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Host traits, identity, and ecological conditions predict consistent flea abundance and prevalence on free-living California ground squirrels

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ABSTRACT

Understanding why some individuals are more prone to carry parasites and spread diseases than others is a key question in biology. Although epidemiologists and disease ecologists increasingly recognize that individuals of the same species can vary tremendously in their relative contributions to the emergence of diseases, very few empirical studies systematically assess consistent individual differences in parasite loads within populations over time. Two species of fleas (*Oropsylla montana* and *Hoplopsyllus anomalous*) and their hosts, California ground squirrels (*Otospermophilus beecheyi*), form a major complex for amplifying epizootic plague in the western United States. Understanding its biology is primarily of major ecological importance and is also relevant to public health. Here, we capitalize on a long-term data set to explain flea incidence on California ground squirrels at Briones Regional Park in Contra Costa County, USA. In a 7 year study, we detected 42,358 fleas from 2,759 live trapping events involving 803 unique squirrels from two free-living populations that differed in the amount of human disturbance in those areas. In general, fleas were most abundant and prevalent on adult males, on heavy squirrels, and at the pristine site, but flea distributions varied among years, with seasonal conditions (e.g., temperature, rainfall, humidity), temporally within summers, and between flea species. Although on-host abundances of the two flea species were positively correlated, each flea species occupied a distinctive ecological niche. The common flea (*O. montana*) occurred primarily on adults in cool, moist conditions in early summer whereas the rare flea (*H. anomalous*) was mainly on juveniles in hot, dry conditions in late summer. Beyond this, we uncovered significantly repeatable and persistent effects of host individual identity on flea loads, finding consistent individual differences among hosts in all parasite measures. Taken together, we reveal multiple determinants of parasites on free-living mammals, including the underappreciated potential for host heterogeneity – within populations – to structure the emergence of zoonotic diseases such as bubonic plague.

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1. Introduction

A key question in biology focuses on understanding why some individuals in populations are more prone to carry parasites, and thus spread diseases, than others (Shaw et al., 1998; Altizer et al., 2003). In structured populations, a trait-based approach may offer some insights into these patterns (Johnson et al.,

2015). Indeed, attributes such as age and sex often co-vary with determinants of parasite presence such as habitat use (Freeland, 1983; Krasnov et al., 1997, 2006a, 2006b, 2012), social factors (Hart, 1994; VanderWaal et al., 2014; Habig et al., 2018), and immunocompetence (Altizer et al., 2003; St. Juliana et al., 2014). Although overlooked in seminal models (Anderson and May, 1992; Arneberg et al., 1998; Arneberg, 2002), epidemiologists and disease ecologists are increasingly recognizing that even individuals belonging to the same age-sex category may vary tremendously in their contributions to the emergence of diseases within a population (Combes, 2001). For example, Typhoid Mary, Patient zero (HIV) and several key SARs patients (super-spreaders) are widely recognized for their disproportional effects (Lloyd-Smith

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et al., 2005; Meyers et al., 2005; Paull et al., 2012). Indeed, a growing literature is revealing key links between social connectivity and parasitism within host populations, attributed to host heterogeneity in contact rates and/or infectiousness (Kilpatrick et al., 2006; Matthews et al., 2006; Clay et al., 2009). Outside the context of social networks, studies on host heterogeneity in parasite loads focus largely on interspecific – rather than intraspecific – variation in parasite preferences for one host species over another (Streicker et al., 2013; García-Longoria et al., 2014; Hammond et al., 2019a). However, relatively few studies explicitly quantify the extent to which parasitism rates consistently differ over time for individual hosts of the same species. Understanding effects of host heterogeneity is complementary to social network studies and crucial for predicting impacts of hosts on disease transmission (Dwyer et al., 1997). Repeated samples from long-term data sets offer opportunities to examine such effects (Smith et al., 2017; Payne et al., 2020).

Understanding intraspecific variation in host heterogeneity is particularly relevant within the context of global flea-borne infections. The Order Siphonaptera (fleas) represents a major group of parasites that is of critical global ecological and public health importance. Of the approximately 2,600 species of fleas, about 90% parasitize mammals (Bourne et al., 2018). Mammalian hosts experience periodic infestations that represent the emergence of unfed immature fleas from the abiotic environment (Krasnov et al., 2004a, 2004b; Bourne et al., 2018). In adulthood, fleas typically parasitize a single host; before dying, female fleas usually drop off the living host and lay eggs in the environment (Bourne et al., 2018). Although fleas may switch hosts if a disease such as plague kills an initial host, this complex life cycle generally results in indirect transmission among hosts (Hudson et al., 2006; Krasnov, 2008; Bitam et al., 2010). As fleas drop off and new ones recolonize, this may give rise to substantial fluctuations in an individual's flea loads over time. However, if individual hosts vary consistently in key characteristics that affect rates of flea infestations, host identity may emerge as a salient predictor of flea community stability and of flea abundance and prevalence. To date, however, the effect of consistent, intraspecific host heterogeneity (e.g., an individual animal's identity) – beyond the effects of host demographic characteristics (e.g., age and sex) and ecological conditions (e.g., seasonal factors, habitat disturbance) – on flea abundance and prevalence has rarely, if ever, been documented in any species, and has not been examined in the classic California ground squirrel (*Otospermophilus beecheyi*, Richardson 1989)-plague system.

Arguably one of the most severe re-emerging infections spread by fleas to humans is plague, caused by the bacterium, *Yersinia pestis*; without treatment, it has a case-fatality ratio in humans of 30% to 100% (Stenseth et al., 2008). In the western United States, the flea *Oropsylla montana* (Baker) is the most important vector of sylvatic plague, particularly in the state of California (Clover et al., 1989; Smith et al., 2010). *Oropsylla montana*, in combination with a second flea, *Hoplopsyllus anomalous* (Baker), and their host, the California ground squirrel, form the principal complex for amplifying epizootic plague in the western United States (Barnes, 1982; Lang, 2004). This complex is also associated with tularemia, a febrile illness that induces sepsis and/or pulmonary infections in humans (Evans and Holdenried, 1943; Roth et al., 2018). Understanding their flea distributions is of public health concern because California ground squirrels regularly interact with humans in parks and exurban communities (Bradley and Altizer, 2007). This is also of considerable ecological importance in North American grasslands (Biggins and Kosoy, 2001; Eads and Biggins, 2015). These native mammals are ecosystem engineers (Swaissgood et al., 2019) and major prey items in California grasslands (reviewed by Smith et al., 2016).

Here, we used 7 years of data from marked California ground squirrels to uncover the determinants of their flea community stability, abundance and prevalence. First, we expected male hosts to harbor more fleas than female hosts because males generally have more compromised immune systems than females (Krasnov et al., 2012). Although this pattern is often reported as the “norm” for mammals, the effects of host sex on flea loads is mixed for California ground squirrels (Holdenried et al., 1951; Lang and Wills, 1991; Lang, 1996; Bursten et al., 1997). Second, we expected the two flea species to occupy contrasting ecological niches. Specifically, each flea species might occur on hosts belonging to different ontogenetic stages (Marshall, 1981; Krasnov et al., 2004a, 2004b). Third, we expected abiotic factors such as temperature (Stewart and Evans, 1941; Lang, 1996) and moisture (Ryckman, 1971; Lang, 1996) to be important determinants of on-host flea loads. Specifically, we predicted high flea loads in conditions with intermediate temperatures and moisture. Cold winter/dry spring conditions can limit flea reproduction (e.g., fleas are only active at >10 °C (50°F; Bibikova et al., 1963)), but excessive summer heat (>26 °C, ~80°F) and relative humidity (>80% RH) can limit flea survival (Krasnov, 2008; Stenseth et al., 2008) and abundance (Stenseth et al., 2006). Although fleas require sufficient moisture to avoid desiccation without favoring mold or flea pathogens (Hirst, 1927; Sharif, 1949; Olson, 1969; Saxena, 1999), these effects are not always straightforward for fleas residing on burrowing rodents; burrows may buffer extreme weather (Ari et al., 2011) and fleas may even prosper if hot/dry aboveground conditions compromise host condition (Eads et al., 2016; Eads and Hoogland, 2017). Finally, because parasites are strong indicators of host fitness (Arnold and Anja, 1993), flea loads might be lowest in pristine areas where hosts are generally of heavier body mass and otherwise in better physiological condition (Hammond et al., 2019b), and therefore less susceptible to flea infestations (Eads et al., 2016). Alternatively, flea loads might be highest on heavier squirrels with more surface area for parasites (Waterman et al., 2014) and at pristine areas if microhabitats more closely match the evolved physiological adaptations of a flea species (Hudson et al., 2006). Overall, we expected the combined effects of host traits, seasonal factors, and site of capture to predict on-host flea community stability, abundance and prevalence in the California ground squirrel. We also investigated whether consistent differences in flea loads for individual California ground squirrel hosts were repeatable over time.

2. Materials and methods

2.1. Long-term study populations and field sites

From 2013 to 2019, we studied free-living California ground squirrels at two distinct locations at Briones Regional Park in Contra Costa County, California, U.S.A. (Latitude: 37° 56' 15.723" North, Longitude: 122° 8' 19.8738" West, elevation: 319 m above mean sea level (Ortiz et al., 2019)). The study area consists primarily of open oak savannah, interspersed with vegetated riparian corridors subject to a relatively mild Mediterranean climate typified by hot, dry summers and cool, rainy winters. The two sites (~9600 m² each) differ in their microhabitat features (Ortiz et al., 2019) and levels of disturbance by humans and dogs (Hammond et al., 2020). Specifically, vegetation height and cover is more intact at the pristine than at the disturbed site (Ortiz et al., 2019). Animals at the disturbed site have higher glucocorticoid levels and poorer body condition than animals at the relatively pristine site (Hammond et al., 2019b).

2.2. Live trapping of free-living squirrels

We live-trapped and released California ground squirrels using Tomahawk Live-Traps (Hazlehurst, Wisconsin, USA) baited with black oil sunflower seeds and peanut butter (Ortiz et al., 2019). Trapping was conducted during the late spring and early summer months (May, June, and July), a time of year when juveniles and adults are most active aboveground (Holekamp and Nunes, 1989; Smith et al., 2018) and most human cases of plague emerge (Smith et al., 2010). Traps were covered with cardboard for shade and checked at intervals of <30 min. Upon first capture in the study, we marked each subject with a Monel ear tag and a Passive Integrated Transponder (PIT) tag (Biomark Inc., Idaho, Nebraska, U.S.A.). Juveniles were weaned young of the year (60–365 days of age) whereas adults were older than 365 days (Hanson and Coss, 1997). Marked individuals were assigned to age categories with high accuracy (Smith et al., 2018). For each capture, we noted the date, time, and site of capture. Using a cone-shaped handling bag (Koprowski, 2002), we recorded each subject's mass and sex. To avoid transfer of fleas among subjects, a series of handling bags was used. After each bag's use, it was briskly shaken inside out and carefully inspected for fleas prior to use on a subsequent subject.

All field methods were approved by the Animal Care and Use Committee of Mills College, USA and consistent with the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes, 2016). Research permits were obtained from the California Department of Fish and Wildlife, USA and the East Bay Regional Park District, USA.

2.3. Flea collection and identification

Upon their first capture in each trapping period (once every 2 weeks), the same handler (J.E. Smith) held each subject in a handling bag while a second handler combed the animal from the nape of the neck to the rump using a standardized combing technique (Stevenson et al., 2005). Specifically, we combed each subject with a flea comb five times to fully sample its dorsal side (few fleas reside on the underside of squirrel hosts) over a white tub sprayed with 90% ethanol. The handler inspected the anogenital regional and hind limbs of each subject for ~45 s, collecting any additional fleas detected on these areas. All fleas were then carefully transferred with forceps into a sterile 2 ml tube where they were fixed in 90% ethanol. Tubes were placed on ice in a cooler and transferred within several hours to a -20 °C freezer for long-term storage at Mills College.

In the laboratory, we used published taxonomic references (Hubbard, 1947; Stark, 1970; Furman and Catts, 1982) to identify each flea to species. We then indexed flea abundance as the total count of all fleas (including zeros; Bush et al., 1997) in three ways: (i) regardless of species, (ii) *O. montana* only, and (iii) *H. anomalous* only. These data were also used to assess the on-host prevalence (presence/absence) of each flea species in a given sampling event, and to calculate community stability for squirrels sampled for fleas on multiple occasions within a year.

2.4. Environmental measures of seasonal conditions

Given the complex life cycles of fleas (Bourne et al., 2018), we expected annual rather than daily environmental measures to most strongly predict the distributions of fleas on rodent hosts (Russell et al., 2018). Specifically, we predicted annual measures of winter precipitation, number of days with temperatures below 50°F (10 °C), number of days with temperatures exceeding 78°F (26 °C) and numbers of days with RH of at least 80% should most strongly predict flea loads (Russell et al., 2018). Based on the literature and our trapping period, we collected weather data for three

seasonal periods, ending on the last day of our “summer” trapping. For convenience, we refer to these as “winter” (20 December of the previous year to 19 March of the current year), spring (20 March to 19 May; prior to start of annual trapping) and “summer” (20 May to 19 July; annual trapping period). We expected that sampling day may also interact with these effects. For example, winter rainfall may have increased effects earlier in the spring/summer while the effects of the number of hot, humid days should be strongest late in the summer. Sensors at Briones Regional Park (Station ID = BNE) collected abiotic measures and these were downloaded from the California Department of Water Resources' website (<http://cdec.water.ca.gov/dynamicapp/wsSensorData>).

2.5. Statistical analysis

We used R version 3.5.1 (R Development Core Team, 2019, Vienna) for all statistical analyses. We applied a principal component analysis (PCA) to reduce the dimensionality of correlated abiotic measures in FactoMineR (Lê et al., 2008) and generated a small number of uncorrelated variables (Table 1). The first principal component (PC1) correlates with cool, humid, and wet conditions in the spring and summer months whereas the second principal component (PC2) reflects cold, humid and rainy conditions in the winter months. Because all seasonal measures loaded highly (\geq absolute value of 0.65) on the first two PCs and these first two PCs together explained 75 % of the total variation (46% and 29%, respectively), only these first two PCs were included as predictor variables in our statistical models. We also implemented the package codyn (Hallett et al., 2016) to calculate annual measures of community stability (Tilman, 1999).

We ran a total of six generalized mixed effect models (GLMMs). First, we modeled the predictors of community stability by fitting a GLMM with a Gaussian distribution in lme4 (Bates, D., Mächler, M., Bolker, B., Walker, S. 2014. Fitting linear mixed-effects models using lme4. arXiv 1406:5823). Second, we fitted separate models to explain each measure of abundance: (i) total (*O. montana* and *H. anomalous* combined), (ii) *O. montana* alone, and (iii) *H. anomalous* alone, each with a negative binomial distribution in the package glmmTMB (Brooks et al., 2017). Shapiro tests ($P < 0.0001$) and visual inspection indicated that the flea count data failed to conform to the normal distribution. To account for over-dispersion, this model was implemented using the “nbinom2” family. Third, to assess the factors predicting on-host prevalence, we fitted a single GLMM with a binomial distribution for each flea species in lme4 (Bates et al., 2014) to examine its presence/absence on a potential host for each capture event. We mean-rescaled data prior to analysis to permit comparisons of effect sizes across variables.

For each model, we entered the fixed effects of host characteristics (age category (juvenile/adult) and sex (male/female)), short-term environmental factors (study site (pristine/ disturbed), day of year, hour of day), and seasonal climate measures (PC1, PC2) as predictor variables. We also included the random effects of year and host identity in each model to account for repeated measures within our data set and to examine inter-individual differences among hosts. Likelihood Ratio Tests (LRTs) were used to assess if the random effect of identity improved each model's fit and calculated unadjusted repeatability for each using the rptR package (Stoffel et al., 2017). Repeatability, defined as the intraclass correlation coefficient, measures the proportion of total variance explained by among (rather than within) individual differences.

Because we expected non-additive effects among seasonal conditions (PC1 versus PC2), day of study and study site (pristine versus disturbed) on fleas, we entered their two-way interactions into each of the five models. Where appropriate, we added a squared term for PCs to examine non-linear effects to test whether intermediate conditions are associated with high flea loads. The interac-

Table 1

Principal components (PC) analysis results for weather conditions at Briones Regional Park in Contra Costa County, USA.

Coefficients	PC1	PC2
Cool Days (#) – Spring	0.98	0.11
Humid Days (#) – Spring	0.83	0.46
Mean Relative Humidity – Summer	0.78	-0.52
Mean Relative Humidity – Spring	0.75	0.60
Total Precipitation (cm) – Summer	0.75	-0.24
Humid Days (#) – Summer	0.73	-0.64
Total Precipitation (cm) – Spring	0.65	0.20
Total Precipitation (cm) – Winter	0.55	0.65
Cool Days (#) – Winter	0.29	0.71
Humid Days (#) – Winter	0.14	0.69
Mean Temperature – Winter	0.11	-0.81
Mean Relative Humidity – Winter	-0.04	0.76
Mean Temperature – Spring	-0.81	-0.17
Hot Days (#) – Summer	-0.83	0.41
Mean Temperature – Summer	-0.87	0.42
Eigenvalues	6.85	4.37
S.D.	2.62	2.09
Proportion of Variance	0.46	0.29

Bolded coefficient names aligned most strongly with PC1 or PC2, respectively.

tion between age and sex category was also examined because we predicted that adult females may respond differently than juveniles of either sex to levels of disturbance; females are most sensitive to levels of anthropogenic disturbance in our study area (Hammond et al., 2019b). Because mass is highly correlated with each age-sex category (Hammond et al., 2019b), the effects of mass on abundance and prevalence were assessed separately for each age and sex category. Finally, we also predicted that sampling day and hour might have interactive effects on flea abundance and prevalence. Statistically significant two-way interactions were retained in the final models and report non-significant ones based on their addition to each final model. No terms in our final models were highly intercorrelated ($R \leq 0.25$ for all terms).

Finally, we tested for relationships between the two flea species to examine the potential for negative abundance correlations between them; we expected that such patterns may be driven by key ecological forces such as interspecific competition (Connell, 1983) and/or physiological constraints (Kearney and Porter, 2009). First, we used a non-parametric Wilcoxon-paired rank test to compare the median abundance and prevalence, respectively, for each flea species on each host. Based on our findings, we next examined whether the common species (*O. montana*) imposed an inhibitory effect on the rare one (*H. anomalous*), by entering *O. montana* abundance for each capture event as a predictor variable in the models explaining *H. anomalous* abundance. $P < 0.05$ was considered statistically significant.

2.6. Data accessibility

Data for this article can be found online at MendeleyData (<https://data.mendeley.com/>) (DOIs: <https://doi.org/10.17632/sv6f563gcp.1>).

3. Results

3.1. Community composition and stability of on-host fleas

We detected 42,358 fleas on hosts for 2,687 out of 2,759 sampling events involving 803 unique California ground squirrels across the 7 year study at two study sites; many individuals were captured multiple times as juveniles and adults (Table 2). All collected fleas were identified as *O. montana* ($N = 35,190$ fleas) or *H.*

anomalous ($N = 7,168$ fleas). On average, we detected 13 ± 0.5 (median \pm S.E.) fleas per first capture event of every 2 week sampling period ($N = 803$ squirrels). The median (\pm S.E.) on-host abundance of fleas was more than three times greater for *O. montana* (10 ± 0.4 fleas; range = 0 to 212 fleas per capture) than for *H. anomalous* (3 ± 0.1 fleas; range = 0 to 54 fleas per capture) and this difference was statistically significant (Wilcoxon-signed rank test: $Z = 27226$, $P < 0.000001$, $N = 803$ squirrels, Table 2).

Annual measures of flea community stability ranged from 0.67 to 44.54, averaging 2.72 ± 0.19 for the 414 unique squirrels sampled for fleas on multiple occasions within at least one season. Each of these hosts were sampled on average over 2 ± 1 years (range: 1–6 annual stability measures per individual). Community stability of on-host fleas was significantly predicted by the interaction of host age (-0.002 ± 0.28 , $P = 0.99$) and sex (0.08 ± 0.42 , $P = 0.85$; Age * Sex: 0.98 ± 0.47 , $P = 0.036$). Notably, adult males had significantly more stable flea communities than juvenile males (0.65 ± 0.31 , $P = 0.034$, Table 2), but community stability did not significantly differ with age for females (0.18 ± 0.29 , $P = 0.52$). We also detected no sex differences in community stability for juveniles (0.0001 ± 0.0001 , $P = 1.000$) or adults (0.99 ± 0.65 , $P = 0.128$) nor did stability vary significantly by study site (0.44 ± 0.33 , $P = 0.18$), weather (PC1: 0.06 ± 0.06 , $P = 0.349$; PC2: -0.08 ± 0.08 , $P = 0.346$), or year (Chi-square = 9.7, degrees of freedom (d.f.) = 1, $P = 1.0000$).

3.2. Determinants of overall abundance of on-host fleas

Host traits, in combination with environmental factors, explained patterns of overall on-host flea abundance. In general, adults (versus juveniles) and individuals at the pristine (versus disturbed) site had the most fleas, but age effects were most pronounced at the pristine site (Age * Site: $P = 0.008$, Table 3, Fig. 1; see Supplementary Table S1 for details). Males also had significantly more fleas than females ($P = 0.004$, Table 3, Fig. 1) regardless of age (Supplementary Table S1). Moreover, heavier squirrels had more fleas than those of lower masses; this pattern was statistically significant for all age-sex categories ($P \leq 0.02$) except for adult males ($P = 0.06$, Supplementary Table S1). In general, overall flea abundances increased over the course of the season (positive day of year effect) and cooler, wetter winters (PC2) tended to be associated with more fleas, but the effects of spring/summer conditions varied between sites such that multiple environmental factors explained total abundance. First, although the influence of winter conditions on the number of fleas sampled generally declined as each summer progressed (PC2 * Day: $P < 0.00001$, Table 3), the effects of spring/summer conditions on total flea abundance were consistent throughout each sampling summer (Supplementary Table S1). Second, total flea abundance varied between sites in response to winter (Site * PC2: $P = 0.003$; Site * PC2²: $P < 0.00001$, Table 3) and spring/summer conditions (Site * PC1: $P < 0.00001$, Table 3). For hosts at the pristine site only, cold/wet winters corresponded with a direct increase in total flea abundance (PC2: 0.04 ± 0.02 , $t = 2.16$, $P = 0.03$), and the magnitude of these effects significantly declined as each summer progressed (PC2 * Day: -0.003 ± 0.001 , $t = -3.96$, $P < 0.00001$). However, intermediate winter conditions had negligible effects on total flea abundance at the pristine site (PC2²: 0.01 ± 0.06 , $t = -0.12$, $P = 0.91$). Total flea abundance at the pristine site also tended to increase with cool and moist spring/summer conditions (PC1: 0.11 ± 0.06 , $t = 1.86$, $P = 0.06$) and intermediate spring/summer conditions (PC1²: -0.05 ± 0.03 , $t = -1.75$, $P = 0.08$), but these effects were not statistically significant. In contrast, the direct and intermediate effects of cool winter (PC2: 0.18 ± 0.17 , $t = 1.12$, $P = 0.27$; PC2²: 0.06 ± 0.07 , $t = 0.88$, $P = 0.38$) and spring/summer (PC1: -0.02 ± 0.08 , $t = -0.23$, $P = 0.82$; PC1²: -0.03 ± 0.03 , $t = 0.85$, $P = 0.39$) conditions were less clear at the disturbed site. Third, total flea abun-

Table 2
Mean \pm S.E. flea abundance, prevalence, and community stability on hosts.

Age-sex category	Unique individuals ¹ (hosts)	Captures (trapping events)	Total flea abundance ¹ (mean \pm S.E.)	<i>Oropsylla montana</i> abundance ¹ (mean \pm S.E.)	<i>Hoplosyllus anomalous</i> abundance ¹ (mean \pm S.E.)	<i>O. montana</i> prevalence ¹ (mean \pm S.E.)	<i>H. anomalous</i> prevalence ¹ (mean \pm S.E.)	Community stability ² (mean \pm S.E.; sample size)
Adult males	N = 203	N = 462	21.5 \pm 1.0 fleas	18.8 \pm 0.9 fleas	2.6 \pm 0.2 fleas	0.98 \pm 0.01	0.56 \pm 0.02	3.6 \pm 0.7, N = 81
Adult females	N = 254	N = 1,013	19.0 \pm 0.6 fleas	16.9 \pm 0.5 fleas	2.1 \pm 0.1 fleas	0.97 \pm 0.01	0.52 \pm 0.02	2.7 \pm 0.3, N = 140
Juvenile males	N = 287	N = 558	11.2 \pm 0.5 fleas	7.8 \pm 0.4 fleas	3.4 \pm 0.2 fleas	0.92 \pm 0.01	0.66 \pm 0.02	2.3 \pm 0.2, N = 130
Juvenile females	N = 300	N = 726	9.7 \pm 0.3 fleas	7.0 \pm 0.3 fleas	2.7 \pm 0.2 fleas	0.92 \pm 0.01	0.64 \pm 0.02	2.2 \pm 0.2, N = 162

¹ Sampling unit is the number of unique hosts sampled for fleas at least once during the seven-year study.

² Sampling unit is the number of unique hosts sampled for fleas on multiple occasions within at least one summer.

Table 3
Factors predicting flea abundance on California ground squirrels.

Overall flea abundance		<i>Oropsylla montana</i> abundance		<i>Hoplosyllus anomalous</i> abundance	
Fixed effects	Estimate \pm S.E.	Fixed effects	Estimate \pm S.E.	Fixed effects	Estimate \pm S.E.
Age-category (adult)	0.70 \pm 0.04^c	Age-category (adult)	0.81 \pm 0.04^c	Age-category (adult)	-0.32 \pm 0.06^c
Sex (male)	0.11 \pm 0.04^b	Sex (male)	0.11 \pm 0.05^b	Sex (male)	0.08 \pm 0.06
Hour of day	-0.05 \pm 0.01^c	Hour of day	-0.06 \pm 0.01^c	Hour of day	-0.43 \pm 0.16^b
Day of year	0.003 \pm 0.0008^c	Day of year	-0.002 \pm 0.001^a	Day of year	0.003 \pm 0.01
Site (pristine)	1.04 \pm 0.11^c	Site (pristine)	0.85 \pm 0.11^c	Day * Hour	0.01 \pm 0.003^c
Age * Site (adult)	-0.22 \pm 0.08^b			Site (pristine)	0.86 \pm 0.07^c
				Day * Site	0.01 \pm 0.003^c
Winter conditions (PC2: cool & wet)	0.54 \pm 0.17^b	Winter conditions (PC2: cool & wet)	0.20 \pm 0.15		
Winter conditions ²	0.06 \pm 0.07	Winter conditions ²	0.05 \pm 0.06	Winter conditions (PC2: cool & wet)	-0.13 \pm 0.06^a
Winter conditions * Day	-0.002 \pm 0.0004^c	Winter conditions * Site	-0.17 \pm 0.05^b	Winter conditions ²	0.09 \pm 0.05
Winter conditions * Site	-0.14 \pm 0.05^b	Winter conditions² * Site	-0.09 \pm 0.02^c		
Winter conditions² * Site	-0.08 \pm 0.02^c			Spring/Summer (PC1: cool & wet)	-0.78 \pm 0.10^c
Spring/summer (PC1: cool & wet)	-0.02 \pm 0.08	Spring/summer (PC1: cool & wet)	-0.01 \pm 0.07	Spring/Summer conditions * Day	0.004 \pm 0.0005^c
Spring/summer conditions ²	-0.03 \pm 0.03	Spring/summer conditions ²	-0.02 \pm 0.03	Spring/Summer conditions²	-0.07 \pm 0.03^b
Spring/summer conditions * Site	0.15 \pm 0.02^c	Spring/summer conditions * Site	0.19 \pm 0.02^c	<i>Oropsylla montana</i> abundance	0.01 \pm 0.01^c
Random effects	X²	Random effects	X²	Random effects	X²
Identity	2795.2^c	Identity	3259.1^c	Identity	5355.3^c
Year	2923.2^c	Year	3351.9^c	Year	5356.6^c

For categorical fixed effects, the level of the variable being compared is shown in parentheses (e.g., for "Adult", adults were compared to juveniles, and a positive estimate indicates that adults had more fleas than juveniles). Statistically significant terms are in bold and superscript letters indicate p-values (a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.00001$).

dance was significantly higher for more easily trapped squirrels (e.g., hosts captured early in the day; Hour: $P < 0.00001$) as well as on squirrels trapped late in the summer (Day: $P < 0.00001$, Table 3); these effects were additive (Supplementary Table S1). Finally, the random effect of year significantly explained flea abundance (Year: $P < 0.00001$, Table 3).

3.3. Determinants of on-host abundance for *Oropsylla montana*

Factors predicting on-host abundance for *O. montana*, the common flea species, largely mirrored those predicting overall flea abundance. First, *O. montana* were significantly more abundant on adults, on males, and on individuals residing at the pristine site, but this time, all of these effects were additive and heavier squirrels of all age-sex categories had significantly more fleas (Tables 3, see Supplementary Table S2 for details). Second, as with total flea abundance, the effects of winter (Site * PC2: $P = 0.0007$; Site * PC2²: $P < 0.00001$) and spring/summer (Site * PC1: $P < 0.00001$; Site * PC1²: -0.02 ± 0.01 , $P = 0.06$) conditions on *O. montana* abundance varied between sites (Table 3, Fig. 2A-B). At the pristine site,

O. montana was most abundant in summers after intermediate winters (PC2²: -0.04 ± 0.01 , $t = -3.38$, $P < 0.001$) rather than in summers after more extreme winter weather (PC2: -0.004 ± 0.03 , $t = -0.14$, $P = 0.89$; Fig. 2A). Also at the pristine site, *O. montana* was most abundant in years with the coolest and wettest spring/summer conditions (PC1: 0.03 ± 0.01 , $t = 2.64$, $P = 0.008$) rather than with intermediate spring/summer weather (PC1²: -0.01 ± 0.01 , $t = -1.44$, $P = 0.15$). At the disturbed site, *O. montana* abundance was greatest in years characterized by intermediate winter (PC2²: -0.03 ± 0.01 , $t = -4.13$, $P < 0.0001$) and spring/summer (PC1²: -0.009 ± 0.003 , $t = -2.63$, $P = 0.008$) conditions rather than years with extreme winter (PC2: 0.004 ± 0.02 , $t = 0.20$, $P = 0.85$) or spring/summer (PC1: 0.004 ± 0.008 , $t = 0.51$, $P = 0.61$) weather. The effects of weather conditions on *O. montana* abundance were consistent throughout each summer (Supplementary Table S2). Third, we again detected significantly more *O. montana* on hosts trapped earliest each day, but it was most abundant early in the summer (Table 3, Fig. 4A); these effects were additive (Supplementary Table S2). Finally, its abundance also varied significantly among years (Year: $P < 0.00001$, Table 3).

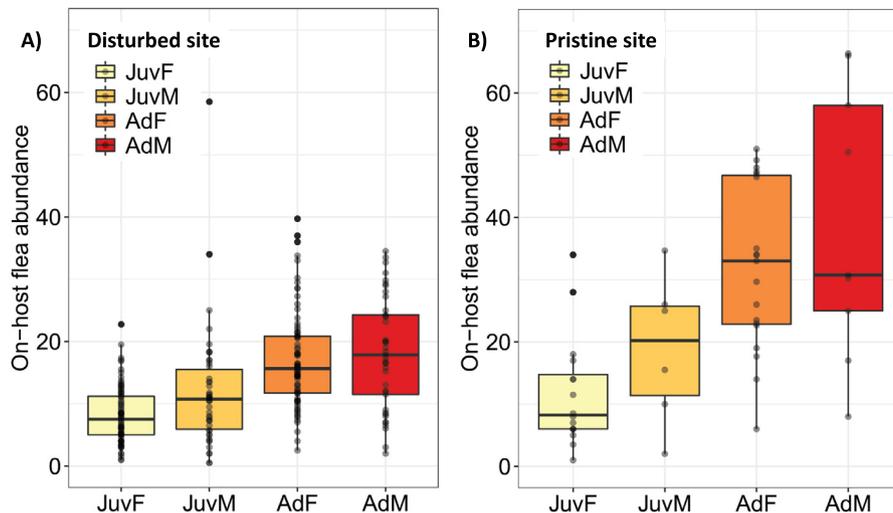


Fig. 1. Mean on-host flea abundance for female (F) and male (M) California ground squirrels sampled multiple times while residing at the (A) disturbed or (B) pristine site at Briones Regional Park in Contra Costa County, USA at two consecutive life history stages: as a juvenile (Juv) and as an adult (Ad). Although statistical models include all individuals from the 7 year study, average values for the subset of individuals sampled across the lifespan (as both juveniles and adults) are plotted here because identity was a strong predictor of on-host flea abundance ($N = 1,322$ captures of the week for 51 unique males and 99 unique females across the lifespan). The central line in each box and whisker plot shows the median value while the bottom and top edges show the 25th and 75th percentiles, respectively. Whiskers extend up to 1.5 times the interquartile range. Individual data points are overlaid.

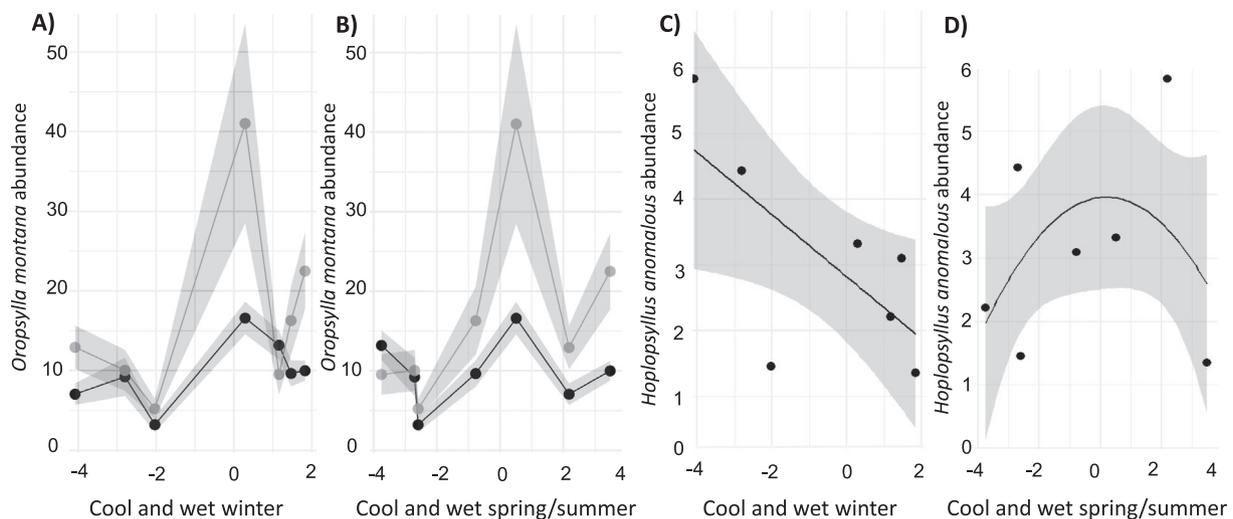


Fig. 2. The effect of seasonal weather conditions on the mean abundance of (A-B) *Oropsylla montana* and (C-D) *Hoplopyssyllus anomalus* sampled from California ground squirrel hosts ($N = 803$ unique individual squirrels) at the pristine (grey points and lines; $N = 551$ captures) and disturbed (black points and lines; $N = 2,208$ captures) sites at Briones Regional Park in Contra Costa County, USA. Principal components load positively with cool and wet conditions in winter (PC2) and spring/summer (PC1), respectively, for this 7 year study. Points represent annual values for each principal component and gray shading indicates 95% confidence intervals for on-host abundance for each flea species.

3.4. Determinants of on-host abundance for *Hoplopyssyllus anomalus*

As with other measures of abundance, the effects of age, sex, mass, and site of residence again influenced the abundance of *H. anomalus*. In some cases, however, the direction and magnitude of these effects differed from those found for *O. montana*. For example, juveniles had significantly higher numbers of *H. anomalus* than adults (Age: $P < 0.00001$, Table 3; see Supplementary Table S3 for details). Moreover, although males generally had more *H. anomalus* than females, this effect was not statistically significant (Sex: $P = 0.21$, Table 3). In addition, although heavier males had significantly more *H. anomalus* than lighter squirrels for both juveniles and males ($P \leq 0.01$), mass had no detectable effect on *H. anomalus* numbers for females belonging to either age category

(Supplementary Table S3). Nonetheless, as with other measures of abundance, residents at the pristine site had the highest numbers of *H. anomalus* (Site: $P < 0.00001$). We found no significant interactions among these effects (Supplementary Table S3).

Hoplopyssyllus anomalus abundance was positively predicted by that of *O. montana*, the most common flea detected on squirrels ($P < 0.00001$, Table 3). That is, individuals with many *O. montana* also had relatively high numbers of *H. anomalus* (and vice versa). Interestingly, however, *H. anomalus* abundance was favored by seasonal conditions that differed from those favoring *O. montana*. Moreover, these strong seasonal effects of winter and spring/summer persisted regardless of capture site (Supplementary Table S3). Specifically, *H. anomalus* was most abundant in years with warm, dry (PC2: $P = 0.02$) and intermediate (PC2²: $P < 0.0001$) winter

weather (Table 3, Fig. 2C). *Hoplopsyllus anomalous* was most abundant in years with intermediate spring/summer weather ($PC1^2$: $P = 0.005$) but constrained by cool and moist spring/summer weather ($PC1$: $P < 0.00001$, Table 3, Fig. 2D). *Hoplopsyllus anomalous* abundance increased as the summer progressed (Fig. 3B), particularly on the squirrels trapped early in the day at end of the season (Day * Hour: $P < 0.00001$, Table 3). Finally, its abundance varied among years (Year: $P < 0.00001$, Table 3).

3.5. Determinants of prevalence for each flea species on hosts

The two species of fleas varied in their overall prevalence as well as in the factors contributing to their distribution. First, when we assessed prevalence of each flea species at the level of each squirrel sampled, the median prevalence of *O. montana* was significantly higher than that of *H. anomalous* (1.0 ± 0.01 vs. 0.7 ± 0.02 captures; range = 0–1; Wilcoxon-signed rank test: $Z = 1701$, $P < 0.000001$, $N = 803$ squirrels, Table 2). Second, when we assessed the multiple factors contributing to the presence/absence of each flea species for each capture event, the results from the linear mixed effects models largely mirrored those revealed for abundance (Supplementary Table S4). As in the abundance models, host age, and to a lesser extent host sex and mass, as well as study site, hour, day, year, and seasonal factors were strong predictors of prevalence (Supplementary Table S4). These models again revealed strong differences in host fidelity and temporal divergence between the flea species. *Oropsylla montana* was significantly more prevalent on adults and *H. anomalous* was significantly more prevalent on juveniles (Supplementary Table S4). *Oropsylla montana* was consistently prevalent throughout each summer, but *H. anomalous* prevalence increased across days (Fig. 4).

3.6. Repeatability of host identity on flea abundance, prevalence and community stability

Beyond the effects of age, sex, site and seasonal conditions, the random effect of host identity significantly predicted on-host community stability (Chi-square = 124.8, d.f. = 1, $P < 0.00001$) as well as abundance for: (i) total fleas (Fig. 5), (ii) *O. montana*, and (iii) *H. anomalous* (Tables 3, $P < 0.00001$ for all three measures). Moreover, repeatability estimates for community stability were particularly high at 0.74 (ranging from 0.7 to 0.8, Table 4). Those for flea abundance ranged from 0.2 to 0.3. The 95% posterior credible intervals for community stability and all three measures of flea abundance did not overlap with zero, thereby indicating their statistical significance (Table 4). With regards to flea prevalence, the effects of host identity were strongest for *O. montana* (Supplementary Table S4). Repeatability measures were statistically significant for both *O. montana* (0.11) and *H. anomalous* (0.07) prevalence (Supplementary Table S5).

4. Discussion

Fleas were ubiquitous on California ground squirrels at our study sites, with 97% of observations over 7 years carrying at least one flea; our sampling regime may have even resulted in occasional false negatives (e.g., Eads et al., 2015, 2013). All squirrels in our study likely harbored fleas. Given that these fleas can carry zoonotic diseases, this very high prevalence is of great interest. In particular, *O. montana* were found in almost every observation, averaging 95% of captures for each individual sampled. *Hoplopsyllus anomalous* was also common but not as ubiquitous (59%). Flea abundances were strongly associated with host traits (age, sex, mass), environmental drivers, and host individual identity. Below, we discuss the implications of each of these patterns.

Overall, we uncovered strong, but complex, effects of host age and sex on flea loads which may be explained by differences in the quality, exposure or immunocompetence among hosts belonging to various age-sex categories (Tinsely, 1989; Zuk and McKean, 1996; Hawlena et al., 2005). Regarding host quality, the “well-fed host” hypothesis predicts parasites should prefer adult rodent hosts, which, on average, are usually larger and, thus, represent a good nutritional resource; the “poorly fed hypothesis” predicts a parasite preference for juvenile hosts who are, on average, more susceptible to infestations (Hawlena et al., 2005). Within each age category, heavier squirrels were generally parasitized by more fleas, particularly by the common flea species, *O. montana*. Interestingly, however, *O. montana* were also most abundant on adult squirrels, but *H. anomalous* was found most often on juveniles. Our findings suggest that the two flea species follow opposing strategies for infesting hosts. These may indeed be attributed to host nutritional value and susceptibility, but alternative sources of ontogenetic variation (e.g., host exposure, sociality) should also be explored before reaching a definitive conclusion. It is worth noting that our findings differ from those in an earlier study, also in Contra Costa county from May to July; both flea species were mainly on juveniles and only an average of two fleas were detected on the 15 adults sampled (Bursten et al., 1997). In contrast, a recent study from April to January in Fresno and Madera counties reported host preferences matching ours (Hubbart et al., 2011).

With respect to the sex differences, we found that males had significantly more fleas than females across the lifespan. These findings are consistent with widely documented patterns of parasitism across vertebrates, including those for multiple rodent species (Morand et al., 2004). Male hosts typically have higher species richness and abundance of parasites than female hosts. Our study partially aids in resolving mixed findings of no sex differences (Bursten et al., 1997), sex differences only for adults (Holdenried et al., 1951; Rutledge et al., 1979), or sex differences for across the lifespan (Lang, 1996) on the California ground squirrel. Our ability to detect sex differences for adults – in addition to juveniles – is likely due to our large sample size ($N = 2,759$ samples, 803 individuals) and ability to detect large numbers of fleas (e.g., 15.4 ± 0.3 fleas/host). However, even with a large sample size, we detected no effect of host sex on the flea prevalence.

Future studies should investigate if our finding that males have the most fleas is best explained by sex differences in parasite exposure and/or immunocompetence (Tinsely, 1989; Zuk and McKean, 1996). With respect to exposure, because females are more strongly connected in their belowground networks (Smith et al., 2018) and have larger home ranges (Boellstorff and Owings, 1995), one would expect females to indirectly spread and pick up flea larvae more often than males. One possibility is that females compensate for increased exposure via grooming (Bursten et al., 2000). In terms of the immune system, our study occurred outside of the breeding season, a period of time when adult females have higher basal stress levels than males (Hammond et al., 2019b), suggesting that high flea loads on males is not attributed to sex differences in glucocorticoid levels. Despite their reduced glucocorticoid levels, males generally maintain higher levels of circulating testosterone during the summer (Holekamp and Talamantes, 1991); this may sufficiently compromise the male immune system to increase susceptibility (Zuk and McKean, 1996; Moore and Wilson, 2002; Morand et al., 2004). Going forward, an integrative approach could disentangle the potentially interactive effects of endocrinology (Folstad and Karter, 1992), movement ecology (Kraft and Stapp, 2013; Dougherty et al., 2018; Sih et al., 2018), sociality (Fenner et al., 2011; Godfrey, 2013; White et al., 2017), and grooming (Hart, 1994; Hawlena et al., 2007; Hillegass et al., 2008) on parasite loads and their associated fitness correlates for California ground squirrels.

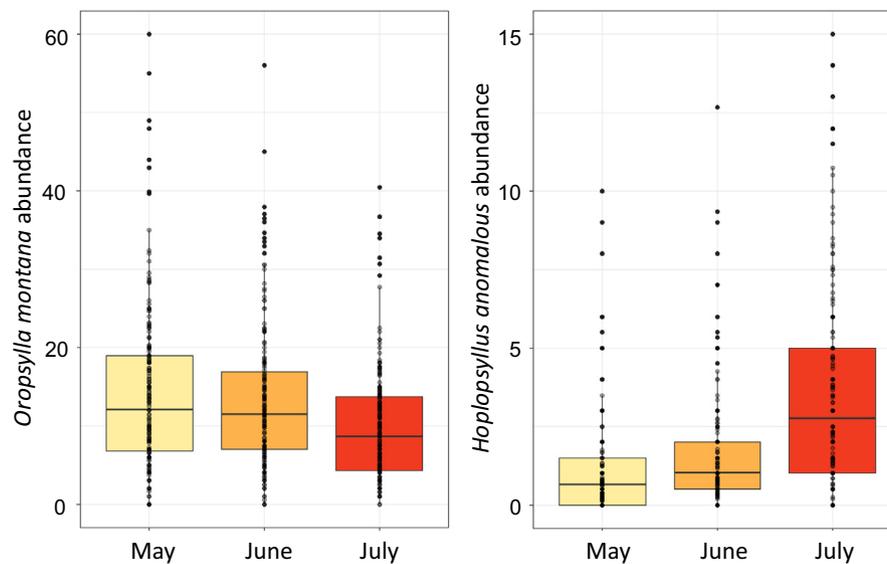


Fig. 3. Mean abundance of (A) *Oropsylla montana* and (B) *Hoplopsyllus anomalus* on California ground squirrels as a function of sampling day across the 7 year study ($N = 2,759$ captures of 803 unique individual squirrels). These continuous data were entered directly into our statistical models. For visual representation only, data are represented graphically based on monthly means for individuals sampled in the months of May ($N = 270$ individuals on 605 occasions), June ($N = 581$ individuals on 1,125 occasions), and July ($N = 519$ individuals on 1,029 occasions). For each box and whisker plot, the central line shows the median value while the bottom and top edges of the box show the 25th and 75th percentiles, respectively. Whiskers extend up to 1.5 times the interquartile range. Individual data points are overlaid.

Theory predicts that: (i) features of the abiotic environment (e.g. moisture, seasonality, darkness, environmental stability; Ryba et al., 1986; Wimsatt and Biggins, 2009) should generally limit parasite numbers and (ii) when two parasite species specialize on the same host species, each parasite should partition themselves temporally to avoid interspecific competition (Lindsay and Galloway, 1997). Indeed, we detected strong but opposing effects of seasonal conditions on the incidence of each flea species on hosts. Although seasonal factors and site differences had major influences on overall flea abundance, they had opposing effects on each flea species. Whereas *O. montana* was more prevalent in cold, wet and humid conditions, the opposite pattern occurred for *H. anomalus*. Specifically, total flea abundance (and that of *O. montana* alone) was greatest in summers following winters with intermediate weather, particularly at the pristine site. In contrast, *H. anomalus* did best at both sites in summers following extremely dry winters and in years with intermediate spring and summer conditions. Our findings extend work suggesting that *H. anomalus* is more tolerant to warm and dry conditions than *O. montana* (Stewart and Evans, 1941; Lang, 1996; Hubbart et al., 2011). Despite this evidence of niche partitioning, the two species were positively associated in their abundances. That is, some hosts simultaneously had high numbers of both flea species while other hosts had relatively few fleas of either species. Taken together, these patterns suggest that differences in the physiological tolerances, rather than competitive ability, most strongly shape distributions of each flea species on California ground squirrels.

The seasonal effects on parasite loads also varied between sites, suggesting that human modification of landscapes can affect the relative abundance of even some of the smallest and seemingly most resilient members of ecological communities – rodents and fleas. Although their physical resilience and potential for rapid population growth makes fleas particularly robust to environmental challenges (Bourne et al., 2018), our finding that hosts had consistently more fleas at the pristine (versus disturbed) site offers several insights about the negative effects of anthropogenic disturbances on fleas. First, our data suggest that vegetative manage-

ment at the disturbed site might reduce flea abundance, perhaps by disturbing microhabitats; consistent conditions within nests and burrows importantly promote parasite abundance on birds (Cantarero et al., 2013) and mammals (Krasnov, 2008; Hubbart et al., 2011). Thus, mowing and other disturbances may offer a useful management tool for reducing flea numbers in areas subject to human foot traffic. Second, it is highly unlikely that squirrels picked up fleas from dogs visiting the disturbed site. Dogs in California usually only harbor cat fleas (*Ctenocephalides felis felis* (Rust and Dryden, 1997)). Third, these differences also fail to correspond with site level differences in glucocorticoids. Squirrels at the disturbed site had fewer fleas despite having higher baseline “stress” levels. Although our current study lacks replication (disturbed versus pristine sites), our long-term data offer important baseline information for understanding the effects of seasonal variation and habitat modification in a plague-relevant system. We contribute to a growing understanding of the effects of human land use patterns on fleas (Durden et al., 2004; Hubbart et al., 2011) and, more broadly, environmental change on the risk of zoonotic transmission (Patz et al., 2000). These findings are of particular importance in the face of climate change as unpredictable periods of rain and prolonged droughts become more common (Patz et al., 2000; Greer et al., 2008; Cizauskas et al., 2017). Our results suggest that dry conditions suppress the more abundant flea species (and flea numbers overall) and therefore reduce host exposure. However, because stress associated with droughts can increase host susceptibility to fleas (e.g., Eads et al., 2016), we expect the effects of drought on parasite loads to be complex. Future efforts to predict flea incidence and mitigate associated plague risk should consider the interplay between seasonal conditions and human land use practices.

Overall, our study reveals significant effects of individuality among free-living hosts on flea community stability and numbers, contributing to the small but growing literature quantifying consistent individual differences in ectoparasite loads for birds (Boulinier et al., 1997), reptiles (Payne et al., 2020), primates (Zohdy et al., 2017), and rodents (Boyer et al., 2010; Bohn et al.,

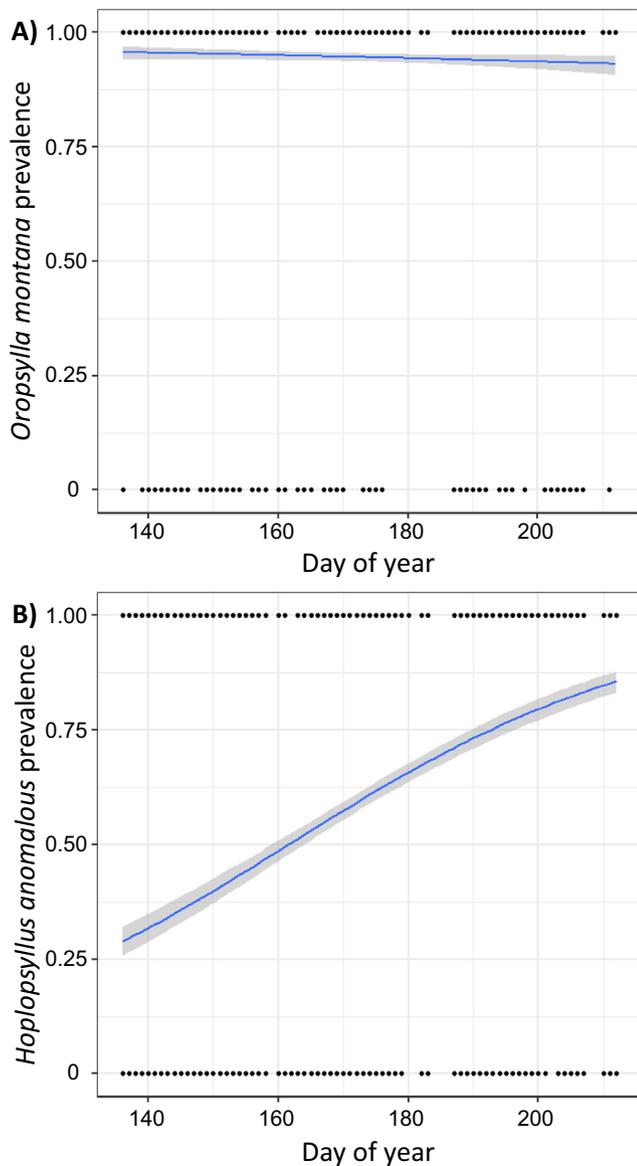


Fig. 4. Mean prevalence of (A) *Oropsylla montana* and (B) *Hoplopsyllus anomalous* detected on California ground squirrels as a function of sampling day across the 7 year study ($N = 2,759$ captures of 803 unique individual squirrels). The smoothed line reflects the mean value for each sampling day of the year and gray shading indicates 95% confidence intervals for the prevalence of each species of fleas detected on potential squirrel hosts.

2017). Interestingly, studies on free-living rock-dwelling gerbils, Cairo spiny mice, and Gunnison's prairie dogs (Krasnov et al., 2006a; Eads et al., 2016) failed to find evidence for this potential phenomenon. Thus, more studies are required to understand the extent to which our findings are generalizable across rodent hosts and to uncover the biological drivers of repeatable flea loads in host populations for which this pattern exists. Nonetheless, the maintenance of consistent individual differences is particularly interesting when it occurs over long enough periods for the parasites to turnover repeatedly. Our sampling found repeatability in flea loads even across years (i.e., across many flea generations) despite the high potential for individuals to vary in flea loads over time and we regularly removed all fleas (without replacement) as part of our sampling regime. Notably, since we removed fleas at each sample point, the fact that some individuals picked up numerous fleas over and over across their lifespan – and that flea

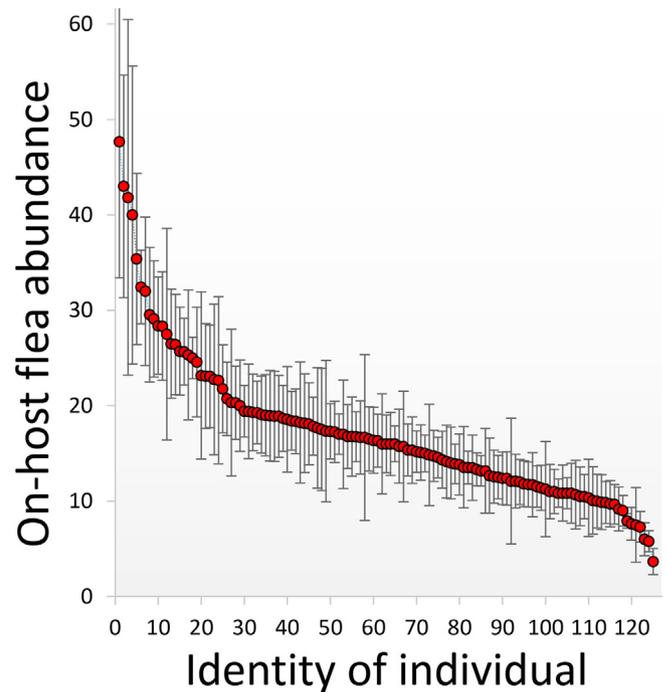


Fig. 5. Mean \pm S.E. on-host flea abundance for California ground squirrels captured. For visual purposes only, we plot the subset of individuals captured on at least five occasions. On average, these 125 individuals were sampled on the first capture of the week on 11 ± 0.5 occasions (range: 6 to 33 samples per individual) over a 3.0 ± 0.1 year-period (range: 1 to 6 years). Individuals are ranked from those with the highest to the lowest mean number of on-host fleas across the 7 year study ($N = 88$ females; 39 males). These data reflect the statistically significant and repeatable effects of individual identity on flea burdens in all of the statistical models even after controlling for the effects of age, sex, site membership, and seasonal conditions.

Table 4

Repeatability estimates of host identity on flea abundance and community stability. Mean repeatability estimates for community stability from 671 annual measures on 414 individuals and abundance measures on 803 individuals (sampled over a total of 2,759 captures). The 95% credible intervals are given in parentheses and significant terms whose 95% credible intervals did not overlap zero are in bold.

	Repeatability (95% credible interval)	P-value
Community stability	0.74 (0.68, 0.79)	<0.0001
Total on-host abundance	0.24 (0.19, 0.28)	<0.0001
<i>Oropsylla montana</i> abundance	0.20 (0.15, 0.24)	<0.0001
<i>Hoplopsyllus anomalous</i> abundance	0.26 (0.19, 0.31)	<0.0001

community stability was highly repeatable across years – is particularly striking in this system. Repeatable flea loads over time may be attributed to particular individuals occupying burrow systems with more favorable conditions for fleas than other burrow systems (Krasnov et al., 2004a, 2004b). Alternatively, these consistent individual differences in parasitism could be attributed to behavioral type (e.g., shy versus bold individuals; Sih et al., 2018), pace of life syndromes (e.g., low versus high investment in immunity; Réale et al., 2010), and social network connectivity (Fenner et al., 2011; Godfrey, 2013; White et al., 2017). These factors could be particularly relevant in this study species; individuals vary consistently in boldness (Holding et al., 2020), physiology (Hammond et al., 2019b), and social connectedness (Smith et al., 2018).

Flea load repeatability on hosts has important implications for epidemiology. First, behavioral factors such as exploration and boldness may explain inter-individual differences in parasite mea-

tures. For example, tree squirrels show a positive association between trappability and gastrointestinal parasites (Santicchia et al., 2019). Because ground squirrels caught early in the day (e.g., most trappable individuals) had the most fleas, a similar pattern might occur in California ground squirrels. Such individual differences may mask effects when researchers only sample a small number of hosts (i.e., short-term sampling may only reflect flea loads of the most easily trapped individuals). Second, repeated measures on known individuals also permitted us to study parasite loads on specific individual hosts across time and environmental conditions. Not only are these approaches more ethical for animal welfare and protective of these native ecosystem engineers, they also permitted inferences unavailable from destructive sampling (i.e., sacrificing hosts or destroying burrows). Finally, the finding that host identity consistently predicted all parasite measures here also has practical management implications for interventions aimed at targeting specific individuals to mitigate disease. More generally, it underscores the need to integrate the explicit effects of host identity into theoretical models aimed at explaining parasite (and disease) dynamics/transmission.

In conclusion, our findings elucidate the multiple factors contributing to community stability, abundance, and prevalence for two flea species that together make up the plague nexus for California ground squirrels. First, applying a trait-based approach (Johnson et al., 2015) revealed the importance of age and sex on the incidence of periodic parasites on a mammalian host. Second, we uncovered divergent niches between the two flea species and their dynamic responses to multiple abiotic factors. Third, despite this variation, we revealed strong evidence for consistent individual differences in flea loads. Our research supports the notion that some hosts may impose a disproportionately large effect on parasite persistence (Modlmeier et al., 2014). Whereas community level studies have made extensive progress identifying variation in parasite loads among different host species (Russell et al., 2018), we are unaware of previous studies that reveal the role of intraspecific host identity in predicting flea loads across time. More broadly, our findings contribute novel insights to the literature by documenting – to our knowledge for the first time – consistent host heterogeneity within a major complex for amplifying epizootic plague on the west coast of the United States, and emphasize the need to incorporate such effects in studies of parasitology going forward.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2020.12.001>.

References

- Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., Dobson, A.P., Ezenwa, V., Jones, K.E., Pedersen, A.B., Poss, M., Pulliam, J.R.C., 2003. Social organization and parasite risk in mammals: Integrating theory and empirical studies. *Annu. Rev. Ecol. Syst.* 34, 517–547.
- Anderson, R.M., May, R.M., 1992. *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford.
- Ari, T.B., Neerincx, S., Gage, K.L., Kreppel, K., Laudisoit, A., Leirs, H., Stenseth, N.C., 2011. Plague and climate: scales matter. *PLoS Pathog.* 7, e1002160.
- Arneberg, P., 2002. Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. *Ecography (Cop)* 25, 88–94.
- Arneberg, P., Skorpung, A., Grenfell, B., Read, A.F., 1998. Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. London B* 265, 1283–1289.
- Arnold, W., Anja, V.L., 1993. Ectoparasite loads decrease the fitness of alpine marmots (*Marmota marmota*) but are not a cost of sociality. *Behav. Ecol.* 4, 36–39.
- Barnes, A., 1982. Surveillance and control of bubonic plague in the United States. In: Edwards, M., McDonnell, U. (Eds.), *Animal Disease in Relation to Animal Conservation*. Academic Press, Zoological Society of London, London.
- Bibikova, V.A., Il'inskaya, V.L., Kaluzhenova, Z.P., Morozova, I.V., Shmutter, M.I., 1963. On biology of the *Xenopsylla* fleas in the Sary-Eshikotrau desert. *Zool. J.* 42, 1045–1051.
- Biggins, D.E., Kosoy, M.Y., 2001. Influences of introduced plague on north american mammals: Implications from ecology of plague in Asia. *J. Mammal.* 82, 906–916.
- Bitam, I., Dittmar, K., Parola, P., Whiting, M.F., Raoult, D., 2010. Fleas and flea-borne diseases. *Int J Infect Dis* 14:e667–e676
- Boellstorff, D.E., Owings, D.H., 1995. Home range, population structure, and spatial organization of California ground squirrels. *J. Mammal.* 76, 551–561.
- Bohn, S.J., Webber, Q.M.R., Florko, K.R.N., Paslawski, K.R., Peterson, A.M., Piche, J.E., Menzies, A.K., Willis, C.K.R., 2017. Personality predicts ectoparasite abundance in an asocial sciurid. *Ethology* 123, 761–771.
- Boulinier, T., Sorci, G., Monnat, J.-Y., Danchin, E., 1997. Parent-offspring regression suggests heritable susceptibility to ectoparasites in a natural population of kittiwake *Rissa tridactyla*. *J. Evol. Biol.* 10, 77–85.
- Bourne, D., Craig, M., Crittall, J., Elsheikha, H., Griffiths, K., Keyte, S., Merritt, B., Stokes, L., Whitfield, V., Wilson, A., Wilson, A., 2018. Fleas and flea-borne diseases: biology, control, and compliance. *Companion Anim.* 23, 204–211.
- Boyer, N., Réale, D., Marmet, J., Pisanu, B., Chapuis, J.L., 2010. Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. *J. Anim. Ecol.* 79, 538–547.
- Bradley, C.A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. *Trends Ecol. Evol.* 22, 95–102.
- Brooks, M., Kristensen, K., van Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Maechler, M., Bolker, B., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378–400.
- Bursten, S.N., Berridge, K.C., Owings, D.H., 2000. Do California ground squirrels (*Spermophilus beecheyi*) use ritualized syntactic cephalocaudal grooming as an agonistic signal? *J. Comp. Psychol.* 114, 281–290.
- Bursten, S.N., Kimsey, R.B., Owings, D.H., 1997. Ranging of male *Oropsylla montana* fleas via male California ground squirrel (*Spermophilus beecheyi*) juveniles. *J. Parasitol.* 83, 804–809.
- Bush, A., Lafferty, K., Shostak, A., Lotz, J., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83:575–583.
- Cantarero, A., López-Arrabé, J., Rodríguez-García, V., González-Braojos, S., Ruiz-De-Castañeda, R., Redondo, A.J., Moreno, J., 2013. Factors affecting the presence and abundance of generalist ectoparasites in nests of three sympatric hole-nesting bird species. *Acta Ornithol.* 48, 39–54.
- Cizauskas, C.A., Carlson, C.J., Burgio, K.R., Clements, C.F., Dougherty, E.R., Harris, N.C., Phillips, A.J., 2017. Parasite vulnerability to climate change: an evidence-based functional trait approach. *R. Soc. Open Sci.* 4, 160535.
- Clay, C.A., Lehmer, E.M., Previtali, A., St. Jeor, S., Dearing, M.D., 2009. Contact heterogeneity in deer mice: implications for Sin Nombre virus transmission. *Proc. R. Soc. B Biol. Sci.* 276, 1305–1312.
- Clover, J.R., Hofstra, T.D., Kuluris, B.G., Schroeder, M.T., Nelson, B.C., Barnes, A.M., Botzler, R.G., 1989. Serologic evidence of *Yersinia pestis* infection in small mammals and bears from a temperate rainforest of north coastal California. *J. Wildl. Dis.* 25, 52–60.

- Combes, C., 2001. Parasitism: The Ecology and Evolution of Intimate Interactions. University of Chicago Press, Chicago.
- Connell, J.H., 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *Am. Nat.* 122, 661–696.
- Dougherty, E.R., Seidel, D.P., Carlson, C.J., Spiegel, O., Getz, W.M., 2018. Going through the motions: Incorporating movement analyses into disease research. *Ecol Lett* 21, 588–604.
- Durden, L.A., Ellis, B.A., Banks, C.W., Crowe, J.D., Oliver, J.H., 2004. Ectoparasites of gray squirrels in two different habitats and screening of selected ectoparasites for *Bartonellae*. *J. Parasitol.* 90, 485–489.
- Dwyer, G., Elkinton, J.S., Buonaccorsi, J.P., 1997. Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. *Am. Nat.* 150, 685–707.
- Eads, D.A., Biggins, D.E., 2015. Plague bacterium as a transformer species in prairie dogs and the grasslands of western North America. *Conserv. Biol.* 29, 1086–1093.
- Eads, D.A., Biggins, D.E., Antolin, M.F., Long, D.H., Huyvaert, K.P., Gage, K.L., 2015. Prevalence of the generalist flea *Pulex simulans* on black-tailed prairie dogs (*Cynomys ludovicianus*) in New Mexico, USA: the importance of considering imperfect detection. *J. Wildl. Dis.* 51, 498–502.
- Eads, D.A., Biggins, D.E., Doherty, P.F., Gage, K.L., Huyvaert, K.P., Long, D.H., Antolin, M.F., 2013. Using occupancy models to investigate the prevalence of ectoparasitic vectors on hosts: an example with fleas on prairie dogs. *Int. J. Parasitol. Parasites Wildl.* 2, 246–256.
- Eads, D.A., Biggins, D.E., Long, D.H., Gage, K.L., Antolin, M.F., 2016. Droughts may increase susceptibility of prairie dogs to fleas: incongruity with hypothesized mechanisms of plague cycles in rodents. *J. Mammal.* 97, 1044–1053.
- Eads, D.A., Hoogland, J.L., 2017. Precipitation, climate change, and parasitism of prairie dogs by fleas that transmit plague. *J. Parasitol.* 103, 309–319.
- Evans, F.C., Holdenried, R., 1943. A population study of the Beechey ground squirrel in central California. *J. Mammal.* 24, 231.
- Fenner, A.L., Godfrey, S.S., Bull, C.M., 2011. Using social networks to deduce whether residents or dispersers spread parasites in a lizard population. *J. Anim. Ecol.* 80, 835–843.
- Folstad, I., Karter, A., 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139, 603–622.
- Freeland, W.J., 1983. Parasites and the coexistence of animal host species. *Am. Nat.* 121, 223–236.
- Furman, D.P., Catts, P.E., 1982. *Manual of Medical Entomology*. Cambridge University Press, New York, New York.
- García-Longoria, L., Garamszegi, L.Z., Möller, A.P., 2014. Host escape behavior and blood parasite infections in birds. *Behav. Ecol.* 25, 890–900.
- Godfrey, S.S., 2013. Networks and the ecology of parasite transmission: a framework for wildlife parasitology. *Int. J. Parasitol. Parasites Wildl.* 2, 235–245.
- Greer, A., Ng, V., Fisman, D., 2008. Climate change and infectious diseases in North America: the road ahead. *CMAJ* 178, 715–722.
- Habig, B., Doellman, M.M., Woods, K., Olanen, J., Archie, E.A., 2018. Social status and parasitism in male and female vertebrates: a meta-analysis. *Sci. Rep.* 8, 1–13.
- Hallett, L.M., Jones, S.K., MacDonald, A.A.M., Jones, M.B., Flynn, D.F., Ripplinger, J., Slaughter, P., Gries, C., Collins, S.L., 2016. CODYN: An R package of community dynamics metrics. *Methods Ecol. Evol.* 7 (10), 1146–1151.
- Hammond, T.T., Hendrickson, C.I., Maxwell, T.L., Petrosky, A.L., Palme, R., Pigage, J.C., Pigage, H.K., 2019a. Host biology and environmental variables differentially predict flea abundances for two rodent hosts in a plague-relevant system. *Int. J. Parasitol. Parasites Wildl.* 9, 174–183.
- Hammond, T.T., Ortiz-Jimenez, C.A., Smith, J.E., 2020. Anthropogenic change alters ecological relationships via interactive responses in stress physiology and behavior within and among organisms. *Integr. Comp. Biol.* 60, 57–69.
- Hammond, T.T., Vo, M., Burton, C.T., Surber, L.L., Lacey, E.A., Smith, J.E., 2019b. Physiological and behavioral responses to anthropogenic stressors in a human-tolerant mammal. *J. Mammal.* 100, 1928–1940.
- Hanson, M., Coss, R., 1997. Age differences in the response of California ground squirrels (*Spermophilus beecheyi*) to avian and mammalian predators. *J. Comp. Psychol.* 111, 174–184.
- Hart, B.L., 1994. Behavioural defense against parasites: interaction with parasite invasiveness. *Parasitology* 109, S139–S151.
- Hawlena, H., Abramsky, Z., Krasnov, B.R., 2005. Age-biased parasitism and density-dependent distribution of fleas (*Siphonaptera*) on a desert rodent. *Oecologia* 146, 200–208.
- Hawlena, H., Bashary, D., Abramsky, Z., Krasnov, B., 2007. Benefits, costs and constraints of anti-parasitic grooming in adult and juvenile rodents. *Ethology* 113, 394–402.
- Hillegass, M.A., Waterman, J.M., Roth, J.D., 2008. The influence of sex and sociality on parasite loads in an African ground squirrel. *Behav. Ecol.* 19, 1006–1011.
- Hirst, L., 1927. Rat-flea surveys and their use as a guide to plague preventive measures. *Trans. R. Soc. Trop. Med. Hyg.* 21, 87–104.
- Holdenried, R., Evans, F.C., Longanecker, D.S., 1951. Host-parasite-disease relationships in a mammalian community in the central coast range of California. *Ecol. Monogr.* 21, 1–18.
- Holding, M.L.M., Putman, B.J.B., Kong, L.M., Smith, J.E., Clark, R.W., 2020. Physiological stress integrates resistance to rattlesnake venom and the onset of risky foraging in California ground squirrels. *Toxins (Basel)* 12, 617.
- Holekamp, K.E., Nunes, S., 1989. Seasonal variation in body weight, fat, and behavior of California ground squirrels (*Spermophilus beecheyi*). *Artic. Can. J. Zool.* 67, 1425–1433.
- Holekamp, K.E., Talamantes, F., 1991. No seasonal variation in circulating testosterone and oestrogens of wild-caught California ground squirrels (*Spermophilus beecheyi*) in reproduction. *Reproduction* 93, 415–425.
- Hubbard, C.A., 1947. *The Fleas of Western North America*. Iowa State College Press, Ames, Iowa.
- Hubbart, J.A., Jachowski, D.S., Eads, D.A., 2011. Seasonal and among-site variation in the occurrence and abundance of fleas on California ground squirrels (*Otospermophilus beecheyi*). *J. Vector Ecol.* 36, 117–123.
- Hudson, P.J., Dobson, A.P., Lafferty, K.D., 2006. Is a healthy ecosystem one that is rich in parasites? *Trends Ecol. Evol.* 21, 381–385.
- Johnson, P.J., de Roode, J.C., Fenton, A., 2015. Why infectious disease research needs community ecology. *Science* 349, 1259504.
- Kearney, M., Porter, W., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol. Lett.* 12, 334–350.
- Kilpatrick, A.M., Daszak, P., Jones, M.J., Marra, P.P., Kramer, L.D., 2006. Host heterogeneity dominates West Nile virus transmission. *Proc. R. Soc. B Biol. Sci.* 273, 2327–2333.
- Koprowski, J.L., 2002. Handling tree squirrels with a safe and efficient restraint. *Wildl. Soc. Bull.* 30, 101–103.
- Kraft, J., Stapp, P., 2013. Movements and burrow use by northern grasshopper mice as a possible mechanism of plague spread in prairie dog colonies. *J. Mammal.* 94, 1087–1093.
- Krasnov, B.R., 2008. *Functional and Evolutionary Ecology of Fleas: A Model for Ecological Parasitology*. Cambridge University Press, Cambridge.
- Krasnov, B.R., Bordes, F., Khokhlova, I.S., Morand, S., 2012. Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. *Mammalia* 76, 1–13.
- Krasnov, B.R., Khokhlova, I.S., Shenbrot, G.I.G., 2004a. Sampling fleas: the reliability of host infestation data. *Med. Vet. Entomol.* 18, 232–240.
- Krasnov, B.R., Mouillot, D., Shenbrot, G.I., Khokhlova, I.S., Poulin, R., 2004. Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: the influence of phylogeny and local environmental conditions. *Ecography (Cop)* 27:787–797.
- Krasnov, B.R., Shenbrot, G.I., Khokhlova, I.S., Hawlena, H., Degen, A.A., 2006a. Temporal variation in parasite infestation of a host individual: Does a parasite-free host remain uninfested permanently? *Parasitol. Res.* 99, 541–545.
- Krasnov, B.R., Shenbrot, G.I., Medvedev, S.G., Vatschnok, V.S., Khokhlova, I.S., 1997. Host-habitat relations as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the Negev Desert. *Parasitology* 114:S0031182096008347.
- Krasnov, B.R., Stanko, M., Miklisova, D., Morand, S., 2006b. Habitat variation in species composition of flea assemblages on small mammals in central Europe. *Ecol. Res.* 21, 460–469.
- Lang, J.D., 1996. Factors affecting the seasonal abundance of ground squirrel and wood rat fleas (*Siphonaptera*) in San Diego County, California. *J. Med. Entomol.* 33, 790–804.
- Lang, J.D., 2004. Rodent-flea-plague relationships at the higher elevations of San Diego County, California. *J. Vector Ecol.* 29, 236–247.
- Lang, J.D., Wills, W., 1991. Ecology of sylvatic plague in the San Jacinto Mountains of southern California. *Bull. Soc. Vector Ecol.* 16, 183–199.
- Lê S, J.J., Husson, F., 2008. FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Software* 25:1–18.
- Lindsay, L.R., Galloway, T.D., 1997. Seasonal activity and temporal separation of four species of fleas (Insecta: Siphonaptera) infesting Richardson's ground squirrels, *Spermophilus richardsonii* (Rodentia: Sciuridae), in Manitoba, Canada. *Can. J. Zool.* 75, 1310–1322.
- Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E., Getz, W.M., 2005. Superspreading and the effect of individual variation on disease emergence. *Nature* 438, 355–359.
- Marshall, A., 1981. *The Ecology of Ectoparasitic Insects*. Academic Press Inc., Limited, London.
- Matthews, L., Low, J.C., Gally, D.L., Pearce, M.C., Mellor, D.J., Heesterbeek, J.A.P., Chase-Topping, M., Naylor, S.W., Shaw, D.J., Reid, S.W.J., Gunn, G.J., Woolhouse, M.E.J., 2006. Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Natl. Acad. Sci.* 103, 547–552.
- Meyers, L.A., Pourbohloul, B., Newman, M.E.J., Skowronski, D.M., Brunham, R.C., 2005. Network theory and SARS: predicting outbreak diversity. *J. Theor. Biol.* 232, 71–81.
- Modlmeier, A.P., Keiser, C.N., Watters, J.V., Sih, A., Pruitt, J.N., 2014. The keystone individual concept: an ecological and evolutionary overview. *Anim. Behav.* 89, 53–62.
- Moore, S.L., Wilson, K., 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* 297, 2015–2018.
- Morand, S., Gouy De Bellocq, J., Stanko, M., Miklisová, D., 2004. Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central Europe? *Parasitology* 129, 505–510.
- Olson, W., 1969. Rat-flea indices, rainfall, and plague outbreaks in Vietnam, with emphasis on the Pleiku area. *Am. J. Trop. Med. Hyg.* 18, 621–628.
- Ortiz, C.A., Pendleton, E.L., Newcomb, K.L., Smith, J.E., 2019. Conspecific presence and microhabitat features influence foraging decisions across ontogeny in a facultatively social mammal. *Behav. Ecol. Sociobiol.* 73, 42.
- Payne, E., Sinn, D.L., Spiegel, O., Leu, S.T., Wohlfeil, C., Godfrey, S.S., Gardner, M., Sih, A., 2020. Consistent individual differences in ecto-parasitism of a long-lived lizard host. *Oikos* 129, 1061–1071.
- Patz, J., Graczyk, T., Geller, N., Vittor, A., 2000. Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol.* 30, 1395–1405.

- Paull, S.H., Song, S., McClure, K.M., Sackett, L.C., Kilpatrick, A.M., Johnson, P.T., 2012. From superspreaders to disease hotspots: linking transmission across hosts and space. *Front. Ecol. Environ.* 10, 75–82.
- Réale, D., Garant, D., Bergeron, P., Careau, V., Montiglio, P.O., 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. Trans. R. Soc. B*, 4051–4063.
- Roth, T., Sammak, R., Foley, J., 2018. Prevalence and seasonality of fleas associated with California ground squirrels and the potential risk of tularemia in an outdoor non-human primate research facility. *J. Med. Entomol.* 55, 452–458.
- Russell, R.E., Abbott, R.C., Tripp, D.W., Rocke, T.E., 2018. Local factors associated with on-host flea distributions on prairie dog colonies. *Ecol. Evol.* 8, 8951–8972.
- Rust, M.K., Dryden, M.W., 1997. The biology, ecology, and management of the cat flea. *Annu. Rev. Entomol.* 42, 451–473.
- Rutledge, L.C., Moussa, M.A., Zeller, B.L., Lawson, M.A., 1979. Field studies of reservoirs and vectors of sylvatic plague at Fort Hunter Liggett, California. *J. Med. Entomol.* 15, 452–458.
- Ryba, J., Rodl, P., Bartos, L., Daniel, M., Cerny, V., 1986. Some features of the ecology of fleas inhabiting the nests of the suslik (*Citellus citellus* (L.)). I: Population dynamics, sex ratio, feeding, reproduction. *Folia Parasitol.* 33, 265–275.
- Ryckman, R.E., 1971. Plague vector studies part II. The role of climatic factors in determining seasonal fluctuations of flea species associated with the California Ground Squirrel. *J. Med. Entomol.* 8, 541–549.
- Santicchia, F., Romeo, C., Ferrari, N., Matthysen, E., Vanlauwe, L., Wauters, L.A., Martinoli, A., 2019. The price of being bold? Relationship between personality and endoparasitic infection in a tree squirrel. *Mamm. Biol.* 97, 1–8.
- Saxena, V., 1999. Mesostigmatid mite infestations of rodents in diverse biotopes of central and southern India. *J. Parasitol.* 85, 147–149.
- Sharif, M., 1949. Effects of constant temperature and humidity on the larvae and the pupae of the three Indian species of *Xenopsylla* (*Insecta: Siphon*). *Philos. Trans. R. Soc. B* 233, 581–635.
- Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117, 597–608.
- Sih, A., Spiegel, O., Godfrey, S., Leu, S., Bull, C.M., 2018. Integrating social networks, animal personalities, movement ecology and parasites: a framework with examples from a lizard. *Anim. Behav.* 136, 195–205.
- Sikes, R.S., 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *J. Mammal.* 97, 663–688.
- Smith, C.R., Tucker, J.R., Wilson, B.A., Clover, J.R., 2010. Plague studies in California: a review of long-term disease activity, flea-host relationships and plague ecology in the coniferous forests of the southern cascades and northern sierra nevada mountains. *J. Vector Ecol.* 35, 1–12.
- Smith, J.E., Gamboa, D.A., Spencer, J.M., Travenick, S.J., Ortiz, C.A., Hunter, R.D., Sih, A., 2018. Split between two worlds: Automated sensing reveals links between above- and belowground social networks in a free-living mammal. *Philos. Trans. R. Soc. B Biol. Sci.* 373, 20170249.
- Smith, J.E., Lehmann, K.D.S., Montgomery, T.M., Strauss, E.D., Holekamp, K.E., 2017. Insights from long-term field studies of mammalian carnivores. *J. Mammal.* 98, 631–641.
- Smith, J.E., Long, D.J., Russell, I.D., Newcomb, K.L., Muñoz, V.D., 2016. *Otospermophilus beecheyi* (*Rodentia: Sciuridae*). *Mamm. Species* 48, 91–108.
- St. Juliana, J.R., Khokhlova, I.S., Wielebnowski, N., Kotler, B.P., Krasnov, B.R., 2014. Ectoparasitism and stress hormones: strategy of host exploitation, common host–parasite history and energetics matter. *J. Anim. Ecol.* 83, 1113–1123.
- Stark, H.E. 1970. A revision of the flea genus *Thrassis* Jordan, 1933 (*Siphonaptera: Ceratophyllidae*), with observations on the ecology and relationship to plague. *Univ. Calif. Publ. Entomol.* 53:1–184.
- Stenseth, N.C., Atshabar, B.B., Begon, M., Belmain, S.R., Bertherat, E., Carniel, E., Gage, K.L., Leirs, H., Rahalison, L., 2008. Plague: past, present, and future. *PLoS Med.* 5, e3.
- Stenseth, N.C., Samia, N.I., Viljugrein, H., Kausrud, K.L., Begon, M., Davis, S., Leirs, H., Dubyanskiy, V.M., Esper, J., Ageyev, V.S., Klassovskiy, N.L., Pole, S.B., Chan, K.-S., 2006. Plague dynamics are driven by climate variation. *Proc. Natl. Acad. Sci.* 103, 13110–13115.
- Stevenson, H.L., Labruna, M.B., Monteneri, J.A., Kosoy, M.Y., Gage, K.L., Walker, D.H., 2005. Detection of *Rickettsia felis* in a New World flea species, *Anomiopsyllus nudata* (*Siphonaptera: Ctenophthalmidae*). *J. Med. Entomol.* 42, 163–167.
- Stewart, M.A., Evans, F.C., 1941. A comparative study of rodent and burrow flea populations. *Exp. Biol. Med.* 47, 140–142.
- Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.* 8, 1639–1644.
- Streicker, D.G., Fenton, A., Pedersen, A.B., 2013. Differential sources of host species heterogeneity influence the transmission and control of multihost parasites. *Ecol. Lett.* 16, 975–984.
- Swaigood, R.R., Montagne, J.-P., Lenihan, C.M., Wisinski, C.L., Nordstrom, L.A., Shier, D.M., 2019. Capturing pests and releasing ecosystem engineers: translocation of common but diminished species to re-establish ecological roles. *Anim. Conserv.* 22, 600–610.
- Tilman, D., 1999. The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* 80, 1455–1474.
- Tinsely, R., 1989. The effects of host sex on transmission success. *Parasitol. Today* 5, 190–195.
- VanderWaal, K.L., Atwill, E.R., Isbell, L.A., McCowan, B., 2014. Linking social and pathogen transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). *J. Anim. Ecol.* 83, 406–414.
- Waterman, J.M., Macklin, G.F., Enright, C., 2014. Sex-biased parasitism in Richardson's ground squirrels (*Urocitellus richardsonii*) depends on the parasite examined. *Can. J. Zool.* 92, 73–79.
- White, L.A., Forester, J.D., Craft, M.E., 2017. Using contact networks to explore mechanisms of parasite transmission in wildlife. *Biol. Rev.* 92, 389–409.
- Wimsatt, J., Biggins, D.E., 2009. A review of plague persistence with special emphasis on fleas. *J. Vector Borne Dis.* 46, 85–99.
- Zohdy, S., Bisanzio, D., Tecot, S., Wright, P.C., Jernvall, J., 2017. Aggression and hormones are associated with heterogeneity in parasitism and parasite dynamics in the brown mouse lemur. *Anim. Behav.* 132, 109–119.
- Zuk, M., McKean, K., 1996. Sex difference in parasite infections: patterns and processes. *Int. J. Parasitol.* 26, 1009–1024.