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A comparison of the diet and fine-scale distribution of sympatric Tibetan and red foxes in Qinghai, PR China

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We compared the diet and the spatial distribution of the Tibetan fox *Vulpes ferrilata* and the red fox *Vulpes vulpes* in the Tibetan plateau, to elucidate mechanisms of coexistence for these two sympatric canids and to clarify their roles as definitive hosts for zoonotic *Echinococcus* parasites. Diet and fine-scale distribution patterns were assessed by fecal DNA analysis. A total of 45 fecal samples (15 belonging to Tibetan fox, 30 belonging to red fox were collected from 15 sites into three of which contained only Tibetan fox feces, six only red fox feces, and six contained feces of both species. The abundance of pika burrows, a key prey item for both species, did not differ among the sites. Food composition analysis, estimated using a point-frame method, revealed slight but insignificant differences between the two species. Tibetan foxes consumed primarily mammals, whereas red foxes consumed primarily insects. The dietary range of the Tibetan fox was narrower than that of the red fox but there was little dietary overlap between the two species. These findings suggest that the weak partitioning of food resources between Tibetan and red foxes can facilitate their coexistence even within the same habitat where they share the same key prey items, i.e. small mammals such as pikas. These dietary differences between the two fox species also suggest that the Tibetan fox is a more important definitive host for *Echinococcus* on the Tibetan plateau than is the red fox.

On the Tibetan plateau, sympatric carnivores play important roles in maintaining the sylvatic cycle of zoonotic parasites, such as *Echinococcus* spp. As this area is one of the most serious endemic regions for echinococcosis (Jenkins et al. 2005), studies on the definitive fox host species – the red fox *Vulpes vulpes* and Tibetan fox *Vulpes ferrilata* – are necessary to clarify the epidemiological status of each parasite species (Wang et al. 2008). As the life-cycle of *Echinococcus* parasites is maintained through fox predation of intermediate host species such as voles and pikas, then understanding the diet and feeding habits of these two sympatric canid species is particularly important.

Previous studies have shown that the red fox is largely an opportunistic forager (Schaller 1998, Lin et al. 2010, Murdoch et al. 2010) while the Tibetan fox is a specialist forager of small mammals such as pikas (Zheng 1985, Schaller 1998, Clark et al. 2008, Liu et al. 2010). Identifying the origins (host species) of fecal samples deposited by sympatric carnivores of similar body size is difficult (Heinemeyer et al. 2008). In the majority of dietary studies on carnivores, field-collected fecal samples have been assigned to host species either by the morphological characteristics of the feces or by virtue of being collected at known locations (e.g. near occupied dens or tracks; Zheng 1985, Lin et al. 2010, Liu et al. 2010, Murdoch et al. 2010). Hence, in the absence of prior information or of species-specific differences in scats, identifying the origin of fecal samples becomes increasingly unreliable. Recently, however, a noninvasive genetic method has been developed that enables accurate identification of species from fecal samples (Nonaka et al. 2009, Jiang et al. 2012). Importantly, such fecal DNA analysis enables more precise comparisons of diet among sympatric carnivores. DNA analysis of field-collected fecal samples can also be used to infer the spatial distributions of sympatric carnivores (Ruiz-González et al. 2008). Although the broad geographical ranges of Tibetan and red foxes are considered to overlap (Schaller and Ginsberg 2004, Clark et al. 2008, Wozencraft 2008), the fine-scale spatial distributions of these species (e.g. home ranges) and their spatial relationship has yet to be determined. In this study, we used fecal DNA analysis
to determine the diet and fine-scale spatial distribution of the Tibetan fox and the red fox where they exist in sympatry on the Tibetan plateau, in Qinghai province, PR China. In addition, we used this information to evaluate the relative roles of these definitive host species in the life-cycle of *Echinococcus* spp. on the Tibetan plateau.

**Material and methods**

**Study site**

Feces were collected from grassland within 100 km of the town of Heka in Xinghai county, Qinghai province, PR China (35°19′N, 99°05′E – 36°06′N, 100°39′E, Fig. 1). The study area lies on the Tibetan plateau at an altitude of 3000–4500 m above sea level, within the eastern part of the geographical distribution of the Tibetan fox (Schaller and Ginsberg 2004, Wozencraft 2008). The site serves as summer and winter grazing areas for yak and domestic sheep of Tibetan pastoralists.

**Fecal sampling**

We collected fox feces in September 2010, August 2011 and August 2012. Sampling sites were selected along roads within a radius of about 100 km from Heka town. Four line transects (about 200 m long and 2 m wide, measured by counting the steps of each investigator) were placed at each sampling site (Fig. 1). Sampling sites were placed at least 4 km apart, as this distance was longer than the length of an individual home range for both Tibetan and red foxes (red foxes = 2.28–8.71 km²; Zhou et al. 1995; Tibetan foxes = 5.2–7.2 km²; Liu et al. 2007). Within each sampling site we sampled along four line transects that covered approximately the area of an individual home range for both Tibetan and red foxes (~400 m diameter). Feces were labeled and held separately in plastic bags in the field, before being stored at −80°C for at least 10 days to kill any *Echinococcus* eggs (Veit et al. 1995). Feces were then stored at −20°C until use.

**Fecal DNA analysis**

Fecal DNA was extracted from washings of the frozen feces using QIAamp DNA Stool Mini Kits according to methods described by Nonaka et al. (2009). Briefly, ASL buffer from the kit was added directly to the frozen fecal samples and used to ‘wash’ the sample, by shaking the plastic bag vigorously 50 times. After removing the feces, we then collected approximately 1.4 ml of the liquid in a tube, to which an EX inhibiting tablet was added. We mixed the mixture vigorously for 1 min and then incubated it at room temperature for 1 min. We centrifuged the sample at 20 000 × g for 3 min and transferred 600 μl of the supernatant to a fresh tube to which 15 mAU of Proteinase K was added. The remaining extraction procedures followed the manufacturer’s instructions, extracting DNA with 50 μl AE buffer. We performed polymerase chain reaction (PCR) amplification for the partial sequence of the D-loop region of the DNA with primers: prL (5′–CACCATTAGCACCAAGCT–3′) and prH (5′–CCTGAAGTAGGAACCAGATG–3′). The sequences of the PCR products were read with a DNA sequencer using Big-Dye terminator cycle sequencing kits ver. 3.1. Sequences were identified to species by alignment to known sequences using the Basic Local Alignment Search Tool (BLAST; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

![Figure 1. Distribution of Tibetan and red fox feces collected around Heka town, Qinghai Province, PR China, in September 2010 (‘10), August 2011 (‘11), and August 2012 (‘12). The locations of Tibetan and red fox feces collected are shown as right-half-filled circles and left-half-filled circles, respectively. The filled circles indicate the locations where both foxes’ feces could be gathered. The squares indicate the locations where fecal sampling were performed. Black and gray lines show a national road and rivers or lakes, respectively.](https://bioone.org/journals/Wildlife-Biology/10.1644/10-083.1?view=download&expiresAt=2022-09-10T14:56:10Z&imageWatermark=true)
Fecal dietary analysis

All but 0.5–1.0 g of each fecal sample that was successfully identified to species was used in the dietary analysis (0.5–1.0 g of each fecal sample was used in concurrent study of fecal parasite load; Li et al. 2013). Feces were washed with tap-water through 1 and 0.5 mm mesh sieves and the fragments remaining on the sieves sorted into broad food categories (mammals, birds, insects and plants). The mammal category was further divided into large or small species according to the thickness of any hairs (<1 or > 1 cm) and bones (<2 or > 2 mm) found within the sample. In addition, samples containing pika teeth were classed as ‘Ochotona sp’. The insect category was divided into Coleoptera, Orthoptera or larvae.

Dietary composition, breadth and similarity

We evaluated diet composition by tallying the frequency of occurrence of food items and by using the point-frame method (Takatsuki et al. 2007). In the point-frame method, the food remains were spread over a petri dish that was placed on a sheet with a 2 mm grid. All items (i.e. bones, teeth and hairs) that covered any of the crossing points of grids were tallied until 200 items of each food had been counted (Takatsuki and Tatewaki 2012). We defined the proportion of each food item in the fecal sample as:

\[
\%P_i = \frac{\sum_{j=1}^{n} \%P_{ij}}{\sum_{j=1}^{n} P_{ij} \times 100}
\]

where \(\%P_{ij}\) is the proportion of total crossing points covered by food item \(i\) in the fecal sample \(j\), \(P_{ij}\) is the total number of crossing points covered by food item \(i\) in feces \(j\), and \(t\) is the number of food items. We compared the dietary composition estimated by the point-frame method between Tibetan and red foxes nested within year using a permutational, non-parametric, multivariate analysis of variance (MANOVA; Anderson 2001, McArdle and Anderson 2001), that can compare groups without calculating the central locations of these groups, does not require specific assumption concerning the number of variables or the nature of their individual distributions or correlations, and is robust under an unbalanced experimental design. For each food item, we compared the difference between the two fox species using a generalized linear model (GLM), with \(\%P_{ij}\) as the response variable and fox species, year of sampling as explanatory variables, and a binomial error structure. We calculated dietary breadth (\(B\)) in each species according to Levin's measure (Krebs 1999):

\[
B = \frac{1}{\sum P_i^2}
\]

where \(P_i\) is the proportion of food item \(i\). The scores potentially ranged from 1 (only one item consumed) to the maximum number of food categories (nine in this case, when all food categories were consumed evenly). We used Schoener’s (1970) index of overlap, \(C_{xy}\), to assess the dietary similarity between two fox species:

\[
C_{xy} = 100 \left( 1 - \frac{1}{2} \sum |P_{xi} - P_{xy}| \right)
\]

where \(P_{xx}\) and \(P_{xy}\) are the proportions of food item \(i\) of species \(x\) and \(y\) obtained by the point-frame method.

Prey abundance

The relative abundance of pikas at each of the sampling sites was evaluated by counting the number of new pika burrows (those with fresh soil and feces) within the line transects. This metric has been shown to be correlated with population density of these pikas (Liu et al. 2003). We used a generalized linear mixed model (GLMM) to compare the total number of new pika burrows among three broad ‘types’ of sampling sites (those where only Tibetan fox feces were collected (T), those where only red fox feces were collected (R), and those where feces of both species were collected (B)). We included ‘fecal sampling site’ as a random effect in the GLMM. We also analyzed the result of the GLMM by Tukey’s multiple comparison test.

Statistical analysis

We conducted all statistical analyses using statistical software R (ver. 2.15.1, <www.R-project.org/>). We used the Adonis function of package ‘vegan’ (Oksanen et al. 2013) for permutational MANOVA and the car package for conducting likelihood-ratio type 2 test (Fox and Weisberg 2011). We used the Imer function of package ‘ImeR’ for GLMM (Bates et al. 2014) and used the glht function of package ‘multcomp’ for conducting Tukey’s multicomparison test (Fox and Weisberg 2011).

Results

We collected 70 fecal samples from 19 sites, but only successfully determined species in 45 of those samples from 15 sites (Fig. 1). In total, 199 to 370 bp sequences obtained from 30 field-collected feces were matched to published sequences of Vulpes vulpes (GenBank accession number AB292754), and 222 to 360 bp sequences from 15 field-collected feces were matched to published sequences of Vulpes ferrilata (JF520840). We collected feces of Tibetan and red foxes exclusively at three and six sites, respectively, with feces of both species present together in the same year at a further six sites (Fig. 1). We found red fox feces predominately in the eastern portion of our study area, while Tibetan fox feces were collected predominately in the western portion of the study area, with both feces present together in the central area of the study site (around Heka town; Fig. 1). The abundance of new pika burrows did not differ significantly among these three site ‘types’ (Tukey’s multiple comparisons test, T–R, \(z = -0.94, p = 0.62\); T–B, \(z = -0.13, p = 0.99\); R–B, \(z = -1.26, p = 0.22\)). However, there was large variation in the average number of burrows they contained: red fox only sites (R) = 1.0 ± 2.1 SE; Tibetan fox only sites (T) = 2.8 ± 3.6; and sites with both species (B) = 31.7 ± 11.4).

Via fecal analysis, we identified nine food categories consumed by both fox species (Table 1). The species differed in the dominant food items they consumed, with Tibetan foxes most frequently consuming mammals, and red foxes most frequently consuming insects. Over the whole study period, the highest \(\%P\) and \(\%F\) value for Tibetan foxes was for small mammals, whereas in red foxes, the highest \(\%P\) and
%F value was for Orthopteran insects. Overall food composition did not differ significantly between the two fox species (permutational MANOVA, F\textsubscript{1,44} = 3.78, p = 0.074). However, for each food item, the %P values of pika, Coleoptera, birds and earthworms did differ significantly between the two species (pika LR test, χ\textsuperscript{2} = 46.6, DF = 1, p < 0.001; Coleoptera LR test, χ\textsuperscript{2} = 323.7, DF = 1, p < 0.001; birds LR test, χ\textsuperscript{2} = 63.4, DF = 1, p < 0.001; earthworm LR test, χ\textsuperscript{2} = 182.6, DF = 1, p < 0.001). Our results showed that the dietary breadth of the Tibetan fox was narrower (B = 2.46 in %P, 4.08 in %F) than that of the red fox (B = 4.51 in %P, 5.52 in %F), particularly in terms of the %P value. Interestingly, dietary overlap between the two fox species was low (C\textsubscript{xy} = 50.2 in %P, 70.3 in %F).

Discussion

In this study, we have shown that the distribution of feces of red and Tibetan foxes overlapped at a scale of 400 m (i.e. twice the length of the 200 m line transect used in this study), which is less than the average home range size for either species. Although it is known that the geographical distributions of Tibetan and red fox overlap at a broad scale throughout China (Schaller and Ginsberg 2004, Clark et al. 2008, Wozenacka 2008), our results provide evidence that the two species occur sympatrically even at a fine scale. Indeed, we found that these two species apparently share the same defecating places within their home ranges in the same year. These shared defecating places may be a by-product of the foraging or scavenging behavior of red foxes because red foxes are known to defecate on food remnants (Henry 1977), around the carcasses of large mammals (Macdonald 1985) and where prey are abundant (Monclus et al. 2009). Previous studies have shown that the Tibetan fox is a specialist predator on small mammals, and especially pikas (Schaller 1998, Liu et al. 2010). Nevertheless, the abundance of pikas (based on a count of new burrows) did not differ significantly among site types, though burrows were most numerous at sites where we found feces of both species together.

The plateau pika Ochotona curzoniae is the dominant small mammalian herbivore on the Tibetan plateau and is regarded as a keystone species in the ecosystem (Smith and Foggin 1999). Additionally to the red fox (Schaller 1998) several other carnivores, including the steppe polecat Mustela eversmanni, the weasel Mustela altaica, M. eversmanni, and Pallas’ cat Otocolobus manul also rely heavily on pika (Smith et al. 1990, Schaller 1998, Smith and Foggin 1999). Our finding that there was likely a relatively high abundance of pikas in areas where both foxes were present suggests that the Tibetan and red fox are able to share foraging areas without excluding each other.

Nevertheless, the distributions of the Tibetan and the red fox showed some dissimilarities that are likely to be correlated with geoenvironmental differences that occur on a larger regional scale. We found red fox feces predominately in low altitude areas in the eastern portion of our study area and Tibetan fox feces in the higher altitude western areas, which suggests key differences in habitat preferences between these two species. Fecal collections over a much larger area will be required to more fully understand the relationship between altitude and the density of each fox species.

Our results showed that while the food items consumed by the two species did not differ significantly, the dietary overlap between them was low (in terms of %P). We found that Tibetan foxes ate more small mammals and fewer orthopteran insects than did red foxes, and showed a more restricted dietary breadth. These results support those of previous feeding studies (Zheng 1985, Schaller 1998, Liu et al. 2010). For example, previous studies have shown that while the diet of the red fox in China and Mongolia varies among regions, small mammals constitute their principal food items, with Coleopteran and Orthopteran insects also an important resource (Schaller 1998, Lin et al. 2010, Murdoch et al. 2010). Interestingly, we found that the proportions of each food item consumed by red foxes changed among years. This also supports previous work showing that that the diet of red foxes varies both seasonally and regionally (Schaller 1998, Lin et al. 2010, Murdoch et al. 2010, Xuanlong et al. 2010).
Interspecies competition among sympatric canids can be greatly reduced via partitioning the use of shared food resources, as has been reported to occur for corsac and red foxes (Murdoch et al. 2010), and for San Joaquin kit foxes and coyotes (Cypher and Spencer 1998). Our results support these previous findings and suggest that the weak partitioning of food resources we observed between these two species can facilitate their coexistence within the same habitats on the Tibetan plateau. Differences in activity patterns may further facilitate coexistence between these two canid species. The Tibetan fox is relatively diurnal, corresponding to the activity of pikas (Schaller 1998, Wang et al. 2004), while the red fox is largely nocturnal (Ables 1969, Eguchi and Nakazono 1980, Weber et al. 1994, Zhou et al. 1995, Doncaster and Macdonald 1997). Indeed, we have observed Tibetan foxes being active in the daytime both directly and using camera traps (Tsukuda et al. unpubl.). Such temporal segregation between Tibetan and red foxes might further facilitate sympatric coexistence between the two fox species.

In this ecosystem, wild foxes are known to be important definitive hosts of Echinococcus multilocularis and E. shiquicus (Jenkins et al. 2005). Echinococcus shiquicus, which exclusively uses the plateau pika O. curzoniae as an intermediate host, has been found solely in the Tibetan fox (Xiao et al. 2005). Echinococcus multilocularis, meanwhile, can use many small mammalian species as intermediate hosts (Giraudoux et al. 2006, Wang et al. 2008). Our results revealed that the Tibetan fox consumed a higher proportion of small mammals than did the red fox. This finding suggests that the Tibetan fox is likely to be a more important definitive host of Echinococcus in the Tibetan plateau because of its high level of predation on infected intermediate hosts. Previous work has shown that the infection rate of E. multilocularis among red foxes is a function of the rate of fox predation on voles, which are key intermediate hosts for E. multilocularis (Saitoh and Takahashi 1998, Yokohata and Kamiya 2004, Tsukada 2005, Tanner et al. 2006, Hegglin et al. 2007, Raoul et al. 2010). Interestingly, prevalence of E. multilocularis infection is broadly similar in Tibetan foxes (33.3–59.1%) and red foxes (15 – 59.3%; Wang et al. 2008). Additionally, a survey of helmint fauna in the Tibetan and red fox also showed no difference in the prevalence of taeniid cestodes, including E. multilocularis, in the two fox species (Li et al. 2013). The seasonal and regional variations in the diets of each fox species might have mitigated any differences between the two fox species in their rates of infection with E. multilocularis. Difference in susceptibility to E. multilocularis infection between the Tibetan and red fox are poorly understood but are also likely to influence infection rates. To more robustly understand the epidemiological risk to the Tibetan and red foxes of echinococcal infections in Tibetan plateau, further ecological and parasitological studies on these species will be needed.

In this study, we have revealed slight partitioning in the diets of the Tibetan and red fox, with the former species being a specialist small mammal predator. In addition, we show that there is significant overlap in the spatial distributions of these two species. Our data support the suggestion that the Tibetan fox is the key definitive host for Echinococcus spp. in this region. Hence, furthe epidemiological surveys should focus on infection dynamics in the Tibetan fox population to elucidate the sylvatic cycle of Echinococcus spp. infection in the Tibetan plateau.

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