Review of: "Developmental genetics of color pattern establishment in cats"

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From parietal wall paintings to Charles Darwin's drawings or Rudyard Kipling stories, human art and science works evidence a long fascination for the colour patterns that adorn animal coats. Interest for their exquisite diversity indeed fuelled more than a century of studies aiming at identifying the mechanisms responsible for their formation and evolution. The study published on September 16th in *Nature Communications* by Christopher Kaelin, Kelly McGowan and Greg Barsh (Stanford University) unveils part of the mystery, revealing the nature and function of a developmental gene in the production of colour pattern differences in domestic cats.

Not only do different breeds of cats display a variety of pigment patterns –from common Tabby markings (stripes or whorls) to freckled, rosette or marbled patterns, they also provide a straightforward technical solution to a long-time challenge in evo-devo and developmental genetics studies, that is, the collection of embryonic specimens from species displaying variation in natural markings. Instead of relying on occasional zoo samples or performing costly and time-consuming fieldwork, Kaelin *et al.* collected embryonic cat specimens by collaborating with a regulation program that captures and sterilises feral cats before releasing them in their environment. This non-invasive approach allowed the rapid collection of roughly 1000 embryos from pregnant females. A golden mine for comparative developmental studies.

By performing histological sections in embryonic skins of Tabby-patterned feral cats, the authors made a striking observation: before hair follicle and pigment production, the overlying epidermal layer is organised in transient, alternating thick and thin regions spatially matching future coloured stripes. This histological inhomogeneity varied in Blotched cats, correlating with colour pattern differences in this breed. Blotched cats indeed have larger stripes due to a loss of function of the Taqpep enzyme, previously shown to locally drive stripe self-organisation (in a study by the same authors). Thus, region-specific epidermis thickness forms a morphological pre-pattern of coloured stripes. This result is a first in the field, as pre-patterns had thus far only been described for later pigment gene expression profiles or pigment cell differentiation.

The presence of this unique morphological pre-pattern was evidenced again using an experiment of single cell sequencing in which fetal skin basal keratinocytes clustered in two subpopulations based on differential expression of 277 genes. Among these: *Dkk4* and *Wif1*, previously involved in self-organised

hair follicle spacing and differentiation. The authors next studied profiles of transcript expression for these candidates in Tabby and Blotched breeds, thereby shedding light on a molecular pre-pattern that precedes the production of thick and thin skin regions –and later, light and dark stripes: *Dkk4* is dynamically expressed in skin embryonic domains that will later form thick epidermal regions. They found that *Dkk4*-positive areas also express higher levels of *Edar*, a target of Wnt signalling.

At this point of the study, authors capitalised on existing variation in domestic cat patterns, for which previous work associated some colour changes to causal genetic loci. In particular, an absence of dark Tabby stripes (e.g., in Abyssinian or Burmese breeds) or a shrinking of dark regions into small spots (e.g., in Savannah cats) had been linked to changes at the “Ticked” locus. By studying these breeds, Kaelin *et al.* found that *Dkk4* is located in a region overlapping with the linkage interval of Ticked, and displays a pattern of variation consistent with colour pattern differences associated to Ticked, which reinforced their conclusion that *Dkk4* plays a key role in the production of colour pattern variation.

Previous work had identified pigmentation genes involved in the production of embryonic blueprints that foreshadow colour patterns (e.g., *Agouti*, *End3b*) and showed how differences in the expression levels or spatial profiles of these genes cause natural variation in colour distribution. However, this study is unique because it genetically links changes in a patterning gene (*Dkk4*), member of the conserved Wnt developmental pathway also involved in hair production and spacing, with strain-specific differences in early histological topologies of putative colour domains, long before pigments or hair appear. It thereby successfully tackles one of the biggest difficulties in pattern-formation studies: untangling the role of developmental molecules on patterning events from their downstream functions on character differentiation. This study also stands out by its broad use of modern genomics and developmental biology techniques in cats, rarely studied despite the attachment of a large public to these animals. In particular, the authors made an impressive use of cat pattern diversity to strengthen and extend their results, establishing these animals as key models in the field and setting the promise of future important discoveries on the genetic and developmental bases of pattern formation.