Climate, soils, and connectivity predict plague epizootics in black-tailed prairie dogs (*Cynomys ludovicianus*)

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Abstract. Outbreaks of plague in wildlife are sporadic and spatially dispersed, and they depend on coincidence of susceptible hosts, flea vectors, the plague bacterium (*Yersinia pestis*), and environmental factors that support pathogen transmission. We fit spatial models of plague outbreaks to a long-term data set (1981–2005) of towns of black-tailed prairie dogs (*Cynomys ludovicianus*) on the shortgrass steppe of northeastern Colorado. We investigated the effects of spatial distribution (town area and connectivity to other prairie dog towns), climate (spring and summer precipitation and temperature), and soil moisture-holding capacity. In logistic regression models, plague epizootics were predicted by connectivity to other towns experiencing plague during periods with relatively low temperatures, in soils with high moisture-holding capacity. After accounting for connectivity between prairie dog towns and current-year climatic conditions, little additional spatial or temporal autocorrelation was detected. Spatial logit association models provided evidence for localized epizootic hotspots and that greater summer rainfall predicted plague events. Plague outbreaks were not predicted by precipitation in the previous year. As such, no evidence was found to support an indirect cascade model of plague outbreaks for black-tailed prairie dogs on the shortgrass steppe in Colorado. Instead, the models suggest that plague occurrence depends upon direct climatic effects on flea vectors and the plague pathogen.

Key words: black-tailed prairie dog; Cynomys ludovicianus; *disease ecology; generalized linear models; spatial autocorrelation; temporal autocorrelation; vector-borne disease;* Yersinia pestis.

Introduction

Plague, a disease caused by the bacterium *Yersinia pestis*, sporadically causes large-scale die-offs in wild rodents and occasionally spills over to other mammals. This includes current human cases and historic pandemics like the medieval Black Death in Eurasia (Gage and Kosoy 2005). Plague is highly virulent in many mammals, and is transmitted both by fleas and direct contact with infected individuals. After its introduction into North America in the late 1800s, plague spread to infect many ground-dwelling rodent species in the western United States and Canada. Black-tailed prairie dogs (*Cynomys ludovicianus*) are diurnal, ground-dwelling, colonial sciurids that live in discrete towns in short-grass and mixed-grass prairies on the Great Plains east of the Rocky Mountains. They were first reported to be plague-infected in the 1940s and provide an example of plague's sporadic nature (Cully et al. 2000, Antolin et al. 2002). Plague epizootics within prairie dog towns result in >95% reduction of populations (Cully and Williams

2001, Stapp et al. 2004) and have converted black-tailed prairie dog populations into classic metapopulations with regular town extinctions and recolonization (Antolin et al. 2006, Augustine et al. 2008). Rapid die-offs in prairie dogs are thought to be facilitated by their social habits, including living in densely populated towns, frequent contact between individuals, and the sharing of burrows where conditions are favorable for flea-borne transmission (Hoogland 1979, Gage and Kosoy 2005).

Both large-scale and local climatic variability provides a partial explanation for the sporadic spatial and temporal patterns of plague outbreaks. In the semiarid and arid western United States, human plague cases (Enscore et al. 2002, Ben Ari et al. 2010) and die-offs of black-tailed prairie dogs are more frequent during years with warmer, wetter springs and cooler summers (Stapp et al. 2004, Snall et al. 2008). In years of drought, which frequently occur in this region, plague outbreaks are uncommon. Similarly, temperature and rainfall variation predicts plague outbreaks in gerbils in Kazakhstan (Stenseth et al. 2006) and human plague cases in Vietnam (Cavanaugh and Marshall 1972).

Associations between climate and plague outbreaks lead to alternative hypotheses explaining reemergence of plague from low background levels in the semiarid ecosystems where it occurs. The first is a cascade model,

in which precipitation increases primary productivity and subsequent growth of rodent populations one to two years later. Higher rodent survival increases overall flea populations, and milder weather leads to temperature-related pathogen survival, resulting in epizootics in rodents and increases in human exposure (Enscore et al. 2002, Collinge et al. 2005b, Ben Ari et al. 2010). This lag between increased precipitation and disease outbreaks has also been implicated in Sin Nombre virus prevalence in deer mice linked to outbreaks of hantavirus pulmonary syndrome in humans in the southwestern United States (Yates et al. 2002). The second hypothesis does not require lags and cascades. Instead, plague transmission may increase as a direct result of climatic effects on *Y. pestis* and fleas. High temperature increases clearance of *Y. pestis* infections from fleas and lowers transmission (Gage and Kosoy 2005), while warm early springs and cool moist summers promote flea survival and reproduction in both seasons, setting up favorable conditions for die-offs within that year (Stenseth et al. 2006). Rodent abundance that exceeds density thresholds and promotes transmission may also increase plague risk (Davis et al. 2004, Salkeld et al. 2010), but direct links between climate, ecosystem productivity, rodent abundance, and plague outbreaks have not been made (Kausrud et al. 2007).

Plague epizootics often occur in clusters of prairie dog towns with short inter-town distances (Cully and Williams 2001, Girard et al. 2004, Stapp et al. 2004, Collinge et al. 2005a), but epizootics can be widely spaced in a region within the same season (Girard et al. 2004, Stapp et al. 2004). Routes of transmission between towns are not well understood, but in our study area in northeastern Colorado the generalist flea *Pulex simulans* infests both prairie dogs (Tripp et al. 2009) and wide-ranging carnivores such as swift fox (*Vulpes velox*). Swift foxes show evidence of exposure to *Y. pestis* during outbreaks in prairie dogs (Salkeld et al. 2007). This co-occurrence suggests a role for carnivores in transmitting plague between towns (Salkeld et al. 2007). Additionally, multiple studies report that large towns are more likely to experience plague die-offs (Stapp et al. 2004, Collinge et al. 2005a), and plague is the only known natural cause of large town extinctions in this area (Barnes 1993, Cully and Williams 2001). As a consequence of the metapopulation dynamics of prairie dog towns, with regular episodes of extinction and recolonization (Roach et al. 2001, Stapp et al. 2004), the area and connectivity of towns changes from year to year and may influence the likelihood of plague epizootics.

The clustering of prairie dog towns and plague epizootics complicates statistical analyses of the data. While prairie dog towns have discrete boundaries, the possibility remains that plague risk is not statistically independent among towns. Spatial autocorrelation occurs when closely spaced sampling points are more similar to each other than distantly spaced ones, for example, when towns with small inter-town distances all undergo epizootics in the same year. Similarly, temporal autocorrelation occurs when sampling points in time series are influenced by the state of the same points at previous times, for example, if precipitation in previous years influences current plague risk. Both types of autocorrelation violate standard assumptions in linear models, with overestimates of degrees of freedom and underestimates of error (Burton et al. 1998). In this study, spatial and temporal autocorrelation were examined by three extensions of logistic regression models. First, yearly spatial and temporal autocorrelation was assessed by spatial logit association models, with observations clustered spatially or temporally assuming that towns close together are alike or are autocorrelated through time. Second, we tested for specific localized hot or cool spots of extinction using spatial logit association models by adding spatial components as independent variables. Third, autoregressive (AR) models predicting the proportion of towns going extinct in a given year were tested for temporal autocorrelation. We analyzed a 25-year data set (1981–2005) of black-tailed prairie dog towns that includes town area, location, and die-offs.

The analysis sheds light on alternative hypotheses for the risk of plague outbreaks—temporal cascades functioning through the ecosystem vs. direct climatic effects on *Y. pestis* and flea vectors. Because soil characteristics and moisture have important ecological implications in this semiarid grassland (Sala et al. 1992), we also examined moisture-holding capacity of soils. Soil moisture affects flea development and survival in the underground burrow environment (Krasnov 2008). We tested the effects of current and lagged spring and summer precipitation, current spring and summer temperature, soil moisture-holding capacity, and spatial factors (town area and connectivity to other prairie dog towns). Models were parameterized using data from 1981 to 2003 and validated with data from 2004 to 2005.

Materials and Methods

Study site

The study site (Fig. 1) is in Weld County, Colorado, USA (elevation 1280–1950 m) on the Pawnee National Grassland (PNG) administered by the U.S. Forest Service, with ~80 000 ha of publicly owned land embedded within a checkerboard of lands under federal, state, and private ownership. The PNG is divided into eastern and western units in different drainage systems separated by a 16 km wide strip of private land. A greater portion of the western unit is contiguous federal grassland (46%) than the eastern unit (18%). Additionally, the northwestern corner of the study area includes the Central Plains Experimental Range (CPER), established in 1939 and administered by the U.S. Department of Agriculture/Agricultural Research Service to study the effects of grazing on the shortgrass steppe. Both the PNG and CPER are research areas of the Shortgrass Steppe Long Term Ecological Research (SGS-LTER) project at Colorado State University.
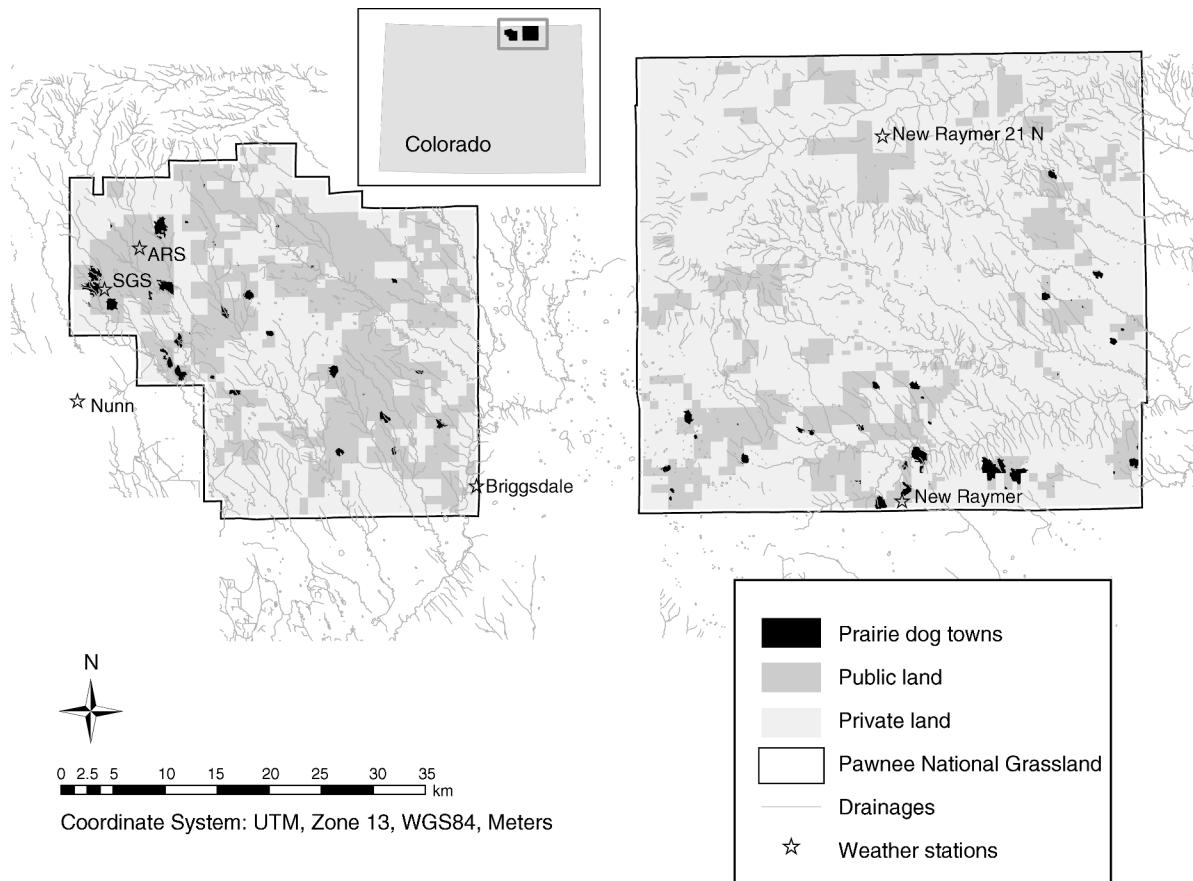
FIG. 1. Largest recorded area of black-tailed prairie dog (*Cynomys ludovicianus*) towns (1981–2005) in relation to weather stations, drainages, and land ownership on the Pawnee National Grassland, Colorado, USA. Prairie dogs probably use drainage networks to move between towns.

The study site is within the shortgrass steppe ecosystem, with 20–50 cm of annual precipitation (34 cm average) falling primarily between April and September. Vegetation is characterized by low-stature plants that tolerate grazing and drought, and is dominated by the C_4 grasses blue grama (*Bouteloua gracilis*) and buffalo grass (*Buchloe dactyloides*), which contribute 60–80% of the plant cover, biomass, and net primary productivity (Lauenroth and Burke 2008). Prairie dog towns on the shortgrass steppe are located within major dry stream channels, have population densities of 25–28 prairie dogs/ha (Koford 1958, McClintock et al. 2009), and are patchily distributed because plague outbreaks generate metapopulation dynamics (Roach et al. 2001).

Prairie dog surveys and mapping

Over the course of the study, 75 prairie dog towns were surveyed by the U.S. Forest Service on the PNG. Beginning in 1995, nine additional towns on the CPER were surveyed by the SGS-LTER after prairie dogs colonized the area. Surveys confirmed whether towns were active or extinct. In the late summer or fall of each

year, extant towns were mapped using the outermost active burrows to determine town area and geographic coordinates of town centroids. Burrows were considered active if they were cleared of vegetation and cobwebs, and had signs of fresh digging and/or fresh scat. From 1981 to 1994 towns were mapped relative to ground control landmarks. Maps were digitized and scaled relative to the ground control points and incorporated into a geographic information system (GIS) using ArcGIS Desktop 9.1 (Environmental Systems Research Institute 2005). After 1995, the coordinates of landmarks and towns were measured using the global positioning system (GPS). All GIS layers used Universal Transverse Mercator projection, Zone 13, datum WGS84, in meters. In a small number of cases town areas were not recorded, especially in the first year or two after colonization was noted. For these cases, town size was estimated from the relationship between town size and age (in years) determined from a town area growth function fitted to the data from all towns (Savage 2007).

Prairie dogs use low-lying drainages as dispersal corridors between towns rather than moving in straight

lines (Garrett and Franklin 1988, Roach et al. 2001). Drainage distance between towns was determined by measuring the length of lowest elevation paths between town centroids, using United States Geological Survey (USGS) hydrography digital line graphs (1:24 000; finer resolution of 1:10 000 when elevation lines were too subtle to be seen at 1:24 000).

Town extinctions and epizootics

In this study, a die-off refers to a dramatic decline in area of a black-tailed prairie dog town, while the term epizootic refers to a verified outbreak of plague. Starting in 2003, when funds for testing were secured, the nine die-offs between 2003 and 2005 were verified to be plague epizootics by detecting the plague bacterium in carcasses or fleas (bacteriologic and serologic testing courtesy of the Center for Disease Control, Fort Collins, Colorado). The term extinction is used to refer to a prairie dog town that had either no evidence of live prairie dogs immediately after its decline, or had only a few survivors. Between 1981 and 2005, 53 extinctions occurred, when towns were active in one year but inactive the next. The extinction was recorded as having occurred in the second year. In six instances, evidence of a die-off was noted during mapping, in which case the extinction was counted in that year. Three towns that had large decreases in area (average loss 86.7%) from the previous year were also counted as extinctions. Not every epizootic causes 100% mortality, but towns with only few surviving prairie dogs were recorded as extinctions.

We are confident that large town extinctions during the 1981–2005 surveys were caused by plague. Black-tailed prairie dogs are highly susceptible to plague, with mortality rates of >95% during plague epizootics (Cully and Williams 2001, Pauli et al. 2006), and plague is the only disease known to cause town extinctions over short time periods (Barnes 1993). While larger towns have a higher probability of experiencing plague epizootics (Cully and Williams 2001, Lomolino and Smith 2001, Collinge et al. 2005a), plague can eradicate both small and large towns (Roach et al. 2001, Stapp et al. 2004). Another cause of town die-offs, poisoning, was banned on the PNG in the 1970s. Die-offs of small towns caused by unauthorized poisoning, predation, demographic stochasticity, or other factors may occur (Stapp et al. 2004), and error was reduced by excluding small town extinctions where poisoning or predation was noted by PNG staff (six instances, average town area = 0.46 ha).

Independent variables

Prairie dog town area and connectivity.—Town area was measured as the minimum convex polygon of the outermost active burrows of each town in each year, and is used as a proxy for abundance because prairie dog density does not change dramatically between towns or between years at this site (McClintock et al. 2009). We calculated connectivity twice for each extant town

during each year, in reference to both extant towns and towns undergoing extinctions. The connectivity of each of the k prairie dog towns in each year was calculated as

$$C_j = \sum_{i=1}^{k} P_i A_i \exp(-d_{ij}), i \neq j$$

where P_i denotes active ($P_i = 1$) or extinct ($P_i = 0$) towns, A_i is the town area (ha) in that year, and d_{ij} is the distance (km) between the centroid of towns j and i (Ovaskainen and Hanski 2004). This measure takes into account that smaller towns that are near may have as large an effect as bigger towns that are farther away. Because the PNG comprises two units, separated by a 16-km strip of private land and located in different drainages (Fig. 1), and because genetic analyses of *Y. pestis* suggest different plague strains circulate within the eastern and western parts of the study area (Lowell 2007), the eastern and western units were analyzed separately. All spatial estimates of inter-town distance, including connectivity, were carried out separately for each unit.

Climate.—Daily and monthly total precipitation (mm) and daily absolute maximum air temperature (°C) were recorded at seven weather stations (Fig. 1; Climate and Hydrology Database Projects 1981–2006, National Climate Data Center 1981–2005). Two weather stations were located on the eastern part of the PNG (New Raymer and New Raymer 21 N) and five were located on or near the western unit of the PNG (SGS11, SGS12, ARS HQ, Briggsdale, and Nunn) (Fig. 1). Because stations SGS11 and SGS12 are located only 122 m apart, their data were averaged.

Average spring and summer precipitation for eastern and western units of the PNG were calculated from total rainfall from 1 March through 15 June and from 16 June through 30 September (Appendix A). Precipitation before March and after September each year is sparse, often as snow that is blown and drifts, and is thus unreliably measured. Including climatic variables from the current year or from the previous year allows testing whether plague outbreaks are consistent with cascades or by direct effects. The current year's precipitation should relate more directly to effects of moisture on plague transmission, while the previous year's precipitation should correlate with the potential of rainfall to increase food availability and thus the abundance of potential rodent hosts (e.g., Collinge et al. 2005b). The current and previous year's precipitation were not tested simultaneously in individual models because of lack of independence (and collinearity) of variables derived from the same data (Legendre and Legendre 1998), but were assessed in models testing for temporal autocorrelation.

Maximum average spring and summer temperatures were calculated for the eastern and western units of the PNG (Appendix A) using daily maximum temperatures.

Four of seven weather stations were not continuously in service, with missing data for 14.9% of observations (New Raymer, missing 1.1 yr; New Raymer 21N, 6.4 yr; SGS12, 6.3 yr; Nunn, 8.9 yr). Strong linear relationships for daily maximum temperature among stations allowed multiple imputations of missing data (e.g., Schafer 1997) implemented in S-Plus, version 6.2 (Insightful Corporation 2003).

Soils.—Hot and dry summer conditions reduce the occurrence of plague epizootics (Stapp et al. 2004, Stenseth et al. 2006), but microclimates within burrow systems buffer both ambient temperatures and loss of humidity (Wilcomb 1954). Under the dry conditions normally found in northeastern Colorado, prairie dog towns on soils with greater capacity to hold moisture may be expected to have greater plague occurrence because relative humidity influences survival of fleas (Krasnov 2008). We created soils maps of the study area using the soil horizon with the maximum 1.5 mPa (millipascals) water-holding capacity, which is the percentage (by mass) of water that soils retain in their pore spaces at 1.5 mPa of pressure. This is commonly called the wilting point of plants (USDA 2005) and measures the soil layer that will hold the most water under dry conditions (maximum soil depth surveyed was 1.52 m). We overlaid prairie dog coverage for each year on this soils map and performed zonal statistics using Spatial Analyst in ArcMap 9.1 (Environmental Systems Research Institute 2005) to find the average maximum 1.5 mPa soil moisture capacity for each prairie dog town in each year. Soil data at different depths were obtained from the Northern Weld County Soil Survey (USDA 1982).

Statistical analysis

We tested for multicollinearity between independent variables by calculating variance inflation factors (VIF), which measure the number of times the variance of a parameter estimate is raised by multicollinearity. In logistic regression, VIF values above 2.5 may be cause for concern (Allison 1999); the maximum VIF for our independent variables was 1.28.

Model selection.—Data were fit to a logistic regression implemented by generalized linear models (GLM) with binomial error terms. Model selection reported here closely agrees with an information-theoretic approach using Akaike's Information Criterion (AIC) to determine the best-fitting, most parsimonious models (Burnham and Anderson 2002). The methods and results for that analysis are in Savage (2007).

Independent variables were: connectivity to extant towns, CE; connectivity to plagued towns, CP; town area, A; 1.5 mPa soil moisture capacity, S; temperature variables, average spring temperature T_{sp}, average summer temperature T_{su}; and four precipitation variables, average spring precipitation in each plague year, P_{sp}, average summer precipitation in each plague year, P_{su}, average spring precipitation lagged one year, P_{sp-1},

and average summer precipitation lagged one year, P_{su-1}. Interactions between soils and current and lagged precipitation were also examined. Eastern vs. western units of the study area were tested separately but no main effects or interactions differed between them.

A backward stepwise model selection procedure was performed in S-Plus version 6.2 (Insightful Corporation 2003) starting with a global model. At each step, partial t tests were performed, variables and interactions with the smallest t statistic were removed from the model, and a chi-square test compared differences between larger and reduced models. Variables and interactions were eliminated until no more could be removed without significant chi-square tests ($\alpha = 0.05$). The procedure was run twice, using the current year's precipitation and the previous year's precipitation.

Generalized linear modeling (GLM).—Logistic regression was used to generate models, with town extinction from plague in each calendar year as the dependent variable. For p independent variables, the model takes the following form (Neter et al. 1996):

$$E\{Y_i\} = \pi_i = \frac{e^{\beta_0 + \beta_1 x_1 + \ldots + \beta_{p-1} x_{p-1}}}{1 + e^{\beta_0 + \beta_1 x_1 + \ldots + \beta_{p-1} x_{p-1}}}$$

where $x_1, \ldots, x_{p-1}$ are independent predictor variables and $\beta_1, \ldots, \beta_{p-1}$ are logistic coefficients. The logit transformation of the probability of town extinction from plague, π_i, produces an alternate linear form of the logistic response function:

$$\text{logit}(\pi) = \log_e(\frac{\pi}{1-\pi}) = \beta_0 + \beta_1 x_1 + \ldots + \beta_{p-1} x_{p-1}.$$

Performance of models was evaluated using receiver operating characteristic (ROC) curves commonly used for models with binary outcomes. ROC curves compare the true positive rate (x-axis: sensitivity) against the false positive rate (y-axis: 1−specificity) at threshold probabilities (Fielding and Bell 1997). The closer the curve is to the upper left corner of the graph, the higher the discrimination accuracy of the model. The area under the curve (AUC) measures overall model accuracy and is independent of the probability threshold that, in this study, determines whether a town has gone extinct from plague. The AUC measures range from 0.5, indicating accuracy no better than chance, to 1.0 for 100% accuracy.

To further evaluate model performance we reserved two years of data (2004–2005) to test GLM models parameterized from 1981 to 2003 data. We computed a threshold-dependent measure of model performance, total model accuracy, which is the proportion of the sample correctly predicted by the model at the decision threshold. The point that maximizes the true positive rate and minimizes the false positive rate was used as the cutoff probability. Model accuracy was calculated for the 1981–2003 "training set" of the data, as well as for the 2004–2005 data reserved for model validation.

TABLE 1. Model coefficients, standard errors, and area under the ROC curve (AUC) for the top GLM logistic regression model describing the probability of extinction from plague of individual prairie dog (*Cynomys ludovicianus*) towns on the Pawnee National Grassland, Colorado, USA.

Variable	Coefficient	SE	AUC
Intercept	5.905	3.310	0.769
Connectivity: extant towns	−1.430	0.633	
Connectivity: extinct towns	1.261	0.461	
1.5 mPa soil moisture capacity	1.249	0.518	
Summer temperature	−0.331	0.117	

Finally, we used *K*-fold cross validation to determine how consistently the GLM models performed. The complete data set *D* is randomly divided into *K*, mutually exclusive sets (D_1, D_2, . . . D_K) of equal size so that each model is trained and tested *K* times. During each replicate, one of the folds (D_i) is withheld, the model is trained on the remaining $D-D_i$, and then tested on the D_i set. The cross-validation accuracy estimate is the average of the *K* individual accuracies (Kohavi 1995). A 10-fold cross validation process was performed using S-Plus version 6.2 (Insightful Corporation 2003).

Testing for spatial and temporal autocorrelation.—We investigated three methods for examining correlation structure in the data beyond the original GLM, to test whether GLM successfully accounted for spatial and temporal autocorrelation. Two approaches, generalized estimation equations (GEE) testing for both spatial and temporal autocorrelation and autoregressive (AR) models testing for temporal autocorrelation, did not improve model fit. The methods and results from these approaches can be found in Appendix B.

The third method used spatial logit association GLM models to identify localized clusters, hot or cool spots of extinction. In spatial logit association GLM (Besag and Newell 1991, Lin 2003) spatial components are added as independent variables. Towns were considered neighbors if drainage distance between them was <10 km, which is longer than the measured dispersal distance of prairie dogs (Knowles 1985, Garrett and Franklin 1988). Other covariates were included in the model to account for global trends:

$$\text{logit}(\pi) = \beta_0 + \sum_{j=1}^{s} \beta_j w_j + \sum_{i=1}^{p} \beta_i x_i$$

where β_j are coefficients for the spatial effects indexed by the *j*th town and its neighbors as defined by spatial weights w_j in the spatial weights matrix **W**. We used a binary spatial weights matrix based on a limiting distance of 10 km. If the distance between two towns was ≤10 km, their weight was 1, otherwise weights were 0. Distances between towns were based on drainage distance (km; Fig. 1).

A scanning algorithm was used to identify spatial association (Lin 2003). Starting with the top GLM with spatial independence, we searched for local spatial association systematically so that hot spots refer to spatial clustering of elevated extinction rates, and cool spots refer to clustering of less frequent extinctions. In the first round of searches, each w_i was entered into the initial spatial independence model and tested for significance. If more than one local association was identified, the w_i with the smallest AIC was selected for inclusion in the model. After the most significant w_i was identified, the w_i vector was dropped for the next round of searches for the second most significant local association term. The process was repeated for each w_i until none significantly improved the fit of models.

RESULTS

Logistic regression via GLM

Backward stepwise model selection of the effects of current-year or previous-year precipitation resulted in the same top model (Table 1). Extinction was more likely for towns that were isolated from extant towns but were connected to towns experiencing plague. Extinction was also more likely on towns with soils with greater moisture-holding capacity, and in years with cool summer temperatures. Although it appeared that warmer springs also increased plague, only summer temperature was significant at α = 0.05. Town area, current and lagged spring and summer precipitation, and the interactions between precipitation and soils were not significant predictors of town extinctions.

For the top GLM, the area under the ROC curve (AUC), a threshold-independent measure of model accuracy, was 0.729 for the 1981–2003 data, and 0.963 for the 2004–2005 validation data (Fig. 2). Overall model accuracy, a threshold-dependent measure of model performance, was 0.723 for the 1981–2003 data and 0.802 for the 2004–2005 validation data. A 10-fold cross-validation predicted an average accuracy of 0.686, lower than the 0.802 accuracy achieved by the top model on the 2004–2005 data.

Tests for spatial and temporal autocorrelation

GEE models accounting for spatial or temporal autocorrelation performed nearly identically to the GLM without autocorrelation. Similarly, in autoregressive models testing for temporal autocorrelation, the autoregressive component of the model was not significant ($P > 0.05$), and thus a simple linear regression model accurately predicted the proportion of towns going extinct in a given year (Fig. 3). This suggests that there was no temporal trend in extinction over time and that plague outbreaks were determined by current conditions. Significant model variables, coefficients, and standard errors can be found in Appendix B.

Spatial logit association GLM and detection of clusters: "hotspots" of extinction

Beginning with a baseline GLM that included connectivity to extant towns, connectivity to plagued towns, 1.5 mPa soil moisture capacity, and summer
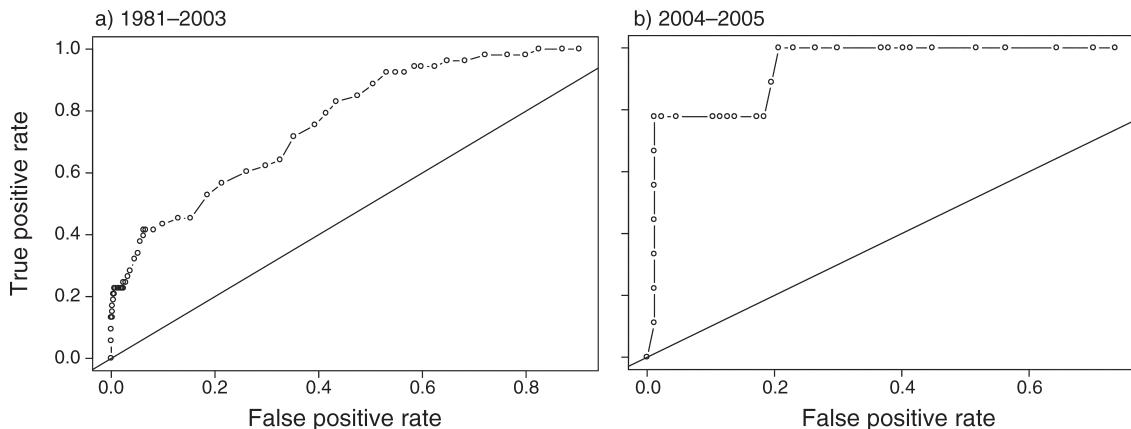
Fig. 2. Area under the receiver operating characteristic (ROC) curve (AUC) for generalized linear models (GLM) predicting extinctions from plague in black-tailed prairie dog towns for data from: (a) 1981–2003 and (b) 2004–2005.

temperature (Table 2), the sequential addition of four towns and their neighbors improved the model fit. The overall accuracy of the model increased from 0.54 for the spatial independence model to 0.90, while the AUC increased from 0.81 to 0.84. The positive and significant parameter estimates indicate that these four towns and their neighbors represent pockets of extinction. The years associated with these local hotspots correspond to the four peaks in Fig. 3 in which a large proportion of towns went extinct. In spatial logit association GEE models where we used Moran's I (Appendix B), we found only two years with spatial autocorrelation. Because the Moran's I statistic is a global test, it could not detect local clustering except in 1992 and 1999. The ability to identify spatially correlated local events may be useful for identifying factors that account for additional spatial and temporal variability in plague outbreaks.

Discussion

Disease transmission is an inherently spatial process. In the case of plague, susceptible hosts, flea vectors, and the plague bacterium must spatially coincide with abiotic conditions that favor plague transmission and outbreaks, and our analyses clearly demonstrate that spatial factors are critical for predicting plague risk in black-tailed prairie dogs. Explicitly including connectivity to extant and extinct towns accounted for most of the spatial variation in plague occurrence. Additional tests of autocorrelation detected little additional large-scale spatial variation. Our analysis reflects the common finding that plague occurs in clusters of prairie dog towns with short inter-town distances, while isolated towns have fewer plague outbreaks (Lomolino and Smith 2001, Girard et al. 2004, Stapp et al. 2004, Cully et al. 2010). Further, spatial logit models identified a number of local hotspots of extinction. Closer examination of clusters of towns could help identify additional factors that increase plague risk, for instance town-specific precipitation and temperature, prairie dog

population density, fine-scale variation in soils, and seasonal flea abundance (Tripp et al. 2009). We have measured such local differences in precipitation and prairie dog thermoregulation at a few sites within our study area where such fine-scale data were available (Lehmer et al. 2006).

At the same time that plague epizootics were clustered, plague was also scattered, with individual towns undergoing epizootics while neighbors were unaffected, also a finding in other studies of plague in prairie dogs (Cully and Williams 2001, Girard et al. 2004). Rather than simple local contagion, this pattern points to the importance of long-distance dispersal of plague-infected fleas by predators such as coyotes (Canis latrans), swift foxes (Vulpes velox), badgers (Taxidea taxus), or raptors (Salkeld et al. 2007). Another possibility is that plague persists in local pockets and concurrent outbreaks among distant colonies are triggered by environmental conditions as described here.
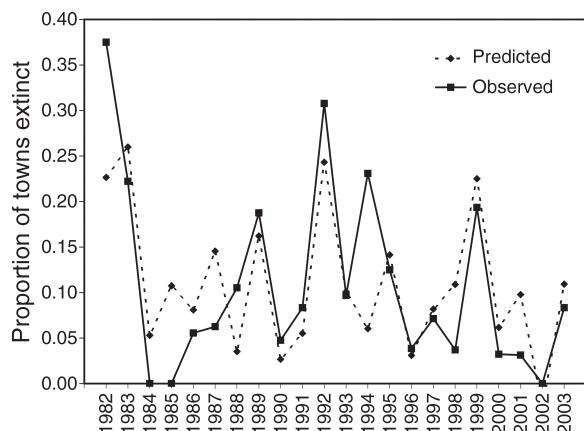

Fig. 3. Observed (solid line) and predicted (dashed line) proportion of prairie dog towns going extinct per year. Predicted values are based on network connectivity to extant towns, summer temperature, and 1.5 mPa soil moisture.

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TABLE 2.   Model coefficients and standard errors for the GLM spatial association logit model describing the probability of extinction of individual prairie dog towns.

Variable	Coefficient	SE	AIC	AUC
a) Independence model				
Intercept	7.1651	6.4734	285.39	0.644
Connectivity: extant towns	−1.1737	0.6024		
Connectivity: extinct towns	1.0402	0.4420		
1.5 mPa soil moisture capacity	1.2797	0.5440		
Summer temperature	−0.1331	0.0779		
b) Spatial association				
1982, town 11	1.9066	0.6610	281.92	0.660
1992, town 7	1.9309	0.8018	277.93	0.676
1994, town 32	2.3326	0.8121	273.70	0.693
1989, town 16	1.9298	0.7626	270.57	0.707

Note: The values for AIC and the area under the ROC curve (AUC) reflect the sequential change in the model as spatial regional effects are added to the model.

A previous analysis based partly on the same data set (Stapp et al. 2004) found plague risk to be higher in years when the El Nino Southern Oscillation (ENSO) was active. Similarly, the best-supported models in the current analysis included climatic variability during summer. Prairie dog town extinctions were associated with cooler summers and, to some extent, warmer early springs. Analyses of human plague cases in the southwestern United States show that both ENSO and the Pacific Decadal Oscillation (PDO: high ocean temperatures in the northern Pacific correlated with higher precipitation in North America) increase human plague occurrence in the western United States when they are active (Ben Ari et al. 2010). Many fewer cases occur when neither ENSO nor PDO is active and the region is in drought.

These results agree with a wealth of research on the influence of temperature on flea development and survival, the ability of fleas to clear plague infections, and the plague bacterium itself. First, temperatures below 10°C and above 35°C adversely affect flea development and survival, and reduce flea population size (Krasnov 2008). This kind of mid-temperature peak in flea abundance has been observed on northern grasshopper mice (*Onychomys leucogaster*), a rodent that commonly associates with prairie dogs in our area and may play a role in plague outbreaks (Salkeld and Stapp 2009, Salkeld et al. 2010). Second, replication and persistence of the plague bacterium in fleas is temperature dependent. At 26°C, genes are expressed that enhance survival and produce biofilms for adhesion of bacteria in the flea midgut (Perry and Fetherston 1997, Hinnebusch 2005). At temperatures above 28°C fleas readily clear infections (Gage and Kosoy 2005). Our own experiments with the two most abundant prairie dog fleas in Colorado, *Oropsylla hirsuta* and *O. tuberculata cynomurus*, show that both fleas can transmit *Y. pestis* within 48 h of taking infectious blood meals, and that they maintain infections for longer periods of time (Wilder et al. 2008). Future studies investigating the effects of temperature and moisture on development and

survival of prairie dog fleas, on the fleas' ability to maintain or clear infections, and on the plague bacterium itself will help elucidate the role of climate in plague transmission and epizootics in prairie dogs.

The relationship between extinctions and summer climate points to two main ideas about plague risk. First, hot, dry weather decreases plague while cool, moist weather promotes extinctions within the same year. Second, we found no evidence for yearly temporal lags in precipitation in either GLM or GEE models, and no evidence of temporal autocorrelation in GEE spatial logit association models or GLM autoregressive models. This implies that past climatic conditions did not strongly influence the probability of extinctions in the current year at our study site. Rather, current climatic conditions, connectivity, and moisture-holding capacity of soils were most predictive of plague epizootics in prairie dog towns. This does not support the cascade hypothesis, which emphasizes the effects of past precipitation on rodent population size as a primary driver of epizootics (Enscore et al. 2002, Collinge et al. 2005b). The cascade hypothesis may explain human plague exposures and cases, which could occur as a lagged spillover from plague-infected rodents. In black-tailed prairie dogs, lagged precipitation was identified as a factor predicting plague outbreaks in Montana but not in Boulder County, Colorado (Collinge et al. 2005b, Snall et al. 2008). This may point to regional differences in the strength of trophic cascades, since we too did not find lagged effects of precipitation at our study sites in Weld County, Colorado. However, the effects of the current year's precipitation were not tested in the analyses of the Boulder County or Montana data. Our results shift emphasis toward the effects of current climatic conditions on flea survival and reproduction, and on the plague bacterium itself.

Plague outbreaks occur when localized abundance, as measured by town size and connectivity, is high. This concurs with a long-term study of plague in great gerbils in Kazakhstan, where gerbil abundance predicted plague outbreaks (Davis et al. 2004). Warmer early spring

temperatures and cooler, wetter summers in the current year also predicted plague outbreaks in great gerbils, supporting the effects of climate on plague outbreaks (Stenseth et al. 2006). Brown and Ernest (2002) found that rodent population dynamics did not exhibit simple "bottom-up" regulation, because of the complexity of interactions between numerous environmental variables (i.e., magnitude and timing of rainfall events, temperature, herbivory, predation). Prairie dog density does not vary dramatically between towns and between years on our study area (McClintock et al. 2009), but plague outbreaks in prairie dogs are more common when the density of an associated rodent, the northern grasshopper mouse, is high (Stapp et al. 2009, Salkeld et al. 2010). Thus, while abundance of rodents and fleas plays a role in plague dynamics (Davis et al. 2004, Webb et al. 2006, Stapp et al. 2009, Tripp et al. 2009, Salkeld et al. 2010), past precipitation may not always predict either rodent or vector abundance.

In addition to climatic and spatial variation, our results suggest that towns on soils that retain moisture under dry conditions are more likely to succumb to plague than towns with drier soils. Soils have the potential to greatly impact flea populations because fleas spend most of their preadult life cycle off hosts in nesting materials. Water content of burrow soils may impact flea populations via the relative humidity of burrows. Flea larvae cannot survive below 40–50% relative humidity, and increasing relative humidity can speed egg and larval development and increase survival (Krasnov 2008). Soil moisture itself impacts flea survival: even at low ambient relative humidity (12%), increasing soil moisture of sand or sandy clay from zero to 1% increased larval survival from zero to 100%. On silty clay soils, larval survival increased above 5% soil moisture because clay binds water, making it unavailable at low levels (Silverman and Rust 1983). The dry conditions and clay soils normally found on the shortgrass steppe clearly have an impact, but the relationship between 1.5 mPa soil moisture capacity and plague transmission and persistence requires further exploration.

The results of this study influenced management of prairie dogs on the Pawnee National Grasslands (PNG). From 1995 to 2005 prairie dog populations expanded steadily. Because diets of prairie dogs and cattle overlap significantly, they have long been viewed as competitors for forage (Derner et al. 2006). The increase in prairie dog acreage resulted in pressure from the local ranching community to control prairie dog towns on the PNG. However, in 2003, after several years of drought and no plague, epizootics began to occur, and by 2007 many of the large towns on our study area had gone extinct. Results from this study showed that extinctions from plague take place on a regular basis, especially during relatively wet years, and poisoning prairie dogs was avoided for several years on the PNG (S. Curry, *personal communication*).

In conclusion, this study emphasizes the spatial nature of plague transmission and that plague outbreaks cluster in space. These data underscore the current reality for black-tailed prairie dogs with repercussions for associated species: plague epizootics are now a regular and integral part of prairie dog population dynamics. While the models presented here suggest that soil moisture and climatic conditions are important predictors of epizootics, and that epizootics greatly amplify plague in the environment leading to clusters of town extinctions, the reservoir that maintains plague between epizootics remains unknown.

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APPENDIX A

Plots of average precipitation and temperature in the spring and summer for the Pawnee National Grassland and Central Plains Experimental Range, Colorado, 1981–2005 (*Ecological Archives* A021-132-A1).

APPENDIX B

Methods and results of tests for spatial and temporal autocorrelation in models that predict extinctions from plague in black-tailed prairie dog towns in northern Colorado, 1981–2005 (*Ecological Archives* A021-132-A2).