Is there reduction in disease and predispersal seed predation at the border of a host plant’s range? - field and herbarium studies of Carex blanda

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Running headline: Disease in peripheral host populations
Summary

1. Small, isolated populations at species’ borders have been postulated to be less likely to have specialist pathogens and predators. Field and herbarium surveys were thus used to determine if two pathogens (a smut and a rust) and a predispersal seed predator were less common at the western range limit of the forest sedge *Carex blanda* in Kansas, USA.

2. Host plant size, reproduction, and density did not decline at the western border of the range. In fact, plants at two western sites had unusually large size and seed production.

3. Host populations at the edge of the range were more likely to be disease-free or lack the predispersal seed predator. Where the smut, seed predator, and rust were found, the proportion of infected or infested plants was not related to longitude, latitude, or percent forest cover.

4. More of the peripheral populations lacked the smut than the rust, as expected given the more localised nature of smut spore dispersal and the limited period when smut infection can occur.

5. In the adjacent, more highly forested state of Missouri, there were no geographic patterns in the incidence of the smut or seed predator in herbarium data.

6. The smut and rust increased in frequency over the 129 year span of herbarium collections.

7. Although field and herbarium distributional data were not identical (for example, smut infection was found much farther west in the field than in the herbarium data), the qualitative agreement between the two datasets suggests herbarium data can be used more broadly for studies of natural enemy distributions.

8. Limited dispersal by pathogens and seed predators is probably the reason why small, isolated western populations were less likely to have natural enemies. Peripheral host populations may thus have different ecological and evolutionary trajectories compared to more central populations. This conclusion, as well as the considerable variation among peripheral
1populations, is relevant to geographical studies of coevolution and to research on climatic effects
2on plants inhabiting ecotonal regions.
3Key-words: Anthracoidea blanda, biogeography, central-marginal model, ecotone, host-
4pathogen interactions, peripheral populations, predispersal seed predator, Puccinia, smut, rust
Introduction

Research on the distributions of species, and their limits, is fundamental to the discipline of ecology. Most distributional studies examine well-known taxa such as birds and plants, and recently, marine invertebrates (Hengeveld & Haeck 1982; Brown 1984; Sagarin & Gaines 2002ab; Gaston 2003). Theoretical advances, however, have also emphasised the importance of exploring distributions of parasites, pathogens, and mutualists (collectively, ‘natural enemies’) and their hosts. With specialists, common sense and theory suggest that natural enemy distributions will be constrained by host distributions, and will depend on colonisation and extinction rates of the natural enemy and its host, as well as gradients in host habitat suitability (Case et al. 2005). Hochberg & Ives (1999), extending Holt (1979), illustrated that parasitoids with high dispersal rates may enforce host range limits. When dispersal is limited, however, general metapopulation theory (Hanski & Ovaskainen 2000) suggests that isolated peripheral host populations are likely to escape colonisation by specialist natural enemies. In such a scenario, Antonovics et al. (2001) revealed that peripheral host populations could act as refugia, and contribute to recolonisation of the center of the host range following an epidemic.

Documentation of host and natural enemy distributions at the borders of host ranges is an essential first step in exploring the above theory. In the case of plants, it is known that herbivorous insects often do not attack hosts across their entire range (Garcia et al. 2000, Brewer & Gaston 2002). We do not know of explicit studies of plant disease in peripheral vs. more central host populations. However, infection levels often vary spatially, and can be related to environmental variables and/or the size and location of host populations (Kirby 1988; Burdon et al. 1995; Ingvarsson & Ericson 1998; Ericson et al. 1999; Thrall et al. 2001; Smith et al. 2003; Laine & Hanski 2006). Invasions of either hosts or pathogens/parasites also provide insight. For
example, peripheral chestnut populations remained free of the introduced chestnut blight fungus for decades (Davelos & Jarosz 2004) and invasive plants may lose some of their pathogens as they expand their range (Mitchell & Power 2003).

To address host/natural enemy distributions, we compared distributions of two pathogens and one predispersal seed predator in peripheral vs. more central populations of the sedge Carex blanda. Carex blanda, an understory plant, is found throughout the eastern forests of North America, and reaches its western limits in the broad Great Plains ecotone where forests disappear and grasslands predominate. Our work was focused in Kansas (KS), USA where precipitation and percent forest cover both decrease from east to west. We explored three topics:

First, we examined the host plant, since variation in host suitability could influence natural enemy distributions. We asked whether plant size and reproduction or plant density are reduced as one reaches the range limits. A reduction in individual or population attributes would be consistent with many studies (Hengeveld & Haeck 1982; Brown 1984; Carey et al. 1995; Hochberg & Ives 1999; McGill & Collins 2003; Holt et al. 2005). However, exceptions also exist, such as higher densities in the annual Hormungia petraea in peripheral populations (Kluth & Bruelheide 2005), or Sagarin & Gaines’ (2002ab) data on high densities of invertebrate species near range edges. Other examples include work by Griggs 1914, Prince et al. 1985, and Stokes et al. 2004.

Second, we explored geographic variation in levels of pathogen infection and seed predation, and predicted that occurrence of these organisms would be reduced at the host range limits for at least two reasons. One explanation focuses on the fact that western host populations occur in a drier climate; precipitation and humidity often plays a key role in pathogen infection (e.g. Huber & Gillepsie 1992). Another explanation, which is not mutually exclusive, is that
western host populations are more isolated, which may reduce the probability of colonisation by pathogens or seed predators. Host population isolation is potentially captured by two geographic variables: longitude (western populations are further from the presumed eastern source populations of the natural enemies) and forest cover (western host populations often occur in small forest patches). Separating the independent roles of all three variables (precipitation, longitude, and forest cover) is challenging since they are intercorrelated. Focusing on two kinds of data on natural enemies may provide contrasts. For example, one might expect all three variables to lead to a lower likelihood of finding a pathogen or seed predator at a western location (i.e. presence/absence data). However, if one focuses on the percentage of plants infected/infested at only those locations where the natural enemy is found, we had different predictions for the precipitation vs. isolation variables. Specifically, if the progressively drier climate is of major importance, the percentage of plants infected/infested would be predicted to decrease as one moves westward. If limits on dispersal are crucial, western populations should often be free of pathogens and seed predators, but if a dispersal event does occur, the build-up of such populations at the site (and thus the observed percentage of infected or infested plants) could be unaffected by the site’s isolation. Finally, we expect that natural enemies may differ in their distributions depending on their modes of dispersal and life histories.

The third goal of this study was methodological in nature. Ideally, biogeographic studies should focus on the entire species range (Sagarin & Gaines 2002a; Gaston 2003). However, such studies may only be feasible if ranges are small or linear (such as invertebrates along coastlines) (Sagarin & Gaines 2002b). Given these challenges, we were intrigued by Antonovics et al. (2003), which showed that herbarium specimens can provide distributional data on
diseased plants. We compared KS distributional data from herbarium specimens to KS field data to evaluate if herbarium data can be used more broadly for studies of natural enemy distributions.
Methods

THE STUDY SYSTEM

Carex blanda Dewey (Cyperaceae) is a common woodland sedge that occurs throughout the eastern deciduous forest in North America (Bryson 1980; Fig. 1). Plants are monoecious, flower in late April through to mid-May, and produce seeds from mid-May through to June. The plant lacks extensive clonal growth by rhizomes and thus occurs in discrete clumps.

We examined three natural enemies of C. blanda. The smut fungus Anthracoidea blanda (Vánky and H. Alexander) causes localised ovary infections (Vánky 2005). Individual infected flowers are sterilised with the ovaries replaced by sori that consist of fungal teliospores around remnant ovary tissue. An infected plant can range from having a single infected flower to over 1150% of flowers sterilised. Based on Scandinavian studies of other Anthracoidea species (Kukkonen 1972; Ericson et al. 1993; Ingvarsson & Ericson 1998, 2000), we expect that sori overwinter in the soil, teliospores germinate in the spring, and basidiospores infect developing flowers. Transmission of Anthracoidea is enhanced in Sweden by mycophagous beetles (Ericson et al. 1993); we have not observed these beetles in KS. Single seeds can also be killed by a seed predator. Infested seeds are greatly enlarged, and contain single white larvae which destroy the developing seed. These larvae were tentatively identified as chalcids (superfamily Chalcidoidea, order Hymenoptera; personal communications, J. Amrine); in this group, female wasps oviposit on developing seeds and the larvae feed and pupate in the seed. Usually < 5% of the seeds on a plant are affected. With the exception of very rare smut infection of C. grisea, smut and enlarged seeds were not observed on other co-occurring sedges.

The third natural enemy is a rust fungus in the Puccinia caricina species complex (based on ITS sequence comparisons with Gen Bank, L. Szabo, personal communications). Uredospores...
are produced on leaves throughout the growing season, with teliospores appearing in the autumn. In general, rusts reduce photosynthetic area (Lopes & Berger 2001), and thus plant growth.

Fifty six other Carex species are listed as hosts of *P. caricina* (Farr *et al.* 1989). However, the four KS species on this list are aquatic and do not co-occur with *C. blanda* (C. Morse, personal communications). The sexual stages of *P. caricina* occur on plants in the Urticaceae and Grossulariaceae (Farr *et al.* 1989); plants in these families (e.g. Ribes, Urtica) have distributions that span eastern – western KS (Great Plains Flora Association 1997) and were common at many of our sites. However, since the rust identification occurred after the field work was completed, we lack data on abundance of the alternate hosts at the sites, or infection levels.

FIELD SURVEYS

In May and early June of 2004 and 2005, we surveyed populations of *C. blanda* from eastern to central KS. Twenty-six sites were surveyed in 2004; 31 sites (including all 2004 sites) were surveyed in 2005 (see Table S1 in Supplementary Material, Fig. 1). We used linear transects since the species often occurs along trails and streams. When *C. blanda* was first encountered at a site, a continuous 30m transect was established, and the number of reproductive plants of *C. blanda* was recorded for a 1m width on either side of the meter tape. Plants were scored for the presence or absence of smut sori, enlarged seeds indicating predispersal seed predation, and rust (only in 2005). Only flowering plants were recorded because sedge identification depends on reproductive characters. Additional transects were established if the first transect had < 60 plants. In 2005, the 30m transect was subdivided into 15 2m x 1m segments, with counts made in each segment to quantify plant density. We measured plant height (base to highest point of plant) and basal diameter (using calipers) on three plants in 2004 and five plants in 2005; in 2005, we also measured the number of inflorescences, and for two inflorescences, the number of
1 seeds per inflorescence. Table S1 describes the 31 sites, their longitude and latitude using a 2 Global Positioning System (GPS) (Garmin GPSMAP 76, accuracy < 15m), their local habitat 3 (whether prone to flooding), and their understory vegetation cover as open (plants easily visible 4 on the forest floor), intermediate, or dense (plants hidden in dense vegetation).

5 We are confident that the central KS sites exist on the western range limit of *C. blanda*. 6 First, although no formal count was kept, we often explored over 10 woodland areas in central 7 KS before a single area with *C. blanda* was found; in eastern KS, the plant is found in virtually 8 every woodland. Second, our central KS locations are similar to the longitude of herbarium 9 specimens (Fig. 1). Finally, Küchler (1974) delineated the pre-settlement western boundary of 10 where prairies had a significant amount of “islands” of forest vegetation; several central KS sites 11 are farther west than this boundary (Fig. 1).

12 For each site, we determined the average annual precipitation in mm (based on longterm 13 (typically 1948-2005) average annual rainfall data at the closest weather station, available 14 through the High Plains Regional Climate Center (www.hprcc.unl.edu/wrcc/states/ks.html), and 15 percent forest cover in a 200m radius circle around each site. The choice of the 200m distance 16 was arbitrary, but covered a wide range (near 0 – 100%) of forest cover (Table S1). Geographic 17 information system (GIS) work was done using Environmental Systems Research Institute’s 18 ArcGIS™, with supplementary work in MicroSoft Excel™. Percent forest cover was based on 19 KS land cover data from the US Geological Survey’s National Land Cover Dataset (NLCD) 20 (Vogelmann *et al.*, 1998). The NLCD is derived from Landsat satellite TM imagery from circa 21 1992. This dataset is in raster (Geo-TIFF) format, and is projected to Albers Conical Equal Area 22 North American Datum 1983; all spatial datasets were converted to this projection for analysis.

23 We used the Spatial Analyst extension to reclassify the 21 land cover classes into water, non-
Forested, or forested areas, maintaining the original 30m resolution in the resulting grid dataset. Forested areas included deciduous woods, evergreen woods, mixed woodlands, and woody wetlands. We used geographic coordinates collected with a GPS to map sedge surveys from 2005, then generated a 200m radius circle around each site using the Buffer tool. To determine percent forest cover within each site ‘area’, we performed a spatial cross tabulation between the land cover data and the 200m radius circles, using the Tabulate Area tool.

7HERBARIUM SURVEYS

A total of 1629 herbarium sheets were examined from KANU, R. L. McGregor Herbarium, University of Kansas, Lawrence, KS; KSP, T. M. Sperry Herbarium, Pittsburg State University, Pittsburg, KS; KSTC, Herbarium of Emporia State University, Emporia, KS; KSC, Herbarium, Kansas State University, Manhattan, KS; MO, Herbarium at the Missouri Botanical Gardens, St. Louis, MO; and MICH, Herbarium at University of Michigan, Ann Arbor, MI. These herbaria are strong in central US and/or Carex collections. For each sheet we recorded the collector, collection date, county and state, and presence vs. absence of smut sori or enlarged seeds characteristic of the seed predator. We were not initially aware of the rust; we thus resurveyed most KS sheets for presence/absence of rust (except those from the Herbarium at the Missouri Botanical Gardens). All putative rust pustules were examined under a dissecting microscope. Mycologists have been known to use herbaria as a source of fungal material. Although no such annotations were evident, we asked herbarium directors whether there was any indication of fungal removal over the history of their collections. All responses were negative.

We used herbarium data to provide presence/absence data by considering the specimen (defined as all plants present on a sheet) infected or infested if any plants showed symptoms.

Further, since collectors sometimes distribute plants collected at a single location to multiple
herbariums, we followed Antonovics et al. 2003 by defining all plants on multiple sheets that had the same collector, date, and location to be a single specimen. We limited analyses to KS or Missouri (MO) specimens; MO is a more heavily forested state in which \textit{C. blanda} occurs throughout. Further, we restricted analyses to specimens for which we had complete location and date information, and for seasons where infection/infestation is detectable. We knew from field work that smut sori are not visible on infected inflorescences until late spring, and that in early and mid summer, respectively, predator-infested seeds and smut sori fall off the plant.

Thus we limited our analyses of smut infection to specimens collected between May 12th – June 9th (KS: n = 116; MO: n = 327), and for seed infestation to collections prior to June 5th (KS: n = 183; MO: n = 414). These dates included the dates of the field surveys. We did not detect a significant seasonal effect on rust presence/absence, so the entire KS data set was used (n = 208).

Finally, for all KANU specimens from KS, we recorded size data: height (cm; maximum height of plant) and inflorescence length (mm, average of two inflorescences). These variables were unlikely to be altered by the process of collecting or mounting a specimen; for example, basal diameter was not used because collectors often divide a large plant into smaller portions that fit on a herbarium sheet (C. Morse, personal communications).

Each herbarium specimen was assigned geographic coordinates (following Antonovics et al. 2003, these were based on the longitude and latitude of the county seat as not all records had specific location information), average annual precipitation (average of longterm data from all county weather stations; from the High Plains Regional Climate Center as noted earlier), and percent forest cover. To estimate forest cover by county, we obtained county boundary data for KS and MO in vector, ESRI shapefile format (U.S. Census Bureau, 2000). We performed a
spatial cross tabulation between the landcover and county datasets to obtain the percent forest cover for each county.

3 ANALYSES

All statistical analyses were done in Minitab 14 (Minitab, Inc.) or SAS 9.1 (SAS Institute, Cary, NC, USA). Initially, we analysed correlations between geographic variables for field sites and counties (for herbarium data). Because of the very high correlation of KS longitude and precipitation (see Results), we only included longitude in subsequent analyses.

8 Analyses of Field Surveys

Unless noted, analyses are for 2005, where we had more sites and more complete data (2004 and 2005 patterns were consistent - see correlation analyses in Results).

11 Plant traits: For individual plant traits and mean site density (number of plants in occupied 2m x 1m segments of the transects), we performed general linear models. Independent variables could include survey date, geographic variables (longitude, latitude, forest cover), and local site variables (habitat, cover, and population density). Plant size was calculated as plant basal area \( \pi r^2 \); this trait is highly correlated with biomass in plants with similar life forms to sedges (i.e. grasses; Malmstrom et al. 2005). Plant size and mean seed production (number of inflorescences x number of seed/inflorescence) were log-transformed to correct for heterogeneity in variance. In these and similar analyses, full models with all predictor variables were run first, and then nonsignificant terms were eliminated in subsequent model runs. Habitat and cover variables were only eliminated if their interaction was not significant. Type III sums of squares were used so that the order variables entered in the model did not alter the results.

22 Pathogen and seed predator levels: We analysed natural enemy distributions in three ways. First, the presence/absence of disease and predation at the sites were examined with logistic
Second, we took a community approach by scoring each site as having 0, 1, 2, or 3 pathogen or predator species present and analysing the data using ordinal logistic regression. Finally, for sites where a particular pathogen or predator was present, we analysed variation in the percentage of plants with infection or predation with general linear models on arcsine-transformed data. The independent variables were the same as described above for analyses of plant traits. In logistic regressions, the vegetation cover variable had to be deleted so that algorithms would converge. We present two tests used in Minitab 14 to describe logistic regressions: a Z test of whether each coefficient for a model predictor was significantly different from zero, and a G statistic which tests whether all slopes are equal to zero.

**Analyses of herbarium surveys**

**Coverage:** To examine the geographic coverage of collections, we used resampling methods for each state for the entire plant collection, as well as specimens with each natural enemy. Following Antonovics et al. (2003), a specimen was randomly sampled without replacement and the county name was noted. This process was repeated for the entire collection, noting whether subsequent specimens provided new county names. The collection of specimens was then randomised, and the process was repeated 100 times. In coverage curves, the x axis is the position of the specimen in the collection (i.e. first, second,...last examined) and the y axis is the proportion of the 1000 randomisations that resulted in a new county at that position.

**Plant traits:** Using general linear models, we explored whether plant height or average inflorescence length was predicted by collection date, longitude, latitude, or forest cover.

**Pathogen and seed predator levels:** We performed multivariate logistic regression on the presence/absence of each natural enemy, with the initial full model using the collection year and geographic variables (see Analyses of Field Surveys for details). Analyses were done by state.
and for a combined data set. To present relationships from these logistic regressions, we divided each data set using the median value of the independent variable, and calculated the percentage of infected/infested individuals in each data set half. The equal sample sizes of the two halves of the data set circumvents issues of whether infection/infestation rates are rare in western specimens because fewer were collected at the range limit. In parallel with community analyses of field data, we used an ordinal logistic regression to analyse whether 0, 1, 2, or all 3 of the natural enemies were detected in each KS county. The number of specimens per county was included in analyses to account for variation in sampling intensity among counties.

In addition to using collection year in the above logistic regressions, we also explored temporal trends by calculating the percent of infected or infested specimens in three time intervals (< 1920, 1920-1960, > 1960). Although our specimens ranged from 1874 to 2003, specimen numbers per decade varied greatly. We chose these three intervals to have sufficient sample sizes per interval and to allow comparisons among the three natural enemies.
Results

2 WHAT ARE THE RELATIONSHIPS BETWEEN GEOGRAPHIC VARIABLES?

As expected, KS locations with more western longitudes had lower precipitation \((r = -0.97, p < 0.0001; r = -0.93, p < 0.0001)\), and lower forest cover \((r = -0.70, p < 0.0001; r = 0.79, p < 0.0001)\), while locations with higher precipitation had higher forest cover \((r = 0.62, p < 0.0001; r = 0.71, p < 0.0001)\). The latitude of a location was not significantly correlated with other variables in the field data; in the herbarium data, southern counties had higher precipitation \((r = -0.37, p < 0.01)\). Because KS longitude and precipitation were so strongly correlated, we only included longitude in KS analyses. Similar, although weaker, correlations occurred in the MO herbarium data \((\text{precipitation and longitude, } r = -0.58, p < 0.0001; \text{precipitation and forest cover, } r = 0.42, p < 0.0001; \text{longitude and forest cover } r = -0.49, p < 0.0001)\); in addition, southern counties had higher forest cover \((r = 0.48, p < 0.0001)\).

3 IS PLANT SIZE OR DENSITY REDUCED AT THE RANGE LIMIT?

KS field surveys

Despite considerable variation, average plant size increased to the west \((F_{1,28} = 6.8, p = 0.01)\) and at sites with lower forest cover \((F_{1,28} = 6.2, p = 0.02)\) \((R^2 = 0.21)\). Average seed number per plant increased to the west also \((F_{1,29} = 6.73, p < 0.01, R^2 = 0.19)\) \((\text{Fig. 2})\). Two peripheral host populations with unusually large plants greatly affected the relationships \((\text{Fig. 2})\). If these two populations were removed from the analyses, plant size increased with population latitude \((F_{1,27} = 4.4, p = 0.05, R^2 = 0.14)\) and no factor predicted seed number per plant. Plant density did not vary geographically but highest densities were found in lowland habitats with open cover \((\text{Fig. 223})\). For the 18 sites examined at comparable census dates, 2004 plant size was positively correlated with 2005 plant size \((r = 0.6, p = 0.02)\).
KS herbarium surveys

Plant height and inflorescence length of herbarium specimens did not vary with latitude, longitude, or forest cover.

AT THE RANGE LIMIT, IS IT MORE LIKELY THAT SITES WILL LACK DISEASE OR SEED PREDATION?

KS field surveys

The 31 sites varied greatly in smut, seed predator, and rust levels (Table S1); such differences appear to be characteristic of the sites given positive correlations between percentage of plants with smut infection \((r = 0.75, p < 0.0001)\) and with seed predation \((r = 0.69, p < 0.001)\) for sites visited in the same two weeks in 2004 and 2005. The percentage of plants with smut or seed predators was correlated across all sites \((r = 0.36, p = 0.04)\), but we found no significant correlation between these organisms when examining the 22 sites that had both organisms. Rust infection was not significantly correlated with either the smut or seed predator. Lack of strong correlations between species led us to analyse each natural enemy separately.

The nine smut-free sites were all located at the western margin, particularly in the south (Table S1, Fig. 1), leading to significant longitude \((Z = 2.17, p = 0.03)\) and latitude \((Z = 2.23, p = 170.03)\) effects in logistic regression (for the model, \(G = 18.978, d. f = 1, p < 0.001\)). The three seed predator-free and two rust-free sites were also located on the range limit. Due to the small sample sizes, the effect of longitude was only marginally significant for the seed predator data \((Z = 1.84, p = 0.07)\), and the logistic regression algorithm did not converge for the rust data.

From a community perspective, the number of natural enemy species/site was reduced at the range limit (Table S1; Fig. 4a), leading to significant coefficients for longitude \((Z = 2.24, p =\)
10.03) and latitude (Z = -2.91, p < 0.01) in multivariate logistic regression (test that all slopes were 0 in the model: G = 25.273, p < 0.001, d.f. = 2).

3Herbarium surveys

Carex blanda occurred in 52 KS counties, with eight, 15, and 48 counties having specimens with smut, seed predators, or rust, respectively (Fig. 1). In MO, 95 of the 114 counties had C. blanda; the smut and seed predator were found in 14 and 32 counties, respectively. The geographic coverage for the plant and rust in KS was excellent (Fig. 5ad). Despite the small number of smutted specimens, smut coverage was fair, with the probability of adding another county to the distribution reduced to 0.26 when all specimens had been examined (Fig. 5c). Coverage was spottier for the seed predator with final probabilities of adding new counties to the distribution near 0.5 (Fig. 5b). MO plant coverage was very similar to KS; coverage for MO smut and seed predator data appears similar to the KS seed predator data.

The presence/absence of one pathogen or predator species on a specimen was statistically independent of the presence/absence of other species (contingency tests; in all cases, p > 0.1).

For KS, 10.3% of the specimens had smut, 10.4% had seed predators, and 75.3% had rust. Smut was more likely to be absent on western KS specimens; the exact significance of this result from the multivariate logistic regression depends on the statistical test (Z test for whether coefficient was different from 0 = -1.77, p < 0.08; G test that all slopes are 0 is G = 5.55, d.f. = 1, p = 0.02).

The likelihood of detecting the seed predator was lower for specimens collected in counties with lower forest cover (Z = 2.20, p = 0.03, G = 5.184, d.f. = 1, p = 0.02). Rust was less likely to be observed in western KS specimens (Z = 2.04, p = 0.04) and has become more common in recent years (Z = 2.85, p < 0.01) (final model, G = 12.526, d.f. = 2, p < 0.01). These spatial and temporal relationships are illustrated in Table 1. In the community analysis, species number
declined as forest cover declined (Fig. 4b, Z = -2.88, p < 0.01). As expected, we detected more
natural enemy species for counties with more specimens (Z = -1.94, p = 0.05); for the final
model, G = 31.872, d.f. = 2, p < 0.001).

The percentage of MO specimens with smut (5.5 %) and seed predators (10.4%) was
generally similar to KS. The geographic variables didn’t explain the presence/absence of the
smut or seed predator in MO or in the combined state dataset; however, there was an increase in
smut infection over time (for combined dataset: Z = 2.02, p = 0.04; G = 5.373, d.f. = 1, p = 0.02)
(Table 1).

WAS THERE A LOWER PERCENTAGE OF PLANTS WITH NATURAL ENEMIES AT
THE RANGE LIMIT?

We found no geographic pattern to the percentage of plants infected or infested when we
restricted analyses to only sites where the pathogen or seed predator was present. In fact, several
western sites had high percentages of plants with disease or seed predation (Table S1).
Discussion

2INDIVIDUAL AND POPULATION LEVEL TRAITS OF PLANTS ACROSS THE EAST-WEST GRADIENT

4We found no evidence for reduced plant size, reproduction, or density at the western range limit. In fact, the largest plants and some of the highest densities occurred at two far western sites, emphasising the value of intensive sampling at a species’ limits (Sagarin & Gaines 2002a).

7These two western sites were not obviously different from other sites. Since data were only taken on flowering plants, one might hypothesise that only the largest plants flowered in the west while a wider range of plant sizes flowered in the east. However, no such patterns in flowering were observed. The reduced incidence of the natural enemies we studied also does not explain plant patterns. The smut and seed predator are limited to individual flowers and shouldn’t affect plant growth. Rust can reduce plant growth, but some western sites had high rust levels and very large plants (site 29, Table S1). Other unmeasured variables may have been important. For instance, trees were sparse at some western sites, allowing greater light to the understory. The absence of pathogens we did not study (e.g. soil-borne organisms, viruses) might also have been important. Regardless of mechanism, we show that range limits per se are not always associated with reduction in plant performance, as also found by Stokes et al. (2004) and Kluth & Bruehlheide (2005). Our results further argue against a westward decline in host suitability for natural enemies; in fact, the larger size and reproduction in some western sites suggest greater host suitability.

21PATHOGEN INFECTION AND SEED PREDATION ACROSS THE EAST-WEST GRADIENT
We found few factors that predict pathogen or predator levels at the local site level; we thus focus on larger spatial scale patterns. All three natural enemies did have widespread distributions (Fig. 1, Table S1). However, if one examines the presence or absence of a pathogen or seed predator at locations, there was a lower probability of finding these three organisms on the western host limit. If one focuses only on sites where the natural enemy was present, we did not discern any geographic patterns to the percentage of plants infected or infested. This latter result suggests that host populations across KS are largely suitable for these natural enemies, and argues against the general westward decline in precipitation as the primary determinant of pathogen and predator distributions. For example, fungal infection can probably occur in the drier western climate because of higher humidity in microclimates in woods and along creeks, where *C. blanda* is often found. Low probability of dispersal to isolated western populations thus probably explains the absence of the smut, and to a less degree, the rust and the seed predator, at the range limit. This conclusion is consistent with the lack of geographic patterns in smut or seed predator incidence in MO, where forest cover is higher (average % forest cover per county = 38%) and understory forest species are likely to have more continuous distributions. Our interpretations of cause and effect at the state-wide level must be tempered by the very high correlation between geographical variables, especially between longitude and precipitation. In our analyses, we also note that we treated field sites as independent data points. We made this decision because even sites separated by short distances could have very different frequencies of natural enemies (Table S1). However, as one goes from MO to central KS, forest fragmentation changes from a patchy landscape to linear forest fragments along creeks and rivers. Possible long-distance dispersal of pathogens or seed predators between connected forest fragments deserves further study.
Dispersal mechanisms and life histories differ among pathogen species, with important ecological and genetic consequences (Thrall & Burdon 1999). For example, in contrast to the smut and seed predator, the distribution of alternate hosts may have major effects on the distribution of *P. caricina*. We lack data to explore such patterns, but as noted in the Methods, data from plant atlases suggest that sexual and asexual hosts of the rust co-occur across Kansas. We did find that the smut had a more restricted KS distribution than the rust, especially in the field data. This result may be explained by different modes of spore dispersal. For instance, teliospores from smutted inflorescences overwinter at the base of plants; the following spring, basidiospores disperse by very local air movement. In contrast, rust urediospores can disperse in the atmosphere over 100 km (Eversmeyer & Kramer 2000). Susceptible tissues are also present on the host for a shorter time for the smut (immature flowers present for 2-3 weeks) vs. the rust (leaf tissue present for months). The limited spore dispersal of the smut, and its absence in several western sites, could be considered in relation to theoretical work by Bahn et al. (2006), who showed that unsuccessful dispersal from isolated peripheral populations can contribute to extinctions and the formation of range limits. However, although smut did disappear at one transect between 2004 and 2005 near the host range limits (site 14, Table S1), transect data on extinctions are inconclusive since these organisms may exist elsewhere in the forest patch.

Smut and seed predation levels in 2004 and 2005 were strongly correlated across the sites. Positive year-to-year correlations were also apparent for similar organisms in Sweden at a smaller spatial scale (infection/infestation of tussocks by a congeneric smut, *A. heterospora* and a mite *Phytoptus caricis*, Ingvarsson & Ericson 1998). These Swedish flower-inhabiting species compete for shared ovary resources, especially when present at high densities (Ingvarsson & Ericson 2000). We found that the levels and distributions of the smut and seed predator of *C.*
**1blanda** were largely independent of each other. Ovary resources may not have been limiting since plants were not completely smutted or infested with seed predators, even when the percentage of infected/infested plants was high.

4USE OF FIELD AND HERBARIUM DATA TO STUDY PATHOGEN AND PREDATOR DISTRIBUTIONS

Recent investigations (Antonovics *et al.* 2003 (and references within); Hood & Antonovics 2003) have used herbaria to study plant-pathogen interactions. Our work is unusual in that we compare data from herbarium and field surveys. Both field and herbarium approaches have pros and cons. In the field, a researcher can examine many plants/site and collect detailed data at each site. However, finding sites and recording data is time-consuming and reduces the number of total sites examined; this was challenging given the narrow temporal window when smut infection and seed predation could be scored. In particular, we are aware that although we did intensive field sampling in the west, we had fewer field sites in eastern KS simply because of the difficulty in visiting many sites within the appropriate timeframe. We doubt if more sampling would alter our overall conclusions (although since our study was completed, we have identified some eastern sites that may be smut-free). In the future, we would like to focus on one latitudinal region and intensively sample sites to determine if correlations between site isolation and smut infection occur.

In contrast to field sampling, surveys of herbarium collections allow one to examine plants from hundreds of locations. However, bias can be introduced depending on where collectors go and which plants they collect. Some biases can be addressed in a qualitative manner by comparing field and herbarium datasets. For example, rust was common in both datasets. Higher rust incidence in the herbarium dataset may reflect the fact that plants were examined
1microscopically; hence even single pustules were detected. Bias may exist against collecting
2smutted plants: smut infection was similar (KS) or smaller (MO) than seed predation in the
3herbarium data set, but was at a similar level or more common at most field sites (Table S1).
4Further, heavily smutted plants were less common in the herbarium data than in the field.
5However, importantly, such biases should not lead to geographic patterns; for example, it is not
6obvious that western collectors would be more likely to avoid smutted plants than eastern
7collectors. Another challenge of herbarium data, incomplete coverage, can be quantified due to
8approaches such as Antonovics et al. 2003. Such resampling methods look at patterns in the
9actual data: incomplete coverage due to collectors never working in parts of a species’ range will
10obviously not be detected. We were pleased by how generally high the coverage was, given the
11limited number of specimens, although distributional data on the smut and seed predator is far
12from complete.
13Herbarium and field surveys also differ in spatial and temporal scales. From a
14methodological perspective, we knew the exact longitude and latitude of a field site, and thus
15investigated whether fine-scale forest cover (200m radius circle) was a predictor in analyses.
16Such fine-scale analyses were impossible for the herbarium specimens, where precise location
17data were often missing. Thus, we analysed the herbarium data at the spatial scale of a county,
18where, due to greater coverage across the state, we could look for larger scale spatial patterns.
19Field surveys also provide an instantaneous “snapshot” of distributions in contrast to the
20cumulative distributions resulting from multiyear herbarium surveys. Antonovics et al. (2001)
21noted chance dispersal events or short-lived persistence of populations at the far margins of a
22species’ range may be sampled by collectors. Thus a cumulative collection may imply a broader
23range distribution than is typically seen in any single year. In contrast, however, the western
boundary for *C. blanda* in our herbarium and field data were similar, and field surveys extended the western limit of the smut and seed predator (but not the rust) (Fig. 1). The narrower spatial distribution of the smut and seed predator in the herbarium vs. the field survey probably reflects sampling intensity. A herbarium sheet has only 1–5 plants; thus even without any bias against plants with disease or predation, the probability of detection of natural enemies at any single site using a herbarium collection is low. Further, the number of herbarium specimens per county declined as one moved from east to west in KS. In contrast, we purposely did intensive field sampling at the western range limit and thus by sampling many populations and plants, were more likely to detect the smut and seed predator. Because of differences between approaches, we did not expect that herbarium distributions of natural enemies would match distributions uncovered in field surveys. However, as emphasised by Antonovics *et al.* (2003), relative comparisons are valuable. Hence we find it encouraging that the western vs. eastern differences in presence/absence of disease and seed predation occurred in both data sets. The somewhat lower percentage of smutted specimens in MO than KS (5.5% compared to 10.3%) was surprising, but given the difference was not significant, it may be due to chance sampling.

An unexpected result was that the percentage of specimens with smut and rust increased significantly over the >100 year span of herbarium collections, and a similar trend was found for seed predation. As noted in the Methods, we lack evidence that herbarium specimens were “mined” by early mycologists. It is also doubtful that any biases against collecting infected material would change across time. However large changes in the landscape and land use occurred over the 129 year span of the collections. By comparing modern forest cover to historical records (Fifth Annual Report of the State Board of Agriculture 1877), we found that there was no net change in forest cover west of 96° W longitude, where peripheral host
populations are located. However, 5-10% of eastern KS was forested in the late 1800’s, while current estimates are 10-20% (Fig. 1). Disease levels possibly have increased in parallel with the increase in forest cover since increased forest cover is likely to be associated with increased abundance and reduced isolation of populations of C. blanda. Grazing pressures or frequencies of the alternate hosts of the rust may also have changed over time. Interestingly, Antonovics et al. (2003) reported a 16% increase in smut infection in Silene over a 100 year span of herbarium collection and Bearchell et al. (2005) inferred that the relative abundance of two wheat pathogens in dried plant material has changed over a 160 year span. The changes in the wheat pathogen population dynamics were correlated with changes in atmospheric pollution.
Conclusion

We found no evidence of reduced size, reproduction, or density at range limits and discovered surprisingly large plants with high seed production at some western sites. Smut, seed predators, and rust were commonly found in much of the host range in KS, but were more likely to be absent in peripheral host populations, probably due to limits on dispersal. In the future, distributional studies need to be paired with analysis of host resistance and pathogen virulence structure. Such work could be particularly informative in light of coevolutionary theory (Nuismer et al. 2003; Thompson 2005) and since peripheral plant populations can have restricted genetic variation (Lesica & Allendorf 1995). We expect that ecological and genetic studies of peripheral populations will become of increasing interest; borders of species’ distributions (Parmesan & Yohe 2003) and disease (Harvell et al. 2002) are predicted to change as a result of global phenomenon and such changes may be more evident at ecotones (Neilson 1993).

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References


Table 1. Spatial and temporal variation in disease and seed predation based on herbarium specimens.  
A. Spatial variation in percentage of specimens with smut, seed predation, and rust in KS for variables that were significant in multivariate logistic regression (see text). The percentage infected or infested was calculated for western vs. eastern longitudes, or lower vs. higher values of percent forest cover by using the median value of the predictor variable to divide the data set in half.  B. Temporal variation in percentage of specimens with smut, seed predation, and rust. Data were divided in 40 year intervals using the combined KS and MO datasets (for smut and seed predator) or the KS data (for rust). Significant temporal variation was found for smut and rust in multivariate logistic regression (see text); For both A. and B., samples sizes are indicated in parentheses; p values listed in tables are based on Chi-square or Fisher’s exact tests.  

<table>
<thead>
<tr>
<th>Longitude</th>
<th>Western</th>
<th>Eastern</th>
<th>χ²</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% with smut</td>
<td>3.4 (n = 58)</td>
<td>17.2 (n = 58)</td>
<td>4.949</td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td>% with rust</td>
<td>68.6 (n=99)</td>
<td>81.8 (n = 99)</td>
<td>4.583</td>
<td>1</td>
<td>0.032</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Forest Cover</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>16% with seed predation</td>
<td>4.4 (n = 91)</td>
<td>16.3 (n = 92)</td>
</tr>
</tbody>
</table>
### Temporal Variation

<table>
<thead>
<tr>
<th>Period</th>
<th>Smut (%)</th>
<th>Seed Predator (%)</th>
<th>Rust (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before or equal to 1920</td>
<td>0 (n = 56)</td>
<td>4.0 (n = 76)</td>
<td>52.2 (n = 23)</td>
</tr>
<tr>
<td>1921-1960</td>
<td>1.8 (n = 55)</td>
<td>12.2 (n = 90)</td>
<td>64.7 (n = 34)</td>
</tr>
<tr>
<td>After 1960</td>
<td>8.7 (n = 332)</td>
<td>11.1 (n = 431)</td>
<td>80.1 (n = 151)</td>
</tr>
</tbody>
</table>

Chi-squared tests:
- \( \chi^2 = 3.9 \), d.f. = 2, \( p = 0.14 \)
- \( \chi^2 = 10.3 \), d.f. = 2, \( p = 0.006 \)
Field and herbarium data displayed on map of KS, USA, with USA map showing distribution of *C. blanda* (http://www.npwrc.usgs.gov/resource/othrdata/plntguid/species/careblan.htm). The 31 field sites from 2005 are indicated by circles; circles with or without a dot indicate locations where smut was present or absent, respectively. All sites had seed predators and rust
1 with the exception of sites indicated along the western border with a 1 (seed predator absent) or a 2 (rust absent). The letters in the
2 counties denote those in which the host plant (H), smut (S), or seed predator (P) occurred at least once in the herbarium dataset.
3 Herbarium rust distributions are not shown, but nearly match the plant distribution (all but 4 counties with plants had at least one
4 rusted specimen). Shading of counties reflects the percent of the county area in forest; see text. The line in central KS was digitised
5 in a GIS from a scanned and georectified map of pre-European settlement vegetation by Küchler (1974), and indicates the western
6 boundary of where prairies had a significant amount of ‘islands’ of forest vegetation.

7

8
Fig. 2. Relationship between individual plant traits and longitude for 31 KS field sites. a), average plant size (basal area, cm$^2$), b) average seed production per plant. The arrows designate a...
site that was grazed; if this site is eliminated from the data, the positive relationship between plant traits and western longitude is stronger.
Fig. 3. Effect of habitat type (lowland vs. upland) and vegetation cover (open, intermediate, and dense) on average density (± standard error) of *C. blandia* in KS field sites. Average density of plants (± standard error) at sites. Significant terms in the analysis were Habitat \( (F_{1,24} = 15.52, p < 0.001) \), Cover \( (F_{2,24} = 1.51, \text{n.s.}) \), Habitat x Cover \( (F_{2,24} = 10.07, p < 0.001) \), and Survey date \( (F_{1,24} = 9.40, p < 0.001) \); \( R^2 = 0.56 \).
Fig. 4. Species richness of natural enemies of *C. blanda* in KS as related to a) longitude for field surveys and b) percent forest cover for herbarium surveys. For a), each point is based on a different field site in 2005 and for b), each point represents a county. A species richness of three indicates that the smut, seed predator, and rust were all found; smaller numbers represent situations where one or more of these species were not present.
Fig. 5. The probability of discovering a new county for each subsequent herbarium specimen examined for KS collections of a) *Carex blanda*, b) individuals of *C. blanda* infested by seed predators, c) individuals of *C. blanda* infected by smut, and d) individuals of *C. blanda* infected by rust. If most specimens are from unique counties, there is a high probability that examination of each subsequent specimen will expand the distribution, and curves will not reach the x axis. In contrast, if multiple specimens from the same county are common, curves will approach the x axis, as there is a lower probability that examination of an additional specimen will add new distributional information. See text and Antonovics et al. 2003 for details.