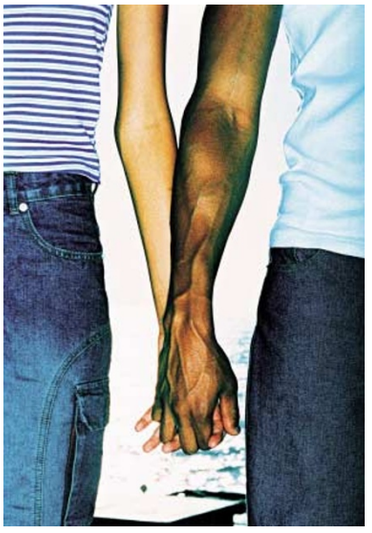
EVOLUTION

A tale of courtship



Far from being a prurient pastime, scrutinizing the interaction between the sexes is the only means of studying the most fundamental problem there is — the propagation of life. Two recent papers on *Drosophila* now highlight two of the topics tackled by this rich area of research: in one, Dauwalder *et al.* describe a gene that is expressed specifically in males and is required for courting behaviour; in the other, Miller and Pitnick reveal, for the first time, how post-copulatory interactions between the sexes drives the evolution of a reproductive trait, in this case longer sperm tails.

It is the male fly that takes the initiative to mate through an elaborate courtship ritual. Like other somatic sexual features, sex-specific behaviours, such as courtship, are controlled by the combined action of the sex-specific forms of Doublesex (Dsx) and Fruitless (Fru) proteins. But how? Dauwalder and colleagues provide part of the answer to this question in their study of the *takeout* (*to*) gene, which they found — in an RNA subtractive hybridization screen — is specifically expressed in male heads. Subsequent mutant studies showed that *to* is required for male courting behaviour: *to* mutant male flies could distinguish between males and females, but courted less often. The expression of *to*, which encodes one in a family of 20 secreted proteins, depends on the male-specific forms of Dsx and Fru; this study therefore identifies the first target of Dsx and Fru that is involved in sex-specific behaviour, as well as providing the curious puzzle of how fat cells (the cells in the head in which *to* is expressed) might control courtship.

One of the driving forces behind the evolution of many male traits is female-driven sexual selection, the peacock's tail being the most famous example. Miller and Pitnick now show experimentally how female choice drives the evolution of one such sexually selected trait: longer sperm tails. Sperm tail length is highly heritable, so populations of flies could be bred that had either giant or very short sperm tails. It was clear from mating these males to females that were bred to have either very long or very short sperm storage organs (seminal receptacles, SRs) that longer sperm were more successful at fertilizing females — especially those with long SRs — when competing with shorter sperm. As well as showing that

GENE EXPRESSION

Making long-distance contact

Enhancer elements are a somewhat mysterious feature of higher eukaryotic genomes, predominantly because how they enhance the expression of genes that lie far away from them remains unknown. Do they, for example, make direct contact with their target gene by looping out the intervening DNA or do they act indirectly by producing a transcriptionally favourable environment?

Answers to these questions now come from a recent paper by David Carter and colleagues who have developed a new technique — RNA TRAP — to investigate enhancer elements. They show, for the first time, that long-range enhancer elements very likely come into physical contact with the genes they regulate — results that shed doubt over non-contact models of enhancer function and demonstrate the usefulness of this technique for exploring transcription-regulating elements.

Step one of RNA TRAP involves localizing horseradish peroxidase (HRP) to oligos that are targeted to an RNA as it is being transcribed. In this study, oligos were directed against two genes that lie in the mouse β-globin cluster, downstream of a locus control region (LCR). This LCR contains six DNase-I hypersensitive sites (HS1–6) and is required for the high-level expression of β-globin locus genes in erythroid cells. In step two, the localized HRP catalyses the covalent deposition of biotin onto chromatin proteins in close proximity to the transcribed gene. The labelled chromatin is then purified by affinity chromatography and the DNA sequences bound to it are identified by PCR.

In their study, Carter *et al.* used mouse E14.5 fetal liver cells, which express only two of the four genes at the *Hbb* locus, *Hbb-b1* and *Hbb-b2*. In their first RNA TRAP experiment, probes were targeted to the 3′ intron of *Hbb-b1*, and the enrichment of sequences across the *Hbb* locus was measured. The sequence around the targeted region was most greatly enriched, as expected, with enrichment dropping off sharply over the silenced regions of the locus. The enrichment picked up again around the LCR, especially at HS2, and to a lesser extent at HS1 and HS3. A similar enrichment pattern was detected when a 3′ intron of *Hbb-b2* was targeted with oligos. Again, HS2 was highly enriched; as was HS4, but to a lesser extent. These findings indicate that certain regions of the LCR, especially HS2, come into close physical proximity to the active *Hbb-b1* and *Hbb-b2* genes. Moreover, these results tie in nicely with previous *Hbb*-locus deletion studies in mice that have shown that gene expression in this region is most drastically reduced by the deletion of HS2.

To rule out the possibility that these results might be caused by the preferential deposition of biotin in certain chromatin regions, Carter *et al.* also ran control experiments in which they omitted the intronic probes at step one, causing biotin to be randomly deposited across the genome. No preferential labelling of *Hbb*-locus sequences occurred as a result, lending further weight to their findings. The authors' planned improvements to this assay should shed more light on the exact nature of the interaction that occurs between enhancers and the genes they regulate.

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References and links

ORIGINAL RESEARCH PAPER Carter, D. *et al*. Long-range chromatin regulatory interactions *in vivo*. *Nature Genet.* **32**, 623–626 (2002)

WEB SITE

Peter Fraser's lab:
http://www.babraham.ac.uk/research/developmental_genetics/chromatin.htm#fraser