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Figure 1 | A sugar glider (*Petaurus breviceps*). A skin membrane called the patagium extends from this marsupial's forefeet to its hindfeet and enables it to glide through the air.

Evolution

How a skin fold for gliding emerged in marsupials

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A parachute-like skin membrane, the patagium, evolved independently in several marsupial species. Genomic analysis suggests that this trait came about through different changes to the regulation of the same gene. **See p.127**

A highly elastic and resistant skin membrane, known as the patagium, has enabled self-powered flight in species such as bats and now-extinct pterosaurs, yet some marsupial species use the patagium in another way: as a gliding parachute. Just like the webbed-sleeved jumpsuit used by a wingsuit skydiver, the design of which was inspired by these natural membranes, the patagium extends from the forefeet to the hindfeet. After jumping from a tree, a marsupial glider will rely on its patagium to glide for up to 50 metres, maneuvering gracefully through the air with only tiny movements of its limbs[1]. This evolutionary adaptation has enabled gliding species to search for food over wide areas because the animals spend much less energy gliding than they would do climbing. It also enables them to avoid land-roaming predators and to escape quickly from aggressive tree-climbers. On page 127, Moreno *et al.*[2] shed light on the elusive molecular mechanisms that allowed this evolutionary adaptation to emerge.

Moreno and colleagues first sequenced and compared the genomes of 15 gliding and non-gliding marsupial species. Patagium formation has emerged independently in at least three marsupial lineages: feathertail gliders (*Acrobates pygmaeus*), southern greater gliders (*Petauroides volans*) and sugar gliders

(*Petaurus breviceps*; Fig. 1). Therefore, the authors proposed that genetic features associated with the patagium might be common to the genomes of these three species. They were particularly interested in finding mutations that affect regions of non-coding DNA called enhancers, which act as 'genomic switches' that determine when and where certain genes should be expressed[3].

The authors looked for candidate enhancers in the developing patagium tissue of newborn sugar gliders using techniques known as ChIP-seq and ATAC-seq, which identify regions of DNA that are transcriptionally active. A subset of enhancers contained sequences that the authors called 'glider accelerated regions'. Accelerated regions, which were originally studied in the context of human evolution[4], are parts of the genome that are highly evolutionarily conserved but show a substantial number of nucleotide changes in particular species. These regions allow scientists to identify genomic changes that are associated with the emergence of species-specific traits. Despite Moreno and colleagues identifying thousands of glider accelerated regions, not even one was shared between the three marsupial glider species.

But genes are rarely controlled by a single enhancer. Instead, enhancers tend to cooperate, which gives robustness to gene-expression programs[5]. Rather than a mutation shared between glider species, perhaps different genomic sequences evolved independently to produce the same effect. The use of a technique called Micro-C, which identifies potential interactions between enhancers and genes, pointed to a location (locus) in the genome that contains the *Emx2* gene. The involvement of this locus in patagium evolution was backed by two key observations: the *Emx2* locus contains at least one glider accelerated region in each of the three glider species, and the *Emx2* gene itself is expressed during patagium formation.

Emx2 is a familiar gene to many developmental biologists because its expression is essential for building the head[6] and the urogenital system[7]. But generating a structure for gliding is a completely different matter. Investigating the function of this gene in an unusual research model, such as the sugar glider, is not trivial because conventional experimental tools that have been established for model organisms, such as mice and fruit flies (*Drosophila melanogaster*), are not necessarily available. The authors overcame this problem by exploiting a special feature of marsupials: after birth, infants (joeys) continue to develop *ex utero*, inside the maternal pouch[8]. The authors designed an 'in pouch' genetic-engineering approach to lower the expression of *Emx2* in developing sugar-glider joeys. They found that this gene is indeed required for proper patagium development in this species.

Moreno and colleagues next asked whether the mechanism that drives *Emx2* expression in the patagium precursor tissue was an evolutionary innovation specific to gliding marsupials or whether it was evolutionarily conserved across mammals — a question that could be addressed using mouse models. *Emx2* expression in mice was localized to the skin between the forelimbs and hindlimbs (the lateral skin), an anatomical region that closely corresponds with the patagium. Strikingly, although *Emx2* was expressed in mice only before birth, its expression was prolonged until post-birth developmental stages in the sugar glider. This phenomenon, known as heterochrony[9], indicates that the mechanisms that underpin expression of *Emx2* in the lateral skin are evolutionarily conserved among mammals, but that there are subtle differences in the timing of expression between species.

After forcing prolonged expression of *Emx2* in the lateral skin of genetically engineered mice, the authors observed an increase in cell proliferation and the density of cells in the tissue — both features that are reminiscent of patagium development. However, a patagium was not fully formed in engineered mice, which could be attributed to the difficulties in accurately reproducing a gene-expression pattern from another species. Additional factors are probably also required to complement the action of *Emx2*, and these would together support patagium evolution through a combination of gradual changes.

"Existing developmental pathways can be repurposed for specific evolutionary adaptations."

This work is a compelling example of how the sequencing of previously unsequenced genomes, coupled with innovative functional experiments and technologies that assess gene transcription and regulation, can be applied to address relevant questions in the field of evolutionary biology. Conventionally, comparative evolutionary genomics has been focused on mutations in DNA sequences that code for proteins, because these mutations are easier to identify than are those in non-protein-coding sequences[10]. But mutations in coding sequences might not be among the most common causes of evolutionary variation, because they can render genes inactive and cause broad effects that might be advantageous in a particular tissue, but detrimental in others[10]. Instead, mutations in enhancer regions can lead to time- and tissue-restricted effects that an organism might be able to tolerate more than they can mutations in coding regions.

Therefore, the enhancer mutations described in the current study would preserve *Emx2* functions in crucial tissues, such as the brain or the urogenital system, while providing room for new functions to evolve. The study also highlights the intricacies of convergent evolution — the independent evolution of similar features in different species — which can happen when unrelated mutations alter a common gene. This demonstrates that existing developmental pathways can be repurposed for specific evolutionary adaptations.

Some interesting questions remain to be answered. Which transcription factors activate *Emx2*? Are these transcription factors also expressed differently in different glider species? What other relevant genes or pathways could be involved in patagium development? The in-pouch genetic-engineering tools that Moreno and colleagues developed provide ways to address these questions, as well as to study other relevant aspects of marsupial biology. For example, gliders have evolved pockets inside their pouches to protect babies from the impact forces of landing on trees[1]. They have also developed flattened tails that act as stabilizers while gliding. It would be interesting to explore how these adaptations emerged and, particularly, how they relate to the evolution of the patagium.

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