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VARIATION IN MITOCHONDRIAL DNA AND PHYLOGENY OF SIX SPECIES OF PIKAS (*OCHOTONA*)

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Phylogenetic relationships of six species of *Ochotona* were investigated using mitochondrial DNA (mtDNA) restriction-site analysis. The phylogenetic tree constructed using PAUP was based on 62 phylogenetically informative sites with *O. erythrotis* designated as an out-group. Two clades were evident. One contained *O. curzoniae*, *O. huangensis*, and *O. thibetana*. In the second, *O. daurica* was a sister taxon of *O. cansus*. The five species appear to come from different maternal lineages. The branching structure of the tree and sequence divergence confirm that *huangensis* and *cansus* are distinct species, and that *morosa* is a synonym of *O. cansus* rather than *O. thibetana*. Divergence time, estimated from genetic distances, indicates that the ancestors of the two maternal lineages diverged ca. 6.5×10^6 years ago. *O. curzoniae* diverged from *O. huangensis*, and *O. daurica* diverged from *O. cansus*, at about the same time (ca. 3.4×10^6 years ago). These data suggest a period of rapid radiation of the genus *Ochotona* in China, perhaps during the late Pliocene. These calculations correspond roughly to tectonic events and environmental changes in China throughout this period, and appear to be substantiated by the fossil record.

Key words: *Ochotona*, pika, China, mitochondrial DNA, phylogeny, restriction maps, taxonomy

The systematic status of the family Ochotonidae (order Lagomorpha), which includes only one living genus, *Ochotona* (pikas), is poorly known. Besides occupying remote and inaccessible habitats, morphological similarities among species of pikas have presented major obstacles to systematists. Most recent treatments have ceased to use the division of subgenus (Corbet, 1978; Hoffmann, 1993; Smith et al., 1990), however, others insist on subgeneric classification. Allen (1938) divided *Ochotona* into three subgenera, while Ellerman and Morrison-Scott (1951), Feng and Zheng (1985), and Yu et al. (1992) recognized two subgenera (*Pika* and *Ochotona*), based on whether or not the incisive and palatal foramina were separated.

Although numerous morphological studies have been conducted during the past

century (Smith et al., 1990), the taxonomic status of some forms of *Ochotona* remain problematic. The number of recognized species varies considerably across treatments. Forms considered as separate species by one author are included in several separate species by others (Smith et al., 1990). For example, *O. daurica* and *O. curzoniae*, meadow-steppe burrowing species, frequently have been regarded as conspecific (Allen, 1938; Ellerman and Morrison-Scott, 1951). Later, however, they have been treated as sibling species (Corbet, 1978; Feng and Zheng, 1985; Smith et al., 1990; Weston, 1982; Yu et al., 1992). *O. cansus* and *O. thibetana* are similar in morphology and habitat (both being shrubland, burrowing species), therefore, there has been confusion concerning the taxonomic position of the form *cansus*. Is it a synonym of *O. thi-*

betana (Allen, 1938; Corbet, 1978; Weston, 1982), or is it a separate species (Feng and Kao, 1974; Feng and Zheng, 1985; Smith et al., 1990; Yu and Zheng, 1992)? In addition, the systematic status of two forms within the *cansus/thibetana* complex has continued to generate controversy. The form *huangensis*, an independent species as originally described by Matschie (1908), was listed as a subspecies of *O. thibetana* (Allen, 1938; Ellerman and Morrison-Scott, 1951; Feng and Kao, 1974; Feng and Zheng, 1985; Shou and Feng, 1984; Smith et al., 1990), and a synonym of *O. daurica* (Gromov and Baranova, 1981; Ognev, 1940). Yu and Zheng (1992), however, separated it as a distinct species. The form *morosa* was described as a subspecies of *O. cansus* (Thomas, 1912), but was placed in *O. thibetana* by subsequent authorities (Allen, 1938; Feng and Kao, 1974; Yu and Zheng, 1992). Recently, it was reallocated to *O. cansus* by Smith et al. (1990).

The phylogeny of pikas is of considerable interest because of their suitability for the study of evolutionary patterns and processes. Unfortunately, few studies on phylogenetic relationships in the genus have been reported. Weston (1982) used a numerical approach in her revision of the genus. Although a comprehensive effort, Weston did not examine all specimens used in her revision. In addition, specimens were used uncritically; several apparently were mislabeled. As a result, aspects of this revision appear inaccurate. For example, Weston (1982) did not recognize *cansus* and *huangensis* as distinct species, and the specimens of *O. thibetana* she examined were actually a mixture of five species: *O. cansus*, *O. huangensis*, *O. forresti*, *O. nubrica* (the form *ihasaensis* only), and *O. thibetana*. Among the eight specimens of *O. erythrotis* she examined were specimens of another species, *O. gloveri*. Each of these errors can be traced to earlier confusion in assignment of specimens due to the high degree of convergence among forms. Smith et al. (1990) contended that because Weston's revision was a phenetic analysis, it was not

useful for inferring phylogenetic relationships.

A phylogenetic tree, for 14 species of the subgenus *Ochotona* in China, was derived from 22 morphological characteristics using a cladistic analysis (Yu et al., 1992). In this study, one minimum-length tree was produced; the shrub-dwelling species (*O. huangensis*, *O. thibetana*, *O. cansus*, and *O. thomasi*) formed one clade, while the steppe-dwelling species (*O. curzoniae* and *O. daurica*) appeared closely related. Yu et al. (1992) proposed that the diversification of this subgenus in China was correlated with the uplifting of the Qinghai-Xizang Plateau.

To date, most systematic treatments of *Ochotona* have been based on morphological characteristics, however, morphology often exhibits phenotypic plasticity and convergent evolution. Smith et al. (1990) suggested that molecular techniques and cladistic analysis would provide the information needed to clarify the systematic relationships of *Ochotona*. Molecular methods offer potentially powerful means to infer phylogenies because they expose genetic variation directly. MtDNA, in particular, is an ideal genetic marker for studying relationships among closely related species due to its simple molecular structure, rapid evolution, maternal inheritance, and absence of recombination (Avice, 1986; Harrison, 1989; Wilson et al., 1985). The purpose of this study was to estimate the relationships among six species of Chinese pikas (*O. erythrotis*, *O. huangensis*, *O. thibetana*, *O. cansus*, *O. curzoniae*, and *O. daurica*) and clarify some of the confusion concerning their taxonomy through mtDNA restriction-site analysis.

MATERIALS AND METHODS

A total of 26 specimens representing six species and eight forms (including subspecies) were used in this study (Appendix I). Animals were sacrificed in the field. Heart, liver, and kidney tissues were stored in liquid nitrogen until DNA was isolated.

MtDNA was isolated following the procedures described by Wang and Shi (1993). Purified mtDNA was digested with 16 hexanucleotide-

specific restriction endonucleases according to the specifications of the manufacturer; *Ava*I, *Bam*HI, *Bgl*II, *Bgl*III, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Sca*I, *Stu*I, and *Xba*I. The fragments produced by digestion were separated by electrophoresis through horizontal 0.8% agarose gels (0.05 Ug/ml ethidium bromide was added to the gel mix) using a TBE buffer system (pH 8.3). The DNA fragments were visualized under UV light.

Lambda DNA fragments, digested with *Hin*dIII, were used as molecular-weight standards. All of the restriction sites were mapped using a double-digestion method (Carr et al., 1987). Genetic distances between each haplotype were evaluated using site methods (Nei and Li, 1979). Using the restriction maps, variable restriction sites of each haplotype were coded as present or absent. A character matrix (Table 1) was formed using 62 phylogenetically informative restriction sites (i.e., those shared by at least two haplotypes).

Cladistic analyses of the mtDNA restriction sites were performed to evaluate relationships among the five species within the subgenus *Ochotona*, with *O. erythrotis* of the subgenus *Pika* designated as the outgroup. The distinguishing characteristics for subgeneric classification were based on those given by Yu et al. (1992). An unweighted parsimony analysis was conducted to construct phylogenetic trees using the PAUP program (Swofford, 1985). Branch-and-bound searches were used to ensure that all minimum-length trees were identified. To estimate the reliability of branches on the most-parsimonious tree, a bootstrap analysis was performed using a heuristic search with 100 replications.

RESULTS

Variation was observed in the length of the mtDNA molecules. The size of mtDNA from the five species in the subgenus *Ochotona* was ca. 17.9 kb, while that from *O. erythrotis* in the subgenus *Pika* was only ca. 16.9 kb.

All of the enzymes used in this study showed restriction-site variation. Sixteen restriction endonucleases surveyed 42–50 restriction sites, which corresponded to 252–300 base pairs, or 1.41–1.68% of the total mtDNA molecule. A total of 109 mtDNA restriction sites was mapped, and 10 haplotypes were identified from the

TABLE 1.—Character matrix for 62 phylogenetically informative restriction-endonuclease sites for six species of *Ochotona*. Presence is indicated by 1, absence by 0. Enzyme abbreviations are as follows: A, *Ava*I; I, *Bam*HI; D, *Bgl*II; Y, *Bgl*III; C, *Cla*I; E, *Eco*RI; R, *Eco*RV; N, *Hin*dIII; T, *Hpa*I; P, *Pst*I; V, *Pvu*II; K, *Sac*I; L, *Sal*I; F, *Sca*I; S, *Stu*I; J, *Xba*I.

Taxa	Restriction-endonuclease site															
	A	I	D	Y	C	E	R	N	T	P	V	K	L	F	S	J
<i>O. erythrotis</i>	000	1111	1100	0	01101	10000	1	10110	11000	01000	0	10	0	0000	01000000	00011000
<i>O. curzoniae</i>	111	1110	1100	1	10100	11110	1	11110	11101	11100	1	11	0	0111	10111110	10011110
<i>O. curzoniae</i>	111	1110	1100	1	10100	11110	1	10110	11100	11100	1	11	0	0111	10111110	10011110
<i>O. c. cansus</i>	110	1011	1111	0	10010	11011	0	11111	11110	11011	1	10	1	1110	11011111	11111101
<i>O. c. cansus</i>	110	1011	1111	0	10010	11011	0	11111	11100	11011	1	10	1	1110	11011111	11111101
<i>O. c. morosa</i>	110	1011	1111	0	00010	11011	1	11111	11100	11011	1	10	1	1110	11011111	11111101
<i>O. c. stovensii</i>	110	1011	1111	0	00111	11011	1	11111	11100	11011	1	10	1	1110	11011111	11111101
<i>O. thibetana</i>	110	1101	1110	1	11101	01100	1	10111	11101	11100	1	10	0	0110	11011111	00100100
<i>O. huangensis</i>	110	0110	1110	1	10000	10100	0	10110	11111	10100	1	10	0	1110	10101110	10100000
<i>O. daurica</i>	110	1011	1010	1	00101	00100	0	11101	11100	11011	1	00	1	1110	11011111	01011000

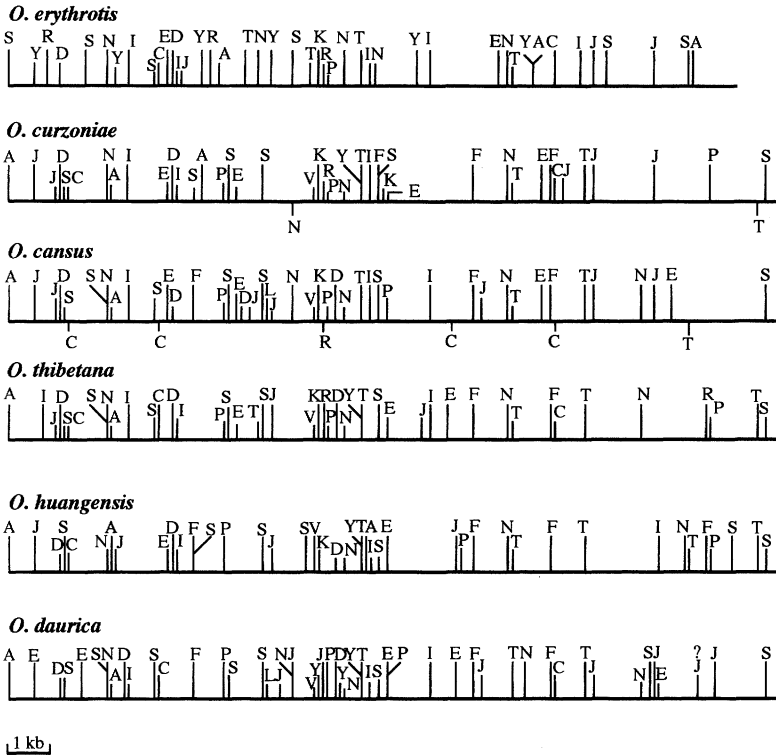


FIG. 1.—Restriction maps of mtDNA for six species of *Ochotona*. Lines above the horizontal indicate sites that are invariant; lines below the horizontal indicate sites that are variable. Enzyme abbreviations are as follows: A, *Ava*I; I, *Bam*HI; D, *Bgl*I; Y, *Bgl*II; C, *Cla*I; E, *Eco*RI; R, *Eco*RV; N, *Hind*III; T, *Hpa*I; P, *Pst*I; V, *Pvu*II; K, *Sac*I; L, *Sal*I; F, *Scal*; S, *Stu*I; J, *Xba*I.

restriction-enzyme analysis. The 10 haplotypes were summarized in six restriction maps based on different species (Fig. 1). Only five sites were invariant in all individuals examined.

Based on restriction maps, the genetic distance (the mean number of nucleotide substitutions per nucleotide site) between

any two mtDNA haplotypes was calculated (Table 2). The percentage sequence divergence among haplotypes ranged from 5.31 (*O. curzoniae* versus *O. thibetana*) to 22.53% (*O. erythrotis* versus *O. huangensis*). As expected from previous morphological studies, the greatest level of divergence of haplotypes was between *O. eryth-*

TABLE 2.—Genetic distances of mtDNA for 10 haplotypes of pikas (*Ochotona*).

Taxa	1	2	3	4	5	6	7	8	9
1. <i>O. erythrotis</i>									
2. <i>O. curzoniae</i>	0.1566								
3. <i>O. curzoniae</i>	0.1527	0.0039							
4. <i>O. c. cansus</i>	0.1705	0.0568	0.0584						
5. <i>O. c. cansus</i>	0.1723	0.0587	0.0603	0.0020					
6. <i>O. c. morosa</i>	0.1604	0.0568	0.0584	0.0037	0.0055				
7. <i>O. c. stevensi</i>	0.1455	0.0568	0.0568	0.0037	0.0090	0.0036			
8. <i>O. thibetana</i>	0.1667	0.0531	0.0545	0.0621	0.0640	0.0621	0.0554		
9. <i>O. huangensis</i>	0.2253	0.0657	0.0676	0.0877	0.0833	0.0942	0.1119	0.0776	
10. <i>O. daurica</i>	0.2001	0.0987	0.1016	0.0676	0.0693	0.0676	0.0609	0.0863	0.1307

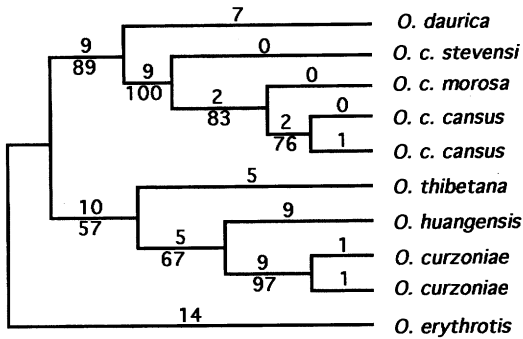


FIG. 2.—Phylogenetic tree of 10 haplotypes of six Chinese species of pikas based on 62 phylogenetically informative, mtDNA, restriction-endonuclease sites. One minimum-length tree was identified through branch-and-bound searches (tree length = 84; CI = 0.597; RI = 0.727). Branch lengths and bootstrap confidence values are indicated above and below the branches, respectively.

rotis (the outgroup) and species of the subgenus *Ochotona*, with a mean divergence of 18.12%. Mean divergence among species of the subgenus *Ochotona* was 7.96% (5.31–13.07%; Table 2).

The branch-and-bound search of the phylogenetic analysis produced a single minimum-length tree (Fig. 2) of 84 steps with a consistency index (CI) of 0.597 and a retention index (RI) of 0.727. A bootstrap analysis with 100 heuristic replications also resulted in a single most-parsimonious tree, which was identical to that obtained from the branch-and-bound search. The bootstrap confidence value suggested that the major branching pattern was relatively robust.

Two main clusters were apparent. One was comprised of *O. cansus* and *O. daurica* in 89% of the replicates. The form *morosa* appeared within the *O. cansus* grouping. The second cluster contained *O. curzoniae*, *O. huangensis*, and *O. thibetana* 57% of the time. Within the second cluster, *O. curzoniae* was the sister taxon to *O. huangensis* in 67% of the replicates.

DISCUSSION

Analysis of mitochondrial-DNA restriction sites from six species of *Ochotona* sug-

gests a completely different picture of systematics of pikas than in prior treatments. The major problem in earlier systematic studies of *Ochotona* has been the strong morphological resemblance between the species and the necessity to rely on morphological characters. Although many different morphological characters have been used over the years to define species and subspecies of pikas, the taxonomic status of some forms remains controversial. Weston's (1982) conclusion that the pikas, while being a morphologically conservative group, also demonstrated considerable intraspecific variation, is apparently inaccurate; her findings were based on inaccurate classification of material.

In this study we examined some of the most problematic cases in the systematics of *Ochotona*. One example concerns the systematic position of *O. cansus*. It frequently has been included in *O. thibetana* (Allen, 1938; Corbet, 1978), because of similarities in pelage color, size, and shape of the skull. Weston (1982) also did not retain *O. cansus* as a valid species, although the distinctiveness of *cansus* and *thibetana* may be found in her revision. However, Feng and Kao (1974), followed by Feng and Zheng (1985), Smith et al. (1990), and Yu and Zheng (1992), recognized it as a distinct species based on its narrow zygomatic width and the absence of intermediate forms in the extensive zone of sympatry between the two species. The two taxa were regarded as sibling species on the basis of both morphological and ecological similarities (Smith et al., 1990). A phylogenetic tree constructed from morphological characteristics using cladistic analysis also demonstrated that *cansus* and *thibetana* were distinct, but closely related species (Yu et al., 1992).

A second case concerns the putative close relationship between *O. curzoniae* and *O. daurica*, both meadow-steppe burrowing species with similar pelage color and a number of shared cranial features. Although they have been regarded as conspecific

(Allen, 1938; Ellerman and Morrison-Scott, 1951), modern studies considered these taxa as distinct sibling species (Corbet, 1978; Feng and Zheng, 1985; Smith, et al., 1990; Weston, 1982; Yu et al., 1992). Studies using serum proteins (Zhou and Xia, 1981) and diploid chromosome number (*O. curzoniae*, $2n = 46$, Tan and Bai, (1987); *O. daurica*, $2n = 50$, (Vorontsov and Ivanitskaya, 1973) also support their separation. Both phenetic (Weston, 1982) and phyletic (Yu et al., 1992) trees demonstrated that these forms are closely related.

In contrast to the currently held opinion that *curzoniae* and *daurica*, and *cansus* and *thibetana*, are pairs of sibling species, the present mtDNA analysis determined that each member of the pair was located on a different branch of the phylogenetic tree (Fig. 2). The branching pattern of the tree indicates that the four taxa are separate species, but not sibling species. The sequence-divergence data also support this point; average divergence between the suggested pairs of sibling species are at the species level (Table 2). For example, mean sequence divergence between *O. thibetana* and subspecies of *O. cansus* was 6.09% (5.54–6.40%). In addition, samples of *O. c. stevensi* and *O. thibetana* were collected from a region of sympatry, yet these forms had a sequence divergence of 5.54%. The sequence-divergence values for these four taxa were undoubtedly at the level of species distinctness (5.31–13.07%). The sequence-divergence values also support *O. curzoniae* as a separate species.

The resolution of subspecies within each of the species considered here also is controversial. The form *huangensis* was first described as a species based on two specimens (Matschie, 1908); the type locality is an unrecorded spot that was later assumed to be the Tsingling Mountains (Qinling, Shaanxi Province) in the vicinity of Sianfu (Xi'an shi) by Allen (1938). He recognized *O. huangensis* as a subspecies of *O. thibetana*, and this placement was followed by Ellerman and Morrison-Scott (1951), Feng

and Kao (1974), Weston (1982), Feng and Zheng (1985), and Smith et al. (1990). However, Ognev (1940), followed by Gromov and Baranova (1981), placed it as a synonym of *O. daurica*, apparently because Thomas (1908) in his original description, compared *O. huangensis* to *bedfordi*, a subspecies of *O. daurica*. They (Gromov and Baranova, 1981; Ognev, 1940), however, did not examine any specimens of *O. huangensis*. Our quantitative examination of many specimens from the assumed type locality, as well as from various sites throughout China, revealed remarkably distinctive skull characteristics between *huangensis* and *O. thibetana*, which suggest that *huangensis* might be a distinct species (Yu and Zheng, 1992). In the present study, *O. daurica* was in one clade, while *O. huangensis* was the sister taxon of *O. curzoniae* following the divergence of *O. thibetana* in the other clade. The analysis of mtDNA restriction sites also indicated a high level of divergence between the *huangensis* and *O. thibetana*. Thirty-two sites, which correspond to 54.2% of the total sites, were variable, and the sequence divergence between them was 7.76%. The divergence between the form *huangensis* and *O. daurica* was even higher (Table 2). Thus, the mtDNA data support the recognition of *huangensis* as a distinct species, rather than as a subspecies of *O. thibetana* or a synonym of *O. daurica*.

The form *morosa* originally was described as a subspecies of *O. cansus* (Thomas, 1912) and was placed as a synonym of *O. thibetana* by subsequent authorities (Allen, 1938; Feng and Kao, 1974; Yu and Zheng, 1992). However, it was reassigned to *O. cansus* by Smith et al. (1990). In our study, *morosa* was the sister taxon to *O. c. cansus* in 83% of the replicates within one clade, while *O. thibetana* was in the other clade. The sequence divergence between *morosa* and *cansus* ranged from 0.36 to 0.55%, while the sequence divergence between *morosa* and *O. thibetana* was 6.21%. The mtDNA data indicate that *morosa*

should be recognized as a subspecies of *O. cansus*, rather than *O. thibetana*. In traditional studies, *stevensi* and *cansus* were regarded as subspecies of *O. cansus*. In the present study, the mtDNA data support the split of these subspecies within *O. cansus*. These subspecific forms constitute clades in 83–100% of the bootstrap replicates.

Perhaps, the most intriguing results from the mitochondrial DNA, restriction-site analysis are the phylogenetic relationships of these species. As previously stated, Weston's (1982) revision did not provide resolution of the phylogeny of *Ochotona*. A phylogeny that included 14 species of the subgenus *Ochotona* in China was constructed from morphological characters using cladistic analysis (Yu et al., 1992). In that phylogeny, shrub-dwelling species, *O. huangensis*, *O. thibetana*, and *O. cansus*, had a close relationship within a more advanced cluster, while steppe-dwelling species, *O. curzoniae* and *O. daurica*, appeared closely related with a number of primitive morphological characteristics. The results of our mtDNA analysis do not support this hypothesis. In the present study, *O. huangensis*, *O. curzoniae*, and *O. thibetana* form a clade, whereas *O. daurica* was the sister taxon to *O. cansus* in the other clade. The two clusters appear to represent two maternal lineages.

Our molecular data also reveal that pikas are not necessarily conservative in morphology. Morphological similarities among the species examined probably are due to convergent evolution, apparently because morphology has tracked the environment and resulted in adaptive modifications in structure that increase the probability of survival. Ecological (Smith, 1988; Smith et al., 1990) and karyological (Vorontsov and Ivanitskaya, 1973) information support this contention. Life-history traits of species of pikas living in particular habitats (rock and talus, meadow-steppe, shrub) are remarkably convergent (Smith, 1988). Similarly, a karyological study of eight taxa of pikas (*O. pusilla*, *O. rutila*, *O. macrotis*, *O. rufescens*,

O. daurica, *O. alpina*, *O. hyperborea*, and *O. pallasi pricei*) suggested that the steppe-dwelling pikas (*pusilla*, *daurica*, and *pallasi*) might have entered the steppe environment three independent times (Vorontsov and Ivanitskaya, 1973). Our data suggest that *O. daurica* and *O. curzoniae* come from different maternal lineages. Therefore, it is not surprising that morphological characteristics might not be suitable to study the phylogeny of pikas, whether by phenetic or cladistic approaches.

Magnitude of molecular evolution has been used to estimate the age at which lineages have diverged (Brown et al., 1979). If the estimate of divergence of mtDNA with time as determined for other mammalian species ($2\%/1.0 \times 10^6$ years) is applied to pikas, *O. erythrotis* may have diverged from the five species in the subgenus *Ochotona* ca. 1.12×10^7 years ago. The ancestors of the two maternal lineages discussed above (Fig. 2) diverged from each other ca. 6.5×10^6 years ago. *O. thibetana* diverged from the *O. huangensis*-*O. curzoniae* clade ca. 3.9×10^6 years ago. *O. daurica* diverged from *O. cansus*, and *O. huangensis* diverged from *O. curzoniae*, at about the same time, ca. 3.4×10^6 years ago. These divergence estimates suggest a period of rapid radiation of *Ochotona* in China, perhaps during the late Pliocene.

Evolutionary diversification of pikas might be influenced by historical episodes of geologic and climatic changes. Previous geological studies indicate that two significant events occurred from the late Pliocene to the beginning of the Pleistocene (3.4 – 2.48×10^6 years ago) in China. The Qinghai-Xizang (Tibet) Plateau was uplifted and the temperature in the region decreased sharply, which resulted in an ice age throughout the Northern Hemisphere. During this period, glaciers developed in major mountain chains of the Plateau (Dong et al., 1995; Fang et al., 1995; Shackleton et al., 1984). During the Pleistocene, the Qinghai-Xizang Plateau was affected extensively by climatic oscillations, glacial-interglacial

transitions, and continued uplift. Moreover, northwestern China was becoming drier as a result of a rain-shadow effect by the Himalaya massif on the Plateau. Therefore, vegetation was significantly altered and isolation of habitats frequently occurred. All of these events provided a good opportunity for a spectrum of diversification and specialization within the genus *Ochotona*.

The earliest emergence of *Ochotona* in the fossil record seems to be the late Miocene in central Asia (Dawson, 1967). During the Miocene, the genus *Ochotona* was fully developed, and many species evolved. Fossil species of *O. largelii*, *O. minor*, and *O. gyizhongensis* were found in late Miocene (Bao-dian, 5.2–12 × 10⁶ years ago) deposits of Inner Mongolia, Shaanxi, Gansu, and Xizang provinces. The fossil form *O. cf. tibetana* was found in late Pliocene and early Pleistocene deposits in Qinghai and Gansu (ca. 1.8 × 10⁶ years ago). Fossils of living species, *O. daurica* and *O. curzoniae*, have been found in late Pleistocene formations in Hebei and Gansu provinces and in Holocene deposits in Xizang, respectively (Qiu and Qiu, 1990; Yu et al., 1992).

Recent geological studies have demonstrated that a unified ice sheet did not cover the Qinghai-Xizang Plateau during the Quaternary Ice Age (Li and Shi, 1995; Li et al., 1995), thus, large-scale biotic extinction was unlikely. Ancestral pikas may have been typical of arid, cold-adapted steppes, but they now are distributed in environments, such as steppe, shrub, coniferous forest, and alpine rocks (Smith, 1988; Smith et al., 1990). The fossil record and the history of climatic change suggest that pikas might have responded to habitat shifts induced by continued uplift of the Qinghai-Xizang Plateau along with glacial cycles from the late Pliocene to the Pleistocene through gradual adaptation to new habitats. As a result, species in the genus *Ochotona* have successfully occupied many habitats. Perhaps this diversification is the reason why so many species currently have sym-

patric distributions, and a vast majority of the species are distributed on the Qinghai-Xiang Plateau and adjacent areas today.

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APPENDIX I

Specimens examined.—All voucher specimens were deposited in the museum of the Northwest

Plateau Institute of Biology, Academia Sinica. Catalog numbers are given in parentheses.

Ochotona erythrotis (subgenus *Pika*).—CHINA (3): Xunhua, Qinghai, 35°45'E, 102°40'N (93J043, 93J044, 93J045).

Ochotona huangensis.—CHINA (1): Xunhua, Qinghai, 35°45'E, 102°40'N (93J046). This locality is the type locality for the form *xunhuaensis*, a synonym of *O. huangensis* (Yu and Zheng, 1992).

Ochotona thibetana.—CHINA (2): Kangding, Sichuan, 30°05'E, 101°45'N (93J001, 93J002). This locality is the type locality of the form *zappeyi*, a synonym of *O. thibetana* (Hoffmann, 1993; Smith et al., 1990).

Ochotona cansus stevensi.—CHINA (1):

Kangding, Sichuan, 30°05'E, 101°45'N (93J003).

Ochotona cansus morosa.—CHINA (5): Mt. Taibai, Shaanxi, 33°55'E, 107°42'N (93J006, 93J008, 93J010, 93J012, 93J014). This is the type locality.

Ochotona cansus cansus.—CHINA (6): Menyuan, Qinghai, 37°20'E, 101°40'N (93J019, 93J020, 93J022, 93J023, 93J024, 93J029).

Ochotona curzoniae.—CHINA (6): Menyuan, Qinghai, 37°20'E, 101°40'N (93J026, 93J027, 93J029, 93J030, 93J035, 93J039).

Ochotona daurica.—CHINA (2): west side of Qinghai Lake, Qinghai, 37°10'E, 99°40'N (94J002, 94J005).