CHROMOSOME COUNTS OF *OPUNTIA* (CACTACEAE), PRICKLY PEAR CACTI, IN THE MIDWESTERN UNITED STATES AND ENVIRONMENTAL FACTORS RESTRICTING THE DISTRIBUTION OF *OPUNTIA FRAGILIS*

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Abstract: *Opuntia fragilis, O. humifusa* s.l., and *O. macrorhiza* s.l. are widely distributed prickly pear taxa that have been studied cytologically mostly in western North America, but upper Midwestern United States populations, which form most of the northeastern-most extent of their distributions, have not been analyzed previously. The wide-ranging and most northern of all cacti, *O. fragilis*, is relatively abundant, at least historically, in the upper Midwestern United States but does not occur further southeast than Jo Daviess County, Illinois, while *O. humifusa* s.l. is found throughout most of the eastern United States. This difference in distribution may indicate that environmental variables impede the establishment of *O. fragilis* in most of the eastern United States. We present the first chromosome counts of *O. fragilis*, *O. humifusa* s.l., and *O. macrorhiza* s.l. for 40 populations over part of their Midwestern range and employ habitat niche modeling using 19 environmental variables to extrapolate potential reasons why *O. fragilis* may not be found in the eastern United States.All twelve populations of *O. fragilis* analyzed were hexaploid, a finding consistent with previous reports, and adding further evidence for its vegetative dispersal from the southwestern United States, where the species likely originated. Populations of *O. humifusa* s.l. and *O. macrorhiza* s.l. were all tetraploid, indicating that the northeastern-most range of those taxa is occupied by polyploid derivatives of their southern diploid relatives. Results from niche modeling suggest suitable habitat for *O. fragilis* in the upper Midwest is strongly predicted for areas with seasonal fluctuations in temperature but annual precipitation homogeneity, variables that may present significant environmental barriers to the existence of the species in the eastern United States.

Resumen: Tres taxa de nopales ampliamente distribuidos por los EE. UU., *Opuntia fragilis*, *O. humifusa*, y *O. macrorhiza* han sido estudiados citológicamente en partes de sus distribuciones, pero las poblaciones del medio oeste que forman las distribuciones más al noreste de estas especies, nunca han sido analizadas. *O. fragilis*, el cactus con un amplio rango de distribución y que se encuentra más al norte que cualquier otra cactácea, es relativamente abundante en el medio oeste de los EE. UU., pero no se encuentra más al sureste del condado Jo Davies en el estado de Illinois; mientras que *O. humifusa* s.l. se encuentra prácticamente por todo el este de los EE. UU. Factores ambientales podrían estar previniendo el establecimiento de *O. fragilis* en el este de los EE. UU. Presentamos los primeros recuentos de cromosomas de poblaciones de *O. fragilis*, *O. humifusa* s.l., y *O. macrorhiza* s.l., colectadas en partes de sus rangos de distribución en el medio oeste, y usamos modelaje de nichos de hábitat integrando diecinueve variables ambientales para extrapolar las posibles razones por las cuales *O. fragilis* esta ausente del este de los EE. UU. Todas las poblaciones de *O. fragilis* analizadas fueron hexaploides, lo cual concuerda con otros reportes de la especie, y añade evidencia sobre su dispersión vegetativa de su sitio de origen probable, el suroeste de los EE. UU. Todas las poblaciones de *O. humifusa* s.l. y *O. macrorhiza* s.l. fueron tetraploides, lo cual indica que la parte noreste de sus rangos de distribución está habitada por derivativas poliploides de sus parientes diploides del sur de los EE. UU. Los resultados del modelaje de nichos de hábitat de *O. fragilis* sugiere que se puede predecir áreas habitables para *O. fragilis* por cambios estacionales de la temperatura y homogeneidad de precipitación anual, variables que no se encuentran en el este de los EE. UU., y que pueden ser una barrera para el establecimiento de *O. fragilis* en esa región.

Key words: Midwest, niche modeling, *Opuntia*, polyploidy.

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Introduction

Opuntia fragilis (Haw.) Nutt., the brittle prickly pear cactus, is one of the most widespread members of the *O. polyacantha* complex (sensu Parfitt 1991), with populations ranging from the southwestern United States north to British Columbia and east as far as eastern Ontario, Canada. *Opuntia fragilis* is the northernmost distributed cactus and the most cold tolerant of those species that have been analyzed thus far (Loik and Nobel 1993). The *O. polyacantha* complex is most species rich in the southwestern United States, where the members of the group are thought to have originated (Pinkava 2002). Diploid members of the complex (e.g., *O. polyacantha* Haw. var. arenaria (Engelm.) Parfitt and *O. polyacantha* Haw. var. polyacantha) are restricted to northern Chihuahua, Mexico, adjacent southwestern Texas, and the southern fourth of New Mexico, while polyploid members of the clade are found mostly to the north of those diploid populations, as far north as Canada for tetraploid *O. polyacantha* and hexaploid *O. fragilis* (Bowden 1945; Parfitt 1991; Pinkava 2002).

Relatively few counts have been reported for *O. fragilis* (Bowden 1945; Pinkava et al. 1977; Löve and Löve 1982; Parfitt 1991), covering a very minor portion of the known range of the species from Arizona to Utah in the United States, with a single count from Manitoba, Canada. All counts for the species thus far have been hexaploid, so Parfitt (1991) considered the species to be an ancient polyploid derived from a common ancestor of the *O. polyacantha* complex, since no other taxa within the complex share the same morphological features of *O. fragilis*. It is widely believed that the facile disarticulation of the cladodes in *O. fragilis* has allowed the species to disperse over such a broad distribution by attaching to migrating bison, for instance, or other large migratory mammals (Ribbens 2008). This mode of dispersal is further corroborated by the usually low frequency of sexual reproduction reported for the species in northern populations (Burger and Louda 1995; Staniforth and Frego 2000; Ribbens 2008; Ribbens et al. 2011). No ploidy determinations, however, have been recorded from the Midwestern United States, where *O. fragilis* is known from numerous localities and represents the majority of the northeasternmost limit of the known distribution of the species (Benson 1982; Ribbens 2008). Thus chromosome counts could be useful to test further the "out-of- the-southwest migration hypothesis," since populations in the Midwest should be expected to be more or less clones of Southwestern-Western populations that were dispersed over the Great Plains and into the upper reaches of the Midwest via large migratory mammals.

Environmental factors affecting the past and present distribution of *O. fragilis* have not been studied in detail on a large scale, although there have been numerous suggestions as to why the species inhabits such a broad distribution and what environmental constraints may be advantageous or disadvantageous to the continued survival of the species in a given area on a regional scale (Frego and Staniforth 1985; Loik and Nobel 1993, Burger and Louda 1995; Staniforth and Frego 2000; Bennett et al. 2003; Ribbens 2008; Ribbens et al. 2011). These studies have been based mostly on local edaphic factors (Frego and Staniforth 1985), competitive interactions with other vegetation and cactophagous insects (Burger and Louda 1995), and even increased reproductive potential in association with lichens and lycophytes (Bennett et al. 2003). So it is very clear that local site characteristics such as substrate type help to drive *O. fragilis* population structure and are extremely important in determining suitable potential habitat for the species (Frego and Staniforth 1985; Ribbens 2008; Ribbens et al. 2011).

Large-scale habitat niche modeling has proven worthwhile, when the proper environmental variables are taken into account (Elith et al. 2011), for the predictions of suitable habitat for a number of rare and endangered species (Williams et al. 2009) or taxa with very few occurrence records (Pearson et al. 2007), as well as for widespread taxa (Saupe et al. 2011), and comparing worldwide climatic niche similarities for comparison with biogeographically disjunct taxa (Smith and Donoghue 2010). Niche modeling methods may prove useful for testing the environmental limits of a species like *O. fragilis*, which has a very broad but restricted distribution, when considered alongside other species of *Opuntia*. For instance, *O. humifusa* s.l. covers a very broad range throughout the eastern United States; however, *O. fragilis* is only naturally found as far south and east as northwestern Illinois in Jo Daviess County. Are dispersal or broad scale environmental factors limiting the success of the species in the eastern United States? Both of these factors have been proposed as explanations for the range of *O. fragilis* (see Ribbens 2008).

Opuntia humifusa (Raf.) Raf. s.l. has been studied cytologically only in the eastern and southeastern United States (Bowden 1945; Doyle 1990; Baker et al. 2009a, 2009b; Majure unpublished data), where both diploids and tetraploids have been found. *Opuntia macrorhiza* Engelm. s.l. has been studied extensively in the southwestern United States (Pinkava and McLeod 1971; Pinkava et al. 1973, 1992, 1998; Powell and Weedin 2001, 2004) and moderately so in the lower Midwestern United States (Kansas and Missouri; Doyle 1990; Pinkava et al. 1998), where it has been reported exclusively as tetraploid.

Circumscription of both *O. humifusa* sand *O. macrorhiza* has been the subject of much contention (Griffiths 1916; Benson 1969; Benson 1982; Voss 1985; Doyle 1990; Majure and Ervin 2008; Majure 2010; Majure unpublished data), and numerous morphotypes have been recognized as different species (Engelmann 1850, 1856; Britton and Rose 1920) or varieties (Weniger 1969; Benson 1982), many of which are now in synonymy with either *O. humifusa* s.l. or *O. macrorhiza* s.l. (Benson 1982; Anderson 2001). The incongruence in species delimitation is the result of presumed reticulate evolution, oftentimes followed by polyploidization, high morphological diversity and morphological plasticity, as well as poor representation of *Opuntia* in herbaria (Rebman and Pinkava 2001; Powell and Weedin 2004; Majure and Ervin 2008; Majure unpublished data).

The current circumscription of *Opuntia humifusa* s.l. encompasses a broad-ranging taxon found throughout the eastern United States, the Midwest, and parts of the southwestern US (Benson 1982; Pinkava 2003; Powell and Weedin 2004). *Opuntia macrorhiza* s.l. consists of a species suggested to be distributed throughout the southwestern, the Midwestern, and parts of the southcentral United States (Arkansas) by some researchers (Benson 1982; Powell and Weedin 2004), while others consider the distribution to barely reach the Midwest (Kansas, Missouri), and consider most Midwestern *Opuntia* populations to be synonymous with *O. humifusa* (e.g., Pinkava 2003). Intermediate morphological characters of both species are observed in many areas of their distribution, which further complicates delimitation of the two species and any morphologically cryptic species that may need to be recognized as segregates of the two taxa (Majure unpublished data).

The circumscription of *O. humifusa* and species limits in the *Humifusa* clade (sensu Majure unpublished data) are being investigated to test whether or not some taxa under synonymy with the species may deserve recognition at the species level (Majure and Ervin 2008; Majure 2010; Majure unpublished data.). Determining ploidy may provide further evidence for species boundaries (Pinkava 2002), which will aid in the delimitation of species in the clade and could illuminate potential introgression among taxa, helping to explain the oftentimes morphologically ambiguous nature of species boundaries in the group. Understanding ploidy also is useful in phylogenetic reconstruction, where problematic taxa may be the result of hybridization with subsequent genome duplication (Soltis et al. 2008).

We report the first counts of *O. fragilis* for populations in Illinois, Iowa, Michigan, Minnesota and Wisconsin, the first counts of *O. humifusa* s.l. from Indiana, Michigan, Missouri, Ohio, and Wisconsin, and the first counts of *O. macrorhiza* s.l. from Iowa and Minnesota. *Opuntia fragilis* is considered rare and endangered in Iowa, Illinois, Michigan, and Wisconsin, while *O. humifusa* s.l. is endangered in Wisconsin, and *O. macrorhiza* s.l. is endangered in Iowa. Knowing the ploidy of these taxa may be beneficial to conservationists in determining how best to manage these declining populations, and ploidy determinations are also complementary to ongoing research regarding the genetic diversity and ecology of *O. fragilis* in the Midwest (Ribbens 2008; Ribbens et al. 2011), and the phylogenetic relationships and species boundaries of taxa in the *Humifusa* clade (see Majure 2010; Majure unpubl. data). Throughout this study we use *O. humifusa* s.l. and *O. macrorhiza* s.l. to delimit Midwestern populations, since species limits in the *Humifusa* clade are not yet completely resolved (Majure unpublished data). We also present results from ecological niche modeling on a small sample (relative to the entire distribution of the species) of *O. fragilis* populations from the upper Midwestern United States and discuss the potential reasons for absence of *O. fragilis* in the eastern United States.

Materials and Methods

Chromosome counts. Root tips were collected from early morning through early afternoon from vegetative propagules planted in the University of Florida, Department of Biology greenhouse. Previous researchers have found that growth rates in root tips are higher during early morning to afternoon hours (i.e., mitoses are more frequent; Remski 1954), and we noticed decreased starch grain production in roots collected in the morning versus those collected later in the day. Root tips were placed in 2-mM 8-hydroxyquinoline (sensu Soltis 1980) for up to eight hours at 4 °C or in nitrous oxide (N2O) for one hour (Kato 1999) and then fixed in a 3:1 absolute ethanol-glacial acetic acid solution for two to twenty-four hours. Root tips then were placed in 70% ethanol for at least 2 hours, then digested in 40% HCl for 5–10 minutes (depending on the size of the root) at room temperature and placed back into 70% ethanol at 4°C until use. Root tips, rinsed in distilled water after each treatment, were squashed in 60% acetic acid, stained with 1% aceto-orcein dye, and then viewed on a Zeiss Photomicroscope III (Carl Zeiss, Oberkochen, Germany).

At least five mitoses were counted per specimen to insure repeatability and confidence in the number of chromosomes counted, since endomitosis in root cells has been reported in the allopolyploid taxon, *Opuntia spinosibacca* (Weedin and Powell 1978), tetraploid *O. humifusa*, and tetraploid *O. pusilla* (Bowden 1945). Occasionally, more than one root per specimen was also counted. Specimens used in this study are listed in Appendix 1, and the geographical distribution of collections is given in Fig. 1. A total of 40 populations were analyzed, including 12 *O. fragilis*, 17 *O. humifusa* s.l., and 11 *O. macrorhiza* s.l. populations.

Habitat niche modeling. We used Maxent (Phillips et al. 2006) to build predictive models of the distribution of *O. fragilis* and to determine factors affecting habitat suitability for the species. We were particularly interested in broad scale environmental factors affecting the distribution of *O. fragilis* and not local microhabitat variables. Therefore, our models are based on nineteen environmental variables (http://www.worldclim.org/bioclim), which are monthly worldwide climate data (Hijmans et al. 2005), and 105 presence points for *O. fragilis* from the Midwestern United States (see Ribbens 2008; Ribbens and Anderson 2009; Ribbens and Geisler 2009; Ribbens and Flores 2009; Ribbens et al. 2010), which represent all known populations of the species from the five states sampled (Iowa, Illinois, Michigan, Minnesota and Wisconsin). We modeled the presence of *O. fragilis* projected to virtually all of North America at a 30 arc-second or approximately 1 km^2 scale resolution raster data set using 25% of the original presence points as test data to better constrain those variables most correlated with the actual distribution of the species. Thus, 75% of the actual data were used as training data for testing the model (Phillips et al. 2006; Phillips and Dudik 2008). Only 100 of the 105 original points were used for modeling, since only one presence point is used for the highest scale of resolution provided
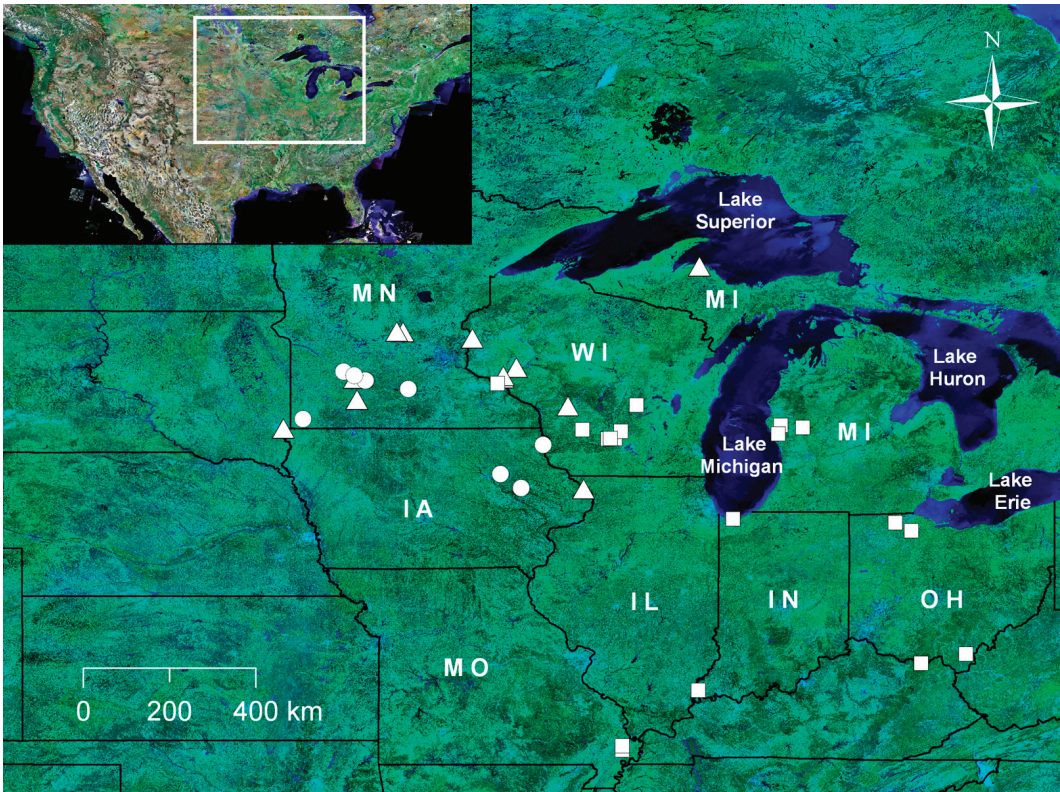
Figure 1. Map of the distribution of the *O. fragilis* (triangles), *O. humifusa* s.l. (squares), and *O. macrorhiza* s.l. (circles) populations analyzed in this study. The insert shows the study area in relation to the rest of the United States.

(i.e., several data points were located within 1 km^2 of other presence points). Maxent uses only presence data to model habitat suitability (Phillips et al. 2006). Jackknife statistics (i.e., data sampling without replacement) were used to calculate the overall effect that each climatic variable had (by itself) on the distribution of *O. fragilis*, and this was evaluated further by comparing the mean output values from Maxent of the percent contribution from each climatic variable over ten replicates.

Results

Chromosome counts. *Opuntia fragilis* was found to be hexaploid (Fig. 2A) in all twelve Midwestern populations surveyed, which is consistent with all other cytological investigations of the species (Bowden 1945; Pinkava et al. 1977; Löve and Löve 1982; Parfitt 1991). *Opuntia humifusa* s.l. and *O. macrorhiza* s.l. were tetraploid in all of the populations studied (Fig. 2B). No obvious endomitotic cells were recorded during evaluation of chromosome number in any of the three taxa. Chromosomes of all three taxa were small and mostly homogeneous in form, ranging from 3 to 5.5 μm in length and typically metacentric (Fig. 2).

Habitat niche modeling. Results from Maxent habitat niche modeling for *O. fragilis* suggest that large fluctuations in temperature and minimal fluctuations in precipitation play a major role in the distribution of the species. According to our jackknife results, seasonal

temperature change (bio4) is the most important variable to determine suitable habitat for *O. fragilis*, when analyzed alone (jackknife = 96%), resulting in a positive correlation with predicting the distribution of *O. fragilis* (i.e., as temperature seasonality increases, the probability of encountering suitable habitat increases). Annual mean temperature (bio1) and temperature of the driest quarter of the year (bio9) constitute the highest percent contribution for describing habitat suitability (36.35% and 21.46% contribution, respectively), as well as precipitation seasonality (14.17% contribution), of which there is a negative effect with predicting the distribution of the species (i.e., as precipitation seasonality increases, habitat suitability decreases). Precipitation of the warmest quarter (bio18) of the year has the least effect of all variables when analyzed alone based on jackknife results (85%), but has a moderate effect on species distribution when analyzed with the other variables (6.67% contribution). Taken altogether, the 19 climatic variables predict a distribution of suitable habitat for *O. fragilis* that is strikingly accurate (Fig. 3), at least for the upper Midwestern and western United States. For instance, there is a strong prediction for suitable habitat in areas of Colorado, Utah, Wyoming and Nebraska where *O. fragilis* is actually known, even though no data points from those locations were used in our analyses. There are strong predictions of suitable habitat for the species in eastern Ontario as well, where the species has been recorded (Consaul et al. 1998). The
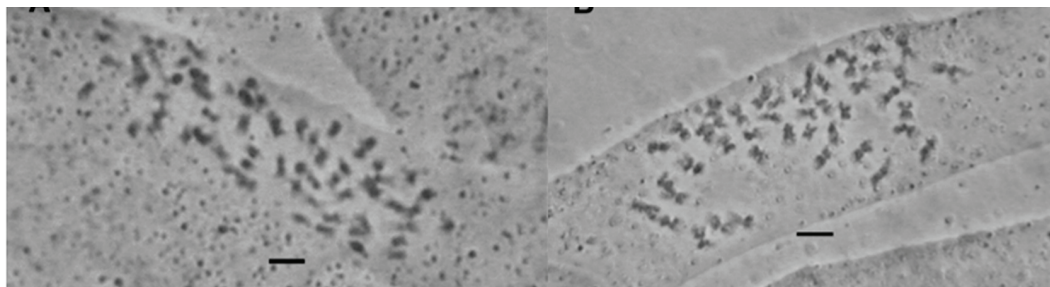
Figure 2. Chromosome squashes of A) *O. fragilis* from E. Ribbens s.n., Marquette Co., MI, and B) *O. humifusa* from L.C. Majure 3274, Porter Co., IN. Scale = 5 µm.

northeastern United States also includes suitable habitat, although the species has never been recorded there (Fig. 3; see discussion).

Discussion

The *O. polyacantha* complex is suggested to have originated in the Chihuahuan Desert (Pinkava 2002) of the southwestern United States and adjacent Mexico, and hexaploid *O. fragilis* was likely derived from lower-ploid members of that clade only (Parfitt 1991; Majure et al. unpublished data). Therefore, it is most probable that *O. fragilis* originated in the southwestern United States and then expanded to its current distribution. Hexaploid counts of the Midwestern *O. fragilis* material add further evidence for this dispersal scenario, since all western populations of *O. fragilis* thus far studied are hexaploid as well (see Parfitt 1991). Also, the paucity of sexual reproduction in the species (Ribbens 2008; Ribbens et al. 2011) makes vegetative dispersal and propagation of *O. fragilis* imperative for the species to have become so broadly distributed throughout its current range.

Cladode disarticulation is a novel feature demonstrated only by *O. fragilis* in the *O. polyacantha* complex, and this very effective method of vegetative dispersal is not a common feature throughout *Opuntia* s.s. in general. As well, very few of those taxa demonstrating easily disarticulating cladodes show distributional success comparable to that of *O. fragilis*. For example, *O. pubescens* Wendl. ex Pfeiff. ranges widely from Mexico to Argentina (Anderson 2001). However, *O. pusilla* (Haw.) Haw. is restricted to the southeastern United States (Benson 1982; Pinkava 2003), and *O. repens* Bello and *O. triacantha* (Willd.) Sweet are restricted to a few islands of the Greater and Lesser Antilles (Anderson 2001). Species with low sexual productivity, such as *O. fragilis*, however, may effectively disperse, given an efficient mode of transport, and thus significantly increase their ranges, through vegetative apomixis (Rebman and Pinkava 2001).

Opuntia fragilis is one of the most widespread species of *Opuntia* in North America and is well known for being the northernmost distributed species of *Opuntia* (Britton and Rose 1920; Benson 1982; Parfitt 1991; Anderson 2001). It is widely believed that this species occupies such a large distribution due to its easily disarticulating cladodes and retrorsely barbed spines that allow

free cladodes to latch on to the fur of grazing animals, which later disperse the vegetative propagules (Britton and Rose 1920; Benson 1982; Ribbens 2008). Vegetative dispersal and favorable environmental conditions could explain the distribution of *O. fragilis* throughout the Great Plains and most of the Midwest, where large herds of buffalo, *Bison bison* L., once roamed (Meagher 1986). Likewise, the oldest fossils of *B. bison* that have been found in North America were determined to be late Pleistocene-early Holocene in age (McDonald 1981; Meagher 1986), which correlates with the end of the last glacial maximum and increasing available habitat northward for *O. fragilis* that would have not been available before that time.

Previous counts of *O. humifusa* s.l. have been either diploid or tetraploid (Bowden 1945; Doyle 1990; Baker et al. 2009a, 2009b), although diploid individuals have been discovered only in the southern portion of the range of the species (Baker et al. 2009a, 2009b; Majure unpublished data), and only tetraploids have been found in northern populations studied so far (Bowden 1945; Doyle 1990; Majure unpublished data), consistent with our study. Likewise, only tetraploids have been found in the species *O. macrorhiza* s.l. in the southwestern United States and other parts of the range of that species (Pinkava and McLeod 1971; Pinkava et al. 1973, 1992, 1998; Powell and Weedin 2001, 2004). Our counts of *O. macrorhiza* from the Midwest confirm the tetraploid nature of that species in its northernmost range, as well.

Our ecological niche modeling for *O. fragilis* suggests that suitable habitat for the brittle prickly pear is mostly predicted by large seasonal shifts in temperature and very small shifts in seasonal precipitation. Thus, the majority of the eastern United States may not provide suitable habitat for the species, due to more heterogeneous precipitation patterns and more homogeneous temperatures throughout the year, especially progressing southward. Data from Ribbens et al. (2011) suggest that the southeasternmost population of *O. fragilis* in Jo Daviess Co., Illinois, is negatively affected by increasing precipitation in that area, further adding evidence for our modeling results regarding precipitation patterns. Granted, our data are also limited to climatic variables, and taking into account edaphic factors along with biological data at a population scale would be even more informative for building a predictive niche model for the species, since substrate, among other mi-
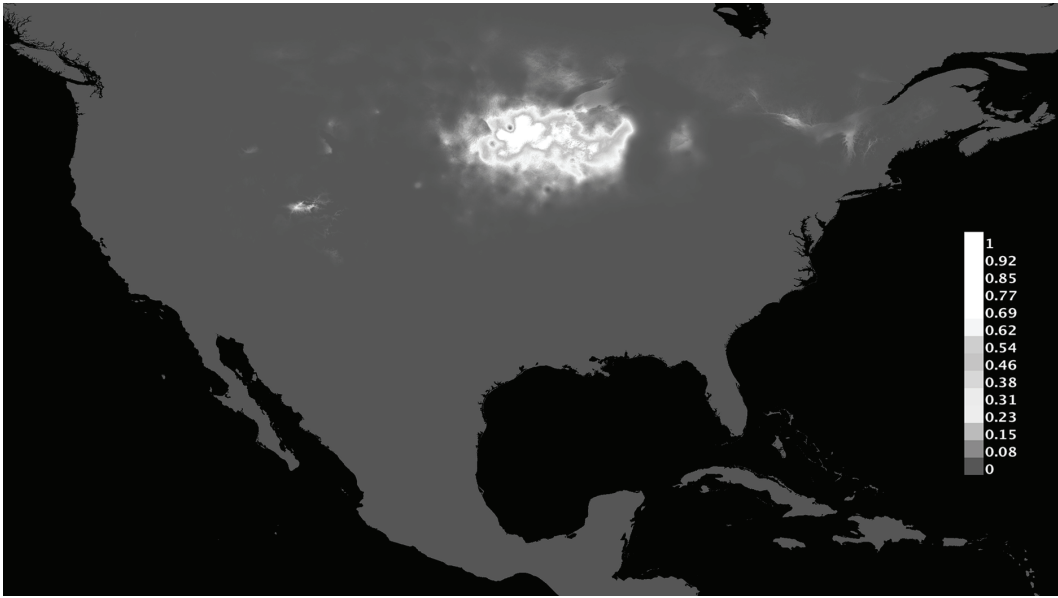
Figure 3. Maxent predicted habitat suitability model for *O. fragilis* (from logistic output). The scale bar denotes the percent probability of habitat suitability with 1 (100 %) equal to the maximum probability of habitat suitability for *O. fragilis* and 0 (0 %) equal to the minimum probability of habitat suitability for the species. Note that the maximum probability of habitat suitability is located in the point-dense area of the Midwest used for model testing, which may indicate slight model bias from sampling bias (Phillips et al. 2006). However, other parts of the known range of *O. fragilis* are also predicted with high probabilities, although no points were used as testing or training data for those areas (e.g., Colorado, Utah, Wyoming). This suggests that the model parameters and environmental variables used for our analyses may be relatively good predictors of habitat suitability for this species.

crohabitat variables, is known to affect the distribution of *O. fragilis* (Ribbens 2008; Ribbens et al. 2011). Our data points are also limited to only upper Midwestern populations, which may bias results from the predictive models (Phillips et al. 2006), so including the full geographic range of the species would possibly expand the possibilities for discovering other niches where *O. fragilis* could potentially occur. Our data are robust, however, in that we were able to extrapolate suitable areas from other parts of the distribution of *O. fragilis* based on just those data points used from the Midwest, so our results make biological sense regarding parts of the true distribution of the species. The predicted suitable habitat revealed in eastern North America east of Michigan is interesting, since *O. fragilis* has been recorded near Ottawa, Canada (Consaul et al. 1998), although the species is considered introduced at that site (Staniforth and Frego 2000). Areas further east from the Ottawa site in the northeastern United States were also revealed to contain suitable habitat for the species (Fig. 3), but *O. fragilis* has never been recorded that far to the east. Those northeastern areas would roughly correspond to the Adirondack Mountains of New York and the Appalachian Mountains of New Hampshire and Maine (i.e., Long Fellow Mountains of Maine). It is possible, however, that the species was never dispersed to those areas, merely has not been discovered, or has since gone extinct in those areas. *Bison bison* did occur in the northeastern United States (Meagher 1986), so if buffalo were the main dispersers of *O. fragilis*, then they likely would have introduced the brittle prickly into

the northeastern United States, as well as the Midwest. There have been reports of a small prickly pear seen in Maine (D. Steadman, FLMNH, pers. comm.). No voucher specimens were gathered at the time of observation, however, so this has not been confirmed. Could *O. fragilis* exist in Maine?

Acknowledgments

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Appendix 1.

Chromosome counts of *O. fragilis*, *O. humifusa,* and *O. macrorhiza*. All voucher specimens collected by E. Ribbens are deposited in the R.M. Meyers Herbarium (MWI), Western Illinois University, and those collected by L.C. Majure are deposited at the University of Florida Herbarium (FLAS) unless otherwise noted. Only county level locality data are given here, since these taxa are considered rare and endangered throughout most of their upper Midwestern range. Chromosome counts were determined by L.C. Majure.

Opuntia fragilis (Nutt.) Haw. — 2n=66; **Illinois**, Jo Daviess Co., *E.Ribbens s.n.*; **Iowa**, Lyon Co., *E.Ribbens s.n.*; **Michigan**, Marquette Co., *E.Ribbens s.n.* Fig, 2a*;* **Minnesota**, Cottonwood Co., *E.Ribbens s.n.,* Redwood Co., *E.Ribbens s.n.*, Sherbourne Co., *E.Ribbens s.n.*, Stearns Co., *E.Ribbens s.n.;* **Wisconsin**, Buffalo Co., *E.Ribbens s.n.*, Dunn Co., *E.Ribbens s.n.*, Monroe Co., *E.Ribbens s.n.*, Pepin Co., *E.Ribbens s.n.*, Polk Co., *E.Ribbens s.n.*

Opuntia humifusa (Raf.) Raf. (s.l.) — 2n=44; **Indiana**, Porter Co., *L.C. Majure 3274* Fig. 2B, Posey Co., *E. Ribbens s.n.;* **Michigan,** Muskegon Co., *L.C. Majure 3259*, Newago Co., *L.C. Majure 3261*, Oceana Co., *L.C. Majure 3262*; **Missouri,** New Madrid Co., *L.C. Majure 2435* (MISSA), Scott Co., *L.C. Majure 2441*(MISSA); **Ohio,** Adams Co., *L.C. Majure 3251*, Gallia Co., *L.C. Majure 3252*, Lucas Co., *L.C. Majure 3254*, Woods Co., *L.C. Majure 3253*; **Wisconsin**, Columbia Co., *E. Ribbens s.n.,* Columbia Co., *E.Ribbens s.n.,* Marquette Co., *E.Ribbens s.n.,* Richland Co., *L.C. Majure 3273*, Sauk Co., *E.Ribbens s.n.,* Sauk Co., *E.Ribbens s.n.*

Opuntia macrorhiza Engelm. (s.l.) — 2n=44; **Iowa,** Allamackee Co., *E.Ribbens s.n.,* Buchanan Co., *E.Ribbens s.n.,* Linn Co., *E.Ribbens s.n.;* **Minnesota,** Brown Co., *E.Ribbens s.n.,* Nicollet Co., *E.Ribbens s.n.,* Pepin Co., *E.Ribbens s.n.,* Redwood Co., *E.Ribbens s.n.,* Redwood Co., *E.Ribbens s.n.,* Redwood Co., *E.Ribbens s.n.,* Renville Co., *E.Ribbens s.n.,* Rock Co., *E.Ribbens, s.n.*