long-standing issues in meiotic recombination. I will focus on three: where recombination occurs; the type of recombination that occurs; and how recombination events are spaced along individual chromosomes.

Meiotic recombination is initiated by self-inflicted breaks in the double-stranded DNA; these breaks are then repaired by recombination with a homologous chromosome (Fig. 1). Genome-wide maps of meiotic double-strand breaks in budding yeast have indicated that most breaks occur in 'hotspots' that are distributed throughout the genome, with an average inter-hotspot distance of less than 10,000 bases[4]. Mancera and colleagues' recombination map[1] closely parallels these maps of double-strand breaks, with recombination occurring at almost all regions. In fact, the only truly recombination-cold regions of the yeast genome are associated with repeated sequences. These include mobile genetic elements, the DNA that encodes ribosomal RNA and sequences close to some chromosome ends.

The authors also investigated the relationship between the two outcomes of inter-homologue recombination: crossovers, where parental sequences flanking the recombination site are swapped; and non-crossovers, in which no swap occurs (Fig. 1). Previous studies[2] of several test regions had shown that crossovers and non-crossovers associated with gene conversion occur with similar frequencies. Although this suggested that crossovers and non-crossovers are alternative outcomes of a common pathway, subsequent studies indicated that they are produced by distinct molecular mechanisms[5].

Mancera and co-workers' data[1] reveal an unanticipated feature of genome-wide recombination patterns, namely the existence of regions (about 1% of the genome — an amount significantly greater than expected by chance) where the ratios of crossovers to non-crossovers differ substantially from the genome-wide average. The identification of hotspots where either non-crossovers or crossovers predominate, if confirmed, will provide regions where each type of recombination can be studied in relative isolation.

The existence of non-crossover hotspots also has implications for the design and interpretation of studies that use linkage disequilibrium (the disproportionate occurrence of certain gene combinations) to determine genetic association. If non-crossover hotspots exist, they would create 'holes' of low linkage disequilibrium within linkage-disequilibrium blocks, and copies of genes near such hotspots would be difficult to track relative to outside markers.

The authors' findings also reveal new features of the spacing between recombination events. It has been well documented (and confirmed by the present study) that crossovers show positive interference — that is, crossover at a locus reduces the likelihood of a second crossover nearby. This results in a more uniform inter-crossover spacing than is expected by chance. Previous studies that analysed thousands of tetrads found no evidence for positive interference between a crossover and a non-crossover[6,7]. By contrast, Mancera and co-workers' analysis of the spacing between non-crossovers and crossovers reveals a modest yet significant level of positive interference. This suggests that the spacing between recombination events is controlled, in part, at a very early step in recombination, perhaps at the time of formation of the double-strand break itself.

These findings, although intriguing, must be interpreted with caution. In particular, it is likely that a high marker density, which is required for high-resolution scoring of recombination, has the collateral consequence of altering the outcome of some events. Sequence differences between parental chromosomes at densities similar to those in this study have been shown to substantially alter the outcome of meiotic recombination[8], most likely by causing the disassembly of intermediates of inter-homologue recombination and redirecting events towards multiple exchange or genetically invisible recombination between sister chromatids. So, although Mancera and colleagues' data are probably representative of some 'real world' meioses — where hybrids with similarly high densities of sequence polymorphism are occasionally found — the above concerns should be kept in mind when considering the implications for recombination mechanisms.

Cautions and cavils aside, this fascinating study opens new doors in the study of meiotic recombination. For years to come, these data will be fertile material for analysis, and the insights gained from this approach in budding yeast should prompt others to develop similar methods to analyse recombination processes genome-wide in other organisms. ■

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ECOLOGY

Forest air conditioning

F. I. Woodward

During the growing season, with photosynthesis at its peak, leaf temperatures remain constant over a wide latitudinal range. This is a finding that overturns a common assumption and has various ramifications.

The wood laid down as tree rings is rich in environmental information. The rings provide measures of annual growth. And the oxygen isotopic composition (^{18}O and ^{16}O) of wood cellulose provides estimates of historical temperature and humidity[1] — take a piece of wood, extract the cellulose, measure the ratio of ^{18}O to ^{16}O, slot the observations into a model of factors that favour one isotope over the other, and out pops a measure of ambient temperature and humidity.

But things are not so simple, say Helliker and Richter (page 511 of this issue)[2]. They have found that the oxygen-isotope ratios in cellulose collected across 50° of northern latitude, ranging from subtropical to boreal forest ecosystems, indicate that growing-season leaf temperatures are virtually constant. This latitudinal range has a 15 °C difference in growing-season temperatures. The more-or-less constant leaf temperatures (21.4 ± 2.2 °C) not only belie the assumption that leaf temperatures are the same as ambient temperatures, but also mean that humidity reconstructions will yield much lower values for cooler climates than would otherwise be expected and higher values for warmer climates.

The ratio of the ^{18}O and ^{16}O isotopes in wood cellulose is determined by the isotopic exchanges in leaves when they are synthesizing the sucrose from which cellulose is produced[3] (Fig. 1). The ratio is largely determined by the periods of maximum photosynthesis (around midday and around midsummer of the growing season), when the greatest amounts of sucrose are made before its incorporation into cellulose. The temperature-sensitive isotopic composition of precipitation and the rate of leaf evaporation (transpiration) both control the ^{18}O and ^{16}O ratio[1,3]. Determining ambient temperature and humidity directly from the ^{18}O:^{16}O composition of wood cellulose[1] depends on the simplifying assumption that leaves are at ambient temperature, and that the relative humidity of the air directly controls evaporation. This convenient assumption is highly unlikely to apply at times of maximum photosynthesis. The leaf temperature will

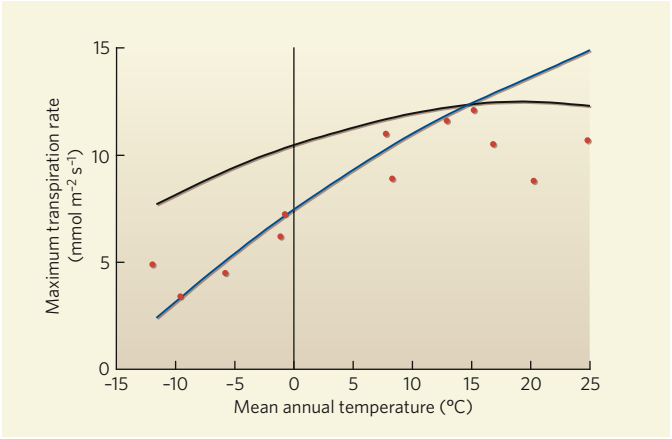
Box 1 | A test of constant leaf temperature

Helliker and Richter[2] provide climatic, isotopic and latitudinal data that can be used to test their conclusions by considering the energy balance of leaves in a canopy.

Convection is one way that leaves exchange energy with the ambient air; another is by evaporation of leaf water (transpiration). The rate of transpiration depends on the difference between leaf and air temperatures, and on the difference in humidity between the surrounding air and the leaf interior (assumed to be saturated at leaf temperature).

When leaf temperature equals air temperature, all energy exchange is assumed to occur by transpiration (black line in the figure). When the leaf temperature is constant and differs from the air temperature, energy exchange occurs by both convection and transpiration (blue line), and so transpiration rates are generally less than when leaf and air temperatures are equal. Observations of maximum rates of canopy transpiration[4,6] (red dots) can be compared with these expectations; all the data points are from forested sites, except the two at the lowest temperatures.

Constancy of leaf temperature[2] fits well with these measurements up to mean annual temperatures of 15 °C. Beyond that point, where the two curves cross, however, leaf temperatures of 21.4 °C will be less than the growing-season air temperatures. In consequence, leaves will be gaining heat from the surrounding air, further increasing transpiration, a response not observed at these forest sites[4]. **F.I.W.**



then exceed air temperature, and the gradient for evaporation is the difference in humidity between saturated air in the leaf and ambient air, which is quite different from the relative humidity of the air.

The approach adopted by Helliker and Richter[2] was to reject the assumption that leaves are at ambient temperature, and to calculate leaf temperatures using site-specific climatic data, the isotopic composition of precipitation and the ^{18}O:^{16}O composition of wood cellulose. The surprising outcome is that the calculated leaf temperatures are virtually constant across all of the sites sampled, contrasting with studies[1] that show large changes in calculated temperature with latitude. So, for example, during the growing season, leaf temperatures in a boreal forest will be on average 10 °C warmer than air temperatures, and the gradient for evaporation may be as much as twice that calculated under the assumption that air and leaf temperatures are equal.

This amount of temperature elevation is quite unexpected, especially for coniferous trees of the boreal zone — the leaves of these trees are needle-like and readily exchange energy with the surrounding air by convection, so that when isolated they closely follow air temperature. The explanation for the effect calculated by Helliker and Richter, as they themselves propose, is that in a forest the leaves are not isolated; rather, they are frequently bunched together in tight canopies (as anyone who has tried to stroll through mature boreal forest, or even a Sitka spruce plantation, will know). These dense and compact structures minimize the rate of convective energy exchange. Leaf temperatures are raised above ambient predominantly as a result of this canopy effect, rather than through a leaf-driven process.

Testing Helliker and Richter's conclusions is difficult, as leaf temperatures of the most actively photosynthesizing leaves in a forest canopy are not measured on a regular basis. An alternative test, which I have carried out, can be achieved by calculating the energy balance of leaves in a canopy over a wide range of growing-season temperatures, as described in Box 1. From this, one can conclude that the constancy in leaf temperature fits well with observations up to the point where the calculated leaf temperature equals the growing-season temperature. The data provided by Helliker and Richter imply that, beyond this point, leaf temperatures are lower than growing-season temperatures. As a consequence, leaves would be gaining heat from the surrounding air, a response not observed at selected forest sites[4].

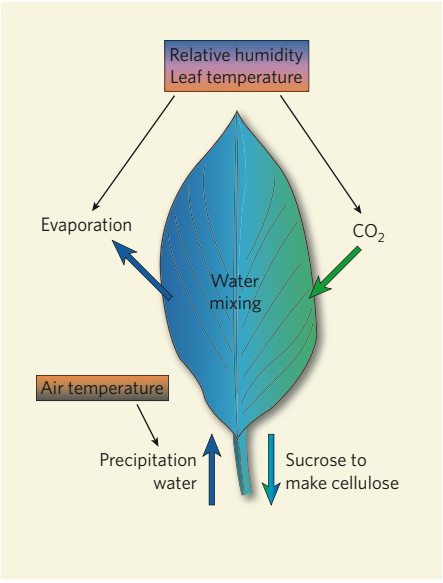


Figure 1 | Factors determining the ^{18}O:^{16}O ratio in wood cellulose. The ratio depends on differential discrimination against ^{18}O and ^{16}O in the leaf, and on the isotopic composition of water supplied by precipitation[2,3]. Evaporation (transpiration), which depends on the relative humidity of the air and on leaf temperature, favours the lighter isotope, and so the leaf becomes enriched in ^{18}O. The ^{18}O:^{16}O ratio of water in the sucrose produced by photosynthesis, which is a substrate for cellulose production, depends on a mix of enriched ^{18}O at the sites of evaporation and unenriched precipitation water. This ratio is influenced by the rate of evaporation and where in the leaf this mixing occurs. ^{18}O and ^{16}O from CO_2 exchange mixes completely with water before sucrose is synthesized.

A result of Helliker and Richter's study is that climatic reconstructions using oxygen isotopes in tree-ring cellulose will require reanalysis that takes into account the effects of leaf temperature and relative humidity on the ^{18}O:^{16}O ratio. Global vegetation models that assume that mean air temperature is the temperature for calculating both transpiration and photosynthesis will likewise need to be reviewed. Observations[4] show that the air temperature for maximum rates of carbon dioxide uptake increases by only 8 °C over a 35 °C change in mean annual temperature, indicating that the temperature response of photosynthesis is conservative, as expected from the constancy of leaf temperature.

Leaf structure and physiology show rather modest variations globally[5]. One cause may be the relative uniformity of leaf temperature — at least in fully forested vegetation. The fact that vegetation canopy rather than leaf morphology dominates temperature control in the forests sampled by Helliker and Richter suggests the need for greater emphasis on understanding how the canopy responds to climate change, and to global warming in particular. ■

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