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The mating system, dispersal behavior and genetic structure of a collared pika (*Ochotona collaris:* Ochotonidae) population in the southwest Yukon, and a phylogeny of the genus *Ochotona*.

By

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Abstract

Pikas (*Ochotona*, Ochotonidae) are small, short-eared lagomorphs that inhabit steppes and mountains in northern and central Asia and alpine regions in western North America. I examined the dispersal patterns, genetic structure and mating system of a collared pika (*O. collaris*) population from the southwest Yukon. Additionally, I reconstructed the phylogeny of the genus *Ochotona* using several mitochondrial genes.

Limited mark-recapture data suggests that juvenile collared pikas seldom disperse over 300 m from their natal dens, and that adults rarely travel off of an established territory. This behaviour could result in frequent inbreeding if individuals tend to mate with their nearest neighbours. However, because collared pikas are difficult to capture before they have dispersed, I decided to examine their dispersal behavior using indirect genetic methods. Pikas were captured within a four-square-kilometer study site from 1999 to 2008, and each pika caught (n=364) was genotyped at fifteen variable microsatellite loci. The data revealed very fine-scale genetic structuring in the population in all but two years, which is consistent with a pattern of limited dispersal. Parentage analyses also demonstrated that collared pikas disperse a mean of approximately 600 m from their natal dens and that they display no sex-biased dispersal. The population was able to maintain its genetic diversity despite undergoing a population bottleneck, likely due to receiving a small number of immigrants

from other populations. The mating system of collared pikas was largely polygynandrous, as there was evidence that both males and females produced offspring with multiple partners. Collared pikas also appear to make breeding forays to mate with individuals up to one kilometer away, and this ensures that their genes disperse and settle farther than they do.

Phylogenetic analyses were conducted using two data sets: one that contained *cyt*B and ND4 sequences from 49 *Ochotona* specimens and another that contained six mitochondrial genes from nine *Ochotona* species. Maximum-likelihood and maximum-parsimony analyses both recovered three main clades within *Ochotona*: one of steppe-dwelling pikas primarily from the Qinghai-Tibet Plateau, one of pikas from mountainous regions surrounding the Qinghai-Tibet Plateau, and one of alpine-dwelling pikas from northern Asia and North America.

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Chapter 1
General Introduction

Background and Context

Dispersal is an important aspect of an animal's life history, and if, when, how far and where an individual disperses will be influenced by its species, sex, body condition, and a variety of environmental cues (Greenwood 1980; Slatkin 1985; Ronce 2007; Long *et al.* 2008; Hawkes 2009). A species' dispersal habits can have important ecological and evolutionary consequences, because they influence how populations respond to bottlenecks, environmental change, and habitat fragmentation (Kokko and López-Sepulcre 2006; Busch *et al.* 2007). Additionally, an understanding of the factors that shape a species' dispersal habits is of great importance to population genetics because dispersal plays a large role in shaping a species' genetic structure (Bohonak 1999).

Populations of organisms with limited dispersal abilities may show genetic structuring at distances as small as one kilometer (*e.g.* common voles, *Microtus arvalis,* Schweizer *et al*. 2007). There has been a recent increase in the number of studies examining the fine-scale genetic structure of populations of several species, particularly through the use of spatial autocorrelation analyses of hypervariable genetic markers (such as microsatellites). In populations of organisms with low mean dispersal distances (< 1 km), such analyses typically reveal a pattern of positive spatial genetic autocorrelation at low distances, which gradually declines to zero and becomes negative at longer distances (Peakall *et al.* 2003; Busch *et al.* 2009).

Additionally, while dispersal can be rare or restricted to one sex in some species, whether or not an individual disperses, or how far it travels, may also be contingent on population density. Positive density-dependent dispersal, where increased competition for resources due to crowding induces animals to migrate, is well documented in birds and small mammals (see Matthysen 2005 for a review). However, dispersal may also be negatively density dependent if animals remain philopatric at high densities because of a lack of territory vacancies (Lucia *et al.* 2008) or if crowding and increased antagonistic encounters with conspecifics prevents animals from dispersing (the “social fence” hypothesis; Hestbeck 1982).

Whether or not a population that undergoes periodic fluctuations displays positive or negative density-dependent dispersal will also influence how its genetic diversity and structure change through time. Under a situation of negative density-dependent dispersal, a population’s genetic diversity and structure may remain temporally stable, as it does in the root vole (*Microtus oeconomus*, Pilot *et al.* 2010). This is because, during low-density phases, migration among populations may restore any genetic diversity previously lost through drift (Pilot *et al.* 2010). In contrast, under a scenario of positive density-dependent dispersal, a fluctuating population will display temporal changes in its level of genetic diversity, although it may maintain a high level of diversity over the long term. Diversity will be lost during low density phases, and depending on the severity of the population decline, genetic differentiation among populations may increase during the low density phase. Once population densities start increasing, migration among populations will increase and restore the genetic diversity lost in each population. This model appears to explain why cyclic water vole (*Arvicola terrestris*) populations retain their genetic diversity through time (Berthier *et al.* 2006).

Frequently, patterns of genetic structuring vary between the sexes, as there is some degree of sex-biased dispersal in most bird and mammal species. Males tend to be the non-dispersing sex in birds while females are often the non-dispersing sex in mammals (Greenwood 1980); thus genetic structuring tends to be more pronounced in females in mammals (Fredsted *et al*. 2005; Nussey *et al.* 2005; Frantz *et al.* 2008; Loison *et al*. 2008; Nituch *et al.* 2008), and males in birds (Double *et al*. 2005; Temple *et al*. 2006), although exceptions to this pattern exist (*e.g.* female-biased dispersal in the tree shrew *Tupaia tana*, Munshi-South 2008)

Patterns of sex-biased dispersal will depend on the costs and benefits of dispersal for each sex, and these typically depend on (or are shaped by) the mating system of the species in question. Greenwood (1980) first hypothesized that the direction of sex-biased dispersal is linked to mating systems in birds and mammals, as species with female-defense polygyny often display male-biased dispersal while species with resource-defense monogamy (or polygyny) often display female-biased dispersal. The former mating system is common in mammals, while the latter mating systems are common in birds. Familiarity with one's territory (and thus philopatry) will benefit males in resource-defence mating systems and females in most female-defense systems; hence it is male mammals and female birds that tend to disperse (Greenwood 1980).

Greenwood's (1980) hypothesis, however, does not explain all patterns of sex-biased dispersal, which vary widely even just among mammals (Lawson Handley and Perrin 2007). Patterns of dispersal in mammals are likely determined by interactions among several of the potential costs and benefits of dispersal. Beyond the lack of familiarity with a new territory, the costs of dispersal in mammals can include increased mortality and a loss of the benefits of kin cooperation, while the benefits can include a potential increase in resource availability, inbreeding avoidance, and avoidance of kin competition (Lawson Handley and Perrin 2007).

Study Species: The Collared Pika (*Ochotona collaris*)

Chapters two, three and four of this thesis focus on the dispersal habits, mating system and genetic structure of a population of collared pikas (*Ochotona collaris*) from the southwest Yukon. The collared pika is a small, short-eared lagomorph that primarily inhabits alpine boulder fields in northwestern North America (Fig. 1.1. Fig. 1.2; MacDonald and Jones 1987). Collared pikas are solitary herbivores that do not hibernate; rather, they spend much of the summer gathering vegetation that they cache and feed on later during the winter months (Morrison *et al.* 2004, 2009; Fig. 1.3). Adults are territorial and typically maintain individual territories of about 0.2 ha (Franken 2002).

I expect that the collared pika population will display a high degree of fine-scale genetic structuring because the dispersal abilities of collared pikas are believed to be quite limited (~ 300 m; Franken 2002). This is because they are generally reluctant to travel far from talus slopes (Morrison *et al.* 2004). Even while foraging, pikas rarely venture more than 6 m from talus, even though better quality forage may be present further out (Morrison *et al.* 2004; McIntire and Hik 2005). Collared pikas likely stay near talus for several reasons. First, they typically escape from predators by fleeing into crevices within the talus (Holmes 1991) and they are more vigilant when far from talus which makes foraging far from talus unprofitable (Roach *et al.* 2001). Additionally, talus provides relief from heat as pikas will stay underneath talus during warm days and they will go underneath talus to cool off after short bursts of activity (MacArthur and Wang 1973).

Little is known about the mating system of the collared pika. However, Smith and Ivins (1984) hypothesize that its close relative, the American pika (*Ochotona princeps*), is monogamous, based on behavioural observations. However, socially monogamous mammals may not necessarily be sexually or genetically monogamous. For example, although mountain brushtail possums (*Trichosurus cunninghami*) display strong pair bonds, Martin *et al.* (2007) found that 35% of juveniles in one population were the results of extra-pair copulations.

Aspects of the collared pika's behaviour indicate that it may actually be polygynous or polygynandrous, rather than monogamous. Franken (2002) noted that, during spring, male collared pikas will travel to the territories of several females in a short period of time, and similar observations have been made for American pikas (Brown *et al*. 1989). Hence, there is a possibility that male pikas go on breeding forays to mate with multiple females. Such behaviour has been seen in the males of several other small mammal species, including the banner-tailed kangaroo rat (*Dipodomys spectabilis*, Winters and Waser 2003). Male banner-tailed kangaroo rats have low mean dispersal distances and individuals often have territories near to those of close relatives. However, males often mate with females that are not their closest neighbours, and this serves to reduce rates of inbreeding in the population (Winters and Waser 2003).

The Evolutionary History and Biogeography of the Pikas

The penultimate chapter of this thesis will address the systematics and phylogenetics of the genus *Ochotona* (Ochotonidae, Lagomorpha). The genus is comprised of least thirty-one species from north and central Asia, western North America and southeastern Europe (Hoffmann and Smith 2005; Table 1.1). However, it was formerly a much more diverse family, in terms of both morphological variability and species richness. The family has its origins in Asia during the middle Oligocene, and by the Miocene, it contained over a dozen genera spread across Eurasia, North America and Africa (Dawson 1981). Some extant pika species, including *O. princeps* and *O. pusilla,* also had much more extensive ranges in the past, and *O. princeps* in particular may be undergoing another range retraction due to recent climatic changes (Beever *et al.* 2003; Galbreath *et al.* 2009).

Because pikas are lagomorphs, their closest living relatives are the rabbits and hares (Leporidae). Most fossil and molecular evidence indicates that Leporidae and Ochotonidae diverged during the late Eocene (McKenna and Bell 1997; Matthee *et al.* 2004; Lopez-Martinez 2008). However, the discovery of a variety of lagomorph ankle bones in early Eocene deposits indicates that the two families may have begun diverging even earlier (Rose *et al*. 2008). These bones, found in Gujarat, India, came from both gracile leporids and more robust ochotonids and, along with both gracile and robust calcanei from China, suggest that the divergence of the leporids and ochotonids was already taking place by the early Eocene (Rose *et al.* 2008).

While leporids and ochotonids may have begun their divergence during the early Eocene (Rose *et al.* 2008), most lagomorph fossils from this time period lack characteristics that are distinctive of either ochotonids or leporids and thus cannot easily be placed in either group (*e.g.* Gawne 1978). It is during the late Eocene and early Oligocene that fossils clearly belonging to the leporid or ochotonid lineages begin appearing in the fossil record (Lopez-Martinez 2008), and the earliest fossils from the family Ochotonidae (*Sinolagomys, Bohlinotona*) are from central Asia (Lopez-Martinez 2008).

Extant pikas are morphologically distinct from the rabbits and hares. Pikas are more rodent like in their superficial form, are smaller (100-200 g), lack the upper third molar (M3) and have shorter ears (Angermann *et al.* 1990). Additionally, because pikas tend to escape from predators by fleeing into burrows or under rock piles, they lack many of the adaptations for a cursorial habit that leporids possess, such as elongated hind limbs. Pika skulls can easily be differentiated from leporid ones as they lack supraorbital bones and have relatively short nasal regions, while leporids have skulls with prominent supraorbital bones and long nasal regions

(Angermann *et al.*1990). While it is unclear whether leporids arose in Asia or North America, pikas almost certainly originated in Asia.

Ochotonids once occurred through several regions they are absent from today, including Africa, Western Europe and the Middle East, and they were at their most diverse during the Miocene. For example, remains of two species of *Australolagomys* (an ochotonid) that date from the lower Miocene have been found in the Namib Desert of southwest Africa (Cooke 1968). Another ochotonid, *Kenyalagomys,* has also been found in early Miocene deposits from Israel (Tchernov *et al.* 1987). Ochotonids present in Asia during the Miocene include *Alloptox* and *Bellatona*, although *Bellatona* went extinct by the mid Miocene and *Alloptox gobiensis* went extinct by the end of the Miocene (Erbajeva 1981). Pikas spread into Europe during the late Oligocene and early Miocene (~25 MYA) and diversified there into several genera, most of which went extinct by the late Miocene (Lopez-Martinez 2008). However, one of these genera, *Prolagus,* persisted in Corsica and Sardinia until the 1700s (Lopez-Martinez 2008). *Prolagus* has been placed in Ochotonidae by some authors but others place it in the family Prolagidae (Lopez-Martinez 2008). The ochotonids *Oreolagus,* and *Hesperolagomys* had also entered North America by the mid-Miocene (Lopez-Martinez 2008).

During the Pliocene, the ochotonids underwent another radiation in Eurasia (Erbajeva 1981). Forms similar to modern *Ochotona* were present in Asia by the beginning of the Pliocene and included *Ochotona, Ochotonoides* and *Prochochotona* (Erbajeva 1981). These ochotonids displayed a larger size range than present day forms. The large Eurasian ochotonids include the species *O. tologoica, O. gromovi,* and *O. complicidens* (Erbajeva 1985). However, by the upper Villafranchian (~1.2 MYA), the larger ochotonids were extinct, and the ranges of surviving forms retracted (Erbajeva 1981). The time period most large ochotonids went extinct corresponds to Eurasia becoming more arid (Erbajeva 1981).

Modern pikas are generally morphologically homogeneous, although there are some major behavioural differences among them. Most pikas either inhabit steppes, shrubby areas, or alpine rockslides, with a few species being somewhat flexible in their habitat choice (Smith *et al.* 1990). Alpine-dwelling pikas are typically solitary and territorial (Broadbrooks 1965; Markham and Whicker 1973), do not burrow, and tend to forage on or near (<6 m from the margin) boulderfields (Roach *et al.* 2001; Morrison *et al.* 2004; Gliwicz *et al.* 2005). However, steppe-dwelling pikas, such as the plateau pika (*O. curzoniae*), are generally highly-social burrow dwellers (Smith and Gao 1991). The steppe-dwelling and alpine-dwelling pikas also differ in several demographic parameters. Steppe pikas typically live at higher densities, undergo more dramatic population fluctuations and have higher fecundity rates than alpine pikas (Smith *et al.* 1990). Based on the current understanding of *Ochotona* phylogeny, pikas appear to have invaded the steppe environment multiple times, so the morphological and behavioural traits that they share are likely the result of convergent evolution, although more research is needed to confirm this (Yu *et al*. 2000).

The diversification of Asian *Ochotona* was likely driven by the uplifting of the Tibetan Plateau (Yu *et al.* 2000). This geologic event, along with the glaciations that occurred with it, diversified the region topographically and likely isolated pika populations from each other, leading to several vicariance events. The climate of the Tibetan Plateau was also extremely unstable during the Pleistocene, which would have resulted in changing selective pressures that resulted in further diversification in the genus (Yu *et al.* 2000).

Most modern *Ochotona* species did not appear until the Pleistocene, although the steppe pika, *O. pusilla*, has an extensive fossil record extending back to the late Pliocene (Erbajeva *et al.* 2001). *O. pusilla* appears to have diverged approximately 2.8 MYA in central Asia (Niu *et al.* 2004) but it later expanded its range into western Europe and Great Britain (Erbajeva *et al*. 2001; Fisher and Yalden 2004; Fostowicz-Frelik 2008), a region where no pika species occur today. *O. pusilla*'s range expansion into Europe was likely triggered by the climatic changes that occurred during the mid to late Pleistocene (Erbajeva 2001). During that time, cold, dry steppes, to which *O. pusilla* are well-adapted, became widespread. Asia was also well-connected to Europe and there were few major barriers to dispersal (such as ice sheets) between the two continents (Erbajeva 2001). This allowed *O. pusilla* to expand its range dramatically.

During the postglacial period, the range of *O. pusilla* began to retract as dry steppe environments were replaced with forest. Today, *O. pusilla* inhabits steppe regions in Kazakhstan and Russia between the Volga and Irtysh rivers, although Holocene fossils of it have been found in Crimea and Hungary (Erbajeva 2001). During historic times, they also occurred in the southern Urals and southeastern Europe (Erbajeva 2001) so much of *O. pusilla's* decline occurred relatively recently.

The oldest North American *Ochotona* fossils, which date from the early Pliocene, were found in Oregon and are from the species *O. spanglei* (Shotwell 1956). Nearctic pikas were slightly more diverse in species number and form in the past than they are today but they never reached the levels of diversity they did in Asia. Nearctic pikas also had a larger North American range during the Pleistocene, as two different forms (large and small) have been found in northeastern North America, well beyond *Ochotona*'s current range (Mead and Grady 1996). A form similar to *O. princeps* lived in the east during the Rancholabrean (300 000 to 11

000 yr BP) and it may have persisted in small relict populations south of Ontario up to about 30 000 yr BP (Mead and Grady 1996). The large form (possibly *O. whartoni*) may have persisted in Ontario until the Holocene (9000 yr BP) although most fossils of *O. whartoni* are from Alaska and the Yukon (Mead and Grady 1996).

The Extant North American Pikas

Two pika species currently occur in North America: the collared pika (*O. collaris*) and the American pika (*O. princeps*). Collared pikas are found in the far northwest corner of British Columbia, most of the Yukon, central and southern Alaska and the Northwest Territories west of the Mackenzie River (Smith *et al.* 1990). American pikas occur discontinuously in mountainous areas from central British Columbia and Alberta south to northern New Mexico and California (Smith *et al.* 1990; Fig. 1.2). The ranges of the two are separated by a gap of about 800 km (Smith *et al.* 1990).

Extant North American pikas are a monophyletic group and appear to be the result of a single invasion of North America across Beringia (Lanier and Olson 2009). Some authors have hypothesized that the split between the two North American species occurred during the Pleistocene glaciations (*e.g.* Weston 1981; MacDonald and Jones 1987). However, Lanier and Olson (2009), using relaxed molecular clock dating techniques, found that the split between *O. princeps* and *O. collaris* predated the Pleistocene glaciations.

The present distribution of pikas in North America has been shaped by a combination of climatic and geographic factors. American pikas typically occur on cool, mesic sites with climatic conditions that favour the formation of talus (Hafner 1994). Such habitats are typically alpine environments characterized by permafrost and cold temperatures that inhibit extensive vegetative growth that would break up the talus (Hafner 1994). However, American pikas occasionally occur on non-alpine talus-like habitats, including lava beds (*e.g.* Rodhouse *et al.* 2010) or anthropogenic settings including mine tailings, stone walls, rock dams and historic foundations (Millar and Westfall 2010; Manning and Hagar 2011).

American pikas have many physiological and behavioural traits that make them well-adapted to cold environments. However, their ability to tolerate high temperatures is limited, as they do not pant to dissipate excess heat. American pikas also have high mean body temperatures of 40.1 °C and this does not change seasonally (MacArthur and Wang 1973; Smith 1974). Their basal metabolic rates are significantly higher than would be predicted based on allometric models, and they also have a lower thermal conductance than predicted based on their body size (MacArthur and Wang 1973). The upper lethal body temperature of an American pika is 43 °C (MacArthur 1973) and death can occur after two hours of exposure to an ambient temperature of 28 °C (MacArthur and Wang 1973). Smith (1974) also found that pikas caged without access to shade died at temperatures as low as 25.5 °C.

Since American and collared pikas are susceptible to hyperthermia and do not burrow (MacArthur and Wang 1973; Smith 1974), they rely on talus for thermoregulation (MacArthur and Wang 1974). American pikas can behaviourally thermoregulate by decreasing their above-ground activity levels and remaining below ground on hot days (MacArthur and Wang 1974; Smith 1974). However, at higher elevations, there may be very few days where pikas must limit their activity level due to heat. For example, American pikas limited the amount of time they spent above ground during the summer at low altitude sites (2550 m) in the Sierra Nevadas (Smith 1974). At higher elevation sites (3400 m), where the average maximum daily temperature was 8.3 °C lower, pikas remained active throughout summer days (Smith 1974). Collared pikas also alter their diurnal patterns of foraging over the course of the summer (Morrison *et al*. 2009).

American pikas have undergone numerous range expansions and retractions during the Pleistocene in response to changes in climate. During the last glacial maximum, they were pushed out of the northern part of their range by continental ice sheets (Galbreath *et al.* 2009), while in the southern part of their range, cool conditions allowed them to exist at lower elevations than they do today (Grayson 1977; Mead 1987; Hafner 1993, 1994; Grayson 2005). They also occurred much farther east than they currently do, including into Ontario, Virginia, Maryland, and West Virginia (Mead 1987). The discovery of a mummified collared pika along with preserved fecal pellets in a lowland part of Alaska also indicates that collared pikas had a more extensive range during the last glacial maxima than they do today (Guthrie 1973).

During the warm altithermal period following the Wisconsin glaciation (~9000 to 5000 yr BP), American pikas shifted their range upslope by an average of 300 m and extended their range north into areas previously covered by the continental ice sheets (Hafner 1994; Grayson 2005; Galbreath *et al.* 2009). Following the altithermal period, American pikas then expanded their range slightly as the climate cooled (Hafner 1994; Grayson 2005). However, they did not recolonize areas vacated during the altithermal period that were more than 20 kilometers from a refuge (Hafner 1994), which indicates that an expanse of lowland habitat more 20 km wide between areas of suitable pika habitat represent a major barrier to dispersal in the species.

Climate Change and North American Pikas

Recently, both American and collared pikas have received some attention from conservation groups. For example, in 2007, the Center for Biological Diversity petitioned the United States Federal Government to list the American pika as endangered or threatened under the United States Endangered Species Act (Wolf *et al.* 2007). COSEWIC (Committee for the Status of Endangered Wildlife in Canada) added the collared pika to the list of species requiring assessment in 2009 and a status report is currently being compiled for this species.

Climate change is the primary factor thought to represent a threat to North American pikas. American pikas in particular may be undergoing another range retraction in response to recent warming. There has been a 0.74 °C increase in the mean global temperature during the past 100 years, and this temperature increase has been greater at higher northern latitudes (IPCC 2007). Some populations of American pikas in the Great Basin region of United States may have already succumbed to climate warming and others have shifted their range upslope (Beever *et al.* 2003, Beever *et al.* 2010). Additionally, in Yosemite National Park in California, the lower elevational range of the species has contracted and moved upslope by 153 m and at least one historic population has become extirpated (Moritz 2007).

Ecological niche models also predict that the American pika will undergo a further significant range retraction in response to climate change. For example, Galbreath *et al*. (2009) developed an ecological niche model for American pikas based on 19 standard bioclimatic parameters. The model had high predictive power with regards to the species’ occurrence under current climatic conditions, and it was then used to predict the species' range under a future hypothetical situation where atmospheric CO_2 levels have doubled. The predictions indicated that the Sierra Nevada subspecies of the American pika (*O. princeps schisticeps*) is at risk of extinction but that complete extinction of the remaining four subspecies (which occur in the Cascades, the northern and southern Rockies and Utah) is unlikely (Galbreath *et al*. 2009).

Other studies on pikas in the Sierra Nevadas and the Great Basin region of the United States also indicate that climate change is a threat to the species in that area. Wilkening *et al.* (2011) found that high mean summer temperatures were associated with a low chance of persistence for pika populations. Beever *et al*. (2010) also found that pika persistence in the Great Basin is strongly influenced by climatic variables, primarily chronic heat stress (as measured by mean summer temperature) and acute cold stress (as measured by the number of days temperatures under talus dropped below – 5 °C or – 10 °C). Cold stress was considered as a variable in the study because levels of mountain snowpack have been declining in the Great Basin region since the mid-20th century. A lack of insulation in the form of a thick layer of snow pack can expose pikas to extreme cold, and there is evidence (though based on a small data set) that pikas exposed to temperatures below – 5 °C are less likely to survive the winter (Beever *et al*. 2010). Morrison and Hik (2007) also note that a low snow pack may increase winter mortality in collared pikas.

Despite studies indicating that American pikas are declining due to climate change, many populations can be considered secure, especially in the northern parts of their range. For example, *O. princeps princeps* occupy 90% of available habitat in Glacier National Park and 96% of suitable habitat in Utah (FWS 2010). *O. princeps saxatilis* still occurs at 58 of 62 historical sites surveyed in Colorado and at 92% of surveyed suitable habitat in Utah (FWS 2010).

Documentation of American pika populations outside their typical bioclimatic envelope indicates that ecological niche models may overestimate future extinction risks for the species. For example, Beever *et al*. (2008) discovered an American pika population at a hot, dry, low elevation site in northwest Nevada and concluded that, while temperature has a strong influence on the distribution of pika populations, it also interacts with several other factors that influence where they can persist. Simpson (2009) also described a population of American pikas from Oregon that occurred outside the species' typical bioclimatic envelope and argued that further investigation is required to determine how local temperatures influence the distribution of American pikas.

American pikas also persist at the Craters of the Moon National Monument and Preserve in southern Idaho, despite the fact that maximum temperatures in July often exceed 29 °C, precipitation is low (< 390 mm annually) and elevations are below the minimum elevations of populations of Great Basin pikas (Rodhouse *et al.* 2010). However, pikas at the site typically occur on complex lava beds with deep crevices in the substrate that allow the pikas to escape into cooler microhabitats on hot days (Rodhouse *et al*. 2010).

Patterns of occupancy in the Great Basin also suggest that pikas in that region tolerate a broader range of precipitation levels and thermal environments than previously thought (Millar and Westfall 2010). Pikas in the Great Basin are strongly associated with periglacial rock-ice-feature till formations, which provide microclimates favourable to pikas (Millar and Westfall 2010). Intra-rock-ice-feature till air circulation can create a negative thermal anomaly on hot days where air temperatures within the rock matrix can be lower than in adjacent bedrock or soil. On cold days, the opposite thermal anomaly can occur, where the air temperature among the rock matrix is warmer than the above ground ambient air temperature (Millar and Westall 2010). Additionally, rock-ice-feature till formations tend to be associated with dense wetland vegetation, a valuable food source for pikas (Millar and Westfall 2010).

Since the factors that determine where American pikas can and cannot persist are numerous, it is difficult to make a conclusion regarding how intense of a decline they are likely to undergo in the foreseeable future. Most studies indicating that they are declining have centered on the Great Basin and American pikas are likely to decline in that region. However, they appear to be secure in most other parts of their range, where it is either still quite cold during most of the year or where topographical features allow them to effectively behaviourally thermoregulate. A conclusion on how climate change is likely to affect collared pikas is even more difficult as most data on the species is from a single population in the Ruby Ranges of the southwest Yukon.

Chapter Descriptions

The following three chapters of thesis will address the dispersal habits, mating system and genetic structuring of a collared pika population from the southwest Yukon. **Chapter Two** describes the microsatellite markers I used to genotype collared pikas caught over a period of twelve years at a site in the southwest Yukon. In **Chapter Three**, I present parentage analyses of the collared pika population from ten different breeding seasons and the results are used to make inferences about the population’s mating system and dispersal habits. In **Chapter Four**, I examine the fine-scale genetic structure of the population, determine if it receives migrants from neighbouring populations, and examine how and if its genetic diversity changed in response to population declines that occurred during 2000 and 2002. In **Chapter Five,** I examine the phylogenetic relationships among the members of the genus *Ochotona* using two data sets of mitochondrial gene sequences. Several of the phylogenetic relationships among members of the genus *Ochotona* are unclear, because several clades of Asian pikas were supported only by low bootstrap support values in both comprehensive, recent studies of pika phylogeny (Yu *et al.* 2000; Niu *et al*. 2004). I hope to clarify some of these relationships. Finally, in **Chapter Six**, I present the major conclusions of this work and make recommendations for future research on the biology of Ochotonidae.

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Figures and Tables

Fig. 1.1: A collared pika (*Ochotona collaris*) from the southwest Yukon. Photo by Kieran O’Donovan.

Fig. 1.2: The range of the collared pika (*O. collaris*, grey) and the American pika (*O. princeps*, black). Modified from Smith *et al.* 1990.

Fig. 1.3: Top: Typical habitat for a collared pika – an alpine boulderfield interspersed with patches of meadow. Bottom: A collared pika haypile.

Table 1.1: A listing of all extant species in Ochotonidae along with their common names, range, habitat, conservation status, and recent taxonomic history. This list follows Hoffmann and Smith (2005). Information on species' ranges and habitats are based on Smith *et al.* (1990) and IUCN (2010) and conservation status listings are from the IUCN Red List of Threatened Species (IUCN 2010). IUCN statuses are abbreviated as follows: LC = Least Concern, DD = Data Deficient, NT = Near Threatened, V = Vulnerable, E = Endangered, CE = Critically Endangered; I = Increasing, D = Decreasing, S = Stable, U = Population Trend Unknown.

Species	**Common name**	**Range**	**Habitat**	**Status**	**Taxonomic Notes**
O. alpina	Alpine or Altai pika	South and east of Lake Baikal east to the upper Amur river drainage and in mountainous areas bordering the Gobi desert.	Rock and Talus	LC, U	Formerly considered to include *O. argentata*, *O. collaris, O. hoffmanni*, *O.hyperborea* and *O. princeps* (Corbet 1978; Formozov *et al.* 1996; Formozov *et al.* 2004).
O. argentata	Helan Shan or Silver pika	Helan-Shan Ridge, Ningxia, China.	Rocky outcrops in a small forest; also mine shaft entrances	CR, D	Formerly a subspecies of *O. alpina;* Formozov *et al*. (2004) elevated it to species status. It is likely more closely related to *O. pallasi* (Lissovsky *et al.* 2007) and has been included in *O. pallasi* as *O. p. helanshanensis* (Smith 2008).
O. cansus	Gansu Pika	Western Shaanxi, southern Shaanxi, southwestern Gansu, eastern and southern Qinghai, northwestern Sichuan.	Alpine shrub	LC, U	Formerly included in *O. thibetana* (Ellerman and Morrison-Scott 1951; Corbet 1978; Honacki *et al.* 1982). Smith *et al.* (1990) consider it a separate species based on skull characteristics and a lack of intermediate forms in zone of sympatry. Yu *et al.* (1997) demonstrated that it is genetically distinct.
O. collaris	Collared pika	Alaska, Yukon Territory, western Northwest Territories, northwest British Columbia.	Alpine talus slopes	LC, U	Formerly included in *O. alpina* (Corbet 1978) or *O. princeps* (Broadbrooks 1965; Banfield 1974; Youngman 1975). Weston (1981; 1982) demonstrated that it is morphologically distinct from *O. princeps* and *O. alpina*.
O. curzoniae	Black-lipped or Plateau pika	Southern Xinjiang, Qinghai, Xizang and western Sichuan, China, trans-Himalayan Nepal and Sikkim, India.	Alpine deserts, steppes and meadows	LC, D	Considered a distinct species by recent treatments (*e.g.* Corbet 1978; Honacki *et al.* 1982; Smith *et al.* 1990); included in *O. daurica* by Ellerman and Morrison-Scott (1951).

Species	**Common name**	**Range**	**Habitat**	**Status**	**Taxonomic Notes**
O. daurica	Daurian pika	The Gobi region in Mongolia and adjacent regions of Russia and China.	Semi-deserts and steppes	LC, U	Considered a distinct species in recent treatments (e.g. Corbet 1978; Honacki *et al*. 1982; Smith *et al*. 1990; included *O. curzoniae* by Ellerman and Morrison-Scott (1951).
O. erythrotis	Red-eared or Chinese red pika	Qinghai to west central Gansu and southern Xinjiang, China.	High elevations; steep rocky terrain adjoining meadows	LC, U	Formerly included *O. gloveri* (Corbet 1978); included in *O. rutila* by Ellerman and Morrison-Scott (1951).
O. forresti	Forrest's pika	Northwest Yunnan and southeast Xizang, China, northern Burma (Myanmar), Assam and Sikkim India, Bhutan.	High elevation mountain slopes	LC, U	Has been included in *O. thibetana* (Honacki *et al.* 1982), *O. pusilla* (Ellerman and Morrison-Scott 1951) and *O. roylei* (Corbet 1978). Smith *et al.* (1990) consider a distinct species based on Feng and Zheng (1985)'s data.
O. gaolingensis	Gaoligong pika	Mount Gaoligong, northwest Yunnan Province, China.	Alpine talus slopes	DD, D	Recently described by Wang *et al.* (1988). Very similar to *O. forresti* (Smith *et al.* 1990).
O. gloveri	Glover's pika	Southwest Qinghai, northeast Xizang, northwest Yunnan and western Sichuan, China.	Alpine talus slopes	LC, U	Included with *O. rutila* by Ellerman and Morrison-Scott (1951), and with *O. erythrotis* by Corbet (1978), Honacki *et al.* (1982) and Weston (1982). Separated by Feng and Zheng (1985). *O. kamensis* (Corbet 1978; Honacki *et al.* 1982) was synonymized with *O. gloveri gloveri* by Feng and Zheng (1985).
O. himalayana	Himalayan pika	Mount Jolmolungma (Everest) area in southern Xizang, China.	Talus piles, walls, precipices.	LC, U	Included in *O. roylei* by Corbet (1978), Honacki *et al.* (1982) and Weston (1982). Yu *et al.* (2000) confirmed that it is genetically distinct from *O. roylei*.
O. hoffmanni	Hoffmann's pika	Hentiyn Nuruu ridge of the Bayan-Ulan range in Mongolia; Erman Mountain range of the Russian Federation.	Talus	E, U	Initially considered a subspecies of *O. alpina;* was elevated to specific rank by Formozov *et al.* (1996).
O. huangensis	Tsling-ling pika	China (Gansu, Henan, Hubei, Qinghai, Shaanxi, Shanxi, Sichuan).	Forests, shrubland and grassland	LC, U	Treated as a subspecies of *O. thibetana* by Ellerman and Morrison-Scott (1951), Weston (1982), Feng and Zheng (1985) and Smith *et al.* (1990). Yu *et al.* (1997) argue it is a distinct species based on genetic data.

Species	**Common name**	**Range**	**Habitat**	**Status**	**Taxonomic Notes**
O. hyperborea	Northern or Siberian pika	Asia, from the Ural mountains to Hokkaido. Also found on several islands in the Bering Sea and the Sea of Okhotsk.	Primarily a rock-dweller; may live around tree stumps, logs or piles of driftwood	LC, U	Included with *O. alpina* by Corbet (1978) and Honacki *et al.* (1982). Smith *et al.* (1990) treat as a separate species, as *O. alpina* and *O. hyperborea* have different chromosome numbers and are morphologically distinct. *O. turuchensis* was formerly included as a subspecies of *O. hyperborea* (Smith *et al.* 1990).
O. iliensis	Ili pika	Borohoro Shan, Xinjiang, China.	Talus	E, D	Recently described (Li and Ma 1986) and not included in treatments prior to its description.
O. koslowi	Kozlov's pika	Arkatag Range, China.	Alpine meadows	E, D	Smith *et al.* (1990) note that this form has generated little taxonomic confusion.
O. ladacensis	Ladak pika	Kashmir (India), Qinghai, Xizang, Xinjiang, China.	Talus	LC, U	Modern treatments treat this form as a distinct species (Smith *et al.* 1990)
O. macrotis	Large-eared pika	The Pamir and Tien Shan Mountains, The Hindu Kush, The Karakoram Range, the Himalayan Mountains and Kunlun Mountains, and the mountains of Sichuan and Yunnan Provinces, China.	Talus; found at elevations up to 6130 m	LC, U	Corbet (1978) included with *O. roylei*. Considered a distinct species by Ellerman and Morrison-Scott (1951), Honacki *et al.* (1982), Weston (1982), Feng and Zheng (1985) and Smith *et al*. (1990).
O. muliensis	Muli pika	Western Sichuan, China.	Shrubs and steppes	DD, U	Included with *O. gloveri* upon its initial description, but Feng and Zheng (1985) considered it distinct based on lack of foraminae on frontals.
O. nigritia	Black pika	Piyanma, Yunnan, China.	Alpine regions	DD, U	Possibly a melanistic form of *O. forresti* (Hoffmann and Smith 2005).
O. nubrica	Nubra pika	The Qinghai Plateau, from Ladak to eastern Xizang.	Subalpine or alpine shrubs or steppes	LC, U	Ellerman and Morrison-Scott (1951) placed in *O. pusilla*. Corbet (1978) placed *O. n. lama* into *O. roylei* and *O. n. nubrica* into *O. thibetana*. Smith *et al.* (1990) treat as a distinct species.
O. pallasi	Pallas's pika or Mongolian pika	The Gobi desert region and east-central Kazakhstan.	Rocky areas and steppes	LC, D	Modern treatments treat this form as a distinct species (Smith *et al.* 1990)

Species	**Common name**	**Range**	**Habitat**	**Status**	**Taxonomic Notes**
O. princeps	American pika	Mountainous areas in southwest Canada and the northwestern United States.	Alpine talus slopes	LC, D	Corbet (1978) placed in *O. alpina*. *O. collaris* has been treated as a subspecies of *O. princeps* (Broadbrooks 1965; Youngman, 1975). Weston (1981, 1982) demonstrated that *O. collaris* is distinct from *O. princeps.*
O. pusilla	Steppe or little pika	The Upper Volga River and Ural mountains in Kazakhstan, and south and east to border with China and Russia.	Steppes	LC, D	Modern treatments treat this form as a distinct species (Smith *et al.* 1990). Ellerman and Morrison-Scott (1951) included *O. nubrica* and *O. forresti* in *O. pusilla.*
O. roylei	Royle's pika	The Himalayan massif from Pakistan to Kashmir, northwestern India, Nepal and Xizang, China.	Talus	LC, S	Modern treatments treat this form as a distinct species (Smith *et al.* 1990). Corbet (1978) placed *O. forresti, O. macrotis,* and *O. nubrica lama* in this species.
O. rufescens	Afghan pika	The Mountains of southern Russia, Turkmenistan, Iran, Afghanistan and Baluchistan, Pakistan.	Rocky deserts, fields	LC, S	Smith *et al.* (1990) note that this form has generated little taxonomic confusion, beyond whether the form *vulturna* should be considered a valid subspecies and whether the form *seiana* belongs in this species or *O. curzoniae.*
O. rutila	Turkestan red pika	The Tien Shan Mountains, the Pamirs, Tajikistan, eastern Xingjiang, China, and possibly northern Afghanistan.	Talus	LC, S	Modern treatments treat this form as a distinct species (Smith *et al.* 1990). Ellerman and Morrison-Scott (1951) included O. *erythrotis* and *O. gloveri* in *O. rutila.*
O. thibetana	Moupin pika	The eastern Tibetan Plateau, along the Himalayan massif in China, northern Burma (Myanmar), and Bhutan.	Bamboo and rhododendron forests	LC, U	Formerly included several forms now considered to belong to *O. cansus* or *O. nubrica* (Ellerman and Morrison-Scott 1951; Corbet 1978; Honacki et al. 1982)
O. thomasi	Thomas' pika	Gansu, Qinghai and Sichuan, China.	Meadow thickets	LC, U	Has generated little taxonomic confusion (Smith *et al.* 1990).
O. turuchanensis	Turuchan pika	The southern expanse of the Yenisei River to Lake Baikal and middle region of Lena River watershed in Russia.	Talus	LC, U	Only recently recognized as a distinct species (Lissovsky *et al.* 2007).

Chapter Two

Isolation and Characterization of Microsatellite Loci for the Collared Pika (*Ochotona collaris*) and their cross-amplification in five other *Ochotona* species[1]

Pikas (*Ochotona*, Ochotonidae) are small lagomorphs that occur in western North America and northern and central Asia. There are approximately thirty species of pika worldwide, although only two, the American pika (*O. princeps*) and the collared pika (*O. collaris*), are found in North America. Both species live in alpine boulderfields, with the collared pika occurring farther north. These pikas occur in naturally fragmented habitats isolated by expanses of meadow, lowland, or forested habitats that they avoid. Due to their fragmented distributions, North American pikas have been used to study metapopulation dynamics (*e.g*. Franken and Hik 2004), but pikas are difficult to capture and mark before they have dispersed, so estimating their dispersal rates can be difficult. To this end, we have developed eight new microsatellite loci to facilitate analysis of population structure, dispersal, and mating habits of the collared pika.

Genomic DNA was extracted from one female collared pika using a standard phenol/chloroform method (Sambrook *et al*. 1989), and an enriched genomic library was constructed using the method described by Hamilton *et al*. (1999). One-hundred eight clones were amplified and 56 clones with inserts greater than 100 bp in size were sequenced. Primers were developed for ten loci with dinucleotide repeats using Primer3 (Rozen and Skaletsky 2000).

We genotyped forty-six pikas from the Ruby Ranges in the southern Yukon (61° 12’ N, 138° 16’ W; 1800-2000 m).Pikas were trapped from

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July-September, 2007, using methods described by Franken and Hik (2004). We extracted DNA from ear plugs using the QIAGEN DNeasy blood and tissue kit (QIAGEN). We also tested all primers that amplified products reliably in *O. collaris* in *O. princeps, O. alpina, O. hyperborea, O. dauurica,* and *O. pallasi.* Additionally, we tested nine primer sets developed to amplify microsatellites in *O. princeps* (Peacock *et al*. 2002) in *O. collaris*, and the above Asian pika species.

PCRs were conducted with 15 µL reactions, which included ~20 ng of DNA in a solution of 0.5 µM of each primer, 200 µM dNTPs, 1X PCR buffer and 1.3-2 mM $MgCl_2$. Most reactions used a concentration of 1.3 mM of $MgCl_2$; however, reactions with primers Ocp4 and Ocp9 contained 2 mM $MgCl_2$. PCR reactions were run on an Eppendorf Mastercycler 96 with conditions as follows: 95 °C for 10 min; 32 cycles of 95 °C for 30 s, annealing at 50-59 °C for 30 s, and elongation at 72 °C for 75 s; and a final step at 72 °C for 10 min. Forward primers were fluorescently labeled with either NED, VIC, 6-FAM, or PET markers (Applied Biosystems).

PCR reactions were run on an Applied Biosystems 3730 DNA Analyzer and sized with GeneScan 500 LIZ markers (Applied Biosystems). Genotypes were scored using Genemapper v. 4.0 (Applied Biosystems). We estimated expected and observed heterozygosities at each locus using the Excel Microsatellite Toolkit (Park 2001), and we used Genepop v. 4.0 (Rousset 2008) to test for deviations from Hardy–Weinberg equilibrium and for linkage disequilibrium between loci. Significance levels were adjusted for multiple comparisons using a sequential Bonferroni correction (Rice 1989). The program Micro-checker (van Oosterhout et al. 2004) was used to check the data for null alleles, alleles that were mis-scored due to stuttering or large-scale allelic dropout.

Eight loci amplified reliably and were polymorphic in both *O. collaris* (Table 2.1) and *O. princeps* (Table 2.2). Additionally, all nine of the loci designed by Peacock et al. (2002) were polymorphic in *O. collaris* (Table 2.1). Most primer pairs designed for North American pikas amplified products in four Asian pika species (Table 2.2) except for *Ocp*9 in *O. dauurica* and *O. pallasi* and *Occ*1 in *O. alpina.*

The number of alleles for each locus ranged from 2-10 (mean = 6.0) and observed heterozygosities ranged from 0.15 to 0.87 (mean = 0.59, Table 2.1). All loci were in Hardy-Weinberg Equilibrium, and no loci were in linkage disequilibrium after a Bonferroni correction was made. No loci displayed evidence of having null alleles, scoring errors due to stuttering, or allelic dropout.

These loci have been used to determine changes in the population genetic structure, dispersal habits and mating behaviour of collared pikas in the southern Yukon over a 13 year period (Chapters Three and Four).

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Tables

Table 2.1: Characterization of seventeen microsatellite loci for the collared pika, *Ochotona collaris. Occ* loci were isolated by the authors, and *Ocp* loci were isolated from American pikas, *O. princeps*, by Peacock *et al*. (2002). Only forward primers were marked with fluorescent dyes. H_o = observed heterozygosity, H_E = expected heterozygosity.

Locus Name	Genbank Accession No.	Primer sequence (5'-3')	Repeat Motif	Annealing Temp.	No. animals typed.	No. of Alleles .	Size Range (bp)	H_o/ H_E
Occ01	EU665183	F:TGACAGGCAACATTTCCTCA R:CAGAAGCCTGCCTTGAGAAG	$[CA]_{10}$	50 °C	29	5	267-277	0.52/0.52
Occ02	EU665184	F:CGAGCATTTCACAGTCCTCA R:TCTCTTTCCTCTTCCCAGCA	$[AC]_{8}$	50 °C	45	5	165-173	0.56/0.61
Occ03	EU665185	F:GAAGCTTTTCTTCATGCAAGGT R:GACTTGAACCGGGAACTTCA	$[CA]_{14}$	50 °C	41	4	229-235	0.24/0.34
Occ04	EU665186	F:TGAACTTACAATGGCCAGAATG R:GGAGTGAACTAGCGGATGGA	$[CA]_{4}CT[CA]_{15}$	59 °C	40	10	251-281	0.70/0.82
Occ05	EU665187	F:TCCCCCATTTCTAACAGTGC R:TGGAAACATAGGAAACCGTGT	$[GT]_{5}AT[GT]_{12}$	50 °C	38	4	221-227	0.61/0.59
Occ07	EU665188	F:CACCAGTTATGCACACAAGC R:GTTCATGCACTTCCATTTGC	$[CA]_{15}$	50 °C	44	5	225-235	0.55/0.50
Occ09	EU665189	F:CAACACATGGTCCTCCAAGTT R:TGCTAAGTTGGTCAGTGAAGTACA	$[AC]_{2}AT[AC]_{5}AT[AC]_{9}$	50 °C	31	5	240-262	0.45/0.52
Occ10	EU665190	F:GTTTATAGAGAGGAAGCGTG R:CTTCTGTTGGTTCATTGCC	$[GT]_{9}[GA]_{13}$	50 °C	33	2	170-172	0.15/0.14
Ocp1	AF487492	F: AGTGACATAAATGACGGGACA R: TCAGACCCAACTCAACACAG	$[AG]_{16}AC[AG]_{4}AC[AG]_{7}$	55 °C	30	4	299-311	0.73/0.68
Ocp2	AF487493	F: AGCCACACTTACAGAACCACCAA R: TCATCACCAATCTGCCAATTACC	$[GATA]_{12}$	55 °C	46	5	417-433	0.70/0.68
Ocp3	AF487494	F: CAGCCATCTGGACAATGAAACTAA R: GGAACATTTGCCGTTGTAGAAAG	$[CTAT]_{8}$	55 °C	42	3	291-299	0.38/0.39
Ocp4	AF487495	F: CACTAGGTTATTGCGCCAGGGT R: CTGCTTCTGGTTTCAGCCTGACT	$[ATAG]_{10}$	59 °C	37	7	208-232	0.87/0.82
Ocp5	AF487496	F: CAAGTTCCGGCTTTGCTCAGTTC R: GTACATGCAGTGGCAAGGGTTGA	$[TATC]_{14}$	55 °C	32	6	331-359	0.78/0.75
Ocp6	AF487497	F: GGCTTCAGATTTCCTCAACACC R: CCACCTGACTTCTGCAACTTTCT	$[TAGA]_{9}TGA[TAGA]_{5}$	55 °C	37	10	315-366	0.84/0.83
Ocp7	AF487498	F: ATCCTGAGCTATCTTTGCCATT R: CCCAAAACTCCTTGAGAGACA	$[AC]_{15}$	55 °C	42	8	225-247	0.38/0.38
Ocp8	AF487499	F: TTCCTCTGGAGTCCTCTAACCC R: CCTCGAGCAAGTTTGGTTGTT	$[AG]_{17}$	55 °C	45	10	282-302	0.84/0.82
Ocp9	AF487500	F: CCTGAATCGCAAACATCATGG R: TGTGGGCATTTGGAGACTGAA	$[TAGA]_{14}$	59 °C	32	7	291-315	0.66/0.75

Table 2.2: Number and size of alleles observed for 17 microsatellite loci developed for North American pikas when used in four Asian pika species. The number of alleles found in a population of *O. princeps* when genotyped with the primers developed in this study is also noted. Tissue from all animals was obtained from museum collections and the museum accession numbers for the specimens is noted under each species' name in the table. MSB = Museum of Southwestern Biology, RAM = Royal Alberta Museum, UAM = University of Alaska Museum of the North, n = number of individuals tested, x = no amplification

	***O. alpina* n=2** MSB 94337-94338		***O. dauurica* n=5** MSB 94340-41; 94346		***O. hyperborea* n=13** MSB 94348-54, UAM 23239-41, 29145, 34205		***O. pallasi* n=2** MSB 94355-56		***O. princeps* n=13** RAM 96911, 96915-17, 96925-26, 96934, 02816, 0284-7, 02810	
	Number of alleles	Size Range	Number of alleles	Size Range	Number of alleles	Size Range	Number of alleles	Size Range	Number of alleles	Size Range
Occ1	X	X	3	255-275	6	275-287	2	269-271	4	269-275
Occ2	2	155-157	3	145-159	8	157-174	4	149-161	3	143-149
Occ3	1	222	1	218	8	224-249	1	235	4	227-256
Occ4	1	247	2	256-281	1	247	1	247	4	256-277
Occ5	2	223-227	3	202-227	8	217-239	2	221-229	5	207-219
Occ7	1	227	5	223-239	8	221-249	2	225-229	5	225-235
Occ9	3	245-261	1	240	5	240-250	2	242-256	4	242-260
Occ10	2	180-186	2	147-165	4	156-174	2	166-186	2	188-195
Ocp1	1	311	2	305-319	9	301-323	1	335	3	309-335
Ocp2	3	381-405	3	376-382	8	382-451	1	442	5	381-405
Ocp3	2	315-323	2	317-321	7	295-319	2	287-303	3	287-303
Ocp4	2	195-207	1	157	5	214-230	2	216-220	4	228-248
Ocp5	2	327-331	4	346-356	7	346-370	4	330-360	3	339-347
Ocp6	2	327-331	1	356	8	318-368	2	350-356	4	310-362
Ocp7	1	271	1	277	8	249-291	3	255-259	8	219-287
Ocp8	1	275	3	277-299	3	278-282	2	298-304	3	282-286
Ocp9	2	222-226	x	x	8	218-232	x	x	6	266-295

Chapter Three

Widespread promiscuity and the absence of sex-biased dispersal in a population of collared pikas, *Ochotona collaris*[1]

Introduction

Mammalian mating systems are diverse and vary widely with respect to the average number of mates each animal may have and the length of time members of a mating pair associate with each other. However, monogamy is rare, as approximately 90% of mammal species show some form of polygyny (Clutton-Brock 1989). Despite this, monogamy occurs in several unrelated mammal species, indicating that it evolved in mammals more than once (Kleiman 1977). Kleiman (1977) divided monogamous mating systems into two types: “obligate,” in species where male parental care is required for optimal offspring survival, and “facultative,” in cases where female ranges are so large that a male can usually only defend the range of a single female (Kleiman 1977). However, a recent phylogenetic study on the evolution of monogamy in mammals indicated that it has evolved more frequently in the absence of parental care and that it tends to evolve in species where females have small, exclusive and widely-dispersed home ranges (Komers and Brotherton 1997). Conversely, a comparative phylogenetic study by Dobson *et al*. (2010) suggested that multiple causes have influenced the evolution of monogamy in mammals, so generalizations about it are difficult to make.

Among monogamous mammals, pairs may be either socially and/or genetically monogamous (Dobson *et al*. 2010). Social monogamy refers to the living arrangement of an opposite-sex pair of animals without reference to their sexual behavior. A socially monogamous pair of animals may frequently associate with each other and cooperate in rearing young,

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or they may simply share an exclusive territory. However, socially monogamous animals are not necessarily genetically monogamous. For example, while approximately 90% of bird species are socially monogamous, less than 25% display true genetic monogamy, where most or all of the young produced by a female are fathered by her social mate (Griffith *et al*. 2002). Extra-pair fertilizations are also common in many socially monogamous mammal species (*e.g.* Goossens *et al.* 1998; Martin *et al*. 2007).

Familiarity with a species' mating system can be valuable in understanding other aspects of its biology, including the direction of sex-biased dispersal (Greenwood 1980, Dobson 1982, Lawson Handley and Perrin 2007). Generally, female-defense polygyny is associated with male-biased dispersal while resource-defense polygyny is associated with female-biased dispersal (Greenwood 1980). However, there are exceptions, and how a species' mating system influences its dispersal habits is complex. The benefits of kin cooperation and the costs of kin competition must be considered in evaluating the evolution of sex-biased dispersal in a species (see Lawson Handley and Perrin 2007 for a review).

Inbreeding avoidance also influences the patterns of sex-biased dispersal in many species, although it likely forms only part of the explanation for it in most mammals (Hoogland 1992, Dobson *et al*. 1997, Lawson Handley and Perrin 2007). Even so, sex-biased dispersal alone can be sufficient for reducing the occurrence of inbreeding in some populations (*e.g.* Lebigre *et al*. 2010). In species where dispersal is limited and animals often establish territories near close kin, individuals may reduce the inbreeding coefficient of their offspring by mating with animals other than their close neighbours (Winters and Waser 2003). Alternative means of inbreeding avoidance may also evolve where dispersal or mating forays do not sufficiently reduce the likelihood of close inbreeding, such as kin recognition mechanisms that allow animals to identify close relatives so they can avoid mating with them (Mateo 2003).

Mammal species with no sex-biased dispersal are rare (Lawson Handley and Perrin 2007). Even in species where members of both sexes are equally likely to disperse, one sex will often disperse farther than the other (*e.g. Dipodomys spectabilis* [Busch *et al*. 2009]). A species with no sex-biased dispersal would be expected to have little social complexity and a simple mating system (Perrin and Goudet 2001; Devillard *et al*. 2004). Additionally, Perrin and Mazalov's (2000) model-based study predicted that there will be no sex-biased dispersal when local competition affects males and females equally and where female fitness is limited by extrinsic factors. Consequently, a mammal that may be predicted to have no sex-biased dispersal is the collared pika (*O. collaris*), a solitary, territorial lagomorph that has a mating system which is likely either facultatively monogamous (Smith and Ivins 1984) or polygynandrous (where males and females may have multiple partners). Like some other polygynandrous mammal species, collared pikas show no sexual dimorphism (Cooper *et al.* 2011). Additionally, collared pikas of both sexes must defend a territory, so both sexes will experience similar levels of local competition. Female fecundity in this species is also strongly influenced by extrinsic factors, primarily winter climate (Morrison and Hik 2007; Patil 2010).

My objectives in this study were to examine the mating system of the collared pika, to determine if it displays sex-biased dispersal, and to analyze mate choice in the species with respect to pairwise relatedness. Pikas (*Ochotona*: Ochotonidae) are small (~160 g), short-eared lagomorphs that occur in the mountains of western North America and the steppes and mountains of northern and central Asia. Both species of North American pikas (*O. collaris* and *O. princeps*) are solitary and territorial, (Broadbrooks 1965) while all Asian rock-dwelling pikas studied to date live in pairs on a shared territory (Smith, 1988; Smith *et al.* 1990; Gliwicz *et al*. 2005). In contrast, steppe-dwelling pikas are typically social burrow dwellers that live in family groups (*e.g. O. curzoniae*, Smith and Wang, 1991; Dobson *et al.* 1998, 2000; Yin *et al*. 2009).

Although collared pikas are solitary and territorial during the summer (Broadbrooks 1965), observations carried out once pikas have stopped gathering food for winter indicate that there is overlap between the territories of neighboring male and female animals (K. O'Donovan unpublished data), suggesting that individual collared pikas may be either monogamous or polygynous (in cases where a male's territory overlaps those of multiple females). However, male collared pikas will stray from their territories during the mating season (Franken 2002), and collared pikas are largely solitary, a characteristic of many species with high extra-pair paternity rates (Cohas and Allainé 2009). Therefore, I expected that there would be significant extra-pair paternity in collared pikas.

The collared pikas in the study population typically disperse short distances, as evidenced by both direct observations (Franken 2002) and on the level of very fine-scale genetic structuring seen in the population in most years (see Chapter 4). Their apparently low mean dispersal distances could leave collared pikas susceptible to inbreeding, if they tend to mate with their nearest neighbors. I determined the inbreeding coefficients in the population for each year, and calculated how far apart the territories of animals that had produced offspring together were. I also determined if animals can reduce the inbreeding coefficients of their offspring by mating with animals that are not their nearest neighbours.

Finally, I predicted that there would be no difference in the average dispersal distance between males and females. The costs of dispersal and benefits of philopatry should not differ between the sexes in collared pikas, as Peacock (1997) concluded that competition for resources is the main factor influencing dispersal of the closely related American pika. Dispersing collared pikas of both sexes must find and defend their own territories and build and defend their own food caches during summer, and since the costs and benefits of dispersal will be similar between the two sexes, the average dispersal distances between them should not differ.

Methods

Study Population

I focused on a population of collared pikas (*Ochotona collaris*) located in the Ruby Ranges east of Kluane Lake in the southern Yukon (61° 12’ N, 138° 16’ W; 1800-2000 m; Fig. 3.1). The population inhabits an approximately 4 km^2 valley that is dissected by shallow, 1-2 m wide streams. The valley consists of meadow and tundra interspersed with patches of talus that range in size from 654 m^2 to over 156,000 m^2 (Franken and Hik 2004a). Collared pikas live primarily on talus, but will venture short distances into meadows to graze or to gather vegetation to cache (Morrison *et al.* 2004). Collared pikas do not hibernate and instead cache vegetation to eat during winter in “haypiles” located in the talus (Morrison *et al*. 2009). At our study site, collared pikas defend a territory of between 0.16 ha (females) and 0.2 ha (males) (Franken 2002).

Sample Collection

Pikas were captured from mid-June to mid-August each summer from 1999 to 2008 using Tomahawk live-capture traps (Tomahawk Live Trap Co, Tomahawk, WI, U.S.A.) baited with fresh native vegetation. In 2006 and 2007, trapping was extended into late September. Each pika caught was weighed, sexed, and marked with numbered ear tags (Monel #1) containing a unique set of colour wires. A small portion of ear tissue was taken from each pika and these ear plugs were stored in 95% ethanol until the DNA could be extracted. I also recorded the location of each pika's capture location and haypile relative to a 50 m by 50 m field grid. Pikas were classified as young of the year or adults (>1 year old) based on their mass and molt pattern (Franken and Hik 2004b). The capture and handling protocols were approved by the Animal Care Committee for the University of Alberta, who follow the Canadian Council on Animal Care guidelines.

Molecular Sexing

In several instances, I was uncertain of the sex of an adult pika, typically because the animal had to be released before it could be sexed. In these cases, the sex was determined through sequences of a zinc finger protein (ZF) gene, copies of which are found on both the X and Y chromosomes. I amplified a portion of the gene using the primers described in Fernando and Melnick (2001).

PCRs were conducted with 50 µL reactions, which included 3 µL of DNA template in a solution of 0.5 µM of each primer, 200 µM dNTPs, 1X PCR buffer (50 mMKCl, 10 mMTris, 0.1% Triton X 100, and 0.16 µg/µL bovine serum albumin), 2 mM $MgCl_2$, and I µL of dimethyl sulfoxide. PCR reactions were run on an Eppendorf Mastercycler 96 (Eppendorf Canada, Mississauga, ON, Canada) with conditions as follows: 95 °C for 10 min; 32 cycles of 95 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 75 s; and a final step at 72 °C for 10 min. PCR products were purified using a QIAGEN PCR purification kit (Qiagen, Valencia, California, USA). Products were sequenced using the amplification primers and the ZFSEQ primer (Fernando and Melnick 2001). Sequencing reactions were set up using the BigDye® Terminator v. 3.1 cycle sequencing kit (Applied Biosystems by Life Technologies, Carlsbad, CA, U.S.A.), using half reactions according to the manufacturer's directions. Sequences were run on a 3730 DNA analyzer (Applied Biosystems) and aligned and edited using the program SeqMan (DNASTAR, Inc, Madison, WI, U.S.A.).

Since females will have only one copy of the ZF gene located on the X chromosome, there will be only one sequence obtained when the gene is amplified and sequenced for a female animal. A male animal will have two copies, one on the X chromosome and one on the Y chromosome, so two sequences will be obtained when the gene is amplified and sequenced for a male animal. Males can thus be identified by the presence of "double peaks" on the resulting sequence chromatograms. The reliability of this technique as used in this study was assessed by sequencing the gene for fifteen animals of known sex (*i.e.* either multiple people sexed the animal or it was an obvious female with swollen nipples).

Microsatellite Genotyping and Analysis

DNA was extracted from ear tissue using the Qiagen DNeasy Blood and Tissue Kit (Qiagen). Animals were genotyped using the seventeen primers and conditions described in Zgurski *et al*. (2009) (See Chapter Two).

I used Genepop v. 4.0 (Rousset 2008) to test each locus in each year for deviations from Hardy–Weinberg equilibrium and to examine each set of loci for linkage disequilibrium for each year. I used the Monte Carlo chain method with 10 000 dememorizations, 1000 batches and 5000 iterations following the method of Guo and Thompson (1992).

Microchecker (van Oosterhout *et al*. 2004) was used to examine data from each locus from each year for the presence of null alleles.

Inbreeding coefficients (F_{IS}, Weir and Cockerham 1984) for each year were calculated using SPAGeDi (Spatial Pattern Analysis of Genetic Diversity; Hardy and Vekemans 2002). Permutation tests (with 20 000 permutations) were used to determine if each value was significantly different from the values expected under a model of random breeding.

Parentage Analysis

I used the program CERVUS (Kalinowski *et al*. 2007) to assign parents to each juvenile in the population during each year, using the “parent pair (sexes known)” option. Candidate parents for each juvenile pika included all adults potentially present in the population when the juvenile was conceived. Thus, each adult pika present during the birth year of the juvenile under question and the pikas present the previous year were considered as candidate parents. The breeding season for collared pikas occurs from April to July, depending on the year (Franken and Hik 2004b). Since the vast majority of pikas captured at the study site were captured post-dispersal, I did not assign mothers to individuals based on a close physical association between the two. Juvenile collared pikas generally disperse very soon after they have been weaned, which occurs about one month after birth.

CERVUS uses a simulation-based approach to assess the confidence of parentage assignments. For each set of simulations (one for each year), I simulated 10 000 offspring from the number of potential parents present, which ranged from 13 fathers and 18 mothers in 2004 to 32 fathers and 50 mothers in 2008 (Table 3.1). I am confident that the majority of potential parents were detected, because collared pikas are conspicuous due to their vocalizations, haypiles, and diurnal activity. The study area was also surveyed weekly for untrapped pikas throughout each summer. However, because there is a possibility of overlooking pikas that migrated into the valley late in the summer, and because not every adult pika was captured and genotyped successfully, I set the proportion of parents sampled in each analysis to 0.90. I also set the genotyping error rate to 0.015, based on the duplicate genotyping of animals caught in 2008, and in assigning parentage to juveniles, I made assignments based on LOD scores. I considered results from both the relaxed (80%) and strict (95%) confidence levels.

Dispersal

The dispersal distance for each juvenile pika was inferred by considering how far its haypile was from its mother’s haypile. This was sufficient because once a pika has dispersed, settled on a territory and started caching vegetation (“haying”), it is extremely unlikely that the animal will select a new territory. Of the 366 animals caught during this study, only three were ever caught haying at a different location from where they were initially observed haying. Thus, determining the distance between a juvenile’s haypile and its mother’s haypile indicates how far the juvenile dispersed from its birthplace. After determining that the data did not fit a normal distribution (Shapiro-Wilk test, $P < 0.001$), I used a Mann-Whitney test to determine if the average dispersal distance between males and females differed. Unless otherwise indicated, all statistical analyses were done using SYSTAT v. 12 (Systat Software, Inc., Chicago, IL, USA).

Mating System

To determine if animals will leave their territories to seek mating opportunities, in cases where both parents of a pika were known, I calculated the distance between the parents' haypiles. I also determined the nearest potential mate for each pika by determining the haypile position of its nearest opposite-sex neighbour. I then calculated the pairwise-relatedness values ("*r*") for each pair of opposite-sex nearest neighbours, and between each pair of that had produced offspring, using the program STORM (Software for Testing Hypotheses of Relatedness and Mating Patterns, Frasier 2008). STORM uses the method described by Li *et al*. (1993) to calculate *r* values.

I also determined whether or not collared pikas choose mates based on pairwise relatedness using the program STORM (Frasier 2008). First, I used STORM to create a distribution of the average expected pairwise relatedness values of mating pairs in the population that would occur under a model of random mating. This was done separately for each year. Two hundred iterations were used for each year and each iteration sampled the pools of male and female breeders (with replacement), randomly created several mating pairs, calculated the pairwise *r* value for each one and calculated the average *r* value between mating pairs for the whole population. These separate distributions (one for each year) were combined to give a distribution of average pairwise *r* values expected for the population under a model of random mating. The true average pairwise *r* value between males and females who were confirmed to have produced offspring in this study were compared to this distribution ($\alpha = 0.05$). The *P*-value was directly calculated by determining where in the distribution of average *r* values under a random mating model the actual average *r* value between all parent pairs was located. A two-tailed test was used.

I also used a Mann-Whitney test (after determining that the nearest-neighbour "*r*" data were not normally distributed, Shapiro-Wilk test, $P = 0.003$) to compare the mean *r* value between opposite-sex nearest neighbours to the mean *r* value between all animals that had actually produced offspring and to animals that had produced offspring who were not nearest neighbors. This was done to determine if pikas avoid inbreeding by preferentially mating with animals that are not their nearest neighbours. I also searched the parentage data obtained by CERVUS for instances of close relatives (parent-offspring, or full- or half siblings) producing offspring.

Results

Three hundred sixty-six collared pikas were genotyped for this study. The number of juveniles captured and genotyped for each year varied from 10 in 2000 to 58 in 2005 (Table 3.1). The number of potentially breeding females ranged from 16 in 2001 to 35 in 2005, and the number of potentially breeding males ranged from 13 in 2004 to 36 in 2005 and 2006 (Table 3.1).

Two loci, *Ocp*3 and *Ocp*7, deviated significantly from Hardy-Weinberg equilibrium during several years. Microchecker (van Oosterhout et al*.* 2004) also indicated that there was evidence for null alleles in *Ocp*3 and *Ocp*7 during five years, so I omitted them from further analyses. After applying a sequential Bonferroni correction, there was no evidence that any loci were in linkage disequilibrium. Additionally, when loci were in apparent linkage disequilibrium (before a sequential Bonferroni correction), the disequilibrium never lasted more than one year. As a result, I did not eliminate any loci due to linkage disequilibrium.

The mean number of alleles for each of the fifteen loci used in this study varied from 5.87 in 2003 to 6.80 in 2007 and 2008 (Table 3.1) and the average polymorphic information content (PIC) for each locus ranged from 0.56 in 2006 to 0.61 in 2008 (Table 3.1). The non-exclusion probabilities for the combined loci were small with a mean of 0.0071 for the first parent, 0.00014 for the second parent and 2.7 X 10^{-7} for the parent pair (Table 3.1).

There was no evidence (based on F-Statistics) for widespread inbreeding in each year, with the exception of 2008. F_{IS} values for each year ranged from 0.0028 (2007) to 0.077 (2008), but only in 2008 did the F_{IS} score differ significantly from the value expected under a model of random mating (Table 3.1).

Molecular Sexing

Partial sequences for the zinc finger protein gene sequenced here for the purpose of molecular sexing collared pikas can be found under Genbank accession numbers HM466972 for ZFX and HM466973 for ZFY. Sequences from the fifteen animals of known sex were accurate (*e.g.* all males had “double peaks,” and no females did).

Dispersal

Maternity was assigned for 198 collared pikas at the 80% confidence level. Female pikas dispersed a mean of 641 m (SD= 546, N = 101) and male pikas dispersed a mean of 605 m (SD = 515, N = 97). There was no significant difference between the average distance dispersed by the two sexes (U = 5045.05, N_1 = 101, N_2 = 97, P = 0.72, Fig. 3.2). Considering males and females together as a group, the mean distance that juveniles dispersed from their natal den was 630 m (SD = 536 m, N = 198). However, the distribution of distances dispersed was skewed towards shorter dispersal distances (Fig. 3.2), and the maximum distance dispersed was 2051 m. Individuals that may have dispersed farther than that would have left the study area so dispersal events > 2 km were not detected.

When considering maternities assigned at the 95% confidence level, the mean dispersal distance was lower ($\bar{X}$ = 536 m, SD = 538 m, N = 84, maximum = 1876 m). However, the results were similar in showing no difference in mean dispersal distances between males and females. Females dispersed a mean of 564 m (SD = 548 m, N = 37) while males dispersed a mean of 513 m (SD = 535 m, N = 47). The difference was not significant (P = 0.33).

Mating System

Both maternity and paternity was assigned for 141 juvenile pikas at the 80% confidence level. Multiple mating was common, as twenty-one out of thirty-nine female pikas with more than one detected offspring in a single year had them with more than one male. These are minimum values, as some offspring may have died or left the valley before we could detect them and some offspring were not genotyped and included in this analysis. There was evidence of multiple mating (by both males and females) during each year of the study.

Pikas did not restrict their mating activities to their nearest opposite-sex neighbours, as the mean distance between pikas who had produced detected offspring together was 668 m (SD = 527 m, N = 141, see Fig. 3.3). The average distance between the closest potential mate for each pika was significantly lower ($\bar{X}$ = 60 m, U = 59106.00, N_1 = 468, N_2 = 141, P<0.0001), so pikas will make forays away from their territories to seek mating opportunities. Even so, 23% of juvenile pikas that could be assigned both a mother and a father were the result of matings between animals whose territories were situated 100 m or less apart, and 19%

were the results of matings between nearest neighbours. The collared pikas in this study did not undergo any permanent mating dispersal, as the vast majority returned to their original haypile after their mating forays. Only three pikas (0.82%) were captured on different haypiles from the ones they were first caught on.

Both maternity and paternity could be assigned at the 95% confidence level for 48 pikas. At this higher interval, the mean distance between pikas that had offspring together was 613 m (SD = 467 m, *N* = 48), which is far lower than the mean distance to the closest potential mate for each pika ($\bar{X}$ = 60 m, P<0.0001). However, a disproportionately large number of offspring were produced by animals less than 100 m apart (Fig. 3.3). There was evidence of multiple mating at the higher confidence interval as well, as six females and nine males had offspring from more than one mate.

There was no indication that collared pikas chose mates based on any indicators of genetic dissimilarity, nor was there any indication that pikas reduced the inbreeding coefficient of their offspring by going on mating forays. The mean pairwise *r* value between pairs that had produced offspring ($\bar{X}$ +SD = 0.083+0.29, *N* = 135) did not differ significantly from those expected under a model of random mating (*P* = 0.11). Additionally, the mean *r* value between opposite-sex nearest neighbours ($\bar{X}$ +SD = 0.052+0.29, *N* = 428) did not differ from those between animals who had produced offspring together (*U* = 27182.50, N_1 = 428, N_2 = 135, *P* = 0.30). Finally, the *r* values between opposite-sex nearest neighbours did not differ from those of pikas who had produced offspring but were not each other's nearest neighbours ($\bar{X}$ +SD = 0.070+0.24, *U* = 20778.50, N_1 = 428, N_2 = 104, *P* = 0.46).

Based on an examination of the parentage assignments made by CERVUS, it is rare for close relatives to produce offspring. However, it clearly does occur on occasion. One pika was the result of a mother-son mating, and two were the result of father-daughter matings.

Discussion

Mating System

The collared pika population studied here generally displayed a polygynandrous mating system, as both males and females would mate with multiple partners. The pikas also did not restrict their mating activities to their nearest neighbours. This differs from the more monogamous mating system proposed for and found in some populations of American pikas. Smith and Ivins (1984) first proposed that American pikas are facultatively monogamous, based on a sexual alternation of territories seen in the species and the inability of single males to monopolize access to multiple females. Subsequent investigations demonstrated that American pikas at the Bodie Hills in California do tend to mate primarily with their nearest neighbours (Peacock and Smith 1997a). Additionally, at a site in the Sierra Nevadas, the mean number of territories separating mated pairs was 1.38 (Peacock & Smith 1997b), and the mean distance between the haypiles of nearest neighbours was 32 m (Peacock 1997). However, animals in the *O. collaris* population studied here sometimes went across several territories to mate, at a higher average distance of about 500 m.

The mating system of North American pikas likely varies across populations and individuals. While the American pika population at Bodie was largely monogamous (Peacock *et al*. 1997a), observations of American pikas elsewhere suggest that males of that species will sometimes travel away from their territories to seek mating opportunities. Barash (1973), Svendsen (1979), Smith and Ivins (1984) and Tapper (1973) all report that male pikas will expand their home range during May and June (mating season) and may wander during this time. This may be the case for some Asian pika species as well, because Gliwicz *et al.* (2005) noted that Kawamichi (1971), Nikolski *et al.* (1990) and Nikolski and Mukhamediev (1997) found that *O. hyperborea* and *O. alpina* males will travel to the territories of several females during the breeding season. While genetic data from more rock-dwelling pika populations would be needed to make any definitive conclusions about their mating systems, the wandering behavior of males combined with genetic confirmation of polygynandry in the collared pika suggests that rock-dwelling pikas are flexible in their mating systems.

While rock-dwelling pikas may generally be polygynandrous, they are often somewhat socially monogamous or polygynous, although the details of male-female interactions vary among species. Pikas tend to be aggressive towards intruders on their territories (Broadbrooks 1965; Svendsen 1979; Smith and Ivins 1983, 1984), but neighbouring male and female American pikas often tolerate each other's presence (Smith and Ivins 1984; Brandt 1989), and will have a greater proportion of overlap between their territories than will neighbouring animals of the same sex (Svendsen 1979; Smith and Ivins 1984). During the late summer and early fall, the territories of male collared pikas may also overlap with those of one or more females, although each animal will still maintain its own haypile (K. O'Donovan unpublished data). However, in American pikas, there is less overlap between the territories of neighbouring heterosexual pairs in the fall than there is during summer (Smith and Ivins 1984), and unlike both North American species, male-female pairs of the Asian rock-dwelling northern pika (*O. hyperborea*) will store food collectively (Gliwicz *et al.* 2005).

While many rock-dwelling pikas will tolerate opposite-sex neighbours on their territory for at least part of the year, there is limited social interaction between them and no evidence for mate guarding behavior. In American pikas, while there can be some affiliative behaviors between neighbouring males and females, there are also agonistic interactions between members of such pairs (Smith and Ivins 1983). The generally asocial nature of pikas may predispose them to a polygynandrous mating system as solitary animals tend to have high rates of extra-pair paternities since the male does not guard the female and prevent her from mating with other males (Clutton-Brock and Isvaran 2006). Male North American pikas are also incapable of securing sufficient resources to attract females they can then monopolize. American and collared pikas of both sexes must build a haypile each summer and each animal will typically cache enough food for itself only (Dearing 1997; Morrison *et al*. 2009).

The mating system of the more social black-lipped or plateau pika (*Ochotona curzoniae*) differs considerably from that of the collared pika. Plateau pikas inhabit high altitude steppes and meadows where they have access to more forage than do rock-dwelling pikas, which have access to forage primarily at the edges of their territories. Because of this, plateau pikas can exist at far higher densities than rock-dwelling pikas. Plateau pika populations can have up to 380 animals/ha (although such high densities are found only at the end of the breeding season, [Smith *et al.* 1990]). In contrast, even during the year where collared pikas at the Ruby Ranges study site were present at their highest density, and on the side of the valley with the highest pika density, there were only 3.81 collared pikas / ha (Morrison and Hik 2007).

Plateau pikas generally live in social groups, and adult females and males often mate multiply (Dobson *et al*. 1998). However, copulations between animals from different social groups are rare, as Dobson *et al*. (2000) found that 89% of copulations in a population of plateau pikas occurred between animals from the same group. In another population, only 1.7% of offspring were found to be the result of a female mating with a male from outside her social group (Yin *et al*. 2009). A mating pair or group of plateau pikas will spend more time in close proximity to each other than will a pair of collared or American pikas, as plateau pikas will allogroom and huddle (Dobson *et al*. 1998). Dominant males in plateau pika groups control access to females, as they generally sire a disproportionately large number of offspring although they do not completely suppress subordinate males from mating (Yin *et al*. 2009). During the mating season, plateau pikas can be very aggressive towards animals from outside their social groups, which serves to keep extra-group paternities very low (Yin *et al.* 2009).

Dispersal

Most animal species display some degree of sex-biased dispersal, with male-biased dispersal and female philopatry being the norm in mammals (Greenwood 1980; Dobson 1982). Collared pikas thus appear to be quite unusual among mammals in displaying no apparent sex-biased dispersal at the scale examined in this study. In this study, the average dispersal distance for males and females did not differ, nor did the distribution of dispersal differences between the two sexes appear to differ (Fig. 3.3). A lack of sex-based differences in dispersal distance has also been documented in American pikas (Smith and Ivins 1983; Smith 1987). The lack of sex-biased dispersal in North American pikas is likely a function of the species’ solitary nature, and the need for both sexes to find their own territories. There is no apparent difference in the level of resource competition between the sexes, and since there is also no evidence for kin-based altruistic behavior in collared pikas, neither sex will benefit from kin cooperation by remaining philopatric.

Unlike North American pikas, there is sex-biased dispersal in plateau pikas. Females of this species often remain philopatric and benefit from this as related females cooperate by uttering alarm calls for each other and caring for each other's offspring (Smith and Wang 1991; Dobson *et al*. 1998). Dispersal in plateau pikas appears to be driven by a trade-off between the benefits of nepotism (which promote philopatry) and resource competition, which may induce animals to migrate to less dense social groups (Dobson *et al*. 1998).

While both collared and American pikas display even-sexed dispersal, the mean dispersal distances in this population of collared pikas differed from those observed in a population of American pikas from Colorado, where most juveniles settled within 50 m of their mother's home range (Smith and Ivins 1983). Additionally, juvenile American pikas may stay on their mother's home range during their first summers (Smith and Ivins 1983) and juveniles can avoid conflict with their parents by restricting their activities to when their parents are inactive (Smith and Ivins 1987). Collared pikas, however, disperse shortly after weaning (Morrison and Hik 2007), and they disperse an average of 500-600 m.

The differences in dispersal habits between American and collared pikas may be related to the lower densities that generally characterize the latter species. This difference may, in turn, be related to the harsher climate collared pikas inhabit. The collared pika population under study here reached its highest density (1.21/ha) in 2007, although the density on the south-facing slope (3.81 pikas/ha) was higher than on the east- or west-facing slopes. However, an American pika population from Colorado had 8.2 pikas/ha at its highest density (Smith and Ivins 1983), and densities in this species generally range from 6-10/ha (Smith *et al.* 1990). The mean distance to an American pika's nearest neighbour (13 m, Smith and Ivins 1983) is also much lower than it is in collared pikas (60 m). Collared pika densities are also likely much lower during the breeding season. The densities and nearest-neighbour distances cited above are based on numbers of animals present at the end of August, but over-winter mortality in collared pikas can be very high. For example, in our population, female over-winter survival ranged from 0.1 to 0.5, depending on the year (Morrison and Hik 2007). During years with low densities and high over-winter mortality rates, the nearest neighbour for many collared pikas in our population may be a few hundred meters.

The differences in dispersal timing and mean dispersal distance between American and collared pikas may be related to differences in population densities that affect the movement patterns of individuals. The high densities that characterize many American pika populations may prevent juveniles from dispersing long distances from their natal den. For a similar reason, high densities may prevent adults in dense populations from mating with animals beyond their nearest neighbours. North American pikas are very aggressive towards unfamiliar animals (Broadbrooks 1965; Smith and Ivins 1983, 1986), and a juvenile in a high-density population may be restricted from wandering due to frequent aggressive encounters with other pikas (*i.e.* a social fence effect; Hestbeck 1982). At the low densities that characterize collared pika populations, individuals will encounter fewer conspecifics and may be less restricted from moving about.

Mate Choice and Inbreeding

If dispersal is related primarily to resource competition in collared pikas, then animals will likely disperse only as far as needed to find a suitable territory. Indeed, in this study, animals dispersed a mean of 536 m, with the distribution of dispersal distances skewed towards shorter distances (Fig. 3.2). This could leave pikas vulnerable to inbreeding although this does not appear to be the case as inbreeding coefficients in the population were low and rarely differed from those expected under a model of random mating (Table 3.1). This differs from the pattern seen in American pikas in California, who mated with second-order relatives more often than by chance (Peacock and Smith 1997b).

Unlike banner-tailed kangaroo rats, (*Dipodomys spectabilis,* [Winters and Waser 2003]), collared pikas do not appear to reduce the inbreeding coefficients of their offspring by undertaking mating forays. Pairs of collared pikas that had produced offspring in this study did not have lower relatedness coefficients than either random pairs or nearest-neighbor pairs. Additionally, pairs of nearest neighbour pikas who had produced offspring did not have significantly lower relatedness coefficients than non-nearest neighbour pairs who had produced offspring. Thus, pikas do not appear to undertake mating forays in order to reduce their chances of inbreeding, but may do so to simply increase the genetic diversity of their offspring (Charmantier *et al*. 2004).

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Figures and Tables

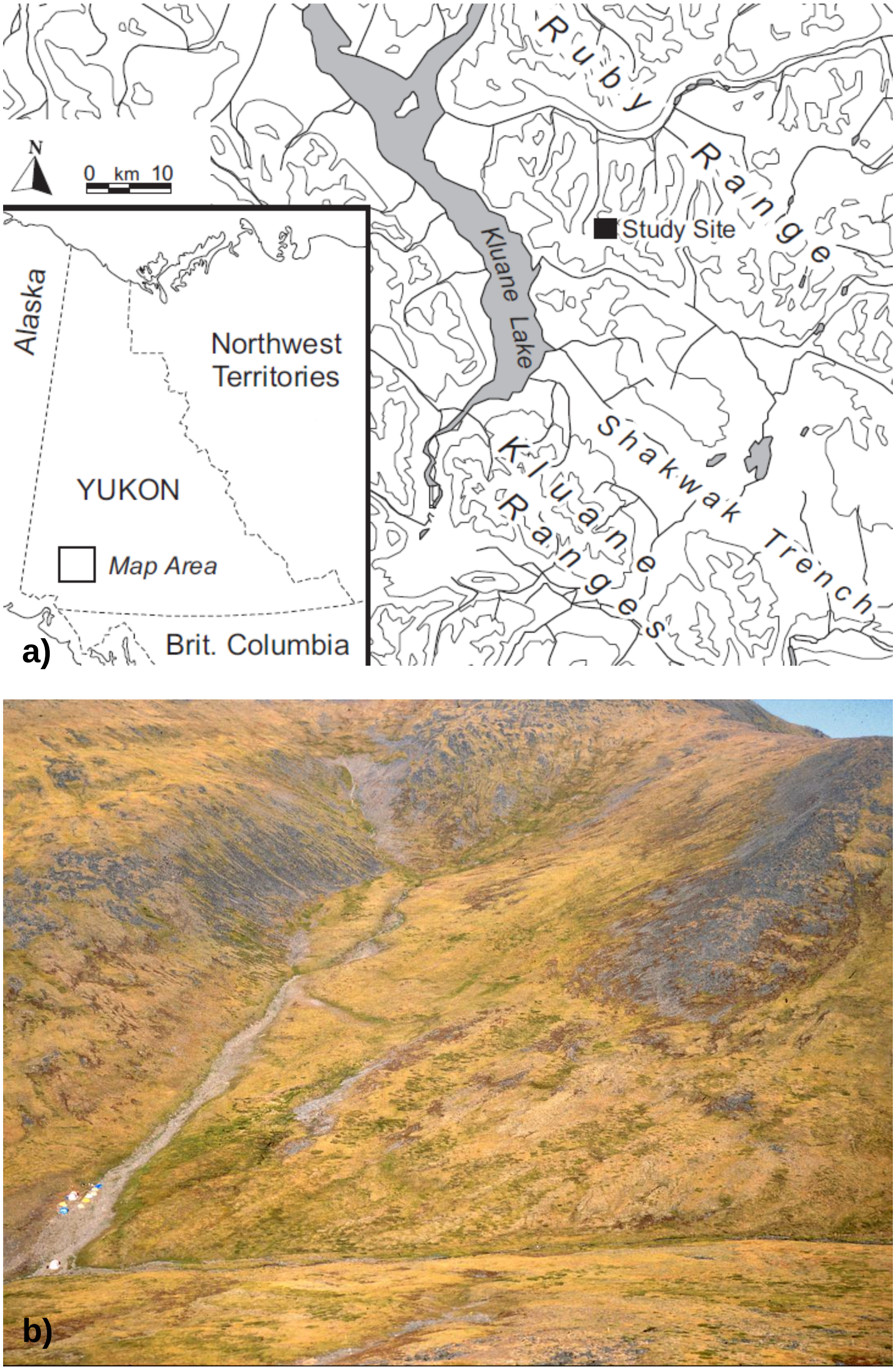

Fig. 3.1: a) A map showing the location of the study site. Modified from Danby (2007). **b)** A photograph of part of the study site.

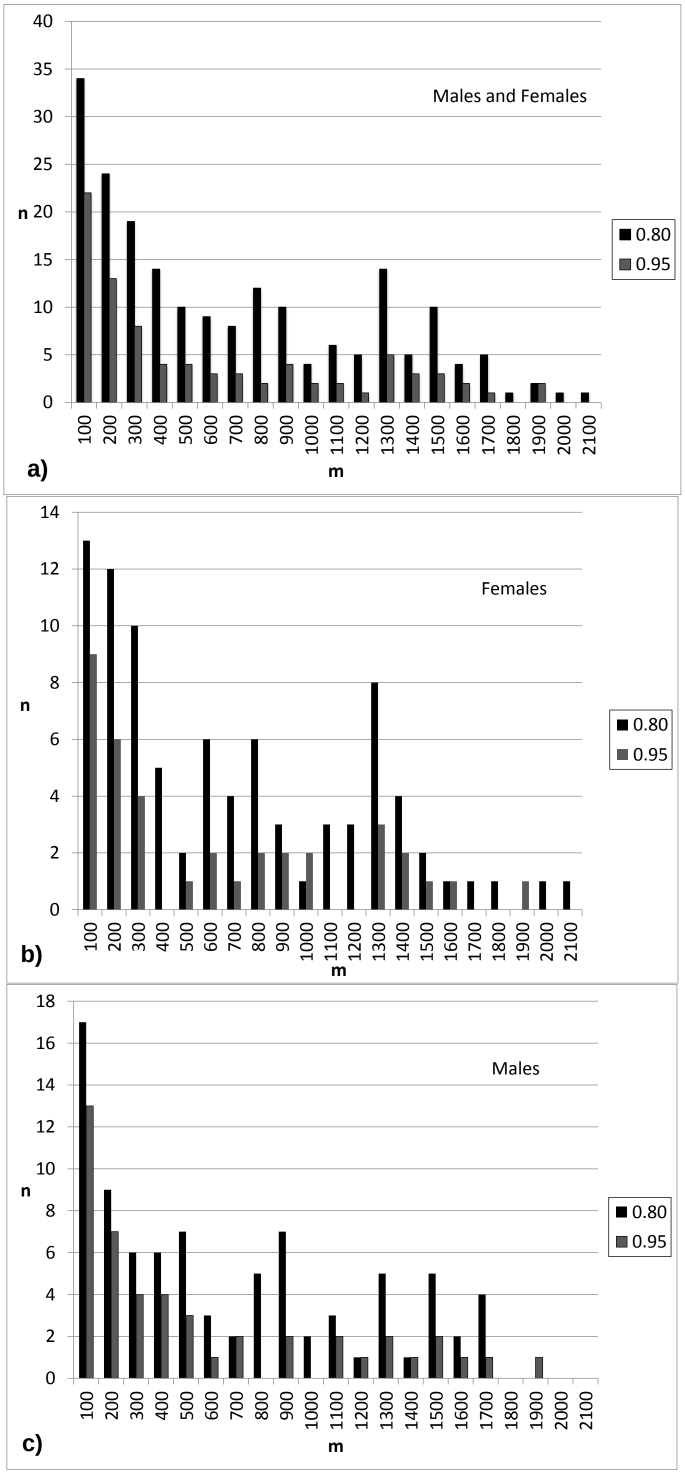

Fig. 3.2: Distribution of dispersal distances of juvenile collared pikas in the Ruby Range, Yukon, during the entire study (1999-2008) for: a) both sexes, b) females, and c) males. Results from using the relaxed (80%) and strict (95%) confidence levels are shown.

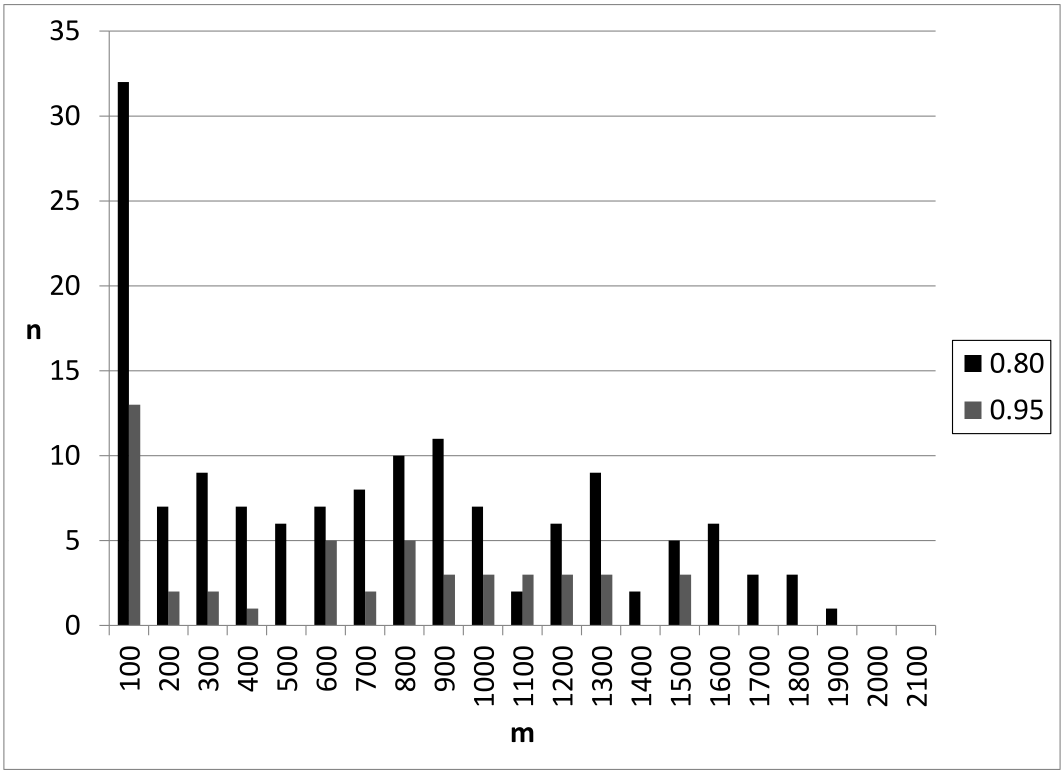

Fig. 3.3: Distribution of distances between the territories of male and female collared pikas that produced offspring during the entire study (1999-2008). Results from both the relaxed (80%) and strict (95%) confidence levels are shown.

Table 3.1: Overall genetic structure of the study population in the Ruby Range, Yukon, from 1999 to 2008. Values indicated are the number of juvenile collared pikas caught and genotyped during each year, the number of potential parents considered for each juvenile, the population size, the mean number of alleles per locus, the polymorphic information content (PIC) for the fifteen loci used in this study, and the average combined non-exclusion probability for the fifteen loci for the first parent (EXC_{SP}), the second parent (EXC_{SP}) and the parent pair (EXC_{PP}) for each year.

Year	No. Offspring	No. Candidate Mothers	No. Candidate Fathers	Population Size[1]	Mean No. Alleles/ Locus	F_{IS}	Mean PIC/Locus	EXC_{FP}	EXC_{SP}	EXC_{PP}
1999	30	32	28	71	6.33	-0.035	0.58	0.0074	0.00014	$2.7X10^{-7}$
2000	10	30	33	29	6.00	-0.039	0.57	0.0077	0.00016	$3.3X10^{-7}$
2001	33	16	19	52	6.00	-0.016	0.59	0.0057	0.00010	$1.6X10^{-7}$
2002	30	20	29	56	6.07	-0.020	0.58	0.0073	0.00014	$2.7X10^{-7}$
2003	13	28	34	32	5.87	0.033	0.57	0.0085	0.00018	$3.9X10^{-7}$
2004	39	18	13	73	6.07	0.013	0.57	0.0085	0.00018	$4.3X10^{-7}$
2005	58	35	36	64	6.67	-0.010	0.57	0.0072	0.00014	$2.7X10^{-7}$
2006	20	34	36	71	6.73	0.037	0.56	0.0074	0.00015	$2.5X10^{-7}$
2007	44	28	33	80	6.80	-0.028	0.57	0.0069	0.00013	$2.2X10^{-7}$
2008	23	50	32	68	6.80	0.077*	0.61	0.0045	0.000069	$8.0X10^{-8}$

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[1] The population size was obtained by a complete enumeration of the population. The numbers presented here are for the population at the end of August.

Chapter Four

The fine-scale genetic structure of a collared pika (*Ochotona collaris*) population before, during and after a population decline

Introduction

Many small mammal populations in northern ecosystems undergo periodic density fluctuations (Krebs 1996). Dramatic changes in a population's density can have a major effect on its genetic structure, both temporally and spatially. During periods of low population density, genetic drift can result in both a loss of genetic diversity within populations and increased differentiation among populations (Wright 1978). Very sharp decreases in a population's effective population size (*i.e.* a population bottleneck) may both decrease a population's allelic diversity and reduce its overall heterozygosity (Nei *et al*. 1975), although there will be a faster reduction of allelic diversity than heterozygosity (Nei *et al*. 1975; Maruyama and Fuerst 1985). A bottlenecked population will therefore display a transient excess of heterozygosity due to the rapid loss of rare alleles (Nei *et al*. 1975; Maruyama and Fuerst 1985; Cornuet and Luikart 1996).

Not all populations that have experienced a demographic bottleneck will show a genetic signature of having undergone one. In fact, many mammal populations that undergo dramatic fluctuations with frequent low phases maintain high levels of genetic diversity over the long term (*e.g.* Burton *et al.* 2002; Berthier *et al.* 2006; Ehrich *et al.* 2009; Pilot *et al.* 2010). Both mutation and gene flow can increase the genetic diversity of a population; however, gene flow will be more important over the time scales considered in most studies. Thus, small mammal populations often recover lost genetic diversity very quickly after a decline if they receive dispersers from neighboring populations (*e.g.* Busch *et al.* 2007).

Populations undergoing changes in density may also differ in how their genetic composition changes through time. A population may maintain consistently high levels of genetic diversity even through periods of low density (*e.g.* Pilot *et al.* 2010) or it may lose diversity during low phrases which is then regained during high phases (*e.g.* Berthier *et al.* 2006). How a population's genetic diversity changes through time can depend on whether it displays positive or negative density-dependent dispersal. Under a situation of negative density-dependent dispersal, there will be little change in the population's genetic variability over time because increased gene flow among populations during low-density periods will compensate for genetic diversity lost in each one by drift (Pilot *et al*. 2010).

In contrast, under a situation of positive density-dependent dispersal, populations will undergo temporal variations in genetic diversity that correspond with changes in density. During low-density phases, populations will lose genetic diversity due to drift, and this may serve to increase genetic differentiation among populations. During the high density phase, gene flow among populations will increase and this will restore diversity lost in each population during the low density phase. Furthermore, if migrants are exchanged among genetically-differentiated populations, then there will be genetic differentiation across years in each population (Berthier *et al.* 2006).

Along with its overall genetic diversity, a population's fine-scale spatial genetic structure can also change through time in concordance with changes in its density. Species characterized by low levels of dispersal often display fine-scale genetic structure within populations at scales of < 1 km (*e.g.* Peakall *et al.* 2003; Double *et al.* 2005; Lee *et al.* 2009; McEachern *et al.* 2007; Schweizer *et al*. 2007). Such structure often varies temporally with changes in density. For example, in banner-tailed kangaroo rats (*Dipdomys spectabilis*), high densities were associated with low levels of fine-scale genetic structure because there was a greater overlap of kin clusters at high densities (Busch *et al*. 2009). However, intermediate densities were associated with increased levels of genetic structuring in woodrats (*Neotoma* spp.) because low or high densities do not facilitate the formation of kin-based clusters while intermediate densities do (McEachern *et al.* 2007). The increased competition for resources that comes with high population densities seems to dissolve kin clusters, while at low densities, there will necessarily be fewer kin clusters present to generate a pattern of positive genetic structure (McEachern *et al.* 2007).

I used microsatellite data from a collared pika (*Ochotona collaris*) population to determine if it shows genetic structuring at a fine scale (< 2 km), and to determine if its genetic structure remains stable over time or varies temporally with the density of the population. The density of the collared pika population under study has fluctuated in response to broad-scale climate patterns (Morrison and Hik 2007), which makes it a useful system with which to examine the influence density has on a population’s fine-scale genetic structure. I also determined if the population recovered its genetic diversity after a decline.

Specifically, I predicted that the population will show genetic structuring on a fine scale because collared pikas and the related American pikas (*Ochotona princeps*) have limited dispersal abilities due to their strong preference for inhabiting alpine boulder fields. This preferred habitat of pikas is patchily distributed and long distance movements often require that pikas travel across habitat that they generally avoid.

Parentage analyses have indicated that the average dispersal distance in this population is about 600 m, with most animals dispersing shorter distances while a smaller number disperse longer distances (1 – 2 km) (see Chapter 3).

I also predicted that the collared pika population will show a higher degree of genetic structuring during low density than high density years, based on the observation that American pikas only disperse as far as they need to in order to find a vacant territory (Smith 1987). During low density years, most pikas will likely disperse very short distances, as vacant territories should be plentiful. Many pikas will then have parents or siblings as their nearest neighbors, which will result in a higher degree of genetic structuring. In high density years, where vacant territories may be scarce, juvenile pikas may have to travel longer distances, or even leave the population, to find a suitable territory. Levels of fine-scale genetic diversity should then be lower during high density years.

Additionally, if dispersal is positively-density dependent, then this collared pika population should regain any genetic diversity lost during the population decline. During the low density period, the population will likely lose genetic diversity due to drift; however, as the population increases, it may begin to receive migrants from surrounding populations, which are approximately one kilometer away (Franken 2002). If these migrants originate from genetically differentiated populations, they may restore genetic diversity lost in the study population, provided that they survive and breed.

Materials and Methods

Field Site

The study site is located in the Ruby Ranges east of Kluane Lake in the southern Yukon (61° 24’ N, 138° 28’ W; 1700-2000 m). The site is approximately 4 km^2 and consists of meadow and tundra interspersed with patches of talus that range in size from 654 m^2 to over 156 000 m^2 (Franken and Hik 2004). The valley is dissected by shallow, 1-2 m wide streams and has three dominant aspects (facing east, west and south) with each side being separated from the others by the streams and 100-300 m of meadow and shrubs that pikas generally avoid (Fig. 4.1). The pikas are capable of dispersing across the streams that dissect the valley.

Sample Collection and Microsatellite Genotyping.

I used genetic data from pikas captured from 1998 to 2009 and the capture methods used are described in Chapter 3. Population densities used in analyses in this study were based on the total number of pikas detected each season by the end of August.

DNA was extracted from ear tissue using the DNeasy Blood and Tissue Kit from Qiagen (Valencia, CA, USA). Animals were genotyped using the primers and conditions described in Zgurski *et al.* (2009) and each locus was tested for deviations from Hardy-Weinberg Equilibrium (HWE) and for the presence of null alleles. Additionally, each pair of loci was tested for linkage disequlibrium for each year (see Chapter 3 for details). Since loci *Ocp*3 and *Ocp*7 deviated from HWE during several years and since there was evidence for null alleles at these two loci, they were omitted from further analyses (see Chapter 3).

Spatial Genetic Structuring

Traditional F-statistics (F_{IS}, F_{ST}, and F_{IT}, Weir and Cockerham 1984) for each year were calculated using SPAGeDi (Spatial Pattern Analysis of Genetic Diversity; Hardy and Vekemans 2002). For the pairwise and global F_{ST} calculations, three subpopulations were defined: the North, the East and the West. Each of these groups was separated by meadow and a shallow creek (Fig. 4.1). Permutation tests (with 20 000 permutations) were used to determine if each value was significantly different from the values expected under a model of random breeding.

I also used the program SPAGeDi to examine the fine-scale genetic structure of the population during each year. The relatedness coefficient (*R*) between each pair of pikas during each year was calculated by SPAGeDi using the method proposed by Wang (2002). I chose this method because it is robust for small sample sizes and for populations with unknown relatives included in it (Wang 2002). I also repeated the analyses using the method described by Queller and Goodnight (1989) to estimate pairwise relatedness coefficients. The distance separating the capture locations of each pika were also calculated by SPAGeDi using the grid co-ordinates of each pika's capture location. To assess the fine-scale genetic structure of the pika population during each year, I performed individual-based statistical correlation analyses between pairwise *R* estimates and the log of pairwise spatial distances. The slope of this relationship can be used to determine if the pairwise relatedness coefficients among the pikas decrease with distance (Hardy and Vekemans 2002). Under an "isolation by distance" model in a two-dimensional space, pairwise relatedness coefficients between animals should be negatively correlated with the log of their pairwise distances (Rousset 1997; 2000). To determine if the slope of the relationship between pairwise *R* and the logarithm of distance was significant, I used a numerical resampling procedure in which spatial locations were randomly permuted twenty-thousand times (a procedure equivalent to a Mantel test, Hardy and Vekemans [2002]). The correlation coefficient was recalculated after every permutation to generate a distribution of coefficients expected under a random distribution of genotypes.

I also examined the genetic structure of the population by grouping each pair of pikas into a distance class based on how far apart their haypiles were. Analyses were set up using six distance classes: 0-250 m, 251-500 m, 551-750 m, 751-1000 m, 1001-1250 m, and >1251 m. These distance classes were chosen because they typically allowed for at least 100 pairs of animals to be included in each class for most years. Only one distance class was used for animals > 1251 m apart due to the smaller number of animals in the higher compared to the lower distance classes during some years. I also did analyses for 2000 and 2003 using three distance classes (1-700 m, 700-1300 m, and 1300-1900 m) because the pika density during these two years was low and when the data was analyzed using six distance classes, several classes contained fewer than the recommended 100 pairs required for a powerful analysis (Hardy and Vekemans 2002). The distribution of expected mean relatedness coefficients for each distance class under a scenario of random genotype distribution was determined using a permutation test with 20 000 permutations. Reference allele frequencies were estimated using data from all years.

Bottleneck

I used the program “Bottleneck” (Piry *et al*. 1999) to determine if the population displayed a genetic signature of having gone through a bottleneck (decrease in N_e) during the study period. Populations that have undergone a recent decrease in N_e generally display an excess of heterozygosity (H_e) compared to the heterozygosity expected under drift-mutation equilibrium (H_{eq}, Nei *et al*. 1975; Maruyama & Fuerst 1985). This is because, while a decrease in N_e leads to a reduction in both allele number and H_e, the allele number will be reduced faster than H_e. Therefore, a bottlenecked population will, for a brief period, have a higher H_e than H_{eq}. Here, a sign test and a Wilcoxon signed-rank test were used to determine if the collared pika population displayed an excess of heterozygosity compared to the heterozygosity expected under a situation of drift-mutation equilibrium. “Bottleneck” generates the distribution of H_{eq} by simulating the coalescent process for *n* genes under a specific mutation model chosen by the user, where *n* = sample size. To generate the distribution of H_{eq} for the population in each separate year, 1000 iterations were used with a two-phase model of microsatellite mutation. This model combines the infinite alleles model and the single-step mutation model (Di Renzo *et al*. 1994). As recommended by Piry *et al* (1999), I specified that 95% of the mutations be single-step and 5% be multi-step, with a variance of 12 for the multi-step mutations.

I also used the mode shift test, as described in Luikart *et al.* (1998), to determine if the distribution of allele frequencies in the population is characteristic of a population that has gone through a bottleneck. A population in mutation-drift equilibrium will have a large proportion of alleles present at a low frequency (<0.1) (Luikart *et al*.1998), and alleles at a low frequency are expected to be more common than alleles present at an intermediate frequency, regardless of the mutation mode (Nei *et al*. 1976). After a bottleneck, there is often a shift in allele frequency distribution towards more alleles being present at an intermediate frequency than at a low frequency (Luikart *et al*. 1998).

Detection of First Generation Immigrants

The program GeneClass2 (Piry *et al*. 2004) was used to detect first generation migrants into the population. Since there was no information on outlying populations, the L_{HOME} statistic was used, which calculates the log-likelihood that an individual originated in the study population. The log-likelihood was calculated using the frequency method of Paetkau *et al*. (1995), and the frequency for missing alleles was set at the default of 0.01. The probability that an individual was a migrant was calculated using the Monte Carlo resampling algorithm of Paetkau *et al*. (2004), using 10 000 replicates. Animals with a $P \leq 0.01$ of belonging to the home population were considered to be migrants. This lower α value was used because some animals identified as migrants at $0.01 < P < 0.05$ were actually the offspring of migrants, as determined from results of a parentage analysis. I also determined whether or not the migrants had detected offspring using the results from a previous parentage analysis (see Chapter 3).

Analysis of Genetic Differentiation Across Time

Since migrants from a differentiated population can gradually alter the genetic composition of a population, I also examined the level of population differentiation in the population between years. The pairwise F_{ST} between populations from different years was calculated and a permutation test (using 20 000 permutations) was used to determine if they differed from those expected if there was no differentiation in the population across years. I also used a Mantel test (as implemented by GenAlex, Peakall and Smouse 2006) to examine the data for a temporal equivalent of an “isolation by distance” effect.

Results

Four hundred forty-two pikas were genotyped, and the number of pikas genotyped each year ranged from twenty-nine (2000) to seventy-four (2007). The densities at the end of August ranged from 0.44 pikas/ha in 2000 to 1.21 pikas/ha in 2007 (Fig. 4.2).

The mean number of alleles across all loci for each year ranged from 4.93 (2000) to 6.75 (2008), and the mean observed heterozygosity ranged from 0.59 (2006) to 0.64 (2001) (Table 4.1). The number of alleles per locus ranged from 2 (*Occ*10) to 13 (*Ocp*8). There was no evidence for widespread inbreeding in each year, with the exception of 2008. F_{IS} values for each year ranged from 0.0028 (2007) to 0.077 (2008), but only in 2008 did the F_{IS} score differ significantly from the value expected under a model of random mating (Table 4.1). Global F_{ST} values indicated that there was a low, but significant, level of genetic differentiation within the pika population in all years except 1998, 2000 and 2002 (Table 4.1). Pairwise F_{ST} values indicated that the subpopulations with the lowest levels of genetic differentiation were the East and West populations in all years except 1998 and 2004 (Table 4.1). F_{IT} values did not deviate significantly from zero except during 2003, 2006, and 2008 (Table 4.1).

Spatial Genetic Structure

Preliminary analyses using the program Structure 2.1 (Pritchard *et al.* 2000) consistently indicated that there were no discrete subpopulations within the valley (data not shown). I therefore used an approach to detect an “isolation by distance” effect, which may not be apparent using an approach that examines the data for discrete clusters of genetically similar individuals.

During 1999, 2001, 2005, 2006, 2007, 2008, and 2009, there was a significantly negative correlation between pairwise relatedness coefficients and the logarithm of the pairwise distances between pikas (Table 4.2), indicating that pikas which are close together in space have, on average, higher relatedness coefficients. However, the correlation coefficients in these years were small and ranged from 0.066 to 0.17 (Table 4.2). During 1998, 2000, 2002, 2003, and 2004, the distribution of genotypes in the population did not differ significantly from a random distribution (Table 4.2), indicating that there was no isolation by distance effect in those years.

During several years (1999, 2001, 2002, and 2004-2008), pika pairs in the lowest distance classes (0-250 m) had average pairwise relatedness coefficients that were higher than those expected under a random distribution of genotypes (Fig. 4.3). In 2000, pikas in the third distance class (551-750 m) also had higher than expected mean relatedness coefficients (Fig. 4.3). During 1998, 2003, and 2009 there was no genetic structuring within the pika population (Fig. 4.3). When analyzed with fewer distance classes, there was no evidence for genetic structuring during 2000 or 2003 (data not shown).

Patterns of spatial genetic structuring inferred using Queller and Goodnight’s (1989) method of estimating pairwise relatedness coefficients were largely congruent with those inferred from using Wang’s (2002) method. However, no significant pattern of genetic structuring was found in 1999 using Queller and Goodnight’s (1989) method, and a weak pattern of structuring was found in 2009 (data not shown). Both methods of estimating pairwise relatedness coefficients found similar patterns of genetic structuring in 2001, 2002 and 2005-2008.

Bottleneck

As indicated by a Wilcoxon signed rank test, there was no evidence for an excess of H_e in any year throughout the study period (Table 4.3). The sign test also failed to indicate that there were more loci with a heterozygosity excess than a heterozygosity deficit. In three years (1998, 2006 and 2007), there were significantly more loci with a heterozygosity deficit than excess (ten loci with a deficit and 5 with excess, Table 4.3). The mode shift test indicated that the distribution of allele frequencies fitted the normal “L-shaped” distribution expected from a population in mutation-drift equilibrium (Table 4.3). Thus, the population showed no genetic signature of having gone through a bottleneck.

Immigration

GeneClass2 detected seventeen individuals (at $P<0.01$) as potential immigrants from genetically differentiated populations (Fig. 4.4). The number of such immigrants varied each year from zero (in 2009) to three (in 2001, 2004, and 2008) (Fig. 4.4). In 1999, 2001, 2004-2006 and 2008, one of the migrants had detected offspring (Fig. 4.4). However, this estimate may underestimate the number of migrants that produced offspring, as some offspring may have not been caught and genotyped. The estimated number of migrants into the population will also be underestimated by this method, as animals from nearby populations may not have been sufficiently genetically distinct to have been identified as migrants, and some migrants may not have been caught and genotyped.

Genetic Variability and Genetic Differentiation Over Time

The genetic diversity and structure of the pika population under study did not remain stable through time. In 1998, the mean number of alleles per locus was 5.87 and the observed heterozygosity was 0.61 (Table 4.1). The mean number of alleles declined to 4.93 in 2000, which was the year that the population density was at its lowest. However, the population recovered, as did its genetic diversity. By 2008, the mean number of alleles and observed heterozygosity was 6.75 and 0.65, respectively, although both declined slightly in 2009 (Table 4.1; Fig. 4.2).

There was usually a small, but significant, degree of genetic differentiation between populations from different years (Table 4.4). Populations without a significant degree of genetic differentiation were usually separated in time by only one year (Table 4.4). However, the value of F_{ST} between populations was not correlated with the amount of time separating them ($P = 0.60$).

Discussion

Fine-scale Genetic Structure

Using spatial autocorrelation analyses, I detected a pattern of fine-scale genetic structuring in a collared pika population for eight out of the twelve years evaluated. The results are consistent with an “isolation by distance” effect, because pikas in lower distance classes during 1999, 2001 and 2004-2008 had higher than expected mean relatedness coefficients than did pikas in larger distance classes (Fig. 4.3). However, a Mantel test did not detect any genetic structure during two of the years (2002 and 2004) where the autocorrelation analyses did detect it. This is likely because the Mantel test is less powerful in detecting genetic structure than autocorrelation analyses that examine structure by grouping individuals into several discrete distance classes (Peakall *et al.* 2003).

The pattern of fine-scale genetic structure found in most years is consistent with observations that collared pikas have low mean dispersal distances. American pikas also have low mean dispersal distances and usually colonize the nearest available territory upon leaving their natal dens (Smith 1987; Peacock and Smith 1997), although in one study, there was enough long-distance migration to prevent populations two km apart from becoming genetically differentiated (Peacock 1997).

The degree and pattern of fine-scale genetic structuring seen in most years of this study also characterizes other populations of small mammals with low average dispersal distances, including the Australian bush rat (*Rattus fuscipes*, Peakall *et al*. 2003) and the banner-tailed kangaroo rat (*Dipodomys spectabilis*, Busch *et al*. 2007). Each of these species shows genetic structuring at scales < 1 km, and animals situated close to each other have a higher degree of genetic similarity than animals situated farther apart. This is because mean dispersal distances are low in each species, making it likely that an individual will be surrounded by close kin.

Factors beyond restricted dispersal may also produce patterns of fine-scale genetic structuring, although none of them seem likely to apply here. For example, capturing a large number of pre-dispersal juveniles may generate a pattern of positive spatial genetic structure that will dissolve once the juveniles disperse (Scribner and Chesser 1993). However, it is unlikely that the genetic structure in the collared pika population under question is due to the sampling of pre-dispersal juveniles, because the vast majority of pikas captured at the study site were captured while they were caching food, which they do not start to do until they have dispersed and secured a territory. Therefore, the genetic structuring seen in most years at this site is likely due to short dispersal distances, not the capture of pre-dispersal juveniles.

Social structure can also generate genetic structure, although it can be difficult to separate the effects of social structure from limited dispersal. In many social species, relatives of either sex may preferentially associate with each other, which generates positive genetic structuring in that sex. For example, male vinous-throated parrotbills (*Paradoxornis webbianus*) associate with each other throughout the year and nest very close to each other during the second laying peak (Lee *et al.* 2009). However, the degree of genetic structuring seen in the collared pika population examined here is not likely to be the result of any social structuring, nor has the clustering of kin resulted in the development of cooperation among neighbors. North American pikas are territorial and interactions between neighbors are generally antagonistic in nature. Both collared and American pikas will chase intruders on their territories and individuals occasionally end up in physical altercations that involve biting, boxing with the forepaws, tumbling, and kicking with the hindfeet (Broadbrooks 1965; Svendsen 1979). The territories of adult males and females often overlap, but the two animals will rarely be simultaneously active on the overlapping parts of the territory at the same time (Svendsen 1979; K. O'Donovan, unpublished data).

Inbreeding

For many species with low mean dispersal distances, individuals are typically surrounded by relatives that they may mate with. Examples include the Seychelle's warbler (*Acrocephalus sechellensis*, Eikenaar *et al*. 2008) and the white-toothed shrew (*Crocidura russula*, Duarte *et al*. 2003). Inbreeding is common in both species and individuals in either one do not suffer any fitness-related consequences when they do inbreed. However, in this study, although there is evidence that collared pikas have low mean dispersal distances, there was no evidence that they commonly inbreed (see also Chapter 3). F_{IS} values did not differ significantly from those expected from a randomly breeding population in any year except for 2008. Even so, the F_{IS} value seen was low ($F_{IS} = 0.077$, Table 4.1).

Analyses of the genetic structure of two other small mammal species have also demonstrated that restricted dispersal, even if it is seen in both sexes, does not necessarily lead to high levels of inbreeding. Banner-tailed kangaroo rats and Pacific jumping mice (*Zapus trinotatus*) are both characterized by low mean dispersal distances, and there is no evidence for widespread inbreeding in either species (Winter and Waser 2003; Vignieri 2007). Banner-tailed kangaroo rats show no signs of inbreeding because they tend not to mate with their closest neighbours (Winter and Waser 2003), although collared pikas do not appear to reduce their chances of mating with a close relative by copulating with animals that are not their nearest neighbours (Chapter 3). In Pacific jumping mice, cryptic components of dispersal serve to reduce the likelihood of inbreeding, as males tend to disperse farther than females and the breeding system is promiscuous (as it is in collared pikas, Chapter 3), which reduces the amount of inbreeding that occurs (Vignieri 2007).

Density and Genetic Structure

Since American pikas tend to colonize the first vacant territory they come across (Smith 1987), I predicted that there would be a higher degree of genetic structuring in the population during the years with the lowest densities. However, in the two years with the lowest pika densities, there was no evidence of fine-scale genetic structure, as determined by either a Mantel test or spatial autocorrelation analysis (Table 4.2, Fig. 4.3). These results contrast with other studies that have examined the genetic structure of mammal populations over several years. For example, in both red deer (*Cervus elaphus*) and banner-tailed kangaroo rats, low population densities are associated with higher levels of genetic structuring (Nussey *et al.* 2005; Busch *et al.* 2009). However, increased levels of genetic structuring can also be associated with intermediate densities, as it is in woodrats (*Neotoma* spp., McEachern *et al*. 2007). Intermediate densities are associated with increased levels of genetic structuring in woodrats because low or high densities do not facilitate the formation of kin-based clusters while intermediate densities do (McEachern *et al.* 2007).

While there was no genetic structuring seen in the two years with the lowest pika densities, there was no clear correlation between the degree of genetic structuring in the population and the population's density. In 2000 and 2003, there were only 29 and 32 collared pikas genotyped, respectively, and there may not have been sufficient statistical power to detect any genetic structuring at these low densities. The low number of animals genotyped in these two years was unavoidable, as every pika in the population (as of the end of August) was sampled during those two years (although a small number of immigrants may have arrived in September after we had left the study site). The lack of genetic structure in 2000 and 2003 may also have been because the pikas would have been able to disperse farther during these low density years, as their movements would have been less restricted by the presence of other pikas, who tend to chase intruders.

Evidence for a Population Bottleneck

Since I had genetic samples from most animals in this population from before, during, and after a population decline, I was interested in determining if the population showed a genetic signature of having gone through a population bottleneck. The pika population declined sharply in 2000, as very few animals (n = 6) trapped in 1999 survived the winter. The population increased during 2001 and 2002, and it then underwent another decline in 2003. Overwinter survival in the population was very poor from 2002-2003, as only 10 pikas survived the winter. However, the percentage of animals that survived from 2002-2003 wasn’t as low as it was in 2000 (8% overwinter survival from 1999-2000, and 18% overwinter survival from 2002-2003). After 2003, the population increased substantially and stayed relatively high for the remainder of the study period (Fig. 4.2).

The observed heterozygosities varied from a low of 0.56 in 2006 to a high of 0.65 in 2008 and they did not decline in response to the population decline. However, the mean number of alleles per locus in the population in 1999 was 5.87 and it declined to 4.93 in 2000 (Table 4.1). However, this decline in allele number was temporary, as the mean allele number per locus increased in 2001 to 5.87. It then declined in 2003 to 5.47, but again increased steadily until 2008, where it reached 6.75, which is higher than it was at the start of the study (Table 4.1).

I used three tests (Wilcoxon signed-rank test, sign test, mode shift test) as implemented in the program “Bottleneck” (Piry *et al.* 1999) to determine if the collared pika population displayed any genetic signatures of having undergone a bottleneck. None of the tests used indicated that the population had undergone a recent bottleneck (Table 4.3), although there was a drop in the average number of alleles per locus from 1999 to 2000 (Table 4.1).

There are two ways that the allele number in a population can increase: mutation and migration. The mutation rate of the loci used here is unknown; however, given the high mutation rate of microsatellites in general (*e.g.* 0.0081 mutants/generation/locus for microsatellites in banner-tailed kangaroo rats, Busch *et al.* 2007), some of the increase in allele number may have been due to mutations. Additionally, the population is not completely isolated from others, so dispersal has likely played a role in maintaining the genetic diversity of this population. Other populations of collared pikas are situated about one kilometer north, south and east from the study site, and these could be sources of migrants (Franken 2002).

The level of fine-scale genetic structure seen in this population indicates that, during most years, collared pikas typically disperse only a few hundred meters from their natal den, although they are capable of dispersing 2 km (Chapter 3). However, even a very small amount of migration can restore the genetic diversity of a bottlenecked population. For example, an insular population of song sparrows (*Melospiza melodia*) recovered lost genetic diversity 2-3 years after a severe bottleneck due to the arrival of three immigrants (Keller *et al.* 2001). That level of immigration would have been impossible to detect had not every bird on the island been marked (Keller *et al.* 2001). Additionally, a severely bottlenecked, inbred wolf population in western Scandinavia was genetically “rescued” by a single immigrant from the east (Vilà *et al.* 2003). At least 17 collared pikas from other populations had immigrated into the study population (Fig. 4.4), although this number represents the minimum number of immigrants, as immigrants from populations genetically similar to the study population would not have been identified as immigrants with the methodology used. At least six of these migrants produced offspring, so there is gene flow into the study population.

Genetic signatures of bottlenecks can be extremely difficult to detect in populations of lagomorphs or rodents because they are rarely isolated enough to completely prevent migration among populations. In a study similar to this one, a population of banner-tailed kangaroo rats also showed none of the genetic signatures of a bottleneck, despite having been through one during the duration of the study (Busch *et al.* 2007). Mark-recapture studies of the same kangaroo rat population showed that they have limited dispersal ranges and that >80% of individuals remained within 100 m of their birthplace (Waser *et al.* 2006). Even so, migration was the most likely explanation for the failure of several methods to detect the allele frequency distortions typically associated with a bottleneck (Busch *et al.* 2007). Although mark-recapture analyses indicated that migration among populations of kangaroo rats was unlikely, genetic parentage analyses documented cases of precapture dispersal in juveniles (Waser *et al.* 2006), showing that a sufficient number of animals disperse to prevent the erosion of genetic diversity despite population declines.

Migration among populations, in addition to genetic drift, is also why cyclic rodent populations often maintain their density despite undergoing periodic low phases. Berthier *et al*. (2006) found that water vole populations (*Arvicola terrestris*) maintain a high level of genetic diversity despite experiencing periodic low numbers, due to two characteristics of their population cycle. First, during the low phase of the population cycle, there is a loss of genetic diversity in each deme, and an increase in genetic differentiation among demes, because genetic drift preserves different alleles in deme. Secondly, during the high density phase, there will be migration among the genetically differentiated demes, restoring the genetic diversity each deme lost during the low-density phase. This can result in genetic differentiation of a population across time (Charnov and Finerty 1980). This occurred in the collared pika population studied here, as there was a significant level of differentiation in the population across years (Table 4.4).

Considering the results from this study in conjunction with others that have looked at the genetic diversity of fluctuating mammal populations through time, it is apparent that even where mark-recapture studies or genetic evidence indicate that a species tends to be have very low mean dispersal distances, there may still be sufficient migration among populations to prevent inbreeding or a loss of genetic diversity after population declines. A limited amount of mark-recapture data (Franken 2002) and examinations of fine-scale genetic structure (this study) indicate that collared pikas usually disperse < 1 km from their natal den. However, there was sufficient migration into the population to quickly restore lost genetic diversity (in the form of lost rare alleles) in the population. Since rare long-distance dispersal events can be difficult to detect, results from single-sample methods used to detect bottlenecks in populations with a lack of historical demographic data should be interpreted cautiously. Only a small number of migrants are needed (provided they reproduce) to prevent a loss of genetic diversity in a population, even after it has suffered a decline. Like many other fluctuating populations, this collared pika population appears resilient to losses of genetic diversity over time.

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Figures and Tables

Fig. 4.1: A diagram of the study site located in the Ruby Ranges in the southwest Yukon, Canada. The gray areas represent talus patches, and the black represents the stream. The matrix in between the talus patches consists of alpine meadow, but only the vegetation immediately surrounding the patches is used by pikas. Adapted from Franken (2002).

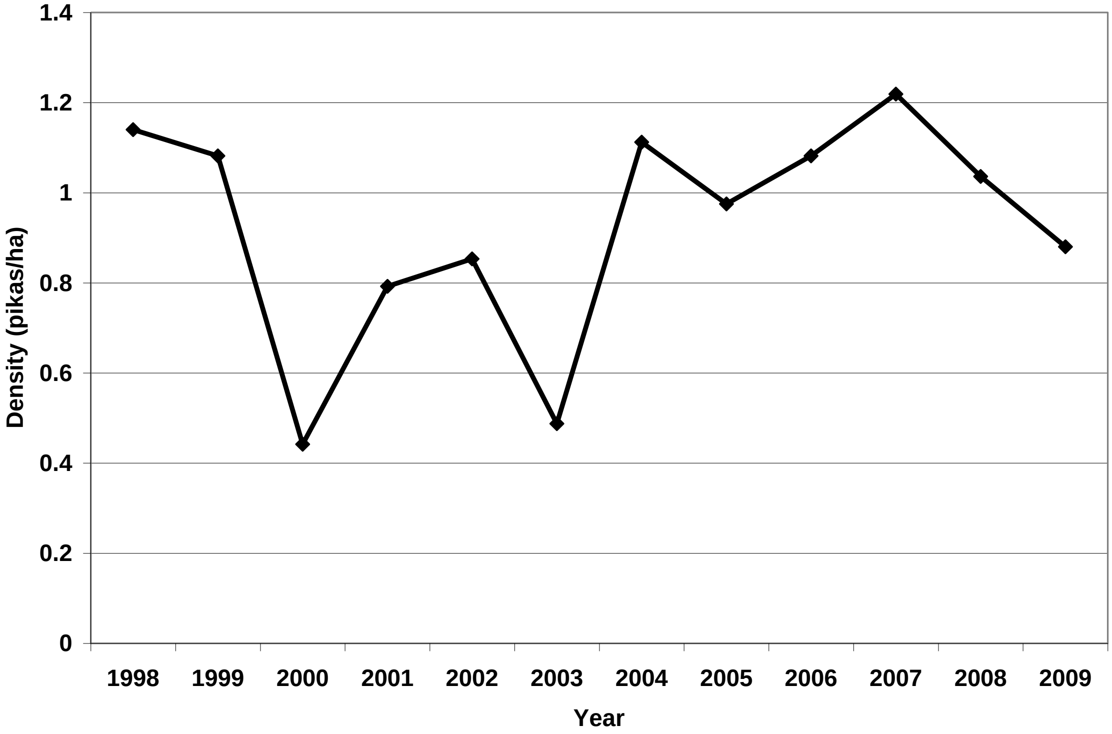
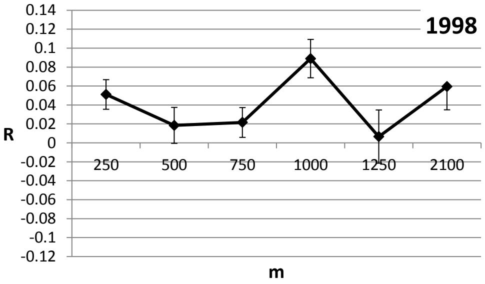
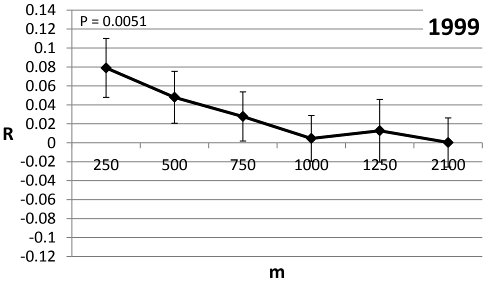
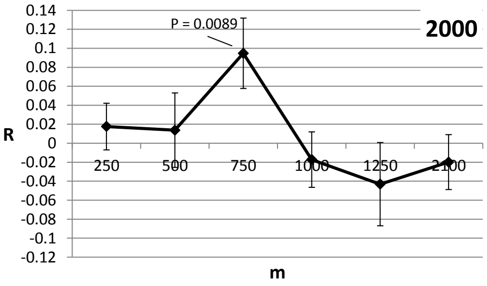


Fig. 4.2: The density at the end of August for a collared pika population at a four square kilometer valley in the Ruby Ranges, Yukon Territory, from 1998-2009. Values are based on a complete enumeration census so there are no associated error bars.


1998
R
m
0.14 0.12 0.1 0.08 0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08 -0.1 -0.12
250 500 750 1000 1250 2100


1999
P = 0.0051
R
m
0.14 0.12 0.1 0.08 0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08 -0.1 -0.12
250 500 750 1000 1250 2100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R
m
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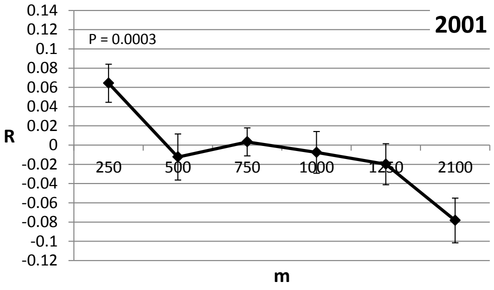
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R
m
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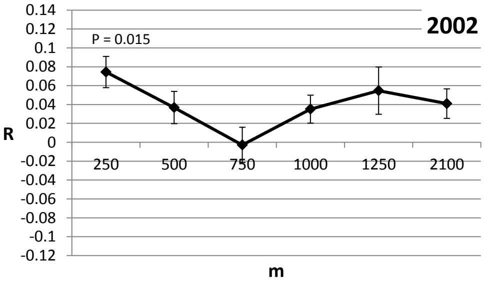
250 500 750 1000 1250 2100


2002
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R
m
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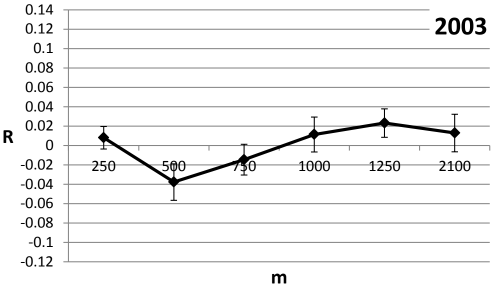
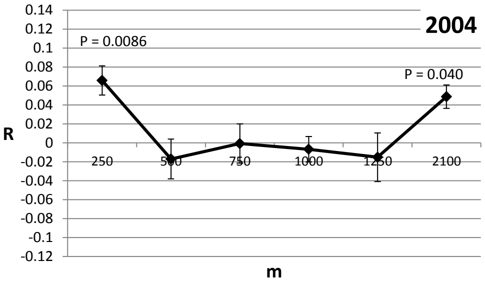
250 500 750 1000 1250 2100


2003
R
m
0.14 0.12 0.1 0.08 0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08 -0.1 -0.12
250 500 750 1000 1250 2100


2004
P = 0.0086
P = 0.040
R
m
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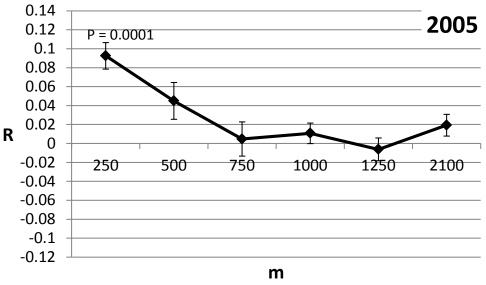
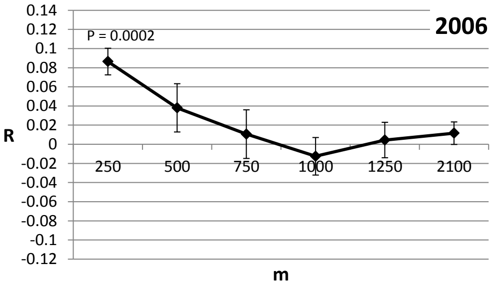
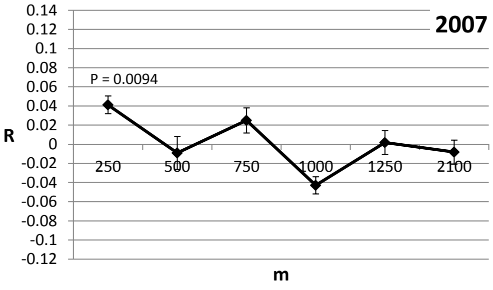
250 500 750 1000 1250 2100


2005
P = 0.0001
R
m
0.14 0.12 0.1 0.08 0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08 -0.1 -0.12
250 500 750 1000 1250 2100


2006
P = 0.0002
R
m
0.14 0.12 0.1 0.08 0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08 -0.1 -0.12
250 500 750 1000 1250 2100


2007
P = 0.0094
R
m
0.14 0.12 0.1 0.08 0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08 -0.1 -0.12
250 500 750 1000 1250 2100

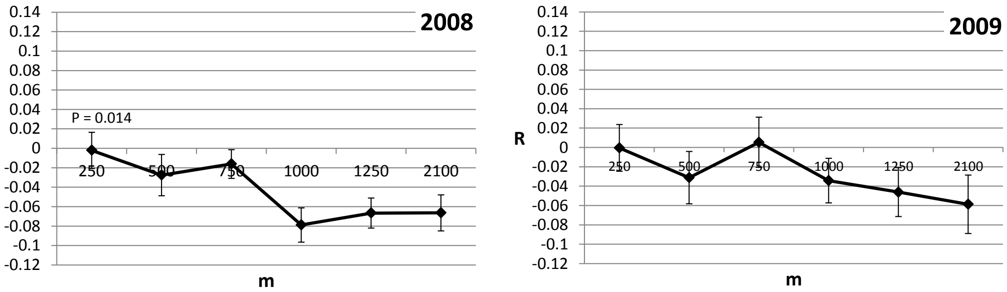


Fig. 4.3 Results of spatial autocorrelation analyses for a collared pika population. Separate analysis was conducted for each year from 1998 to 2009. Error bars indicate standard error as determined by a jackknife procedure. *R* = relationship coefficient as measured by Wang (2002). Statistical significance was determined by a permutation test with 20 000 permutations. Where $P < 0.05$ (one-tailed test), the exact *P*-value is noted on the figure.

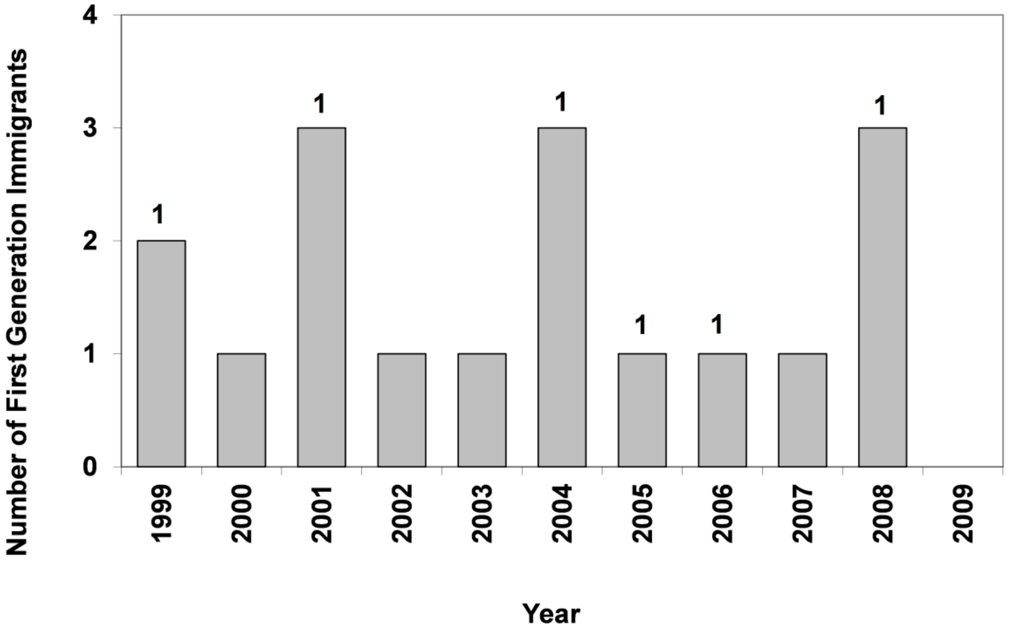


Fig. 4.4: The number of first generation migrants into a collared pika population from 1999-2009, as detected using the L_{HOME} statistic from the program GeneClass2 (Piry *et al*. 2004). Individuals with a $P \leq 0.01$ of belonging to the home population were considered to be migrants. The numbers above the bars indicate the number of migrants with detected offspring, as determined by a parentage analysis.

Table 4.1: Population size, diversity indices, and F-statistics of a collared pika population over ten years based on fifteen microsatellite loci. H_e = expected heterozygosity, H_o = observed heterozygosity. The significance of each global F statistic is based on 20 000 permutations and a one-tail test (obs>exp). One asterisk indicates that $0.01 < P < 0.05$ and two asterisks indicates that $P < 0.01$. N = North subpopulation, E = East subpopulation, W = West subpopulation. For pairwise F_{ST}, N/A indicates that the value was not calculated because the West subpopulation was extinct or contained only 4 individuals.

	1998	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
Population Parameters												
Total (August census)	75	71	29	52	56	32	73	64	71	80	68	58
Genetic Diversity												
Mean number of alleles	5.87	5.87	4.93	5.87	5.53	5.47	5.87	6.07	6.13	6.27	6.75	6.21
H_e	0.61	0.62	0.60	0.64	0.60	0.61	0.61	0.60	0.59	0.63	0.60	6.30
H_o	0.61	0.63	0.61	0.64	0.61	0.58	0.60	0.60	0.56	0.61	0.65	6.30
Global F Statistics												
F_{IS}	0.018	-0.035	-0.039	-0.016	-0.02	0.033	0.013	-0.0097	0.037	-0.0028	0.077**	0.0061
F_{ST} (global)	0.0028	0.017*	0.016	0.021*	0.011	0.028*	0.019*	0.022**	0.015*	0.020**	0.017*	0.025*
F_{IT}	0.021	-0.017	-0.026	0.0053	-0.0084	0.060*	0.032	0.013	0.051*	0.018	0.092**	0.031
Pairwise F_{ST}												
F_{ST} (pairwise, N & W)	0.010	0.025	N/A	0.041	0.0070	N/A	0.018	0.023	0.019	0.019	0.016	0.038
F_{ST} (pairwise, N & E)	-0.0044	0.013	0.016	0.019	0.017	0.028	0.017	0.022	0.021	0.021	0.027	0.024
F_{ST} (pairwise, W & E)	0.025	0.0084	N/A	0.032	0.014	N/A	0.041	0.022	0.0062	0.0062	-0.0055	-0.0026

Table 4.2: Results of twelve Mantel tests measuring the degree of correlation between the log pairwise geographic distances and pairwise relationship coefficients (as measured by Wang, 2002) of collared pikas from a 4 km^2 valley. Separate tests were done for each year of data. The P-values are for one-tailed tests. R = correlation coefficient.

Year	**P-value**	**R**	**Slope**
1998	0.53	0.0049	0.0015
1999	0.0099	0.098	-0.025
2000	0.068	0.071	-0.016
2001	0.00020	0.17	-0.044
2002	0.12	0.067	-0.011
2003	0.63	0.014	0.0048
2004	0.19	0.051	-0.0091
2005	0.00030	0.087	-0.025
2006	0.011	0.069	-0.027
2007	0.0083	0.066	-0.020
2008	0.0012	0.11	-0.032
2009	0.011	0.070	-0.022

Table 4.3: Results from three bottleneck detection methods (Wilcoxon signed-rank test, sign test, and mode shift test) used to determine if a collared pika population that had undergone a decline in 2000 and 2003 showed a genetic signature of having undergone a population bottleneck. The tests were implemented using the program BOTTLENECK (Piry *et al.* 1999). H_{def} = Heterozygote deficiency. H_{exc} = Heterozygote excess.

Year	**P (one tail), Wilcoxon signed-rank test**	**Sign Test**	**Expected No. of Loci with H_{exc}**	**H_{def}:H_{exc}**	**Evidence for Mode Shift**
1998	0.98	0.047	8.70	10:5	No
1999	0.64	0.43	8.82	7:8	No
2000	0.28	0.45	8.73	7:8	No
2001	0.72	0.12	8.78	9:6	No
2002	0.72	0.24	8.80	8:7	No
2003	0.74	0.25	8.77	8:7	No
2004	0.81	0.13	8.68	9:6	No
2005	0.90	0.13	8.68	9:6	No
2006	0.98	0.048	8.69	10:5	No
2007	0.91	0.045	8.75	10:5	No
2008	0.64	0.26	8.72	8:7	No
2009	0.90	0.11	8.87	9:6	No

Table 4.4: Top diagonal: Pairwise F_{ST} values between collared pika populations from different years, based on fifteen microsatellite loci. Bottom diagonal: The significance of each F_{ST}, as determined by a permutation test using 20 000 permutations. Non-significant results (at P<0.05) are highlighted in grey.

	1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
1999		>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001
2000	0.026		>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001
2001	0.013	0.016		0.4537	0.0248	>0.001	0.0011	>0.001	0.0018	>0.001	>0.001
2002	0.019	0.034	0		0.3776	>0.001	0.0001	>0.001	0.0001	>0.001	>0.001
2003	0.020	0.038	0.0060	0.0006		0.1078	0.0002	>0.001	0.0052	>0.001	>0.001
2004	0.032	0.040	0.012	0.0131	0.0034		0.0002	>0.001	0.0001	>0.001	>0.001
2005	0.013	0.031	0.0070	0.0177	0.0132	0.0094		0.4477	0.3669	>0.001	>0.001
2006	0.020	0.041	0.014	0.0304	0.0265	0.0218	-0.003		0.0304	>0.001	>0.001
2007	0.011	0.032	0.0064	0.0142	0.0068	0.0099	0.0004	0.0034		>0.001	>0.001
2008	0.02	0.040	0.0156	0.0179	0.0151	0.0212	0.0199	0.0290	0.0069		>0.001
2009	0.054	0.087	0.0913	0.1012	0.0819	0.0984	0.0805	0.0855	0.0604	0.0607	

Chapter Five

An Updated Phylogeny of the Genus *Ochotona*

Introduction

Pikas (*Ochotona*, Ochotonidae) are small, short-eared lagomorphs that inhabit northern and central Asia, southeastern Europe, and the mountains of northwestern North America. The earliest fossils from the family (*Sinolagomys, Bohlinotona*) are from central Asia, where the group likely has its origins (McKenna and Bell 1997; Lopez-Martinez 2008). Today, twenty-nine of the thirty-one recognized species occur in Eurasia, with only two occurring in North America (Hoffmann and Smith 2005; Table 1.1, Table 5.1). However, the genus was much more diverse during the Miocene when multiple genera were found throughout Europe, the Middle East, Asia, North America, and Africa (Cooke 1968; Tchernov *et al.* 1987; Erbajeva 1988; McKenna and Bell 1997; Lopez-Martinez 2008). At least twenty-five fossil ochotonid genera exist; however, all were extinct by the Pliocene, with the exception of *Prolagus*, which persisted in Corsica and Sardinia until the 1700s (Lopez-Martinez 2008).

The taxonomy of the genus *Ochotona* has been a difficult subject due to the lack of morphological diversity in the genus. Generally, the number of species recognized has increased through time; for example, Corbet (1978) recognized 13 species, Smith *et al.* (1990) recognized 23, and Hoffmann and Smith (2005) recognized 31 (Table 5.1)

The increase in the number of *Ochotona* species recognized is due both to the discovery of new forms in remote areas and the elevation of several subspecies to species status. For instance, the Ili Pika (*O. iliensis*), which has a restricted range in the Tien Shan Mountains, Xinjiang, China, was discovered and described only in 1986 (Li and Ma, 1986). The recently recognized silver pika (*O. argentata*) has been included in past treatments as a subspecies of both *O. alpina* and *O. pallasi* (as *O. p. helanshanensis*) (Smith *et al.* 1990) but it has recently been treated as a separate species based on karyological, morphological and acoustic data (Formozov *et al.* 2004). Several other species recognized by Hoffmann and Smith (2005), including *O. cansus, O. erythrotis, O. forresti, O. gaoligongensis, O. himalayana, O. hoffmanni, O. huangensis, O. muliensis, O. nubrica,* and *O. turuchanensis* were formerly included as subspecies in previous treatments.

Subgeneric classification schemes for the genus have also varied among authors. Ellerman and Morrison-Scott (1951) recognized 12 Asian species, and notes that they could be divided into two groups of possible subgeneric value (*Pika* and *Ochotona*) based on whether or not the incisive and palatal foramina are completely or practically separate (*Pika*) or form a single foramen (*Ochotona*) (Table 5.1). Corbet (1978) recognized 14 species in the genus (12 in Asia, plus two in North America), but commented that while the division of the palatal foramina is a useful character for separating species, there were no grounds for recognizing subgenera in the genus.

The phylogeny of the genus *Ochotona* has recently been examined using mtDNA sequences, and these studies have clarified some outstanding issues in pika systematics and have left others still open to investigation. Yu *et al.* (2000) examined the molecular systematics of the genus using sequences of mitochondrial cytochrome *b* (cyt*b*) and NADH dehydrogenase subunit 4 (ND4) genes from nineteen pika species. Rather than finding two major clades, three were found, which Yu *et al.* (2000) termed the Shrub-Steppe Group, the Northern Group and the Mountain Group (see Table. 5.1). The Shrub-Steppe Group included species that have been placed in the subgenus *Ochotona* (Ellerman and Morrison-

Scott 1951; Yu *et al.* 1992) and the Mountain Group included species from the subgenus *Pika* (Yu *et al.* 2000). However, the Shrub-Steppe Group contained members of both subgenera (Yu *et al.* 2000). Yu *et al.* (2000) did not suggest a subgeneric classification for *Ochotona* although Hoffmann and Smith (2005) placed the members of the “Mountain Group” in the subgenus *Conothoa. O. huangensis* was not placed consistently in any of the three clades (Yu *et al.* 2000), although Hoffmann and Smith (2005) placed it in the subgenus *Ochotona.*

Niu *et al*. (2004) examined the phylogeny of the genus using partial mitochondrial cytochrome *b* sequences (~400 bp) from thirty-five individuals from twenty-seven species. Three major clades were recovered: the Qinghai-Tibet Plateau Group, the Surrounding Qinghai-Tibet Plateau Group, and the Northern Group (Table 5.1). However, neither *O. pusilla* nor *O. huangensis* placed within either of these groups, and *O. pusilla* appeared to be the sister group of the remaining members of the genus (Niu *et al.* 2004). *O. huangensis* placed as the sister group of the Qinghai-Tibet Plateau group, although only with very low statistical support (Niu *et al.* 2004). Additionally, three species in the Qinghai-Tibet Plateau Group, *O. cansus O. curzoniae,* and *O. thibetana,* were not monophyletic, indicating that further work on species delimitation in that group is required.

The subgenus *Pika* has also been taxonomically difficult. Within this subgenus, Corbet (1978) placed *O. collaris, O. princeps* and *O. hyperborea* within *O. alpina*, although Weston (1982), using morphological data, demonstrated that the two North American taxa (*O. collaris* and *O. princeps*) are morphologically distinct from either two Asian taxa. Additionally, *O. alpina* and *O. hyperborea* are morphologically distinct from each other (Weston 1982), a result consistent with karyological data (Vorontsov and Ivanitskaya 1973). Lissovsky (2003) found three distinct species (based on skull measurements) within the *alpina-hyperborea* complex, including *O. alpina, O. hyperborea* and *O. turuchanensis*. Two other taxa were also postulated as distinct entities: *O. mantchurica* and *O. scorodumovi* (Lissovsky 2003). The former taxon is typically treated as a junior synonym of *O. alpina* and the latter is typically treated as a subspecies of *O. hyperborea.* Acoustic data, however, indicate that there are four distinct taxa in the *alpina-hyperborea* complex: *O. alpina, O. hyperborea, O. turuchanensis* and *O. scorodumovi* (Lissovsky 2005).

Lissovsky *et al.* (2007) further examined the phylogeny of the subgenus *Pika* using partial sequences of the cytochrome *b* and cytochrome oxidase subunit I genes. The results indicated that the two North American taxa (*O. collaris* and *O. princeps*) are monophyletic, a result also found by Lanier and Olsen (2009), who included multiple samples of *O. collaris, O. princeps* and *O. hyperborea* in their analyses. *O. scorodumovi*, typically treated as a subspecies of *O. alpina* (Smith *et al.* 1990), was also revealed to actually be a closer relative of *O. hyperborea*, although it may be the sister taxon of *O. hoffmanni* (Lissovsky *et al.* 2007). As both morphological and acoustic data also demonstrated the distinctiveness of *O. scorodumovi*, Lissovsky *et al.* (2007) argued that it should be considered a separate species but that the taxonomic composition and valid name await further study, as populations assigned to *O. mantchurica* may belong within *scorodumovi. O. argentata*, which has been placed as a subspecies of *O. alpina* (*e.g.* Smith *et al.* 1990) was found to be more closely related to *O. pallasi* (Lissovsky *et al.* 2007). The results also supported treating *O. turuchanensis* as a separate taxon, as all sequences from it formed a monophyletic group. The genetic distance between *O. turuchanensis* and *O. alpina* was also similar to the distances typically seen between *Ochotona* species (Lissovsky *et al.* 2007).

Despite the many recent advances made towards understanding the evolutionary history of *Ochotona*, some outstanding issues still remain. The taxonomic relationships among the major clades in the genus remain unclear, likely due to the very short internodes separating taxa on very long branches (see Lanier and Olsen 2009). Additionally, relationships recovered with high statistical support in some studies have not been recovered by others. For example, the sister-group relationship between *O. alpina* and *O. pallasi* found by Niu *et al.* (2004) was not found by Lanier and Olsen (2009), who instead found *O. alpina* to be more closely related to *O. hyperborea.* The placement of *O. pusilla* also requires further investigation. It appears to be the sister taxon to the rest of the genus, but this result was based on a limited amount of sequence and received only low statistical support (Niu *et al.* 2004).

The purpose of this study was to examine the phylogenetic relationships among species in the genus *Ochotona* by sequencing ND4 and *cyt*B for several pika specimens. These two genes have also been used by Yu *et al.* (2000) and Lanier and Olsen (2009) to infer relationships among pika species. The new sequences generated here were used to supplement those already available. Additionally, four additional mitochondrial genes were sequenced for a smaller number of species to infer the relationships among the three major *Ochotona* clades recovered by both Yu *et al.* (2000) and Niu *et al.* (2004).

Materials and Methods

Taxa selection and DNA sequencing

NADH dehydrogenase subunit 4 (ND4) and cytochrome *b* (*cyt*B) sequences from Genbank used in this study are listed in Table 5.2. New sequences generated came from both *O. collaris* specimens collected in the field and from frozen, ethanol-preserved tissue from museum specimens (Table 5.3). ND4 and *cyt*B were sequenced for three specimens of *O. collaris*, one of *O. thibetana nangqenica,* one of *O. thibetana thibetana* and one of *O. curzoniae melanostoma*. ND4, NADH dehydrogenase subunit 2 (ND2), *cyt*B, cytochrome c oxidase subunit I (*COX*1), 12S rDNA, and 16S rDNA were also sequenced for *O. alpina*, *O. daurica, O. hyperborea, O. pallasi, O. rufescens,* and *O. thibetana.* The collection location of each pika whose DNA was used in this study (including sequences from Genbank) is shown in Fig. 5.1.

Genomic DNA was extracted using the Qiagen Blood and Tissue Kit (Qiagen Inc.), with a final elution volume of 100 µL. Primers used to amplify sequences were those from Luo *et al.* (2008) along with several designed from the mitochondrial genomes of *O. collaris*, *O. curzoniae*, and *O. princeps* (Genbank accession numbers NC003033, EF535828, NC005358, respectively) (Table 5.4).

PCRs were conducted with 30 µL reactions, which included 2.4 µL of DNA in a solution of 0.5 µM of each primer, 200 µM dNTPs, 1X PCR buffer and 2 mM $MgCl_2$. PCR reactions were run on an Eppendorf Mastercycler 96 with conditions as follows: 95 °C for 10 min; 32 cycles of 95 °C for 30 s, annealing at 50-53 °C for 30 s, and elongation at 72 °C for 2 min; and a final step at 72 °C for 10 min. Reactions were cleaned with a Qiagen PCR Purification kit, following the manufacturer`s directions, and they were quantified using a Nanodrop ND-1000 (Nanodrop Products, Thermo Fisher Scientific, Inc).

Sequencing reactions were set up using the BigDye® Terminator v. 3.1 cycle sequencing kit (Applied Biosystems by Life Technologies) using 1 µL of BigDye® solution, 3 µL of 2.5 X sequencing buffer, 75 ng of PCR reaction, 2.5 pmol of primer and water to 10 µL. Sequences were purified using a standard ethanol precipitation protocol and were then run on a 3730 DNA analyzer (Applied Biosystems by Life Technologies) and edited using the program Sequencer 4.10 (GeneCodes Corporation). Final sequence alignment was done by eye using the program BioEdit (Hall 1999).

Data Analysis

The program MEGA version 5 (Tamura *et al.* 2011) was used to carry out maximum-parsimony analyses. Two data sets were analyzed: one containing ND4 and *cyt*B sequences for 49 *Ochotona* specimens (Data Set One) and one containing 12S rDNA, 16S rDNA, *COX*1, ND4, ND2 and *cyt*B sequences for 9 *Ochotona* species (Data Set Two). Sequences from *Lepus europaeus* and *Oryctolagus cuniculus* (Genbank Accession Numbers NC004028 and NC001913) were used as outgroups. Analyses were carried out for each separate gene included in each data set, although only combined results will be presented and discussed here. All three codon positions were used for protein-coding data.

Maximum-parsimony trees were obtained using the Close-Neighbour-Interchange algorithm (Nei and Kumar 2000) with search level three and ten random addition search trees. Support for each phylogenetic relationship found was assessed using a bootstrap analysis with 500 replicates.

The program PhyML 3.0 (Guindon *et al.* 2010) was used to infer the maximum likelihood tree for both data sets. A general-time-reversible (Tavaré 1986) plus gamma model of sequence evolution was used in the maximum-likelihood analysis for Data Sets One and Two. This model was selected based on the output from FindModel, which is a web-based implementation of the ModelTest script (Posada and Crandall 1998).

Branch support was assessed using a bootstrap analysis with 200 replicates. Trees were visualized using TreeView (Page 1996).

Results

Data Set One was 2523 bp long. The maximum-parsimony analysis found eleven most-parsimonious trees of length 4672 (Fig. 5.2). The MP trees had (considering only parsimony-informative sites) consistency indices of 0.35, retention indices of 0.70, and composite indices of 0.25.

O. pusilla and *O. huangensis* were the successive sister taxa of the remainder of the genus. Three main clades were also recovered, which will be referred to here as the *Conothoa, Pika,* and *Ochotona* clades, corresponding to subgenus names used by Hoffmann and Smith (2005). The *Pika* clade comprised *O. alpina, O. collaris, O. mantchurica, O. hyperborea, O. pallasi, O. princeps, O. scorodumovi* and *O. turuchanensis.* The *Conothoa* clade comprised *O. erythrotis, O. forresti, O. himalayana, O. koslowi, O. ladacensis, O. macrotis, O. roylei,* and *O. rufescens,* and the *Ochotona* clade included *O. annectens, O. cansus, O. curzoniae, O. daurica, O. nubrica, O. thibetana,* and *O. thomasi.* These largely correspond to the subgenera grouping used by Hoffmann and Smith (2005) (see Table 5.1) with the exception that here, *O. rufescens* places in the *Conothoa* clade, while Hoffmann and Smith (2005) placed it in the genus *Ochotona.* The relationship among these three clades was not well resolved by the maximum-parsimony analysis.

Three species from the *Ochotona* group with multiple specimens included were not monophyletic: *O. cansus O. curzoniae,* and *O. thibetana* (Fig. 5.2). *O. annectens* grouped within *O. cansus* with high bootstrap support and *O. curzoniae melanostoma* appears to be more closely related to *O. thibetana* than to other *O. curzoniae. O. thomasi* placed within the *Ochotona* clade as the sister taxon to the rest of the group with high bootstrap support.

Few relationships within the *Conothoa* group were resolved with high bootstrap support in the maximum parsimony analysis (Fig. 5.2). However, the sister-group relationship between *O. koslowi* and *O. ladacensis* and between *O. macrotis* and *O. roylei* were supported by bootstrap values of 99 and 97, respectively.

Within the *Pika* clade, two main monophyletic groups were recovered with high support values: a North American clade (composed of *O. collaris* and *O. princeps*) and a northern Asian clade (composed of *O. alpina, O. hyperborea, O. mantchurica, O. pallasi, O. scorodumovi* and *O. turuchanensis*). Within the northern Asia group, *O. pallasi* was the sister group to a clade composed of *O. alpina, O. hyperborea, O. mantchurica*, *O. scorodumovi*, and *O. turuchanensis* (Fig. 5.2).

The maximum-likelihood (ML) tree found from an analysis of Data Set One (Fig. 5.3) had a log likelihood score of -23565. It was largely congruent with the MP tree, although *O. pusilla* and *O. huangensis* were the successive sister taxa to the *Ochotona* group, rather than the rest of the genus. However, this relationship was very poorly supported by the bootstrap values. *O. erythrotis* was the sister taxon to the remainder of the genus, but again, this relationship was not well supported.

Data Set Two was 7690 bp long. One MP tree of length 5794 was found (Fig. 5.4a). Considering only parsimony-informative sites, the tree had a consistency index of 0.59, a retention index of 0.54 and a composite index of 0.32.

O. rufescens (*Conothoa* group) was the sister taxon to the remaining species included in the analysis. Within the *Ochotona* group, *O. curzoniae* was the sister group to *O. daurica* + *O. thibetana* and within the

Pika group, the two North American species formed a clade that was sister to a clade composed of *O. pallasi, O. alpina* and *O. hyperborea. O. alpina* and *O. hyperborea* were sister groups. The ML tree (Fig. 5.4b), which had a log-likelihood score of -34396, was similar to the MP tree, although the ML tree placed *O. rufescens* as the sister group to the *Pika* clade, albeit with very low bootstrap support.

Discussion

O. pusilla has previously included forms now considered to belong to *O. nubrica, O. forresti,* and *O. thibetana* (Ellerman and Morrison-Scott 1951; Hoffmann and Smith 2005). However, Erbajeva (1988) (as cited in Sokolov *et al.* 2009) concluded from a study on tooth and skull characteristics from both extinct and extant taxa that *O. pusilla* is distinct from other *Ochotona* and suggested that it be placed in its own subgenus, *Lagotona*. Niu *et al.* (2004), who analyzed partial *cyt*B sequences (402 bp) for several *Ochotona* taxa, found that *O. pusilla* is the sister taxon to the remainder of the group. Here, this result was confirmed through the use of a complete *cyt*B sequence in the MP analysis (Fig. 5.2), although the ML analysis placed *O. pusilla* within the *Ochotona* group (Fig. 5.3).

Results from morphological and molecular phylogenetic analyses that place *O. pusilla* at the base of the *Ochotona* phylogenetic tree are consistent with the fossil record of the genus. *O. pusilla* has an extensive fossil record extending to the late Pliocene (Erbajeva *et al.* 2001), although most modern *Ochotona* species did not appear until the Pleistocene. *O. pusilla* appears to have diverged approximately 2.8 MYA in central Asia (Niu *et al.* 2004) but it later became widespread in western Europe and Great Britain (Erbajeva *et al*. 2001; Fisher and Yalden 2004; Fostowicz-Frelik 2008), a region where no pika species occur today. *O. pusilla*'s range expansion into Europe was likely triggered by the climatic changes that occurred during the mid to late Pleistocene (Erbajeva *et al.* 2001). During that time, cold, dry steppes, to which *O. pusilla* are well-adapted, became widespread. *O. pusilla* are today most common in semi-desert environments (Sokolov *et al.* 2009).

O. huangensis may also have diverged early in the genus' evolutionary history, as it was shown by the MP analysis to be the sister group of the all other species in the genus, minus *O. pusilla* (Fig. 5.2). Other molecular studies have also uncovered this result (Yu *et al.* 2000; Niu *et al.* 2004). O*. huangensis* was formerly classified as a subspecies of *O. thibetana* (Ellerman and Morrison-Scott 1951; Smith *et al.* 1990), as the two species are morphologically similar and are widely sympatric (Smith 2008). However, Yu and Zheng (1992) (as cited by Smith 2008) suggested that the species is sufficiently distinct to be given species status. Results of molecular analyses of Niu *et al* (2004) and Yu *et al.* (2000) are congruent with this.

Subgenus Ochotona

Niu *et al.* (2004) and Yu *et al.* (2000) found that the steppe-dwelling pika species of the Qinghai-Tibet Plateau form a monophyletic group, and Hoffmann and Smith (2005) placed these in the subgenus *Ochotona.* The results of this study are largely congruent with this classification, with one exception. Hoffmann and Smith (2005) placed the Afghan pika (*O. rufescens*) in the subgenus *Ochotona*, although in no analyses here did *O. rufescens* group with this subgenus. Instead, it was resolved as belonging to the *Conothoa* group (Figs. 5.2, 5.3). Niu *et al.* (2004) found that it belonged to what they termed the "Surrounding Qinghai-Tibet Plateau Group." Indeed, unlike the other members of the subgenus *Ochotona* (which largely inhabit the Qinghai-Tibet Plateau), *O. rufescens* occurs in the mountains of Afghanistan, Pakistan, Armenia, Iran and southwestern Turkmenistan.

In the past, *O. daurica* and *O. curzoniae* have been treated as the same species (*e.g.* Ellerman and Morrison-Scott 1951), but the two taxa are clearly distinct species (Fig. 5.2, 5.3). However, this study again places the status of *O. curzoniae melanostoma* into question. *O. curzoniae melanostoma* was first described as a separate species (*O. melanostoma*), although most authors place it within *O. curzoniae* (Smith *et al.* 1990). Here, this subspecies of *O. curzoniae* is revealed to be potentially more closely related to *O. thibetana* than to other *O. curzoniae* (Fig. 5.2, 5.3). Such a result could be the result of a misidentification; however, I consider this unlikely given that the collector of the specimen used is the author of several works on pika taxonomy.

The status of the Gansu pika, *O. cansus*, has varied across treatments. Corbet (1978) and Honacki *et al.* (1982) placed it within *O. thibetana,* although Hoffmann and Smith (2005) list it as a separate species. Although *O. cansus* and *O. thibetana* are broadly sympatric (Smith 2008), they are morphologically distinct, and occupy different ecological niches (Hoffmann and Smith 2005). Here, *O. cansus* was resolved as the sister taxon to *O. curzoniae*, rather than being a close relative of *O. thibetana.* The form *O. annectens*, however, grouped within *O. cansus*, rendering *O. cansus* polyphyletic. Yu *et al.* (2000) and Niu *et al.* (2004) treated *O. annectens* as a distinct species, although Hoffmann and Smith (2005) treated it as a subspecies of *O. daurica*. The results of analyses presented here suggest that *O. annectens* is best treated as a subspecies of *O. cansus* (Figs. 5.2, 5.3).

Subgenus *Conothoa*

Within the subgenus *Conothoa*, *O. ladacensis* and *O. koslowi* form a well-supported clade, as do *O. roylei* and *O. macrotis*. However, the remainder of the relationships within this subgenus remain unresolved, likely because they are separated from each other by very short branches (Fig. 5.3). A larger amount of sequence will be needed to determine the phylogenetic relationships among the members of this genus.

Subgenus *Pika*

Relationships among the members of the subgenus *Pika* were generally well-resolved here (Figs. 5.2, 5.3). The analysis presented by Niu *et al.* (2004) indicated that *O. alpina* and *O. pallasi* are sister taxa, although in all analyses presented here (which used both more sequence and taxa) *O. alpina* was the sister species to *O. hyperborea* and its close relatives (*O. mantchurica* and *O. scorodumovi*) (Figs. 5.2, 5.3).

O. alpina appears to be non-monophyletic in Figs. 5.2 and 5.3; however, the taxon *O. alpina hoffmanni* is treated as a distinct species by Hoffmann and Smith (2005), based on morphological, acoustic and karyological evidence presented by Formozov and Baklushinskaya (1999). Treating *O. alpina hoffmanni* as a distinct species would leave *O. alpina* as a monophyletic taxon (Lissovsky *et al.* 2007), furthering the case for treating it as a distinct species. Additionally, the new sequence from *O. alpina hoffmanni* added here show that it is distinct from *O. alpina* and is more closely related to *O. turuchanensis. O. turuchanensis* was also only recently recognized as a distinct species (Lissovsky 2003; Lissovsky *et al.*2007).

Lissovsky *et al.* (2007) commented that *O. turuchanensis* appears to be a very young species that underwent a rapid divergence, as it has a wide geographic range and a very low level of sequence divergence, particularly compared to other *Ochotona* species with similarly broad ranges. Lanier and Olsen (2009) found that *O. collaris* also has a very low level of intraspecific genetic variation, particularly compared to *O. princeps* and *O. hyperborea*. In this study, I added additional *O. collaris* sequences to those presented by Lanier and Olsen (2009). These sequences also demonstrate a low level of divergence from other *O. collaris* sequences. The very low level of genetic variation in *O. collaris* suggests that it underwent a recent and rapid population expansion, although insufficient data exists to determine the origin and direction of this expansion.

O. argentata (*O. pallasi helanshanensis*) represents another recently described species. This taxon has been placed at times with *O. alpina* (Smith *et al.* 1990), although molecular evidence places it closer to *O. pallasi* (Lissovsky *et al.* 2007; Fig. 5.2, 5.3). Formozov *et al.* (2004) argued for its recognition as a distinct species based on morphological, acoustic and karyological evidence. It is likely a close relative of *O. pallasi*, rather than *O. alpina.*

Biogeography of Ochotona

The large number of cryptic species in *Ochotona* is likely a result of the geographic isolation and small sizes of many pika populations, two factors that can promote vicariance speciation. Many populations of alpine-dwelling pika species occur on mountain ranges that are separated from others by warmer, lowland areas pikas rarely cross. However, currently isolated populations may have been closer to others during the past (Smith *et al.* 1990). Pikas are well-adapted to cold regions, and during warm, interglacial periods in the Pleistocene, the zone in which pikas could inhabit decreased and pikas were pushed into higher elevation regions, which isolated formerly well-connected populations and allowed for population divergence (Smith *et al.* 1990). For example, during the warm altithermal period following the Wisconsin glaciation, *O. princeps* shifted its range upslope by an average of 300 m (Hafner 1994; Grayson 2005), which lead to the isolation of many populations and a corresponding high degree of genetic differentiation among them (Hafner 1994).

Yu *et al.* (2000) hypothesize that the uplifting of the Tibetan Plateau, which occurred in phases from 3.4 to 1.2 MYA, also promoted speciation in *Ochotona.* With this series of geological events came major environmental changes and the formation of numerous geological barriers that would prevent gene flow among lineages. This environmental and topographical diversification of the landscape could promote adaptation and differentiation among different lineages.

As is the case for much of the biota of the Northern Hemisphere, current phylogeographic patterns in many pika species also appear to have been shaped by the Pleistocene glaciations. For example, like *O. collaris* and *O. turuchanensis*, there is little sequence divergence among populations of *O. curzoniae* (Fig. 5.3; with the exception of the form *O. curzoniae melanostoma*), which is consistent with a recent population expansion. Ci *et al.* (2009) examined the patterns of genetic structure among populations of *O. curzoniae* in detail and also found that patterns of genetic diversity in this species were consisted with a recent, rapid population expansion.

Ci *et al.* (2009) offered the following interpretation of their results: During an extensive glacial period (0.50-0.17 MYA), much of the central and highest altitude areas of the Qinghai-Tibet Plateau (QTP) would have been covered in ice, which would have led to the extirpation of *O. curzoniae* populations there. During the subsequent interglacial period, which lasted from 0.17-0.03 MYA, populations from the peripheral areas of the QTP would have expanded and rapidly colonized areas in the central area of the plateau.

During the last glacial period, the arid conditions on the central region of the QTP would have restricted glacier growth. However, the humid conditions in areas surrounding the interior of the plateau would have allowed for glacier formation. The glaciers would have prevented gene flow between peripheral and interior QTP populations of *O. curzoniae* (Ci *et al.* 2009). Glaciers in the humid areas surrounding the plateau may also have led to the extirpation of many *Ochotona* populations from that area. The rapid expansion of other *Ochotona* lineages out of the arid region of the QTP (which would have acted as a glacial refugium) following the last glacial period may be responsible for the very short branches separating several clades in the subgenera *Ochotona* and *Conothoa*.

Directions for Further Study

Although many studies indicate that *O. pusilla* and *O. huangensis* are the successive sister groups to the remainder of the genus, this result should be considered preliminary until more data is examined. Only *cyt*B sequences are available for these two taxa and more sequence data should be generated for these two species to determine their correct phylogenetic position.

Further work on species delimitation in the genus *Ochotona* is also needed, as both this study and the study by Lanier and Olsen (2009) indicate that there are species in this subgenus that are polyphyletic.

Ideally, a combination of behavioural, morphological and genetic data should be used to examine species boundaries in this group. To date, the majority of sequence data used to infer phylogenetic relationships among pikas has been from the mitochondrial genome, which is maternally inherited. However, data from this single source is often insufficient in resolving the phylogenetic relationships among taxa with a complex evolutionary history (Ben Slimen *et al.* 2008). For example, there has been extensive mitochondrial introgression in the genus *Lepus*, so relationships among species in that genus are not accurately reflected in trees constructed from mitochondrial sequences alone (Ben Slimen *et al.* 2008). Thus, the next step in resolving the evolutionary history in the genus *Ochotona* would be to include additional data from both genomes.

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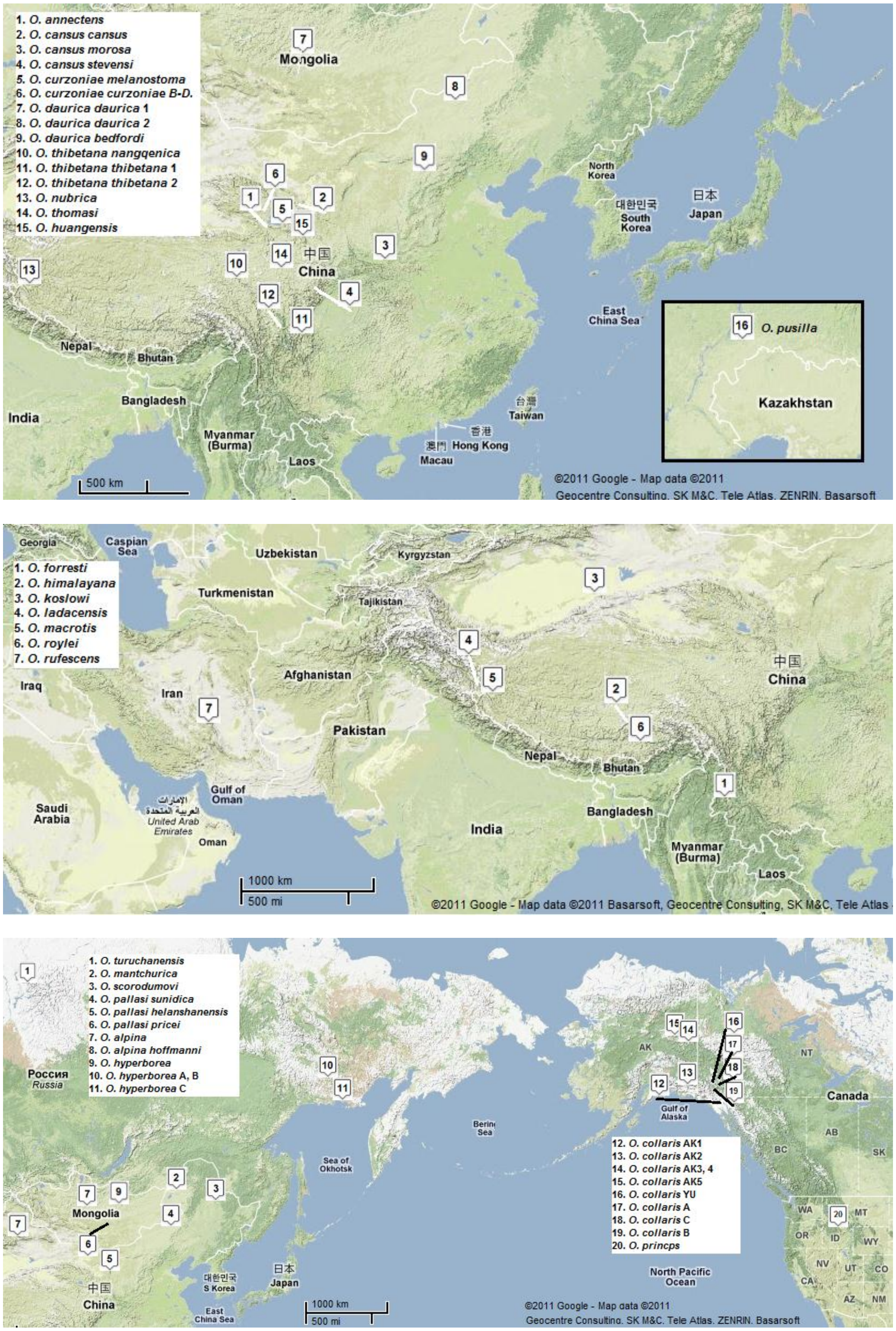

Fig. 5.1: Collection locations for the pikas used in this study.

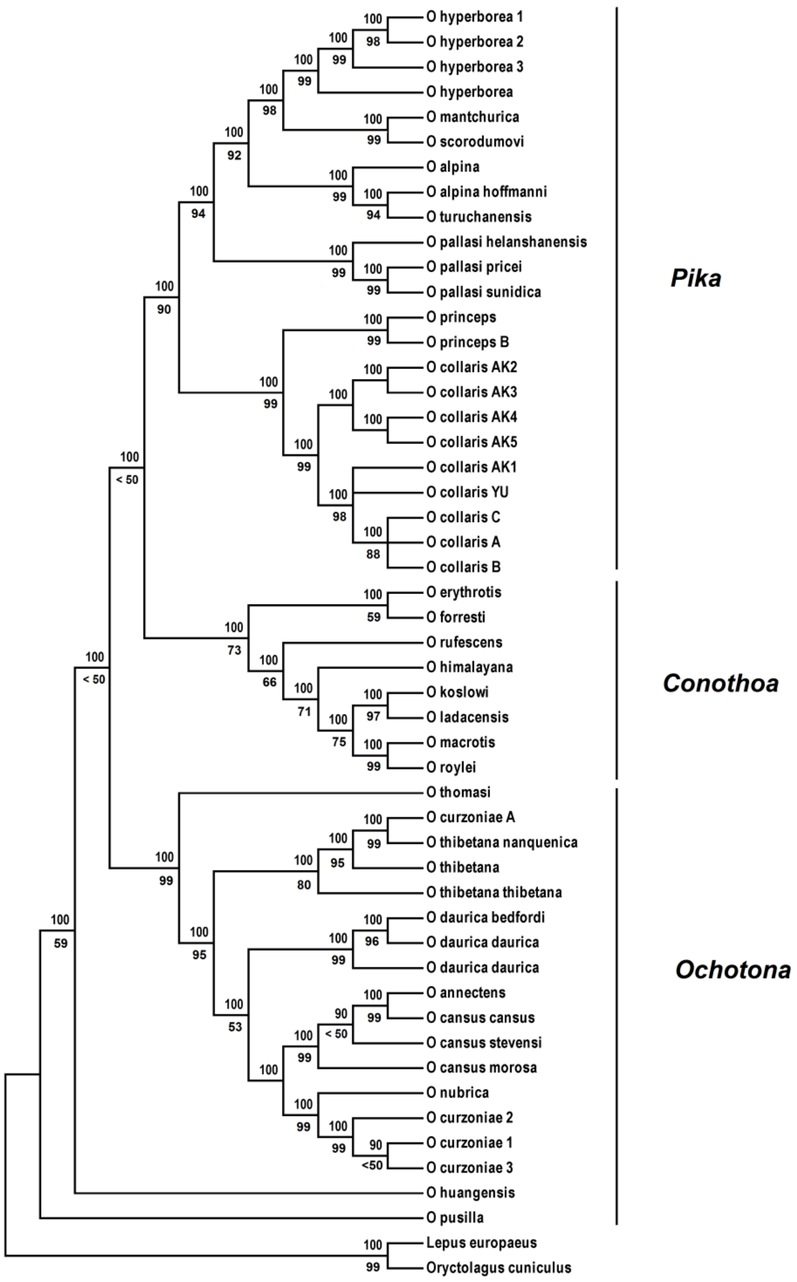

Fig. 5.2: A majority-rule consensus tree of eleven most-parsimonious (MP) trees from an analysis of ND4 and *cyt*B sequences from 49 *Ochotona* specimens and two outgroup taxa (*Lepus* and *Oryctolagus*). Values above the branches indicate the percentage of MP trees that contained the clade and values below the branches are bootstrap support values based on 500 replicates. Labels to the right indicate the subgeneric classification based on Hoffmann and Smith (2005).

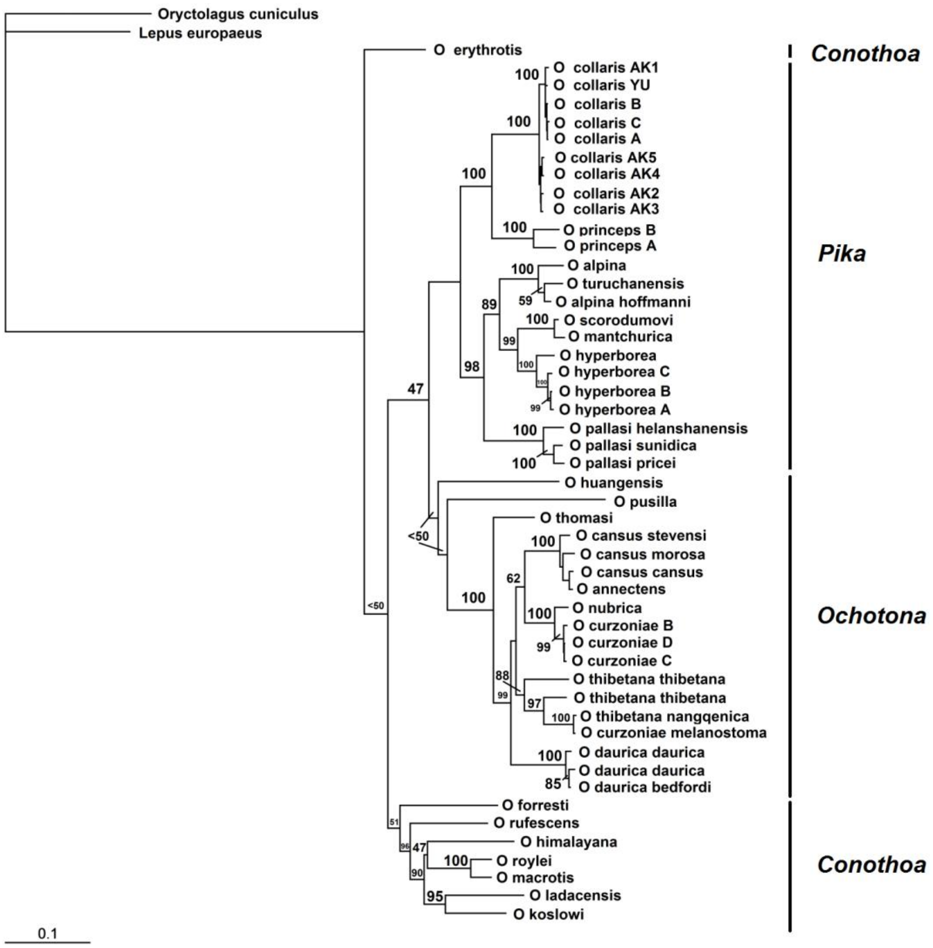

Fig. 5.3: A maximum-likelihood tree from an analysis of ND4 and *cyt*B genes from 49 *Ochotona* specimens. The values above the branches are bootstrap values based on 200 replicates. Labels to the right indicate the subgeneric classification based on Hoffmann and Smith (2005).

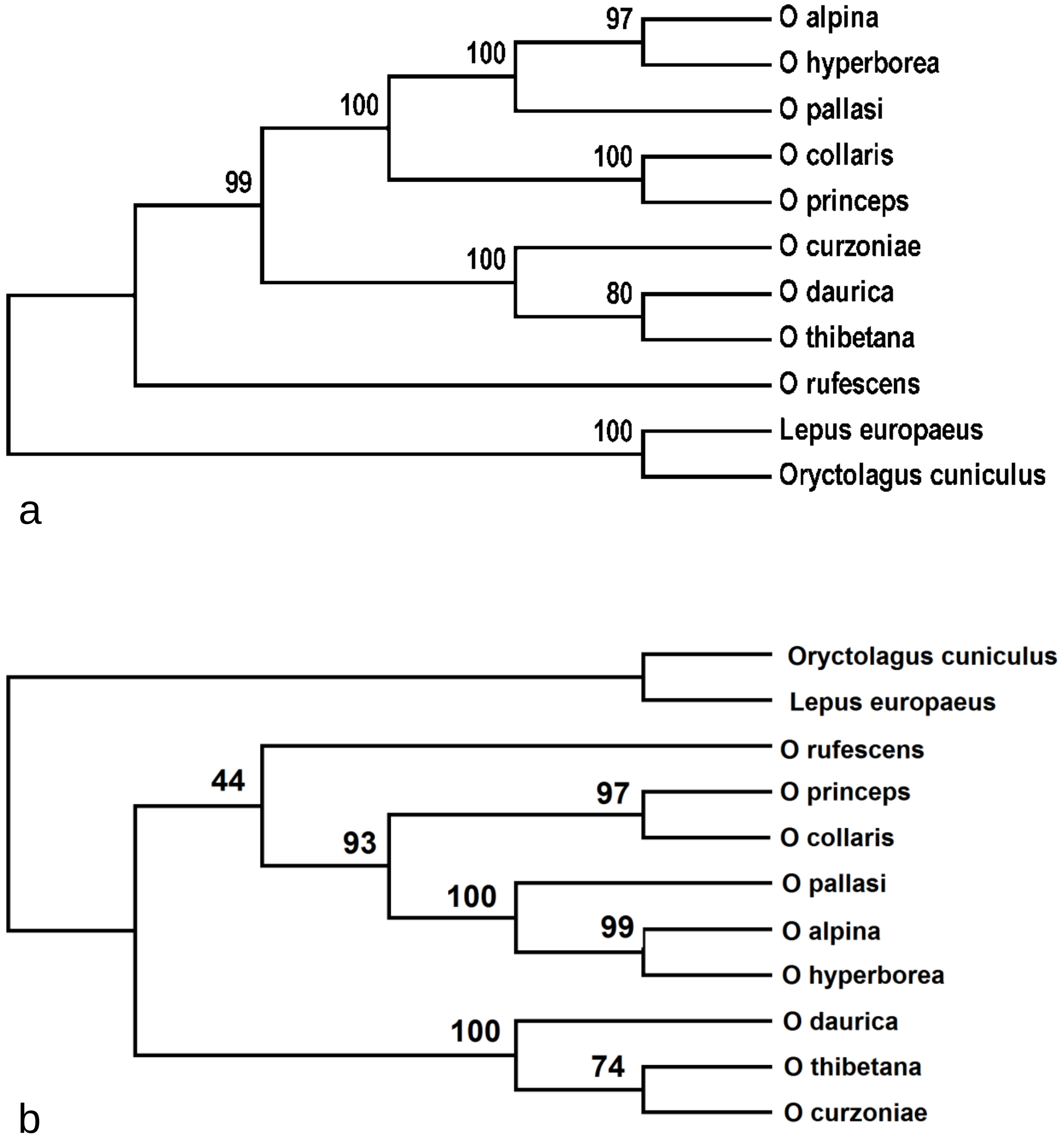

Fig. 5.4: The most-parsimonious (MP) tree (a) and maximum-likelihood (ML) tree (b) found from analyses of ND2, ND4, *COX*1, 12S rDNA, 16s rDNA and *cyt*B sequences from 9 *Ochotona* specimens and two outgroup taxa (*Lepus* and *Oryctolagus*). Values above the branches are bootstrap support values based on 500 (MP) or 200 (ML) replicates.

Table 5.1: Classification schemes for the genus *Ochotona*. Numbers in parentheses indicate number of subspecies recognized, where applicable.

Ellerman and Morrison-Scott 1951[1]	**Smith *et al*. 1990**	**Yu *et al.* 2000**[2]	**Niu *et al.* 2004**[2]	**Hoffmann and Smith 2005**
Subgenus *Ochotona*	*O. alpina* (7) *O. cansus* (4)	**Shrub-Steppe Group**	**Qinghai-Tibet Plateau Group**	***Conothoa***
	O. collaris			
O. daurica (7)	*O. curzoniae*	*O. annectens*	*O. annectens*	*O. brookei*
O. koslowi	*O. daurica* (5)	*O. cansus*	*O. cansus*	*O. erythrotis*
O. macrotis (2)	*O. erythrotis*	*O. curzoniae*	*O. curzoniae*	*O. forresti*
O. pusilla (5)	*O. forresti*	*O. daurica*	*O. daurica*	*O. gaoligongensis*
O. roylei (4)	*O. gaoligongensis*	*O. nubrica*	*O. nubrica*	*O. gloveri* (3)
O. rufescens (4)	*O. gloveri* (3)	*O. thibetana*	*O. thibetana*	*O. himalayana*
O. thibetana (6)	*O. himalayana*	*O. thomasi*	*O. thomasi*	*O. iliensis*
	O. hyperborea (11)			*O. koslowi*
Subgenus *Pika*	*O. iliensis*	**Northern Group**	**Northern Group**	*O. ladacensis*
	O. koslowi			*O. macrotis* (5)
O. alpina (6)	*O. ladacensis*	*O. alpina*	*O. alpina*	*O. muliensis*
O. hyperborea (8)	*O. macrotis* (5)	*O. hyperborea*	*O. collaris*	*O. nigrita*
O. ladacensis	*O. muliensis*	*O. pallasi*	*O. hyperborea*	*O. roylei* (5)
O. pallasi (3)	*O. nubrica* (2)	*O. princeps*	*O. pallasi*	*O. rutila*
O. rutila (4)	*O. pallasi* (4)		*O. princeps*	
	O. princeps (36)			
	O. pusilla (2)	**Mountain Group**		***Pika***
Corbet 1978	*O. roylei* (3)		**Surrounding Qinghai-Tibet Plateau Group**	
	O. thibetana (7)	*O. erythrotis*		*O. alpina* (4)
O. alpina (6)	*O. thomasi*	*O. forrest*		*O. argentata*
O. curzoniae		*O. himalayana*		*O. collaris*
O. daurica (5)		*O. ladacensis*	*O. brookei*	*O. hoffmanni*
O. erythrotis		*O. koslowi*	*O. erythrotis*	*O. hyperborea* (9)
O. koslowi		*O. macrotis*	*O. forresti*	*O. pallasi* (5)
O. ladacensis (1)		*O. roylei*	*O. gloveri*	*O. princeps* (5)
O. pallasi (4)			*O. himalayana*	*O. turuchanensis*
O. pusilla (2)		**Unplaced**	*O. iliensis*	
O. roylei (4)			*O. koslowi*	***Ochotona***
O. rufescens (4)		*O. huangensis*	*O. ladacensis*	
O. rutila			*O. macrotis*	*O. cansus* (4)
O. thibetana (2)			*O. muliensis*	*O. curzoniae*
O. thomasi			*O. roylei*	*O. daurica* (4)
			O. rufescens	*O. huangensis*
			O. rutila	*O. nubrica* (2)
				O. pusilla (2)
			Huange Group	*O. rufescens* (3)
				O. thibetana (5)
			O. huangensis	*O. thomasi*
			Central Asia Group	
			O. pusilla	

1. This work addressed the classification of Asian species only. 2. Study did not include all pika species.

Table 5.2: ND4 and *cyt*B Genbank sequences used in this study.

Species	**ND4**	**cytB**
Lepus europaeus	NC004028	AF421471
Oryctolagus cuniculus	NC001913	NC001913
Ochotona alpina	AF273130	AF273009
O. annectens	AF273129	AF273008
O. cansus cansus	AF273129	AF273003
O. cansus morosa	AF273127	AF273007
O. cansus stevensi	AF273127	AF273005
O. collaris AK1	EU54742	EU549742
O. collaris AK2	EU549748	EU549743
O. collaris AK3	EU549749	EU549738
O. collaris AK4	EU549750	EU549739
O. collaris AK5	EU549753	EU549736
O. collaris YU	EU549753	EU549737
O. curzoniae 1	AF273122	AF273001
O. curzoniae 2	AF273123	AF273002
O. curzoniae 3	AF273004	AF273124
O. daurica bedfordi	AF273135	AF273000
O. daurica daurica	AF273134	AF273011
O. erythrotis	AF273121	AF272999
O. forresti	AF273120	AF272998
O. himalayana	AF273119	AF272997
O. huangensis	AF273117	AF272995
O. hyperborea 1	EU549754	EU549744
O. hyperborea 2	EU549755	EU549746
O. hyperborea 3	EU549756	EU549745
O. koslowi	AF273116	AF272993
O. ladacensis	AF273114	AF272993
O. macrotis	AF273133	AF273010
O. nubrica	AF273113	AF272991
O. roylei	AF273131	AF272988
O. pallasi helanshanensis	AF273118	AF272996
O. pallasi sunidica	AF273132	AF272990
O. princeps A	NC005358	NC005358
O. princeps B	AF272989	AF273112
O. pusilla	NA	HM366945
O. scorodumovi	AF273115	AF272994
O. thibetana	AF273110	AF272986
O. thomasi	AF273111	AF272987
O. turuchanensis	EF567056	NA
O. mantchurica	EF567058	NA

Table 5.3: Museum catalogue numbers and location of collection for specimens sequenced for this study. NMNH = Smithsonian Museum of Natural History, MSB = Museum of Southwestern Biology, University of New Mexico, MVZ = Museum of Vertebrate Biology, University of California, Berkeley.

Taxon	**Country**	**Province and Locale**	**Museum**	**Catalogue Number**
O. alpina hoffmanni	Mongolia	Ovorkhangay, Ulaan Tsutgalan	MSB	94337
O. collaris	Canada	Yukon Territory, Ruby Ranges	NA	NA
O. collaris	Canada	Yukon Territory, Front Ranges	NA	NA
O. collaris	Canada	Yukon Territory, Columbia Icefields	NA	NA
O. curzoniae melanostoma	China	Qinghai, Gonghe County	NMNH	USNM 449083
O. daurica daurica	Mongolia	Ovorkhangay	MSB	94346
O. hyperborea	Mongolia	Tov, Gorkji Terelj National Park	MSB	94348
O. pallasi pricei	Mongolia	Ovorkhangay, Ulziyt Uul	MSB	94356
O. rufescens	Iran	Kerman Province, mountains of east Kerman.	MVZ	191919
O. thibetana nangqenica	China	Qinghai, Nangqen County	NMNH	USNM 449092
O. thibetana thibetana	China	Sichuan, Shimian County	NMNH	USNM 574319

Table 5.4: The primers used to amplify and sequence mitochondrial genes for species of *Ochotona* in this study.

Primer Name	**Sequence 5'-3'**	**Gene Amplified/Sequenced**
curz1F[a]	AAAGCAAAGCACTGAAAAATG	12S rDNA
curz1R[a]	ATGCTAGAGGTGATGTTTTTG	12S rDNA
12S1F[b]	GCRAGGCACTGAAAATGCCTAGATGAG	12S rDNA
12S1R[b]	CCAAGCACACTTTCCAGTATACTTACC	12S rDNA
16S2F[b]	CAGCCTGTATACCGCCATCTTCAGC	16S rDNA
16S2R[b]	GGTGGCTGCTTTTAGGCCAACTATGG	16S rDNA
16S3F[b]	CGAGCCTAGTGATAGCTGGTTGTCCAG	16S rDNA
16S3R[b]	CGTTGAACAAACGAACCTTTAATAGCG	16S rDNA
curz3F[a]	AGTTACTTTGATAGAGTAAA	ND2
curz3R[a]	TTCTTACCAAGCCCTGAGGT	ND2
ND6R[b]	AGGTTRAGGAGTATGATGTCTGGGTT	ND2
curz4F[a]	AATACCCTAATCAACTGGCTTCAATCTA	COX1
curz4R[a]	GTCTTCATAGTCGGTATATTCATAGCTTCA	COX1
COX6550R[b]	GGT CAA GGG TGT AGC CTG AGA ATA	COX1
curz6F[a]	CAACAAGCCCTACTAAT	ND4
curz6R[a]	TTTGGATAACTAGGAAG	ND4
curz7F[a]	TAAACGCAGGAACCTACTT	ND4
curz7R[a]	GGTTCCTAAGACCAACGGA	ND4
ND16F[b]	AGAACGACTAAACGCAGGAACC	ND4
cytb-F[b]	GAC TCA GGA TAC TCC TCC GTA GCC	cytB
cytb-R[b]	GAA TTT CAG CTT TGG GTG CTG ATG G	cytB

[a] primers described by Luo *et al.* (2008). b = primers designed for this study.

Chapter Six

Conclusions

In this thesis, I addressed several aspects of the population genetic structure and behaviour of the collared pika (*Ochotona collaris*), a little-studied lagomorph native to north-western North America. I also examined the phylogeny of the genus *Ochotona* as a whole, using sequences from several mitochondrial genes.

In Chapter 2, I presented eight new microsatellite markers used in Chapters 3 and 4 (along with markers presented by Peacock *et al.* 2001) to examine the changing genetic structure, dispersal habits and mating system of a collared pika population in the southwest Yukon. Little is known about these aspects of collared pika biology, although the closely-related American pika appears to be monogamous, as adjacent male-female pairs often have some overlap in their territories (Smith and Ivins 1984). However, genetic parentage analyses often reveal that socially monogamous species are actually sexually polygynandrous (*e.g.* Wolff *et al.* 2002; Solomon *et al.* 2004). Thus, genetic data are often needed to determine the mating system of a species.

Parentage analyses can also be invaluable in studying dispersal, particularly for animals that are difficult to capture before they have dispersed (Waser and Hadfield 2011). Collared pikas in particular are very challenging to capture before they have dispersed since they do so very soon after weaning. Additionally, they will rarely enter traps unless they are caching food, which they do not start to do until they have dispersed and found a vacant territory. However, since collared pikas tend to center their territories on a single point (their food cache), and only very rarely change territories, parentage analyses can be used to infer how far a pika has dispersed from its natal den.

The Mating System and Dispersal Habits of the Collared Pika

The genetic parentage analyses presented in Chapter 3 revealed that collared pikas are generally polygynandrous, although a disproportionately large number of animals that produced offspring together were each other's nearest neighbours. However, animals did not restrict themselves to mating with animals on nearby territories, as pikas with territories over one kilometer apart did produce offspring together on occasion. This finding is consistent with many aspects of pika behaviour and ecology. For example, collared pikas do not appear to mate guard. Even though a male and female pika may have some territory overlap during the fall, they will rarely be seen in close proximity to each other (Kieran O'Donovan, unpublished data). In contrast, the males and females of many socially monogamous mammals with low rates of extra-pair paternity will spend most of their time close to each other, which reduces the ability of either member of the pair to seek out extra-pair copulations (*e.g.* Schubert *et al.* 2009; Wright *et al.* 2010). Collared pikas also wander from their territories during spring (Franken 2002) and northern pikas (*O. hyperborea*) will even travel widely above the snow during the breeding season in early spring (Sokolov *et al.* 2009). Male little pikas (*O. pusilla*) are also very active during the breeding season and move about extensively while females tend to remain sedentary (Sokolov *et al.* 2009). Thus, both behavioural observations and genetic data indicate that male pikas will wander during the breeding season to seek out extra mating opportunities.

Polygynandrous (or promiscuous) mating systems are common in mammals and occur in a wide variety of taxa (Wolff and MacDonald 2004). Under such a system, males can generally increase their fitness by mating with multiple females, although this can be costly when it requires that the male undergo extraterritorial movements. For example, increased mortality in wandering males has been documented in red foxes (*Vulpes vulpes*, Baker *et al.* 2003) and grey mouse lemurs (*Microcebus murinus,* Kraus *et al.* 2008). While a male's reproductive success has long been acknowledged to increase with the number of mates he has (*e.g.* Bateman 1948), under some circumstances, females may also increase their fitness by mating multiply. Documented benefits of multiple mating for females include lower rates of reproductive failure, increased offspring viability and postcopulatory inbreeding avoidance (Keil and Sachser 1998; Stockley 2003; Firman and Simmons 2008). However, I could not determine which, if any, of these benefits apply to female collared pikas that mate multiply, as determining the number of mates each animal had was not possible.

Mating systems where females mate multiply can also provide benefits to females beyond increases in offspring viability. For example, Wolff and MacDonald (2004) note that males of many species are less likely to kill the offspring of females they have mated with and thus females may mate multiply to reduce the chances their offspring will be killed by conspecific males. However, infanticide has not been documented in any pika species and is unlikely to have influenced the evolution of mating patterns in pikas. Finally, it is possible that multiple mating in female pikas is not a genetic adaptation and is largely influenced by environmental factors. For example, the trait has a very low heritability in red squirrels (*Tamiasciurus hudsonicus*) and is instead influenced primarily by environmental factors (McFarlane *et al.* 2011). In some small mammals, such as pygmy field mice (*Apodemus microps*, Bryja and Stopka 2005), the number of mates an individual has is dependent on population density and habitat type. Since some American pika populations are largely monogamous (Peacock and Smith 1997), the mating system of North American pika populations may be flexible and dependent on environmental factors such as population density.

With maternity data, I could also examine the dispersal habits of the collared pika and test the hypothesis that they display no sex-biased dispersal. Although the majority of mammals display some degree of sex-biased dispersal (Lawson Handley and Perrin 2007), collared pikas have many characteristics associated with species without sex-biased dispersal, including a lack of social complexity (Devillard *et al.* 2004). Collared pikas were indeed found to have no sex-based differences in their dispersal behaviour, as both males and females dispersed the same average distance.

The mean dispersal distance in collared pikas was higher than expected (about ½ kilometer) and some individuals dispersed two kilometers from their natal den. This mean distance is relatively high compared to other pika species. For example, one study on the dispersal habits of black-lipped pikas (*Ochotona curzoniae*) found that 57% of individuals were philopatric (Dobson *et al.* 1998). American pikas also have limited dispersal habits, because juveniles often remain on their natal territory or an adjoining home range, and only 25% of animals in one population attempted to disperse farther than that (Smith and Ivins 1983; Smith 1987). North American pika females (*O. collaris* and *O. princeps*) often become aggressive towards their weaned offspring; however, juvenile American pikas can remain on their mother’s territory by staying near the edge of her territory and becoming active when she is inactive (Smith and Ivins 1987). However, collared pika juveniles do not appear to do this and will disperse very shortly after weaning.

The Genetic Structure of a Collared Pika Population

Many studies on the genetic structure of populations are limited to one or a few years. This study is one of a few that has tracked the changes in the genetic structure of a mammal population for a period of over a decade. I found that both the genetic structure and the genetic diversity of the pika population fluctuated with the density of the population

(Chapter 4). The population lost some of its genetic diversity when it declined; however, this was restored once the population started to recover. Although pikas have been thought to be quite restricted in their dispersal abilities and therefore prone to inbreeding (Smith *et al.* 1990), I demonstrated that some populations may actually be quite resilient against losses in genetic diversity despite undergoing fluctuations in density.

Although I had limited information on neighbouring populations, an analysis using GeneClass2 (Piry *et al.* 2004) allowed me to identify individuals that were unlikely to have originated in the study population and were thus likely immigrants from neighbouring populations. The study population received at least ten such migrants (both males and females), which likely explains why the population regained genetic diversity lost during the population decline. Without genetic data on these neighbouring populations, I could not determine how far immigrants may have travelled, nor could I know exactly how many animals immigrated into the population each year. Immigrants from nearby populations may not have been sufficiently genetically distinct to have been identified as immigrants. However, the pattern of genetic change seen in the study population is consistent with positive density-dependent dispersal (Berthier *et al.* 2006), as populations with negative density-dependent dispersal typically have stable levels of genetic diversity (Pilot *et al.* 2010). Some Asian pika species also display positive density-dependent dispersal, including *O. hyperborea* and *O. pusilla* (Sokolov *et al.* 2009).

Collared Pika Life History

Until recently, relatively little information has been available about the biology of the collared pika; however, this study and others conducted at the same field site have now provided a nearly complete view of its life history.

Collared pikas first reproduce during the spring after their birth and there is very little variation in age at first reproduction (Franken 2002). Collared pikas appear to wander from their territories during the breeding season in order to find mates and they are generally polygynandrous (Chapter Three). The date that litters are conceived varies across years, from late March to early June, and parturition occurs about thirty days after conception (Franken and Hik 2004). The earliest that young may be born is thus late April, although such early births are rare and most young are born between early June and July (Franken and Hik 2004). Late births are associated with a late snowmelt, although parturition date does not appear to affect litter survival rate (Franken and Hik 2004). The mean litter size in collared pikas is 2.2-3.0 offspring (Smith *et al.* 1990) with a maximum of six offspring being reported (MacDonald and Jones 1987).

Collared pikas are born blind and hairless at and reach adult size about 40-50 days after birth (MacDonald and Jones 1987). They disperse quickly after weaning (which takes thirty days) and disperse a mean of about half a kilometer from their birth site (Chapter Four). The date that collared pikas start haying varies and ranges from mid-June to early September, with juveniles starting to hay about ten days later than adults (Morrison *et al.* 2009). The date that an individual starts haying influences survival rates in some years, with earlier haying dates often improving survival rates (Morrison *et al.* 2009). Haying rates start off low and increase as summer progresses, to a maximum of about three hundred haying trips per day in August (Morrison *et al.* 2009).

Collared pikas will stop haying once the vegetation has started senescing, although they will continue to forage on vegetation and lichen during early fall (K. O’Donovan, personal observation). Collared pikas do not hibernate, and during the winter, they presumably rely on their haypiles and lichen for sustenance. Morrison *et al.* (2009) found that approximately 75% of haypiles contain sufficient food for 90 days and 50% contain sufficient food for 177 days.

Climate Change and the Collared Pika

Some environmental groups and scientists are concerned that climate change will put the continued persistence of North American pikas at risk (Wolf *et al.* 2007, Beever *et al.* 2011). Additionally, Wei-Dong and Smith (2005) suggest that climate change (along with increased grazing pressure) may have caused declines in Ili Pika (*O. iliensis*) populations from the Tien Shan Mountains, China. There is evidence that large-scale climate patterns influence the population growth rates of the collared pika population examined in this study, likely through variation in snow melt timing (Morrison and Hik, 2007). Furthermore, climate warming could increase mortality in collared pika populations through decreases in winter snowpack and increases in winter freezing rain events (Morrison and Hik 2007). However, warming could also improve survival and growth rates in pika populations by causing an earlier snow melt and a longer growing season (Morrison 2007). More data is needed to tease these effects apart.

Although my work did not deal directly with the potential effects of climate change on collared pikas, I presented some demographic data in Chapter 4. The study population underwent a decline in 2000 and again in 2003 and a population viability analysis on this population (assuming no immigration) suggested that it has a high probability of going extinct in the next fifty years (Morrison 2007). However, this estimate may be somewhat pessimistic, as the population appears to receive immigrants from neighbouring populations and it has recovered genetic diversity lost during the population decline (Chapter 4).

Nonetheless, it may be prudent to continue to monitor populations of collared pikas, since they are known to be sensitive to changes in the climate (Morrison and Hik 2007) and Flato *et al.* (2008) predict that the mean annual temperature for the south-western Yukon will increase by 3.4°C by 2080. The range of the related American pika also appears to be retracting in response to recent climate warming (Beever *et al.* 2011). Additionally, beyond data from the population examined in this work, there is little data on population sizes and trends for collared pikas, either in the Yukon or elsewhere.

The Systematics and Evolution of *Ochotona*

In Chapter 5 of this thesis, I used a mitochondrial data set to infer the phylogenetic relationships among species in the genus *Ochotona*. Results confirmed the sister group relationship between *O. alpina* and *O. hyperborea*, and suggested that the Afghan pika (*O. rufescens*) belongs in the subgenus *Conothoa*, rather than the subgenus *Ochotona*. The steppe-dwelling pikas from the Tibet-Qinghai Plateau all formed a monophyletic group, but some species in this group appeared to be non-monophyletic, including *O. curzoniae.* In particular, the subspecies *O. curzoniae melanostoma* appears to be more closely related to *O. thibetana* than *O. curzoniae* (Chapter 5).

Generally, the taxonomy of *Ochotona* has been a difficult topic, as highlighted by the many changes that have been made to pika classifications during the past century (see Table 1.1). Numerous cryptic species in *Ochotona* have been described recently (*e.g.* Formozov *et al.* 2004), making it plausible that *O. curzoniae melanostoma* is a separate species more related to *O. thibetana* than *O. curzoniae*. However, phylogenetic trees inferred from single genetic sources do not always reflect the true species trees (Degnan 1993). The next step in unravelling the evolutionary history of the pikas and devising a classification scheme consistent with it should be to include nuclear markers in datasets used to examine their population structure and phylogeny.

General Conclusions

Sex-based differences in dispersal habits are nearly universal in mammals, and their direction and magnitude will be influenced by a species' social structure and mating system (Lawson Handley and Perrin 2007). This study has provided a rare example of a mammalian species (the collared pika) that lacks sex-biased dispersal. This is likely a result of the species' very simple social system: individuals are asocial and polygynandrous, and juveniles leave their parents' territory very quickly after weaning. This simple system was likely shaped by the extreme environment collared pikas live in. Juveniles must disperse quickly to find a territory and build a food cache before the end of the very short growing season. Since individual collared pikas are typically capable of gathering only enough food for themselves (Morrison *et al.* 2009) males cannot monopolize sufficient resources to attract multiple females, and females are not distributed in a way such that males can defend groups of them. This precludes the development of a polygynous mating system, which is generally common in mammals. Since there are also no apparent sex-based differences in resource or mate competition in collared pikas, selection pressures on males and females should be similar, leading to even rates of dispersal between males and females.

This study also highlights the importance of long-term studies in answering questions related to the ecology and evolution of small mammals. For example, the genetic diversity and structure of this population did not remain stable but fluctuated with the density of the population. I also demonstrated that the population was able to regain genetic diversity lost after a population decline. Numerous other studies on the genetic structure and diversity of fluctuating small mammal populations have also demonstrated that many such populations typically retain high levels of diversity via movement of individuals among populations (Berthier *et al.* 2006; Dong *et al.* 2010; Pilot *et al.* 2010; McEachern *et al.* 2011). In such populations, the effects of drift can act to decrease genetic diversity during low-density phases, while increased immigration (in species with positive density-dependent dispersal) during high-density phases can counteract the loss of diversity through drift.

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