The past population dynamics of *Ochotona curzoniae* and the response to the climate change

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Abstract. Plateau pikas (*Ochotona curzoniae*) are widespread on the Qinghai-Tibetan Plateau. To study the past population dynamics of this species, we used a method based on the Bayesian coalescent theory to analyze the mitochondrial DNA of pikas sampled from the Qinghai-Tibetan Plateau. The Skygrid model was selected as optimal model for estimating population demography. The simulation results under the optimal model showed two distinct changes in effective population size since 1 million years ago. The first population expansion coincided with the Paleo-Lakes period. Subsequent population decline was related to the shrinking of the lakes and the last glacial maximum. Changes in climate, due to the appearance of the paleo-lakes and the glaciers, created more or less suitable habitats for the pika, which corresponded with changes in effective population sizes over time. The second population increase was related to the Kunlun-Yellow River Movement: the uplift of the Qinghai-Tibetan Plateau resulted in a wet and cold climate, and subsequent vegetation changes provided more habitats for the pika.

Key words: *Ochotona curzoniae*, past population dynamics, demography, Qinghai-Tibetan Plateau, climate.

Introduction

The Qinghai-Tibetan Plateau has experienced several significant geological uplifts since the Quaternary period, including the Qingzang Movement, the Kunlun-Yellow River Movement, and the Gonghe Movement (Ma et al. 2005, Zheng & Yao 2006). The Kunlun-Yellow River Movement had a profound impact on the plateau. The plateau altitude exceeded 3,000 meters after this event, causing the plateau to enter the cryosphere. Combined with global climate changes, this uplift effectively caused the glacial period of the Pleistocene (Liu 1999). In addition, the Paleo-Lakes period is another event that affected the climate of the plateau in the late Pleistocene (Zhu et al. 2004). These climatic and environmental changes played an influential role in the distribution and diversity of plants, leading to changes in the diversity of animals living in different habitats (Wang et al. 2001, Shapiro et al. 2008, Nater et al. 2015).

The plateau pika (*Ochotona curzoniae*) is widely distributed across the Qinghai-Tibetan Plateau (Lai & Smith 2003). As a keystone species, this small diurnal mammal has important implications for maintaining the stability of the alpine meadow ecosystem by constructing burrows that are used as shelter and nest sites by other species, providing food resources for a suite of top predators, and contributing to plant community dynamics (Smith & Foggin 1999, Arthur et al. 2008). This species lived through the Pleistocene (Lanier & Olson 2009), such that climate change likely influenced the geographic distribution and effective habitat of plateau pikas during this period. In addition, the distribution of plateau pikas largely coincides with the range of the Qinghai-Tibetan Plateau, making it an ideal candidate for understanding the relationships between climatic changes and the population dynamics of species of the Qinghai-Tibetan Plateau (Ci et al. 2009).

Coalescent theory has been commonly used as a foundation to estimate past population dynamics using molecular sequence data. Early coalescent-based estimation algorithms required a priori parametric settings, leading to difficult and time-consuming calculations (Minin et al. 2008). Over time, many nonparametric models have been developed, such as the Bayesian Skyline Plot (BSP; Drummond et al. 2005), a method that uses Bayesian coalescent inference together with a Markov chain Monte Carlo sampling algorithm (MCMC; Metropolis et al. 1953, Hastings 1970). However, the smoothness of the plot remains problematic. In recent years, the Gaussian Markov random field (GMRF) Bayesian Skyride model was proposed, which first exploits a Gaussian Markov random field to achieve temporal smoothing. Based on the Skyride model, the Bayesian Skygrid was developed, offering several improvements (Gill et al. 2012, Horn et al. 2014).

In this study, we used mitochondrial DNA (mtDNA) data from pikas to determine their effective population size over time using the BSP, Skyride, and Skygrid models, comparing their plots. Ultimately, to understand the effect of climatic changes on the pika population demographic history, we estimated the timing of specific demographic events.

Materials and Methods

Sequence acquisition

A total of 246 samples were collected from 37 locations (Table1, Fig. 1). The sequences analyzed included the mtDNA sequences of Cyt*b* (1140bp), tRNA-Thr and tRNA-Pro (135bp), 12SrRNA (946bp), and the partial D-loop of all specimens. Their haplotypes have been published in GenBank by Liu et al. (2013) and Ci et al. (2009), accession numbers FJ227330–FJ227482 and KF038167–KF038213, including Cyt*b*, tRNA-Thr, tRNA-Pro, partial D-loop, and 12SrRNApika haplotypes. Additional sequences that have never been amplified from fifty-four Cyt*b*, tRNA, and D-loop samples and 71 12SrRNA samples were supplemented for this study. The haplotypes of 12SrRNA and concatenated sequences of other three genes were deposited in GenBank under accession numbers KM225715–KM225732 and KM225702–KM225714, respectively. The Cyt*b*, tRNA, 12SrRNA and D-loop sequences from each specimen were concatenated into a 2561bp sequence alignment for further analysis.

Table 1. Details of sampling sites.

| Sampling site code	Sampling site	Latitude (°N)	Longitude (°E)	Altitude (m)	Sample size
YSL	Yelashan	30.187	97.295	4338	14
BD	Bangda	30.529	97.128	4348	10
JZ	Jiangzi	28.901	90.101	4660	10
LKZ	Jungong	34.647	100.592	3467	6
HB	Haibei	37.685	101.274	3238	6
NQ	Naqu	31.441	92.277	4420	6
QL	Qilian	37.959	100.255	3645	6
GC	Gangcha	37.503	100.482	3164	6
EL	ElingLake	35.074	97.716	4234	8
T1	Tibet1	29.237	87.218	4481	5
T2	Tibet2	31.355	80.577	4753	7
T3	Tibet3	30.578	82.536	4944	8
T4	Tibet4	29.493	85.089	4607	4
TJ	Tianjun	37.174	99.283	3275	6
CD	Chengduo	33.356	97.236	4348	8
ZD	Zhiduo	33.54	96.064	4308	7
KLS	Kunlunshan	35.734	94.310	4110	7
SQ	Shiqu	33.033	98.015	4254	8
SD	Senduo	35.440	101.00	3389	4
TT	TuotuoRiver	34.330	92.591	4533	5
NM	Nimu	29.502	90.270	3908	10
AK	Kaerdun	36.929	90.242	4100	7
AT	TuziLake	36.800	87.308	4750	8
AQ	AqikeLake	37.003	88.610	4250	6
AA	Kaerqiuer	37.043	90.755	4184	6
AD	Anduodong	32.309	91.732	4675	5
AX	Anduoxi	32.178	91.593	4586	3
NMC	NamCo	30.721	91.035	4807	6
XDT	Xidatan	35.712	94.058	4532	8
XX	XingxingSea	34.833	98.135	4194	8
HSX	Huashixia	35.082	98.855	4237	7
BL	BeiluRiver	34.862	92.942	4590	5
CM	ChumaerRiver	35.356	93.386	4520	2
LKZ	Langkazi	29.109	90.417	4425	8
GL	Geladandong	33.589	91.652	4873	4
QML	Qumalai	34.142	95.884	4389	7
QHL	QinghaiLake	36.630	100.107	3175	5
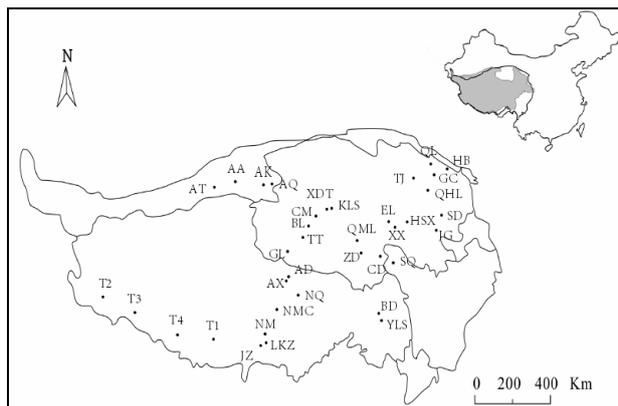
Figure 1. Map of the sampling sites. Approximate geographic distributions of the plateau pika (gray area) are shown on the outline map of China (Smith & Johnston 2008).

DNA extraction and sequencing

Total genomic DNA was extracted from ~15mg of muscle tissue collected from study animals using a TIANamp Genomic DNA Kit (Tiangen Inc., Beijing, China). Polymerase chain reaction (PCR) amplifications were conducted in total reaction volumes of 50 μL, containing 5.0 μL 10×Taq buffer (TaKaRa, Dalian, China), 2.5U Taq DNA polymerase (TaKaRa), 1.5mM MgCl$_2$, 0.1mMdNTPs, 0.4μM each primer, 40–60 ng genomic DNA, and sufficient ddH$_2$O to achieve the final volume. A 1,140-bp fragment of Cyt*b* was amplified using the primers L14724 (5′-CGAAGCTTGATATGAAAAA CCATCGTTG-3′) and H15915 (5′-CGGAATT CCATTTTTGGTTTA CAAGAC-3′) (Kocher et al. 1989). The 12S rRNA sequence was amplified with the primers 12F (5′-AAAGCAAAACACTGAA AATG-3′) and 12R (5′-TTTCATCTTTTCCTTGCGGTAC-3′) (Liu et al. 2013). The remaining nucleotide sequences (tRNA-Thr, tRNA-Pro, and D-loop) were amplified with the primers FR (5′-ATGTTCCGCCCAATCAGCCAAT-3′) and RV (5′-GTTGCTGGTTT CACGGAGGATGG-3′) (Ci et al. 2009). PCR was performed as follows: initial denaturing at 95°C for 8 min; followed by 35 cycles of 95°C for 45 s, 55–57°C for 45 s, and 72°C for 80 s; and a final extension at 72°C for 7 min. Thermocycling was conducted in a Bio-Rad S1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA). PCR products were purified with the TIANgel Midi Purification Kit (Tiangen Inc., Beijing, China). PCR products were sent to Sangon Biotech Co. (Shanghai, China) for sequencing.

Sequence analysis

DNAStar was used to merge the forward and reverse sequences. Then, the spliced sequences were aligned using Mega 5.05. BEAST v1.8.0 was run to simulate the demographic history over time (Gill et al. 2012). Reconstruction of the effective population size (N$_{ef}$) can be described as N$_{ef}$g (τ) over time, where g denotes the generation length, and time is estimated based on molecular clock calibration (Grant et al. 2012, Lanier & Olson 2009).

Generation time of *O.curzoniae*
Female plateau pikas will typically give birth to 1–4 litters from April to August each year in different areas (Qu et al. 2012). Most juveniles become mature in the next breeding season. In a rare case (e.g. low population density and a low altitude of ~3,100 m above sea level), a few of the juveniles from the first litter may participate in breeding 3 months after birth. Hence, one year is regarded as the typical generation time of the plateau pika. Therefore, estimates of τ were converted to N_{ef} by dividing by a generation time of 1 year (Kitchen et al. 2008).

Estimation of population demography
We chose three tree models for estimating demographic change using BEAST v1.8.0 (Drummond et al. 2012), including BSP, GMRF Bayesian Skyride, and Bayesian Skygrid. The dataset was run under an HKY+G+I model of nucleotide substitutions suggested by the jModelTest 2.1.8 (Posada 2008). The analyses were based on a strict molecular clock, with substitution rates of 4.8% and 6% per million years based on the average mutation rate of the Cyt*b*/D-loop of American pika (*Ochotona princeps*) and the higher mutation rate of mtDNA proposed for other small mammals (Galbreath et al. 2009), respectively. The BSP, Skyride and Skygrid models were set with default priors for model parameters in BEAUti. Each MCMC sample was based on a run of 100,000,000 generations, sampled every 1,000 generations. All samples were then examined in Tracer v1.6 (Rambaut & Drummond 2013) with the first 10% discarded as burn-in to produce the final population trajectory. Operators were tuned with successive runs of the program under the chosen model until the effective sample size for each parameter exceeded 200 (Drummond & Rambaut 2007). An analysis of a posterior simulation-based analog of Akaike's information criterion through Markov chain Monte Carlo (AIC$_M$) was used to select the most likely model of demographic change in Tracer.

Results

We analyzed the mtDNA data to estimate the effective population size of the plateau pika over time using the BSP, Skyride and Skygrid models under a mutation rate of 4.8%. The estimated trajectories all show a spike around 70–80 thousand years ago (kya). However, the trends are different in all three trajectories prior to 0.08 million years ago (mya). Figure 2a depicts the simulation results using a BSP for the simulation dates from 0.67 mya to present. In the plot, the effective population size remained stable until 76 kya years, and then increased rapidly until it reached a maximum at ~17–18 kya, followed by an apparent decrease. Figure 2b shows the demographic history using the Skyride model, which only goes as far back as 0.25 mya. The trajectory shows a very slow increase between 0.09–1 mya, followed by a distinct increase at ~90 kya. The N_{ef} reached a maximum between 20–23 kya. The time scale reconstructed by the Skygrid model covers 1mya (Fig. 2c). The trajectory shows two peaks that are separated by a long period of relatively little change. One peak appears at ~80 kya, reaching a maximum at ~20 kya. The other peak arises at 0.36–0.9 mya, with the maximum appearing at 0.59 mya.

The shapes of the estimated trajectories under a rate of 6% were the same as those observed at a rate of 4.8%, though the timing of the changes in effective population size were slightly later. The first population expansion occurred at ~60–66 kya and reached a maximum at about 12–17 kya. The second effective population size change happened at 0.35–0.89 mya under the Bayesian Skygrid model.
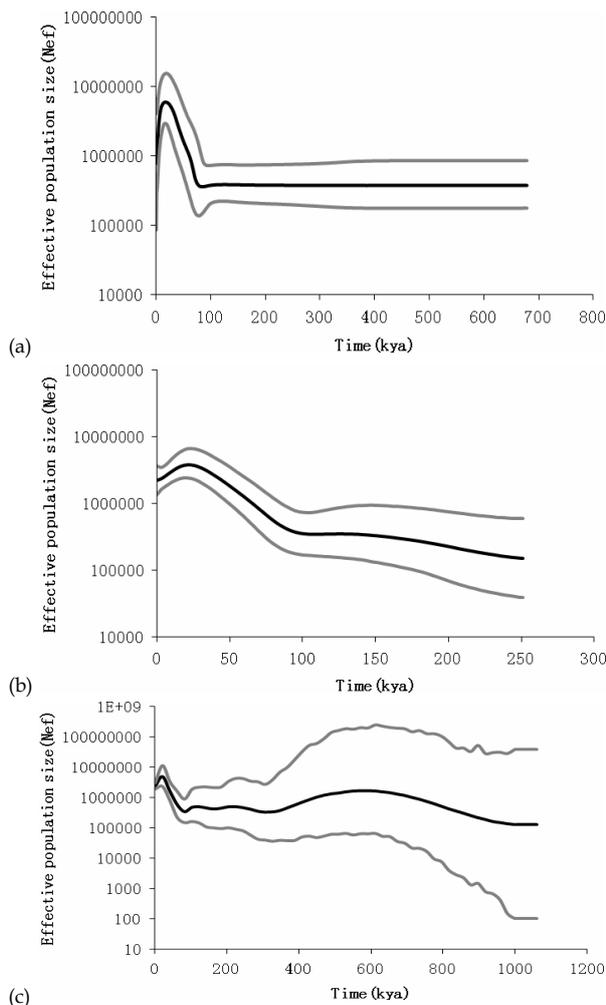

Figure 2. Simulation of the demographic history of pikas using coalescent-based models. (a) Bayesian Skyline plot of effective population size over time. (b) Skyride plot of effective population size over time. (c) Skygrid plot of effective population size over time. Values on the y-axis are logarithmic values. Black lines represent median posterior effective population sizes over time. Grey lines delimit the 95% highest posterior densities of the effective population size, accounting for uncertainty in the reconstructed substitution model parameters.

We then compared the models by AIC$_M$ using the results calculated with a rate of 4.8%. AIC$_M$ is a method that compares the marginal likelihood estimations to calculate reliable Bayes factors. Lower AIC$_M$ values indicate a better model fit, and positive values indicate a better relative model fit of the row's model compared to the column's model (Baele et al. 2012). A comparison of the models showed that the Skygrid simulation had the lowest AIC$_M$ value (Table 2), indicating a better relative model fit. Therefore, the estimated trajectory using the Skygrid model is predicted to be the best among the three trajectories.

Discussion

Incongruence among the three models
When the three models were compared in our study, we found that the Skygrid model had the best fit, with the evidence indicating that the simulations using BSP and

Table 2. Models comparison based on AIC$_M$.

Trace	AIC$_M$	S.E.	Skyride	BSP	Skygrid
Skyride	21043.00	1.004	-	-448.14	-449.89
BSP	20594.86	0.349	448.14	-	-1.75
Skygrid	20593.11	0.277	449.89	1.75	-

Skyride were not ideal for uncovering population dynamics in the distant past. This is a limitation of the algorithms in both the BSP and Skyride analyses (Grant et al. 2012). Grant et al. (2012) proposed that climatic deteriorations during glacial maxima lead to population declines that can erase previous demographic histories, with the exception of information from the last glacial cycle, when using the BSP model. Skyride incorporates the GMRF prior, which does not provide an overall estimate of the effective population size by only affects the smoothness of the trajectory. Therefore, this model was unable to uncover the true distant demographic history of the plateau pika. Skygrid is an improvement over the BSP and Skyride models, as it can recover true population trajectories (Gill et al. 2012, Kuzmina et al. 2013). Although the data show a piecewise constant function among the three models, Skygrid estimates the effective population size trajectory, which changes values at previously specified times called grid points; this enables the analysis to capture as much information on population dynamics as possible, while the other models merely estimate trajectory changes at coalescent times (Gill et al. 2012). Our results further support the notion that the Skygrid model can overcome the algorithm limitations of BSP and Skyride to effectively estimate population sizes over a considerable amount of time.

The effect of climate change on the pika

The climatic and environmental changes during the Pleistocene epoch played an important role in the distribution and diversity of plants and animals (Yang et al. 2006, Jin et al. 2008). According to the trajectory simulated using the Skygrid model under a rate of 4.8%, the demographic history of the plateau pika showed two changes at 76 kya and 0.9 mya.

The first population expansion happened at about 76 kya. According to the record of the Guliya ice core, the plateau entered into the early stadial of the last glacial at 75 kya, resulting in a gradual temperature decline (Yao et al. 1997). Paleoclimatic evidence shows that vast pan-lakes were formed in the plateau during at 37–116 kya, reaching a peak at 80 kya and then shrinking again at 45 kya (Yu 2008). The formation of the pan-lakes resulted in a shift to a humid climate at 76 kya. Sporopollen analysis suggests that the vegetation changed to a sparse forest steppe in northern Tibet and alpine shrubby steppe and alpine meadow in some areas of southern Tibet in the late Pleistocene (Shen & Tang 1994, Wu et al. 2004). Pikas prefer to inhabit alpine grasslands with little brushwood and shorter broad-leaved plants (Wang et al. 2004). The forest receding in the hinterland of the Qinghai-Tibetan plateau provided a more suitable habitat for the increasing pika population. The effective population size rapidly declined following the expansion at 17–18 kya. Although the climate appeared to fluctuate during the stadial-interstadial period, the overall shift was towards a drier climate (Wang et al. 1996). The

paleo-lakes shrank in this cold environment, while the Chin-sha River and the Nu River ran through the Tuotuo River paleo-lake and north Tibet pan-lake, respectively, resulting in the rapid escape of water from the paleo-lakes around 36–37 kya; in turn, the lakes shrank or vanished(Wu et al. 2009). The paleo-lakes degenerated completely and settled into their present pattern at about 17 kya. The shrinking of the paleo-lakes may have caused the plateau climate to become drier overall. As the last glacial maximum arrived (about 20 kya), the climate became drier and colder in the plateau (Zheng et al. 2002). As the climate deteriorated, pikas lost their suitable habitat, resulting in stiff competition for food resources (Liu 2008). Desertification even occurred in the north plateau around this time (Wan et al. 2008). Due to the lack of food in the desert, the habitat was not suitable for pikas, causing them to scatter across the meadows existing in the desert. This is likely to account for the apparent drop in the population size of the pika after 17–18 kya.

The second population increase occurred at 0.9 mya. This demographic change may be related to the Kunlun-Yellow River Movement, which occurred at 0.6–1.2 mya. As the plateau was lifted to 3000-3500m, major mountainous regions entered the cryosphere. The increase in reflectivity caused by several glaciations formed a cold anticyclone, which cooled the plateau (Li et al. 1998). The climate gradually cooled during this period, leading to a significant change in the vegetation in the plateau (Kong & Du 1996). The vegetation of the northern Tibetan plateau was coniferous with a broadleaf mixed forest before the Kunlun-Yellow River Movement (Kong & Du, 1996); as the climate changed, the forest gradually degenerated. At the same time, sediment cores from the hinterland of the plateau indicate that in general, the climate was humid in the early middle Pleistocene (Tian et al. 2011). Sporopollen evidence also shows that herbaceous levels reached 44±16%, of which Cyperaceae spores accounted for 25% between 0.5 and 0.85mya, reflecting a humid environment in northern Tibet (Lv et al. 2001, Wu 2002). Therefore, the cool and wet climate provided a more suitable habitat for the pika, resulting in an increase in its effective population size. Sporopollen records also show that the vegetation type was mainly grassland, represented by the presence of Cyperaceae under cold and wet conditions in the north-eastern plateau and the east Kunlun Mountains around 0.65 mya (Liu et al. 1994, Xu et al. 2001). This increased area of steppe vegetation led to an increase in the pika population.

Estimating the mutation rate of combined mtDNA sequence

In this study, we applied two mutation rates to estimate the demographic history of plateau pikas, as the rate of molecular evolution is time-dependent. Many studies have suggested that discrepancies in evolutionary rates can occur when they are calibrated by different time points. Usually, the short term (<1–2 mya) mutation rate is higher than the long-term substitution rate (Ho et al. 2005, Ho et al. 2007). However, there is no suitable fossil record available to calibrate the evolutionary rate at short timescale; therefore we adopted the strict molecular clock model to analyze the demographic history.

The short-term mutation rates for mammals range from 0.11 to 0.96 as proposed by Ho *et al.* (2007); these were

estimated for non-coding control regions, which have much higher rates those of mtDNA coding regions. The control region in our analysis was 342bp, which is smaller than the length of sequence we used. Moreover, there are large confidence intervals on the rate estimates obtained from recent calibration points (Ho et al. 2005). Considering all of the above factors, we applied a conservative mutation rate of 6% to assess the demographic history of pikas.

The patterns of the demography curve estimated by the rate of 6% were not different from those under the 4.8% rate in the three models. Despite the fact that the population expansions occurred slightly later under the rate of 6% than under the rate of 4.8%, these findings did not contradict our results indicating a change in the population demography. However, if we want to better understand the precise relationship between climate and the demographic history of pikas, it will be necessary to acquire reliable time points in short-term or intermediate time frames for calibration.

The plateau pika serves as the main prey for many predatory animals on the plateau. Some predatory animals such as *Mustela eversmanni, Mustela altaica,* and *Otocolobus manul* rely heavily on plateau pikas for food (Smith & Foggin 1999). The Tibetan fox (*Vulpes ferrilata*) is known to be an obligate predator of pikas (Harris et al. 2014). Populations of these types of predators are always linked to the dynamics of pika populations (Wei et al. 1994, Smith & Foggin 1999). However, studies of the demographic histories of animals that prey on plateau pikas are lacking at long timescales, making it difficult to assess whether the population history dynamics of predators have had an effect on the effective population size of the pika, and this should thus be investigated further in the future.

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