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Genetic diversity and reproductive biology in *Warea carteri* (Brassicaceae), a narrowly endemic Florida scrub annual[1]

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Carter's mustard (*Warea carteri*) is an endangered, fire-stimulated annual endemic of the Lake Wales Ridge, Florida, USA. This species is characterized by seed banks and large fluctuations in plant numbers, with increases occurring in postdisturbance habitat. We investigated the mating system, patterns of isozyme variation, and effective population sizes of *W. carteri* to better understand its population biology and to comment on reserve designs and management proposals relevant to this species. *Warea carteri* is self-compatible and autogamous, and probably largely selfing. Measures of genetic variation in *W. carteri* were lower than values reported for species with similar ecological and life history traits (6.6% of loci polymorphic within populations, 1.87 alleles per polymorphic locus, and 0.026 and 0.018 expected and observed heterozygosity, respectively). The high average value for Nei's genetic identity (0.989) reflects the paucity of genetic diversity. Genetic variation within populations was not correlated with aboveground population size, effective population size estimates (N_e), or recent disturbance history. Much of the diversity detected was found among populations ($F_{ST} = 0.304$). A significant cline in allele frequencies at one locus and a significant negative correlation between geographic distance and Nei's genetic identity also point to spatial organization of genetic diversity. As a result we propose that reserve design should include the entire geographic range of *W. carteri*. We also recommend that the natural fire regime be mimicked.

Key words: Brassicaceae; effective population sizes; genetic diversity; mating system; *Warea carteri*.

The Lake Wales Ridge (LWR; Highlands and Polk Counties) of south-central Florida is both a hotspot of biodiversity in the continental United States and highly endangered (Noss and Peters, 1995). Among higher plants, there are 16 federally listed species endemic to the LWR. The ongoing creation of an archipelago of fire-managed reserve "islands" is key to their conservation. Designing a reserve system and land management program that will support viable populations or metapopulations of these species requires knowledge of their autecology and population structure. *Warea carteri* (Brassicaceae) is one of seven LWR endemics for which we are gathering detailed genetic and demographic data to evaluate current reserve designs and make recommendations about fire management.

Warea carteri is a federally endangered annual occurring in xeric sandhills and scrubby flatwoods (community delineations follow Abrahamson, 1984). There are historical records of *W. carteri* from outside the LWR, including Brevard, Broward, Dade, De Soto, and Glades Counties (Al-Shebaz, 1985). In his description of the species, Small (1909) stated that *W. carteri* was the "most common species of *Warea,* occur[ring] in great abundance." *Warea carteri* has almost certainly been extirpated from these areas, indicating it has already suffered a reduction in its range. The xeric communities of the LWR were historically distributed in over 200 patches ranging in area from four to 1200 hectares (ha) along the ~160 × 20 km north-south ridge (Christman, 1988). Peroni and Abrahamson (1985) estimated that 84% of the presettlement extent of xeric, upland communities in Highlands County (which contains close to half the LWR) had been converted to citrus groves or residential developments by 1981.

Demographic data indicate that *W. carteri* thrives in a temporally restricted, postdisturbance niche (Menges and Gordon, 1996). Large fluctuations (up to three orders of magnitude) in the number of plants are associated with fire, with increases the year after fire followed by decreases the second year after fire (Menges and Gordon, 1996). Oscillations in 2-yr cycles often occur, damping over time. *Warea carteri* may appear to be extirpated

from sites for years, only to have aboveground individuals recruit after fire. Sites associated with anthropogenic disturbance often support more stable and persistent but small populations. Historically, fires were frequent in peninsular Florida. Estimates of the natural fire return intervals of the communities *W. carteri* inhabits span 2–20 yr, based on the life histories of plants in these communities and their patterns of abundance in response to fire (Abrahamson and Hartnett, 1990; Myers, 1990; Ostertag and Menges, 1994; Menges and Kohfeldt, 1995; Menges and Hawkes, 1998). Fires have now been suppressed on the LWR for ~60 yr.

The ephemeral nature of populations of *W. carteri* suggests that seed banks are present. Seed dormancy of at least 2 yr has been demonstrated under laboratory conditions (N. Kohfeldt and E. Menges, Archbold Biological Station [ABS], unpublished data). Fruits and seeds of *W. carteri* do not have any specialized structures to facilitate dispersal, and spatial congruence of aboveground populations over time suggests limited dispersal (E. Menges, ABS, personal observations). Seeds of *W. carteri* average 0.282 ± 0.067 mg, and their potential horizontal travel is 1.89 m in a wind speed of 2.17 m/s (D. Gordon, The Nature Conservancy [TNC], unpublished data).

Conservation of genetic diversity is considered to be important for the long-term evolutionary potential of species (Barrett and Kohn, 1991; Huenneke, 1991). The primary purpose of this study was to assess genetic diversity in *W. carteri,* by surveying isozymes in 23 populations throughout the species' range (Fig. 1), and make recommendations about how to capture this diversity in reserves. Since genetic diversity is a dynamic, not a static phenomenon, we assessed processes and traits that might affect the population genetics of *W. carteri.* Using Wright's *F* statistics, we assessed population substructure and gene flow. Since the mating system of *W. carteri* was unknown and likely to influence patterns of genetic diversity (Loveless and Hamrick, 1984; Hamrick and Godt, 1990), we determined this trait and discuss its implications for demographic and genetic dynamics. Given the large fluctuations of populations with fire and persistence between fires via in situ seeds, we estimated effective population sizes of *W. carteri* and relate population-level genetic diversity statistics with census population sizes, effective population sizes, and recent history of disturbance. Thus our approach to the conservation of *W. carteri* has been to obtain genetic, ecological, and demographic data to reconstruct the dynamics of past and present populations and evaluate reserve designs and fire management regimes that will affect population dynamics and genetic variation in the future.

MATERIALS AND METHODS

Floral biology—Flowers of *W. carteri* are perfect and offer both nectar and pollen as floral rewards. They are open and radially symmetrical, with four white petals and six white stamens of equal length. The flowers are clustered in round, dense racemes, and there are several inflorescences per plant. By observing marked flowers we found that the flowers are protandrous. The stamens abscise before stigmas become receptive. Stigmatic receptivity was assessed using hydrogen peroxide, a standard test (Kearns and Inouye, 1993), and by observing changes in the surface area and texture of the stigma.
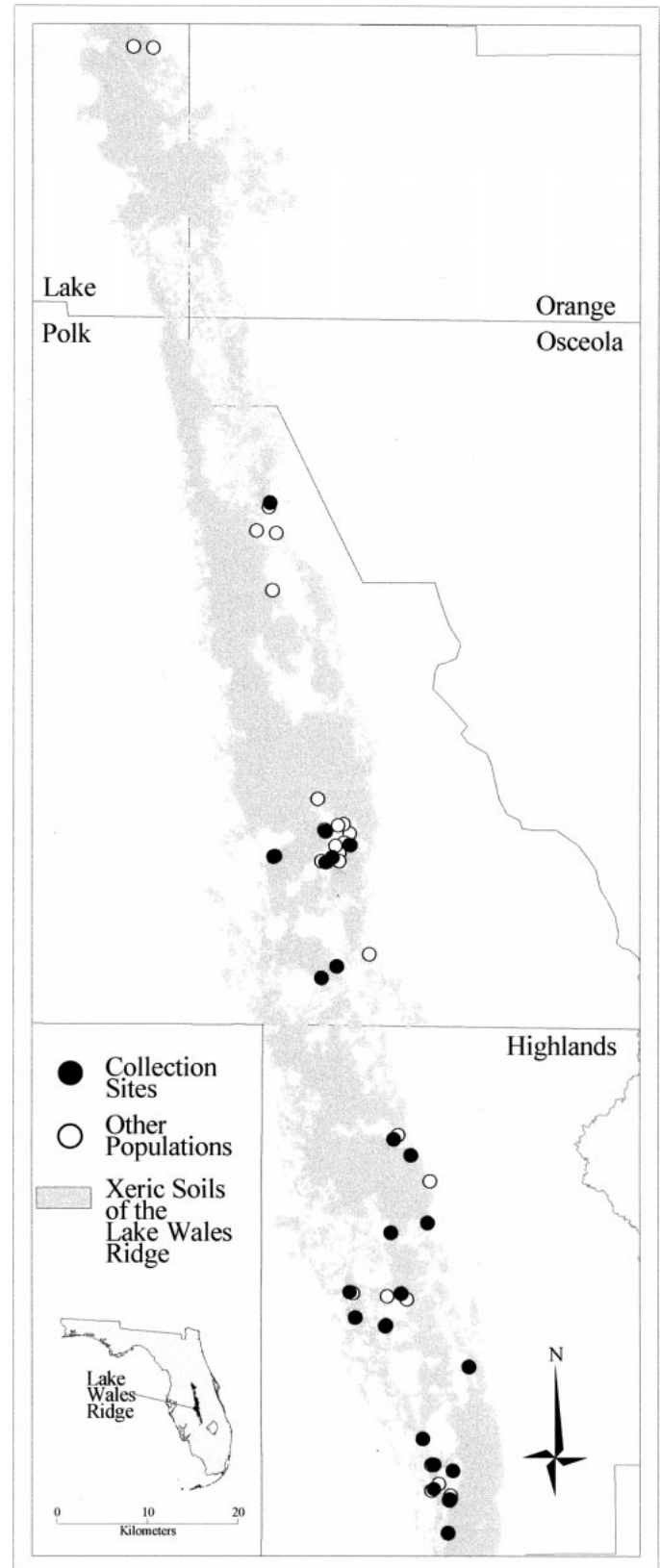

Fig. 1. Known populations of *Warea carteri* on the Lake Wales Ridge (LWR, shaded) in Lake, Polk, and Highlands Counties, Florida, USA. Populations sampled for isozymes are shown with a filled circle and populations not sampled are shown with an open circle.

Mating system—We tested the mating system of *W. carteri* in a natural population at the Lake Placid Scrub Wildlife and Environmental Area (LPSWEA) south of Lake Placid, Florida, in the 2nd and 3rd wk of October 1994. This population consisted of ~300 individuals bordering a sand road in scrubby flatwoods that had not burned in about ten years. The mating system experiment tested for agamospermy (asexual reproduction via seeds), autogamy (spontaneous self-pollination), and self-compatibility. Outcross-pollinated and open-pollinated flowers served for comparisons. We applied each pollination treatment to one inflorescence per plant, such that all 20 plants in the experiment received each pollination treatment.

We removed all flowers and fruits from the inflorescences that would be treated so that only flower buds remained and randomly assigned each to a pollination treatment. Plastic mesh bags (Delnet nonwoven polyolefin, Applied Extrusion Technologies, P.O. Box 852, Middletown, Delaware, 19709; mesh size 0.8–0.9 mm) secured with twist-ties were used to isolate all but the control inflorescences. Newly opened flowers in the agamospermy, self, and outcross treatments were emasculated before anther dehiscence daily for 4 d. Flowers in the self-compatibility and outcross treatments were pollinated as their stigmas matured, by direct contact between anthers of first-day flowers protected from insect visitation and the stigmas. Generally, we used a single pollen donor per plant in the outcross treatment. The average distance between the plants donating and receiving pollen was 6.0 m (SE = 0.8, range = 1.5–13.1, $N = 20$). On the fourth day, we removed the remaining buds and apical meristem from all inflorescences in all pollination treatments, ensuring that no additional flowers would release self-pollen and confound the treatments.

Fruits were harvested as they matured and scored under a dissecting microscope for number of aborted and full-sized, mature seeds. Seeds were considered aborted if they appeared to have less than half the endosperm of a full-sized seed. All full-sized seeds from each fruit were weighed together to obtain a mean seed mass. We used a Kruskal-Wallis test to detect pollination treatment effects in the proportional fruit set data, since these data could not be transformed to approximate a normal distribution (Sokal and Rohlf, 1995). Proportional seed set data were arcsine transformed and a nested analysis of variance was performed to test for treatment effects. We tested for differences in mean seed mass per fruit among the pollination treatments with a one-way ANOVA. All ANOVA analyses were evaluated using Type IV sum of squares since different fruit numbers were treated in each plant and pollination treatment combination, resulting in empty cells in the data matrix (SAS, 1989). Pairwise differences among treatments were evaluated using Tukey's Honestly Significant Difference. Significance was evaluated at $P < 0.05$ throughout.

Electrophoresis—We visited all known extant sites for *W. carteri* on the LWR for which we were able to obtain permission to collect leaf material during the 1994 or 1995 flowering season (October); additional areas of suitable habitat were also surveyed for populations. Populations were defined by isolation from conspecifics by at least 50 m. Leaf collections were made from populations that were at least 1.6 km distant from other sampled populations. We sampled a total of 23 populations throughout the range *W. carteri* currently occupies, with the exception of an isolated site known from Lake County, Florida, that we were not able to access (Fig. 1). The northernmost sampled population was 115 km from the southernmost sampled population. Aboveground population size averaged 93 plants (median = 37, range = 18–703). History of fire and anthropogenic disturbance were perfectly correlated among the populations we studied: all five populations occurring in natural vegetation had burned recently, and all 18 populations occurring along sand roads had not.

For genetic analysis, we collected a small number of leaves from every plant in populations containing between 15 and 29 plants, and from a spatially stratified random sample in populations with 30 or more plants. The stratified samples were collected in the following manner:

from an initial survey of a population we obtained an estimate of the population size, and on a second sweep through the population we sampled an appropriate fraction of the plants to obtain a total sample of 30 individuals. Leaves were shipped to the laboratory within 24 h, where they were processed for horizontal starch gel electrophoresis. Tissue was extracted in a modified Wendel's sorghum buffer (Morden, Doebley, and Schertz, 1987). Gels stained for acid phosphotase (ACP, Enzyme Commission designation 3.1.3.2), alcohol dehydrogenase (ADH, 1.1.1.1), colorimetric esterase (EST, 3.1.1.-), peroxidase (PER, 1.11.1.7), and phosphoglucomutase (PGM, 5.4.2.2) were run in sodium borate buffer pH 8.6 (Kephart, 1990) at 200 v for 2.5 h. Glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH, 1.2.1.12), isocitrate dehydrogenase (IDH, 1.1.1.41), glucose-6-phosphate isomerase (GPI, 5.3.1.9) and 6-phosphogluconic acid dehydrogenase (6-PGD, 1.1.1.44) gels were run in a histidine/citrate buffer system (Ellstrand, 1984) at 25 mA for 3 h. Aconitase (ACO, 4.2.1.3), malate dehydrogenase (MDH, 1.1.1.37), mendadione reductase (MNR, 1.6.99.-), and triosephosphate isomerase (TPI, 5.3.1.1) gels were run on tris-citrate pH 8.0 (Kephart, 1990) at 50 mA for 5 h.

When more than one putative locus was observed for an enzyme, loci were numbered sequentially, with the most anodally migrating locus designated "one." We assigned sequential letters to enzyme variants for individual loci in the same manner. No formal genetic analyses were conducted to document the pattern of inheritance of putative alleles. Allelic assignments were based on the observed pattern of population variation, the known subunit structure of the enzymes, and the cellular compartmentalization generally observed for plant enzymes (Kephart, 1990). Standard measures of levels of genetic variation (percentage of loci polymorphic, number of alleles per locus, and observed and expected heterozygosity) were calculated for populations and for the species as a whole. Wright's F statistics (Wright, 1965, 1978) were calculated for all loci, and deviations from Hardy-Weinberg expectations were detected using chi-square tests. Pairwise genetic similarity between populations was calculated using Nei's unbiased genetic identity (Nei, 1978). Hierarchical cluster analysis of genetic identity values was performed using arithmetic averaging (UPGMA; Sneath and Sokal, 1973).

Effective population sizes—Because *W. carteri* has both overlapping generations and fluctuating population sizes, we used models that correct for these factors sequentially. Hill (1972) demonstrated that the effective size per generation of an equilibrium population with overlapping generations is equivalent to the effective size of a population with discrete generations. We used the following form of the "Hill" model (Eq. 11 in Caballero, 1994) to correct for variance in reproductive success and the mating system in populations with overlapping generations:

$$N_V = (4N_C L)/[2(1 - F_{IS}) + S_k^2(1 + F_{IS})] \qquad (1)$$

where N_C is the cohort size, L is the generation length, S_k^2 is the variance in reproductive success and F_{IS} is Wright's (1969) statistic that measures the deviation from Hardy-Weinberg expectation within populations. It is important to note that we used the flowering plant stage of the life cycle as the reference point for our calculation of N_C and S_k^2 (this issue is discussed clearly by Rockwell and Barrowclough, 1995). Specifically, "cohort size" refers to the number of flowering plants expected to result from a cohort of seeds, and "reproductive success" refers to the number of flowering plants produced per flowering plant. The average effective size per generation (N_V) was converted to an average number per year (N_V^*) by multiplying by the generation length (Hill, 1972; Lande and Barrowclough, 1987). We then took the harmonic mean of these values to correct for fluctuation in population size and age structure (Lande and Barrowclough, 1987), giving our final estimate of effective size (N_e).

The data required for the Hill model include the number of individuals (here, flowering plants) entering the population per generation, their variance in reproductive success, and F_{IS}. Following Heywood (1986)

TABLE 1. Census data and estimated average effective population sizes for selected populations of *Warea carteri* at Archbold Biological Station (ABS) and The Nature Conservancy's Tiger Creek Preserve (TCP). These are populations censused for at least 6 yr and for which the maximum aboveground population size among all census years was at least 20 individuals. For this analysis, we defined populations as groups of plants that were isolated from conspecifics by at least 50 m and had the same fire history. N_H, N_A and N_e are defined in the text.

| Population | Census | | | | | | | | N_H | N_A | N_e |
	1988	1989	1990	1991	1992	1993	1994	1995			
TCP 1	—	458[b]	0	23[b]	4	1	0	78[b]	2.1	174.7	4.6
TCP 4[a]	—	—	2	47[b]	0	1	25[b]	7	2.2	29.9	4.8
TCP 11	—	—	1	25	0	41[b]	11	3	2.4	29.5	5.2
TCP 21	—	11[b]	17	43[b]	2	11	0	9[b]	3.7	29.0	8.1
TCP 10[a]	—	—	2	10	5	0	703[b]	164	3.3	318.7	7.2
ABS 7[a]	—	—	18	0	21	4	23	11	4.0	28.1	8.7
ABS 2/3[a]	64	2	35	4	54	30	70	0	4.3	70.2	9.3
ABS 4	16	11	0	72[b]	6	135	13	88	5.6	92.4	12.1
ABS 1	58	2	44	12	18	30	7	10	8.4	48.9	18.1
TCP 5	—	—	12[b]	19	15	7	50[b]	7	11.8	39.6	25.5
ABS 10	—	—	77	18	134	16	45	21	28.8	112.0	62.2
TCP 3[a]	—	140	95	223	213	32	45	94[b]	77.0	259.9	166.3
ABS 12/13[a]	—	—	49	326	161	64	352	59	83.0	34.0	199.1

[a] These populations were also surveyed for isozyme diversity.
[b] Fire occurred the previous year.

and Husband and Barrett (1992), we obtained a species-wide estimate of variance in reproductive success from data on variance in fecundity from 11 populations in 3 yr. Only one and two of these populations could be sampled for three and two sequential years, respectively; the remaining populations did not appear aboveground more than one of the three years they were sampled. Our estimate of seed production per plant was the product of the number of fruits per plant (N = 8–30 plants per population and year) and the average number of full-sized seeds per fruit per population and year (N = 1 fruit per plant). Species values for the mean and variance of seed production per plant were obtained by averaging values across years within populations and then across populations. We then used the analytical solution described by Barrowclough and Rockwell (1993) to estimate variance in reproductive success:

$$S_k^2 = p^2 V_F + X_F V_p \qquad (2)$$

where p is the probability of surviving from seed to flowering plant (0.068; E. Menges and C. Weekley, ABS, unpublished data), V_p is the variance associated with this probability [$V_p = p(1 - p)$], and X_F and V_F are species-level estimates of the mean and variance in seed production per plant described above, respectively. We obtained an equilibrium estimate of the number of flowering plants entering the population per generation (N_cL) with the following expression:

$$N_cL = NX_F pL \qquad (3)$$

where N is the number of flowering plants per year and L is the generation length (mean age of parents, including residence time of seeds in the soil, 4 yr). We used census data from selected populations of *W. carteri* at ABS and The Nature Conservancy's Tiger Creek Preserve (near Frostproof in Polk County, Florida) for values of N (Table 1). Aboveground population sizes of zero were replaced with ones in these calculations. A species-wide value for F_{IS} was obtained from the isozyme data presented here.

We used a nonparametric Mann-Whitney U test to detect differences between estimates of average effective population size in populations with and without fire management (where fire management includes prescribed burning). We used Pearson and Spearman correlations to test for relationships between census population size, effective population size, or disturbance history and the proportion of loci polymorphic, the number of alleles per polymorphic locus, and expected heterozygosity per locus. The correlation between these population genetic statistics and effective population sizes was tested with data from the six populations for which we had both types of data. The effective population

size estimates tested were the arithmetic and harmonic means of the average effective population sizes per year (N_v^*), described above, and the harmonic mean of census population sizes (N_A, N_e, and N_H, respectively). Fire was considered recent if it had occurred within 3 yr before leaves were sampled.

RESULTS

Mating system—*Warea carteri* is self-compatible and autogamous, but not agamospermous (Fig. 2). Ninety-two percent of fruit and 61% of the ovules in these fruit developed in response to hand-pollination with self pollen. Autogamous fruit set averaged 51%, and seed set within these fruits averaged 33%. Few fruit developed in the agamospermy treatment (2%), and these fruits set no seeds. Proportional fruit set was significantly lower in the autogamy treatment compared to either of the hand-pollinated treatments (self and outcross); autogamous proportional seed set was significantly lower than self, outcross, and control treatments. The proportion of fruit and seed set in the self and outcross treatments was significantly higher than in the open-pollinated control treatment. Self and outcross pollination treatments were not significantly different for fruit or seed set. Differences in mean seed mass per fruit were not significantly related to pollination treatment.

Electrophoresis—Of 25 putative loci that could be clearly and consistently resolved, six (24%) were polymorphic at the species level: *Acp-2, Est-1, Idh-1, Idh-2, Pgm-2,* and *Pgm-3.* Three of these, *Est-1, Idh-1,* and *Idh-2,* had only one allele present at >5% frequency. Allele frequencies varied more widely for the remaining three loci (Table 2). Nei's genetic identity values for all populations averaged 0.989 (range = 0.946–1.00). Within this range, the populations grouped into three main clusters and one outlier (Fig. 3). Banding patterns were consistent with the number of loci usually associated with diploids (Kephart, 1990), with the exception of PGM, which had three staining loci, representing an apparent duplication. No private alleles were found. Three addi-
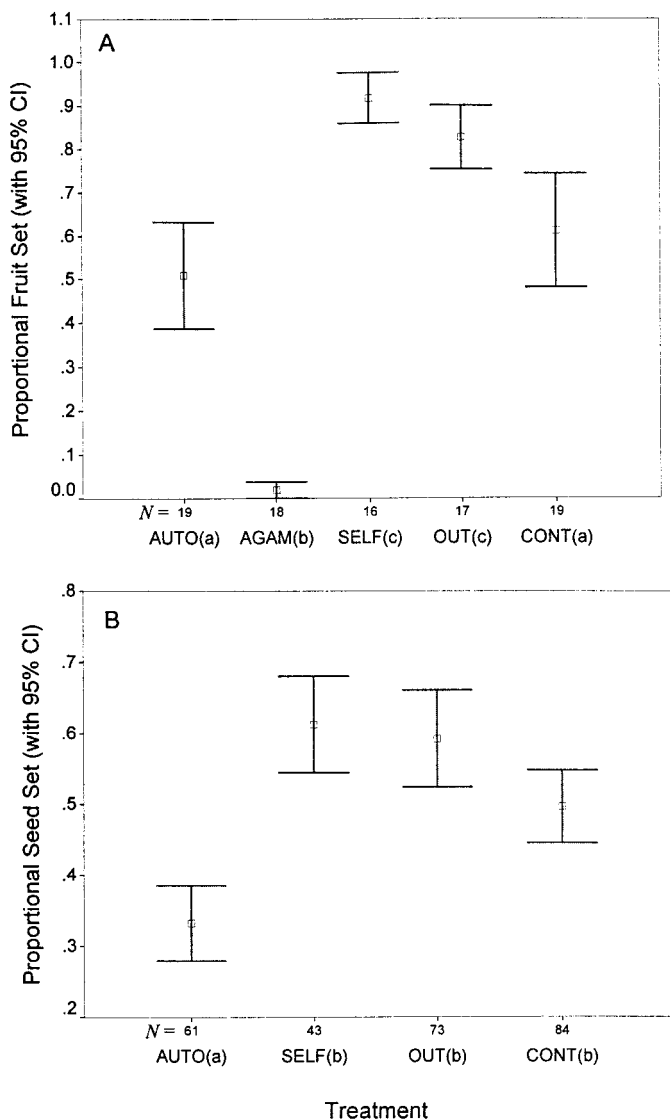
Fig. 2. Results of the breeding system experiment of *Warea carteri*: proportional fruit set (A) and proportional seed set (B) by pollination treatment with 95% confidence intervals. AUTO = autogamy, AGAM = agamospermy, SELF = self-pollination, OUT = outcross pollination, CONT = open pollination. Different letters beside the treatment labels denote significant differences in fruit or seed set ($P < 0.05$). Sample size (N) is the number of plants for fruit set and the number of fruits for seed set.

cies in the number of heterozygotes. The mean value of F_{IS} across all loci was 0.325.

Genetic differentiation among populations of *W. carteri,* assessed with Wright's (1969) statistic F_{ST}, was moderate. Mean F_{ST} averaged across all polymorphic loci was 0.304, indicating that ~30% of the total genetic variance we detected in *W. carteri* is found among populations. Based on this value, *Nm,* the number of migrants per generation is 0.57 (where $Nm = [(1/F_{ST}) - 1]/4$, from Wright [1951]). This expression for *Nm* measures historical, not current, gene flow and assumes populations are at equilibrium with respect to migration and drift (Slatkin and Barton, 1989). We found a weak but significant negative correlation between geographic distance and Nei's genetic identity ($r^2 = -0.147$; $P < 0.001$), and a strong association between relative population location on the north-south axis of the LWR and a cline in the allele frequencies at one locus (Fig. 4). This relationship is significant for two of three alleles at this locus (Spearman's rank order correlation coefficient $= -0.784$, $P < 0.001$ and 0.709, $P < 0.001$, respectively).

Effective population sizes—Estimates of average effective population size were quite small for most populations (Table 1), though there are unresolved problems with the models and estimation of parameters (see Discussion). Although the Mann-Whitney U test did not show any significant difference in estimates of average effective aboveground population size based on fire-management ($P = 0.11$, $N = 13$), the fire-managed populations tended to have lower estimates for average effective aboveground population size than populations that did not experience fire management (Table 1).

There were no significant correlations between within-population genetic statistics (proportion of loci polymorphic, alleles per polymorphic locus, or expected heterozygosity per locus) and aboveground population size, the three estimates of effective population size, or history of fire or anthropogenic disturbance. However, average values for these statistics from the recently burned populations in natural vegetation were consistently higher than average values from the roadside populations that had not burned recently (7.2 vs. 6.4% of loci polymorphic, 1.12 vs. 1.09 alleles per polymorphic locus, and 0.030 vs. 0.024 expected heterozygosity, respectively).

DISCUSSION

Mating system, fecundity, and inbreeding—Given that *Warea carteri* is semelparous and the number of mates available varies in response to a stochastic phenomenon (fire), we expected to find evidence of selection for reproductive assurance. Self-compatibility and autogamy are features of the mating system of *W. carteri* that confirm this expectation. Reproduction via selfing allows contributions to the seed bank between fires. Still, insects do play a role in maintaining high fecundity in *W. carteri*. Lower fruit and/or seed set in the autogamy treatment compared to the hand-pollinated and open-pollinated treatments suggests that insect-facilitated pollination contributes significantly to fecundity (26% total reduction in fecundity without insects). Lower fruit and seed set in the open-pollinated (control) treatment compared to the

tional loci (*Gdh-1, Pgi-1,* and *Acp-1*) were apparently variable, but could not be consistently resolved and were not scored.

The mean number of alleles per polymorphic locus within populations was 1.87 (range 1.00–2.67) and, on average, only 6.6% of loci were polymorphic (the most common allele being present at <95% frequency; range 0–12%). Average expected heterozygosity was 0.026 (range 0.000–0.045); average direct-count heterozygosity was 0.018. Eight populations had genotype frequencies that deviated significantly from Hardy-Weinberg equilibrium for all three polymorphic loci; another five populations deviated for at least one locus (R. Dolan, unpublished data). In each case the loci examined had deficien-

TABLE 2. Allele frequencies by population for the three loci with more than one allele present at >5% frequency detected in *Warea carteri*. Populations are arranged from north to south.

Population	Status[a]	Acp-1			Pgm-2			Pgm-3		
		a	b	c	a	b	c	a	b	c
Horse Cr	1, 5	0.81	0.15	0.04	0.7	0.83	0.10	1.00	—	—
TCP-Libby	1, 3	0.43	0.57	—	—	1.00	—	1.00	—	—
TCP-CH	2, 3	0.63	0.37	—	0.20	0.47	0.33	1.00	—	—
Hillcr Hts	2, 4	0.57	0.43	—	—	0.95	0.05	0.98	0.02	—
TCP-Pfund	2, 3	0.17	0.77	0.07	—	1.00	—	1.00	—	—
TCP-Gate	2, 3	0.70	0.30	—	—	1.00	—	1.00	—	—
LASF-Tr	1, 3	0.40	0.60	—	0.07	0.77	0.16	1.00	—	—
LASF-Z Tr	1, 3	0.33	0.67	—	0.03	0.93	0.03	0.97	0.03	—
Carter Cr	1, 5	0.27	0.74	—	—	1.00	—	1.00	—	—
Van Pelt	1, 4	0.08	0.92	—	0.33	0.60	0.07	1.00	—	—
F Villas	1, 5	0.06	0.94	—	0.02	0.61	0.37	1.00	—	—
T Pouch	1, 4	0.31	0.69	—	—	0.81	0.19	1.00	—	—
J Creek	1, 5	0.27	0.67	0.06	0.13	0.78	0.09	0.98	0.02	—
Grand C	1, 4	—	1.00	—	—	0.86	0.14	1.00	—	—
L L No	1, 4	—	1.00	—	—	1.00	—	1.00	—	—
L L New	1, 4	—	1.00	—	0.19	0.09	0.72	0.91	—	0.09
High P Est	1, 4	0.06	0.94	—	—	0.72	0.28	0.88	—	0.12
Lakeview	1, 4	—	1.00	—	0.72	0.28	—	0.96	—	0.04
Lk Pl Scrb	1, 3	—	1.00	—	0.62	0.34	0.04	0.88	—	0.22
ABS-Hufty	2, 3	0.28	0.62	0.10	0.03	0.76	0.21	1.00	—	—
ABS-McJ	1, 3	0.37	0.63	—	0.40	0.50	0.10	1.00	—	—
ABS-W	1, 3	—	1.00	—	—	1.00	—	1.00	—	—
ABS-S	1, 3	—	1.00	—	0.03	0.97	—	1.00	—	—

[a] History of disturbance (1 = roadside, without recent fire, 2 = natural vegetation, recently burned), and current protection status (3 = protected, 4 = not protected, 5 = protection planned) of the populations.

hand-pollinated treatments is suggestive of pollen limitation (30 and 21% reduction in fruit set and 11 and 9% reduction in seed set, compared to self and outcross treatments, respectively). If insect visitation is positively related to plant density, fire could positively affect fecundity in a density-dependent manner (i.e., Allee effect, see Lamont, Klinkhamer, and Witkowski, 1993; Widen, 1993; and Groom, 1998).

Several aspects of the reproductive biology of *W. carteri* suggest that selfing is the common mode of reproduction. *Warea carteri* is protandrous, but because there are many flowers densely clustered within inflorescences, pollen and receptive stigmas are present simultaneously in close proximity. In addition, many inflorescences flower simultaneously on a plant. In preliminary observations (620 min), only a small fraction (3%) of the insect travel occurred between flowers on different plants of *W. carteri* (M. Evans, ABS, unpublished data). *Warea carteri* was found to be highly self-compatible, and autogamous fruit set (51%) was not significantly different from open-pollinated fruit set, suggesting autogamy naturally occurs at a relatively high rate. All of these factors suggest that pollen transfer within plants followed by fertilization (autogamy and geitonogamy) is common in *W. carteri*. The deficiencies of heterozygotes found in the isozyme survey could be the result of selfing, among other causes (e.g., Wahlund effect). The short- and long-term negative impacts of selfing include inbreeding depression and the loss of genetic diversity (Barrett and Kohn, 1991; Huenneke, 1991). The lack of a significant difference in either proportional fruit or seed set between self and outcross pollinations indicates that inbreeding depression does not impact early life history stages, although inbreeding depression is expected to be expressed in later life history stages in selfing species (Husband and Schemske, 1996). The low level of isozyme diversity we found within populations of *W. carteri* is consistent with habitual inbreeding, among other possible causes (such as population fluctuation).

Species and within-population patterns of isozyme variation—Several of the life history traits in *W. carteri* are associated with little genetic diversity, including the annual habit, selfing, insect pollination, gravity-dispersed seeds, as well as rarity and a postdisturbance niche (Hamrick et al., 1991). The isozyme analysis of *W. carteri* revealed levels of variation that are lower than average values for species grouped on the basis of each of these traits. The proportion of loci polymorphic in *W. carteri* is less than six of seven average values found in a more recent study that grouped species on the basis of two life history traits (Hamrick and Godt, 1996). The high average value of Nei's genetic identity (0.989) in *W. carteri* reflects the paucity of genetic variation at the species and population levels. The suite of traits combined in *W. carteri* may cause its species- and population-level statistics of genetic diversity to be lower than most of the values reported in other studies. Our results contrast strongly with published studies of isozyme variation in some of the perennial herbs endemic to the LWR. These studies reported surprisingly high levels of genetic variance from smaller samples (Lewis and Crawford, 1995; McDonald and Hamrick, 1996). In *Polygonella* spp., these relatively high levels were attributed to the LWR serving as a glacial refugium for some of the species studied (Lewis and Crawford, 1995). A recent study of genetic diversity in another Florida scrub endemic, using randomly amplified
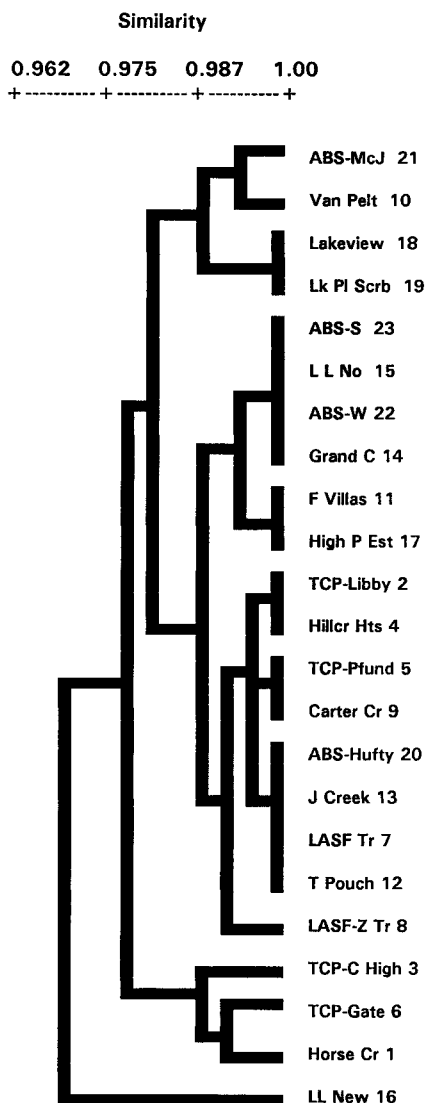
Fig. 3. Cluster diagram generated by the UPGMA method (Swofford and Selander, 1989) based on Nei's unbiased genetic identity values for 23 populations of *Warea carteri*. The cophenetic correlation coefecient is 0.70. Numbers following population names correspond to Table 2 and indicate relative position north to south in ascending order.
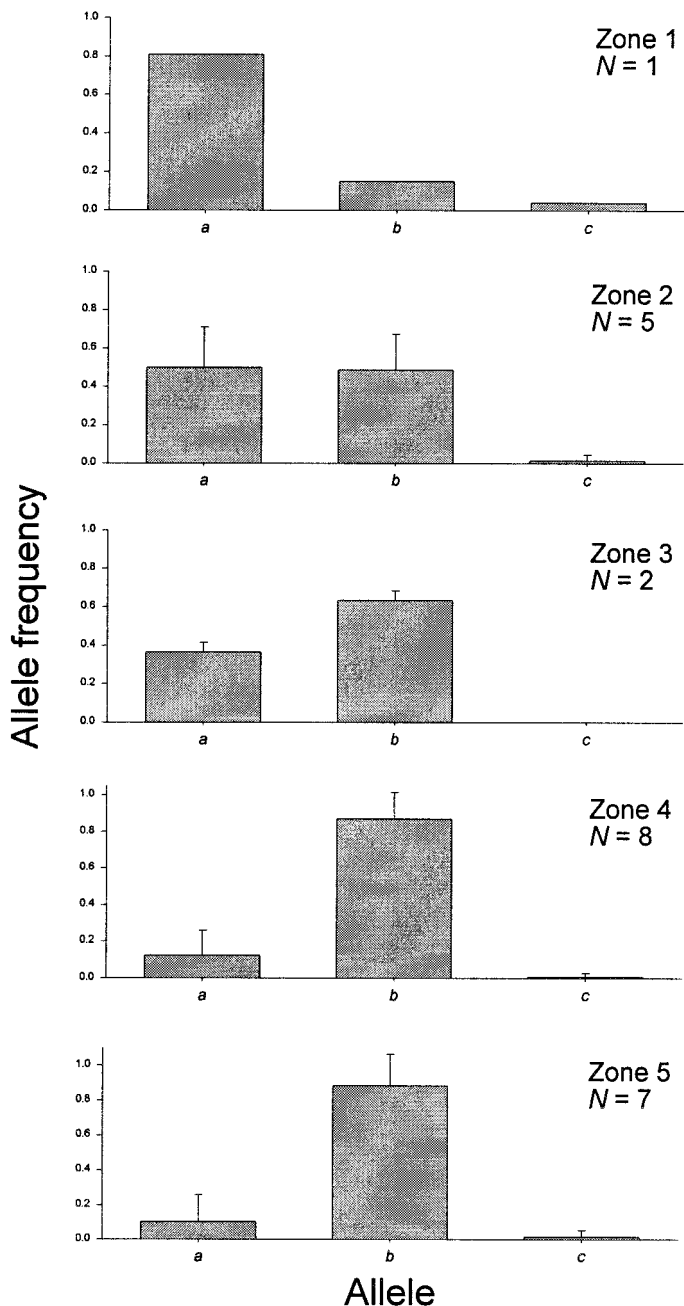

Fig. 4. Histograms illustrating north–south clines in the allele frequencies at the locus ACP-1 in *Warea carteri*. The populations sampled for isozyme diversity were divided into five zones of equal distance from north to south. The histograms indicate the average allele frequencies (with 95% confidence intervals) of the populations in each zone. The frequencies of alleles *a* and *b* were significantly related to population position.

polymorphic DNA, found no variation (G. Romano, University of Florida, unpublished data).

Census and effective population sizes and genetic diversity statistics—The lack of significant relationships between population-level patterns of genetic variation and census population sizes or effective population sizes estimated from census data reflects the difficulty of predicting the population genetics of a species that has a complex life history and is responsive to a stochastic ecological phenomenon. Several plant studies have found a significant positive relationship between census population size and percentage loci polymorphic, number of alleles per polymorphic locus, and/or gene diversity (i.e., expected heterozygosity; see references in Table 1 in Ellstrand and Elam, 1993; Prober and Brown, 1994; Raijmann et al., 1994; Sun, 1996). Census population sizes

were probably not good predictors of population genetic statistics (percentage of loci polymorphic, number of alleles per polymorphic locus, and expected heterozygosity per locus) in our species because of its seed banks. There is need, both theoretically and empirically, for a better understanding of how seed banks may influence plant population genetic dynamics.

Because of its seed banks, we used effective popula-

tion sizes to try to link the demographic and genetic behavior of populations of *W. carteri.* Unfortunately, the theory for calculating effective population size from census data for populations with overlapping generations and fluctuating population sizes is poorly developed. An important assumption of the ''Hill'' model (Hill, 1972) that we used is that the population is at equilibrium. It is assumed that neither cohort size, inbreeding, nor variance in family size changes from year to year; under this assumption, the effective size per generation is the same as the effective size for a population with discrete generations. This was not the case in our species. We used constant values for inbreeding and variance in family size, summarized from data that varied among loci, years, and/or populations. Our estimates of cohort size varied because of strong fluctuations in reproduction. There is no indication in the theoretical literature as to how inaccurate estimates of effective size per generation may be for populations experiencing such fluctuation. In addition, we found that summarizing the fecundity data in different ways and different estimates of *p* led to very different estimates of variance in family size, cohort sizes, and effective sizes (M. Evans, unpublished data). These reasons may explain the lack of significant relationships between effective population sizes and population genetic statistics.

Gene flow and drift—We found evidence that genetic variance is structured in *W. carteri,* but the data are subject to alternative explanations other than limited gene flow. The life history traits found in *W. carteri* are expected to limit gene flow among populations and are associated with greater values of F_{ST}, the proportion of the genetic variation found among populations. The value we found for *W. carteri* ($F_{ST} = 0.304$) is moderate relative to other species with each of these traits (Hamrick et al., 1991), though an even higher proportion might be expected since these traits are combined in *W. carteri.* Estimated gene flow ($Nm = 0.57$) based on this value of F_{ST} suggests random differentiation could occur among populations at neutral loci (Wright, 1951). However, using F_{ST} to estimate gene flow assumes the populations are in migration-drift balance. Whitlock (1992) found that temporal fluctuations in demographic parameters, including population size and migration rate, increased both the mean and variance of F_{ST}. He concluded estimates of F_{ST} and *Nm* from single samples of genetic markers should be viewed conservatively in species experiencing demographic stochasticity. The cline in allele frequencies along the north–south axis of the LWR is another form of spatial organization we detected in the isozyme data. However, a cline at one locus could be the result of selection. This cline in turn was responsible for another spatial pattern we found: the negative correlation between geographic distance and Nei's genetic identity. These indications that genetic diversity is spatially structured in *W. carteri* are consistent with our understanding of its autecology (selfing and limited seed dispersal), but because they are subject to other explanations and are based on few loci, we cannot make strong conclusions about gene flow.

The data we present here also do not provide a clear picture of the role of drift in populations of *W. carteri.*

The lack of diversity we found within populations is consistent with drift, especially in combination with the evidence for inbreeding. The fluctuation in aboveground population sizes of *W. carteri* suggests its effective population sizes could be low, which would lead to drift. However, divergence among populations that is not excessive and the cline we observed at one locus contradict a strong role for drift. N_e remains a critical parameter to estimate in order to understand how significant drift was or is in populations of *W. carteri.* We review alternative calculations of N_e in a separate manuscript (M. Evans, unpublished data), including molecular and demographic estimates for the seven listed endemic plants of the LWR we are studying.

Past, present, and future—It is likely that both pollination and seed bank dynamics of *W. carteri* have been affected by recent habitat loss and fire suppression. Populations have become more isolated both spatially and temporally, constricting gene flow. Fire suppression has probably depleted seed banks of *W. carteri,* both as a source of genetic variation and demographic stability. Different types of disturbance (anthropogenic vs. fire) probably also affect demographic dynamics, effective population sizes, and population genetic dynamics, though it is not clear how. For example, we might expect less genetic diversity in the fire-disturbed populations that we sampled, since they experienced stronger population fluctuation and smaller minimum population sizes, but the average values for genetic diversity statistics (percentage of loci polymorphic, number of alleles per polymorphic locus, and expected heterozygosity per locus) were consistently higher in the fire-disturbed populations than in the roadside populations. Our conservative recommendation is that the natural fire regime, which is still under investigation (Abrahamson and Hartnett, 1990; Myers, 1990; Ostertag and Menges, 1994; Menges and Kohfeldt, 1995; Menges and Hawkes, 1998), be mimicked. The spatial organization of genetic diversity found in this study suggests that preservation of scrub habitat throughout the range of *W. carteri* is needed to adequately capture the current distribution of genetic variation. Similar results and recommendations were made for three other LWR endemics (Lewis and Crawford, 1995; McDonald and Hamrick, 1996).

Summary—We found that *W. carteri* is autogamous and self-compatible and predict its mating system includes habitual selfing. These traits, especially combined with seed banks, probably promote persistence in the face of spatial and temporal heterogeneity. We found little genetic variation in *W. carteri* and what variation we did detect was spatially organized, thus we recommend range-wide representation in reserves. Further evaluation of reserve design and management with respect to genetic diversity in *W. carteri* will require better estimates of effective population sizes and gene flow. A better understanding of the seed biology of *W. carteri,* including the cues for germination, germination fraction, and the dynamics of seed viability in the soil, will allow us to better model both effective population sizes and demographic dynamics. Our work highlights the importance of incorporating seeds into demographic and ge-

netic modeling of certain plant populations and the equilibrium assumptions of models that have commonly been used to estimate essential microevolutionary parameters (Nm and N_e).

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