Genetic Effects of Post-Plague Re-colonization in Black-Tailed Prairie Dogs

End-of-year report for summer 2008 field research

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Abstract

Due to the recent introduction of pathogens such as *Yersinia pestis,* the causative agent of the plague, natural populations of black-tailed prairie dogs (*Cynomys ludovicianus*) are increasingly threatened. Prairie dogs are extremely vulnerable to plague, and colonies may be extirpated following exposure to the bacterium. Repopulation of extinct colonies allows immigrants to leave nearby crowded colonies, and leads to questions about the influence of plague on the evolution of prairie dog populations. The goal of this project is to explore the genetic effects of re-colonization after plague in prairie dogs. Specifically, I will determine the colony of origin of all founders, which will allow estimates of the degree of inbreeding in newly founded colonies. I will also estimate the extent of founder effects, which arise from small populations with low genetic diversity that are subject to inbreeding, disease, and a suite of other stochastic environmental and genetic processes. Finally, I will examine the effect of plague on genetic diversity by comparing heterozygosity of colonies before and after plague events. Prairie dogs were trapped at 10 colonies in Boulder County, and blood, tissue and ectoparasites were collected. Each animal was marked with a unique tag for future identification and estimates of survival and dispersal. Using markers developed previously in our lab, all individuals will be genotyped at 15 microsatellite loci. Comparisons will be made among colonies founded since extirpation in 2005 or 2006, populations re-colonized after a plague epidemic in 1994, and those that have no record of being affected by plague.

Objectives

1) Identify whether founders are immigrants or individuals that escaped exposure to plague, and thereby assess the degree of founder effects in colonies extirpated by plague.
2) Determine whether heterozygosity differs between recently founded and already established prairie dog colonies.

Hypothesis

I hypothesize that plague extirpations decrease genetic diversity via founder effects. I predict that colonies recently re-colonized after plague die-offs will exhibit a pattern of decreased heterozygosity compared with colonies where plague appears to be historically absent.

Methods

Data were collected from 10 sites in Boulder County in 2008 (table 1), five of which were extirpated by plague in 2005-2007 and subsequently re-colonized. Prairie dogs were trapped for one week at all control sites and two weeks at all post-plague site.. Because of low population densities at re-colonized sites, and in an effort to capture all individuals in a colony, trapping was performed by targeting active burrows with one to four traps (Hoogland 1995). To standardize trapping effort across colonies, targeted trapping was also performed at control colonies.

Table 1. Trapping dates and plague history of 10 prairie dog colonies in Boulder County. No data on plague occurrence are available prior to 2003 for Hall Ranch or Aweida II.

Prairie dog colony site / Ownership	**Plague history**	**2008 Trapping dates**
Hall Ranch / Boulder County POS		July 25 – August 1
Dover/Blacker / City of Boulder OSMP	1994, 2006	June 23 – July 3
Aweida II / City of Boulder OSMP		July 10 – July 15
Belgrove/McKenzie / City of Boulder OSMP	1994, 2006	July 7 – July 18
S. Dam Boulder Reservoir / City of Boulder Parks & Recreation	(Survived 86, 91, & 94 plague) 2007	July 17 – July 31
Ute Industrial Park / City of Boulder OSMP	(Survived 1994 plague)	July 7 – July 9
Klein / City of Boulder OSMP	(Survived 86, 91, & 94 plague)	June 30 – July 3
Dowe Flats / Boulder County POS	1994, 2006	July 25 – August 1
Rock Creek Farm / Boulder County POS	1994	June 23 – June 27
Beech / City of Boulder OSMP & Boulder County POS	1986, 1994, 2006	July 21 – July 30

Prairie dog trapping and processing were conducted in accordance with protocols approved by the University of Colorado's Institutional Animal Care and Use Committee (Sackett 2008, unpublished) and are described in detail therein. Briefly, in order to accustom animals to traps and thereby increase trap success, traps were held open and baited for five days prior to sampling. Once the trapping session began, traps were baited at one site at 6:30 a.m. and left open until 9 a.m.; traps at the second site were baited at 8:30 a.m. and left open until 11 a.m. During unusually hot weather, traps were left open for less time. Captured prairie dogs were collected and placed in the shade until processing, during which time they were anesthetized. Processing involved collection of tissue for DNA; collection of blood for pathogen screening; determination of age, sex and size information for demographic analysis; removal of ectoparasites for estimates of potential health and pathogen presence; and insertion of a Passive Integrated Transponder (PIT) tag for future identification. After processing, animals were placed back into the traps until the anesthesia wore off and they became alert, at which time they were returned to their capture locations.

Administration of anesthesia involved placing animals from their traps directly into a cone-shaped canvas bag that physically restrains the animals, thereby keeping them calm (Hoogland 1995). The bag zips from both ends, allowing for easy handling of animals and placement of an anesthesia cone (with 2 mL of isoflurane on a cotton ball) above each prairie dog's mouth and nose. Fleas were collected by spraying prairie dogs with permethrin and then removing fleas with tweezers. Tissue for DNA analysis was collected using a 2-mm diameter ear punch (Braintree Scientific). Approximately 0.5mL blood was collected from each individual, except juveniles weighing less than 500 grams.

Blood samples were sent to the Centers of Disease Control and Prevention in Fort Collins for identification of plague antibodies. Presence of these antibodies would indicate exposure to *Y. pestis*, and allow for evaluation of survival capabilities of prairie dogs exposed to plague. In 2007, five animals in two colonies tested positive for plague antibodies.

DNA from prairie dogs at all re-colonized sites has been extracted, and individuals are currently being genotyped at 15 microsatellite loci developed in our lab (Jones et al. 2005, Sackett et al. 2009). Individual and colony-average heterozygosity will be calculated and compared between

previously established (i.e. not affected by plague in 2005-2007) and recently re-colonized (i.e. after 2005) colonies. Using an assignment program (Microsatellite Analyzer) and genetic data from 2003-2006, founders of re-colonized sites will be assigned to a colony of origin. This information will allow estimation of migration and colonization rates. Additionally, through identification of residents in post-plague colonies as descendants of immigrants vs. long-time residents, it will provide estimates of potential inbreeding and future genetic diversity loss, which will determine the evolutionary potential of the species.

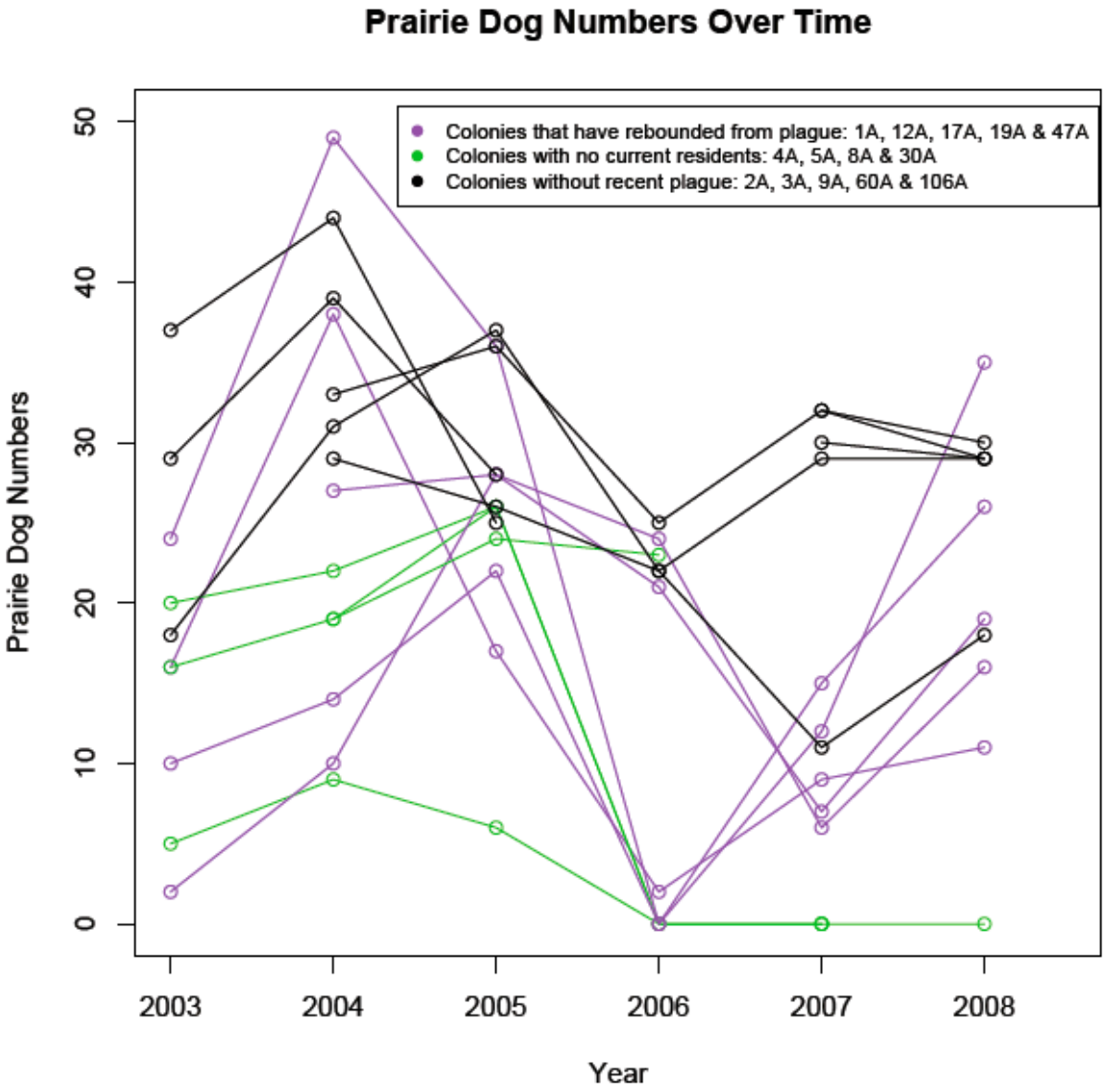
Results

During the 6-week field season, we captured 279 prairie dogs and processed 242, 56 of which (23.1%) had been tagged in previous years. Capture rates were lower at most re-colonized sites when compared with control sites (table 2). Between-year recapture rates varied from 22.2% at Dowe Flats (where only 2 of the 9 animals tagged in 2007 were captured in 2008) to 100% at Boulder Reservoir (where all animals tagged last summer were captured and processed again in 2008). The majority of individuals in repopulated colonies (estimated by visual counts and within-trapping session recapture rates) were captured. Rates of re-colonization appear to vary dramatically among colonies, estimated from 11 individuals trapped at Dowe Flats to 35 individuals trapped at Dover/Blacker (Figure 1). Prairie dogs in post-plague sites did not exhibit visual signs of poor health as they had last year. Additionally, animals seemed to harbor fewer fleas at post-plague sites than at control sites, probably because of the lethality of plague to fleas (analysis pending). Blood samples from all prairie dogs at post-plague sites have been sent to the CDC for plague screening; fleas from these sites will be tested for plague at Northern Arizona University.

Table 2. Summary of prairie dog captures at each site sampled summer 2007 (left 2 columns) and 2008 (right three columns). Colonies that experienced plague in 2005-2007 are in boldface type.

Property Name	Individuals Processed (2007)	Previously Tagged (prior to 2007)	Individuals Processed (2008)	Previously Tagged (prior to 2008)	Rate of Recapture (2008 from 2007)
Klein	32	5	30	6	0.1875
Dover/Blacker	12	0	**35**	**4**	**0.33**
Ute Industrial	29	2	29	6	0.207
Dowe Flats	9	0	**11**	**2**	**0.22**
Rock Creek	11	4	18	4	0.3636
Beech	15	0	**26**	**5**	**0.33**
Hall Ranch	30	5	29	8	0.267
Belgrove	7	0	**19**	**3**	**0.429**
Aweida	32	10	29	12	0.375
S. Dam Boulder Reservoir	6	0	**16**	**6**	**1**
TOTAL			**242**	**56**	

Figure 1. Number of prairie dogs per colony over time. Black lines represent control colonies, green lines represent colonies that were eliminated by plague and have not been repopulated, and purple lines represent colonies that have been re-colonized after plague. Trapping effort was not equal among colonies.



In 2007, we captured five prairie dogs from two colonies (two from the Boulder Reservoir and three from Belgrove) that tested positive for plague antibodies at the CDC. In 2008, we recaptured four of those individuals (two at the Reservoir and two at Belgrove). Therefore, four prairie dogs that were exposed to plague subsequently recovered and survived another year. Antibodies in these animals persisted in 2008, when they tested positive again. In addition, two other prairie dogs from Beech also tested positive in 2008 (one of which was captured last year but did not then have antibodies). Although the survival of these animals is intriguing, more research is needed to determine whether some level of resistance to plague may be possible in prairie dogs. Additionally, whether this resistance has a genetic component and is capable of proliferating among colonies is unknown. Furthermore, the ability of this characteristic to persist depends on stochastic

and non-random factors in a population, including whether resistant individuals are preyed upon or experience differential mating success from other individuals, and whether resistance confers a cost to the animal. Future research on reproductive success of individuals with antibodies, as well as the survival of these animals and their offspring, is needed to determine the potential ability of prairie dogs to respond to the disease over meaningful spatial and temporal scales.

Prairie dogs in re-colonized sites were trapped in spatially distant portions of their colonies, indicating either that they use larger areas when they live at lower densities, or that they may represent distinct family groups and separate immigration events. Multiple immigrations of prairie dogs from different areas of origin, probably accompanied by a higher rate of population growth, would suggest less inbreeding occurs in newly re-colonized populations; conversely, colonization based on only one immigration event implies a higher risk of inbreeding. The extent to which inbreeding occurs influences populations' ability to respond to novel evolutionary challenges such as introduced pathogens and climate change. Determining the levels of inbreeding within newly founded colonies will require: 1) genetic analysis of all captured animals, and 2) future trapping seasons to estimate rate of population increase and immigration. Genetic analysis has begun, and is expected to conclude in March 2009; subsequent field seasons will take place during the summers of 2009 and 2010.

Conclusions

Variation exists in re-colonization time and population growth of prairie dog colonies extirpated by plague. Capture rates at sites that had been colonized in 2007 or before ranged from 11 to 35, and averaged 21. The number and origins of founders dictate the extent of inbreeding that will occur as the colony expands to its previous size. Inbreeding, in turn, influences the overall health and genetic diversity of a population, and affects prairie dogs' ability to respond to changes in their environment. This field research was the second season of data collection a five-year dissertation project on the genetic consequences of disease in prairie dogs. Similar trapping will be conducted through 2010, and re-colonized populations will be followed over time to estimate rates of increase, fitness, and genetic variation. Regular reports documenting results of genetic analyses will be submitted annually. Knowledge gained from this research will help us understand the factors affecting the health of this important species and many others that rely on prairie dogs for habitat or food. Such information is crucial to the protection of this keystone species, an important component of grassland ecosystems in Boulder and across its range. Furthermore, as wildlife disease prevalence and human-wildlife interactions increase, information about evolutionary responses to disease in wildlife are increasingly important.

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