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Short Communication

Parasitism of Black-Tailed Prairie Dogs by *Linognathoides cynomyis* (Phthiraptera: Polyplacidae)

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Abstract

The following study investigates louse parasitism of black-tailed prairie dogs (*Cynomys ludovicianus* (Ord, Rodentia: Sciuridae)) on 20 plots at 13 colonies in the short-grass prairie of New Mexico, USA, June–August, 2011–2012. Among 124 lice collected from 537 prairie dogs during 1,207 sampling events in which anesthetized animals were combed for ectoparasites, all of the lice were identified as *Linognathoides cynomyis* (Kim, Phthiraptera: Polyplacidae). Data were analyzed under an information-theoretic approach to identify factors predicting louse parasitism. Lice were most prevalent on plots with high densities of prairie dogs. At the scale of hosts, lice were most abundant on prairie dogs in poor body condition (with low mass:foot ratios) and prairie dogs harboring large numbers of fleas (Siphonaptera, mostly *Oropsylla hirsuta* (Baker, Siphonaptera: Ceratophyllidae) and *Pulex simulans* (Baker,Siphonaptera: Pulicidae)). Lice have been implicated as supplemental vectors of the primarily flea-borne bacterium *Yersinia pestis* (Yersin, Enterobacteriales: Yersiniaceae), a re-emerging pathogen that causes sylvatic plague in prairie dog populations. Coparasitism by lice and fleas, as found herein, might enhance plague transmission. *L. cynomyis* deserves attention in this context.

Key words: flea, louse, Phthiraptera, prairie dog, Siphonaptera, *Yersinia pestis*

The black-tailed prairie dog (BTPD, *Cynomys ludovicianus* (Ord, Rodentia: Sciuridae) Ord 1815) is a colonial burrowing rodent in the Great Plains of North America. Since the late-1800s, BTPDs have undergone substantial declines due to poisoning campaigns, recreational shooting, habitat conversions, and disease (Hoogland 1995). When abundant, BTPDs structure grassland ecosystems as foundation and keystone species (Kotliar et al. 2006). Moreover, like all wildlife, including hundreds of vertebrates and invertebrates that associate with them, BTPDs have intrinsic value (Miller and Reading 2012).

Mostly due to the ecologic importance of BTPDs, the scientific community has studied ectoparasites and vector-borne diseases among their populations. Fleas (Insecta: Siphonaptera) have received the most attention due to the catastrophic effects of *Yersinia pestis* (Yersin, Enterobacteriales: Yersiniaceae), the bacterial agent of sylvatic plague, on BTPDs (Eads and Biggins 2015). Less attention has been directed at other ectoparasites. Blood-sucking lice (Insecta: Phthiraptera) of the suborder Anoplura have received little attention.

The majority of lice collected from BTPDs are probably *Linognathoides cynomyis* (Kim, Phthiraptera: Polyplacidae) (Kim 1986, Kim et al. 1986). McCampbell (1931) reported lice from BTPDs in Colorado. Menzies et al. (1951) reported lice from BTPDs in Texas. McKenna et al. (1977) reported lice from BTPDs

in North Dakota. Hoogland (1979) found more lice on BTPDs in South Dakota than white-tailed prairie dogs (*Cynomys leucurus*) in Wyoming. Kietzmann (1987) found *L. cynomyis* (Kim 1986) on BTPDs in South Dakota. Kucera et al. (2007) reported *L. cynomyis* from BTPDs in Nebraska.

Little is known about the ecology of lice on BTPD colonies. Further, it remains unknown if louse parasitism negatively affects BTPD populations. Lice have been implicated as supplemental vectors of *Y. pestis* to rodents (Blanc and Baltazard 1942, Houhamdi et al. 2006) and humans (Dean et al. 2018). Continued research on louse parasitism of BTPDs is therefore warranted.

Here, I report on lice from BTPDs in New Mexico. The primary objective was to study flea parasitism of BTPDs, with implications for the maintenance and spread of plague (Eads 2014). Trapping and sampling of BTPDs allowed for an investigation of louse parasitism. Colleagues and I 1) collected lice and keyed them to species, 2) identified factors predicting the occurrence of lice on BTPD colonies, and 3) identified factors predicting the abundance of lice on BTPDs.

Materials and Methods

Field research was completed under Colorado State University Institutional Animal Care and Use Committee Protocol #10-1785A.

We studied BTPDs on the short-grass prairie of Vermejo Park Ranch, Colfax County, New Mexico (36° 32′ N, 104° 45′ W). Sampling occurred on 1.54 and 2.25 ha plots (*n* = 20) distributed among 13 colonies during June–August, 2010–2012. BTPD burrows on 10 plots had been treated with 0.05% deltamethrin dust 4–11 yr before our study began (Eads et al. 2013, 2016).

We live-trapped BTPDs in Tomahawk traps (Hazelhurst, Wisconsin) distributed on plots at a density of 16 ha^{-1}. We anesthetized each BTPD using isoflurane in an induction chamber and combed it with a fine-toothed comb for 45 s to remove ectoparasites (Eads et al. 2013, 2016). Each BTPD was visually classified by agesex (i.e., adult female, adult male, juvenile female, juvenile male). We measured each BTPD's body mass and right hind foot, allowing for calculation of mass:foot ratios that assume lower mass:foot ratios represent lower body condition, and mass and foot length scale isometrically (Schulte-Hostedde et al. 2001).

Lice were collected during 2011 (20 plots) and 2012 (19 plots) and were identified to species using a stereo zoom microscope and keys (Kim 1986, CDC (https://www.cdc.gov/nceh/ehs/docs/pictorial_keys/lice-anoplura.pdf). Fleas were also identified to species (Eads et al. 2016).

In the assessment louse occurrence on BTPD colonies, we ran regression models with individual plots as sampling units and a binomial variable for louse detection in a given year as a response ('GLIMMIX', SAS version 9.3, SAS Institute Inc.). Three independent variables were considered: *YEAR* of sampling (to assess louse phenology), the historical use or nonuse of deltamethrin *DUST* on plots (because lice are susceptible to deltamethrin; Kim 2006), and the *DENSITY* of BTPDs (indexed as the number of unique BTPDs captured on a plot divided by the area of a plot). Eight possible models, including an intercept-only model, were ranked by Akaike's Information Criterion (i.e., AICc; Burnham and Anderson 2002). AICc values were used to calculate model weights, which range from 0 (no support) to 1 (strong support; Burnham and Anderson 2002). We calculated evidence ratios for all models, using the evidence ratios for model selection (Anderson 2008). Upon model selection, if the 90% confidence interval of a variable's parameter (β) encompassed 0, the associated variable was considered a poor predictor of louse occurrence given the data (Burnham and Anderson 2002).

In the assessment of louse abundance on BTPDs (i.e., counts of lice, including zeroes), the data were restricted to plots on which ≥1 louse was collected in a given year, thereby concentrating on plots known to be occupied by lice. Four variables were of interest, including *AGE-SEX* and body *CONDITION*. We included a variable for *MONTH* to evaluate the phenology of lice at a scale finer than *YEAR*, and a variable for the abundance of *FLEAS* on BTPDs to assess coparasitism by lice and fleas. *YEAR*, *DUST*, and *DENSITY* were poor predictors of louse abundance and were excluded from this assessment. We ran 16 possible models, including an intercept-only model, using negative binomial regression ('GLIMMIX' in SAS) and evaluated the models using AICc, model weights, and evidence ratios. Results remained the same when the analysis corrected for BTPD body size (lice per unit mass$^{-0.67}$; Heusner 1985).

In a few cases, we measured the aggregation of lice on individual BTPDs using the negative binomial parameter *k*. The parameter converges toward 0 as aggregation increases and a smaller number of BTPDs carry the largest numbers of lice (Wilson et al. 2002). We estimated *k* using the MASS package and 'theta' library in R version 2.13.2 (R Development Core Team 2011).

Results

During 2011 and 2012, we sampled 537 individual BTPDs during 1,207 sampling events and collected 124 lice, all of which were identified as *L. cynomyis*. Lice were found during 7% of processing events. The abundance of lice on BTPDs ranged from 0 to 6 in 2011 and from 0 to 10 in 2012. We found lice on 13 of 20 plots and 10 of 13 colonies in 2011, and on 8 of 19 plots and 7 of 13 colonies in 2012. Lice were aggregated on particular BTPDs (*k* = 0.08, *SE* = 0.02) (Fig. 1). Greater than 99% of the 4,993 fleas considered herein were identified as *Oropsylla hirsuta* (Baker, Siphonaptera: Ceratophyllidae) (a *Cynomys* specialist) and *Pulex simulans* (Baker, Siphonaptera: Pulicidae) (a generalist of many mammals).

In the assessment of louse occurrence on plots, four models received AICc weights ≥ 0.14 and their evidence ratios were similar (Table 1). Lice were predicted to be less prevalent in 2012 than in 2011 and less prevalent on plots with a history of DUST treatment. However, in the most supported model, *YEAR* (β = 1.28, –0.03–2.59) and *DUST* (β = 1.45, 0.00–2.90) were poor predictors of louse occurrence. Lice were predicted to be more prevalent on plots with higher *DENSITY* of BTPDs; this variable was in the top four models and was an important predictor of louse occurrence in the most parsimonious model, which included *DENSITY* (β = 0.20, 0.03–0.37) and was four times more supported than an intercept-only model.

In the assessment of louse abundance on BTPDs at plots where lice were found, lice were aggregated on particular BTPDs (*k* = 0.15, *SE* = 0.03, *n* = 721 combings). The most supported model received a weight of 0.43; it was 2 to 72 times more supported than any other model and was used for interpretation (Table 2). Lice were most abundant in the *MONTH* of June (β = 1.97, 1.34–2.60) and less abundant in July and August. Lice were more abundant on BTPDs in lower *CONDITION* (β = –0.01, –0.02––0.01) and BTPDs harboring more *FLEAS* (β = 0.04, 0.02–0.06).

Discussion

It seems probable that *L. cynomyis* occurs throughout much of the BTPD range (Kim et al. 1986). However, unless hosts are anesthetized and examined intensively for lice, or hosts are killed and inspected, lice are notoriously difficult to detect (Kim et al. 1986). To date, lice have not been reported for BTPDs in New Mexico (Ford et al. 2004). Here, we report on *L. cynomyis* parasitism of BTPDs in northeastern New Mexico, with lice collected using a standardized procedure (Eads et al. 2013).
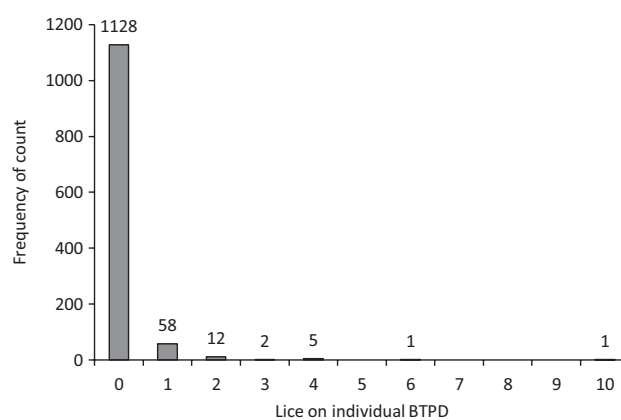

Fig. 1. Frequency distribution for the abundance of lice (*Linognathoides cynomyis*) on 537 black-tailed prairie dogs (*Cynomys ludovicianus*) during 1,207 sampling events at the Vermejo Park Ranch, New Mexico, June–August, 2011–2012.

Table 1. Relative support (weights) and evidence ratios for models of the occurrence (detection/nondetection) of lice (*Linognathoides cynomyis*) on plots where we sampled black-tailed prairie dogs (*Cynomys ludovicianus*), Vermejo Park Ranch, New Mexico, June–August, 2011–2012

Model	AICc	ΔAICc	Weight	Evidence ratio
Intercept + YEAR + DUST + DENSITY	52.99	0	0.27	1
Intercept + DUST + DENSITY	53.49	0.50	0.21	1.28
Intercept + YEAR + DENSITY	53.77	0.78	0.18	1.48
Intercept + DENSITY	53.80	0.81	0.18	1.50
Intercept	55.94	2.95	0.06	4.37
Intercept + YEAR	56.10	3.11	0.06	4.74
Intercept + DUST	57.54	4.55	0.03	9.73
Intercept + YEAR + DUST	57.72	4.73	0.02	10.64

Models are ranked from low to high by Akaike's Information Criterion for small sample sizes (AICc). Also presented are differences between AICc values (ΔAICc), which are used to calculate model weights (ranging from 0 = no support to 1 = strong support) and evidence ratios (using model weights and the most supported model for reference).

Table 2. Relative support (weights) and evidence ratios for models that evaluated the abundance of lice (*Linognathoides cynomyis*) on black-tailed prairie dogs (*Cynomys ludovicianus*), Vermejo Park Ranch, New Mexico, June–August, 2011–2012

Model	AICc	ΔAICc	Weight	Evidence ratio
Intercept + MONTH + CONDITION + FLEAS	611.59	0	0.43	1
Intercept + MONTH + AGESEX + CONDITION + FLEAS	612.40	0.81	0.29	1.50
Intercept + MONTH + AGESEX + CONDITION	615.85	4.26	0.05	8.41
Intercept + MONTH + CONDITION	619.65	8.06	0.01	56.26
Intercept + MONTH + FLEAS	620.13	8.54	0.01	71.52

Models are ranked from low to high by Akaike's Information Criterion for small sample sizes (AICc). Also presented are differences between AICc values (ΔAICc), which are used to calculate model weights (ranging from 0 = no support to 1 = strong support) and evidence ratios (using model weights and the most supported model for reference). This table is limited to models with a weight >0.

It is perhaps unsurprising that host-related factors correlated with louse parasitism of BTPDs. Most lice spend the majority or entirety of life on hosts (Kim 2006, Monello and Gompper 2009). In our study, lice tended to occur on plots with high densities of BTPDs. Where BTPDs are abundant, more patches of hosts (habitat) and food resources (blood) are available to *L. cynomyis*.

Like fleas (Eads et al. 2016), lice were most abundant on BTPDs in poor condition. When in poor condition, rodents are malnourished and their immunological defenses against ectoparasites and their behavioral defenses are compromised. In such times, ectoparasites may proliferate (Lochmiller and Deerenberg 2000); host grooming is an important defense against lice (Murray 1987). Ectoparasites may cause a further decline in host condition, creating a feedback cycle that benefits the ectoparasites (Beldomenico and Begon 2010).

Lice were most abundant on BTPDs carrying large numbers of fleas. Particular BTPDs may be especially prone to louse and flea parasitism (e.g., BTPDs in poor condition, as found herein). On hosts with large numbers of lice and fleas, the ectoparasites may behave in ways that help to reduce competition. For example, although not quantified herein, observations from our study suggest lice preferentially inhabit areas, and lay eggs under BTPD legs. In contrast, observations suggest fleas commonly inhabit the leg axillae, dorsum, and rump (Eads et al. 2017). Spatial separation might help to reduce competition for sites that are used for feeding and breeding, and reduce competition for blood meals.

The study herein provides new information on louse parasitism of BTPDs but continued study is warranted. We studied BTPDs at one site during short periods of time (June–August 2011 and 2012). Louse parasitism of BTPDs is expected to vary regionally. Moreover, louse parasitism can vary seasonally (as found herein for summer) and may oscillate substantially when studied for periods >2 yr. Louse parasitism might also vary with host-related factors not evaluated herein (e.g., BTPD reproduction in spring and microclimates within BTPD burrows).

We found only 124 lice, with an observed maximum of 10 lice on one BTPD. Prior research demonstrates that lice can attain high densities on BTPDs, raising suspicion that louse parasitism might affect BTPD populations. In South Dakota, Hoogland (1979, 1995) found that lice were sometimes so numerous on BTPDs that only estimates of 25, 50, 75, and 100 lice were made (Hoogland 1979). Perhaps when abundant, lice negatively affect BTPDs, for example by transmitting pathogens.

Lice are capable of vectoring several types of pathogens, including *Y. pestis*, the plague bacterium. Dean et al. (2018) studied lice in the context of plague transmission (to humans) and aptly noted, 'it is crucial that we understand the full spectrum of capabilities' of plague, a 'versatile, pandemic disease'. In populations of rodents, coparasitism by lice and fleas might increase the probability of *Y. pestis* transmission, facilitated by exchange of parasites among hosts in the confines of burrows and nests (Durden 1983, Krasnov 2008). *L. cynomyis* deserves attention in this context.

Acknowledgments

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