than in control cells, and this effect should be more obvious for chromosome IV than III. Indeed, both predictions were met, providing strong evidence that the checkpoint controls the segregational fate of homologs with distal exchanges.

Pas de deux

To test the model more directly, the authors decided to take matters into their own hands by artificially lassoing the chromosomes together. Specifically, they inserted tandem repeats of the Lac operator at different sites on chromosome IV and used a tetramerizing form of the Lac repressor fused to GFP to cross-link the DNA, creating an artificial tether between the homologs. This not only worked, but provided further evidence for the importance of location. Nondisjunction levels were threefold lower in *mad2*Δ cells with a functional form of the tether than in cells with a nonfunctional tether. Further, the closer the tether was placed to the centromere, the better it worked—when it was 50 kb or less from the centromere, nondisjunction rates were minimal (3–5%), but at 100 kb or more, they increased to rates comparable to those observed for the nonfunctional tether. When placed near the centromere, the tether even facilitated segregation of chromosomes that normally do not recombine, either because of sequence divergence (that is, the chromosomes are 'homeologs') or because of a mutation affecting the catalytic portion of the double strand break–inducing protein Spo11p. All in all, these man-made links were not as good as natural crossovers, but not bad either—as long as the tether was placed in the proximal region of the chromosome.

What do these observations mean for nondisjunction in other organisms, including humans? For example, do cell-cycle control mechanisms—specifically, those associated with the spindle checkpoint—deteriorate with age and, if so, do they preferentially affect homologs with certain types of crossover configurations (for example, those held together by distal exchanges)? There is evidence from studies of female mice linking cell-cycle disturbances to age-dependent meiotic abnormalities[4] and a suggestion that levels of Mad2 and BubR1 decline with age[5,6]. However, the jury is still out on whether the rules of spindle checkpoint control differ in the mammalian oocyte or are affected by maternal age[7]. What about distal exchanges? Are they more common in trisomies involving older than younger women, as would be predicted if there were an age-dependent decline in spindle-checkpoint function (that is, distal exchanges would be 'handled' in the young but not the old ovary)? Evidence from studies of recombination in human trisomies suggests that the answer is yes…and no. For trisomy 16, the most commonly occurring human trisomy, the answer may be yes. Distally located exchanges are a major contributor regardless of maternal age (ref. 8; H. Hall and T. Hassold, unpublished observations) and, in absolute terms, are more common in older women. In contrast, for trisomy 21 (Down's syndrome), the chromosome with the largest body of data, the answer seems to be no. Distal exchanges appear to be an important factor in the genesis of trisomy 21 in young women, but less so—if at all—in older women[9].

These human data should not detract from the main message provided by Lacefield and Murray[3]: that is, it is best to hold your partners close, lest they stray in an undesired direction. Nevertheless, it seems unlikely that the results, intriguing as they are, provide the long-sought one-size-fits-all answer for human aneuploidy. Like many recent findings in this field, they have deepened our understanding of the meiotic process, but we will likely still have to unravel human age-related aneuploidy one chromosome at a time.

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Adaptive drool in the gene pool

John Novembre, Jonathan K Pritchard & Graham Coop

A new study finds that copy number variation in the salivary amylase gene in humans is associated with amylase concentration in saliva and average starch consumption in populations. This provides a striking example of the role of copy number variants (CNVs) in adaptive evolution, and of diet in producing selective pressures.

Human populations have adapted to a wide range of environments, and one important component of this process has been changes in diet. For example, with the onset of the first agricultural revolution, about 10,000 years ago, populations faced selective pressures from huge changes in diet. On page 1256 of this issue, a new study by Nathaniel Dominy and colleagues[1] highlights the role diet can play in human evolutionary history, providing evidence that shifts to starch-rich diets may have led to selection of copy-number variation in a key enzyme.

John Novembre, Jonathan K. Pritchard and Graham Coop are in the Department of Human Genetics, University of Chicago, Chicago, Illinois 60637, USA.
e-mail: jnovembre@uchicago.edu or gcoop@uchicago.edu.

Genes, geography and gastronomy

Geneticists have long been interested in cheek swabs as a simple protocol for isolating DNA from humans, but Perry *et al.*[1] are intrigued by another aspect of the human mouth, our saliva. Food metabolism begins in saliva, where amylase, a starch-digesting enzyme, plays a key role. The copy number of the salivary amylase gene is known to be variable, and Perry *et al.* now find that concentrations of salivary amylase are proportional to gene copy number.

Having established a functional consequence of genetic variation in amylase copy number, the authors turned to population genetic patterns to learn about the evolution of this locus. They examined samples from seven populations, three with high-starch diets and four with low-starch diets. They found that amylase copy number is higher, on average, in the set of populations with starch-heavy diets (**Fig. 1**). Importantly, neither the study populations with

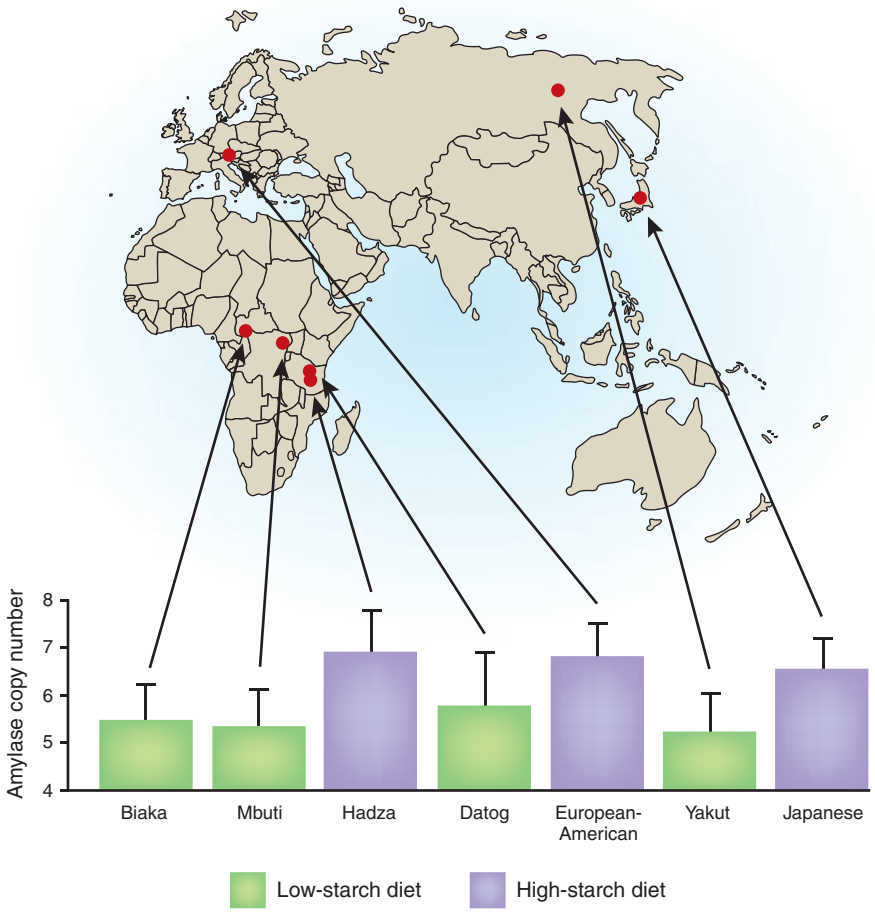

Figure 1 The distribution of salivary amylase copy number in the seven samples from Perry *et al.*[1] The bar chart depicts the mean copy number per sample, with an interval of two standard errors above the mean. Mean copy number is found to be higher in populations with high-starch diets, even when samples are relatively near one another geographically (for example, comparing Hadza and Datog or Yakut and Japanese populations).

high-starch diets nor those with low-starch diets are geographically clustered, which reduces the concern that the observed associations between copy number and diet are simply due to shared ancestry (**Fig. 1**). The authors also show that the differences in copy number between one low-starch population (the Yakut) and one high-starch population (the Japanese) are significantly larger than differences at most other CNV loci, supporting the hypothesis that local diets create strong positive selection on amylase copy number. Finally, using patterns of amylase CNVs in chimpanzees and bonobos, as well as sequence divergence among copies of the amylase gene, Perry *et al.* show that expansions of amylase copy number seem to have occurred recently in the human lineage (perhaps within the last 200,000 years).

Chewing it over

Taken together, the data from Perry *et al.* on amylase variation in humans suggest that higher copy number may be selected for in populations that consume large amounts of starch. The study also raises a number of interesting questions about the details of when and how this selection may have occurred. In Japanese and European-American populations with high-starch diets, are there signs of a recent selective sweep that might indicate that selection pressure began with the domestication of grains (~10,000 years ago)? Or did the selection pressure begin earlier, as early hominids shifted towards foraging on plant underground storage organs (as suggested in ref. 2)? Also, is the relatively large amount of variation in copy number observed within the study populations a sign either that the selective pressure is relatively recent (and thus has not had enough time to reduce variation) or that migration or copy-number mutation introduce alternative copy number variants at a rate that is large relative to the strength of selection? More detailed studies of haplotype variation in the salivary amylase region would help address these questions and might also reveal whether high-copy-number alleles arose independently in each of the high-starch populations, or whether they have shared descent.

An appetite for answers

Elucidating the selective pressures that have acted on the human genome is a key challenge in population genetics. Although the current study suggests that changes in diet provide the selective pressure underlying changes in copy number at amylase, there are many putatively selected regions where the causes of selection are unknown. By understanding the geographic extent of a selective sweep—that is, the populations in which the sweep has occurred—we can improve our understanding of the selective pressure that has shaped variation. As Perry *et al.* show, correlating the frequency of a selected allele to a putative selection pressure offers a helpful way of learning whether that selection pressure can explain the frequency differences of the allele (see also ref. 3).

However, this task is complicated by difficulties in comparing selective pressures across populations. For example, pastoral populations have different diets *and* pathogens than hunter-gatherer populations, making it difficult to pinpoint the exact selective pressure except where there is a clear prior hypothesis about the function of the putatively selected locus. The task is also complicated by the dynamic interaction of selective sweeps and human population history. In particular, a beneficial mutation can take considerable time to move from one region to another if rates of gene flow are low, so that the absence of a beneficial mutation in a region does not imply the absence of the selective pressure. Parallel mutation, as in the case of lactose tolerance[4], can also complicate the picture by allowing populations to become similarly adapted via different genetic routes.

Understanding the basis of human adaptations presents such a fascinating challenge that these difficulties will surely be overcome where possible. Association studies offer a promising way of directly relating selected alleles to phenotypes[4,5] and will also allow differences across populations to be related to phenotypes. Studying patterns of variation in closely related species is another promising approach, as shown in the amylase example.

Savory implications for adaptive change

Changing gene copy number, as a crude mechanism for adjusting gene expression levels, may prove to be a widespread mode of adaptive change. It has recently been shown that a nontrivial fraction of genetic variation in gene expression levels is due to copy number variation[6,7]. Also, Redon *et al.*[8] highlighted several examples of CNV loci that show extreme dif-

ferences in average copy number between the HapMap populations, notably including *CCL3L1*, which is involved in HIV susceptibility[9]. Unfortunately, there may be difficulties in detecting selection on CNV loci: the standard pattern of a selective sweep in surrounding SNPs may not be very strong, as higher rates of mutation make selected CNV alleles more likely appear on multiple haplotypes[10]. To adjust for this, scans for selection between populations should therefore include direct targeting of CNV loci, as they are excellent candidates for selection but may not be well tagged by surrounding variation.

The example of amylase might also be representative of the importance of diet as a widespread selective pressure in human evolution. For example, it is already well known that the selective sweep associated with lactase persistence is one of the strongest signals of selection in the genome[11,12], and multiple populations have evolved lactase persistence independently[4]. So, as far as our evolutionary history is concerned, it seems we really are what we eat.

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Rhythm is not enough

Bernhard Horsthemke

***MAGEL2* is located in a cluster of imprinted genes on human chromosome 15 that is implicated in Prader-Willi syndrome (PWS). A new study shows that mice deficient for this gene show altered behavioral rhythmicity that resembles some features of PWS.**

The jet lag we feel after crossing several time zones reminds us that many of our bodily functions are under the control of a biological clock. The central component of this clock, located in the hypothalamus, is a transcriptional-translational feedback loop that generates circadian (~24 h) oscillations (**Fig. 1**). The clock can be reset by light via the input pathway, and the output pathway translates the oscillations into physiological and behavioral rhythms. As shown by Kozlov *et al.* on page 1266 of this issue[1], the imprinted gene *Magel2* seems to be an important component of the output pathway in mice.

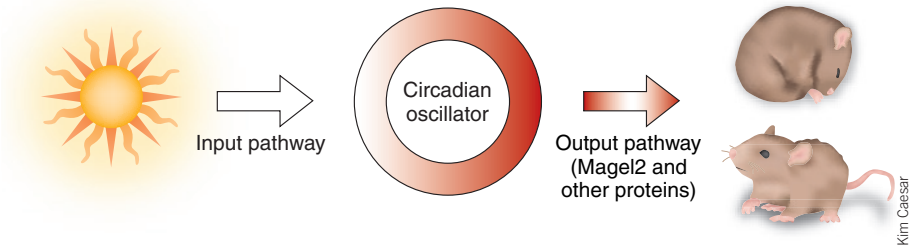

Figure 1 An autonomous, self-sustained oscillator acts as the core of the biological clock. The clock can be reset by light and controls various bodily functions, including wakefulness and sleep.

The hypothalamus and PWS

In a screen for cycling transcripts in the hypothalamus of mice, Panda *et al.*[2] found that a relatively small number of output genes are directly regulated by the oscillator, and *Magel2* is among them. The human ortholog, *MAGEL2*, is located on the proximal long arm of chromosome 15 (15q11-q13) and is expressed from the paternal allele only. Lack of a paternal copy of 15q11-q13 causes PWS, a neurobehavioral disease characterized by neonatal muscular hypotonia and failure to thrive, hyperphagia and obesity starting in early childhood, hypogonadism, short stature, behavioral problems and mental retardation. As *MAGEL2* is highly expressed in the hypothalamus, and a hypothalamic defect most likely underlies PWS, *MAGEL2* deficiency may account for some of the clinical features of PWS.

Kozlov *et al.*[1] generated mice that do not express *Magel2*. These mice were weaned at a somewhat lower frequency than expected, but they did not show any substantial alterations in size or weight, even by two years of age. However, although the mice had a normal circadian rhythm, they ran noticeably less during the night (when mice should be awake) and had increased activity during the day (when mice should be asleep). These changes were associated with reductions in food intake and male fertility, and they establish *Magel2* as a circadian-output gene.

Circadian rhythmicity and imprinting

The work raises three important questions: (i) how does *Magel2* translate the oscillations into behavioral and physiological rhythms, (ii) why is a circadian-output gene subject to genomic imprinting and (iii) what is the role of *MAGEL2* deficiency in PWS?

The function of the Magel2 protein is unknown, but Kozlov *et al.*[1] found reduced levels of the neuropeptides orexin A and B in the hypothalamus of *Magel2*-deficient mice, whereas the levels of prepro-orexin, from which the orexins are derived, were elevated. Orexin-expressing neurons project throughout the brain, particularly in regions implicated in regulating sleep-wakefulness, body temperature and feeding. The authors speculate that Magel2 may modulate orexin signaling by affecting the post-translational processing of prepro-orexin to orexin. This effect, however, must be indirect, because Magel2 is not a prepropeptide-converting enzyme.

Magel2 maps to a chromosomal domain that is differentially marked (imprinted) by DNA methylation during oogenesis and spermatogenesis. Consequently, the maternal allele is

Bernhard Horsthemke is at the Institut für Humangenetik, Universitätsklinikum Essen, Germany.
e-mail: bernhard.horsthemke@uni-due.de