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PATTERNS OF ISOZYME VARIATION IN RELATION TO POPULATION SIZE, ISOLATION, AND PHYTOGEOGRAPHIC HISTORY IN ROYAL CATCHFLY (*SILENE REGIA*; CARYOPHYLLACEAE)[1]

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The distribution of genetic variation within and among plant populations is influenced by both contemporary and historical factors. I used isozyme analysis of band phenotypes to examine genetic structure in the rare prairie forb *Silene regia*. Relationships between current-day population size, isolation, and phenotypic variation were assessed for 18 populations in two regions with differing postglacial history. Western populations from unglaciated southern Missouri and Arkansas were more genetically diverse based on the Shannon-Weaver index (H) and a polymorphic index than were more eastern populations. These differences may be due to loss of variation with repeated founding of new populations in previously glaciated sites in Indiana and Ohio. Within the western region, population size was not significantly correlated with genetic variation. In the east, size was correlated with Shannon-Weaver diversity. There was no relationship between variation and isolation in either region, but eastern populations were slightly more differentiated. Greater among-population differentiation and the demonstrated connection between population size and variation in the eastern sites may reflect lower levels of interpopulation gene flow in the fragmented remnant prairies of Indiana and Ohio.

Population genetic theory posits a direct, positive relationship between genetic variation and population viability. Genetic variation provides the resources on which populations draw for short-term adaptation to environmental change and for longer-term evolutionary change (Beardmore, 1983; Frankel and Soule, 1981). Genetically depauperate populations are in general thought to be at greater risk of extinction than populations rich in genetic variation. Processes that erode genetic variation, such as drift, are more likely to impact small, geographically isolated populations that are unable to ameliorate negative consequences of small size through gene flow from adjacent populations (Falk and Holsinger, 1991). However, empirical studies have rarely demonstrated the nature and strength of the relationships between population size, isolation, and genetic variation in natural populations of plants (Barrett and Kohn, 1991).

A better understanding of these interactions would benefit conservation biologists seeking to preserve genetic diversity in rare species. A case in point is the royal catchfly, *Silene regia* Sims (Caryophyllaceae). There is evidence that small populations of *S. regia* may be suffering adverse genetic effects; Menges (1991) demonstrated a positive relationship between population size and germination percentage of seeds in the laboratory for *Silene regia*, suggesting inbreeding depression as a possible cause of lower germination rates in smaller populations.

Silene regia is a tap-rooted perennial that grows in mesic or wet-mesic prairies, dry woods and glades, especially on calcareous soils (Fernald, 1950; King, 1981). It produces perfect red flowers that are primarily hummingbird pollinated. It is a tetraploid with $2n = 48$ and is believed to be an allopolyploid with a complicated, reticulate evolutionary origin (Heaslip, 1951). Geitonogamous fertilization results in fertile seed set, but the plants are protandrous and primarily outcrossing (E. Menges, Archbold Biological Station, Lake Placid, FL, unpublished data). The species does not spread clonally, although older plants can produce a dozen or more stems. Seeds are gravity-dispersed, have no known dormancy requirement, and do not accumulate in the soil (E. Menges, unpublished data). The geographic distribution of this showy plant is well documented. *Silene regia* is found in every county in southwestern Missouri (Fig. 1), with scattered populations to the northeast, suggesting possible persistence in an Ozarks region glacial refugium with subsequent postglacial migration into areas of Illinois, Indiana, and Ohio following retreat of the Wisconsin glaciation 10,000–12,000 yr ago (Palmer and Steyermark, 1935; King, 1981). In the western part of its range, *Silene regia* is currently found primarily in prairies managed for hay production or pasturage and along roadsides. In the eastern parts of its range where most native prairie has been converted for agriculture, *Silene regia* is restricted to remnants of prairie vegetation, such as pioneer cemeteries and railroad rights-of-way, some of which are now managed to promote prairie plants.

The species is believed extirpated in the southeastern parts of its historical range. There have been no recent sightings in Tennessee, Georgia, Alabama, or Florida, based on interviews with state heritage programs.

The purpose of the present study was to determine levels

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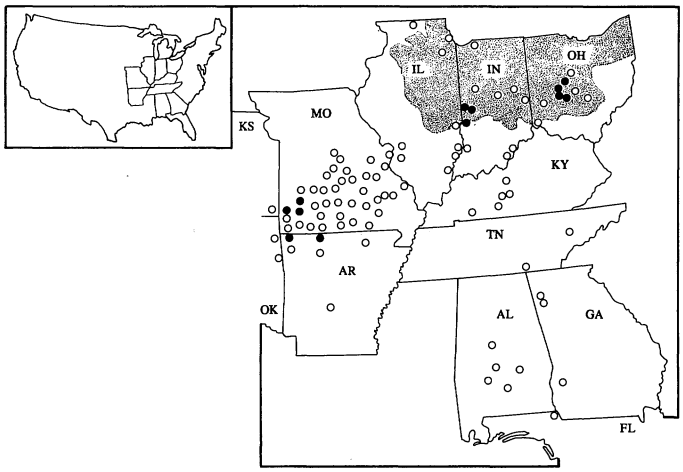

Fig. 1. Historical distribution of royal catchfly (*Silene regia*; adapted from King, 1981). The shaded area is the continental ice sheet of late Wisconsin time. The solid dots are counties with study populations.

TABLE 1. Abbreviations and locations for 18 populations of *Silene regia* from which material was collected for electrophoretic analysis.

Code	Location (county, state)
MIL	Union, OH
BIG	Madison, OH
PCR	Madison, OH
MAR	Champaign, OH
SEL	Clark, OH
SMI	Vermillion, IN
RUP	Fountain, IN
WAB	Warren, IN
SBL	Fountain, IN
NIA	Dade, MO
COX	Lawrence, MO
MRV	Lawrence, MO
NRE	Lawrence, MO
KEN	Jasper, MO
REA	Lawrence, MO
NMA	Lawrence, MO
DEC	Benton, AR
BAK	Boone, AR

of genetic variation as detected by starch gel electrophoresis of soluble enzymes for 18 prairie populations with a range of sizes and degrees of geographic isolation. Because of the proposed postglacial migratory history of the species (King, 1981), populations were also sampled from across its east-west range. *Silene regia* is an ideal species for study of genetic structure in rare plants with glacial/nonglacial distributions.

MATERIALS AND METHODS

Field sampling and electrophoresis—I used analyses of electrophoretic phenotypes to quantify genetic variation in individuals from 18 populations of differing size and degree of isolation across the geographic range of *Silene regia* (Table 1). Population sizes were estimated by visual census at the time of peak flowering during 1992. Determination of the degree of isolation of each population was made by consulting state heritage databases, interviews with agency personnel, and scouting of all roads and public access sites within 2.5 km of each study population.

Single mature leaves were collected from up to 50 plants in each field population for electrophoresis. Leaves were stored in zip-lock bags and were kept on ice or in a refrigerator. Tissue processed this way gave viable staining for up to 2 wk following collection. Two discs of leaf tissue were cut with a paper hole punch and ground in six drops of extracting buffer (0.1 M tris-HCL pH 8.0 with 1.5 mg dithiothreitol per ml).

Malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.27), and 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44) were resolved using the morpholine-citrate (MC) continuous buffer system of Clayton and Tretiak (1972) with an additional three drops of morpholine added to the gel buffer (S. Kephart, Willamette College, Salem, OR, personal communication). Gels prepared with this buffer system were run at either a pH of 6.1 or 7.0 to enhance resolution of different regions of the gel, believed to be the products of different loci. Phosphoglucose isomerase (PGI, EC 5.3.1.9) and alcohol dehydrogenase (ADH, EC 1.1.1.1) were resolved on a discontinuous sodium-borate (Na-B) buffer system (Poulik, 1957). MC gels were run for 5 hr at 50 mA and NaB gels were run at 75 mA for 2.5 hr.

Data analysis—Identification of alleles and their assignment to specific loci proved problematic for *Silene regia*. Tetraploidy, overlapping loci, and additional gene duplication all appear to interact to make genetic interpretation difficult, even when knowledge of enzyme subunit structure and intercellular compartmentalization (Kephart, 1990) are employed. Analysis of segregation patterns on offspring of controlled crossed did not simplify interpretation (Dolan, unpublished data). Therefore, for each enzyme system studied, individual band presence/absence and overall multiband phenotype were determined for each individual in each population (as in Chung et al., 1991 and references therein) instead of the standard measures of genetic variation (number of alleles, percent of loci polymorphic) that require a genetic interpretation. Although banding patterns observed were complex, individuals consistently produced the same patterns through several repeat runs across several growing seasons.

All five enzyme systems scored are generally dimeric enzymes in plants (Weeden and Wendel, 1989; Crawford, 1990; Kephart, 1990). This has the advantage of facilitating visualization of potential unbalanced heterozygote phenotypes, but does result in the scoring of inter- and intralocus heterodimer bands during band presence/absence counting.

Individuals were scored for the presence (1) or absence (0) of putative identical bands. Differences in band intensity, perhaps reflecting dosage effects, were not considered in the presence/absence scoring, but were considered in analysis of phenotype frequencies. For each enzyme studied, unique combinations of band presence and intensity were scored as unique phenotypes. These data were used to determine the number of different bands and phenotypes present in each population.

TABLE 2. Number of different multi-band phenotypes by population.

Enzyme	MIL	BIG	PTC	MAR	SEL	SMI	RUP	WAB	SBI
				East					
MDH	7	8	5	5	7	8	9	8	7
PGI	8	5	8	4	1	11	8	11	6
ADH	4	5	5	3	4	4	4	7	9
IDH	2	2	3	4	3	4	5	1	2
6-PGD	4	3	3	4	3	4	4	3	1
Total	25	23	24	20	18	31	30	30	25

Enzyme	NIA	COX	MAR	BAK	NRE	KEN	REA	DEC	NMA
				West					
MDH	10	16	6	18	13	12	13	9	5
PGI	18	28	8	20	15	19	22	17	12
ADH	7	11	2	9	10	12	10	10	5
IDH	5	4	1	6	3	3	5	4	5
6-PGD	11	9	2	6	10	9	4	7	6
Total	51	68	18	59	51	55	54	47	33

Phenotypic variation in populations was estimated from the above data in two ways. Shannon-Weaver Diversity Index values (H) (Shannon and Weaver, 1949) were calculated from the phenotype data. The band presence/absence data were used to calculate a polymorphic index (PI) (Singh and Jain, 1971) based on the frequency of occurrence of each band:

$$PI = \sum_{i=1}^{N} R_i(1 - R_i),$$

where R_i is the frequency of individuals possessing a given band in the population and N is the number of bands observed. Both measures integrate data on presence and frequency. The polymorphic index is less sensitive to rare alleles (Brown and Weir, 1983).

TABLE 3. Levels of phenotypic variation and total numbers of individuals present in each population, with means within regions. All measurements are significantly greater in the western region ($P > 0.001$). Numbers following means are standard errors.

Population	# Plants	# Bands	# Phenotypes	PI	H	Isolated?
Ohio/Indiana						
SMI	895	34	31	3.604	1.443	Yes
MIL	418	33	25	2.574	1.131	Yes
BIG	376	31	23	2.914	1.251	No
WAB	165	32	30	3.144	1.098	Yes
PCR	149	30	24	2.088	1.104	No
MAR	70	33	20	2.478	0.880	Yes
SBL	64	17	25	2.941	1.098	Yes
RUP	58	34	30	2.904	1.260	No
SEL	45	25	18	2.295	0.841	Yes
Mean		29.9	25.1	2.771 (0.154)	1.123 (0.062)	
Missouri/Arkansas						
COX	1302	49	68	5.208	2.061	Yes
MRV	200	29	18	2.628	0.947	Yes
NRE	191	48	51	4.318	1.817	No
NIA	178	49	51	4.662	1.795	No
BAK	166	50	59	4.469	1.926	Yes
NMA	161	31	33	3.888	1.381	Yes
REA	150	40	54	4.879	1.803	No
KEN	77	44	55	4.620	1.875	No
DEC	41	40	47	4.278	1.739	Yes
Mean		42.2	48.4	4.328 (0.247)	1.705 (0.113)	

TABLE 4. Shannon-Weaver diversity (H) values by enzyme.

State	Population	MDH	PGI	ADH	IDH	6-PGD
Ohio	MIL	1.179	1.704	1.122	0.330	1.320
	BIG	1.675	1.768	1.260	0.688	0.864
	PCR	1.411	1.768	1.428	0.465	0.447
	MAR	1.185	0.980	0.729	0.588	0.916
	SEL	1.526	0.000	1.276	0.444	0.960
	Mean	1.395	1.244	1.163	0.503	0.901
Indiana	SMI	1.797	2.002	1.286	0.804	1.328
	RUP	1.815	1.296	1.262	0.896	1.210
	WAB	1.354	2.288	1.641	0.000	0.209
	SBL	1.716	1.701	1.713	0.362	0.000
	Mean	1.671	1.822	1.476	0.516	0.687
Missouri/Arkansas	NIA	1.858	2.538	1.463	1.145	1.969
	COX	2.227	3.270	1.925	0.964	1.917
	MRV	1.663	1.928	0.482	0.000	0.662
	NRE	2.064	2.535	1.914	0.861	1.710
	KEN	2.043	2.755	2.088	0.570	1.917
	REA	2.236	2.838	1.840	0.992	0.111
	DEC	1.873	2.754	2.064	1.088	0.917
	NMA	0.938	1.991	1.389	1.150	1.439
	BAK	3.551	2.660	1.746	1.150	1.524
	Mean	1.938	2.584	1.657	0.880	1.463

The distribution of total variation within and among populations can be calculated using the Shannon-Weaver diversity index based on frequencies of phenotypes (Chung et al., 1991). Total phenotypic diversity, H_T, was considered equivalent to the total Shannon-Weaver Diversity Index and was partitioned into diversity within populations (H_S) and among populations (D_S). G_S, the proportion of total diversity found among populations, was calculated from the relationship D_S/H_T. These statistics were calculated for the species as a whole using values from all 18 populations surveyed, and separately for each region (east and west) using constituent populations.

Analysis of covariance and correlation analysis were used to examine the relationships between population size and isolation and the various measures of genetic variation.

RESULTS

Fifty-nine different bands were detected among the enzyme systems as follows: ADH seven bands, IDH 12 bands, MDH 21 bands, PGI ten bands, and 6-PGD nine bands. Band frequencies, averaged by state, are presented in Appendix 1. There were significantly more bands (42.2 vs. 29.9) and phenotypes (48.4 vs. 25.1) detected in western populations (those from Missouri and Arkansas) than in eastern populations (Indiana and Ohio) (Table 2). Analysis of band frequencies shows that those bands present in the west but absent in the east are rare in the west, with an average frequency of 3.8%. Bands that are "fixed" or present in every individual in eastern populations are common in the west, being present at an average frequency of over 95%. No bands were "fixed" in western populations. In most cases, east-west clines exist in band frequencies, with Indiana populations having values inter-

TABLE 5. Distribution of genetic variation between and among populations for *Silene regia* as a whole, and for eastern and western populations calculated separately. Values are based on Shannon-Weaver Diversity Index values calculated from frequency of phenotypes (Chung et al., 1991).

	Total				East				West			
Enzyme	H_T[a]	H_S[b]	D_S[c]	G_S[d]	H_T	H_S	D_S	G_S	H_T	H_S	D_S	G_S
MDH	2.679	1.668	1.011	0.377	2.223	1.533	0.689	0.310	2.480	1.938	0.542	0.219
PGI	3.245	1.883	1.362	0.420	2.184	1.533	0.651	0.300	3.534	2.584	0.950	0.269
ADH	2.071	1.432	0.639	0.309	1.661	1.320	0.341	0.205	2.204	1.657	0.547	0.248
IDH	1.008	0.632	0.376	0.373	0.690	0.510	0.180	0.261	1.160	0.880	0.280	0.241
6-PGD	1.476	1.017	0.459	0.311	1.050	0.794	0.256	0.244	1.692	1.463	0.229	0.135
Mean	2.095	1.326	0.770	0.350	1.562	1.136	0.423	0.264	2.214	1.704	0.510	0.222

[a] H_T = total phenotypic diversity.
[b] H_S = diversity within populations.
[c] D_S = diversity among populations.
[d] G_S = proportion of total diversity found among populations (D_S/H_T).

mediate between those in Missouri/Arkansas and Ohio (Appendix 1). The number of different phenotypes observed for each enzyme in each population is presented in Table 2.

The Polymorphic Index (PI), based on presence and frequency of bands, is significantly greater for populations in the west (Table 3.). The Shannon-Weaver Diversity Index (H) also shows eastern populations to be significantly less diverse than western populations. An east-west cline in average H values was detected for every enzyme except 6-PGD (Table 5). This enzyme system exhibited a fixed pattern (H=0.000) for one Indiana population, lowering the average in the state.

Levels of phenotypic population differentiation calculated from Shannon-Weaver Diversity Index values (Table 4) show that 35% of the total species diversity is found among populations. When the data are considered separately by region, slightly greater differentiation is detected among eastern populations (26.4%) than among western ones (22.2%).

There was no significant relationship between population size and any of the measures of phenotypic variation when all 18 populations are considered. When population size and number of bands are compared for each region separately, there is still no consistent relationship detectable between number of bands, number of different phenotypes, or PI and population size. H is not significantly correlated with population size in western populations ($r = 0.136$;$P = 0.727$), but there is a significant correlation for eastern populations ($r = 0.712$; $P = 0.031$). These relationships are presented graphically for PI and H for each region in Fig. 2.

There was no significant relationship between population isolation status and genetic variation once population size was accounted for in analysis of covariance using H as the dependent variable. For all 18 populations, $r^2 = 0.240$ with isolation added as an additional term (F-value = 3.504; $P > 0.05$). Similar values were obtained when each region was considered separately, and when the other measures of genetic variation were used as the dependent variable.

DISCUSSION

Overall levels of genetic variation in Silene regia—The results show *Silene regia* to be a species with considerable genetic variation, with very few individuals sharing the same multienzyme phenotypes. In contrast, many studies of rare plants have revealed little or no variation (e.g., *Pedicularis furbishiae* [Waller, O'Malley, and Gawler, 1988]. *Howellia aquatilis* [Lesica et al., 1988], *Aster furcatus* [Les, Reinartz, and Esselman, 1991], *Limnanthes floccosa* subsp. *californica* [Dole and Sun, 1992]) as a cause or consequence of their rarity. Species with narrowly restricted geographic ranges generally have lower levels of genetic variation than their wider-ranging congeners (see reviews by Hamrick et al., 1991 and Karron, 1987; also Hickey, Vincent, and Guttman, 1991; Bayer, 1992). Past population bottlenecks and/or currently high levels of

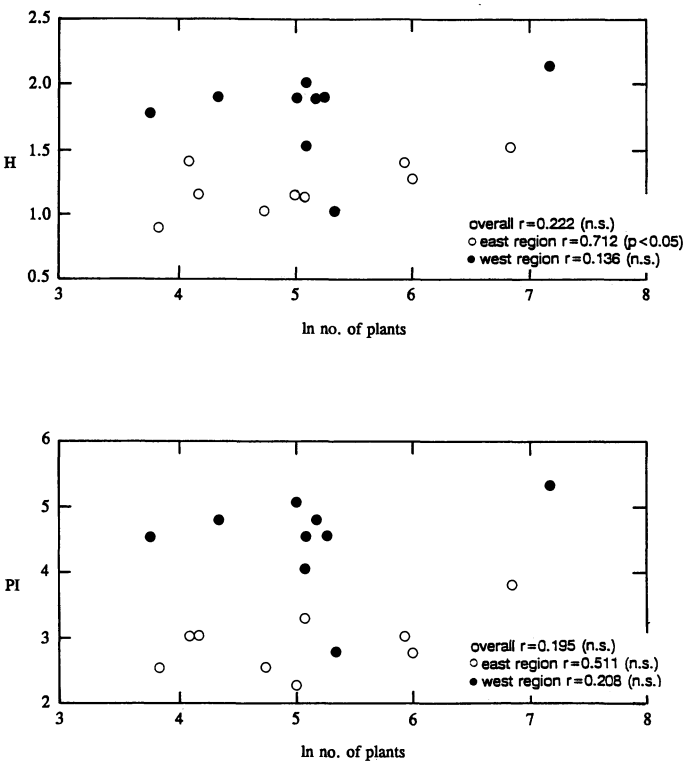

Fig. 2. Population size vs. Shannon-Weaver diversity (H) and the Polymorphic Index (PI) for 18 populations of *Silene regia*. Solid dots indicate western populations.

inbreeding caused by small population size and lack of gene flow into populations are the most commonly offered explanations for low levels of genetic diversity. For *Silene regia*, a long-lived, perennial life history and a primarily outcrossing breeding system with hummingbird pollinators are traits that may promote maintenance of genetic variation in the face of rarity and limited geographic distribution (Hamrick, 1989).

Analysis of genetic differentiation showed that 35% of the variation was found between populations. Although the potential for long-distance gene dispersal via pollen has been documented for hummingbird-pollinated species (Campbell and Dooley, 1992), little is known about patterns of between-population genetic variation in hummingbird-pollinated plants. *S. regia* populations overall show high levels of differentiation compared with an average value for Nei's G_{ST} of 22% (Hamrick et al., 1991; based on a review of data for 406 plant taxa). This may reflect lower than average levels of interpopulation gene flow.

Genetic structure across the east-west geographic range of the species —Genetic structure in *Silene regia* is strongly associated with the geographic location of populations. All measures of variation show populations in the eastern portions of the range to be genetically depauperate compared to western populations. Several alternative and nonmutually exclusive explanations may account for lower levels of genetic variation detected in eastern populations of *Silene regia*.

The data support the hypothesis that *Silene regia* could have migrated into northeastern parts of its range following glaciation from an Ozarkian refugium. Such a migration would likely have resulted in the loss genetic variation (mostly rare alleles [Lande, 1980]) through repeated founding of small populations toward the northeast as the environment became suitable for prairie vegetation. Ten of 59 bands recorded were found only in the west (Appendix 1).

Patterns of current-day genetic variation have been used to support ideas of postglacial migration patterns for other plants and fish; historically unglaciated regions containing present-day populations with high genetic diversity are envisioned as refugia. Populations in once-glaciated areas with lower diversity are envisioned as of migrant origin, having lost variation through the sampling process of founder effect and genetic drift and inbreeding in small, newly founded populations (*Sullivantia* spp., Soltis, 1982; *Sarracenia purpurea,* Schwaegerle and Schaal, 1979; *Cirsium pitcheri*, Loveless and Hamrick, 1988; and for northern pike, Seeb et al., 1987). Glover and Barrett (1986) found a similar pattern of genetic variation between continental and island populations of the plant *Eichhornia paniculata*. Soltis et al. (1991) have identified chloroplast-DNA patterns that are geographically structured in *Tellima grandiflora*.

An alternative explanation for the observed lower levels of genetic variation in eastern states is that extreme fragmentation of prairie habitat and the restriction of *Silene regia* to primarily cemeteries and rights-of-way have eroded variation in the these populations. In addition, the proportion of genetic diversity among populations (G_S) when regions are considered separately shows slightly more variation among eastern populations than among those in the west. This may reflect less gene flow between populations in the east. If eastern populations are more insular, genetic variation may have been lost due to inbreeding and drift (Barrett and Kohn, 1991).

A more speculative hypothesis is that fewer bands and phenotypes were detected in eastern populations due to a higher frequency of gene silencing in these populations. Studies of other species, plant and animal, have shown that "diploidization" of tetraploid genomes is more likely to occur (or at least become fixed as the result of founder effect) in populations at the margins of species ranges (Wilson, Barber, and Walters, 1983). For *Plantago cordata*, a rare plant with historical distribution similar to that of *Silene regia*, Mymudes and Les (1993) found more apparent gene silencing in older, more isolated populations. Support for this hypothesis would require documentation of the genetic basis of inheritance of banding patterns in *S. regia*.

Relationships between population genetic variation and population size and isolation —The large difference in variation detected in the eastern and western regions of the range of *Silene regia* may obscure relationships between population size, isolation, and genetic variation when data from all 18 populations are considered. When each region (east and west) is compared separately, potential negative impacts of small population size are apparent in the east, but not in the west.

Links between population size and genetic variation suggest variation is lost in small populations through inbreeding and drift. No consistent relationship was found between heterozygosity and estimated adult population size in a study of 38 populations of the herbaceous perennial northern monkshood (*Aconitum noveboracense*) by Dixon and May (1990). A similar study of two outbreeding perennials, *Salvia pratensis* and *Scabiosa columbaria*, found significant correlations between population size and the mean proportion of polymorphic loci, and between population size and the number of alleles present (Van Treuren et al., 1991). In addition, no correlation between gene diversity and population size was detected, although it was noted that gene diversity values were high in small populations due to increased frequency of rare alleles that inflated values; genetic differentiation was substantially larger among small populations than among large.

It should also be noted that for polyploids like *Silene regia*, it is difficult to tell how much the predictions from theoretical work and modeling will reveal about functioning in the "real world." Most theoretical work in this area has been based on "animal models . . . diploid, outbreeding organisms with separate sexes . . . that modify the stochastic influence on patterns of genetic variation" (Barrett and Kohn, 1991). For autopolyploids, population size restrictions will have less effect on loss of genetic variation because high levels of segregational heterozygosity are maintained. Moody, Mueller, and Soltis (1993) demonstrated heterozygosity will be lost at a slower rate in autopolyploid populations than in diploid populations for species with mixed ploidy levels. For allopolyploids, loss of heterozygosity is impossible for loci that are fixed. For *S. regia*, an apparent allopolyploid (Heaslip, 1951),

it appears that some loci exhibit fixed heterozygosity, while some segregate.

While the data presented here on *Silene regia* describe the current patterns of genetic distribution in the species, populations and individual plants appear to be long-lived (E. Menges, unpublished data) and genetically diverse compared to many rare species. These patterns reflect the influences of historical and contemporary factors in shaping the genetic structure of the species. It is possible that only a few generations have passed since the fragmentation of the prairie habitat in the eastern portion of the range. Erosion of genetic variation in these sites may just be beginning. The concomitant reduction in fitness and increased chances of extinction brought about by decreased genetic variation is just one of a suite of potential negative consequences of small population size. Other influences on *Silene regia* are being examined with further studies.

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Appendix 1. Average band frequencies by state.

PGI	Ohio	Indiana	Missouri/ Arkansas
1	-	-	0.089
2	-	-	0.054
3	0.050	0.110	0.510
4	0.319	0.236	0.347
5	0.981	0.866	0.802
6	0.375	0.520	0.300
7	0.087	0.354	0.555
8	-	0.071	0.072
9	-	0.142	0.116
10	-	-	0.018

6-PGD	Ohio	Indiana	Missouri/ Arkansas
1	-	-	0.102
2	0.056	0.110	0.391
3	1.000	1.000	0.970
4	0.306	0.228	0.405
5	1.000	1.000	0.997
6	0.013	0.016	0.015
7	0.994	1.000	0.985
8	0.025	0.016	0.046
9	0.013	-	0.029

MDH	Ohio	Indiana	Missouri/ Arkansas
1	0.019	-	0.013
2	1.000	1.000	0.996
3	1.000	1.000	0.989
4	-	0.008	0.083
5	0.831	0.567	0.096
6	-	0.016	0.017
7	0.169	0.283	0.068
8	0.006	0.220	0.396
9	0.387	0.071	-
10	-	0.024	0.051
11	0.169	0.283	0.188
12	0.094	0.244	0.540
13	0.094	0.039	0.172
14	0.550	0.118	0.323
15	-	-	0.005
16	-	-	0.008
17	-	-	0.015
18	-	-	0.015
19	1.000	1.000	0.996
20	1.000	1.000	0.996
21	1.000	1.000	0.996

IDH	Ohio	Indiana	Missouri/ Arkansas
1	-	-	0.005
2	-	0.008	0.024
3	0.981	0.992	0.959
4	0.969	0.984	0.988
5	0.038	0.102	0.231
6	0.044	0.102	0.231
7	0.006	-	0.027
8	-	-	0.027
9	0.081	-	0.042
10	0.087	-	0.058
11	0.081	-	0.045
12	0.087	-	0.061

ADH	Ohio	Indiana	Missouri/ Arkansas
1	0.025	0.102	0.117
2	0.025	0.079	0.097
3	0.931	0.874	0.944
4	0.406	0.575	0.806
5	0.456	0.709	0.882
6	-	-	0.046
7	-	0.008	0.048