UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

Instituto de Ecología

Filogeografía del armadillo de nueve bandas (*Dasypus novemcinctus*) en México

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTORA EN CIENCIAS

P R E S E N T A

MARIA CLARA ARTEAGA URIBE

TUTOR PRINCIPAL: Dr. Rodrigo A. Medellín Legorreta

COMITÉ TUTORAL: Dr. Luis Enrique Eguiarte Fruns
Dr. Joaquín Arroyo-Cabrales

MÉXICO, D.F. **Mayo de 2011**

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

Instituto de Ecología

Filogeografía del armadillo de nueve bandas (*Dasypus novemcinctus*) en México

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTORA EN CIENCIAS

P R E S E N T A

MARIA CLARA ARTEAGA URIBE

TUTOR PRINCIPAL: Dr. Rodrigo A. Medellín Legorreta

COMITÉ TUTORAL: Dr. Luis Enrique Eguiarte Fruns
Dr. Joaquín Arroyo-Cabrales

MÉXICO, D.F. **Mayo de 2011**

UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 8 de noviembre de 2010, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **ARTEAGA URIBE MARÍA CLARA** con número de cuenta **507452553** con la tesis titulada: **"FILOGEOGRAFÍA DEL ARMADILLO DE NUEVE BANDAS (*Dasypus novemcinctus*) EN MÉXICO."**, realizada bajo la dirección del **DR. RODRIGO A. MEDELLÍN LEGORRETA:**

Presidente:	DR. GERARDO PÉREZ PONCE DE LEÓN
Vocal:	DR. ENRIQUE MARTÍNEZ MEYER
Vocal:	DR. ANTONIO GONZÁLEZ RODRÍGUEZ
Vocal:	DRA. SUSANA AURORA MAGALLÓN PUEBLA
Secretario:	DR. JUAN PABLO JARAMILLO CORREA

De acuerdo con lo anterior, la alumna se acogió a la nueva normatividad, con base en el artículo QUINTO TRANSITORIO en apegó a lo establecido en el Artículo 31 del Reglamento General de Estudios de Posgrado.

Sin otro particular, me es grato enviarle un cordial saludo.

A t e n t a m e n t e
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F. a 11 de abril de 2011.

Dra. Maria del Coro Arizmendi Arriaga
Coordinador del Programa

c.c.p. Expediente de la interesada

Edif. de Posgrado P. B. (Costado Sur de la Torre II de Humanidades) Ciudad Universitaria C.P. 04510 México, D.F.
Tel. 5623-0173 Fax: 5623-0172 http://pcbiol.posgrado.unam.mx

AGRADECIMIENTOS

Quiero agradecer al posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México (UNAM) por la oportunidad que me dio para realizar mis estudios de doctorado y todo el apoyo durante los mismos.

La Dirección General de Estudios de Posgrado de la UNAM me otorgó la beca durante cuatro años para la realización de mis estudios y también la beca de fomento a la graduación otorgada en la fase final.

A Rodrigo, mi asesor, un agradecimiento muy especial. Él siempre confió en mi, me acogió en su laboratorio y me hizo sentir muy querida. Gracias por sus enseñanzas, por las oportunidades que me dio de ir al campo con él y aprender un poco de todo lo que sabe. Gracias por las puertas que me abrió, por el entusiasmo que le puso a este trabajo y el apoyo que siempre me brindó.

Gracias a los miembros de mi comité. A Luis, un agradecimiento muy especial por abrirme las puertas de su laboratorio y permitirme trabajar en él. Por su entusiasmo con todos los proyectos y sus enseñanzas teóricas. A Joaquín, gracias por su disposición de ayuda y apoyo durante el desarrollo de este trabajo.

A los revisores de la tesis, Juan Pablo Jaramillo, Antonio González Rodríguez, Enrique Martínez Meyer, Gerardo Ponce de León y Susana Magallón. La lectura cuidadosa y los valiosos comentarios aportados por ellos me ayudaron a mejorar y enriquecer este manuscrito.

Un profundo agradecimiento a Daniel Piñero. Gracias por creer en este proyecto y por comprometerse de la manera en que lo hizo. Gracias por siempre apoyarme. Por transmitirme calma y confianza cuando ya yo la iba perdiendo. Por discutir los datos y creer en ellos. Por sentarse conmigo muchas horas durante todos los años de mi doctorado y haber participado en mi formación académica con discusiones enriquecedoras sobre temas de genética y filogeografía. Su presencia fue fundamental en esta etapa de mi vida.

Quiero agradecer a los directores de los laboratorios de Ecología y Conservación de Vertebrados Terrestres (Rodrigo Medellín), Evolución Molecular y Experimental (Luis Eguiarte y Valeria Souza) y Genética y Evolución (Daniel Piñero), así como al Instituto de Ecología por el apoyo financiero que me brindaron para la realización de este proyecto. Igualmente a Sigma Xi y The American Society of Mammalogists por las becas recibidas.

A mi laborotario querido. A Osi siempre sonriente y dispuesta a ayudar, a Angélica tan alegre, mi compañera de canto; a Antonio (ñoñis) por la paciencia siempre que me reía “con” él; a Rafa por su nobleza y su disposición de ayuda permanente; a Lacitooo el entusiaste y noble lacitoo, por todo; a Horacio siempre tan abierto a aprender y a ayudar; a Pau, por su ternura. A los que ya se fueron Tania, Leo, Gabi, Arturo, por su cariño y a los que llegan por su buena disposición de sonreir y trabajar……para mi fue fundamental encontrar un ambiente tan calido y acogedor en el lab...

A los vecinos…especialmente a Heliot, por siempre ayudarme con mis dudas técnicas de Arc View y a Emmanuel, por su asesoría en programas de dibujo y detalles importantes de gráficos, figuras e imágenes.

A Jaime Gasca por su ayuda en los procedimientos de laboratorio. Su colaboración fue fundamental para obtener los datos de esta tesis. A Alex por su ayuda con los micros. A Erika y Laura por su cuidado para que no faltara nada en el laboratorio. A Diego Ortega, por tantas horas que me dedicó ayudándome a correr programas y siempre con tan buena disposición.

A todas las personas que me ayudaron a conseguir pedacitos de armadillos por cada rincón de México…A Leo, Elizabeth y sus alumnos, Sol de Mayo y sus alumnos, Edgar, Horacio, Arturo, Paty y todas las colecciones biológicas citadas en las tablas de los artículos. Sin su ayuda no hubiera sido posible obtener los datos para construir este trabajo.

Y los agradecimientos más especiales son para las cinco personas que más amo. Mis papás, Nelly y Victor, tan amorosos y siempre apoyando mis deseos. Gracias por todo el amor transmitido en estos años. Por su impulso cada que quería llegar a un nuevo destino en mi vida. Por su tolerancia con mis decisiones que a veces no eran las mejores para ellos, pero que ellos sabían que eran las mejores para mí. A mis hermanos adorados, Andrés y Camilo, que también sin entender muy bien porque voy persiguiendo armadillos por tantas esquinas del mundo, me han apoyado siempre. Estos cuatro personajes son mi ejemplo de vida. Por la honestidad con la que viven, por su emprendimiento, por la buena manera de llevar sus días y por el inmenso amor que le tienen a la vida. Finalmente quiero agradecerle a Rafael, quien alegra cada uno de mis días. Por haber llegado a mi vida, por darme todo el amor que me da y por dejarse querer tanto. Porque es un ejemplo para mí con su dedicación a lo que hace y eso me impulsa, porque me devuelve la credibilidad y me enseña como mejorar cada día. Porque su amor es tranquilo, alegre y lleno de felicidad.

ÍNDICE GENERAL

RESUMEN

Las especies ampliamente distribuidas, como el armadillo de nueve bandas (*Dasypus novemcinctus*), ocupan una extensa variedad de hábitats a lo largo del paisaje. Esto posibilita la diferenciación genética por separación física o por razones ecológicas, y genera que a lo largo de su distribución pueda existir más de un linaje genético. La conexión establecida en el Pleistoceno entre América del Norte y del Sur, a través del Istmo de Panamá, permitió al armadillo expandir su área de distribución hacia el norte. En este estudio evalué si las poblaciones de armadillos mexicanos son derivadas de varios linajes genéticos y examiné si estos linajes presentan expansión demográfica, como es lo esperado para áreas recientemente colonizadas. Además, determiné si el nicho de los linajes es conservado o divergente y evalué el efecto de estas afinidades climáticas en el flujo génico. Evalué la estructura y diversidad genética de los armadillos analizando 408 pb de la región de control de la mitocondria y seis microsatélites nucleares de 157 y 116 individuos, respectivamente. Detecté dos linajes mitocondriales divergentes, con alta variación genética y distribución geográfica alopátrica en México. Ambos linajes sufrieron expansiones demográficas históricas, sin embargo, ambos presentaron una disminución poblacional reciente, posiblemente relacionada con actividades antrópicas. Mientras que el ADNmt indica una fuerte estructura genética, los microsatélites nucleares muestran poca diferenciación. Este patrón contrastante puede resultar de una mayor dispersión de los machos. Para evaluar la similitud en las afinidades climáticas, usé modelos estadísticos nulos y encontré conservadurismo de nicho entre los linajes. El análisis de componentes principales muestra un sobrelapamiento asimétrico del espacio ambiental ocupado por los linajes. Los niveles de mezcla genéticos evaluados con los microsatélites, fueron mayores en áreas del espacio ambiental compartido por ambos linajes. Esto sugiere que el conservadurismo de nicho puede facilitar el flujo génico entre grupos con distribución disyunta. En conclusión, las poblaciones de armadillo mexicano derivan de dos linajes divergentes. La distribución alopátrica que estos presentan en el Oeste y Este de México es probablemente mantenida por el conservadurismo del nicho, ya que el altiplano mexicano no presenta condiciones climáticas adecuadas para esta especie.

ABSTRACT

Widely distributed species such as the nine-banded armadillo (*Dasypus novemcinctus*) can occupy a variety of habitats across the landscape. Such distributional pattern can lead to genetic differentiation by physical or ecological factors, favouring the generation of more than one genetic lineage across the geographical range of these species. The connection established during the Pleistocene between North and South America allowed the nine-banded armadillo to expand northward its distributional range. I tested the hypothesis that Mexican armadillos were derived from several founding lineages and assessed whether the most recently colonized region shows demographic signatures of expansion as might be expected for a recent colonization. In addition, I tested whether the climatic niches of these lineages are conserved or divergent and addressed the role of niche similarity in gene flow. To determine the genetic structure and diversity of Mexican armadillos, I sequenced 408 bp of the mitochondrial control region and six nuclear microsatellites of 157 and 116 individuals, respectively. I observed two divergent mitochondrial lineages with high genetic variation and allopatric distribution. While lineages showed a historical demographic expansion, I also detected evidence of a recent decline, which could possibly be related to human-induced causes. Clear genetic structure was observed with mtDNA, whereas microsatellites indicated low levels of genetic differentiation. This contrasting pattern can be caused by male-biased dispersal. To assess the similarity in the ecological affinities, I used statistical null models and found evidence of niche conservatism between the two lineages. In the Principal Component analysis, I observed an asymmetrical pattern of overlap of the environmental spaces of the two lineages. Genetic admixture levels, assessed using the microsatellite data, were significantly higher in shared portions of environmental space. This suggests that niche similarity can facilitate gene flow among disjunct groups. I conclude that Mexican populations of nine-banded armadillo are derived from two founding lineages. The allopatric distribution of the lineages in West and East of Mexico probably is maintained by the niche conservatism, since the Mexican Plateau does not show suitable climatic conditions for the lineages.

1. INTRODUCCIÓN

La variación genética intraespecífica es una importante medida del potencial evolutivo y la viabilidad a largo plazo de las especies. La cantidad de variación presente en una especie y su distribución espacial son influenciadas por factores históricos, como procesos de deriva génica y eventos de cuello de botella en las poblaciones (Avise 2004). Esta variación es moldeada también por factores ecológicos, como las interacciones bióticas, las condiciones ambientales y las características del paisaje que actúan como barreras al movimiento de los individuos y que afectan la demografía y el flujo génico. Finalmente, algunos atributos de las especies como las conductas de dispersión, las estructuras sociales y la selección de hábitat, pueden determinar patrones asimétricos de dispersión y afectar de esta manera la distribución de la variación (Smith *et al.* 2007). Evidencias del efecto de estos factores pueden encontrarse a lo largo del área de distribución de una especie.

El área de distribución de una especie es el espacio físico en el cual la tasa de establecimiento de poblaciones locales no excede la tasa de extinción de las mismas y donde hay una cohesión entre ellas como resultado del movimiento de individuos (Gaston 2003). Los patrones de variación temporal en la demografía de las poblaciones, resultado de factores intrínsecos o extrínsecos, determinan el carácter dinámico del tamaño del área de distribución (Holt 2003). El límite de la distribución frecuentemente coincide con una combinación particular de condiciones climáticas que pueden llevar a los individuos a sus límites fisiológicos y esto puede afectar las tasas de supervivencia y de reproducción (Gaston 2003), disminuyendo la adecuación individual y consecuentemente influyendo en el tamaño de las poblaciones. Entre tanto, las variaciones temporales y espaciales en las características bióticas y abióticas que enfrentan los individuos pueden generar que las

poblaciones localizadas en el centro de las áreas de distribución de una especie, presenten variaciones demográficas similares a las de la periferia (Holt 2003). Por ello, no puede establecerse un patrón de variación en las densidades poblacionales desde el centro del área de distribución hacia los bordes de la misma (Sagarin & Gaines 2002; Sagarin 2006). Esto a su vez dificulta la asociación de un patrón de pérdida de la variabilidad genética en un sentido espacial ya que ésta es influenciada por las fluctuaciones del tamaño poblacional y por las tasas de flujo génico (Vucetich & Waite 2003).

Las poblaciones que se encuentran en el frente de colonización de nuevas áreas en general presentan bajos niveles de variación genética. Esto es consecuencia de la pérdida estocástica de alelos (deriva génica) por la fundación de dichas poblaciones a partir de pocos individuos (Austerlitz *et al.* 1997) y como resultado de los altos índices de endogamia que éstas puedan presentar (Gaston 2003). Durante el Plio-Pleistoceno (Fig. 1), muchas especies sufrieron fluctuaciones en sus áreas de distribución debido a los cambios que los ciclos glaciales (Hewitt 2004) y los eventos tectónicos (Webb 1976; Tosdal *et al.* 1984) causaron sobre la disponibilidad de hábitats adecuados. La ampliación del área de distribución de muchos mamíferos presentes en América se dio con la aparición de conexiones terrestres entre el norte de América del Sur y sur de América Central. Dichas conexiones posibilitaron la dispersión entre estos dos continentes que habían permanecido separados por casi 100 millones de años (m. a.) (Pascual & Ortíz-Jaureguizar 2007).

1.1 El Gran Intercambio Biótico entre las Américas

El intercambio de faunas que ocurrió entre América del Norte y América del Sur anterior al Holoceno, fue un acontecimiento que influyó en la dinámica y la estructura actual de las comunidades de mamíferos en el continente. Después de estar separados aproximadamente por 100 millones de años (m. a.), desde la fragmentación de la última Pangea (Pascual & Ortíz-Jaureguizar 2007), una serie de conexiones llevaron a la migración de especies en ambas direcciones. Este intercambio no fue un único evento (Pascual & Ortíz-Jaureguizar 2007). En depósitos paleontológicos de América del Norte y del Sur, correspondientes al Cretácico tardío e inicios del Paleoceno, se han registrado fósiles de miembros de los órdenes Didelphiomorphia, Leptictida, Pantodonta y Xenungulata (Marshall & Sempere 1993), que indican la ocurrencia de dispersión a finales del Mesozoico e inicios del Cenozoico (Fig. 1). Durante esta época, las islas de las Antillas y el ya sumergido Arco de las Aves formaron un archipiélago de islas volcánicas que probablemente funcionó como puente para el primer evento de migración entre ambos continentes (Marshall *et al.* 1982).

Posteriormente, durante el Terciario temprano, el nivel del mar se elevó y ambos continentes quedaron nuevamente separados hasta el final del Plioceno. Hace aproximadamente 10 m. a. (12.8 - 9.5 m. a.) comenzó la colisión del arco sur de Panamá con América del Sur (Coates *et al.* 2004). Los registros de Procyonidae, Cricétidae (Marshall *et al.* 1979) y Gomphothereriidae (Prado *et al.* 2005) que alcanzaron América del Sur hace aproximadamente 7- 9 m. a., así como la presencia de especies de Muridae, Glyptodontidae, Megalonychidae y Mylodontidae en América del Norte (Marshall *et al.* 1979), sugieren una dispersión en el Mioceno, probablemente por un archipiélago de islas entre los continentes (Webb 1976; Simpson 1980), resultante de una disminución en el

nivel del mar (Marshall & Sempere 1993) y de la actividad geomorfológica causada por el choque de las placas.

Era	Periodo	Época		m. a.
Cenozoico	Cuaternario	Holoceno		0.01
		Pleistoceno	Ta	1.8
			Te	2.6
	Terciario	Plioceno	Ta	3.6
			Te	5.3
		Mioceno	Ta	11.6
			M	16
			Te	23
		Oligoceno	Ta	28.4
			Te	33.9
		Eoceno	Ta	40.4
			M	48.6
			Te	55.8
		Paleoceno	Ta	58.7
			M	61.7
			Te	65.5
Mesozoico	Cretácico		Ta	99.6
			Te	145.5

Figura 1. Escala geológica del Cenozoico y parte del Mesozoico. m. a.: millones de años. Ta: tardío, M: medio, Te: temprano. Modificado de Gradsteins *et al.* 2004

Sin embargo, el tercer y mayor flujo de fauna proveniente de las dos Américas no comenzó sino hasta hace 3 m. a., una vez que el Istmo de Panamá se había formado completamente. Los inmigrantes de América del Norte a América del Sur incluyeron Mustelidae y Tayassuidae para el Plioceno tardío; Canidae, Felidae, Ursidae, Camelidae, Cervidae, Equidae, Tapiridae y Gomphoteriidae durante el Pleistoceno temprano y Heteromyidae, Sciuridae, Soricidae y Leporidae durante el Holoceno. Los inmigrantes de América del Sur hacia América del Norte incluyeron miembros de las familias Dasypodidae, Glyptodontidae, Hydrochaeridae y Erethizontidae durante el Plioceno tardío.

Didelphidae y Megatheriidae durante el Pleistoceno temprano y medio y Toxodontidae durante el Pleistoceno tardío. Finalmente, Callitrichidae, Cebidae, Choloepodidae, Bradypodidae, Cyclopedidae, Myrmecophagidae, Dasyproctidae y Echimyidae son conocidos solo de depósitos recientes de América del Norte en el Holoceno (Marshall *et al.* 1982).

La formación del Istmo de Panamá creó una fuente potencial de migración entre ambos continentes. Sin embargo, las áreas tropicales de América del Norte y del Sur parecen haber actuado como barreras a la dispersión de algunos taxa (Marshall *et al.* 1982). Solo las familias con algún miembro distribuido en áreas tropicales o subtropicales tomaron parte del intercambio (las mencionadas anteriormente). Además, el patrón de ocupación de las Américas no fue simétrico. Mientras los grupos del norte llegaron a América del Sur ocupando grandes áreas y diversificándose (Ferrusquía-Villafranca 1978), como los Cervidae, Canidae y Camelidae, los grupos sureños en América del Norte permanecieron en áreas restringidas y no tuvieron altas tasas de diversificación (Webb 1976). De estos últimos, solo Pilosa (perezosos), Cingulata (armadillos), Didelphidae (tlacuache) y roedores Caviomorpha lograron colonizar con éxito la zona templada de América del Norte.

Cuando el Gran Intercambio Biótico entre las América comenzó, el paisaje geológico mexicano estaba en formación. En el Cretácico tardío, el nivel del mar era mucho más elevado que actualmente, y el mar epicontinental cubría gran parte del interior del territorio. Hacia el final de este período, el altiplano sufrió continuas elevaciones provocando su drenado, mientras la parte sur del territorio aun estaba sumergida, a la vez que una intensa orogénesis daba origen a las montañas de la Sierra Madre Occidental. Posteriormente, en el Paleoceno se inició la orogénesis que formó la Sierra Madre Oriental. En esta época el mar ya había desaparecido del interior del territorio, y las montañas

jóvenes, depresiones y planicies costeras siguieron su formación hasta el Terciario medio. En el Mioceno, la subducción de la placa de Cocos en la placa de América del Norte generó un fuerte vulcanismo, dando como resultado la elevación del eje neovolcánico y la formación Sierra Madre Sur. Todavía en el Holoceno, los episodios de vulcanismo eran evidentes en la región Central (Graham 2010). Todos estos procesos orogénicos tuvieron drásticas consecuencias en las comunidades que ocupaban este territorio y en los migrantes que llegaban desde América de Sur.

1.2 El género *Dasypus* y los eventos Plio-Pleistocénicos que pudieron afectar su diversificación.

Los armadillos (Cingulata) representan uno de los órdenes más importantes de mamíferos que migraron al hemisferio norte, tanto por la diversidad de taxa que se dispersaron, así como porque participaron en diferentes etapas de este intercambio (Marshall *et al.* 1979). La hipótesis de un origen intertropical de la subfamilia Dasypodinae (Carlini *et al.* 1997) a la que pertenece el género *Dasypus*, es apoyada por el registro fósil. *Nanoastegotherium,* es el miembro conocido más antiguo de la tribu Astegetheriini y sus características anatómicas lo colocan como probable grupo hermano de la subfamilia Dasypodinae. Este taxón, así como el más basal Dasypodinae conocido, *Anadasypus*, ocurren en el mismo nivel estratigráfico en depósitos paleontológicos de Colombia (Carlini *et al.* 1997). La emergencia temprana de esta subfamilia se estimó durante el Eoceno medio (aproximadamente hace 40 m. a.) y la separación de dos de sus especies, *Dasypus kappleri* y *D. novemcinctus*, en el Mioceno tardío (aproximadamente hace 7 m. a.; Delsuc *et al.* 2004). Los eventos relacionados con la elevación de los Andes en América del Sur y la

actividad volcánica de estas cadenas montañosas han sido relacionados con la diversificación del orden Cingulata (Delsuc *et al.* 2004).

El registro fósil de Cingulata en México comprende desde el Mioceno tardío hasta el Pleistoceno tardío y se conocen actualmente un género de Glyptodontidae y dos géneros de Pampateridae (Shaw & McDonald 1987; McDonald 2002). La familia Dasypodidae solo se registra en México en depósitos del Holoceno. *D. novemcinctus* ha sido encontrado en sitios arqueológicos de 25 diferentes localidades (Arroyo com. pers. 2009), pero no hay ningún registro de la especie en América del Norte ni América Central anterior al Holoceno. Una especie morfológicamente semejante, *D. bellus*, tiene un amplio registro fósil, pero está restringido a Estados Unidos de América (EUA). Se registra desde el Mioceno tardío y el Pleistoceno, a lo largo de una gran extensión geográfica, desde Florida hasta Kansas (Shubert & Graham 2000). Hasta el momento no se ha encontrado registro fósil de esta especie en depósitos de ninguna etapa en México, América Central ni América del Sur.

1.3 El armadillo de nueve bandas *(Dasypus novemcinctus)*

El armadillo de nueve bandas es actualmente la especie más ampliamente distribuida del orden Cingulata. Se registra desde el norte de Argentina hasta el centro-sur de los EUA (Wetzel 1985), ocurriendo en una gran variedad de ambientes, como el bosque húmedo tropical, el bosque seco subtropical y las sabanas, y desde el nivel del mar hasta los 3000 msnm (McBee & Baker 1982).

Es un insectívoro generalista (Redford 1985). Posee una de las tasas metabólicas más bajas entre los mamíferos (McNab 1980) y por consecuencia, tiene poca capacidad de termorregular. Humphrey (1974) y Taulmann & Robbins (1996) sugieren que la velocidad

de colonización de esta especie en EUA ha sido limitada por factores climáticos. Específicamente, indican que el armadillo no consigue sobrevivir en regiones donde el suelo permanece congelado más de nueve días seguidos y áreas donde llueva menos de 380 mm al año, ya que en el primer caso los animales no pueden forrajear, y en el segundo, no se mantienen poblaciones de insectos suficientes para mantener a las poblaciones de armadillos. Sin embargo, Freeman & Genoways (1998) mencionan un registro de un macho adulto en una región de Nebraska, donde hubo 176 días de suelo congelado, indicando que este no sería un factor limitante al avance de la especie. En relación con la dieta, se ha registrado la inclusión de vertebrados en ella (revisado por McDonough & Loughry 2008), demostrando su capacidad de consumir otras presas y no siendo sólo la abundancia de insectos un factor limitante a la ocupación de nuevas áreas. Es probable que los individuos registrados en regiones extremas como las mencionadas, no consigan fundar poblaciones permanentes y dependan de otras fuentes para mantener dichas poblaciones. Por ello se puede considerar a las características climáticas como una barrera a la expansión del área de distribución de esta especie.

El armadillo de nueve bandas presenta poliembrionía monocigótica como una característica reproductiva fija (Galbreath 1985; Prodölh *et al.* 1996), produciendo en promedio cuatro hijos. Prodölh *et al.* (1996) registraron pocos adultos con sus hermanos genéticamente idénticos en la población y los juveniles que aún no se habían dispersado, los encontraron distanciados 456 m en promedio. Las explicaciones evolutivas de la poliembionía no parecen residir en aspectos de selección de parentesco (*Kin selection*; es decir, individuos que ayuden a otros relacionados genéticamente a fin de aumentar su adecuación), sino que pueden estar asociadas a peculiaridades reproductivas de la especie (Prodölh *et al.* 1998). Algunos estudios sobre la variación genética de las poblaciones de

armadillo muestran que esta característica reproductiva no afecta el nivel de diversidad genética, ya que el polimorfismo de proteínas y marcadores mitocondriales encontrado está dentro del intervalo esperado para los Eutheria (Huchon *et al.* 1999; Frutos & Van den Bussche 2002).

Fidelidad de los individuos adultos a su territorio y bajo reclutamiento de los juveniles fue registrada en un estudio de campo de una población de armadillos en Florida (Loughry & McDonough 1998; Loughry & McDonough 2001). Sin embargo, Frutos & Van den Bussche (2002) trabajando en cinco poblaciones de armadillos en Paraguay, encontraron una baja estructuración genética en el Citocromo B de la mitocondria y sugirieron que esto podía corresponder a una alta movilidad de individuos entre las poblaciones. Entonces, los movimientos limitados de los armadillos adultos residentes pueden promover la adaptación local e incrementar la diferenciación genética entre las poblaciones, pero los movimientos a larga distancia de individuos dispersándose puede homogenizarla. Éstos últimos pueden ser los responsables de la rápida expansión de esta especie en EUA, sumado a los cambios en el paisaje generados por el hombre como la apertura de carreteras y las modificaciones de los tipos de vegetación (Humphrey 1974). El primer registro del amadillo de nueve bandas en los EUA fue hecho por Audubon & Bachman (1854) en el extremo sur de Texas y, en sólo 160 años ha ocupado gran parte del centro y del sur este de los Estado Unidos (Taulmann & Robbins 1996).

Las poblaciones de armadillo en América del Sur (Huchon *et al.* 1999; Frutos & Van den Bussche 2002) presentan altos niveles de variación genética en los marcadores neutrales y funcionales, a diferencia de los bajos niveles registrados en las poblaciones de EUA (Ramsey & Grigsby 1985; Moncrief 1988, Huchon *et al.* 1999). Sin embargo, no está claro si esta falta de variación está confinada a los EUA, como resultado de la reciente

fundación de sus poblaciones, o si se extiende a las poblaciones de América Central y México.

A lo largo de su distribución, el armadillo de nueve bandas presenta una amplia variación fenotípica, tanto en su color como en el tamaño corporal. Algunas de las diferencias morfológicas, principalmente las craneales y el tamaño corporal total, han sido usadas para proponer a las subespecies, las cuales presentan una distribución alopátrica (McBee & Baker 1982; Wetzel *et al.* 2007). Estas son: (1) *D. n. aequatorialis* se distribuye en el lado oeste de la cordillera de los Andes de Perú y Colombia. (2) *D. n. novemcinctus* ocupa el otro lado de la cordillera Andina y el resto de Sur America, menos el noreste de Venezuela y norte de Colombia. Allí se distribuye (3) *D. n. fenestratus*, quien se extiende por América Central hasta el sur de Honduras. Desde este límite hasta el centro-sur de EUA se encuentra (4) *D. n. mexicanus*. Finalmente, en el oeste de México, desde el norte de la cuenca del Balsas hasta Morelos se distribuye la subespecie (5) *D. n. davisi*. Además de éstas cinco subespecies, Wetzel *et al.* (2007), pero no McBee & Baker (1982), reconocen a *D. n. mexianae* ocupando el delta del Río Pará, en el Amazonas brasilero (Fig. 2; Loughry & McDonough *in press*).

Sin embargo, la información genética indica que las subespecies definidas morfológicamente no representan unidades evolutivas independientes. Los análisis moleculares realizados en este trabajo, han identificado a las poblaciones del Oeste y del Este de México como altamente divergentes con los marcadores mitocondriales (ver capítulo 1), pero los marcadores nucleares indican que ambos grupos están genéticamente conectados. Además, el análisis de muestras de América del Sur ha identificado la presencia de un grupo divergente en la Guyana Francesa, lo que no concuerda con ninguna de las subespecies descrita hasta ahora.

Figura 2. Distribución geográfica aproximada de las subespecies de *Dasypus novemcinctus*. Modificado de Loughry & McDonough *in press.*

1.4 La Filogeografía

La filogeografía estudia los procesos que explican la distribución geográfica de los linajes genealógicos (Avise 2000) a escala microevolutiva y utiliza como unidad de análisis los linajes monofiléticos (Avise 2004). Se ha considerado una subdisciplina de la biogeografía histórica porque enfatiza los aspectos históricos de la distribución actual de los linajes (Lanteri & Confalonieri 2003; Lomolino *et al.* 2005) y como parte de la genética de poblaciones con un énfasis en la geografía y la filogenia. Sin embargo, aborda mucho más que esto. La definición conceptual de esta disciplina incluye implícitamente los factores que modelan los niveles de diversidad, incluyendo los históricos, los ecológicos y algunos atributos de la especie y además aborda su distribución en el espacio geográfico.

El primer paso en un estudio filogeográfico es la definición de los linajes monofiléticos. Un aspecto novedoso de la filogeografía es el hecho de usar segmentos de ADN provenientes de individuos como la unidad taxonómica operacional en el análisis filogenético (Hickerson *et al.* 2010). Estos segmentos de ADN son llamados haplotipos y son definidos como una secuencia de alelos o genes ligados en una copia simple de un cromosoma o en el ADN mitocondrial (Hedrick 2005). Dada una muestra de haplotipos, la relación entre ellos puede ser trazada hacia atrás en el tiempo (hacia el haplotipo ancestral común) a través de una genealogía de genes y esta genealogía puede reconstruirse con métodos filogenéticos (Hey & Machado 2003). Otra herramienta usada para estudiar las relaciones entre ellos es la red de haplotipos. Ésta es un gráfico de círculos conectados entre sí, donde cada círculo representa un haplotipo y su tamaño corresponde la frecuencia del mismo. En ella es posible tener múltiples conexiones entre los diferentes haplotipos dando un patrón reticulado específico. La posición de los haplotipos dentro de la red puede ser interpretada como ancestral o derivado y las relaciones establecidas entre ellos son útiles para inferir procesos demográficos ocurridos en el pasado. Dado que mantienen la información histórica de los datos (Posada & Crandall 2001), las redes tienen ventaja sobre los árboles bifurcados. El análisis de la estructuración genética en el espacio geográfico y de los niveles de diversidad está basado en los linajes reconocidos a través de estos métodos gráficos.

El segundo paso es evaluar los niveles de variación genética presentes en los linajes y para ello la filogeografía se apoya en métodos que derivan de la teoría clásica de genética de poblaciones (Diniz-Filho *et al.* 2008). Existen pruebas que definen si existe una estructura espacial de la variación (e.g. Fst, Gst, Dest; Wright 1951; Nei 1975; Jost 2008), determinan la tasa de flujo entre las poblaciones (usualmente a partir de derivaciones de

Fst), los patrones de aislamiento por distancia (con Prueba de Mantel; Mantel 1967) y explican la varianza entre y dentro de las poblaciones (e.g. AMOVA; Excoffier *et al.* 1992). Estas pruebas se basan en la distribución actual de la frecuencia de alelos (Crandall *et al.* 1999). Además de estos métodos tradicionales, se han propuesto varias maneras de estimar los parámetros poblacionales, basándose en la teoría de la coalescencia (ver abajo).

El tercer paso es abordar los factores que han moldeado la distribución de la variación genética y su estructuración en el espacio geográfico. Es en este punto que la filogeografía trasciende desde la descripción de los patrones, y pasa a inferir los procesos que los generan. Aquí es donde implícitamente se evalúan procesos o explícitamente se proponen hipótesis, como en la filogeografía estadística (Knowles & Maddison 2002). Para esto existen métodos que exploran la historia de los linajes. Muchos de ellos usan la teoría de la coalescencia que se basa en la estocasticidad de los procesos evolutivos (Rosenberg & Nordborg 2002) considerando que bajo neutralidad, a lo largo de sucesivas generaciones y en forma permanente, surgen nuevos alelos por mutación y otros se pierden por deriva (Nordborg 2001). A diferencia de la genética de poblaciones tradicional que permitía hacer predicciones de las frecuencias alélicas en futuras generaciones, la teoría de la coalescencia utilizando sólo una muestra de alelos, genera genealogías de genes bajo diferentes historias demográficas y así estima parámetros poblacionales (Hickerson *et al.* 2010), como la tasa de migración, el tiempo de divergencia, la tasa de mutación, el tiempo al ancestro común más reciente y además asocian la significancia estadística a las hipótesis sugeridas. Los eventos demográficos pueden inferirse también con análisis basados en la coalescencia. Rogers & Harpending (1992) demostraron cómo la forma de la distribución de las diferencias pareadas (*Mismatch Distribution*) entre secuencias puede usarse para detectar los eventos de expansión o contracción de las poblaciones en el pasado. Además, existen

métodos para evaluar las fluctuaciones en el tamaño efectivo de las poblaciones en el tiempo [(como el análisis Skyline Plot (Strimmer & Pybus 2001)].

Cuando la teoría de la coalescencia se convirtió en la herramienta fundamental de la filogeografía, el uso tradicional de un solo marcador fue insuficiente para obtener estimadores precisos de los parámetros poblacionales y se comenzaron a usar marcadores multilocus (Hickerson *et al.* 2010). Durante la década de los 90, la base de los estudios filogeográficos fue el análisis del ADN mitocondrial (ADNmt). Su herencia materna, su recombinación casi nula y su alta tasa de variación (Avise 2009) hicieron de éste la principal fuente de información para reconstruir la historia de las relaciones de manera lineal. Sin embargo, dada la estocasticidad de los procesos evolutivos, la estimación de parámetros demográficos a partir de un sólo marcador incluía un gran sesgo que pudo ser amortiguado al considerar otras partes del genoma, las cuales si eran neutras, debían reflejar la misma historia. Además, la estructura y variación genética de las poblaciones no fue completamente explicada por los procesos inferidos a partir del ADNmt. Existen factores que moldean en otras partes del genoma, las señales dejadas por la historia en el ADNmt, como las presiones selectivas del ambiente y los atributos de la especie (patrones de dispersión, comportamiento social, sistema reproductivo, etc).

La filogeografía integra conceptos y técnicas de genética de poblaciones, demografía, sistemática filogenética, paleontología y biogeografía (Avise 2000; Knowles 2009). En los últimos años, la información espacial esta siendo incluida en los estudios filogeográficos a través de diversas herramientas (Chan *et al.* 2011). Con este enfoque tan diverso se están abordando preguntas que ligan la historia de los linajes, las presiones ambientales y los atributos de las especie con los patrones actuales de diversidad genética.

En el clásico trabajo de Avise *et al.* (1987), los autores mencionan "*This review will be a success if it stimulates further dialogues in these areas (systematic and population genetics)"*. Sin embargo, se generó más que eso. Esta disciplina permitió agregar un nivel más al conocimiento de la diversidad biológica, el de los linajes evolutivos presentes dentro del área geográfica ocupada por una especie.

1.5 El nicho ecológico

El nicho de una especie es el espacio multidimensional cuyos ejes comprenden los recursos y las condiciones que limitan la adecuación de los organismos (Hutchinson 1957). Los recursos son aquellos sobre los que la población tiene efecto (i.e. interacciones bióticas), mientras que las condiciones se refieren a las características ambientales (i.e. condiciones abióticas). Soberón (2007) menciona que el nicho puede ser dividido en dos clases: Eltoniano y Grinelliano. La primera clase considera a los recursos, es decir, a aquellas variables relacionadas con las interacciones bióticas y en este sentido se semeja al nicho realizado descrito por Hutchinson (1957). La segunda, Grinelliano, se asemeja al nicho fundamental. Esta clase corresponde a las condiciones ambientales que limitan la distribución de una especie, y por lo tanto puede considerarse el nicho abiótico o ecológico (*Grinellian niche*, *sensu* Soberón 2007).

La tendencia de una especie a retener aspectos de su nicho fundamental a lo largo del tiempo evolutivo es llamada conservadurismo de nicho (Peterson *et al.* 1999; Wiens & Graham 2005). Esto concepto ha sido aplicado al estudio de la especiación en linajes relacionados (Peterson *et al.* 1999; Graham *et al.* 2004; Kozak & Wiens 2006; Rissler & Apodaca 2007), y esto se ha hecho usando herramientas que modelan de manera predictiva la distribución de las especies (Anderson *et al.* 2003). Estas herramientas combinan los

datos de presencia de los individuos con variables ambientales y ecológicas (temperatura, precipitación, elevación, geología, etc.) y se obtienen modelos que son proyectados en un mapa, donde se indica la distribución geográfica potencial del nicho de esas especies.

La disponibilidad de información sobre variables ambientales y topográficas en diferentes regiones es cada vez mayor. Entre tanto, datos sobre las interacciones biológicas de las especies son escasos y requieren estudios de campo detallados. Esto ha generado que la mayor parte de los modelos de distribución potencial se basen en variables climáticas, y por ello sean modelos de nicho ecológico (o estrictamente climático). Como se mencionó anteriormente, la proyección geográfica de estos modelos de nicho se ha utilizado para formular hipótesis de especiación (Graham *et al.* 2004; Kozak & Wiens 2006; Rissler & Apodaca 2007). Sin embargo, una distribución alopátrica de ellos no necesariamente es un indicador de diferencias en las afinidades ecológicas de los linajes considerados. En la actualidad se están desarrollado herramientas que permiten evaluar estadísticamente la divergencia o el conservadurismo del nicho climático (Warren *et al.* 2008; McCormack *et al.* 2010) y de esta manera permitir una interpretación más acertada de los patrones observados en las proyecciones geográficas de los modelos.

El armadillo de nueve bandas (*Dasypus novemcinctus*) ocupa una extensa variedad de hábitats a lo largo de su área de distribución, posibilitando la diferenciación genética por separación física o por razones ecológicas. Más de un linaje puede formarse y puede presentar afinidades climáticas propias. Esto afectará los patrones de flujo, los niveles de diversidad y la estructuración genética de la especie a lo largo de su distribución geográfica. La conexión establecida en el Pleistoceno entre América del Norte y del Sur permitió al armadillo expandir su área de distribución hacia el norte, y más de un linaje

pudo participar en este proceso. Dada su amplia distribución y su participación en el intercambio de fauna del Pleistoceno, el armadillo es un bueno modelo para abordar la influencia de factores históricos y ecológicos sobre su estructura filogeográfica. En este estudio evalué si las poblaciones de armadillos mexicanos son derivadas de uno o varios linajes y examiné si estos linajes presentan expansión demográfica, como es lo esperado para áreas recientemente colonizadas. Además, determiné el efecto de las afinidades climáticas de los linajes sobre los patrones de flujo génico.

1.6 Objetivos

Objetivo General:

Estimar los niveles y la distribución de la variación genética del armadillo de nueve bandas (*D. novemcinctus*) en México y determinar el efecto de factores históricos, ecológicos y características de la especie sobre los patrones filogeográficos.

Objetivos específicos

1. Determinar cuántos linajes monofiléticos del armadillo de nueve bandas ocurren en México.
2. Hacer inferencias sobre el origen y la historia demográfica de los linajes detectados.
3. Estimar la diversidad genética, la estructuración y las tasas de flujo génico entre los linajes.
4. Evaluar las afinidades ecológicas de los linajes y su influencia en la estructura genética a lo largo del paisaje.

1.7 Estructura de la tesis

En este estudio complemento el escenario de la diversidad genética del armadillo de nueve bandas en el norte de su distribución. En el primer capítulo realizo un análisis detallado de los factores históricos que han contribuido a los patrones de estructura y diversidad genética de esta especie en México. Además, a través del uso de marcadores nucleares y mitocondriales, infiero el comportamiento filopátrico y de dispersión de la especie y abordo la demografía histórica y reciente de dichos linajes. De esta manera cubro los tres primeros objetivos específicos. El resultado de este capítulo es el artículo "**Phylogeography of a widely distributed species in a colonized area: the nine-banded armadillo in Mexico"**

Ya abordado el efecto de la historia y de algunos atributos de la especie sobre los patrones filogeográficos que exhiben las poblaciones de armadillo en México, en el segundo capítulo evalúo si existen diferencias en las afinidades climáticas de los linajes detectados. Esto con el fin de determinar el efecto de algunos factores ecológicos en los patrones de estructura y diversidad genética. Cubro así el cuarto objetivo específico y el resultado de este capítulo es el artículo "**Genetic admixture in multidimensional niche space: asymmetrical niche similarity promotes gene flow in armadillos (*Dasypus novemcinctus*)".**

En la discusión general, hago una integración de los resultados generales. Abordo también otros elementos considerados dentro de los factores históricos, ecológicos y atributos de la especie que pueden estar influenciando los patrones de estructura y diversidad genética y finalmente propongo perspectivas que surgieron a partir de los resultados obtenidos.

Phylogeography of a widely distributed species in a colonized area: Nine-banded armadillo in Mexico

M. C. Arteaga,[a, 1] D. Piñero,[b] L. E. Eguiarte,[b] J. Gasca, [b] R. A. Medellín[a]

[a]Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México, Distrito Federal 04510, México

[b]Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México, Distrito Federal 04510, México

Keywords: Great American Biotic Interchange; demography; *Dasypus novemcinctus*; gene flow; microsatellites; mitochondrial control region; genetic structure.

[1] Maria Clara Arteaga: mcarteaga@ecologia.unam.mx;

Telephone number: (5255) 56229042;

Fax number: (5255) 56228995

Running title: Phylogeography of the nine-banded armadillo

ABSTRACT

The phylogeographic pattern of a widely distributed species in a recently colonized area is influenced by the colonizing lineages, life history traits, and biotic and abiotic factors. The connection established during the Pleistocene between North and South America allowed the nine-banded armadillo (*Dasypus novemcinctus*) to expand its distributional range northward. High levels of genetic diversity have been recorded in South America, whereas low levels have been detected in populations in the United States, perhaps due to a founder effect during colonization. By sampling animals from Mexico and a few other areas, we test the hypothesis that armadillos in North America were derived from a single founding lineage, and assess whether this newly colonized region shows demographic signatures of expansion. We sequenced mitochondrial DNA of 157 and genotyped microsatellites of 116 individuals. Our results showed two divergent mitochondrial lineages with high genetic variation when compared to US populations, suggesting that this species has a higher effective population size in Mexico. Outgroup samples from Central and South America indicate that both lineages differentiated prior to their arrival in Mexico. While lineages showed a historical demographic expansion, due probably to the large area they colonized, we also detected evidence of a recent decline, which could possibly be related to human-induced causes. Clear genetic structure was observed with mtDNA, whereas microsatellites showed low levels of genetic differentiation. This contrasting pattern can be caused by male-biased dispersal. We conclude that North American populations of *D. novemcinctus* are derived from two founding lineages. The phylogeographic structure of nine-banded armadillos in Mexico is strongly influenced by these two lineages and male-biased dispersal.

INTRODUCTION

Patterns of genetic structure and variation of widely distributed species in areas of recent colonization are influenced by many factors, including the number and genetic composition of colonizing lineages [1,2], disturbances such as climate change [3], exploitation and human-mediated habitat perturbations [4,5], and by life-history traits peculiar to each species [6]. During natural colonizations, species gradually expand their geographic range by migrating to adjacent regions [7], facilitating the spread of genetic lineages to new regions [8,9].

At the end of the Pliocene and the beginning of the Pleistocene, the connection established between North and South America through the Isthmus of Panama allowed many species to expand their range [10], in a phenomenon known as the Great American Biotic Interchange (GABI [11,12]). The GABI is recognized as a major biogeographic event that influenced patterns of genetic diversity in many taxa [11, 13-15]. However, the GABI occurred several times during the Pliocene and Pleistocene [12,16]. Consequently, for many species, it remains unclear how many lineages contributed to colonization [17-19].

Geological dynamics, coupled with climate change, and more recently human-related impacts, radically modified the landscape in both North and South America since the Tertiary. Molecular markers have greatly facilitated the timing of divergence events [20] and demographic events [6] so that they can be attributed to one or several of these major Earth history events. Also, with these markers, it is possible to infer the role of life-history traits such as ecology affinities and dispersal patterns in contemporary genetic

distribution of species [21]. For example, in mammals, the combination of sex-linked markers showing genetic structure and autosomal markers lacking structure has been interpreted as evidence that females tend to be more philopatric, while males are the dispersing gender [22,23].

Cingulata was one of the most representative mammal orders that migrated into North America during the GABI. This order is currently composed of 21 species of armadillos, most of which are confined to various parts of South America [24]. The nine-banded armadillo (*Dasypus novemcinctus*) has the broadest distribution. Currently, it ranges from northern Argentina to the southern US [25], and occurring across a wide variety of environmental conditions, such as dry, moist, and cloud forests, savannas, and from sea level up to 3000 m [26]. Only a few American mammal species have such a broad range, including the puma (*Puma concolor*) and possibly the Brazilian free-tailed bat (*Tadarida brasiliensis*). Morphological [27] and molecular [28] evidence supports the early emergence of the subfamily Dasypodinae (ca. 30-33 million years ago [MYA]), which includes the genus *Dasypus*. Within this genus, Molecular data suggest that *D. novemcinctus* originated about 7 MYA [29]. Paleontological data indicate that this probably occurred in northern South America [30]. The earliest remains of *D. novemcinctus* were found in Uruguay and date back to the late Pleistocene (i.e., the Sopas Formation; [31]). The oldest record of *Dasypus* in Mexico corresponds to the Holocene (*ca*. 8000 years ago; [32]). *D. novemcinctus* was first recorded in the lower Rio Grande valley of Texas in 1849 [33], but rapidly colonized much of the southern US subsequently. Due to the wide distribution of *D. novemcinctus* and its clear South American origin, this species is a good model to examine genetic structure in newly colonized areas (e.g. North America during

the GABI), because many genetic lineages could exist across the original range, and one or many of them might have participated in the colonization of North America. Consistent with expectations of a single founding lineage, previous studies showed that South American populations of nine-banded armadillos have higher levels of mitochondrial and nuclear genetic diversity [34,35] than their US counterparts [34,36]. This has been interpreted as the result of a founder effect during the colonization of North America [34]. However, this conclusion is based solely on data from populations in the US. It remains unknown whether similar patterns occur farther south, for example, in Mexico.

We used nuclear and mitochondrial markers to screen a large number of nine-banded armadillos from Mexico, and a few other areas, to further test the hypothesis that armadillos in North America are derived from a single founding lineage. Additionally, we assessed whether the newly colonized region of North America shows demographic signatures of expansion (as might be expected for a recent colonization), or whether colonizing populations have had time to differentiate in the topographically diverse setting of MesoAmerica. Finally, because this species occurs across human-modified habitats and is commonly used by rural populations as a food source [37,38], we also sought to examine whether populations show genetic signatures related to anthropogenic pressures over more recent time scales.

RESULTS

Phylogeographic structure and genetic diversity in mtDNA

Sixty-nine polymorphic sites were identified within 408 bp of the control region, resulting in 77 unique haplotypes in the 138 Mexican samples (GenBank [accession number], see Table S1, supporting information). Nucleotide diversity (π) and haplotype diversity (h) were high, 0.030 ± 0.007 and 0.979 ± 0.005, respectively. Thirteen haplotypes were identified in 19 samples from other countries. Two of these (from Colombia and the US) were shared with the Mexican samples (Table S1).

The phylogenetic tree (Fig. 1) and haplotype network (Fig. 2) separated the haplotypes from Mexico into two major monophyletic lineages corresponding to samples from the central-western (hereafter called West) part of the country, and the southeast (hereafter called East). In the phylogenetic tree, both lineages were supported by high posterior probabilities. One haplotye from Costa Rica and five from Colombia were nested within the West lineage, while one haplotype from Guatemala, one from Costa Rica, two from Nicaragua, two from the US, and nine from Colombia nested within the East lineage.

In Mexico, the two lineages exhibited an almost allopatric distribution, apparently separated by major mountain chains (Fig. 3). Four haplotypes from the East lineage were recorded in the geographic area occupied by the West lineage, and one haplotype from West was recorded in the area occupied by East lineage. Mitochondrial genetic diversity within each lineage was high (Table 1), and the average *p*-distance between them was 4.90%. The AMOVA showed that most of the genetic variance was found between lineages (71%) whereas within-lineage variance was relatively low (29%; Table 2a).

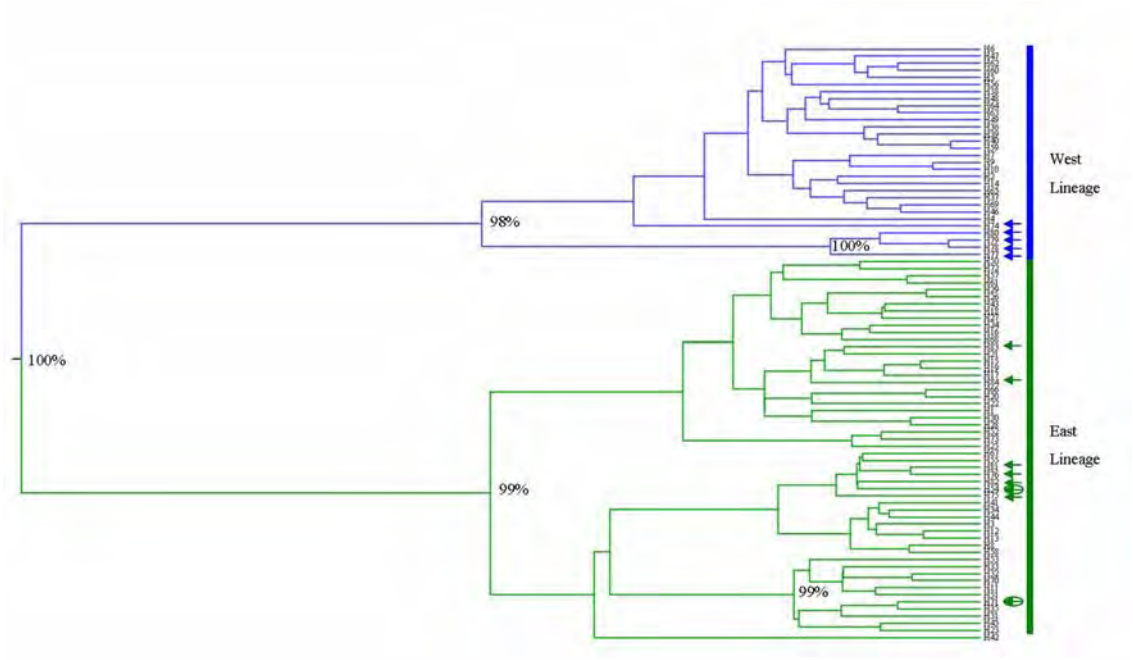

Figure 1. Bayesian phylogenetic tree of *D. novemcinctus* haplotypes based on 408 bp of the mitochondrial control region, showing the two major lineages in Mexico: "West" (blue) and "East" (green). Labels are haplotype identification numbers (see Table S1, supporting information). Numbers above the branches indicate that the Bayesian posterior probabilities for the key node are ≥80%. Arrows indicate haplotypes from Guatemala, Nicaragua, Costa Rica, Colombia and the USA. Arrows within circles indicate haplotypes shared between Mexico and other countries.

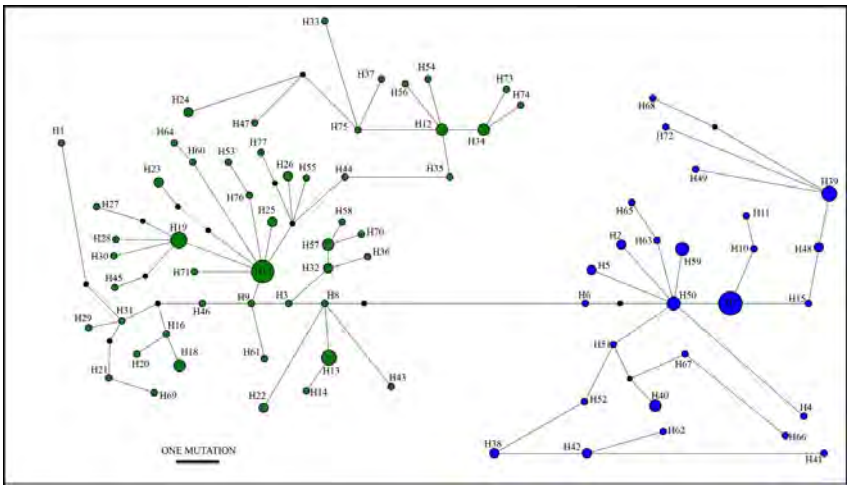

Figure 2. Median-joining network based on 408 bp of the mitochondrial control region from 138 Mexican samples of *D. novemcinctus*, showing the two major lineages: "West" (blue circles) and "East" (green circles). Circle size is proportional to haplotype frequencies; line length represents the number of mutations between haplotypes. Black solid circles represent unsampled or extinct haplotypes.

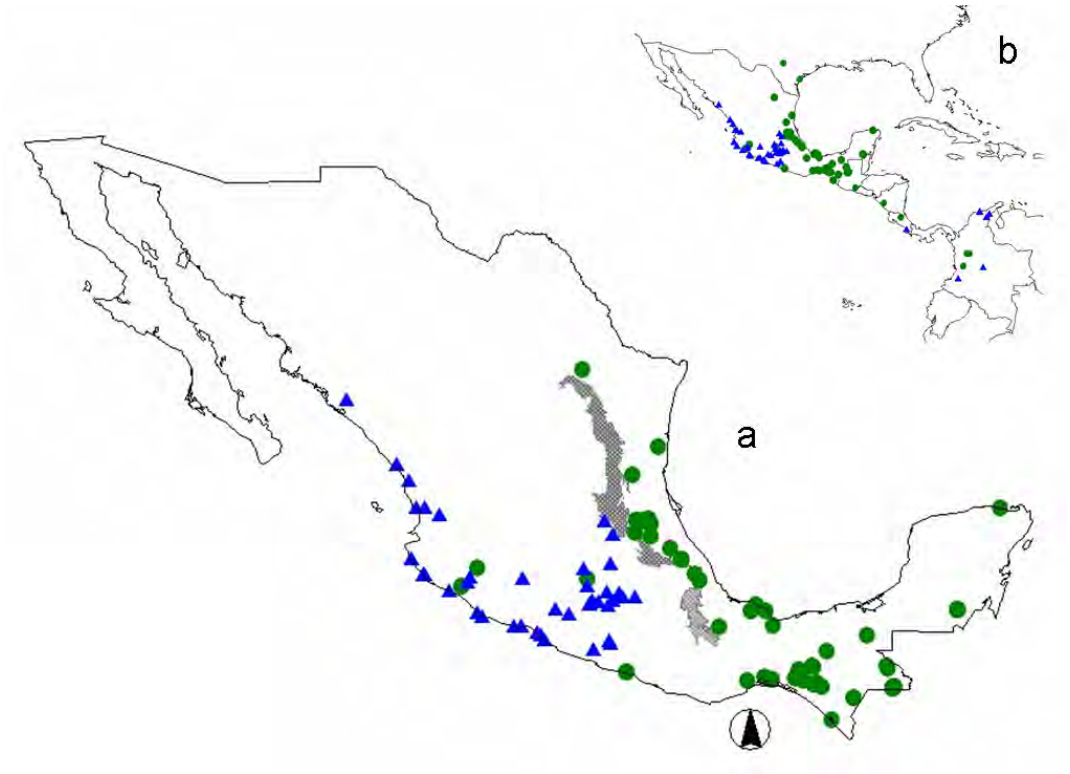

Figure 3. Spatial location of 138 Mexican samples (a) and 19 samples from other countries (b) from *D. novemcinctus* analyzed for the mitochondrial control region (408 bp). Green triangles: West lineage samples. Blue dots: East lineage samples. The geographic position of the Sierra Madre Oriental and the Sierra of Oaxaca in Mexico are suggested as boundaries between these lineages in Mexico.

Table1. mtDNA genetic variation estimates and Fu´s *F*s test in the two major lineages. Based on 408 bp of the mitochondrial control region from 138 Mexican samples of *D. novemcinctus*.

Mitochondrial control region	**West lineage**	**East lineage**
Sample size (n)	54	84
Nucleotide diversity (π)	0.011	0.015
Haplotypic diversity (h)	0.932	0.971
Number of haplotypes	26	51
Mean number of pairwise difference (K)	4.427	6.324
Fu´s Fs test (P value)	-12.96 (0.0001)	-25.14 (0.0001)

Table 2. Hierarchical analysis of molecular variance (AMOVA) on mtDNA (a) and nDNA (b). Based on (a) 408 bp of the mitochondrial control region and (b) five nuclear microsatellite loci. Percentage of variation associated to each level and fixation index (*P* values are given in parenthesis).

(a) Source of Variation	**Mitochondrial control region**
Major lineages (mtDNA)	
Among lineages (West *vs* East)	71.01 (F_{ST} = 0.71, P < 0.0001)
Within lineages	28.99

(b) Source of Variation	**Nuclear microsatellite loci**
Major lineages (mtDNA)	
Among lineages (West *vs* East)	2.74 (F_{ST} = 0.027, P < 0.0001)
Among individuals within lineages	24.17 (F_{IS} = 0.248, P < 0.0001)
Within individuals	73.09 (F_{IT} = 0.269, P < 0.0001)
Clusters defined by Geneland (nDNA)	
Among clusters (K=10)	12.67 (F_{ST} = 0.153, P < 0.0001)
Among individual within clusters	13.36 (F_{IS} = 0.126, P < 0.0001)
Within individual	73.97 (F_{IT} = 0.260, P < 0.0001)

Phylogeographic structure, genetic diversity and gene flow using nuclear microsatellites

Sixty three alleles were found for the five nDNA microsatellites (12, 20, 9, 10, and 12 for *Dnov1*, *Dnov6*, *Dnov7*, *Dnov16*, and *Dnov24* respectively). Overall allelic richness was 11.7. No evidence of scoring errors due to stuttering or large allele dropout was found [39]. Null alleles, however, were detected in two loci (*Dnov16*, *Dnov24*), and significant linkage disequilibrium (P < 0.05) was found in 53% of all possible combinations.

The posterior distribution of the estimated number of clusters in Geneland revealed 10 distinct clusters (Fig. 4a). Individuals assigned to the same genetic cluster were not

always spatially contiguous; seven clusters contained members that were isolated by large intervening areas (Fig. 4b). A significant difference in the proportion of individuals from each mitochondrial lineage in the 10 Geneland clusters was also found (χ^2 = 64.335, P < 0.0001; Table S2).

Overall, the observed heterozygosity values were lower than expected, and high allelic richness was observed in the two lineages (Table 3). The inbreeding coefficient was positive and significant for each of the lineages, and for the total sample (Table 3). The presence of null alleles within the sample did not solely generate positive inbreeding coefficients because F_{IS} estimates in loci that did not show null alleles were also positive (*Dnov1*: 0.006; *Dnov6*: 0.100; *Dnov7*: 0.232). The F_{ST} between lineages was small and different from zero, even when correcting for bias (with ENA correction: F_{ST} = 0.020, 95% confidence intervals: 0.013–0.029; without correction: F_{ST} = 0.027, 95% confidence intervals: 0.014–0.043). Global R_{ST} between lineages was 0.066 and D_{est} was 0.100. For the two different division scenarios (two mitochondrial lineages and the clusters defined by Geneland), AMOVA revealed that most of the genetic variance in the nuclear microsatellites was explained by differences within individuals and among individuals within lineages or clusters, whereas the difference between mitochondrial lineages and among nuclear clusters was lower, but still significant (Table 2b).

High rates of nuclear gene flow between the two mtDNA lineages were found. The estimated number of immigrants from East to West was 34.234 (CI 95% = 5.395–59.341) individuals per generation, and from West to East was 20.01 (CI 95% = 1.10–39.560) individuals per generation.

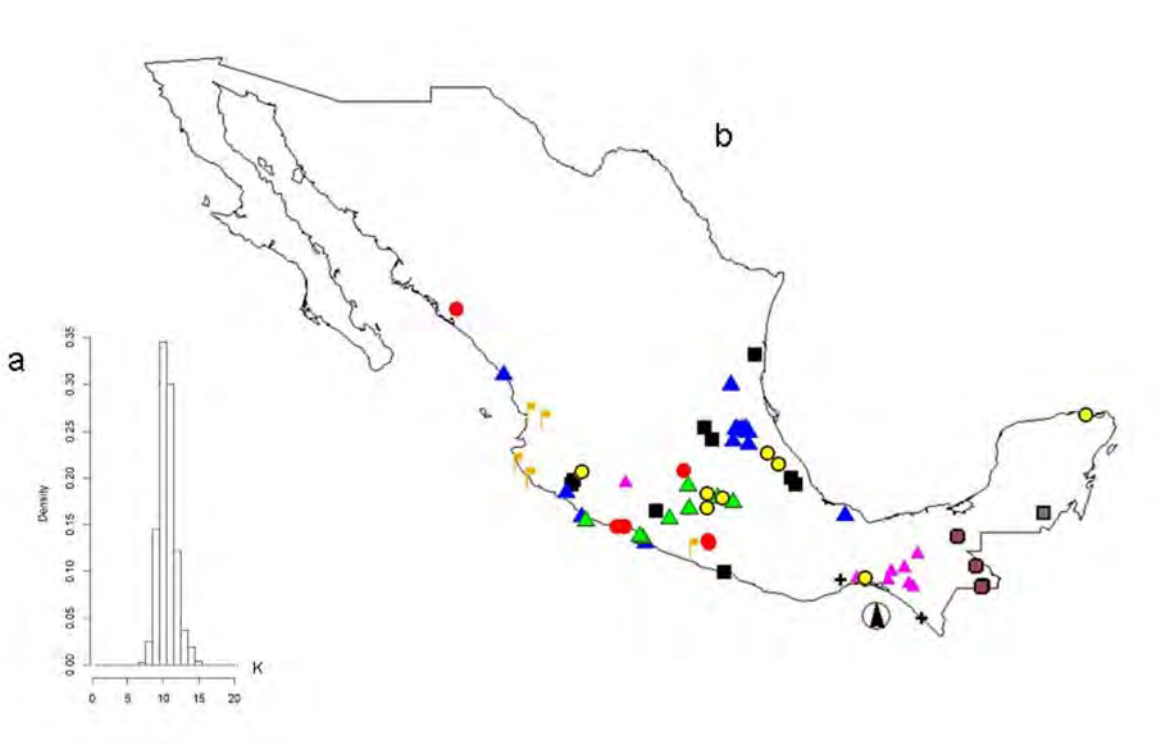

Figure 4. Data from five nuclear microsatellite loci showing the posterior distribution of the estimated number of populations (50 000 000 MCMC in Geneland). Ten genetically distinct populations were found (a). Map shows geographic distribution of each cluster (b: in different colors and marks).

Table 3. nDNA genetic variation estimates in the two major lineages based on five autosomal microsatellite loci from 116 Mexican samples of *D. novemcinctus*.

Nuclear microsatellites	**West lineage**	**East lineage**	**Total**
Sample size	42	74	116
Allelic richness	10.16	11.54	12.6
Inbreeding coefficient	0.314**	0.264**	0.249**
Mean observed heterozygosity	0.551**	0.619**	0.617**
Mean expected heterozygocity	0.8	0.84	0.821

**Significance level, $p < 0.005$

Demography

The number of nucleotide substitutions per site (*d*) in the mtDNA control region was 0.264 (*tv* = 33, *R* = 1.86, *m* = 357 pb). Based on the average divergence time calculated by Delsuc et al. [29], the rate of nucleotide substitutions per site per lineage per year (λ) was 1.90×10^{-8} (interval $2.6 \times 10^{-8} - 2.4 \times 10^{-8}$). This value is similar to the average estimate (1.94×10^{-8}) obtained by Pesole et al. [40] for the same domain (ETAS: Extended Termination Associated Sequences, corresponding to the 3' end of the D-loop region; [41]) in other mammal species. Considering a generation time of four years, the rate of nucleotide substitutions per site per generation (μ) was 7.6×10^{-8}, and, for the 408 bp used, the nucleotide substitutions per haplotype per generation (u) was 3.1×10^{-5}. Finally, for the microsatellites, the mutation rate was 2.7×10^{-4} (Table 4), and it was similar to that recorded in other mammals [42-44].

With mitochondrial data, the two lineages showed a demographic expansion, which accelerated approximately 500 000 years ago (Fig. 5), according to the BSPs. Fu's *F*s values were extremely negative and significantly different from zero (Table 1), further supporting a demographic expansion for both lineages. However, the nuclear data analyzed with MSVAR indicated a more recent population decline (Table 4). According to these results, the East lineage has decreased about fifteen-fold in the last 900 years. Meanwhile, the West lineage showed a five-fold reduction of its ancestral population size, with this decline occurring in the last 5000 years.

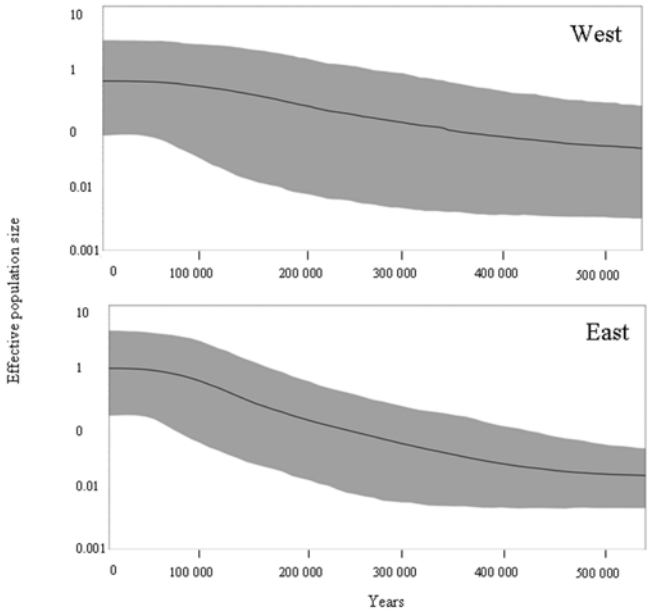

Figure 5. Bayesian skyline plots showing the historical demographic trends for the two main mitochondrial lineages detected. Along the *y*-axis the estimated population sizes are expressed in units of $Ne\tau$, the product of the effective population size per generation time. Solid lines are median estimates, whereas shaded areas represent confidence intervals.

Table 4. Demographic parameters and the corresponding 95% confidence intervals estimated by the MSVAR procedure (Beaumont 1999, 2004; Storz & Beaumont 2002), based on five autosomal microsatellite loci. These estimates were based on Hierarchical Bayesian MCMC simulations using the exponential assumption of population change.

	Mean	**Median**	**Lower 95% HPD**	**Upper 95% HPD**
West lineage				
Current *Ne* (N_0)	3365	11324	177	310455
Historical *Ne* (N_1)	17660	18749.945	1321	233345
Mutation rate (μ)	2.7×10^{-4}	2.7×10^{-4}	8.9×10^{-5}	8.1×10^{-4}
t_a (time in years)	4932	5584.7	24	428548
East lineage				
Current *Ne* (N_0)	2636	3176	49	130016
Historical *Ne* (N_1)	38815	39445	6854	245470
Mutation rate (μ)	2.6×10^{-4}	2.6×10^{-4}	8.4×10^{-5}	7.6×10^{-4}
t_a (time in years)	874	966	8	80909

DISCUSSION

Phylogeographic structure and genetic diversity using mtDNA

The genetic structure of a species in a newly colonized area is strongly influenced by the different lineages that arrived during the increase in the distributional range. Several introductions from multiple founding sources have been detected in a wide range of invasive animals, plants, and fungi [45]. However, information is scarce for species dispersing naturally into new areas. Our study shows that the phylogeographic structure of the nine-banded armadillo in Mexico corresponds to two divergent mitochondrial lineages that are reciprocally monophyletic. The presence of haplotypes from both lineages in Central America and Colombia reveals a wider distribution of these genetic groups. Given the clear South American origin of the nine-banded armadillo [30, 31], such evidence implies that these lineages differentiated prior to their arrival in Mexico. Colonization could have occurred independently or, alternatively, the lineages could have arrived as a single, mixed group of individuals. Multiple colonizations seem more plausible than a single colonization combined with subsequent stochastic sorting and fixation of alleles. As a final point, the current allopatric distribution in Mexico suggests independent northward colonization of each lineage. However, broader sampling is needed to test hypotheses about migration patterns, and to estimate divergence time between the two lineages.

Founder events are a hallmark of colonization and are expected to result in reduced genetic variation of founding populations [7]. However, the nucleotide and haplotype diversity of the mtDNA control region of Mexican armadillos was high. It was also much greater than that recorded in US populations [34]. According to our phylogeny, US populations appear to be derived from the East lineage of Mexico. The low diversity levels reported for populations in the US could be explained by a recent founder event in which

there has been little time for mutation to generate new diversity. In contrast, the lineages in Mexico probably had either an initially large population size, or they recovered from their own founder event a long time ago.

Phylogeographic structure and genetic diversity using nuclear microsatellites

A weak genetic structure in Mexican armadillos was revealed by microsatellites. Recent migration between the two mitochondrial lineages was inferred from the few differences in allelic frequencies between them (F_{ST}). Having a long time to accumulate differences between lineages can generate many private alleles (D_{est}), and a large variance in allele size in each lineage (R_{ST}), just as we observed. The significant relationship estimated between the assignments of individuals to mitochondrial and nuclear clusters suggests that the patterns of genetic structure we uncovered are an accurate record of the species history and not a reflection of stochastic effects limited to one gene, although some marker-specific effects are probable (see below).

The high nuclear variation found in Mexican armadillos supports the idea that there is a large effective population size in Mexico and/or that the lineages have already recovered from a founder effect. Each major lineage occurs across a large geographic area in Mexico, and shows substructure within it. The mixing of individuals from populations with different allelic frequencies can lead to a Wahlund effect, generating Hardy-Weinberg disequilibrium, and a positive inbreeding coefficient [46], as was found in both lineages. This same pattern has been recorded in other mammals (*Gorilla gorilla diehli*, [47]; *Canis aureus*, [48]). The positive inbreeding coefficients also can be generated by endogamy [46]. This possibility should be explored in future studies of these populations. Regarding the linkage disequilibrium detected, in an analysis of seven populations of armadillos in the

US, Loughry et al. [49] observed no evidence of genotypic disequilibrium in the five autosomal microsatellites used in this study. Thus, it seems plausible that disequilibrium was due to demographic changes unique to Mexico.

Contrasting patterns of structuring

Different patterns of genetic structure among different markers can be caused by numerous factors, including those specific to the marker (mutation rate, ploidy, etc.) and those related to the life-history of the organism. mtDNA, because it is matrilinearly inherited, is expected to show different patterns than nuclear genes if females and males have different dispersal patterns or rates. In our study, we found less evidence for genetic structure in microsatellites compared to mtDNA. Although genetic structure between the two types of markers was correlated, there was evidence for more gene flow in nuclear microsatellites. There are at least three non-exclusive explanations for the discrepancy between nuclear and mitochondrial patterns. First, the larger effective population size of nuclear DNA (nDNA) compared to mtDNA could lead to less gene fixation through drift, and therefore less divergence and genetic structure in nDNA compared to mtDNA [50]. While this could partially explain the lack of clear structure in microsatellites, the coalescence-based program MIGRATE (which assesses lineage sorting issues versus migration) indicated high levels of gene flow, suggesting that differences in effective population size alone was not the only explanation. Second, a slower mutation rate in microsatellites could lead to less genetic structure in this marker; however, our data support the opposite prediction, that microsatellite mutation rates are higher than those of mtDNA. Finally, in our opinion, the most likely explanation is the differential mode of inheritance of the markers, combined with sex-biased dispersal. Because mtDNA is inherited through the

female, our results suggest philopatric females and male-biased dispersal. Male-biased dispersal could increase the probability of finding reproductively active females and of mating several times in this mildly polygynous animal [51]. However, a mark-recapture study [52] suggested that, in armadillos, juvenile males were actually more likely to remain in their natal populations than were females, although differences in dispersal compared to philopatry were not specifically tested. Perhaps futures studies using markers associated with the Y chromosome will provide additional insight into dispersal patterns. Finally, the finding that a handful of haplotypes of a lineage were observed in the geographic area occupied by the other lineage could be attributed to rare, long-distance female dispersal events.

The mountain chains of central Mexico have clearly played an important role in the genetic structuring of armadillos, consistent with phylogeographic studies of other mammals [53, 54] and birds [55]. Mammalian studies, however, have only used mitochondrial markers, making it difficult to assess to what degree gene flow in nuclear genes might occur. As shown by our study, an assessment that also includes nuclear markers is necessary to fully understand whether the observed pattern is a consequence of stochastic factors or life history traits. Our data suggest that the Sierra Madre Oriental and the Sierra Madre de Oaxaca affected the genetic structure of armadillos.

Demography

Population dynamics during the increase of a species' range involves a change in effective population size. When a species arrives in a new area, it usually goes through demographic expansion once individuals have overcome potentially stressful biotic and abiotic environmental conditions [56]. But, in recently colonized areas, factors such as

climate change, landscape structure, exploitation and human-mediated habitat perturbations can also affect the genetic diversity of arriving lineages. The high genetic diversity in the two Mexican lineages of armadillos, despite the demographic changes detected in them, suggests the presence of a moderately large ancestral population size. In the last 500 000 years, both lineages underwent a constant expansion, supporting our prediction that this species colonized North America from South America. The nine-banded armadillo is an ecologically tolerant species that occurs in a variety of environmental conditions, which suggests that climate cycling in the Pleistocene did not strongly influence its demographic history. However, despite the ancient expansion, microsatellites provided a signal of demographic decline in both lineages during the Holocene. The decline causes are unknown, but they could be related to habitat alteration and human hunting [37,38]. The interplay between habitat reduction and overharvesting has caused significant population declines and extinction in several species [4,5,57,58]. Paleolimnological evidence suggests widespread deforestation by the ancient Maya and soil erosion periods that transformed original habitats [59]. In addition, zooarcheology studies document hunting of species such as the nine-banded armadillo, deer and tapir [60].

In conclusion, our results demonstrate that at least two different lineages of *D. novemcinctus* arrived in Mexico from the south and colonized disjunct parts of North America. We suggest that females are philopatric, while males maintain a connection among populations through individual dispersal. Further studies that include samples from South American regions would be useful to complete the continent-wide phylogeography of armadillos. This could reveal important information about the time and place of their evolutionary origin. Such sampling might also help to determine the time when armadillos crossed the Isthmus of Panama [18,19], and when they arrived in Mexico. The Great

American Biotic Interchange influenced community and genetic structure of many species in the Americas through extended bouts of migration in both directions [11,12,16]. This study clearly documents the consequences of the GABI in influencing genetic patterns in the nine-banded armadillo.

MATERIALS AND METHODS

Ethics statement

All samples were collected in accordance to the IACUC guidelines of the American Society of Mammalogists [66]. All fresh samples were collected in accordance to Mexico's wildlife legislation under the Wildlife Department permit FAUT-0001.

Sample collection

We collected samples from 157 *D. novemcinctus* specimens at 110 localities, including 138 from Mexico (92 localities in 18 states), 12 from Colombia, 2 from Costa Rica, 1 from Guatemala, 2 from Nicaragua and 2 from the USA. Among them, 86 samples were museum specimens, and 71 were collected in the field from wild populations (see Table S3, supporting information). Samples were maintained in PBS solution at 1% (8 g of NaCl, 0.2 g of KCl, 1.44 g of NaHPO, 0.24 g of KH_2PO_4, pH 7.4), for 24-72 h at 56°C. Total genomic DNA was extracted from each sample using a DNeasy Kit (Qiagen®), and stored at 4°C.

PCR amplification and sequencing of the mtDNA control region

A segment of the mitochondrial control region (408 bp) from 157 samples was amplified via polymerase chain reaction (PCR) using primers D2 (5'–

ATTTYGGCGCTATGTAATTCG–3'; F. Delsuc, Lab. Paleontologie, Phylogenie et Paleobiologie, Université Montpellier II) and N4 (5´–GGCATAAGTCCATCGAGATGTC–3´). 2.5 U of *Taq* polymerase was added per 25 µl of reaction volume. The final concentration was 1X buffer, 0.4 µM of each primer, 0.15 mM of dNTP and 2 mM of $MgCl_2$. The thermal profile for amplification consisted of an initial denaturation cycle at 95°C for 3 min, followed by 30 cycles at 94°C for 30 s, 62°C for 45 s and 72°C for 120 s, and a final extension at 72°C for 10 min. All amplifications were performed in a Perkin–Elmer GeneAmp PCR system 9600 (Applied Biosystems, Foster City, CA).

Each product was examined in a 1.5% agarose gel, stained with ethidium bromide and sequenced in both directions by the Sanger method (FinchLab, University of Washington, Seattle) using forward and reverse primers described above. MtDNA sequences were aligned using ClustalW [62] available in BIOEDIT 7.0.5 [63]. The Akaike Information Criteria in JModeltest 0.1.1 [64] was used to determine that the control region of *D. novemcinctus* best fits a HKY + I + G evolution model.

Microsatellite genotyping

Five nuclear (nDNA) autosomal microsatellite loci previously reported for this species (*Dnov1, Dnov6, Dnov7, Dnov16, Dnov24;* [65]) were analyzed in the 116 individuals from Mexico. Due to DNA degradation in some museum samples, not all individuals clearly amplified for all microsatellite loci. This explains the difference in the number of samples for mitochondrial mtDNA versus microsatellites.

Individuals were genotyped in a 10-µl reaction containing 1X of buffer, 0.3 µM of each primer, 0.15 mM of dNTP, 1–2 mM of $MgCl_2$ and 1.5 U of *Taq* DNA polymerase. The thermal profile for amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 15 s at 94°C, 30 s at 56-58°C and 1 s at 72°C, with a final extension of 7 min at 72°C. All reaction products were run on an ABI PRISM genetic analyzer with a four-capillary system (Applied Biosystems, Foster City, CA) and sized with an internal lane standard using GENEMAPPER 4.0. The presence of null alleles was determined using MICRO-CHECKER [39]. These alleles may affect estimations of population differentiation, by reducing genetic diversity within populations [66].

Phylogeographic structure and genetic diversity in mtDNA

A phylogenetic tree was inferred from unique mtDNA haplotypes using Bayesian analysis implemented in BEAST 1.5.4 [67]. Because *D. novemcinctus* migrated from South to North America [11], haplotypes from Colombia, Central America, and the USA were added in the tree to examine whether the structure detected in Mexico was exclusive to this region or whether is also present in other places (samples from countries other than Mexico were considered only for this analysis). We used an uncorrelated lognormal distribution to describe the variation in the molecular clock rate, as recommended by Drummond et al. [68]. This implies that there is no *a priori* correlation between the molecular clock rate of a lineage and that of its ancestor. Expansion growth was assumed, because other analyses of our data suggested this pattern (see Results). We used a UPGMA tree as the starting tree. The other priors were set at default, and the program was repeatedly run to optimize the scale factors of the *a priori* function. After optimization, ten million generations were run,

with trees sampled every 1000 generations. The first 1000 trees were discarded as burn-in. Convergence on the parameter estimates was checked using Tracer version 1.5 [69].

For more closely related alleles, genealogical relationships among Mexican haplotypes were estimated using the median-joining algorithm of NETWORK 4.516 (http://www.fluxus-engineering.com). The median-joining method uses a maximum parsimony approach to search for the shortest and least complex phylogenetic trees for a given dataset [70]. When internal node haplotypes are not sampled, the median-joining method provides the best estimate of the true genealogy [71].

The distribution of mitochondrial variation was analyzed considering the major mtDNA lineages identified. An AMOVA [72] was performed with ARLEQUIN 3.11 [73] by partitioning the total sum of the squares into components representing variation among the major mitochondrial lineages and within them. The significance of F_{ST} was evaluated by comparing the observed value with the distribution of the values obtained from 1000 random permutations between-lineages. Finally, estimates of nucleotide diversity (π), haplotypic diversity (h), number of haplotypes and mean number of pairwise differences (K) were calculated for all samples and for each one of the major mitochondrial lineages identified with DnaSP 5.0 [74].

Phylogeographic structure, genetic diversity and gene flow in nuclear microsatellites

To explore the phylogeographic structure revealed by the five microsatellites, we used the software Geneland 3.2.2 [75,76]. This Bayesian approach groups individuals into clusters by minimizing Hardy-Weinberg disequilibrium and incorporating the spatial information concerning the origin of the samples [77]. We tested values of K ranged from 1

to 20 with five replicates each of 750 000 MCMC iterations (with thinning = 750 and a burn-in of 1000 for each). We set the model to co-dominant data, correlated allele frequencies, a spatial Dirichlet model, a maximum rate of Poisson process of 100, and a maximum number of nuclei of 300. The number of clusters (*K)* was inferred from the modal value of *K* for these five runs, using the highest posterior probability. The process follows Coulon et al. [77], who suggested inferring *K* in the first run and then running the algorithm again with the *K* fixed at the previously inferred value in order to estimate other parameters. Thus, after *K* was defined by the mode of the posterior distribution of the MCMC chains, this was fixed and we then ran 500 000 MCMC iterations, 100 times, with settings equal to the previous runs. The mean logarithm of posterior probability for each of the 100 runs was calculated, and the 10 runs with highest values were selected. The geographical distribution of the subpopulations was reconstructed from the plot of posterior probabilities of each individual and their assignment to one or more of the genetic clusters. Independence between mitochondrial and nuclear phylogeographic structure was assessed with a contingency table and χ^2 analyses.

Nuclear genetic diversity parameters were estimated for the total sample and for the major mtDNA lineages identified. Estimates of observed (H_O) and expected (H_E) heterozygosities and tests for departure from the Hardy-Weinberg equilibrium were obtained with ARLEQUIN 3.11 [73]. In addition, inbreeding coefficients, defined as a measure of deficit of heterozygotes inside the population [78] and allele richness were calculated in FSTAT 2.9.3 [79]. Linkage disequilibrium tests between pairs of loci with each lineage and in overall individuals were calculated with GENEPOP 3.4 [80] using 10 000 permutations.

We calculated F_{ST}, R_{ST}, D_{est} to identify the contribution of historical and more current factors in the structure of the major mtDNA lineages. F_{ST} evaluates the difference in allelic frequencies between lineages, R_{ST} [81] is the fraction of the total variance of allele size between lineages and D_{est} estimate [82] considers the proportion of each lineage's alleles that are unique. ARLEQUIN 3.11 [73], FSTAT 2.9.3 [79] and SMOGD 1.2.5 ([83]; http://www.ngcrawford.com/django/jost/) were used to calculate these parameters, respectively. To avoid potential bias in F_{ST} due to the presence of null alleles in two microsatellite loci, F_{ST} estimation was performed following the 'Excluding Null Alleles' (hereafter called ENA) correction proposed by Chapuis and Estoup [66] and estimated with the software FreeNA (http://www1.montpellier.inra.fr/URLB/). The distribution of nuclear variation (AMOVA, [68]) was analyzed considering the major lineages identified and the clusters detected with Geneland.

Nuclear gene flow between mtDNA lineages was estimated using the Bayesian inference available in MIGRATE-n 3.0 [84] and our five microsatellite loci. The parameters estimated with this software were θ_i (which is $xN_e\,\mu$) and M_i (which is the ratio of immigration and mutation rates, m_i/μ) and the migration estimate is expressed as $4\theta M$ [84]. The mutation rate was assumed to be constant for all microsatellite loci, and initial values for theta ($\theta = 4N_e\mu$) and migration (M, the ratio of immigration and mutation rates, m/μ) were obtained from F_{ST} estimates. The Bayesian run consisted of one long chain with one million recorded steps and sampling increments of 100 generations. A total of 10 million genealogies (recorded steps multiplied by the sampling increment) were sampled, and the first 10 000 were discarded (burn-in).

Demography

The historical and current demography of the lineages was explored with mtDNA and microsatellite data independently. The mutation rate of each marker is important to make the estimation of demography processes, and they were calculated. The *D. novemcinctus* mitochondrial control region mutation rate was approximated using the formula $d = (t_v + t_vR) / m$, where d is the number of nucleotide substitutions per site, t_v is the number of transversions between species, R is the transversion/transition ratio within the focal species and m is the length of the sequence (bp) [85]. *D. kappleri* was used as outgroup to estimate the d-value. The rate of nucleotide substitution per site per year is $\lambda = d/2T$, where T is the divergence time between the ingroup and the outgroup species, and was calculated using the average divergence time of 7 MYA calculated by Delsuc et al. [29]. The rate of nucleotide substitution per site and generation is $\mu = \lambda g$, where g is the generation time, which in nine- banded armadillos has been estimated at approximately four years [86]. Finally, the nucleotide substitution rate per haplotype (u) was calculated by multiplying the length (m) of the sequence by μ.

The historical demographic trends within the main lineages were estimated with mitochondrial data and the Bayesian skyline plot implemented in BEAST 1.5.4 (BSP; [87,67]). We performed the BSP for each lineage using the complete haplotypes dataset with the HKY + I + G model and used the uncorrelated lognormal relaxed clock model of rate variation [88]. The default priors were used for other parameters and the program was repeatedly run optimizing the scale factors of the *a priori* function. MCMC tests were run for 1×10^7 steps and sampled every 1000 steps. Convergence of the chains and effective sample size of each parameter were evaluated with the program Tracer 1.5 [69]. The rate of

nucleotide substitution per site estimated previously was used to calculate the time of the demographic change. Furthermore, Fu's neutrality F_S test [89] was computed for each lineage with ARLEQUIN 3.11 [73] to assess possible changes in 'population' size. Negative values are expected in lineages that have undergone recent expansion, while positive values are expected in lineages that have recently experienced bottlenecks. Values are expected to be near zero in stable lineage sizes.

The demography of each lineage was also estimated using nuclear data in the coalescence-based method implemented by MSVAR 1.3 [90-92]. The most important output parameters are (i) N_0 , the current effective number of individuals, and N_1, the number of individuals at the time where the expansion/decline began (in a declining population, N_0/N_1 is smaller than 1); (ii) t_a, the number of generations since the beginning of the expansion/decline; and (iii) μ, the mutation rate. The analyses were performed using the exponential growth model, which is more suitable for modeling changes in population size on a shorter time scale [95], and four independent replications were conducted on each lineage using different starting values. Each run for each lineage consisted of 2×10^8 steps and sampled every 10 000 steps. Posterior density for each individual run was examined to check for overall consistency in shape, using Tracer 1.5 [74]. For each lineage, the priors for the first run were $N_1 = 1 \times 10^2$, $N_0 = 1 \times 10^4$, mutation rate (μ) varied among loci (1×10^{-4}, 2×10^{-4}, 1×10^{-5}, 1×10^{-4}, 2×10^{-4}), and t_a varied among loci (1×10^3, 5×10^2, 2×10^3, 3×10^3, 4×10^3); the priors for the second run were $N_1 = 1 \times 10^3$, $N_0 = 1 \times 10^4$, $\mu = 1 \times 10^{-4}$, $t_a = 1 \times 10^3$; the priors for the third run were $N_1 = 1 \times 10^3$, $N_0 = 1 \times 10^2$, $\mu = 1 \times 10^{-4}$, $t_a = 1 \times 10^3$; and the priors for the fourth run were $N_1 = 1 \times 10^5$, $N_0 = 1 \times 10^3$, $\mu = 1 \times 10^{-4}$, $t_a = 1 \times 10^3$.

ACKNOWLEDGEMENTS

We wish to thank all the persons and institutions that provided biological samples for this study. They are listed in Table S3 (supporting information). We thank the Dirección General de Vida Silvestre for the correct permits to collect samples of armadillos. We are grateful to Osiris Gaona, Erika Aguirre and Laura Espinosa for their help with laboratory analyses and technical support. We thank John McCormack, Mariella Superina, and Rafael Bello for valuable comments and English editing. This work is part of M.C.A.'s PhD dissertation in biological sciences at the Universidad Nacional Autónoma de México (UNAM). M.C.A. is grateful to the graduate program Doctorado en Ciencias Biológicas, to UNAM for the scholarship granted for doctoral studies, and to Sigma Xi and The American Society of Mammalogists for research grants. This is a contribution of the EcoHealth Alliance. The manuscript was finalized when R. Medellin was on sabbatical at the Arizona-Sonora Desert Museum and the University of Arizona and he thanks DGAPA-UNAM for support.

REFERENCES

1. Ficetola GF, Bonin A, Miaud C (2008) Population genetics origin and number of founders in biological invasion. Mol Ecol 17: 773–782.
2. Virgilio M, Fauvelot C, Costantini F, Abbiati M, Backeljau T (2009) Phylogeography of the common ragworm Hediste diversicolor (Polychaeta: Nereididae) reveals cryptic diversity and multiple colonization events across its distribution. Mol Ecol 18: 1980–1994.
3. Hewitt GM (2004) Genetic consequence of climatic oscillation in the Quaternary. Philos T R Soc B 359: 183–195.

4. Hájková P, Pertoldi C, Zemanova B, Roche K, Hájek B, et al. (2007) Genetic structure and evidence for recent population decline in Eurasian otter populations in the Czech and Slovak Republics: implications for conservation. J Zool 272: 1–9.

5. Mondol S, Karanth KU, Ramakrishnan U (2009) Why the Indian Subcontinent Holds the Key to Global Tiger Recovery. PLoS Genetics 5: e1000585.

6. Braaker S, Heckel G (2009) Transalpine colonisation and partial phylogeographic erosion by dispersal in the common vole (*Microtus arvalis*). Mol Ecol 18: 2518–2531.

7. Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. Trends Ecol Evol 24: 136–144.

8. Ruedi M, Walter S, Fischer MC, Scaravelli D, Excoffier L, et al. (2008) Italy as a major Ice Age refuge area for the bat Myotis myotis (Chiroptera: Vespertilionidae) in Europe. Mol Ecol 17: 1801–1814.

9. Teacher AGF, Garner TWJ, Nichols RA (2009) European phylogeography of the common frog (*Rana temporaria*): routes of postglacial colonization into the British Isles, and evidence for an Irish glacial refugium. Heredity 102: 490–496.

10. Webb SD (1991) Ecogeography and the Great American Interchange. Paleobiology 17: 266–280.

11. Webb SD (1976) Mammalian faunal dynamics of the Great American Interchange. Paleobiology 2: 216–234.

12. Marshall LG, Webb SD, Sepkoski JJ, Raup DM (1982) Mammalian evolution and the Great American Interchange. Science 215: 1351–1357.

13. Culver M, Johnson WE, Pecon-Slattery JS, O'Brien J (2000) Genomic ancestry of the American puma (*Puma concolor*). J Hered 91: 186–197.

14. Eizirik E, Kim J, Menotti-Raymond M, Crawshaw PG, O'Brien SJ (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Mol Ecol 10: 65–79.

15. Wüster W, Ferguson JE, Quijada-Mascareñas JA, Pook CE, Salomão MG, et al. (2005) Tracing an invasion: landbridges, refugia and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). Mol Ecol 14: 1095–1108.

16. Pascual R, Ortiz-Jaureguizar E (2007) The Gondwanan and South American Episodes: Two major and unrelated moments in the history of the South American mammals. J Mamm Evol 14: 75–137.

17. Daza JM, Smith EN, Páez VP, Parkinson CL (2009) Complex evolution in the Neotropics: the origin and diversification of the widespread genus Leptodeira (Serpentes: Colubridae). Mol Phylogenet Evol 53: 653–667.

18. Weir JT, Bermingham E, Schluter D (2009) The Great American Biotic Interchange in Birds. Proc Natl Acad Sci U S A 106: 21737–21742.

19. Tilston B, Klicka J (2010) The profound influence of the Late Pliocene Panamanian uplift on the exchange, diversification, and distribution of New World birds. Ecography 33:333–342.

20. McCormack JE, Heled J, Delaney KS, Peterson AT, Knowles LL (2011) Calibrating divergence times on species tree versus gene trees: Implications for speciation history of *Aphelocoma* jays. Evolution 65: 184-202.

21. Burney CW, Brumfield RT (2009) Ecology predicts levels of genetic differentiation in

Neotropical birds. Am Nat 174:358–368.

22. Storz JE (1999) Genetic consequences of mammalian social structure. J Mammal 80: 553–569.

23. Prugnolle F, De Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. Heredity 88: 161–165.

24. Gardner A (2005) Order Cingulata. in Wilson DE, Reeder DM, eds. Mammal Species of the World, a taxonomic and geographic reference. The John Hopkins University Press. Baltimore, Maryland. pp. 94–99.

25. Wetzel RM (1985) Taxonomy and distribution of armadillos, Dasypodidae. in Montgomery GG, ed. Evolution and Ecology of Sloths, Armadillos and Vermiliguas. Smithsonian Institute Press, Washington. pp. 23–46.

26. McBee K., Baker R.J. 1982. *Dasypus novemcinctus*. Mammalian Species 162: 1-9.

27. Delsuc F, Scally M, Madsen O, Stanhope MJ, de Jong WW, Catzeflis FM, Springer MS, Douzery EJP (2002) Molecular phylogeny of living xenarthrans and the impact of character and taxon sampling on the placental tree rooting. Mol Biol Evol 19: 1656–1671.

28. Cetica PD, Solari AJ, Merani MS, de Rosas JC, Burgos MH (1998) Evolutionary sperm morphology and morphometry in armadillos. J Submicr Cytol Path 30: 309–314.

29. Delsuc F, Vizcaíno SF, Douzery EJP (2004) Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within Xenarthrans. BMC Evol Biol 4: 11–24.

30. Carlini AA, Vizcaíno SF, Scillato-Yané GJ (1997) Armoured xenarthrans: a unique taxonomic and ecologic assemblage. in Kay RF, Cifelli RL, Flynn JJ, Madden RH, eds.

Vertebrate Paleontology of the Miocene Honda Group, Republic of Colombia. Smithsonian Institution Press, Washington. pp. 213–226.

31. Vizcaíno SF, Perea D, Ubilla M (1995) Presencia de Dasypus (Mammalia, Edentata, Dasypodidae) en la formación Sopas (Pleistoceno tardío) de Uruguay. Distribución cronológica del género. Rev Chil Hist Nat 68: 95–99.
32. Álvarez T (1974) Identificación de los huesos rescatados en la segunda temporada en Santa Martha Chiapas (Cala HH/KK). in Excavaciones en el abrigo de Santa Marta Chiapas. Serie Informes, Departamento de Prehistoria, Instituto Nacional de Antropología e Historia, México D.F. pp. 24–29.
33. Audubon JJ, Bachman J (1854) Quadrupeds of North America. New York: Privately published by J. J. Audubon. 348 p. Vol III.
34. Huchon D, Delsuc F, Catzeflis FM, Douzery EJP (1999) Armadillos exhibit less genetic polymorphism in North America than in South America: nuclear and mitochondrial data confirm a founder effect in *Dasypus novemcinctus* (Xenarthra). Mol Ecol 8: 1743–1748.
35. Frutos S, Van den Bussche R (2002) Genetic diversity and gene flow in nine-banded armadillo in Paraguay. J Mammal 83: 815–823.
36. Moncrief ND (1988) Absence of genic variation in a natural population of nine-banded armadillos, *Dasypus novemcinctus* (Dasypodidae). Southwest Nat 33: 229–231.
37. Escamilla A, Sanvincente M, Sosa M, Galindo-Leal C (2000) Habitat mosaic, wildlife availability and hunting in the tropical forest of Calakmul, Mexico. Conserv Biol 14: 1592–1601.
38. León P, Montiel S (2008) Wild Meat Use and Traditional Hunting Practices in a Rural

Mayan Community of the Yucatan Peninsula, Mexico. Hum Ecol 36: 249–257.

39. Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535–538.

40. Pesole G, Gissi C, Chirico AD, Saccone C (1999) Nucleotide substitution rate of mammalian mitochondrial genomes. J Mol Evol 48: 427–434.

41. Sbisa E, Tanzariello F, Reyes A, Pesole G, Saccone C (1997) Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene 205: 125–140.

42. Dallas JF (1992) Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mamm Genome 3: 452–456.

43. Whittaker JC, Harbord RM, Boxall N, Mackay I, Dawson G, et al. (2003) Likelihood-based estimation of microsatellite mutation rates. Genetics 164: 781–787.

44. Hoelzel AR, Hey J, Dahlheim ME, Nicholson C, Burkanov V, Black N (2007) Evolution of Population Structure in a Highly Social Top Predator, the Killer Whale. Mol Biol Evol 24:1407–1415.

45. Dlugosch, KM, Parker, IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17: 431–449.

46. Hedrick PW (2005) Genetics of Populations. Third Edition. Massachusetts: Jones and Bartlett Publishers. 737 p.

47. Bergl RA, Vigilant L (2007) Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). Mol Ecol 16: 501–516.

48. Zachos F, Cirovic D, Kirschning J, Otto M, Hartl GB, et al. (2009) Genetic Variability, Differentiation, and Founder Effect in Golden Jackals (*Canis aureus*) from Serbia as Revealed by Mitochondrial DNA and Nuclear Microsatellite Loci. Biochemical Genetics 47: 241–250.

49. Loughry WJ, Truman RW, McDonough CM, Tilak M, Garnier S, et al. (2009) Is leprosy spreading among nine-banded armadillos in the southeast united states?. J Wildlife Dis 45: 144–152.

50. Hare MP (2001) Prospects for nuclear gene phylogeography. Trends Ecol. Evol. 16: 700–706.

51. McDonough CM (1997) Pairing behavior of the nine-banded Armadillo (*Dasypus novemcinctus*). Am Midl Nat 138: 290–298.

52. Prodöhl PA, Loughry WJ, McDonough CM, Nelson WS, Thompson EA, et al. (1998) Genetic maternity and paternity in a local population of armadillos assessed by microsatellite DNA markers and field data. Am Nat 151: 7–19.

53. Sullivan J, Arellano E, Rogers DS (2000) Comparative phylogeography of Mesoamerican highland rodents: concerted versus independent response to past climatic fluctuations. Am Nat 155: 755–768.

54. Leon-Paniagua L, Navarro-Siguenza AG, Hernandez-Banos BE, Morales JC (2007) Diversification of the arboreal mice of the genus Habromys (Rodentia: Cricetidae: Neotominae) in the Mesoamerican highlands. Mol Phylogenet Evol 42: 653–664.

55. McCormack JE, Peterson AT, Bonaccorso E, Smith TB (2008) Speciation in the highlands of Mexico: genetic and phenotypic divergence in the Mexican jay (*Aphelocoma ultramarina*). Mol Ecol 17:2505-2521

56. Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and ecology of species range limits. Annu Rev Ecol Evol Syst 40:415–436.

57. Lucchini V, Galov A, Randi E (2004) Evidence of genetic distinction and long-term population decline in wolves (*Canis lupus*) in the Italian Apennines. Mol Ecol 13*:* 523–536.

58. Craul M, Chikhi L, Sousa V, Olivieri GL, Rabesandratana A, et al. (2009) Influence of forest fragmentation on an endangered large-bodied lemur in northwestern Madagascar. Biol Conserv 142: 2862–2871.

59. Rosenmeier MF, Hodell DA, Brenner M, Curtis JH, Guilderson TP (2002) A 4000-year lacustrine record of environmental change in the southern Maya lowlands, Petén, Guatemala. Quaternary Res 57: 183–190.

60. Emery KF (2007) Assessing the impact of ancient Maya animal use. J Nat Conserv 15: 184–195.

61. Gannon WL, Sikes RS 2007 Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research. J Mammal 88: 809–823.

62. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882.

63. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids S 41: 95–98.

64. Posada D (2008) JModelTest: Phylogenetic Model Averaging. Mol Biol Evol 25: 1253–1256.

65. Prodöhl P, Loughry WJ, McDonough CM, Nelson WS, Avise J (1996) Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine-banded armadillo, *Dasypus novemcinctus*. Philos T R Soc B 263: 1643–1649.

66. Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24: 621–631.

67. Drummond AJ, Rambaut A (2007) "BEAST: Bayesian Evolutionary Analysis by Sampling Trees". BMC Evol Biol 7: 214.

68. Drummond AJ, Ho SYW, Rawlence N, Rambaut A (2007) A rough guide to Beast 1.4. Department of Computer Science. The University of Auckland, New Zealand.

69. Rambaut A, Drummond AJ (2007) Tracer v1.4. Available: http://beast.bio.ed.ac.uk/Tracer

70. Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16: 37–48.

71. Cassens I, Mardulyn P, Milinkovitch MC (2005) Evaluating Intraspecific "Network" Construction Methods Using Simulated Sequence Data: Do Existing Algorithms Outperform the Global Maximum Parsimony Approach?. Syst Biol 54: 363–372.

72. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distance among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

73. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinf 1: 47–50.

74. Librado P, Rozas J (2009) DnaSP ver. 5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

75. Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. Mol Ecol Notes 5: 708–711.

76. Guillot G, Santos F (2009) A computer program to simulate multilocus genotype data with spatially auto-correlated allele frequencies. Mol Ecol Resour 9: 1112–1120.

77. Coulon A, Guillot G, Cosson JF, Angibault MA, Aulagnier S, et al. (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. Mol Ecol 15: 1669–1679.

78. Wright S (1951) The genetical structure of populations. Ann Eugenic 15: 323–354.

79. Goudet J (2001) FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices Ver. 2.9.3. Available:
http://www2.unil.ch/popgen/softwares/fstat.htm

80. Raymond M, Rousset F (1995) GENEPOP ver. 1.2: Population genetics software for exact tests and ecumenicism. J Hered 86: 248–249.

81. Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457–462.

82. Jost L (2008) G_{ST} and its relatives do not measure differentiation. Mol Ecol 17: 4015–4026.

83. Crawford NG (2010) SMOGD: software for the measurement of genetic diversity. Mol Ecol Resour 10: 556–557.

84. Beerli P (2008) Migrate version 3.0: A Maximum Likelihood and Bayesian estimator of gene flow using the coalescent. Available: http://popgen.sc.fsu.edu/Migrate/Migrate-n.html

85. Rooney AP, Honeycutt RL, Derr JN (2001) Historical population size change of bowhead whales inferred from NDA sequence polymorphism data. Evolution 55: 1685–1687.

86. Abba AM, Superina M (2010) The 2009/2010 Armadillo Red List Assessment. Edentata 11: 135–187.

87. Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. Mol Biol Evol 22: 1185–1192.

88. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. PLoS Biology 4: e88.

89. Fu Y (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–925.

90. Beaumont MA (1999) Detecting population expansion and decline using microsatellites. Genetics 153: 2013–2029.

91. Beaumont MA (2004) msvar 1.3. Available: http://www.rubic.rdg.ac.uk/mab/stuff/

92. Storz JF, Beaumont MA (2002) Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. Evolution 56: 154–166.

Table S1. Control region haplotypes identified in 157 *D. novemcinctus* samples from Mexico, Colombia, Costa Rica, Guatemala, Nicaragua, and US.

Haplotype ID	GeneBank Access number	Number of samples	Mexican states from samples origin (other countries from samples origin)
H1		1	Quintana Roo
H2		2	Jalisco
H3		1	Veracruz
H4		1	Jalisco
H5		2	México, Guerrero
H6		1	Guerrero
H7		12	Morelos, Hidalgo, Colima, México, Guerrero, Querétaro
H8		1	Jalisco
H9		1	Oaxaca
H10		1	Sinaloa
H11		1	Sinaloa
H12		3	Tamaulipas, Hidalgo, México
H13		5	Colima, Chiapas
H14		1	Colima
H15		1	Nayarit
H16		1	Quintana Roo
H17		11	Quintana Roo, Chiapas, Veracruz
H18		3	Quintana Roo
H19		6	Quintana Roo, Chiapas
H20		1	Quintana Roo
H21		1	Chiapas
H22		2	Chiapas
H23		2	Chiapas
H24		2	Nuevo León, Chiapas
H25		2	Chiapas
H26		2	Chiapas
H27		1	Chiapas
H28		1	Chiapas
H29		1	Chiapas
H30		1	Chiapas
H31		1	Chiapas
H32		2	Hidalgo
H33		1	Hidalgo
H34		3	Hidalgo
H35		1	Hidalgo
H36		1	Hidalgo
H37		1	Hidalgo
H38		2	Hidalgo, Michoacán
H39		5	Jalisco, Colima
H40		3	México, Guerrero, Morelos
H41		1	México
H42		2	México, Michoacán

H43	1	Oaxaca
H44	1	Oaxaca
H45	1	Oaxaca
H46	1	Oaxaca
H47	1	Oaxaca
H48	2	Nayarit
H49	1	Nayarit
H50	4	Guerrero, Michoacán
H51	1	Michoacán
H52	1	Michoacán
H53	1	Veracruz
H54	1	Veracruz
H55	1	Veracruz
H56	1	Tamaulipas
H57	8	Puebla (Colombia)
H58	1	Puebla
H59	4	Guerrero
H60	1	Guerrero
H61	1	Guerrero
H62	1	Guerrero
H63	1	Guerrero
H64	1	Guerrero
H65	1	Guerrero
H66	1	Guerrero
H67	1	Morelos
H68	1	Sinaloa
H69	1	Yucatán
H70	1	Veracruz
H71	1	Veracruz
H72	1	Jalisco
H73	1	Hidalgo
H74	1	Hidalgo
H75	3	Hidalgo (US)
H76	1	Veracruz
H77	1	Veracruz
H78	1	(Costa Rica)
H79	1	(Costa Rica)
H80	1	(Nicaragua)
H81	1	(Colombia)
H82	1	(Colombia)
H83	1	(Colombia)
H84	2	(Colombia)
H85	1	(Colombia)
H86	1	(Colombia)
H87	1	(Nicaragua)
H88	1	(Guatemala)

Table S2. Contingency table where columns are cluster-derived from Geneland analyses and rows are the two identified mitochondrial lineages. The absolute numbers of individuals are included.

	Cluster-derived from Geneland										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
West lineage	1	8	11	5	0	10	4	0	0	4	43
East lineage	8	1	0	5	2	2	19	18	7	11	73
Total	9	9	11	10	2	12	23	18	7	13	116

Table S3. *D. novemcinctus* samples obtained from the mammal collection of different museums.

Collection names	Collection numbers of specimens	Total samples
Mammal collection of American Museum of natural History, US (AMNH)	26007, 171919, 171921, 207420, 24053, 24054, 176676, 176675, 7278, 182075, 14663, 33148, 15463, 139318	14
Colección Zoológica Regional, Instituto de Historia Natural y Ecología, México (CZRMA)	1581, 2255, 43, 50	4
Colección de la Reserva de la Biósfera Los Tuxtlas, México	a, b	2
Colección Mastozoológica del Sureste de México, México (ECOSUR-SC)	52, 575, 980, 1270, 1570, 1572	6
Colección de Mamíferos del Museo de Zoología "Alfonso L. Herrera", México (MZFC)	4254, 5078, 5079, 5075, 4900, 5077	6
Colección Mastozoológica HMAN Instituto Tecnológico Agropecuario de Hidalgo, México (HMAM)	540, 544, 536, 543, 547, 534, 541, 535, 545, 531, 539, 537, 546, 532, 548	15
Colección Nacional de Mamíferos, Instituto de Biología, UNAM, México (IBUNAM)	1153, 3592, 10069, 11535, 17037, 16559, 31600, 43121, 16520, 15583, 27275, 16558, 14528, 43122, 14527, 37069, 16551, 16496	18
Colección de mamíferos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México (ENCB)	40868, 16019, 21096, 39110, 35629, 15099, 26577, 21669, 26067, 36004, 34214	11
Colección de Mamíferos de la Universidad Michoacana San Nicolás de Hidalgo, México (UMSNH)	1323, 394, 887, 1983, c	4
Mammal collection of Smithsonian National Museum of Natural History, US (USMN)	553928, 281290, 281285, 281291, 281288, 337563	6
TOTAL		86

a,b,c specimens without assign number

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Genetic admixture in multidimensional environmental space: asymmetrical niche similarity promotes gene flow in armadillos (Dasypus novemcinctus)

Journal:	*Evolution*
Manuscript ID:	11-0147.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	20-Mar-2011
Complete List of Authors:	Arteaga, Maria; Universidad Nacional Autónoma de México, Departamento de Ecología de la Biodiversidad McCormack, John; Louisiana State University, Museum of Natural Science Eguiarte, Luis; Universidad Nacional Autónoma de México, Departamento de Ecología Evolutiva Medellin, Rodrigo; Universidad Nacional Autónoma de México, Departamento de Ecología de la Biodiversidad
Keywords:	genetic connectivity, niche conservatism, microsatellites, climatic niche, Dasypodidae, niche modelling

Genetic admixture in multidimensional environmental space: asymmetrical niche similarity promotes gene flow in armadillos (*Dasypus novemcinctus*)

Maria Clara Arteaga,[a, 1] John E. McCormack,[b, 2] Luis E. Eguiarte,[c, 3] Rodrigo A. Medellín[a, 4]

[a]Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México, Distrito Federal 04510, México. [b]Museum of Natural Science, Lousiana State University, Baton Rouge, LA 70803; United States of America [c]Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México, Distrito Federal 04510, México

[1] *Corresponding author*: mcarteaga@ecologia.unam.mx; Phone (5255) 56229042

[2] jmccormack@lsu.edu; Phone: (734) 358-6886

[3] fruns@servidor.unam.mx; Phone: (5255) 56229006

[4] medellin@miranda.ecologia.unam.mx; Phone number: (5255) 56229042

Running title: Niche similarity promotes genetic admixture

Keywords: genetic connectivity, niche modelling, niche conservatism, microsatellites, climatic niche, Dasypodidae

ABSTRACT

We unite genetic data with a robust test of niche divergence to test the hypothesis that patterns of gene flow between two lineages of the nine-banded armadillo are influenced by their climatic niches. We collected GIS data on climate using locality information from 111 individuals from two lineages that had associated genetic material. We tested whether niches of these lineages were more conserved or divergent than the background environments of their geographic ranges and found evidence for niche conservatism on two axes and no evidence for divergence on any axis. To address the role of niche similarity in gene flow, we genotyped the 111 individuals at five microsatellite loci and tested whether admixed individuals tended to be located in parts of multidimensional environmental space (E-space) shared between the two lineages. We observed an asymmetrical pattern of overlap, in which the West lineage's E-space was almost completely included inside East lineage's E-space. Genetic admixture levels were significantly higher in the West lineage and, for both lineages, in shared portions of E-space. This suggests that niche similarity can facilitate gene flow among disjunct groups with moderate to good dispersal capabilities, contrasting with the prevailing view of niche conservatism as a diversifying force.

INTRODUCTION

Integrating environmental and genetic data allows researchers to ask how ecology influences evolutionary patterns in the present (Storfer 2006; Kozak et al. 2008) and past (Hugall et al. 2002; Richards et al. 2007; McCormack et al. 2008). With regard to divergence and speciation, this interdisciplinary approach has been strongly influenced by the concept of niche conservatism (Peterson et al. 1999; Wiens and Graham 2005), or the idea that recently-derived taxa tend to be similar in their ecological niches. While there has been conceptual debate about whether niche conservatism better describes a pattern or process (Losos 2008), it has nevertheless been linked to the process of speciation through the idea that lineages that are strongly conserved in their niches are unlikely to cross areas of unsuitable habitat, facilitating reproductive isolation (Wiens 2004) through processes that are undescribed, but would seem to involve genetic drift, mutation-order speciation (Mani and Clarke 1990), or the accumulation of Bateson-Dobzhansky-Muller incompatibilities (Gavrilets 2003). This hypothesis has found some support in poorly dispersing organisms (Kozak and Wiens 2006). Other studies incorporating genetic and environmental data have also suggested speciation via niche divergence (Graham et al. 2004; Rissler and Apodaca 2007). While the preceding studies have tackled the role of niche divergence or conservatism from a phylogenetic perspective, population genetic studies are needed to examine exactly how niche conservatism or divergence might influence the all-important process of gene flow.

The integration of environmental data with genetics to address speciation questions is currently being driven by conceptual and methodological advances in quantifying and comparing ecological niches among species. GIS data from weather stations and remote-sensing satellites provide a trove of environmental information on the Earth's surface, but

because they show strong spatial autocorrelation (Dormann et al. 2007), they pose daunting and very specific challenges for researchers attempting to compare these data between species. For example, strong spatial autocorrelation in GIS data can lead to patterns of niche divergence among allopatric lineages that are likely to be exaggerated and of no greater import than the divergence of random points from the geographic ranges of the two lineages (Godsoe 2010). Several recent studies have promoted the idea of comparing observed niche divergence between lineages to null models of divergence generated from random points from the geographic ranges of the lineages in order to robustly test for niche divergence and conservatism (for a method using principal components analysis, see McCormack et al. 2010; for a method using niche models, see Warren et al. 2008). Visualization of species' available background environments has also illuminated several recent theoretical (Soberón and Nakamura 2009) and empirical (Broennimann et al. 2008; Godsoe et al. 2009) treatments of niche divergence in an evolutionary context. The next challenge is to unite these robust tests with explicit predictions of how niche conservatism or divergence influence landscape-level gene flow.

With this study, we seek to unite robust tests of niche divergence with population genetic data to test the prediction that patterns of dispersal and gene flow between two lineages of the nine-banded armadillo (*Dasypus novemcinctus*) are influenced by their climatic niches as quantified using GIS data. The nine-banded armadillo is distributed in a wide variety of environmental conditions from northern Argentina to the central United States (McBee and Backer 1982). Genetic study indicates that Mexican populations consist of two highly divergent mtDNA lineages (4.5% divergence) with allopatric distributions in Eastern and Western Mexico (Arteaga et al. in review). Given that armadillos are derived from South America (Simpson 1980; Eisenberg 1981), the occurrence of different South

American haplotypes within each of the Mexican lineages suggests that they originated from two separate founding events, with the Eastern Mexican lineage later providing the ancestral stock for northern population expansion into the United States. A small number of mismatched mtDNA haplotypes between East and West (Fig. 1) and higher levels of gene flow in nuclear markers suggest long-distance, and potentially male-biased, dispersal (Arteaga et al. in review). Regardless of the precise details of how gene flow occurs, judging from the wide distribution of armadillos in North and South America, their recent expansion into the United States (Taulman and Robbins1998), and the aforementioned genetic results, nine-banded armadillos appear to be fairly good dispersers. Gene flow among the East and West Mexican lineages also suggest that after dispersal to the geographic range of the other lineage, individuals find their new environs reasonably suitable (i.e., there appears to be mating and introgression). For this reason we made the prediction that genetic admixture among the two lineages might actually be *facilitated* by niche conservatism. We tested for niche conservatism using a new method that constructs null models of niche divergence on orthogonal environmental axes using background environments available to the two lineages (McCormack et al. 2010). Further, by visualizing genetically admixed individuals in multivariate environmental space (E-space, sensu Soberón and Nakamura 2009), we were able to test a resulting prediction that regions of shared E-space should show the highest incidence of genetic admixture compared to E-space that is unique to a particular lineage.

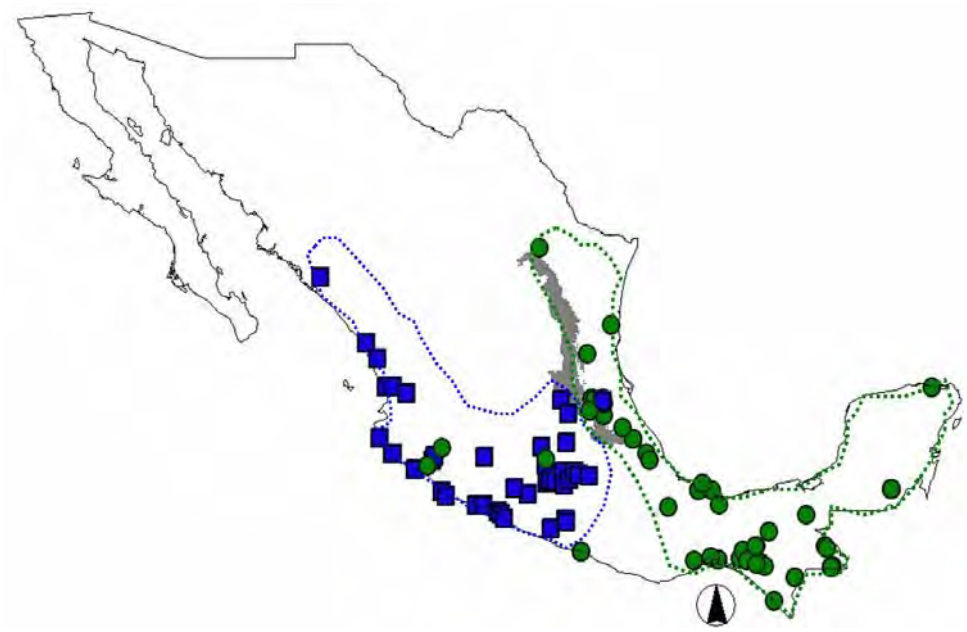

Figure 1. Geographical distributions of the West (blue) and East (green) mtDNA lineages, including a region in southern Mexico between the two lineages where mtDNA affiliation is not known. Note the five geographically mismatched mtDNA haplotypes, four of which occur in the West, which suggests dispersal. The Sierra Madre Oriental, a putative vicariant barrier between the lineages is shown in gray.

METHODS

Environmental data and test for niche conservatism

We collected GIS data on climate using 85 unique *D. novemcinctus* localities comprising 39 individuals from the West lineage and 72 individuals from the East lineage. Locality information came from GPS devices for wild-caught individuals and from georeferenced museum specimens (Tables S1, S2). Because our focus was on the two allopatric mtDNA lineages in Mexico with known gene flow and distinct geographic limits, we did not consider the whole geographic range of *D.novemcinctus* in the United States, Central, and South America. Importantly, genetic results indicate that the two Mexican lineages are

evolutionary distinct from one another. Within the two considered lineages, our occurrence points were well-distributed both geographically and in the wide variety of environmental conditions in Mexico where they occur, with the exception of a small gap in southern Mexico where lineage affinity is unknown (Fig. 1).

GIS data included BioClim climate layers that have a resolution of 1 km^2, describing aspects of temperature, precipitation, seasonality, as well as potentially biologically limiting extremes of these variables (e.g., Bio6, minimum temperature of coldest month). Using ArcView v3.2, we first extracted GIS data from 19 BioClim layers (*Bio1-Bio19*; http://worldclim.org/bioclim) at armadillo occurrence points. Using JMP 5.01 software (SAS Cary, New Jersey, USA), we then conducted a correlation test to remove highly correlated variables, which could bias subsequent analyses (Graham et al. 2004; Walker et al. 2009). Specifically, if two variables showed a correlation coefficient higher than 0.75, we considered them highly correlated, and for each pair of correlated variables, we selected the variable that was more temporally inclusive (e.g., preferring mean temperature over mean temperature of driest quarter) or those likely to be most relevant to armadillos (temperature variables over precipitation variables given that armadillos are poor thermoregulators (McNab 1980) and their distribution might be partially limited by environmental temperatures). The remaining six climate variables included *Bio2* = mean diurnal range of temperature; *Bio5* = minimum temperature of warmest month; *Bio6* = minimum temperature of coldest month; *Bio14* = precipitation of driest month; *Bio15* = precipitation seasonality; and *Bio18* = precipitation of warmest quarter.

Geographically-explicit predictions of climatic niches can often provide a good starting point for exploring niche differences and locating regions of niche similarity, so we generated environmental niche models (ENMs) for both lineages. ENMs are geographic

predictions of lineage distributions based on environmental data from known sampling points (Peterson 2001). We constructed ENMs for each lineage from occurrence points and the six climate layers using MAXENT 3.2.1 (Phillips et al. 2006). We used the default convergence threshold (10^{-5}) and 500 iterations (Pearson et al. 2007), using 25% of localities for model training and 75% for model testing. To assess model performance, we used the area under the receiver operating characteristic curve (AUC, Mertz 1978) as a measure of overall classification accuracy (capacity to discriminate between occupied and unoccupied records). The AUC can vary from 0.5, indicating no discrimination capacity, to 1, indicating perfect discrimination capacity. We produced a map of niche overlap by converting each lineage's niche model to a binary prediction of presence/absence with a prediction threshold of 10% (Pearson et al. 2007). Both binary prediction models were then summed and overlapping niche predictions were retained.

To test for niche conservatism, we employed the multivariate method introduced in McCormack et al. (2010) that compares niche divergence to a null hypothesis of divergence in available background environments on several orthologonal axes of E-space. This method does not actually involve ENMs *sensu stricto*, but rather uses principal components analysis (PCA) to reduce the raw GIS data to a smaller, uncorrelated set of axes. We chose this method over the method of Warren et al. (2008), which uses ENMs to test for niche divergence and conservatism, because our initial results indicated that ENMs for the different lineages were strongly influenced by differing climatic variables (see Results), which makes comparing ENMs difficult. In contrast, the multivariate method leads to readily interpretable axes, whose interpretation is the same for both lineages. Furthermore, the description of multiple niche axes in the multivariate method allows for the kind of

detailed study we wished to undertake, as opposed to the broader, joint estimation of the niche afforded by ENMs.

The general idea behind the McCormack et al. (2010) method is that a pattern of divergence in GIS data could be attributable either to meaningful niche divergence between species (or lineages) or to the fact that GIS data are strongly spatially autocorrelated. Therefore, a strong test of niche divergence or conservatism must compare niche divergence between species to baseline levels of divergence drawn from the background of available habitat contained within each species' geographic range (see next paragraph for detail). The null hypothesis is rejected when niches are more similar (niche conservatism) or more different (niche divergence) between species than the null model of background divergence. It is very important to note that if the null hypothesis is not rejected, this does *not* mean that there is *no meaningful niche divergence* between the species (i.e., failure to reject the null hypothesis does not mean that the null hypothesis is true). Rather, it means that whether divergence between species is meaningful or due to spatial autocorrelation both remain plausible alternatives.

To conduct the multivariate test for niche divergence/conservatism, we extracted raw data from our occurrence points for the two armadillo lineages and, to generate the background predictions, from 1000 random points from within the geographic ranges of each lineage. We drew the random points from within a minimum convex polygon drawn around our occurrence sites (Warren et al 2008; McCormack et al 2010) using ArcView v3.2 and the Hawth's Tools package. Next, we conducted a PCA on these data, extracting the first three PC (niche) axes for further consideration since they comprised the bulk of the variation and were readily interpretable (see Results). Niche divergence or conservatism was evaluated on each niche axis by comparing the observed difference between the means

for each lineage on that axis to the mean difference in their background environments on the same axis. A null distribution of background divergence was created by recalculating the background divergence score over 1000 jackknife replicates with 75% replacement. Significance for rejecting the null was evaluated at the 95% level. All analyses were conducted using Stata version 10 (StataCorp 2003). To provide a rough measure of how spatially autocorrelated an axis is, we assessed the correlation between our three niche axes and longitude/latitude.

Genetic analysis

To test whether genetically admixed individuals were located in parts of E-space that were more similar between the two armadillo lineages, we first visualized this E-space within the envelope of the background E-space available to each of them by making a bivariate plot using the first two PC axes. The first two axes were used because they explained the most variation and because our initial analyses did not detect niche conservatism on PC1, suggesting that this niche axis might be good for observing patterns of partially, but not wholly overlapping niches.

We genotyped 111 individuals (72 from East and 39 from West) from the 85 unique localities using five microsatellite loci in the DNA was extracted from tissue using a DNeasy Kit (Qiagen®). Microsatellites were amplified at five loci (autosomal: *Dnov1*, *Dnov6*, *Dnov7, Dnov16*, *Dnov24;* Prodöhl et al. 1996). Individuals were genotyped in a 10-µl reaction containing 1X of buffer, 0.3 µM of each primer, 0.15 mM of dNTP, 1–2 mM of $MgCl_2$ and 1.5 U of *Taq* DNA polymerase. The thermal profile for amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 15 s at 94°C, 30 s at 56-58°C and 1 s at 72°C, with a final extension of 7 min at 72°C. All reaction products

were run on an ABI PRISM genetic analyzer with a four-capillary system (Applied Biosystems, Foster City, CA) and alleles were scored using GENEMAPPER 4.0. Because loci showed deviations from Hardy-Weinberg equilibrium (HWE) likely due to a Wahlund effect (see Results), we also tested for deviations from HWE and linkage by locus at a smaller geographic scale (eight populations grouped using natural breaks in the sampling distribution) using Genepop On the Web (Raymond and Rousset 1995) and significance was assessed after correction for multiple tests (Rice 1989). Diversity statistics for the two lineages were calculated with ARLEQUIN 3.11 (Excoffier et al. 2005).

We assessed levels of genetic admixture using the Bayesian clustering algorithm implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000), which clusters individuals into groups minimising HW disequilibrium. Individuals are assigned a probability to one or more clusters if their genotype indicates that they are admixed (Pritchard et al. 2000). Because we were interested in levels of admixture between two geographically-isolated lineages with strong structuring in mtDNA, we set the number of clusters to $K = 2$. We did not attempt to detect further genetic structure within the East and West lineages, which was the goal of another study describing broad-scale phylogeographic patterns in this species (Arteaga et al. in review). The rationale for setting $K = 2$ is that, although genetic structure in the nuclear microsatellites does not fall into two clear clusters, there is a statistically significant association between mtDNA haplotypes and microsatellite cluster assignment (Arteaga et al. in review). We ran 10 iterations of STRUCTURE at $K = 2$, using the admixture model and correlated frequencies. Runs had a burn-in period of 100 000 followed by 1 000 000 Markov chain Monte Carlo replicates.

To explore how genetic admixture levels were related to areas of overlap between the niches of the two lineages, we calculated an admixture score using our STRUCTURE

results. We first averaged the assignment scores for individuals across the ten runs. A value that represented the level of admixture was then created by taking the absolute value of the difference between assignment of an individual to one cluster and 0.5. Because this resulted in a counter-intuitive scaling where low admixture individuals had high values, we reversed the scale by taking the absolute value of the score minus 0.5. The final values were admixture scores ranging from 0 (very pure) to 0.5 (very admixed). We then plotted the locations of these individuals with their admixture score in two-dimensional E-space using the first two niche axes. We tested the hypothesis that there should be more admixture in regions of E-space that are shared between the two lineages. We conducted ANOVA with partial Student's *t*-tests on the admixture scores for the two lineages and for the four different groups that are defined by their location in E-space: (1) West individuals in E-space unique to the West lineage, (2) West individuals in E-space shared with the East lineage, (2) East individuals in E-space unique to the East lineage, (4) East individuals in E-space shared with the West lineage (see Fig. 2). Because admixture scores appeared to be non-normally distributed (see Results), we also assessed differences with a non-parametric Wilcoxon test. The predictions were that the lineage with the most overlapping E-space would be the one with the most genetic admixture and, further, that, within a lineage, there would be more genetic admixture in portions of E-space shared with the other lineage compared to individuals in E-space unique to that lineage. The statistic analyses were conducted using JMP 5.01.

RESULTS

Niche models and tests of niche conservatism

Niche models for the two armadillo lineages showed largely disjunct distributions that in general agreed with the known distributions of these lineages (Fig. S1). However, the niche model for the East lineage showed some evidence for prediction into the West lineage's range along the Pacific coast of Mexico. The most relevant environmental variable for predicting the climatic distribution for the West lineage was rainfall seasonality (45.5%) and for the East lineage was minimum temperature in the coldest month (68.2%). The predictive power of occurrence was high for the two models (the AUC of test data were 0.90 and 0.92 for West and East lineages, respectively).

The PCA of raw GIS data indicated three main niche axes that together explained 87.7% of the variation. The first niche axis was associated with temperature and precipitation seasonality and had a high correlation with latitude (Table 1). The second niche axis was associated with temperature extremes, while the third niche axis was associated with rain seasonality. Niche axes 2 and 3 had smaller, but still significant correlations with latitude. Correlations with longitude were lower, but still significant in the first and the third axes (Table 1).

Table 1. Loadings of the environmental variables for each recovered PC axis and Spearman correlations with longitude/latitude.

	PC1	PC2	PC3
BIO15: Precipitation Seasonality	-0.41429	0.37099	0.49605
BIO14: Precipitation of Driest Month	0.49001	-0.32944	-0.08548
BIO6: Min Temperature of Coldest Month	0.44895	0.49786	0.07745
BIO5: Max Temperature of Warmest Month	0.23905	0.69663	-0.21358
BIO18: Precipitation of Warmest Quarter	0.26705	-0.11417	0.82953
BIO2: Mean Diurnal Range	-0.50818	0.08744	-0.08296
Longitud	R2 = 0.027*	R2 = 0.00006	R2 = 0.036*
	F = 57.59	F = 0.88	F = 76.18
Latitud	R2 = 0.458*	R2 = 0.020*	R2 = 0.130*
	F =1632.55	F = 43.235	F =299.371

*Significance level, p = 0.0001

Tests of niche divergence and conservatism on these three niche axes showed evidence for niche conservatism on niche axis 2 and 3 (Table 2). Niche axis 1 did not significantly differ from the null expectation of background divergence (although it was closer to the threshold for niche conservatism), indicating that it was not possible to know if the observed divergence was meaningful or resulted from spatial autocorrelation (Table 2).

Table 2. Tests of niche divergence and conservatism. Observed differences in the climatic niche of the two armadillo lineages on each PC axis compared to the middle 95th percentile of a null distribution of the differences between their environmental backgrounds. Bold values indicate niche conservatism.

	PC1	PC2	PC3
Observed difference	2.1392	**0.0016***	**0.5009***
Null distribution	2.1246 – 2.2380	0.3551 – 0.5093	0.6451 – 0.7146
% variance explained	42.79%	33.05%	11.84%

*Significance level, p < 0.05

Genetic admixture in regions of niche overlap

Visualization of climatic E-space of the West and East lineages and their background E-space showed an asymmetric pattern of overlap where the West lineage's niche and background was smaller and almost completely included inside the niche and background of the East lineage, which showed a much larger unique area (Fig. 2). This pattern was largely driven by niche axis 1. While our earlier tests did not conclude that this pattern on niche axis 1 was consistent with either niche divergence or conservatism (the null hypothesis could not be rejected), it nonetheless presented an opportunity to test the relationship between niche similarity and genetic admixture because some parts of this E-space were overlapping between the two lineages.

When analyzed at the lineage scale, most loci fell outside HWE (Tables S3, S4). However, when analyzed at a smaller geographic scale, there were few deviations and no consistent patterns across populations, suggesting the lineage-level HWE results were caused by a Wahlund effect. No statistically significant patterns of linkage disequilibrium were observed. Locus *Dnov16* showed heterozygote deficiency in several populations, but, following recent recommendations (Selkoe and Toonen 2006), we retained this locus in our analysis. Assignment probabilities were highly correlated in STRUCTURE runs with and without this locus ($R = 0.9$). For each lineage, allelic number by locus was higher and observed heterozygosity was similar to previous results from US populations using the same loci (Loughry et al. 2009) (Table S4).

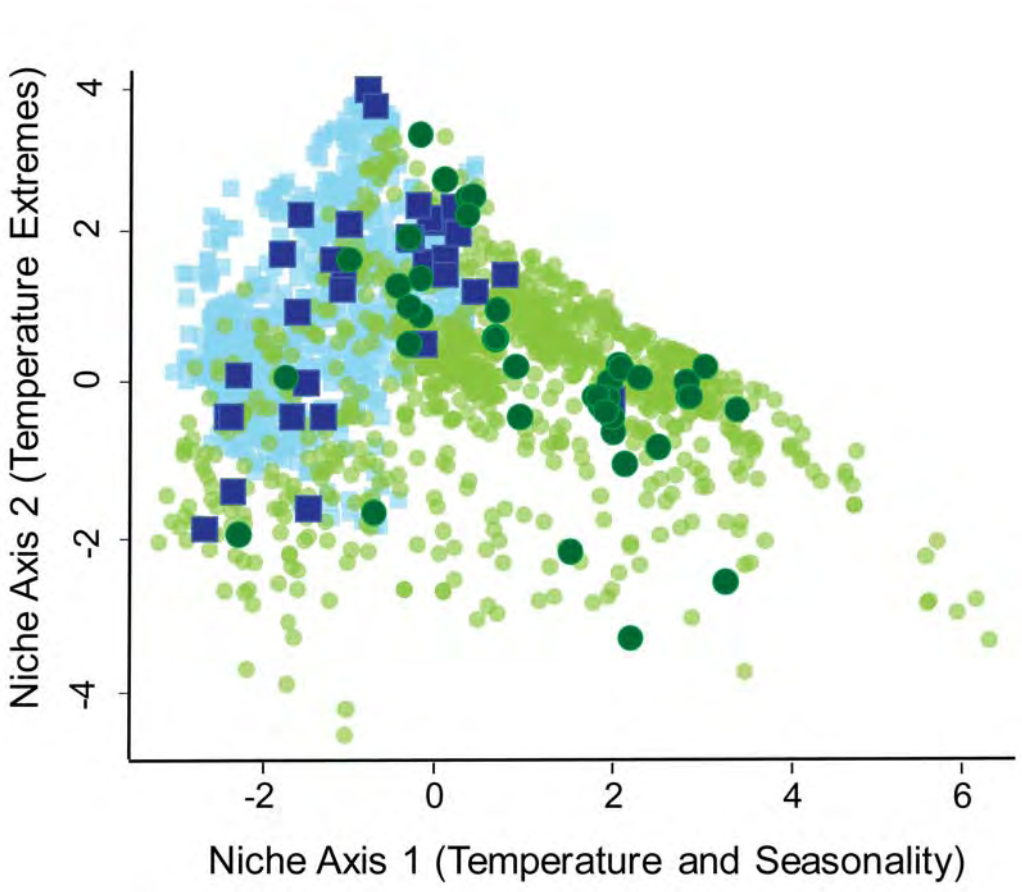

Figure 2. The E-spaces of West (dark blue) and East (dark green) lineages visualized within their respective available background environments (light blue and light green).

Under the assumption of K = 2, 61 of 111 individuals were assigned with probability higher than 0.9 to one of the two clusters, whereas the rest of the individuals were more admixed. Translated to our admixture scores, 61 individuals were "pure", having admixture scores lower than 0.1, while 50 individuals were highly admixed. The ranges of the admixture scores for the two lineages were similar, but the frequency distributions were very different (Fig. S2). The data were obviously not normally distributed and attempts to transform them to normality were unsuccessful, so all further comparisons used both *t*-tests and non-parametric Wilcoxon tests. The genetic admixture

level of the West lineage as a whole was significantly higher than the East lineage as a whole (Table 3).

Table 3. Tests of differences in admixture scores for individuals in different regions of environmental space (see Fig. 2). Wilcoxon test is recorded in the last two columns.

Level *i*	Level *j*	Means *i* - *j*	Difference	t-test t- ratio	Prob < t	Wilcoxon ChiSquare	Prob < t
East	West	0.128-0.200	-0.071	-2.61	**0.01**	9.469	**0.002**
East_unique	West_mixed	0.097-0.205	-0.108	3.84	**0.0002**	15.43	**0.0001**
East_unique	East_mixed	0.097-0.235	-0.138	-3.694	**0.0003**	6.345	**0.011**
West_mixed	East_mixed	0.205-0.235	-0.029	-0.754	0.452	0.023	0.878
East_unique	West_unique	0.097-0.199	-0.081	1.0478	0.297	3.515	0.06
West_unique	East_mixed	0.199-0.235	-0.056	-0.677	0.499	0.158	0.69
West_unique	West_mixed	0.199-0.205	-0.026	-0.331	0.74	-0.331	0.74

West_unique: West individuals in E space unique to the West lineage; West_mixed: West individuals in E space shared with the East lineage; East_unique: East individuals in E space unique to the East lineage; East_mixed: East individuals in E space shared with the West lineage.

When visualized in E-space (Fig. 3), 56 individuals from the East lineage occurred inside unique E-space for the East lineage, whereas 16 East individuals occurred in areas of E-space that were shared between the West and East lineage. Given that the E-space of the West lineage was almost completely subsumed within the E-space of the East, only three individuals from the West lineage occur inside its own unique E-space, whereas 36 West individuals occur inside E-space shared with the East lineage (Fig. 3).

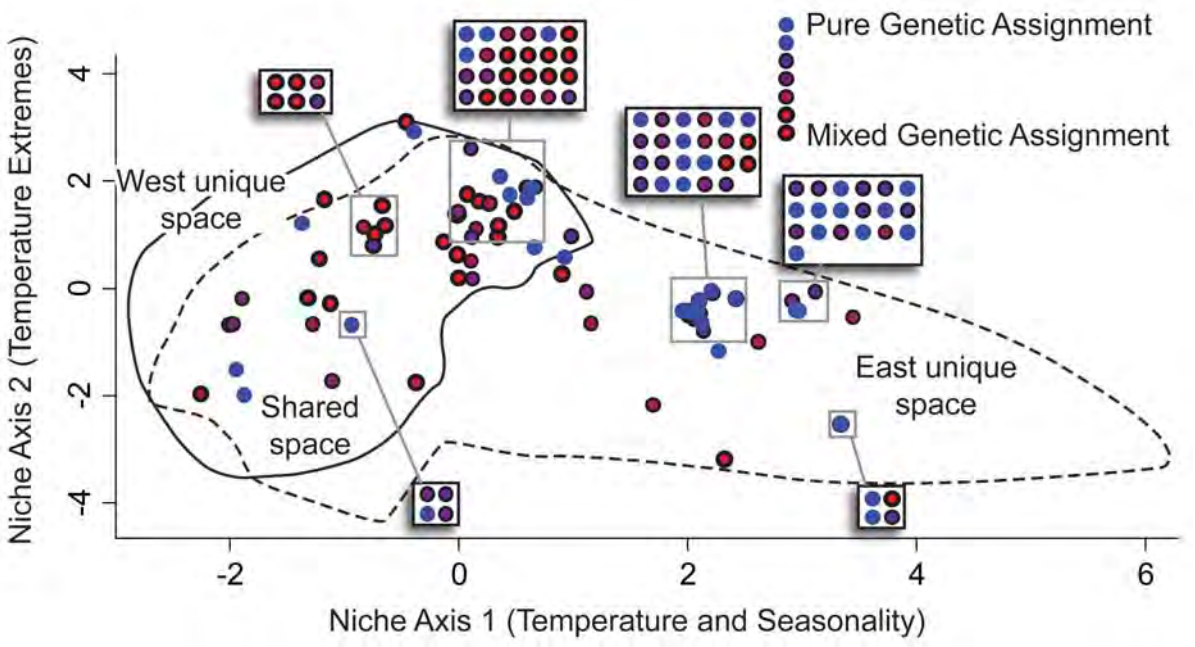

Figure 3. Visualization of the genetic admixture of individuals in regions of unique and shared E-space between the East and West lineages. Where multiple individuals fall into the same location, the individuals have been separated and expanded in the outlined boxes. Note that the blue color denotes genetically pure individuals and does not necessarily signify a relationship to the West lineage shown in blue in Figure 2.

Overall, there was less admixture in portions of E-space that were unique to the two lineages compared to those niche regions that were shared. Individuals from the East lineage that occur in E-space unique to the East lineage showed significantly lower admixture levels than both individuals from the West and East that are found in regions of overlapping E-space (Table 3). While our statistical power to detect differences using individuals from the West in unique E-space was low due to low sample size (n = 3), the mean admixture score for these three individuals was larger than that of either lineage in the region of niche overlap (Table 3). There was not a significant difference between admixture scores for the individuals from the two lineages occurring in the overlapping regions of E-space (Table 3).

DISCUSSION

The integration of ecology with historical biogeography will require both the union of niche data with divergence processes at deep time scales (i.e., cladogenesis) in addition to contemporary studies of how ecology affects dispersal and gene flow at the population level (Wiens and Donoghue 2004). GIS data are ideal for bridging this gap because they are so versatile (Swenson 2008): from the perspective of cladogenesis, they can be combined with phylogenies to explore how niche evolution (or lack of niche evolution) correlates with speciation (Evans et al. 2009; Kozak and Wiens 2006; Graham et al. 2004), but they are also amenable to recent or contemporary studies of the ecological factors influencing dispersal and gene flow (e.g., landscape genetics; Manel et al. 2003). By considering niche evolution at both, the phylogenetic level (between two mtDNA lineages) and population-genetic level (individual-based estimates of genetic admixture), our study provides a crucial link between genetic divergence and climatic niche evolution at the interface of recent and older time scales. The most important conclusion of our study is that climatic niche similarity between two armadillo lineages seems to have facilitated gene flow in regions of E-space that are shared. The finding that similarity in niches is associated with greater gene flow contrasts with the depiction of niche conservatism as a diversifying force that leads to reproductive isolation by reducing gene flow between populations or species separated by regions of inhospitable habitat (Wiens 2004). While the latter may be true for species that disperse poorly or that are reluctant for behavioral reasons to cross regions of less hospitable habitat (e.g., Kozak and Wiens 2006), niche similarity (or conservatism) for other more vagile species may equate to enhanced opportunities for contact and genetic admixture, thereby reducing the likelihood of speciation.

Niche conservatism and the importance of null models

We originally predicted that the two armadillo lineages would show niche conservatism because of evidence from mtDNA and nuclear genes that individuals disperse between the geographic ranges of the two lineages and apparently survive to mate (as documented by mtDNA mismatch and coalescent-based estimates of migration rates described in Arteaga et al. in review), long-distance feats which would be facilitated if their niches were similar. Results from a test that compared the amount of climatic divergence between armadillo lineages to the null expectation of background climatic divergence validated this prediction by showing niche conservatism on two axes of E-space related to temperature extremes (PC2) and rain seasonality (especially rain in the warmest month, PC3). Armadillos are poor thermoregulators (McNab 1980), so it makes sense that their distribution would be partially limited by temperature extremes. Rainfall seasonality could be related to change in insect populations, which are an important food resource for armadillos (Humphrey 1974; Taulman and Robbins 1996). Although we could not reject the null hypothesis in favour of niche conservatism on the first niche axis, which was related both to temperature and rain seasonality, the amount of observed divergence fell toward the conserved side of the null distribution. It is thus unlikely that the observed difference on niche axis 1 translates to meaningful climatic niche divergence, and spatial autocorrelation – which is expected to be acute with climatic data (Soberón 2007) – cannot be ruled out as the driving force behind this pattern.

The results of niche conservatism from tests using null models stand in contrast to the map-based projections of the climatic niche models themselves (Fig. S1). Despite some projection of the East lineage into the range of the West lineage, based simply on the disjunct patterns, one might be tempted to draw the conclusion that these armadillo lineages

are strongly divergent in their climatic niches. This contrast further serves to highlight the problems with drawing conclusions about niche divergence between species from ENM projections on a map or in multivariate E-space in the absence of controls for spatial autocorrelation or, in the case of multivariate E-space, without simultaneous visualization of available background environments. The question is not whether the two lineages live in places that differ in some environmental attributes (one can know that simply by looking at a map), but rather if these differences are large enough to reject a model created by drawing background points that, because they are random, are dissociated from any specific biological relevance to armadillos. Recent conceptual work (Soberón and Nakamura 2009; Soberón 2007; Soberón and Peterson 2005), simulation studies (Godsoe 2010), and methodological advances (McCormack et al. 2010; Warren et al. 2008) are beginning to address the problem of spatial autocorrelation of GIS data for comparing species' niches, but further advances and refinements of the methods will undoubtedly be necessary before speciation studies can reap the full benefits afforded by GIS data.

Asymmetrical niche overlap and genetic admixture in especially similar parts of E-space

Given our prediction that niche conservatism would facilitate dispersal and gene flow between armadillo lineages, a subsequent hypothesis is that greater genetic admixture should be observed in regions of E-space that are especially similar between the two lineages. In thinking about these patterns it is important to note that disjunct and overlapping regions of multivariate E-space (e.g., Figs. 2 and 3) do not directly correspond to geographic space, which is entirely alloparapatric between the two lineages (we discuss the geographic position of admixed individuals in greater detail below). Our hypothesis was

supported by higher estimates of genetic admixture from nuclear microsatellites in regions of overlapping E-space (Fig. 3 and Table 3). Additionally, we found an intriguing pattern of niche overlap that could explain the genetic admixture patterns documented in this study and a previous study. The fact that the E-space of the West lineage was a subset of the E-space of the East lineage (Figure 2) suggests that individuals from the East lineage might find dispersal to the West's geographic range especially tolerable. This pattern of asymmetrical overlap might explain three genetic patterns in this system, the first documented in this study and the last two from a previous study: (1) why the West lineage as a whole showed more genetic admixture (because it would receive more individuals from the East than it sent to the East), (2) why of the five individuals found with mtDNA haplotypes that did not match their geographic location, four of these indicated dispersal from East to West, and (3) why coalescent-based estimates of migration rates from microsatellites suggested higher gene flow from East to West (although the 95% confidence intervals were overlapping; Arteaga et al. in review). These results show how more detailed information on the link between niche characteristics and dispersal and gene flow can be obtained when broad tests of niche conservatism/divergence between lineages are coupled with individual-based genetic information.

Consequences of niche conservatism for connectivity and divergence of armadillos on the Mexican landscape

How the asymmetrical pattern of overlap in multivariate E-space between the two armadillo lineages has influenced their dispersal and evolutionary history is further elucidated by visualizing the geographic locations of individuals from shared and unique niche space (Fig. 4a) and their admixture levels (Fig. 4b). East individuals in niche space shared with

the West are numerous near a well-known lowland corridor linking the Mexican coasts, the Isthmus of Tehuantepec. This suggests that niches for the two lineages, while broadly conserved throughout their distributions, are especially similar in this geographic location. However, geographic proximity, while undoubtedly important, does not seem to be the sole driver of admixture patterns because in this region gene flow appears largely unidirectional from East to West (or more accurately near the Isthmus, north to south) into regions of niche overlap (Fig. 4b). Meanwhile, regions near the Isthmus that comprise part of E-space unique to the East (i.e., north of the Isthmus) show low levels of admixture (Fig. 4a and b; also see Table 3 for quantitative validation of these patterns). Additionally, within the geographic range of the West lineage (whose E-space is almost entirely subsumed within the E-space of the East lineage), highly admixed individuals (red dots in Fig. 4b) do not occur exclusively near the contact zone with the East near the Isthmus, but rather occur mostly along the West coast, almost exclusively in geographic regions where both lineages are predicted according to their ENMs (Fig. 4b). In fact, of the 23 individuals from both East and West that show the most genetic admixture (red dots in Fig. 4b), 16 occur in the relatively narrow geographic zone where ENMs for both lineages overlap.

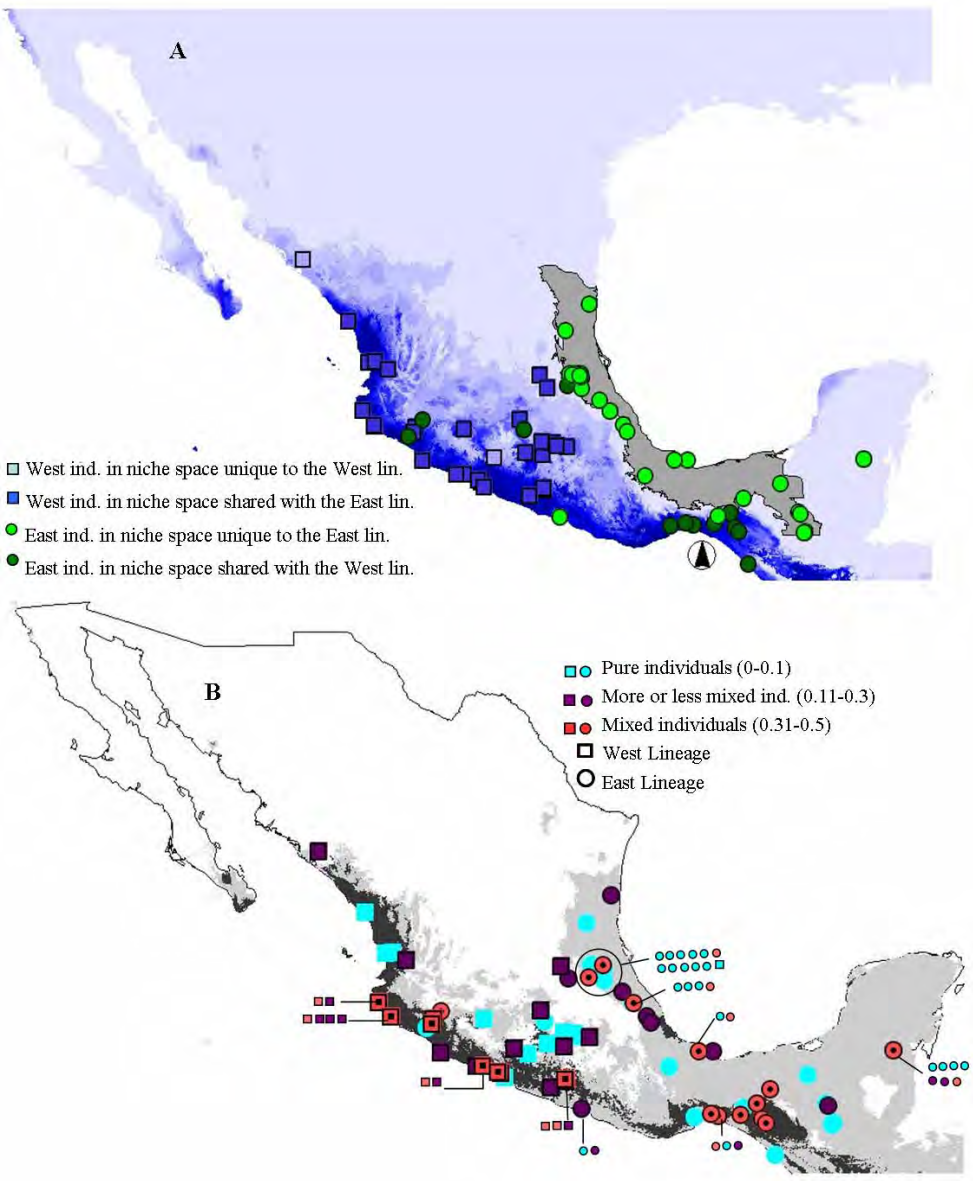

Figure 4. A) Visualization of the geographic locations of individuals from the four groups created by considering their positions in climatic E-space (see Fig. 3). East individuals in shared E-space occur primarily south of the Isthmus of Tehuantepec, whereas most West individuals occur in E-space shared with the East lineage. The biogeograhical region known as the Mexican Gulf Province is shown in gray. B) Genetic admixture of individuals visualized in geographic space. Regions where ENMs for East and West overlap are shown in black. A strong role for niche similarity in promoting gene flow, as opposed to geographic distance alone, is suggested by the large proportion of admixed East individuals to the south of the Isthmus of Tehuantepec, where East and West niches overlap, but not to the north of the Isthmus, where niches do not overlap. Additionally, highly admixed West individuals occur primarily in regions of niche overlap that are not necessarily in close proximity to geographical areas of contact. This results in a pattern of gene flow from East to West, toward regions of shared E-space, as validated quantitatively by the *t*-test results comparing admixture levels between East and West (Table 3).

Niche similarities seem to play an outsized role in genetic admixture among armadillo lineages, but the geography of dispersal should not be neglected. Our results suggest that admixture between armadillo lineages has likely not occurred by long-distance dispersal through the high mountain chains of Central Mexico, but rather via a habitat corridor through the Isthmus of Tehuantepec farther to the south. It is also possible that the East lineage originally occupied regions both north and south of the Isthmus after their original colonization, with the southern population becoming more admixed by influx of West individuals unimpeded by a physical barrier into suitable habitat. Meanwhile, the part of the niche most unique to the East lineage, which contains a high proportion of the genetically pure East individuals, lies in the coastal biogeographical unit known as the Mexican Gulf Province (Fig. 4a). This region has been very important in the biogeographical history of angiosperms (Méndez-Larios and Villaseñor 1995), insects (Hamilton 1994; Llorente-Bousquets et al. 1997), fish (Lydeard et al. 1995), amphibians (Arriaga et al. 1997), reptiles (Campbell and Lamar 1989), birds (Arriaga et al. 1997), and mammals (Müller 1973). Connectivity between these largely genetically pure East populations and those more admixed East populations on the other side of the Isthmus will require more detailed population-level study.

In conclusion, we have presented a novel combination of lineage-level tests of niche conservatism with more detailed individual-based estimates of the role of niche similarity in genetic admixture that could be applied to any study seeking to evaluate the role of niche evolution in diversification. The surprising conclusion that niche conservatism is facilitating gene flow is not so surprising when the dispersal capabilities of armadillos are considered, in addition to the fact that some of the regions of E-space that are especially similar occur close to a major lowland corridor linking the Mexican coasts. None of these

conclusions would have been possible without a conceptual framework and methodology that considers the duality of the niche (sensu Soberón and Nakamura 2009) as an abstract environmental space in addition to a geographically explicit realization of this environmental space on a map.

ACKNOWLEDGEMENTS

We wish to thank all the persons and institutions that provided biological samples for this study; they are listed in Table S1 (supporting information). We are grateful to Daniel Piñero, Osiris Gaona, Erika Aguirre, Laura Espinoza, Jaime Gasca and Alexander Correa for their help with laboratory analyses, technical support and advice in the data analysis. This work is part of M.C.A.'s PhD dissertation in biological sciences at the Universidad Nacional Autónoma de México (UNAM). M.C.A. is grateful to the graduate program Doctorado en Ciencias Biológicas and to UNAM for the scholarship granted for doctoral studies.

CITED LITERATURE

Arriaga, L., C. Aguilar, D. Espinosa-Organista, and R. Jiménez. 1997. Regionalización ecológica y biogeográfica de México. Taller de la Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), México, D.F.

Arteaga, M. C., D. Piñero, L. E. Eguiarte, J. Gasca, and R. Medellín. Phylogeography of a widely distributed species in a colonized area: nine-banded armadillo in Mexico. In review.

Broennimann, O., U. A. Treier, H. Müller-Schärer, W. Thuiller, A. T. Peterson, and A. Guisan. 2008. Evidence of climatic niche shift during biological invasion. Ecol. Lett. 10:701–709.

Campbell, J. A., and W. W. Lamar. 1989. The venomous reptiles of Latin America. Comstock, Cornell University Press, Ithaca and London.

Dormann, C. F., J. M. McPherson, M. B. Araújo, R. Bivard, J. Bolliger, G. Carlo, R. G. Davies, A. Hirzel, W. Jetz, W. D. Kissling, I. Kühn, R. Ohlemüller, P. R. Peres-Neto, B. Reineking, B. Schröder, F. M. Schurr, and R. Wilson. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography 30**:**609–628.

Eisenberg, J. F. 1981. The Mammalian Radiations. Chicago University Press, Chicago.

Escalante, T., G. Rodríguez, and J. J. Morrone. 2004. The diversification of the Nearctic mammals in the Mexican Transition Zone. Biol. J. Linn. Soc. 83:327–339.

Evans, M., S. Smith, R. Flynn, and M. Donoghue. 2009. Climate, niche evolution, and diversification of the "Bird-Cage" Evening Primroses (Oenothera, Sections Anogra and Kleinia). Am. Nat. 173:225–240.

Excoffier, L., G. Laval, S. Schneider .2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinf 1:47–50.

Graham, C. H., S. R. Ron, J. C. Santos, C. J. Schneider, and C. Moritz 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in Dendrobatid frogs. Evolution 58:1781–1793.

Gavrilets, S. 2003. Models of speciation: what have we learned in 40 years? Evolution 57:2197–2215.

Godsoe, W., E. Strand, C. Smith, J. Yoder, T. Esque, and O. Pellmyr. 2009. Divergence in an obligate mutualism is not explained by divergent climatic factors. New Phytol. 183:589–599.

Godsoe, W. 2010. Regional variation exaggerates ecological divergence in niche models. Syst. Biol. 59:298–306.

Hamilton, R. W. 1994. Revision of the New World genus *Pilolabus* Jekel (Coleoptera: Attelabidae). T. Am. Entomol. Soc. 120: 369–411.

Hugall, A., C. Moritz, A. Moussalli, and J. Stanisic. 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc. Nat. Acad. Sci. USA 99:6112–6117.

Humphrey, S. R. 1974. Zoogeography of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States. BioScience 24:457–462.

Kozak, K. H., and J. J. Wiens. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. Evolution 60:2604–2621.

Kozak, K. H., C. H. Graham, and J. J. Wiens. 2008 Integrating GIS-based environmental data into evolutionary biology. Trends Ecol. Evol. 23:141–148.

Llorente-Bousquets, J., L. Oñate, A. Luis, and I. Vargas. 1997. Papilionidae y Pieridae de México: distribución geográfica e ilustración. CONABIO y UNAM, México, D.F.

Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol. Lett. 11:995–1007.

Loughry W. J., R. W. Truman, C. M. McDonough, M. Tilak, S. Garnier S, and F. Delsuc. 2009. Is leprosy spreading among nine-banded armadillos in the southeast United States? J. Wildlife Dis. 45:144–152.

Lydeard, C., M. C. Wooten, and A. Meyer. 1995. Molecules, morphology, and area cladograms: a cladistic and biogeographic analysis of *Gambusia* (Teleostei:Poeciliidae). Syst. Biol. 44: 221–236.

Manel, S., M. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol. Evol. 18:189–197.

Mani, G. S., and B. C. Clarke. 1990. Mutational order: a major stochastic process in evolution. Proc. R. Soc. Lond. B 240: 29–37.

McBee K., and R. J. Baker 1982. *Dasypus novemcinctus*. Mamm. Species 162:1–9.

McCormack, J. E., B. S. Bowen, and T. B. Smith. 2008. Integrating paleoecology and genetics of bird populations in two sky island archipelagos. BMC Biology 6:28.

McCormack, J. E., A. J. Zellmer, and L. L. Knowles. 2010. Does niche divergence accompany allopatric divergence in *Aphelocoma* Jays as predicted under ecological speciation?: insights from tests with niche models. Evolution 64:1231–1244.

McNab, B. K. 1980. Energetics and the limits to a temperate distribution in armadillos. J. Mammal. 61:606–627.

Méndez-Larios, I., and J. L. Villaseñor. 1995. Revisión taxonómica del género *Tetranema* (Scrophulariaceae). Acta Bot. Mex. 32: 53-68.

Mertz, C. E. 1978. Basic principles in ROC analysis. Sem. Nucl. Med. 8:283–298.

Müller, P. 1973. The dispersal centres of terrestrial vertebrates in the Neotropical realm: a study in the evolution of the Neotropical biota and its native landscapes. Junk, La Haya.

Pearson, R. G., C. J. Raxworthy, M. Nakamura, and A. T. Peterson. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. J. Biogeogr. 34:102–117.

Peterson, A. T., J. Soberón, and V. Sánchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Peterson, A. T. 2001 Predicting species geographic distributions based on ecological niche modeling. Condor 103:599–605.

Phillips, S., R. Anderson, and R. Schapire. 2006. Maximum entropy modelling of species geographic distributions. Ecol. Model. 190:231–259.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Prodöhl, P., W. J. Loughry, C. M. McDonough, W. S. Nelson, and J. Avise. 1996. Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine-banded armadillo, *Dasypus novemcinctus*. Proc. R. Soc. Lond. B 263:1643–1649.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity 86:248-249.

Rice, W.R. 1989. Analyzing table of statistical tests. Evolution 43: 223–225.

Richards, C. L., B. C. Carstens, and L. L. Knowles. 2007. Distribution modeling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. J. Biogeogr. 34:1833–1845.

Rissler, L. J., and J. J. Apodaca. 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). Syst. Biol. 56:924–942.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol. Lett. 9: 615–629.

Simpson, G. G. 1980. Splendid Isolation. The Curious History of South American Mammals. Yale University Press, New Haven, Connecticut.

Soberón, J. 2007 Grinnellian and Eltonian niches and geographic distributions of species. Ecol. Lett. 10:1115–1123.

Soberón, J., and M. Nakamura. 2009. Niches and distributional areas: concepts, methods, and assumptions. Proc. Nat. Acad. Sci. USA 106:19644–19650.

StataCorp. 2003. Stata Statistical Software. StataCorp LP, College Station, TX.

Storfer, A., M. Murphy, J. Evans, C. Goldberg, S. Robinson, S. Spear, R. Dezzani, E. Delmelle, L. Vierling, and L. Waits. 2006. Putting the 'landscape' in landscape genetics. Heredity 98:128–142.

Swenson, N. G. 2008. The past and future influence of geographic information systems on hybrid zone, phylogeographic and speciation research. J. Evol. Biol. 21:421-434.

Taulman, J. F., and L. W. Robbins. 1996. Recent range expansion and distributional limits of the nine-banded armadillo *(Dasypus novemcinctus)* in the United States. J. Biogeogr. 23:635–648.

Walker, M. J., A. K. Stockman, P. E. Marek, and J. E. Bond. 2009. Pleistocene glacial refugia across the Appalachian Mountains and coastal plain in the millipede genus *Narceus*: evidence from population genetic, phylogeographic, and paleoclimatic data. BMC Evol. Biol. 9:25.

Warren, D. L., R. E. Glor, and M. Turelli. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883.

Wiens, J. J. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. Evolution 58:193–197.

Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology, and species richness. Trends Ecol. Evol. 19:639–44.

Wiens J. J., and C. H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Annu. Rev. Ecol. Evol. Syst. 36**:**519–539.

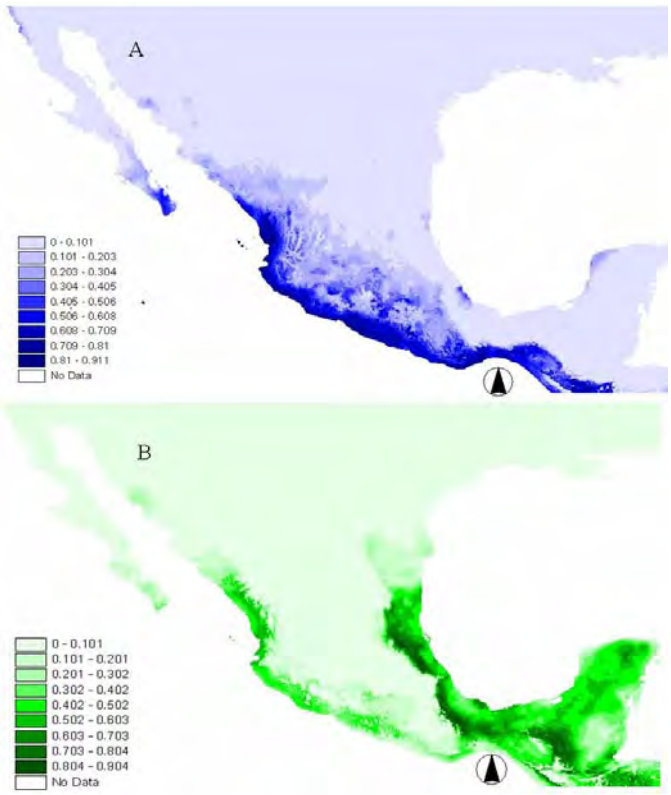

Figure S1. Environmental niche models for both mtDNA lineages (A: West lineage = blue; B: East lineage = green). High levels of predicted habitat suitability are indicated with darker colors.

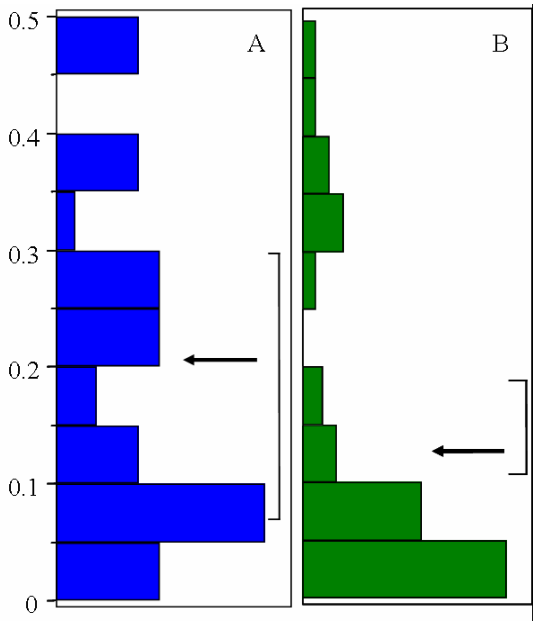

Figure S2. Frequency distributions of admixture scores of (A) the West (blue) and (B) the East (green) lineages. The arrows indicate the position of the mean, and the brackets indicate 95% credible intervals on the means of the admixture scores.

Table S1. *D. novemcinctus* samples obtained from mammal collection of different museums.

Collection names	Collection numbers of specimens	Total samples
Mammal collection of American Museum of natural History, US (AMNH)	26007, 207420, 24054, 176676	4
Colección Zoológica Regional, Instituto de Historia Natural y Ecología, México (CZRMA)	1581, 2255, 43, 50	4
Colección de la Reserva de la Biósfera Los Tuxtlas, México	a, b	2
Colección Mastozoológica del Sureste de México, México (ECOSUR-SC)	52, 575, 980, 1270, 1570, 1572	6
Colección de Mamíferos del Museo de Zoología "Alfonso L. Herrera", México (MZFC)	4254, 5078, 5079, 5075, 4900, 5077	6
Colección Mastozoológica HMAN Instituto Tecnológico Agropecuario de Hidalgo, México (HMAM)	540, 544, 536, 543, 547, 534, 541, 535, 545, 531, 539, 537, 546, 532, 548	15
Colección Nacional de Mamíferos, Instituto de Biología, UNAM, México (IBUNAM)	1153, 10069, 11535, 17037, 16559, 31600, 43121, 27275, 14528, 43122, 14527, 37069, 16496	13
Colección de mamíferos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México (ENCB)	21096, 39110, 35629, 26577, 36004	5
Colección de Mamíferos de la Universidad Michoacana San Nicolás de Hidalgo, México (UMSNH)	1323, 394, 887, c	4
TOTAL		59

a,b,c specimens without assign number

Table S2. Genetic variation estimates in the two major lineages at each of the five loci examined and their combination.

	Number of individuals	Number of alleles	Ho	He
West lineage				
Dnov1	39	9	0.850*	0.848
Dnov6	39	15	0.800*	0.892
Dnov7	39	5	0.375	0.51
Dnov16	39	7	0.175*	0.799
Dnov24	39	10	0.625*	0.855
All loci	39	9.2	0.565*	0.781
East lineage				
Dnov1	72	12	0.873*	0.873
Dnov6	72	17	0.816*	0.9
Dnov7	72	9	0.591*	0.74
Dnov16	72	10	0.211*	0.796
Dnov24	72	11	0.760*	0.85
All loci	72	11.8	0.650*	0.832

* Departures from HW at the $p<0.05$ level

4. DISCUSIÓN

4.1 Factores históricos

Para especies ampliamente distribuidas y que se encuentran a lo largo de diversas condiciones climáticas como *D. novemcinctus*, se esperan patrones simples de aislamiento genético por distancia geográfica, posiblemente solo alterados por barreras físicas a la dispersión (Slatkin 1993). Sin embargo, los eventos ocurridos a lo largo de la historia evolutiva de dichas especies, como los cambios en la demografía y la conectividad de sus poblaciones, pueden dejar huellas en la estructura genética de éstas (Avise 2000). Los resultados de este estudio indican la presencia de dos linajes divergentes de armadillo en México, que llegaron a esta región después de la formación del Istmo de Panamá. Estos linajes sufrieron expansiones demográficas históricas, esperadas para poblaciones en expansión y aparentemente arribaron de manera independiente. El linaje presente en el Este de México se postula como fuente de origen de las poblaciones de EUA. En esta área, que es el extremo norte de su distribución, la especie ha sido particularmente exitosa, mostrando una rápida colonización de nuevos territorios (Taulman & Robbins 1996) desde su primer registro ocurrido en el siglo XIX (Audubon & Bachman 1854).

El proceso de expansión del área de distribución de una especie puede generar bajos niveles de diversidad genética, ya que las poblaciones del frente de colonización generalmente son creadas por pocos inmigrantes (Austerlitz *et al.* 1997). Además, en las áreas periféricas del intervalo de distribución, las poblaciones pueden estar bajo presiones de selección ejercidas por las nuevas condiciones bióticas y abióticas a las que están expuestas. Es muy probable que esto explique los bajos niveles de variación genética registrados en las poblaciones de armadillo de EUA, comparados con las poblaciones de America del Sur y México (Huchon *et al.* 1999; Frutos & Van den Bussche 2002; Arteaga

et al. en revisión). Entre tanto, con el tiempo, el flujo génico entre poblaciones favorecerá un incremento en la diversidad local porque moverá los alelos de una población a otra (Austerlitz *et al.* 1997; Excoffier 2004; Excoffier *et al.* 2009). Asimismo, si las condiciones de los nuevos ambientes son adecuadas, los tamaños poblacionales podrán aumentar y aparecerán nuevas mutaciones que elevarán también los niveles de diversidad.

Los linajes registrados en México presentan una expansión demográfica desde hace ca. 500 000 años y probablemente nuevos haplotipos han surgido. Además, el flujo génico detectado con los marcadores nucleares indica un intercambio de alelos entre poblaciones, favoreciendo así el incremento de la diversidad de la especie en esta región. Su presencia probable desde el Pleistoceno y las altas tasas de migración pueden mitigar los efectos de la deriva génica causados durante la invasión de estos nuevos territorios. Sin embargo, a pesar de la expansión demográfica detectada con el ADNmt, los microsatélites nucleares muestran una reducción reciente de estos linajes, iniciándose para el linaje Oeste, hace aproximadamente 5000 años y para el linaje Este, hace 800 años. En este período, los tamaños efectivos de ambos han decrecido intensamente. Las posibles causas de esta disminución son la cacería a la que la especie ha sido sujeta, dado que las comunidades rurales la usan como fuente de proteína (Escamilla *et al.* 2000; León & Montiel 2008) y la alteración del hábitat por las actividades humanas. Ambos factores se han relacionado con el declive de las poblaciones de diversas especies (Luchini *et al.* 2004; Hájkova *et al.* 2007; Zhang *et al.* 2007; Mondol *et al.* 2008; Craul *et al.* 2009). Mientras que su larga presencia en esta región y las altas tasas de migración pueden amortiguar el efecto de la deriva génica, la disminución del tamaño efectivo por sobreexplotación, pone en riesgo el potencial evolutivo de esta especie.

Con relación al tiempo en que la especie llegó a México, podemos considerar los tiempos de expansión histórica como indicativo de esto y observamos que dichos tiempos anteceden el registro fósil de la especie en esta región. Para América Central y del Norte, los fósiles de *D. novemcinctus* corresponden al Holoceno (aproximadamente hace 8000 años; Álvarez 1974; Kurten & Anderson 1980). Sin embargo, una especie congenérica, *D. bellus*, ocurre en depósitos paleontológicos del Mioceno tardío y Pleistoceno de EUA (desde 2.5 m. a.), desde Nuevo México hasta Missouri y Florida (Schubert & Graham 2002), sin ningún registro para otros países. La relación evolutiva entre estas dos especies es aún desconocida y entenderla será fundamental para establecer una conexión histórica que probablemente ayude a explicar la discontinuidad entre los registros fósiles de ambas.

4.2 Atributos de la especie

El genoma del núcleo y de la mitocondria difiere en sus tasas de mutación, su modo de herencia y su tamaño efectivo poblacional (Hare 2001; Hedrick 2005). Estas diferencias determinan que sus niveles de variación sean interpretados en diversas escalas de tiempo y como consecuencia de atributos de la especie. La región control de la mitocondria, al presentar una tasa de mutación menor (7.6×10^{-8} substituciones por sitio por generación) que los microsatélites nucleares (2.7×10^{-4} substituciones por sitio por generación), revela los procesos históricos más antiguos, mientras que los últimos informan de procesos recientes. Por ello, la historia de la invasión de los dos linajes fue reconstruida con los datos de la mitocondria y las tasas de flujo génico se infirieron a partir de la poca estructuración observada en los datos del núcleo.

Algunos atributos de la especie pueden ser inferidos de las diferencias observadas en las estructuras genéticas entre ambos marcadores. Dado que estos difieren en su modo

de herencia, las especies con un patrón de filopatría asimétrica exhiben tasas de dispersión mayores en un género y esto produce diferentes patrones de estructuración genética en estos marcadores (Prugnolle & Meeus 2002). La fuerte estructura genética observada en la mitocondria y la estructura débil detectada con los microsatélites nucleares, sugieren que los machos se dispersan preferencialmente, manteniendo el flujo génico entre las poblaciones, mientras que las hembras permanecen en su población natal.

Otro factor que tiene influencia en los patrones de diversidad y estructuración es el sistema de apareamiento (Chepko-Sade & Halpin 1987), que en el caso de los armadillos es poligínico (McDonough 1997; McDonough & Loughry 2008). Este patrón involucra un muestreo no aleatorio del acervo genético de los machos en cada generación (Storz 1999). En especies con poliginia y filopatría por parte de las hembras, como el armadillo, las relaciones de parentesco entre los neonatos son menores, debido a que los machos generalmente son individuos inmigrantes que no poseen ninguna relación de parentesco con las hembras del grupo donde se reproducirán (Ross 2001). Esto reducirá la endogamia y su efecto sobre la variación genética de las poblaciones. La información genética obtenida en este estudio, permite hacer inferencias acerca de características del comportamiento de esta especie, siendo de gran utilidad para proponer hipótesis ecológicas e incrementar el conocimiento de su historia natural.

4.3 Factores ecológicos

La incorporación de las características abióticas a la interpretación de los patrones de diversidad genética comenzó recientemente, cuando los modelos de nicho ecológico (Richards *et al.* 2007) y las pruebas estadísticas (Warren *et al.* 2008; McCormack *et al.* 2010), fueron conjugadas con los patrones filogeográficos. Esta integración ha permitido la

formulación de hipótesis evolutivas relacionadas con modos de especiación y grados de conectividad genética ancestral. Mi predicción de conservadurismo de nicho entre los linajes mitocondriales de armadillo fue apoyada por el análisis estadístico usando los modelos nulos. Además, la exploración de los patrones de dispersión considerando los espacios ambientales ocupados por ambos linajes, proporcionó un acercamiento detallado sobre la biología de la especie y dio un marco enriquecedor sobre la unión de datos climáticos con la información genética.

La variación genética y su distribución en el espacio geográfico pueden ser también influenciadas por la disponibilidad de recursos, la densidad e identidad de competidores, los depredadores y los parásitos (Gaston 2003). La integración de estos factores apenas está comenzado (e.g. McCormack *et al.* 2010). Se requiere información que aún no están disponible y que es difícil de obtener, dado que las interacciones biológicas no son constantes en el espacio ni en el tiempo, sino que dependen del contexto comunitario donde la especie se encuentre (Thompson 2005). Estos factores generan diferentes intensidades en las presiones de selección a las que los individuos están sujetos e influyen en las tasas de crecimiento poblacional, repercutiendo finalmente en los niveles de diversidad genética de las poblaciones.

El uso simultáneo de marcadores moleculares de la mitocondria y el núcleo, así como la integración de las herramientas espaciales y estadísticas, ha permitido reconocer el aporte de diferentes factores relacionados con la historia, la ecología y los atributos de la especie, en los patrones de estructuración genética y de diversidad registrados en el norte del área de distribución del armadillo de nueve bandas. La información obtenida del material genético enriquece la reconstrucción histórica de los eventos ocurridos antes del Holoceno y aporta bases importantes para los futuros estudios ecológicos de la especie. Los

cambios climáticos que se están presentando en la actualidad pueden estar modificando el límite geográfico de la especie, manteniendo el carácter dinámico de su área de distribución.

4.4 Perspectivas

La distribución geográfica total de los dos linajes genéticos divergentes registrados en México y la presencia de otros linajes monofiléticos a lo largo de la distribución de la especie será posible abordarlos con el análisis de muestras de América Central y del Sur. La estimación del tiempo de origen de éstos permitirá relacionar su formación con eventos geológicos o climáticos ocurridos en sus áreas de distribución. La filogeografía estadística es una herramienta útil para proponer hipótesis concernientes con dicho origen.

El tiempo de entrada de los linajes a México será resuelto con muestras de América Central que se están recolectando actualmente. Con ello se propondrán nuevas inferencias acerca de su distribución geográfica actual y su participación en diferentes olas de invasión en los nuevos territorios. El uso de métodos de Coalescencia puede contribuir a determinar cómo se dio el avance de los armadillos después de cruzar el Istmo de Panamá y cuando ingresaron a México.

Otro aspecto que podrá abordarse con el análisis genético y que completará la historia de este género al norte del continente, será la relación filogenética entre *D. bellus* y *D. novemcinctus*. La reconstrucción a partir de datos morfológicos del primero indica su semejanza con *D. novemcinctus*, difiriendo principalmente en su tamaño corporal, ya que es mucho mayor (Kurten & Anderson 1980). Determinar la relación evolutiva entre estas dos especies es fundamental para establecer una relación histórica y buscar posibles razones a la discontinuidad entre los registros fósiles de ambas. Hoy existen métodos novedosos

usados para extraer DNA de fósiles (Hofreiter *et al.* 2001) y que pueden ser usados para conseguir secuencias cortas del DNA de *D. bellus*.

Con relación a los atributos de la especie inferidos en este estudio, se requiere hacer una evaluación de marcadores moleculares ligados al cromosoma Y para confirmar el patrón de dispersión sugerido por las diferencias en la estructuración genética de los diferentes marcadores usados.

La aproximación usada en este estudio puede ser aplicada para reconstruir el proceso de colonización de muchas otras especies que participaron en el intercambio de fauna del Pleistoceno. En especial, los diferentes enfoques usados, el genético y el ecológico, pueden ser aplicados al estudio de la estructura filogeográfica de especies que presenten amplia distribución ecológica y geográfica como el puma (*Puma concolor*) y el tlacuache (*Didelphis marsupialis*). El uso de diferentes herramientas permite tener un escenario más completo acerca de los procesos que explican la distribución geográfica de los linajes genealógicos

5. Literatura Citada (Introducción y Discusión)

Álvarez T. 1974. Identificación de los huesos rescatados en la segunda temporada en Santa Martha Chiapas (Cala HH/KK). In: *Excavaciones en el abrigo de Santa Marta Chiapas*, pp. 24–29. Serie Informes, Departamento de Prehistoria, Instituto Nacional de Antropología e Historia, México D.F.

Anderson R.P., Lew D., Peterson A.T. 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. Ecological Modelling, 162:211-232.

Audubon J.J., Bachman J. 1854. *Quadrupeds of North America, III.* Privately published by J. J. Audubon, New York.

Austerlitz F., Jung-Muller B., Godelle B., Gouyon P.-H.1997 Evolution of coalescence times, genetic diversity and structure during colonization. Theoretical Population Biology, 51:148–164.

Avise J.C., Arnold J., Ball Jr. R.M., Bermingham E., Lamb T., Neigel J.E., Reeb C.A., Saunders N.C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics, 18:489–522.

Avise J.C. 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.

Avise J.C. 2004. *Molecular Markers, Natural History, and Evolution* (Second Edition). Sinauer, Sunderland, MA.

Avise J.C. 2009. Phylogeography: retrospect and prospect. Journal of Biogeography, 36:3–15.

Carlini A.A, Vizcaíno S.F., Scillato-Yané G.J. 1997. Armoured xenarthrans: a unique taxonomic and ecologic assemblage. In: *Vertebrate Paleontology of the Miocene Honda Group, Republic of Colombia.* Kay R.F., Cifelli R.L., Flynn J.J., Madden R.H. (eds.). Pp. 213–226. Washington, Smithsonian. Institution Press.

Chan, L.M., Brown, J. L., & A. D. Yoder. 2011. Integrating statistical genetic and geospatial methods brings new power to phylogeography. Molecular Phylogenetic and Evolution, 59:523–537.

Chepko-Sade B.D., Halpin Z.T. 1987. M*ammalian Dispersal Patterns: The Effects of Social Structure on Population Genetics.* University of Chicago Press.

Coates A.G., Collins M-P. Aubry W.A. Berggren. 2004. The Geology of the Darien, Panama, and the late Miocene-Pliocene collision of the Panama arc with northwestern South America. Geological Society American Bulletin, 116:1327–1344.

Crandall K.A., Posada D, Vasco D. 1999. Effective population sizes: missing measures and missing concepts. Animal Conservation, 2: 317–319.

Craul M., Chikhi L., Sousa V., Olivieri G.L., Rabesandratana A., Zimmermann E., Radespiel U. 2009. Influence of forest fragmentation on an endangered large-bodied lemur in northwestern Madagascar. Biological Conservation, 142: 2862–2871.

Delsuc F., Vizcaíno S.F., Douzery E.J. 2004. Influence of tertiary paleoenvironmental changes on the diversification of South- American mammals: a relaxed molecular clock study within Xenarthrans. BMC Evolutionary Biology: 1-13.

Diniz-Filho J.A.F., Telles M.P.C., Bonatto S., Eizirik E., Freitas T.R.O., de Marco P., Santos F.R., Solé-Cava A., Soares T.N. 2008. Mapping the evolutionary twilight zone: molecular markers, populations and geography. Journal of Biogeography, 35: 1–11.

Escamilla A., Sanvincente M., Sosa M., Galindo-Leal C. 2000. Habitat mosaic, wildlife availability and hunting in the tropical forest of Calakmul, Mexico. Conservation Biology, 14: 1592–1601.

Excoffier L., Smouse P.E., Quattro J.M. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics, 131: 479–491.

Excoffier L. 2004. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. Molecular Ecology, 13:853–64.

Excoffier L., Foll M., Petit R.J. 2009. Genetic consequences of rangeexpansions. Annual Review of Ecology, Evolution, and Systematics, 40: 481–501.

Ferrusquía-Villafranca I. 1978. Distribution of Cenozoic vertebrate faunas in Middle America and problems of migration between North and South America; In*: Conexiones terrestres entre Norte y Sudamérica (Simposio Interdisciplinario sobre Paleografía Mesoamericana)*. Ferrusquía-Villafranca I. (ed.). Pp 193–321. UNAM, Instituto de Geología, Boletín 101.

Freeman P.W., Genoways H.H. 1998. Recent northern records of the nine-banded armadillo (Dasypodidae) in Nebraska. The Southwestern Naturalist, 43: 491-495.

Frutos S., Van den Bussche R. 2002. Genetic diversity and gene flow in nine-banded armadillo in Paraguay. Journal of Mammalogy, 83: 815–823.

Galbreath G.J. 1985. The evolution of monozigotic polyembriony in *Dasypus*. In: *Evolution and ecology of sloths, armadillos and vermiliguas.* Montgomery G.G. (Ed). Pp. 243–245. Smithsonian Institute Press, Washington, EUA.

Gaston K.J. 2003. *The Structure and Dynamics of Geographic Ranges.* Oxford University Press, Oxford.

Graham A. 2010. Late Cretaceous and Cenozoic history of Latin American vegetation and terrestrial environments. Missouri Botanical Garden.

Graham C.H., Ron S.R., Santos J.C., Schneider C.J., Moritz C. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. Evolution, 58:1781–1793.

Gradstein F.M., Ogg J.G., Smith A.G., Bleeker W., Lourens, L.J. . 2004. A new geologic time scale, with special reference to Precambrian and Neogene. Episodes, 27: 83–100.

Hájková P., Pertoldi C., Zemanova B., Roche K., Hájek B., Bryja J., Zima J. 2007. Genetic structure and evidence for recent population decline in Eurasian otter populations in the Czech and Slovak Republics: implications for conservation. Journal of Zoology, 272: 1–9.

Hare M.P. 2001. Prospects for nuclear gene phylogeography. Trends in Ecology and Evolution, 16: 700–706.

Hedrick P.W. 2005. *Genetics of Populations.* Third Edition. Jones and Bartlett Publishers, Massachusetts.

Hey J., Machado C.A. 2003. The study of structured populations—new hope for a difficult and divided science. Nature Reviews Genetics, 4:535–43.

Hewitt G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 359:183–195.

Hickerson M.J., Carstens B.C., Cavender-Bares J., Crandall K.A., Graham C.H., Johnson J.B., Rissler L., Victoriano P.F. Yoder A.D. 2010. Phylogeography´s past present and future: Ten years after Avise, 2000. Molecular Phylogentics and Evolution, 54: 291–301.

Hofreiter M., Serre D., Poinar H.N., Kuch M., Pääbo S. 2001. Ancient DNA. Nature Reviews Genetics, 2: 353–359.

Holt R.D. 2003. On the evolutionary ecology of species´s range. Evolutionary Ecology Research, 5: 159–178.

Huchon D., Delsuc F., Catzeflis F.M., Douzery E.J.P. 1999. Armadillos exhibit less genetic polymorphism in North America than in South America: nuclear and mitochondrial data confirm a founder effect in *Dasypus novemcinctus* (Xenarthra). Molecular Ecology, 8: 1743–1748.

Humphrey S.R. 1974. Zoogeography of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States. BioScience, 24:457–462.

Hutchinson G.E. 1957. Concluding remarks. *Cold Spring Harb.* Symp. Quant. Biol., 22: 415–427.

Jost L. 2008. *G*ST and its relatives do not measure differentiation. Molecular Ecology, 17: 4015–4026.

Knowles, L.L., Maddison W.P. 2002. Statistical phylogeography. Molecular Ecology, 11: 2623–2635.

Knowles L.L. 2009. Statistical phylogeography. Annual Review of Ecology, Evolution, and Systematics, 40: 593–612.

Kozak, K.H., Wiens J.J. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. Evolution 60:2604– 2621.

Kurten, B., Anderson E. 1980. *Pleistocene Mammals of North America.* Columbia University Press; New York.

Lanteri, A.A., Confalonieri V.A. 2003. Filogeografía: objetivos, métodos y ejemplos. In: *Una perspectiva latinoamericana de la Biogeografía.* Llorente J., Morrone J. J. (eds.). Pp 185–193. Facultad de Ciencias, UNAM, México.

León P., Montiel S. 2008. Wild meat use and traditional hunting practices in a rural mayan community of the Yucatan peninsula, Mexico. Human Ecology, 36: 249–257.

Lomolino M.V., Riddle B.R., Brown J.H. 2005. *Biogeography,* 3rd edition, Sinauer Associates, 845 pages.

Loughry W.J., Prodohl P.A., McDonough C.M., Avise J.C. 1996. Polyembryony in armadillos. American Scientist, 86: 274–279.

Loughry W.J., McDonough C.M. 1998. Spatial patterns in a population of nine-banded armadillos (*D. novemcinctus*). American Midland Naturalist, 140:161–169.

Loughry W.J., McDonough C.M. 2001. Natal recruitment and adult retention in a population of nine-banded armadillos. Acta Theriologica, 46: 393–406.

Loughry W.J., McDonough C.M. (in press). The nine-banded armadillo. University of Oklahoma Press, Norman, Oklahoma.

Lucchini V., Galov A., Randi E. 2004. Evidence of genetic distinction and long-term population decline in wolves (*Canis lupus*) in the Italian Apennines. Molecular Ecology, 13: 523–536.

Mantel, N.A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research, 27: 209–220.

Marshall L.G., Butler R.F., Drake R.E., Curtis G.H., Tedford R.H. 1979. Calibration of the Great American Interchange. Science, 204: 272–279.

Marshall L.G., Webb S.D., Sepkoski J.J., Raup D.M. 1982. Mammalian Evolution and the Great American Interchange. Science, 215: 1351–1357.

Marshall L.G., Sempere T. 1993. Evolution of the Neotropical Cenozoic Land Mammal Fauna in its geochronologic, stratigraphic, and tectonic context, In: *Biological Relationships between Africa and South America.* Goldblatt. P. (ed). Pp. 329-392. New Haven, Yale University Press.

McBee K., Baker R.J. 1982. *Dasypus novemcinctus*. Mammalian Species, 162: 1–9.

McCormack J.E., Zellmer A.J., Knowles L.L. 2010. Does niche divergence accompany allopatric divergence in *Aphelocoma* Jays as predicted under ecological speciation?: Inside from test with niche models. Evolution, 64: 1231–1244.

McDonald H.G. 2002. Fossil Xenarthra of Mexico: A review. In: *Avances en los estudios paleomastozoologicos en México.* Ballesteros M.M., Arroyo-Cabrales J. (eds). Pp 2-13. Serie Arqueologica, Instituo Nacional de Antropologia e Historia.

McDonough C.M. 1997. Pairing behavior of the nine-banded Armadillo (*Dasypus novemcinctus*). American Midland Naturalist, 138**:** 290–298.

McDonough CM., W.J. Loughry. 2008. Behavioral ecology of armadillos. In: *The Biology of the Xenarthra.* Vizcaíno S.F., Loughry W.J. (eds.). Pp. 281–293. University Press of Florida, Gainesville.

McNab B. K. 1980. Energetics and the limits to a temperate distribution in armadillos. Journal of Mammaloly, 61: 606–627.

Moncrief N.D. 1988. Absence of genic variation in a natural population of nine-banded armadillos, *Dasypus novemcinctus* (Dasypodidae). The Southwestern Naturalist, 33: 229–231.

Mondol S., Karanth K.U., Ramakrishnan U. 2009. Why the Indian Subcontinent Holds the Key to Global Tiger Recovery. PLoS Genetics, 5: e1000585.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321–3323.

Nordborg M. 2001. Coalescent theory. In: *Handbook of statistical genetics.* Balding D.J., Bishop M.J., Cannings C. Pp. 179–212. Chichester, United Kingdom, John Wiley and Sons.

Pascual R., Ortiz-Jaureguizar E. 2007. The Gondwanan and South American Episodes: Two major and unrelated moments in the history of the South American mammals. Journal of Mammalian Evolution, 14: 75–137.

Peterson A.T., Soberón J., Sánchez-Cordero V. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Posada D., Crandall K.A. 2001. Intraspecific gene genealogies: trees grafting into cladograms. Trends in Ecology and Evolution, 16:37–45.

Prado, J.L. Alberdi M. T., Azanza B., Sanchez B., Frassinetti D. 2005. The Pleistocene Gomphotheriidae (Proboscidea) from South America. Quaternary International, 126–128: 21–30.

Prodöhl P., Loughry W.J., McDonough C.M., Nelson W.S., Avise J. (1996) Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine-banded armadillo, *Dasypus novemcinctus*. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 263: 1643–1649.

Prodöhl P.A., Loughry W.J., McDonough C.M., Nelson W.S., Thompson E.A., Avise J.C. 1998. Genetic maternity and paternity in a local population of armadillos assessed by microsatellite DNA markers and field data. American Naturalist, 151: 7–19.

Prugnolle F., De Meeus T. 2002. Inferring sex-biased dispersal from population genetic tools: a review. Heredity, 88: 161–165.

Ramsey P.R., Grigsby B.A. 1985. Protein variation in populations of *Dasypus novemcinctus* and comparisons to *D. hybridus, D. sabanicola* and *Chaetophractus villosus*. In: *Evolution and ecology of sloths, armadillos and vermiliguas.* Montgomery G.G. (ed). Pp. 131–141. Smithsonian Institute Press, Washington, EUA.

Redford K.H. 1985. Food habits of armadillos (Xenarthra: Dasypodidae), In: *The evolution and ecology of armadillos, sloths, and vermilinguas.* Montgomery G.G. (ed.). Pp. 429-437. Smithsonian Institute Press, Washington, EUA.

Richards C.L., Carstens B.C., Knowles L.L. 2007. Distribution modelling and statistical phylogeography: an integrative framework for testing biogeographic hypotheses. Journal of Biogeography, 34:1833-1845.

Rissler L.J., Hijmans R.J., Graham C.H., Moritz C., Wake D.B. 2006. Phylogeographic lineages and species comparisons in conservation analysis: a case study of California herpetofauna. American Naturalist, 167:655–666.

Rogers A.R., Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution, 9:552–569.

Rosenberg N.A., Nordborg M. 2002. Genealogical trees, Coalescent theory and the analysis of genetic polymorphisms. Nature Reviews Genetics, 3: 380-390.

Ross K.G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Molecular Ecology, 10: 265-284.

Sagarin R.D., Gaines S.D. 2002. The `abundant centre' distribution: to what extent is it a biogeographical rule?. Ecology Letters, 5: 137–147.

Sagarin R.D. 2006. Recent studies improve understanding of population dynamics across species ranges. Oikos, 115: 386–388.

Shaw C.A., McDonald H.G. 1897. First record of giant ant-eater (Xenarthra, Myrmecophagidae) in North America. Science, 236: 186–188.

Shubert B.W., Graham R.W. 2000. Terminal pleistoncene armedillo (*Dasypus*) remains from the ozark plateau, Missouri, USA. PaleoBios, 20: 1–6.

Simpson G.G. 1980. *Splendid Isolation.* Yale Univ. Press, New Haven.

Slatkin M. 1993. Isolation by Distance in Equilibrium and Non-Equilibrium Populations. Evolution, 47: 264–279.

Smith H.A., Robins T. J., Van Viuren J. 2007. Coalescence methods reveal the impact of vicariance on the spatial genetic structure of *Elephantulus edwardii* (Afrotheria, Macroscelidae). Molecular Ecology, 16: 2680–2692.

Soberon J. 2007 Grinnellian and Eltonian niches and geographic distributions of species. Ecology Letters, 10: 1115–1123.

Storz J.E. 1999. Genetic consequences of mammalian social structure. Journal of Mammalogy, 80:553–569.

Strimmer K., Pybus O.G. 2001. Exploring the demographic history of DNA sequences using the generalized skyline plot. Molecular and Biological Evolution, 18: 2298–2305.

Taulman, J.F., Robbins L.W. 1996. Recent range expansion and distributional limits of the nine-banded armadillo *(Dasypus novemcinctus)* in the United States. Journal of Biogeography 23: 635–648.

Thompson J. 2005. *The Geographic Mosaic of Coevolution.* University of Chicago Press.

Tosdal R.M., Clark A.H., Farrar R.E. 1984. Cenozoic polyphase landscape and tectonic evoltion of the cordillera occidental, southern- most Peru. Geological Society American Bulletin, 95: 1318–1382.

Vucetich J.A., Waite T.A. 2003. Spatial pattern of demography and genetic processes across the species´s range: null hypoteses for landscape conservation genetics. Conservation Genetics, 4: 639–645.

Warren D.L., Glor R.E., Turelli M. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution, 62:2868–2883.

Webb S.D. 1976. Mammalian faunal dynamics of the great american interchange. Paleobiology, 2: 216–234.

Wetzel R.M. 1985. Taxonomy and distribution of armadillos, Dasypodidae. In: *The Evolution and Ecology of Sloths, Armadillos, and Vermilinguas,* G.G. Montgomery (ed.), Pp. 23–46. Smithsonian Institute Press, Washington, EUA.

Wetzel R.M., Gardner A.L., Redford K.H. J.F. Eisenberg. 2007. Order Cingulata. In: *Mammals of South America, Volume 1. Marsupials, Xenarthrans, Shrews, and Bats,* A.L. Gardner (ed.), Pp 128–156. The University of Chicago Press, Chicago.

Wright S. 1951. The genetical structure of populations. Annals of Eugenics, 15: 323–354.

Wiens J.J., Graham C.H. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution and Systematic, 36: 519–539.

Zhang B., Li M., Zhang Z., Goossens B., Zhu L., Zhang S., Hu J., Bruford W., Wei F. 2007. Genetic viability and population history of the giant panda, putting an end to the 'Evolutionary Dead End'. Molecular Biology and Evolution, 24: 1801–1810.