Consequences of Habitat Fragmentation for the Prairie-Endemic Weevil *Haplorhynchites aeneus*

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**ABSTRACT** Widespread destruction of tallgrass prairies in the midwestern United States has fragmented plant communities with the result that populations of endemic animal species have become geographically isolated from one another. The goal of the research summarized here was to evaluate the potential for conserving endemic prairie species of herbivorous insects by managing their host plants. Our study species was the weevil *Haplorhynchites aeneus* (Boehman), adults of which feed on pollen of plants in the genus *Silphium* (Asteraceae: Heliantheae). The female weevils clip the peduncles of flower heads and oviposit into the heads, where the larvae feed on the ovules. The research was conducted in 12 prairie sites in eastern Illinois. An allozyme analysis revealed that most populations of *H. aeneus* at the various prairie sites were genetically differentiated from one another, but the degree of differentiation was not associated with geographic distance between sites. Adult *H. aeneus* fed and oviposited on the plant species *Silphium laciniatum* L., *S. integrifolium* Michx., and *S. terebinthinaceum* Jacq, which differ in bloom phenology. There was no evidence of genetic differentiation of weevil populations with respect to host plant species, and adult weevils strongly preferred *S. terebinthinaceum*. We conclude that the oligophagous nature of the weevil assures its survival in small prairie remnants even where some of the host plant species are absent. Although *H. aeneus* can have a significant impact on reproduction of host plants by clipping flower heads, the perennial nature of *Silphium* species prevents their local extinction.

**KEY WORDS** plant-insect interaction, phytophagy, insect conservation, Curculionidae
The focus of our study was the prairie-endemic weevil *Haplorhynchites aeneus* (Boehman), which depends for its survival on prairie plants in the prairie ecosystem from Heliantheae (for biology of the weevil, see Hamilton 1973, 1981, 1994). The adults aggregate on plants and not multiple genetically-distinct races, or even genetically differentiated across patches of prairie habitats. We first used allozyme analysis to test the hypothesis that populations of the weevil are genetically differentiated across patches of prairie habitat because of geographic isolation. That hypothesis was supported (see Results). We also used allozymes to confirm that *H. aeneus* comprises a single species, and not multiple genetically-distinct races, or even cryptic species that are specialized to feed on different *Siphiium* species. That hypothesis also was supported (see Results): *H. aeneus* is oligophagous across all three *Siphiium* species with their different host plant species (Tooker et al. 2004). Antistrophus rufus Gillette originally was considered oligophagous across multiple *Siphiium* species, but is now known to comprise at least three cryptic species, each of which apparently is monophagous on different *Siphiium* species in prairie habitats of eastern Illinois (Tooker et al. 2004). The season-long flight period of the gall wasps therefore is because of synchronization of multiple *Antistrophus* species with their different host plant species (Tooker et al. 2004).

We examine the *H. aeneus* and *Siphiium* system from a conservation biology perspective by conducting experiments in 12 prairies in eastern Illinois that varied in the species composition and density of their plant communities. We first used allozyme analysis to test the hypothesis that populations of the weevil are genetically differentiated across patches of prairie habitat because of geographic isolation. That hypothesis was supported (see Results). We also used allozymes to confirm that *H. aeneus* comprises a single species, and not multiple genetically-distinct races, or even cryptic species that are specialized to feed on different *Siphiium* species. That hypothesis also was supported (see Results): *H. aeneus* is oligophagous across all three *Siphiium* species. Finally, we conducted an experiment to determine whether the weevil prefers certain host species over others, which could have important implications for conserving weevil populations in prairie habitats.

### Materials and Methods

**Study Sites.** Study sites were 12 areas of prairie habitat in eastern Illinois (Table 1; Fig. 1), five of which were remnants of virgin tallgrass-mesic prairies, prairie remnants’ areas that have never been cropped, whereas restorations’ have been replanted with prairie vegetation after crop production.
and seven were "restored" prairies: farmland or fallow fields to which endemic plant species have been reintroduced. All sites were geographically isolated from one another by at least 2 km (Fig. 1) and were surrounded by agricultural fields (primarily rotated corn [Zea mays L.] and soybean [Glycine max (L.) Merr.]) urban landscape, or both. All three species were present at the study sites, except for Sundrop and Windfall prairies, each of which were missing one plant species (Table 1). Few weevils were seen on plants other than Silphium species throughout the season.

The Silphium species grew in discrete patches at most of our study sites, probably because of limited dispersion of seeds from parent plants (Pleasants and Jurik 1992). Population densities of each host plant species, per study site, were estimated by sampling with 2- by 2-m squares of PVC pipe (four samples per patch, positioned arbitrarily), counting the number of plants within the square, and estimating the total area of the patches (m²) based on approximate patch shape (usually roughly circular or oval). Densities of the three plant species (log₁₀; from Table 1) differed across study sites, averaging (±SE) 2.04 ± 0.27, 4.03 ± 2.4, and 2.69 ± 0.39 plants/m² for S. laciniatum, S. integrifolium, and S. terebinthinaceum, respectively (overall analysis of variance [ANOVA] \( F_{3,35} = 3.26, P = 0.007 \), plant species term \( F_{2,35} = 12.7, P = 0.0002 \)). Silphium integrifolium was significantly more abundant than the other two species (REGWQ means separation test; SAS Institute 2001). Study sites did not differ significantly in numbers of plants across Silphium species because of this great amount of variation (same ANOVA, study site term \( F_{1,35} = 1.55, P = 0.19 \)).

**Genetic Structure of Populations: Geographic and Host Plant Effects.** Horizontal starch gel electrophoresis (Murphy et al. 1996) was used on larvae of H. aeneus to provide allozyme markers for the genetic analysis. We dissected as many as 15 weevil larvae of unknown age from flower heads of Silphium species in September and October 2006 at ten of the 12 study sites (\( N = 37-131 \) larvae per site, total = 823 larvae; Table 1; excluding Sundrop and Meadowbrook prairies, where weevils were scarce). Larvae were stored at −80°C until being electrophoresed. Loci were initially selected that were known to be polymorphic in other beetle species (Crouau-Roy 1989, Briese et al. 1996, Gonzalez-Rodriguez et al. 2002). Eight loci that yielded consistent stains were surveyed, of which seven were polymorphic in at least one population: aconitate hydratase (Acon, EC 4.2.1.3), alcohol dehydrogenase (Adh, EC 1.1.1.1), aspartate aminotransferase (Aat, EC 2.6.1.1), esterase (Est, EC 3.1.1.1), beta-hydroxycid dehydrogenase (Had, EC 1.1.1.30), isocitrate dehydrogenase (Idh, EC 1.1.1.42), and phosphoglucomutase (Pgm, EC 5.4.2.2). One enzyme, malate dehydrogenase NAD⁺ (Me, EC 1.1.1.40) was monomorphic in all populations. The following four gel buffer systems were used (Murphy et al. 1996): 1) tris/citrate pH 8.6 for Est and Me; 2) tris/citrate pH 8.6 with 0.5 ml NAD⁺ added to the gel buffer before degassing for Adh, Had, Idh, and Pgm; 3) lithium hydroxide/citrate pH 8.1/8.5 for Aat and Acon; and 4) morpholine/citrate pH 6.1 for Had.

The genetic structure of populations was analyzed with Arlequin 3.1 (Excoffier et al. 2005), or Microsoft Excel and a Monte Carlo simulator of an R x C exact test (MONTE CARLO RxC 2.2, Macintosh OS nine program provided by W. Engels, Department of Genetics, University of Wisconsin, Madison, WI). Hardy–Weinberg equilibrium was assessed using exact tests. Because only small numbers of weevils had complete genotypes (see Results), linkage disequilibrium was tested using exact R x C tests on genotypes for pairs of loci. Hypothesis 1 (weevil populations are genetically differentiated across patches of prairie) was tested by first carrying out exact tests for significant allele frequency differences between populations. Geographical variation was further characterized using analysis of molecular variance (AMOVA), a Mantel test for
isolation by distance, and regression of allele frequencies on latitude (using Excel).

Hypothesis 2 (H. aeneus comprises a single species that feeds on all three Silphium species) was tested with exact R x C contingency tests on allele counts from samples of weevil larvae taken from different Silphium species at the same study site. At least six weevil larvae were dissected from flower heads of each Silphium species that were collected in each of five study sites where all three plant species were blooming in 2006 (Wolf Road, Goose Lake, Loda Cemetery, Prospect Cemetery, Lincoln Prairie Grass Trail; Table 1).

Bloom Phenology of Silphium Species. Bloom phenology of the three Silphium species was characterized by determining, for each species and study site, the ordinal date on which the greatest number of new flowers were open (i.e., peak bloom), then used ANOVA to compare mean date of peak bloom for each species, blocked by study site and including a latitude covariate (to determine whether bloom phenology was correlated with latitude).

Utilization of Floral Resources by H. aeneus. Utilization of host plants by adult H. aeneus was examined by monitoring the bloom phenology of individual plants (as a measure of resource availability) and also the abundance of weevils on those plants (as a measure of the response of the beetle population). At the beginning of the 2006 growing season, 25-m transects were established, intersecting and at approximate right angles to one another, through patches of the plants at each study site. For each species, 12 plants were tagged that were separated by at least 2 m within the transects. Study sites were visited 13 times (every 8–11 d from mid-June through October 2006) to record, for each tagged plant, the number of flower buds, open flower heads, senesced flower heads (indicated by wilted petals), the number of adult weevils on the plant, and the number of flower heads that had been clipped.

Host species preference in adult H. aeneus was examined by comparing the percentage of weevils that were on plants of one species with the relative abundance of the flowers of that species (i.e., the percentage of all open flowers of Silphium species that were of that species; logistic regression, PROC GENMOD, SAS 2001). This test was conducted for pairs of Silphium species during periods in which they overlapped in blooming period (see Results): S. laciniatum and S. integrifolium (samples between ordinal dates 180 and 200, when only these two Silphium species were in bloom; see Fig. 1); and S. integrifolium and S. terebinthinaceum (sample dates after date 230, when those two species accounted for most of the flower heads). This analysis included data from all sample dates and study sites for which there was an average of at least five weevils on the plant species in question (including multiple dates for some study sites; N = 30 for the comparison between S. laciniatum and S. integrifolium; N = 18 for the comparison of S. integrifolium and S. terebinthinaceum). Regression analysis (PROC REG; SAS Institute 2001) was used to determine whether the number of weevils on a particular plant species was correlated with the relative abundance of its flower heads on certain dates.

ANOVA (blocked by study site) also was used to test differences between plant species in the percentage of flower heads that beetles had clipped by the end of the season: assuming a binomial distribution, PROC GENMOD, SAS 2001).

Results

Genetic Structure of Populations: Geographic and Host Plant Effects. Although we electrophoresed 823 larvae of H. aeneus (37–131 per study site), sample sizes for individual loci are in many cases smaller because of difficulties in scoring loci for all individuals on some gels (a spreadsheet with genotypes for each individual weevil is available from the corresponding author). Nevertheless, there was sufficient allozyme variation for population genetic analysis, with an average of 3.5 alleles across the eight loci, and an overall average heterozygosity of 0.213. With the exception of one test for which the P value was <0.05 (Fgn at Wolf Road, P = 0.047, well within the range of Bonferroni correction), weevil populations conformed to Hardy–Weinberg expectations at the seven variable loci, indicating that mating was random within each population. There was no evidence of significant linkage disequilibrium.

The allozyme data supported hypothesis 1 (weevil populations are genetically differentiated across patches of prairie). The exact tests of population differentiation revealed significant (P < 0.05), to highly significant (P < 0.0005) differences in allele frequencies between populations for 41 out of 45 comparisons (Table 2). Although the allele frequency differences were certainly significant, the magnitudes of the differences at all loci were small, as measured by FST (Table 3). The overall FST of 0.016 (P < 0.001) is in the range that Wright (1978) characterized as “slight”. Using the well-known equation of Wright (1978), Nm = (1/4 FST) − 1, the estimated number of migrants is 14.6 per generation, a large number for such geographically isolated populations. No regressions of allele frequency on latitude were significant, nor was there evidence of isolation by distance between sites (Mantel test P = 0.19).

These results also supported hypothesis 2 (H. aeneus comprises a single species that feeds on all three Silphium species). There were no significant genetic differences between weevil subsamples from different host plant species at any of the study sites. Hypothesis 2 also is supported by the excellent fit to Hardy–Weinberg expectations in all populations where multiple host plant species were present.

Bloom Phenology of Silphium Species. The three Silphium species differed significantly in the timing of peak bloom across study sites, with ordinal peak bloom dates averaging (±SE) 205.1 ± 1.3, 218.8 ± 1.9, and 239.0 ± 2.5 for S. laciniatum, S. integrifolium, S. terebinthinaceum, respectively (mean significantly different; overall ANOVA F3,33 = 55.3, P < 0.0001; plant
species term $F_{2,33} = 79.2, P < 0.0001$). The timing of peak bloom was not significantly influenced by latitude (ANOVA covariate $P > 0.05$).

Even though the *Silphium* species differed in timing of peak bloom, they overlapped broadly over the season in the periods that they were in bloom (Fig. 2, solid lines), with *S. lacinatum* and *S. integrifolium* coming into bloom first and nearly synchronously, but *S. integrifolium* blooming for a longer period and overlapping more broadly with *S. terebinthinaceum*. Flowers of *Silphium* species in general were present continuously from late June through early September.

**Utilization of Floral Resources by** *H. aeneus*. Adult weevils were present on both *S. lacinatum* and *S. integrifolium* throughout the early bloom period (ordinal dates $\approx 180-210$; Fig. 2, top and middle, dashed line). In fact, the number of weevils on plants appeared to anticipate blooming phenology by $\approx 3$ wk for both plant species. The weevils began aggregating on plants when only a few flowers had opened and were available for feeding and oviposition. Weevils were randomly distributed across *S. lacinatum* and *S. integrifolium* during the period when those were the only two species of host plants in bloom, and regardless of their relative density (Fig. 3, top; logistic regression not significant, $P = 0.44$).

The numbers of weevils on *S. lacinatum* and *S. integrifolium* began to decline rapidly before either plant species had reached peak bloom, clearly as a result of their shifting to *S. terebinthinaceum* (Fig. 2). Weevil adults colonized *S. terebinthinaceum* very early, anticipating the onset of blooming by $\approx 7$ wk (Fig. 2, bottom), suggesting that weevils had a strong preference for that host species. During the period when both *S. integrifolium* and *S. terebinthinaceum* both were in bloom, nearly all weevils were on the latter species, irrespective of the relative abundance of plant species (Fig. 3, bottom; logistic regression not significant, $P = 0.46$), with the exception of one date for one site where flower heads of *S. terebinthinaceum* were scarce.

The preference of weevils for *S. terebinthinaceum* also was reflected in their cumulative impact on plants. The plant species differed significantly in the percentages of flower heads that were clipped by the end of the season (PROC GENMOD $F_{12,29} = 3.91, P = 0.0042$), being higher in *S. terebinthinaceum* ($58.3 \pm 5.7$) than in the other two species ($28.3 \pm 4.4$ and $16.5 \pm 2.6$ for *S. integrifolium* and *S. lacinatum*, respectively). Study sites did not differ significantly in the percentage of flower heads that were clipped (ANOVA site effect, $P = 0.62$).

### Discussion

It is not surprising that populations of *H. aeneus* have become genetically differentiated, because the remnant and restored prairies that were our study sites were small in size and isolated within unsuitable agricultural or urbanized landscapes. Moreover, many of the prairie habitats were subjected periodically to prescribed burning that is intended to maintain the endemcity of the plant community (Steinauer and Collins 1996, Pauly 1997), but may have a devastating impact on communities of herbivorous insects (Swengel 2001, Tooker and Hanks 2004a). It is therefore likely that population densities of the weevil have at intervals been greatly reduced, resulting in population bottlenecks (Nei et al. 1975, Templeton 1980, Crouau-Roy 1989).

Nevertheless, two aspects of the genetic data suggest that weevils can move between patches of prairie, and that gene flow is not prohibited by habitat fragmentation. First, the low level of $F_{ST}$ strongly suggests that ongoing gene flow is relatively high. Although inferring number of migrants from $F_{ST}$ is fraught with a number of assumptions and complications, the inferred number of migrants per generation, 14.6, is well above what would be considered negligible. Second,

### Table 2. Global tests of allele frequency differences in pair-wise comparisons among ten populations of *H. aeneus* (site numbers as in Table 1; Fig. 1) by using exact $P$ values

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* Significant differences from exact tests of population differentiation are indicated as follows:
- "-" ($P > 0.05$); "*" ($P < 0.05$); "**" ($P < 0.005$); "***" ($P < 0.0005$).

### Table 3. $F_{ST}$ values for the seven polymorphic allozyme loci of *H. aeneus*, and the overall $F_{ST}$

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<th>Locus</th>
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<td>Aat</td>
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<td>Overall</td>
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the lack of evidence for isolation by distance suggests that some weevils must indeed travel long distances.

There are several possible explanations for the high rate of gene flow between populations of *H. aeneus*. Delayed diapause may result in an accumulation of weevils in the soil, essentially a “seed bank” of weevils, that would buffer populations from the effects of genetic drift. Such delayed diapause has been reported for other weevil species (Menu and Desouhant 2002, Maeto and Ozaki 2003). The weevil species may be less strongly influenced by prairie burning than suspected. *Silphium* and other species of host plants scat-

Fig. 2. Relationship between ordinal date and mean (±1 SE) percentage of flower heads that were in bloom, and log$_{10}$ density of adult *H. aeneus* at 12 study sites in east central Illinois for the host plant species (a) *Silphium laciniatum*, (b) *Silphium integrifolium*, and (c) *Silphium terebinthinaceum*. Means for the same sample date are shifted 1 d to avoid overlap of error bars.
tered along roadsides and railway right-of-ways may serve as partial corridors for gene flow between prairie remnants along which weevils could travel (Hamilton 1973). Finally, weevils may be accidentally transported by people, such as by alighting on the vehicles of prairie tourists.

The sequential blooming phenology of the *Silphium* species, broadly overlapping throughout the season, presents a continuous resource for adults and larvae of the oligophagous *H. aeneus*, allowing adults to shift from one species to the next as fresh flower heads become available. Other types of herbivorous insects similarly shift between host plant species that bloom sequentially (Floate et al. 1993, Hodkinson 1997). Adult *H. aeneus* used all three species of *Silphium* that were available at our study sites, although with a marked preference for *S. terebinthinaceum*. Nevertheless, oligophagy assures survival of the weevil even in small prairie remnants that do not have the complete set of host plant species. In fact, weevils were present at two of our study sites that were missing one of the *Silphium* species, including Sundrop Prairie where the favored *S. terebinthinaceum* was entirely absent (Table 1).

Although the weevil had a significant impact on reproduction of host plants by clipping flower heads, *Silphium* species are unlikely to be driven to local extinction because they are perennials: some plants eventually will succeed in producing enough seed to assure survival of the population, despite the great numbers of flower heads that are sacrificed in sustaining weevil populations. However, the damage inflicted by *H. aeneus* may have a significant impact on other arthropod species that rely on *Silphium* resources. Pollinators in particular are vital to the survival of
endemic plant communities and are likely to be especially sensitive to the sudden decline in flower heads where weevils are abundant. By clipping flower heads, the weevil also is likely to influence the diverse, but poorly documented community of insects that live within flower heads of Silphium plants, including larvae of gall wasps, other species of weevils, caterpillars, and the natural enemies of these species (J.F.T., unpublished data). Selective pressures associated with *H. aeneus* therefore may have played a significant role in shaping the community of insects that rely on *Silphium* flower heads for their survival.

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