

Threatened rattlesnakes' inbreeding makes species more resistant to bad mutations

October 4 2021, by Emily Caldwell



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The first look at a threatened rattlesnake species' recent genetic history suggests that inbreeding necessitated by limited habitat may not be as detrimental as theory would predict it to be.



In fact, scientists speculate that Eastern massasauga rattlesnakes may have pre-adapted to living in small, isolated populations—where the most dangerous genetic mutations that arose could be easily exposed and purged.

Researchers sequenced the genomes of 90 Eastern massasauga rattlesnakes, which were listed as threatened under the Endangered Species Act in 2016 because of loss and fragmentation of their wetland habitat. For comparison, the researchers also sequenced 10 genomes of a close relative, the Western massasauga rattlesnake, a <u>common species</u> with no limitations on breeding opportunities and large populations.

The Ohio State University team found that the most potentially damaging gene mutations were less abundant in the Eastern than the Western <u>species</u>. This finding suggests the breeding limitations of small, isolated populations might be accompanied by an <u>evolutionary advantage</u> of being able to elbow out genetic variants that get in the way of survival, said H. Lisle Gibbs, professor of evolution, ecology and organismal biology at Ohio State and senior author of the study.

"This is something that has been reported very recently in other endangered species, but it's the first time it's been shown in a reptile," Gibbs said. "We always worry about genetics and the loss of variation and what it means to be in a small <u>population</u> in which there's lots of inbreeding. At least in this species, maybe it's not such a big deal.

"From a conservation perspective, perhaps we can downplay genetics and say ecology—such as habitat restoration—is more important."

Gibbs completed the study with Alexander Ochoa, a former postdoctoral researcher at Ohio State who is now a postdoctoral scholar at the University of Central Florida. The research is published in the journal *Molecular Ecology*.



Eastern massasauga rattlesnakes live in isolated spaces in midwestern and eastern North America, and evolutionary theory posits that the inevitable inbreeding in such populations threatens species with extinction as genetic mutations accumulate. The smallest populations might reach 30 snakes, but Ohio's Killdeer Plains Wildlife Area is home to one of the most genetically diverse and largest populations in the country, numbering in the thousands.

Gibbs has studied Eastern massasaugas for over two decades and, as director of the Ohio Biodiversity Conservation Partnership, advises the Ohio Department of Natural Resources on management of the species.

"Through years and years of study, we know that most populations are isolated, like little natural zoos scattered throughout the landscape," Gibbs said. "Due to habitat degradation, we've known they show little variation—but we've never actually looked at variation in genes that code for things that matter to a rattlesnake."

Only recently has it been possible to apply the research techniques perfected with the <u>human genome</u> to work with this species. Gibbs and Ochoa zeroed in on identifying mutations in genes that may affect survival and reproduction to gauge how hazardous inbreeding might be to Eastern massasaugas.

Though a higher overall number of potentially deleterious mutations were found in the common Western massasaugas, that didn't translate to more threats to their survival because most troublesome gene copies were offset by protective copies. That can happen only in heterozygotes, which have two different copies, or alleles, of a particular gene—one inherited from each parent. Because of generations of inbreeding, Eastern massasaugas are much more likely to have two copies of the same allele.



"That's why inbreeding has impacts—because that's when you get two bad alleles showing up together, with no good allele to compensate, so there is a negative effect," Gibbs said. "There's more inbreeding, so overall you get more mostly bad mutations together, but the really bad ones, because they're exposed, are also eliminated at a much greater rate."

Through another analytical technique comparing the narrowing of the Eastern and Western massasauga genetic makeup over several hundred years, Gibbs and Ochoa confirmed the impact human activity has had on the Eastern massasauga's swampy habitat. Unlike the Eastern species, Western massasaugas live in grassy and woodland regions of the southcentral United States that are less densely populated by humans.

"We looked at what has happened in these snakes and their population sizes over the last 300 years, which is when humans have been tromping all over North America, impacting the landscape," Gibbs said. "The impacts in terms of reducing population sizes are greater in Eastern than in Western massasaugas over this period."

The findings could influence management decisions. A common conservation practice would involve introducing snakes from a more genetically diverse population into a highly isolated group to counter the effects of inbreeding. But it turns out the Eastern massasauga might benefit more from preservation of its habitat while the genetics takes care of itself.

"This counterintuitive result makes us rethink what living in a small population is, and whether genetic problems are as important as we think they are," Gibbs said. "This is certainly not to say living in a small population isn't bad—it just may be that the genetic effects are not as bad as we thought."



More information: Alexander Ochoa et al, Genomic signatures of inbreeding and mutation load in a threatened rattlesnake, *Molecular Ecology* (2021). DOI: 10.1111/mec.16147

Provided by The Ohio State University

Citation: Threatened rattlesnakes' inbreeding makes species more resistant to bad mutations (2021, October 4) retrieved 27 June 2024 from <u>https://phys.org/news/2021-10-threatened-rattlesnakes-inbreeding-species-resistant.html</u>

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