Richard A. Sturm

Melanogenix Group, Institute for Molecular Bioscience, University of Queensland, Brisbane, Qld 4072, Australia

Can blue-eyed parents produce brown-eyed children?

Genes for Human Eye Colour

What is the colour of your eyes?

For identification purposes this is a simple question commonly asked as one of the defining physical features, along with skin and hair colour. How an answer is phrased to this question is not so straightforward as you may think, for it immediately brings in the contentious issue of how to classify eye colouration. The topic is of general community interest and some of the most frequently asked questions of the popular USA-Today newspaper Wonderquest web site (http://www.wonderquest.com) relate to eye colour. It is the optical effects produced by the reflection of light on different materials present in the iris (Figure 1A), which is the feature of the eye we refer to when we say a person has blue, green-hazel or brown eye colour. The iris is a small connective tissue and muscular structure of around 12 mm in diameter with a circular opening in the middle, called the pupil. It controls the amount of light entering the eye which is focused by the lens onto the retina so as to provide the sense of vision. The iris operates like the aperture on a camera. It contracts in bright light making the pupil smaller and dilates in dark conditions making the pupil larger.

Melanin pigment and eye colour

The iris appears as a flat conical disc comprising of the anterior (Front-view) and posterior (Back-view) border layers that can each contribute to eye colour, however the anterior layer is by far the more important. In the cross sectional view of the iris in Figure 1B, the anterior layer shown in blue is composed of a translucent stromal tissue that is a mixture of connective proteins, blood vessels, fibroblast, macrophage and melanocyte cells. The melanocyte is the cell that produces the melanin pigment in the iris. The innermost layer of the iris is a tightly fused posterior pigmented epithelium. It is illustrated as an opaque brown circle in Figure 1B when viewing the iris from the retina at the back of the eye. These layers together determine eye colour through a combination of light absorbance, reflectance and scattering dependent upon the degree of melanin pigmentation within the melanocytes of the stroma, and to a much lesser degree the posterior pigmented epithelium. Therefore eye colour is largely dependent upon the amount of melanin produced by the melano-

CORRESPONDENCE TO
Richard A. Sturm
Melanogenix Group, Institute for Molecular Bioscience, University of Queensland, Brisbane, Qld 4072, Australia
Email: R.Sturm@imb.uq.edu.au

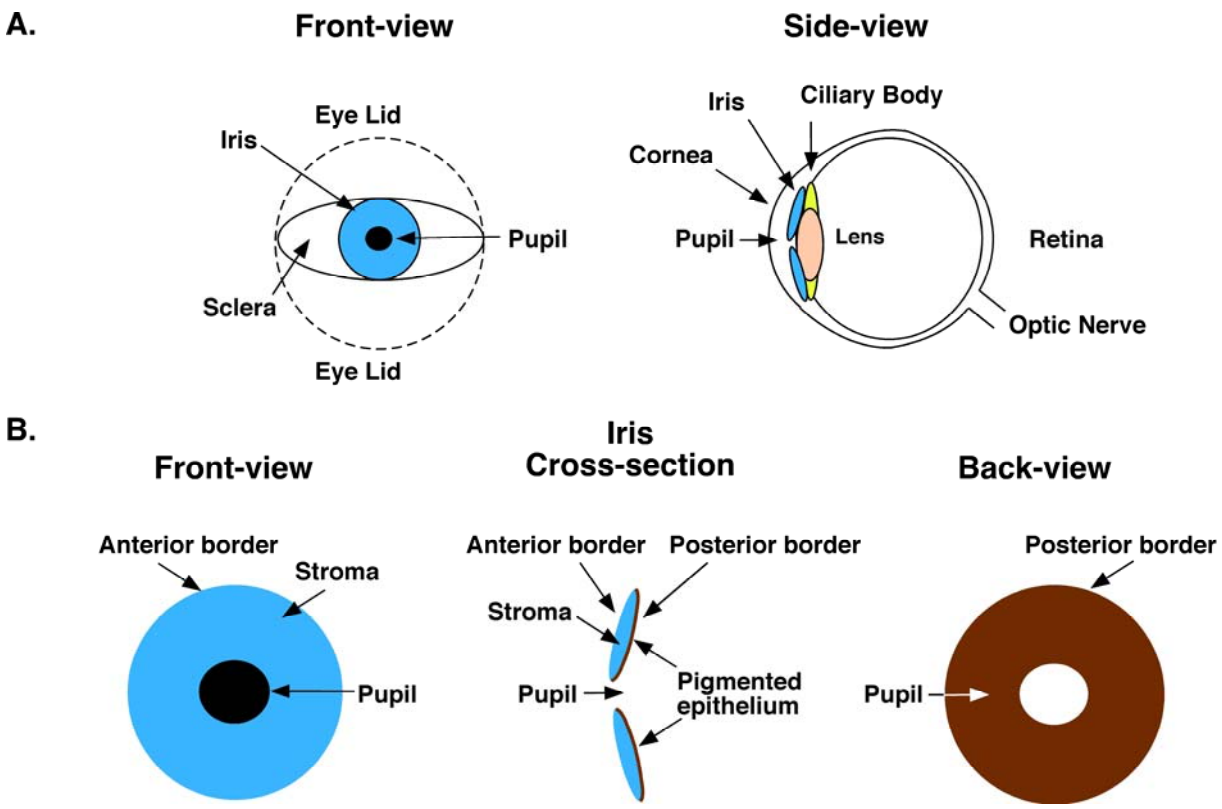

Figure 1.Structure of the eye and iris pigmentation.

cytes. Brown eyes come from heavy pigmentation deposits within the anterior border layer whereas blue eyes come from a lightly pigmented iris. Only if the stroma is thin can some contribution to eye coloration be made from the brown pigment contained within the posterior iris. Light can only pass through the pupil as it is blocked by the heavy pigmentation of the posterior layer, similarly once it has entered the chamber of the eye it is not able to be reflected back out (Figure 1).

Having defined that eye colour specifically refers to the patterns and appearance of the iris, the problem of classification becomes what system may be used to describe these colours. Different lightning conditions may affect how the eye colour is classified. For example, the bright sunlight encountered outdoors may bring out translucent characteristics of the iris that may not be apparent in the relatively dim indoors. Classification is not only difficult because the iris colours, hues and textures change dependent on how eye colour is examined, but because they exist on a continuum from the lightest shades of blue to the darkest of brown or black. A number of genetic studies have usually categorised eyes as a range from blue, grey, green, yellow, hazel, light brown and dark brown, with Figure 2 showing only a small fraction of the differences that can appear within the human population. Whatever classification system may be chosen, standard lighting conditions are essential and observer bias should be avoided. This is the basis for much of the argument and confusion over telling someone what you believe your eye colour to be.

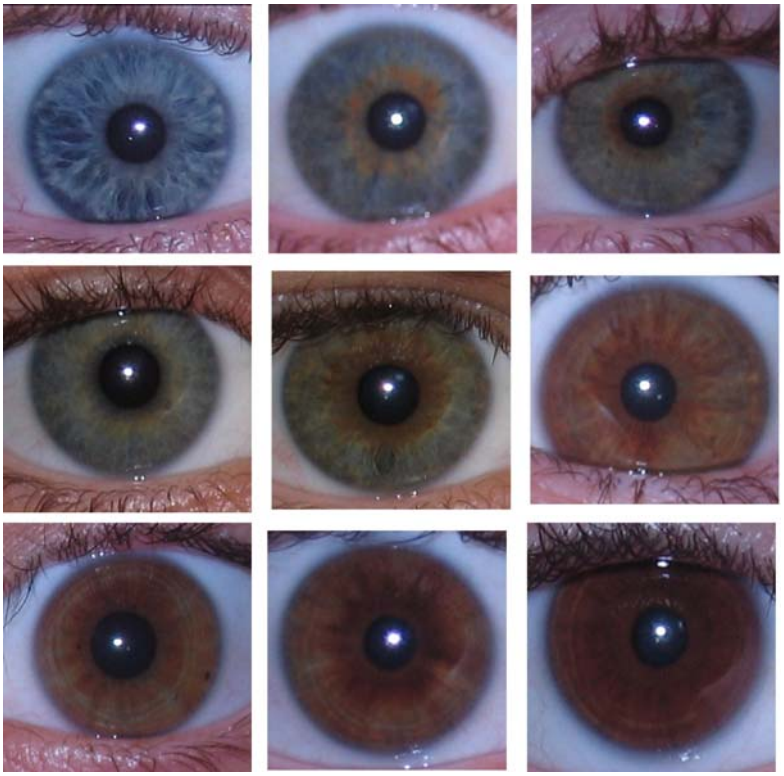

Figure 2. Representative eye colours showing a range from blue to brown.

It is a case of what you or another observer may perceive it to be and the classification system chosen. Observer bias can be removed by using photographic techniques for the comparisons of eye colouration.

Melanin pigment is formed within melanosome particles of the melanocyte cell

Melanin is produced by the melanocyte cell in a specialised subcellular organelle called the melanosome. Two discrete forms of melanin pigment particles have been described which differ in their chemical and protein composition. Eumelanin is a brown/black form of pigment responsible for dark colouration and is packaged in eumelanosomes which are striated, whereas pheomelanin is a red/yellow pigment produced in pheomelanosomes which are granular. Both forms can be found in the skin and hair melanocytes which are transported out of the cell and secreted into the surrounding keratinocytes [1]. Eumelanin usually predominates and it is the amount of melanin that determines the darkness of the skin and the hair. However, melanocytes in red-haired people predominately produce pheomelanin, which explains their light skin colour and lack of ability to get tanned. Unlike the skin and hair in which melanin is made continuously, the pigment particles of the eye are formed and degraded more slowly, retained within the melanocyte cells of the iris, and only contain eumelanin [2]. The melanosomes of the eye are not usually transported and deposited within the iris stroma but can appear in macrophages which are scavenger cells.

Melanin is produced via an oxidative process based on a complex chemical pathway that is not entirely understood. These reactions are partitioned within the melanosome of the melanocyte and begin with the amino acids tyrosine and cysteine. Tyrosine is the building block which is first converted to dopaquinone by a key enzyme known as tyrosinase (TYR). Dopaquinone is an intermediate substrate that goes onto form either eumelanin, when cysteine is absent, or pheomelanin when cysteine is present. Several other enzymes have been isolated which help produce the brown and black eumelanins including tyrosine-related protein-1 (TYRP1) and dopachrome tautomerase (DCT). Many other proteins are also necessary to help form the mature melanosome particle and some of these are listed in Table 1.

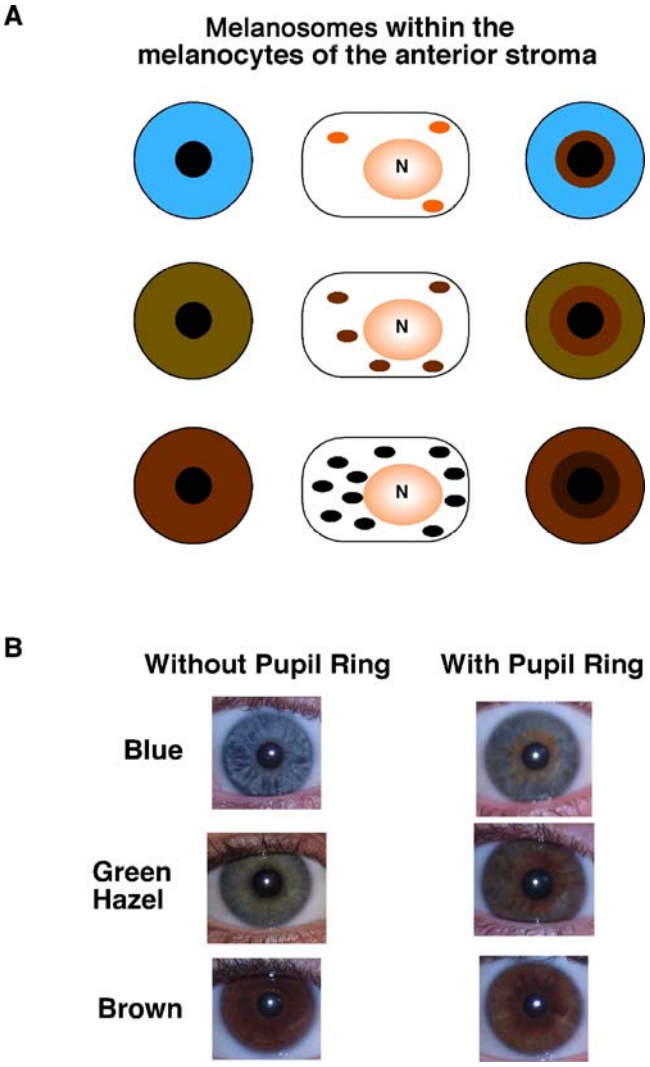

Figure 3.
A. The melanosomal basis of human eye colour.

B. Examples of blue, greenhazel and brown with and without a pigmented pupil ring.

The presence and distribution of melanin is a major determinant of human eye colour

The physical basis of eye colour is determined by the distribution and content of the melanocyte cells in the anterior stroma of the eye (Figure 3). In the brown iris there is an abundance of mature melanosomes within the melanocytes of the stroma, whereas the melanocytes in the blue iris contains a very small number of melanosomes and as such little total melanin. As light traverses the anterior border the minute melanosomal particles of the iris scatter the short blue wavelengths to the surface. Thus, blue eye colour is a consequence of structures in the iris, not of major differences in chemical composition of the melanin – there is no such thing as blue melanin. It is important to understand that the number of melanocytes does not appear to differ in eyes of different colours, but the melanin pigment quantity, packaging and quality does vary, giving rise to a range of eye shades. The pattern of eye colour can although be affected by the density and cellular composition of the connective tissue in the iris stroma. It can give rise to crypts of white that appear in the eye (See Figure 2). Even if the posterior epithelium is heavily pigmented, it is similarily pigmented in irises of different colour and does not exert any major influence of the eye colour. People with albinism lack pigment in the iris and have eyes that may appear pink due to the reflection of light from blood vessels.

White light entering the iris can absorb or reflect a spectrum of wavelengths giving rise to the three common iris colours referred to as blue, green/hazel and brown as shown to the left of panel A in Figure 3, but it must be emphasised again that these broad classifications are simplistic and that there is actually a continuum in the range of eye colours seen. The middle of the panel illustrates the intracellular distribution and content of the melanosome particles within the iridial melanocytes (N, cell nucleus). Although blue eyes have similar numbers of melanocyte cells they contain minimal pigment and few melanosomes, green/hazel irises are the product of moderate pigment levels and melanosomes, and brown irises the result of high melanin levels and melanosomal particle numbers. Each of these eye colours can occur with or without a darker pigmented ring around the pupil, represented to the right of the panel A in Figure 3. Insufficient anatomical studies have been performed into the nature of this

ring. Surprisingly our own work on eye colour classification using digital photographs of adolescent teenagers has found that as many as 70% of those called with green-hazel iris colour have the brown pupillary ring, which maybe a characteristic of non-blue/non-brown eye colours. This major pattern may explain a lot of eye colour that is commonly referred to as hazel.

Identifying genes involved in melanin pigment formation

The study of visible traits, such as colouration in animals, has a long and rich history in genetics. The myriad of coat colours seen in the laboratory mouse and the study of their inheritance during breeding experiments have allowed the classification of at least 127 independent variations in coat pattern. A study of these coat colour mutations has in turn allowed the identification of some 60 genes that are responsible and contribute to the melanin formation of melanocyte cells [3]. Comparison of different forms of these genes, known as polymorphisms, has provided one of the means to identify genes involved in natural variation of pigmentation within human populations [4, 5]. Another way in which genes important to pigmentation have been found is the study of human genetic diseases in which skin, hair or eye colour has been affected and these are known as albinism conditions (http://albinismdb.med.umn.edu/). Table 1 lists the genes responsible for human oculocutaneous albinism types 1 to 4 as TYR, OCA2, TYRP1 and SLC45A2 respectively. Now that some of these major genes responsible for melanin pigment formation have been discovered, the goal of understanding the differences and similarities of human eye, skin and hair colour, which are all interlinked, has become more complex. We need to determine the role the proteins encoded by these genes play to learn how they help in melanin synthesis or melanosome formation, and if they may physically interact. Only upon a full investigation of the action of these pigmentation genes will we truly understand the inheritance of physical traits such as human eye colour.

Figure 4. Heterochromia shows different coloured eyes .

The genetics of eye colour

As an easily scored sign of difference and individuality, but perhaps not classification, it is not surprising that one of the first investigations in human genetics was

the examination of inheritance of eye colour [6]. A century ago, the Davenport's reported in 1907 that brown eye colour was a dominant genetic trait to blue eye colour and it has now become accepted folklore that two blue eyed parents always produce a blue-eyed child, never one with brown eyes. Unfortunately, as with most human physical traits, this simplistic Mendelian model does not convey the complexity of real life. We now know that eye colour is inherited due to the action of multiple genes (Table 1) and not the result of a single gene. Thus eye colouration is referred to as a polygenic trait. Moreover, in a condition know as heterochromia an individual can have eyes of different colour (Figure 4), showing that the amount of melanin contained in the iris can be modified by congenital defects that arise through abnormal development of cells of the eye, physical damage or disease, as well as be determined by genetics. There are also normal changes associated with development and aging, all babies are born blue-eyed with mature eye colour not set until past six years old whereafter most individuals have stable eye colour [7]. In a long term study it was reported that up to 11% of white adults have eye changes when examined approximately 16 years apart. Thus eye colour seems to change in some individuals during later years and most of this is a lightening of colour towards blue. A similar age correlation is seen with hair greying.

What is not often discussed is that almost as soon as the Davenport's announced the conclusions of their familial studies on eye colour there were multiple other reports in the years shortly following that challenged the broad and absolute description of brown eye colour genetic dominance, or at least found exceptions to it. Although not common, two blue-eyed parents can produce children with darker eye colours. More commonly reported was the inheritance of brown eye colour in children from two parents classified with green-hazel eye colour. Although eye colour is commonly used as a easy introduction to human genetics in discussing it as a dominant-recessive model of inheritance of brown-blue, this is too simplistic and should not be used. This model is only a rough approximation and causes some consternation for the families where children of dark eye colour have parents of lighter eye colour. Such families are to be expected and not treated as the exception, it is far better to admit the genetic complexity involved than compromise the true nature of its inheritance for simple explanation.

The OCA2 locus is the major gene for blue / brown eye colour

Our understanding of the genes that contribute to the physical appearance of human eye colour have progressed remarkably. Many different proteins are involved; signal proteins that affect the melanocyte to either increase or decrease synthesis of melanin, melanosome proteins of different kinds that either are

enzymes involved in melanin synthesis or proteins that in different ways affect the synthesis. In skin and hair colour there are also important proteins that are involved in melanosome transport within the cells or in uptake of melanosomes in surrounding skin cells. The latter proteins do however not seem to be involved in eye colour since no such transport has so far been observed in the iris melanocytes. The challenge to predict the colour of a person's eyes from the combination of genes they may inherit from their parents will always be constrained on how these genes act in concert together within the progeny. Genetics will play its part and strongly influence the appearance of eye colour in families but don't be surprised by the diversity since other factors may affect the colour.

It has been shown that the OCA2 gene on chromosome 15 is the major candidate responsible for eye colour [9, 10]. In a study of 502 families it was estimated that it could explain up to 74% of blue/brown eye colour variance contained in this one chromosomal region [10]. However, there are other genes that can modify the major influence that the OCA2 gene plays on eye colour determination. TYR, TYRP1, DCT, SLC42A5, ASIP and MYO5A loci also have effects on eye colour variation. Of all these genes it was found that the TYRP1 polymorphisms were the next strongest, followed by SLC42A5 [9].

How the combination of these other genes, known as the genetic background, effects the major role OCA2 plays in determining blue/brown eye colour can be explained through the direct interaction that occurs at the protein level between OCA2, TYRP1, SLC42A5 and other proteins that help make the melanin pigment. The predominant version, referred to as an allele, of the OCA2 gene in white populations produces the P-protein in low amounts compared to the levels required for full activity. This leads to less production of melanin and gives rise to the recessive blue eye colour. Another OCA2 allele can produce the P-protein very efficiently giving rise to the dominant brown eye colour. The other genes involved in eye colour may compensate for the less efficient OCA2 allele if the other eye colour genes are themselves strongly functional or overactive because in the end it is only the final amount of produced melanin that matters. This is why eye colour is a polygenic trait. It is how the genes coordinate themselves, and the proteins they encode, to act in unison to determine the density and content of melanosomes that ultimately creates a persons eye colour.

Finally, after over a century of study the genetic details in the determination of eye colour are becoming better understood. Additional studies performed on the same families used for the eye colour mapping studies to chromosome 15 [10], using SNP (Single Nucleotide Polymorphism) markers spanning the entire OCA2 gene, has located a region at the start of the gene that is highly associated with blue eye colour [11]. The posi-

tion of these SNPs within the beginning of the gene immediately suggests it is the regulation of the OCA2 protein production (the P-protein which alters the pH of the melanocytes) that is the mechanism for diminished melanin content in the blue eyes of white populations. The three SNP changes "T-G-T" that are close together within the weak OCA2 allele are themselves probably not the cause of blue eye colour, but are located very close to the changes in the DNA sequence that are responsible. They serve only as markers to indicate but not in themselves determine eye colour. From these association studies what can be said is that up 90% of people with an OCA2 "TGT/TGT" genotype will have non-brown eye colour and most of those will be blue eyed. Any of the changes to those three letters will occur more frequently in those of brown eye colour and are associated with the strong OCA2 allele. Further studies are needed to define the actual DNA changes of the OCA2 gene responsible for blue/brown eye colouration, the effects on the P-protein and its cellular role.

Table 1 Human pigmentation-related genes, proteins and function

Locus	Chromosome	Protein	Mutation/Phenotype	Function
Melanosome Proteins				
TYR	11q14-q21	Tyrosinase	Oculocutaneous albinsim type 1	Oxidation of tyrosine,dopa,
TYRP1	9p23	TRYP1	Oculocutaneous albinsim type 3	DHICA-oxidase, TYR stabilisation
DCT	13q32	DCT, TRYP2	?	Dopachrome tautomerase
SILV	12q13-q14	Silver	?	DHICA-polymerisation and melanosome striations
OCA2	15q11.2-q12	P-protein	Oculocutaneous albinsim type 2; eye colour	pH of melanosome and melanosome maturation
SLC45A2	5p13.3	MATP	Oculocutaneous albinsim type 4; skin colour	Melanosome maturation
SLC24A5	15q15.2	NCKX5	Skin colour	Ion transport into the melanosome
Signaling				
ASIP	20q11.2-q12	Agouti signal protein	?	MC1R antagonist
MC1R	16q24.3	MSH receptor	Red hair/skin type	G-protein coupled receptor
POMC	2p23	POMC, MSH, ACTH	Red hair	MC1R agonist
GRP143	Xp22.3	OA1-protein	Ocular albinsim 1	G-protein coupled receptor
MITF	3p12.3-14. 1	MITF	Waardenburg syndrome type 2	Transcription factor
Melanosome transport/uptake by Keratinocyte				
MYO5A	15q21	MyosinVa	Griscelli syndrome	Motor protein
RAB27A	15q15-q21.1	Rab27a	Griscelli syndrome	RAS family protein
HPS1	10q23.1-q23.3	HPS1	Hermansky-Pudlak syndrome 1	Organelle biogenesis and size
HPS6	10q24.32	HPS6	Hermansky-Pudlak syndrome 6	Organelle biogenesis

References

1. Wallin, M. (2002) Nature's palette: How animals, including humans, produce colours. Bioscience Explained 1 (2), 1-12.

2. Wielgus, A.R. and Sarna, T. (2005) Melanin in human irides of different color and age of donors. Pigment Cell Research 18, 454-464.

3. Bennett, D.C. and Lamoreux, M.L. (2003) The color loci of mice--a genetic century. Pigment Cell Res 16, 333-344

4. Sturm, R.A., Teasdale, R.D., Box, N.F. (2001) Human pigmentation genes: Identification, structure and consequences of polymorphic variation. Gene 277, 49-62.

5. Sturm, R.A. (2006) A golden age of human pigmentation genetics. Trends in Genetics 22, 464-468.

6. Davenport, G.C. and Davenport, C.B. (1907) Heredity of eye-color in man. Science 26, 590-592.

7. Bito, L.Z. (1997) Eye color changes past early childhood: The Louisville twin study. Archives of Ophthalmology 115, 659-663.

8. Sturm, R.A. and Frudakis, T.N. Eye colour: portals into pigmentation genes and ancestry. Trends in Genetics 20, 327-332 (2004).

9. Frudakis, T. et al. (2003) Sequences associated with human iris pigmentation. Genetics 165, 2071-2083.

10. Zhu, G. et al. (2004) A genome scan for eye colour in 502 twin familles: most variation is due to a QTL on chromosome 15q. Twin Research 7, 197-210.

11. Duffy, D.L. et al. A three-SNP haplotype in the intron 1 of OCA2 explains most human eye color variation. American Journal of Human Genetics, 80: 241-252 (2007).

Further reading

Barsh, G.S. (2003) What controls variation in human skin color?, PLoS Biol 1, 19-22.

Imesch, P.D. et al. (1997) The color of the human eye: a review of morphologic correlates and of some conditions that affect iridial pigmentation. Surv Ophthalmol 41 Suppl 2, S117-123.

Sturm, R.A., Box, N.F. and M. Ramsay. Human pigmentation genetics: the difference is only skin deep. Bioessays, 20: 712-721 (1998).

Web sites

http://albinismdb.med.umn.edu/

This is the web site hosted by the International Albinism Center at the University of Minnesota and tabulates all the known human mutations associated with albinism.

http://www.wonderquest.com

April Holladay writes for USA Today and has many questions directed to her about eye colour.

Photo credits

Figures 2, 3B and 4 are digital images from the South-east Queensland twin collection provided with the permission of Prof. N. Martin, Queensland Institute of Medical Research, Brisbane, Australia.

Acknowledgment

The Volvox project is funded by the Sixth Framework Program of the European Commission.

