J Vector Borne Dis 48, September 2011, pp. 125–132

Research Articles

Gene flow in a *Yersinia pestis* vector, *Oropsylla hirsuta*, during a plague epizootic

Philip H. Jones[1], Leigh R. Washburn[2] & Hugh B. Britten[3]

[1]*Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, Iowa;* [2]*Basic Biomedical Sciences, Sanford School of Medicine,* [3]*Department of Biology, University of South Dakota, Vermillion, South Dakota, U.S.A.*

ABSTRACT

Background & objectives: Appreciating how *Yersinia pestis*, the etiological agent of plague, spreads among black-tailed prairie dog (*Cynomys ludovicianus*) colonies (BTPD), is vital to wildlife conservation programs in North American grasslands. A little-studied aspect of the system is the role of *Y. pestis* vectors, i.e. fleas, play in the spreading of plague in natural settings. We investigated the genetic structure and variability of a common prairie dog flea (*Oropsylla hirsuta*) in BTPD colonies in order to examine dispersal patterns. Given that this research took place during a widespread plague epizootic, there was the added advantage of gaining information on the dynamics of sylvatic plague.

Methods & Results: Oropsylla hirsuta were collected from BTPD burrows in nine colonies from May 2005 to July 2005, and eight polymorphic microsatellite markers were used to generate genotypic data from them. Gene flow estimates revealed low genetic differentiation among fleas sampled from different colonies. Nested-PCR plague assays confirmed the presence of *Y. pestis* with the average *Y. pestis* prevalence across all nine colonies at 12%. No significant correlations were found between the genetic variability and gene flow of *O. hirsuta* and *Y. pestis* prevalence on a per-colony basis.

Conclusion: Oropsylla hirsuta dispersal among BTPD colonies was high, potentially explaining the rapid spread of *Y. pestis* in our study area in 2005 and 2006.

Key words Dispersal; flea; microsatellites; PCR; prairie dog; sylvatic plague

INTRODUCTION

Black-tailed prairie dogs (BTPD), *Cynomys ludovicianus* (Rodentia: Sciuridae) have become a major focus in wildlife conservation because they are considered a 'keystone' species in North American grasslands[1]. Over 200 prairie species are supported by BTPDs including the endangered black-footed ferret, *Mustela nigripes* (Carnivora: Mustelidae)[1]. One of the most devastating diseases currently impacting BTPDs is sylvatic plague[2] making an understanding of plague dynamics on the landscape vital to the conservation of the prairie ecosystem.

Sylvatic plague, caused by *Yersinia pestis* (a gram-negative bacterium), is mainly transmitted via bites from infected fleas. More rarely, *Y. pestis* is transmitted by a bite from an infected rodent or through inhalation of the bacterium (pneumonic) itself from an infected host. These various modes of transmission provide *Y. pestis* with the ability to persist in the environment and infect multiple hosts[3]. Of the several fleas that serve as vectors for *Y. pestis* to BTPDs, the genus *Oropsylla*, particularly, *O. hirsuta* (Siphonaptera: Ceratophyllidae) dominates the flea fauna[3-4]. *Oropsylla hirsuta* was selected as the focal species for this study because of its common occurrence in the BTPD colonies.

Many studies have used molecular techniques such as microsatellite markers to elucidate how vectors of infectious diseases disperse among wildlife populations[4-7]. Hypervariable microsatellites are excellent for population genetic studies of this type[8-9]. For example, studies that made use of microsatellite variability in disease vectors have uncovered patterns of genetic differentiation that have given insight into how ectoparasitic vectors cause outbreaks[5,6, 10-13]. Antonio-Nkondjio *et al*[5] reported that the mosquito *Anopheles moucheti* (Diptera : Culicidae), a malarial vector, shows low genetic differentiation among populations across large geographic ranges. This indicates that the species has few ecological barriers to dispersal which would limit its ability to carry malaria to new areas. Studies using molecular markers have also shown that

humans have the potential to spread new disease vectors into uninfected areas. For example, Bataille *et al*[6] proposed that mosquito movements between the Galápagos Islands could not have occurred without the assistance of the human settlers. Other genetic studies elucidated how vectors, such as the tsetse fly (*Glossina palpalis*), can display the Wahlund effect while thriving in small populations[11]. This can create a situation in which the species does not disperse over large distances and has a lower potential to spread diseases like dengue over broad areas[11]. These examples illustrate the utility of studying ectoparasitic vector population genetic structures as a potential means to understand how diseases appear in new areas and may suggest ways to prevent, or at least limit the spread of pathogens. The microsatellite markers for *O. hirsuta* were recently developed[13]; therefore, this study was one of the first that elucidated the population genetic structure of this common BTPD flea.

The aim of this study was to use the genetic structure of *O. hirsuta* to understand its dispersal pattern among BTPD colonies, in an approach similar to studies of Bataille *et al*[6] and Failloux *et al*[7] for other ectoparasitic disease vectors. These studies used molecular markers to elucidate the population genetic structure of ectoparasites of interest and to study how that population genetic structure might have contributed to the spread of disease. In addition, we wanted to determine whether *Y. pestis* prevalence was related to *O. hirsuta* genetic structure and variability, and therefore, the flea's ability to disperse among BTPD colonies. Because this study took place during an unexpected epizootic; we were able to investigate the landscape genetic patterns of this common plague vector during an active outbreak potentially enhancing our understanding of the plague transmission.

MATERIAL & METHODS

Study site, flea collections & genomic DNA extractions

The study was conducted from May–July 2005 on nine BTPD colonies in north-central Montana (Fig. 1). The colonies were delineated into two regions, six were located on the Fort Belknap Indian Reservation (FBIR) in Blaine County, Montana, and three were located at 46 km south-east on land administered by the U.S. Bureau of Land Management (BLM) in Phillips County, Montana. Because the entire area experienced a widespread plague epizootic that began in fall of 2005, planned resampling efforts for 2006 were impossible.

Fleas were collected from BTPD burrows using methods described by Jones and Britten[4]. Fleas were identified to species using light microscopy using criteria described in Lewis[14]. All fleas swabbed from burrows were tested for the presence of *Y. pestis*, but only those identified as *O. hirsuta* were used in this genetic study. Genomic flea DNA was extracted using the Easy-DNA kit™ (Invitrogen Life Technologies, Carlsbad, California) following a modified mouse tail extraction procedure[13,15].

Polymerase chain reactions & Yersinia pestis assays

Oropsylla hirsuta used for the genetic study were genotyped using eight microsatellite markers: *Orop*12, 13, 84, 158, 369, 586 from Jones *et al*[13] and Cga4, 14 from Binz *et al*[16]. Polymerase chain reactions (PCR) and genotyping were done using the methods described in Jones *et al*[13]. The same fleas used in this genetic study were used to confirm the presence of *Y. pestis* and to calculate *Y. pestis* prevalence.

Hanson *et al*[15] developed a nested-PCR based diagnostic test to confirm the presence of *Y. pestis* in burrow-collected fleas. The nested-PCR protocol yields high sensitivity for detecting *Y. pestis* allowing detection from small volumes of sample[15]. The primers target a 110 bp segment of the plasminogen activator gene (*pla*) found only

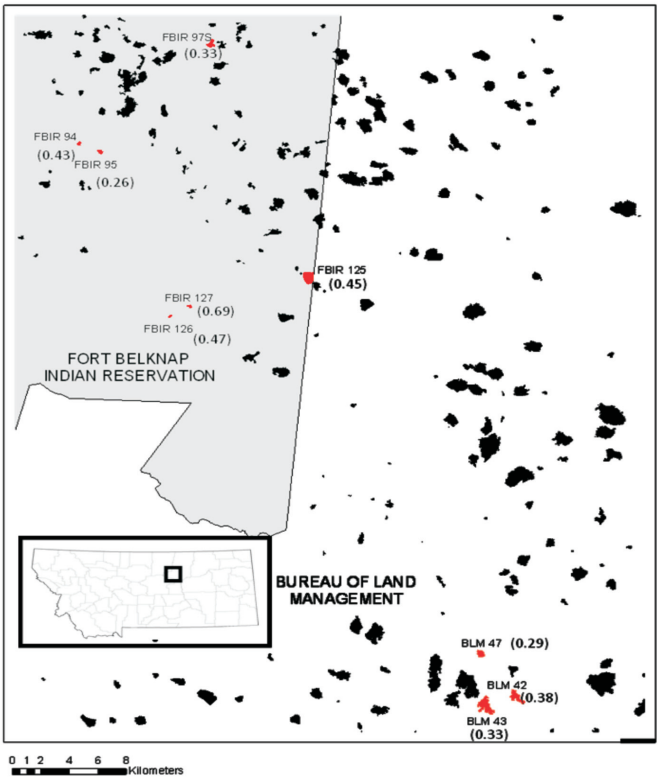

Fig. 1: Black-tailed prairie dog colonies where *Oropsylla hirsuta* were collected are labeled. Mean F_{IS} estimates are in parentheses near each identified sample site. Sample sizes per colony are: FBIR 97S: 16; FBIR 94: 14; FBIR 95: 14; FBIR 126: 14; FBIR 127: 9; FBIR 125: 15; BLM 42: 11; BLM 43: 19; and BLM 47: 17. Inset map is the State of Montana with a box showing the location of the study area.

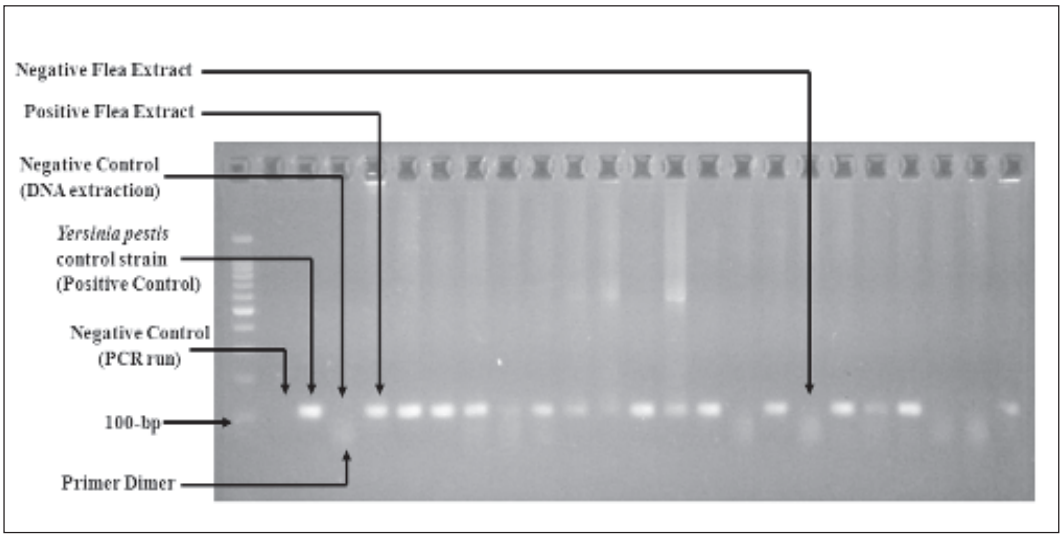

Fig. 2: 1.5% agarose gel of a nested-PCR run showing flea DNA extract positive and negative for the presence of *Y. pestis* together with positive control *Y. pestis* A1122 and negative controls (DNA extraction reagents and deionized water)

on the pPCP1 plasmid of *Y. pestis*[17]. PCR protocol and gel electrophoresis procedures are described in Hanson *et al*[15]. As with Hanson *et al*[15], a blank extraction used as a negative control was included with each set of flea DNA extractions. Negative extraction controls verified that the extraction reagents were not contaminated with *Y. pestis*. If the negative extraction controls yielded positive result for *Y. pestis*, the entire flea DNA set extracted with that negative extraction control was excluded from any plague assay data analysis. A PCR-negative control consisting of deionized water and a positive control consisting of an avirulent *Y. pestis* strain (A1122 var. *Orientalis,* the Centers for Disease Control and Prevention in Fort Collins, Colorado) were used to control for false positive and false negative results, respectively, for each PCR run (Fig. 2).

Data analysis

CONVERT (ver. 1.2)[18] was used to create input files. MICRO-CHECKER (ver. 2.2.3)[19] checked for allelic dropout among the loci. Linkage disequilibrium and null allele frequencies were calculated in GENEPOP (ver. 4.0.7)[20], while Weir and Cockerham's[21] θ fixation (F_{ST}) and inbreeding coefficients (F_{IS}), the number of alleles per locus, allelic richness (mean ± SE), and Nei's[22] gene diversity indices were estimated using FSTAT (ver. 2.9.3)[23]. The means ± SE for F_{ST}s were calculated by Jackknifing. Observed (H_O) and expected (H_E) heterozygosities, exact tests for Hardy-Weinberg Equilibrium (HWE), and hierarchical Analysis of Molecular Variance (AMOVA)[24] were calculated using ARLEQUIN (ver. 3.0)[25]. For the AMOVA, colonies were partitioned into two areas (FBIR vs. BLM). *P*-value approximations for F_{IS} and F_{ST} were estimated with parameters consisting of 100,000 dememorizations and 1,000,000 Markov chain Monte Carlo (MCMC) steps. We delineated $p \leq 0.01$ for our tests for deviations from HWE as described in Lauretto *et al*[26]. We implemented BAYESASS+ (ver. 1.3)[27] to estimate recent directional migration rates with 95% confidence intervals among all nine colonies. This program uses a Bayesian approach to estimate the likelihood of fleas recently immigrating into each colony from each of the other sampled colonies using multilocus genotypes[27]. BAYESASS+ was implemented with 3,000,000 MCMC iterations, a burn-in of 1,000,000 iterations, a sampling frequency of 2,000 and a delta value of 0.15. Estimates of historic effective number of migrants per generation (MPG) between colonies were calculated using the private allele method[28] implemented in GENEPOP and hand calculations using F_{ST} values averaged across all colonies according to Wright's method[29]. These methods have generally proven equivalent in providing measures of the levels of genetic differentiation among colonies[30].

Yersinia pestis assay analysis

Following the nested-PCR assays, fleas were referenced to their respective burrows to compute *Y. pestis* prevalence per BTPD colony. Individual burrows were designated positive when at least one flea tested positive for *Y. pestis*. For each colony, *Y. pestis* prevalence was estimated as the proportion of burrows containing *O. hirsuta* positive for *Y. pestis* divided by the total number

Table 1. Genetic variability of *Oropsylla hirsuta* from nine black-tailed prairie dog colonies

LOCUS		Black-tailed prairie dog colonies from which *O. hirsuta* were sampled								
		FBIR 97S	FBIR 94	FBIR 95	FBIR 125	FBIR 126	FBIR 127	BLM 42	BLM 43	BLM 47
OH12	N_A	2	5	2	4	4	2	4	3	4
	H_O	0	0.21	0.08	0.15	0.18	0	0.30	0.17	0.46
	H_E	0.15	0.75	0.08	0.50	0.57	0.36	0.36	0.41	0.43
	F_{IS}	1	0.72	–	0.70	0.69	1	0.18	0.60	–0.08
	P-value (HWE)	0.04	0	1	0.002	0.002	0.11	0.11	0.01	0.05
OH13	N_A	10	8	16	10	9	9	7	14	12
	H_O	0.18	0.55	0.36	0.27	0.29	0.43	0.71	0.46	0.45
	H_E	0.89	0.85	0.95	0.91	0.95	0.93	0.81	0.95	0.94
	F_{IS}	0.80	0.37	0.63	0.71	0.71	0.56	0.13	0.52	0.53
	P-value (HWE)	0	0	0	0	0	0	0.07	0	0
OH84	N_A	13	9	11	12	7	7	5	12	13
	H_O	0.75	0.57	0.79	0.86	0.54	0.25	0.64	0.71	0.33
	H_E	0.91	0.81	0.85	0.93	0.80	0.88	0.77	0.89	0.89
	F_{IS}	0.18	0.30	0.08	0.08	0.34	0.73	0.18	0.20	0.63
	P-value (HWE)	0.03	0.03	0.68	0.25	0.02	0	0.48	0.004	0
OH158	N_A	5	5	1	4	4	2	6	4	3
	H_O	0.71	0.21	–	0.30	0.50	0.25	0.55	0.17	0.12
	H_E	0.59	0.27	–	0.55	0.44	0.25	0.59	0.16	0.12
	F_{IS}	–0.22	0.21	–	0.47	–0.14	–	0.08	–0.03	–0.02
	P-value (HWE)	1	0.21	–	0.03	1	1	0.41	1	1
OH369	N_A	6	6	6	7	5	6	6	6	6
	H_O	0.67	0.29	0.50	0.73	0.38	0.50	0.18	0.22	0.50
	H_E	0.79	0.84	0.80	0.85	0.81	0.78	0.76	0.77	0.73
	F_{IS}	0.16	0.67	0.39	0.14	0.55	0.38	0.77	0.72	0.32
	P-value (HWE)	0.80	0	0.03	0.05	0.01	0.01	0	0	0.01
OH586	N_A	3	6	9	7	4	4	4	4	3
	H_O	0.29	0.33	0.36	0.09	0	0	0	0.25	0
	H_E	0.47	0.76	0.71	0.80	0.60	0.86	0.86	0.44	0.39
	F_{IS}	0.41	0.58	0.50	0.89	1	1	1	0.45	1.00
	P-value (HWE)	0.44	0.004	0.001	0	0	0.01	0.01	0.14	0.004
CGA4	N_A	6	10	8	6	8	4	6	5	5
	H_O	0.56	0.42	0.85	0.33	0.33	0	0.29	0.60	0.80
	H_E	0.82	0.90	0.81	0.82	0.86	0.80	0.87	0.73	0.76
	F_{IS}	0.33	0.55	–0.04	0.61	0.63	1	0.69	0.19	–0.05
	P-value (HWE)	0.17	0	0.004	0.002	0	0.003	0.01	0.01	0.003
CGA14	N_A	5	6	5	7	6	3	3	7	9
	H_O	0.25	0.20	0.08	0.27	0.20	0.25	0.13	0.27	0.25
	H_E	0.81	0.76	0.64	0.77	0.84	0.61	0.49	0.81	0.86
	F_{IS}	0.70	0.75	0.88	0.66	0.77	0.63	0.76	0.67	0.72
	P-value (HWE)	0	0	0	0	0	0.14	0.02	0	0
All loci	N_A	6	7	7	7	6	5	5	7	7
	H_O	0.43	0.35	0.43	0.38	0.30	0.21	0.35	0.36	0.36
	H_E	0.68	0.74	0.69	0.77	0.73	0.68	0.69	0.65	0.64
	F_{IS}	0.33	0.43	0.26	0.45	0.47	0.69	0.38	0.33	0.29
	P-value (HWE)	0	0	0	0	0	0	0	0	0

N_A= Number of alleles; H_O = Observed heterozygosity; H_E = Expected heterozygosity; F_{IS} = Inbreeding coefficient; HWE = The probability of rejecting Hardy-Weinberg equilibrium (significant *p*-values <0.01; Lauretto *et al*[26]). Several HWEs were recorded up to three decimal places when an integer was present.

of burrows from which *O. hirsuta* were tested. To determine if any significant relationship between *O. hirsuta* genetic structure per colony and the *Y. pestis* prevalence existed, correlation analyses were conducted. The means of Nei's gene diversity and allelic richness were compared to plague prevalence by individual colonies using Pearson correlation coefficients. A test of significance ($p = 0.05$) was performed using Bonferroni probabilities. The means of the posterior probability distribution of migration rates of the proportion of residents as inferred by BAYESASS+ (see diagonal values in Tables) were regressed against the corresponding *Y. pestis* prevalence. The idea was that the ratio of non-migrants represents the inverse of the number of migrants into each respective colony. Our objective was to test the assumption that colonies exposed to more immigrating fleas would have higher *Y. pestis* prevalence. Colonies may display asymmetrical flea migration which then may be proportional to the number of infected fleas exhibited within the colonies. All correlation and regression analyses were conducted with MYSTAT™ software (ver. 12.02.00; SYSTAT™).

RESULTS

Oropsylla hirsuta landscape genetics

A total of 129 *O. hirsuta* were used in the genetic study. All eight loci used were unlinked and showed no indications of allelic dropout. One colony was monomorphic for one locus (F95: locus OH158); otherwise, all loci were polymorphic among the nine colonies (Table 1). Estimated frequencies of null alleles were from 0–0.55. Significant heterozygote deficiencies from HWE were observed in 34 of 71 tests (47.9%). Inbreeding coefficients were high (mean $F_{IS} = 0.52 \pm 0.07$, Fig. 1), while F_{ST} estimates showed a consistent pattern of low genetic heterogeneity across all loci (mean $F_{ST} = 0.04 \pm 0.01$). This finding was supported by the hierarchical AMOVA which showed 92.8% of the variation in allele frequencies was within the colonies and 5.7% variation was among the colonies between both regions (FBIR and BLM).

Patterns of recent gene flow were asymmetrical (Table 2). Out of 72 pairwise comparisons (excluding residents), 58 had high migration rates (>0.05 ± SE). Historical gene flow (N_M) based on Slatkin's method was 1.15 MPG. Mean N_M estimate from F_{ST} values was 8.13 MPG. Lastly, allelic richness for all colonies ranged from 3.80–4.55 with gene diversity varying from 0.62–0.79 (Table 3).

Yersinia pestis prevalence

Yersinia pestis prevalence ranged from 0 (colonies FBIR 94 and 125) to 44.4% (colony BLM 42) with a mean ± SE of 12.06 ± 4.57% (Table 3). Results from the

Table 2. Means (95% confidence intervals) of the posterior distribution of the recent migration rates for *Oropsylla hirsuta* derived from BAYESASS + across all black-tailed prairie dog colonies. Individuals migrating into colonies listed in columns and those migrating from colonies in rows. Bold values on the diagonal are the proportions of derived (nonmigrants) *O. hirsuta* from their source colonies.

Colony	FBIR 97S	FBIR 94	FBIR 95	FBIR 126	FBIR 127	FBIR 125	BLM 42	BLM 43	BLM 47
FBIR 97S	**0.69 (0.67-0.75)**	0.01 (0-0.06)	0.01 (0-0.06)	0.01 (0-0.07)	0.009 (0-0.06)	0.01 (0-0.05)	0.009 (0-0.05)	0.01 (0-0.06)	0.23 (0.14-0.31)
FBIR 94	0.01 (0-0.05)	**0.70 (0.67-0.86)**	0.01 (0-0.05)	0.10 (0-0.22)	0.02 (0-0.13)	0.01 (0-0.06)	0.01 (0-0.06)	0.01 (0-0.06)	0.12 (0.04-0.21)
FBIR 95	0.009 (0-0.05)	0.008 (0-0.05)	**0.69 (0.67-0.75)**	0.01 (0-0.07)	0.01 (0-0.05)	0.009 (0-0.06)	0.009 (0-0.05)	0.009 (0-0.05)	0.24 (0.15-0.31)
FBIR 126	0.008 (0-0.05)	0.01 (0-0.10)	0.007 (0-0.05)	**0.88 (0.67-1.00)**	0.008 (0-0.05)	0.006 (0-0.04)	0.006 (0-0.05)	0.008 (0-0.05)	0.06 (0-0.23)
FBIR 127	0.02 (0-0.09)	0.02 (0-0.09)	0.01 (0-0.08)	0.05 (0-0.17)	**0.74 (0.67-0.90)**	0.02 (0-0.08)	0.02 (0-0.10)	0.02 (0-0.10)	0.10 (0.02-0.22)
FBIR 125	0.01 (0-0.06)	0.01 (0-0.06)	0.009 (0-0.05)	0.03 (0-0.12)	0.01 (0-0.09)	**0.70 (0.67-0.76)**	0.01 (0-0.07)	0.01 (0-0.05)	0.20 (0.10-0.30)
BLM 42	0.01 (0-0.06)	0.01 (0-0.07)	0.01 (0-0.07)	0.04 (0-0.13)	0.01 (0-0.07)	0.01 (0-0.07)	**0.70 (0.67-0.79)**	0.01 (0-0.07)	0.18 (0.08-0.29)
BLM 43	0.007 (0-0.04)	0.007 (0-0.04)	0.007 (0-0.04)	0.04 (0-0.13)	0.007 (0-0.04)	0.006 (0-0.03)	0.008 (0-0.05)	**0.70 (0.67-0.79)**	0.22 (0.12-0.31)
BLM 47	0.003 (0-0.03)	0.003 (0-0.02)	0.003 (0-0.02)	0.004 (0-0.03)	0.003 (0-0.02)	0.003 (0-0.02)	0.003 (0-0.02)	0.003 (0-0.03)	**0.98 (0.91-1.00)**

Table 3. Mean (S.E.) *Yersinia pestis* prevalence, allelic richness, and gene diversity per colony. Standard errors are included with the gene diversity and allelic richness for *O. hirsuta* from the black-tailed prairie dog colonies. The mean ± (SE) of *Y. pestis* prevalence = 12.06 ± 4.57%

Colony	Prevalence (%)	Allelic richness	Gene diversity
FBIR 97S	11.8	4.03 (0.53)	0.69 (0.09)
FBIR 94	0	4.30 (0.38)	0.76 (0.07)
FBIR 95	20	3.94 (0.69)	0.62 (0.13)
FBIR 126	7.1	4.55 (0.43)	0.79 (0.06)
FBIR 127	11.1	4.29 (0.44)	0.76 (0.07)
FBIR 125	0	3.84 (0.53)	0.74 (0.10)
BLM 42	44.4	3.80 (0.35)	0.72 (0.08)
BLM 43	3.4	3.92 (0.61)	0.66 (0.10)
BLM 47	10.7	3.94 (0.63)	0.65 (0.11)

correlation analysis between the genetic structure of *O. hirsuta* samples and *Y. pestis* prevalence indicated that gene diversity ($p = 0.13$) and allelic richness ($p = 0.62$) were not correlated with the number of *Y. pestis* positive burrows on a per-colony basis. Regression analysis between the mean proportion of inferred residents from BAYESASS+ and *Y. pestis* prevalence by colony was non-significant ($r^2 = 0.02$, $p = 0.76$).

DISCUSSION

We estimated genetic variability and gene flow among *O. hirsuta* on nine BTPD colonies at the onset of a widespread plague outbreak. We also investigated whether *O. hirsuta* landscape genetic structure influenced *Y. pestis* prevalence within the study colonies. We found that low levels of genetic differentiation among fleas on BTPD colonies were consistent with studies of other ectoparasitic vectors of infectious zoonotic diseases[12]. Additionally, high gene flow estimates and little genetic differentiation among fleas from different colonies suggest that fleas readily move between neighboring colonies[4]. The lack of correlation between per-colony *Y. pestis* prevalence and *O. hirsuta* genetic variability is consistent with a scenario of readily dispersing fleas[4]. At this spatial scale, movements of hosts other than BTPDs were likely providing dispersal opportunities for these vectors allowing the potential for fleas to move between colonies[4]. Gene flow estimates and results from AMOVA and BAYESASS+ (64.2% of the pairwise estimates had ≥ 5% immigration [95% CI] into colonies) suggest that *O. hirsuta* were moving between colonies, but high inbreeding estimates (F_{IS}) indicated the possibility that intra-colony gene flow might have been somewhat restricted. These results were consistent with several studies which investigated vector genetic variability[5, 12,13, 16, 31-34] that found vectors like *O. hirsuta* are highly polymorphic across all loci and populations.

These results have strong implications for our understanding of how *Y. pestis* disperses across the prairie landscape. Consistent with our results, Tripp *et al*[35] found an increase in the number of fleas on BTPD colonies and an increase in the number of *Y. pestis*-infected fleas on individual prairie dogs during outbreaks in north-central Colorado. *Oropsylla hirsuta* was the most common flea on these colonies[35]. These findings suggest that flea vagility and/or fecundity may increase just prior to, or during, epizootics. We have no data on flea fecundity from the study colonies, but if increased flea vagility drives the increase in flea abundance, we would expect the high gene flow and genetic variability estimates indicative of flea dispersal that we observed in our study colonies. Additionally, if flea vagility increases during plague epizootics, we expect to find a positive correlation between flea gene flow and *Y. pestis* prevalence. In order to test this hypothesis, we estimated the correlation between the percent of *Y. pestis*-positive burrows and the genetic structure of *O. hirsuta* on a per-colony basis. Significant positive correlations would suggest that increased flea vagility influences the spread of *Y. pestis* among BTPD colonies. We found that 12% of burrows (range 0–44.4%) harboring infected *O. hirsuta* before the epizootic became apparent on individual colonies and that the landscape genetic variability and structure of *O. hirsuta* were not correlated with *Y. pestis* prevalence. The enzootic nature of *Y. pestis* on these colonies[15] and the high vagility of *O. hirsuta*[4] may have swamped out our ability to detect this hypothesized correlation.

Mollison and Levin[36] described circumstances under which diseases like sylvatic plague could be maintained in a colony. Plague outbreaks would persist on colonies, if: (i) a number of 'infected' vectors (in this case, fleas) dispersed to the colonies; (ii) those infected fleas were maintained on the colony until a 'threshold' number was reached; and (iii) not all of the hosts (i.e. BTPDs, badgers, coyotes, etc.) succumbed to plague[36]. Thus, prairie dog colonies could maintain low levels of infected fleas as 'reservoirs' without an epizootic transpiring. This would allow infected fleas to be dispersed during enzootic phases between plague outbreaks. Hanson *et al*[15] further supported this hypothesis finding no apparent epizootic even with up to 20% of sampled fleas *Y. pestis*-positive. Our study showed colonies succumbing to the plague outbreak near colonies showing no signs of a pending outbreak. However, by the following season, all the colonies were extirpated, which appeared to follow the pattern suggested by

Mollison and Levin[36]. The genetic structure of *O. hirsuta* from our nine colonies supports the findings of Mollison and Levin[36] in that fleas readily dispersed among colonies potentially spreading *Y. pestis* to unaffected BTPD colonies before a widespread epizootic was initiated.

In summary, our study demonstrated that *O. hirsuta* were able to disperse between BTPD colonies potentially spreading *Y. pestis* throughout the prairie ecosystem. This suggests that once they are started, epizootics are likely to become widespread as fleas move readily among BTPD colonies.

ACKNOWLEDGEMENTS

The authors wish to thank the Assiniboine Tribal Authorities at the Fort Belknap Indian Reservation and the U.S. Bureau of Land Management for granting permission to conduct research on their BTPD colonies. We also thank G. Gerrish and A. Willuweit for their field assistance, S. Story from the Montana Department of Fish, Wildlife, and Parks and Geo Science Associates, Inc. in Billings, MT for GIS datasets, and G. Kaiser from the U.S. Bureau of Indian Affairs for logistical support. We thank E. Monroe for comments on the manuscript and R. Matchett for preparing Fig. 1. Partial funding was provided by the Nelson Foundation and The University of South Dakota's Office of Research and Sponsored Projects. This research was based upon work supported by the National Science Foundation/EPSCoR Grant No. 0091948 and by the State of South Dakota.

REFERENCES

1. Miller B, Reading R, Hoogland J, Clark T, Ceballos G, List R, *et al*. The role of prairie dogs as a keystone species: response to Stapp. *Conserv Biol* 2000; *14:* 318–21.
2. Salkeld DJ, Stapp P. Prevalence and abundance of fleas in black-tailed prairie dog burrows: implications for the transmission of plague (*Yersinia pestis*). *J Parasitol* 2008; *94:* 616–21.
3. Wilder AP, Eisen RJ, Bearden SW, Montenieri JA, Tripp DW, Brinkerhoff RJ, *et al*. Transmission efficiency of two flea species (*Oropsylla tuberculata cynomuris* and *Oropsylla hirsuta*) involved in plague epizootics among prairie dogs. *Eco Health* 2008; *5:* 205–12.
4. Jones PH, Britten HB. The absence of concordant population genetic structure in the black-tailed prairie dog and the flea, *Oropsylla hirsuta*, with implications for the spread of *Yersinia pestis*. *Mol Ecol* 2010; *19:* 2038–49.
5. Antonio-Nkondjio C, Ndo C, Awono-Ambene P, Ngassam P, Fontenille D, Simard F. Population genetic structure of the malaria vector *Anopheles moucheti* in south Cameroon forest region. *Acta Trop* 2007; *101:* 61–8.
6. Bataille A, Cunningham AA, Cedeño V, Cruz M, Eastwood G, Fonseca DM, *et al*. Evidence for regular ongoing introductions of mosquito disease vectors into the Galápagos Islands. *Proc R Soc Lond. Ser B: Biol Sci* 2009; *276:* 3769–75.
7. Failloux A-B., Vazeille M, Rodhain F. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. *J Mol Evol* 2002; *55:* 653–63.
8. Slatkin M. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 1995; *139:* 457–62.
9. Balloux F, Lugon-Moulin N. The estimation of population differentiation with microsatellite markers. *Mol Ecol* 2002; *11:* 155–65.
10. Donnelly MJ, Simard F, Lehmann T. Evolutionary studies of malaria vectors. *Trends Parasitol* 2002; *18:* 75–80.
11. Ravel S, de Meeus T, Dujardin JP, Zézé DG, Gooding RH, Dusfour I, *et al*. The tsetse fly *Glossina palpalis palpalis* is composed of several genetically differentiated small populations in the sleeping sickness focus of Bonon, Côte d'Ivoire. *Infect Genet Evol* 2007; *7:* 116–25.
12. Bello F, Becerra V. Genetic variability and heterogeneity of Venezuelan equine encephalitis virus vector *Ochlerotatus taeniorhynchus* (Diptera: Culicidae) populations of the Colombian Atlantic coast, based on microsatellite loci. *Gen Mol Res* 2009; *8:* 1179–90.
13. Jones PH, Wallace L, Britten HB. Isolation and characterization of 11 microsatellite loci from *Oropsylla hirsuta*, a vector of sylvatic plague. *Mol Ecol Resour* 2009; *9:* 1041–4.
14. Lewis RE. A review of the North American species of *Oropsylla* Wagner and Ioff, 1926 (Siphonaptera: Ceratophyllidae: Ceratophyllinae). *J Vector Ecol* 2002; *27:* 184–206.
15. Hanson DA, Britten HB, Restani M, Washburn LR. High prevalence of *Yersinia pestis* in black-tailed prairie dog colonies during an apparent enzootic phase of sylvatic plague. *Conserv Biol* 2007; *8:* 789–95.
16. Binz T, Bonfils D, Balloux F, Richner H. Isolation and characterization of 23 microsatellite loci in the hen flea *Ceratophyllus gallinae*. *Mol Ecol Resour* 2003; *3:* 215–7.
17. Hinnebusch BJ, Fischer ER, Schwan TG. Evaluation of the role of the *Yersinia pestis* plasminogen activator and other plasmid-encoded factors in temperature-dependent blockage of the flea. *J Infect Dis* 1998; *178:* 1406–15.
18. Glaubitz JC. CONVERT: a user friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol Ecol Resour* 2004; *4:* 309–10.
19. Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Resour* 2004; *4:* 535–8.
20. Raymond M, Rousset F. GENEPOP: Population genetics software for exact tests and ecumenicism. *J Hered* 1995; *86:* 248-249.
21. Weir BS, Cockerham CC. Estimating F-statistics for the analysis of population structure. *Evolution* 1984; *38:* 1358–70.
22. Nei M. Molecular evolutionary genetics. New York : Columbia University Press 1987; p. 208–53.
23. Goudet J. FSTAT (version 1.2): a computer program to calculate F-statistics. *J Hered* 1995; *86:* 485–6.
24. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 1992; *131:* 479–91.
25. Excoffier L, Laval G, Schneider S. ARLEQUIN ver. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinf Online* 2005; *1:* 47–50.
26. Lauretto MS, Nakano F, Faria Jr SR, Pereira CAB, Stern JM. A

straightforward multiallelic significance test for the Hardy-Weinberg equilibrium law. *Genet Mol Biol* 2009; *32:* 619–25.
27. Wilson GA, Rannala B. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 2003; *163:* 1177–91.
28. Slatkin M. Rare alleles as indicators of gene flow. *Evolution* 1985; *39:* 53–65.
29. Wright S. The interpretation of population structure by F-structuring with special regard to systems of mating. *Evolution* 1965; *19:* 395–420.
30. Slatkin M, Barton NH. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 1989; *43:* 1349–68.
31. Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, Costero A, Clark GG, *et al.* Dispersal of the dengue vector *Aedes aedgypti* within and between rural communities. *Am J Trop Med Hyg* 2005; *72:* 209–20.
32. Pérez de Rosas AR, Segura EL, Fichera L, García BA. Macrogeographic and microgeographic genetic structure of the Chagas' disease vector *Triatoma infestans* (Hemiptera: Reduviidae) from Catamarca, Argentina. *Genetica* 2008; *133:* 247–60.
33. Pizarro JC, Gilligan LM, Stevens L. Microsatellites reveal a high population structure in *Triatoma infestans* from Chuquisaca, Bolivia. *PLoS Neglect Trop Dis* 2008; *3:* e202.
34. Julio NB, Chiappero MB, Rossi HJ, Dueòas JCR, Gardenal CN. Genetic structure of *Aedes aegypti* in the city of Córdoba (Argentina), a recently reinfested area. *Mem I Oswaldo Cruz* 2009; *104:* 626–31.
35. Tripp DW, Gage KL, Montenieri JA, Antolin MF. Flea abundance on black-tailed prairie dogs (*Cynomys ludovicianus*) increases during plague epizootics. *Vector Borne Zoonotic Dis* 2009; *9:* 313–21.
36. Mollison D, Levin SA. Spatial dynamics of parasitism. In: Grenfell BT, Dobson AP, editors. *Ecology of infectious diseases in natural populations*. United Kingdom : Cambridge University Press 1995; p. 384–449.

Correspondence to: Philip H. Jones, Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, Iowa 52246, U.S.A.
E-mail: Philip-Jones@uiowa.edu

Received: 26 October 2010 *Accepted in revised form:* 13 June 2011