

it! This has challenged our predictions of how sexual conflict could influence the evolution of genital morphology — until now. The new study of the African bed bug, *Afrocmex constrictus* (Cimicidae) by Reinhardt *et al.* [3], exemplifies the extreme and somewhat bizarre consequences of arms races between males and females.

In *A. constrictus*, as in all Cimicids, females have responded to traumatic insemination from males by evolving ‘paragenitalia sinuses’. These are structural modifications of the abdominal cavity, which develop in addition to their ‘normal’ genitalia [11]. These paragenitalia sinuses guide the male intromittent organ into an organ called the mesospermalege, which is packed with immune cells or haemocytes. The whole structure functions to localize the site of insemination and reduce the physical trauma of insemination. Interestingly, in *A. constrictus*, males mimic females by also having paragenitalia sinuses, although the male form is slightly different to that of the female, with a more open morphology that exposes the site of piercing. To further confuse the issue, some females in this species appear to mimic the male expression of the paragenitalia sinuses by exhibiting the male open-form.

To gain insight into the selection for paragenitalia in males and polymorphic paragenitalia in females in *A. constrictus*, Reinhardt *et al.* [3] measured the intensity of the scarring of paragenital sinuses in males and ‘open’ and ‘closed’ morphs in females. Males were found to have fewer scars than females, irrespective of morph. Cimicids only appear to recognise gender after genital contact, thus Reinhardt *et al.* [3] suggest that the distinct form of the male paragenitalia (open) signals their gender to other males and thereby reduces the incidence of male–male traumatic insemination. In addition, females of the ‘open’ morph had fewer scars than females of the ‘closed’ morph, so that by mimicking males, females appear to suffer less male-induced harm.

The expression of sex-limited traits in both sexes, more commonly known as cross-sexual transfer, is an unusual but relatively widespread phenomenon. However, the revelation that *A. constrictus* are polymorphic in both the form (open or closed) and number of their paragenitalia sinuses, is the first documented account of polymorphic female genitalia within a species. The authors [3] suggest that such genetic differentiation in both male and female traits has the potential to result in speciation via sexual conflict, as predicted by recent theoretical models [13,14].

Males emulating females, and females emulating males: this exciting new study blurs the battle lines between the sexes in the sexual competition arena. Reinhardt *et al.* [3] show us that genital evolution via sexual conflict is more than a theoretical possibility. The bottom line is that the interactions between males and females under sexual conflict are likely to be as important as male or female biased processes — sperm competition or cryptic female choice — in shaping genital morphology. Anyone for sexual equality?

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Evolution of Protein Expression: New Genes for a New Diet

A new study identifies gene duplication of a salivary enzyme as a recent adaptation to changes in diet among human populations, highlighting the diverse ways that gene regulation can evolve.

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Evolution is a contingent process, dependent on the vagaries of the environment, history, and whatever mutations happen to appear.

Consequently, there are few strict ‘laws’ of evolution; ours is instead a science of generalizations. One search for regularities has focused on the molecular basis of adaptation. A widespread but somewhat controversial view is

that important adaptations may be due largely to changes in the expression of rather than the sequences of genes themselves [1–3]. This idea was first proposed by King and Wilson [4] to explain large morphological changes (but low divergence in protein sequence) between humans and chimpanzees.

With the rise of genomics and its vast storehouse of DNA sequences, work has intensified to determine what sorts of mutations cause adaptive change. Much effort has been focused on identifying mutations that make us uniquely human (for example [5,6]), or those that differentiate human populations from each other. A recent study by Perry *et al.* [7] describes a novel case of altered gene expression that has evolved in response to changes in human diet. This result is exciting because it identifies one of only a handful of known genes whose differentiation among populations of our species was driven by natural selection. The study also highlights the diverse and unpredictable ways that evolution can alter gene expression.

In principle, changes in gene regulation — which we define as mutations that alter the amount, timing, or location of a gene's expression — can occur in several ways. These include changes in amino-acid coding sequence of genes, changes in the noncoding sequences, or duplication/loss of genes (Figure 1). While speculation about the genetics of adaptation has centered on the relative importance of 'structural' mutations — those affecting amino acid sequence of proteins — versus *cis*-regulatory mutations [1–3,8], the categories shown in Figure 1 imply that this dichotomy is too simplistic: changes in protein sequence and gene number can affect gene expression as well. Indeed, the work of Perry *et al.* [7] shows that adaptive change in gene expression was achieved by repeated duplication of an enzyme-coding locus.

One of the many differences between humans and chimps is our diet (we are far more omnivorous),

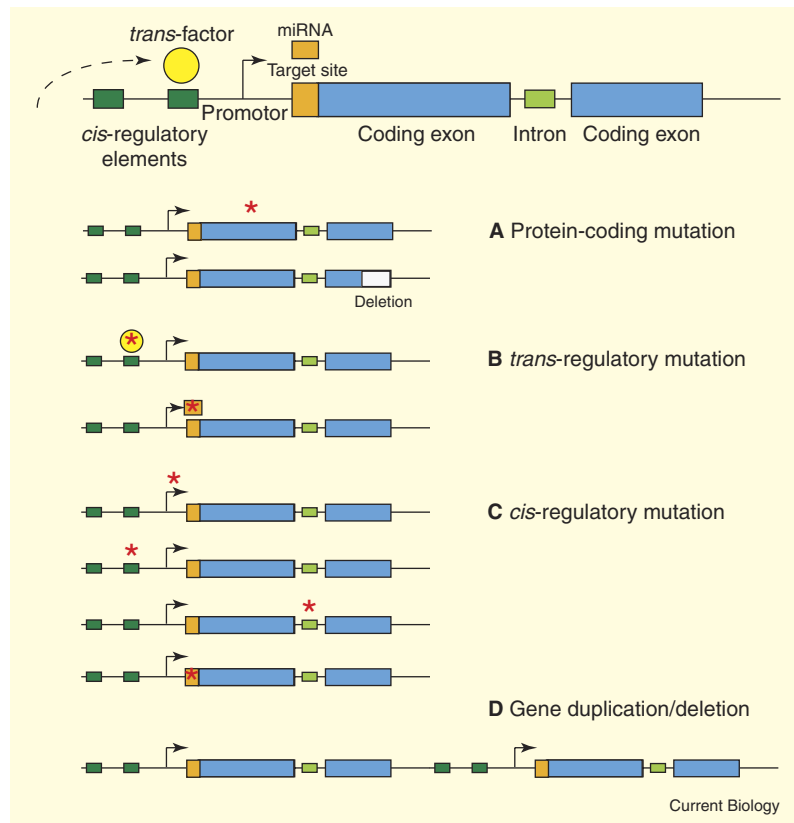


Figure 1. A diversity of mutations can cause changes in gene expression.

A schematic of gene structure showing: protein-coding exons (blue); 3' untranslated region with miRNA target site (orange); *cis*-regulatory elements, such as enhancers (green) and a promoter (arrow). *Trans*-acting factors, such as transcription factors (yellow) and miRNAs (orange), are shown. Possible mutations include single-nucleotide changes (red asterisk), deletions (white box) and whole-gene duplications. Changes in gene regulation fall into four main categories: (A) Changes in amino-acid sequences. These can change expression of the protein itself. This has been shown for both nonsynonymous mutations (for example [13]) and deletions (for example, deletion of a sequence motif can change the localization of the gene product [14]). (B) *Trans*-regulatory mutations. Mutations in 'regulatory proteins', such as transcription factors, can cause cascading effects on gene expression downstream in the pathway. (Some transcription factors are known to evolve rapidly in the human lineage [15].) Mutations in miRNAs, short, single-stranded RNAs, may have similar regulatory effects [16]. (C) *Cis*-regulatory mutations. We know that about 98.5% of our genome is non-coding, but mutations in these regions, including silent substitutions in exons [17], can change expression patterns. Most research has focused on changes in *cis*-regulatory elements of genes (promoters and enhancers) as primary sources of variation in gene expression [1]. But evolution may be more opportunistic: recent work has suggested that mutations in miRNA target sites can also alter protein expression [16,18]. (D) Gene duplication. Although per-gene-duplication rates are low, these rates become appreciable when we consider the entire genome [19]. Duplications can potentially play an important role in gene regulation by increasing the amount of protein produced, a change that can have important fitness consequences (for example, duplication of an esterase gene in aphids confers resistance to insecticide [20]).

but diets also vary among human populations, as does the amount of starch we consume. Perry *et al.* [7] found that variation among human populations in the number of copies of the human salivary amylase gene (*AMY1*) is correlated with starch consumption. Amylase is an enzyme that breaks down starch to

simple sugars in our mouths. Testing undergraduates at Arizona State University, Perry *et al.* [7] first found that the amount of salivary amylase was highly correlated with gene copy number — the more gene copies, the more protein. They then extended their study to seven human groups characterized by

either 'high-starch' diets (populations depending on agriculture: Japanese, Hadza, and European Americans) or 'low starch' diets (hunter-gather or pastoral populations: Biaki, Matubit, Datag, and Yakut). The two groups showed a significant difference in mean number of amylase copies in the expected direction (6.7 *versus* 5.4, respectively). It is clear that copy number has increased in the human lineage as compared to our more frugivorous cousins (chimps have only one copy), but it is not yet known whether differences among human populations reflect the gain or loss of ancestral human gene copies. In one comparison, that of Japanese to Yakut populations, positive selection may have increased copy number: relative to other genes having multiple copies, *AMY1* has duplicated much faster.

What type of selection could have acted here? Perry *et al.* [7] suggest that copy number rose in the starch-eating populations, and did so for two reasons: a general increase in fitness due to greater efficiency of starch digestion, and greater oral digestion of starch that could provide nutrients during episodes of diarrhea, a historically significant cause of human mortality. This hypothesis is difficult to test directly because we are unable to do controlled crosses, *in vivo* studies, or transgenics in humans. But DNA sequencing of the multiple *AMY1* copies could answer several critical questions. Is the pattern of nucleotide variation among homologous gene copies consistent with the action of natural selection? Were the duplication events coincident with changes in foraging behavior and/or the consumption of domesticated grains? And did increases in copy number occur independently in these (and other) high-starch populations, or did gene flow distribute new copies widely?

Compared to single nucleotide changes in individual genes or the regulatory elements that control them, the loss and duplication of genes has been less appreciated

as a source of adaptive novelty in humans. Yet differences in the number (or presence) of gene copies between even closely related species are striking: at least 6.4% of genes in the human genome are not found in chimpanzees [9]. Moreover, humans show substantial polymorphism in gene copy number [10].

The amylase results follow a related study on the genetics of human dietary differences. In 2006, Tishkoff and colleagues [11] identified a mutation in the upstream regulatory region of the gene for lactase, an enzyme important for digesting milk, in pastoral African populations. Using an *in vitro* system, they showed that this mutation could increase gene expression. The relevant mutation, however, is not a duplication, but probably a change in *cis*-regulation. (An independent *cis*-regulatory mutation at this locus, also conferring lactose tolerance, was identified earlier in European populations [12].)

Even in the simplest cases of adaptation, then — increased enzyme production to handle new diets — evolution works in multiple ways. Obviously, no amount of *a priori* speculation will tell us which sorts of mutations will be important; the answer, unfortunately, requires meticulous, case-by-case analysis of putative adaptations.

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