation among all sampled individuals across subpopulations. We used the GenAlEx analysis of molecular variance (AMOVA; Peakall and Smouse, 2006) to estimate values of F_{ST} across subpopulations combined. We also used GenAlEx software to estimate Nei's (1972) genetic distance between members of each of the 10 pairs of subpopulations. We implemented a linear regression of geographic distance on genetic distance using R version 2.9.2 (R Development Core Team, 2009). We used FSTAT (J. Goudet, http://www.unil.ch/izea/softwares/fstat.html) to compare allelic richness among populations by standardizing for differences in size of populations. We applied a test for allelic evidence of any recent reduction in size of population to the dataset of alleles for each subpopulation and for the entire population to calculate *M*, defined as the mean ratio of the number of alleles to the range in size of alleles (Garza and Williamson, 2001).

RESULTS—All seven loci in the population-genetic analysis were polymorphic, exhibiting a total of 69 alleles. Number of alleles per locus was 6–14, with a mean of 9.9. In all individuals, we observed a maximum of two alleles at each locus, indicating diploidy. In a comparison of the five subpopulations, SSW had the lowest expected heterozygosity (H_E = 0.631), the lowest observed heterozygosity (H_O = 0.488), the lowest (and the only statistically significant) *P*-value for deviation from Hardy-Weinberg equilibrium ($P < 0.01$), and, with NE, the lowest mean number of alleles observed per locus (A_O = 4.8; Table 1).

Results of analysis of allelic data using the Bayesian method of STRUCTURE provided mean estimates of the natural logarithm of the posterior probability, Ln P(D), that the genotype data fit a given assumed value of *K*, the number of well-differentiated populations encompassing all 142 individuals sampled. Values of Ln P(D) for the five assumed values of *K* were: 1, −2,740; 2, −2,767; 3, −2,861; 4, −2,977; 5, −3,027. These results support a value of *K* = 1.

Significant linkage disequilibrium between two of the seven microsatellite loci was detected using the method of Garnier-Gere and Dillmann (1992). We excluded the less variable of the two linked loci (*AaA3*) as in Terry et al. (2006) from subsequent analyses, with the exception of the *M*-ratio test as in Garza and Williamson (2001).

When a test for estimating exact *P*-values for deviation from Hardy-Weinberg equilibrium (Guo and Thompson, 1992) was applied to each locus for each subpopulation, we observed two deviations within SSW. The loci *AaH11* ($P < 0.001$) and *AaC3* ($P = 0.014$) had significant deviation from Hardy-Weinberg equilibrium. When we applied the same test to each subpopulation across all loci, only SSW showed significant ($P = <0.001$) deviation from Hardy-Weinberg equilibrium.

The Hardy-Weinberg test for deficiency of heterozygotes yielded significant deviation for locus *AaH11* in SSW ($P = <0.001$) and SE ($P = 0.001$), as well as locus *AaC3* in SE ($P = 0.020$). The corresponding test for excess of heterozygotes revealed no significant difference for any locus in any subpopulation.

SSW had the highest values of Wright's (1965) inbreeding coefficient F_{IS} for four of six loci (Table 2), and the average F_{IS} across all loci for SSW was 0.23, which was more than three times the F_{IS} values of the other subpopulations (Table 2). The overall value of F_{ST} across all loci and all subpopulations was 0.05. Although not

TABLE 1—Summary of data for five subpopulations of the star cactus *Astrophytum asterias* in Starr County, Texas: n, size of sample; A_O, mean number of alleles per locus; H_E, mean expected heterozygosity across all loci; H_O, mean observed heterozygosity across all loci; H-W P-value, all-locus P-value for deviation from Hardy-Weinberg equilibrium (** signifies highly significant); and M-ratio for detecting recent reductions in size of subpopulation.

Subpopulation	n	A_O	H_E	H_O	H-W P-value	M-ratio
SE	84	8.7	0.689	0.659	0.26	0.899
SSW	14	4.8	0.631	0.488	<0.01**	0.713
SW	14	5.8	0.694	0.650	0.41	0.800
N	22	5.7	0.651	0.629	0.58	0.849
NE	8	4.8	0.811	0.775	0.46	0.774

statistically significant, SSW had arithmetically low levels of allelic richness, relative to those of other subpopulations, in the FSTAT analysis. SSW also had, with NE, an average of 4.8 alleles/locus, the lowest value among the five subpopulations.

In the test for allelic evidence of a recent reduction in size of the population, the average value of M for the entire population was 0.894. The lowest value of M for a subpopulation was 0.713 in SSW (Table 1).

DISCUSSION—One negative consequence of sampling only individuals in flower was that it limited sampling to a minority of individuals in each population. Specifically sampling tepals excluded all sexually immature individuals and all adults not in flower on the day of sampling. This is an important consideration because any genetic change (e.g., loss of alleles, decreased heterozygosity) that could be attributed to a recent or incipient contraction in size of the population might be manifested only in individuals young enough to be the progeny of extant sexually mature adults; such a contraction might escape detection in our analysis, as in Friar et al. (2000).

We have no reliable data on average number of years it takes a newly germinated seedling of *A. asterias* to reach sexual maturity in its natural habitat, or on average lifespan of individuals, but Martínez Ávalos et al. (2004) reported that seedlings observed for 5 years in Mexican populations require ≥3 years to reach reproductive maturity. In cultivation, seedlings attain sexual maturity in 2–5 years and can live for ≥30–39 years (S. Brack, pers. comm.; G. Koehres, pers. comm.). The implication is that a fraction of the mature plants that we sampled could have germinated prior to the 1980s; thus, our data may not reflect recent changes in genetic status of this species.

The natural logarithm of the posterior probability of a good fit of the allelic dataset consistently decreased as the assumed value of K increased from 1 to 5, indicating that $K = 1$ is the most likely of the five values of K examined. This analysis supports the conclusion that the five geographic clusters of plants sampled in our study constitute subpopulations of a single ($K = 1$) large population. This is compatible with the low (ca. 0.05) F_{ST}-values between subpopulations and with the hypothesis that a large, spatially continuous population has undergone recent fragmentation such that the subpopulations (with the exception of SSW) have had insufficient time to undergo dramatic genetic differentiation.

The small population of SSW (14 individuals that were reproductive at time of sampling) and the small land area (ca. 0.5 ha) to which SSW is now confined are conducive to inbreeding and loss of alleles. As an illustrative comparison, SSW can be contrasted with the considerably larger population and much larger land area (ca. 400 ha) occupied by SE, where inbreeding would not be expected. When we compared numbers of alleles detected in these two subpopulations, the combined number of alleles for all six loci in SSW was 29, compared with 52 alleles in SE. This difference in number of alleles must be due in part to the larger number of individuals

TABLE 2—Allele frequency-based correlation and variation (F_{IS}, Wright's inbreeding coefficient) among individuals within each subpopulation of the star cactus *Astrophytum asterias* in Starr County, Texas. The matrix consists of F_{IS}-values for each of the six loci in each of the five subpopulations. Numbers in the bottom row are F_{IS}-values for all individuals sampled from all subpopulations combined. Numbers in the last column are F_{IS}-values for all loci combined, for each subpopulation.

Subpopulations	Locus						All loci
	AaB6	*AaH11*	*AaG3*	*AaC3*	*AaD9*	*AaG4*	
SE	0.04	0.13	−0.10	0.11	0.03	−0.00	0.04
SSW	0.10	0.75	−0.09	0.36	0.47	−0.12	0.23
SW	0.01	0.13	0.21	−0.10	−0.03	0.11	0.07
N	0.26	0.19	−0.04	−0.05	0.11	−0.08	0.04
NE	−0.11	0.15	0.10	−0.05	—	0.12	0.05
All	0.07	0.20	−0.04	0.08	0.03	−0.01	

sampled in SE (84) compared to SSW (14). When we standardized allele frequencies for differences in size of populations with the FSTAT allelic-richness program, allelic richness of SSW was arithmetically less for all six loci than allelic richness of SE, although the reduction was not statistically significant. The data for SW, N, and NE revealed a consistently lower allelic richness in SSW for the majority of loci compared to those of other subpopulations. These results are compatible with the inference of loss of alleles in SSW.

It is not surprising that the Hardy-Weinberg test for deficiency of heterozygotes was significant at the same loci (*AaH11* and *AaC3*) that had significant *P*-values for disequilibrium of Hardy-Weinberg in the small, spatially compressed SSW. Less obvious were significant *P*-values in the Hardy-Weinberg test for deficiency of heterozygotes at the same two loci in the largest subpopulation, SE, which had substantially more alleles and allelic richness than SSW. This is expected in these subpopulations, given the history of adverse changes in use of land that have partially destroyed and fragmented the habitat. In the remaining areas of viable habitat, where small numbers of *A. asterias* survive in relative isolation, inbreeding is inevitable, as in the small subpopulation at SSW. The statistically significant Hardy-Weinberg test for deficiency of heterozygotes in two loci in the large and geographically extensive SE may reflect a similar process of fragmentation within a large subpopulation. During the process of collecting samples, we noticed that distribution of individuals within SE was not regular or random, but it was clumped. Indeed, 82% of individuals sampled at SE were clustered in two areas that totaled <1 ha. The observed deficiency of heterozygotes can be attributed exclusively to the area with the most individuals sampled (Terry, 2005).

Despite the relatively large number of plants in SE and the large expanse of land over which SE is distributed, most individuals occur and most breeding takes place in a few small enclaves of densely concentrated plants in proximity to each other. Such a situation is highly conducive to inbreeding that would contribute to significant deficiency of heterozygotes with no evident loss of alleles in SE.

At least on a fine scale, *A. asterias* is characterized by high volatility in size of populations. Martínez Ávalos et al. (2007) have reported mortality of 59–66% due to herbivores and a fungal pathogen during a 2-year period (2005–2006) in transects in four populations in northeastern Mexico. Similarly G. K. Janssen et al. (in litt.) observed 56% mortality due to herbivory in an 8-month period in 2005–2006 in the population in southern Texas. This plant also appears to have a remarkable reproductive capacity that is fully expressed only when environmental conditions are optimal. During a 12-month period in 2004–2005, one transect in southern Texas had a 64% increase in number of individuals (G. K. Janssen et al., in

litt.). It is reasonable to expect that there will be an occasional rapid increase in size of populations within a small area where environmental conditions temporarily are optimal. Such proliferation results in a dense cluster of individuals that are descended from a modest number of ancestors that survived the last period of reproductively unfavorable environmental conditions. It is notable that members of such clusters currently are breeding primarily with their closely spaced and closely related neighbors. Such a situation is conducive to inbreeding that would be observed as a Hardy-Weinberg deficiency of heterozygotes at some loci. This explanation is compatible with genotypic results we encountered in the largest subgroup in the largest subpopulation, SE.

In outcrossing species, decreased heterozygosity often is associated with an increase in expression of deleterious recessive genes, which may bring about a decrease in fitness. This may result in a decreased rate of survival. If allowed to continue unabated, these phenomena would push the population into a self-reinforcing causal chain of events known as the extinction vortex (Soulé and Orians, 2001). The deficiency of heterozygotes detected in SSW is typical in a small, and therefore, likely inbred, group of individuals. If this subpopulation is largely reproductively isolated from other subpopulations, then there may be insufficient gene flow into SSW to mitigate effects of inbreeding. This may not be true with subgroups within SE, as these appear to be close enough to each other to benefit from gametic gene flow, which would be expected eventually to counterbalance inbreeding in those subgroups. Deficiency of heterozygotes in SSW is reflected in the low value of H_O compared to H_E for that population (Table 1). It is notable that heterozygosity (H_O) values for other subpopulations in Table 1 are typical of genetically healthy populations of an outcrossing species, and thus, population-genetic data independently confirm the conclusions of Strong and Williamson (2007), based on pollination experiments, that the breeding system of *A. asterias* is that of an obligate outcrosser.

The average *M*-value of 0.894 for the entire population was not indicative of a recent contraction in overall size of population (Garza and Williamson, 2001). However, the method of sampling may have precluded detection of a recent contraction, i.e., within the past generation. The lowest *M*-value (0.713) was for SSW. *M*-values <0.68 have been associated with reduction in size of populations, island populations, and founder effect (Garza and Williamson, 2001). While the *M*-value for SSW is above the threshold for a reduction in size of population, it is low relative to *M*-values of other subpopulations, and any of these explanations could apply to SSW.

The elevated F_{IS}-values for several loci in SSW, including the value of 0.75 for the *AaH11* locus and the value of 0.23 for all loci combined, reflect a high degree of inbreeding in that subpopulation. Such high values of

TABLE 3—Comparison of pairwise estimates of intersubpopulational genetic differentiation among five subpopulations of the star cactus *Astrophytum asterias* in Starr County, Texas.

Pair of subpopulations	F_{ST}	Nei's genetic distance (D)	Geographic distance (km)
SE–SSW	0.029	0.116	9.6
SE–SW	0.043	0.230	9.7
SSW–SW	0.067	0.324	1.8
SE–N	0.031	0.143	6.2
SSW–N	0.056	0.239	10.2
SW–N	0.046	0.216	9.0
SE–NE	0.035	0.121	2.8
SSW–NE	0.039	0.136	11.7
SW–NE	0.060	0.250	11.4
N–NE	0.053	0.189	5.4

F_{IS} are compatible with the low heterozygosity and low average number of alleles per locus observed in SSW.

The global F_{ST}-value of 0.05 for all subpopulations combined places the degree of genetic differentiation among subpopulations on Wright's (1978) threshold between the upper extreme of little genetic differentiation and the lower extreme of moderate genetic differentiation. This reflects the fact that some genetic divergence among subpopulations has occurred, but much of it can be attributed to the elevated F_{ST}-value for SSW, which was 0.23, a level that indicated great genetic differentiation to Wright (1978). Because SSW is a small subpopulation represented in this study by a relatively small number of samples, the overall F_{ST}-value for the entire population does not exceed the upper limit of Wright's (1978) interval of F_{ST}-values indicating little genetic differentiation.

Among the pairwise values of F_{ST} for all pairs of subpopulations, values indicating moderate genetic differentiation between subpopulations by Wright's (1978) criterion ($F_{ST} > 0.05$) occur for pairs SSW–SW, SSW–N, SW–NE, and N–NE. The pairwise F_{ST}-values in Table 3 constitute a means of recognizing subpopulations that are genetically most different from each other. A conspicuous feature of these results is that F_{ST}-values are all <0.1. These results correspond to support of one large population by the Bayesian method of structure analysis and suggest that gene flow is occurring among the five subpopulations. However, these also could be the result of contractions of populations, incomplete lineage sorting of alleles, or presence of ghost subpopulations (Slatkin, 2005).

Gametic gene flow among subpopulations of *A. asterias* we studied must be attributable to insect pollinators (Blair and Williamson, 2008). In regard to zygotic gene flow (dispersal of seeds) in *A. asterias*, the situation is less clear. There is a robust literature on frugivores as dispersers of seeds of cacti that produce palatable, fleshy fruits (e.g., Godínez-Alvarez and Valiente-Banuet, 1998;

Montiel and Montaña, 2000), but nothing has been reported for *Astrophytum*. Fruits of *Astrophytum* are not fleshy, they are almost dry, and they are covered with dense areolar wool and spinescent bracteoles (Anderson, 2001; Powell et al., 2004). Despite these physical characteristics that would presumably discourage frugivory, we have observed what we interpreted as tooth marks of small mammals that had gnawed on fruits of *A. asterias* in southern Texas. This raises the possibility of endozoochory, although simple predation and destruction of seeds appears more likely from fragments of seeds observed. We have seen evidence of what we presumed were ants opening fruits, suggesting synzoochory. Based on a comparative analysis of morphology of seeds and empirical determination of duration of floating in water, Bregman (1988) reported that seeds of *Astrophytum* (and several other genera of cacti) are of a type compatible with hydrochory. This also has been observed in eastern Nuevo León, Mexico, where summer rains dislodged seeds from dehisced fruit and created flow of water among adult plants such that seeds were transported by water.

Genetic distance and F_{ST}-values for SSW and SW in Table 3 were unexpected aspects of pairwise estimates of genetic differentiation. These subpopulations had the highest pairwise value of F_{ST} (0.067) and the highest value of Nei's (1972) genetic distance D (0.324) of all pairs of subpopulations, but SSW and SW had the smallest geographic distance between them (1.8 km) of all the pairs of subpopulations. Results of regression analysis revealed that all pairs of subpopulations, with the exception of those involving SSW, had a direct correlation of genetic and geographic distances, while SSW is closest genetically to the most distant populations. The unusual genetic makeup of SSW relative to the other subpopulations may be explained as a result of inbreeding and loss of alleles due to destruction and fragmentation of habitat, or possibly as a founder effect by postulating its recent anthropogenic origin from propagules obtained from subpopulations in the eastern part of the range.

The star cactus is a rare plant with a scattered distribution across a fragmented habitat and (with the exception of the one small subpopulation, SSW) with subpopulations that have unexpectedly low F_{ST}-values. How did such a situation arise? One hypothesis is that we are simply looking at ancient history, i.e., persistence of the genetic composition of previous generations, which seems possible in light of documented longevity of some *A. asterias* in cultivation. Another hypothesis is that plant-specific, long-distance insect pollinators are continuing to bridge gaps between fragments of the ancestral population thereby producing sufficient gene flow to diminish effects of inbreeding and genetic drift in isolated subpopulations. However, the longest distance recorded between two *A. asterias* that were visited by a flying insect pollinator was <200 m (G. K. Janssen et al., in litt.),

whereas subpopulations we sampled were kilometers apart. Thus, pollinator-mediated gene flow seems an unlikely explanation of the low F_{ST}-values.

There also is the hypothesis that ghost subpopulations (Slatkin, 2005) between the extant subpopulations that we sampled are, or were, serving as genetic bridges between subpopulations. This would shorten distances that winged pollinators would have to span to effect adequate gene flow, which would keep F_{ST}-values low. This hypothesis will be tested by the success or failure of future analyses of new subpopulations that are geographically intermediate between the currently known subpopulations examined in our study.

One way to simultaneously test all three hypotheses is to genotype every reproductive individual in a subpopulation within a year, collect and germinate seeds from fertilizations for that year, and genotype the progeny. Then, one could look for novel rare alleles coming from ghost subpopulations or long-distance pollination. Such data would provide a good estimate of the real number of migrants; a measure of gene flow in that year (Slatkin, 1985). If the real number of migrants was low or none, that would support the hypothesis that ancient gene flow accounts for the present genetic composition of reproductively mature adults.

Our research offers conclusions useful to planning conservation measures to implement the recovery plan for *A. asterias* (United States Fish and Wildlife Service, 2003). All but SSW appear to be genetically robust in terms of expected levels of heterozygosity and low levels of genetic differentiation among subpopulations, indicating that this species is an efficient outcrosser. SSW is in need of restoration-augmentation. This is evident from its high degree of homozygosity at several loci and elevated levels of F_{IS} and pairwise F_{ST}, indicating inbreeding and some degree of genetic isolation. This is consistent with a small population and the small, island-like area of suitable habitat currently available. The contrapositive conclusion is that SSW would not be genetically suitable as a source of seed for reintroductions to historical habitat or augmentations of other populations. The more efficacious direction for managed flow of genes would be from the larger, more-heterozygous subpopulations with low F_{IS}-values and F_{ST}-values (such as SE and SW) to smaller, more homozygous subpopulations with high F-values, such as SSW.

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