

After the JMIH banquet on 17 July, the CAH/ACH executive (Pat Gregory, David Green, and Jackie Litzgus) had an impromptu meeting over beers by a fountain in the Sheraton hotel lobby. Secretary Jackie took the minutes on her beer napkin. Our agenda covered two topics: the distinguished Canadian herpetologist award, and the need for a CAH/ACH website.

The CAH/ACH has historically had a distinguished Canadian herpetologist award, and the award has previously been given to Francis Cook and Jim Bogart. Given the recent passing of one of our distinguished Canadian herpetologists, Michael Rankin, the executive voted unanimously to rename the award the Michael Rankin Distinguished Canadian Herpetologist Award. The award is given in recognition of outstanding service to the advancement of herpetology in Canada, and each recipient will receive a framed certificate.

As mentioned above, we need a webmaster. The executive discussed the location of the website and it was decided that David would look into the possibility of having the Redpath Museum as the CAH/ACH website home base. We hope to include on the website links to all previous issues of the BULLETIN.

FEATURE ARTICLE

On the Origins and Dispersal of Neotropical Rattlesnakes in South America

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Three major biogeographical events have been proposed as cause of present-day patterns of distribution and diversity in the Neotropics: The final uplift of the Andes in the late Tertiary, the uplift of the Isthmus of Panamá above sea level approximately 3.5 million years ago (Mya), and the series of global climatic fluctuations occurring throughout the late Pliocene and Pleistocene (reviewed in Wüster et al., 2002; 2005a). The uplift of the Isthmus of Panama resulted in a dramatic large-scale dispersal of terrestrial faunal elements, a phenomenon referred to as the Great American Biotic Interchange or GABI (Stehli & Webb, 1985; Wüster et al., 2005). Among the faunal elements involved in GABI, several groups of pit vipers dispersed in both directions between North and South America (Wüster et al., 2002; 2005a). The best documented example of a pit viper with a pattern describing the dispersal event associated with GABI constitutes the phylogeography of the Neotropical rattlesnakes (Wüster et al., 2002; Quijada-Mascareñas, 2005; Wüster et al., 2005a; 2005b). Our research shows a comprehensive story of the dispersal of these rattlesnakes into South America and its implications in Neotropical biogeography.

Rattlesnakes are represented by approximately 30 species primarily of Nearctic distribution that probably originated in north-central Mexico (Campbell and Lamar, 2004; Place and Abramson, 2004). Neotropical rattlesnakes are the only representatives that extend south of Mexico and are the most widely distributed rattlesnakes in the Americas, exhibiting considerable morphological variation. These snakes occur in seasonally dry formations from Mexico to northern Argentina, but are absent from the Central American and Amazonian rainforests, resulting in a highly discontinuous distribution (Campbell and Lamar, 2004; Wüster et al., 2005a).

The Neotropical rattlesnake group includes large snakes that commonly reach more than 1.5 m in length in most of the known distribution, but some South American populations apparently are dwarfed, including those in the inland savannas and highlands of Venezuela and Guyana and on the island of Aruba, where the largest specimens do not exceed 1 m in length (Campbell and Lamar, 2004). On the other hand, these snakes exhibit a considerable amount of ground colour variation, which hinders the taxonomy of this species. As a broad generality, the ground colour of snakes from the Pacific lowlands of Guatemala and parts of northern South America tends to be considerably darker than in snakes from more arid regions, such as the Rio Balsas or Rio Motagua valleys in Mexico and Guatemala, respectively (Klauber, 1972; Campbell and Lamar, 2004). Other characters like body blotches, head pattern, paravertebral stripes, and head scutellation exhibit variation, but the extent and amount by which these characters help to distinguish the different populations of these rattlesnakes still needs to be explored.

Members of the Neotropical group of rattlesnakes are usually found in semi-arid regions, including dry to very dry tropical forest and thorn woodlands, but also in relatively dry open areas within wet forests (Campbell and Lamar, 2004). It is largely absent from tropical rainforests. Interestingly, in parts of Brazil, the species has been able to expand its range due to the clearing of the rainforests for agriculture, for instance in the state of Rio de Janeiro (Bastos et al., 2005). Activity patterns vary according to latitude and habitat. In North and Central America, activity depends largely on season, due to hibernation in winter (Klauber, 1972; Campbell and Lamar, 2004). The rattlesnakes' prey consists mainly of rodents and birds. Some degree of ontogenetic change in diet has been observed in the North and Central America populations, but in South

America this pattern is absent (Campbell, 1998; Salomão et al., 1997).

The highly discontinuous distribution of the Neotropical rattlesnake in South America, including open habitats both north and south of the Amazon rainforest as well as isolated open formations within it, but avoiding the rainforest itself, offers an interesting system to study past changes in their distribution and associated habitat. The large size of these rattlesnakes makes them unlikely candidates of wind dispersal. The prevailing North Brazil current runs southeast to northwest, against the direction of dispersal, making oceanic dispersal improbable. Therefore, their discontinuous distribution is most likely due to vicariance, the timing of which had been inferred using molecular methods and calibrating points of geological events (Wüster et al., 2002; 2005a). By using these approaches, we were able to not only describe the dispersal pattern, but also make inferences on the possible changes of the Amazon rainforest cover during the Pleistocene.

The results of our research may have implications in medical grounds: Neotropical rattlesnakes are responsible for substantial numbers of snakebite cases in South America; an improved understanding of the species' biology and evolution will provide a framework for venom research and improved antivenom design. Several types of venom variation in the Neotropical rattlesnakes have been reported, including geographic and ontogenetic variation (Gutierrez et al., 1990; 1991; Saravia et al., 2002; Warrell, 2004). This is of considerable importance to snake bite treatment because venom therapy depends greatly on the antivenom, so the antivenom prepared for one variant might not provide adequate protection against a bite by another (Wüster et al., 1999; Warrell, 2004).

Systematics of the Neotropical Rattlesnakes.-

Due to their morphological variation, the systematics and taxonomy of the Neotropical

rattlesnakes have been difficult to resolve and the group is considered a taxonomic complex: the *Crotalus durissus* complex (Murphy et al., 2002; Campbell and Lamar, 2004; Wüster et al., 2005a). Only recently, our investigations based on phylogenetic analysis of DNA data have revealed more details on the phylogenetic relationships between the different populations of Neotropical rattlesnakes (Wüster et al., 2005a; 2005b).

In attempt to simplify the complexities of the taxonomy of the *C. durissus* complex, Campbell and Lamar (2004) and Savage et al. (2005) redefined the nominate form of *C. durissus* as that occurring in the coastal areas of the Guyanas, and resurrected the name *Crotalus simus* for the Central American populations. This means that the Guyanan snakes known until now as *C. durissus dryinas* are now *C. d. durissus*, whereas the Central American snakes previously known as *C.d. durissus* are now *C. simus*.

Our research, based on sequences of several DNA regions (Wüster et al., 2005a), provided a phylogenetic background for the resolution of problems affecting Neotropical rattlesnake taxonomy. Our main conclusion is that Mexican and Central American populations correspond to very old lineages with divergences equivalent to full species level, whereas all South American rattlesnakes are closely related, the result of a recent radiation in South America. In particular, the northernmost population from north-eastern Mexico, previously known as *C. durissus totonacus*, was found to be more closely related to the black-tailed rattlesnake, *C. molossus*, than to the other populations of the *C. durissus* complex, and clearly represents a separate species, *Crotalus totonacus*, supporting previous views. However, the populations from the Yucatan Peninsula and from southwestern Mexico also represent two different species, *Crotalus tzabcan* and *C. culminatus*, respectively (Fig. 1). This suggests that the taxon *C. simus* may

be restricted to those populations located in Southern Mexico and Central America.



Figure 1. Specimens of *Crotalus tzabcan* (top) and *C. culminatus* (bottom) representing the ancestral old lineages of Neotropical rattlesnakes that posteriorly dispersed into South America. Photos by Wolfgang Wüster and Adrian Quijada Mascareñas, respectively.

In contrast, our molecular data show all South American populations of the Neotropical rattlesnake to be closely related, including those taxa considered separate species by some authors (e.g., Klauber, 1972). For instance, the populations from Aruba (*C. unicolor*) and the Maturín savanna of NW Venezuela (*C. vegrandis*), often recognised as distinct species based primarily on differences in coloration and pattern, would be better placed among the other South American populations of *C. durissus*, as was previously proposed (Wüster et al., 2002; 2005a; Campbell and Lamar, 2004; Savage et al., 2005).

As remarks on venom variation, populations from southern Brazil (*C. d. terrificus*) have long been notorious for their extremely lethal venom, with potent neuro-myotoxic activity, resulting in paralysis, muscle breakdown and kidney failure, and a correspondingly high fatality rate from bites, whereas the clinical picture following bites by Central American populations is generally similar to that of most North American rattlesnakes, with extensive local tissue damage and haematological abnormalities (Warrell, 2004). Interestingly, the differences between Central and South American snakes appear to be in part due to ontogenetic variation, (i.e.) variation that occurs during the growth of the rattlesnakes. Gutiérrez et al. (1991) and Saravia et al. (2002) compared the venoms of adult specimens and newborns of Central American *C. simus* from and South American *C. durissus*. Using experimental mice, they found that venoms of *C. d. terrificus*, *C. d. cumanensis* (Brazil and Venezuela) and newborn *C. simus* (Costa Rica) induced higher lethal and myotoxic effects than those of adult *C. simus* (Guatemala and Costa Rica). In contrast, adult *C. simus* and *C. d. cumanensis* venoms induced haemorrhage, whereas venoms of *C. d. terrificus* and newborn *C. simus* lacked this effect. The similarity of the venoms of young *C. simus* and adult *C. d. terrificus* suggests that the highly lethal neuro-myotoxic venoms of the South American rattlesnakes of all sizes may be a case of neoteny, where juvenile traits are retained into adulthood, an interesting cause of geographic variation in snake venom composition.

Natural History of Dispersal of Neotropical Rattlesnakes into South America.-- Our recent research on the phylogeography and systematics of Neotropical rattlesnakes using DNA regions has produced a clear picture of the events associated with the origin and dispersal of these snakes to South America.

Here we summarize the main events in the evolution of the *Crotalus durissus* complex. The rattlesnake genus *Crotalus* almost certainly originated somewhere in Mexico approximately 20-30 million years ago (Klauber, 1972; Knight et al., 1993; Place and Abramson, 2004). It has been proposed that the highlands of Mexico are the centre of rattlesnake diversification (Place and Abramson, 2004). The ancestor of the Neotropical rattlesnakes probably originated as a consequence of mountain formation in central Mexico and the uplift of the Isthmus of Tehuantepec (Flores, 1993; Graham, 1993). During the last 50 million years, the formation of the Sierra Madre Oriental mountain range provided a diversity of upland habitats which facilitated the evolution of many new species including rattlesnakes (Flores, 1993; Graham, 1993).

There was a decline in global temperatures during the mid-Miocene, approximately 16 million years ago, with documented effects on the biota of southern Mexico. Such physiographic and climatic fluctuations not only affected speciation rates but also provided a diversity of habitats for the perpetuation of new forms of reptiles (Flores, 1993). These changes probably produced speciation events from which the different old lineages of Neotropical rattlesnakes originated. An extensive new habitat area to the south became increasingly available as South America moved closer to North America and the Panamá land bridge became established (Flores, 1993). These biogeographic conditions probably produced the Neotropical rattlesnake lineages of Pacific Mexico and Central America (Quijada-Mascareñas, 2005). The phylogenies obtained from our analysis of mitochondrial sequences indicates that the Neotropical group separated from the *C. molossus* and *C. basiliscus* clades about 13 millions years ago, and during the subsequent nine millions year

the lineages from Yucatan and the Pacific coast diversified.

The rise of the Isthmus of Panamá was the most important event to structure the biogeography of the formerly isolated continents of North America and South America. Our investigations indicate that the Neotropical rattlesnake was an element of the Great American Biotic Interchange and dispersed towards South America shortly after the final emergence of the Panamá land bridge (Wüster et al., 2002; 2005a). The monophyly and the low levels of sequence divergence among the South American populations are consistent with the hypothesis that this species invaded the South American continent 2-1.5 million years ago, after the uplift of the Panamá land bridge (Bermingham and Martin, 1998; Wüster et al., 2002; 2005). Presumably, prior to this dispersal event, the Central American lineages had already evolved (e.g. *C. simus*), and the lineage that dispersed into South America was present near the primitive Panamanian land bridge and crossed during the early Pleistocene period, when presumably a savannah corridor was established throughout the isthmus (Vanzolini and Heyer, 1985).

After the dispersal of the Neotropical rattlesnake into northern South America, the species rapidly invaded dry savannah habitats throughout the continent in a relatively short period of time, and established itself both north and south of the Amazon forest approximately 1 million years ago. This discontinuity and the low genetic differentiation of the South American rattlesnake populations have provided new insights into the history of the Amazonian forest. During the late Pliocene and Pleistocene (i.e., the last 2-3 million years), a series of global climatic fluctuations affected patterns of Neotropical diversity. The Pleistocene refugia hypothesis argues that drier climatic phases caused a fragmentation

of the Amazonian rainforest into forest refugia isolated by savannahs and seasonal dry forests, leading to increased speciation among forest species (Haffer and Prance, 2001). However, the notion of Pleistocene rainforest fragmentation remains highly controversial (Gosling and Bush, 2005; Wüster et al., 2005a; 2005b; Quijada-Mascareñas et al., 2006).

Based on our phylogenetic research on Neotropical rattlesnakes, the pattern of evolution of the South American populations suggests that wet and dry cycles and Amazon rainforest contraction probably did occur. The lack of genetic differentiation between rattlesnake populations from north and south of the Amazon supports the hypothesis that the Amazon forests have gone through at least one cycle of fragmentation during the Pleistocene since the colonisation of South America. Furthermore, our calculated molecular divergence times indicate that the Trans-Amazon dispersal across the Amazon Basin can be dated to the early to mid-Pleistocene, approximately 1 million years ago (Wüster et al., 2005a; 2005 b). Furthermore, new analysis of sequences using haplotype networks and historical demography methods indicates that North Amazon populations were genetically connected with central south Amazon, strongly suggesting the existence of an extensive central Amazon corridor, evidence of Pleistocene fragmentation of the rainforest (Quijada et al., 2006). Our study also illustrates that, somewhat ironically, research on species that currently live *outside* of the Amazonian forests may tell us as much about the history of those forests as species currently living *inside* them.

Future Research.--Our collaborative research is moving in new directions in order to test our earlier views regarding pit viper biogeography in the Neotropical region. We are now exploring phylogenetic relationships

in other species that apparently dispersed and colonized new regions during GABI. The genus *Bothrops* is a good candidate, whose distribution and hypothetical dispersal pattern is similar to the Neotropical rattlesnake but in the opposite direction. A clear prediction rises from the hypothesis generated according to *Bothrops*' geographical distribution: Old ancestral lineages in South America forests and newer lineages in the northern part of it distribution, especially in the regions of Mexico and Guatemala.

Regarding the Neotropical rattlesnakes, we still need phylogenetic information from isolated populations within the Amazon basin, particularly those isolated deep inside Amazonia and in the path of a likely trans-Amazonian dispersal corridor, like Humaitá, Santarém, and Serra do Cachimbo (Campbell and Lamar, 2004). The phylogenetic relationships of these populations with those from north and south of the Amazon basin would provide powerful additional evidence on the distributional history of *C. durissus* in South America and changes in the distribution of the Amazon rainforest in the past.

Finally, we are researching possible constraints in habitat and ecological preferences of Neotropical rattlesnakes using niche modeling (Ortega-Rodriguez J.M., Wüster W., and Quijada-Mascareñas, in prep.). This approach will allow us to combine our phylogenetic-historical information with ecological variables associated with the distribution of these rattlesnakes. This research is of pivotal importance due to our perception that habitat preferences of Neotropical rattlesnakes are historically quite conserved. This will not only support our inference about Pleistocene distributional changes due to climatic cycles in the past, but also will be the baseline to predict future changes in the distribution of the Neotropical rattlesnakes which may be the product of anthropogenic alteration of habitat and current climate changes.

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FIELD NOTES

Interesting Nesting Behaviour in a Northern Population of Wood Turtles (*Glyptemys insculpta*)

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Introduction

Every year from May until late July (Powell, 1967), depending on weather conditions and geographic location (Ernst et al., 1994), female wood turtles (*Glyptemys insculpta*) begin movements towards their nesting grounds. Nesting can occur within meadows, on railroad beds, logging roads, forest openings, river banks (Harding and Bloomer, 1979) or within gravel pits (Foscarini, 1994; Walde, 1998), and fidelity to these nest sites has been previously reported (Walde, 1998). In Michigan, female wood turtles chose nesting sites that supplied ample exposure to direct sunlight, sand or soil substrate that retained moisture yet was well-drained and not subject to flooding, and a