cousins gives exactly the conditions most likely to enable a rare, and usually recessive, character to show itself. If the bearers of such a gamete mate with individuals not bearing it the character will hardly ever be seen; but first cousins will frequently be the bearers of similar gametes, which may in such unions meet each other and thus lead to the manifestation of the peculiar recessive characters in the zygote".

We now know that alkaptonuria is a defect in the enzyme homogentisate dioxygenase (HGO) that catalyzes the conversion of homogentisic acid to maleylacetoacetate. Cloning of the human HGO gene has led to the characterization of at least 20 different loss of function mutations in the HGO enzymes of alkaptonuric patients. When this enzyme is defective, the buildup and oxidation of homogentisic acid leads to a blackening of the urine and to deposits in connective tissues, resulting in degenerative arthritis.

Now, on page 542 of this issue, Timm and coworkers provide another chapter in the story of alkaptonuria — they present the first crystal structure of human HGO. The structure and mechanism they propose may help in the design of HGO inhibitors that could be used to treat hereditary tyrosinemia type I, a fatal disease that is caused by defects in an enzyme downstream of HGO, which results in the abnormal accumulation of tyrosine metabolites. *Boyana Konforti*

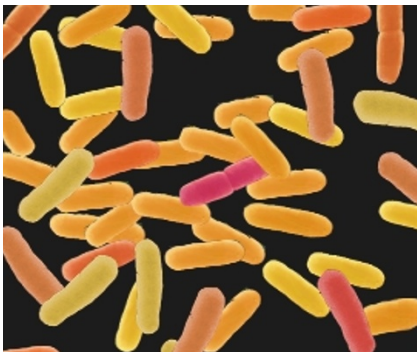
1. W. Bateson. In *Mendel's principles of heredity*. 1–212 (Cambridge University Press, London, England; 1902).
2. A.E. Garrod. The incidence of Alkaptonuria: a study in chemical individuality. *Lancet* **2**, 1616–1620 (1902).

Color me red

Carotenoids are natural pigmented compounds that are widely used as food additives and pharmaceuticals. They are produced mainly by bacteria, fungi, and plants but often cannot be purified in useful quantities from their natural sources. Thus, there is much interest in finding other ways to make them. Now, in the July issue of *Nature Biotechnology* (**18**, 750–753) Claudia Schmidt-Dannert, Daisuke Umeno, and Frances Arnold report the successful 'molecular breeding' of a particular carotenoid biosynthetic pathway in *Escherichia coli*. This is the first example of directed evolution applied to a biosynthetic pathway, rather than a single enzyme.

Carotenoids are composed of long carbon chains, with various modifications. They contain different numbers of carbon–carbon double bonds, and the number of double bonds can be increased by enzymes called desaturases. Other enzymes, called cyclases, also increase the diversity of carotenoids by cyclizing the ends of the carbon chains. Carotenoids with many double bonds and cyclic ends are darker in color than their simpler counterparts.

Schmidt-Dannert *et al.* took advantage of these color differences to create new pathways for making specific carotenoids in *E. coli*, which does not normally produce these compounds. They generated a library of desaturase genetic variants *in vitro* — by 'shuffling' related desaturase genes from two carotenoid-producing species of *Erwinia* bacteria — and transformed this library into an *E. coli* strain that had been engineered to make the appropriate precursor compound, phytoene.



Next, they screened through the colonies, looking for ones displaying darker pigmentation than a colony containing a wild type desaturase gene. The expectation was that some of the desaturase variants in the library, unlike the wild type desaturase enzyme, would be capable of producing additional, darkly colored carotenoids. The picture shows an artistic rendition of individual *E. coli* cells producing a sampling of carotenoids, ranging in color from pale yellow to reddish-purple.

This procedure worked. Schmidt-Dannert *et al.* were able to isolate a pink colony containing the carotenoid tetradehydrolycopene, which has six more double bonds than phytoene, the starting substrate. Using the same basic approach, but with a different enzyme, this group was then able to extend the biosynthetic pathway to include a cyclase. They obtained a mutant cyclase that could act on the new tetradehydrolycopene substrate to produce torulene, a bright red carotenoid.

The selected desaturase and cyclase genes have been sequenced, but it is not yet understood how the observed mutations affect the structures of the proteins. Nonetheless, it is evident that the torulene-producing *E. coli* strain uses a brand new pathway to make the carotenoid — in organisms, such as yeasts, that naturally produce torulene, it is made using different enzymes and starting substrates. These results suggest the exciting possibility that entirely novel compounds could be produced by these combinatorial molecular breeding methods. *Tracy Smith*