What's next in this quest? First, it would be valuable to carry out X-ray observations along other intergalactic sightlines, because Markarian 421 may probe an atypical region of the IGM. Unfortunately, most other quasars are not bright enough to provide such high-quality data. Astronomers have had considerable success probing more than 40 sightlines for O VI absorption lines ($10^{5.5}$ K gas) using the Far Ultraviolet Spectroscopic Explorer (FUSE). But current X-ray and ultraviolet observatories lack the light-gathering power to complete the baryon inventory properly.

That task awaits a new generation of larger space observatories, including NASA's Constellation-X mission[10] and the European Space Agency's XEUS mission for X-ray spectroscopy. Large ultraviolet and optical space telescopes are in the planning stages[11], as astronomers seek to replace the spectroscopic capabilities of the Hubble Space Telescope. New ultraviolet and X-ray observatories are needed to complete the inventory of missing baryons. But they will do much more, allowing astronomers to map out the cosmic web of filamentary intergalactic matter from which the first galaxies and stars were formed. ■

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Evolutionary developmental biology

How and why to spot fly wings

Paul M. Brakefield and Vernon French

How can different species evolve different physical features despite using similar molecular toolkits? Studies of wing colour development in fruitflies point to specific changes in a gene's regulatory region.

Over the past two decades, comparative studies have shown that the development of even widely disparate organisms uses a surprisingly similar set of mechanisms. The process of development is controlled largely by where and when key genes are switched on or off — events that are in turn determined by the binding of available proteins (transcription factors) to the genes' regulatory regions. From an evolutionary perspective, we need to know which of these developmental components have varied during evolution, providing the raw material that is sieved by natural selection to remould organisms[1]. Identifying such components of change requires us to be able to link naturally occurring mutations closely to specific examples of morphological variability, both within species and between related species[2,3].

Several studies have begun to reveal that variation in the regulatory regions of particular genes is associated with species differences in insect bristle or pigment patterns[4–7]. Now, on page 481 of this issue[8], Gompel *et al.* show how specific changes in the regulatory elements of the gene *yellow* have contributed to the evolution of differences in wing pigmentation among related species of fruitfly.

Yellow is one of the key genes needed to make the melanin pigments in insects. In fruitflies, it is characteristically expressed in regions that will form darkly pigmented patches on the adult cuticle[7,9]. In the fruitfly species *Drosophila melanogaster*, *yellow* is expressed at low levels in the wings, which develop a uniformly grey colour. Males of the related species *D. biarmipes*, by contrast, have dark wing tips that are displayed to potential mates during courtship, and the male pupae express *yellow* in matching distal–anterior spots (that is, lying towards the front and tip of the developing wing).

So what determines the difference in the expression of *yellow* between *D. melanogaster* and *D. biarmipes*? Gompel and colleagues[8] show that the answer lies in differences in the flanking regulatory region of the *yellow* gene. They have found that if this regulatory region from *D. biarmipes* is inserted into the developing wing of *D. melanogaster*, it drives the expression of gene constructs in the distal–anterior region of the wing. This property resides in a discrete regulatory element, known as an enhancer, that drives wing-specific gene expression. The enhancer is also present in the non-spotted species but, in *D. biarmipes*, it contains a short sequence that binds unknown transcription factors responsible for driving distal expression. The enhancer also contains defined sites that bind the transcription factor Engrailed, which restricts expression to the anterior side. Further research may reveal additional modifications in other regulatory regions of *yellow*.

Unfortunately, it is not yet possible to produce genetically modified variants of *D. biarmipes*, so the authors have been unable to carry out the reciprocal experiment: transferring the regulatory regions of *D. melanogaster yellow* into *D. biarmipes*. There may also be relevant differences between the species in the distribution of transcription factors — perhaps explaining why the expression of the *D. biarmipes* gene constructs in *D. melanogaster* is not as sharply defined as that of the normal *yellow* gene in *D. biarmipes*.

Nonetheless, the results so far show that evolution of the dark wing spot in *D. biarmipes* males has involved alterations in an ancestral regulatory element of the *yellow* gene. As a result, the regulatory element has gained multiple binding sites for transcription factors whose distributions define the new region of expression. These distributions, including that of Engrailed, may be deeply conserved features of wing development, and Gompel *et al.*[8] argue that this process could generate similar spot patterns in distantly related fly species (see, for example, ref. 10). More generally, the evolutionary lability of short protein-binding sequences in the regulatory regions of other key developmental genes might explain the repeated patterns of disappearance and reappearance

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Figure 1 **Insects displaying the melanin-based colour patterns in their wings. Left, fighting male fruitflies (*Drosophila silvestris*) on Hawaii. Right, mating butterflies (*Ornipholidotos* species) in Kibale Forest, Uganda.**

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of particular morphological traits in other lineages, such as the horns of dung beetles[11].

An intriguing point is that, although Gompel *et al.* show that transfer of the *yellow* gene from *D. biarmipes* to *D. melanogaster* resulted in distal–anterior gene expression, it did not produce a dark wing tip. So, although the regulatory changes that generate a new expression pattern for *yellow* may have been central to the evolution of the *D. biarmipes* wing spot, they are far from the whole story. Expression of the gene *ebony*, for example, is reduced in the spot region of the *D. biarmipes* wing[9], and when Gompel *et al.* introduced the *D. biarmipes yellow* gene into *D. melanogaster* bearing mutations in *ebony*, a faint wing spot was produced. Clearly, changes in the regulation of *ebony* and other genes involved in the branching pathways of melanin synthesis[12] must contribute to formation of the full wing spot in *D. biarmipes*. By identifying all of these changes and their separate effects, it may be possible to reconstruct the pathway by which the wing spot evolved.

It is not known how the wing patterns of *D. biarmipes* and related species contribute to the performance of these flies in their natural environments. However, behavioural observations on sexual selection in other species, including Hawaiian *Drosophila*[13] (Fig. 1), encourage the hope not only of identifying the genetic changes that have become fixed during evolution, but also of providing explanations for the morphological changes in terms of natural selection. Related studies of the control of hair (trichome) and bristle development in fruitflies have also begun to reveal some of the underlying regulatory changes in key developmental genes[4–7]. However, unravelling the adaptive significance of the altered morphologies is likely to prove too great a challenge, as their functions in natural environments remain obscure.

Revealing exactly how diversity in the number, position and shape of patches of wing pigmentation has evolved across the many species of fruitfly will be a challenge for the next decade. However, fruitflies — as well as other insects such as butterflies, with their much richer diversity in wing pigmentation[3,12] — provide wonderful opportunities for tracing evolution all the way, from specific changes in gene regulation through to the performance of the altered morphologies in nature and to their diversity among related species. ■

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Cell biology

Holding sisters for repair

Tatsuya Hirano

When a DNA molecule breaks, its complementary copy can be used as a template for repair. A familiar protein complex is recruited to the damaged site, keeping it close to the undamaged copy.

Before a cell divides, it must replicate its DNA, producing two identical copies of each chromosome, known as sister chromatids. When one of the sister chromatids suffers a break that affects both of its DNA strands, it is mended by 'homologous recombination'. In this process, information on the undamaged sister is used as a template for repair. This mechanism is essential for cell survival and genome stability. But how can the repair machinery find the proper template in the crowded environment of the cell nucleus? Writing in *Molecular Cell*, Ünal *et al.*[1] and Ström *et al.*[2] provide compelling evidence that a protein complex called cohesin has a crucial role.

The cohesin complex was originally identified as a protein component that ensures the proper segregation of sister chromatids during cell division. It does so by holding them together from the time that they are produced (during the 'S phase' of the cell-division cycle) until the mid-stage of mitosis — the phase in which the sister chromatids of each pair are separated in preparation for making two cells[3].

However, previous genetic studies also implicated cohesin in the repair of double-strand breaks (DSBs) between S phase and mitosis (in the post-replicative, or G2, phase). In fact, one of the cohesin subunits had been found to be involved in DSB repair in fission yeast[4] long before its essential role in chromosome segregation was recognized.

Although subsequent studies in budding yeast[5] and vertebrates[6] also supported a requirement for cohesin in post-replicative DSB repair, it remained unclear how the complex participates in this process at a mechanistic level. In principle, two models can be considered. During S phase, cohesin complexes — spaced 10–15 kilobases apart in yeast — build up a physical linkage along the length of the sister chromatids; this genome-wide linkage may be sufficient to keep sisters close enough to allow repair

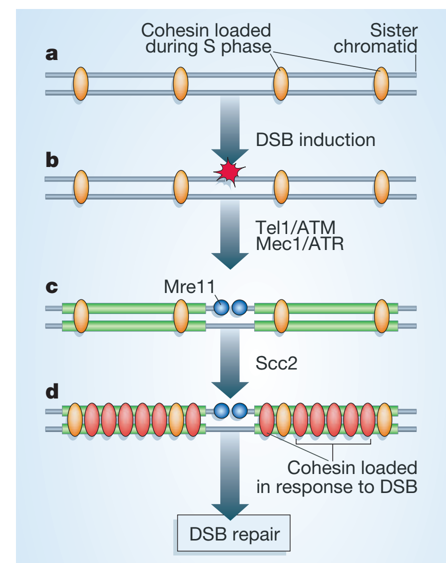

Figure 1 **Local recruitment of cohesin for DNA repair. The model shown is based on the findings of Ünal *et al.*[1] and Ström *et al.*[2].** a, **During the DNA-replication (S) phase of cell division, cohesin (orange ovals) forms links between sister chromatids along their lengths.** b, c, **When a double-strand break (DSB) is induced in one of the sister chromatids** (b), **enzymes of the DNA-damage checkpoint — such as Tel1/ATM and Mec1/ATR — are activated. The DSB-repair protein Mre11 (blue circles) binds to the damaged site, and histone protein H2AX is phosphorylated (green bar) in a large chromosomal region surrounding that site** (c). d, **The phosphorylation of H2AX allows the *de novo* loading of more cohesin (red ovals), a process that also requires Mre11 and the cohesin-loading factor Scc2. The consequent enhanced linkage between the damaged and undamaged DNA strands allows efficient repair of the DSB.**

by homologous recombination when a DSB appears in G2 phase. Alternatively, cohesin may have a more active, more specialized and more local role around the damaged sites.

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