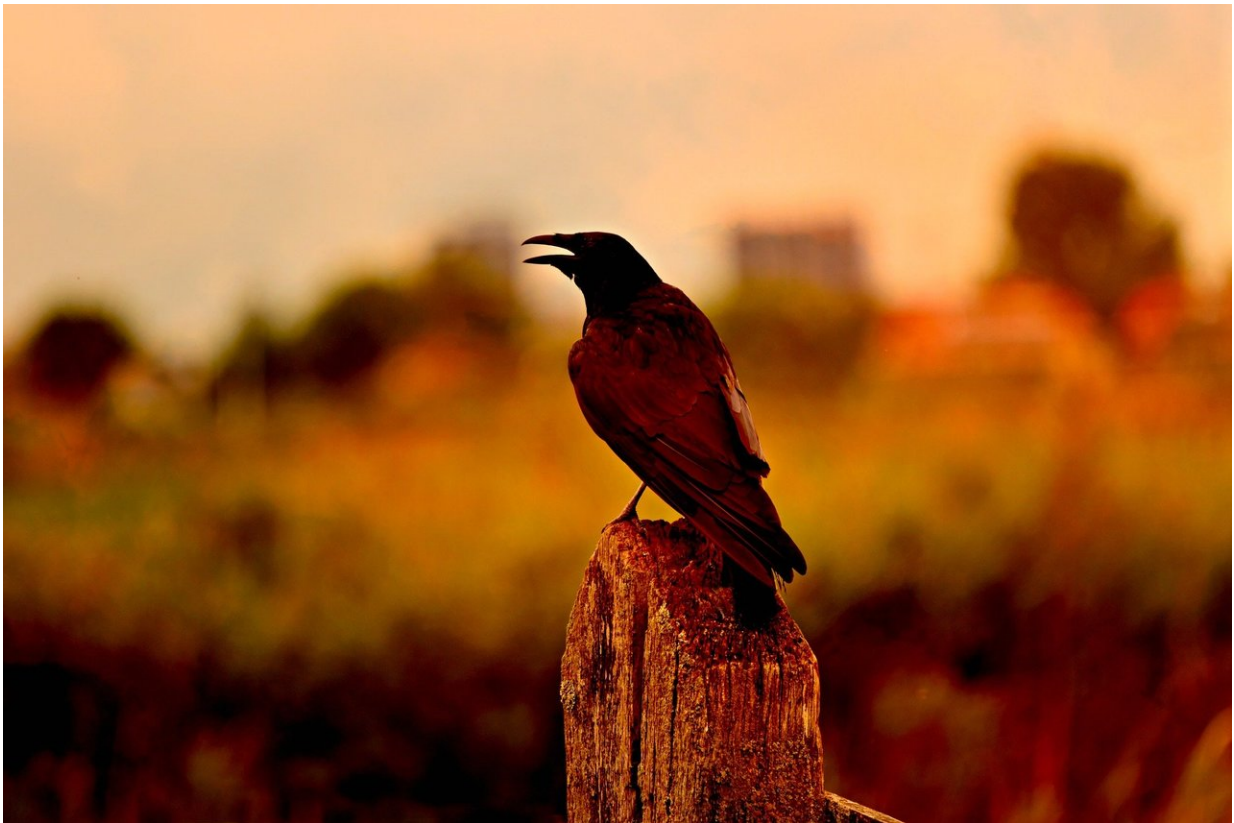


Uniform vs. particolored plumage leads to reproductive isolation among crows, study finds

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Although carrion crows and hooded crows are almost indistinguishable genetically, they avoid mating with each other. LMU researchers have

now identified a mutation that appears to contribute to this instance of reproductive isolation.

The carrion [crow](#) and the hooded crow are genetically closely related, but they are distinguishable on the basis of the color of their plumage. The carrion crow's feathers are soot-black, while the hooded crow's plumage presents a particolored combination of black and light gray. Although crosses between the two forms can produce fertile offspring, the region of overlap between their geographical distributions in Europe is strikingly narrow. For this reason, the two forms have become a popular model for the elucidation of the processes that lead to species divergence. LMU evolutionary biologist Jochen Wolf and his team are studying the factors that contribute to the divergence of the two populations at the molecular level. Genetic analyses have already suggested that differences in the color of the plumage play an important role in limiting the frequency of hybridization between carrion and hooded crows. The scientists have now identified a crucial mutation that affects this character. Their findings appear in the online journal *Nature Communications*, and imply that all corvid species were originally uniformly black in color.

The ancestral population of crows in Europe began to diverge during the Late Pleistocene, at a time when the onset of glaciation in Central Europe forced the birds to retreat to refuge zones in Iberia and the Balkans. When the climate improved at the end of the last glacial maximum, they were able to recolonize their original habitats. However, during the period of their isolation, the populations in Southwestern and Southeastern Europe had diverged from each other to such an extent that they no longer interbred at the same rate, i.e. became reproductively isolated. In evolutionary terms, the two populations thereafter went their separate ways. The Western European population became the carrion crow, while their counterparts in Eastern Europe gave rise to the hooded crow. The zone in which the two now come into contact (the "hybrid

zone") is only 20 to 50 km wide, and in Germany it essentially follows the course of the Elbe River. "Within this narrow zone, there is a low incidence of interbreeding. The progeny of such crosses have plumage of an intermediate color," Wolf explains. "The fact that this zone is so clearly defined implies that hybrid progeny are subjected to negative selection."

Wolf wants to understand the genetic basis of this instance of reproductive isolation. In previous work, he and his group had demonstrated that the two populations differ genetically from each other only in segments of their genomes that determine plumage color. Moreover, population [genetic studies](#) have strongly suggested that mate selection is indeed based on this very character—the two forms preferentially choose mating partners that closely resemble themselves. These earlier studies were based on the investigation of single-base variation, i.e. differences between individuals at single sites (base-pairs) within the genomic DNA. "However, we have never been able to directly determine the functional effects of such single-base variations on plumage color," says Matthias Weissensteiner, the lead author of the study. "Even when we find an association between a single-base variant and plumage color, the mutation actually responsible for the color change might be located thousands of base-pairs away."

To tackle this problem, the researchers have used a technically demanding method to search for interspecific differences that affect longer stretches of DNA. These "structural" variations include deletions, insertions or inversions of sequence blocks. "Up until recently, high-throughput sequencing technologies could only sequence segments of DNA on the order of 100 bp in length, which is not long enough to capture large-scale structural mutations," says Wolf. "Thanks to the new methods, we can now examine very long stretches of DNA comprising up to 150,000 base pairs."

The team applied this technology to DNA obtained from about two dozen birds, and searched for structural variations that differentiate [carrion crows](#) from hooded crows. The data not only confirmed the results of the single-base analyses, they also uncovered an insertion mutation in a gene which is known to determine plumage color by interacting with a second gene elsewhere in the genome. In addition, phylogenetic analysis of DNA from related species revealed that their common ancestor carried the black variant of the first of these genes. The variant found in the hooded crow represents a new mutation, which first appeared about half a million years ago. "The new color variant seems to be quite attractive, because it was able to establish itself very quickly, and therefore must have been positively selected," says Wolf. How the variant accomplished this feat is not yet clear.

The evidence suggests that it first appeared in the region which now encompasses Iran and Iraq, and there are some indications that the lighter [plumage](#) confers a selective advantage in hot regions, because it effectively reflects sunlight. This supports the idea that the mutation might have initially been favored by natural selection. "Once it had reached a certain frequency within the local population, it would have been able to spread because parental imprinting, which enables nestlings to recognize their parents, also causes mature birds to choose mates that resemble their parents in appearance," Wolf explains. However, other possible scenarios, such as random genetic drift in small populations or the involvement of selfish genes (which promote their own propagation), are also conceivable and have yet to be ruled out.

More information: Matthias H. Weissensteiner et al. Discovery and population genomics of structural variation in a songbird genus, *Nature Communications* (2020). [DOI: 10.1038/s41467-020-17195-4](https://doi.org/10.1038/s41467-020-17195-4)

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