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RESEARCH ARTICLE

Investigation of the geographic origin of burrowing owl fleas with implications for the ecology of plague

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ABSTRACT

Host-parasite relationships between Western Burrowing Owls (*Athene cunicularia hypugaea*) and the fleas (*Pulex irritans,* Siphonaptera:Pulicidae) they harbor were studied to understand the extent to which migratory Burrowing Owls translocated fleas from wintering grounds to breeding grounds. This has implications for host-parasite relationships in Burrowing Owls and also potentially for the dynamics of plague, as Burrowing Owl distributions overlap plague foci, owls inhabit fossorial mammal colonies where epizootic outbreaks of plague occur, and owls may harbor species of flea that are competent plague vectors. We used hydrogen stable isotope analysis to help elucidate geographic origins of fleas collected from adults and nestlings in 2 migratory populations of Burrowing Owls in Idaho and Oregon, USA. For adults, we posited that bird-mediated dispersal would impart flea isotopic compositions representative of southern latitudes and be similar to owl toenail tissue recently grown on wintering grounds, but they would differ from contour feathers presumably grown on breeding grounds the previous year. We assumed nestling feathers and toenails would have isotopic compositions representative of the breeding grounds. We analyzed contour feathers and toenails from adults collected shortly after they arrived in breeding grounds following spring migration and from nestlings later in the breeding season, to which we compared isotopic compositions in fleas collected from individuals of both age classes. Fleas on nestlings in both populations had isotopic compositions that did not differ from nestling feathers and toenails, suggesting that nestling fleas had breeding ground origins. Fleas on adults in one population (Oregon) had breeding ground isotopic signatures, as flea compositions did not differ from nestling feathers or toenails. Adult owls in Idaho had fleas that similarly did not express a wintering ground signature, but they were enriched in the heavy isotope (deuterium) relative to nestling feathers and toenails. Therefore, we discuss the possibility that adult owls in Idaho acquired fleas at migratory stopover sites. While the latter indicates that Burrowing Owls have the potential to disperse fleas, there was no evidence of continent-wide movement of fleas by owls from wintering grounds to breeding grounds.

Keywords: Athene cunicularia, deuterium, ectoparasites, long-distance dispersal, *Pulex irritans*, stable isotopes, USA

Investigación del origen geográfico de las pulgas de *Athene cunicularia* con implicancias para la ecología de plagas

RESUMEN

La relación hospedero-parásito entre *Athene cunicularia hypugaea* y las pulgas (*Pulex irritans,* Siphonaptera:Pulicidae) que albergan fueron estudiadas para entender el grado con que los individuos migratorios de *A. c. hypugaea* transportaron pulgas desde los sitios de invernada a los sitios reproductivos. Esto tiene implicancias para las relaciones hospedero-parásito en *A. c. hypugaea* y potencialmente para la dinámica de la plaga, ya que las distribuciones de *A. c. hypugaea* se superponen con los focos de las plagas, los búhos habitan colonias de mamíferos fosoriales donde se producen los brotes epizoóticos de la plaga y los búhos pueden albergar especies de pulga que son vectores de plaga competentes. Usamos análisis de isótopos estables de hidrógeno para ayudar a dilucidar los orígenes geográficos de las pulgas colectadas de adultos y polluelos en dos poblaciones migratorias de *A. c. hypugaea* en Idaho y Oregón, EEUU. Para los adultos, postulamos que la dispersión mediada por las aves generaría composiciones isotópicas de la pulga representativas de las latitudes del sur, que serían similares al tejido crecido recientemente en los sitios de invernada de las uñas de los búhos, pero que serían diferentes de las plumas de contorno crecidas presumiblemente en los sitios reproductivos del año anterior. Asumimos que las plumas y las uñas de los polluelos tendrían composiciones isotópicas representativas de los sitios reproductivos. Analizamos las plumas de contorno y las uñas de adultos colectados poco después de que llegaron a los sitios reproductivos luego de la migración de primavera y de polluelos al final de la estación reproductiva, a las que comparamos con la composición isotópica de las pulgas colectadas de individuos de ambas clases de edad. Las pulgas de los polluelos de ambas poblaciones tuvieron composiciones isotópicas que no difirieron de las plumas y las uñas de los polluelos, sugiriendo que las pulgas de los polluelos tienen su origen en los sitios reproductivos. Las pulgas en los adultos de una población (Oregón) tuvieron las firmas isotópicas del sitio reproductivo, ya que las composiciones de las pulgas no variaron de las de las plumas o las uñas de los polluelos. Los búhos adultos en Idaho tuvieron pulgas que tampoco expresaron una firma del sitio de invernada, pero estuvieron enriquecidas en el isótopo pesado (deuterio) con relación a las plumas y las uñas de los polluelos. Por ende, discutimos la posibilidad de que los búhos adultos en Idaho adquieran sus pulgas en los sitios de parada migratoria. Mientras que esto último indica que los individuos de *A. c. hypugaea* tienen el potencial de dispersar pulgas, no hubo evidencia de movimiento de pulgas a escala continental por parte de los búhos desde los sitios de invernada a los sitios reproductivos.

Palabras clave: Athene cunicularia, deuterio, dispersión de larga distancia, ectoparásitos, EEUU, isótopos estables, *Pulex irritans*

INTRODUCTION

Birds disperse propagules such as seeds or parasites after ingesting them or, externally, when propagules adhere to feathers or to the body surface (Costa et al. 2015). Migratory birds may be the most abundant and competent vertebrate vectors facilitating long-distance dispersal (Viana et al. 2016), and bird-mediated dispersal can occur with high frequency and facilitate colonization, range expansion and biological invasions (Coughlan et al. 2017, Ricklefs et al. 2017). Bird-facilitated dispersal of infectious organisms is of special interest because it can have far-reaching and rapid consequences for public health (Hubálek 2004, Altizer et al. 2011). For example, migratory birds carrying ticks spread Lyme and other diseases (Smith et al. 1996, Palomar et al. 2012, Cohen et al. 2015), and migratory birds have mediated the proliferation of both West Nile virus and avian influenza (Reed et al. 2003). There is evidence that migratory species themselves encounter a broader range of pathogens from inhabiting more diverse environments than counterparts that do not migrate (Waldenström et al. 2002), and the number of parasites per host is sometimes related to migratory distance (Figuerola and Green 2000). However, studies of pathogen dynamics in migratory species are still needed to help understand present and future disease risks for wildlife and humans alike (Altizer et al. 2011).

Plague is a flea-borne, zoonotic disease caused by the etiological agent *Yersinia pestis*. It was introduced to North America, and first reported in non-native rats in San Francisco, California, in 1900 (Kellogg 1920, Simpson 1920). Disease dynamics are characterized by quiescent periods interspersed with rapidly spreading outbreaks (Abbott and Rocke 2012, Eisen and Gage 2012). Commensal rodents are especially susceptible, as are some rodent-consuming carnivores (Abbot and Rocke 2012). Humans, in which the disease can be fatal, also contract plague through flea bites or exposure to infected individuals (Dennis and Gage 2003). Most cases of plague in wildlife and humans occur during sporadic epizootics when *Y. pestis* spreads rapidly between reservoir and incidental hosts through the bites of infectious fleas (Eisen et al. 2015). Predatory birds are hypothesized to play some role in epizootics of plague by moving infected prey or fleas that carry *Y. pestis* between populations (Gage and Kosoy 2005, Belthoff et al. 2015).

In western U.S. areas with plague, important candidates for investigation include avian species that inhabit prairie dog (*Cynomys* spp.) colonies or frequent other fossorial mammal burrows within plague systems, and those that have the potential to harbor fleas. Western Burrowing Owls (*Athene cunicularia hypugaea*; hereafter Burrowing Owls) have a geographic range that closely overlaps areas with plague (Klute et al. 2003, Poulin et al. 2011, Abbott and Rocke 2012, Kugeler et al. 2015; Figure 1), and they nest widely in prairie dog colonies (Desmond et al. 2000, Restani et al. 2001, Lantz et al. 2007, Alverson and Dinsmore 2014). Outside the range of prairie dogs, the owls use burrows of other fossorial mammals with frequent exposure to *Y. pestis* (e.g., American badgers [*Taxidea taxus*] Messick et al. 1983 and a variety of other Sciurids). The owls hunt rodent prey and harbor fleas (Brown 1944, Smith and Belthoff 2001a) such that widespread migratory (e.g., Holroyd et al. 2010, Holroyd and Trefrey 2011) movements could disperse fleas, perhaps even from plague foci in the southern United States or Mexico to owl breeding grounds further north (or vice versa).

The common flea species *Pulex irritans* (Siphonaptera: Pulicidae) infests Burrowing Owls as well as a variety of mammalian host species (Hopla 1980, Smith and Belthoff 2001a, Belthoff et al. 2015). Sometimes referred to as the human flea, *P. irritans* is a competent plague vector (Burroughs 1947, Eisen and Gage 2012, Ratovonjato et al. 2014), has a cosmopolitan distribution, and occurs in both the breeding and wintering grounds of Burrowing Owls in western North America (Hopla 1980). Burrowing Owls are not simply a phoretic host, as *P. irritans* may obtain blood meals from the owls they infest (Graham et al. 2016). Although the potential effects of fleas on Burrowing Owl health and physiology requires further study, harboring *P. irritans* does not appear to elevate stress hormones or reduce hematocrit levels in Burrowing Owls, but owls with fleas preen significantly more than owls where *P. irritans* numbers have been reduced via fumigation (C. Nellis and J. Belthoff personal observation). Additional flea species that are competent plague vectors also occur in owl burrows or on the owls themselves (Jellison 1939, Wheeler

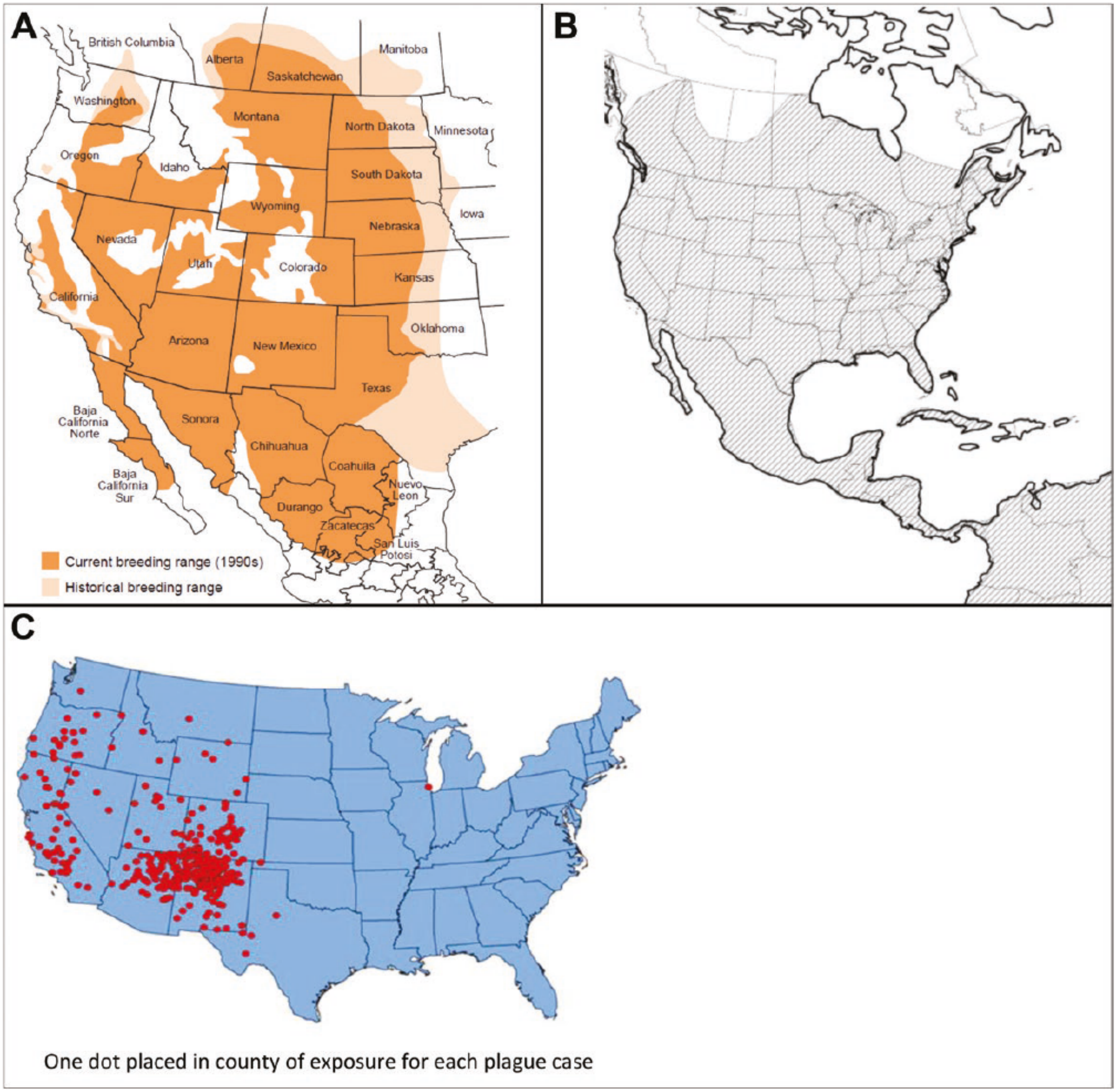

FIGURE 1. (**A**) Burrowing Owl range in western North America (from Klute et al. 2003, courtesy of U.S. Fish and Wildlife Service), (**B**) North American distribution of *Pulex irritans* (adapted from Hopla 1980), and (**C**) distribution of human plague cases (1970–2012) in the U.S. (U.S. Centers for Disease Control and Prevention).

1941, Brown 1944, Smith and Belthoff 2001a, Belthoff et al. 2015). In fact, Wheeler et al. (1941) described the first example of a bird host as a carrier of plague-infected ectoparasites when sticktight fleas (*Echidnophaga gallinacea*) collected from a Burrowing Owl during an epizootic outbreak of plague in California caused plague infection in a laboratory guinea pig (*Cavea porcellus*). The owls are generally considered non-susceptible to plague (Conrey 2010).

We were interested in the host-parasite relationships between Burrowing Owls and *P. irritans* in relation to owl migration for its implications on potential plague dispersal via long-distance transport of infected fleas from wintering grounds to breeding grounds. To investigate these relationships, we captured owls, collected their fleas, and examined stable isotopes of hydrogen in owl and flea tissues to estimate biogeographic origins of the fleas. Stable isotopes of hydrogen reflect average seasonal rainfall and vary geographically in predictable fashion in North America (Sheppard et al., 1969, Macías-Duarte and Conway 2015). Growing feathers acquire stable isotope values in their non-exchangeable hydrogen fractions that reflect those values of their diet. Because migratory birds in North America, including Burrowing Owls (Pyle 1997), typically grow their feathers on or near their breeding grounds, they carry with them isotopic information derived from the foodweb at the breeding site in the inert feathers until a subsequent molt (Hobson 1999, Wassenaar and Hobson 2000). For more than 2 decades ornithologists have estimated molt origin and therefore breeding or wintering latitudes of manifold bird species by analyzing hydrogen stable isotope ratios in feathers (e.g., Hobson and Wassenaar 1997, Hobson 1999, Wassenaar and Hobson 2000, Meehan et al. 2001, De Ruyck et al. 2013, Ress Wittenberg et al. 2013, Knick et al. 2014, Domenech et al. 2015, Macías-Duarte and Conway 2015, Werner et al. 2016, Fox et al. 2017). Other avian tissues such as toenails also contain isotopic signatures useful for establishing geographic origins (Clark et al. 2006, Catry et al. 2012, Hahn et al. 2014). Unlike feathers, toenails grow continuously, thus hydrogen isotope ratios in toenails may reflect dietary information over a window of several weeks to a few months and therefore, at least for birds that molt just once per year, likely reflect more recent locales than where feathers were formed depending upon the timing of sample collection (Lourenço et al. 2015). Thus, both feather and toenails can be used to infer geographic origin of migrant birds but on potentially different temporal extents. Insect chitin, such as that in flea exoskeletons, is similarly useful for analyzing geographic origin using stable isotopes of hydrogen approaches, as isotopes in chitin reflect locales in which the molt has occurred (Wassennaar and Hobson 1998, Rubenstein and Hobson 2004, Hobson et al. 2018).

We addressed the question of geographic origins of fleas on Burrowing Owls by measuring stable isotopes of hydrogen in feathers, toenail, and fleas from adult and nestling owls captured during the breeding season from 2 migratory populations. We anticipated (i) that stable isotope ratios in the feathers of both adults and nestlings would express a breeding ground signature because of the typical patterns of prebasic molt in owls (Pyle 1997), and (ii) that toenails would reflect a wintering ground signature in adults but not nestlings (Figure 2). We could then use these relationships to examine hydrogen isotope ratios in fleas collected from both adult owls and nestlings to help deduce their geographic origins, i.e. breeding grounds or wintering grounds sources. We hypothesized that fleas collected from adult owls soon after arrival from spring migration would be the most likely to express a wintering ground signature if the owls transported them rather than acquired them in the breeding grounds.

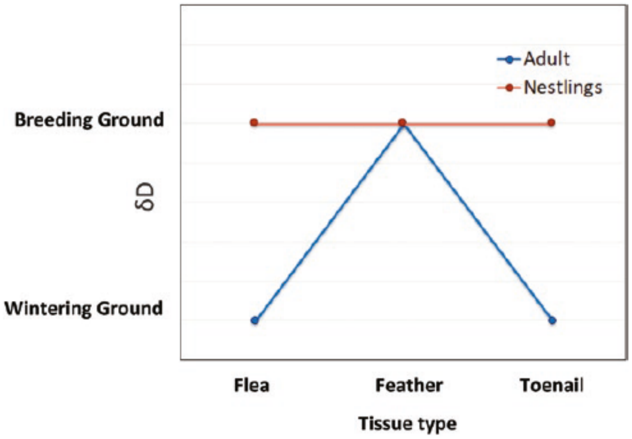

FIGURE 2. Predicted relationships in δD among fleas, feathers and toenails from adult and nestling Burrowing Owls during the breeding season if migratory adults have fleas with wintering grounds origin. The vertical axis is inverted to reflect breeding ground δD compositions which are expected to be more negative to the north (top of figure).

METHODS

Study Areas and Owl Capture

We studied Burrowing Owls and their fleas in 2 northwestern U.S. breeding populations located ~440 km apart, each of which receives annual monitoring via mark-recapture studies: (i) Morley Nelson Snake River Birds of Prey National Conservation Area, Ada and Elmore Co., Idaho (NCA: 43.19083°N, 116.150277°W); and (ii) Umatilla Army Depot, Morrow and Umatilla Co., Oregon (DEPOT: 45.84222°N, 119.440555°W). Owls in these breeding populations are annual migrants, and leg-band recoveries and telemetry studies indicate that individuals in each may winter in or travel through portions of California or Mexico (J. Belthoff personal observation; D. H. Johnson personal observation; see also Holroyd and Trefrey 2011). Based on satellite- and geolocator-tagged adult Burrowing Owls (n = 11), the mean time between arrival in and departure from wintering areas is 137 days (range: 85–190 days) (D. H. Johnson personal observation). Spring (northbound) migration of adults (n = 3 male and n = 7 female) averages 27 days (range: 8–42 days, D. H. Johnson personal observation). Data concerning stopover locations and duration during spring migration for owls returning to northern tier U.S. states and Saskatchewan, Canada (D. H. Johnson personal observation), are available for 3 adult females tracked with satellite transmitters. One had 3 stopovers of 4, 6 and 3 days in duration; the other 2 had 1 stopover each (4 and 3 days in duration). Although information is limited, fidelity to wintering sites is strong in Burrowing Owls (Woodin et al. 2007). Owls return to the NCA and DEPOT to breed in March–April and frequently nest in artificial burrows provided by researchers (Smith and Belthoff

2001b, Belthoff and Smith 2003, Johnson et al. 2010). They lay 8–12 eggs that females incubate for ~22 days (Conway et al. 2012), and nestlings remain in nests for ~1 mo before making increased use of nearby satellite burrows for roosting. Nestlings gradually disperse from nest locations in late summer (King and Belthoff 2001) and adults and nestlings initiate autumn (southward) migration a number of weeks thereafter.

We captured adult owls at nest sites using wire traps placed at the mouths of burrows frequently in combination with an mp3 player and small speaker that broadcasted the owl's primary ("coo-coo") call. Nestlings were captured by hand from nest chambers when ~2–4 wk of age after opening the artificial burrows. We classified sex of adult owls by the presence/absence of a brood patch, which develops only in females, combined with behavior near nests. Although plumage differences distinguish nestlings from adults, nestling sex cannot be distinguished based on morphology or plumage and so was unknown for the owls in our sample. Captured owls already possessed or received a U.S. Geological Survey metal leg band for individual identification.

Sample Collection: Feathers, Toenails and Fleas

At each nest, we collected 2–3 contour feathers from the flanks of adult owls and from up to 3 nestlings and stored them separately by individual in coin envelopes. Using pet toenail scissors, we also obtained ~0.5 mm of the distal tip of a toenail from each captured adult and from up to 3 nestlings at a nest (the same individuals from which feathers were collected) and stored them in 1.5 mL plastic centrifuge tubes. Immediately upon capture we also searched the plumage of adult and nestling owls for fleas and aspirated them into 50 mL scintillation vials, to which we added ~5 mL of ethanol. We collected and analyzed fleas separately for each adult owl. Fleas from nestlings within a nest were pooled for analysis because nestlings from a nest were comingled upon capture, so all fleas could not be assigned to individuals as in adults. Sample sizes for fleas varied based on infestation level and our ability to capture fleas from owls, but we analyzed up to 10 fleas per adult and up to 10 fleas from pooled nestlings at a nest. We inspected each flea under 10–20 X magnification to distinguish species and sex. All male fleas keyed morphologically to *P. irritans*. Because female *P. irritans* and a closely related congener, *P. simmulans*, cannot typically be distinguished morphologically, we presumed that because all the males were *P. irritans* that all the females were also (Belthoff et al. 2015). Feather, toenail and flea samples were stored at room temperature until stable isotope analysis.

Stable Isotope Analysis

Hydrogen isotopic compositions were analyzed in Burrowing Owl contour feathers and toenail clippings, and in the whole fleas collected from owls. Samples were washed with a chloroform: methanol (2:1) solution, oven-dried overnight at 70°C, and allowed to equilibrate with laboratory air for 96 hr (Wassenaarr and Hobson 2006). Samples were then cut using surgical scissors, packaged into silver capsules and stored in 96-well microtiter plates (Wassenaar and Hobson 2003). We prepared 0.1–0.2 mg samples of feather barbs obtained from the distal 1–2 cm tips of feathers and 0.2 mg of toenail clippings for isotopic analysis. Whole fleas analyzed averaged 0.20 ± 0.09 (SD) mg in mass. Hydrogen isotope compositions were obtained using a Thermo Delta V CF-IRMS coupled with TC/EA. All deuterium data are reported in ‰ in standard delta notation (δD) and were normalized to VSMOW using CBS and KHS reference materials (Wassanaar and Hobson 2006, Soto et al. 2017).

Many fleas exhibited visual evidence for blood meals, but for 2 reasons we did not expect this blood to affect isotope compositions or our interpretations significantly. First, our sample processing involved soaking in alcohol, so some blood was probably washed away. Second, the mass of protein (the main solid H-carrier) in a blood meal is a tiny fraction of the total H we measured. Approximately 20% of blood mass is protein, so if a blood meal constitutes 10–20% of the body mass of a flea, only 2–4% of the total mass of blood protein plus chitin proteins could have been derived from blood. We estimated that the isotope composition of blood protein (derived from owls) differs from chitin by ~50‰ based on the Hobson et al. (1999; blood protein relative to local water) and chitin compositions of fleas that we measured (chitin protein relative to local water; local water composition from Tappa et al. 2016). If so, the bias to chitin composition from owl blood protein is only 1–2‰. Such a small correction is well within the noise of our data.

Statistical Analysis

We used mixed models to examine the potential effects of age class (adult, nestling), tissue type (flea, feather, toenail) and the interaction of these 2 factors on δD for each Burrowing Owl population (NCA, DEPOT) separately. We considered the effects significant when $P < 0.05$ and used Tukey's HSD for pairwise comparisons among means when effects were judged as significant. Because we analyzed >1 feather and flea from individuals, and up to 2 adults and 3 nestlings per nest site, we used individual and nest site as random effects. For adult owls, we also evaluated the potential effect of sex, tissue type, and the interaction of these 2 factors for the 2 populations separately in a mixed model using individual as a random effect. Neither sex nor its interaction with tissue type was significant, so we pooled adult males and females for analysis of deuterium in relation to age class and tissue type. Means are reported ± 1 SD unless otherwise noted, and all statistical analyses were completed using JMP Pro 14.2 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Between April 1 and June 29, 2017, we captured 30 adult Burrowing Owls from 28 nest sites and 75 nestlings from 30 nest sites in the NCA and DEPOT populations (Table 1). Six of the 15 adults (40%) from the NCA were recaptures of individuals originally captured and leg-banded in the NCA in a previous breeding season, and all but one adult in the DEPOT (93%) sample were recaptures of individuals captured and leg-banded at the DEPOT during a previous breeding season. All nestlings in both populations were captured for the first time during our study in 2017. We analyzed a toenail sample from all adults and all but 2 of the nestlings (for whom the sample was inadvertently lost during laboratory preparation) and 2 feathers from each owl, except for 4 adults where 3 each were analyzed. We analyzed 149 fleas obtained from Burrowing Owls in the NCA (2.7 ± 2.4 per adult, range: 1–10) and 6.4 ± 3.9 fleas per nest from nestlings (range: 1–10, Table 1). For the DEPOT, there were 122 fleas (1.8 ± 1.3 per adult, range: 1–6) and 7.3 ± 2.7 (range: 3–10) per nest from nestlings (Table 1).

Deuterium in Feathers, Toenails and Fleas

Mean δD in Burrowing Owl tissues (feathers and toenails) ranged between -91.9‰ (NCA nestling feathers) and -29.6‰ (NCA adult toenails, Table 2). Feathers and toenails typically were more depleted in the heavy isotope in the NCA relative to the DEPOT for both adults and nestling owls (Table 2). Mean δD in fleas ranged less among age-population classes than either feathers or toenails (Table 2).

There was a significant interaction between tissue type and age of owl on δD in both the NCA and DEPOT populations (Table 2). As expected, δD in nestling feather and toenail samples did not differ, and this pattern was similar in each population (Figure 3, Table 3). Toenail samples in adults from both populations were more enriched in the heavy isotope than toenails in nestlings (Figure 3, Table 3), which is consistent with a more southern signature in the adults, as expected under migration. However, in contrast to predictions, feathers from adults were more enriched than in nestlings in each population, so they also seemed more representative of possible wintering grounds (Figure 3, Table 3). Finally, δD for fleas at the DEPOT did not differ between adults and nestlings (Figure 3, Table 3), but fleas from adults were more enriched in the heavy isotope than those from nestlings in the NCA (Figure 3, Table 3), although differences were much smaller than between nestlings and adults for feathers and toenails.

DISCUSSION

Stable isotopes have previously been useful for mapping distribution and movement of Burrowing Owls under the assumption that deuterium ratios in precipitation and owl feathers are correlated across broad geographic clines (Macías-Duarte and Conway 2015). However, rather than identify the exact source locations where tissues were grown, our objective was to compare δD in owl fleas to those from owl tissues to evaluate the extent to which patterns were consistent with long-distance movement of fleas by migrating Burrowing Owls. Such a pattern would be reflected by disparate isotopic compositions between the fleas and owl tissues. Migratory birds indeed move a variety of ectoparasites among populations and between wintering and breeding grounds as up to 39 million Neotropical ticks are transported to the U.S. annually on migratory songbirds (Cohen et al. 2015). Fleas can adhere to hosts via mouthparts, embed in the skin, or otherwise exist within plumage and then translocate over long distances while associated with migrating birds (Schwan et al. 1983). For instance, after becoming infested with parrot sticktight fleas (*Hectopsylla psittaci*) while overwintering in South America, Cliff Swallows (*Petrochelidon pyrrhonota*) introduced this species of flea into North America when returning to breed in California (Nelson et al. 1979, Schwan et al. 1983). Adult fleas

TABLE 1. Location, timing and the number of adult and nestling Burrowing Owls and their fleas captured in 2 populations during 2017 and analyzed for investigation of geographic origin of tissues to examine the potential for long-distance dispersal of fleas (*Pulex irritans*, Siphonaptera: Family Pulicidae) by migratory owls. The number of adults that are male and female is indicated, whereas all nestlings are unknown sex.

State	Population	Age class of owl	Number of owls (Sex ratio: M:F)	Capture dates	Number of owl nests	Number of fleas
Idaho	NCA	Adults	15 (11:4)	April 1–May 18	14	40
		Nestlings	46	May 30–June 29	17	109
Oregon	DEPOT	Adults	15 (8:7)	May 7–May 14	14	27
		Nestlings	29	May 27–June 2	13	95

TABLE 2. Results of linear mixed model analysis for potential effects of tissue type and age of owl on δD (‰) in fleas, feathers and toenails collected from adult and nestling Burrowing Owls from the (A) NCA (Idaho) and (B) DEPOT (Oregon) populations in 2017. Individual and nest site were random effects because >1 feather and flea from individuals and up to 2 adults and 3 nestlings per nest site were examined.

(A) NCA

Fixed effects tests

Factor	df	*F* ratio	*P*-value
Tissue type	2 and 294.9	88.04	< 0.001
Age	1 and 37.0	346.98	< 0.001
Tissue type*Age	2 and 296.0	118.03	< 0.001

Random effects covariance parameter estimates

Variance component	Estimate	SE	Wald *P*-value
Individual	26.93	13.31	0.043
Nest site	66.09	26.23	0.012
Residual	109.11	9.29	

(B) Depot

Fixed effects tests

Factor	df	*F* ratio	*P*-value
Tissue type	2 and 228.1	64.52	< 0.001
Age	1 and 37.3	129.01	< 0.001
Tissue type*Age	2 and 228.1	71.16	< 0.001

Random effects covariance parameter estimates

Variance component	Estimate	SE	Wald *P*-value
Individual	6.62	10.41	0.524
Nest site	30.29	16.33	0.064
Residual	116.76	11.21	

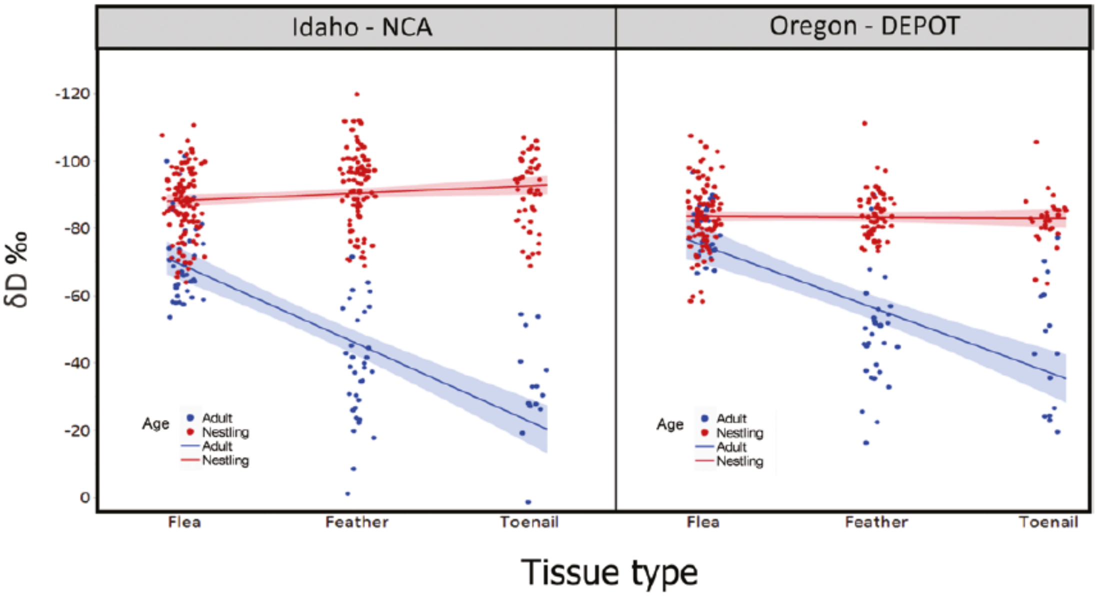

FIGURE 3. Deuterium (δD, ‰) in flea, feather and toenail samples from adult (blue) and nestling (red) Burrowing Owls in 2 migratory populations. Data points are jittered for presentation. Regression lines with confidence bounds are for illustrative purposes only. See Table 3 for mean values and the results of statistical comparisons within populations. The vertical axis is inverted to reflect breeding ground compositions to the north (top of figure).

TABLE 3. Least squares mean δD (‰) in fleas, feathers and toenails collected from adult and nestling Burrowing Owls from the NCA (Idaho) and DEPOT (Oregon) populations in 2017. Number of samples (*n*) analyzed is indicated for each age class. Separately for each population, means with the same letters (in parentheses) do not differ significantly ($P > 0.05$) from one another; see also Figure 3.

Population	Age class	Flea (x̄ ± SE)	Feather (x̄ ± SE)	Toenail (x̄ ± SE)
NCA	Adults	−69.5 ± 2.5	−38.3 ± 2.5	−29.6 ± 3.1
	95% CI	−74.6 to −64.4	−43.4 to −32.2	−35.6 to −23.5
	n	40	33	15
		(A)	(B)	(C)
	Nestlings	−88.1 ± 2.1	−91.9 ± 2.0	−89.1 ± 2.2
	95% CI	−92.3 to −83.9	−95.9 to −87.8	−93.6 to −84.7
	n	109	94	45
		(D)	(D)	(D)
DEPOT	Adults	−79.1 ± 2.3	−48.4 ± 2.1	−44.9 ± 2.7
	95% CI	−83.6 to −74.6	−52.7 to −44.2	−50.3 to −39.5
	n	27	31	15
		(A)	(B)	(B)
	Nestlings	−82.6 ± 1.7	−84.0 ± 1.9	−82.3 ± 2.2
	95% CI	−86.1 to −79.0	−87.8 to −80.3	−86.8 to −77.8
	n	95	58	28
		(A)	(A)	(A)

live from several months to more than a year (Lahane 2005), which is sufficient time to accompany a Burrowing Owl on migration as migration movements are accomplished in shorter durations than flea lifespans (Holroyd et al. 2011, D. H. Johnson personal observation). As some of the flea species that occur on Burrowing Owls are competent plague vectors, long-distance movements would have implications for the ecology of plague in western North America.

Despite the potential for birds to mediate dispersal of fleas, our study did not reveal evidence of continent-wide movement of *P. irritans* such as between Burrowing Owl wintering grounds and breeding grounds. Instead, isotopic compositions of fleas on adult owls in the DEPOT population closely resembled those in nestling feathers and toenails, which suggests local breeding ground geographic origin for these fleas. Similarly, fleas on adult owls in the NCA did not indicate a wintering ground source, as they were more like those in nestling tissues than isotopic compositions in either adult feathers or toenails. However, even though they did not appear to reflect a wintering ground signature, fleas on NCA adults had significantly different isotopic compositions than fleas, feathers and toenails from NCA nestlings; this suggests a possible different source location than for fleas from nestlings. One potential explanation for this pattern is the acquisition of fleas by adults at stopover sites that owls use during migration, which presumably would have a somewhat more southern isotopic signature than breeding grounds based on patterns of observed migration (Holroyd et al. 2010, Holroyd and Trefrey 2011, D. H. Johnson personal observation). The limited available data on stopover biology of Burrowing Owls indicate that owls may stopover at more than one location and for more than one day in each location while undertaking northward migration. If they make use of burrows at these stopover locations or encounter different sources of fleas, such as from hunted prey, then there is the potential to acquire fleas. Nonetheless, our data are clear in that there is no isotopic evidence that owls transport fleas from wintering areas. Owls may be devoid of fleas in at least some wintering areas (Sissons et al. 2006, Woodin et al. 2007), which also may indicate that owls do not disperse fleas from breeding grounds to wintering grounds via autumn migration.

P. irritans is widespread in distribution and has adapted to many host species, including carnivores, rodents, ungulates and humans (Lewis et al. 1988, Hopla 1980). Hopla (1980: p. 195) wrote that "Ordinarily, when dealing with the genus *Pulex* one does not expect to be involved in a host association involving Aves. However, in the Pacific Northwest, this unique phenomenon is encountered....Whatever the processes involved in such a unique adaptation, they are truly remarkable ones." Thus, while this peculiar association has been recognized for decades, much about this host-parasite relationship remains poorly known. Roost, cache and nest burrows are the most likely source of Burrowing Owl fleas, just as they are for many species of flea that infest birds (Whiting et al. 2008). During winter, Riding and Belthoff (2015) microwaved nest debris in owl burrows in Idaho to kill flea eggs, larvae and/or pupae before the onset of springtime nest site selection and breeding. Owls used burrows with and without the microwave treatment with similar frequency, so the absence of fleas in these burrows did not appear to influence nest site reuse. But fleas ultimately infested nestlings at the microwaved sites during the ensuing nesting season (Riding and Belthoff 2015). It is not clear to what extent these fleas were reintroduced by previously parasitized adults arriving to nest in the burrows, upon rodent prey that owls delivered to the nests, through carnivores or other mammals visiting the previously microwaved burrows, or by flea movements among nearby burrows, so these dynamics of the host-parasite relationship require further investigation.

Other species of flea do occur in Burrowing Owl nests or on the owls. For instance, Jellison (1939) recovered 109 live rodent fleas of 6 species from a Burrowing Owl nest in a plague area near Dillon, Montana. Wheeler et al. (1941) retrieved 70 sticktight fleas from a single Burrowing Owl following a plague outbreak in California. Brown (1944) recovered a single flea from each of 2 live Burrowing Owls in Alberta, Canada, and identified them as *Oropsylla idahoensis* and (tentatively) *Rectofrontia fraternal*, noting that *O. idahoensis* was a known plague vector. Smith and Belthoff (2001a) found 3 species of flea on owls in Idaho, although almost 90% of individuals were *P. irritans*. Belthoff et al. (2015) found *P. irritans* strongly associated with Burrowing Owls in Oregon, Idaho, and Washington, but not in Colorado or South Dakota. Thus, even though *P. irritans* occurs in other portions of the Burrowing Owl range, the association of this flea species with Burrowing Owls remains most pronounced in the northwestern U.S. despite more widespread distribution of both Burrowing Owls and *P. irritans*.

Finally, an unexpected result was that contour feathers of adult Burrowing Owls in both owl populations did not present a breeding ground δD signature. Instead, δD was more similar to that of adult toenails, which suggests more of a wintering ground origin, or at least an origin that is different than from the breeding grounds. One potential explanation for this pattern is that the owls from which we collected contour feathers in the spring of 2017 were breeders or were hatched in different populations in 2016, and thus deuterium values reflected those geographic locations rather than current breeding sites. Dispersal among populations is reported in Burrowing Owls (e.g., Duxbury 2004, Holroyd et al. 2011). However, this potential explanation is weakened because (i) breeding dispersal distances typically average only <0.5–4 km (De Smet 1997, Catlin et al. 2005, Riding and Belthoff 2018), and (ii) almost all adults in the DEPOT population and many of those in the NCA were recaptures of owls captured previously in the respective study areas. A second explanation is that the prebasic molt of body feathers was not complete by the time that adult or juvenile owls left breeding grounds for migration the previous year, although Pyle (1997) notes that the prebasic molt in both adults and hatch year individuals is completed on the summer grounds. Lastly, it is possible that the isotopic signatures from contour feathers in adults reflect occurrence of pre-alternate molt. Pyle (1997: p. 85) describes the absence of or limited scope of pre-alternate molt in Burrowing Owls, commenting that it may include "just a few breast feathers." Poulin et al. (2011) discuss that reports of a pre-alternate molt (e.g., Thomsen 1971, Butts 1973), including replacement of some body feathers in March–May, requires confirmation as it may instead be part of early prebasic molt related to the formation of brood patches. However, the contour feathers we analyzed in adult owls in spring had distinctly different isotopic compositions than feathers in nestlings, and the fact that many of the contour feathers presented similarly among owls may suggest a pattern that included more than a few feathers involved in a pre-alternate molt and more contour feathers than just those on the breast. While we collected only fully grown contour feathers, some male and female owls in our study populations had body feathers in sheaths indicating obvious molting (J. Belthoff, personal observation), and therefore in the case of males, not in relation to a development of a brood patch. Had we collected remiges rather than contour feathers, we believe it is likely that they would have reflected a breeding ground signature, as breeding adults in both the DEPOT and NCA populations show breeding site fidelity (J. Belthoff and D. H. Johnson, personal observation) and flight feather molt occurs in July–September while on the breeding grounds (Pyle 1997).

In conclusion, birds are hypothesized to be among the possible agents for spreading plague between rodent populations (Stenseth et al. 2008, Gage 2012). Gage (2012) explains that in the course of capturing, killing and feeding on rodents, birds of prey especially may acquire fleas that are carried to new sites where the fleas leave their accidental host and find new rodent hosts. Burrowing Owls do not appear to be an accidental host for *P. irritans*, as this flea species often parasitizes the owls, and Burrowing Owls are not simply a phoretic host (Graham et al. 2016). Although *P. irritans* is thought to be a competent plague vector, we detected no evidence of long-distance dispersal of fleas between wintering grounds and breeding grounds by Burrowing Owls. The lack of pronounced continent-level movement of fleas suggests that even during epizootic outbreaks of plague the chances of such long-distance dispersal of fleas carrying plague bacteria would be low. However, the data for one population of owls was consistent with the possibility that smaller spatial extent, inter-population movements of fleas by a raptor is possible, such as between potential stopover sites and breeding areas. While stable isotopes are useful for addressing broad scale movements, molecular studies to examine genetic structure in flea populations and to more narrowly define their sources are important next steps in understanding the dynamics of the host-parasite relationships involving Burrowing Owls and *P. irritans*.

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