Post-Plague Re-colonization in Black-Tailed Prairie Dogs

End-of-year report for summer 2009 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. All individuals were genotyped at 18 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.

Introduction

Highly social species such as prairie dogs are hypothesized to have higher disease transmission, thus suggesting an important role of disease in the evolution of social species (). More (). Introduced pathogens offer a unique opportunity to examine how naïve species will respond to a novel threat, and whether social species are indeed more vulnerable to pathogens. The existence of plague in prairie dogs thus provides a means of testing whether a novel pathogen provokes an evolutionary response in a colonial species.

The dynamics of plague are poorly understood, but what is clear is the rapidity with which it extirpates prairie dog colonies. Plague is transmitted by fleas, which prairie dogs may harbor in high numbers, and once the first prairie dog contracts the disease, it is only a matter of weeks before the entire colony dies out. Given that plague decimates entire colonies in such a short time, it seems likely that plague can be considered an evolutionary force in prairie dogs.

Because plague eliminates entire populations, which are then recolonized, populations undergo extreme bottlenecks as a result. Plague is therefore expected to have genetic consequences on the affected colonies, including a decrease in genetic diversity both at the colony level due to the bottleneck and founder effects, and at the individual level due to inbreeding that likely results from a founder effect. This depression of genetic diversity may be exacerbated if continued immigration occurs from only one or few source colonies. Populations that have been subjected to repeated plague outbreaks are expected to possess lower diversity than those that have undergone bottlenecks only once, and colonies that have never experienced plague should be unaffected.

The aim of this study is to determine the extent to which plague influences the evolution of prairie dogs on short time scales. To answer this question, I will investigate changes in genetic diversity after recolonization following plague extirpations. I will also determine the origin of population founders and immigrations rates to estimate the degree of inbreeding expected in recolonized populations.

Methods

Data were collected from 10 sites in Boulder County in 2009 (Table 1, Figure 1), six of which were extirpated by plague in 2006-2007 and subsequently re-colonized. Traps were pre-baited

at each colony for at least five days, and each day after trapping concluded. By targeting active burrows with one to four traps (Hoogland 1995), prairie dogs were trapped for one week at all control sites and two weeks at all post-plague sites.

Table 1. Plague history of 11 prairie dog colonies in Boulder County. No data on plague occurrence are available prior to 2003 for Hall Ranch or Aweida II. Asterisk indicates that a colony died out in 2009.

Prairie dog colony site / Ownership	**Plague history**
Dowe Flats / Boulder County POS	1994, 2006
Hall Ranch / Boulder County POS	
Rock Creek Farm / Boulder County POS	1994
Aweida II / City of Boulder OSMP	
Dover/Blacker / City of Boulder OSMP	1994, 2006
Belgrove/McKenzie / City of Boulder OSMP	1994, 2006
Beech / City of Boulder OSMP & Boulder County POS	1986, 1994, 2006
*Waneka / City of Boulder OSMP	1994, 2009
Johnson/Dawson / City of Boulder OSMP	2006
S. Dam Boulder Reservoir / City of Boulder Parks & Recreation	2007 (Survived '86, '91, & '94 plague)
Klein / City of Boulder OSMP	(Survived '86, '91, '94 & '06 plague)

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 2009) and are described in detail therein. Briefly, traps were baited at one site at 6:00 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 above each prairie dog's mouth and nose. The cone was connected to a regulator that delivered 1 – 4% isoflurane in oxygen (precise amount to be determined by individual animals' reactions to isoflurane). 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. Although survival is extremely rare, it has been occasionally observed (Sackett unpublished data).

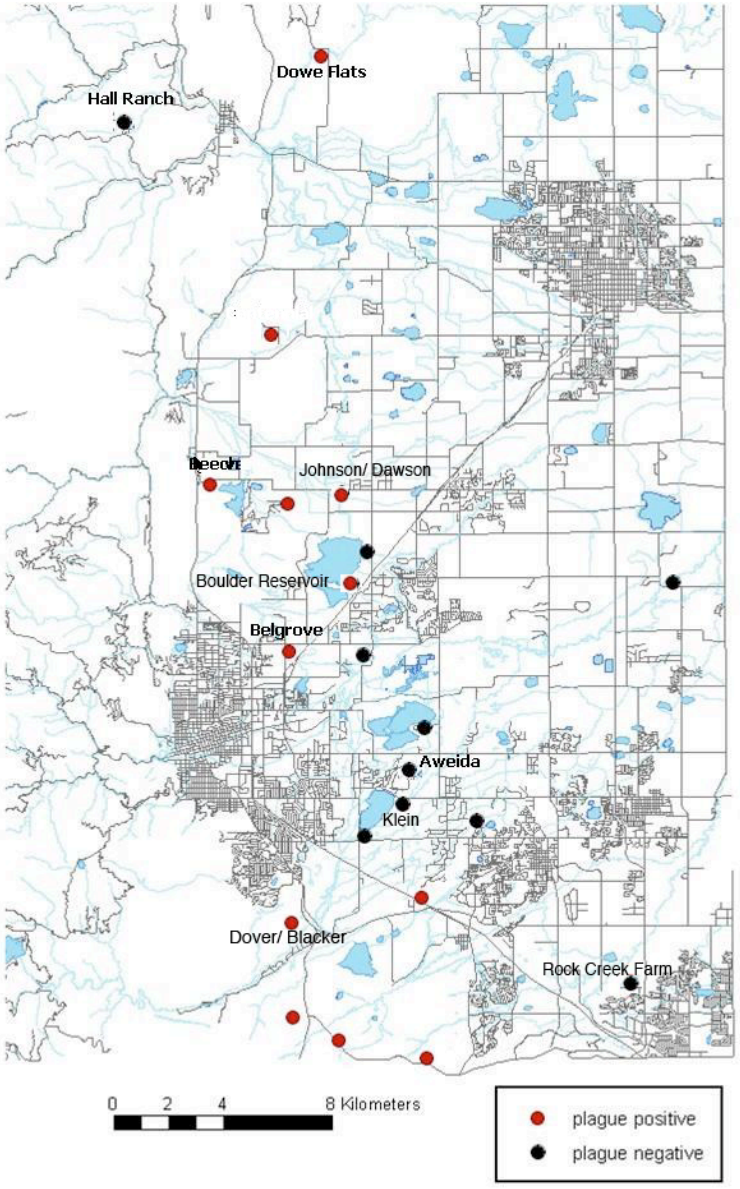


Figure 1. Map of study colonies in Boulder County. Red dots represent colonies extirpated by plague in 2006-2007; black dots represent control colonies.

DNA from prairie dogs at all re-colonized sites collected from 2007-2009 was extracted, and individuals were genotyped at 18 microsatellite loci developed in our lab (Jones et al. 2005, Sackett et al. 2009). Microsatellites are rapidly-evolving neutral markers that are used to infer genome-wide patterns among individuals and populations. Individual and colony-average heterozygosity were calculated using Arlequin (Schneider et al. 2000) and compared between recently re-colonized (i.e. after 2006) populations and those not exposed to plague. To assess whether residents were genetically different before and after plague, I calculated differentiation between the two temporally segregated colonies in the program Genepop (Rousset 1997). Using the likelihood-based assignment program Structure (Pritchard et al. 2000) 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 versus 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 and Discussion

During the 6-week field season, we processed 235 prairie dogs, 58 of which (24.7%) had been tagged in previous years. Capture rates were lower at most re-colonized sites when compared with control sites (table 2), but the difference was less pronounced than in previous years, and some post-plague colonies (e.g. Beech, Boulder Reservoir) had higher capture rates than control colonies. Between-year recapture rates varied from 15.8% at Belgrove (where only 3 of the 19 animals tagged in 2008 were captured in 2009) to 63.6% at Dowe Flats (where 7 of the 11 animals tagged in 2008 were captured and processed again in 2009). Interestingly, Belgrove had the highest recapture rate in

2008 but the lowest in 2009. The majority of individuals in repopulated colonies (estimated by visual counts and within-trapping session recapture rates) were captured.

Table 2. Summary of prairie dog captures at each site sampled summer 2007-2009. Colonies that experienced plague in 2006-2007 are in boldface. N/A indicates colonies that were not visited, whereas zeroes denote extirpated colonies or those where no individuals were caught. §Trapping occurred for only 3 days at Klein in 2009. *Waneka was extirpated by plague in 2009. ‡Ten previously-tagged individuals were released unprocessed. ∂One individual from Aweida survived since tagging in 2005. Two-year recapture rates were determined by calculating the proportion of individuals captured in 2009 that were originally tagged in 2007 and survived to be captured in 2008.

Property Name	Individuals Processed in 2007 (# previously tagged)	Individuals Processed in 2008 (# previously tagged)	Rate of Recapture (2008 from 2007)	Individuals Processed in 2009 (# previously tagged)	Rate of Recapture (2009 from 2008)	Individuals processed in 2009 tagged in 2007 (Proportion recaptured in 2009 from 2007)†	Estimated 2yr survivorship (based on Proportion of individuals tagged in 2007 recaptured in 2009)
Dover/Blacker	12 (0)	**35 (4)**	**0.333**	**21 (9)**	**0.257**	**1 (0.250)**	**0.083**
Dowe Flats	9 (0)	**11 (2)**	**0.222**	**20 (7)**	**0.636**	**1 (0.500)**	**0.111**
Beech	15 (0)	**26 (5)**	**0.333**	**49 (9)**	**0.346**	**2 (0.400)**	**0.133**
Belgrove	7 (0)	**19 (3)**	**0.429**	**16 (3)**	**0.158**	**1 (0.333)**	**0.143**
S. Dam Boulder Reservoir	6 (0)	**16 (6)**	**1**	**39 (7)**	**0.438**	**4 (0.667)**	**0.667**
Johnson/Dawson	**0**	**0**	**0**	**11 (0)**	**0**	**0**	**0**
Hall Ranch	30 (5)	29 (8)	0.267	40 (4‡)	0.138/0.483	4 (0.500)	0.133
Klein	32 (5)	30 (6)	0.188	15 (3)§	0.100	2 (0.333)	0.0625
Ute Industrial	29 (2)	29 (6)	0.207	N/A	N/A	N/A	N/A
Rock Creek Farm	11 (4)	18 (4)	0.364	0	0	0	0
Aweida	32 (10)	29 (12)	0.375	24 (10)	0.345	8∂ (0.667)	0.250
Waneka*	35 (4)	N/A	N/A	0	0	0	0
TOTAL	**218**	**242**		**235**			

Rates of re-colonization appear to vary dramatically among colonies, ranging from 16 individuals trapped at Belgrove, at least two years after plague, to 49 individuals trapped at Beech (Figure 2). Prairie dogs in post-plague sites did not exhibit visual signs of poor health as they had in 2007, immediately following plague. This was true even at Johnson/ Dawson, which was recolonized more recently; this colony was interesting in that 100% of our captures were juveniles. Sex ratios were even at all colonies. Finally, 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).

Diversity within individuals in a population was assessed by measuring heterozygosity, the average proportion of loci with different alleles within individuals. Heterozygosity at 18 microsatellite loci in newly founded colonies varied from 0.615 (Dowe Flats, with 20 individuals captured and recolonization beginning in 2007) to 0.713 (Dover/ Blacker, with 21 individuals captured and recolonization also beginning in 2007). Average heterozygosity in recently founded colonies was 0.661—a value which, contrary to predictions, represents a slight (but not significant, $p = 0.112$) increase in heterozygosity compared to the average pre-plague heterozygosity of the same colonies. Five out of six populations (with the exception of Dowe Flats) saw an increase in heterozygosity following recolonization after plague (Figure 3). In contrast, during the same time

frame, control colonies experienced a nonsignificant decline in heterozygosity (from 0.641 to 0.619, $p = 0.466$), with two out of three colonies following this pattern.

Figure 2. 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.

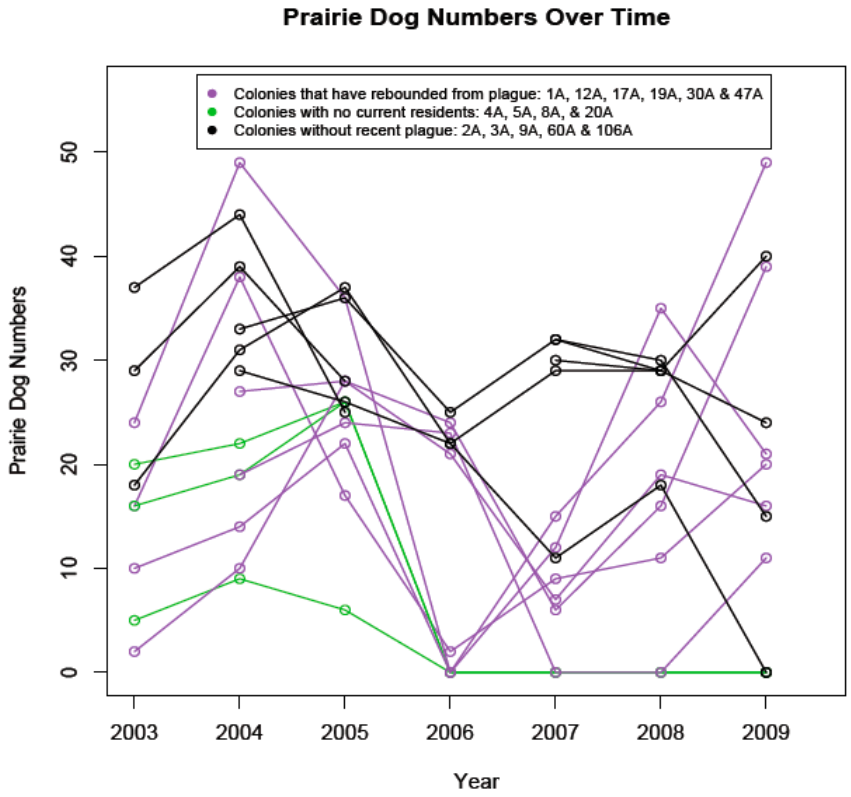
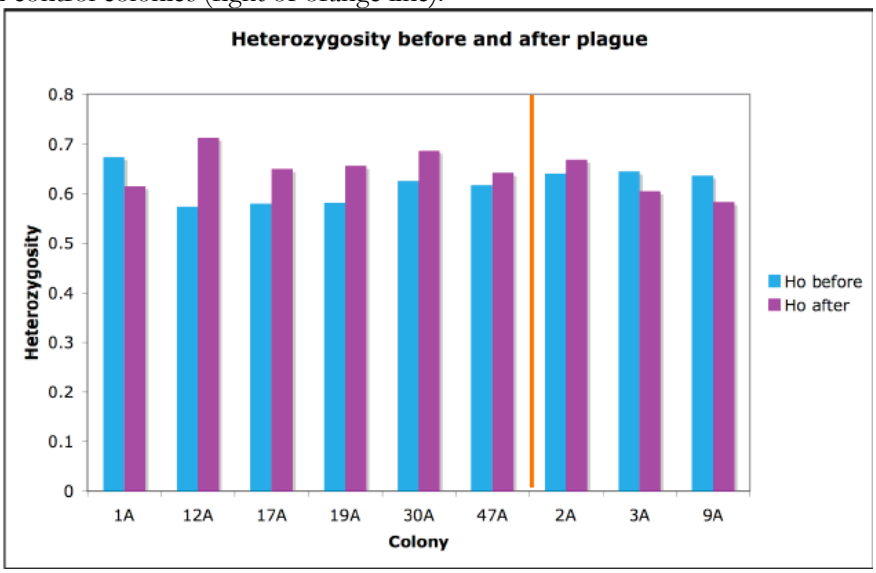


Figure 3. Heterozygosity (H_0) before and after plague in extirpated colonies (left of orange line) or before and after 2006 in control colonies (right of orange line).



Stochastic factors such as predation or environmental constraints may cause heterozygosity to fluctuate slightly between years, and is likely the driver of the patterns observed in control colonies. Stochasticity could also explain the increase in heterozygosity in post-plague colonies; however, it is also possible that these colonies see a large influx of immigrants into the empty niche vacated by prairie dogs due to plague. Prior to plague outbreaks, colonies have high population density; thus, immigrants are likely experience to tough competition with residents. After plague, this competition is eliminated, and immigrants may be more successful at surviving—and introducing new diversity into the population.

Table 3. Colony heterozygosity before & after plague. Asterisks represents colonies that were extirpated by plague in 2006-2007.

Colony	H_o before	H_o after	Increase/decrease?
*1A	0.674	0.615	decrease
*12A	0.574	0.713	increase
*17A	0.580	0.650	increase
*19A	0.582	0.656	increase
*30A	0.626	0.687	increase
*47A	0.617	0.642	increase
2A	0.641	0.668	increase
3A	0.645	0.605	decrease
9A	0.637	0.583	decrease

Prairie dogs in re-colonized sites were located in spatially discrete 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 would introduce greater genetic diversity into newly recolonized populations, thus mitigating founder effects that occur with colonization. The increase in heterozygosity in post-plague colonies may support this idea that immigrants are arriving from multiple source colonies. If immigration is indeed occurring from multiple locations, we would expect to see not only an increase in heterozygosity, but an increase in overall diversity within a population (e.g. number of alleles per locus), and a change in diversity before and after plague (e.g. a change in allele frequency).

Diversity within populations, both before and after plague, was assessed in two different ways in Arlequin (Schneider et al. 2000): the average number of alleles per locus and gene diversity. In colonies experiencing plague followed by recolonization, there was a decline in average number of alleles per locus when compared with control colonies, although the difference was not significant (average change -0.021 plague, +0.259 control, $p = 0.366$; Figure 4). Post-plague colonies had significantly lower gene diversity than control colonies (0.538 and 0.617, respectively, $p = 0.027$), but the change in gene diversity was not significant ($p = 0.529$), indicating that post-plague colonies also had lower diversity before being extirpated by plague. The reason for this lower level of diversity is unclear, but suggests there may be spatial variation in gene diversity that can be attributed to environmental factors not tested in this study.

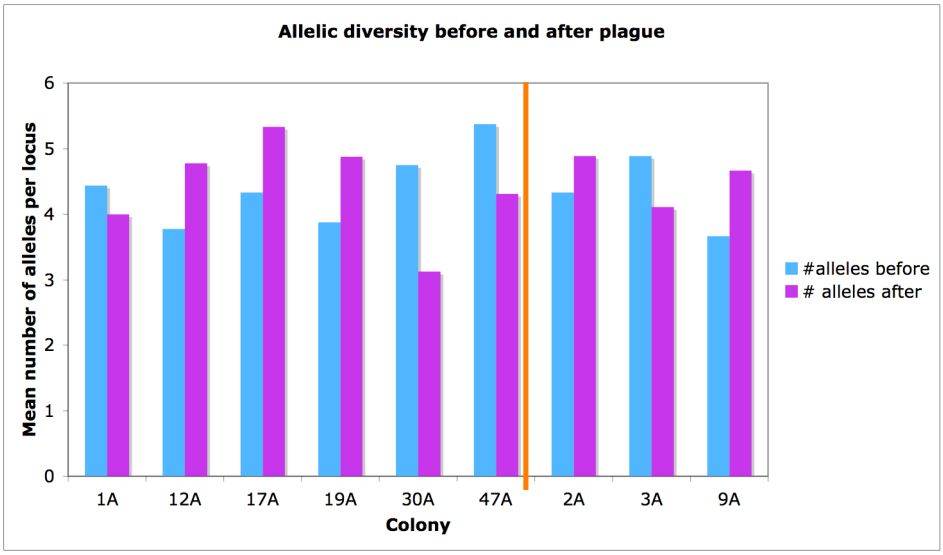


Figure 4. Average number of alleles per locus before and after plague in 2006 (extirpated colonies; left of orange line) or before and after 2006 (control colonies; right of orange line).

To estimate whether founders of new populations represented similar ancestry as those occupying the colony before plague extirpation (as might be expected if migration corridors are stable over time), I calculated F_{ST} values in Genepop (Rousset 1997). F_{ST} is a measure of differentiation between two populations, and is commonly used to estimate differences between discrete populations in space, but can also be used to distinguish between discrete populations in time, as in before and after extirpation/recolonization. Differentiation between populations before and after 2006 was an order of magnitude higher in plague-experiencing versus control colonies (F_{ST} = 0.068 plague, 0.005 control, p = 0.009; Figure 5).

Temporal differentiation between colonies before and after plague represents a change in allele frequency over a short period of time. This may occur for several reasons. First, migration routes may vary over time, causing new genetic inputs to a population to also change over time. This possibility seems likely if the habitat is continuous and individuals can use one of a number of routes. If migration routes are dynamic in time, however, we would expect temporal fluctuation of genotype frequencies in control colonies. Although no significant departure from Hardy-Weinberg equilibrium is observed, it is possible that small departures exist. Further analysis is needed to determine whether variable migration routes are a possible source of the change in genetic composition.

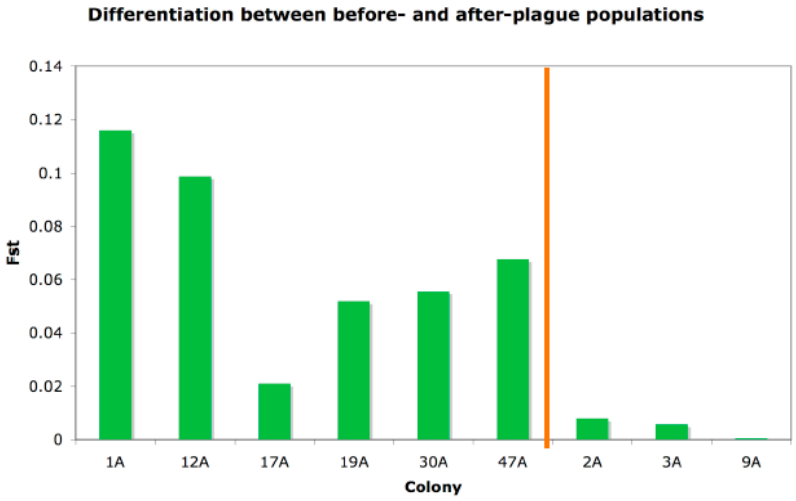


Figure 5. Temporal differentiation (F_{ST}) between individuals in colonies before and after plague for extirpated colonies (left of orange line), or before and after 2006 for control colonies (right of orange line).

Second, temporal differentiation within colonies before and after plague could arise from stochastic demographic fluctuations. During colony formation, one or two genotype groups could become prevalent due to colonization order or other non-adaptive effects. After plague, when all genotype groups are eliminated, the next wave of colonization will

also be governed by some non-adaptive processes allowing new genotypes to increase in frequency. It is possible that changes in road location and size change migration corridors so that new waves of colonization can occur via different routes.

A third potential explanation for temporal differentiation in colonies experiencing plague is that only plague-resistant genotypes remain (or are able to colonize) after plague. If this is the case, we might expect to see an increase in plague resistance among prairie dogs. However, an increase in resistance among populations requires that several assumptions be met: 1) plague resistance occurs, 2) resistance is heritable, and 3) the benefits conferred by resistance outweigh any costs incurred. The first assumption can be confirmed (see below), but the other two are untested.

In 2007, five animals in two colonies (two from the Boulder Reservoir and three from Belgrove) tested positive for plague antibodies, and in 2008, four of these animals tested positive again (one was not recaptured), and two new animals from a third colony (Beech) also tested positive. Both of these new individuals were recaptured in 2009, indicating that they, along with the other four recaptured animals, survived exposure to plague. Assessment of plague antibodies for animals captured in 2009 is pending.

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. As stated above, whether this resistance has a genetic component and is capable of proliferating among colonies is unknown. Identifying the genetic basis of resistance, if there is one, will contribute greatly to our understanding of plague dynamics. Finally, the ability of this characteristic to persist, if it does have a genetic basis, 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.

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 evolutionary 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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