MOLECULAR PHYLOGEOGRAPHY OF THE AMERICAN BEAVER (*Castor Canadensis*): IMPLICATIONS FOR MANAGEMENT AND CONSERVATION

By

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DEDICATION

To my parents Valentina Serrano Cardenas and Ricardo Pelz Marin, my aunt Ana Pelz Marin, and my siblings Lariza, Fanto, Laika and Jacko.
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ABSTRACT

The American beaver, the largest rodent of North America, is distributed in ponds, lakes, and streams from Alaska to northern Mexico. This semi-aquatic mammal is considered an ecosystem engineer because beavers modify the landscape by cutting trees and by creating dams and ponds that have important effects on the aquatic community structure, providing habitat for aquatic invertebrates, fish, and birds. The American beaver has played an important socioeconomic role in the history of North America due to beavers’ fur value, which caused the near extirpation of this mammal at the beginning of the 1900s due to overharvest by early Europeans. Because of the highly specific habitat requirements of beavers, this mammal also suffers the effects of habitat loss in some areas where riparian ecosystems are now scarce. My objectives in this study were to assess how climatic and geological events affected the current distribution of the American beaver in North America, and how the management actions to restore and control beaver populations have affected the genetic structure and conservation of beaver populations. Specifically, I addressed four aspects of the management and conservation genetics of the beaver: 1) a literature review of the management of beavers in the past 100 years; 2) the development of novel microsatellite DNA markers to address the population genetic structure aspects of the study; 3) the use of these microsatellite DNA markers to assess genetic diversity of current populations of beavers and to detect past population bottlenecks; and 4) the use of two mitochondrial DNA genes to resolve the current phylogeography of the American beaver in order to better understand how historical factors have affected the beaver’s current distribution and genetic structure in
North America. The results from this study provide information of the effects that management actions and climatic events can have on the genetic structure of beavers. This information can be used by wildlife biologists, and land managers, to develop future strategies for management and conservation of the American beaver.
INTRODUCTION

The genus *Castor* arrived in the New World approximately 7.6 to 8 million years ago (mya) through the Beringian land bridge (Horn et al. 2011). After the land bridge between Eurasia and North America disappeared, the American beaver (*Castor canadensis*) and the Eurasian beaver (*Castor fiber*) were completely isolated from each other and diverged into separate species (as long as 24,000 years ago; Müller-Schwarze and Sun 2003). Currently, the American beaver is distributed in ponds, lakes, and streams from Alaska to northern Mexico, but is absent in Arctic tundra and peninsular Florida (Jenkins and Busher 1979). Beavers depend on riparian vegetation because they feed on bark, cambium, leaves, and roots of aspen, poplar, birch, willow, and alder (Müller-Schwarze and Sun 2003). Beavers are considered ecosystem engineers because they modify their environment by cutting trees and building dams. Beaver dams create ponds that have important effects on the stream community structure and on the entire riparian ecosystem by providing habitat for aquatic invertebrates, fish, and birds (Wright et al. 2002, Naiman et al. 1988).

The association between beavers and riparian vegetation has shaped the distribution of this species in North America. Because of their riparian association, along with climatic and geological events, the distribution of the American beaver has changed over time. During the last glacial maximum, approximately 18,000 to 21,000 years ago, ice sheets extended throughout much of the northern portion of the continent, the lowered sea levels exposed land bridges that connected land masses, such as the Beringian land bridge that connected North America with Siberia, when the climate was much colder.
and drier (Jackson et al. 2000, Waltari et al. 2007). Consequently, the distribution of plant and animal species was different from their current distribution. For instance, there is evidence that dusky willow (*Salix melanopsis*) and black cottonwood (*Populus trichocarpa*) were restricted to areas in the Cascades of the Pacific Northwest and the Rocky Mountains (Brunsfeld et al. 2007, Jaramillo-Correa et al. 2009). This could have affected the distribution of species associated to these riparian trees, such as the beaver. After approximately 4,000 years, when the climate warmed and ice sheets started to melt and retreat, plant and animal species found new available habitats and started to expand their distributions (Waltari et al. 2007).

Another factor that affected the distribution of the beaver in North America is their management by humans. For example, beaver pelts were an important fur-trading item between North America and Europe. This resulted in the overhunting and near extirpation from North America of the beaver by the late 1800’s (Müller-Schwarze and Sun 2003). The serious decimation of beaver populations rose concern among pelt traders and naturalists at the beginning of the 1900’s. This was followed by a series of efforts to restore beaver populations by translocating and reintroducing individuals throughout North America. More recently, the management of beavers has shifted to control of their population sizes and occurrence. The modifications that beavers cause to their environment often brings them in conflict with people. Thus, to control their populations, and mitigate conflicts with humans, beavers have been managed by removing individuals and translocating them to different water systems, or by eradicating certain populations (Müller-Schwarze and Sun 2003).
Therefore, the management of beavers in the last century has been either to restore, or to control their populations. However, these management actions do not always consider ecological or genetic aspects of the species, and there has not been an evaluation of the ecological and genetic consequences of some of the methods to manage beavers. In addition, there has not been a study of how past climatic and geologic events affected the current distribution of American beavers. The study to examine some climatic and geological events have affected historical beaver distributions will aid our understanding of the present biogeographical patterns, current populations’ genetic structure, and potential species responses to future climate and landscape changes. This information is important for the management and conservation of species that are habitat specialist and are at constant risk to loss of habitat. It is also important to develop appropriate management and conservation plans for populations that face a higher risk of extinction, such as beaver populations in northern Mexico, where the American beaver is considered in danger of extinction (SEMARNAT 2010).
PRESENT STUDY

The following is a summary of the objectives, methods, and most important findings of the studies in this document. The complete description of methods, results, and conclusions of this study are presented in the papers appended to this dissertation.

Objectives

My objectives were to design and use molecular markers to address phylogenetic relationships among beaver populations in North America, to understand how climatic and geological events affected the current distribution of the beaver, and learn how the management actions to restore and control beaver populations have affected the geographic distribution, genetic structure, and conservation of current beaver populations.

Methods

In order to understand the effect of climatic and geological events, as well as management actions on the genetic diversity, phylogenetic relationships, and current distribution of the American beaver, I conducted a literature review of the different methods that have been applied for the management of the beaver in North America. I focused on two aspects of beaver management: 1) the control of beaver population densities, and 2) the conservation of beaver populations. I carried out a search of peer-reviewed papers and official reports published between 1900 and 2011 using the databases ISI Web of Knowledge, and BIOSIS. I used as keywords “beaver management”, “beaver control”, and “beaver conservation”.
For the genetic analyses, I developed microsatellite DNA markers, by constructing a genomic library from tissue collected from wild-caught beavers in Tres Rios Wetlands, Arizona, following the protocol described by Glenn & Schable (2005). To obtain specific microsatellite fragments, the genomic library was hybridized to biotinylated microsatellite oligonucleotide probes (GT)15 (CT)15 and (GATA)8. The recovered microsatellite-enriched fragments were cloned and sequenced, and I was able to design 40 primers pairs using Primer 3 software (Rozen & Skaletsky 2000). Of the primer pairs designed, 31 failed to amplify, one locus was monomorphic (Cca 151), and eight loci amplified and were polymorphic.

Seven of these microsatellite loci, along with two other microsatellite loci previously described by Crawford et al. (2008) for the American beaver, were used to infer genetic diversity measures of beaver populations in areas of the United States where the beaver was depleted in the late 1800’s, and where translocations and reintroductions occurred during the 1900’s. I collected tissue samples in collaboration with Wildlife Services (USDA) of wild beavers captured from Alabama, Arizona, Maine, Minnesota, South Carolina, Texas, and Wisconsin. I extracted genomic DNA from these tissue samples using a DNeasy blood and tissue kit following the manufacturer’s protocol (Qiagen Ltd., Valencia, CA).

To assess genetic variation within populations, I estimated observed heterozygosity ($H_o$), expected heterozygosity ($H_E$), mean observed number of alleles ($N_a$), number of private (unique) alleles per population ($P_A$), mean allelic richness per locus ($N_r$), and levels of inbreeding $F_{IS}$ using FSTAT software (Goudet 2001). The levels of population
subdivision were estimated by values of $F_{ST}$, $G'_{ST}$, and standardized $R_{ST}$ (Wright 1951, Hedrick 2005, Goodman 1997, respectively). Values of $F_{ST}$, and $R_{ST}$ were applied in a Mantel test to assess correlation between genetic and geographical distances of beaver individuals. Geographic structure among beaver populations was assessed using the software programs STRUCTURE 2.3.2 (Pritchard et al. 2000), and GENELAND (Guillot et al. 2005a, Guillot et al. 2005b). To detect the presence of a past population bottleneck I performed a test of excess heterozygosity using the BOTTLENECK software (Cornuet and Luikart 1996).

To infer phylogenetic relationships among beaver populations I used a 520 base pair (bp) fragment of the mitochondrial DNA hypervariable domain I (HV I) of the control region (CR), and a 307 bp fragment of the mitochondrial DNA cytochrome $b$ (cyt $b$) gene. I obtained 195 tissue samples from 12 different US states, from wild beavers, captured in the field by Wildlife Services (USDA), and 44 samples of tissue, skin, or bone from several museum collections, which represented ten US states and seven Canadian provinces. From the total of 239 samples obtained, I successfully extracted DNA from 189 samples using a DNeasy blood and tissue kit following the manufacturer’s protocol (Qiagen Ltd., Valencia, CA). I used the extracted DNA to PCR amplify and sequence the CR and cyt $b$ fragments using the primers and conditions described by Cheney (1995), and Montgelard et al. (2002), respectively.

I performed two analyses, first, the phylogenetic analyses for the CR and the cyt $b$ separately. Second, I conducted the phylogenetic analyses for both sequences (CR and cyt $b$) concatenated. I used both, Maximum Likelihood (ML) and Bayesian Inference
(BI) approaches to reconstruct phylogenetic relationships among the haplotypes. I estimated measures of genetic diversity such as gene diversity ($h$), and nucleotide diversity ($\pi$) for both gene fragments and for the combined dataset. For the combined dataset I performed an Analysis of Molecular Variance (AMOVA Excoffier et al. 1992) to assess the differentiation among geographic regions, among populations within regions, and within populations (Weir & Cockerham 1984). Specifically, I compared three major geographic regions structured as follow: West (Arizona, Idaho, Alaska, Wyoming, Ne Mexico, Washington, and Canada), Central (Texas, Oklahoma, Minnesota, Wisconsin, Missouri, Kentucky, Tennessee, and North Dakota), and East (Maine, Alabama, South Carolina, and Virginia). I also used the combined dataset to estimate the time to the most recent common ancestor (MRCA) for all haplotypes and several monophyletic clades, and to assess the demographic history (e.g. expansion) of each major clade in the phylogenetic tree using Tajima’s $D$ (Tajima 1989) and Fu’s $F_S$ (Fu 1997) tests for neutrality, as well $D^*$ and $F^*$ statistics (Fu and Li 1993).
CONCLUSIONS

The effects that beavers have on their ecosystem are remarkably important for other riparian species, however, these same effects on their surroundings can cause the development of conflict with people, which changes the perception of beavers from an ecosystem engineer to a pest. The methods to control beaver populations were well represented in my literature review (see Appendix A). However, there is also a vast number of studies that discussed the conservation of the beaver and suggested the use of this species as a tool for ecosystem and biodiversity enhancement and conservation. Overall, the management of beavers needs to consider both the positive and negative effects of beaver activity on a specific ecosystem. Managers need to develop a holistic plan that includes ecological information specific to the region, the other species that are benefited by the presence and activity of beavers, the areas where beavers can cause damage to anthropogenic structures, the areas where beaver can expand their populations, and the long-term consequences of these expansions.

The analyses of the microsatellite loci (see Appendix B and C) show that despite the almost complete eradication of beavers from North America, this species still has high levels of genetic diversity, and significant levels of population subdivision. However, the genetic differentiation does not correlate with geographic distance. There is also lack of evidence of past population bottlenecks.

The phylogenetic analyses using mitochondrial DNA (see Appendix D) show that climatic and geologic events did affect past and present distribution of the beaver in North America, as well as the phylogenetic relationships among beaver populations.
The phylogenetic trees obtained in this study show the presence of multiple Pleistocene refugia for the beaver, probably in areas of the Appalachian Mountains, the Rocky Mountains and Texas. This evidence is supported by the grouping of individuals by geographic areas, such as the case of individuals form the eastern (i.e. South Carolina, Alabama, Virginia, and Maine), and south-central (i.e. Texas, Missouri, Oklahoma, Kentucky, and Tennessee) regions of the United States. Moreover, divergence times of major clades and subclades, which date to the middle and late Pleistocene, and the signal of population expansion based on the neutrality tests further support a refugia past for the American beaver.

In addition to the combination of major climatic fluctuations, topographic and vegetation variations, anthropogenic actions during the last 200 years have had an impact in the genetic structure, and distribution of the beaver. Despite the high genetic diversity found for neutral markers in this study, beavers might have in other regions of their genome the consequences of the almost eradication of their populations. Further studies that look at genes under selection such as the major histocompatibility complex are needed to elucidate the impact of past demographic events on the genetic structure of the beaver.

The tight association that the beaver has to riparian ecosystems can often cause conflict with people. However, beaver contributions to the ecosystems are irreplaceable. This mammal provides habitat for other species associated to wetlands and riparian areas. There is evidence that beavers increase vegetation heterogeneity and diversity. Thus, the results from these studies provide information that can aid scientists and
managers in the development of appropriate conservation and management strategies that ensure the long-term survival of beaver populations, the maintenance of wetlands with the presence of beavers, and the reduction of beaver-human conflicts.
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APPENDIX A

A CENTURY OF NORTH AMERICAN BEAVER MANAGEMENT: A REVIEW

“The following paper is pending submission to the Journal of Wildlife Management”

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**ABSTRACT** The American beaver (*Castor canadensis*) is a semi-aquatic mammal found in lakes and rivers throughout North America. The beaver is considered an ecosystem engineer because it modifies the ecosystem cutting trees and sometimes creating dams and ponds. This species has had important interactions with humans due to the value of its fur, and because of the conflicts originated by the modifications beavers cause to their surroundings. These interactions have promoted the application of different management methods, either to control or conserve beaver populations. In this study we reviewed scientific literature and official reports published between 1900 and 2011 to evaluate the ecological and evolutionary consequences of such management actions on the beaver. From the references reviewed, 54% discussed methods to control beaver population densities and to reduce damage, while 72% considered ecological aspects of the beaver in the methods suggested. Forty six percent of the references suggested the conservation of the beaver, but only three references specifically focused on the conservation of the beaver. Mostly, the references that mentioned the conservation of the beaver, suggested this action as a way to conserve riparian ecosystems and water levels. The majority of studies reviewed considered some ecological aspects of the beaver, but evolutionary considerations were not well represented, and no study had a holistic approach to manage the beaver. Some of the methods to control beaver populations can have important impacts on the evolutionary pathway of these populations and need to be considered. We recommend that managers need to compile ecological, genetic, and behavioral information of each particular population before any strategy is designed and
applied, to ensure a holistic management and with it the long term survival of the beaver and wetlands.

**KEY WORDS:** American beaver, beaver management, beaver control, beaver conservation, riparian ecosystems.

The American beaver is considered the largest rodent of North America; it is distributed in ponds, lakes, and streams from Alaska to northern Mexico, except in the Arctic tundra and peninsular Florida (Jenkins and Busher 1979). Beavers live in small family groups (colonies) usually consisting of a breeding pair, one to two yearlings and one to two kits (RIC 1998). Beavers are considered ecosystem engineers because they modify their habitat to meet their requirements. Beavers cut trees to construct dams, lodges, and as a major food source (Naiman et al., 1986). The construction of dams raises the water level, which changes the position of the water’s edge (Hodgdon and Lancia, 1983). These hydrogeomorphological modifications created by beavers have important effects not only on the aquatic community structure, but also on the entire riparian ecosystem that provides habitat for aquatic invertebrates, fish, and birds (Wright et al. 2002, Gurnell 1998, Naiman et al. 1988).

In addition to the ecological importance of the American beaver, this species had a substantial influence on the Native American culture, and on the establishment of human settlements (Martin 1978). For instance, for the Micmac Indians of northeastern United States and eastern Canada, the beaver was admired for their industry and
abounding genius, and represented an important food source during the winter. When Europeans arrived to North America, the beaver became an essential part of Europe’s economic and geographic expansion into the northern reaches of the New World of the 17th century (Moloney 1967). From the mid-1600s to the late 1850s, beaver pelts represented an important fur-trading item between North America and Europe. In some areas, high beaver abundance determined where Europeans founded their first cities and fur-trading ports (Fig. 1) that in return, had a dramatic effect on the beaver populations, driving American beaver close to extirpation throughout North America (Burroughs 1961, Moloney 1967, Hammond 1993). By the late 1800s, the serious decline in beaver populations caused concern among the fur traders and other early North Americans about the status of the beaver. As a result, during the early 1900s, several translocations and reintroductions took place in the US to restore beaver populations (Müller-Schwarze and Sun 2003). For instance, between 1901 and 1906, 20 beavers from the Province of Ontario were released in Adirondacks, New York State. After the success of this reintroduction, 14 beavers from Yellowstone National Park were translocated to several areas of the Adirondacks (Radford 1907). In Massachusetts, beavers were reintroduced between 1928 and 1932 after having been extirpated from the region in the early 1700s (Shaw 1984, DeGraaf 2001). In South Carolina, between 1939 and 1941, six beavers from Georgia were reintroduced to the Sandhills Wildlife Refuge in Chesterfield County (South Carolina Department of Natural Resources 2010). In Arizona, between 1940 and 1950 a few beavers were translocated from Springerville and Pima, Arizona, to the
Chiricahua Mountains and from the White Mountains to the Graham Mountains (Carrillo et al. 2009).

The beaver translocations and reintroductions carried out during the early 1900s did not take into account the ecological and evolutionary background of the source and recipient populations (Müller-Schwarze and Sun 2003). Such management actions resulted in mixing of populations which may have resulted in important ecological, genetic, and evolutionary consequences for the American beaver (Hall and Kelson 1981). Nevertheless, the American beaver recovered from near extirpation. Restored populations increased their size, and currently the beaver is found throughout most of North America. Many beaver populations continue to expand, both geographically and demographically, such as populations in North Carolina (Woodward et al. 1985) and Mississippi (Bullock 1982), whereas other populations such as in Arizona and northern Mexico remain small (Pelz-Serrano et al. 2005, Carrillo 2009). Thus, different beaver populations have required different management actions. For instance, in areas where beavers are highly abundant, the development of conflict with humans often occurs due to the damage that beavers can cause to croplands, roads, and water flow; consequently, control of beaver population sizes is required. Conversely, in areas where beaver populations are small, such as in northern Mexico where beavers are considered in danger of extinction, the populations need to be managed to increase their number and secure conservation for their populations and habitats. However, the different management actions that are being enacted under each of the above scenarios may be having important ecological and evolutionary consequences on the beaver populations. Therefore, our objectives were to
review the historical and current management actions for the control and conservation of American beaver populations in light of potential genetic consequences, and to evaluate the ecological and evolutionary consequences of such management actions.

METHODS

We reviewed the information available about the historical translocations and reintroductions of beavers throughout North America to evaluate the potential ecological and genetic consequences these actions might have had on beaver populations. Furthermore, we conducted a survey of peer reviewed papers and official reports published between 1900 and 2011 using the databases ISI Web of Knowledge, and BIOSIS. We used as keywords “beaver management”, “beaver control”, and “beaver conservation”. From the references obtained, we discarded those publications not relevant to the American beaver, such as studies about the mountain beaver (Aplodontia rufa), muskrat (Ondatra zibethicus), and the Eurasian beaver (Castor fiber). From the papers reviewed, we recorded the management action employed, the efficacy and success of the management methods to achieve the objectives stated in each study.

RESULTS

We found 30 records of beaver translocations or reintroductions throughout North America reported on peer reviewed papers and on official reports (Table 1). All of these management actions occurred in the 20th century, but 86.6% (26) of these actions were
carried out between 1901 and 1957. While the most recent record of beaver translocations across geographic regions is from 1999 (Table 1).

The search using ISI web of knowledge and BIOSIS returned 92 citations from keywords “beaver management”. Only 30.4% (28) of the references were relevant to American beaver management, the remaining papers referred to Eurasian beaver ecology and management or to other species such as the muskrat and the river otter (*Lontra canadensis*). For the keywords “beaver control” the search returned 43 citations, from this total, 48.8% (28) were relevant to the American beaver, and of these papers, nine overlapped with the “beaver management” search. The search using keywords “beaver conservation” returned 40 citations and after discarding those not relevant to the American beaver we ended with a total of 17 (42.5%) papers. From this total, 8 were also present under the “beaver management” search, and 4 under the “beaver control” search.

Among the 45 papers reviewed, we identified different management goals for the American beaver that we classified into three categories: 1) management for beaver conservation, 2) beaver population density and damage control, 3) beaver management for ecosystem restoration and biodiversity enhancement. Within each category, several methods or strategies were identified and are further discussed below. The citations returned in the search for “beaver control” were all included in the second management category, while those citations obtained in the search of “beaver conservation” were included, as appropriately, into each category.

*Management for beaver conservation*
Of the 45 papers reviewed, 21 considered and suggested the conservation of beaver habitat to protect this species (Table 2). In addition, of those 21 papers, 13 articles suggested that conservation of the beaver and its habitat would aid in the conservation of other species associated with riparian ecosystems (Table 2). In summary, a management plan to protect beaver habitat in order to conserve other riparian species, will depended on the target species to be conserved and/or the physical characteristic (i.e. water level, canopy coverage) to be managed. Five papers (Bradt 1947, Grasse and Putnam 1950, Yeager and Rutherford 1957, Longley and Moyle 1963, Bloomquist and Nielsen 2009) recognized that, in addition to protecting the beaver and its habitat, beaver populations need to be controlled to prevent overpopulation and the subsequent conflicts with people. The primary control method mentioned in these papers was harvesting of populations.

*Management for beaver population density and damage control*

Twenty-five of the papers discussed the management of beavers to control their populations size and/or mitigate damage to humans caused by beaver activity (Table 2). From these papers, only one (DeStefano et al. 2006) considered the reintroduction or relocation of beavers for riparian restoration, in addition to population control. Two papers (King et al. 1998, Collen and Gibson 2001) recognized that although beaver density needs to be controlled, the ecosystem benefits provided by beavers to certain other species needs to be considered in the management plans, and beaver population control should be specific for each system and species to be conserved. Methods to control beaver populations included: livetrapping and relocation, harvesting, shooting,
poisoning, surgical and chemical sterilization, biological control, physical and chemical protection of trees, and flooding control devices.

**Management of beaver for ecosystem and biodiversity enhancement and conservation.**

The management of beaver for riparian restoration and for the conservation of biodiversity was also well represented in the reviewed papers. Twenty-two articles discussed different methods (e.g. riparian vegetation protection, delimitation of buffer zones) to manage beavers for these objectives. Depending on the study focus, some authors also suggested methods to control beaver population density as a tool for biodiversity enhancement (Table 2).

**DISCUSSION**

The beaver has had an important role in the history of North America human settlements (Martin 1978). Furthermore, beavers have been key components of riparian ecosystems shaping biodiversity heterogeneity of these ecosystems (Naiman et al. 1986). Nevertheless, due to the over-trapping of the beaver during the 1800’s, the beaver was almost extirpated from North America at the end of the 19th century (Burroughs 1961, Moloney 1967, Hammond 1993). Efforts to recovered beaver populations throughout North America began in the 1900’s. In some states were few beaver populations remained small, such as in Wisconsin and Kansas, beaver trapping was banned for several years until the populations recovered. Then, in some regions, beavers from recovered populations were used to restock beaver populations throughout the state from
where the animals were taken from (Henserson 1960, Jackson 1961). This practice was appropriate to boost beaver populations and to maintain beaver populations’ genetic integrity and evolutionary pathways. However, in some states from which beavers were extirpated, such as New York and Massashussets, people reintroduced beavers from other geographic regions to repopulate the area. Some of these reintroductions used the same subspecies to be reintroduced as the original resident subspecies, and the reintroduced animals were taken form close geographical areas (Radford 1907, Hensley 1946, Henderson 1960, Woodward et al. 1985,ermont Fish and Wildlife Department 2004, Carrillo et al 2009, South Carolina Department of Natural Resources 2010, Mississippi Fish and Game 2011). This mangement action ensured the evolutionary pathway of the subspecies involved, however, there is not enough information regarding the individuals reintroduced to better evaluate the effect the introduced indiviuals could have had on the resident populations. On the other side, in eight of the 30 records of beaver reintroductions found in this review, the animals used in the reintroduction were from a different subspecies from the original resident beaver population (Radford 1907, Pietsch 1956, Sealander 1956, Jackson 1961, Barbour and Davis 1974, Woodward et al. 1985, Longcore et al. 2007, Indiana Department of Natural Resources 2011). For instance, in 1907, beavers from Yellowstone National Park were translocated to several areas of the Adirondacks, New York (Radford 1907). The subspecies described for the state of New York is C. c. acadicus, whereas the translocated individuals belonged to C. c. missouriensis (Jenkins and Busher 1979). Another example is the translocation of beavers from Wisconsin, corresponding to the subspecies C. c. canadensis, to North Carolina,
where beavers belong to the subspecies *carolinensis* (Woodward et al. 1985).

Furthermore, in nine of the 30 translocations reviewed, there is no information about the origins of the translocated individuals. These management actions could have had important implications for the genetics and evolutionary history of the resident populations. Translocation of individuals can increase the potential for intraspecific hybridization, or mixing of previously differentiated gene pools (Stockwell et al 1996, Olden et al. 2004). Intraspecific hybridization can promote genetic homogenization compromising the unique characteristics of geographically distinct populations, and the fitness of individuals by altering local adaptations (Stockwell et al. 1996).

Nevertheless, the translocation and reintroduction of beavers, as well as restrictive trapping seasons during the 1900’s contributed to the recovery of the beaver throughout North America. Currently the beaver is widely distributed and the management of this mammals has turned to mostly control population sizes and in some areas to restore and conserve riparian ecosystems. The effects that beavers have on their ecosystem are important for other riparian species (Naiman et al. 1986, Barnes and Dibble 1988), however, these same effects on their surroundings can result in conflicts with people, which frequently changes the perception of beaver from an ecosystem engineer to a pest. These differing attitudes towards beavers has generated differing strategies and methods for management of beaver populations, under different scenarios, from lethal control and eradication, to conservation and use in ecosystem restoration. All of these management actions can have repercussions on the genetic and evolutionary characteristics of beaver populations.
Management for beaver conservation

As many as 40.3% ($n = 21$) of the articles reviewed suggested and discussed the importance of conserving the American beaver, yet only three studies (Smith and Peterson 1991, Hood and Bayley 2008a, Hay 2010) had their major focus as management for the conservation of the beaver. All these authors recognized the importance of assessing habitat requirements and population dynamics for the development of appropriate management plans. In addition, they suggest evaluating the local effect this mammal has on its specific ecosystem in order to more effectively manage and conserve the beaver.

Such local ecosystem effects include variables such as pond water levels, wetland-behavior interactions, and successional vegetation. Water level had an effect on the use of lodges during winter months and their abandonment in the spring, as well as on mortality during the winter (Smith and Peterson 1991). These results allowed to suggest a management plan to enhance beaver populations using a strategy to control water levels, which should decline during the summer months then reach a stable level in the fall (Smith and Peterson 1991). Determining the importance of beaver activity for the creation and maintenance of open wetlands in western Canada suggested the conservation of the beaver in the region by conserving the wetlands and native riparian vegetation (Hood and Bayley 2008a). A long-term study conducted to evaluate the process of succession in beaver ponds after removal of beavers showed how rapidly beavers can recolonize available habitat and the regeneration process of riparian forest with beaver
activity (Hay 2010). In this study, beavers recolonized the habitat within 2-3 years following a removal event. Another important outcome of this study is the identification of cattle grazing as a major impediment to regeneration of riparian vegetation. In order to restore beaver habitat, cattle grazing restrictions need to be implemented (Hay 2010).

**Management for beaver population density and damage control**

Due to the increase in human populations over the last century, beavers and humans interact more often than in the past. Some of the modification beavers do to their ecosystem can directly or indirectly affect human populations. Beaver dams can change the flow or level of water causing floodings on roads or private property (Longcore et al. 2007, Jonker et al. 2009). Herbivory by beavers can have damage crops or can create clearcuts that in turn might affect fish populations with important economic or recreation value to people (Collen and Gibson 2001, Longcore et al. 2007). Therefore, most management of the beavers is focused on the control of their populations in order to mitigate conflict with humans.

**Trapping and Hunting**

Historically beavers have been an important furbearer with a high direct economic value (Müller-Schwarze and Sun 2003). However, after beavers were almost extirpated from North America, trapping seasons were modified several times, depending on the status of beaver populations in each state. Currently trapping of beavers is allowed in most of Canada and the United States, but regulations vary among provinces and states.
For instance, in California, beaver trapping is allowed only in 42 counties, in New Hampshire and Washington there is only one open trapping season per year, and in Arizona trapping is not allowed due to the small number of beavers in the state (U.S. Fish and Wildlife Service 2011). Furthermore, in Mexico, because of the small number of beaver populations remaining in the country, and their small population sizes, this mammal is considered at risk of extinction therefore taking beavers is forbidden (SEMARNAT 2010).

Nevertheless, in the areas of North America where beaver trapping is allowed, this represents a major method to control beaver populations, especially in areas of human-beaver conflict (Müller-Schwarze and Sun 2003). Since the late 1940’s researchers have suggested harvest of beaver populations as an efficient method to reduced population sizes. However, harvest of individuals can have genetic consequences of beaver populations. Harvesting can alter the gene flow among populations, it can alter the rate of genetic drift due to the direct effect on effective population size, and it can also reduce fitness of the population by the removal of potentially important individuals for the deme (Harris et al. 2002). Therefore, managers need to consider these potential consequencnes in their trapping regulations.

Monitoring beaver populations every two years to determine which populations needed harvesting of surplus individuals can be effective, however, the first priority of beaver management needs to conserve the genetic structure and evolutionary pathway of each beaver population, and indirect contributions of beavers to riparian ecosystems (Harris and Aldous 1946, Grasse and Putnam 1950, and Patric and Webb 1954. The
incorporation of colonies’ carrying capacity into management plans of beavers is important to determine the number of animals, and the frequency, for beaver harvest. This plan avoids overexploitation of vegetation, allows the persistence of beaver colonies in areas already occupied, and prevent the expansion of the population into areas where beavers are unwanted (Yeager and Rutherford 1957).

More recently, demographic and ecological information has been incorporated into management plans for beaver harvest, to secure the ecological integrity of beaver populations, along with a balance between people and the presence of beavers, and to assure the benefits they provide to riparian ecosystems. A bioeconomic model that incorporates beaver population dynamics (i.e. growth and migration) into the design of a cost-minimizing trapping strategy is important because this model considers the cost of beaver damage (the cost to a government agency to trap the beavers), how fast the beaver population is growing, the migration patterns, and provides the least costly strategy to trap beavers which determines the colonies to be harvested, the number of individuals to be harvested, the time of the year, and how often to harvest (Bhat et al. 1993). However, this model has limitations, it requires abundant information on beaver population dynamics (which is not easy to obtain), each model is specific for one area and one point in time, and it assumes that all landowners involved in the development of the strategy agree to cooperate (often is not the case). The consideration of beaver activity and intensity is important for the management of beaver and riparian ecosystems. The estimation of the effect of beaver activity on different habitats in the Tierra del Fuego Archipelago, Argentina, suggested an intensive harvest of beavers in areas where
vegetation impacts were greater, and in the best areas for timber exploitation if densities of beavers were reaching carrying capacity (Lizarralde 1993). A comparison between the efficacy of night-vision surveys versus removal trapping to estimate colony size and composition of beaver populations in central Illinois revealed night-vision surveys to be helpful for monitoring spatial and temporal variations in colony size, but not for estimating composition of beaver colonies (McTaggart and Nelson 2003). Removal trapping is effective to obtain population trends of beavers, but because this technique is time consuming and expensive, a combination of live-trapping, marking of individuals, and observing individuals with night-vision equipment to monitor the populations is more effective (McTaggart and Nelson 2003). Another method to obtain demographic information of beaver colonies is the use of remote videography. This technique was used to obtain complete counts of kits, natality data, colony sizes, and interesting behavioral observations of six beaver colonies along the Mississippi River (Bloomquist and Nielsen 2009).

Today, the economic value of beaver and the market value of their pelts has decreased compared to a century ago. Currently beaver trapping is more for recreation or removal of nuisance individuals. However, there are still different methods reported for trapping beavers or for killing the animals without trapping them. A management plan to maintain beaver populations and harvest them for maximum sustainable yield was suggested as a way to preserve the art of hunting, however, this management plan had no ecological or evolutionary considerations for the beaver other than those necessary to maintain the populations throughout the years (Longley and Moyle 1963). There are also
different methods to shoot beavers without altering the pelt or meat quality (Parker et al. 2006). However, these methods do not suggest a specific management plan to apply these strategies, nor do they consider the ecological consequences of using these methods. Furthermore, shooting was not a very effective method as it is time consuming and requires special equipment such as boats, guns, river access (Hammerson 1994). In addition, shooting is not allowed in all 50 states, for this reason trapping is a more effective method to control beaver populations (Hammerson 1994).

Live trapping of beavers has also been suggested to control population densities by trapping the animals and translocating them to new areas (Harris and Aldous 1946, Grasse and Putnam 1950, Yeager and Rutherford 1957, DeStefano et al. 2006). However, translocations might not be as efficient to control beaver populations and mitigate conflicts with people if they are not carried out appropriately. A detailed protocol to conduct beaver translocations should consider the number of beavers to be relocated, and the number of colonies to be established, according to the total area for the relocation (Harris and Aldous 1946). In addition, to enhance the success of relocations there needs to be an a priori preparation of the release sites. For instance, one should locate a release site with ample food and building materials for the beavers, and consider the level and flow of water. Demographic, genetic, and ecological information is required for successful beaver relocations (Bradt 1947, Grasse and Putnam 1950, Yeager and Rutherford 1957, DeStefano et al. 2006, Padhi and Balakrishnan 2006). The population density of the original population, its reproductive rate, mortality, dispersal patterns, the carrying capacity of the release sites, distance of release sites from original colony
locations, and degree of harvest to maintain a stable population are factors that need to be considered in the management plans for beavers. Particularly, incorporating demographic and dispersal patterns into management plans to account for future increases in the population, to account for individuals that will be colonizing new areas, and to account for individuals that may cause conflicts with humans is important to assure the success of the management plan (Bradt 1947, DeStefano et al. 2006), Padhi and Balakrishnan 2006). However, the criteria to select the animals to be released at each site are not specified in the management plans reviewed. And, how many animals are suggested to be released per site, the sex ratio, or the relatedness among such individuals are not clear. The structure of new colonies could have important implications for relocation success and for the ecological and evolutionary pathway for the populations. As mentioned before, translocation of individuals can have important genetic consequences that need to be considered in the management plans of wildlife (Olden et al. 2004).

Additional information important in the development of beaver management plans is public perspective towards beavers and their effects on riparian ecosystems. Surveys conducted in different states, regarding public attitudes toward beavers and their management (McKinstry and Anderson 1999, Jonker et al. 2006, Jonker et al. 2009) have revealed the concerns people may have when beavers are found on their properties. In Wyoming, the surveys showed that landowners have conflicts with beavers when the croplands are either flooded or not irrigated as a result of beaver activity. However, landowners also acknowledged that beavers were important in Wyoming to increase the water reservoirs (McKinstry and Anderson 1999). Moreover, although landowners were
in favor of harvesting nuisance beavers, they also showed interest in the distribution of information on how to manage the beavers without killing them (McKinstry and Anderson 1999). In Massachusetts, people had a good attitude toward the aesthetic value of beavers (e.g. enjoyed seeing beavers in their backyard), and understood some of the benefits beavers provide (e.g. increased of water table). However, these attitudes changed when the number of beavers on their properties increased and damage to roads or crops occurred. When this situation arose, people were willing to pay licensed “nuisance” trappers or wildlife control agencies to remove or harvest the nuisance beavers from their properties (Jonker et al. 2006 and 2009). In order to reduce the conflicts between beavers and people it is important to gather more information regarding the ecology of the beavers in each region as well as the dynamics of each beaver population to be managed into the development of a plan to either reduce their population size or to conserve them and their habitat (McKinstry and Anderson 1999).

*Other methods to control beaver populations*

Other methods to eliminate or control beaver populations without trapping the animals include, poisoning, biological control (e.g. gray wolf, alligators), fertility control, and interspecific competition. In Quebec, Canada, beaver mortality due to wolf predation was 15%, whereas mortality due to trapping was slightly higher (20%; Potvin et al. 1992). The combined effect of trapping and predation could be used to manage beaver populations were high. On the other hand, predation of beavers by alligator in
southeastern United States has not been that effective to control beaver populations (Hammerson 1994).

Surgical sterilization of one adult per colony has been used as a method to control beaver populations. However, when both adult breeders remained in the colony and the sterilization method did not affect hormonal levels (i.e. tubal ligation) of the breeding pair, reproduction was partially inhibited (Brooks et al 1980). However, in those colonies in which one of the breeding adults was removed instead of sterilized, the progeny within the colony reproduced. Therefore, surgical sterilization is an ecologically feasible method to control beaver populations (Brooks et al. 1980).

The competition between beavers and other herbivores has also been suggested as a method to control beaver populations. In a mixed-wood boreal forest in Canada the competition between beavers and ungulates, was evaluated as a method to control beaver populations (Hood and Bayley 2008b). Beavers were able to modify and adapt their foraging behaviors to the presence of ungulates and competition. However, there should be a threshold at which the coexistence between ungulates and beavers does control beaver populations as it has been observed in other systems such as in Yellowstone National Park in the US (Hood and Bayley 2008b).

**Damage control**

In addition to methods to control beaver numbers, other methods have been suggested to reduce damage to roads, croplands etc. caused by beavers. For instance, exclusions have been suggested as an effective method to protect trees from beaver...
herbivory (Dudderar 1977, King et al. 1998). In Texas, most of the visible damage caused by beavers in a baldcypress (*Taxodium distichum*) ecosystem was on mature trees (King et al. 1998). Therefore, for long term management of the baldcypress, and to avoid further damage to mature trees, beaver exclusions needed to be implemented, and beaver colonies needed to be monitored every 3 to 5 years to estimate population size and the damage they might cause to the baldcypress ecosystem (King et al. 1998).

Another method tested to reduce damage to trees due to beaver herbivory is the use of chemicals (i.e. polyethylene glycol and fructose) to modify the palatability of the species beavers use for food, or the use of a deterrent treatment (i.e. casein hydrolysate) applied to the species of trees to be protected from beaver herbivory. A combination of these two methods is a feasible approach to decrease the use of important riparian species, and can facilitate the use of invasive species such as the tamarisk (Kimball and Perry 2008).

Another important consideration in the management plan of beaver, to reduce damage to anthropogenic structures, is the consideration of beaver habitat requirements, and beaver activity patterns. Including beaver ecological and behavioral information in the planning of city roads, bridges, etc., and in the elaboration of management plans to control beaver damage is important to assure an appropriate and successful beaver management plan (Curtis and Jensen 2004). When evaluating habitat use by beavers along roadsides, and the cues of those areas to serve as habitat for beavers in New York State, the probability of roadside beaver occupancy was inversely correlated to the proportion of roadside area devoid of woody vegetation. In addition, major limiting
factors for beaver occupancy were highways, agricultural areas, and residential areas (Curtis and Jensen 2004). Therefore, to generate a proactive management plan for beavers, highway departments and corresponding government agencies need to maintain open areas along highway corridors to discourage beavers from colonizing the area. In addition, assessment of beaver habitat at stream crossings on existing roads will aid highway departments to identify potential problematic areas before beaver colonization occurs (Curtis and Jensen 2004).

Other methods to discourage beavers from establishing a colony in a specific area are the removal of dams and the use of devices to maintain water at an undesirable level for beavers. However, these methods are not very effective. The removal of dams just makes the beavers build the dam again, or if the beavers decide to move from that location, they may relocate to a nearby area where they can still cause damage (Hammerson 1994, Loates and Hvenegaard 2008). In the case of the devices to control water level or to keep beavers away from culverts, these methods can be effective if they are installed adequately, however, most devices need to be installed by professionals and required monthly maintenance (Hammerson 1994).

**Management of beaver for ecosystem restoration and biodiversity enhancement**

After the serious depletion of beaver populations in the late 19th century, naturalists and researchers from the beginning of the 1900’s began to document the consequences of absent beaver activity in riparian ecosystems, and the benefits that beavers brought to such ecosystems (Müller-Schwarze and Sun 2003). In 1946,
reintroductions of beavers into specific locations of the Black Hills Mountains in South Dakota were conducted not only to restore beaver populations, but also to restore water levels and provide habitat for trout (Harris and Aldous 1946). Reintroductions took place in other regions of North America since the early 1900’s, but the protocol followed for the 1946 reintroductions in South Dakota was the first one to present a thorough method to conduct beaver reintroductions and monitor the success of the colonies, and provided valuable information on the benefits beavers bring to the ecosystem.

Beaver ponds enhanced edge formation within the floodplain mosaic promoting landscape heterogeneity and biodiversity in the Roanoke River floodplain of eastern North Carolina (Townsend and Butler 1996). In the same way, beavers promoted higher species diversity in the riparian areas of a boreal mixed-wood forest in Alberta, Canada, and beaver activity played an important role as a structuring agent of riparian vegetation (Martell et al. 2006). Therefore, for beaver and forest conservation, a forest buffer area of 30 m adjoining the water needs to remain intact, to provide long-term forage for beavers and good forest conditions (Martell et al. 2006). Beavers have also an effect in structuring baldcypress forests in Canada (King et al. 1998). Maintaining a balance between the presence of beaver and forest dynamics using beaver exclusions to protect mature trees with constant monitoring and control of beaver populations is needed to conserve baldcypress forests (King et al. 1998). Other studies have looked at the influence of beaver activity on riparian vegetation dynamics. Beaver activity is also an important factor for riparian trees recruitment, and establishment, in rivers with water flow and flooding regulation (Breck et al. 2003, Andersen and Shafroth 2010). Therefore, a
management plan for riparian vegetation should consider the relationship between water flow, flooding patterns, and beaver herbivory to enhance the recruitment of trees, and still have the benefits beavers bring to wetlands. Furthermore, monitoring density of beaver food caches (i.e. beaver relative abundance) can give trends in beaver abundance that could be incorporated into a better management plan for the riparian vegetation (Beck et al 2010).

Other environmental factors should be considered for the management and conservation of beavers and riparian ecosystems. Prescribed fire can have an impact on riparian vegetation and on beaver populations. In Elk National Park in Canada, repeated burning decreased beaver occupancy and number of beaver colonies. When fire was combined with drought and herbivory by ungulates, the results were also negative, beaver habitat was not enhanced and riparian biodiversity decreased (Hood et al. 2007). Even though prescribed fire is necessary for the regeneration of some woody plant species, such benefits depend on many factors (e.g. herbivory, fire severity, fire frequency) that need to be considered before fires are implemented. Therefore, managers need to understand the relationship between beaver and fire coexistence to allow a broad range of species existence in ever changing environments (Hood et al. 2007).

In addition to the influence beavers have on the vegetation, they also have an important impact on the water level. In southwestern Quebec, oscillations between water flooding and beaver activity were both important in shaping the wetland (Reddoch and Reddoch 2005). In a riparian area of the Rocky Mountain National Park beavers had a strong effect on the hydrology of riparian areas. Beaver dams and ponds increased the
depth, extent, and duration of floodings, and reduced the water table decline in the drier summer months (Westbrook et al. 2006). Considering the hydrological effects of beavers in management plans can aid the conservation of riparian vegetation, and the mitigation of conflicts between humans and beavers (Reddoch and Reddoch 2005, Westbrook et al. 2006).

Beavers can create and maintain habitat for species of fish (Collen and Gibson 2001). However, beavers can also not be so beneficial for fish in some situations, such as upstream migration, which can be difficult with the presence of beaver dams, or the warm temperature of water might not be suitable for certain species of fish. Because of such positive and negative effects for some species of fish and other riparian species, managers need to take into consideration and weigh both the positive and negative effects for the entire ecosystem, and elaborate a management plan that can balance such effects and conserve the target species of fish while maintaining beavers in the ecosystem (Collen and Gibson 2001). Beaver activity enhanced habitat for native puye (Galaxias maculates) in Cape Horn Reserve in Chile (Moorman et al. 2009). The native puye increased in abundance while invasive trout decreased. The challenge for managers in Cape Horn Reserve is to manage the beaver, an invasive species in Chile, in such a way that their numbers are reduced and it does not affect habitat for native species, but also maintaining the areas that have enhanced habitat for native puye (Moorman et al. 2009).

Beaver ponds have also been found to be beneficial for different species of amphibians such as the boreal chorus frog (Pseudacris maculata; Stevens et al. 2007), the wood frog (Rana sylvatica; Stevens et al. 2006 and 2007, Karraker and Gibbs 2009), the
mink frog (*Rana septentrionalis*; Popescu and Gibbs 2009), and the spotted salamander (*Ambystoma maculatum*; Karraker and Gibbs 2009). An increase in amphibian abundance, productivity, and recruitment occurs in areas with beaver activity. Management plans to conserve beaver habitat, which would provide habitat for amphibians and would aid in the conservation of amphibian species under conservation concern need to be created. In the same way, a higher duck number occurs in areas with beavers (7.5 ducks/km) compared to areas without beavers (0.1 ducks/km; McKinstry et al. 2001). In addition, the reintroduction of beavers to 13 different locations throughout Wyoming also promoted an increase in waterfowl habitat and in waterfowl numbers (McKinstry et al. 2001). Also a higher number of bird species occurred at abandoned beaver ponds than at active beaver ponds (Aznar and Desrochers 2008). Therefore, beaver cycles are needed to maintain vegetation cycles, and the promotion of species diversity in riparian areas. In western Massachusetts, numerous scrub–shrub bird species of conservation concern, such as the Chestnut-sided Warbler (*Dendroica pensylvanica*) and Gray Catbird (*Dumetella carolinensis*), use large beaver meadows (Chandler et al. 2009). Conservation of beavers is important to aid conservation of scrub–shrub bird species. However, managers need to generate management plans that prioritize conservation of mostly large beaver meadows for the conservation of scrub–shrub birds and reduce conflicts with people.

As described by the different management categories in this review, beaver management is not an easy task due to the different goals managers might have. Unquestionably, beavers have been key participants in structuring riparian ecosystems in
North America, however, because of the increase in human populations and the landscape changes humans have generated, the effects of beavers in shaping wetlands communities have also changed. Current management plans for beavers need to manage for the conservation of beaver populations and their contributions to riparian ecosystems, but also for the conservation of other species that might be affected by high beaver activity, and for the effects of beaver activity near human settlements. Managers need to follow an Adaptive Resource Management when elaborating a management plan for wildlife (Longcore et al. 2010). This method involves several steps, first the compilation of information about the issue to be managed, the ecosystem where the plan will be applied, and the scientific results from previous management plans. Then the specific objectives and goals need to be established, and based on such objectives a model to meet the objectives needs to be developed. After these steps are complete, the management plan can be implemented and after a given time, which has been previously establish in the model, the system needs to be monitored to evaluate the effectiveness of the management plan, and the data obtained need to be analyzed and evaluated. If these steps are not followed correctly, serious damage to an ecosystem can be done (Longcore 2009). In the Southwestern Riverside County Multi-Species Reserve in California, beavers were considered a disturbance for the habitat of least Bell’s vireo (*Vireo bellii pusillus*) and southwestern willow flycatcher’s (*Empidonax traillii extimus*). Due to that belief, and without further scientific research, beavers were removed with the purpose of protecting habitat for both endangered songbirds. Unfortunately the removal of the beavers did not directly result in an increase in abundance of the songbird species, the wetland water
level decreased at some locations, vegetation has changed since the removal of the beavers, numerous beavers were killed, and four beavers were translocated to a reserve in Texas (Longcore 2009). This study shows one example of the consequences of development and implementation of an uninformed management plan.

CONCLUSIONS

The American beaver has an important effect on riparian ecosystems in North America. Many species of animals and plants depend on beaver activity for habitat creation. Humans also benefit from beaver activity by an increase in the water level and water retention during dry months. Nevertheless, because beavers can significantly modify their surroundings they can cause damage to roads, croplands, fish populations, etc., which creates conflict with people. Beaver management needs to consider both the positive and negative effects of beaver activity on specific ecosystems. Managers need to develop a holistic approach that includes ecological, genetic and evolutionary information of beaver populations in the region, species that are benefited by the presence and activity of beavers, areas where beavers can cause damage to anthropogenic structures, areas where beavers can expand their populations, and long-term consequences of such expansions. Furthermore, it is important that managers have all the scientific information required to understand the ecological and evolutionary consequences of implementing management plans. In the case of the American beaver this should not be an impediment, as described in this review, beaver management has been carried on since the beginning of the 19th century. As a result, a vast amount of
information is available to determine the best methods to obtain demographic
information, estimate carrying capacity of beaver populations, and prevent and minimize
conflict with people. Genetic information of the beaver populations to be managed also
needs to be considered, currently there is not a wide amount of genetic information of
beaver populations, therefore more research on this matter is needed to assure an
appropriate management of the beaver, and to preserve the evolutionary characteristics of
the species. In addition, throughout the different management plans in the history of the
beaver in North America, the importance of considering the ecological and evolutionary
processes of populations has been shown repeatedly, therefore, managers need to
consider these processes a priority when managing beavers.

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Figure 1. Map of European settlements based on beaver trapping or fur trading activities during the 17th, 18th, and 19th centuries. Circles indicate the settlements established due to beaver populations. Shaded areas indicate the area explored by Europeans during that period of time in search of beaver populations.
Table 1. Beaver reintroductions and translocations in North America reported in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Source location</th>
<th>Source subspecies</th>
<th>Final destination location</th>
<th>Resident subspecies</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radford 1907</td>
<td>Ontario, Canada</td>
<td><em>C. c. acadicus</em></td>
<td>Adirondacks, New York</td>
<td><em>C. c. acadicus</em></td>
<td>1901-1906</td>
</tr>
<tr>
<td>Radford 1907</td>
<td>Yellowstone, Wyoming</td>
<td><em>C. c. missouriensis</em></td>
<td>Adirondacks, New York</td>
<td><em>C. c. acadicus</em></td>
<td>1907</td>
</tr>
<tr>
<td>Shaw 1984, DeGraaf 2001</td>
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Table 1. References resulting from the literature search and discussed in the text. The management categories follow those described in the text.

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APPENDIX B

DEVELOPMENT OF NINE NEW MICROSATELLITE LOCI FOR THE AMERICAN BEAVER, *CASTOR CANADENSIS* (RODENTIA: CASTORIDAE) AND CROSS-SPECIES AMPLIFICATION IN THE EUROPEAN BEAVER *CASTOR FIBER*


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Development of nine new microsatellite loci for the American Beaver, Castor canadensis (Rodentia: Castoridae) and cross-species amplification in the European Beaver Castor fiber

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Keywords: Castor canadensis, Castor fiber, cross-species amplification success, microsatellite, beaver

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Abstract

We developed nine new nuclear dinucleotide microsatellite loci for *C. canadensis*. All loci were polymorphic, except for one. The number of alleles ranged from two to four and from five to twelve in populations from Arizona and Wisconsin respectively. Average heterozygosity ranged from 0.13 to 0.86 per locus. Since cross-species amplification in *Castor fiber* was successful only in four loci, we tested also nine recently published *Castor canadensis* loci in the Eurasian species. Eight of the published loci amplified, however, three were monomorphic. The number of alleles was lower in *C. fiber* than in *C. canadensis* at all loci tested.
The American beaver (\textit{Castor canadensis}) is broadly distributed throughout North America and is considered to be an ecosystem engineer because it modifies surrounding habitat (Wright et al. 2002, Müller-Schwarze and Sun 2003). Ponds created by beaver can increase the number of species of herbaceous plants, provide habitat for waterfowl broods and fish reproduction (Naiman et al. 1988, Nummi 1992, Schlosser 1995, Barnes and Mallik 2001). However, alterations that beavers cause to their environment also can have negative economic impacts due to flooding (Müller-Schwarze and Sun 2003) and habitat modification. As a consequence, translocations and lethal methods have been used to control beaver populations. In addition, beavers have been hunted for their pelts since the early 1800s and in many regions of North America were virtually extirpated by 1900 (Müller-Schwarze and Sun 2003). Although there have been many studies of beaver ecology, little is known about the effects of hunting and management plans on the genetics of this mammal (Müller-Schwarze and Sun 2003). The number of microsatellite loci available is limited to ten in the American beaver (Crawford et al. 2008) and no loci have been described so far in the European sister species \textit{Castor fiber}. Therefore, we developed and characterized microsatellite DNA markers to investigate population structure, connectivity, and dispersal patterns of \textit{C. canadensis} populations. We also tested the cross-species amplification success of the published and the newly developed American Beaver loci in the European Beaver.

We constructed a genomic library following the protocol described by Glenn and Schable (2005). Genomic DNA was extracted using the DNeasy tissue kit (QIAGEN) from 1 cm$^3$ of tissue collected from beaver tails from Tres Rios Wetlands in Phoenix, Arizona (AZ).
The DNA was partially restricted with the enzyme RSAI and fragments were ligated (using T4 DNA ligase) to double-stranded SNX-24 linkers (Glenn and Schable 2005). To create a whole genome PCR library, linker-ligated fragments were PCR amplified using a SNX-24 forward primer and high fidelity DNA polymerase (Invitrogen). This library was hybridized to biotinylated microsatellite oligonucleotide probes (GT)$_{15}$, (CT)$_{15}$ and (GATA)$_8$. Hybridized fragments were captured on streptavidin-coated paramagnetic beads (Dynal). Microsatellite-enriched fragments were recovered by PCR and products were ligated and transformed using a TOPO TA cloning kit (Invitrogen). Approximately 270 colonies were amplified using M13 forward and reverse primers. We selected 200 non-redundant clones ranging from 500–1200 bp, as visualized on 1.5% agarose gels. Clones were sequenced using M13 primers on an ABI 3730xl genetic analyzer (PE Applied Biosystems) using BigDye Terminator. Ninety clones had recognizable microsatellite sequences, of which 44% (40) had adequate flanking regions to design primers. Forty primer pairs were designed using PRIMER 3 software (Rozen & Skaletsky 2000). The designed primer pairs were double-checked for homodimers, hairpins and heterodimers using OLIGO ANALYZER software (Integrated DNA Technologies; http://www.idtdna.com/analyser/Applications/OligoAnalyzer).

Of the initial 40 primer pairs designed, 31 failed to amplify, one locus was monomorphic (Cca 151), and eight loci amplified and were variable (Table 1). We assessed variation among 55 individuals from the Tres Rios population and 30 individuals from northcentral Wisconsin (WI). M13 primer sequences were added to the 5’ end of each forward primer (Schuelke 2000), and were used in combination with M13 fluorescently labeled primers.
PCR was performed in 15 μL volumes using 50-100 ng of genomic DNA, 1X PCR buffer (20 mM Tris-HCL, pH 8.4, 500 mM KCl, Invitrogen), 0.2 mM of each dNTP, 0.2 μM of each primer, 1 U of Taq DNA polymerase (Invitrogen), and a locus-specific conditions (Table 1). All PCRs were conducted using a Mastercycler Gradient (Eppendorf, Germany) and the thermal profile for loci Cca56, Cca76 and Cca112 was an initial denaturation at 94°C for 2 min followed by 30 cycles of 94°C for 30s, 30 s at the locus-specific annealing temperature (Table 1) and 72°C for 30s, followed by a final extension of 3 min at 72°C. The rest of the loci were amplified using a touch down protocol that consisted of an initial denaturation at 94°C for 2 min followed by 3 cycles at 94°C for 30 s, annealing at 60-50°C for 30 s (2°C decrease every three cycles), extension at 72°C for 30 s, followed by 30 cycles at 94°C for 30 s, 90 s at 50°C and 72°C for 30s, followed by a final extension for 2 min at 72°C. All PCR products were analyzed using an ABI 3730 DNA Analyzer with Gene Scan-500 LIZ size standard (Applied Biosystems).

We used the GENOTYPER (ABI) software to determine allele size, and genotypic disequilibrium between pairs of loci was tested using FSTAT 2.9.3 (Goudet 2001) and GENEPOP 1.2 (Raymond and Rousset 1995). Other population genetic parameters were estimated in ARLEQUIN (Excoffier et al. 2005) and loci were tested for null alleles using MICRO-CHECKER (Van Oosterhout et al. 2004). No linkage disequilibrium was observed in either the AZ or WI population after Bonferroni correction (α = 0.05, P > 0.05; Rice 1989). The number of alleles ranged from two to four per locus in the AZ population and from five to 12 in WI (Table 1). Average
heterozygosity ranged from 0.27 to 0.65 in the AZ population and from 0.13 to 0.86 in WI. No significant deviations from Hardy-Weinberg Equilibrium (HWE) were observed in the AZ population. However, six loci (Table 1) showed significant deviations from HWE ($\alpha = 0.05, P > 0.05$; Rice 1989) for the WI individuals due to a deficiency of heterozygotes, which may be due to the Wahlund effect (Templeton 2006, Table 1). No loci in the AZ population showed evidence of null alleles (all $P > 0.05$) but four loci in the WI individuals may have null alleles ($Cca15 P < 0.001$, $Cca20 P < 0.001$, $Cca56 P < 0.01$ and $Cca92 P < 0.01$).

It is known from mitochondrial DNA analyses (Piaggio AJ et al., unpublished) that the AZ population was established by closely related individuals, which could explain the relatively low polymorphism observed. The WI samples demonstrate that the new markers developed have reasonable diversity in other populations.

Cross-species amplification was performed for these primers and the Crowford et al. (2008) primers in the Eurasian beaver ($C. fiber$). Four newly developed and eight previously published loci successfully amplified (Table 2); all newly developed loci and 5 previously published loci were polymorphic in 10 $C. fiber$ individuals.

The development of these markers, and the cross species utility, will allow population level studies to be conducted to allow better management of these species and potentially allow for comparative studies between the species.
References


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Table 1 Characteristics of the nine microsatellites loci developed and optimized for *Castor canadensis*. Locus name, GeneBank accession number, primer sequences, cloned repeat, magnesium chloride concentration, Annealing temperature ($T_a$), size of cloned repeat, mean number of alleles per locus ($N_A$), observed ($H_o$), and expected ($H_E$) heterozygosities are reported. TDN indicates the use of touch down protocol.

<table>
<thead>
<tr>
<th>Locus</th>
<th>GeneBank Accession Number</th>
<th>Primer sequence (5'-3') F, forward; R, reverse</th>
<th>Repeat Motif</th>
<th>Mg$_2^+$Cl$_2$ (mM)</th>
<th>$T_a$ (°C)</th>
<th>Size (bp)</th>
<th>Allelic range</th>
<th>$N_A$</th>
<th>$H_o$</th>
<th>$H_E$</th>
<th>Allelic range</th>
<th>$N_A$</th>
<th>$H_o$</th>
<th>$H_E$</th>
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<td>EU703999</td>
<td>F:/PET/AAGTGAC TTTGGGCATTAA CC R: GGACACCTGGTG CAATCC F:/VIC/ CACTTGTC</td>
<td>(GT)$<em>{21}$ ATGTAT (GT)$</em>{3}$</td>
<td>3.0</td>
<td>TDN</td>
<td>249</td>
<td>241-243</td>
<td>2</td>
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<td>7</td>
<td>0.13</td>
<td>0.69</td>
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<td>EU704000</td>
<td>F:/VIC/ CCTCTGGGATCT GCACTGTC R: GTGGTTCAAGTG CAAGCAC</td>
<td>(CA)$_{14}$</td>
<td>3.0</td>
<td>TDN</td>
<td>305</td>
<td>303-309</td>
<td>2</td>
<td>0.42</td>
<td>0.48</td>
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<td>(CA)$<em>9$ TATA (CA)$</em>{10}$</td>
<td>3.2</td>
<td>54</td>
<td>262</td>
<td>218-256</td>
<td>4</td>
<td>0.65</td>
<td>0.65</td>
<td>224-250</td>
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<td>Accession</td>
<td>Primers</td>
<td>Forward/Reverse</td>
<td>Sequence</td>
<td>Product Size</td>
<td>Genotype</td>
<td>Heterozygote</td>
<td>Allele 1</td>
<td>Allele 2</td>
<td>Genotype 1</td>
<td>Genotype 2</td>
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<tr>
<td>Cca62</td>
<td>EU704003</td>
<td>F: NED/GCAAAGG ATTGAATAACC AAGTG R: CCAGGGTGGATG AATT TG</td>
<td>(GT)_3 GTTGCC (GT)_11</td>
<td>2.5 TDN 329 341-349</td>
<td>3 0.63 0.63 303-353</td>
<td>7 0.46</td>
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<td>EU704004</td>
<td>F: PET/CCCATAGAACC AAGCAG R: CAACTGACTGCA AATAGCTACAGC</td>
<td>(GT)_20</td>
<td>2.5 52 173 154-172</td>
<td>2 0.40 0.41 149-189</td>
<td>12 0.56</td>
<td>0.78</td>
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<tr>
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<td>EU704005</td>
<td>F: NED/TCTTGACTCTG GGCA TG R: CAGAGCTTCCACTATG TACAC GAC</td>
<td>(CA)_20</td>
<td>3.5 TDN 204 198-202</td>
<td>3 0.54 0.65 196-208</td>
<td>6 0.56</td>
<td>0.76</td>
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<td>(GT)_18</td>
<td>3.2 55 198 192-194</td>
<td>2 0.27 0.24 186-204</td>
<td>7 0.86</td>
<td>0.75</td>
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<td>(GT)_22</td>
<td>3.5 TDN 176 164-172</td>
<td>3 0.65 0.62 154-188</td>
<td>9 0.3</td>
<td>0.85</td>
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</tbody>
</table>

* - locus not in Hardy-Weinberg Equilibrium (P < 0.05).

**Bold** H_0 - indicates the likely presence of null alleles.
Table 2 Cross-species amplifications of the *Castor canadensis* loci in *Castor fiber*, including the number of individuals genotyped (N) and the number of alleles (N\textsubscript{A}).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele sizes (bp)</th>
<th>N</th>
<th>N\textsubscript{A}</th>
<th>N\textsubscript{Crawford et al.2008}</th>
<th>N\textsubscript{A Crawford et al.2008}</th>
</tr>
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<tbody>
<tr>
<td>Cca56</td>
<td>242-250</td>
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<tr>
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<td>157-189</td>
<td>10</td>
<td>3</td>
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<td>200-204</td>
<td>10</td>
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<td>Cca4*</td>
<td>384-396</td>
<td>57</td>
<td>6</td>
<td>60</td>
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<td>Cca5*</td>
<td>145-166</td>
<td>57</td>
<td>4</td>
<td>60</td>
<td>11</td>
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<tr>
<td>Cca8*</td>
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<td>7</td>
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<td>10</td>
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<td>57</td>
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<td>217-219</td>
<td>57</td>
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</table>

* - loci described in Crawford et al. 2008.
APPENDIX C

GENETIC DIVERSITY AFTER EXTIRPATION AND RECOLONIZATION OF POPULATIONS: THE CASE OF THE AMERICAN BEAVER (CASTOR CANADENSIS)

“The following paper is pending submission to the Journal of Mammalogy”

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Abstract    The American beaver is a semi-aquatic mammal found in lakes and streams throughout North America. The beaver is considered an ecosystem engineer due to the modification this mammal does to its environment. However, in the 1600’s the beaver was also considered an important fur-trading item which resulted in the overexploitation and near eradication of the species in the late 1800’s. By the beginning of the 20th century pelt traders and biologist conducted translocations and reintroductions to restore beaver populations. The purpose of this study was to assess if there was an impact of overexploitation and different management actions on the genetic diversity of the beaver in seven areas across North America. We used nine microsatellite loci to assess genetic diversity measures of 153 individuals from Alabama, Arizona, Maine, Minnesota, South Carolina, Texas, and Wisconsin. Our results show high genetic diversity (average observed heterozygosity was 0.613, and expected heterozygosity was 0.740), and high genetic differentiation (average $F_{ST}$ =0.128, 95% CI from 0.093 to 0.182; average $R_{ST}$ =0.199, $P <0.001$) across populations. No evidence of past population bottlenecks or isolation by distance could be detected. Despite the fact that beavers demonstrate a high genetic diversity in neutral markers, we recommend assessment of genetic diversity of other parts of the genome of this species, and that the substantial differentiation found among the populations studied be considered for future management and conservation plans of the beaver.

KEY WORDS: American beaver, bottleneck, ecosystem engineer, gene diversity, microsatellite loci
INTRODUCTION

The American beaver is the largest rodent of North America; its distribution includes ponds, lakes and streams from Alaska to northern Mexico, and all habitat types except arctic tundra and the Peninsular Florida ecosystems (Jenkins and Busher 1979). The beaver lives in colonies that usually consist of four to eight related individuals occupying a section of stream or a lake (Jenkins and Busher 1979). Beavers have been considered monogamous based on behavior and colony composition (Sun 2003). However, genetic data have showed that beavers are not always genetically monogamous (Crawford et al. 2008). In the same way, beavers present variation in their dispersion patterns. There are reports in which females show higher philopatry than males (Bergerud and Miller 1977, Crawford et al. 2008), whereas in other studies dispersal rate and distance travel between sexes was nearly equal (Bloomquist 2007, Havens 2006). Average natal dispersal also shows a wide range, from 4 to 30 km (Sun et al. 2000, Havens 2006). However, there have been reports in which relocated beavers have traveled 238 km (Hibbard 1958) and 241 km (Libby 1957) from the release points.

Beavers are herbivores that have the ability to cut trees which allows them to build dams and lodges (Jenkins and Busher 1979). Because of this ability to modify their habitat to meet their requirements, beavers are considered ecosystem engineers (Naiman et al 1986, Naiman et al 1988). Dams and ponds created by beavers have important effects not only on the aquatic community structure, but also on the entire riparian ecosystem by
providing habitat for invertebrates, fish, and birds (Wright et al. 2002, Naiman et al. 1988).

In addition to the ecological importance of the American beaver, this species had a substantial influence on the Native American culture, and on the establishment of human settlements (Martin 1978). For instance, for Native Americans of northeastern United States and eastern Canada, the beaver was considered the sacred center of the land because this mammal created habitat for the rest of the wetland community (Martin 1978). When Europeans began to settle in North America, beaver pelts represented an important fur trade item between North America and Europe from the mid-1600s to late 1850s. In some areas, high beaver abundance determined where Europeans founded their first cities and fur trading ports, which in turn, had a dramatic effect on beaver populations, driving the American beaver near extirpation throughout North America (Müller-Schwarze and Sun 2003). By the late 1800s, the serious decline in beaver populations caused concern among fur traders and other early North American settlers regarding beaver status in many areas. As a result, during the early 1900s, several translocations and reintroductions took place in different areas of North America to restore beaver populations (Appendix A). For instance, between 1901 and 1906, 20 beavers from the Province of Ontario, Canada, were released into the Adirondacks, New York (Radford 1907). After the success of this reintroduction, 14 beavers from Yellowstone National Park, Wyoming were translocated to several areas of the Adirondacks (Radford 1907). In New England, beavers where extirpated in the late 1700’s and there were no signs of beaver presence in the region until 1928, when a
couple of beavers naturally re-colonized an abandoned pond in Massachusetts. After this event, inhabitants of Massachusetts and nearby states started reintroductions of beavers from New York into New England in 1932 and in following years (Shaw 1984, DeGraaf 2001). In Wisconsin, between 1850 and 1947, trapping seasons were closed and beaver translocations were implemented to replenish the extirpated populations in that state. In South Carolina, between 1939 and 1941, six beavers from Georgia were reintroduced to the Sandhills Wildlife Refuge in Chesterfield County (South Carolina Department of Natural Resources 2010). And in Arizona, between 1940 and 1950, a few beavers were translocated from Springerville City and Pima City, Arizona, to the Chiricahua Mountains, and from the White Mountains to the Graham Mountains (Carrillo et al. 2009).

These management actions certainly recovered beaver populations throughout the United States and Canada. However, little is known about the effects of these translocations and reintroductions on the genetic diversity of the American beaver. Translocation of individuals can increase the potential for intraspecific hybridization, or mixing of previously differentiated gene pools (Stockwell et al. 1996, Olden et al. 2004). Molecular techniques have aided to study species-level genetic diversity to elucidate the effects of translocation and reintroduction on genetic diversity of recipient populations (Stockwell et al. 1996, Moritz 1999). For instance, the bridled nailtail wallaby (Onychogalea fraenata) in Australia, showed a significant reduction in allelic diversity of all translocated animals and their wild-born progeny compare to remnant natural populations (Sigg 2005). Another example is the Florida crocodile (Crocodylus acutus)
where human-mediated migration of crocodiles from Latin America and the Greater Antilles caused inter- and intra-specific hybridization and unexpected population structure (Rodriguez et al. 2010). These hybridization events can contribute to the loss of genetically distinct populations and a reduction in individual fitness within affected populations (Rhymer and Simberloff 1996).

Therefore, understanding the impact of genetic diversity on the persistence of populations post-translocation or post-reintroduction has become an important consideration to guarantee the success of such management actions (Stockwell et al. 1996). Translocation programs should include a genetic assessment of the selected animals for release, and genetic monitoring of the established population over time to detect and evaluate potential changes in genetic diversity and their effect on the long term survival of the population (Goossens et al. 2002).

In the case of the American beaver, the translocations and reintroductions carried out during the early 1900s did not take into account population genetics of both the source animals and recipient populations (Müller-Schwarze and Sun 2003). Such management actions resulted in some mixing of non-closely-related populations, which potentially could have resulted in negative ecological, genetic, and evolutionary consequences for the American beaver (Hall and Kelson 1981). The genetic diversity of beaver populations following former management actions is not known. Therefore, the objectives of this study were to examine genetic diversity of beavers in seven geographic areas across North America (Alabama, Arizona, Maine, Minnesota, South Carolina, Texas, and Wisconsin; Fig. 1) where translocations and reintroductions took place during
the 1900’s. This assessment of genetic diversity can aid evaluating the genetic effects of future population changes, and can guide conservation management actions that intend to conserve defined target levels of genetic diversity (Lawrence et al. 2008).

**METHODS**

*Samples collection*

In collaboration with Wildlife Services (USDA), we collected a total of 153 tissue samples from wild beavers captured in field ecology projects from Alabama, Arizona, Maine, Minnesota, South Carolina, Texas, and Wisconsin (Fig. 1). We collected all tissue samples in either blood buffer (1M Tris-HCl, 0.5M EDTA, 4M NaCl, 0.5M SDS) or 95% ethanol and stored all samples at -20°C until DNA extraction was performed.

*DNA isolation and amplification*

Genomic DNA from tissue samples was extracted using approximately 25 mg of tissue and a DNeasy blood and tissue kit following the manufacturer’s protocol (Qiagen Inc., Valencia, CA). We used nine microsatellite DNA loci developed for the American beaver (Crawford et al. 2008, Pelz et al. 2009). All microsatellite loci contain dinucleotide repeats except Cca8, which has a tretranucleotide repeat (Table 1). PCR amplification of the microsatellites followed the conditions described by Crawford et al. (2008) and Pelz et al. (2009).
From the 153 samples collected, 117 individuals successfully amplified for all nine loci. Microsatellite allele sizes were analyzed and genotyped using the ABI software program GENOTYPER (version 1.1). To assess genetic variation within populations, we estimated observed heterozygosity ($H_o$), expected heterozygosity ($H_E$), mean observed number of alleles ($N_a$), number of private (unique) alleles per region ($P_A$), mean allelic richness per locus ($N_r$), and levels of inbreeding $F_{IS}$, using the software program FSTAT (Goudet 2001). We estimated population subdivision using $F_{ST}$ as implemented in ARLEQUIN 3.1 (Excoffier, et al. 2005). However, because $F_{ST}$ values have been questioned as accurate measures of differentiation for highly polymorphic loci such as microsatellites (Jost 2008), we also calculated a standardized measure of genetic differentiation ($G'ST$) (Hedrick 2005) using the software program SMOGD 1.2.5 incorporating 1,000 bootstrap replicates (Crawford 2009). In addition, we used RSTCALC (Goodman 1997) to estimate standardized $R_{ST}$, which corrects for variance among loci and sample sizes among populations.

To further estimate population differentiation we applied a Mantel test to assess correlation between genetic and geographic distances among beaver individuals. Mantel tests assume that a single process generates the pattern of correlation between variables. However, this assumption may be violated, in a population genetics context, if different processes among distinct genetic groups control gene flow and genetic distances (Fortin and Dale 2005). We used pairwise estimates of $F_{ST}$ and $R_{ST}$ among populations to perform the Mantel test as implemented in GENEPOP 4.1 (Raymond and Rousset 1995, Rousset 2008).
**Population structure analyses**

The extent of genetic structure among the beaver populations was assessed using program STRUCTURE 2.3.2 (Pritchard et al. 2000), which uses a Bayesian approach to identify populations or genetic clusters \( K \), and assigns individuals to groups. Analyses were conducted with 100,000 iterations of burn-in followed by 200,000 repetitions of Markov Chain Monte Carlo (MCMC) for 10 independent runs from \( K = 1 \) to \( K = 8 \). The analyses were ran using four different models: 1) admixture with correlated allele frequencies, 2) admixture with uncorrelated allele frequencies, 3) non-admixture with correlated frequencies, and 4) non-admixture with uncorrelated frequencies. To determine the optimal number of populations we selected the number of clusters by looking at the highest likelihood values (from the mean of the 10 runs) as well as the highest \( \Delta K \) value. We used both values because some evidence has suggested the likelihood method is not always accurate (Evanno et al. 2005). The value of \( \Delta K \) is based on rate of change in the log probability of data between successive \( K \) values, which provides a better estimate of the true number of genetic clusters (Evanno et al. 2005).

We also used R and GENELAND (Guillot et al. 2005a, Guillot et al. 2005b) to infer the number of populations. This method uses georeferenced multilocus genotypes to infer the number of populations, and the spatial location of spatial discontinuities between the populations found (Guillot et al. 2005b). For this analysis we used 10 independent MCMC runs of the spatial model using 100,000 iterations for \( K = 1 \) to \( K = 8 \). We ran the model with uncorrelated and correlated allele frequencies.
To detect the presence of past population bottleneck we performed a heterozygosity excess test using the software program BOTTLENECK (Cornuet and Luikart 1996). This test is based on the fact that during a population bottleneck, allelic diversity is reduced faster than heterozygosity, because the loss of rare alleles does not make a large contribution to overall expected heterozygosity (Cornuet and Luikart 1996). This test assumed three different mutation models, the infinite allele model (IAM), the two-phased model (TPM), and the stepwise mutation model (SMM; Piry et al. 1999). The significance of the deficiency was calculated using a Wilcoxon singed rank test (Piry et al. 1999).

RESULTS

Sample collection and DNA amplification

We successfully extracted DNA from 153 samples from Alabama (AL), Arizona (AZ), Maine (ME), Minnesota (MN), South Carolina (SC), Texas (TX), and Wisconsin (WI). From the total number of samples, 76.5 % ($n = 117$) successfully amplified for all nine microsatellites used in this study.

Genetic diversity

The nine microsatellite loci surveyed in 117 individuals were highly polymorphic and informative. The mean number of alleles per locus was 7.2 (range from 5.77 to 9.66). Mean $H_E$ across populations was 0.740, with SC showing the lowest genetic diversity ($H_E = 0.717$) and WI the highest ($H_E = 0.773$) (Table 2). Mean allelic richness, which is a
measure of allelic diversity correcting for differences in sample size using rarefaction (Kalinowski 2004, Salgueiro et al. 2008), also indicated lower genetic variability in SC (Table 2). In the same way, a linear regression between sample size and $H_E$ ($r^2 = 0.246$, df = 5, $P=0.257$) further supports the lower genetic diversity of SC regardless of sample size. Mean $H_O$ across populations was 0.613. Alabama showed the lowest $H_O$ (0.522) with TX the highest ($H_O = 0.679$) (Table 2). Private alleles ($PA$) were found in all sampled populations with a total of 31 private alleles, WI had the highest number of $PA$ (13), whereas SC and TX only presented one private allele each (Table 2).

The pairwise $F_{ST}$ estimates suggest genetic structure ($P < 0.001$). Average $F_{ST}$ across all loci and all populations was 0.128 (95% CI from 0.093 to 0.182). The largest pairwise $F_{ST}$ estimate was between AL and ME ($F_{ST} = 0.163$), while the smallest pairwise $F_{ST}$ estimate was between MN and ME ($F_{ST} = 0.073$) (Table 3). The values for $G'_{ST}$ also suggested structure across geographic regions ($P < 0.001$) (Table 4). However, using the $G'_{ST}$ estimator, the largest genetic differentiation was between AL and WI ($G'_{ST} = 0.594$), and the smallest was between AL and TX ($G'_{ST} = 0.276$) and also TX and SC ($G'_{ST} = 0.278$). The standardized values of $R_{ST}$ genetic differentiation were smaller than those obtained by $F_{ST}$ estimates. Nevertheless, all $R_{ST}$ values were statistically significant ($P < 0.05$) (Table 3), except for the pairwise value between TX and ME ($R_{ST} = 0.058$, $P = 0.066$). The average $R_{ST}$ value across all loci and all populations was 0.199 ($P < 0.001$). The largest pairwise $R_{ST}$ value was between AL and WI ($R_{ST} = 0.222$) while the smallest $R_{ST}$ value was between ME and MN ($R_{ST} = 0.047$).
The Mantel test for correlation between linear distance (km) among the seven populations and genetic distances (using two different distance estimators, $F_{ST}$ and $R_{ST}$) showed that genetic differentiation did not increase with geographical distance ($r = 0.317, P = 0.274; r = 0.253, P = 0.134$, respectively) (Fig. 2).

**Population structure**

The Bayesian population assignment analyses using STRUCTURE 2.3.2 showed that individuals in this study are most consistent with six genetic groups, based on the combined microsatellite genotypes, following the mean likelihood and $\Delta K$ analyses (Evanno et al. 2005) (Fig. 3). The six genetic groups were consistent among the four models used (i.e. admixture-correlated, admixture-non correlated, non-admixture-correlated, non-admixture-non correlated) in the analyses. The six groups structured as follows: 1) South Carolina, 2) Texas and Alabama, 3) Maine, 4) Minnesota, 5) Arizona, and 6) Wisconsin (Fig. 5). Similar analyses performed using GENELAND gave slightly different results (Fig. 4). The model of correlated allele frequencies resulted in the seven genetic groups (with Texas and Alabama being separated), while the model of uncorrelated allele frequencies gave four genetic groups structured as follow: 1) South Carolina, Texas and Alabama, 2) Maine and Minnesota, 3) Arizona, and 4) Wisconsin.

The analyses performed using BOTTLENECK did not show significant recent bottlenecks in any of the sampling populations used in this study. The results of the one-tailed Wilcoxon signed-rank test to detect heterozygote excess were not significant for the three models ($P > 0.05$; Table 5) used in these analyses. For TPM, the default
condition of the BOTTLENECK software (proportion of 70% SMM in TPM) resulted in zero loci with excess of heterozygotes for either population. After parameter corrections, 2 loci were detected with excess of heterozygotes under IAM, and TPM only with a proportion of 10% SMM in TPM for Wisconsin, and Arizona. However, the one-tailed Wilcoxon signed-rank test was still not significant ($P > 0.05$; Table 5).

**DISCUSSION**

The American beaver was almost extirpated from North America at the beginning of the 20th century. In some areas beavers were not seen in riparian ecosystems for several decades (Müller-Schwarze and Sun 2003). This decrease in population size could have impacted the survival of the species because small populations lose genetic diversity more rapidly due to genetic drift and inbreeding (Frankham 2005). However, our analyses showed the American beaver did not follow this pattern.

Despite the near eradication of the beaver from North America, the results from our study show this species recovered rapidly from low population numbers and retained high levels of genetic diversity. Populations that expand rapidly after a population bottleneck do not lose heterozygosity because they only lose rare alleles. This lost does not make a large contribution to overall expected heterozygosity, and this excess in heterozygosity can be an indicator of population bottlenecks (Cornuet and Luikart 1996). However, our analyses fail to detect a recent bottleneck in any of the analyzed sampling locations. This could be explained because the detection of an excess of heterozygotes is higher when only few generations have passed since the bottleneck (Luikart and Cornuet
In the case of the American beaver, the bottleneck caused by the over harvesting of the species was around 100 years ago, which is about 30 to 40 generations (Müller-Schwarze and Sun 2003). Therefore, the evidence of the bottleneck may not be detectable using excess heterozygotes due to an increase in population size, gene flow levels, and new mutations in these rapidly evolving loci since the time of the bottleneck (Nei et al. 1975). This method to detect bottlenecks has limitations and caution should be applied when interpreting the results if the demographic history of populations is unknown (Whitehouse and Harley 2001).

The high heterozygosity present in American beaver individuals surveyed for this study (Average $H_E = 0.740$) agrees with a previous study of two beaver populations in Illinois, which analyzed seven microsatellite loci and showed a mean heterozygosity of 0.663 (Crawford et al. 2009). The high genetic diversity retained by the American beaver is remarkable for a species that was seriously depleted. Usually, species that have undergone severe bottlenecks, such as the Florida panther ($Puma concolor coryi$; Driscoll et al. 2002), the northern elephant seal ($Mirounga angustirostris$; Weber et al. 2000) or the greater prairie chicken ($Tympanuchus cupido$; Bellinger et al. 2002), exhibit low genetic diversity and evidence of past bottlenecks in rapidly evolving genetic markers such as microsatellites. However, the degree of loss of genetic diversity in a species depends on the bottleneck size (number of individuals during the bottleneck), the duration of the bottleneck in generations, and the intrinsic rate of growth for the species (Nei et al. 1975). In the case of the American beaver, there is not precise information of the number of remaining beaver populations in North America at the end of the 19th
century, nor how large those populations were. The population sizes could have been large enough to prevent the American beaver from suffering genetic effects from bottleneck events. In addition, beaver populations across their geographic range were extirpated at different points in time which might have allowed some individuals to migrate to inaccessible areas where hunters were not able to find them, which was also aided by high levels of connectedness among few major drainages throughout their range, such as the St. Lawrence seaway, the Mississippi and Missouri Rivers. This could have given the beaver time to colonize new areas and recover in numbers. Moreover, the efforts to recover beaver populations in the early 1900’s boosted some remaining populations, reducing the duration of some bottleneck event, bringing population above a critical population size (Amos and Harwood 1998). Other species have showed similar patterns after population bottlenecks. For instance, elephants (*Loxodonta africana africana*) in the Kruger National Park, South Africa, suffered a sever bottleneck by 1900, however, this population increased rapidly due to immigration from Mozambique which allowed this population to retain a higher genetic diversity compared to other elephant populations in South Africa (Whitehouse and Harley 2001). White-tailed eagles (*Haliaeetus albicilla*) in Europe have also retained high genetic diversity after sever population bottlenecks during the twentieth century (Hailer et al. 2006). In the case of white-tailed eagles migration had a minor impact on the retention of genetic diversity, but the long generation time of eagles allowed to buffer and slow the loss of genetic diversity (Hailer et al. 2006). The American beaver has showed long distance dispersal events
(Libby 1957, Hibbard 1958) which could have helped in boosting population size after the bottleneck.

Although we found high genetic diversity among our analyzed individuals, there is no evidence presently of high gene flow among the geographic regions (average $F_{ST} = 0.128$, $R_{ST} = 0.199$). Additionally we did not detect a pattern of isolation by distance, another indicator of gene flow between nearby population, so the lack of isolation by distance is consistent with the lack of gene flow among the geographic regions. The pattern of limited gene flow observed could be a sampling effect, and due to some populations having small sample sizes (9, 10, 13 for TX, AL, ME respectively), or due to not having a continuous distribution of beaver samples throughout their range. The lack of high gene flow among populations is also supported by the population structure analyses. The analyses in STRUCTURE identified six major genetic groups, while GENELAND identified seven groups when allele frequencies were considered correlated and four groups when allele frequencies were considered uncorrelated. When GENELAND identified four groups, SC, TX, and AL formed a group. This seems possible as the Alabama population is largely composed of alleles from the TX and SC populations, and does not contain its own unique combinations of alleles (Figure 5). The model with allele frequencies uncorrelated assumes the allele frequencies are statistically independent across populations. This is a computationally convenient assumption, but might not accurately reflect the statistical distribution of allele frequencies (Guillot 2008). The model that considers allele frequencies to be correlated across populations assumes that present time populations are the result of the split of a putative ancestral population
into $K$ populations that then underwent random and independent drift (Guillot 2008). The advantage of this model is that biologically it is more accurate. In the case of the beaver individuals in this study, given the low gene flow estimated among geographic regions, the models that are biologically more reasonable are the presence of six or seven groups, the difference between these two scenarios is whether AL is combined with TX or not.

The high level of genetic structure found among beaver populations can be explained by the dispersal ability of the beaver. Even there have been records of remarkable long distances traveled by beavers (Hibbard 1958, Libby 1957), the average dispersing distance traveled by beavers ranges from 4 to 30 km (Sun et al. 2000, Havens 2006). Dispersing individuals of the American beaver follow the rivers or wetlands, however, habitat loss and fragmentation can reduce the possibilities for dispersing individuals to survive and settle in new areas, which reduces gene flow among populations (Lande and Barrowclough 1987, Lande 1999). In addition, beavers are social animals that form small colonies, also reducing the number of migrants. (Müller-Schwarze and Sun 2003). This could further explain the low levels of gene flow detected among geographic regions in this study, particularly for neighboring geographic areas such as MN and WI. On the other hand, the fact that MN and ME show the smallest genetic distance indicates some gene flow between these two non-neighboring areas. This pattern could be explained by looking at the record of beaver translocations at the beginning of the 1900’s (Appendix A). Beavers from Ontario and Wyoming were reintroduced into New York State (Radford 1907) repopulating this and nearby areas, transferring genetic variation from the central plains into the northeastern part of the
United States. The genetic consequences of these reintroductions might still be reflected in the current genetic variation and structure of North American beaver populations (Appendix A).

Our results show that genetic diversity in the American beaver is not impoverished. Despite the almost extirpation of this species from North America, the beaver managed to recover from this widespread demographic event. Lizarralde et al. (2008) reported high genetic diversity for two mitochondrial DNA regions ($H_E = 0.758$ for the cytochrome $b$ gene, and $H_E = 0.87$ for the control region D-loop) in the population of exotic American beavers in Tierra del Fuego, Argentina, exemplifying the ability of beavers to recover from a population bottleneck in the form of a founder event. This population was founded in 1946 by 25 pairs of beavers and currently has a population size of 100,000 individuals. Apparently the founding population size did not negatively affect the viability and fertility of individuals (Lizarralde et al. 2008). Our findings also indicate the American beaver has made a successful genetic recovery following a recent population bottleneck. However, the fact that the American beaver shows high genetic diversity at the mitochondrial DNA and microsatellite loci levels, does not mean that dramatic reductions in population size did not affect other regions of their genome such as the Major Histocompatibility Complex (MHC) a series of genes important for immunity and disease resistance. Russian populations of the Eurasian beaver (*Castor fiber*) show substantial polymorphism at minisatellite DNA loci but not at MHC loci. In the same way, Scandinavian beaver populations show low polymorphism at minisatellite loci, and complete monomorphism at MHC class I and II loci (Ellegren et al. 1993).
Our study had limitations such as small sample sizes for some populations analyzed and we did not have the entire representation of the American beaver range throughout North America. Beaver populations from the central plains and the west coast of the United States, as well as samples from Canada, need to be included to have a full representation of the genetic diversity of the American beaver. Nevertheless, the high genetic diversity and differentiation among populations found in this study provides important insights on the ecology and evolution of the beaver. The beaver does not have a long generation time to buffer the effects of population bottlenecks like other species such as the white-tailed eagle (Hailer et al. 2006), black rhinoceros (Swart et al. 1994) or elephants (Whitehouse and Harley 2001), therefore the high genetic diversity found in our study could be explained by other factors such as migration patterns, a extraordinary genetic diversity prior the bottleneck, or a fast reproductive rate and high reproductive success (Nei et al. 1975). These characteristics show the ecological plasticity of the beaver, which can be critical to the evolutionary survival of a species, particularly in unstable environments (Smith and Skúlason 1996, Michaux et al. 2005). The understanding of the ecological plasticity of a species is essential for its appropriate management. Thus, the information provided in this study can help in the management and conservation of the beaver. For instance, translocation of individuals did help in the recovery of the beaver after the extreme reduction of their populations as it has been also observed in the recovery of other species (Madsen et al. 1999). However, long distance translocations may not preserve the distinct evolutionary pathways and potential ecological adaptations of current beaver populations in North America. Therefore, we
suggest that if beavers are translocated, these actions need to consider the genetic characteristics of the animals to be moved, as well as the genetic structure of the recipient population. Furthermore, understanding the evolutionary processes contributing to the maintenance of genetic diversity in North American beavers will aid the conservation of small and threatened beaver populations, such as the remnant populations in northern Mexico, where the beaver is considered in danger of extinction (SEMARNAT 2010).

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SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES. 2010. Life’s better outdoors. Beaver in South Carolina. South Carolina Department of Natural Resources. South Carolina.


Figure 1. Sampling locations (dots) of the seven geographic regions in this study for the American beaver (*Castor canadensis*).
Figure 2. Isolation by distance analyses for the seven populations of the American beaver. a) The $F_{ST}$ genetic distances against the transformed geographic distances (km). b) The $R_{ST}$ genetic distances against the transformed geographic distances (km).
Figure 3. Results showing the most likely number of genetically distinct groups ($K$) within the dataset according to the analyses of STRUCTURE 2.3.2. a) Shows the mean log-likelihood $\text{Ln}(K)$ for each estimated $K$. b) Shows the estimated Delta $K$ ($\Delta K$) statistic for each genetically distinct group.
Figure 4. Results showing the most likely number of genetically distinct groups ($K$) within the dataset according to the analyses of GENELAND. a) Shows the estimated number of populations ($K$) according to the model of correlated allele frequencies. b) Shows the estimated number of populations ($K$) according to the model of uncorrelated allele frequencies.
Figure 5. Major six genetic groups identified by STRUCTURE for the seven populations of American beaver. The numbers indicate the original populations as follows 1. SC, 2. TX, 3. ME, 4. MN, 5. AL, 6. AZ, and 7. WI.
## Table 1. Microsatellite DNA loci used for the population genetic structure analyses.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Developed by</th>
<th>Primer sequence (5'-3') F, forward; R, reverse</th>
<th>Repeat Motif</th>
</tr>
</thead>
</table>
| Cca15 | Pelz et al. 2009 | F: AAGTGACCTTTGGGCATTAACC  
 R: GGACACCTGGTGCAATCC  
 (GT)₂₁ ATGTAT (GT)₃ |  |
| Cca20 |  | F: CCTCTGGGATCTGCACTGTC  
 R: GTGGTTCAAGTGCAAGCAG  
 (CA)₁₄ |  |
| Cca56 |  | F: GCAGAGCACCAATAAAATCC  
 R: CTGGACTCTTTGGAAACGCC  
 (CA)₉ TATA (CA)₁₀ |  |
| Cca76 | Pelz et al. 2009 | F: CCCATAGAACCACCAAAGCAG  
 R: CAACTGACTGCAAATAGCTACAGC  
 (GT)₂₀ |  |
| Cca92 |  | F: TCCTTGACTCTGGGCATG  
 R: CAGAGCTTCCACAGTATCTGG  
 (CA)₂₀ |  |
| Cca112 |  | F: CCCCCTTTAGTGGTTCTTT  
 R: GACTTTGCTGGTTTAGAGGTTG  
 (GT)₁₈ |  |
| Cca122 |  | F: ACCAAGTGAATACATACCCTAC  
 R: CAAGTATACAGTTGTCAGCATGTTAC  
 (GT)₂₂ |  |
| Cca8  | Crawford et al. 2008 | F: GGGGCTCAGAGGAAAAAGGAG  
 R: GATCAGGCAAAAGGCTGGTA  
 GATA₁₂ |  |
| Cca10 |  | R: TGCAGAACAAGAAAATTTGAAAG  
 TC₁₉ |  |
Table 2. Genetic diversity measurements for the seven populations. N denotes sample size, \( N_a \) indicates mean number of alleles, \( PA \) is the number of private alleles, \( N_e \) is the effective number of alleles, \( H_O \) is the observed heterozygosity, \( H_E \) is the expected heterozygosity, \( N_r \) is the mean allelic richness per locus, and \( F_{IS} \) is the level of inbreeding.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>( N_a )</th>
<th>( PA )</th>
<th>( N_e )</th>
<th>( H_O )</th>
<th>( H_E )</th>
<th>( N_r )</th>
<th>( F_{IS} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>18</td>
<td>5.889</td>
<td>1</td>
<td>3.507</td>
<td>0.623</td>
<td>0.717</td>
<td>4.935</td>
<td>0.134</td>
</tr>
<tr>
<td>TX</td>
<td>9</td>
<td>5.777</td>
<td>1</td>
<td>3.842</td>
<td>0.679</td>
<td>0.743</td>
<td>5.778</td>
<td>0.091</td>
</tr>
<tr>
<td>ME</td>
<td>13</td>
<td>7.222</td>
<td>3</td>
<td>4.279</td>
<td>0.590</td>
<td>0.747</td>
<td>6.353</td>
<td>0.218</td>
</tr>
<tr>
<td>MN</td>
<td>18</td>
<td>8.000</td>
<td>7</td>
<td>4.951</td>
<td>0.648</td>
<td>0.749</td>
<td>6.410</td>
<td>0.138</td>
</tr>
<tr>
<td>AL</td>
<td>10</td>
<td>6.222</td>
<td>2</td>
<td>3.743</td>
<td>0.522</td>
<td>0.719</td>
<td>5.940</td>
<td>0.285</td>
</tr>
<tr>
<td>AZ</td>
<td>22</td>
<td>7.667</td>
<td>4</td>
<td>4.024</td>
<td>0.626</td>
<td>0.733</td>
<td>5.786</td>
<td>0.149</td>
</tr>
<tr>
<td>WI</td>
<td>27</td>
<td>9.667</td>
<td>13</td>
<td>4.953</td>
<td>0.601</td>
<td>0.773</td>
<td>6.537</td>
<td>0.226</td>
</tr>
</tbody>
</table>

Table 3. \( F_{ST} \) values (below diagonal) and standardized \( R_{ST} \) values (above diagonal) for all pairwise comparisons between the seven populations of \( C. canadensis \).

<table>
<thead>
<tr>
<th>Population</th>
<th>SC</th>
<th>TX</th>
<th>ME</th>
<th>MN</th>
<th>AL</th>
<th>AZ</th>
<th>WI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>-</td>
<td>0.052</td>
<td>0.107</td>
<td>0.127</td>
<td>0.144</td>
<td>0.192</td>
<td>0.183</td>
</tr>
<tr>
<td>TX</td>
<td>0.082</td>
<td>-</td>
<td>0.058*</td>
<td>0.109</td>
<td>0.112</td>
<td>0.193</td>
<td>0.157</td>
</tr>
<tr>
<td>ME</td>
<td>0.161</td>
<td>0.082</td>
<td>-</td>
<td>0.047</td>
<td>0.138</td>
<td>0.102</td>
<td>0.089</td>
</tr>
<tr>
<td>MN</td>
<td>0.135</td>
<td>0.101</td>
<td>0.073</td>
<td>-</td>
<td>0.108</td>
<td>0.103</td>
<td>0.079</td>
</tr>
<tr>
<td>AL</td>
<td>0.111</td>
<td>0.09</td>
<td>0.163</td>
<td>0.111</td>
<td>-</td>
<td>0.055</td>
<td>0.222</td>
</tr>
<tr>
<td>AZ</td>
<td>0.161</td>
<td>0.135</td>
<td>0.13</td>
<td>0.089</td>
<td>0.098</td>
<td>-</td>
<td>0.212</td>
</tr>
<tr>
<td>WI</td>
<td>0.161</td>
<td>0.154</td>
<td>0.143</td>
<td>0.129</td>
<td>0.158</td>
<td>0.155</td>
<td>-</td>
</tr>
</tbody>
</table>

All comparisons for \( F_{ST} \) were significant at a \( P < 0.001 \)
All comparisons for \( R_{ST} \) were significant at a \( P < 0.01 \), except for the comparison with the *

Table 4. \( G'_{ST} \) values for all pairwise comparisons between the seven populations of \( C. canadensis \).

<table>
<thead>
<tr>
<th>Population</th>
<th>SC</th>
<th>TX</th>
<th>ME</th>
<th>MN</th>
<th>AL</th>
<th>AZ</th>
<th>WI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX</td>
<td>0.278</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td>0.582</td>
<td>0.342</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN</td>
<td>0.484</td>
<td>0.42</td>
<td>0.338</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>0.364</td>
<td>0.276</td>
<td>0.565</td>
<td>0.428</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZ</td>
<td>0.561</td>
<td>0.514</td>
<td>0.47</td>
<td>0.368</td>
<td>0.398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WI</td>
<td>0.588</td>
<td>0.537</td>
<td>0.473</td>
<td>0.41</td>
<td>0.594</td>
<td>0.554</td>
<td></td>
</tr>
</tbody>
</table>

All comparisons were significant at a \( P < 0.001 \)
Table 5. Results of the test for a population bottleneck for the seven populations of *C. canadensis*. SMM denotes stepwise mutation model, TPM two-phased model, and IAM infinite allele mode.

<table>
<thead>
<tr>
<th>Mutation model</th>
<th>Proportion of SMM (%)</th>
<th>One-tailed Wilcoxon signed-rank test for heterozygote excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMM</td>
<td></td>
<td>SC</td>
</tr>
<tr>
<td>70 (default)</td>
<td></td>
<td><em>P</em>=1.0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td><em>P</em>=1.0</td>
</tr>
<tr>
<td>IAM</td>
<td></td>
<td><em>P</em>=1.0</td>
</tr>
</tbody>
</table>
APPENDIX D

PHYLOGEOGRAPHY OF THE AMERICAN BEAVER (CASTOR CANADENSIS)

“The following paper is pending submission to the Journal of Wildlife Management”

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Running Heading: American beaver molecular phylogeography

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Glacial cycles during the Plesitocene played an important role in structuring the evolutionary pathways and distributions of species. The Pleistocene refugium
hypothesis states that populations suffered contractions of their distributions during glacial periods and were isolated in single or multiple refugia. Then, when the glacials contracted, such isolated populations rapidly expanded into deglaciated areas. The purpose of this study was to investigate if the American beaver (*Castor canadensis*) follows the refugia hypothesis and to assess the influence of past climatic and geologic changes on the phylogenetic relationships of this species. We used mitochondrial control region and cytochrome *b* sequences to perform Maximum Likelihood and Bayesian Inference analyses. The phylogenetic analyses revealed the presence of multiple Pleistocene refugia for the beaver in areas of the Appalachian Mountains, the Rocky Mountains and Texas. This was further supported by the demographic analyses, which suggest a history of population expansion, and by the estimated divergence times of major clades and subclades in the phylogenetic tree, which dates correspond to the middle and late Pleistocene. Climatic fluctuations did have an influence in the distribution of the beaver in North America. This species is tightly associated to wetlands and riparian ecosystems, thus, the understanding of how climatic fluctuations can affect the beaver and wetlands is essential for the conservation of this species and its habitat.

**KEY WORDS:** American beaver, glacial refugia, mitochondrial DNA, phylogeography, Pleistocene

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Between 18,000 and 21,000 years ago the landscape and climate of North America was considerably different from the present day. Ice sheets extended throughout much of the northern portion of the continent, the lowered sea levels exposed land bridges that connected land masses, such as the Beringian land bridge that connected North America with Siberia, and the climate was much colder and drier (Jackson et al. 2000, Waltari et al. 2007). Consequently, during this period, plant and animal species’ distributions were different from their current distributions. Species faced a reduction and fragmentation of their ranges due to inevitable movement and contact with ice sheets, which provoked shifts in the distribution of their habitats, such as forests and deserts (Grimm et al. 1993, Hewitt 1996, 2004). These shifts were the result of unfavorable climate conditions for species that were not physiologically tolerant to the changes. Some intolerant species became isolated in refugia where vegetation remained relatively unchanged. After approximately 4000 years, when the climate began to change to warmer and wetter, these species began to expand their geographic distribution as new suitable areas became available (Waltari et al. 2007).

Understanding the influence of geological and climate events, and Pleistocene refugia on the distribution of species, has been essential to understanding the historical and present biogeographical patterns, current populations’ genetic structure, and species responses to climate and landscape changes. Phylogenetic analyses can help discern the influence and impact of such changes on the biogeographical pattern of species, and
their genetic diversity (Haenel 2007, Waltari et al. 2007).

In general, there are signals left in an extant population that permit to predict if a species existed in a refugia in the past (refugia hypothesis; Haenel 2007). In the case of a refugium, as ice sheets expanded southwards, most species experienced reduction and fragmentation of their ranges due to climatic changes and distributional changes of their habitats. Then, as temperatures warmed the populations that remained isolated in single or multiple refugia often expanded their geographic areas to new available habitats (Haenel 2007, Waltari et al. 2007). Under this hypothesis one would expect to see taxa grouped by regions including derived populations that expanded from the same region or refugium. These population expansions should be reflected in a unimodal distribution of pairwise differences. The observed groups should share a high proportion of haplotypes and should show a low genetic variance among populations within regions. And the divergence (coalescent) time of populations should date at most to the last glacial maxima (≤18,000 years; Haenel 2007). These phylogeographic patterns are seen in several organisms that reflect the presence of a Pleistocene refugium, such as the willow flycatcher (Empidonax traillii; Paxton 2000), the gorilla (Gorilla beringei; Anthony et al. 2007), the red fox (Vulpes vulpes; Aubry et al. 2009), and the collared lemming (Dicrostonyx groenlandicus; Fedorov and Stenseth 2002).

Numerous phylogeographic studies of plant and animal species on the North American continent have showed the existence of refugia in the Appalachian Mountains, the Rocky Mountains, the Pacific Northwest, and Beringia (Avise 2000, Hewitt 2004, Shafer et al. 2010). More recently, geological studies have showed that a
portion in central United States (i.e. Kansas, Oklahoma, Texas) was free of ice during the last glacial maximum despite the expansion of the Laurentide ice sheet farther to the south (Holliday et al. 2002). Therefore, the central United States might represent other potential refugia for species that were tolerant to the environmental conditions in such an area. Species from different taxa, such as the American marten (*Martes americana*, Stone et al. 2002), the western diamondback rattlesnake (*Crotalus atrox*, Castoe et al. 2007), and the mountain chickadee (*Poecile gambeli*, Spellman et al. 2007), used some of these regions as Pleistocene refugia. Moreover, some species that show evidence of a refugial past, are also species associated with very restricted distributions due to a high specificity in habitat requirements, for example, species from high elevation forests such as the pygmy salamander (*Desmognathus wrighti*; Crespi et al 2003), or species associated with grasslands such as the black-footed ferret (*Mustela nigripes*; Wisely et al. 2008). Thus, it is important to mention that the phylogeographic study of endemic or habitat dependent species is essential for the further understanding of species’ responses to changes in the environment because it can facilitate hypothesis testing of the formation and evolution of ecosystems as well as for the understanding of how these ecosystems responded to late Quaternary events (Carstens et al. 2005, Wisely et al. 2008).

The American beaver is a semiaquatic rodent that can be found along stream banks and the shores of lakes and mashes, which feeds on bark, cambium, leaves and roots of poplar (*Populus* spp.), birch (*Betula* spp.), willow (*Salix* spp.) and alder (*Alnus* spp.; Müller-Schwarze and Sun 2003). These narrow habitat requirements could have
led this mammal to seek refuge where the appropriate conditions remained as their primary strategy to survive the last glaciation (Hewitt 2004). Furthermore, the geological changes in the major drainages of North America affected the structure of geographic patterns of stream-dwelling organisms which promoted divergence and speciation (Kozak et al. 2006). For instance, drainage patterns west of the Eastern Continental Divide, which separates eastern-flowing drainages of the Atlantic Slope from those that drain into the Gulf of Mexico, were different before the Plesitocene glacial advances. As a result of such glacial movements, flow patterns of rivers were reversed to the south creating the fusion of formerly isolated drainages and originating modern river basins, such as the Ohio River basin and the Tennessee River basin (Kozak et al. 2006). Before the Pleistocene the Missouri River flowed northeast to Hudson’s Bay, but during the Pleistocene as most of North Dakota was covered with ice, the water flowed along the ice margins to the south. This ice-marginal drainage is what constitutes the present Missouri River, other ice-marginal drainages also formed present rivers in North Dakota (Bluemle 1972). On the northwestern region of North America the Cordilleran ice sheet engulfed major drainages of the United States originating current lakes and rivers (Waitt 1983). For instance, on the western side of Washington, the Puget Sound is a large body of water that was formed by glaciers extending over this area (Waitt 1983). The drainage formed by the Cordilleran ice sheet flowed south in the Cascade and Olympic Mountains and merged with meltwater streams flowing toward the Pacific Ocean (Waitt 1983). In addition to the changes to major drainages, glaciation movements also generated changes to vegetation that could
have affected the distribution of species associated to such taxa. Plant taxon suffered
distributional changes that involved individualistic changes in population abundance
and ranges which included east-west and northward movements of taxa (Williams et al.
2004). Between 13 000 and 11 000 years before the present (yr BP) in the Great Lakes
region, species of spruce (Picea) were replaced by pine species (Pinus) whereas
between 18 000 and 15 000 yr BP in Beringia there was an increase in pollen
abundances of birch species (Betula) and decreases in Salix and Poaceae which
indicates a shift in individual species abundance (Williams et al. 2004).

At present, the phylogeographic history of the American beaver is unknown,
however, the complex history of drainage basins formation along with vegetation
changes due to geological and climatic changes provide the base to understand the
phylogeographic structuring of beaver populations throughout their evolutionary
histories. Because the beaver is a species that depends on riparian vegetation and
wetlands, the shifts in abundance and distribution of riparian vegetation as well as the
change in flow of drainages, and creation and destruction of rivers and streams could
have determined the use of multiple refugia to cope with the substantial changes during
the Pleistocene. Moreover, the study of a riparian-dependent species can aid the
understanding of species’ responses to historical environmental and geological changes.
Therefore, the objectives of this study are to assess the phylogeography of the American
beaver, in an effort to evaluate whether genetic patterns and divergence times support
the refugia hypothesis for this species, and to make inferences regarding their historical
and current distributions based on the genetic patterns and divergence observed.
METHODS

Sample collection

We obtained 195 tissue samples of 12 different US states, from wild beavers captured in the field by Wildlife Services (USDA; see Table 1). In addition, we requested, and obtained 44 samples of tissue, skin or bone from several museum collections. These samples originated from ten US states and seven Canadian provinces (Appendix 1).

DNA isolation

Genomic DNA was successfully extracted from a total of 189 samples (188 tissue samples and one bone sample). For tissue samples we used approximately 25 mg of tissue and the Qiagen DNeasy blood and tissue kit following the manufacturer’s protocol (Qiagen Ltd., Crawley, West Sussex, United Kingdom). In the case of genomic DNA extraction from hide and hair we followed a used-developed protocol adjustment. Instead of using the ATL buffer recommended by the protocol as step one, we used X1 buffer (10mM Tris-HCl buffer, pH 8.0, 10mM EDTA, 100mM NaCl, 40mM dithiothreitol, 2% SDS, 250μg/mL Proteinase K; Suenaga and Nakamura 2005), and the cell lysis step was allowed to continue until all cells were completely lysed which often took 24-48 hours. An additional 20 microliters of proteinase K was added after 24 hours if the lysis took longer than 24 hours. For genomic DNA extraction from bone samples, bones were pulverized using a SPEX SamplePrep Freezer/Mill 6770 cryogenic grinder (Spex CertiPrep, Metuchen, NJ, USA) following the manufacturer’s instructions. We
decalcified the pulverized bone samples by adding 1ml of EDTA (0.5 M pH 8.0 Promega, Madison, WI, USA) to 30m g of bone powder. The samples were incubated on a rocking-platform for 4 days in a refrigerator at 4 °C. Every 24 hours, the samples were centrifuged to pellet bone powder and the EDTA buffer was replaced with fresh buffer. On the final day the bone powder was pelleted and EDTA buffer removed. DNA from the decalcified bone powder was extracted following the protocol described previously for tissues.

**DNA amplification**

**Control Region**

We amplified and sequenced a 520 base pair (bp) fragment of the mitochondrial DNA hypervariable domain I (HV I) of the control region (CR). We used the universal primers Thr-L15926 (5′-CAATTCCCCGGTCTTGTAAACC-3′) and DL-H16340 (5′-CCTGAAGTAGGAACCAGATG-3′; Cheney 1995, Vila et al. 1999, Durka et al. 2005). Polymerase chain reactions (PCRs) were performed in 20 μL volumes using 1X PCR buffer (20 mM Tris-HCL, pH 8.4, 500 mM KCl, Invitrogen), 0.2 mM of each dNTP, 0.3 μM of each primer, 1 U of Taq DNA polymerase (Invitrogen), 1.5 mM of MgCl₂. All PCR amplifications were conducted using a Mastercycler Gradient (Eppendorf, Germany); the thermal cycling profile was 4 min at 96 °C, 35 cycles (40 s at 96 °C, 45 s at 55 or 56 °C, 1 min at 72 °C) plus 10 min at 72 °C. Purified PCR amplicons were directly sequenced in both forward and reverse directions using the same primers used for amplification. We edited and aligned each sequence using
Sequencher 4.6 (Genes Codes Corporation 2006) and uploaded them to the NCBI web page (www.ncbi.nlm.nih.gov) for confirm that the sequences obtained belong to the American beaver. We aligned the resulting edited sequences using CLUSTAL as implemented in Mesquite 2.73 (Madison and Madison 2010). Geographic location, haplotype number, and GeneBank Accession number for each haplotype can be accessed in Appendix 2.

**Cytochrome b**

We amplified and sequenced a 307 bp fragment of the mitochondrial DNA cytochrome b (cyt b) gene using the primers L7 (5′ACCAATACCAATGACATGAAAAATCATCGTT-3′) and H6 (5′-TCTCCATTTCTGGTTTACAAGAC-3′; Montgelard *et al.* 2002, Durka *et al.* 2005). PRC reactions were performed as described above, and the thermal cycling profile was 4 min at 94 °C, 35 cycles (30 s at 94 °C, 30 s at 58 °C and 70 s at 72 °C) plus 7 min at 72 °C. The sequences obtained were edited and aligned as described for the CR sequences. Geographic location, haplotype number, and GeneBank Accession number for each haplotype can be accessed in Appendix 3.

**Combined data**

For all individuals that successfully amplified for both the CR and the cyt b regions, we concatenated the sequences using Mesquite 2.73 (Madison and Madison 2010) and analyzed them together to boost the resolution of our findings and
corroborate the resolution from using the two data sets alone. Geographic location, and haplotype number for each haplotype can be accessed in Appendix 4.

**Phylogenetic analyses**

We performed two analyses, first, the phylogenetic analyses for the CR and the cyt b separately. Second, we conducted the phylogenetic analyses for both sequences (CR and cyt b) concatenated.

We used both, Maximum Likelihood (ML) and Bayesian inference (BI) approaches to reconstruct phylogenetic relationships among the haplotypes. We determined the model of nucleotide substitution that best fit our data by using the Akaike’s information criteria as implemented in MODELTEST (version 3.7; Posada and Crandall 1998) and Mr.MODELTEST (version 2.0; Nylander 2004). For the ML analyses, the MODELTEST analysis showed that the model that best fit the data for the CR is the Hasegawa-Kishino-Yano (HKY+\(\Gamma\)) model with gamma-distribution among-site rate variation (\(\Gamma =0.1008\)). Under the Bayesian Inference approach, MrMODELTEST showed that the model selected for the CR is the general time reversible (GTR+\(\Gamma\)) with a gamma distribution shape parameter \(\Gamma =0.6650\).

For the cytochrome b analysis, the MODELTEST showed that the best fit model for the data was the unequal-frequency Kimura 3-parameter (K81uf+I) model with a fraction of invariable sites. The proportion of invariable sites (I) was 0.8028. Under the BI approach the selected model by MrMODELTEST was the general time reversible (GTR+I) model with a fraction of invariable sites I =0.5409.
For the CR and cyt b concatenated data, the model selected for the ML analyses was the Transversion (TVM+I+Γ) model with a fraction of invariable sites (I =0.7475) and gamma distribution (Γ =0.8648). Under the BI, the selected model was the general time reversible (GTR+I+Γ) model with a fraction of invariable sites (I =0.7460) and gamma-distribution among-site rate variation (Γ =0.8782).

Measures of genetic diversity such as gene diversity (h, the probability that two randomly chosen mtDNA sequences are different) and nucleotide diversity (π, the probability that two randomly chosen copies of a nucleotide site are different) were estimated using the program ARLEQUIN 3.1 (Excoffier, et al. 2005). In addition, for the concatenated data, we used an Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) to generate a matrix of pairwise estimates of ΦST to assess the differentiation among geographic regions, among populations within regions, and within populations (Weir & Cockerham 1984). Specifically, we compared nine major drainage basins of North America including sampling locations as follow: Pacific Ocean Seaboard (Alaska), Columbia Puget Sound Region (Washington, Idaho, and Alberta, Canada), Colorado Basin (Arizona), West Gulf of Mexico Drainage (New Mexico), Arkansas-Red River Drainage (Oklahoma, northern Texas, and southern Missouri) Ohio Drainage (Kentucky, Tennessee), Upper Mississippi Drainage (Minnesota, and Wisconsin), Missouri Drainage (Wyoming, North Dakota), and Atlantic Slope Drainage (Alabama, Georgia, South Carolina, Virginia and Maine).

For each mtDNA gene segment and for the combined data, we performed a Bayesian inference analysis using MrBAYES (version 3.1.2; Ronquist and Huelsenbeck
2003) with 4 separate runs implementing MCMC simulation starting from a random
tree. Each run was conducted with $10 \times 10^6$ generations and sampled at interval of 1,000
generations. The first 25% of the samples of each run were discarded as burn-in, and all
remaining sampled trees were used to find the posterior probability of clades. A
consensus tree was generated with the 50% majority rule algorithm. We used PHYML
(version 3.0 Guindon et al. 2010) to perform ML analyses with searches consisting of
1,000 random replicates to evaluate the nodal support.

We used haplotypes with frequency $>1$ for each of the individual genes and the
combined dataset to perform independent network analyses for each data set. We used the
median-joining (MJ) network algorithm as implemented in NETWORK 3.5.1.0 (Bandelt
et al. 1999) with the default weight (10) and epsilon value of 10.

**Time divergence estimation**

To estimate divergence time for the American beaver lineages we calculated the
mutation rate for the CR and the cyb sequences separately from $\mu = k/(2t + 4N_e)$. Where $k$
is the amount of divergence between the two species, *C. fiber* and *C. canadensis*, $t$ is the
time since the species have diverged measured in generations, and $N_e$ is the ancestral
effective population size (Kimura 1983). Divergence between *C. fiber* and *C.
canadensis* was calculated as the average pairwise difference between the sequences
analyzed using Kimura’s two-parameter model (Kimura 1980). The divergence time, $t$,
between the two species was previously estimated by Horn et al. (2011), and $4N_e$ was
considered the same as the nucleotide diversity ($\pi$) of the sequences analyzed. For the
cyb sequences the divergence between *C. fiber* and *C. canadensis* was zero, thus we could not estimate the mutation rate for the cyb fragment.

We used the estimated mutation rate for the CR to calculate the time to the most recent common ancestor (MRCA) for all CR haplotypes using BEAST 1.6.1 (Drummond and Rambaut 2007). We used the model of nucleotide substitution selected by MrMODELTEST described above. In addition, we determined from a likelihood ratio test that a relaxed molecular-clock model with an uncorrelated lognormal tree prior best accounted for the variation in rates of evolution among the different lineages in our data set. The analyses were run 5 times with different random seeds, each for 20 million generations, with samples logged every 10,000 generations. After a visual inspection of the trace revealed that the initial 10% of samples had not reached stationarity, these samples were discarded as burn-in. We combined results from each run in LOGCOMBINER 1.6.1 from the BEAST package and analyzed them in TRACER 1.5 (Rambaut and Drummond 2007).

**Demographic history**

To address the demographic history (e.g. expansion) of each major lineage, we estimated Tajima’s *D* (Tajima 1989) and Fu’s *F*<sub>S</sub> (Fu 1997) tests for neutrality. DnaSP version 5.0 (Librado and Rozas 2009) was used to perform these tests and calculate the corresponding *P*-values. These tests assume that the population has been in mutation–drift balance for a long period of evolutionary time (Nei and Kumar 2000). Therefore, large and negative values of *D*, and significant negative *F*<sub>S</sub> values may indicate
historical population expansion. However, these tests are incapable of distinguishing between recent expansion and selection (Fu 1997). Therefore, we also estimated Fu and Li’s (1993) $D^*$ and $F^*$ statistics to distinguish background selection from population growth or range expansion. If $D$ and $F_S$ are significant and $D^*$ and $F^*$ statistics are not, population growth or range expansion is suggested. The reverse situation suggests selection. Furthermore, we evaluated patterns of historical demographic expansion by assessing a mismatch distribution analysis for each of the major lineages using ARLEQUIN. A mismatch distribution analysis is the tabulation of all the pairwise differences among all DNA sequences in a sample (Harpending 1994). A population that has undergone rapid and sudden expansion is expected to have a Poisson-shaped (unimodal) mismatch distribution, whereas a population that is either subdivided or in demographic equilibrium is expected to exhibit a more ragged (multimodal) distribution (Slatkin and Hudson 1991). We assessed the statistical significance of these distributions with sum of squared distances and Harpending’s raggedness index (Harpending 1994). In addition, we also performed an AMOVA among the nine drainages to determine if most of the genetic variation lies among drainages, among populations within drainages, or within populations.

RESULTS

Sample collection and DNA amplification

We collected 239 samples of beaver tissue/bone from wild caught animals or from museum specimens. From the samples obtained from Wildlife Services ($n = 195$),
we successfully extracted DNA from 158 (83.6%). While from the museum samples ($n$ =44), we were able to extract DNA only from 31 (70.4%). From these 189 samples, 97 (51.3 %) successfully amplified for the CR and 109 (57.6 %) for the cyt b.

**Phylogenetic analyses of individual genes**

The 97 CR sequences produced 34 haplotypes. Haplotypes 16 and 18 were more common than the others with a proportion of 8.82% respectively. Haplotype 16 was found in individuals from the Columbia Puget Sound Region and the Pacific Ocean Seaboard. While haplotype 18 was found in individuals from the Arkansas-Red River Drainage and from the Ohio Drainage (Fig. 1). The remainder of the haplotypes occurred in only one or two individuals. The haplotype diversity was high ($h = 0.95$), while the mean nucleotide diversity was moderate ($\pi = 0.015$), and the majority of observed nucleotide substitutions were mostly transitions ($\bar{X} = 6.71$).

The models of nucleotide substitution selected by MODELTEST and by Mr.MODELTEST were used to perform the Maximum Likelihood analyses and the Bayesian Inference analyses respectively for each of the datasets. The specific parameters for each model are listed in Table 1.

The Bayesian Inference phylogeny shows six main lineages, each with posterior probability support $> 0.75$ (Fig. 2). Only one clade had posterior probability support $> 0.95$, which includes haplotypes from the Atlantic Slope Drainage (Fig. 2). This phylogeny shows another group of haplotypes from the Atlantic Slope drainage, and one group of haplotypes from the Arkansas-Red River Drainage, however, these groups
have low posterior probability and bootstrap support (Fig. 2). The Maximum Likelihood analyses revealed the same phylogeny as the Bayesian Inference with the exception of the grouping of haplotypes from Maine which was not recovered by the Maximum Likelihood topology, and of the subgroup that contains haplotypes WI17, MN9, MO1, which in the Maximum Likelihood topology does not include haplotype MN9.

In the cyt b analyses, the 109 samples amplified resulted in 19 haplotypes. The most common were haplotypes 1, 8 and 6. Haplotype 1 occurred only in the Upper Mississippi Drainage, the Arkansas-Red River Drainage, the Colorado Basin, and the Atlantic Slope Drainage. Haplotype 8 occurred in all drainages except in the West Gulf of Mexico Drainage and the Colorado Basin. Haplotype six was present in all drainages except in the Pacific Ocean Seaboard and the Colorado Basin (Fig. 1). The haplotype diversity was high ($h = 0.826$), the mean nucleotide diversity was low ($\pi = 0.0046$), and the observed substitutions were mostly transitions ($\bar{X} = 1.65$). The Bayesian Inference and the Maximum Likelihood analyses show one main lineage in the cyt b phylogenetic tree and several unresolved polytomies. The main lineage has a posterior probability with support of 0.75, and a Maximum Likelihood bootstrap support after 1000 iterations of 35%. However, a subclade supported by Bayesian Inference with a posterior probability of 0.73 had a Maximum Likelihood bootstrap support $< 30\%$ (Fig. 3).

**Phylogenetic analyses of combined data**

From the 97 and 109 individuals that amplified for the CR and cyt b respectively, only 57 individuals amplified successfully for both genes. These
individuals were used for the combined dataset and analyses. The combined dataset produced 50 haplotypes. Haplotype 32 was the most common, but only occurred in two drainages, the Arkansas-Red River Drainage and the Ohio Drainage (Fig. 1). Haplotype diversity was high \( (h = 0.994) \), mean nucleotide diversity was moderate \( (\pi = 0.015) \), and the observed substitutions were mostly transitions \( (\bar{X} = 7.75) \).

The AMOVA results from the nine drainages indicated that 45.67% of the molecular variance resided within populations \( (P < 0.0001) \), 29.81% of variation was apportioned among population within groups \( (P < 0.0001) \), and 24.52% distinguished among drainages \( (P =0.00196; \text{Table 3}) \). In addition, pairwise estimates of gene flow showed low to high differentiation among populations, with values of \( \Phi_{ST} \) ranging from 0.005 to 0.797 (Table 5).

Both, the Maximum Likelihood and Bayesian Inference phylogenies showed five major haplotype groups (Fig. 4). Only one clade shows posterior probability support > 0.95 and bootstrap support > 50%. This clade includes five haplotypes from the Ohio Drainage, one from the Arkansas-Red River drainage, four haplotypes from the Missouri Drainage and one haplotype from the Columbia Puget Sound Region (Fig. 4). The phylogeny shows two other groups of haplotypes from the Atlantic Slope Drainage and one group with haplotypes from the Arkansas-Red River Drainage, but these groups have low posterior probability \( (< 0.95) \) and bootstrap support \( (< 50\%) \). Furthermore, the phylogeny shows an unresolved polytomy with haplotypes from all drainages except from the Atlantic Slope Drainage (Fig. 4).
**Time divergence estimation**

The estimated mutation rate for the CR sequences was $2.83 \times 10^{-8}$. The estimation of the time indicates that *C. canadensis* began diverging into different lineages $\sim 0.764$ (95% C.I. 0.44 – 1.1) my BP. And the divergence time between *C. canadensis* and *C. fiber* lineages was $\sim 1.231$ (95% C.I. 0.71 – 1.8) my BP. We did not estimate the divergence time of each specific lineage of the *C. canadensis* phylogeny due to the lack of genetic structure present on the phylogeny.

**Demographic history**

For the entire data set, both Tajima’s and Fu’s neutrality tests were negative, but only $F_S$ was significant ($D = -0.92, P > 0.10; F_S = -34.25, P = 0.00$). Some of the lineages presented negative values for these statistics, but only lineage E (Fig. 4) presented significant outcomes for both $D$ and $F_S$ statistics, and lineage C presented only a significant value for $F_S$ statistic (Table 2). The mismatch distribution analysis for the whole data showed a bimodal distribution and a high average number of pairwise differences ($k = 9.832$; Fig. 5a). However, the distribution was not different from the one generated under the null model of population expansion. The raggedness index value was non-significant ($rg = 0.010, P > 0.05$) and failed to reject the hypotheses of population expansion. In the case of lineage E, the mismatch distribution showed a unimodal distribution and a low number of pairwise differences ($k = 9.832$; Fig. 5f). And the raggedness index value was non-significant ($rg = 0.038, P > 0.05$). In the same
way, lineage C showed a unimodal mismatch distribution indicated by a low level of pairwise differences ($k = 9.832; \text{Fig. 5d}$) and by a non-significant value of the raggedness index ($rg =0.113, P > 0.05$). Lineages A, B, and D showed a multimodal mismatch distribution (Fig. 5).

**DISCUSSION**

In this study we used fresh tissue samples and museum samples to address the objectives. However, the success of DNA extraction was higher for the fresh tissue samples collected by Wildlife Services than for the museum samples. The 13 museum samples from which we could not extract DNA were hides ($n =9$) and bones ($n =4$). Extracting DNA from museum samples is not always possible because the preservation chemicals, or other preservation methods used may have degrade the DNA. Nevertheless, museum samples are an important source of samples from areas where we could not obtain fresh tissue samples.

Regions of the mtDNA are often used to reconstruct historic patterns of species distributions and relationships among populations (Avise 2000, Wisely et al. 2008). Previous studies have described glacial refugia in North America based on mtDNA phylogeographies of several species (Lessa et al 2003, Jaramillo-Correa et al. 2009). These Pleistocene refugia include eastern Beringia (central Alaska and far western Yukon), the Pacific Northwest, the Rocky Mountains, the Southwest and the south-central United States (Kansas, Oklahoma, Texas), and the Appalachian Mountains (Lessa et al 2003, Jaramillo-Correa et al. 2009, Crespi et al. 2003). In this study we used one
non-coding and one protein-coding gene of the mtDNA to infer the Pleistocene history of
the American beaver and its relationship with major drainages and vegetation changes in
North America. The phylogenetic tree reconstructed from the combined dataset has a
basal-most lineage (lineage A, Fig. 4) composed of haplotypes from the Arkansas-Red
River Drainage, which corresponds to the south-central Pleistocene refugium mentioned
above. During the Last Glacial Maximum (LGM) the Laurentide ice sheet covered most
of Canada and northern United States. Thus, because the Arkansas-Red River drainage is
located south of where the limits of the Laurentide ice sheet were, this unglaciated area
could have worked as a refugium for species of plants and animals such as loblolly pine
(\textit{Pinus taeda}; Schmidtling 2007) and the eastern fox squirrel (\textit{Sciurus niger}; Moncrief et
al. 2010). There is pollen evidence that species of Birch were present in low abundance
south of the ice sheet between 18 000 and 15 000 yr BP (Prentice et al. 1991). Then
between 12 000 and 9000 yr BP when temperature and rainfall increased there was an
expansion of Birch throughout northeastern United States (Prentice et al. 1991). The
American beaver could have used areas along the Arkansas-Red River basin where
vegetation such as Birch was present during the LGM. Furthermore, it has been
suggested that boreal woodland extended as far as south-central United States probably
westward from Georgia (Anderson et al. 2007). This indicates a moist and cooler
environment, most likely with the presence of water pools suitable for semiaquatic
species such as the beaver. In the same way, the taxa grouped in lineages B and D of the
combined dataset correspond to the Atlantic Slope drainages. This drainage system was
composed by the former Appalachian and Lower Tennessee-Old Cumberland drainage.
However, the Atlantic Slope drainage system changed after the Pleistocene and currently this drainage system includes the Tennessee, Cumberland, James, Roanoke, Santee, and Mobile Basin drainages (Kozak et al. 2006). This change in the drainages promoted the contact of previously isolated populations (Kozak et al. 2006), which is evident in the phylogeny of the American beaver in this study. The Atlantic Slope drainage system has been suggested as a Pleistocene refugium for other species such as the millipede (Narceus sp.; Walker et al. 2009), the pygmy salamander (Desmognathus wrighti; Crespi et al. 2003), the two-lined salamanders (Eurycea bislineata; Kozak et al. 2006), and the wood frog (Rana sylvatica; Lee-Yaw et al. 2008), which are also species associated with wetlands and cooler environments. Further, Phylogeographic studies of forest trees and annual plants have suggested Texas, southern Florida and the southern Appalachians as Pleistocene glacial refugia (Jaramillo-Correa et al. 2009). The beaver is a semiaquatic mammal closely associated with riparian vegetation which may have restricted its distribution to some of these Pleistocene refugia. This pattern has been uncovered in other species closely associated with specific habitats such as the black footed ferret (Wisely et al. 2008), tree squirrels (Tamiasciurus spp.; Arbogast et al. 2001), and the North American desert spider, (Agelenopsis aperta; Ayoub and Riechert 2004).

On the other hand, lineages C and E of the phylogenetic tree are composed of a mixture of haplotypes corresponding to different drainage systems. This could be explained by the hydrological and geological events of the Pleistocene. Throughout the different glaciation events that occurred during the Pleistocene the course of the rivers changed and new rivers were formed (Bluemle 1972). These events caused the beaver
to move into new available areas and leave other regions were conditions were no longer suitable. In addition to cope with the changes in hydrology during the Pleistocene, the beaver also had to find suitable vegetation. Arboreal taxa expanded north after the last glacial maximum in response to increasing temperatures and rainfall. For instance, northern populations of boreal taxa (e.g., *Picea*, *Pinus*, *Abies*, *Larix*, and *Betula*) expanded in areas vacated by the retreating ice sheets while southern populations declined (Williams et al. 2004). However, the patterns of population expansion also differed among taxa, which might have had an effect on the expansion of the beaver during this period. The beaver has considerable migration ability as well as a notable ecological plasticity, which allows beavers to thrive in a variety of habitats (Appendix B). This might be one of the reasons why the pattern of a Pleistocene refugium for the beaver is not as clear as it is for other species.

Nevertheless, it is worth noting that despite the lack of high support from the Bayesian Inference and Maximum Likelihood analyses for haplotype grouping into lineages corresponding to drainage systems, we found other genetic signals that support the presence of Pleistocene refugia for the American beaver. Overall, we found high haplotype diversity and moderate to low nucleotide diversity, which indicates range expansion. This pattern is shown for all the three topologies (i.e. CR, cyt *b*, and combined dataset); however, the cyt *b* topology lacks phylogenetic structure, while the CR and the combined data set topologies had similar patterns. Moreover, in the phylogenetic tree from the combined dataset, the significant value of Fu’s *F*$_S$ and the non-significant values of *D* and *F*$_*$ suggest a range expansion after a period of low
effective population size, which is expected under the refugia hypothesis. In the same way, clade C which is the only clade with enough posterior probability and bootstrap support, showed evidence of population expansion from the significant values of the neutrality test statistics and from mismatch distributions; this suggests a recent population expansion into areas that were previously glaciated or unsuitable for the American beaver. In the case of clade C, the population expansion was probably east of the Rocky Mountains into the Mississippi Valley region. Prior to the Pleistocene the Missouri River flowed northeast to Hudson's Bay, but during the Pleistocene the upper Missouri River was separated from the northwestern branch of the Mississippi River by an ice lobe (Cross et al. 1986). This prevented species to disperse and have contact with other populations. However, the intermittent connections between drainages during the multiple glaciation events allowed beaver populations to have contact. Beavers from the Missouri Drainage could have found several routes throughout the Upper Mississippi and Ohio Drainages to colonize areas of southern and eastern United States as can be seen in the phylogenetic tree. There is also evidence that by the late Pleistocene and early Holocene, spruce, balsam poplar, and aspen trees had returned to the Midwest region of the US, which allowed the expansion of species associated with moister and cooler environments (Levin 1988). Therefore, the American beaver was able to expand into areas that previously were glaciated or did not harbor riparian vegetation. The very shallow genetic distances observed and reflected in a lack of phylogenetic structure as further supports a rapid growth from a small ancestral population and post-Pleistocene dispersal of beaver populations. This pattern can be expected if time has been sufficient
to recover haplotype variation through mutation but is too short for the accumulation of a large amount of sequence differences (Avise 2000).

Moreover, a moderate genetic variance among populations within the nine drainages, and a significant genetic distance among these regions support a refugial past for the American beaver. Furthermore, the results of applying a molecular clock to the CR haplotypes indicate that the time to the most common recent ancestor for all lineages of *C. canadensis* corresponds to the middle Pleistocene (~0.76 my BP), and the divergence times of the different lineages corresponds to middle and late Pleistocene (~0.12- 0.011 my BP), which is expected under the refugia hypothesis. Additionally, there is evidence that some of these lineages persisted in distinct refugia despite the repeated range expansion and contractions during the glacial cycles of the late Pleistocene. Different geographic features could have acted as physical barriers that prevented the mixing of lineages (Chavez and Kenagy 2010). This is the case for beaver lineages showing a refugial past in the Arkansas-Red River and the Atlantic Slope drainages. Despite the lack of evidence for a population or range expansion of these lineages, the regional grouping of their haplotypes, the high haplotype diversity, and low nucleotide diversity is consistent with the refugia hypothesis. In addition, the phylogenetic tree shows that the lineages that correspond to the Atlantic Slope drainage system diversified within this region, but only as the Laurentide ice-sheet expanded and contracted throughout the remainder of the Pleistocene, multiple climatic and physical changes occurred to the landscape and vegetation around the Appalachian Mountains (Soltis et al. 2006) which might have prevented beavers from dispersing further west.
In the last 15 years, the field of phylogeography has advanced at a fast pace providing vast evidence of the phylogeographic history of different organisms. Also, the increasing climatologic and geographic information has aided the understanding of phylogeographic patterns within an evolutionary context, including how environmental changes have affected such patterns (Riddle 2009). In this study we have shown that a combination of major climatic fluctuations and topographic and vegetation variations have had an important influence in shaping the phylogeography and genetic structure of the American beaver. However, it is worth noting that in addition to environmental events, anthropogenic actions during the last 200 years have had an impact in the genetic structure of *C. canadensis*. The American beaver was once almost extirpated from North America due to the extreme hunting pressure that early Europeans put on this species (Müller-Schwarze and Sun 2003). This marked an important demographic event for beaver populations and is also exhibited in the American beaver phylogeny.

Our research limitations include the lack of samples from areas of the American beaver’s distribution range such as northern Mexico, the Pacific Coast, the Great lakes region, and Canada, particularly samples from northern Mexico are important because this region represented Pleistocene refugia for several species such as the Shortleaf pine (Schmidtling 2007) and the black bear (*Ursus americanus*; Lessa et al. 2003) and might have worked as a refugium for the beaver as well. Thus, reconstruction of an American beaver phylogeography might be partial and adding samples from these regions would aid resolution for some lineages that currently have low support. Also, patterns of population expansion and recolonization during post-glaciation events might be further
resolved. Nevertheless, the phylogeny described in this study shows that despite natural migratory movements that the American beaver underwent, as well as multiple translocations conducted by humans in the last 200 years, there is still a signal of a phylogeographic pattern and the presence of glacial refugia during the Pleistocene glacial period. Revealing the complex historical patterns that shaped the current phylogeography and genetic diversity of a species is crucial to understanding the demographic and ecological responses each species has to environmental changes, and can aid our knowledge of how future changes can affect this species. In the case of the American beaver, it is important to recognize the tight association this mammal has to riparian ecosystems to understand how environmental changes will affect beaver populations, to effectively manage their populations in the future. Global climate-change models predict that during the next 20 years temperatures will increase and patterns of precipitation will change. This will alter the flow of the rivers and steams that will have a direct effect on the distribution of riparian vegetation and the species associated to them (Primack 2000, Palmer et al. 2008). The current trend with riparian systems is one of habitat loss due to direct anthropogenic activities which is a negative factor in beaver conservation. If climate related environmental change also has a negative effect on current riparian systems, then the combined effect may cause another significant demographic reduction in beaver populations if additional riparian ecosystems are not available.
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Drainage Systems

- Pacific Ocean Seaboard
- Columbia Puget Sound Region
- Colorado Basin
- West Gulf of Mexico
- Arkansas-Red River
- Ohio Drainage
- Upper Mississippi
- Missouri Drainage
- Atlantic Slope Drainages

Fig. 1. Median-joining network for haplotypes with frequency >1 found among the nine drainages described in the text. Circles are proportional to haplotype frequencies; numbers inside circles correspond to haplotypes; numbers adjacent to branches indicate the number of mutations between connected haplotypes; a) network from CR haplotypes, b) network from cyt b haplotypes, c) network from combined data set haplotypes.
Drainage Systems

- Pacific Ocean Seaboard
- Columbia Puget Sound Region
- Colorado Basin
- West Gulf of Mexico
- Arkansas-Red River
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Fig. 2. Bayesian Inference tree for *C. canadensis* constructed from the CR data set of 34 haplotypes (520 base pairs) using *C. fiber* as outgroup. The phylogeny inferred with maximum-likelihood (ML) methods yielded similar topology. Numbers to the right of the diagonal indicate ML bootstrap support and those to the left of the diagonal indicate Bayesian posterior probabilities. Sampling localities shown in Appendix I are indicated at branch tips. Roman numbers indicate major lineages discussed in the text.
Fig. 3. Bayesian Inference tree for *C. canadensis* constructed from the cyt *b* data set of 19 haplotypes (307 base pairs) using *C. fiber* as outgroup. The phylogeny inferred with maximum-likelihood (ML) methods yielded a similar topology. Numbers to the right of the diagonal indicate ML bootstrap support > 50%, and those to the left of the diagonal indicate Bayesian posterior probabilities > 0.5. Sampling localities shown in Appendix I are indicated at branch tips. Roman numbers indicate major lineages discussed in the text.
Fig. 4. Bayesian Inference tree for *C. canadensis* constructed from the combined (CR + cyt b) data set of 50 haplotypes (827 base pairs) using *C. fiber* as outgroup. The phylogeny inferred with maximum-likelihood (ML) methods yielded a similar topology. Numbers to the right of the diagonal indicate ML bootstrap support, and those to the left of the diagonal indicate Bayesian posterior probabilities > 0.5. Sampling localities shown in Appendix I are indicated at branch tips. Letters A through E refers to lineages discussed in the text.
Fig. 4. Mismatch distribution for the *Castor canadensis* sequences. Dashed line indicates the observed distribution of pairwise differences, and solid line indicates the expected distribution based on a population expansion. a) Mismatch distribution of all lineages combined, b) distribution of lineage A, c) distribution of lineage B, d) distribution of lineage C, e) distribution of lineage D, f) distribution of lineage E.
Table 1. Parameters of the model of nucleotide substitution selected using MODELTEST for Maximum likelihood analyses and for Bayesian Inference using Mr. MODELTEST. $\Gamma$ denotes the gamma distribution shape parameter, and $I$ denotes the proportion of invariable sites.

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Table 2. Number of sequences, polymorphic sites, haplotype and nucleotide diversity, and values of Tajima’s $D$ and Fu’s $F_S$ tests from each of the lineages of the combined data set phylogeny. See Fig. 4 for drainages within each lineage.

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<th>Fu &amp; Li’s statistics</th>
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Table 3. Components of analyses of molecular variance (AMOVAs) of the combined sequences of mitochondrial DNA from the nine drainages described in the text.

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<th>Source of Variation</th>
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Table 4. Pairwise estimates of the concatenated mitochondrial DNA sequences $\Phi_{ST}$ for all populations and the nine drainages described in the text. $\Phi_{ST}$ values significantly greater than zero ($P < 0.05$) are in boldface type.

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*POS: Pacific Ocean Seaboard
CPSR: Columbia Puget Sound Region
COB: Colorado Basin
WGMD: West Gulf of Mexico Drainage
ARRD: Arkansas-Red River Drainage
OD: Ohio Drainage
UMD: Upper Mississippi Drainage
MD: Missouri Drainage
ASD: Atlantic Slope Drainage
Table 5. Pairwise estimates of the concatenated mitochondrial DNA sequences $\Phi_{ST}$ for the nine drainages described in the text. All comparisons for $\Phi_{ST}$ were significant at a $P < 0.05$, except for the comparison with the *.

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<th>COB</th>
<th>WGMD</th>
<th>ARRD</th>
<th>OD</th>
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*POS: Pacific Ocean Seaboard  
CPSR: Columbia Puget Sound Region  
COB: Colorado Basin  
WGMD: West Gulf of Mexico Drainage  
ARRD: Arkansas-Red River Drainage  
OD: Ohio Drainage  
UMD: Upper Mississippi Drainage  
MD: Missouri Drainage  
ASD: Atlantic Slope Drainage
Appendix 1. Number of samples obtained per geographic region and source of samples.

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* WS: Wildlife Services. USDA  
UWBM: Burke museum of Natural History and Culture  
MSB: Museum of Southwestern Biology  
SNOMNH: Sam Noble Oklahoma Museum of Natural History  
KUNHM: Kansas University Natural History Museum  
BYU: Bringham Young University Museum  
USNM: Smithsonian Institution National Museum of Natural History  
MCZ: Museum of Comparative Zoology  
RAM: Royal Alberta Museum  
RM: Redpath Museum McGill University
Appendix 2. Number of CR haplotypes, geographic location, and GeneBank Accession numbers for each haplotype.

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<th>GeneBank Accession number</th>
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Appendix 3. Number of cyt *b* haplotypes, geographic location, and GeneBanck Accession numbers for each haplotype.

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Appendix 4. Number of haplotypes of the concatenated dataset, and geographic location for each haplotype.

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