**How the emu lost its wings**

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When groups of animals or plants independently evolve similar behaviors, shapes, sizes or colors, a process known as convergent evolution, it’s likely that these traits are adaptations to a shared environment and strongly favored by natural selection. In winter, hares and birds like ptarmigans both adopt an all-white coat, strongly suggesting that camouflage is an excellent way to avoid predation. But do different convergent lineages use the same genes or genetic pathways to achieve such adaptations? And are such changes in these traits driven more by changes in genes themselves or by changes in the molecular ‘switches’, or gene regulators, that turn genes on or off? These are perennial questions in evolutionary biology, yet scientists have surprisingly few examples of the molecular basis of convergence in traits, especially complex traits like the size and shape of bones. We wanted to see whether we could understand the genetic basis of a complex morphological trait in a group of animals whose genetics was difficult to study by conventional means of breeding in the lab or conducting crosses between species. After all, most species on Earth can’t be studied by such means, yet we still need to examine such species to understand general rules by which evolution produces the extraordinary diversity of the natural world.

The group of flightless birds known as ratites – including well-known birds such as the ostrich, emu, cassowaries, rheas, kiwi and the extinct moa – provide a dramatic example of convergent loss of wings and reduction of bones of the forelimb, along with traits such as large body size and loss of the keel, a projection of the sternum that is essential for flight. Whereas ostriches, and most other ratites have forelimbs that are reduced to varying degrees, the extinct moa of New Zealand have lost their forelimbs entirely. Moreover, recent research showed, and our work confirmed, that the loss of flight in the ratites likely occurred multiple times, despite their belonging to a single group of birds. We therefore used a variety of molecular and computational approaches to understand the genomic basis of loss of flight in ratites, which, together with a group of flight-capable chicken-like birds called tinamous, comprise the paleognathous birds (distinguished from other birds by the unique configuration of bones in the palate of the mouth).

Our team first sequenced complete genomes of 11 ratites and tinamous, including the genome of an extinct moa. We then conducted extensive computational analyses of the genes of ratites and other birds and found that, although a handful of genes did show signatures of rapid and convergent change specifically along ratite lineages, these genes had relatively little in common and didn’t suggest any common molecular pathways. We then studied a class of regulators of genes known as conserved non-exonic elements – regions of the genome that are highly conserved between species, implying an important function, yet do not encode a protein. Scouring through hundreds of thousands of such elements, we found that those few regulators that changed convergently on ratite lineages disproportionately occurred near genes with clear roles in limb development, cell proliferation and other processes we might expect to drive morphological change in the ratite forelimb. We also found that many of these noncoding elements evolving quickly in ratites were also in regions of the genome that were active during early development of the forelimb, keel and other flight-related anatomical regions in chickens – a good sign that these regions were in fact regulators, or enhancers, of other genes. Finally, we were able to demonstrate that the chicken and tinamou versions of one of these noncoding enhancers could drive gene expression in the developing forelimb, whereas the version found in flightless rheas could not, suggesting that the accelerated evolution of these regulators had functional consequences for gene expression, and presumably morphology. Our study only scratched the surface in terms of cataloging the many genetic loci that presumably underlie morphological changes in ratites, and we didn’t demonstrate any specific changes in forelimb morphology due to changes in gene regulation. Still, the pattern of conservation and acceleration throughout the genome suggested that the gene regulators, rather than genes themselves, appear to be more important in the evolution of the flightless phenotype in ratites, and possibly other birds. Flightless birds do seem to use similar genetic pathways as they loose the capacity to fly, and our study provides the foundation for more detailed study of the many gene regulators that are likely involved in these transformations.