PERSPECTIVES

GENETICS

Decoding the evolution of species

Mutations in protein-coding regions are implicated in the evolution of a flightless cormorant

By **Kimberly L. Cooper**

In the middle of the 20th century, researchers identified DNA and proteins and began to understand how they shape organisms. At first, scientists assumed that differences in protein sequence, structure, and function uniquely define any animal, including humans. Yet these building blocks are extraordinarily similar among animals, and human proteins can substitute for their counterparts in far simpler species (*1*–*3*). The modern dogma is that differences between species are largely a result of mutations in the noncoding instructions that determine when, where, and how much of a gene is expressed. On page 921 of this issue, Burga *et al*. (*4*) show that scientists may have underestimated the contribution of protein-coding mutations to evolution.

Some protein-coding mutations are known to have caused evolutionary change, affecting attributes such as hair and fur color (*5*, *6*), the orientation of feathers (*7*), or animal behavior (*8*). However, the functions of each of these genes are limited to specific aspects of development. Many more genes, in contrast, are reused again and again during development. Therefore, a mutation that might, for example, reshape the limb would give the animal no advantage if it also prevents the formation of a functioning heart. The assumption has been that most protein-

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A flightless cormorant spreads its tiny wings. Burga *et al.* report mutations in protein-coding sequences in the flightless cormorant's genome.

coding sequences are constrained in order to preserve functions necessary for survival, health, and reproduction of the organism.

Some scientists have questioned whether this prevailing view is premature, given the relatively few naturally occurring mutations with adaptive consequence, either coding or noncoding, that have been identified (*9*). Many more, however, have accepted the growing body of evidence at face value and have focused on identifying noncoding regulatory sequence differences between species. Individual examples of coding sequence mutations that contributed to evolutionary change continue to be cataloged, but whole genome scans for evolutionary differences have largely focused on the noncoding regulatory DNA.

The results reported by Burga *et al*. show that this view may need to be revisited. They report the whole genome sequence of four species of cormorant, including a flightless species found on the neighboring islands of Isabela and Fernandina in the Galapagos archipelago (see the photo). Identifying mutations in noncoding regulatory DNA sequences that have evolved a new function can be like finding a needle in the proverbial haystack, but changes to sequences that are constant in many other species are suggestive. Burga *et al.* found few alterations to evolutionarily conserved noncoding sequences and no smoking gun pointing to an obvious candidate for wing reduction in the flightless cormorant.

They therefore looked throughout the flightless cormorant genome for mutations predicted to affect the function of the proteins themselves. As the results show, several hundred protein-coding sequences in the flightless cormorant genome contain mutations at sites that are consistently the same between other bird species and even mammals. Such strong evolutionary conservation is commonly accepted as evidence that changes to these protein sequences are not consistent with their normal function. Genes associated with human developmental disorders were overrepresented, including a group of genes that act together to make the primary cilium. Many cells, including the cartilage cells that produce the limb skeleton, rely on these hairlike protrusions to receive signals from neighboring cells (*10*).

Are these protein sequence changes responsible for reduction of the wing skeleton and loss of powered flight in the Galapagos cormorant? To definitively assign cause and effect requires making the same mutation in another species to see whether it is sufficient to change that individual in a similar way. Burga *et al*. achieved this in two systems: in *Caenorhabditis elegans*, a soil roundworm commonly used by geneticists because it is small and easy to keep in the lab, and in isolated mouse cartilage cells in culture.

Although worms lack wings or even limbs, replacement of the worm *Ift122* gene, which is necessary for cilia formation, with the mutated flightless cormorant version reproduced a cilia-dependent behavior consistent with a partial loss of cilia function. The transcription factor Cux1, which appears to control the expression of several genes required for cilia formation, has also acquired a protein-coding mutation in the flightless cormorant. Whereas the ancestral Cux1 promotes differentiation of cultured mouse cartilage cells, their differentiation is impaired in the presence of the mutated flightless-cormorant version of Cux1. Together, the worm and cartilage cell data provocatively suggest that cilia function is compromised and skeletal cartilage differentiation is impaired in the flightless cormorant.

It remains to be shown whether any of the protein-coding mutations found in the flightless cormorant are sufficient to reduce the limb skeleton in a vertebrate animal. It is not yet clear which mutations act together to replicate wing reduction in this flightless bird, or whether wing reduction and flightlessness are necessarily advantageous to this species. Alternatively, these mutations and their effects might be the by-product of genetic drift in a very small island population where chance events can prevail.

Questions also remain about how protein-coding mutations in genes that participate in multiple developmental events—especially those related to disease in humans—are tolerated during evolution. This is particularly puzzling for mutations that affect development of the cilia, given that genetic disorders affecting cilia in humans (ciliopathies) can cause neurological, vision, and renal malfunction in addition to limb malformations (*11*). Future studies should aim to determine whether the flightless cormorant protein mutations specifically affect the wing skeleton, or whether these birds have effects in other organs that are consistent with human disease. If not, then how are these mutations accommodated to preserve the function of other organs while allowing for change in the limb?

Burga *et al*. have demonstrated the importance of searching for protein-coding sequence changes that may have accumulated during the course of evolution. Thousands of animal and plant genomes are becoming available, and similar approaches can be applied to other species. A deeper catalog of the types of protein-coding mutations that are tolerated and their impact on the form and function of an organism will reveal more of the mysteries of evolution. ■

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