DNA testing settles 70-year mystery over possible conjoined twins buried at ancient Angel Mounds site

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A portion of the East Village of Angel Mounds near Evansville, Ind., under excavation in 1941 by Works Progress Administration workers led by Indiana archaeologist Glenn A. Black. Black proposed that a pair of co-buried infant skeletons excavated at the time were conjoined twins. Credit: Indiana University

(PhysOrg.com) -- A mystery revealed 70 years ago when archaeologist Glenn A. Black suggested the ancient remains of two infants buried at Southern Indiana's Angel Mounds archaeological site were conjoined twins has been solved through DNA analysis at Indiana University.

When Black and a Works Progress Administration excavation crew in 1941 discovered the unique grave -- two infants buried in a single

interment -- the position of the skeletons relative to one another led Black to hypothesize they were conjoined. Even though inspection showed no shared elements of conjoined twinning or fused skeletal elements, Black's field interpretation of the double burial still led him to suspect that the two were flesh-joined twins.

"The 'conjoined twins' are well known at the Glenn Black Laboratory (at IU) and also within the Department of Anthropology," said Charla Marshall, an adjunct professor of anthropology at Indiana University-Purdue University Indianapolis who received her Ph.D. from IU Bloomington this year. "They are pretty legendary, as such interesting case studies often come to be."

Legendary enough, too, for Marshall to propose a test of Black's hypothesis by using recoverable maternally inherited mitochondrial DNA, or mtDNA, to compare genotypes of the co-buried infants, which in and of itself was a unique find: Of the 310 mostly adult burial sites discovered at the Middle Mississippian Age (A.D. 1050-1400) village near Evansville, Ind., only 3 to 5 percent contained two or more nearly complete individuals.

Even if the tests showed the remains to be those of ordinary twins, the dual interment was still unique, Marshall noted. Many societies at the time viewed twin births negatively and one or both twins would be killed, while in other Eastern North American societies a twin birth was accorded high status and deaths would have warranted excessive ceremony. This burial, Black noted at the time, was otherwise unremarkable with no adornment and a location in the common burial area at Angel Mounds.

Using an automated DNA sequencing system at the Indiana Molecular Biology Institute at IU Bloomington, the team led by Marshall analyzed the mtDNA of each infant, passed down only through the maternal lines

to offspring of both sexes, to determine whether the two infants belonged to the same haplogroup (common ancestors identified through similar DNA sequence variations called single-nucleotide polymorphisms).

Not only were the two infants not twins, and therefore not possibly conjoined, but the two were not even maternal siblings, test results found. One infant belonged to Haplogroup C, an mtDNA lineage believed to have arisen geographically between the Caspian Sea and Lake Baikal about 60,000 years ago, and the other belonged to Haplogroup A, which is thought to have come from Asia between 30,000 and 50,000 years ago.

"In addition to using molecular genetics analysis to shed light on this 70-year-old mystery, we also make one more case in all of the evidence against a requirement for a maternal relationship for co-burial in Midwestern societies," Marshall said.

Co-authors on the findings that appeared in the *American Journal of Physical Anthropology* were IU Anthropology professors Della Collins Cook and Frederika A. Kaestle, and Patricia A. Tench, an adjunct associate professor at the University of Cincinnati.

Provided by Indiana University