

LANDSCAPE ECOLOGY OF THE CAPYBARA (*Hydrochoerus hydrochaeris*) IN THE
CHACO REGION OF PARAGUAY

by

JUAN MANUEL CAMPOS KRAUER

D.V.M., Universidad Nacional de Asuncion, 1998

AN ABSTRACT OF A DISSERTATION

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ABSTRACT

Habitat fragmentation and destruction are the most ubiquitous and serious environmental threats confronting the long-term survival of plant and animal species worldwide. However, some native or exotic species can take advantages of these alterations and expand their range, placing endemic species at risk of extinction by changing the composition of biotic communities and altering ecosystem.

Capybara (*Hydrochoerus hydrochaeris*) are a widely distributed rodent throughout most of South and Central America, but restricted to areas of standing water. As the Gran Chaco ecosystem of Paraguay has been converted from dry tropical forest to pastureland, I hypothesized that this habitat alteration created potential for invasion by capybara into newly fragmented areas. I used ecological niche modeling to generate hypotheses about how the distribution of capybara has been affected by land use change, and tested those hypotheses with phylogeographic analyses. To understand the mechanisms that have allowed the invasion, I investigated home range, habitat use and thermoregulation of capybara via radiotelemetry in a deforested area in which capybara had recently invaded.

Genetic analyses confirm a rapid range expansion scenario with evidence of secondary contact between two distinct phylogroups which had previously been disjunct. Modeling results indicated that conversion of forest to pastureland allowed the expansion to occur. Capybara selected water significantly more than it was available to them, and avoided shrub forest. I found a significant positive correlation between body temperature and distance from water, and a significant negative correlation between distance from water and Chaco ambient temperature. Capybara proximity to water appeared to be

tightly linked to body thermoregulation. These results suggest that although capybara have expanded into the Chaco forest as it is converted to pastureland, the presence of permanent water sources in those pastures are the mechanism that allow capybara to persist in this habitat.

This is the first study to characterize capybara in a xeric habitat without a year round water source, and scarce natural grasslands. My results show how anthropogenic habitat modification has allowed capybara to thrive. Understanding how capybara invade and utilize the deforested Central Dry Chaco will provide valuable information for the future management of the species and the Chaco ecosystem.

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Chapter I

Natural History of the Capybara and the Ecology of the Gran Chaco Region

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The purpose of this first chapter is a general introduction to the known biology of capybara and the history and ecology of the Gran South American Chaco with emphasis in the Paraguayan portion of the Chaco region.

Natural history of capybara (*Hydrochoerus hydrochaeris*)

The capybara (*Hydrochoerus hydrochaeris*) (Linnaeus 1766) or carpincho, as locally known in southern South America, is a rodent in the suborder Caviomorpha, Superfamily Cavoidea (Nowak 1991). It is the largest extant rodent in the world. The head and body length average 100-130 cm and shoulder height is up to 50 cm. Weight varies regionally from 27-91 kg (Mones 1986; Nowak 1991; Ojasti 1973). The body is large and heavy with a vestigial tail. Limbs are short heavy and strong; the forefeet possess four digits and hindfeet have three toes with thick nails. All front and hind digits are united by moderate webbing which is used to help propulsion in water. The head is heavy and broad, with short rounded ears with a large snout. Capybara coat has relatively long hairs, ranging from 30 to 120 mm with the distinctive characteristic of being accumulated in sparse clumps of three to four (Pereira 1980). The coat varies in coloration from a dark reddish brown all the way to light yellowish (Figure 1). Capybara has an extensive distribution from Panama south into northern Argentina. In southern South America it is found in Paraguay, Uruguay, and in the northern and northeastern provinces of Argentina (Redford 1992a). Temperature, water and altitude are believed to be important factors in their distribution (Mones 1986; Ojasti 1973), (Figure 2).

Capybara is a semi-aquatic rodent that inhabits densely vegetated areas around ponds, lakes, rivers, streams, marshes, and swamps (Krieg 1929; Macdonald 1981; Ojasti 1973). The densest populations have been recorded on seasonal floodplains and savannas of the Venezuelan Llanos and the Pantanal region of Brazil (Schaller 1981). Capybara is found throughout Paraguay except for extremely dry habitat in the northern Chaco region (Neris 2002; Wetzel 1974; Ziegler 2002). Capybara requires sufficient water for protection, wallowing and drinking, as well as dry ground for resting and grazing. These factors limit the number of animals that a

region can support (Ojasti 1978; Schaller 1981). Herds suffer greatly in areas where dry seasons are severe; concentrating around remaining water and succumbing to starvation, or being susceptible to predation and disease (Ojasti 1991; Quintana 2003).

In areas where capybara is not harvested, it is active in the morning and evening, resting during the heat of the day in a shallow bed in the ground. In areas with high human density and hunting pressure, the species is nocturnal (Barreto and Herrera 1998; Herrera 1989; Lord 1991; Murphey et al. 1985; Ojasti 1991). Capybaras are slow moving animals, however when alarmed on land it will race toward the nearest water body in which it will enter diving head first. Capybaras are capable swimmers and can remain underwater for a considerable length of time. Although closely associated with aquatic environments, capybara use water primarily as a place of refuge, and most normal activity is on land (Herrera 1989; Lord 1991; Macdonald et al. 1984; Schaller 1976; Schaller 1981).

Capybaras live in herds ranging from a pair or family to complex groups of several adults of both sexes and their offspring. Groups of 20 and up to 100 or more have been reported, but such large congregations are apparently not stable. A population density of about 12.5 animals/km² in one part of Venezuela was reported by Schaller (1981). A typical capybara herd is a closed society formed by a dominant male, some adult females with their offspring of different ages, and a few subordinate males on the periphery of the herd (Azcarate 1980). The dominant male often expels other males from the herd. Solitary individuals, normally comprising 5 to 10 % of the population, are males. A dominance hierarchy is assumed to exist in wild capybara herds (Azcarate 1980; Ojasti 1973; Schaller 1981).

Capybara reaches sexual maturity at about 1.5 years of age or a weight of 30 kg depending on the habitat quality (Ojasti 1973). Animals are capable of breeding year round, but

mating increases during the rainy season (Schaller 1981). The gestation period is approximately 150 days (Zara 1973), and one litter per year is most common. However, if conditions are favorable, females may breed twice in a year (Lombardero 1955; Moojen 1952; Ojasti 1971; Schaller 1981). Capybara litter size can vary greatly depending on the habitat quality, ranging from 1 to 8 pups and averaging of 4.8 pups in Venezuela. In Brazil and Paraguay the range is from 1 to 4 (Krieg 1929; Ojasti 1973; Rengger 1830; Wetzel 1974). Capybara birth takes place anywhere near water in their habitat; however, lack of cover increases the risk of predation of newborns.

High quality capybara habitat is found near cattle ranches. Capybara diet consists mainly of aquatic vegetation and grasses; it is not uncommon to see capybara grazing with cattle (Quintana 1998). Capybaras appear to take advantage of pasture maintenance, water provision and predator control to greatly improve their welfare factors (Quintana 2002). In areas not overexploited, capybara populations may be increasing locally. Capybaras are known to feed in crop fields to the point of being considered pests in some areas of Brazil (Paschoaletto 2003).

Home range size of a capybara herd varies according to the availability of sufficient resources, and averages about 10 ha for high-density populations, but areas up to 200 ha have been reported in the Pantanal region of Brazil (Schaller 1981). Herds do not wander far from water and tend to spend most of their time on a small core area of < 1 ha (Azcarate 1980). The home range is marked with scent stored in (nasal and anal glands), and conflicts between residents and intruders take place. However, home ranges seem to be related more to the maintenance of group integrity and organization than to land tenure (Schaller 1981).

Capybara is common throughout its distribution and its conservation status is considered to be “low risk”. Information about distribution and population status varies greatly among

countries and monitoring methods range from census-based direct observations, to hunting records, to no information at all. Harvesting capybara is permitted in Venezuela and Argentina and was allowed in the recent past in Paraguay. However, hunting is prohibited in the great majority of the South American countries. Nevertheless, capybara is still an economically important species and is illegally hunted for its hide which is prized for its softness, and the thick grease from the hide is used in the pharmaceutical trade (Federico 2005). Capybara is also one of the principal species hunted by indigenous peoples and local settlers for subsistence.

Capybara conservation status varies among South American countries; however, capybara is one of the few large mammal species to take advantage of anthropogenic land transformation. Capybara is an efficient herbivore and as a rodent, it has unique characteristics such as quick maturation and a high reproductive rate. These facts make capybara suitable for commercial captive breeding. In Venezuela, Colombia, Brazil and Argentina, efforts are underway to create environmentally sound and sustainable ranching with capybara (Federico 2005).

The Gran Chaco of South America

The Gran Chaco region of South America gets its name from the Quechua word Chacu meaning lowland or land of good hunting. The Chaco is a vast plain measuring about a million square kilometers, of which 46% extends through northern Argentina, 32% in northwestern Paraguay, 15% in southeastern Bolivia, and 7% in southwestern Brazil (Hueck 1972; Figure 3). Paraguay has the largest remaining tracts of intact Chaco habitat in South America. The Chaco, although not a rainforest ecosystem, is as rich as tropical rainforest in terms of numbers of large mammal species (Redford 1990). The Gran Chaco is an almost flat alluvial plain with a constant slope on the east side of the Andes Mountains. It is almost unbroken by hills with elevation ranging from 100 – 500 m above sea level. The Chaco is considered one of the largest wooded grasslands in South America. Recently it has suffered from intense degradation through persistent forest clearing and overgrazing. For these reasons, the Gran Chaco ecosystem of Paraguay has received the highest global priority for conservation by the United Nations (Green 1996).

The Chaco climate is classified as semi-arid and arid (Fatecha 1988). It is characterized by having two major seasons (wet and dry), with climatic conditions being relative stable due to the absence of major topographic relief. Average ambient temperature in the Chaco rises from south to north and rainfall decreases westward (Gorham 1973). The highest rainfall values are along the Paraguayan River with a maximum of 1,300 mm in Asunción (Paraguay), diminishing to the south near Santa Fe (Argentina), with annual rainfall of 1,000 mm. The lowest rainfall levels are found between the northern border of Argentina, Paraguay and Bolivia and can be as low as 200 mm per year (Gorham 1973; Redford 1990). The wet season coincides with the austral summer; this provides favorable conditions for forage species to thrive. Conversely,

during winter months, frost occurrence is frequent toward the southwest and less frequent to the northeast. Due to its geographic location, the Chaco has large variations in temperature between summer and winter with temperature during the summer reaching highs of 47°C, and below freezing during winter; with an annual mean temperature of 24°C to 25.5°C (Savaria-Toledo 1993).

The Chaco soils are mostly alluvial deposits of material originating in the Andes Mountains and transported by the rivers Bermejo and Pilcomayo, which have the characteristic of changing their courses over time due to their shallowness and the extremely flat Chaco topography (Glatzle 1999). Eastern and western Chaco regions have significant differences in soil; the eastern soils are dominated by clay and have poor drainage; western soils, on the other hand, have high concentrations of loam and sand and relatively good drainage (FAO 1964; FAO/UNESCO 1971).

The Gran Chaco is a unique ecosystem with mosaic of grasslands, savannas, open woodlands and xeric thorn forest comprised of cacti and bromeliads with grass ground cover (Bucher 1982). Other typical vegetation types are palm savannas, savannah parkland, low tree and shrub savannah, with halophytic shrubs on saline patches (Fretes 1970; Hueck 1966; Mereles 1992). The Chaco is dominated by large tracts of woodland characterized by the tree quebracho blanco (*Aspidosperma quebracho-blanco*) covering most of the southwest Bolivian, and the northeastern Argentinean Chaco, as well as the central and north Paraguayan Chaco. To the southeast, large areas are covered by low woodland shrubs, characterized by being almost impenetrable. This area is dominated by the presence of the tree guaimipire (*Ruprechtia triflora*), distributed also in the humid southern Chaco (Mereles 1992). Gallery forest is also common regularly in the vicinity of watercourses with richer soil and greater humidity. Periodically

flooded lands are dominated by palm groves (*Copernicia australis*) and species of algarrobo (*Prosopis alba*, *P. nigra*), with the ground covered with species of *Paspalum* and *Cynodon* grass.

The Chaco ecosystem harbors some of the highest densities known for many mammalian species such as the jaguar (*Panthera onca*), puma (*Felis concolor*), Chacoan guanaco (*Lama guanacoe*), giant anteater (*Myrmecophaga tridactyla*), tapir (*Tapirus terrestris*) and the rare giant armadillo (*Priodontes maximus*) (Redford 1992b). The richness and wildness of the Gran Chaco is illustrated by the discovery of a new species of large mammal, the Chacoan peccary in the 1970's (Wetzel 1974). The Chaco is also an important stopover for Austral and Neotropical migrant birds (Brooks 1998). In total, the Chaco is home for approximately 500 bird species, 150 species of mammals, 120 species of reptiles and over 100 species of amphibians.

It is thought that the first humans arrived to the Chaco about 8,000 years ago. Since then, the Chaco region has been inhabited by several indigenous tribes living by hunting, gathering and, for some, fishing. Most were traditionally nomadic, moving in seasonal patterns, and their impact to the ecosystem was strong mainly as a consequence of the use of fire. Arrival of the Spanish was late and settlements were small and limited to the Chaco shoreline of the major rivers such as the Paraguay and Parana River. Only during the nineteenth century did settlements begin in the Chaco of Argentina, Bolivia and Paraguay. New settlements were based on extensive ranching and forest-related activities. Industrialization of ranching and agriculture began in the 1970's, and since that time, the Chaco region has been rapidly modified. As a consequence, large native animals such as guanacos, tapirs, pampas deer (*Ozotoceros bezoarticus*) and swamp deer (*Blastocerus dichotomus*), jaguar and giant armadillo have almost completely disappeared from the Chaco ecosystems (Cabrera 1973).

Rapid and large scale agricultural and cattle ranching development has transformed and degraded the central and southern Gran Chaco of Paraguay. In Argentina, the Chacoan Forest has almost entirely been converted to pasture or logged and left fallow. The northern Chaco of Paraguay and southern Bolivia still contain large tracts of intact Chaco forest; however, significant modification of the landscape is in progress. Recently published data estimate an astonishing 500 ha of forest being cleared daily in Paraguay (Huang 2009). It is a fact that the fragile Chaco ecosystem is facing intense transformation. We hope that governments, land owners and residents are aware that if conservation and rational policies are not implemented, the Chaco region will in the near future become a sterile dry landscape.

The Paraguayan Chaco region

Paraguay is a land locked country in south-central South America. The north to south flowing Paraguay River divides the country into two distinct areas: the southern Oriental region and northern Chaco region. The Oriental region is characterized by mostly subtropical humid forest, marshy plains, and grasslands (Myers 1982). The mean annual precipitation increases in a northwest southeast direction from 1,200 to 1,760 mm/year. The Oriental region has a large number of rivers, streams, lakes and natural ponds. The topography is mainly plains to the southwest with hills distributed throughout the northeast of the region; the highest altitude above sea level is 850 meters. The main conservation issue in the Oriental region is that it holds 96% of the population of Paraguay. It is a region that has been intensively deforested; remaining forest exists as small isolated fragments.

The Chaco region of Paraguay is a vast alluvial plain virtually unbroken by hills, situated in the rain shadow of the Andes Mountains; It stretches from about 19° to 25° South latitude and

between 62° and 57° West longitude, politically demarked to the east by the Paraguay River and to the south-west by the Pilcomayo River (Figure 4). Altitudes range from 380 m in the north-west and 80 m in the south-east, where the Paraguay and Pilcomayo Rivers join (FAO 1964; Hueck 1966; Ramella 1989). The Paraguayan Chaco can be divided into two zones. The Humid Chaco or Low Chaco is a plain predisposed to flooding and greatly influenced by the periodical flooding of the Paraguay, Pilcomayo and other tributary rivers (Mereles 1992). The area is mostly characterized by wide-open natural humid grasslands, annually flooded savannas and swamps (Redford 1990). On the other hand, the High Chaco also known as the Chaco Boreal, is much drier, characterized by abundant water during the rainy season (November to May) but diminishing greatly or even disappearing completely during the dry season, that extends from June to October (Hueck 1966). The landscape is predominantly thick desert xeric thorn forest (Gorham 1973; Holdridge 1969; Redford 1990). According to Glitzle (1999), this region can further be subdivided into three major sub regions. The Central Chaco is derived from a former inland delta with a mosaic of old riverbeds with coarse sandy sediments contrasting with areas with fine textured soils, which were deposited by an ancient river system. The second region is the Northern Chaco, most of which is more homogeneous and is comparable with the coarse sandy sediment system of the Central Chaco. The third region is the driest region forming the Sand Dune areas in the far north-west of the Paraguayan Chaco, formed by old alluvial deposits remodeled by winds (Glatzle 1999). In the Paraguayan Chaco, it is not uncommon to see as much as 80 percent of the rainfall during the summer (Eidt 1968). The Pilcomayo River is the principal river running southward from its origin in the Andes Mountains to the Chaco basin; this river has an extraordinary capacity to produce sediments, some 60 million m³ annually on average and a great variation in flow, with peaks of 2,000 m³/second and lows of only

3 m³/second. Due to the unique characteristics of the Paraguayan Chaco ecosystem, European colonization was curtailed until recent years, remaining mostly undeveloped and immune to modern anthropogenic land modification. More recently, the Mennonite community has had a strong influence on the development of the Paraguayan Chaco. The first settlers arrived in the 1920's and more followed in the 1930s's. Currently, the Central Chaco of Paraguay is almost totally settled by Mennonites. At present, the human population estimate is 142,000 in the region, with the majority of settlements established less than 50 years ago. The main economic source of income is cattle production. The estimated cattle population is approximates 2,000,000 with nearly 60,000 horses (SENACSA 2008). The cattle population has dramatically increased since the 1970's, as well as the rate of deforestation of native forest which has been transformed into introduced pasture land (Benirschke 1989; Huang 2009). To accommodate cattle year-round, artificial ponds have been constructed throughout the Central Chaco.

Anthropogenic land transformation of the Paraguayan Chaco has drastically transformed the area, changing the forest to open field. This land change has brought new species to the area as well as greatly damaged the ecological balance of the region. This has caused outbreaks of diseases such as hanta virus and Chagas disease (*Trypanosoma cruzi*). Because the Gran Chaco ecosystem is poorly understood, little is known how wildlife species interact with the new matrix of habitat patches. The Chaco region is rapidly changing. If no action is taken to implement sustainable development polices, the damage will be irreversible. Luckily, for the Paraguayan Chaco, it is still not too late for it too be saved for future generations.

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Figure 1

Chaco capybara in water, surrounded by aquatic vegetation.



Figure 2

Geographical distribution of capybara: 1, *Hydrochoerus hydrochaeris*; 2, *H. isthmus*. Black circle depicts the study area; the question mark represents the unknown capybara distribution in the Chaco region of Paraguay. Figure modified from Mones (1981, 1986).



Figure 3

Gran Chaco region of South America, including Argentina, Bolivia, Paraguay and Bolivia. Map modified from wikimedia.org.



Figure 4

Gran Chaco region of Paraguay. Map modified from wikimedia.org.



Chapter II

Deforestation and cattle ranching drive rapid range expansion and secondary contact of vicariant populations of a semiaquatic rodent in the Gran Chaco ecosystem

Running head: Range expansion of capybara in Paraguay

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Keywords: Capybara, cattle ranching, deforestation, ecological niche modeling, Gran Chaco Ecosystem, phylogeography, range expansion, secondary contact, Trypanosoma spp.

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Abstract

Anthropogenic habitat alteration has the capacity to alter the distribution of species. Capybara are a widely distributed rodent throughout most of South and Central America, but restricted to areas of standing water. As the Gran Chaco ecosystem of Paraguay is converted from dry tropical forest to pastureland, we hypothesize that this alteration creates potential for invasion by capybara into newly fragmented areas. We used ecological niche modeling to generate hypotheses about how the distribution of capybara has been affected by land use change, and then tested those hypotheses with phylogeographic analyses. We surveyed throughout the Chaco to estimate the distribution of capybara, and we collected noninvasive genetic samples. Using program GARP, we modeled habitat suitability with both current and historical land use patterns. GARP models were based on 6 environmental or climatic variables, and we modeled both the current distribution of capybara and the distribution of capybara 80 years ago. We then verified the hypothesized demographic signal generated with our model using phylogeographic analyses of 386 bp of the mtDNA control region. Comparison of present and past models suggested that populations expanded into the Gran Chaco after forests were converted to pastureland. Analyses of the mitochondrial D-loop supported the rapid range expansion scenario. We also found evidence of secondary contact of two distinct phylogroups which had previously been disjunct. Anthropogenic land transformation appeared to be a major factor influencing the distribution, as predicted by the niche model and confirmed by genetic data. Habitat modification altered connectivity of populations across the landscape allowing long separated clades of capybara to invade previously unoccupied areas and come into contact with one another. Because capybara is the reservoir host of a trypanosomal disease, the expansion and contact of two previously disjunct populations has implications for disease emergence.

Introduction

Anthropogenic land transformation is a worldwide phenomenon which changes and remodels ecosystems at an unprecedented rate (National Academy of Sciences 2000). The expansion of native species and the invasion of exotic species places endemic species at risk of extinction (Aguirre & Tabor 2008), changes the composition of biotic communities, and alters ecosystem services (Lockwood *et al.* 2007). In addition, land use change brings previously disjunct populations and species together to create no analog ecosystems. Naïve host are exposed to new pathogens which provides opportunities for the emergence of infectious diseases (Daszak 2000). Thus, understanding the ecological drivers of species distribution is fundamental to forecasting future distributions and vulnerabilities in light of human activities.

Ecological niche modeling (Busby 1991; Stockwell & Peters 1999) uses the fundamental niche of a species (Hutchinson 1957) to determine suitable habitat and distribution of species by correlating occupancy and environmental data to predict the distribution of a species (Franklin 1995; Hirzel *et al.* 2002). Ecological niche modeling has been successfully used to predict the distributions of vectors, parasites and pathogens important to human health, threatened plants and animals, as well as invasive species (Peterson 2001; Peterson & Vieglais 2001; Soberon & Peterson 2005; Graham *et al.* 2006; de Barros *et al.* 2007; Costa *et al.* 2008). These new modeling techniques incorporate explicit spatio-geographic data that make it possible to generate alternative hypotheses that can then be tested with phylogeographic data (Walker *et al.* 2009). Using independent datasets to test biogeographic hypotheses is a powerful new approach to inferring the drivers of ecological change (Richards *et al.* 2007). In the current paper, we used niche modeling as a hypothesis generator and then tested our hypothesis with an independent

phylogeographic dataset to validate the model and to better understand the demographic history and population trends during the modeled time period (Weaver *et al.* 2006).

We investigated the past and present distribution of the semi-aquatic rodent, capybara (*Hydrochoerus hydrochaeris*), in the Chaco region of Paraguay to determine the ecological drivers which have allowed this species to expand its distribution. The Paraguayan Chaco is a vast alluvial plain bordered on the west by the Pilcomayo River and on the east by the Paraguay River and is divided into two ecoregions (Fig. 1). The Low Chaco, a plain predisposed to flooding (Redford *et al.* 1990; Mereles *et al.* 1992) is characterized by palm forests with natural humid grasslands, and annually flooded savannas and swamps (Redford *et al.* 1990). The High Chaco is characterized by thick, xeric thorn forest (Holdridge 1969; Gorham 1973; Redford *et al.* 1990; Glatzle 1999) with abundant standing water during the raining season (November to May) which diminishes greatly or disappears entirely during the dry season (Hueck 1966; Eidt 1968). The uniquely harsh environment of the Paraguayan Chaco ecosystem has allowed it to remain mostly undeveloped and immune to modern anthropogenic land modification until 50 years ago when deforestation began for cattle production (Servicio Nacional de Calidad y Salud Animal 2008). Anthropogenic activities dramatically changed the landscape of the High Chaco. Approximately 500 ha of forest are cleared daily for pasture, and year round water sources have been established in this seasonally dry habitat (Fig. 1). Conversion of dense forest to pasture and year-round water availability greatly alters the resource potential of the ecosystem and may permit the invasion of species into the landscape.

Capybara (*Hydrochoerus hydrochaeris*) are the world's largest living rodent (Nowak 1991). These semi-aquatic mammals are distributed throughout most of South America, from Panama to northern Argentina, Paraguay and Uruguay (Redford *et al.* 1990). Although closely

associated with aquatic environments, capybara use water primarily as a place of refuge, and most normal activity is on land (Schaller 1976). Nevertheless, a year-round standing water source is essential for capybara to be present in an area. Capybara are an efficient grazing herbivore that shares pasture with livestock (Mones & Ojasti 1986; Ojasti *et al.* 1991; Quintana *et al.* 1998; Quintana 2003). Populations are increasing in South America; and in Brazil, capybara are considered a local pest (Paschoaletto *et al.* 2003). Capybaras destroy crops and compete with livestock for forage. In addition, they are the reservoir host of a trypanosome parasite (*Trypanosoma evansi*) responsible for livestock disease (Franke *et al.* 1994; Herrera *et al.* 2004). It appears that capybaras are taking advantage of land transformation occurring throughout South America and expanding its range as deforestation for agriculture and pasturelands occurs. This scenario appears to be occurring regionally in the central Chaco region of Paraguay. We used the dual approach of ecological niche modeling and phylogeography to investigate the drivers of range expansion of capybara in the Chaco region of Paraguay.

Using ecological niche modeling, we predicted the past and present dry season distribution of capybara in the Chaco of Paraguay to generate hypotheses about the ecological drivers that have allowed the invasion of capybara into the dry tropical forest region of the Chaco. We then used a phylogeographic approach to test this hypothesis. By incorporating genetic data, we tested whether the distribution models provided an accurate picture of the demographic history of capybara, and identified the drivers of demographic change to capybara populations. This combined approach improves our understanding of how land transformation affects the invasibility of an ecosystem and provides a more robust method for identifying ecosystems at risk.

Materials and methods

Animal surveys

We surveyed for capybara at 121 locations throughout accessible parts of the Chaco region of Paraguay during the dry season, in austral winters from 2005 to 2007. We combined landowner interviews with on the ground surveys to determine the presence of capybara. We surveyed for capybara on foot walking throughout the area; capybaras were considered present when they were directly observed, or tracks or fecal pellets were located. Capybara tracks and fecal pellets are distinctive from those of other large herbivores which allowed us to accurately identify the species. In 86 of the 121 locations, we surveyed for capybara and interviewed landowners to determine capybara presence; at 35 locations, landowners were not available for interviews. Locations were recorded in UTM coordinate system using Geographical Positioning System (GPS) at all sites. We also included presence data from two recently published capybara surveys in the region (Neris *et al.* 2002; Ziegler *et al.* 2002). As part of our model validation, we identified locations from which capybara were absent. We considered capybara to be absent from an area if there was a standing water source, yet no sign (fecal or tracks) could be found. We also considered an area to have no capybara if there was no permanent water source within a minimum of a 20 km radius.

Ecological niche modeling

Predictive modeling of species distributions represents an important tool in biogeography, evolution and conservation (Peterson & Vieglais 2001). The Genetic Algorithm for Rule-Set Prediction (GARP: <http://biodi.sdsc.edu/>; see <http://beta.lifemapper.org/desktopgarp> for software download) is an expert-system, machine-learning approach to predictive modeling

(Stockwell & Peters 1999). Ecological niche modeling uses environmental data to model the presence of suitable habitat and the potential distribution on a geographic scale (Soberon & Peterson 2005). To predict capybara distribution in the Gran Chaco of Paraguay, and to better understand factors affecting the distribution of capybara, we used six environmental datasets. All environmental layers were resolved to a 1 km pixel resolution by using ArcGIS version 9.2. Datasets included vegetative landcover of South America (Eva *et al.* 2002), soil type, average annual precipitation, average annual temperature (The Food and Agriculture Organization of the United Nations 1964; Holdridge 1969), rivers and altitude (Dirección del Servicio Geografico Militar 1998). Eighty percent of the locations were used for model training and the rest were retained to test model accuracy.

We developed 1250 distribution models of capybara occupancy using a subset of 85 locations. Desktop GARP was run for 1000 iterations or until a model convergence of 0.01 was reached. From our 1250 distribution models, subsets of best models were selected following the criteria of Anderson *et al.* (2003). A total of 114 models with the highest commission and lowest omission (commissions higher than 50 ± 5 , omission less than 10) were selected from the pool. Second, models with the highest test accuracy (over 0.68%) were selected, accounting for a total of 122 models. From these, only models that were unanimously selected by the two approaches were selected as the final pool of best models. From the mutual set, a subset of 20 best models was selected. These 20 models were concatenated to form a single distribution.

The final best ecological niche models were projected onto a digital map, exported as an ASCII raster grid, and imported into ArcView 9.2 using the Spatial Analysis Extension for visualization. The number of concordant models was summed; this procedure produced a composite map comprised of pixels with values depicting the percentage of models that

predicted the presence of capybara in the pixel. To validate the GARP model, we used 24 locations where capybara were present, but were not included in model construction, and 39 locations where we found no capybara or considered them absent. We calculated the proportion of locations where the model accurately predicted capybara to be present or absent.

Using the same model selection and concatenation procedure as described for the current survey data, we constructed a best concatenated model for the hypothesized recent past (80 years ago) capybara distribution. To create our distribution map of the past, we allowed all of the environmental datasets to remain the same, except the landcover layer. We modified the vegetation layer, reclassifying introduced pastureland to the predominant landcover type surrounding the pasture. We identified pastureland in our landcover map layer and by satellite imagery (Eva *et al.* 2002). We modified our survey data by excluding locations that were found adjacent to man made ponds and cattle reservoirs.

Sample collection and molecular methods

Genetic samples were collected during surveys. We used a non-invasive approach by extracting DNA from capybara fecal pellets. Pellet size varies among age class and sex; we used this characteristic to avoid re-sampling individuals by collecting different sized pellets in each sampling location (Murphy *et al.* 2003; Wehausen *et al.* 2004; Hibert *et al.* 2008; McCann *et al.* 2008). In total, we collected 110 samples for the genetic analysis; each sampling location was > 50 km from each other to reduce pseudoreplication (Table 1). A total of thirteen populations were considered for analysis. Collected fecal samples were stored dry in individual sterile plastic containers with silica to absorb humidity. Samples that were not dried sufficiently for direct storage were dried with an electric heater device overnight at a maximum temperature of 40° C.

We also opportunistically collected muscle or skin tissue samples from road kills, hunters, or during live-trapping. Samples were preserved in 2 ml plastic tubes with 95% ethanol. Hair samples were divided in two, one preserved dry and the other in 95 % ethanol in 15 ml sterile plastic tubes. Fecal pellets were stored at room temperature in closed boxes to prevent any light exposure. Samples stored in 95% ethanol were maintained in their original containers at 4° Celsius. Dry hair samples were stored in a -20° Celsius freezer. Extractions and amplification were carried out at the Conservation Genetics and Molecular Ecology Lab at Kansas State University.

Cross contamination is a common risk when using noninvasive samples such as fecal pellets (Deuter *et al.* 1995; Taberlet *et al.* 1996; Reed *et al.* 1997). To prevent this from occurring, DNA was extracted in a room where no PCR products were stored. Fecal material is known to yield low quality and quantity DNA making the extraction procedure challenging (Taberlet *et al.* 1996; Murphy *et al.* 2003; Nsubuga *et al.* 2004; Wehausen *et al.* 2004); however, we obtained results using a combination of several extraction protocols (Boom *et al.* 1990; Eggert *et al.* 2000; Wehausen *et al.* 2004; Eggert *et al.* 2005).

Four primers were designed to amplify a 386 base pair (bp) fragment of the hypervariable mitochondrial (mtDNA) D-loop region, in two overlapping sets. Primer CapyD 2 (5'-TAATGCATGTCCCATGAAC-3') and CapyD 3R (5'-TGGTGCATGTCTAACGATGG-3') amplified a 245 bp region. Primer CapyD 5F (5'-TTCCCATGAATATTTAGCATGT-3') and CapyD 2R (5'-TTTAGAAACCCACGAGTT-3') amplified a 292 bp region. Primers were designed based on published capybara D-Loop sequences obtained from GeneBank (accession numbers EU149776 and EU149773; Perdomo *et al.* 2007). Polymerase Chain Reaction (PCR) was performed in an Eppendorf Mastercycler (Eppendorf, Wesbury, NY, USA) in 20 µl volumes containing 1 U

AmpliTaq Gold DNA Polymerase (Applied Biosystems, Inc., Foster City, CA, USA), 1X AmpliTaq Buffer II, 1 μ M forward primer, 1 μ M reverse primer, 2 mM MgCl₂, 0.2 mM each dNTP, 1.5 ug/ μ l BSA (New England Biolabs, Ipswich, MA, USA), and 3 μ l of the DNA extract. The PCR profile consisted of a single denaturation step at 95° C for 10 min, followed by 45 cycles of denaturation at 95° C for 45 s, annealing at 56° C for 45 s and primer extension at 72° C for 45 s, and a final extension at 72° C for 8 min. All PCR's involving extracts from fecal material were run with a positive control (Tissue) and all PCR's were run with a negative control. Products were verified on a 2% agarose gel. Samples were sequenced in both directions by an ABI 3100 at the GTCA Kentucky Sequencing Facility, University of Kentucky. Sequences were manually analyzed and evaluated by eye using program Sequencher 4.7. A first alignment was done with program Se-AL V2.0a Carbon (Rambaut 2002) and finalized with CLUSTALW (Tamura *et al.* 2007) implemented in program MEGA4 (Higgins *et al.* 1994). Representative haplotype sequences were deposited in GenBank under accession numbers xxx-xxx.

Population structure analyses

Patterns of genetic divergence among and within capybara populations were assessed by analysis of molecular variance (AMOVA) implemented by program Arlequin 3.1 (Hartl & Clark 1997; Excoffier & Schneider 2005). AMOVA components can be used to calculate Φ -statistics, which summarize the degree of differentiation among hierarchical population divisions and are analogous to *F*-statistics (Wright 1951). We compared the explanatory power of four hypothetical subdivisions of capybara in the Chaco. The first scenario grouped all Chaco populations as a single pandemic population. The second scenario divided capybara in to three populations in the east, central and west. The third scenario consisted of four different subgroups

(North Central Chaco, South Central Chaco, West Chaco and East Chaco), and the fourth scenario subdivided the Chaco region as follows ((West Chaco + North Central Chaco), South Central Chaco, East Chaco). The best grouping was considered to be the one that maximized significance values among group variation Φ_{CT} . We also calculated haplotype diversity (h) (Nei 1987) and nucleotide diversity (π) (Tajima 1983; Nei 1987) measured in terms of number of polymorphic sites; the number of private haplotypes per population was computed using Arlequin 3.1 (Excoffier & Schneider 2005).

To assess whether population structure was in mutation-migration equilibrium, we tested for isolation by distance among populations. We correlated population pairwise genetic distance Φ_{ST} to pairwise geographic distance using partial correlation analysis. We tested for significance with a Mantel test (Mantel 1967) using 10,000 random permutations of the data to test for a significant correlation.

Population demography analyses

Deviation from selective neutrality was tested by Fu's F_s (Fu 1997) and Tajima's D (Tajima 1989). Neutrality tests can be used as an indication of recent population expansion when the null hypothesis of neutrality is rejected. Nonsignificant values for Tajima's D combined with significantly negative F_s values have been interpreted as evidence supporting demographic population expansion (Fu 1997).

As an additional test of population expansion, we calculated the distribution of pairwise differences between pairs of haplotypes (mismatch distribution) (Rogers & Harpending 1992). This distribution is usually multimodal in populations at demographic equilibrium, but it is unimodal in populations which have recently expanded (Hudson 1990; Excoffier & Smouse

1994; Slatkin 1991; Ray *et al.* 2003). We calculated the ruggedness index (r) of the mismatch distribution. The ruggedness index is expected to be greater in older stationary populations than in younger expanding populations (Rogers & Harpending 1992). From the mismatch distribution we were able to estimate the time since expansion $\tau = 2\mu t$, and the population size before and after the expansion $\theta_0 = 2\mu N_0$ and $\theta_1 = 2\mu N_1$ (Rogers & Harpending 1992). To estimate these three parameters, we used a range of mutation rates (3, 6 and 12%) (Pesole *et al.* 1999; Spradling *et al.* 2001) to account for uncertainty on the exact mutation rate of capybara mitochondrial d-loop, and we used an estimated generation time of three years for capybara (Colin 1991; Nowak 1991).

Phylogenetic and nested clade analysis

To evaluate evolutionary relationships among haplotypes, two approaches were used. First, a minimum spanning network (MSN) of haplotypes was constructed according to Templeton *et al.* (1992) using TCS version 1.21 (Clement *et al.* 2000). TCS Program uses statistical parsimony to define the parsimony limit consisting in the maximum number of differences among haplotypes as a result of single substitution with a 95% statistical confidence. Haplotypes with the smallest differences are first connected, then more divergent haplotypes are added until all haplotypes are included in the network. Second, we used program Network 4.5 (Bendelt *et al.* 1999) which uses the median joining approach to combine a minimum spanning tree within a single network and then by parsimony criterion, median vectors are added to the network (Bendelt *et al.* 1999).

To better understand the historical connectivity among haplotypes in the network, we estimated the divergence time among the major phylogroups and clades using program Network

4.5 with the time estimate option. To account for uncertainty of the exact mutation rate of capybara mitochondria d-loop, we used the average of a range of mutation rates (3, 6 and 12%) per million years.

To better understand the demographic and evolutionary mechanisms responsible for spatial distribution of observed genetic variation, we conducted a nested clade analysis (NCA) using our sequence data to differentiate population history from population structure (Templeton 1998). We nested clades by hand according to the rules described by Templeton *et al.* (1987) and Templeton & Sing (1993). The nested clade design was used to assess geographic distances among haplotypes, which in turn were used to test for geographical association between clades. D_c measures the geographical range of the clade, and D_n measures the geographical distance between a haplotype and the geographical center of the haplotype at the next level of nesting (Templeton *et al.* 1995). A matrix of Euclidian distances between all populations was obtained using ArcMap version 9.2. The association between the geographical location and genetic distance was tested statistically with 10,000 random permutations using program GeoDis ver 2.5 (Posada *et al.* 2000).

To further understand the relationship between haplotypes, we carried out phylogenetic analysis using the neighbor-joining (NJ) method (Saitou & Nei 1987) executed through program MEGA 4 (Tamura *et al.* 2007), maximum parsimony (MP) implemented through PAUP ver. 4.0 (Swofford 1998), and Bayesian inference (BI) analysis carried out in MrBayes 3.1 (Ronquist & Harpending 1992). (We included a haplotype sequence from a capybara from Venezuela as an outgroup (GenBank accession number EU149773)). To find the substitution model that best fit the data, we used program Modeltest (Posada *et al.* 1998). Using Akaike's information criterion

(AIC), (Akaike 1974) the best model was TIM+I with base frequency of A: 0.3477, C: 0.2671, G: 0.1131, T: 0.2715 (-lnl = 712.9, AIC = 1439.9, I = 0.88, G = equal rate for all sites).

For the Bayesian inference analysis, we carried out two independent runs of 1,000,000 generations with four simultaneous, incrementally heated Markov chains, with sampling every 100 generations. The first 2,500 sampled trees were discarded as burn-in to avoid the possibility of including random unconverged trees by the Markov chain. The remaining trees were used to construct a 50% majority rule consensus tree and displayed using program MEGA 4 (Tamura *et al.* 2007). Posterior probabilities for individual clades were used as nodal confidence estimates. Likewise, our MP trees were inferred with a general heuristic search and 10,000 random addition sequences with tree-bisection reconnection branch swapping. Robustness of branching pattern in the MP tree was tested using non-parametric bootstrapping with 1,000 bootstrap replicates of 100 random sequence addition replicates.

Results

Ecological niche modeling

Using program GARP, we generated two distribution maps based on present and past ecological variables and capybara locations. Of our six environmental layers used to model capybara distribution, soil type, average annual rainfall, and altitude were significant, single variable predictors of presence with high test accuracy. However, the best fitting models were obtained using all environmental layers.

For our map of current distribution, all models predicted the presence of capybara in the southeastern Low Chaco region with 100% agreement (Fig. 2). Similarly, most human made pasturelands of the central Chaco were considered suitable habitat by all 20 models. The second

most probable area of finding capybara, with 75 % model agreement, was a stretch of land at the boundary between the High Chaco and the Low Chaco which is the transition zone between dry and humid tropical forest. This area was believed to be the northwest extent of the species range as described by early collectors (Wetzel & Lovett 1974). Areas with 50% model agreement were found in the central and west Chaco region. This region is characterized by sandy soil and little to no pasturelands or natural grasslands, which limited the presence of capybara. The northwest Chaco had only 25% model agreement for capybara presence. This area is characterized by sandy soils and little permanent standing water other than the Pilcomayo River. The northern High Chaco also had 25% model agreement of capybara presence; this area has pockets of permanent water but is dominated by virgin dry tropical forest with little anthropogenic disturbance. During our survey, we found no sign of capybara in this area. In the northern most region of the High Chaco, GARP found a zero percent probability of capybara inhabiting this densely forested region.

Our ecological niche model accurately predicted the known capybara distribution. Only a few validation locations of presence and absence were incorrectly classified. From 39 absence data points (not used for model construction by GARP); 12 (30%) fell in areas that predicted capybara to be present. Of these, 6 (50%) fell in an area with 25% model agreement of capybara presence; 5 (13%) fell in an area with 100% model agreement and 1 (2%) fell in an area with 50% model agreement. Nine of the absence data points were in the Central Chaco region where rapid land conversion is occurring and colonization by capybara is ongoing. It should be noted that this distribution map was based on the dry season distribution of capybara; during the rainy season, it is likely that the range of this species expands and the probability of finding this animal in seasonally flooded areas likely increases.

Our second distribution map corresponded to the hypothesized past distribution of capybara. GARP predicted a much more restricted distribution of capybara 80 years ago, based on difference in land cover. The most suitable habitat, based on model agreement, was limited to the southern Low Chaco and the two major rivers that converge in this region. Unlike the present distribution, the past distribution had a low probability of occupancy by capybara in the central Chaco (Fig. 2). Thus, the difference between the past and present distribution maps suggests that capybara have rapidly expanded north into the central Chaco region as dry tropical forest was converted to pastureland and year round permanent water sources were established.

Population structure analysis

For genetic analyses, of population structure, we averaged 8 samples per population; however, for two populations, Mariscal and Laguna Rey, we had fewer than 5 samples. In total, we found 21 polymorphic sites, with an average of 5.5 haplotypes per population. The mean transition rate was 5.3 with a transversion rate of 0.15. Of the four AMOVA models, the model which divided capybara populations into three subgroups from east to west explained the most genetic variation: 27% of the variation could be explained by among group variation (East, Central, West); variation among populations within groups accounted for 17.7% of the variation, and within population variation accounted for a total of 55.6% of variation (Table 2). Nucleotide diversity (π) was similar among East, Central, and West Chaco populations (global mean = 0.016); the average number of pairwise differences was substantially greater in the East Chaco than in the West or Central Chaco; and haplotype diversity was substantially lower in the Central Chaco (Table 3). Population BN, located in the northeast Chaco had the highest diversity (π = 0.018, mean number of pairwise differences = 6.78). Our global pair-wise population

differentiation index (Φ_{ST}) among populations was 0.44, with the most differentiation found between the populations of Sauces and Jerovia ($\Phi_{ST} = 0.84$). This value stands in contrast to the populations of Toledo and Maroma which had $\Phi_{ST} = 0.07$, yet was separated by almost 300 km (Table 4). We did not find a significant correlation of geographic distance and genetic differentiation, indicating that the genetic pattern on the landscape could not be explained by equilibrium evolutionary processes.

The disruption of isolation by distance equilibrium likely resulted from a strong signal of population range expansion. With one exception, (Sauces, $D = -1.85$, $P = 0.01$), Tajima's D was not significant; however, nearly all populations had significant negative values for Fu's F_s test, suggesting a signal of population expansion. Results of the mismatch test suggested that the distribution of allele frequencies was not significantly different from a unimodal distribution. We infer from this result that the East Chaco (sums of squared deviation (SSD) = 0.04, $P = 0.09$; $r = 0.07$, $P = 0.13$) and the Central Chaco groups (SSD = 0.05, $P = 0.21$; $r = 0.07$, $P = 0.34$) had recently expanded their range. In contrast, the West Chaco group deviated significantly from unimodal distribution (SSD = 0.08, $P = 0.04$; $r = 0.47$, $P = 0.44$) implying that populations in this group were likely in equilibrium (Fig. 3).

Phylogenetic and nested clade analysis

Sampled capybara populations in the Gran Chaco had 14 unique haplotypes, with two dominant haplotypes corresponding to 2 major phylogroups. The haplotype network was dominated by these two haplotypes, separated by long branches with short internal branches within phylogroups (Fig. 4). This pattern of haplotypic distribution has previously been attributed to isolated populations that have undergone recent range expansion (Slatkin 1991;

Rogers & Harpending 1992) and then come into secondary contact within the sampled area (Avice 2000).

The construction of the haplotype network gave similar results using programs TCS and Network. The two haplotypes at the center of each cluster corresponded to the ancestral haplotypes from which all surrounding haplotypes in the clade diverged. Haplotype 2 was the center of clade 3-1 which formed phylogroup AB; haplotype 1 formed the center clade 3-2 which comprised phylogroup CD. The molecular clock analysis estimated that these two phylogroups diverged 19,700 years Before present (Bp). This time corresponded with the height of the last glacial maximum. Subclade formation within phylogroup 3-2 occurred more recently, approximately 2,700 years Bp near the end of the Holocene.

The phylogenetic trees created using neighbor-joining (NJ), maximum parsimony (MP) and Bayesian inference (BI) gave similar results, with different bootstrap and posterior probability values. Our phylogenetic analysis divided our 14 haplotypes into two main clades which were further subdivided into four subclades A, B, C and D (Fig. 5). Subclade A was found throughout the Central, East and West Chaco. Subclade B, sister clade to Subclade A, was composed of haplotypes 10 and 11, which were present only in the East Chaco group. Subclade C also had representatives from all three geographic areas. But subclade D, which is most closely related to Subclade C, was formed by three haplotypes (4, 6 and 7), all of which were found only in the East Chaco.

Discussion

We have demonstrated a powerful approach to testing the effects of land use change on the distribution of species. We used ecological niche modeling to generate hypotheses about the spatial extent and drivers of distributional change, and tested those hypotheses with molecular genetic data. We believe that using ecological niche modeling not as the final analysis, but as a hypothesis generator, provides a powerful use of this biogeographic approach. Non-invasive sampling of capybara assisted in providing location data for the survey and in providing genetic data for phylogeographic testing of our hypothesis.

Using ecological niche modeling to generate hypotheses

Although the Spanish conquistadors were probably the first Europeans to see capybara, Azara (1802) was the first naturalist to fully describe the species in the Gran Chaco region. Current distribution maps indicate that capybara inhabits the Low Chaco region, but the northern extent of the distribution within the Chaco region is unclear and has changed through time. Mones *et al.* (1986) classified most of the Central and the entire High Chaco region as an area in which capybara were unlikely to be found. Wetzel & Lovett (1974) collected specimens in the transition zone of the central Chaco, and proposed that the area was the distributional edge of capybara in the Gran Chaco ecosystem. More recently, Brooks (1998) surveyed large mammals in the Chaco from 1989 to 1990 and stated that capybara was not found in the dry central Chaco region. In contrast, the most recent published surveys (Neris *et al.* 2002; Ziegler *et al.* 2002) support our findings that capybara are recently present in the dry central Chaco.

We used survey data and ecological niche modeling to create a spatially explicit hypothesis that addressed the drivers behind observed recent range expansion of capybara. By

first creating and validating a model with current survey data, we were able to predict capybara distribution with increased confidence. Past and current niche models both predicted high probabilities of capybara throughout the Low Chaco region and along the two major rivers drainages; however, only the current niche model predicted a high probability of capybara in the fragmented landscape of the central Chaco. The difference in the distribution of capybara between the past and present models was driven by the presence of recently created pasturelands and year round water catchments in the dry tropical forest of the central Chaco. The expanding ranching industry of this area appears to have created suitable habitat for capybara by extending human-made grasslands and previously unavailable water sources into the dry tropical forest of the central Chaco. These findings suggest that rapid deforestation in the dry central Chaco has allowed capybara to invade this ecoregion from the Low Chaco region, the Pilcomayo River and the Paraguay River. This hypothesis was supported by our phylogeographic and population genetic analyses.

A test of the hypothesis with genetic data

Our genetic data strongly supported the hypothesis of recent range expansion into the central Chaco region. Multiple analyses, including the use of Fu's F_s , mismatch analysis, and coalescent methods, reveal the genetic signal of demographic expansion. Source populations for this expansion were hypothesized to have come from the Pilcomayo River, the southern Low Chaco, and the Paraguay River, all of which have year round standing water. In support of this hypothesis, we found moderate to high levels of connectivity and haplotype sharing among populations from the Pilcomayo River, the southern Low Chaco and the newly invaded central Chaco. To the east, however, populations along the Paraguay River, (Bahia Negra, Olimpo),

were the most genetically diverse and differentiated from all other populations. Contrary to our hypothesis, these findings suggest that little migration has occurred between these northern populations and southern populations in the central Chaco. We infer, based on modeling and genetic results, that large scale range expansion was prevented from the east into the central Chaco because tropical dry forest on the eastern edge of the Gran Chaco has not yet experienced the level of deforestation and modification found in the west and central Chaco region. The presence of large areas of primary growth tropical dry forest likely serves as a natural barrier to capybara dispersal. Thus, we have identified contemporary processes of isolation that support our inferred mechanism of isolation that prevented range expansion prior to anthropogenic land change.

Secondary contact of two isolated clades

In addition to supporting our hypothesis of range expansion, the genetic data uncovered evidence of secondary contact of two distinct phylogroups of capybara in the Gran Chaco region. Although the geographic origin of these phylogroups was outside the scope of this study, the domination of two haplotypes which are the ancestral haplotypes for distinct phylogroups strongly suggests that descendants from two refugial populations have expanded into the central Chaco to form sympatric populations of two ancestral groups. Molecular clock estimates suggest that these groups diverged approximately 19,700 years BP, during the Last Glacial Maximum (LGM).

The LGM had important effects in the regional climate of South America and the species occupying this region. Temperatures during the LGM are believed to have been several degrees cooler, and characterized by frequent cycles of drought and flooding (30,000 to 25,000 years BP)

(Clapperton 1993; May *et al.* 2007) which continued throughout the late Pleistocene – Holocene transition period (Kruck 1996; Barboza *et al.* 2000; Pasig 2005; May *et al.* 2007). During this period, evidence suggests that other regional species associated with water, such as marsh deer (Marquez *et al.* 2006) and aquatic birds such as the roseate spoonbill, wood stork and jabiru stork (Lopes *et al.* 2006; Lopes *et al.* 2007) expanded their distributions and diverged genetically. More specifically, Lopes *et al.* (2006) found that in the Pantanal of Brazil, two colonies of Wood Storks diverged approximately 6,250 years BP with the most recent common ancestor approximately 18,900 years BP. Temporal and spatial patterns of phylogeography for the semi-aquatic capybara suggest that climatic fluctuation and the resulting ecological changes facilitated similar trends of vicariance and divergence across multiple members of the South American megafaunal wetland community.

The two divergent phylogroups of capybara currently have representatives throughout the newly invaded central Chaco. Indeed, within the central Chaco, land use and land cover changes have maintained high rates of migration and connectivity among populations in this area (e.g. Toledo, Loma Plata and Mariscal) to create an admixture of descendants from the two phylogroups. Although no rivers flow through this area to facilitate connectivity during the dry season, regular placement of water reservoirs and wet season flooding likely maintain connectivity of these newly invaded populations.

Consequences of deforestation

Current land use practices have greatly modified the Gran Chaco ecosystem and created conditions which have allowed capybara to invade areas formerly inhospitable to the species. This phenomenon has also brought into contact two populations that were genetically divergent

and likely evolved with their own community of parasites and pathogens. Indeed, patterns of biodiversity and divergence of pathogens often parallel the patterns found in their host (Holmes 2004). When vicariant populations come into contact with one another, the resulting sympatry can greatly increase the population size of naïve hosts and create an optimal situation for epizootic spread (Daszak *et al.* 2001). In the Paraguayan Chaco, contact of disparate populations of capybara could have a profound economic impact. Because capybara are the reservoir host of *Trypanosoma evansi*, which severely affects horses and domestic dogs (Franke *et al.* 1994; Herrera *et al.* 2004), their invasion into a newly created habitat coupled with the admixture of two previously isolated populations has the potential to increase the threat of *T. evansi* in this region.

Although the Gran Chaco region has experienced many climatic variations during the Quaternary period, with interchanging humid and dry climatic conditions, never before have capybara been directly affected by such rapid and pervasive land transformation. It appears that that land use changes in the Chaco are providing the conditions necessary for capybara to expand its range into previously uninhabitable areas of dry tropical forest, and indeed throughout forested regions of South America. This phenomenon is bringing into contact long separated host populations and their protozoan blood-borne diseases which has potentially wide-ranging economic impacts to the livestock industry. Indeed, environmental change, especially deforestation, has been implicated for the increase in human cases of the congener, *T. cruzi*, the protozoan parasite responsible for Chagas disease in Brazil (Briceho-Leon 2007). Further expansion of ranching and agriculture throughout South America not only has devastating consequences to biodiversity on the continent, but may also create disease highways for invading host populations for which the potential risks are poorly understood.

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Table 1

Populations sampled in phylogeographic study of capybara in the Chaco region of Paraguay. Geographic coordinates, number of samples per site (n), haplotype present in population and percent haplotype frequency in each population are presented.

Population	Geographic Location	n	Haplotype present in population	Percent haplotype frequency in population
Toledo	S22° 21' 16.0" W60° 19' 52.5"	13	H1, H2, H3, H5, H8, H13	15, 8, 23, 31, 8, 15
Mariscal	S22° 01' 24.9" W60° 37' 21.7"	3	H3	100
Lapacho	S22° 13' 28.8" W60° 11' 28.0"	5	H1	100
Loma Plata	S22° 23' 16.8" W59° 50' 07.1"	10	H1, H2, H3	50, 40, 10
Laguna Rey	S22° 54' 06.9" W58° 42' 42.1"	4	H1, H14	75, 25
Jerovia	S23° 23' 35.6" W59° 12' 07.5"	8	H2, H3	75, 25
Arizona	S23° 53' 24.3" W58° 24' 09.1"	10	H1, H2, H3, H9	50, 30, 10, 10
Maroma	S23° 32' 46.4" W57° 54' 24.0"	6	H2	100
Eñe	S24° 17' 51.9" W58° 34' 43.0"	8	H2, H3	63, 37
Loreto	S23° 52' 09.4" W59° 27' 33.1"	11	H1, H2, H3	73, 9, 18
Los Sauces	S23° 18' 01.8" W61° 25' 30.7"	11	H1, H2	91, 9
Olimpo	S21° 09' 41.3" W57° 56' 09.4"	12	H1, H2, H4, H6	8, 17, 67, 8
Bahia Negra	S20° 09' 55.7" W58° 08' 12.6"	9	H2, H6, H7, H10, H11, H12	11, 23, 33, 11, 11, 11

Table 2

Analysis of molecular variance for four models in 13 capybara populations. Variance components: AG = among groups; AP = among populations within groups; WP = within populations.

Population Grouping	Variance component	% Total variance	Φ Statistics	P
1- All Chaco populations	AP	37.75	FST: 0.38	0.000
	WP	62.25		
2- East Chaco, Central Chaco, West Chaco				
Chaco	AG	26.70	FCT: 0.27	0.008
	AP	17.73	FSC: 0.24	0.000
	WP	55.57	FST: 0.44	0.000
3- East Chaco, South Central Chaco, North Central Chaco, West Chaco				
Chaco	AG	20.57	FCT: 0.20	0.008
	AP	19.69	FSC: 0.25	0.000
	WP	59.74	FST: 0.40	0.000
4- (West Chaco + North Central Chaco), South Central Chaco, East Chaco				
Chaco	AG	19.32	FCT: 0.19	0.014
	AP	22.22	FSC: 0.28	0.000
	WP	58.46	FST: 0.41	0.000

Table 3

Summary statistics of genetic analysis of 386 bp of the mitochondrial d-loop region for capybara in the Chaco region of Paraguay. Populations are arranged by geographic groups. Genetic diversity indices include Population nucleotide diversity (π), pairwise differences, and haplotype diversity (H). For tests of population expansion, we report Tajima's D (D) and Fu F (Fs) probabilities, estimated time since expansion (τ), population size before expansion (θ_0), population size after expansion (θ_1) among thirteen capybara populations. (NS) stands for not significant.

Geographic group	π	Pairwise differences	H	D	Fs	τ	θ_0	θ_1
East Chaco								
Olimpo	0.01	3.08	0.16	-0.29 NS	-11.14	0.00	0.00	99,999
Bahia Negra	0.02	6.78	0.20	0.73 NS	-3.88	8.84	0.02	65
Mean	0.01	4.93	0.18	0.22	-7.51	4.42	0.01	50,032
Central Chaco								
Toledo	0.01	3.46	0.15	0.76 NS	-11.87	8.18	0.00	5.44
Mariscal	0.00	0.00	0.00	0.00 NS	0.00 NS	0.00	0.00	0.00
Lapacho	0.00	0.00	0.00	0.00 NS	0.00 NS	0.00	0.00	0.00
Loma Plata	0.01	3.31	0.12	2.32 NS	-11.25	7.05	0.00	6.64
Laguna Rey	0.01	3.00	0.14	-0.80 NS	-1.23 NS	0.00	0.00	99,999
Jerovia	0.00	0.43	0.05	0.33 NS	-15.45	0.62	0.00	99,999
Arizona	0.01	1.73	0.13	0.86 NS	-11.44	3.34	0.00	3.53
Maroma	0.01	2.57	0.12	0.51 NS	-6.21	3.00	0.00	0.49
Eñe	0.00	0.54	0.05	1.16 NS	-14.11	0.83	0.00	99,999
Mean	0.01	1.55	0.09	0.57	-10.16	2.56	0.00	33,335
West Chaco								
Loreto	0.01	2.36	0.12	0.60 NS	-11.25	0.00	0.00	99,999
Sauces	0.00	1.09	0.12	-1.85	-16.81	3.00	0.00	0.12
Mean	0.00	1.73	0.12	-0.62	-14.03	1.50	0.00	50,000

Table 4

Pairwise difference Φ_{ST} among 13 capybara populations sampled in the Chaco region. Values near zero indicate high levels of gene flow; values closer to 1 represent population differentiation. Shaded rectangles correspond to samples with significant P-values.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Toledo	0.000												
2 Mariscal	0.008	0.000											
3 Lapacho	0.533	1.000	0.000										
4 Loma Plata	0.106	0.233	0.317	0.000									
5 Laguna Rey	0.186	0.474	0.147	-0.083	0.000								
6 Jerovia	0.207	0.593	0.953	0.358	0.646	0.000							
7 Arizona	0.203	0.394	0.190	-0.072	-0.133	0.514	0.000						
8 Maroma	0.079	0.172	0.655	0.032	0.223	0.077	0.182	0.000					
9 Eñe	0.172	0.414	0.940	0.337	0.622	-0.102	0.500	0.079	0.000				
10 Loreto	0.308	0.531	0.074	0.029	-0.133	0.650	-0.062	0.348	0.631	0.000			
11 Saucés	0.505	0.812	-0.089	0.254	0.039	0.845	0.113	0.586	0.834	0.005	0.000		
12 Olimpo	0.496	0.603	0.511	0.395	0.336	0.692	0.391	0.520	0.682	0.412	0.510	0.000	
13 Bahía Negra	0.278	0.259	0.289	0.160	0.080	0.415	0.176	0.240	0.404	0.219	0.328	0.156	0.000

Figure 1. Chaco region of Paraguay with pie chart of haplotype distribution in each capybara population. Colors correspond to haplotypes in nested clade analysis (Fig. 3). Numbers correspond to 13 populations: 1 Bahia Negra, 2 Olimpo, 3 Toledo, 4 Mariscal, 5 Lapacho, 6 Loma Plata, 7 Jerovia, 8 Laguna Rey, 9 Arizona, 10 Eñe, 11 Maroma, 12 Loreto, and 13 Sauce. Background colors represent land cover type: light gray for pasturelands, medium dark periodically inundated grasslands, dark gray dense deciduous forested areas.

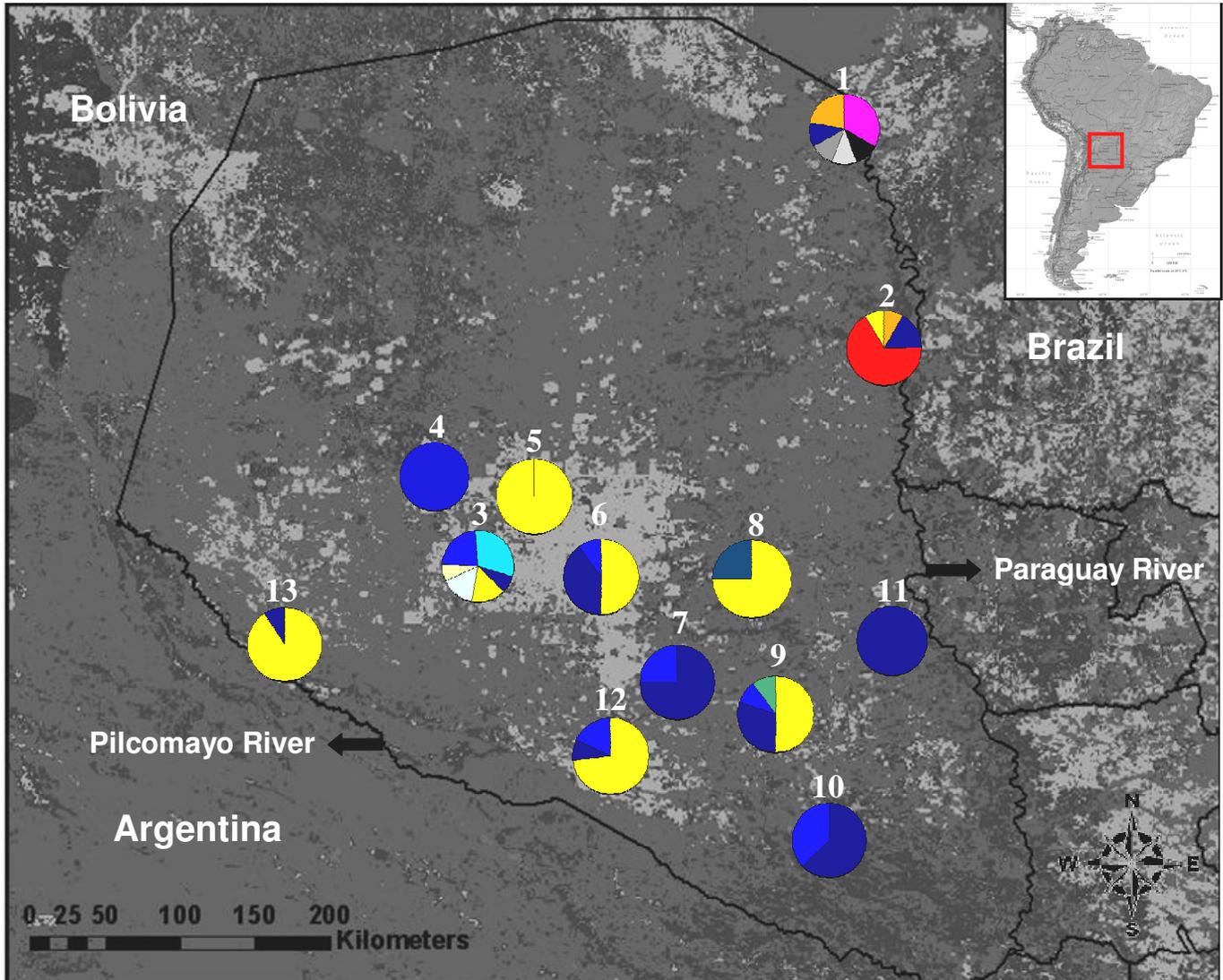
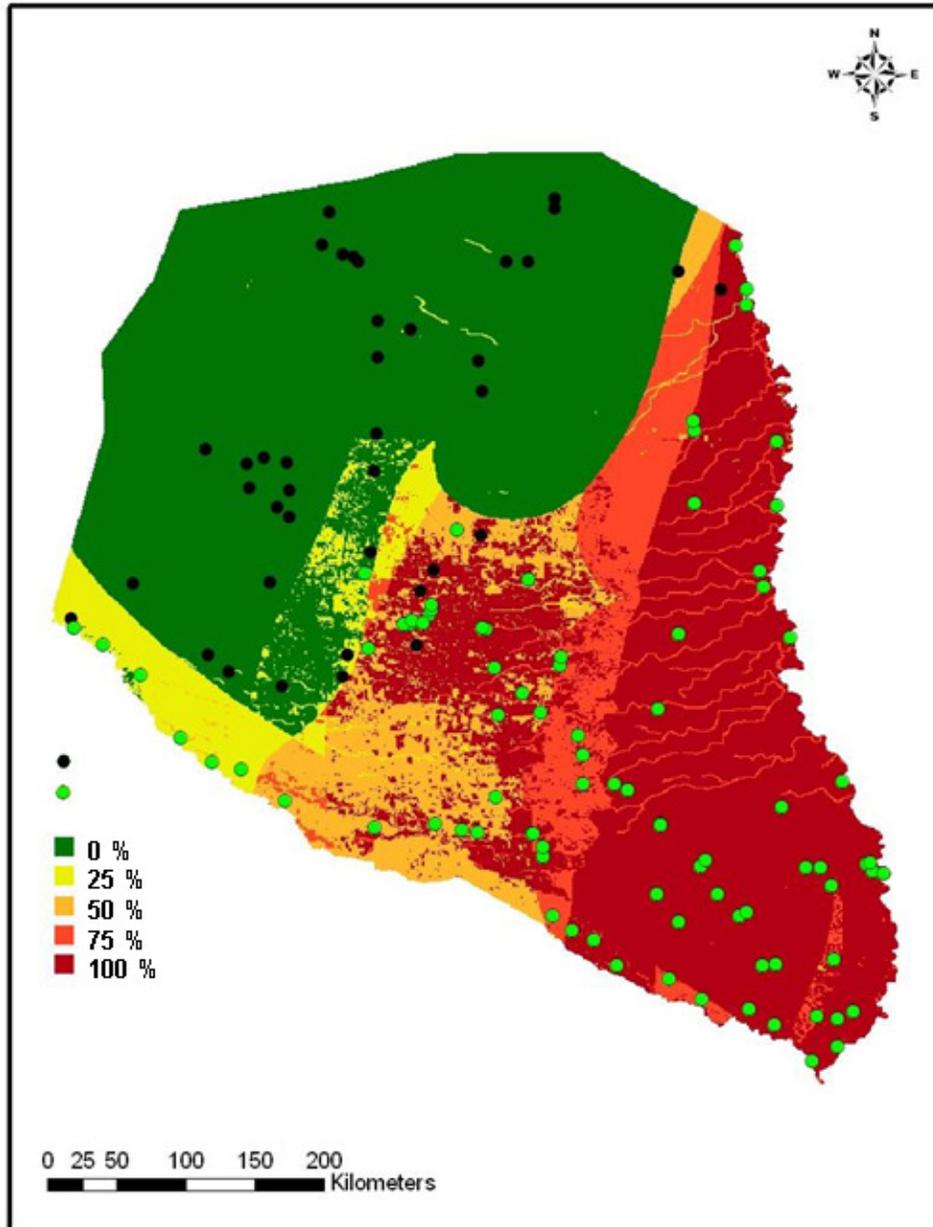


Figure 2

Results of the GARP model predicting the likely distribution of capybara in the Gran Chaco ecosystem of Paraguay. Color specifies the percentage of models that are in agreement with capybara presence. The top map represents the current distribution; black circles correspond to locations where capybaras were absent and green represents locations where capybaras were present. The bottom map represents the likely distribution of capybara 80 years ago prior to intensive cattle ranching in the Central Chaco.



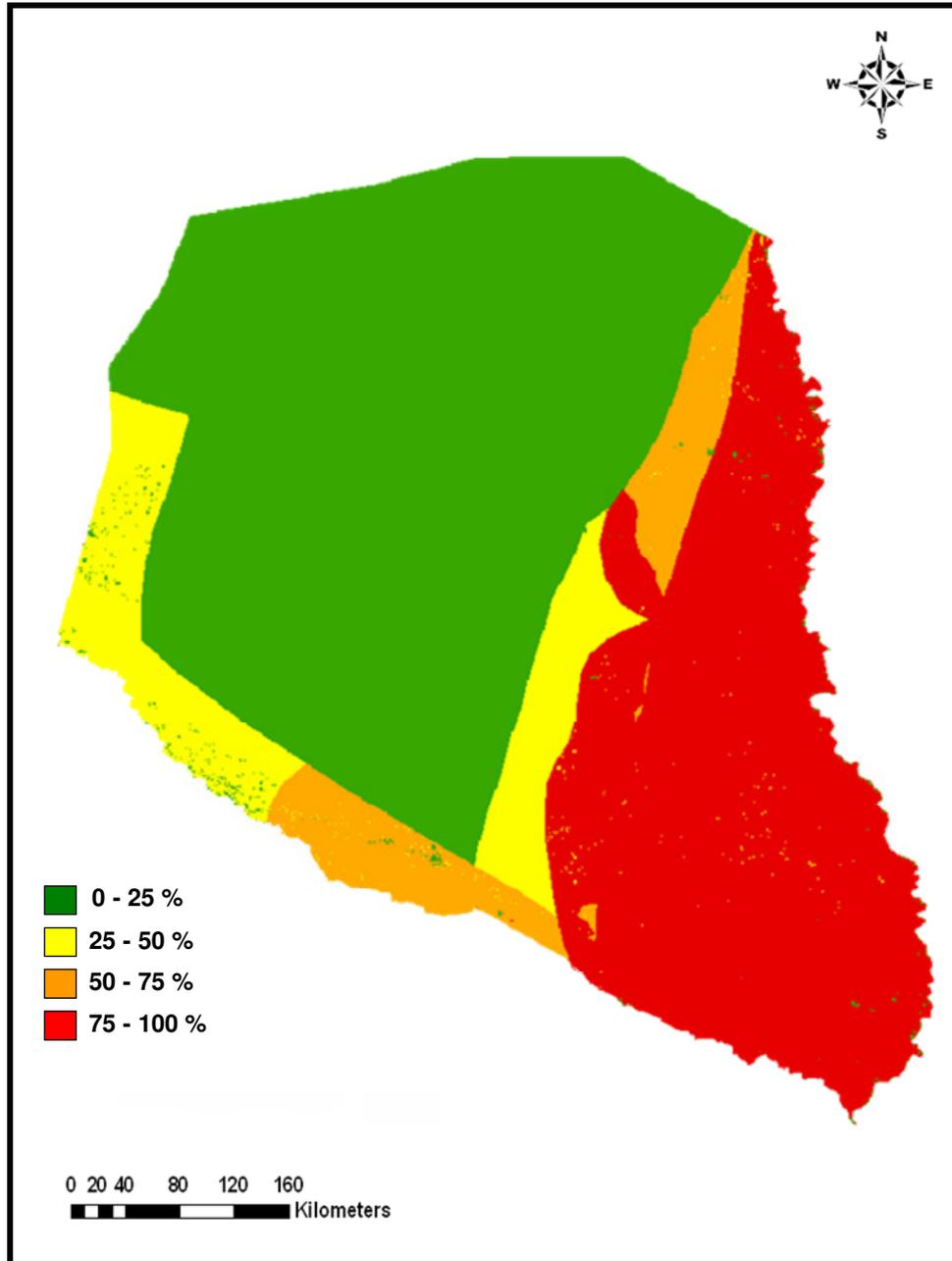


Figure 3

Mismatch distribution of pairwise nucleotide differences among capybara in the Central, West and East Chaco. Solid lines indicate expected distribution under Rogers (1995) sudden population expansion model.

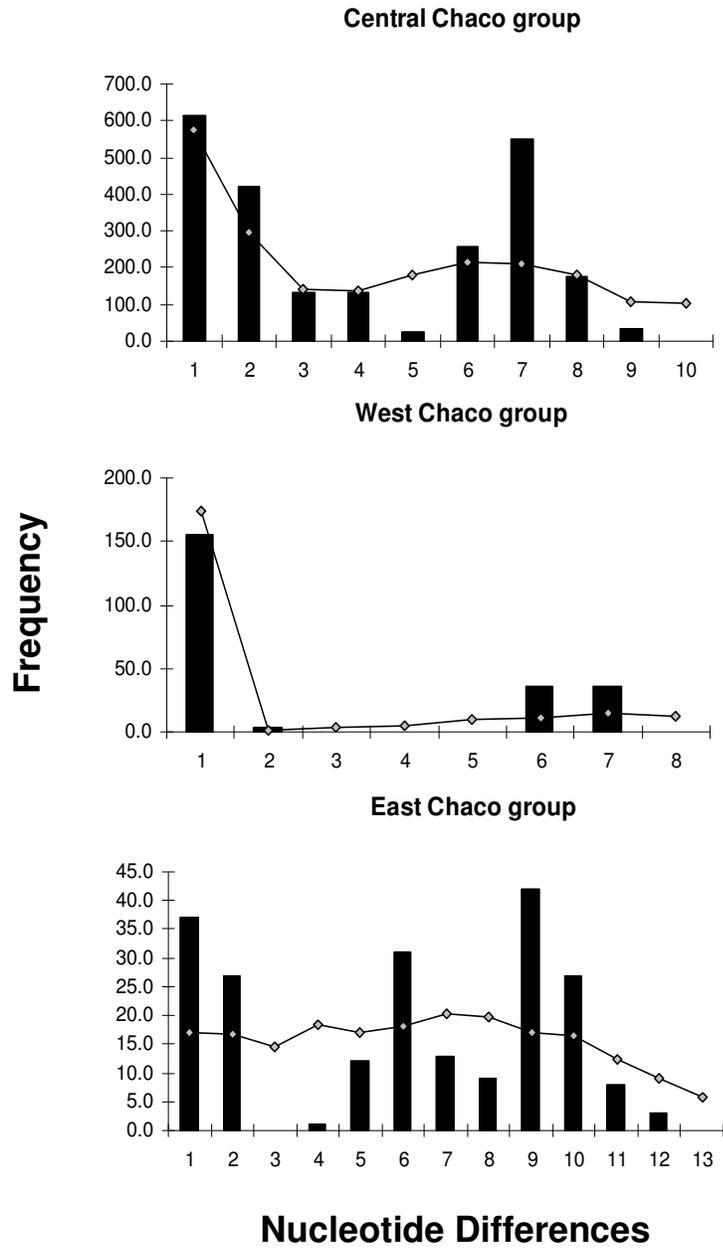


Figure 4

Unrooted nested clade inferred from maximum parsimony and derived from 14 capybara haplotypes. Circles represent unique haplotypes identified by an H followed by the haplotype identification. The size of the circle depicts the number of capybara individuals with that haplotypic sequence; colors correspond with Figure 5 haplotype pie charts. Black dots represent missing haplotypes.

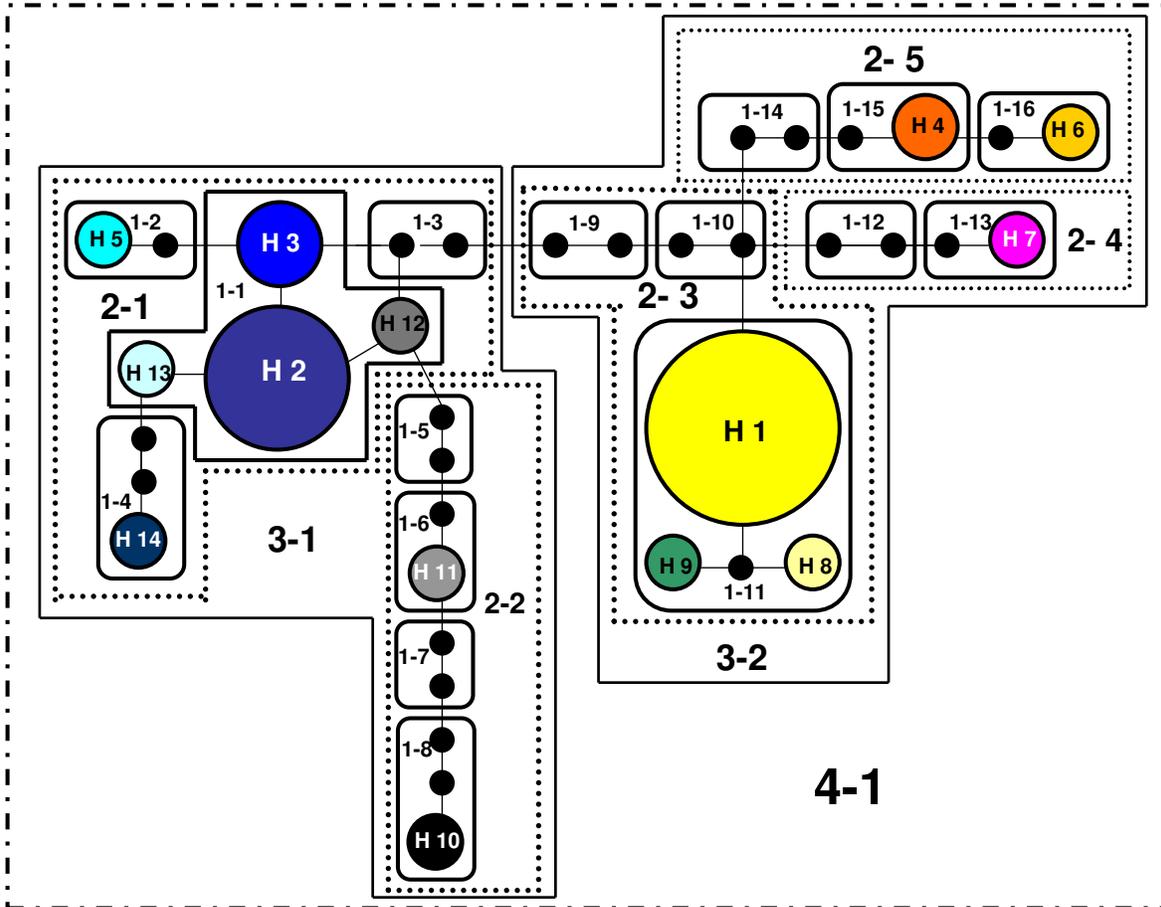
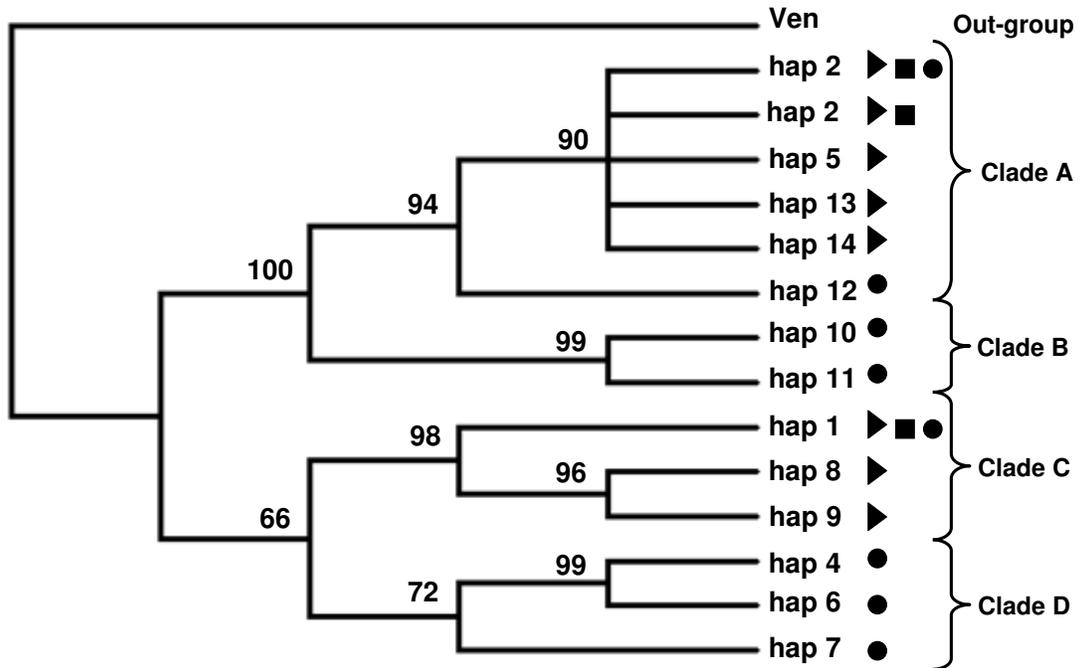


Figure 5

Topology of the Bayesian tree with posterior probabilities. Two major clades and 4 subclades are resolved (Clades AB and CD). Symbols represent geographic region in which the haplotype was found; East Chaco = ●, Central Chaco = ►, West Chaco = ■.



Chapter III

Home Range and Habitat Use of Capybara in Newly Invaded Pastureland in the Dry Chaco Region of Paraguay

Running head: Home Range and Habitat Use of Capybara

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Abstract

Deforestation is one of the primary causes of species decline worldwide. Some species, however, can take advantages of this change and expand their ranges, placing endemic species at risk of extinction, changing the composition of biotic communities, and altering ecosystems. From June 2005 to July 2007, we investigated home range, habitat use and body temperature dynamics of six capybara (*Hydrochoerus hydrochaeris*), individuals via radiotelemetry collars and temperature sensitive implants in a deforested area of the Central Dry Chaco region of Paraguay in which capybara had recently invaded. Our results show that the average home range of capybara was 183 ha, similar to home ranges of capybara in the Brazilian Pantanal. Within the study area, capybara used and selected water significantly more than its availability, followed by Chaco forest and pasture land; and had lower than expected use or avoidance of shrub forest. Overall, capybaras were located 95% of the time < 500 m from permanent water, with the greatest distances from water occurring between 1:00 and 6:00 AM. The peak of activity occurred during evening and early night. Average year round body temperature was 36.15°C. We found a significant positive correlation between body temperature and distance from water, and a significant negative correlation between distance from water and Chaco ambient temperature. These findings suggest that capybara use water to thermoregulate. This study is the first to characterize capybara in a xeric habitat without a year round natural water source, and scarce natural grasslands. Our results show how anthropogenic habitat modification has allowed capybara to thrive, and assist us in understanding how capybara invade and utilize deforested areas. This study will provide valuable information for the future management of the species; however, the consequences of the invasion of capybara into the Dry Chaco to locally native species are still unknown.

Introduction

Anthropogenic habitat destruction and land fragmentation are the main cause of biodiversity loss worldwide (National Academy of Sciences 2000; Fahrig 2003). The expansion of exotic species places endemic species at risk of extinction (Aguirre and Tabor 2008), changes the composition of biotic communities, and alters ecosystem services (Lockwood et al. 2007; Vitousek et al. 1997). However, not only can exotic species benefit from land use change, native species can take advantage and expand into ecosystems where they historically did not occur. The expansion of native species has been driven by land use and land management changes such as agriculture, game exploitation, predator and poaching controls (Acevedo et al. 2006). For example, coyotes (*Canis latrans*) were historically restricted to central North America, but have expanded and colonized most of the North American continent in less than two centuries (Fener et al. 2005). Many more native species are known to have taken advantage of anthropogenic land modification and expanded their range (barred owl, Livezey 2009; collared peccary, Steven et al. 2004; nutria, Guichon et al. 2003).

Capybara (*Hydrochoerus hydrochaeris*) is the world's largest living rodent (Mones and Ojasti 1986; Nowak 1991). They are semi-aquatic mammals, extensively distributed throughout most of South America, from Panama south into northern Argentina, Paraguay and Uruguay (Redford et al. 1990). Capybara inhabit open flooded grasslands as well as densely vegetated areas around permanent water sources such as ponds, rivers, marshes, and swamps. Although closely associated with aquatic environments, capybara use water primarily as a place of refuge, and most normal activity is on land (Schaller 1976). Nevertheless, year-round water is essential for capybara to be present in an area. Capybara lives in herds ranging from a pair to complex groups of several adults of both sexes and their offspring (Azcarate 1980; Herrera 1992; Mones

and Ojasti 1986; Schaller and Granshaw 1981). These giant rodents are efficient grazing herbivores, feeding primarily on aquatic plants and grasses that grow in or near water (Barreto and Herrera 1998; Gonzalez and Parra 1972; Lord and Lord 1988; Macdonald 1981; Quintana and Malvarez 1994, 1998). Capybara have been documented to share grazing area with livestock; it is not uncommon for populations of capybara to benefit from pasture management, predator control and provisioning of water by ranchers (Mones and Ojasti 1986; Ojasti and Robinson 1991; Quintana 2003). In certain regions of Brazil, capybara has become an agricultural pest due to changes in habitat as a result of ranching and agriculture (Paschoaletto et al. 2003).

Capybara ecology home range and habitat use have been previously studied throughout the core of its distribution, in habitats such as tropical forest and seasonally flooded plains where the species is locally abundant (Alho et al. 1987; Barreto and Herrera 1998; Cordero and Ojasti 1977, 1981; Herrera 1992, Herrera and Macdonal 1989; Lord 1991; Quintana 2002, 2003; Quintana and Malvarez 1994). By contrast, our study system is in the central Gran Chaco ecosystem of Paraguay at the periphery of its historical distribution. Recent deforestation in this region has facilitated the expansion of this species from more mesic habitat into the central Chaco (Campos-Krauer and Wisely, in review), where populations densities are low (Brooks 1998).

The central Chaco is dominated by thick tropical xeric thorn forest (Gorham 1973; Holdridge 1969; Redford et al. 1990) characterized by high temperatures and abundant water during the raining season. As much as 80% of the annual precipitation occurs from November to May (Eidt 1968). Precipitation and standing water diminish greatly or even disappear completely during the colder dry season that extends from June to October (Hueck 1966)

Due to these harsh characteristics of the Paraguayan Chaco ecosystem, it has remained mostly undeveloped and immune to modern anthropogenic land modification until recent years. The rate of deforestation has dramatically increased since the 1970's (Benirschke et al. 1989). Currently, approximately 500 ha of native forest are cleared daily and transformed into pastureland (Huang 2009). To support a growing cattle population, approximately one pond every 100 ha of pasture is constructed to maintain year-round water. The conversion of dense xeric thorn forest to pasturelands and the availability of year-round water supply have greatly altered the resource potential of the ecosystem.

The Chaco region is an area in which capybara has been present for thousand of years. However, much of the best habitat for capybara is located in the southern Low Chaco region throualong the shorelines of rivers and in marshes. It is clear that capybara are taking advantage of land use change to expand their range to the central Chaco region (Campos-Krauer and Wisely, in review). Understanding the factors and mechanisms that have allowed this expansion requires a detailed understanding of how capybara colonizes areas on a fine scale. To this end, we examined movement patterns, habitat use, and thermal ecology in several groups of capybara in the central Chaco region to better understand the ecological drivers of species range expansion. Currently, little is known about how these newly established capybara populations use the xeric Central Chaco. In this paper, we describe and compare home range and habitat use of capybara in the Chaco and compare our results with observations bean other studied regions. Understanding the spatial requirements of individuals is essential to predicting and understanding the capacity of the habitat. In particular, we determined the role of permanent water as a critical factor for capybara. Ultimately, we determined what factors are critical for capybara to expand, persist and survive in this seasonally dry habitat. Fine scale understanding of

how habitat modification and land fragmentation can affect the species movements and dispersal pattern will significantly increase our knowledge of the ecology of the capybara in a region where rapid deforestation dominates land use patterns. This information will be valuable to forecasting future distributions and vulnerabilities in light of human activities.

Methods

Study area

The research was carried out from June 2005 to August 2007, at Fortín Toledo, Boquerón Dept., Paraguay (22°21'05'' N, 60°19'35'' W Figure 1). The study area was in and around a biological reserve of approximately 180 ha surrounded by private ranches encompassing a total area of 3,500 ha. Vegetation on the study area consisted primarily of five types: 1) Primary Chaco forest was virgin tropical xeric thorn forest characterized by thick understory vegetation with an abundance bromeliads (*Bromelia sp.* and *Dyckia sp.*), Trees were less than 15 m tall with many spines, dominated by species such as Quebracho tree (*Schinopsis quebracho-colorado*), bottle tree (*Chorisia insignis*), palo santo tree (*Bulnesia sarmientoi*), mistol (*Zizyphus mistol*) and verde olivo (*Cercidium praecox*). This habitat was distributed across the landscape as remnant forest islands surrounded by pasture or secondary growth. 2) Secondary Chaco forest consisted of secondary forest regrown from 20 year-old, introduced pastures. This habitat was characterized by a wide variety of thorny shrubs, dominated by species of *Mimosa*, *Acacia* and *Prosopis alba*, and *P. nigra*. Cacti were also diverse and abundant; taxa such as *Opuntia*, *Cleistocactus* and several tree cactus such as *Cereus sp.* were present. Within this habitat there was little to no understory vegetation due mostly to soil compaction. 3) Introduced pasture was cleared forest characterized by introduced non-native grass dominated by gatton panic (*Panicum*

maximum) and to lesser extent star grass (*Cynodon dactylon*). These grasslands were cleared with few trees remaining and divided into large paddocks in which cattle were maintained year-round with a water source. 4) Seasonally flooded wetland occurred as natural depressions which could hold water during the rainy season, but dried out during the dry season. These areas had a mix of introduced and native grass species (*Cynodon sp*) together with palm trees (*Copernicia alba*). In addition to natural wetlands, areas surrounding man-made ponds had a high abundance of grass. 5) Open water from man-made ponds was present in each pasture paddock. Each reservoir was at least 625 m² and 1.5 m in depth and many were covered by aquatic plants such as *Eichhornia crassipes* and *Pistia stratiotes*. Ponds were generally built without modifying the surrounding forest, which was left to prevent eolic sand deposits and to provide shade for cattle. These forest patches and ponds remained as islands most of the time surrounded by grassland. During the dry season, standing water was found only in these man made ponds.

Capture and radiotelemetry

From June 2005 to August 2007, we located and monitored a maximum of 6 capybara year round. Radio collars (Telonics, Mesa, Arizona 85202, USA) were placed on four capybara (2 adult males, 2 adult females) and intra-abdominal implants (Telonics, Inc) were placed in two capybara (1 adult male and 1 juvenile male). The small sample size reflects the low density of capybara in this study area and is representative of the patchily distributed populations within this region.

Animals were trapped with box-style live traps and chemically immobilized using a combination of Ketamine HCl (Ketalar, 4.7 mg/kg), and Tiletamine HCl/Zolazepam HCl (Telazole, 1.17 mg/kg; Kreeger 2002). Drugs were delivered intramuscularly by a blowgun

(Telinject, U.S.A., Inc., Agua Dulce, CA) using 3 cc plastic dart (Telinject) or a standard 3 cc pole syringe. Animals were considered fully immobilized when they did not respond to external stimulus. Each animal was sexed, weighed and marked with ear-tags. We collected pinnae epithelial tissue from ear plugs and/or blood for genetic analysis. All captured animals received a general examination to evaluate body condition, external parasites and possible wounds. Individuals were classified as juvenile if < 25 kg, animals > 25 kg were considered adults. We used two different sizes of implants depending on the body size of the animal to be implanted (IMP/300L or IMP/400L with MS4 temperature sensor, Telonics, Mesa, Arizona 85202, USA). For details on the implant procedure see Campos-Krauer et al. (in prep). Each transmitter was equipped with a mortality sensor which activated when animals had not moved for > 6 hours. All procedures were in compliance with the Secretaría del Medio Ambiente, Paraguay; Kansas State University Institutional Animal Care and Use Committee (Protocol No. 2362); and guidelines approved by the American Society of Mammalogists (Gannon et al. 2007).

We estimated independent telemetry locations (White and Garrott 1990) using triangulation with a minimum of three vectors per location. We marked and georeferenced > 50 fixed reference locations every 500 m in the study area from which a vector bearing was collected. We used vehicles to move among reference locations to reduce triangulation time. We used a 3 element folding yagi antenna (Advanced Telemetry Systems, Inc., Isanti, Minnesota), attached to a 3 m long aluminum pipe to maximize signal reception. Locations were estimated using a minimum of three vectors by using the maximum likelihood estimation option (Lenth 1981) in program LOAS (LOAS™ 2005, Ecological Software Solutions LLC). We calculated an average location error from reference collars placed in random locations approximately 1 m from

the ground. Animals were located at six hour intervals with the location time moving ahead one hour daily to cover a 24 hour period every 12 days.

Observers were able to detect animal movement due to differences in signal strength during data collection at a single vector. We recorded activity patterns from two capybaras using this approach. Patterns of signal reception other than a consistent pulse intensity were considered to be an active animal. These data indicated time of day during which animals tended to move. Activity patterns were analyzed as the number of individuals active per hour of day over the total number of locations for each hour of the day (Figure 1).

Home range analysis

We determined individual home range size using 95%, 75% and 50% fixed Kernel home ranges (Worton 1989), using the Animal Movement Analysis extension to ArcView, version 3.2 (ESRI, Environmental Systems Research Institute Table 1). All animals had >70 locations and a minimum of two months of data (Table 2); however, of six animals, only three survived or retained their radio tag year round to estimate wet and dry season home ranges. We compared average home range size during the wet and dry season and compared the average of our home range estimates to other published estimates of home range for capybara using a Student's *t*-test (Zar 1996). Due to the semi-aquatic nature of capybara we calculated the distance from the estimated location to the nearest permanent water source using ArcView.

Habitat use analysis

We compared used to available habitat to determine which habitats were preferred or avoided. Habitat types were identified and delimited from high resolution satellite images (eMap

International, Boulder Colorado, USA). We created polygon shape files by hand corresponding to the 5 land cover types by using Geographic Information System (GIS). We delimited the study area as a 5 x 7 km rectangle (3,500 ha) which incorporated the six minimum convex polygon (MCP) home range estimations (Mohr 1947) of our six study animals (Figure 2). We carried out a compositional analysis to identify proportion of used and available habitat types that were used significantly more or less than expected by chance at two scales of use (Aebischer et al. 1993). First, we compared the proportion of habitat in the study area (the available habitat) with the proportion of habitat in each MCP home range (the used habitat). Second, we carried out a finer scale analysis by comparing the estimated proportion of habitat found in each MCP home range (the available habitat) to the proportions of observed animal locations within each habitat type (the used habitat) using ArcView, version 3.2 (Environmental Systems Research Institute). When no locations were recorded in a specific habitat type, a value of 0.01% replaced the zero as suggested by Aebischer et al. (1993).

To test the null hypothesis of random use, we calculated the difference in log-ratios between matching pairs of used and available habitats. This hypothesis was tested by a multivariate analysis of variance (MANOVA). If the hypothesis of random use was rejected, we then ranked the habitat types in order of relative use. For this analysis, we calculated the mean log-ratio difference for all possible pairs of habitat types, and compared them to zero using a t-test (Aebischer *et al.* 1993). Compositional analysis makes it possible to rank habitat types in order of relative use; although in our case, seasonal (dry and wet) sample sizes were too small to produce clear patterns of significant differences between ranks. Statistical tests were carried out with program SAS 9.1 (SAS Institute, Inc., Cary, North Carolina).

Body temperature analysis

Two capybara received internal radio-implants with temperature sensitive pulse rates. The temperature sensor monitored body temperature in the proximity of the implant. Special circuitry provided approximately 0.1° C resolution; a self calibration pulse interval eliminated calibration drifts due to aging and battery voltage changes over time. Transmitters were calibrated at the factory and retested at the field station to maximize accuracy. Temperature data were recorded as the number of pulses per minute. We also collected ambient temperature, humidity and wind direction for each triangulation animal for the two animals with implants. We assessed the relationship of body temperature with distance from water, ambient temperature, time of day and season using a multivariate analysis of variance (MANOVA) and conducting posterior univariate linear regressions implemented by program JMP-IN 4.0.4 (SAS 2001, SAS Institute Inc., Cary, North Carolina).

Genetics analysis

Microsatellite molecular marker data provided valuable information to identify relatedness among capybara in the study area. Tissue samples from six capybara were stored in 95% ethanol (epithelium) or lysis buffer (blood) and stored at -4 °C until DNA extraction. Total genomic DNA was extracted using the tissue or blood protocol of the QIAGEN DNeasy DNA extraction kit (Qiagen Inc., Valencia, California). We used polymerase chain reaction (PCR) to amplify 8 microsatellite loci previously developed for capybara (Herrera et al. 2004). To gain a better understanding of the spatial distribution of animals in the Fortin Toledo area we included eight additional samples opportunistically collected from locally depredated capybara.

Polymerase Chain Reaction (PCR) was performed in an Eppendorf Mastercycler (Eppendorf, Wesbury, NY, USA) in 20 μ l volumes containing 1 U Taq DNA Polymerase (Applied Biosystems, Inc., Foster City, CA, USA), 1X AmpliTaq Buffer II, 0.5 μ M forward primer with an M-13 tail on the 5' end, 0.5 μ M reverse primer, and 0.3 μ M of an M-13 reverse complement primer, 2.25 mM MgCl₂, 0.2 mM each dNTP, 1.6 μ g/ μ l BSA (New England Biolabs, Ipswich, MA, USA), and 1 μ l of the DNA extract. The PCR profile consisted of a single denaturation step at 94° C for 5 min, followed by 30 cycles of denaturation at 94° C for 30 s, annealing at locus-specific temperatures at a range of 56° to 61° C for 45s and primer extension at 72° C for 45 s. A second round followed by 10 cycles of denaturation at 94° C for 30s, annealing at 55° C for 45s primer extension at 72° C for 45 s followed by final extension at 72° C for 10 min was used to incorporate the fluorescent dye third primer. All PCR's were run with a negative control. Products were verified on a 2% agarose gel. Genotypes were visualized on an LICOR 4300 DNA Analysis System. All genetic work was conducted in the Kansas State University, Conservation Genetic and Molecular Ecology Lab.

Genotypic data from microsatellite loci were assessed for Hardy-Weinberg equilibrium using an exact probability test generated by a Markov chain analysis in GENEPOP 3.3 software (Raymond and Rousset 1995). Linkage disequilibrium was tested by the genotypic equilibrium test in GENEPOP 3.3. All tests were adjusted for multiple comparisons using a sequential Bonferroni correction (Rice 1989).

We used program Cervus 3.0.3 (Marshall et al. 1998) for parentage analysis. Cervus uses a likelihood-based approach based on the genotypes of individuals in the population, the proportion of the population sampled, and typing errors. One advantage of using this program in parentage assignments is that it allows for genotyping errors and mutations, such that parents are

not excluded on the basis of a single mismatch with offspring. Another advantage of using this program for parentage assignment is that it can distinguish between multiple nonexcluded parents and identify the most likely parent from among them. We ran the program without identifying any known parents. This estimate was based on our six capybara trapped considering that the population in the area is low. We also carried out a second run which included the nine capybara from the study area, but were not radio-marked. Each individual was typed at every locus with an error rate of 0.02, as determined by error rate in Cervus. The strict level of confidence was 95%; the relaxed level of confidence was 80% in all assignments. For all the analyses we ran 10,000 simulations.

We used Cervus to identify the most likely mother of our trapped juvenile capybara (copy 43), from two adult female capybara (copy 2, copy 50) trapped in the study area. Similarly, we carried out the same approach to identify the most-likely father from three trapped adult male capybaras (copy 1, copy 41, and copy 45) in the study site. Finally, we also attempted to identify the most likely parents by comparing all genotyped animals against our two youngest animals (copy 45 and copy 43) sampled in the study area.

We estimated pairwise genetic relatedness and examine the relationship between genetic relatedness as a function of geographic distance by performing a regression analysis and permutation test among our six trapped capybara and all genotyped animals with program GenAlEx (Peakall and Smouse 2006). This program employs multivariate analysis methods (Smouse and Peakall 1999) to calculate a correlation coefficient (r , range -1 to $+1$) between genetic and geographic distances for all pairs of individuals within user specified distance classes. For example, r in the distance class 100–1000 m would represent the correlation between genetic and geographic distances calculated between all pairs of individuals within 100–1000 m

of one another. We used the genotypic distance option to calculate linear genetic distances between all possible pairs of collections and selected distance classes that produced an even number of pairwise comparisons across all classes.

Results

Home range analysis

Over the study period we collected total of 1,781 locations with a minimum of 71 and a maximum of 600 locations per animal; with an estimate average location error rate of (41 ± 12 m, mean \pm SE, $n = 9$; Table 2). We found that there were patterns of spatial overlapping among individual capybara locations points among capybaras 95% kernel home range (Table 3). We were only able to estimate temporal overlap among four capybaras. We found 82% temporal and similar spatial overlap among capybara 43 and 41 demonstrating that they were in the same group. We found zero temporal overlap among capybara 43 and 41 group with capybara 50 and 45. Thus, we infer that capybara 50 and 45 were from a separated group. Among capy 50 and 45 we were not able to estimate temporal overlap because they were radiomarked at different times during the study; however we did find a spatial overlap of 19 and 80%; which suggests that these animals were likely from the same group. We were not able to estimate temporal overlap between capybara 1 and 2, because they were captured and subsequently went missing, before other animals were radiomarked.

Based on our spatial and temporal overlap data and confirmed by direct observation we were able to confirm that two capybara (capy 43 and 41) were in the same group. From observational data we found the group was formed by one adult male (capy 41), one untagged adult female, and four juveniles including capy 43. We found that capy 1 (adult male) and capy 2

(adult female) had a high percent of spatial overlap between them and between capy 43 and 41. These data suggest that after capy 1 and capy 2 were possibly part of the same group, but that capy 1 and 2 either dispersed, or were depredated. We further infer that their home range was reoccupied by the group dominated by capy 41. On the other hand, capybara 45 an adult male had little overlap with any other capybara during the tracking period. This male was observed most of the time alone. However, on few occasions it was observed together with a second capybara but with no juveniles. Home range analysis indicated that he was not part of the previously described group. This animal disappeared before capy 50 (an adult female) was trapped, which made it impossible to corroborate any temporal overlap among them. However, these animals had high spatial overlap, which suggests that this female was the one observed with capy 45.

In general, the great majority of capybaras were observed near or on the shore of permanent ponds singly or in small groups, up to a maximum of six animals. Capybaras were often observed resting in the shade or walking near the ponds. Activity patterns of two capybaras revealed that daytime activity was higher than at night, with peaks of activity at 16:00 and 20:00 hrs (Figure 3).

Using measures of adaptive kernels, the average 95% kernel home range size was 183 ± 54 ha, ($n = 6$), the 75% kernel = 64 ha, ± 22 ha, and the 50% kernel = 28 ± 9 ha (Table 1). We found no significant difference in home range size among seasons.

Across seasons, the average distance from water for all capybaras was 126 ± 4 m ($n = 1,781$), with the distance from water increasing during the night from 1:00 to 6:00 AM ($t = 2.01$, $P < 0.01$; mean 1 = 214, Mean 2 = 132; Figure 3). We found that 95 % of the locations were <

500 m from a permanent water source, 84 % were < 250 m, 64 % were < 100m and 46 % were < 50 m from water.

Habitat use analysis:

The average MCP home range size for all capybara was (583 ± 97 ha, mean ± SE, n = 6). Using our five habitat classifications (Primary Chaco forest, Secondary Chaco forest, Introduced pasture, seasonally flood wetland and Open water), we compared habitat use at two scales: the overall study area and within the MCP home range. Our results suggest that capybara did not establish home ranges at random ($\Lambda = 0.023$, $\chi^2_2 = 74.16$, $P < 0.0001$). Ranking 4 for the habitat that significantly was use the most and 0 to the habitat that was significantly used the less (Table 4). At the study area scale habitat types were used in the following hierarchal sequence; Primary Chaco forest > Introduced Pasture > seasonally flood wetland > Open water > Secondary Chaco forest (Table 4a). Secondary Chaco forest was used significantly less than available, followed by water surface and flooded grass area which were uses significantly less in proportion to there availability. It is important to clarify that water surface and flooded grass area had a small proportion of the overall study area. The two top-ranking habitats, Chaco forest and pasture, were used significantly more than what was available within the study area, and Chaco forest was selected over pasture land. On the smaller scale of home range, overall use of the five habitat types based on proportion of radio locations in each habitat type differed significantly from the proportion of habitat available within the MCP home ranges ($\Lambda = 0.037$, $x^2_2 = 44.31$, $P < 0.0001$). The ranking matrix indicated that the following habitat type followed sequence; Water > Chaco forest > Pasture > Flooded grass area > Shrub forest (Table 4b). Within the home range, water surface was the most highly preferred habitat. Chaco forest and pasture had

no detectable difference in use from availability. Followed by flooded grass area with a value of one, significantly less used than available and finally; Shrub forest was used less than its availability, suggesting that capybara were avoiding this habitat type.

Body temperature analysis

The average ambient temperature during the study area was 28°C, with an average day time temperature during dry season of 27.26°C and night 24.64°C. The average day time wet season was of 31.88°C and night 29.52°C.

The average body temperature for capybara was $36.15 \pm 0.27^\circ\text{C}$ ($n = 1021$); with no significant difference among dry and a wet season. We found a significant positive correlation between body temperature and distance of the animal from water ($r^2 = 0.004$, $P = 0.02$; $F = 4.51$, $DF = 1008$). We found significant negative correlation between ambient temperature and distance of the animal from water ($r^2 = 0.02$, $P < 0.0001$; $F = 29.01$, $DF = 1017$), (Figure 4).

Genetics results

Among the 8 microsatellite markers we found no evidence of linkage disequilibrium. Similarly the second group (13 animals) also was out of Hardy-Weinberg equilibrium at five out of eight loci with excessive homozygosity ($P < 0.0001$) and heterozygosity deficit ($P < 0.0001$). The overall Expected Heterozygosity was (0.60) with an Observed Heterozygosity of (0.45).

Parentage analysis did not suggest any of the radiocollared adults or other sampled individuals in the Fortin Toledo area as possible parents of the radio-collared juvenile.

Similarly, we found no first order relatedness among all capybara trapped. Likewise, we found second order relatedness between capybara 45 and 43 ($r = 0.24$) (Table 5). We found that

9 pairwise estimates of relatedness were substantially negative and thus animals were less related than expected by chance. The spatial autocorrelation analysis of genetic distance showed that the six radiomarked capybara had no significant spatial autocorrelation nor did any animals from the Fortin Toledo area (Figure 5). Overall capybara in the Fortin Toledo area had no spatial structure. Genetic relatedness did not drive membership of groups. Paternity results among capy 43 (juvenile) and capy 41 (adult) from the same group indicated that they were not related even though they were in the same group.

Low genetic diversity and excessive homozygosity are characteristics commonly observed in populations at range margins during range expansion. These genetic changes can arise as a consequence of repeated founder events, genetic drift in small populations or population bottlenecks. Our results demonstrate that capybara populations in the study area have characteristics of a recent expanding population. The expansion process has created a zone of secondary contact between distinct capybara phylogroups.

DISCUSSION

Our study was conducted out in a newly invaded area in which population densities were low, thus our sample size was representative of the population at the leading edge of this invasion. Based on our direct observations and overlapping home range data, we confirmed that we had three groups of capybaras. All groups were small, ranging from a pair to a family group of six. Each group had two to three ponds within their home ranges, near which they remained the majority of the time. Groups moved together to feed, rest or hide in surrounding habitats and always maintained the pond as the center point. We found some spatial overlap but little temporal overlap among individuals from different groups. The exception was capy 2 (female) who had high spatial overlap with two groups, and made occasional short solo trips to a

neighboring pond. We found low genetic relatedness within the sampled members of groups. Dispersal patterns and patterns of social hierarchy were beyond the scope of this study, but could explain the lack of relatedness within groups.

Activity patterns were similar to those of other populations in Venezuela (Herrera et al. 1989) and Brazil (Alho et al. 1987). We found the highest activity of capybara during the crepuscular hours with little activity during the middle of the day or at night. During the height of their activity, distance from water was less than 150 m indicating that activity was mostly in habitat directly surrounding or in ponds (Figure 1 and 3). On the other hand, activity diminished during the night and distance from water increased. Groups moved during late evening and early night away from water ponds, occasionally moving to a different water pond or more commonly to a bedding area in the dense Chaco forest where they would spend the night. It appears that capybara are using the cover of the darkness and the dense Chaco forest as protection during the night, moving closer to water the next morning (Figure 3).

Capybara populations in the study area were not intensely persecuted by humans; occasional poaching was possible but rare. However, capybara remained cautious of humans and as soon as presence was detected, capybaras would silently flee toward the nearest ponds. Only when an animal was surprised by a human or a predator would they loudly jump in the water. Interestingly, capybara remained in the water only for a few minutes where they would hide under aquatic vegetation. Shortly after entering the water, they would emerge and escape into the dense Chaco forest using it as the final hiding spot.

Predators such as jaguar (*Panthera onca*) and puma (*Felis concolor*) were common in the region and study area. One radiocollared capybara was confirmed to have been killed by a puma at the shore of a pond. Tracks and signs of predators were not uncommon surrounding the ponds.

Similarly, the great majority of ponds in the study area had black caimans (*Caiman yacare*) that could easily depredate juvenile and subadult capybaras. Their presence in all of the ponds in our study area could explain why capybara tended to bed down in forested areas during the night, and use water as an escape mechanism for only a short while.

The overall average estimated 95% Kernels of 183 ha were similar to the estimates of home range for populations of capybara in the Brazilian Pantanal (Alho et al. 1987, 1989; Schaller and Cranshaw 1981). These home range sizes were considerably larger than those found in Venezuela in which capybara home range are much smaller with higher population densities. Small home ranges and higher densities in the llanos of Venezuela could be due to the higher productivity of the grasslands than in the Pantanal or the Gran Chaco (Herrera et al. 1989).

In general, capybaras were found surrounding man made ponds. In the Gran Chaco, capybara presence and survival directly depends on the availability of permanent water sources and introduced pastureland (Campos-Krauer and Wisely, in review). Natural permanent water sources in the region are scarce, with the great majority of water sites drying out during the dry season. Man made ponds are reliable year round sources of water that are regularly distributed in or surrounding pasturelands. As a result, capybara home ranges in the central dry Chaco included two or three ponds separated by approximately 1 km, that were typically used by multiple groups, but only one group at a time. Despite the considerable distance covered by capybara, 95 % of all location points were < 500 m from a permanent water source demonstrating the necessity of these ponds for capybara. A tight association with water is in agreement with previous reports (Azcarate 1980; Barreto and Herrera 1998; Cordero and Ojasti 1981; Herrera and Macdonals 1989; Lord 1991; Mones and Ojasti 1986; Murphey et al. 1985; Quintana and Malvarez 1994, 1998). Although we found no significant difference among wet

and dry season home ranges when we considered all animals, two capybara which were members of a group of six had larger home ranges during the dry season than during the wet season.

Expansion during the dry season could be due to a need for additional food resources to support the group as productivity declined during the dry season. The dry season home ranges of solitary or paired capybara were smaller than for the large group.

Considering that capybara is a semi-aquatic rodent, it was not unexpected that water surface was a preferred habitat type. Also preferred, however, was the primary Chaco forest. Primary Chaco forest surrounded the great majority of ponds in the study area; we believe that the dense vegetation of the forest plays a significant role for capybara survival in the region. The forest likely served as a cool shaded area during the hot summer and provides shelter from the cold south winds during winter, and provided suitable protection from predators. Introduced pastureland was also preferentially used, providing extensive high quality forage year round for capybara. Without pasturelands, capybara foraging habitat would be confined to small, periodically flooded natural pasture, or the vegetation immediately surrounding man made ponds. Interestingly, capybara avoided secondary Chaco forest. The lack of understory vegetation provided few food resources and little protection against extreme climatic conditions and predators, making this habitat risky for capybara.

Average body temperature of capybara in our study (36.15° C) was similar to other findings (López-Barbella 1982). Body temperature was positively correlated with distance from water. These findings suggest that capybara use water to thermoregulate as well as for predator avoidance. Our interpretation is further supported by the observations that capybara ventured further away from water only when ambient temperatures were low. Although capybara have been anecdotally reported to use water for thermoregulation, this is the first study documenting

this phenomenon. Other semi aquatic rodents such as the European beaver (*Castor fiber*), American beaver (*Castor canadensis*), and the Australian water rat (*Hydromys chrysogaster*) also use water as a thermoregulatory tool and have developed physiological adaptations to optimize the benefits of water high cooling power (Fanning and Dowson 1980; Hart 1971; Steen and Steen 1965). Similarly, the capybara has evolved skin and hair that enhances the ability of water to regulate body temperature. Capybara epidermis is covered in folds of tissue which creates a larger surface area; additionally, the hair emerges at an acute angle and is clumped in groups of three or four follicles with each clump spaced relatively sparsely which speeds the drying process (Pereira et al. 1980).

Our genetic results are consistent with the hypothesis that capybara populations in the Central Dry Chaco region have recently expanded into this region and that they are at the leading edge of the expansion. The homozygosity of individuals is indicative of a population originating from a small number of individuals which colonized the area recently. Surprisingly we were not able to identify any first order relatedness among our trapped capybara. On the other hand, we found a second order relatedness among two sampled capybara, both of which were found in different groups a juvenile male (capy 43) and adult male (capy 45). The lack of genetic structure and relatedness could be related to sample size, or to the fact that the local capybara populations have relatively recently established in the area, without sufficient time to demonstrate signals of genetic structure at the analyzed scale. On the other hand, capybara could be facing intense predation which would explain the sudden disappearance of our monitored animals and the lack of detecting parents in the groups.

Worldwide, anthropogenic land use change and fragmentation is implicated in the establishment of invasive species (Lockwood et al. 2007; Peterson and Vieglais 2001). Capybara

invaded the Central Dry Chaco after large-scale deforestation occurred for ranching (Campos-Krauer and Wisely submitted). It appears that ample forage and suitable cover were created when primary Chaco forest was fragmented. Perhaps more important to the expansion of capybara into this region was the creation of regularly distributed, permanent water sources in this otherwise xeric habitat. As a taxon, rodents are highly successful invaders. They exhibit demographic traits necessary for invasion such as high fecundity, short generation time and opportunistic breeding which can provide advantages over local fauna. Rodents cause losses to harvest world-wide and are the most significant crop pests (Singleton et al. 1999). Where they have invaded they often further alter habitat [e.g. semi-aquatic European beaver (*Castor fiber*), American beaver (*Castor Canadensis*) and the South American nutria (*Myocastor coypus*)]. With voracious appetites and high reproductive rates, these species are of great concern world wide.

Capybara also appears to be a species capable of taking advantage of anthropogenic land management and transformation. Large scale land cover change from Chaco forest to a patchwork of pasture and forest appears to be a critical factor allowing capybara to invade these regions. The central dry Chaco region has been intensively transformed during the last 70 years. This habitat transformation has drastically changed the structure and distribution of primary Chaco forest, as well as significantly modified the water availability in the area. This transformation has created conditions which have allowed capybara to expand its range into an area previously inhospitable to this species. Establishment in this region, however, is driven by the modification of water availability in the area.

The expansion of capybara into the central dry Chaco is a clear consequence of the great land transformation that has affected the area. Its appearance in the region should alert those responsible for the health of the environment that the Gran Chaco ecosystem is being irreparably

altered. Invasive species can outcompete native species and alter ecosystem services. Indeed, the presence of capybara in the central Chaco may already be altering the epizootiological landscape of the region. Capybaras are reservoir hosts of *Trypanosoma evansi*, a protozoan parasitic infection that infects both domestic and wild mammals (Franke et al. 1994). At present, capybara population numbers are still small and have not been linked to any agriculture damage or disease reservoir in the central dry Chaco, but as livestock and capybara densities increase in this region, the potential for disease outbreak is high. This study increases our understanding of the extent of the spatial distribution and ecological requirements of capybara which will be essential for effective management of this newly established species.

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Table 1

Overall capybara home range estimates

Name	MCP	95% Kernel	75% Kernel	50% Kernel
Capy 1	437	193	75	42
Capy 2	492	421	168	67
Capy 45	737	198	58	24
Capy 43	484	52	17	10
Capy 41	997	62	27	10
Capy 50	352	176	40	16
Average	583 ha	183 ha	64 ha	28 ha

Table 2

Column 1, represents name of capybara trapped and group to which it belonged; Column 2 and 3, show period of time during which it was monitored; Column 4 and 5 show sex and weight when captured; Column 6, 7 and 8 show transmitter type, total location points and final report of the animal.

1	2	3	4	5	6	7	8
Capy 1 Group 1	03-07-2005	14-10-2005	Male	82 kg	Collar	71	Collar destroyed
Capy 2 Group 1	21-09-2005	13-12-2005	Female	56 kg	Collar	185	Disappeared
Capy 45 Group 2	02-03-2006	8-09-2006	Male	52 kg	Collar	405	Disappeared
Capy 43 Group 3	02-06-2006	28-2-2007	Male	17 kg	Implant	435	Disappeared
Capy 41 Group 3	28-06-2006	10-10-2007	Male	64 kg	Implant	600	Killed by puma
Capy 50 Group 2	10-06-2007	28-8-2007	Female	56 kg	Collar	85	Disappeared

Table 3

Asymmetrical matrix of percent data point spatial overlap among six capybara monitored in the study area; Columns correspond to percent overlap of corresponding capybara with different capybaras.

	capy 1	capy 2	capy 45	capy 43	Capy 41	capy 50
capy 1		66	1	49	49	6
capy 2	56		20	29	29	0
capy 45	38	79		0	0	19
capy 43	83	90	12		84	1
capy 41	78	94	9	86		2
capy 50	0	1	80	0	0	

Table 4

A. Ranking matrices for the overall comparison of habitat type use from minimum convex polygon home range versus habitat availability in the entire study area. B. Ranking matrices for capybara based on comparing proportion of radio locations for each animal in each habitat type within MCP home ranges per season. Each average element in the matrix was replaced by its sign. Sign represents level of significant deviation from random at $P < 0.05$. A value of 4 corresponds to the highest significantly used habitat, a value of 0 correspond to the lowest significant used habitat type.

A

	Water	Flooded area	Pasture	Shrub forest	Chaco Forest	Rank
Water		-	---	+	---	1
Flooded area	+		-	+	-	2
Pasture	+++	+		+++	---	3
Shrub Forest	-	-	---		---	0
Chaco Forest	+++	+	+++	+++		4

ChF > P > FG > W > ShF

B

	Water	Flooded area	Pasture	Shrub forest	Chaco Forest	Rank
Water		+++	+++	+++	+++	4
Flooded area	---		-	+	-	1
Pasture	---	+		+++	-	2
Shrub Forest	---	-	---		---	0
Chaco Forest	---	+	+	+++		3

W > ChF > P > FG > ShF

Table 5

Mean pairwise relatedness estimates of trapped capybara; based on Queller and Goodnight (1989) estimator. Capy 45 and capy 43 have a second order relatedness.

	Capy 1	Capy 2	Capy 45	Capy 50	Capy 41	Capy 43
Capy 1	0.0000					
Capy 2	-0.1871	0.0000				
Capy 45	-0.2149	-0.3333	0.0000			
Capy 50	-0.1214	-0.0454	-0.2789	0.0000		
Capy 41	0.0307	0.0417	-0.2500	-0.7100	0.0000	
Capy 43	-0.0055	-0.4581	0.2437	-0.0558	-0.6557	0.0000

Figure 1

Capybara study long average percentage activity by time of day; based on two capybara in the Study area, Central Dry Chaco region.

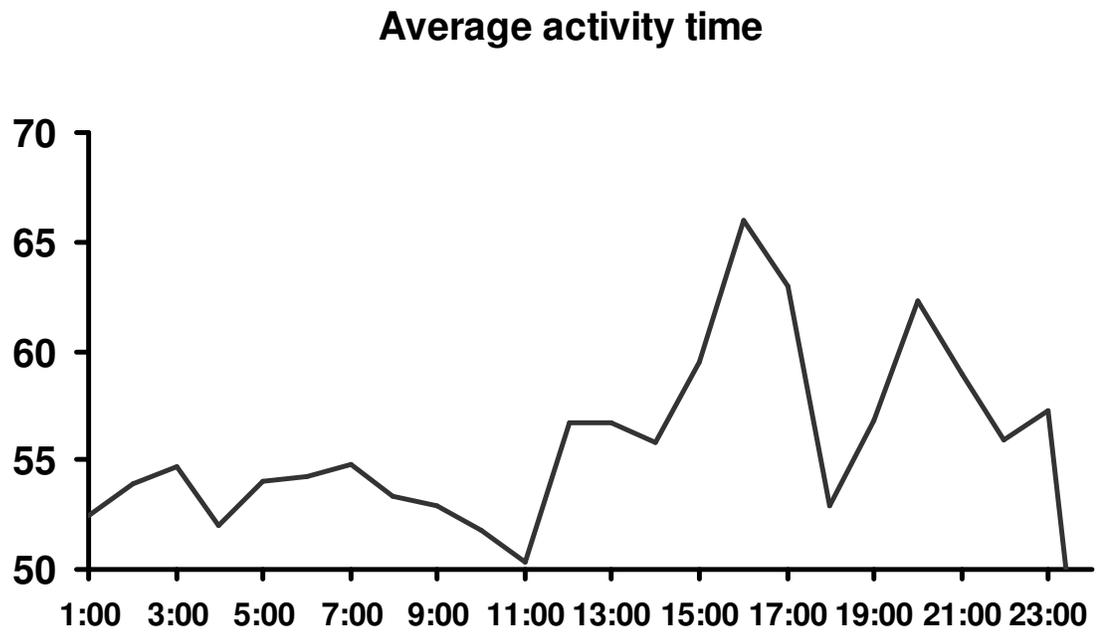


Figure 2

Our study area, located in the Central Dry Chaco region of Paraguay. Background shading represents habitat types. Black represents water surface, dark gray represents primary Chaco forest, medium dark gray represent secondary Chaco forest, Light gray represents periodically flooded land and white represents open pasture. Color lines represent overall 95 and 50% Kernels for six capybaras; each color represents an individual; black for capybara # 1 ♂, green for capybara # 2 ♀, red for capybara # 45 ♂, blue for capybara # 41 ♂, pink for capybara # 43 and yellow for capybara # 50 ♀.



Figure 3

Capybara average percent distance from water; study long based on six capybara in the Central Dry Chaco region.

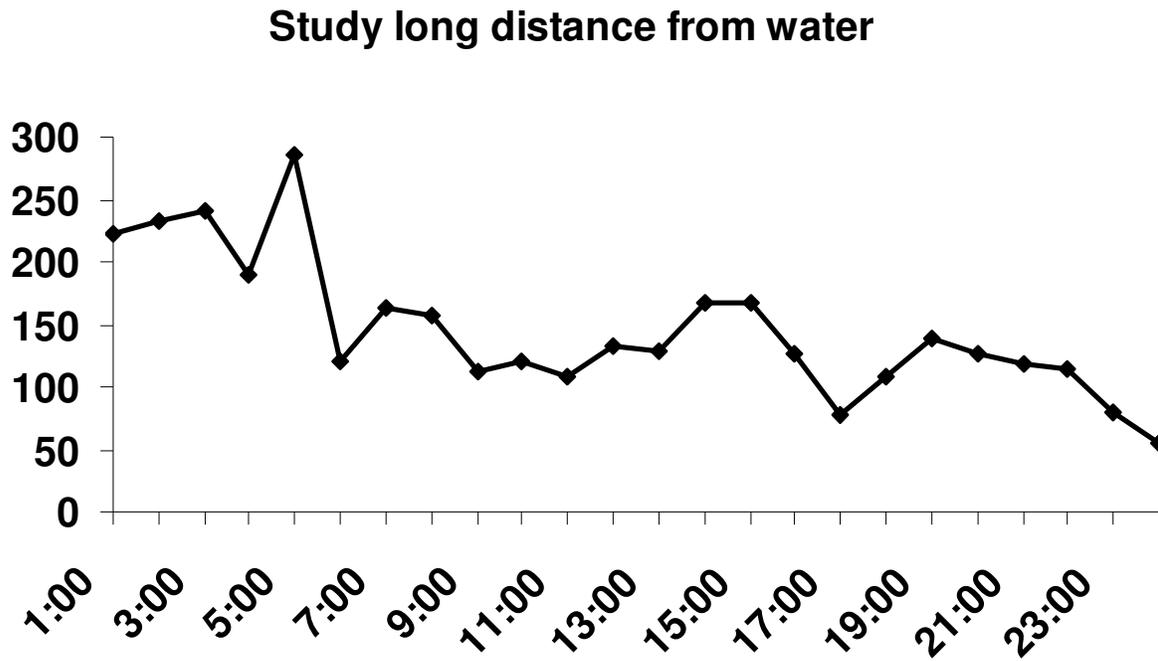
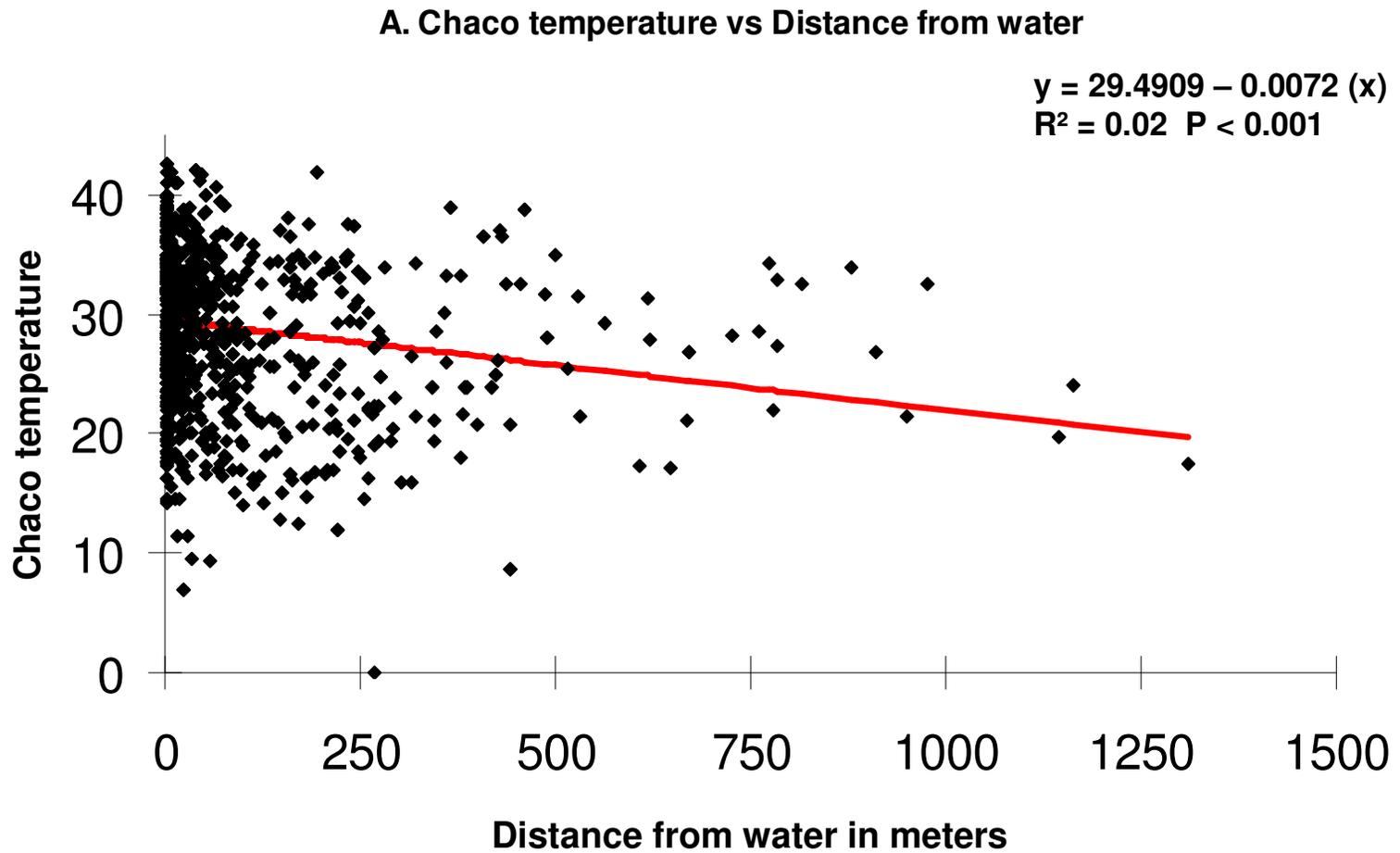


Figure 4

A scatterplot linear regression of Chaco temperature vs. Distance from water. **B** scatterplot linear regression of capybara body temperature vs. Distance from water.



B. Capybara body temperature vs Distance from water

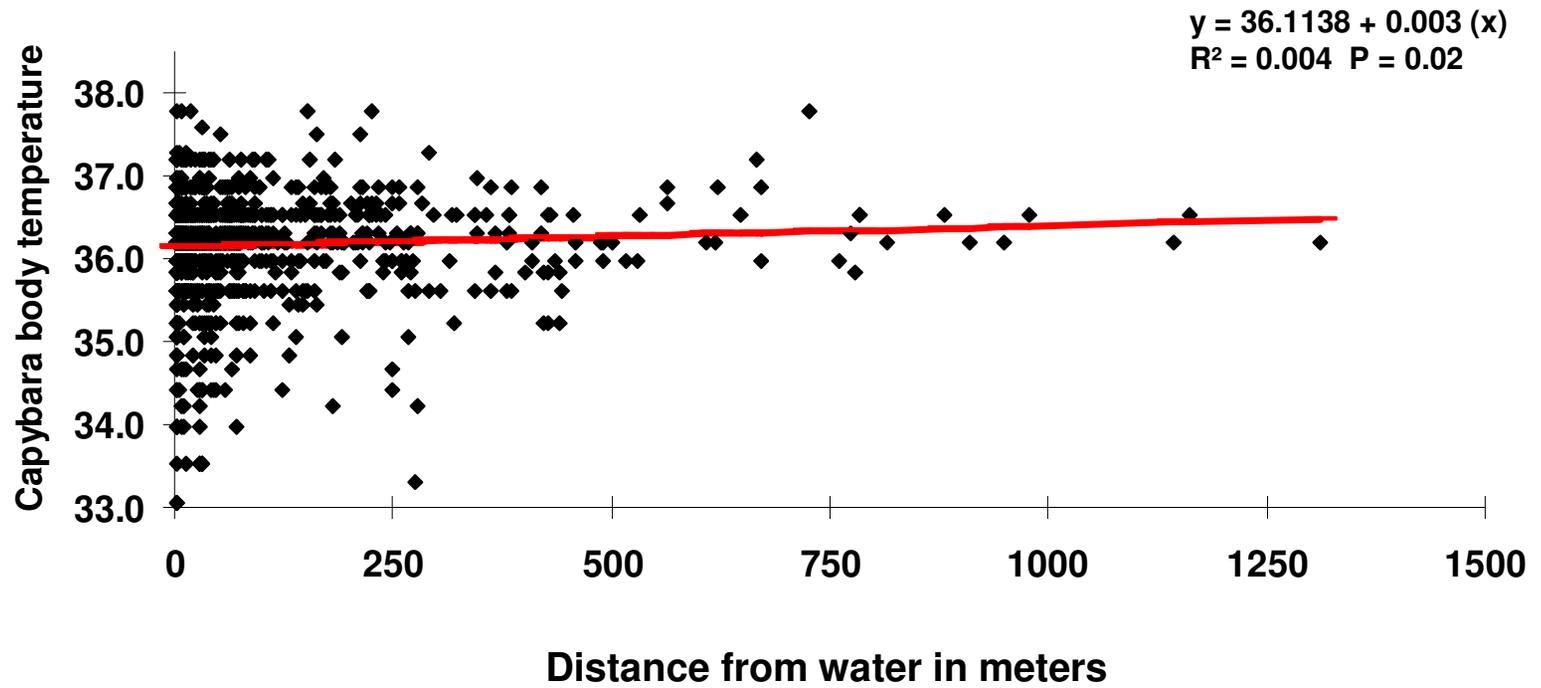
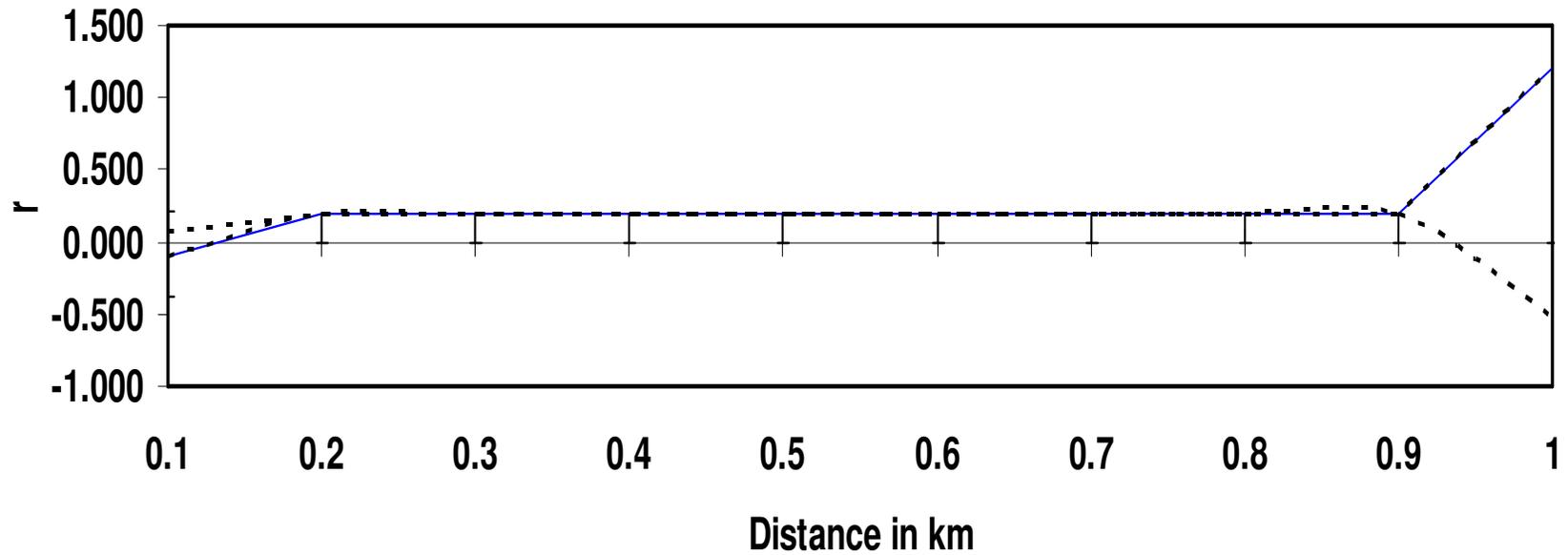


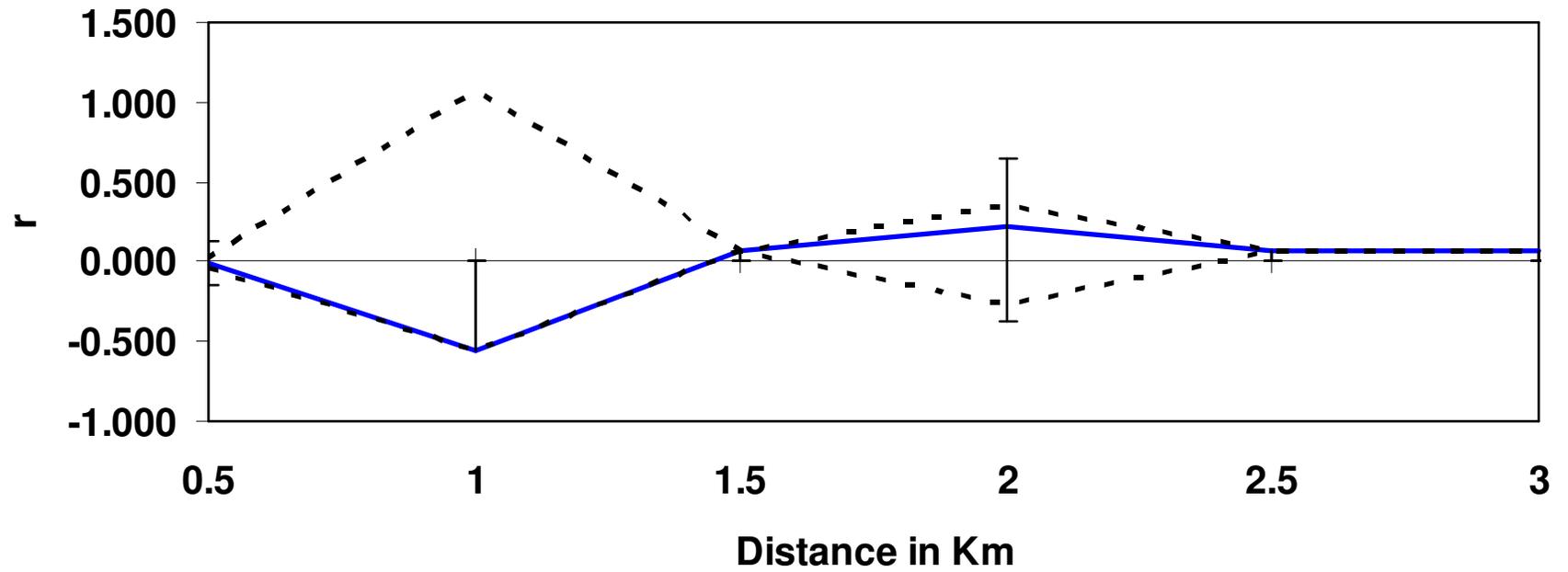
Figure 5

Spatial structure analysis of capybara population in the study site. First, capybara trapped (n = 6) little confidence limits, no significant values. Second, All capybaras genotyped from the study site and surrounding (n = 13) no significant values found.

Results of Spatial Structure Analysis



Results of Spatial Structure Analysis



Chapter IV

Final Conclusions

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This chapter is based on years of personal experience and hundreds of articles read. Comments provided in this chapter are personal thoughts and thinking of the author. The intention is to briefly describe the past, current and possible future of the Chaco region of Paraguay. At present it has not been and is not being considered for publication in any scientific journal.

Overview of the Paraguayan Chaco land Change

It is not surprising that the Quechua Indians named this wide plain at the foothill of the Andes, the land of great hunting, “The Chaco”. This vast region is diverse as a tropical forest. However, climatic conditions such as the very high temperatures and humidity during the summer months followed by the extreme dry seasons and the dense vegetation in the Chaco have always seemed to be more favorable to wildlife than to humans; this factor being the reason why the Chaco remained almost immune to modern human transformation until recent times. After European colonization began, the Chaco received several new names such as the Infierno Verde (Green Hell), and was considered more a place of punishment than a home. Only after the nineteenth century did interest in the colonization of the Chaco begin, mostly as a consequence of growing border conflicts between Bolivia and Paraguay. Due to this political strife, the Paraguayan government wanted to establish a presence in the wild desolated area. Soon most of the southeast of the Paraguayan Low Chaco became an area for extensive cattle production, taking advantage of the abundant open grass lands characteristic of the area. Similarly, settlements grew up along the banks of the Paraguay River, associated with the tannin industry, based on wild stands of Quebracho Colorado tree (*Schinopsis lorentzii*). The same policy of colonization encouraged thousands of Mennonite immigrants from Canada and later from the Soviet Union, to occupy the distant and desolate central Chaco area. Finally, in 1932 the conflict over the Chaco region brought Bolivia and Paraguay to a declared war for the territory. The Chaco War ended three years later with near 100,000 people dead. Paraguay ended victorious and was able to maintain the great majority of the Chaco. This new circumstance completely would change the Chaco history.

After the conflict ended, many military bases were established and interconnected with new roads. This situation started a new colonization event with settlements reaching areas never before reached and increased the need of communication between the Chaco and the rest of the country. This resulted in the construction of the trans-Chaco road in the late 1960's. This road was one of the first large scale land transforming projects in the Chaco. Still today the real ecological impact the construction had to wildlife, water flow and the habitat is unknown. However, it served its role and soon there was intense communication and connection between the rest of the country and the Chaco region.

Following the new global trend of development, which sadly was measured by deforestation, the Chaco started intense clearing of its natural forest. Aided by new, state-of-the-art bulldozers; clearing forest by directly stepping on it or by working in combination clearing one hectare at a time by using extremely large chains. Forest was cleared with no consideration toward the wildlife or the environment. First, land was used for croplands and later for introduced pasturelands. The region that most suffered from extensive clearing was the central Chaco region in and surrounding the large Mennonite communities. The low Chaco region was less affected by forest clearing due to the extensive natural pasturelands. However, the impacts of humans were also large, due to the inappropriate use of fire to clean extensive areas and overgrazing.

During the same time, the international demand for wildlife fur was high, and this situation had a direct impact to the Chaco wildlife. As a consequence, many hunters were exploiting all areas of the territory. The principal species hunted were felids, foxes and peccaries. However, surely all large mammal species must have suffered from uncontrolled and indiscriminate hunting. Nonetheless, the wildlife richness and isolation of the Chaco region was

once again reaffirmed in 1972 by the rediscovery of the Chacoan peccary (*Catagonus wagneri*) by Ralph Wetzel. The species was thought extinct for more than 10,000 years and had only been known from fossil bones from nearby Argentina. Ironically, soon after its discovery, this unique mammal would suffer great population reduction as a direct consequence of over hunting and rapid habitat loss.

The land clearing process in the Chaco has not stopped; the most recent estimates are of an average of 500 ha daily. The Chaco soil and vegetation, due to its alluvial formation, are thought to be recent and still adapting to the changing Chaco. This situation places the region at the highest risk in the world of rapid desertification if land use and management is not optimal. Water is one of the scarcest resources in most of the Chaco, with the exception of the low Chaco. However, long atypical dry seasons can also affect the mentioned area. Most of the fresh water is harvested by capturing rainfall in man made ponds and special cisterns for human use. In the Chaco region it is not common to find fresh underground water with most underground reservoirs having high levels salinity. All of these harsh conditions have not stopped the growth of the cattle business in the region, with significant numbers of new ranches formed yearly. At present the Chaco region is one of the highest meat producing regions of Paraguay, with an estimated population of approximately two million cattle.

Human population increase, intense land clearing and the modification of water availability have transformed extensive areas of the Chaco landscape. In areas in which there were thousands hectares of extensive thick almost impenetrable forest, now it is dominated by open fields with almost no trees left and a monoculture of introduced grass. This situation has drastically changed the habitat for hundreds of species of animals. Having to emigrate or die, they are faced with the ever smaller patches of remaining degraded forest. On the other hand,

with the new habitat and water availability new species adapted or benefited by the new habitat configuration are starting to increase.

Drastic changes in the landscape always have consequences in the short or long term, including soil degradation, erosion, water salination due to loss of the vegetative cover and local extinction of native species. However, I believe that the Chaco is not completely lost, even as large areas are still being cleared; there still is hope and time to save the last remaining pieces of intact Chaco forest. If strict conservation policies benefiting wildlife are implemented and enforced, there is a high possibility of saving the remaining forest and recover species numbers and diversity.

For this to be possible, I believe that there are several key factors that need to be evaluated. First, there has to be rational policies for exploitation of natural resources with a coordinated development integrating all land use in a single final conservation goal. Second, forest and wildlife need to receive an economic value to stimulate private conservation. If forest is no longer perceived as an unproductive marginal land, then a conservation plan can be implemented successfully. This can be accomplished by tax incentives or the emerging international carbon market. Third, degraded and damaged habitat areas need to be restored and improved for native wildlife re-colonization. Finally, the fundamental factor is education at all levels, from primary schools to conservation courses at the community level.

Overview of Capybara (*Hydrochoerus hydrochaeris*) in the Paraguayan Chaco

Capybara have been in the low Chaco region for hundreds of thousand of years. The native Indians knew the species well and considered a important recourse. In fact the name capybara comes from the Guarani word capifí-y-gua, meaning the animal from the grass and the water. Capybara has a conservation status of “low risk” although information on its distribution and population numbers are unknown for the Chaco region. It is a common inhabitant of the low Chaco region, being present in almost every marsh, swamp or river bed. However, capybara in the low Chaco is intensively poached and persecuted. Nevertheless, populations still seem to thrive, probably due to the presence of large ranches with low human populations, and the high reproductive potential of the species. On the other hand, in more populated areas surrounding small settlements and towns; capybara populations have become almost completely nocturnal and their numbers are reduced.

In general, capybara is one of the few big mammals that has benefited from anthropogenic land transformation and management. Natural predator populations such as Jaguars (*Pantera onca*), puma (*Felis concolor*) have been drastically reduced by hunting and predator control implemented by ranchers. Pasture land has been improved and managed. However, population numbers of capybara in the low Chaco region never reached the immense populations such as those described in the Venezuelan Llanos. There have been reports of great numbers of capybara mortality due to trypanosomosis in Venezuela as well as, in the low Chaco region. Capybara are known to be host reservoirs to (*Trypanosoma evansi*) an African trypanosoma introduced by infected horses brought by the early Spanish settlers. At present the disease is common in the low Chaco region. It is locally named Mal de caderas (Spanish) or

tymbya (Guarani). This disease can severely affect horses and domestic dog populations. However, its impact on capybara populations in the low Chaco region is completely unknown.

According to results of my dissertation, the capybara population is one species taking direct advantage of the anthropogenic land fragmentation in the Chaco. I have evidence that capybara is expanding its range within the central dry Chaco region. Capybara have moved from the low Chaco region to new pasturelands and ponds in the central Chaco, using ponds as connecting stations from which they expand northward. The central Chaco region due to its climatic and soil structure has never supported capybara populations. Empirical evidence of this is that native Indian tribes from the Central Chaco have no place in their culture for capybara which is not the case of low Chaco tribes in which capybara is probably the most commonly represented animal species in wood carvings.

The arrival of capybara to the Central Chaco is one more sign of the extent to which the land has been modified. Capybara are taking advantage of permanent water ponds which is the essential factor for their survival. The combination of three factors: permanent water, introduced pasture and remnant forests seems to cover the essential needs for capybara, allowing it to establish permanent populations in the area.

Through my work, I was able to better understand the expansion process at two hierarchical scales. At the largest scale, I found that capybara in the Chaco are not from a single population, but the result of an admixture of 2 geographically and genetically distinct populations which had been isolated from one another until very recently. Populations that were separated for thousands of years are now coming together in the Central Chaco as a consequence of new pathways created by anthropogenic land change. To present this new frontier has created a contact zone for capybaras to intermix and breed with long isolated populations. Considering

that capybara are reservoirs of *Trypanosoma evansi*, it is unknown what possible consequence this may have to capybara with naïve immune systems or to the local native species. To date, there has not been a report of Mal de caderas (trypanosomosis) in the newly invaded area, however there is no active surveillance, and its occurrence is likely just a matter of time.

At a finer scale, we found that capybara presence in the Central Chaco region is tightly tied to man made ponds and patchy deforestation. The recent invasion of capybara into this area has remained generally unnoticed to most local land owners. With small groups inhabiting local ponds, their presence is difficult to detect. However, if an outbreak of trypanosomosis should occur, it is unknown what consequences this may have for the newly established capybara populations.

The expansion of capybara to the Central Chaco is a direct consequence of land change. I believe that it is possible to take advantage of the situation. Capybara is known for having fast reproductive rates and for being very efficient herbivores. If populations are monitored and a species management plan for the region implemented, a regulated harvest of these newly invading animals can serve as an extra resource for land owners. This will give value to the animal, provide a vehicle for more accurate monitoring of the expanding population, and allow for surveillance of emerging diseases.

As a final conclusion, the Chaco region of Paraguay is suffering from intense land change that is greatly reshaping the landscape. These changes have resulted in immense damage to local native wildlife and plant species. As a consequence, some species are taking advantage of the new landscape and expanding their range. Capybara is now present in areas in which it was previously very rare or completely absent. This situation should serve as a warning that the ecosystem is becoming unstable.