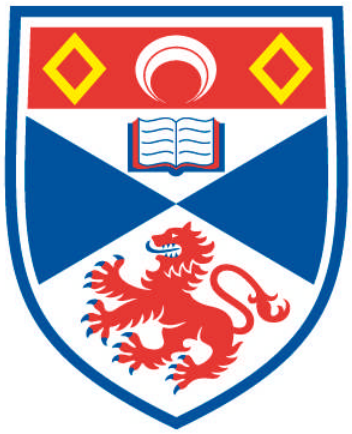
SKIN COLOUR, PIGMENTATION AND THE PERCEIVED HEALTH OF HUMAN FACES

Ian D. Stephen

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



2009

Skin Colour, Pigmentation and the Perceived Health of Human Faces

Ian D. Stephen

This thesis was submitted for the degree of Doctor of Philosophy in December 2008

I, Ian David Stephen, hereby certify that this thesis, which is approximately 29,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date **Signature of Candidate**

I was admitted as a candidate for the degree of Doctor of Philosophy in January 2006; the higher study for which this is a record was carried out in the University of St Andrews between 2006 and 2008.

Date **Signature of Candidate**

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date **Signature of Supervisor**

Embargo on both all or part of printed copy and electronic copy for the same fixed period of two (2) years on the following ground:

Publication would preclude future publication.

Date **Signature of Candidate**

Signature of Supervisor

Acknowledgements

I would like to thank first of all Prof. Dave Perrett for supervision, guidance and support, and without whom this project would not have been possible. I would also like to thank the other members of the Perception Lab for their help and inspiration, through the many stimulating conversations that occur in the laboratory. Especially, Dr Jamie Lawson, Michael Stirrat, Vinet Coetzee, Dr Janek Lobmaier, and Dr Johannes Schindelin.

Special thanks to Lesley Ferrier for her organisational support and guidance; Michael Stirrat, Dr Johannes Schindelin and Pete Wilcox for technical support and expertise; Prof Anya Hurlbert and Dr Yazhu Ling for guidance and advice on colour calibration; Vinet Coetzee and Jaco Greeff for cross-cultural assistance; Dr Joanne Cecil for diet assessment advice

Thanks also to my friends, family and fellow PhD students in the department, and most importantly to Marissa Parkin for supporting me and waiting (mostly) patiently for all this work to be completed.

This work was supported by the BBSRC and Unilever Research.

Table of Contents

Acknowledgements iii
Abstract 1
Section 1: Literature Review 3
1.1 Introduction 4
1.2 Sexual Selection 5
1.3 Parental investment and who chooses 7
1.4 Health Signalling 8
1.5 Handicap Hypothesis 9
1.6 Heterozygosity 13
1.7 Sexual selection in humans 15
1.8 Facial Attractiveness 17
1.8.1 Symmetry 18
1.8.2 Averageness 21
1.8.3 Sexual Dimorphism 23
1.8.4 Masculinity as a handicapping signal 25
1.9 Skin Colour 26
1.9.1 Structure and function of human skin 27
1.9.2 Skin Pigmentation 28
1.9.2.1 Blood 28
1.9.2.2 Carotenoids 29
1.9.2.3 Melanin 30
1.10 The evolution of Primate Trichromatic Colour Vision 33
1.11 Summary 36
Section 2: Carotenoids Affect Skin Colour 38
2.1 Section 2 Introduction 39
2.2 Study 1 – Natural dietary carotenoid intake contributes to skin yellowness 41
2.2.1 Introduction 41
2.2.1.2: Predictions 42
2.2.2 Methods 42
2.2.2.2: Statistical Methods 43
2.2.3 Results 44
2.2.4 Discussion 46
2.3 Study 2 – Reflectance Spectra 47
2.3.1 Introduction 47
2.3.2 Methods 49
2.3.3 Statistical Methods and Results 49
2.3.4 Discussion 56
2.4 Section 2 Discussion 57
Section 3: Pure Colour Transforms 59
3.1 Section 3 Introduction 60
3.1.1 Lightness 61
3.1.2 Redness 66
3.1.3 Yellowness 67
3.1.4 Contrast between skin and lips 68
3.2 Methods 69
3.2.1 Photography 69
3.2.2 Colour Transformations 70
3.2.3 Experimentation 73

3.2.4 Statistical Methods 74
3.3 Results 75
3.3.1 Relative Lip Colour 75
3.3.2 Lightness 75
3.3.3 Redness 77
3.3.4 Yellowness 78
3.4 Section 3 Discussion 79
3.4.1 Contrast between Skin and Lips 80
3.4.2 Lightness 80
3.4.3 Redness 81
3.4.4 Yellowness 82
3.4.5 Gradients 82
Section 4: Blood Colour Transforms 83
4.1 Introduction 84
4.1.1 Blood Perfusion 85
4.1.2 Blood Oxygenation 86
4.1.3 Primate Colour Signals 86
4.2 Methods 87
4.2.1 Photography 87
4.2.2 Empirical Measurement of Deoxygenated Skin Blood Colour 87
4.2.3 Empirical Measurement of Oxygenated Skin Blood Colour 88
4.2.4 Image Manipulation 89
4.2.5 Experimentation 91
4.2.6 Statistical Methods 92
4.3 Results 93
4.3.1 Single-Axis Transforms 93
4.3.2 Two-dimensional Colour Transform 96
4.4 Section 4 Discussion 97
Section 5: Melanin and Carotenoid Transforms 100
Carotenoid and melanin provide perceptible cues to health in human faces 101
5.1 Introduction 101
5.1.1 Carotenoids 102
5.1.2 Melanin 106
5.1.3 Predictions 108
5.2 Methods 109
5.2.1 Photography 109
5.2.2 Empirical Measurement of Carotenoid Colour 109
5.2.3 Empirical Measurement of Melanin Colour 109
5.2.4 Image Manipulation 110
5.2.5 Experimentation 112
5.2.6 Statistical Methods 113
5.3 Results 114
5.3.1 Single-Axis Transforms 114
5.3.2 Two-Dimensional Transform 116
5.4 Discussion 117
5.4.1 Carotenoids 117
5.4.2 Melanin 118
5.4.3 Two-Dimensional Transforms 119
5.4.4 Conclusion 119
Section 6: Cross-Cultural Studies 120

6.1 Introduction....121
6.2 Methods....124
6.2.1 Photography....124
6.2.2 Image Manipulation....125
6.2.3 Experimentation....125
6.2.4 Statistical Methods....125
6.3 Results....126
6.4 Discussion....129
Section 7: General Discussion....130
7.1 Effects on Evolution....137
7.2 Conclusions....141
References....143
Appendix A: Graphs showing relationship between correlation coefficients and carotenoid absorption spectra....159
Appendix B: Display Accuracy....163

Abstract

Many non-human animal species use colour to signal dominance, condition or reproductive status. These signals have not previously been noted in humans. This thesis investigates the effects of skin colouration and pigmentation on the apparent health of human faces.

Section 2 showed that individuals with increased fruit and vegetable and carotenoid consumption have yellower skin (Study 1) due to increased carotenoid pigmentation in the skin (Study 2).

In Section 3, participants enhanced the redness, yellowness and lightness of the skin portions of colour-calibrated facial photographs to optimise healthy appearance. This suggests roles for blood (red) and carotenoid/melanin (yellow) colouration in providing perceptible cues to health. The contrast between lips and facial skin colour was not found to affect the apparent health of the faces, except in the b* (yellowness) axis, where enhanced facial yellowness caused an apparent blue tint to the lips.

In Section 4 participants enhanced empirically-derived oxygenated blood colour more than deoxygenated blood colour to optimise healthy appearance. In two-dimensional trials, when both blood colour axes could be manipulated simultaneously, deoxygenated blood colour was removed and replaced with oxygenated blood colour. Oxygenated blood colouration appears to drive the preference for redness in faces.

In Section 5 participants increased carotenoid colour significantly more than they increased melanin colour in both single-axis and two-dimensional trials. Carotenoid colour appears to drive the preference for yellowness in faces.

In a cross-cultural study (Section 6), preferences for red and yellow in faces were unaffected by face or participant ethnicity, while African participants lightened faces more than UK participants. A preference for more redness in East Asian faces was explained by this group's lower initial redness.

The thesis concludes that pigments that provide sexually-selected signals of quality in many non-human animal species – carotenoids and oxygenated blood - also provide perceptible cues to health in human faces.

Section 1:

Literature

Review

1.1 Introduction

This thesis investigates the role of skin colour and pigmentation in the perception of health in human faces.

It is known that colour is used to signal condition (Martinez-Padilla *et al.*, 2007; Olson & Owens, 1998), sexual status (Setchell *et al.*, 2006), dominance (Setchell & Wickings, 2005) and hormonal status (Rhodes *et al.*, 1997) in many non-human animal species. There is evidence that colourful ornaments are condition dependent, with better fed (Perez-Rodrigues & Vinuela, 2008) and less parasitized (Martinez-Padilla *et al.*, 2007; Olson & Owens, 1998) individuals exhibiting larger and brighter colourful ornaments. Section 2 of this thesis examines the role of diet in determining the colour of the skin, particularly in relation to carotenoid pigments (which provide the colour in many of the animal kingdom's colourful ornaments). The remainder of this thesis will address the effect of skin colour and pigmentation on the apparent health of human faces.

Colourful signals are often sexually selected, with bigger and brighter ornaments being preferred by the opposite sex (while it is more common for males to exhibit these ornaments, and females to be the choosy sex, examples of female ornamentation and male choice exist; Massaro *et al.*, 2003; Waitt *et al.*, 2003). Many of the colourful displays made by non-human animals involve the bare skin (Perez-Rodrigues & Vinuela, 2008). Skin colour in humans is one of the most widely varying physical characteristics, both between and also within populations (Jablonski, 2006; Robins, 1991). Skin colour can communicate a number of social and biological messages. People associate more negative stereotypes such as aggression and criminality with

black people with darker skin than they associate with black people with lighter skin (Maddox & Gray, 2002). Blushing is a reddening of the face that is associated with social embarrassment (Leary, *et al.*, 1992) and also with sexual attraction (Bogels *et al.*, 1996). However, while the *distribution* of pigmentation colour in human faces has been shown to affect perceptions of age, health and attractiveness (Matts *et al.*, 2007), the *overall* skin colour has not previously been studied with respect to perceptions of health. I will examine the role of skin colour and pigmentation in providing a perceptible cue to human health, and its possible role in sexual selection.

In this Section, I review the relevant literature relating to sexual selection, face perception and skin colour.

1.2 Sexual Selection

Many species exhibit phenotypic and behavioural traits that appear to be deleterious in terms of survival. The peacock's tail is large, brightly coloured and cumbersome, simultaneously making the bearer more conspicuous to predators and less capable of evading predation. Why then should such phenotypes evolve? Darwin (1871) proposed a theory of sexual selection to explain them. Perhaps individuals with larger and brighter ornaments are preferred as mates by the opposite sex. Under this hypothesis, the increased reproductive success from sexual selection outweighs the handicap to survival caused by having such ornaments. In this way, larger and brighter ornaments may be selected for in the population.

Many animals exhibit traits that appear maladaptive to survival, such as the bright plumage of birds, which may attract predators. These ornaments can be maintained through sexual selection if the bearer has increased reproductive success (Darwin, 1871; Trivers, 1972). Since males have greater variation in their reproductive success than females in most species (Bateman, 1948), access to females is a limiting resource. Females invest more in each offspring than males, right from the initial difference in gamete size (anisogamy) to mammalian gestation and, in many species, post-partum care (Trivers, 1972). Males should therefore be expected to compete for access to females (male-male competition), and females are expected to be choosy, in order to obtain the best mates (female choice; Trivers, 1972). Several studies have confirmed that males who are best able to compete with other males achieve preferential access to females (e.g. the best fighters in elephant seals achieve almost all of the copulations in a colony; Le Boeuf, 1974). Also, males who are best able to attract females by being, for example, the house finch with the brightest red head and throat plumage (McGraw *et al.*, 2001) or the sage grouse with the most extravagant displays (Gibson, 1996), obtain greater numbers of matings.

Ornamentation may be preferred by females for a number of reasons. Fisher (1958) proposed that once a male characteristic is preferred, for any reason, the very fact that it is preferred provides an impetus for the continuation and accentuation of that male trait and the corresponding heritable (genetically or culturally; Laland, 1994) female preference for the trait. If bright-coloured males are preferred, a selection pressure exists for males to evolve ever brighter plumage. Females who prefer bright plumage will also obtain a reproductive advantage for their male offspring who will, in turn, obtain more reproductive success. This “sexy sons” hypothesis is known as Fisherian

runaway selection (Fisher, 1958). This hypothesis relies on the heritability of preference as well as the heritability of trait

1.3 Parental investment and who chooses

Sexual selection is thought to originate from the difference in the parental investment made by the two sexes (Trivers, 1972) and is a phenomenon that is likely to have existed since very early in the evolution of life. The emergence of anisogamy meant that the sex producing the larger gamete (the female) has made a larger initial investment in the embryo than the sex producing the smaller gamete (the male). Indeed, a male can impregnate a female and walk away with very little potential loss of investment. However, in many mammalian species, the female is committed to a substantial investment for at least the length of the gestation period. At birth, the mother must either continue to invest in the offspring or kill or abandon it, which would represent a significant loss of investment (Trivers, 1972). This greater investment by females means that females are expected to be choosier about both mate quality, so as not to waste investment on weak offspring, and also to be choosier about the male's potential and willingness to invest in the offspring.

This difference in initial investment means that the resources limiting the reproductive output of the two sexes are different (Bateman, 1948). In Bateman's (1948) original experiments with *Drosophila melanogaster*, females were found to be limited by the number of eggs they could produce. Males, with much cheaper gametes, were limited by their access to females. This finding has been replicated in a number of other species (see Trivers, 1972).

In many species, however, investment does not end at birth, and includes "any investment by the parent in an individual offspring that increases the offspring's chances of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring", such as incubation, protection, feeding, and teaching. Investment in securing a mate is not included (Trivers, 1972). Many species, including humans, show significant male parental investment. As male investment increases, so male choosiness will increase (at least for females with whom he will invest and not simply desert) as having high quality offspring becomes more important. In species such as humans, where males invest more than half but less than equal to the female investment, selection favours a mixed strategy for both sexes (Parker & Simmons, 1996). Females should be choosy and select a male who has good genes, high investment potential and low likelihood of desertion, but may also increase their fitness through extra-pair copulations (EPCs) with males who are genetically superior to their partner. Similarly, males are expected to invest in the offspring of a single female, whilst competing to inseminate females in whose offspring he will not invest, particularly if those offspring will be raised by another male. Males are expected to be choosy about the female in whose offspring he will invest (Parker & Simmons, 1996; Trivers, 1972). The risk of sexually transmitted pathogens and, in some species, social risks associated with EPCs will induce choosiness in extra-pair partners. In humans, therefore, both males and females are expected to be choosy, and both males and females are expected to compete for mates.

1.4 Health Signalling

Attraction is a way to ensure that individuals reproduce with the most beneficial mate and to raise the most successful offspring. Direct benefit models of sexual selection

suggest that individuals choose mates that offer the most benefit directly to themselves. This may involve nuptial gifts, territory and parental care (Trivers, 1972). Indirect benefit models of sexual selection suggest that individuals choose mates based on genetic quality, gaining advantages associated with “good genes” in their offspring (Fisher, 1958). Choosing mates based upon their health potentially provides both direct and indirect benefits. A healthy mate will be better equipped to provide more parental care and is also likely to have a lower parasite load, exposing the individual to a lower chance of infection (direct benefits). Additionally, any heritable aspect of increased health, such as a better immune system or more efficient heart and lungs, may be passed on to offspring (indirect benefits). The identification of health, and signalling of health, should therefore play a role in mate selection.

1.5 Handicap Hypothesis

Another hypothesis for the evolution of seemingly maladaptive traits is known as the handicap hypothesis. Traits which are preferred by one sex advertise quality by imposing a handicap on the bearer (Zahavi, 1975). By investing in a costly ornament, the bearer is advertising that he has the resources to waste, and more. This is a restatement of the economic concept of conspicuous consumption (Getty, 2002; Veblen, 1899). The development of male secondary sexual characteristics requires significant investment in terms of resources (Zahavi, 1975), including energy and useful compounds such as carotenoids (carotenoid-based ornamentation requires the deposition of carotenoids in the skin, hair or feathers, making it unavailable to other processes such as immune function; Saks *et al.*, 2003). The development and maintenance of these costly ornaments can only be achieved by high-quality

individuals. Females that choose males with large ornaments can be assured that they are obtaining high quality mates (Zahavi, 1975).

It was proposed that the quality being advertised by sexual ornaments is freedom from parasites and pathogens (Hamilton & Zuk, 1982). Those males who can better resist parasites and pathogens, or through behaviour encounter fewer parasites and pathogens, can afford to invest more in costly ornamentation to advertise their quality (Hamilton & Zuk, 1982). American goldfinches artificially infected with intestinal coccidians had less saturated carotenoid-based bill and plumage colouration than individuals who were free from infection (McGraw & Hill, 2000), supporting this hypothesis.

Folstad and Karter (1992) proposed testosterone as a mediating pathway for the relationship between parasitism and ornamentation. The immunocompetence handicap hypothesis (ICH) proposes that testosterone - required for the development of secondary sexual characteristics - is an immunohandicap, and is affected by nutritional and disease status. It may therefore provide a mechanism for the trade-off between investment in secondary sexual characteristics and reproductive effort on the one hand and immunocompetence on the other (Folstad & Karter, 1992). Only high-quality males, or those who pay a lower cost for higher testosterone levels, will therefore be able to produce and maintain large secondary sexual characteristics without compromising their immune system excessively. Ornamentation could then be an indicator of “good genes” (Folstad & Karter, 1992).

By mating preferentially with males that display indicators of immunocompetence and freedom from parasites, females obtain direct benefits such as protection from other males (e.g. Utami *et al.*, 2002) and increased resources, or improved breeding grounds (e.g. Parker, 1983) and avoidance of parasites and infectious diseases (Hamilton & Zuk, 1982). They may also obtain indirect benefits in the form of good genes, improved parasite resistance and increased reproductive potential that will only be expressed in the offspring (Fisher, 1958; Grammer *et al.*, 2003).

Hamilton and Zuk (1982) suggested that their hypothesis would be supported if individuals with larger ornaments had lower parasite loads. This also extends to individuals with higher testosterone having lower parasite loads (Folstad & Karter, 1992). Studies attempting to find these patterns found mixed results, with some studies finding negative associations between ornament size and parasite load (Moller *et al.*, 1999) and some finding positive associations (Gonzalez *et al.*, 1999; Hamilton & Zuk, 1982). Both have been interpreted as supporting the handicap hypothesis. However, Getty (2002) showed that the ICH does not make predictions in either direction, since the resources of the individual and the magnitude of the handicap compared to these resources will determine the penalty paid for the ornament. Even though larger ornaments result in larger handicaps, high-quality individuals may begin with greater resources and pay a lower relative cost of ornamentation. The relative effects of ornament size, initial resources and relative cost all determine parasite load. Therefore, individuals with large ornaments may have high or low parasite loads (Getty, 2002).

Two testable predictions are made by the ICH. Increases in testosterone level should inhibit immune function and increase reproductive effort (Folstad & Karter, 1992), and higher quality males should pay a lower immunocompetence price for increased testosterone (Grafen, 1990). Several studies have investigated these predictions, using testosterone implants, and again found mixed results. High testosterone male red-winged blackbirds and males with testosterone implants did not exhibit reduced antibody production in response to inoculation with keyhole limpet haemocyanin in a free-ranging population (Hasselquist *et al.*, 1999). However, testosterone-implanted sand lizards exhibited greater mobility and higher mating success. They also exhibited greater mass loss during the mating season and increased tick load, supporting the ICH (Olsson *et al.*, 2000). In barn swallows, testosterone-implanted males suffered increased ectoparasite load, though this increase was smaller in individuals with longer tails, supporting the hypothesis that testosterone has an immunosuppressive effect and that higher-quality males pay a reduced cost of testosterone (Saino *et al.*, 1995). Testosterone-implanted male red grouse suffered increased parasite load and reduced condition, but also showed increased comb size (Mougeot *et al.*, 2004). Lindstrom *et al.* (2001) found that testosterone-implanted greenfinches exhibited increased immune response to the common Sindbis virus early in infection, but decreased response later on and overall. There was also weak evidence that males with larger tail patches paid a reduced cost of testosterone, providing mixed results (Lindstrom *et al.*, 2001). These findings provide some support for the hypothesis that high-quality individuals pay a lower immunocompetence cost of bearing large ornaments.

1.6 Heterozygosity

One potential explanation of the "good genes" that are suggested to be sought in mate choice is heterozygosity (Brown, 1997). Heterozygosity is beneficial in a number of species, as a greater degree of heterozygosity reduces the number of deleterious recessive alleles that are likely to be expressed. Heterozygous salmonid fish exhibit increased growth rate, disease resistance and developmental stability (Brown, 1997; Leary *et al.*, 1985; Mulvey *et al.*, 1994). Red deer show a correlation between heterozygosity at a number of microsatelites and birth weight (Slate & Pemberton, 2002). Females should be expected to prefer dissimilar mates (who may be expected to have different alleles to oneself at a larger number of loci), in order to increase heterozygosity in their offspring. Additionally, particularly in species with high paternal investment, preferences are expected for males with high levels of heterozygosity, as more heterozygous mates are more likely to be healthy, expose the female to lower levels of pathogens and be better able to provide paternal investment in offspring. These preferences have been found in several species (Brown, 1997).

The major histocompatibility complex (MHC, in humans known as the human leukocyte antigen, HLA) is a highly polymorphic region of the genome that is involved with parasite and pathogen resistance and self-non-self recognition. It has therefore been studied as a possible location for the action of sexual selection, based on inbreeding avoidance and heterozygote advantage (Brown, 1999; Thornhill *et al.*, 2003; Thornhill & Grammer, 1999). MHC heterozygosity is associated with developmental stability (low fluctuating asymmetry, FA) in many animals and plants, and individuals with low FA are preferred as mates in many species (Brown, 1999), including humans (e.g. Brown *et al.*, 2008; Gangestad *et al.*, 2005; Gangestad *et al.*,

1994; Grammer & Thornhill, 1994; Mealey *et al.*, 1999; Perrett *et al.*, 1999). Additionally, it has been found that women prefer the scent of men who are heterozygous at MHC alleles (Thornhill *et al.*, 2003) and men who are heterozygous at more MHC loci are rated as more facially attractive by women, perceived to be healthier and perceived to have healthier skin (as rated on patches) than men who are homozygous at certain MHC loci (Roberts *et al.*., 2005). This suggests that women prefer heterozygosity, perhaps as a mechanism for avoiding catching diseases from parasitized mates, as a way of ensuring better paternal care from healthier partners or for obtaining rare alleles for offspring. Further, there is evidence that people prefer partners who are genetically dissimilar to themselves, which may indicate that they are seeking heterozygosity in their offspring. Hutterites were found to have significantly fewer marriages matching for certain HLA loci than expected by chance (Ober, 1999), and several studies have shown that people prefer the scent of opposite sex individuals with dissimilar MHC to their own (Penn, 2002; Penn & Potts, 1999; Thornhill *et al.*, 2003; Wedekind *et al.*, 1995), though other studies have failed to find these patterns (Hedrick & Black, 1997; Ihara *et al.*, 2000).

One prediction of the MHC hypothesis that does not appear to have been addressed relates to the fact that MHC heterozygosity is thought to be an aspect of parasite resistance and good genes (Brown, 1999). If this is the case, MHC heterozygous men should be able to have increased testosterone and increased masculinity, whilst paying a lower fitness cost, though this has yet to be demonstrated. MHC heterozygosity could be a source of quality that the handicap hypothesis can operate on. Since symmetry is thought to be reduced by problems such as illness during development (though direct evidence of this is yet to emerge; Rhodes *et al.*, 2001b), it may be that

symmetry is a check on the honesty of signals of quality. For example, individuals developing large ornaments without the quality that they are advertising may exhibit greater fluctuating asymmetry. This would explain the greater attractiveness of symmetrical individuals.

1.7 Sexual selection in humans

Several studies have shown that humans exhibit many of the behaviours associated with sexual selection, including intrasexual competition and intersexual choice (Symons, 1979). Male-male violence over access to women is widespread. High levels of intra-group male-male violence to obtain and defend access to women has been reported in Aboriginal Eskimos (Rasmussen, 1931). The Yanomamo tribe from Venezuela fight wars with other villages, in order to obtain and defend access to women (Chagnon, 1968). The Mae Enga tribe of Papua New Guinea fight wars over land in order to raise crops and livestock, but the tribesmen report that the reason for obtaining the land is in order to provide food to obtain more wives and raise more children (Meggitt, 1977).

Intrasexual competition may also take place in non-violent ways. Politics may provide a way for men to raise their social status and increase access to women (Symons, 1979). Hunting also provides a mechanism for men to compete over women. Despite the relatively small contribution that hunting makes to the nutritional intake of most small societies, men invest significant time and effort into obtaining meat – time that, nutritionally, would be better spent foraging for other foods (Symons, 1979). However, meat is exchanged for sex in many societies, with the most successful hunters obtaining the most sexual access to women (Siskind, 1973; Gurven, 2004).

Indeed, food is implicitly or explicitly required to obtain access to sex in many cultures (Knight, 1991). This suggests that men are using hunting to compete over mates.

Intersexual choice also operates in humans, though men and women have been shown to desire different qualities in the opposite sex. Men are consistently more interested than women in the physical attractiveness of potential partners, whereas women are consistently more interested than men in social status and ability and willingness to invest (Buss, 1989). Women become more interested in physical attractiveness when seeking extra-pair copulations (EPCs) and in the fertile phase of the menstrual cycle (Penton-Voak & Perrett, 2000; Penton-Voak *et al.*, 1999). This may be a mechanism to allow women to increase the likelihood of becoming pregnant from an EPC, maximising the good genes benefits, whilst minimising the risk of being caught and punished. The increased preference for masculine men when seeking short term relationships, and preference for feminine men for long term relationships may allow women to maximise the good genes benefits from masculine men, whilst obtaining better parenting and reduced risk of violence from feminine men long term (Little *et al.*, 2002).

Men have considerably more variability in their reproductive success than women (a survey of the Yanomamo tribe found that the most reproductively successful man had 43 children, while the most successful woman had 14; Chagnon, 1968). However, the relatively high parental investment made by men in at least some of their offspring means that men are also expected to exercise intersexual choice, especially in women with whom he will invest (Trivers, 1972). Physical attractiveness is a major factor in

the choice of mates by men (Buss, 1989). While rates of female-female violence are low compared with men, high quality men are a limited resource over which competition takes place, including competition through maximising ones own attractiveness (Buss & Dedden, 1990) and competitor derogation – reducing the perceived attractiveness of competitors (Fisher, 2004).

Arranged marriages are common in many societies, seemingly removing intersexual choice, especially from women (Crook, 1972). However, intersexual choice is likely to operate in these societies through various mechanisms. EPCs are outside of the control of the elders, and would be subject to the same sexual choice mechanisms that operate in other societies. In addition, younger and more attractive women command higher dowry payments in Kenyan Kipsigis (Borgerhoff Mulder, 1988) and more expensive engagement rings in Americans (Cronk & Dunham, 2007). Families of men therefore prefer to arrange marriages with attractive, young women. Families of women will prefer to arrange marriages with richer men. Pressure to obtain an attractive and wealthy spouse is also likely to be applied by the individuals on their parents or the elders arranging the marriage.

1.8 Facial Attractiveness

An important mechanism for mate choice in humans is attractiveness. Whilst the phrase “beauty is in the eye of the beholder” suggests that social conditioning and individual preferences control attractiveness, a view held by some feminist writers (Wolf, 1991), a growing body of literature suggests that people generally agree on which faces are attractive and which are unattractive, including cross-culturally and in societies with little access to Western media (Cunningham *et al.*, 1995; Langlois *et al.*,

2000). Additionally, research has identified several specific aspects of faces and bodies that contribute to attractiveness and the aspects of quality that they may signal. Studies on the question of whether aspects of facial attractiveness signals health have yielded mixed results.

1.8.1 Symmetry

Fluctuating asymmetry (the deviance from symmetry in an individual in features that are symmetrical on average across the population) is thought to reflect developmental instability (Van Valen, 1962). Developmental instability is a measure of the ability of an individual to develop stably in the given environmental conditions, with less favourable environmental and genetic factors increasing instability (see Moller & Thornhill, 1998). Indeed, ratings of symmetry have been found to correlate with ratings of attractiveness. Controlling for rated health eliminated most of these effects however, suggesting that the relationship between symmetry and attractiveness is mediated by the healthy appearance of symmetry (Jones *et al.*, 2001; Rhodes *et al.*, 2007). Men with low FA were found to have more sperm per ejaculate, higher sperm speed and better sperm migration (Manning *et al.*, 1998). However, MHC heterozygosity and MHC allelic rarity (thought to be indicators of genetic and immune quality) have been found not to correlate with symmetry (Thornhill *et al.*, 2003).

Symmetry (low fluctuating asymmetry) has been found to correlate with attractiveness in several studies in non-human animals (Swaddle & Cuthill, 1994) as well as in human faces (Gangestad *et al.*, 1994; Grammer & Thornhill, 1994; Mealey *et al.*, 1999; Penton-Voak *et al.*, 2001; Perrett *et al.*, 1999) in several cultures (Rhodes

et al., 2001a) and bodies (Brown *et al.*, 2008; Møller *et al.*, 1995; Singh, 1995). More symmetrical men in a natural fertility population were found to live longer, have more offspring, begin reproduction younger and have more lifetime sexual partners (Waynforth, 1998). Women with more symmetrical partners experience more copulatory orgasms (Thornhill *et al.*, 1995). Since female orgasm is not required for conception, several hypotheses have been advanced to explain its existence (Thornhill *et al.*, 1995). Female orgasm is thought to be a mechanism for moving sperm into the uterus and increasing conception chances (Fox *et al.*, 1970). By having orgasms preferentially with certain men, women can exert some additional influence over the paternity of her offspring (Smith, 1984). The increased frequency of copulatory orgasm with men of low fluctuating asymmetry suggests that symmetry signals good genes (Thornhill *et al.*, 1995).

Attraction to other men during the fertile phase of the menstrual cycle is thought to be an adaptation allowing women to maximise genetic fitness benefits by mating with high quality partners during ovulation, whilst minimising the risks of infidelity, and maintaining a long-term relationship. Women with partners with high fluctuating asymmetry experience more attraction to other men during the fertile phase of the menstrual cycle (Gangestad *et al.*, 2005). This suggests that the benefits obtained from extra-pair mating may be higher to those women with more asymmetrical partners. This provides additional evidence that symmetry may signal good genes.

Symmetry shows cyclical variation, with women becoming more symmetrical at the follicular (fertile) phase of the menstrual cycle (Manning *et al.*, 1996; though this finding was not replicated in one study of breast volume; Hussain *et al.*, 1999). Men

who are sensitive to this variation and are more attracted to women at the fertile phase may have more reproductive success. This mechanism also allows women to become more desirable and receive more sexual attention when at their most fertile (Manning *et al.*, 1996). Facial photographs of women taken during the fertile phase of the menstrual cycle are rated as more attractive than those taken in the luteal phase (Roberts *et al.*, 2004). While initial studies found that women were better able to detect facial symmetry in men during menses (women were better able to identify the more symmetrical of two faces during menses than during the luteal phase of the menstrual cycle, suggesting that high progesterone levels may inhibit facial symmetry detection), and failed to find any cyclical change in preference for male facial symmetry (Oinonen & Mazmanian, 2007), another study found that women showed a stronger preference for male facial symmetry during the fertile phase of the menstrual cycle, but only if they already had a partner or were looking for a short-term relationship. This suggests that women are seeking to maximise good genes benefits of short term and extra pair copulations (Little *et al.*, 2007).

While a study of medical records failed to link facial symmetry with actual medical health (Rhodes *et al.*, 2001b), a study based on reported number of illnesses found that more symmetrical people had fewer respiratory infections in the preceding three years (Thornhill & Gangestad, 2006b).

There have been suggestions that symmetry itself may not be attractive and that it may simply be correlated with another attractive trait. Women prefer the odour of men who are more symmetrical and attractive (Rikowski & Grammer, 1999). Female Japanese scorpionflies show a preference for the pheremones of more symmetrical

males. When the males were manipulated to be more or less symmetrical, the females chose to copulate with males that were *originally* more symmetrical regardless of their current symmetry, suggesting that pheremones and not symmetry are controlling mate preference (Thornhill, 1992). A study in humans found that symmetrical faces were more attractive. However, showing only half the face, thereby hiding many cues to symmetry, yielded very similar results, suggesting that symmetry merely correlated with another attractive trait (suggested to be masculinity, Scheib *et al.*, 1999, though this is unclear, Penton-Voak *et al.*, 2001). A further study suggested that asymmetry may in fact be more attractive, perhaps because of an unnatural or unexpressive appearance of symmetrical faces (Swaddle & Cuthill, 1995), though doubt has been cast upon the validity of the methods used in this study, as the mirrored blending method used may have smoothed imperfections in the skin or mirrored blemishes that would not be perfectly symmetrical in real faces (Perrett *et al.*, 1999).

1.8.2 Averageness

Another aspect of facial attractiveness is averageness. The most common form of natural selection is stabilising selection, in which selection operates against extremes and maintains an equilibrium about the mean (Dobzhansky, 1970; Langlois & Roggman, 1990). Individuals who exhibit characteristics about the population mean are likely to have fewer deleterious mutations and a higher level of heterozygosity than individuals with extreme characteristics (Symons, 1979). Indeed, individuals with more average (less distinctive) faces have been found to have had better childhood health (Rhodes *et al.*, 2001b), though perceived health does not account for all of the attractiveness of average faces (Rhodes *et al.*, 2007).

Additionally, people respond to prototypical (average) examples of objects as though they were familiar (Strauss, 1979). Familiarity, in turn, increases attraction to faces (Peskin & Newell, 2004). Average objects other than faces (dogs, wristwatches, birds) have also been found to be seen as more attractive, suggesting an inherent attraction to prototypical objects (Halberstadt & Rhodes, 2000).

Average composite images are rated as more attractive than most individual images, and the more individual images contribute to the composite image, the more attractive the composite is considered (Langlois & Roggman, 1990), though this finding has not always replicated successfully (Grammer & Thornhill, 1994). However, it has subsequently been suggested that other aspects of the composite images may have contributed to their attractiveness. The process of averaging faces used by Langlois & Rogmann (1990) causes a smoothing of blemishes and an apparent improvement in the skin condition of the faces. However, combining 32 photographs of the same face produced less attractive composites than 32 different faces, suggesting that this cannot explain all of the attractiveness of averageness (Langlois *et al.*, 1994). Composite images are also more symmetrical than individual faces, though controlling for symmetry fails to remove the effect of averageness on attractiveness (Langlois *et al.*, 1994; Rhodes *et al.*, 1999). The effect of averageness on attractiveness has been observed cross-culturally, with both Chinese and Japanese participants preferring faces that had been transformed towards the population mean. Indeed, it made little difference whether the population mean used was of the same or other race (Rhodes *et al.*, 2001a).

While average faces are attractive, the most attractive faces are not average. Composite faces composed of individual female faces that were rated as highly attractive are found to be more attractive than average composites. The face produced by exaggerating the difference between the average composite and attractive composite to create a face with exaggerated attractive features is found to be more attractive than both the average and attractive composites (Perrett *et al.*, 1994). These attractive composites have more feminine features and may indicate a higher oestrogen/androgen ratio (Johnston & Franklin, 1993). It has been claimed that the attractive composites may be more average than the normal composites. The attractiveness transform may thus be a transform towards more averageness, and this may explain the preference for the attractive transformed faces (Rubenstein *et al.*, 2001). However, another study manipulated faces along an attractiveness dimension, and found that the average composite was rated as the most normal face, while the most attractive face was one that was manipulated towards the attractive composite (though not the most caricatured attractive face; DeBruine *et al.*, 2007).

1.8.3 Sexual Dimorphism

The degree of sexual dimorphism in faces contributes to their attractiveness. Feminine features of the body develop under the influence of female reproductive hormones, such as oestrogen (Barber, 1995; Singh, 1993), and ratings of femininity correlate with oestrogen levels, suggesting that femininity is a signal of reproductive health (Law Smith *et al.*, 2006). Studies have found that female faces with a high degree of femininity are considered more attractive (Perrett *et al.*, 1998; Rhodes *et al.*, 2003; Rhodes *et al.*, 2007). Perceived health also contributes to the attractiveness of

feminine female faces (Rhodes *et al.*, 2007) and feminine-faced women experience fewer and less severe respiratory illnesses (Thornhill & Gangestad, 2006a).

There have been suggestions that femininity may be a handicapping signal, with oestrogen influencing the development of feminine features and having an immunosuppressant effect (Service, 1998; Singh, 1993). However, since higher oestrogen levels are associated with increased fecundity (Stewart *et al.*, 1993), it is more parsimonious to suppose that feminine features are attractive due to an association with fecundity.

Male facial masculinity has a more complicated relationship with attractiveness. The immunocompetence handicap hypothesis suggests that testosterone is an immunosuppressant. Only males with strong immune systems should therefore be able to sustain a high level of testosterone and develop masculine traits (Kirkpatrick & Ryan, 1991). Facial masculinity has been found to be associated with lower levels of respiratory illness and antibiotic use in men (Thornhill & Gangestad, 2006a) and also to appear healthier (Rhodes *et al.*, 2003), supporting the hypothesis and suggesting that masculinity is a signal of immunocompetence that should be considered attractive. Increased testosterone levels are also associated with troubled relationships including increased levels of infidelity, domestic violence and divorce (Booth & Dabbs, 1993), and more masculine faces are perceived as less warm, emotional, honest, cooperative and less good fathers (Perrett *et al.*, 1998). While initial studies failed to find masculine male faces to appear more attractive (Rhodes *et al.*, 2003) or found that women considered more feminine male faces more attractive (Perrett *et al.*, 1998), another study suggests that women's preference for masculinity

changes across the menstrual cycle, with masculinised faces preferred in the follicular (fertile) phase and feminised faces preferred in the luteal phase (Penton-Voak & Perrett, 2000). Since women's interest in extra-pair copulations also peaks in the fertile phase (Gangestad *et al.*, 2005), this suggests that women may be attracted to faces that signal good genes and immunocompetence when conception is most likely (Penton-Voak & Perrett, 2000), but to faces that indicate more prosocial and parental abilities when conception is less likely. This hypothesis is lent further support by the finding that women prefer more feminine men for long term relationships and more masculine men for short term relationships, perhaps in order to maximise parental investment from feminine men, whilst gaining good genes and immunocompetence benefits from masculine men (Little *et al.*, 2007). Further, women who consider themselves to be more attractive (Little *et al.*, 2001), or who have lower waist-to-hip ratio (a more attractive body shape) or who are rated by others as more attractive prefer more masculine male faces for a long term relationship than their less attractive peers (Penton-Voak *et al.*, 2003). This result is driven by the less attractive women, who prefer more masculine men for short term relationships and more feminine men for long-term relationships. In this way, less attractive women (who may be more at risk of desertion by their partners) may be reducing the risk of desertion by long-term partners by selecting more feminine men (who may be less likely to desert their partners).

1.8.4 Masculinity as a handicapping signal

In humans, testosterone influences the growth and masculinising of facial structures in men (Thornhill & Gangestad, 1993), and male facial masculinity may be an indicator of good genes, since it is associated with increased disease resistance and

developmental stability (Thornhill & Gangestad, 2006a). Masculinity may therefore be a handicapping signal, indicating good genes (Folstad & Karter, 1992). Facial dominance, which is closely related to masculinity (Mazur & Booth, 1998) and pubertal testosterone levels (Swaddle & Reierson, 2002), may be considered an indicator of competence and social dominance (Mueller & Mazur, 1998). In a sample of American military officers, men with higher rated facial dominance were found to achieve more career success, achieving more promotions and a higher maximum rank (Mueller & Mazur, 1998). However, this was only true of men with good cognitive, social and athletic skills. For men who lacked these attributes, facial dominance was a significant hindrance to career success. Further, men with more career success had more children and grandchildren. This suggests that facial dominance is a handicapping signal. Those who have significant positive attributes reap the rewards of exhibiting facial dominance signals, whilst less endowed men pay a high cost of bearing facial dominance signals, both in terms of career advancement and in terms of reproductive success (Mueller & Mazur, 1998).

1.9 Skin Colour

Though a number of different colour spaces may be used to measure and describe colour, the CIELab 1976 colour space is used throughout this thesis. This colour space is modelled upon the human visual system and is designed to be perceptually uniform (a change of one unit will appear to be of approximately the same magnitude regardless of the dimension of the change). The CIELab colour space is commonly used in human perceptual work (Martinkauppi, 2002), and is defined by L* (luminance), a* (red-green) and b* (yellow-blue) dimensions (Fig 1.1).

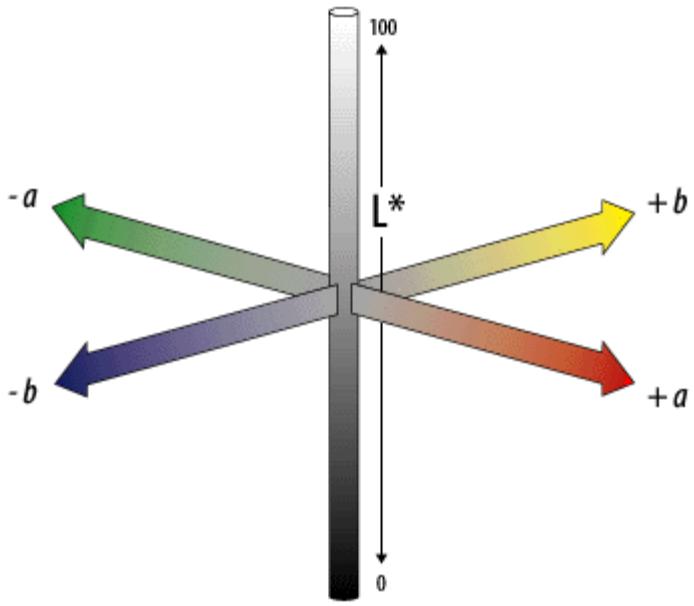


Fig. 1.1: A graphic representation of the CIELab colour space. From www.adobe.com

1.9.1 Structure and function of human skin

The skin is the largest and most visible organ in the human body. It serves to protect the body from the environment, including UV radiation, and to regulate body temperature and the amount of water and other chemicals entering and leaving the body (Jablonski, 2006). The skin is made up of a number of layers, which are considered in two main components, the dermis and the epidermis (Robins, 1991). The dermis, the deeper component, is a layer of connective tissue, made up of collagen, elastin and reticular fibres. These fibres give the skin its strength and elastic properties. The dermis contains a number of structures, including hair follicles, sweat glands, sebaceous glands, *arrector pili* muscles (which cause the hairs to stand on end in some circumstances) and a network of lymphatic and blood vessels (Robins, 1991).

The epidermis is the component of the skin closer to the surface. It is made up of four sub-layers of skin cells know as keratinocytes, which are embedded in a matrix of lipids and proteins (Elias & Feingold, 2003). Keratinocytes contain keratin filaments

embedded in a gelatinous matrix. This construction gives the epidermis its strength and durability (Jablonski, 2006). The deepest of these layers of keratinocytes is called the basal layer or *stratum basale*. This is a single layer of columnar keratinocytes in which mitosis (cell division) takes place, interspersed with a number of melanocytes, which produce melanin. Superficial to the basal layer, the *stratum spinosum* is composed of several layers of keratinocytes. Together, the *stratum basale* and the *stratum spinosum* are referred to as the Malpighian layer. Superficial to this is the *stratum granulosum*, which consists of several layers of keratinocytes, which gradually become flattened and die. The *stratum corneum* is the most superficial layer of the skin and it consists of several layers of dead, flattened keratinocytes. New keratinocytes are produced by mitosis in the *stratum basale* and migrate towards the surface, where they are lost from the *stratum corneum* by abrasion, in a process known as “desquamation”. The time taken for a cell to be lost from the skin surface from its production in the *stratum basale* is typically four to six weeks (Robins, 1991).

1.9.2 Skin Pigmentation

A number of pigments within the dermis and epidermis give the skin its colour.

1.9.2.1 Blood

In order to keep the skin supplied with oxygen, the dermis contains a rich network of blood vessels. Arterioles bring oxygenated blood from the heart, and venules remove deoxygenated blood from the skin. Capillaries allow the diffusion of oxygen and carbon dioxide across their thin membranes, supplying cells with oxygen and removing carbon dioxide. These capillaries also join arterioles to venules.

The effect of the blood on the colour of the skin depends upon both the amount of blood present (which is dependent on the degree of vascularisation and vasodilation) and the oxygenation state of the haemoglobin in the blood (Zonios *et al.*, 2001). Blood containing a high level of oxygenated haemoglobin has a bright red colour, impacting on the a* (red-green) dimension of the CIELab colour space (Fig 1.1), whereas blood with high levels of deoxygenated haemoglobin has a duller, bluish-red colour (Pierard, 1998), increasing a* whilst decreasing b* (yellow-blue) and L* (light-dark) and this is reflected in the skin colour (Changizi *et al.*, 2006). Additionally, vasodilation, which occurs in order to promote heat loss (Charkoudian, 2003) and also to regulate blood pressure (Drummond & Quah, 2001), causes oxygenated arterial blood to flow into the skin, increasing the oxygenation levels of the skin blood (Liu *et al.*, 1992).

1.9.2.2 Carotenoids

Carotenoids are a group of yellow-red pigments that are found in the skin (Edwards & Duntley, 1939). They are obtained from the diet, chiefly from fruit and vegetables, and cannot be synthesised *de novo* in the body (Alaluf *et al.*, 2002b). Carotenoids require the presence of lipids in the diet to be absorbed (Prince & Frisoli, 1993) and are transported in the blood serum bound to lipoproteins (Krinsky *et al.*, 1958). Carotenoid supplements have been shown to increase the levels of carotenoids in the blood serum and in the skin (Stahl *et al.*, 1998). Skin carotenoid levels affect skin colour, contributing to the b* (yellowness) component of skin colour (Alaluf *et al.*, 2002b) and very high carotenoid intake can lead to a harmless yellow discolouration of the skin (Monk, 1983). In Section 2 I investigate the hypothesis that natural dietary intake of carotenoids and fruit and vegetables is related to the yellowness of the skin.

Carotenoids are found in subcutaneous fat (Edwards & Duntley, 1939), and have been detected in all layers of the skin (Alaluf *et al.*, 2002b). Carotenoid concentrations are particularly high in the *stratum corneum* as they are secreted through the sebaceous (oil secreting) glands and reabsorbed by the *stratum corneum* (Edwards & Duntley, 1939; La Placa *et al.*, 2000). Thus the concentrations of carotenoids vary around the body, and are especially high in the palms of the hands and soles of the feet, due to their unusually thick *stratum corneum*.

Carotenoids have a role in photoprotection in the skin. Skin samples with higher levels of carotenoids have been found to have a higher minimum erythemal dose (MED; the minimum amount of UV radiation required to cause erythema or skin reddening; Alaluf *et al.*, 2002b), and carotenoid supplementation has been found to reduce UV radiation-induced erythema in healthy skin (Stahl *et al.*, 2000). This mechanism is probably related to the carotenoids' ability to protect against reactive oxygen species (free radicals that are produced in the body during UV irradiation or by immune defence mechanisms, and may damage molecules such as DNA or proteins; Stahl *et al.*, 2000). Carotenoids have also been found to protect the skin from visible light in photosensitive individuals (Matthews-Roth *et al.*, 1974).

1.9.2.3 Melanin

Melanin is the pigment that most people would think of when considering skin colour. Melanin is a dark brown pigment and impacts negatively on the L* dimension (darkens the skin) and positively on the b* dimension (increases skin yellowness) in the CIELab colour space (Alaluf *et al.*, 2002c; Shriver & Parra, 2000).

Melanin is produced in melanosomes on the lamellae of skin cells called melanocytes (Billingham, 1948), before being deposited in the surrounding keratinocytes (Robins, 1991). Larger melanosomes remain as individual structures whilst smaller melanosomes are packaged together into melanosome clusters surrounded by a membrane (Toda *et al.*, 1972).

While the skin of different ethnic groups varies considerably in terms of colour, and most of this variation is attributable to melanin, the preponderance of melanocytes is similar between the ethnic groups (Alaluf *et al.*, 2002a). The difference in colour is produced by the increased activity of melanocytes in darker skin, producing more melanin (Iozumi *et al.*, 1993) and the relatively larger size and increased melanisation of melanosomes in darker skin (Toda *et al.*, 1972). Additionally, melanosomes in darker skin contain more dihydroxyindole (DHI)-enriched (darker brown) eumelanin and less 5,6-dihydroxyindole-2-carboxylic acid (DHICA)-enriched (lighter brown) eumelanin and yellow-red pheomelanin than lighter skin (Alaluf *et al.*, 2002a), though pheomelanin contributes a very small amount to skin colour, even in lightly pigmented groups (Alaluf *et al.*, 2002a).

UV light exposure causes sun tanning in most people (notably not in fair skinned, red headed individuals), which consists of both an immediate and a delayed tanning response. The immediate response involves the oxidation of existing melanin, causing it to become darker (Robins, 1991), but no increase in the number or size of melanosomes (Jimbow *et al.*, 1974; Robins, 1991). The delayed tanning response involves the production of more melanosomes and changes in the proportions of the melanin types present. While phaeomelanin (yellow-red) increases very little,

DHICA-enriched melanin (light brown) increases rather more and DHI-enriched melanin (dark brown) increases much more markedly (Alaluf *et al.*, 2001).

Photoprotection is usually considered to be the primary role of melanin, which it achieves by scattering and absorbing UV radiation in the epidermis (Kollias *et al.*, 1991). Melanin also acts as a stable (less reactive and therefore less damaging to cells) free radical, "soaking up" damaging reactive oxygen species (more reactive free radicals that cause cell damage) liberated by UV radiation (Robins, 1991). Darker skinned people suffer less DNA damage after UV irradiation than light skinned people (Tadokoro *et al.*, 2003), and are much more resistant to UV-induced erythema and skin cancer (Robins, 1991). However, phaeomelanin releases free radicals during UV irradiation (Hill & Hill, 2000), which may partially explain why individuals, with high levels of phaeomelanin (such as red-heads) experience higher levels of skin cancer and erythema than individuals with more eumelanin, though other factors may also contribute (Hennessy *et al.*, 2005).

The melanin-producing cascade plays a significant role in the immune defence of insects (Boman & Hultmark, 1987). In humans, melanocytes have phagocytic function (engulfing invading pathogens) and melanosomes have lysosomal function (destroying invading cells, once they have been engulfed by the melanocyte; Burkhart & Burkhart, 2005). It has been suggested that the primary role of melanin is immune defence rather than photoprotection, as geographical areas with darker-skinned people have higher parasite loads as well as higher UV radiation loads (Burkhart & Burkhart, 2005). However, levels of UV radiation are much higher at equatorial latitudes than towards the pole. The skin tone of indigenous people becomes lighter away from the

equator, leading to suggestions that the reduced availability of UV light at high latitude led to the evolution of light skin (Jablonski & Chaplin, 2000).

1.10 The evolution of Primate Trichromatic Colour Vision

The light-sensitive cones in the retina detect different specific wavelengths of light, depending upon the specific photopigments they contain (Jacobs, 1993). In most mammals, which have dichromatic vision, the retina contains two types of cone: one that responds to short (blue) wavelengths (S cones), and one that responds to long wavelengths (L cones). This gives dichromatic, yellow-blue colour vision. The S photopigment is encoded by a gene on an autosome, whereas the L photopigment is encoded by a gene on the X chromosome (see Surridge *et al.*, 2003). In Old World (catarrhine) primates and apes, the L gene has been duplicated on the X chromosome, and one copy has mutated to encode for a third photopigment, sensitive at intermediate wavelengths (M cones), giving trichromatic vision, in which red and green are also distinguishable (e.g. Dulai *et al.*, 1999). In most species of New World monkeys, however, this duplication has not taken place. Instead, the L gene is polymorphic, with several different alleles encoding for L photopigments. Since the gene is X-linked, males and homozygous females are dichromatic, whereas heterozygous females are trichromatic (in heterozygous females, X inactivation causes the production of different photopigments in different cone cells; Regan *et al.*, 2001).

Different theories have been advanced to explain the selective advantage that may have caused the evolution of trichromatic colour vision in primates, and also the point in time that such an evolutionary event occurred. Explanations for the evolution of

trichromatic colour vision in primates tend to focus on increased ability to identify red and orange fruit against a background of green leaves (Allen, 1879). This advantage has been confirmed by modelling (Regan *et al.*, 2001; Riba-Hernandez *et al.*, 2004), though field studies have failed to find an advantage for trichromatic individuals over dichromatic individuals in finding these fruits (Dominy *et al.*, 2003; Hiramatsu *et al.*, 2008).

Another hypothesis suggests that folivory is more important that frugivory in the evolution of trichromatic colour vision in primates. In several species of plant, leaves become lighter and greener as they get older. As these leaves age, they become tougher and lower in protein. When young, these leaves are also dappled with red. To dichromats, these leaves appear to be a similar colour to old leaves, whereas trichromats can discern the red dappling and therefore can better identify younger, more tender leaves (Lucas *et al.*, 1998). Indeed, it has been observed that trichromatic species eat these red leaves more frequently than dichromatic species, while similar results were not found for red vs green fruits (Lucas *et al.*, 2003).

A third hypothesis that has been advanced to explain the evolution of primate trichromatic vision is the use of colour in social and sexual signalling. It has been shown that trichromatic primate species have areas of bare skin that become redder with increased dominance rank (Setchell & Dixson, 2001) and increased testosterone in male (Setchell & Dixson, 2001) and reproductive quality in female mandrills (Setchell *et al.*, 2006). Additionally, redness is interpreted as a badge of status by other male mandrills (Setchell & Wickings, 2005) and are preferred as mates by females (Setchell, 2005). Male macaques become redder in the mating season, in

response to increased testosterone levels (Rhodes *et al.*, 1997), and redder males are preferred by females (Waitt *et al.*, 2003).

Changes in the amount of blood in the skin and the degree of oxygenation of the blood alter the light reflectance spectrum of the skin in predictable ways. It has been shown that the wavelengths to which the long- and medium-wavelength photopigments in the cones of primates' eyes respond maximally is closely matched to the parts of the spectrum that change with changing levels of blood perfusion and oxygenation. The eyes of primates with habitually trichromatic colour vision are therefore ideally suited to discerning differences in the perfusion and oxygenation of blood in the skin of conspecifics (Changizi *et al.*, 2006). Additionally, trichromatic species are more likely to have patches of bare skin, where such changes in skin blood perfusion and oxygenation will be visible to conspecifics (Changizi *et al.*, 2006). Humans become facially flushed with blood when angry (Drummond & Quah, 2001), when experiencing unwanted social attention (Leary *et al.*, 1992) and in a range of social situations, including talking to someone whom the blusher finds attractive (Bogels *et al.*, 1996), indicating a role for redness in human social and sexual signalling.

It has therefore been suggested that the detection of socio-sexual signals may have provided the selective advantage for the evolution of trichromatic colour vision (Changizi *et al.*, 2006), as being sensitive to these cues could allow an individual to avoid violent conflict with high ranking or very angry individuals, or to detect higher quality mates. Phylogenetic study has shown that primate trichromatic colour vision is likely to have evolved well before the evolution of skin and pelage redness,

suggesting that such signalling did not drive the evolution of trichromatic vision, and instead trichromatic vision enabled colour signalling to evolve (Fernandez & Morris, 2007). However, it is still possible that subtle differences in skin blood colour existed prior to the evolution of trichromatic vision. Detection of such cues could still have provided a selective advantage to those able to detect them, providing selective pressure for the evolution of trichromatic vision. The evolution of trichromatic vision, in turn, would then allow the evolution of conspicuous skin redness signals. Changizi *et al.* (2006) also present data showing that more routine trichromacy is associated with an increased percentage of facial bareness in the primate order, providing further support for the evolution of trichromacy to detect socio-sexual signals.

Finally, while dichromatic mammals can see yellow and blue, analysis of the wavelength responses of their cones suggest that they are unable to distinguish yellow from red or green (Carroll *et al.*, 2001). This means that signalling based on yellow pigmentation is also unlikely to be useful in such animals, as it will be easily confused with other yellow, red or green pigmentation.

1.11 Summary

From this review of the relevant literature, it can be seen that several aspects of the human face play a significant role in attraction and mate selection, potentially signalling different aspects of mate quality, including health. The face is also the most habitually exposed skin, and contains the most social information. Skin colour varies widely both within and between populations, and is controlled by a number of pigments. These pigments have associations with health, and are used to signal condition in a number of non-human animal species. It is likely, therefore, that human

skin colour will be affected by the health of the individual, and that it will be perceived as a cue to health by observers. In the remainder of this thesis, I will examine the effect of diet on skin colour, and the effect of skin colour on the perceived health of human faces.

Section 2:

Carotenoids

Affect Skin

Colour

2.1 Section 2 Introduction

This section describes two studies examining the effect of natural daily fruit and vegetable and carotenoid intake on the colour of human skin. Study 1 shows that skin yellowness is greater in individuals that consume more fruit and vegetables and carotenoids. Study 2 uses spectral analysis to confirm that this effect is caused by increased carotenoid levels in the skin.

Many species of birds and fish display brightly coloured, sexually-selected ornaments, based on carotenoids. These yellow-red ornaments are thought to signal condition and bigger, brighter carotenoid ornaments are preferred by the opposite sex. Greenfinches with brighter carotenoid-based plumage colouration have been found to have more effective immune responses, as measured by a range of metrics (Blount *et al.*, 2003). Carotenoid levels are lower in chickens and guppies infected with parasites (Olson & Owens, 1998) and nematode infection reduces the brightness of the supra-orbital comb carotenoid ornament in red grouse (Martinez-Padilla *et al.*, 2007), suggesting that carotenoid ornaments signal a reduced parasite load.

Females of the livebearing fish *Poecilia parae* prefer to mate with males with carotenoid colouration (red and yellow males) than with female-coloured, dull-coloured or blue males, all of which live in the same populations (Bourne et al, 2003). Female house finches prefer to associate with males with brighter carotenoid pigmentation (Hill, 1990). Yellow-eyed penguins mate assortatively by the brightness of their yellow, carotenoid-based eye flashes, suggesting that individuals of both sexes prefer mates with brighter eye flashes (Massaro *et al.*, 2003).

Carotenoids cannot be synthesised *de novo* in the body, being obtained primarily from fruits and vegetables in the diet (Alaluf *et al.*, 2002b), and are therefore a limiting factor for organisms (Goodwin, 1984). Carotenoid ornaments may signal nutritional status and, by extension, foraging ability. Supplementing the diet of guppies with carotenoid-rich algae or with a carotenoid supplement was found to enhance the sexually-selected orange colouration of males (Karino & Haijima, 2004). In red grouse, a period of restricted feeding (thereby reducing nutritional intake and carotenoid intake) was found to reduce the redness of carotenoid ornaments (Perez-Rodrigues & Vinuela, 2008). Further, Perez-Rodriguez & Vinuela (2008) found that the carotenoid colouration of exposed skin (eye ring) was reduced more quickly than that of the beak.

It is likely, therefore, that the increased yellow colouration associated with carotenoids in human skin will increase with increased dietary intake of carotenoids and will be perceived as healthier and more attractive. In this section, I investigate the relationship between natural dietary carotenoid intake and human skin colour. In later Sections, I will investigate the effects of skin colour and pigmentation on the perceived health of faces.

2.2 Study 1 – Natural dietary carotenoid intake contributes to skin yellowness

2.2.1 Introduction

As discussed in Section 1, carotenoids are present in human skin, particularly in the *stratum corneum.* Though the levels of carotenoids in human skin are small (Robins, 1991), levels of carotenoids in the skin correlate with skin yellowness (CIELab b* values), and are therefore thought to contribute to normal human skin colour (Alaluf *et al.*, 2002b). Additionally, supplementation of the diets of individuals with Betatene, a carotenoid-rich algal extract, over a period of 12 weeks increased levels of carotenoids in the blood serum (measured from blood samples). Measurements of skin carotenoid levels were made using reflectance spectrophotometry, and found to closely track blood serum values, also increasing with supplementation (Stahl *et al.*, 1998). Unusually high levels of carotenoids can occur in the body, either due to excessive intake or pathological mechanisms such as a failure to convert carotenoids to vitamin A. This can lead to skin yellowing, and is known as carotenaemia (Monk, 1983).

Alaluf *et al.* (2002) found that levels of carotenoids in the skin correlate with skin yellowness (CIELab b*), and suggested that this was attributable to normal dietary intake. However, Alaluf *et al.* (2002b) did not examine dietary intake of carotenoids and no direct connection from natural dietary intake of carotenoids to skin yellowness (b*) has yet been established.

2.2.1.2: Predictions

Carotenoids cannot be synthesised *de novo* in the body, and are obtained from the diet, particularly from fruit and vegetables (Alaluf *et al.*, 2002b). It is hypothesised therefore that normal dietary carotenoid intake will contribute to normal human skin colour. Those individuals with higher carotenoid intake are likely to have yellower skin (as measured by the CIELab b* component). Further, this increased skin yellowing is likely to be more detectable in more photoprotected areas where melanin pigmentation (which impacts on the L* and b* components, and may therefore interfere with yellowness measurements, Stamatas *et al.*, 2004, is less pronounced, Alaluf *et al.*, 2002c). The palm of the hand is likely to be a region that is particularly well suited to detecting the hypothesised relationship, due to its low melanin content and thick *stratum corneum* (Edwards & Duntley, 1939). High intake of fruit and vegetables may be expected to be associated with other aspects of a healthy lifestyle, such as taking more exercise. Individuals who take more exercise may also be expected to be more tanned, due to more time spent out of doors, exposed to the sun. The amount of exercise taken by participants will therefore be measured controlled.

2.2.2 Methods

82 Caucasian participants (aged 18–26, 48 female, all of whom reported not having used artificial tanning products in the preceeding month) completed a diet questionnaire (Margetts *et al.*, 1989) from which daily intake frequency for food items was calculated. The daily intake frequencies of the fruit and vegetable items from the questionnaire were summed for each participant. This gave a number of fruit and vegetable portions consumed per day for each participant. 76 (43 female) of these participants completed the Godin Leisure-Time Exercise Questionnaire. The Weekly Leisure Activity Score (WLAS) was calculated, giving a validated estimate of

exercise frequency (Godin & Shephard, 1997). 22 further participants (aged 19–28; 11 female) were shown examples of standard serving sizes of fruits and vegetables (NHS, UK) and asked the number of portions (or fraction) they ate at a sitting. From these, the mean portion size per food was calculated, and used as an estimate for all participants. Item frequency was multiplied by mean portion size consumed to obtain an estimate of daily intake per food for the 82 participants. β-carotene content for each food item was retrieved from the Canadian Nutrient File (version 2007b). These values were multiplied by the total amount of β-carotene obtained from each food item by each participant. For each participant, the β-carotene intake was summed across all food items to give a total natural daily intake of β-carotene for each participant. Fruit and vegetable, and β-carotene intake was summed across items. A Konika Minolta CM2600d reflectance spectrophotometer was used to obtain measurements of skin colour (in CIELab colour space; see section 1.9) from the left outer shoulder, outer upper arm, inner upper arm, and ventral interosseous region of the palm of the hand. The CIELab colour space was used in preference to the RGB or other alternative colour spaces because the CIELab colour space is modelled on the human visual system, and is perceptually uniform (a change of one unit in CIELab colour space is of the same magnitude perceptually regardless of the initial colour and regardless of the dimension in which the change was made; see section 1.9).

2.2.2.2: Statistical Methods

Some dietary variables were not normally distributed (Kolmogorov-Smirnov tests $p<0.05$), therefore non-parametric statistical tests were used in the analysis. Correlations (Spearman's ρ) were used to test for a relationship between daily intake of fruit and vegetable portions and skin yellowness (b*) and also between daily β-

carotene intake and skin yellowness (b*). Non-parametric partial correlations (Spearman's ρ) were used to test for these relationships, whilst controlling for the amount of exercise engaged in by the participants (WLAS).

2.2.3 Results

The palm of the hand is a skin area that shows carotenoid colour well (Stahl *et al.*, 1998), and is subject to minimal melanisation. I found that palm skin yellowness (b*) correlated with dietary intake of fruit and vegetables ($\rho=0.451$; $p<0.001$; $n=82$, Fig. 2.1A), and with estimated natural β-carotene intake ($\rho=0.471$; $p<0.001$; $n=82$, Fig. 2.1B). This relationship was apparent at other skin locations, but was strongest in skin regions that are not habitually exposed to sunlight (Table 2.1). Such topographic variation is expected because melanisation, which also affects yellowness, would degrade but not eliminate correlations between dietary carotenoid and skin yellowness. Though a relationship was found between exercise level (WLAS) and the natural daily intakes of fruit and vegetables ($\rho=0.328$; $p=0.002$; $n=76$) and β-carotene ($\rho=0.286$; $p=0.012$; $n=76$), controlling for exercise level of participants did not weaken the correlation between skin yellowness and fruit and vegetable ($\rho=0.428$; $p<0.001$; $df=73$) and β-carotene ($\rho=0.451$; $p<0.001$; $df=73$) intake. Hence the relationship between skin colour and diet does not appear to be a by-product of other aspects of a healthy lifestyle, as measured by the WLAS.

I have shown here that natural dietary fruit and vegetable and β-carotene intake affects the colour of human skin, making it more yellow.

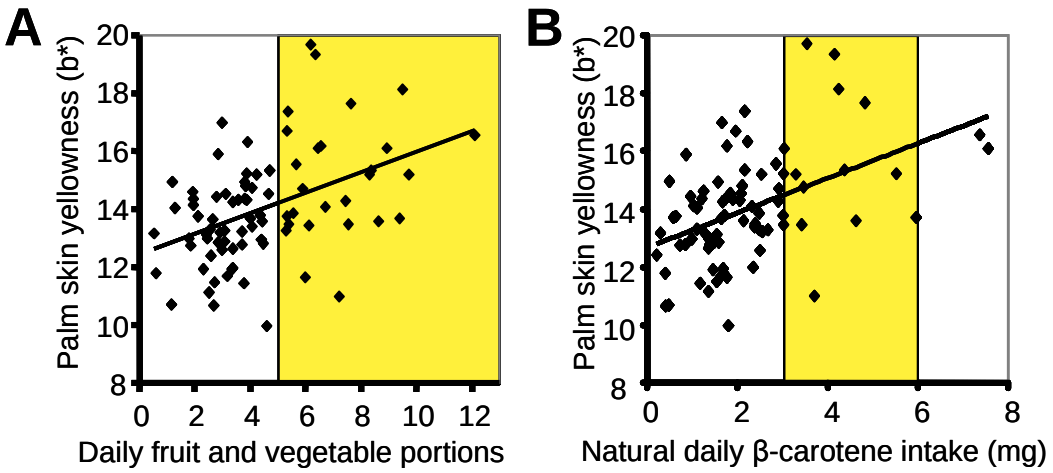


Fig. 2.1. Diet and skin colour.
(**A**) Daily fruit and vegetable intake correlates with palm skin yellowness (b*; ρ=0.451; p<0.001; n=82). Yellow zone indicates US recommended daily intake range of 5-13 servings (Institute of Medicine, 2000). (**B**) Natural daily β-carotene intake correlates with palm skin yellowness (b*; ρ=0.471; p<0.001; n=82). Yellow zone shows recommended intake to reduce the incidence of cancer (Dietary Guidelines Advisory Committee, 2005).

	Daily intake							
	Fruit & vegetables (n=82)		β-carotene (n=82)		Fruit & vegetables, (controlling exercise, df=73)		β-carotene (controlling exercise, df=73)	
	ρ	p	ρ	p	ρ	p	ρ	p
Shoulder	0.211	0.057	0.229	0.039	0.216	0.062	0.232	0.045
Upper arm	0.171	0.124	0.192	0.082	0.174	0.135	0.193	0.096
Under arm	0.195	0.079	0.267	0.015	0.203	0.080	0.275	0.017
Palm	0.451	<0.001	0.471	<0.001	0.428	<0.001	0.451	<0.001
Mean	0.246	0.026	0.273	0.009	0.245	0.034	0.287	0.013

Table 2.1. Relationships between daily fruit and vegetable and β-carotene intake and skin yellowness (b*) at various body locations. Spearman's rho (ρ) and probability (p) are given. Non-parametric partial correlations are used to control for exercise level. Shaded cells indicate statistically significant relationships.

2.2.4 Discussion

My results demonstrate that skin yellowness is affected directly by natural fruit and vegetable and β-carotene intake. Further, I have shown that the palm of the hand, with its thick stratum corneum (Stamatas *et al.*, 2004) and minimal melanisation exhibits the strongest correlation, and more photoexposed areas such as the upper arm show less strong correlations. Since carotenoids are especially prevalent in the stratum corneum (Edwards & Duntley, 1939), and the yellow carotenoid signal is expected to be degraded by melanisation (Alaluf *et al.*, 2002c), this adds weight to the argument that it is the carotenoid content of the diet that is contributing to skin yellowness.

Further, since controlling for exercise levels did not remove the relationship of fruit and vegetable and β-carotene intake with skin yellowness, it is unlikely that other lifestyle factors explain the relationship.

Palm skin yellowness thus has potential as an accessible biomarker of diet composition similar in power to serum β-carotene measurement (skin yellowness $\rho=0.45$ in this study; serum β-carotene measurement $r=0.48$, Polsinelli *et al.*, 1998). I have shown that skin yellowness is a reliable indicator of a healthy diet, high in fruit and vegetables. It may be hypothesised, therefore, that skin yellowness may provide a perceptible cue to health.

2.3 Study 2 – Reflectance Spectra

2.3.1 Introduction

In Study 1, I showed that skin yellowness is predicted by natural dietary intake of fruit and vegetables and carotenoids. Further, since controlling for exercise did not remove the correlation, it is unlikely that the relationship is attributable to other lifestyle factors. In this section, I will use scanning reflectance spectroscopy to confirm that the relationship between skin yellowness and natural dietary intake of fruit and vegetables and carotenoids is caused by dietary carotenoids in the skin.

It is possible to detect carotenoids in the skin by non-invasive reflectance spectrophotometry (Stahl *et al.*, 1998). This method compares the reflectance of light from the skin at different wavelengths to the reflectance spectra of skin pigments to determine the pigment content of the skin. These methods have shown that carotenoid supplementation causes increased carotenoid levels in the skin (Stahl *et al.*, 1998), and that carotenoid levels in the blood serum are correlated with levels in the skin (Stahl *et al.*, 1998). Excessive intake of carotenoids, and certain metabolic disorders can lead to an abnormal but harmless yellowing of the skin (Monk, 1983). Further, Alaluf *et al.* (2002b) found that levels of carotenoids in the skin correlate with skin yellowness (CIELab b*), and suggested that this was attributable to normal dietary intake. However, Alaluf *et al.* (2002b) did not examine their participants' diets, and the connection of natural dietary intake of carotenoids to skin colour has not yet been reliably established.

The different pigments in the skin have different absorption and reflectance spectra, absorbing light at certain wavelengths and reflecting light at others. It is these unique spectra that give the pigments their unique colours. In the visible spectrum, carotenoid absorption is best seen in the range 400-540nm (Miller, 1937; Stahl *et al.*, 1998). It is this absorption in the short wavelength (violet, blue, green) part of the spectrum and reflectance at longer wavelengths (green, yellow, red) that gives carotenoids their yellow-red colour.

β-carotene absorption peaks at 453nm (in the violet range), and 480nm (blue), α-carotene absorption peaks at 447nm (violet) and 475nm (blue) and lycopene absorption peaks at 447nm (violet), 473nm (blue) and 505nm (green) (Miller, 1937). In contrast, melanin shows a smooth increase in absorption towards the short wavelength (violet) end of the spectrum, peaking at 335nm (ultraviolet) (Kollias, 1995). Oxyhaemoglobin shows absorption peaks at 415nm (violet), 542nm (green) and 576nm (yellow) and deoxyhaemoglobin shows peaks at 439nm (violet) and 556nm (green) (Edwards & Duntley, 1939).

Increases in the levels of carotenoids in the skin are expected, therefore, to increase the absorption, and to produce a corresponding decrease in reflectance, of light at the wavelengths associated with carotenoid absorption. In other words, a negative relationship between skin reflectance and dietary carotenoid intake should be observed at the wavelengths associated with carotenoid absorption. This effect should be strongest at the wavelengths with the greatest carotenoid absorption values. Therefore, the correlation coefficients should themselves be negatively related to the absorption spectra of carotenoids.

2.3.2 Methods

Thirty-seven participants (29 female, 8 male) completed food frequency questionnaires (Margetts *et al.*, 1989), and estimated daily β-carotene intake was calculated, as described in Study 1. Spectral reflectance measurements of skin colour at seven locations on the arm, hand and face (table 2.2) were made using a Konika Minolta CM-2600d spectrophotometer, sampling every 10nm from 360-740nm (a range that encompasses the entire visual spectrum and extends into ultraviolet at the short wavelength end and infrared at the long wavelength end). To control for overall lightness of the skin, reflectance values were divided by the total reflectance for each individual.

Standard absorption coefficient values for three common carotenoids (α-carotene, β-carotene and lycopene) were derived from Miller (1937). Mean absorption coefficient values at each wavelength were also computed to give an estimate of a typical carotenoid absorption spectrum. Melanin absorption coefficient values were derived from (Sarna & Swartz, 1988), and haemoglobin values were obtained from Scott Prahl at omlc.ogi.edu.

2.3.3 Statistical Methods and Results

Since some variables were non-parametrically distributed, Spearman's rank correlations were used for data analyses. To test for the presence of a carotenoid signal, Spearman's rank correlations were used to test for relationships between daily β-carotene intake and skin reflectance values at the wavelengths associated with carotenoid absorption (400-540nm). Significant relationships were found at

wavelengths corresponding to peak carotenoid absorption coefficients (table 2.2). These correlation coefficients were plotted and compared to carotenoid absorption coefficients (fig. 2.2). To test for relationships between the curves plotted, Spearman's rank correlations were used. Significant correlations were found, particularly for β-carotene and lycopene, and for the mean signals (table 2.3). Similar results for the relationship between fruit and vegetable intake and skin reflectance were also found (tables 2.4-2.5) Examination of the plots of correlation coefficients overlaid with the absorption coefficients of common carotenoids and other common skin pigments strongly supports the connection of natural daily β-carotene and fruit and vegetable intake with skin colour (figs 2.2-2.5).

	Inner Arm	Right Cheek	Left Cheek	Forehead	Upper Arm	Shoulder	Palm
400	ρ=-0.351 p=0.033	ρ=0.059 p=0.730	ρ=-0.021 p=0.901	ρ=-0.236 p=0.160	ρ=-0.253 p=0.131	ρ=-0.333 p=0.044	ρ=-0.044 p=0.797
410	ρ=-0.363 p=0.027	ρ=0.028 p=0.867	ρ=-0.005 p=0.976	ρ=-0.252 p=0.132	ρ=-0.251 p=0.134	ρ=-0.333 p=0.044	ρ=-0.113 p=0.505
420	ρ=-0.394 p=0.016	ρ=0.064 p=0.709	ρ=0.004 p=0.983	ρ=-0.230 p=0.170	ρ=-0.246 p=0.142	ρ=-0.328 p=0.047	ρ=-0.175 p=0.299
430	ρ=-0.403 p=0.014	ρ=0.053 p=0.756	ρ=0.007 p=0.967	ρ=-0.249 p=0.137	ρ=-0.251 p=0.135	ρ=-0.337 p=0.041	ρ=-0.225 p=0.180
440	ρ=-0.409 p=0.012	ρ=0.028 p=0.868	ρ=-0.024 p=0.887	ρ=-0.299 p=0.072	ρ=-0.277 p=0.097	ρ=-0.344 p=0.037	ρ=-0.266 p=0.111
450	ρ=-0.416 p=0.010	ρ=-0.009 p=0.959	ρ=-0.028 p=0.868	ρ=-0.334 p=0.043	ρ=-0.282 p=0.090	ρ=-0.375 p=0.022	ρ=-0.313 p=0.060
460	ρ=-0.364 p=0.027	ρ=0.008 p=0.963	ρ=-0.075 p=0.659	ρ=-0.346 p=0.036	ρ=-0.289 p=0.082	ρ=-0.394 p=0.016	ρ=-0.321 p=0.053
470	ρ=-0.349 p=0.035	ρ=-0.040 p=0.814	ρ=-0.103 p=0.545	ρ=-0.319 p=0.054	ρ=-0.295 p=0.076	ρ=-0.411 p=0.012	ρ=-0.295 p=0.077
480	ρ=-0.361 p=0.028	ρ=-0.089 p=0.601	ρ=-0.156 p=0.357	ρ=-0.346 p=0.036	ρ=-0.303 p=0.068	ρ=-0.416 p=0.010	ρ=-0.324 p=0.050
490	ρ=-0.372 p=0.024	ρ=-0.131 p=0.438	ρ=-0.176 p=0.297	ρ=-0.342 p=0.039	ρ=-0.317 p=0.056	ρ=-0.431 p=0.008	ρ=-0.363 p=0.027
500	ρ=-0.397 p=0.015	ρ=-0.096 p=0.574	ρ=-0.103 p=0.545	ρ=-0.315 p=0.058	ρ=-0.321 p=0.053	ρ=-0.422 p=0.009	ρ=-0.346 p=0.036
510	ρ=-0.321 p=0.053	ρ=0.062 p=0.715	ρ=-0.029 p=0.866	ρ=-0.223 p=0.185	ρ=-0.285 p=0.087	ρ=-0.385 p=0.019	ρ=-0.279 p=0.095
520	ρ=-0.251 p=0.134	ρ=0.292 p=0.080	ρ=0.246 p=0.142	ρ=-0.055 p=0.746	ρ=-0.231 p=0.170	ρ=-0.372 p=0.023	ρ=-0.195 p=0.248
530	ρ=-0.116 p=0.494	ρ=0.346 p=0.036	ρ=0.309 p=0.063	ρ=-0.023 p=0.895	ρ=-0.188 p=0.264	ρ=-0.344 p=0.037	ρ=-0.133 p=0.431
540	ρ=-0.130 p=0.442	ρ=0.344 p=0.037	ρ=0.240 p=0.152	ρ=0.010 p=0.953	ρ=-0.172 p=0.308	ρ=-0.299 p=0.073	ρ=-0.099 p=0.560

Table 2.2: Correlations between natural daily β-carotene intake and reflectance at each wavelength, for different body locations. Significant relationships highlighted in dark grey. Marginally significant relationships highlighted in light grey.

	Inner Arm	Right Cheek	Left Cheek	Forehead	Upper Arm	Shoulder	Palm
β-carotene	ρ=-0.676 p=0.006	ρ=-0.717 p=0.003	ρ=-0.611 p=0.015	ρ=-0.882 p<0.001	ρ=-0.579 p=0.024	ρ=-0.402 p=0.137	ρ=-0.606 p=0.017
α-carotene	ρ=-0.696 p=0.004	ρ=-0.582 p=0.023	ρ=-0.421 p=0.117	ρ=-0.735 p=0.002	ρ=-0.393 p=0.148	ρ=-0.177 p=0.529	ρ=-0.387 p=0.154
Lycopene	ρ=-0.307 p=0.265	ρ=-0.757 p=0.001	ρ=-0.821 p<0.001	ρ=-0.725 p=0.002	ρ=-0.861 p<0.001	ρ=-0.824 p<0.001	ρ=-0.850 p<0.001
Mean	ρ=-0.625 p=0.013	ρ=-0.739 p=0.002	ρ=-0.639 p=0.010	ρ=-0.850 p<0.001	ρ=-0.629 p=0.012	ρ=-0.486 p=0.066	ρ=-0.654 p=0.008

Table 2.3: Correlations between correlation coefficients given in table 2.2 and absorption coefficients of common carotenoids, for different body locations. Significant relationships highlighted in grey.

	Inner Arm	Right Cheek	Left Cheek	Forehead	Upper Arm	Shoulder	Palm
400	r=-0.319 p=0.053	ρ=0.044 p=0.794	ρ=-0.052 p=0.756	ρ=-0.258 p=0.117	ρ=-0.285 p=0.086	ρ=-0.317 p=0.055	ρ=-0.063 p=0.704
410	ρ=-0.348 p=0.034	ρ=-0.007 p=0.966	ρ=-0.055 p=0.745	ρ=-0.308 p=0.059	ρ=-0.279 p=0.094	ρ=-0.323 p=0.050	ρ=-0.120 p=0.471
420	ρ=-0.371 p=0.023	ρ=0.007 p=0.963	ρ=-0.054 p=0.748	ρ=-0.300 p=0.066	ρ=-0.281 p=0.091	ρ=-0.310 p=0.061	ρ=-0.178 p=0.282
430	ρ=-0.382 p=0.019	ρ=0.020 p=0.905	ρ=-0.038 p=0.819	ρ=-0.306 p=0.061	ρ=-0.284 p=0.087	ρ=-0.317 p=0.055	ρ=-0.222 p=0.178
440	ρ=-0.384 p=0.018	ρ=0.009 p=0.955	ρ=-0.084 p=0.618	ρ=-0.293 p=0.073	ρ=-0.311 p=0.060	ρ=-0.324 p=0.050	ρ=-0.238 p=0.149
450	ρ=-0.383 p=0.019	ρ=-0.038 p=0.819	ρ=-0.092 p=0.584	ρ=-0.284 p=0.083	ρ=-0.316 p=0.056	ρ=-0.350 p=0.033	ρ=-0.277 p=0.091
460	ρ=-0.346 p=0.035	ρ=-0.022 p=0.894	ρ=-0.140 p=0.406	ρ=-0.311 p=0.056	ρ=-0.327 p=0.047	ρ=-0.367 p=0.025	ρ=-0.289 p=0.077
470	ρ=-0.324 p=0.049	ρ=-0.073 p=0.664	ρ=-0.170 p=0.312	ρ=-0.285 p=0.082	ρ=-0.332 p=0.044	ρ=-0.381 p=0.019	ρ=-0.255 p=0.122
480	ρ=-0.324 p=0.049	ρ=-0.105 p=0.533	ρ=-0.218 p=0.193	ρ=-0.307 p=0.060	ρ=-0.336 p=0.041	ρ=-0.386 p=0.018	ρ=-0.257 p=0.117
490	ρ=-0.342 p=0.037	ρ=-0.149 p=0.378	ρ=-0.251 p=0.132	ρ=-0.277 p=0.092	ρ=-0.348 p=0.034	ρ=-0.394 p=0.015	ρ=-0.300 p=0.066
500	ρ=-0.365 p=0.026	ρ=-0.11 p=0.501	ρ=-0.178 p=0.290	ρ=-0.233 p=0.157	ρ=-0.350 p=0.033	ρ=-0.394 p=0.015	ρ=-0.319 p=0.050
510	ρ=-0.326 p=0.048	ρ=0.016 p=0.923	ρ=-0.107 p=0.525	ρ=-0.153 p=0.356	ρ=-0.322 p=0.051	ρ=-0.364 p=0.026	ρ=-0.257 p=0.118
520	ρ=-0.301 p=0.069	ρ=0.226 p=0.177	ρ=0.148 p=0.381	ρ=0.024 p=0.885	ρ=-0.273 p=0.101	ρ=-0.359 p=0.029	ρ=-0.222 p=0.178
530	ρ=-0.191 p=0.256	ρ=0.284 p=0.088	ρ=0.207 p=0.218	ρ=0.077 p=0.642	ρ=-0.240 p=0.151	ρ=-0.340 p=0.039	ρ=-0.186 p=0.261
540	ρ=-0.163 p=0.333	ρ=0.298 p=0.072	ρ=0.162 p=0.337	ρ=0.106 p=0.523	ρ=-0.222 p=0.185	ρ=-0.288 p=0.083	ρ=-0.154 p=0.354

Table 2.4: Correlations between natural daily fruit and vegatable intake and reflectance at each wavelength, for different body locations. Significant relationships highlighted in dark grey. Marginally significant relationships highlighted in light grey.

	Inner Arm	Right Cheek	Left Cheek	Forehead	Upper Arm	Shoulder	Palm
β-carotene	r=-0.608 p=0.016	r=-0.688 p=0.005	r=-0.630 p=0.012	r=-0.759 p=0.001	r=-0.592 p=0.020	r=-0.337 p=0.219	r=-0.509 p=0.052
α-carotene	r=-0.658 p=0.008	r=-0.533 p=0.041	r=-0.440 p=0.101	r=-0.735 p=0.002	r=-0.404 p=0.136	r=-0.121 p=0.668	r=-0.279 p=0.313
Lycopene	r=-0.300 p=0.277	r=-0.761 p=0.001	r=-0.850 p<0.001	r=-0.336 p=0.221	r=-0.875 p<0.001	r=-0.814 p<0.001	r=-0.804 p<0.001
Mean	r=-0.586 p=0.022	r=-0.718 p=0.003	r=-0.668 p=0.007	r=-0.693 p=0.004	r=-0.650 p=0.009	r=-0.429 p=0.111	r=-0.546 p=0.035

Table 2.5: Correlations between correlation coefficients given in table 2.4 and absorption coefficients of common carotenoids, for different body locations. Significant relationships highlighted in grey.

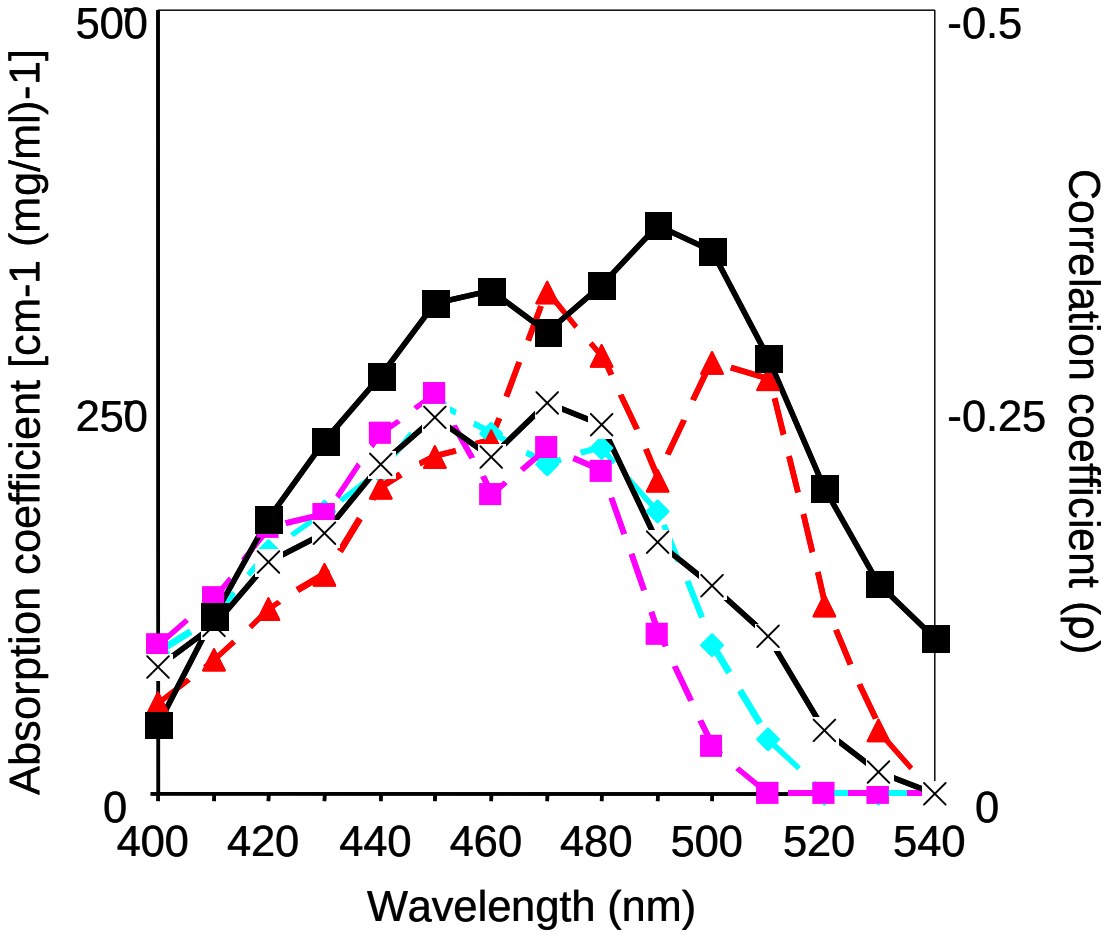


Fig. 2.2: Correlation coefficients (solid black line) of the relationship between dietary β-carotene intake and skin reflectance values at 10nm intervals, at the palm of the hand. Similar graphs for the other skin areas can be found in Appendix A. Dashed lines show absorption spectra for common carotenoids. Red triangles=lycopene, blue rhombi=β-carotene, purple squares=α-carotene. Black dashed line shows mean absorption spectrum for the three carotenoids.

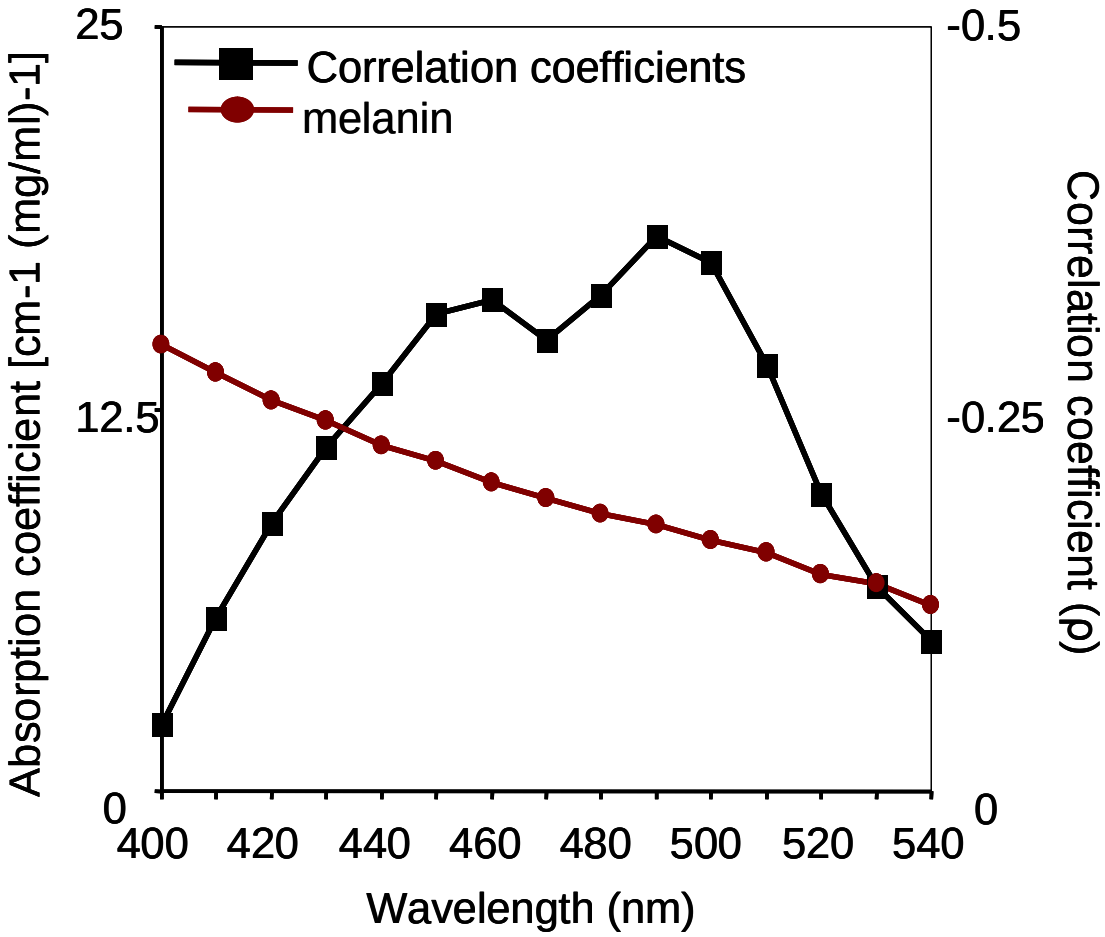


Fig. 2.3: Correlation coefficients (solid black line) of the relationship between dietary β-carotene intake and skin reflectance values at 10nm intervals, at the palm of the hand. Brown line (circular markers) shows absorption spectrum for melanin.

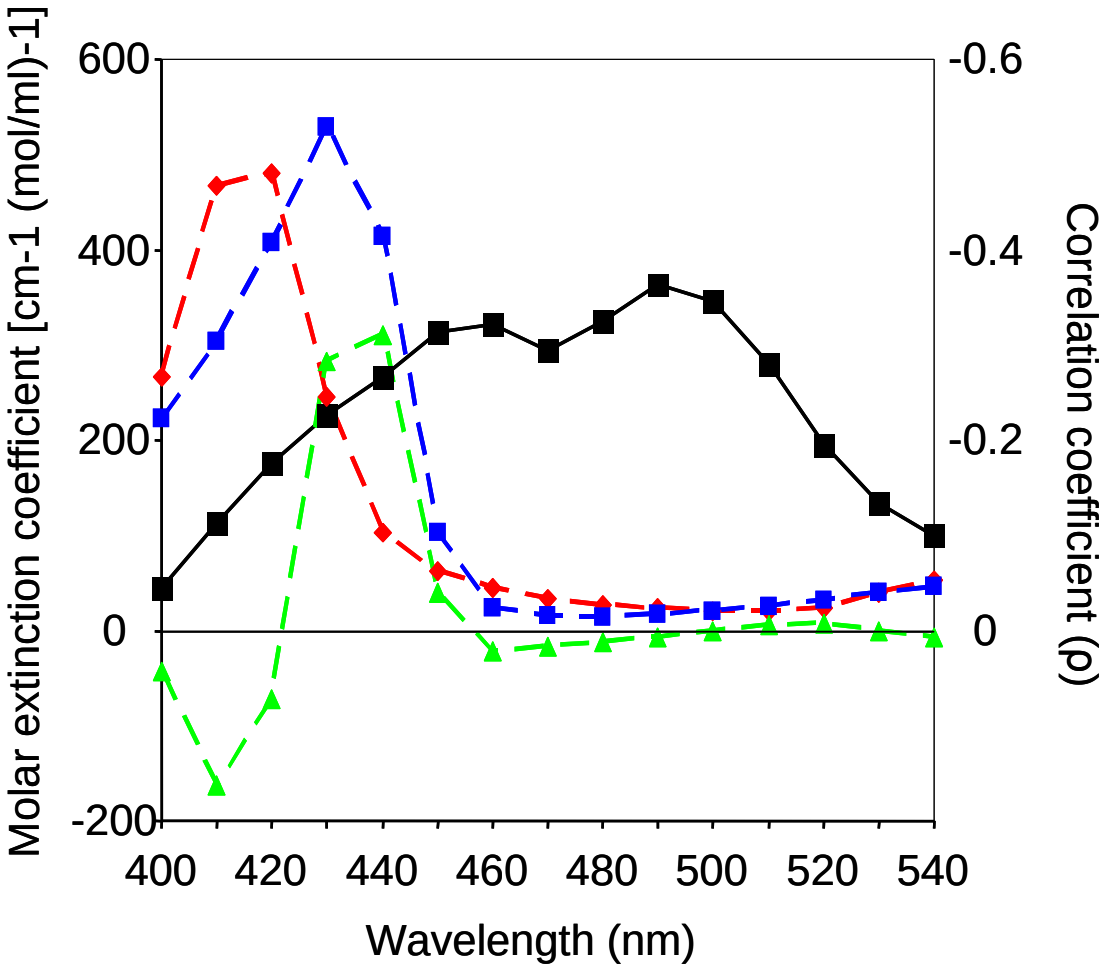


Fig. 2.4: Correlation coefficients (solid black line) of the relationship between dietary β-carotene intake and skin reflectance values at 10nm intervals, at the palm of the hand. Dashed lines show absorption spectra for oxygenated (red rhombi) and deoxygenated (blue squares) haemoglobin (from Scott Prahl, omlc.ogi.edu). Green dashed line (triangular markers) shows absorption difference between oxygenated and deoxygenated blood (deoxygenated minus oxygenated blood)

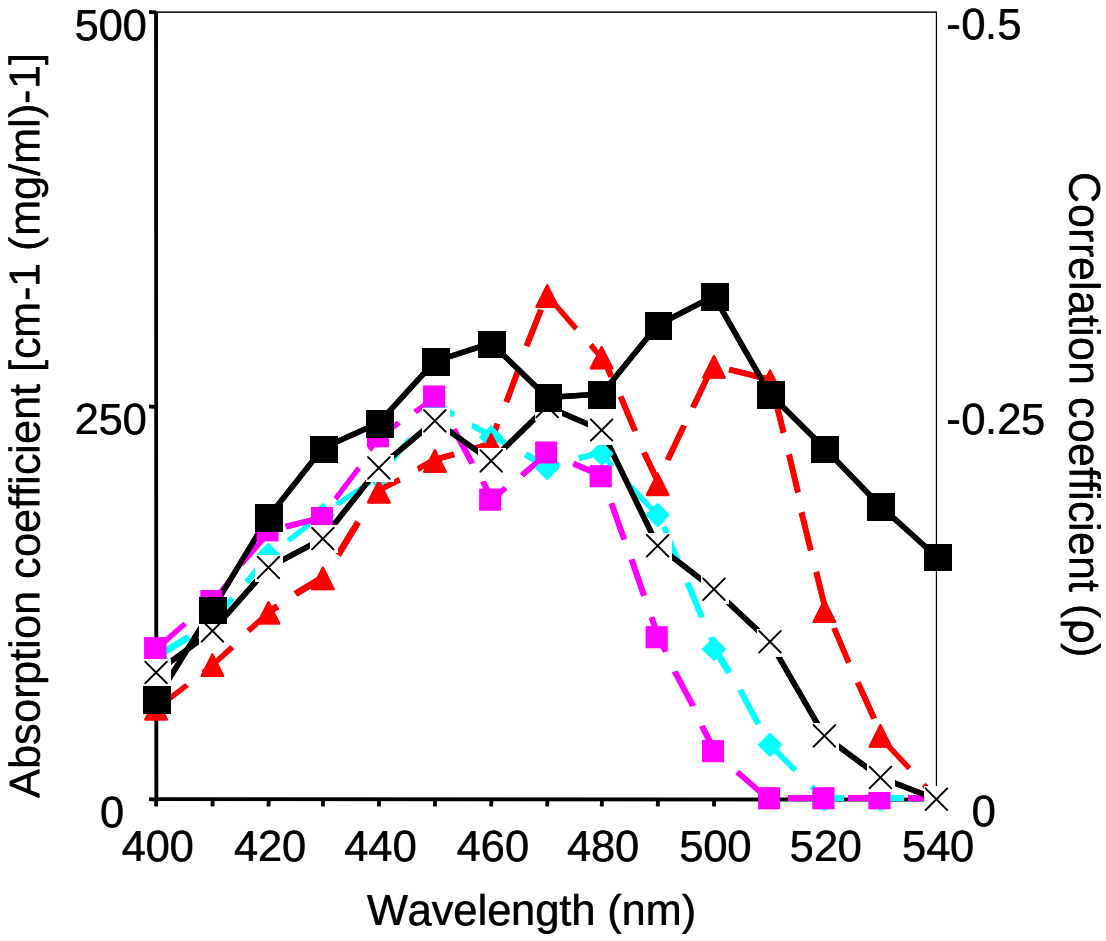


Fig. 2.5: Correlation coefficients (solid black line) of the relationship between dietary fruit and vegetable intake and skin reflectance values at 10nm intervals, at the palm of the hand. Similar graphs for the other skin areas can be found in Appendix A. Dashed lines show absorption spectra for common carotenoids. Red triangles=lycopene, blue rhombi=β-carotene, purple squares=α-carotene. Black dashed line shows mean absorption spectrum for the three carotenoids.

2.3.4 Discussion

The relationship between the natural daily β-carotene intake and the reflectance at each wavelength in the relevant range is highly similar to the inverse of the absorption spectra of common carotenoids. This contrasts with the lack of similarity between the correlation curve and the absorption spectra of the other major yellow pigment, melanin and also from the curves of oxygenated and deoxygenated haemoglobin, and the absorption spectral change associated with a change in the ratio of oxygenated to

deoxygenated haemoglobin. This evidence provides strong support for the hypothesis that increased natural dietary intake of fruit and vegetables and carotenoid pigments affects the natural colour of people's skin. The peak absorption of light by carotenoids is in the short wavelength (violet, blue and part of green) area of the visible spectrum (Miller, 1937), giving the skin a yellowish colour.

There is little similarity between the absorption spectra of other common skin pigments and the plotted correlation coefficients. This suggests that carotenoid intake, and not some other lifestyle correlate (such as exercise or time spent out of doors) is responsible for the increased yellowness of the skin of individuals with high natural daily fruit and vegetable and carotenoid intakes (see Study 1).

2.4 Section 2 Discussion

Studies 1 and 2 demonstrate that individuals with higher natural dietary intake of fruit and vegetables and carotenoids have increased levels of carotenoids in the skin. These carotenoids are detectable by reflectance spectrophotometry. Further, individuals with higher natural dietary intake of fruit and vegetables and carotenoids have increased skin yellowness.

Species of birds and fish display colourful, sexually selected, carotenoid based ornaments. These ornaments become larger and brighter in response to increased dietary intake of carotenoids (Karino & Haijima, 2004). This response is particularly rapid and pronounced in fleshy ornaments, such as the eye-ring skin of red grouse (as opposed to keratinised ornaments such as the beak; (Perez-Rodrigues & Vinuela, 2008). Human skin therefore has a similar relationship with nutritional factors to

those seen in species for whom carotenoid colouration is a sexually selected, condition dependant, condition signalling ornament.

It is known that the carotenoid content and colour of human skin is dependant on the nutritional state of the individual. CIELab b* values are associated with carotenoid levels in the skin and serum of individuals (Alaluf *et al.*, 2002c). I have shown that the carotenoid colour of human skin is directly dependant upon the nutritional composition of the diet. Individuals with a higher natural dietary intake of carotenoids show increased skin carotenoid levels and increased skin yellowness (CIELab b*).

Given the condition-dependent nature of the carotenoid colouration of human skin, and the direct relationship between carotenoid ornament brightness and mate choice in many species of birds and fish (Blount *et al.*, 2003; Bourne *et al.*, 2003; Saks *et al.*, 2003), the relationship of skin carotenoid colouration (and indeed colouration in general) to perceptions of health (and therefore attractiveness) in humans is worthy of study. The remainder of this thesis will examine the role of skin colouration in health perception in humans.

Section 3:

Pure Colour Transforms

3.1 Section 3 Introduction

This Section describes a suite of three experiments, each examining the effect of skin colour in one of the CIELab colour dimensions (lightness, redness and yellowness) on the apparent health of human faces, and a fourth examining how the colour contrast between the lips and the facial skin affects the apparent health of human faces. These experiments are described together in this section to allow a more cohesive treatment of the results.

The colour and texture of facial skin are associated with apparent health in humans (Fink *et al.*, 2006; Jones *et al.*, 2004). Fink *et al.* (2006) measured the distribution of colour in the faces of women of different ages and applied these distributions to standard-shaped three-dimensional faces. These stimuli therefore differed only in colour distribution. When presented to participants for rating, the older faces were perceived as older, less attractive and less healthy than the younger faces, showing that colour distribution alone contains enough information to make these judgements. Jones *et al*. (2004) found that ratings of health of skin patches taken from face photographs correlated positively with ratings of attractiveness of the whole faces. Jones *et al.* (2004) also found that manipulating the colour and texture of faces to look more or less healthy while shape information remained constant changed the attractiveness of the faces. The effect that overall skin colour has on the apparent health of faces has not been previously reported. This section investigates the relationship between perceived health and skin colour by allowing participants to manipulate colour calibrated facial photographs along CIELab L* (lightness), a* (redness) and b* (yellowness) axes to optimise healthy appearance.

3.1.1 Lightness

The darkness of skin is primarily dependent on the level of melanin present in the epidermis. Melanin is a dark brown pigment that impacts positively on the b* (yellow) component and negatively on the L* (lightness) component of skin colour. Darker skin contains larger, more mature (stage 3 and 4) and more individually dispersed melanocytes than lighter skin (Toda *et al.*, 1972).

Many primate groups, including chimpanzees, have light skin under dark hair. Exposed skin in these species is lightly pigmented and becomes darker with photoexposure and with increasing age (Post *et al.*, 1975). It is often assumed, however, that the original colour of modern human skin was black (Hamilton, 1973). As migration to the savannah took place, hominins are thought to have lost their hair as part of a suite of thermoregulatory adaptations. It is suggested that dark skins evolved to protect against the high levels of UV radiation in the African savannah, in the absence of large amounts of body hair (Jablonski & Chaplin, 2000). This hypothesis is supported by the fact that dark skin is less susceptible to sunburn (symptoms of which include erythema – skin reddening - and oedema - inflammation) and skin cancer than light skin, and has higher minimum erythemal doses (MED) of UV radiation (the amount of UV radiation required to cause erythema – this is the standard way of expressing tendency to burn; Robins, 1991). Therefore, dark skin is important where levels of UV radiation are high, such as near the equator (Jablonski & Chaplin, 2000).

The selective pressures influencing the evolution of light skin are less clear, however. As levels of UV radiation are reduced further away from the equator, the selective pressure to maintain high levels of the dark pigment melanin in the skin are reduced

(Jablonski & Chaplin, 2000). However, population genetic studies suggest that there has not been enough time since the divergence of European and Asian populations from African populations for the high frequency of certain variants of the MC1R gene (partly responsible for skin colour) present in light skinned populations to be explained by genetic drift alone. Selective pressures must have been acting to select for light skin at high latitudes (Aoki, 2002).

The vitamin D hypothesis aims to provide a selective mechanism for the evolution of light skin at high latitude (Murray, 1934). Vitamin D is synthesised in the dermis (lower level) of the skin. This synthesis requires UV light. Vitamin D is required for the normal absorption of calcium from the gut and its deposition in bones (Loomis, 1967; Neer, 1975), and vitamin D deficiency leads to rickets or osteomalacia (Schultz, 1932). Individuals with rickets show symptoms including bowed legs, which impair locomotion, and a deformed pelvis (Loomis, 1967; Neer, 1975). Pelvic deformity may also impair locomotion and, in women, can prevent natural childbirth, leading to death in the absence of Caesarian section (Jablonski & Chaplin, 2000). Individuals with rickets also have weak bones which are liable to breaking, and weaker muscles (Neer, 1975).

Near to the equator, levels of UV radiation are high and vitamin D can be synthesised in sufficient amounts even by individuals with very dark skin. Away from the equator, however, the increased filtering effects of the atmosphere (Park, 1932; Neer, 1975) mean that UV radiation is reduced by up to 5-fold. Individuals with dark skin can therefore be unable to synthesise sufficient vitamin D (Jablonski & Chaplin, 2000). This lack of UV radiation is compounded by the increased seasonality that is

introduced at high latitudes. At 41ºN (the Mediterranean Sea), due to changes in the angle of the sun and day length, there is a 16-fold decrease in available UV light from summer to winter (Neer, 1975).

Melanin filters out large amounts of UV radiation, limiting the amount that reaches the dermis and is therefore available for the synthesis of vitamin D (Loomis, 1967). Levels of UV radiation reduce dramatically as latitude increases. It is therefore expected that selective pressure would favour individuals with lighter skin away from the equator. Combined with the selective pressure for darker skin near the equator to protect against sunburn and skin cancer, a selection gradient is expected, from very dark skin at the equator to very light skin far from the equator. This is indeed the general pattern that is observed in indigenous people around the world (Jablonski & Chaplin, 2000).

Objections to the vitamin D hypothesis have been voiced, however. Robins (1991) points out that rickets are associated less with latitude and more with behavioural patterns, such as urbanisation, spending large amounts of time indoors and wearing clothes that expose very little of the skin to the sun. These behaviours prevent UV light from reaching the skin. In the Asian community in the UK, the phytate content of chapatti flour has been suggested to inhibit the absorption of calcium in the intestine, leading to the relatively higher levels of rickets that are observed in this population (Goswami *et al.*, 2000; Shaw, 2003). Further, though dark skinned individuals cannot synthesise vitamin D throughout the year at high latitude (Jablonski & Chaplin, 2000), vitamin D can be stored in the fat and muscle tissue

(Rosentreich *et al.*, 1971) and over the whole year, UV levels are sufficient to produce enough vitamin D even at high latitudes (Beadle, 1977).

Another hypothesis for the evolution of light skin at high latitude is sexual selection. This hypothesis suggests that sexual selection for light skin provides the selection pressure for light skin to evolve away from the equator (Aoki, 2002; Darwin, 1871). Van den Berghe & Frost (1986) reviewed evidence from the Human Relations Area File and found that, out of 51 societies that cited skin colour as a component of attractiveness, 47 expressed a preference for lighter skin. Of these, 30 mentioned light skin in relation to women only and 14 in relation to both sexes. American college students have also been found to prefer the lighter categories when asked to express a preference for descriptions of skin colour (Feinmann & Gill, 1978), though using descriptions is a questionable methodology.

A number of hypotheses have been advanced to explain the preference for light skinned females. In most populations, women have lighter skin than men (Hulse, 1967; van den Berghe & Frost, 1986), even in cases where women spend more time in the sun than men (Byard, 1981). Since femininity is strongly preferred in female faces (Perrett *et al.*, 1998), an exaggeration of this aspect of femininity may also be preferred as a feminine trait. Some evidence suggests that women prefer femininity in male faces (Perrett *et al.*, 1998), particularly when the observer is in the luteal (infertile) phase of the menstrual cycle (Penton-Voak & Perrett, 2000; Penton-Voak *et al.*, 1999) and for long-term relationships (Little *et al.*, 2002), so lightness could also be preferred in male faces.

Lightness of skin may be associated with fecundity. The sexual dimorphism present in skin lightness appears at puberty, when other aspects of sexual dimorphism become more pronounced (van den Berghe & Frost, 1986). Skin darkens during pregnancy and during the infertile phase of the menstrual cycle (van den Berghe & Frost, 1986). Areolar skin also darkens during pregnancy and, after approximately three births, fails to return to its previous colour (Pawson & Petrakis, 1975). Men who are sensitive to these shifts in colour, and prefer lighter skinned women may have a reproductive advantage. However, since women become lighter with senescence, and are darkest during the reproductive years (Jablonski, 2006), these cues may need to be interpreted in conjunction with cues to youth, such as smooth, unwrinkled skin in order to provide useful cues to fecundity.

Skin lightness is also associated with higher socioeconomic status in many societies (Hulse, 1967; Jones, 2000; van den Berghe & Frost, 1986) and is considered more attractive in racially stratified societies – societies where wealth, health and power are associated with white skin (Jones, 2000).

People may also take skin lightness or darkness as a sign of health, lightness indicating an ability to produce sufficient vitamin D in high latitude areas, and darker skin indicating increased resistance to the negative effects of UV radiation. In this case, it would be expected that lighter skin would be preferred at high latitude areas with low levels of UV radiation, and darker skin would be preferred near the equator where UV radiation levels are high. However, there is evidence that light skin is preferred even close to the equator where dark skin would be advantageous (van den

Berghe & Frost, 1986). Section 6 will investigate the affect of ethnicity on colour preference.

Following previous studies, I hypothesise that light skin will be preferred by participants, particularly in female faces. A preference for averageness in faces (Langlois & Roggman, 1990) may limit the preference for extremely light faces.

3.1.2 Redness

Skin redness is affected by the amount of blood in the skin, and also by the oxygenation state of the blood (Zonios *et al.*, 2001), with oxygenated blood having a brighter red colour and deoxygenated blood having a darker, bluer red colour (Pierard, 1998). Increased facial redness is associated with higher dominance rank and increased testosterone levels in male non-human old world primates (that have trichromatic colour vision; Rhodes *et al.*, 1997; Setchell & Dixson, 2001), and is preferred by females (Waitt *et al.*, 2003). In female mandrills, facial redness varies across the menstrual cycle, brightening during the follicular (fertile) phase of the menstrual cycle and also with maturity (Setchell *et al.*, 2006). Sexual skins such as the perineum, genitalia and surrounding areas also signal oestrus in several species of primate (Dixson, 1983). Indeed, it has been suggested that primate colour vision evolved to allow the detection of socio-sexual signals based on skin blood perfusion and oxygenation (Changizi *et al.*, 2006). Increased skin blood flow is also associated with physical fitness (see Section 4 for a more detailed discussion of blood colour). It is therefore hypothesised that increased levels of redness at rest will be considered healthier in human faces.

Sexual dimorphism in skin redness has been reported, with men being redder than women (Edwards & Duntley, 1939; Tarr *et al.*, 2001). It may be hypothesised, therefore, that participants will enhance an existing dimorphism by reddening male faces more than female faces. Extremes of facial redness may not be preferred because of the preference for averageness (Langlois & Roggman, 1990), so it may be expected that very red faces will be reduced in redness to optimise healthy appearance.

3.1.3 Yellowness

Increased skin yellowness is associated with increased levels of carotenoids (Alaluf *et al.*, 2002b; Section 1) and melanin (Stamatas *et al.*, 2004) in the skin. Increased carotenoid levels are associated with increased reproductive health and immunocompetence in many species, including humans. Preference for carotenoids may be expressed as a preference for yellowness in the skin. Melanin may have health benefits or costs. Preference for increased melanin will result in a preference for yellower (and darker) faces, if melanin is driving the relationship between yellowness and apparent health (see Section 5 for a more detailed discussion of carotenoids; melanin is discussed above). It is hypothesised that increased skin yellowness will appear healthy.

To test the hypotheses discussed in this section, a computer programme will be used to allow participants to manipulate the colour of the skin portions of colour calibrated photographs along CIELab L* (lightness), a* (redness) and b* (yellowness) axes to optimise the healthy appearance of the faces.

3.1.4 Contrast between skin and lips

In light-skinned faces, the features, especially the eyes and lips, are darker in colour than the surrounding areas. Increasing this contrast increases the attractiveness of female faces, but decreases the attractiveness of male faces (Russell, 2003). The contrast between the features and the surrounding areas is naturally greater in women than in men, and images with increased facial contrast are judged to be more feminine (female faces) or less masculine (male faces). Women's use of makeup (presumably to enhance the healthy, attractive appearance of the face) has also been found to increase this contrast (Russell, pers comm..). These studies used greyscale images in much of the work, and only considered luminance, not colour differences. Additional conditions in the current study will involve the manipulation of the facial skin both while the lips remain unchanged and while the lips are colour manipulated along with the rest of the facial skin. This will allow the investigation of the effects of the contrast between the lips and the facial skin on all three CIELab colour axes.

Increasing the luminance of the facial skin while maintaining the original luminance of the lips will increase the contrast between the facial skin and the lips. If the luminance of the lips is manipulated along with the facial skin, the contrast of the skin with the lips will remain unchanged. Following Russell's hypothesis, it may be predicted that the lips constant condition will increase apparent health more than the lips manipulated condition in female faces. Male faces are expected to show the opposite pattern.

Increasing the redness of the facial skin while the lips remain constant will reduce the relative redness of the lips, or even make them appear green in comparison. Further, many lipsticks used by women exaggerate the redness of the lips (perhaps as a sexual

signal that imitates the labia of the female genitalia; Low, 1979). This may restrict the amount of redness added, particularly to female faces, to optimise healthy appearance. In this case, participants are expected to increase facial redness less in the lips constant condition than the lips changing condition, particularly for female faces.

Increasing yellowness of the facial skin when the lip colour remains constant may give the lips a blue appearance. In this case, more participants are expected to increase the yellowness of the facial skin less to optimise healthy appearance when the lips remain constant as the rest of the skin changes around it than when the lips change along with the rest of the facial skin.

3.2 Methods

3.2.1 Photography

51 Caucasian participants (30 female, 21 male, aged 18–22) from the University of St Andrews were photographed with neutral expressions and were not wearing makeup. The camera was a Fujifilm FinePix S2Pro digital SLR, fitted with a Nikon 60mm fixed length lens. Photography took place within a booth, painted with a standard Munsell N5 grey paint (Verivide, UK). Illumination was provided by three d65 daylight simulation tubes fitted into high frequency fixtures (Verivide, UK) to reduce the effects of flicker on photography. A GretagMacbeth Mini ColorChecker colour chart was included in the frame. Matlab was used to read RGB values for each of the 24 colour squares on the photograph of the colour chart. A least-squares transform from an 11-expression polynomial expansion of these RGB values to manufacturer stated CIELab values of the colour patches of the colour chart was used to colour correct the photographs (Hong *et al.*, 2001). A mean ΔE value of 2.44 was achieved

(ΔE is the Euclidean distance between two points in CIELab colour space). This value is within the acceptable colour calibration limits for accurately displaying complex colour images on a cathode ray tube (CRT) monitor (Stokes *et al.*, 1992). Additionally, displayed faces were within the colour range of the population, meaning that participants were presented with accurate and realistic images to manipulate (Appendix B). The images were delineated in Psychomorph (after Burt & Perrett, 1995). Matlab was used to read the mean CIELab L*, a* and b* values of the skin portions of each colour calibrated face to give initial CIELab values.

3.2.2 Colour Transformations

Face-shaped masks were created representing a neutral skin tone, one with increased a* and one with decreased a*. The masks were delineated in Psychomorph, marking the same points as on each of the images. The masks were then warped to the shape of each facial image, and the images manipulated by the colour difference between the two masks (fig. 3.1; Burt & Perrett, 1995). This gave a series of 13 images from +16 to -16 units of a*. This process was repeated for b* and L*. Only the facial skin was manipulated. Eyes, hair, clothing and background remained constant. Because the colour masks used for the transform were even in colour distribution, no change in colour distribution occurred on the images. Two sets were produced – one with the lips remaining constant and one where they changed along with the facial skin (Fig.3.2-3.4).

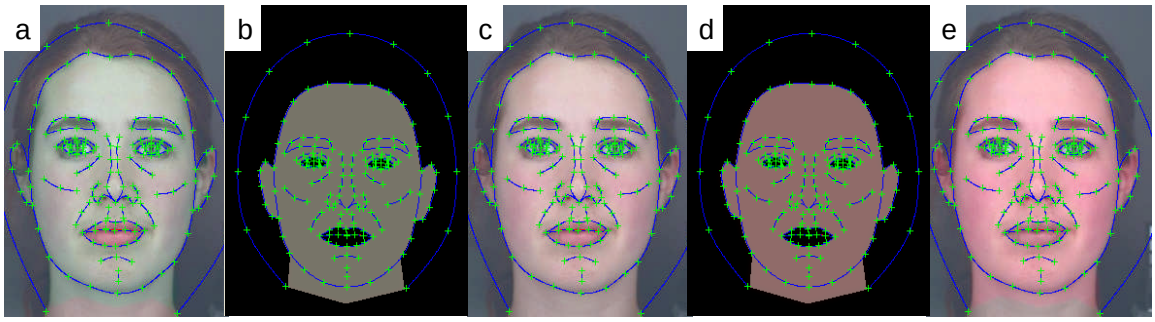


Fig. 3.1: delineated images and masks. The original image (c) is transformed by the difference between the two colour masks (b) and (d), giving facial photographs with redder (e) or greener (a) skin portions.

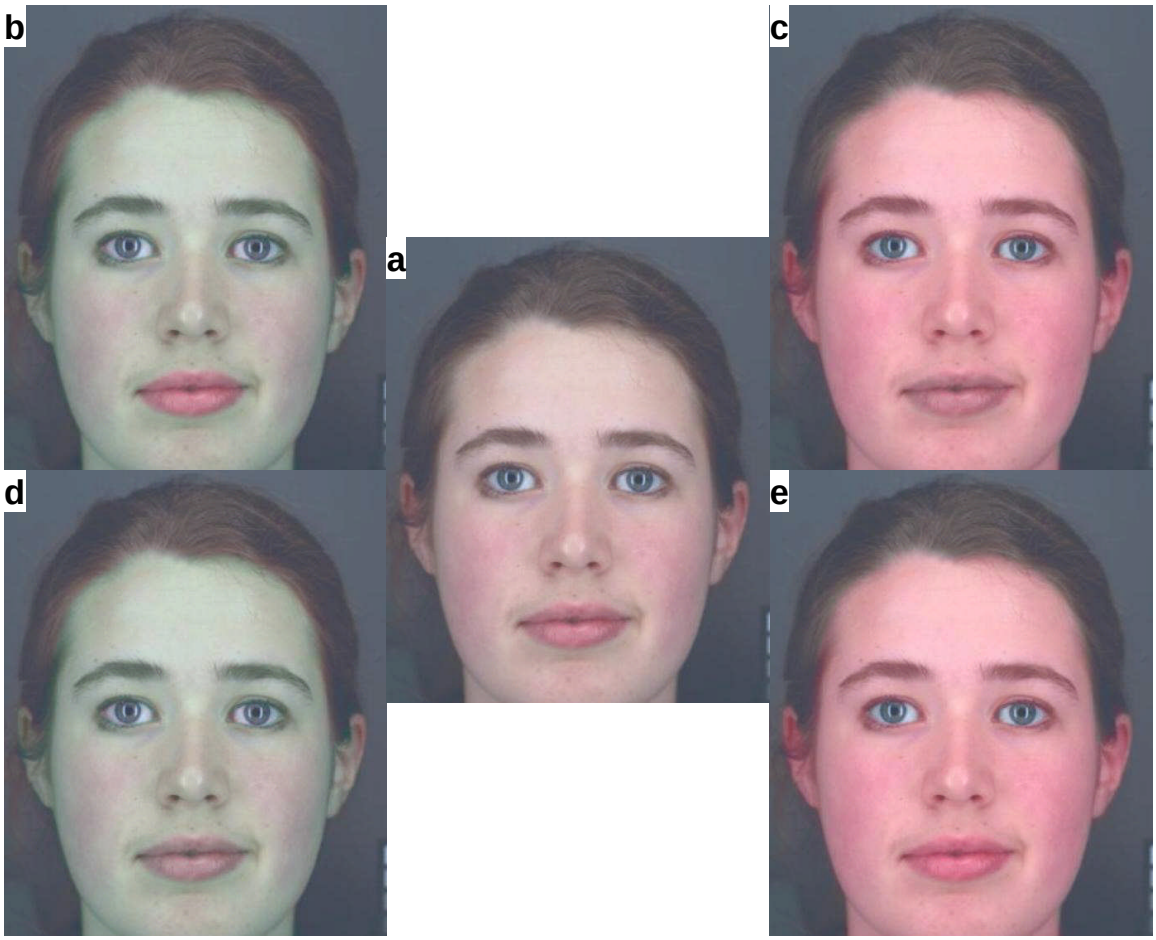


Fig. 3.2: a* (red-green) colour transforms. Original image (a) and images with skin portions transformed by -16 (b) and +16 (c) units of a* with lips remaining constant and with lips manipulated along with the rest of the face (d, e).

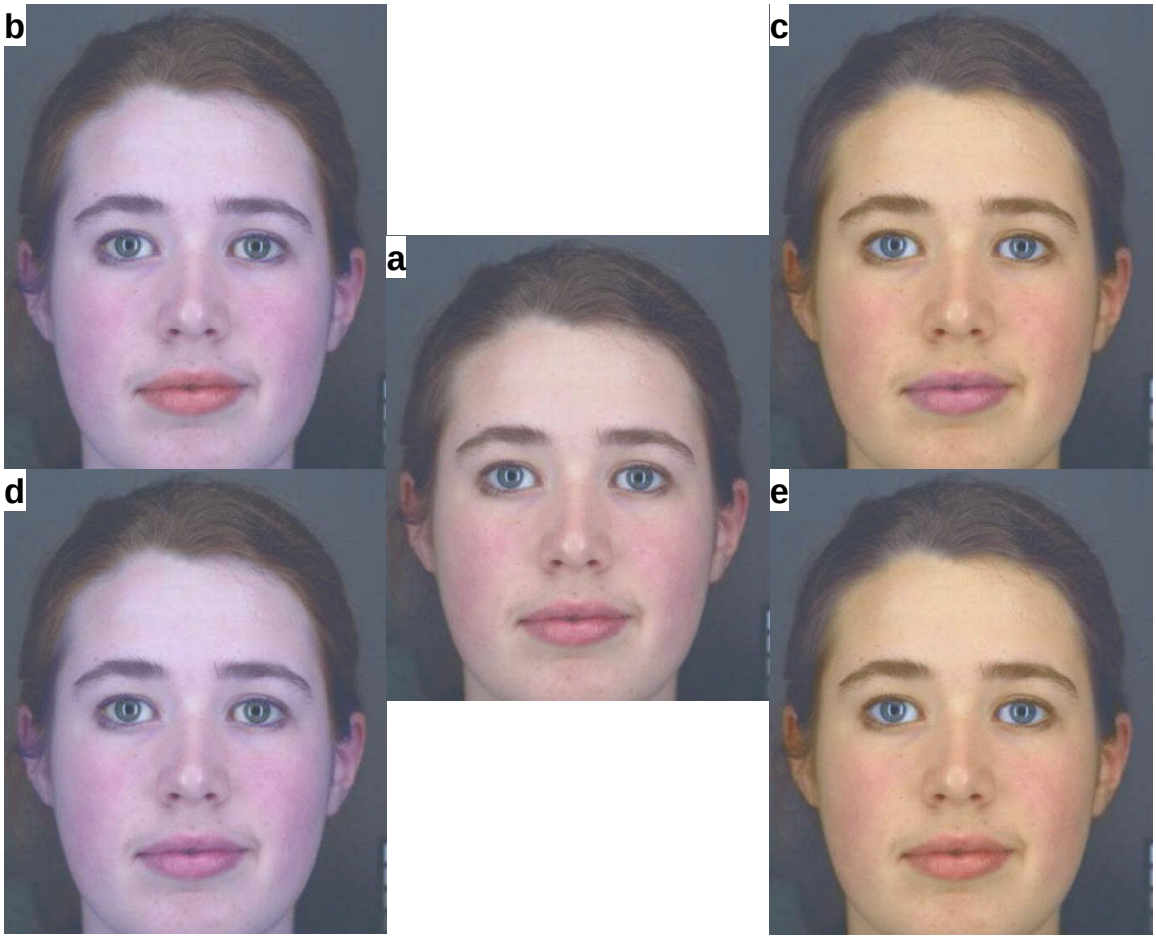


Fig. 3.3: b* (yellow-blue) colour transforms. Original image (a) and images with skin portions transformed by -16 (b) and +16 (c) units of b* with lips remaining constant and with lips manipulated along with the rest of the face (d, e).

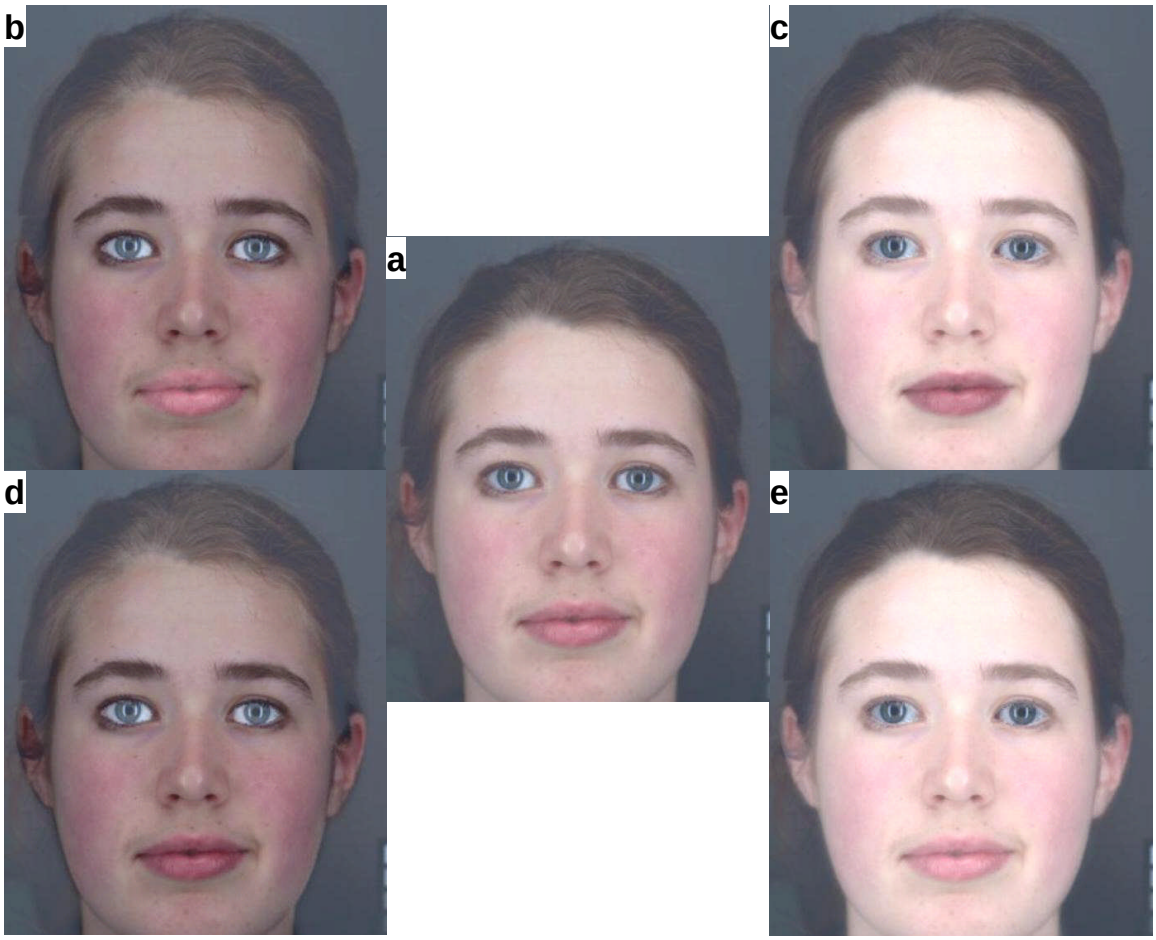


Fig. 3.4: L* (light-dark) colour transforms. Original image (a) and images with skin portions transformed by -16 (b) and +16 (c) units of L* with lips remaining constant and with lips manipulated along with the rest of the face (d, e).

3.2.3 Experimentation

32 Caucasian participants (12 male, 20 female, aged 19–26) from the University of St Andrews were presented with the stimuli on a CRT, colour calibrated using a ColorVision Spyder 2Pro to mean ΔE of 2.323 for skin tones. A computer program allowed them to manipulate the colour of the facial skin to achieve optimum healthy appearance. Moving the mouse horizontally across the screen changed the colour of the face. Participants were presented with the 51 faces, one at a time and asked to "make the face as HEALTHY as possible". Participations manipulated the colour of

the face until healthy appearance was optimised and then clicked the mouse to move on to the next face. Male and female faces were presented in separate sets of trials (blocks). Order of presentation of faces and manipulations (L*, a*or b* dimension; with or without lips) were randomised within these blocks. Each participant was randomly assigned the lips manipulated or lips constant version of each face (no participant saw both versions of a face). Each participant was presented with 306 trials.

3.2.4 Statistical Methods

Mean colour changes applied to each face were calculated (by face dataset). Mean colour changes applied by each participant were also calculated (by participant dataset). In the by face dataset, one-sample t-tests (H_0: no colour change) were used to test whether the faces were changed in colour to optimise healthy appearance. Univariate ANOVAs were used to test for the effect of face sex on colour change, as this test allows initial face colour to be controlled as a covariate (by face dataset; dependent variable=colour change applied; fixed factor=face sex; covariate=initial face colour: L*, a* or b*). Pearson's r was used to test for correlation between initial face colour and colour change applied. Independent samples t-tests were used in the by participant dataset to test for differences in amount of colour change applied by male and female participants. To test for an interaction between sex of face and sex of participant, repeated-measures ANOVAs were used (dependent variable=colour change applied; within-subjects factor=participant sex; between-subjects factor=face sex). When one or more variable was not normally distributed, Mann-Whitney U tests were used in place of t-tests. Paired-samples t-tests were used to test for differences between lips changing and lips constant conditions.

3.3 Results

3.3.1 Relative Lip Colour

No difference in amount of colour transform applied to optimise healthy appearance was found between the two lip conditions (lips changing with the rest of the facial skin and lips remaining constant as the rest of the skin changes) for the luminance (L*; paired samples t test t_{50}=0.187, p=0.852) or redness (a*; t_{50}=0.855, p=0.397) transforms. However, participants increased the yellowness (b*) of faces more when the lips changed with the rest of the facial skin than when the lips remained constant (t_{50}=2.793; p=0.007). Repeated measures ANOVAs showed no interaction between sex of face and lip condition affecting the amount of L* ($F_{1,49}$=0.025, p=0.876) , a* ($F_{1,49}$=0.438, p=0.511) or b* ($F_{1,49}$=0.525, 0.472) change applied to optimise healthy appearance, suggesting that the effect of lip condition on amount of colour change applied was not affected by the sex of the faces.

The results of the analyses described above were not qualitatively different for the lips constant and lips changing conditions (i.e. direction and significance of analyses were not qualitatively different when repeated for each of the two conditions). Results were therefore pooled for the two conditions (lips manipulated or not manipulated) in the analyses below.

3.3.2 Lightness

To optimize healthy appearance, faces were lightened in the L* transform (ΔL*=1.21$\pm$0.23, t_{50}=5.200, p<0.001), and the amount of lightening applied was negatively related to the initial lightness (L*) of the face (Fig. 3.5A, R^2=0.50; Table 3.1).

Colour axis	Correlation with initial facial colour (n=51)		
	L*	a*	b*
L*	r=-0.708; p<0.001	r=0.231; 0.103	r=-0.509; p<0.001
a*	r=0.242; p=0.087	r=-0.906; p<0.001	r=0.125; p=0.381
b*	r=-0.116; p=0.419	r=0.010; p=0.945	r=-0.772; p<0.001

Table 3.1. Correlations (Pearson's r) of colour transform with initial facial colour.

Female faces were initially lighter than male faces (van den Berghe & Frost, 1986; female L*=67.79±0.54; male L*=65.86±0.60; t_{49}=2.342; p=0.023). Controlling for initial facial lightness, female faces were lightened more than male faces ($F_{1,48}$=27.398; p<0.001; Fig. 3.5B). Thus participants lightened the faces of both sexes, but increased an existing sexual dimorphism (Perrett *et al.*, 1998) to optimise healthy appearance by lightening female faces more than male faces.

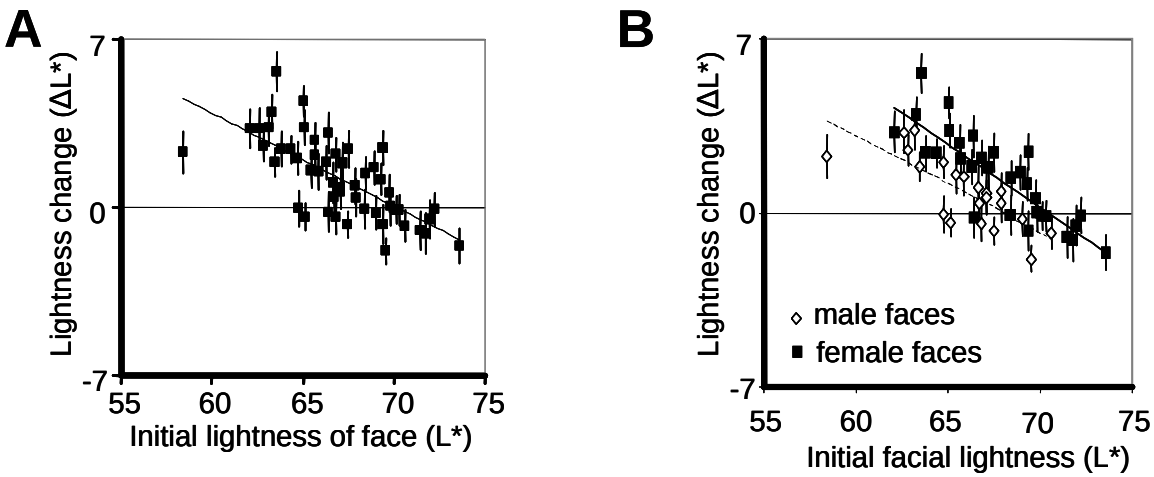


Fig. 3.5: (A) Amount of lightness change applied to optimise healthy appearance. (B) Female faces (solid symbols) are lightened more than male faces (hollow symbols) for a given value of initial face lightness (L*). Each point represents one face. Error bars show standard error of the mean across all participants.

A non-significant trend suggested that male participants may prefer lighter skin than female participants (independent samples t test; t_{30}=2.032; p=0.051), suggesting a greater preference for feminine light skin by men than by women. However, this preference was not limited to female faces, as no interaction between the sex of the face and the sex of the participants was found to affect the amount of lightness change applied to optimise healthy appearance ($F_{1,49}$=0.005; p=0.941).

3.3.3 Redness

Participants increased the redness of faces to optimise healthy appearance (Fig. 3.6A; Δa^*=1.62±0.19; t_{50}=8.714, p<0.001). The initial redness of the face was negatively related to the amount of redness applied to optimise healthy appearance, with initially less red faces increased in redness more than initially redder faces (r=-0.906; p<0.001; Fig 3.6A; Table 3.1).

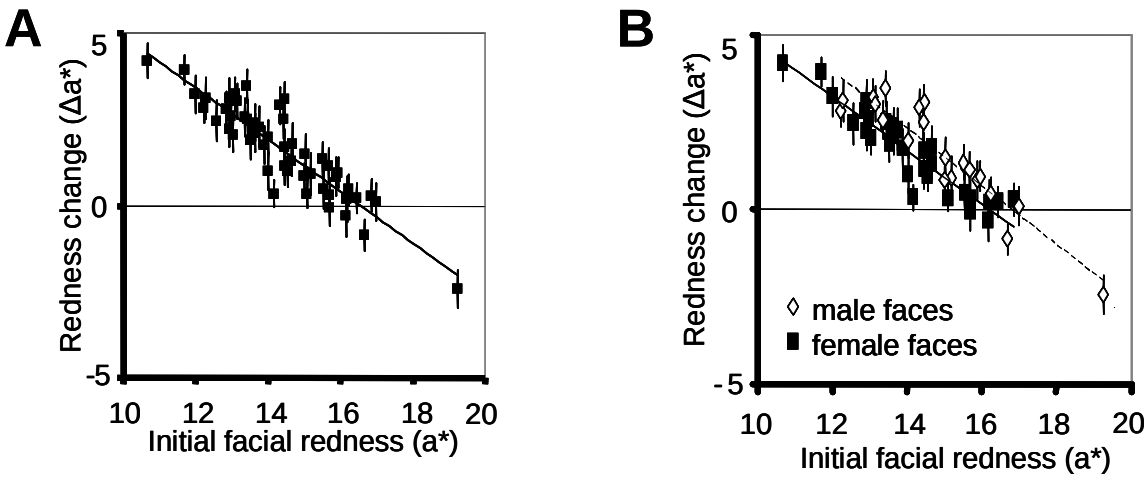


Fig. 3.6: (A) Amount of redness change applied to optimise healthy appearance. (B) Male faces (hollow symbols) are increased in redness more than female faces (solid symbols) for given values of initial face redness (a*).

Male faces showed a trend to be initially redder than female faces (male a*=14.86 ±0.37; female: a*=14.07±0.27; t_{49}=1.765, p=0.084) and were significantly redder than female faces after participants adjusted a* (male: a*=16.46±0.63; female: a*=15.72±0.56; t_{49}=4.422; p<0.001). Controlling for initial colour of the face, male faces were increased in redness more than female faces (Fig. 3.6B; $F_{1,48}$=15.708; p<0.001). Thus redness appears healthy for both male and female faces but participants enhance the natural sexual dimorphism in face colour when judging optimal appearance; participants associate a higher level of redness with optimal health in men than in women.

The sex of the participants was not found to affect the amount of redness change applied to optimise healthy appearance (Mann-Whitney U test; U=93; p=0.307; n=32). No interaction between the sex of the face and the sex of the participants was found to affect the amount of redness change applied to optimise healthy appearance ($F_{1,49}$=0.067; p=0.798).

3.3.4 Yellowness

Participants increased the yellowness of faces to optimise healthy appearance (Fig. 3.7 Δb*=5.25±0.18; t_{50}=29.639, p<0.001). The increase in yellowness was negatively related to the starting yellowness (b*) of the face (Fig. 3.7, R^2=0.60; Table 3.1). Faces initially low in yellow were altered more than faces initially high in yellow.

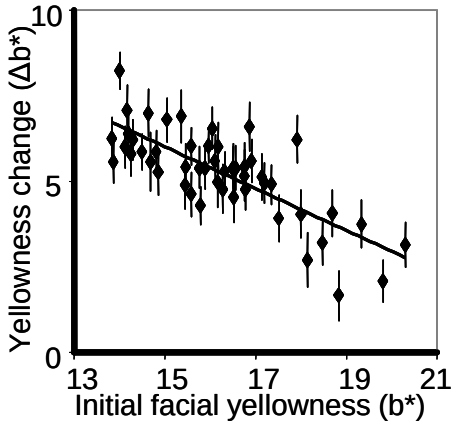


Fig. 3.7: Amount of yellowness change applied to optimise healthy appearance. Yellowness change applied correlates negatively with initial face yellowness (b*).

Male and female faces did not differ in initial yellowness (t_{49}=1.079; p=0.286) and sex of face did not affect yellowness change applied to optimise healthy appearance ($F_{1,48}$=0.382; p=0.540).

The sex of the participants was not found to affect the amount of yellowness change applied to optimise healthy appearance (t_{30}=0.690; p=0.495). No interaction between the sex of the face and the sex of the participants was found to affect the amount of yellowness change applied to optimise healthy appearance ($F_{1,49}$=0.676; p=0.415) dimensions.

3.4 Section 3 Discussion

Participants increased red (a*), yellow (b*) and lightness (L*) to optimise the healthy appearance of faces.

3.4.1 Contrast between Skin and Lips

Participants added more yellow to the face when the lips changed than when they remained unchanged. This may be because increasing the b* component of surrounding areas will make the lips look bluer. Blue lips are a sign of cyanosis (a lack of oxygenated blood), which is associated with respiratory and cardiac illnesses (Ponsonby *et al.*, 1997). There was no interaction of sex of face and lip condition affecting the amount of colour change applied to optimise healthy appearance. Since the contrast between the lips and the skin is affected differently depending on whether the lips also change in colour, no support was found for Russell's (2003) hypothesis that the relative luminance or relative colour of facial skin and facial features (i.e. the contrast between facial skin and the lips and eyes) affects the healthy appearance or attractiveness of a face. Russell's (2003) results may differ from mine because of his use of black and white photographs, and only three levels of contrast.

3.4.2 Lightness

Skin lightness is associated with high social status in some populations (Hulse, 1967; Jones, 2000), and is useful in preventing vitamin D deficiency in populations far from the equator (Jablonski & Chaplin, 2000). It is also associated with femininity and fecundity in women (van den Berghe & Frost, 1986). In agreement with other studies (Hulse, 1967; also van den Berghe & Frost, 1986 list 32 such studies), there was also sexual dimorphism in initial and final skin lightness found in the current study, with female faces lighter than male faces. Controlling for initial lightness of face, more lightness was added to female faces. The existing sexual dimorphism in the lightness of faces was enhanced to optimise healthy appearance. The preference for lighter skin in female than male faces (the optimal lightness of female faces is higher than the

optimal lightness of male faces) may indicate an association between lightness, femininity and sexual dimorphism.

3.4.3 Redness

The a* dimension is associated with haemoglobin levels, and the present results are consistent with the hypothesis that oxygenated haemoglobin and increased skin blood flow look healthy because they are associated with athletic health (Armstrong & Welsman, 2001) and physical training (Johnson, 1998), and with increased levels of reproductive hormones (Rhodes *et al.*, 1997). The increase in redness to optimise healthy appearance is also consistent with the observation that reduction in skin blood flow is caused by illness and health factors such as diabetes (Charkoudian, 2003), smoking (Richardson, 1987) and hypertension (Panza *et al.*, 1990). A more detailed examination of the role of blood colour in the perception of health in human faces is warranted and can be found in Section 4.

A trend was found in the current study to suggest that male faces are initially redder than female faces. Controlling for initial redness of face, participants reddened male faces more than female faces to optimise healthy appearance. The optimal redness of male faces was higher than for female faces. Participants therefore enhanced the existing sexual dimorphism of redness in faces to optimise healthy appearance. These enhancements of sexual dimorphism indicate a role of skin colour in sexual selection (Andersson, 1994).

3.4.4 Yellowness

Increased skin yellowness (b*) increases apparent health, but decreased skin lightness (low L*) does not. It is likely that increased yellowness appears healthy because of its association with increased carotenoid (yellow; increased b*) levels rather than with increased melanin levels (dark and yellow; increased b*, decreased L*). This conclusion is consistent with the evidence that carotenoids are associated with improved reproductive health (Brief & Chew, 1985) and immunocompetence, including increased number and activity of T lymphocytes (Alexander *et al.*, 1985; Chew, 1993). It is also consistent with the fact that illness, including malaria and HIV infection is associated with low carotenoid levels (Friis *et al.*, 2001). Further consideration of the role of carotenoid and melanin in the perception if health in human faces is warranted and can be found in Section 5.

3.4.5 Gradients

The negative correlation between starting colour (starting a* or b*) with colour added (Δa* or Δb*) supports a hypothesis that low colour looks unhealthy. Participants add colour to remedy the situation. The regression lines (that relate colour change applied to faces in order to optimise healthy appearance to initial face colour) cross the x-axis (a*), or would do so if extrapolated (b*), indicating that individual faces that are very red or very yellow will have colour removed to optimise healthy appearance. Similarly, faces that are very light are darkened to optimise healthy appearance, suggesting that there is an optimum colour range for healthy facial skin appearance.

Section 4:

Blood Colour Transforms

Blood colouration provides perceptible cues to health in human faces

A version of this section has been published as:

Stephen, I.D., Coetzee, V., Law Smith, M.J., Perrett, D.I. (2009) Skin blood perfusion and oxygenation colour affect perceived human health. *PLoS ONE, 4,* e5083.

4.1 Introduction

This Section describes a suite of three experiments examining the effect of blood colouration on the apparent health of human faces. The first experiment examines the effect of oxygenated blood colour, the second deoxygenated blood colour. The third experiment uses two-dimensional colour transforms to further examine the relationship of these pigment colours with apparent health. These experiments are described together to allow a more thorough and cohesive treatment and discussion of the results.

There have been suggestions that reddening is considered attractive, both across the face (Fink *et al.* 2001), and specifically in the cheeks (Zahavi & Zahavi, 1997). In Section 3, I showed that increased facial skin redness increased the apparent health of faces.

The red pigment, haemoglobin in blood is one of the main contributors to human skin colour (Zonios *et al.*, 2001). Skin redness is caused by skin blood perfusion, controlled by vascularisation and vasodilation. In addition, blood oxygenation state

affects the colour of the skin, oxygenated blood being a bright red colour and deoxygenated blood being a darker, bluer shade of red (Pierard, 1998). This experiment investigates the pigment basis of the preference for redder facial skin by manipulating the oxygenated and deoxygenated blood colour of the facial skin. To allow the further investigation of the relationship between oxygenated and deoxygenated blood colour, additional experiments were performed to allow the manipulation of both oxygenated and deoxygenated blood colour simultaneously.

4.1.1 Blood Perfusion

In women, high oestrogen levels are associated with increased vascularisation of the skin (Thornton, 2002) and increased oestrogen and progesterone are associated with an increased vasodilatory response to heating (Charkoudian *et al.*, 1999), which causes arterialisation of the blood in the skin (Liu *et al.* 1992). Increased progesterone and oestrogen in the luteal phase of the menstrual cycle (Baird *et al.*, 1997) and increased oestrogen in the follicular phase (Lipson & Ellison, 1996; Stewart *et al.*, 1993) are associated with increased chance of embryo implantation. Increased oxygenated blood colouration may therefore be a good indicator of reproductive health in women.

Physical training increases the responsiveness of the vasodilator mechanism as well as reducing the activity of the vasoconstrictor mechanism, providing improved heat loss during exercise (Johnson, 1998). Skin vasodilation is impaired in patients with type 2 diabetes mellitus (Charkoudian 2003), with senescence (Tankersley *et al.*, 1991) and in smokers (Richardson, 1987). Hypertension (high blood pressure) is associated with decreased reactivity of the vasodilatory system (Panza *et al.*, 1990) and causes

capillary rarefaction (the loss of capillaries in the skin; (Serne *et al.*, 2001). A slightly reddish skin colour is expected to increase healthy appearance, as a cue to normal blood circulation (Fink *et al.*, 2001).

4.1.2 Blood Oxygenation

Increased blood oxygenation is associated with aerobic fitness in 11-17 year old boys and peak oxygen uptake (VO_2max), positively associated with haemoglobin levels, is the most common measure of athletic fitness (Armstrong & Welsman 2001). Increased oxygenation of the blood in the skin causes reddening (Changizi *et al.*, 2006). Increased levels of deoxygenated blood are associated with hypoxia and can lead to cyanosis (a blue tint to the skin), which is associated with several respiratory and coronary illnesses (Pierard, 1998).

4.1.3 Primate Colour Signals

Red-green colour vision in primates may have evolved to allow the interpretation of socio-sexual colour signalling, since the spectral sensitivities of components of the primate retina are matched to the spectra needed to identify skin blood concentration and oxygenation (Changizi *et al.*, 2006). Reddening of the facial skin occurs with increased dominance rank in male mandrills (Setchell & Dixson 2001). In the mating season, male rhesus macaques experience facial reddening associated with increased testosterone (Rhodes *et al* 1997). This colour is preferred by female macaques (Waitt *et al.*, 2003). Male macaques have also been found to prefer red colouration in female anogenital regions (Waitt *et al.*, 2006).

The handicap hypothesis suggests that testosterone is detrimental to the immune system (Folstad & Karter, 1992), so facial reddening due to increased blood perfusion may be an honest signal of quality and health. In female mandrills, facial reddening increases during the follicular (fertile) phase of the menstrual cycle, and with sexual maturity, suggesting that redness may also indicate female reproductive quality and fertility (Setchell *et al.*, 2006). In non-human primates, it appears that increased levels of oxygenated blood colouration signal reproductive status in both sexes.

Wearing red increases success in sporting contests (Hill & Barton, 2005a, 2005b), possibly through an association with anger and dominance (Drummond & Quah, 2001) or factors such as the relative visibility of competitors (Rowe *et al.*, 2005).

To summarise the predictions, it is expected that increased skin blood colour, particularly oxygenated blood colour, will increase the healthy appearance of faces.

4.2 Methods

4.2.1 Photography

The same facial photograph set was used as in Section 3.

4.2.2 Empirical Measurement of Deoxygenated Skin Blood Colour

10 further participants (5 male, 5 female; aged 24–35) were recruited. A spectrophotometer (Konika Minolta CM2600d) reading (CIELab colour space, d65 illuminant, 10º illumination angle, SCI) was taken of the first dorsal interosseous region of the left hand (chosen for relative lack of visible tendons and veins; fig. 4.1),

after they had stood with hands by their sides for 30 seconds (hyperaemia – high blood content), and again after holding their hands above their heads for 10 seconds (hypoaemia – low blood content). The elevated blood content of the lowered arm is due largely to deoxygenated blood (Feather *et al.*, 1988).

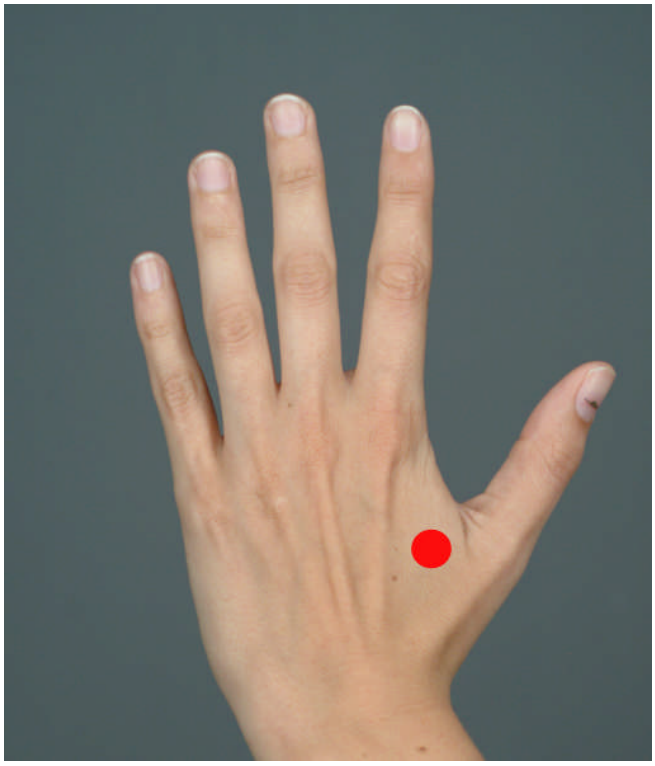


Fig. 4.1: Red circle shows location of the first dorsal interosseous region.

4.2.3 Empirical Measurement of Oxygenated Skin Blood Colour

10 further participants (2 male, 8 female; aged 19–25) were recruited.

Spectrophotometer measurements were made of the first dorsal interosseous region of the left hand, after 2 minutes sitting with hands resting on a desk (hypoaemia), and again after five minutes of sitting with their left hand in hot water (45–50ºC), causing a high degree of hyperaemia with arterial blood (Liu *et al.*, 1992). The measured skin region was gently and quickly dried before measurement.

4.2.4 Image Manipulation

Matlab was used to make two pairs of face-shaped masks, as described in Section 3. One pair was the colour of the mean high and low oxygenated blood measurements. The other pair was the colour of the mean high and low deoxygenated blood colour measurements. For each of the 51 faces, thirteen images were generated in equal steps from high to low colour, for each of the pigment dimensions (Fig. 4.2). For 2D transforms, each of the thirteen images was transformed along the second colour dimension, giving 169 images per face (Fig. 4.3). Hair, eyes, clothing and background remained constant. Table 4.1 gives the values of the transformations in CIELab colour space. Since the effects of lip condition in Section 3 were small and did not qualitatively affect the results, in this set of experiments, lips were manipulated along with the rest of the facial skin in all cases.

	L*	a*	b*	Colour change (ΔE)
Oxygenated blood colour	+2.25	+5.36	-0.17	5.81
Deoxygenated blood colour	-4.98	+4.29	-1.57	6.75

Table 4.1. Colour transform applied to produce the high colour endpoint image.

The sign is changed for the low colour endpoint image.

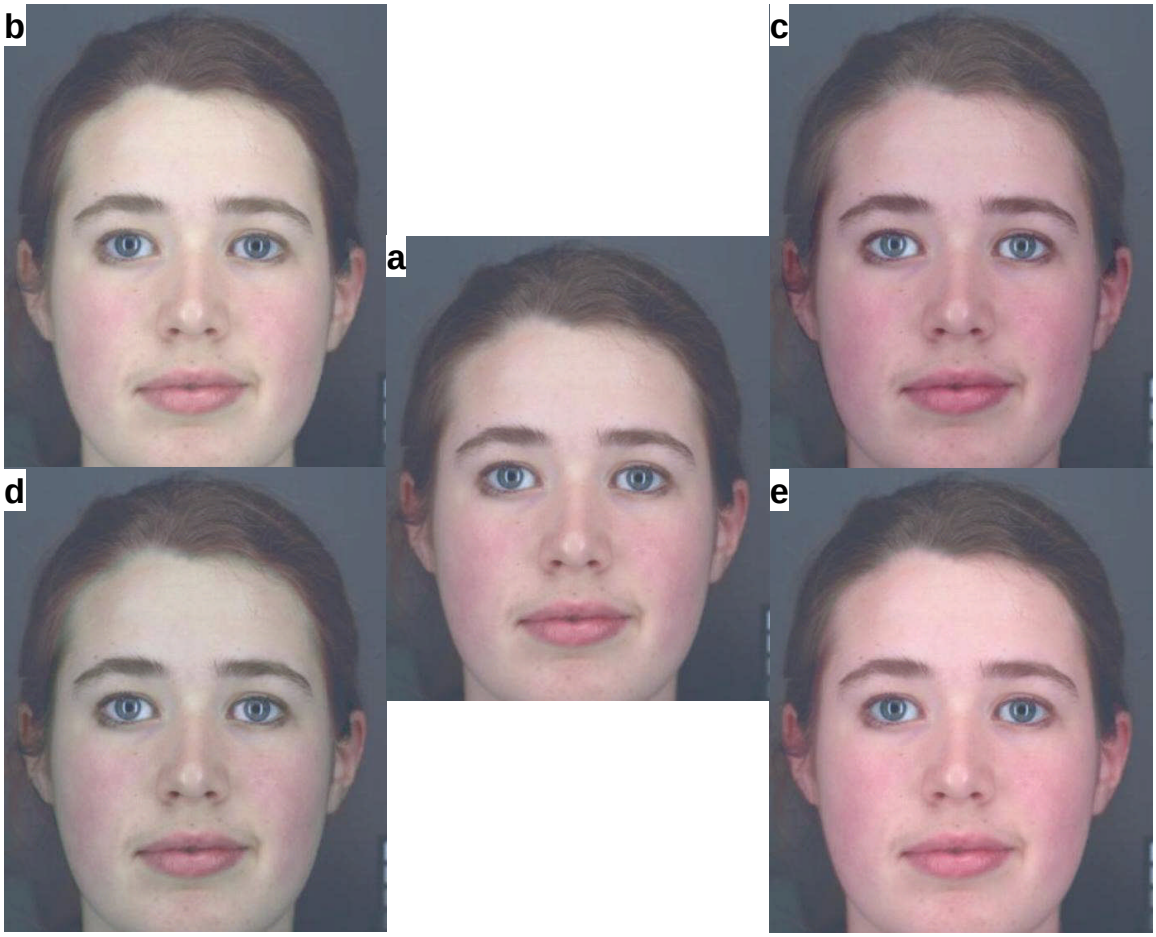


Fig. 4.2: Blood colour transforms. Original image (a) and endpoint images with skin portions transformed along blood colour axes. (b) lowest deoxygenated blood colour, (c) highest deoxygenated blood colour, (d) lowest oxygenated blood colour, (e) highest oxygenated blood colour. Table 4.1 shows the magnitude of the colour transformations in CIELab colour space.

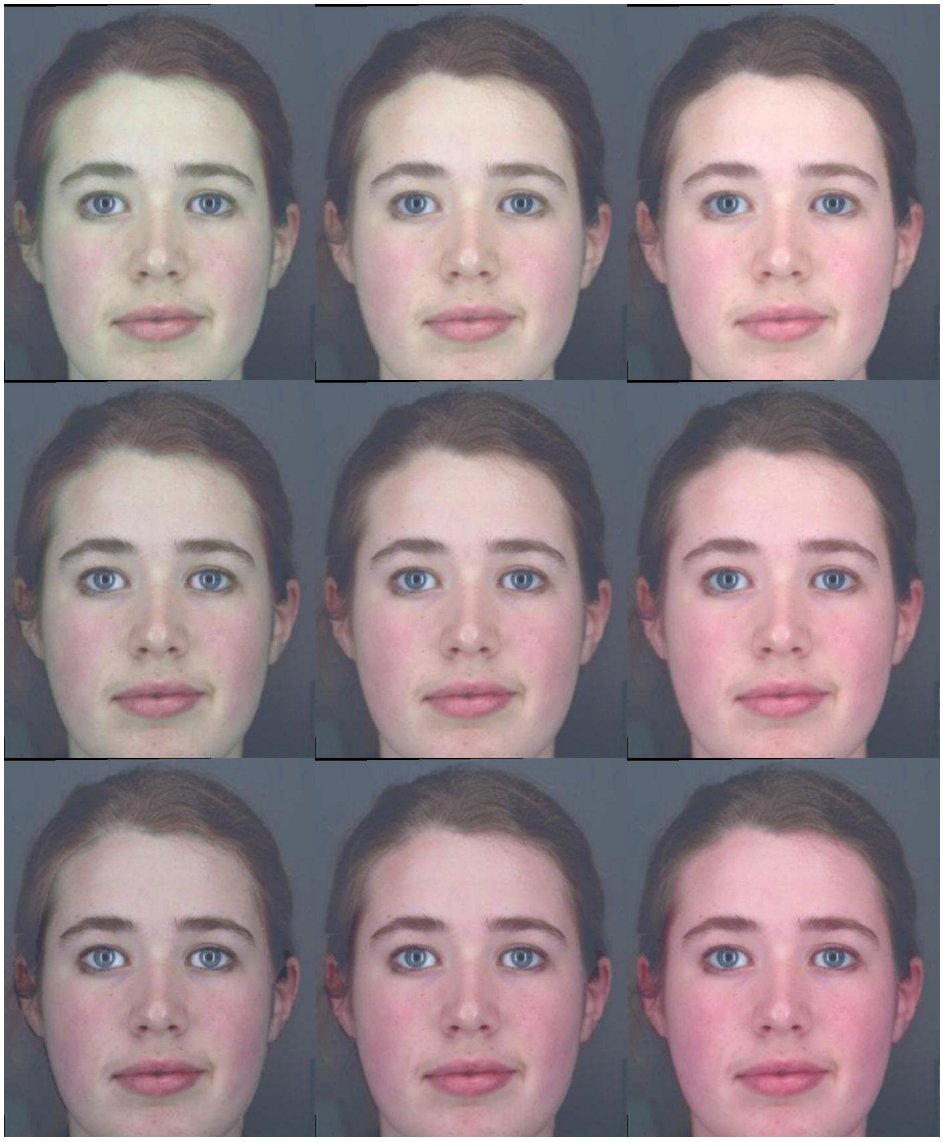


Fig 4.3. End-points of the two-dimensional blood colour transform. Original image (centre) and low oxygenated blood colour (left) to high oxygenated blood colour (right) and low deoxygenated blood colour (top) to high oxygenated blood colour (bottom).

4.2.5 Experimentation

For manipulations along single pigment colour axes, 30 participants were recruited (14 male, 16 female, aged 18–24). All participants were Caucasian and recruited from the University of St Andrews. Participants were presented with the stimuli, one face at a time, in random order on a CRT monitor (colour calibrated using a ColorVision Spyder 2Pro to mean ΔE of 2.32 for a range of skin tones reflecting faces of various

ethnicity). A computer program allowed participants to manipulate the colour of the facial skin along a single CIELab or pigment colour axis to achieve optimum healthy appearance.

For the two-dimensional transforms, 29 further Caucasian participants (15 male; 14 female; aged 18–25) were recruited. Two-dimensional trials allowed participants to manipulate colour along the oxygenated and deoxygenated blood colour axes simultaneously. Moving the mouse horizontally across the screen changed the colour of the face in one colour dimension, and moving the mouse vertically changed the colour in the other colour dimension. Both dimensions could be changed simultaneously.

4.2.6 Statistical Methods

Mean colour changes applied to each face were calculated (by face dataset). Mean colour changes applied by each participant were also calculated (by participant dataset). In the by face dataset, one-sample t-tests (H_0: no colour change) were used to test whether the faces were changed in colour to optimise healthy appearance. Univariate ANOVAs were used to test for the effect of face sex on colour change, as this test allows initial face colour to be controlled as a covariate (by face dataset; dependent variable=colour change applied; fixed factor=face sex; covariate=initial face colour: L*, a* or b*). Pearson's r was used to test for correlation between initial face colour and colour change applied. Independent samples t-tests were used in the by participant dataset to test for differences in amount of colour change applied by male and female participants. To test for an interaction between sex of face and sex of participant, repeated-measures ANOVAs were used (dependent variable=colour

change applied; within-subjects factor=participant sex; between-subjects factor=face sex). In the two-dimensional trials, one-sample t-tests (H_0: no colour change) were used to test whether the faces were changed in each colour dimension to optimise healthy appearance. When one or more variable was not normally distributed, Mann-Whitney U tests were used in place of t-tests.

4.3 Results

4.3.1 Single-Axis Transforms

To optimise healthy appearance, participants increased oxygenated (Fig. 4.4; $\Delta E=1.52\pm0.13$; $t_{50}=12.101$, $p<0.001$) and deoxygenated blood colour (Fig. 4.4; $\Delta E=0.51\pm0.19$; $t_{50}=2.702$, $p=0.009$), respectively (table 4.2). Participant sex was not found to affect the amount of oxygenated (independent samples t-test $t_{28}=1.246$, $p=0.223$) or deoxygenated (Mann-Whitney $U=91$, $p=0.400$, $n=30$) blood colour change applied to optimise healthy appearance. Face sex, participant sex and the interaction between the two was not found to affect the amount of colour change applied to optimise healthy appearance (table 4.3). However, a trend of marginal significance suggests that, for each given value of initial redness (a*), participants increased the amount of deoxygenated blood colour more for male faces (estimated marginal mean$\pm$SE=0.757$\pm$0.164) than for female faces (estimated marginal mean$\pm$SE=0.337$\pm$0.136) to optimise healthy appearance (table 4.3). Since male faces show a trend to be initially redder than female faces (male a*=14.86 $\pm$0.37; female: a*=14.07$\pm$0.27; $t_{49}=1.765$, $p=0.084$), this may represent an exaggeration of sexual dimorphism, in line with the results in Section 3.

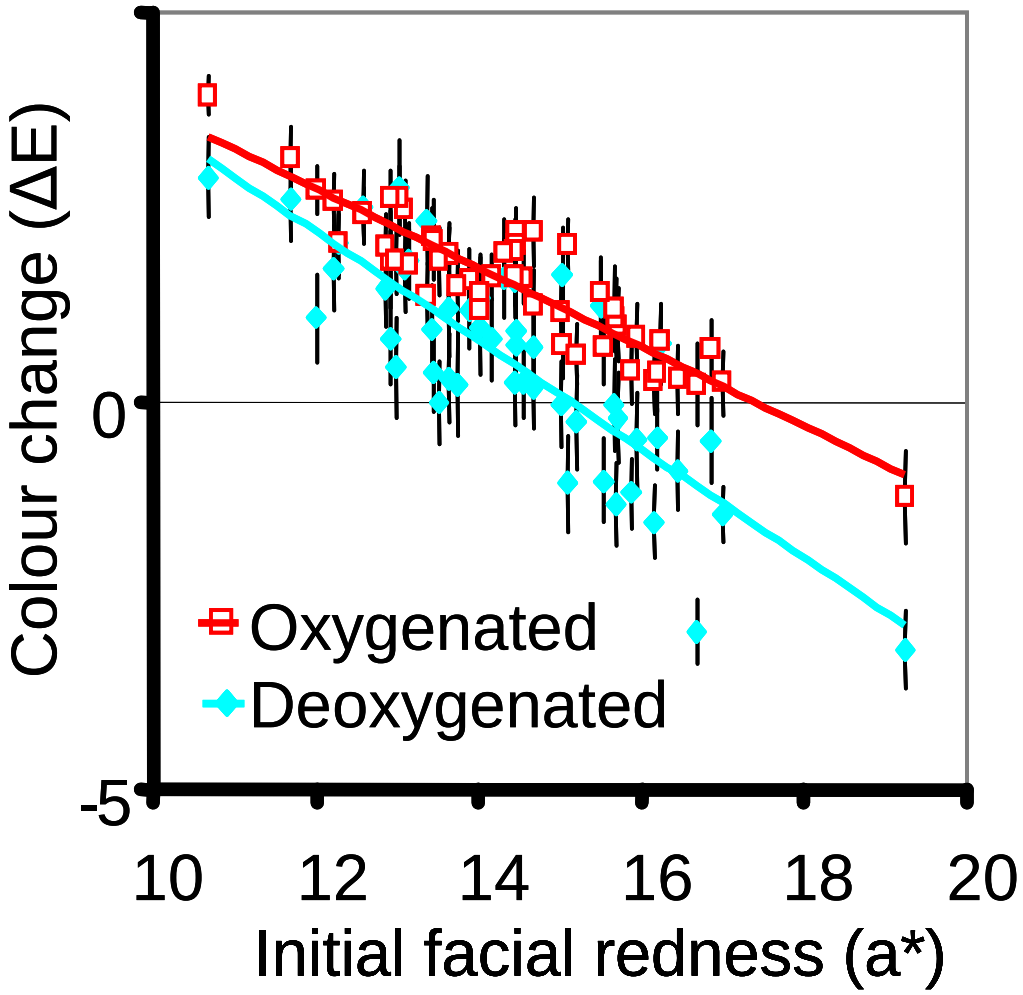


Fig. 4.4: Effect of blood colour on apparent health of faces. Participants add oxygenated (red symbols) and deoxygenated (blue symbols) blood to faces to optimise healthy appearance. Initial face redness (a*) correlates with oxygenated (R^2=0.83) and deoxygenated (R^2=0.69) blood colour added to optimise healthy appearance.

Transform	ΔL* component	Δa* component	Δb* component	Total colour change (ΔE)
Oxygenated blood	0.57±0.05	1.36±0.12	-0.04±0.00	1.52±0.13
Deoxygenated blood	-0.39±0.14	0.34±0.12	-0.12±0.04	0.51±0.19

Table 4.2. Colour changes along component CIELab axes and total colour change (ΔE). Mean change across participants and faces (±SE) in the single-axis pigment transforms to maximize health.

Colour axis	Effect of participant sex	Interaction between face sex and participant sex	Effect of sex of face (initial redness controlled)
Oxygenated blood	t_{28}=1.246; p=0.223	$F_{1,49}$=0.026; p=0.872	$F_{1,48}$=1.868; p=0.178
Deoxygenated blood	U=91; p=0.400; n=30	$F_{1,49}$=0.077; p=0.783	$F_{1,48}$=3.791; p=0.057

Table 4.3. Effects of sex on colour transformations. Column 2: Differences in colour change made by male and female participants (by participant dataset), examined with independent samples t-test or Mann-Whitney U test for deoxygenated blood colour, as data is non-normal. Column 3: Interaction between sex of face and sex of participant on colour transformation, examined with repeated-measure ANOVAs (by face dataset; dependent variable=colour change, within-subjects factor=participant sex, between-subjects factor=face sex). Column 4: Univariate ANOVAs were used to test for the effect of face sex on colour change, as this test allows initial face colour to be controlled as a covariate (by face dataset; dependent variable=colour change; fixed factor=face sex; covariate=initial face redness (a*).

Blood colour is enhanced most in faces initially low in redness (i.e. appearing low in skin blood perfusion; table 4.4; fig. 4.4). Enhancing blood colour increases apparent health only up to a point, above which increases in blood colour detract from apparent health, and several faces which start out high in redness are reduced in blood colour to improve appearance, particularly deoxygenated blood colour.

Colour axis	Correlation with initial facial colour (n=51)		
	L*	a*	b*
Oxygenated blood	r=0.288; p=0.041	r=-0.911; p<0.001	r=0.090; p=0.531
Deoxygenated blood	r=0.395; p=0.004	r=-0.831; p<0.001	r=0.323; p=0.021

Table 4.4. Correlations (Pearson's r) of colour transform with initial facial colour.

Oxygenated blood colour was more beneficial to apparent health than deoxygenated blood colour. All but one of the faces (98%) appeared healthier when oxygenated blood colour was elevated, whereas 66% appeared healthier with elevated

deoxygenated blood colour (Fig. 4.4). Overall, oxygenated blood colour was enhanced more than deoxygenated (matched pairs t-test t_{50}=8.753; p<0.001).

4.3.2 Two-dimensional Colour Transform

In the two-dimensional transform (where participants could manipulate oxygenated and deoxygenated blood colour axes simultaneously, Fig. 4.3), participants decreased deoxygenated blood colour (ΔE=-3.10±0.11, t_{50}=29.295, p<0.001) and increased oxygenated blood colour (ΔE=3.03±0.07; t_{50}=41.473, p<0.001; Fig. 4.5). This represents an increase in the ratio of oxygenated to deoxygenated blood colour in the face. The combined colour change in the two-dimensional transform included a large increase in the overall redness of the faces, an increase in the lightness of the face, and a small increase in the yellowness of the face (table 4.5). This is consistent with an increase in the blood content of the facial skin, as well as with a change from deoxygenated to oxygenated blood.

Colour axis	Colour change in 2D blood trials	
	Significance	Mean±SE
ΔL*	t_{50}=44.659; p<0.001	1.53±0.03
Δa*	t_{50}=54.343; p<0.001	2.21±0.04
Δb*	t_{50}=7.356; p<0.001	0.06±0.01
Overall ΔE	t_{50}=64.033; p<0.001	2.70±0.04

Table 4.5. Overall CIELab and ΔE colour change applied to the faces in two-dimensional pigment trials.

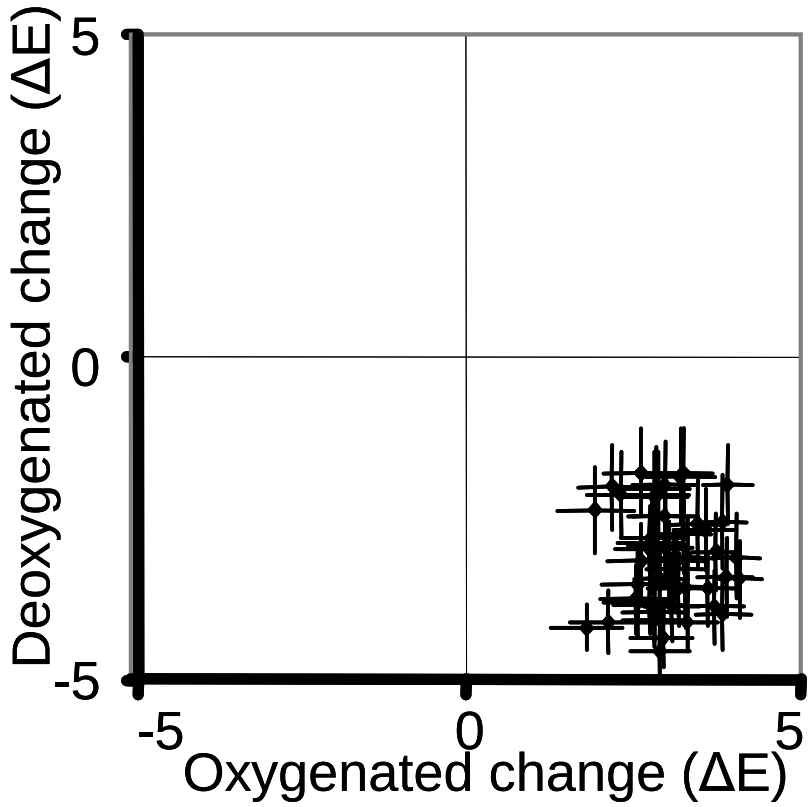


Fig. 4.5: Two-dimensional blood colour transform applied to optimise healthy appearance. Participants increase oxygenated blood colour and decrease deoxygenated blood colour to optimise healthy appearance. Data points show mean±SE ΔE colour change along each axis (oxygenated and deoxygenated blood colour). Each point represents one face, and standard errors are calculated across participants.

4.4 Section 4 Discussion

These results suggest that skin redness enhances healthy appearance through its association with the colour of blood. For human faces, skin that is rich in blood appears healthier than skin that is drained of blood. My results also show that oxygenated blood looks healthier than deoxygenated blood despite the subtlety of the colour difference. This is consistent with the association of blood oxygenation and skin vasodilation and vascularisation with increased sex hormone levels (Charkoudian

et al., 1999) and physical fitness (Armstrong & Welsman, 2001) and the absence of a range of illnesses (Charkoudian, 2003; Panza *et al.*, 1990; Ponsonby *et al.*, 1997).

Redness has importance for primate social signals, and is suggested to have played a role in the evolution of primate colour vision (Changizi *et al.*, 2006). I note that redness also acts as a health index by providing sensitivity to blood circulation which is influenced by reproductive hormonal status, and coronary and respiratory fitness.

In line with my predictions, I have shown that increased skin blood colour increases the apparent health of human faces (as does increased redness – see Section 3). Increased blood colour is associated with vasodilation and vascularisation. These mechanisms are made more responsive by increased levels of reproductive hormones (Charkoudian *et al.*, 1999) and physical training (Johnson, 1998). Low levels of blood colour in the skin may suggest poor health as they may indicate anaemia (Muhe *et al.*, 2000) or capillary rarefaction, which may be caused by hypertension (Panza *et al.*, 1990), diabetes (Charkoudian, 2003), old age (Tankersley *et al.*, 1991) or smoking (Richardson, 1987). Thus, skin blood colour reflects skin blood perfusion (Zonios *et al.*, 2001) and provides a perceptible cue to health.

I have also shown that oxygenated blood in the skin appears healthier than deoxygenated blood. In the single axis transforms, participants increase oxygenated blood colour more than deoxygenated blood colour. In the two-dimensional transforms, when participants could manipulate both oxygenated and deoxygenated blood colour axes simultaneously, participants removed deoxygenated blood colour and increased oxygenated blood colour. This colour change simulated an increase in

blood oxygenation. Oxygenated blood is associated with health and physical fitness (Armstrong & Welsman 2001), and is a bright red colour. Deoxygenated blood has a slightly bluish red colour and is associated with ill health (Ponsonby *et al.*, 1997). In humans, as in other primate species, skin colour variation provides a perceptible cue to health and condition in a way that is relevant for sexual selection.

The preference for increasing deoxygenated blood colour when oxygenated blood colour is not available suggests that increased perfusion with deoxygenated blood, though not as beneficial to health appearance as oxygenated blood colouration, is preferable to pallor.

I have provided the first evidence in mammals of the use of blood colour as a perceptible cue to health condition. I have shown that the colour relating to skin blood perfusion and oxygenation has a strong effect on health perception. Despite the subtlety of the colour difference between skin perfusion with oxygenated and deoxygenated blood, significant differences were found in the effects on perceived health, suggesting a high degree of perceptual sensitivity to these colour differences (Changizi *et al.*, 2006). These perceptible cues to health are relevant for mate selection and hence may play a role in evolution.

Section 5: Melanin and Carotenoid Transforms

Carotenoid and melanin provide perceptible cues to health in human faces

5.1 Introduction

This Section describes a suite of experiments examining the relationship between skin carotenoid and melanin colour and apparent health of human faces. The first experiment examines the effect of carotenoid colour, the second melanin colour. The third experiment uses two-dimensional transforms to further examine the relationship of these pigment colours to apparent health. These experiments are described together to allow a more thorough and cohesive treatment and discussion of the results.

In Section 3, I showed that participants increased yellowness to optimise healthy appearance of faces. The main pigments that contribute to skin yellowness are melanin and carotenoids (Edwards & Duntley, 1939). In this section, participants are allowed to manipulate colour-calibrated facial photographs along empirically-derived melanin and carotenoid colour axes to optimise healthy appearance. Participants manipulate faces along these axes individually (single-axis transforms) and simultaneously (two-dimensional trials). I find that participants increase carotenoid and melanin colour in single axis transforms. However, carotenoid colour is found to be preferentially increased in the two-dimensional transforms, suggesting that the apparent health benefit from increased melanin colour may be partly attributed to the yellow content of the pigment simulating increased carotenoid levels.

5.1.1 Carotenoids

Carotenoids are a group of yellow-red pigments. They are obtained from the diet, primarily from fruit and vegetables (Polsinelli *et al.*, 1998), and cannot be synthesised *de novo* in the body (Alaluf *et al.*, 2002b). Dietary supplementation with carotenoids increases the levels of carotenoids in the blood serum and can cause skin yellowing (Stahl *et al.*, 1998). It is also possible to predict serum carotenoid levels by analysis of spectrophotometric measurements of skin colour (Stahl *et al.*, 1998). Section 2 of this thesis establishes a connection between natural dietary intake of carotenoids and skin yellowness.

Carotenoids are associated with immunocompetence and disease resistance in humans and other animals, both directly and through conversion to vitamin A (Bendich & Olson, 1989). β-carotene supplementation is associated with increased lymphocyte blastogenesis in cows (Tjoelker *et al.*, 1990) and pigs (Hoskinson *et al.*, 1989, 1992), has a marked beneficial effect on the growth of the thymus gland in children (Seifter *et al.*, 1981), and increases the number and activity of T lymphocytes in healthy human adults (Alexander *et al.*, 1985). Levels of β-carotene and serum retinol ("the most widely used measure of vitamin A status" and a metabolite of many carotenoids; Friis *et al.*, 2001) are lower in women with HIV and/or malaria infection, and in women with raised levels of serum α_1-antichymotrypsin (a proteinase enzyme that is produced in the liver during the inflammation response and can be used as an indicator of infection; Friis *et al.*, 2001). Carotenoid levels are also known to be lower in chickens and guppies infected with parasites (see Olson & Owens, 1998). Increased β-carotene levels have been associated with reduced incidence of lung cancer (McClarty *et al.*, 1995), UV-induced erythema (Alaluf *et al.*, 2002b), photoaging of the skin and skin cancer (Stahl *et al.*, 2000; Taylor *et al.*, 1990), possibly through its

antioxidative properties (Alaluf *et al.*, 2002b). The immune system generates reactive oxygen species, including free radicals, and therefore may be particularly sensitive to oxidative stress (Chew, 1993), so may receive additional benefits from high levels of carotenoids. Several other mechanisms have also been suggested to explain the immune-enhancing properties of carotenoids (such as influencing the function of antigen-presenting cells), independent of their antioxidant properties (see Hughes, 2001 for a review). Low levels of carotenoids are therefore associated with reduced health status and increased levels with elevated health status.

Carotenoids are associated with reproductive health in humans and other animals. Sows given supplements of β-carotene and vitamin A had higher litter weights, greater numbers of live piglets in a litter, reduced incidence of still-born piglets (Coffey & Britt, 1993) and reduced embryonic mortality (Brief & Chew, 1985). Carotenoid-supplemented blue tits had higher reproductive output and their offspring had faster-developing immune systems and brighter yellow plumage than non-supplemented controls (Biard *et al.*, 2005). Vitamin A and carotenoids have been shown to play important roles in spermatogenesis and testosterone production in boars, and in progesterone production in sows (see Chew, 1993). Women who failed to conceive during *in-vitro* fertilisation (IVF) were found to have levels of carotenoids in the follicular fluid that fluctuated to an unusually high degree (possibly indicating a disturbed sieving effect of the blood-follicular barrier, affecting the control of carotenoid flow across this barrier) suggesting that carotenoids may be important for conception in humans (Schweigert *et al.*, 2003). It has also been demonstrated that plasma carotenoid levels vary across the menstrual cycle in women, being lowest at menses. β-carotene concentration is highest in the late follicular (most fertile) phase

(Forman *et al.*, 1996). Carotenoids are therefore positively associated with immunity and reproductive status.

Several species of birds and fish use carotenoid-based ornaments as a sexual signal, and it has been suggested that these ornaments act as handicapping signals (Lozano, 1994). Carotenoids are a limited resource for animals, as they cannot be synthesised in the body (Goodwin, 1984). Large, brightly-coloured carotenoid ornaments may indicate superior foraging ability (Endler, 1980). Also, using carotenoids in sexual ornaments diverts these resources away from their other roles in the immune system and protection from oxidative stress (reviewed in Hughes, 2001), so that only individuals with good immune systems can afford to use their carotenoid resources in this way. Carotenoid ornamentation may therefore be an honest signal of health and immunocompetence (Lozano, 1994).

Male and female animals show preferences for mating with carotenoid-coloured individuals. Male carotenoid colouration is preferred by female zebra finches (Blount *et al.*, 2003), house finches (Hill, 1990), and greenfinches (Saks *et al.*, 2003). Female fish from the genus *Poecilia* prefer males with red and yellow (carotenoid and pteridine-based) colouration to males with blue colouration (Bourne *et al.*, 2003). Female sticklebacks prefer males with greater access to dietary carotenoids (Pike *et al.*, 2007a). Males also exhibit preference for female carotenoid colouration in gobies (a small marine fish), performing more displays towards more colourful females (Amundsen & Forsgren, 2001). Female as well as male yellow-eyed penguins have bright carotenoid ornamentation that predict body condition and are preferred by the opposite sex (Massaro *et al.*, 2003). American goldfinches mate assortatively by

intensity of carotenoid plumage colour (MacDougall & Montgomerie, 2003). Thus carotenoid-based pigmentation has an important role in sexual selection of both males and females in many animal species.

Carotenoid ornament size and colouration relate positively to immune function in several species. Bright carotenoid coloured male house finches were found to be more likely than drab males to survive an epidemic of *Myoplasma gallisepticum* (Nolan *et al.*, 1998). Male zebra finches with carotenoid supplemented diets showed parallel increases in cell-mediated immune function and sexual attractiveness (Blount *et al.*, 2003). Carotenoid colouration also relates positively to immune function in zebra finches (Blount *et al.*, 2003; McGraw & Ardia, 2003), mallards (Peters *et al.*, 2004), greenfinches (Saks *et al.*, 2003) and house finches (in one study, Nolan *et al.*, 1998; though not in another, Navarra & Hill, 2003). In male sticklebacks, dietary supplementation with other antioxidants increased the colour intensity of carotenoid ornaments, suggesting that carotenoid ornamentation may signal total antioxidant reserves (Pike *et al.*, 2007b).

Mate quality, as measured by a variety of indicators, has been found to correlate positively with carotenoid levels and colouration in a number of species. Male northern cardinals with brighter red pigmentation were found to produce more offspring, pair with earlier-breeding females, and obtain higher quality territories (Wolfenbarger, 1999). Female house finches prefer to mate with colourful males, brighter males provide more food during nesting and there was a correlation between father and son colouration, suggesting a possible genetic element (Hill, 1991). Carotenoid colouration and ornamentation has been found to predict sperm quality in

male mallards (Peters *et al.*, 2004), though not in guppies (Skinner & Watt, 2006). Carotenoid levels in the plasma of female red-legged partridges correlated positively with carotenoid levels in their eggs (Bortolotti *et al.*, 2003), and blue tit nestlings from nests with carotenoid-supplemented mothers had faster developing immune systems, brighter yellow feathers and longer tarsi than nestlings from unsupplemented nests (Biard *et al.*, 2005).

In women, carotenoids have been reported to be concentrated in sexual-signalling areas of the body, such as the buttocks and breasts (Edwards & Duntley, 1939), suggesting a possible sexual signalling role in humans. Since information about reproductive status is visible in the face (Law Smith *et al.*, 2006), it may be expected that increased β-carotene colouration in the facial skin will increase healthy appearance. While skin yellowness does not differ between the sexes (see Section 3), skin carotenoid levels may be higher in women than in men (Edwards & Duntley, 1939), and it may be expected that this sexual dimorphism will be exaggerated, with carotenoid colour enhanced more in women than in men.

5.1.2 Melanin

Melanin impacts primarily on the yellow (b*) and luminance (L*) axes, being a dark yellow colour (Stamatas et al., 2004). Melanin functions to protect the skin from ultraviolet (UV) light (Daniels *et al.*, 1973), preventing skin cancer and sunburn (Robins, 1991) by scattering and absorbing UV radiation in the epidermis (Kollias *et al.*, 1991). In dark skin, the action of melanin can reduce the amount of UV radiation reaching the dermis by 90% (Daniels *et al.*, 1973). UV radiation can produce reactive oxygen species, such as free radicals, which in turn can damage complex molecules

such as DNA and proteins. Melanin also has an antioxidant effect, absorbing these free radicals and preventing damage (Robins, 1991).

Melanin's role in preventing the penetration of UV radiation into the dermis also protects against the photolysis of folate (Branda & Eaton, 1978). Folate is required for the normal development and function of several systems in the body, and deficiency particularly affects reproductive capacity. During pregnancy, when twice the normal levels of folate are required, deficiency has been associated with several birth defects including malformation of the eye, palate, lip, gastrointestinal system, aorta, kidney and skeleton (Omaye, 1993), and neural tube defects, which can lead to disability or miscarriage of the foetus (Jablonski & Chaplin, 2000).

Conversely, the prevention of UV penetration into the skin prevents the formation of vitamin D (Murray, 1934). Vitamin D is primarily obtained from photosynthesis in the skin and is present in large amounts in relatively few foods (Loomis, 1967). Vitamin D deficiency can lead to rickets in children and osteomalacia in adults (Murray, 1934), as well as reduced muscle strength (Neer, 1975). Dark skin (containing more melanin) allows less of the UV light to pass than does light skin: African skin filters out 50-95% of the UV light available to European skin, and has been shown to synthesise less vitamin D than light skin *in vitro*.

The melanin-producing cascade plays a significant role in the immune defence of insects (Boman & Hultmark, 1987). In humans, melanocytes have phagocytic function (engulfing invading pathogens) and melanosomes have lysosomal function (destroying invading cells; Burkhart & Burkhart, 2005). It has been suggested that the

primary role of melanin is immune defence rather than photoprotection, as geographical areas with darker-skinned people have higher parasite loads as well as higher UV radiation loads (Burkhart & Burkhart, 2005). However, levels of UV radiation are much higher at equatorial latitudes than towards the pole. The skin tone of indigenous people becomes lighter away from the equator, leading to suggestions that the reduced availability of UV light at high latitude led to the evolution of light skin (Jablonski & Chaplin, 2000).

It has also been suggested that the evolution of light skin can be attributed to protection from the cold in high latitude regions (Post *et al.*, 1975), since melanocytes are found to be particularly susceptible to cold damage (Mikhail, 1963) and depigmentation damage is more frequent in black sufferers of frostbite than white patients (Blair *et al.*, 1957).

Melanin may therefore provide health costs and benefits depending on different levels of UV radiation.

5.1.3 Predictions

It is hypothesised that increased carotenoid colour in the skin will increase the healthy appearance of individuals. This may be greater in female faces. The expected effects of melanin colouration, which may have health advantages and disadvantages, on the apparent health of faces are less clear.

5.2 Methods

5.2.1 Photography

The same facial photograph set was used as in Section 3

5.2.2 Empirical Measurement of Carotenoid Colour

10 participants (2 male, 8 female; aged 19–22) completed an eight-week course of β-carotene supplementation (15mg/day; Holland and Barrett Ltd). Periodic spectrophotometer measurements of skin colour were taken on the outer left shoulder, inner left upper arm, and left ventral interosseous region of the palm of the hand. These regions are less exposed to the sun than other skin regions, and hence less subject to melanin change through seasonal tanning. The mean changes (week 0–8) in spectrophotometer readings across the three regions (table 5.1) were used to generate the carotenoid colour transform (Table 5.2).

	b* before supplementation	b* after supplementation	b* change
Shoulder	16.39	17.65	t_9=-2.607; p=0.028
Inner arm	15.48	16.20	t_9=-1.433; p=0.186
Palm	13.22	14.44	t_9=-5.359; p<0.001

Table 5.1. Yellowness (b*) change over β-carotene supplementation period.

	L*	a*	b*	Colour change (ΔE)
β-carotene colour	+1.3	+4.3	+10.5	11.42
Melanin colour	-5.20	-1.21	+7.10	8.88

Table 5.2. Colour transform applied to produce the high colour endpoint image.

The sign is changed for the low colour endpoint image.

5.2.3 Empirical Measurement of Melanin Colour

Spectrophotometer measurements were taken at two locations on the dorsal side of the upper arm; 2cm proximal to the elbow (‘upper arm’) and 2cm distal to the shoulder

('shoulder'). Nine (6 male; aged 20-23) of the 19 participants were more tanned at the elbow than the shoulder. Mean CIELab values for skin areas with high and low melanisation from these participants were used to produce the melanin transform (table 5.2).

5.2.4 Image Manipulation

Matlab was used to make two pairs of face-shaped masks, as described in Section 3. One pair was the colour of the mean high and low carotenoid colour measurements. The other pair was the colour of the mean high and low melanin colour measurements. Thirteen images were generated in equal steps from high to low colour, for each of the pigment dimensions (Fig. 5.1). For 2D transforms, each of the thirteen images was transformed along a second colour dimension, giving 169 images per face (Figs 5.2). Hair, eyes, clothing and background remained constant.

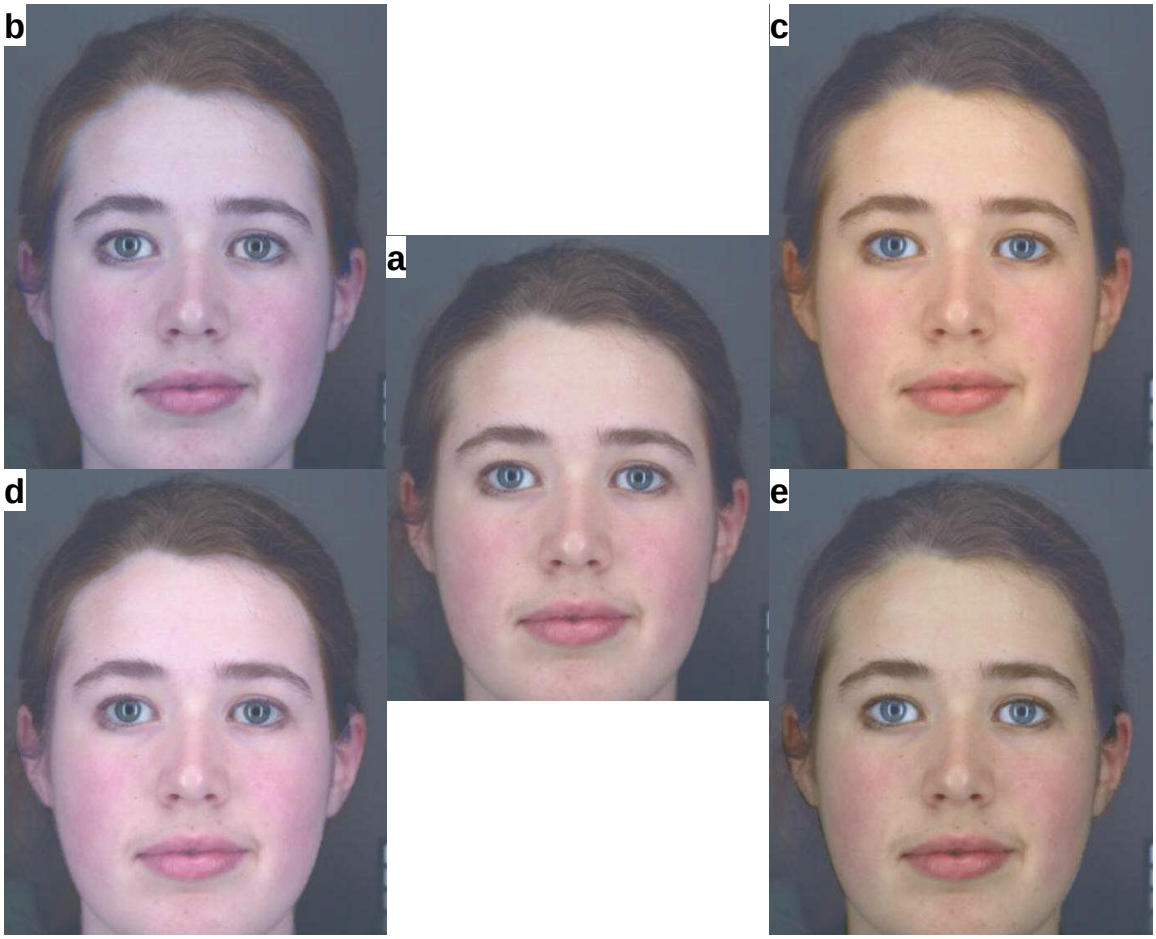


Fig. 5.1: Melanin and carotenoid colour transforms. Original image (a) and images with skin portions transformed along melanin and carotenoid colour axes. (b) low carotenoid colour, (c) high carotenoid colour, (d) low melanin colour, (e) high melanin colour.

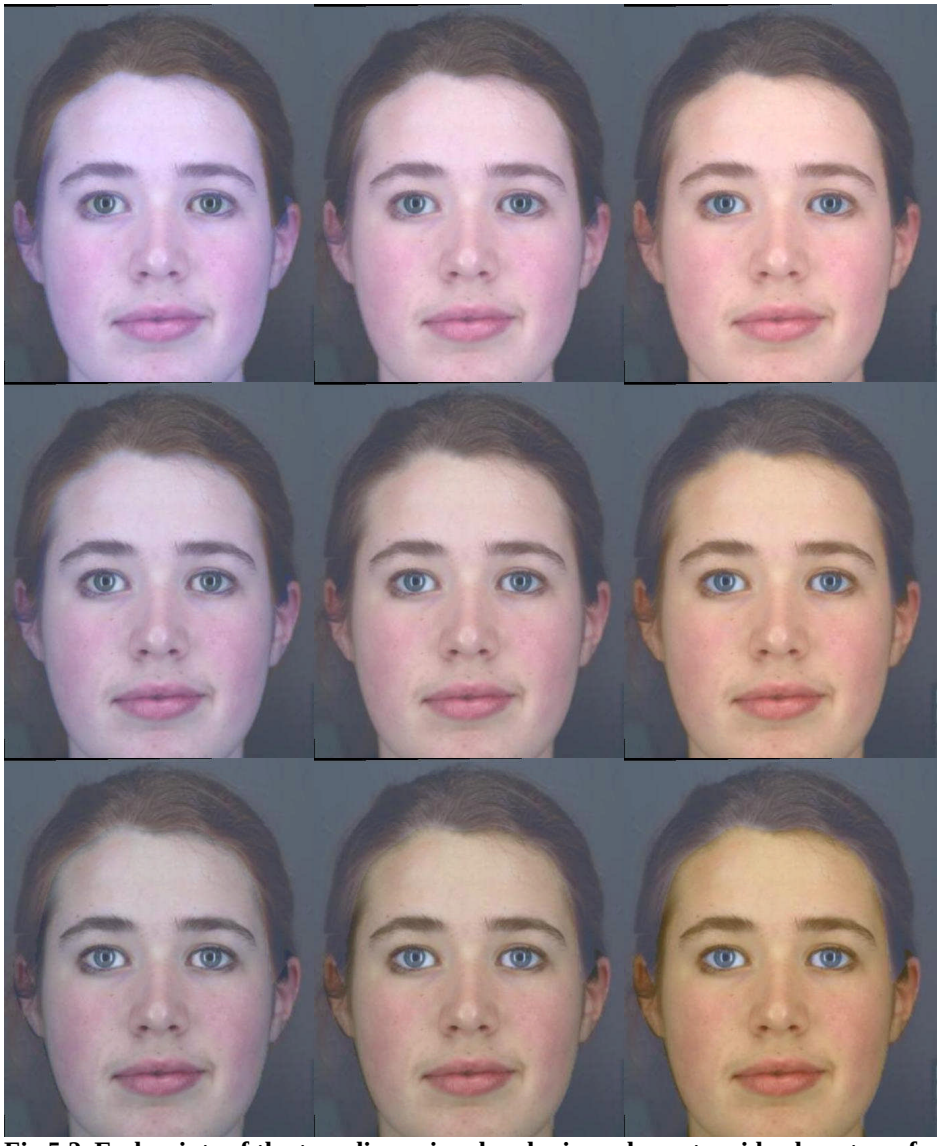


Fig 5.2. End-points of the two-dimensional melanin and carotenoid colour transform. Original image (centre) and low carotenoid colour (left) to high carotenoid colour (right) and low melanin colour (top) to high melanin colour (bottom).

5.2.5 Experimentation

Further Caucasian participants were recruited from the University of St Andrews: 22 (12 male, 10 female, aged 18-25) for the two-dimensional transform, 22 (10 male, 12 female, aged 20–26) for the β-carotene single-axis transform and 26 (12 male, 14 female, aged 18–24) for the melanin transform.

Participants were presented with the stimuli, one face at a time, in random order on a CRT monitor (colour calibrated using a ColorVision Spyder 2Pro to mean ΔE of 2.32 for a range of skin tones reflecting faces of various ethnicity). A computer program allowed participants to manipulate the colour of the facial skin along a single CIELab or pigment colour axis to achieve optimum healthy appearance. 2D trials allowed participants to manipulate colour in two dimensions simultaneously.

5.2.6 Statistical Methods

Mean colour changes applied to each face were calculated (by face dataset). Mean colour changes applied by each participant were also calculated (by participant dataset). In the by face dataset, one-sample t-tests (H_0: no colour change) were used to test whether the faces were changed in colour to optimise healthy appearance. Univariate ANOVAs were used to test for the effect of face sex on colour change, as this test allows initial face colour to be controlled as a covariate (by face dataset; dependent variable=colour change applied; fixed factor=face sex; covariate=initial face colour: L*, a* or b*). Pearson's r was used to test for correlation between initial face colour and colour change applied. Independent samples t-tests were used in the by participant dataset to test for differences in amount of colour change applied by male and female participants. To test for an interaction between sex of face and sex of participant, repeated-measures ANOVAs were used (dependent variable=colour change applied; within-subjects factor=participant sex; between-subjects factor=face sex). In the two-dimensional trials, one-sample t-tests (H_0: no colour change) were used to test whether the faces were changed in each colour dimension to optimise healthy appearance. When one or more variable was not normally distributed, Mann-Whitney U tests were used in place of t-tests.

5.3 Results

5.3.1 Single-Axis Transforms

In the single pigment transforms, faces were increased in carotenoid colour (Fig. 5.3A, $\Delta E=6.39\pm0.15$; $t_{50}=43.208$, $p<0.001$) and melanin colour (Fig. 5.3B, $\Delta E=3.51\pm0.12$; $t_{50}=29.584$, $p<0.001$) to improve healthy appearance (Table 5.3). The colour change to maximize apparent health for both carotenoid and melanin transforms is negatively correlated with initial facial yellowness; faces starting low in yellowness received more carotenoid ($R^2=0.38$, Fig. 5.3A) and melanin ($R^2=0.15$, Fig. 5.3B) colour transformation (table 5.4). Neither initial face lightness nor redness related to the amount of melanin or carotenoid colour transform applied to optimize healthy appearance (Table 5.4). Participant sex and the interaction between face and participant sex was not found to affect the amount of colour change applied to optimise healthy appearance (table 5.5). A non-significant trend suggested that female faces (estimated marginal mean$\pm$SE=6.58$\pm$0.15) may be enhanced in carotenoid colour more than male faces (estimated marginal mean$\pm$SE=6.13$\pm$0.18), enhancing a possible pre-existing dimorphism (Edwards & Duntley, 1939).

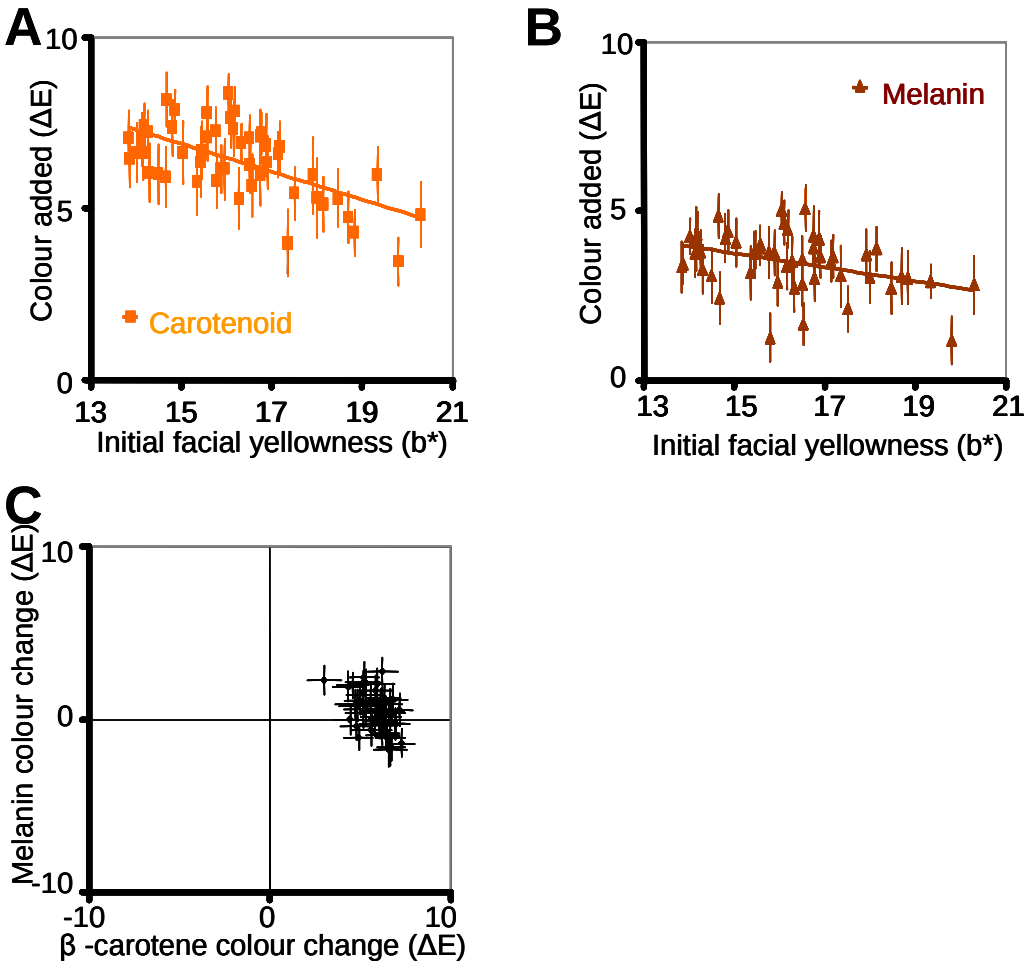


Fig. 5.3. Effect of carotenoid and melanin pigment colours on apparent health of faces. (A) Initial facial yellowness (b*) correlates with the carotenoid (R^2=0.38) transform applied to optimize healthy appearance for 51 Caucasian faces (ΔE = CIELab distance between initial and optimized face colour mean±SE). **(B)** Initial facial yellowness (b*) correlates with the melanin colour change (R^2=0.15) applied to optimize healthy appearance. (**C**) Two-dimensional colour change applied to optimize healthy appearance (ΔE mean±SE). All faces are increased in β-carotene colour to optimize healthy appearance.

Transform	ΔL* component	Δa* component	Δb* component	Total colour change (ΔE)
Carotenoid	0.69±0.02	2.27±0.06	5.54±0.13	6.39±0.15
Melanin	-2.11±0.07	-0.49±0.02	2.88±0.10	3.51±0.12

Table 5.3. Colour changes along component CIELab axes and total colour change (ΔE). Mean change across participants and faces (±SE) in the single-axis pigment transforms to maximize health.

Colour axis	Correlation with initial facial colour (n=51)		
	L*	a*	b*
β-carotene	r=-0.064; p=0.655	r=-0.240; p=0.089	r=-0.616; p<0.001
Melanin	r=0.159; p=0.265	r=0.121; p=0.397	r=-0.388; p=0.005

Table 5.4. Correlations (Pearson's r) of colour transform with initial facial colour.

Colour axis	Effect of participant sex	Interaction between face sex and participant sex	Effect of sex of face (Initial yellowness controlled)
β-carotene	t_{20}=-1.204; p=0.243	$F_{1,49}$=0.301; p=0.586	$F_{1,48}$=3.613; p=0.063
Melanin	t_{24}=0.443; p=0.663	$F_{1,49}$=0.029; p=0.866	$F_{1,48}$=0.079; p=0.781

Table 5.5. Effects of sex on colour transformations. Column 2: Differences in colour change made by male and female participants (by participant dataset), examined with independent samples t-tests. Column 3: Interaction between sex of face and sex of participant on colour transformation, examined with repeated-measure ANOVAs (by face dataset; dependent variable=colour change, within-subjects factor=participant sex, between-subjects factor=face sex). Column 4: Univariate ANOVAs were used to test for the effect of face sex on colour change, as this test allows initial face colour to be controlled as a covariate (by face dataset; dependent variable=colour change; fixed factor=face sex; covariate=initial face yellowness (b*).

5.3.2 Two-Dimensional Transform

When participants could manipulate melanin and β-carotene colour simultaneously (two-dimensional trials Fig. 5.3C) they induced an overall colour change ($\Delta E=5.07\pm0.08$; $t_{50}=61.417$; $p<0.001$; Table 5.6). This was comprised of a small change in melanin colour ($\Delta E=0.45\pm0.16$; $t_{50}=2.875$; $p=0.006$) and a significantly larger ($t_{50}=22.803$; $p<0.001$) change in carotenoid colour ($\Delta E=5.88\pm0.12$; $t_{50}=49.264$; $p<0.001$).

Colour axis	Colour change in 2D β-carotene vs melanin trials	
	Significance	Mean±SE
ΔL*	t_{50}=35.483; p<0.001	1.09±0.03
Δa*	t_{50}=62.720; p<0.001	2.18±0.03
Δb*	t_{50}=56.873; p<0.001	4.44±0.08
Overall ΔE	t_{50}=61.417; p<0.001	5.07±0.08

Table 5.6. Overall CIELab and ΔE colour change in two-dimensional pigment trials.

5.4 Discussion

5.4.1 Carotenoids

A preference for the yellow hues associated with carotenoids in the skin is consistent with a preference for individuals with a diet naturally high in fruit and vegetables. These findings have public health utility: knowledge that increase in fruit and vegetable consumption benefits health appearance will help promote healthy eating (Chung *et al.*, 2006) in line with governmental recommendations (Institute of Medicine, 2000; Dietary Guideline Advisory Committee, 2005).

Carotenoid colouration in humans could indicate several health attributes including enhanced immune function (Hughes, 2001) and reproductive health (Schweigert *et al.*, 2003). Since it contributes to apparent health, skin colour may have a role in sexual selection in humans (Buss, 1989). Past or present mate choice based on health appearance may translate to selection for individuals who are most capable of acquiring healthy food resources and/or are most able to resist disease (and can therefore expend the nutrient benefits gleaned from diet on skin colouration). I show that carotenoid-colour – which signals health in many animals with trichromatic (or greater) vision (Massaro *et al.*, 2003) – provides a perceptible cue to health in humans.

5.4.2 Melanin

Melanin has a complex role in health perception. Participants increase melanin colour in faces to optimize healthy appearance, but this may be driven by the yellow aspect of the pigment imitating carotenoid colour. Melanin darkens the skin, yet lightness is preferred by participants. When participants can adjust both carotenoid and melanin levels, melanin is increased only slightly but carotenoid level is increased considerably, maintaining skin lightness and increasing skin yellowness.

Associations between skin lightness and health differ across different populations; particularly in multi-ethnic societies where wealth and health care are associated preferentially with lighter skin and European ethnicity (Jones, 2000). Despite this, other data indicate similar perception of skin colour-health relationships for faces of different ethnicities (Asian, African and Caucasian) and by different participant populations (white European and black South African) even when there are cultural differences in skin lightness preference (see Section 6 of this thesis).

The impact on apparent health from an increase in melanin (which increases yellowness but decreases lightness) reflects a balance between costs and benefits. The benefit from melanisation can be attributed to the increased skin yellowness, which simulates a high carotenoid intake and good diet. Costs follow a decrease in skin lightness which can affect apparent socio-economic status (Jones, 2000) and impair vitamin D synthesis (Murray, 1934; Jablonski & Chaplin, 2000).

5.4.3 Two-Dimensional Transforms

Whilst 68% of faces were increased in melanin, all were increased in carotenoid colour. Collectively these results indicate a greater role of carotenoid pigment than melanin in health perception. The gain in apparent health from increasing skin yellowness in the CIELab b* transforms appears to be attributable to the effects that dietary carotenoids have on skin yellowness and apparent health.

5.4.4 Conclusion

I have provided the first evidence in mammals of the use of yellow colouration to provide a perceptible cue to health condition. I have shown that human skin colour reflects healthiness of diet and controls health perception. This cue may be relevant for sexual selection and hence may play a role in the evolution of human skin colour.

Section 6: Cross-Cultural Studies

Cross-cultural study

6.1 Introduction

This Section describes a suite of three experiments, each examining the effects of one of the three CIELab colour axes (lightness, redness and yellowness) on the apparent health of human faces, and the effects of the ethnicity of the faces and the participants upon these results. These experiments are described together to allow a more thorough and cohesive treatment and discussion of the results.

Traditionally, the study of attractiveness has held two hypotheses. The social expectancy theory has suggested that attractiveness (or "beauty") is a social construct that is imposed by society and culture (Wolf, 1991). This theory notes that different cultures have their own standards of beauty (Darwin, 1871), and that individuals' attitudes and behaviour towards themselves and others is a response to this imposed standard of beauty (Langlois *et al.*, 2000). In contrast, adaptationist, evolutionary theories note the connection between attractiveness and mate choice in humans (Symons, 1979). They suggest that aspects of attractiveness should provide honest indicators of mate quality (Barber, 1995). In this case, certain aspects of attractiveness should be universal, differing little within and between cultures (Langlois *et al.*, 2000). Perceptions of aspects of attractiveness may vary between populations because of different evolutionary histories (Cunningham *et al.*, 1995) and cultures. Skin colour is a physical trait that is thought to be important both in an evolutionary context (Jablonski & Chaplin, 2000) and has impacts on the perception treatment of

individuals societally (Maddox & Gray, 2002). Similarities and differences in cross-cultural preferences for skin colour may be expected.

Research has supported evolutionary frameworks of attractiveness. Strong agreement about who is attractive exists both within and between populations (Cunningham *et al.*, 1995), and several studies have found agreement between populations about specific aspects of attractiveness, while different populations place different emphasis on specific aspects of attractiveness. Symmetry and averageness contribute to attractiveness ratings in Asian and Caucasian faces by Asian and Caucasian participants (Rhodes *et al.*, 2001a). Japanese and Caucasian participants responded in similar ways to exaggerations of attractiveness in Japanese and Caucasian faces (Perrett *et al.*, 1994). Preference for neotenous female faces has been found in a diverse range of cultures, including traditional societies (Jones, 1995). Further support for an evolutionary, rather than social, origin of attractiveness comes from a study that found that infants preferred to look at attractive faces, regardless of ethnic group, gender or age of the face groups (Langlois *et al.*, 1991). Further, broad agreement is found between cultures in the attributes that are sought in a partner, with women valuing "good financial prospect" more highly than men in 36 of 37 cultures and "ambition-industriousness" in 34 of 37 cultures. Men value attractiveness more highly than women in all 37 cultures examined. Further, men were found to universally prefer younger women and women universally to prefer older men (Buss, 1989).

Cultural differences in mate preferences have also been found. Whereas Greek and British (relatively wealthy societies) men preferred lighter female body shapes and a waist to hip ratio (WHR) of 0.7, Ugandan (relatively poor society) men were found to

prefer heavier female body shapes and a WHR of 0.5 – heavier but with a narrower waist relative to hips (Furnham *et al.*, 2002). It has also been suggested that more conservative cultures prefer more gender-stereotyped (lower WHR in women) figures than more liberal societies (Furnham & Nording, 1998). Additionally, Asian participants have been found to be less favourably disposed to sexual maturity (high cheek bones, narrower faces) and expressiveness (large smile, dilated pupils, high eyebrows) aspects of women's faces than Caucasian and Hispanic participants, though all groups liked these features to some degree (Cunningham *et al.*, 1995). This may be attributed to a cultural preference for "sexual immaturity, modesty and inexpressiveness in the public appearance of women" (Cunningham *et al.*, 1995). Asian participants were also more accepting of women with wider faces (Cunningham *et al.*, 1995).

While preferences for lighter skin, especially in women, have been found in many diverse cultures (van den Berghe & Frost, 1986), social factors have been suggested to play a role in preferences for skin colour. A study of attractiveness in Bahia, Brazil found that women with African features and dark skin are considered less attractive than women with European features and light skin (Jones, 2000). This is attributed to racial stratification in the society, whereby light skin and European features are associated with high status and wealth, whereas dark skin and African features are associated with low status and low wealth (Jones, 2000). It has also been shown that skin colour can produce responses associated with societal groups. For example, African Americans with very dark skin are assigned many of the negative attributes that are associated with that ethnic group more so than light skinned African

Americans (i.e. people are more likely to describe darker skinned individuals as "aggressive" or "criminal" than lighter skinned individuals (Maddox & Gray, 2002).

The current study repeats the CIELab colour transforms described in Section 3, for faces of various ethnicity and with participants from the UK and South Africa. It may be predicted, therefore that individuals from different cultures will retain the same broad preferences for skin colour, though some variation in the magnitude of these preferences may be found. South Africa is a significantly more racially stratified society than the UK, with whites earning five times more than non-whites in 1991 (Treiman *et al.*, 1996) and more than four times as much spent on healthcare for whites as for blacks (Yach & Harrison, 1995). Additionally, suntanning is fashionable in the UK (Melia & Bulman, 1995), perhaps reflecting an ability to pay for foreign holidays and health clubs. This suggests that the South African participants may prefer lightness more than the UK participants. On the other hand, the somatic norm theory of attraction suggests that faces closer to the population average will be preferred (Langlois & Roggman, 1990). This theory predicts that the South African participants should prefer darker faces than the UK participants.

6.2 Methods

6.2.1 Photography

Photographs were taken of undergraduate students (10 Caucasian male, 10 Caucasian female, 7 East Asian male, 9 East Asian female, 6 African female, 5 South Asian female, 3 mixed ethnicity female), using a Fujifilm FinePix S2Pro digital SLR camera, fitted with a Nikon 60mm fixed length lens, and a Nikon Canfield lens-mounted flash. Participants were seated, facing the camera, in front of a grey screen, and asked to

maintain a neutral expression. Additional images were taken with a GretagMacbeth Mini ColorChecker chart in the frame. Colour measurements (in the CIELab colour space) were taken of the colour patches in the image and directly with a Konica Minolta CM2600d spectrophotometer. Linear transforms were used to colour calibrate the images (achieving a post-calibration mean colour error [ΔE] of 6.704). Whilst colour calibration methods are not as accurate in this Section as in previous Sections, this Section does not attempt to link the preferred colour results to actual physiological phenomena and therefore accurate representations of faces are less important than in previous chapters. The focus of this Section is the similarities and differences between the skin colour preferences of different populations of faces and of participants.

6.2.2 Image Manipulation

CIELab L*, a* and b* transforms of each face were produced, using the same methods as in Section 3.

6.2.3 Experimentation

One-dimensional colour transformation trials were presented in random order to black South African (10 male, 10 female) participants at the University of Pretoria, South Africa and white UK-based (7 male, 11 female) participants at the University of St Andrews, UK.

6.2.4 Statistical Methods

Mean colour changes applied to each face were calculated (by face dataset). Mean colour changes applied by each participant were also calculated (by participant

dataset). In the by face dataset, one-sample t-tests (H_0: no colour change) were used to test whether the faces were changed in colour to optimise healthy appearance. One-way ANOVAs (dependent variable=colour change applied; independent variable=face ethnicity) were used to test for differences in the amount of colour change applied to faces of different ethnicities to optimise healthy appearance. One-way ANOVAs (dependent variable=initial face redness; independent variable=face ethnicity) were used to test for differences in the initial facial redness in faces of different ethnicity. Due to some non-normally distributed variables, Mann-Whitney U tests were used to test for differences in amounts of colour change applied by South African and UK-based participants.

6.3 Results

Statistical results are summarized in Table 6.1. As in Section 3, to optimize healthy appearance, facial images were increased in red, yellow and lightness (Fig. 6.1). Redness change was negatively related to initial face redness (Fig. 6.1A), yellowness change to initial face yellowness (Fig. 6.1B), and lightness change to initial face lightness (Fig. 6.1C).

For analysis of the effect of face ethnicity on colour manipulations, mixed and south Asian groups were excluded due to small sample size. One-way ANOVAs showed that there was no difference between the amount of yellowness [$F_{2,41}$=0.616; p=0.545] or lightness [$F_{2,41}$=2.659; p=0.083] change applied to different ethnic faces. More redness change was applied to East Asian faces than to African [mean difference±SE=1.49±0.54; Tukey post-hoc test p=0.023] or Caucasian [mean

difference±SE=1.12±0.38; p=0.013] faces [$F_{2,41}$=5.953; p=0.006]. This difference can be attributed to variation in initial redness of the three face types ($F_{2,41}$=9.259; p=0.001), whereby the East Asian faces are initially the least red. All of the faces, independent of their ethnicity, lie along the same regression line relating initial facial redness to redness transformation (Fig. 6.1A).

		L*	a*	b*
Colour change to optimize healthy appearance		t_{49}=4.623; p<0.001	t_{49}=13.661; p<0.001	t_{49}=23.741; p<0.001
ΔE (mean±SE)		0.73±0.16	2.39±0.18	3.42±0.14
Correlation between initial facial colour and colour change (n=50)	**L***	r=-0.436; p=0.002	r=0.129; p=0.373	r=-0.159; p=0.271
	a*	r=0.082; p=0.573	r=-0.832; p<0.001	r=-0.342; p=0.015
	b*	r=0.058; p=0.690	r=-0.388; p=0.005	r=-0.592; p<0.001

Table 6.1. Colour change applied to faces of different ethnicities.

No difference between the African and UK participants was found in the colour change applied to optimize healthy appearance in the redness (Mann-Whitney U=129; p=0.141) or yellowness (U=171; p=0.806) dimensions. African participants lightened faces more than European participants (U=90; p=0.008; Fig. 6.1D).

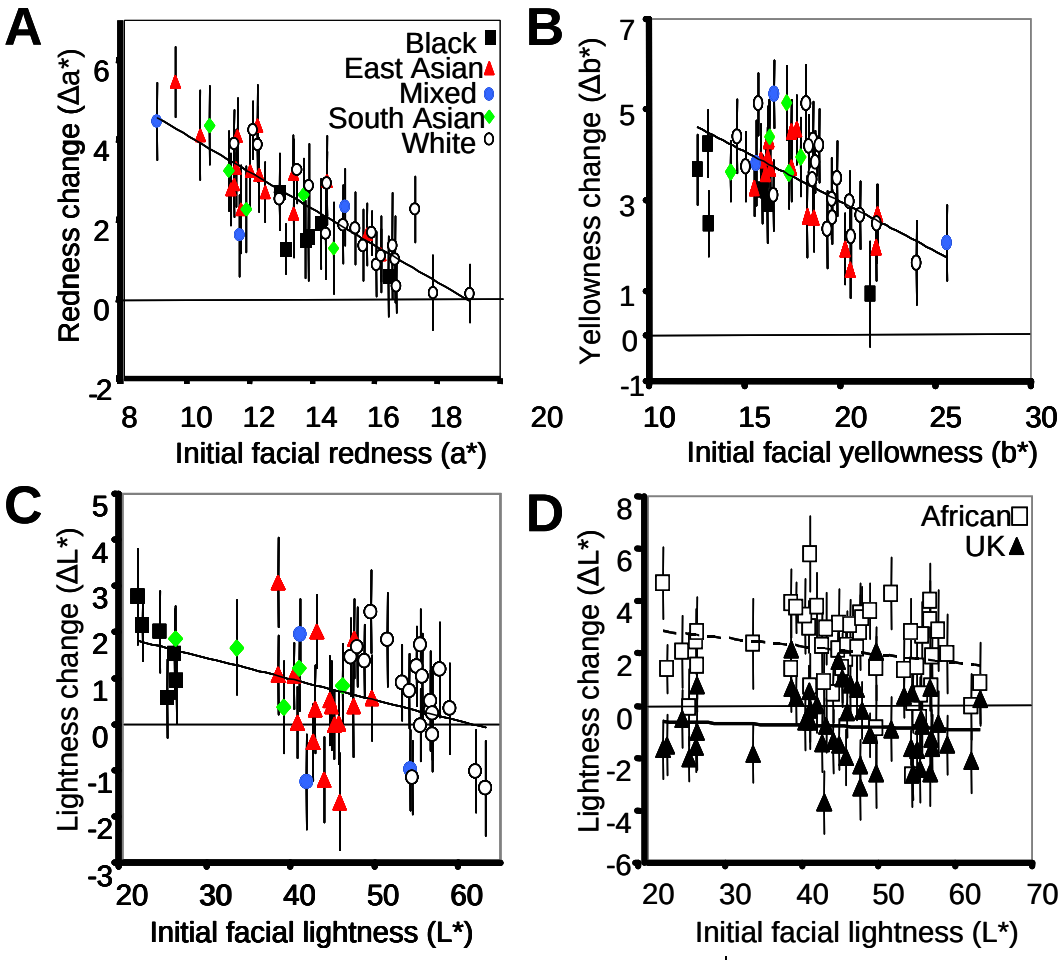


Fig. 6.1. Effect of CIELab skin colour components on perceived health of faces.

(A) Initial facial redness (a*) correlates with change in redness (Δa* mean±SE) applied to optimize healthy appearance (R^2=0.69). For each value of initial redness (a*), male faces were increased in redness (Δa*) more than female faces. Results pooled across African and UK participants. **(B)** Initial facial yellowness (b*) correlates with the change in yellowness (Δb* mean±SE) applied to optimize healthy appearance (R^2=0.35). Results pooled across African and UK participants. **(C)** Initial facial lightness (L*) correlates with change in lightness (ΔL* mean±SE) applied to optimize healthy appearance (R^2=0.19). Results pooled across African and UK participants. **(D)** African participants (hollow symbols; R^2=0.05) lighten faces more than UK participants (solid symbols; R^2=0.004). Each point represents a single face. Error bars represent standard error of the mean across all participants.

6.4 Discussion

Previous Sections showed that variation in the amount of colour change applied to different faces can largely be attributed to the initial colour of the faces. This Section shows that faces from several different ethnic groups lie along the same regression line relating initial face colour to colour change applied to optimise healthy appearance. No difference was found between different ethnic groups of faces in the amount of lightness or yellowness change applied to optimise healthy appearance. Participants increase the redness of East Asian faces more than faces of other ethnic groups. However, East Asian faces lie along the same regression line relating initial facial redness to redness change applied to optimise healthy appearance. The additional reddening applied can be attributed to the lower initial redness values of East Asian faces.

Black South African and white UK-based participants show preferences for redness and yellowness in skin colour, with no difference in preference found between the two ethnicities. African participants increase lightness more than UK participants. This suggests that a cultural element is involved. This is consistent with Jones' (2000) study that shows relative social status of ethnic groups affecting attractiveness judgements in ethnically stratified societies. In South Africa, a society that is ethnically stratified with white people being both wealthier (Woolard, 2002) and healthier (Yach & Harrison, 1995) than black people, lightness (which can be treated as a cue to ethnicity independently of other cues; Maddox & Gray, 2002) affects health perception. In the UK, a less racially stratified society (Smith, 2000), increased lightness does not increase perceived health to the same degree.

Section 7:

General

Discussion

7 General Discussion

This thesis has addressed the role of skin colour in providing a perceptible cue to health in human faces.

In Section 2, I investigated the impact of natural, unsupplemented dietary consumption of fruit and vegetables and carotenoids on the yellowness of human skin. In Study 1, I showed that individuals with higher natural dietary intakes of fruit and vegetables and carotenoids had yellower skin. In Study 2, I showed that the relationship between skin reflectance and fruit and vegetable and carotenoid intake shows the characteristic double peaks in the 400-540nm part of the visible spectrum that is associated with carotenoid light absorption. In contrast, there is little resemblance with the absorption spectra of the other main skin pigments: melanin, haemoglobin and oxygenated haemoglobin. These results fit in with a body of literature that show that carotenoid supplementation leads to skin yellowing (Stahl *et al.*, 1998), and that individuals with elevated levels of carotenoids in the serum have yellower skin (Alaluf *et al.*, 2002b). My results are the first to show that natural, unsupplemented dietary intake of carotenoids correlate with skin yellowness. My results indicate that palm skin yellowness is as powerful a predictor of fruit and vegetable intake as blood serum measurements of carotenoids (Polsinelli *et al.*, 1998).

Carotenoid pigments play a significant role in signalling condition and sexual selection in a number of non-human animal species, particularly birds (Saks *et al.*, 2003) and fish (Pike *et al.*, 2007a), and are implicated in immune and reproductive health in humans and other animals (Hughes, 2001; Brief & Chew, 1985). It was expected, therefore, that skin carotenoid colour would act as a perceptible cue to

health in human faces. Sections 3 to 6 therefore investigated the role of skin colour and pigmentation in influencing the apparent health of faces.

In Section 3, I showed that skin colour affects the perceived health of human faces. Participants were allowed to manipulate the colour of the skin portions of colour calibrated facial photographs along CIELab L* (light-dark), a* (red-green) and b* (yellow-blue) axes separately to optimise healthy appearance. Participants increased the lightness, redness and yellowness.

The preference for redness in human facial skin was in agreement with a literature that shows redness acting as a sexually selected signal in non-human primates and skin blood perfusion being associated with health in humans. In non-human primate species, redness signals increased dominance testosterone levels in males (Rhodes *et al.*, 1997; Setchell & Wickings, 2005) and reproductive status in females (Setchell *et al.*, 2006), and is preferred by the opposite sex (Waitt *et al.*, 2006; Waitt et al., 2003). In humans, increased skin blood flow is associated with increased levels of sex hormones (Charkoudian *et al.*, 1999; Thornton, 2002), physical fitness (Johnson, 1998) and the absence of a range of illnesses (Charkoudian, 2003; Panza *et al.*, 1990). Skin redness is influence by skin blood perfusion and oxygenation. I predicted, therefore, that skin blood colour would explain the relationship between skin redness and apparent health of human faces. This hypothesis is addressed in Section 4.

The preference for yellowness in human faces is consistent with a body of literature that describes the use of yellow, carotenoid-based, condition-dependent, sexually-selected signals in non-human animals, particularly birds (Saks *et al.*, 2003) and fish

(Pike *et al.*, 2007a). These carotenoid compounds are also positively associated with reproductive (Chew, 1993; Coffey & Britt, 1993; Schweigert *et al.*, 2003) and immune health (Hughes, 1999, 2001) in humans and other animals. I therefore hypothesised that carotenoid pigments may explain the relationship between skin yellowness and apparent health of human faces. This hypothesis is addressed in Section 5.

Whilst increased melanin levels are consistent with increased skin yellowness, melanin also darkens the skin (Stamatas *et al.*, 2004). The preference that was seen for increased skin lightness is consistent with a preference for a reduction in melanin colour, while the preference for increased skin yellowness is consistent with a preference for an increase in melanin colouration. Melanin has a complex relationship with health, with increased melanin levels associated with increased protection from UV radiation (Daniels *et al.*, 1973), protecting against skin cancer, sunburn (Robins, 1991) and folate photolysis (Omaye, 1993); increased melanin also prevents the photosynthesis of vitamin D in the skin (Murray, 1934), potentially leading to rickets. The expected relationship of melanin colour to apparent health is therefore unclear. Section 5 addresses the way in which melanin pigmentation affects apparent health.

Whether or not the lips were transformed in colour along with the rest of the facial skin did not affect the amount of redness or lightness change applied to optimise healthy appearance. Participants added more yellowness to the faces when the lips were manipulated along with the facial skin than when they remained constant. This is likely to be explained by the fact that the yellowing of skin while the lips remain constant gives the lips a bluish appearance. No support is found by this study for

Russell's (2003) hypothesis that the contrast between the skin and the facial features affects the apparent sexual dimorphism and attractiveness of the face (although my results address perceived health rather than attractiveness and dimorphism directly).

In Section 4, I investigated the effect of skin blood colouration on the apparent health of human faces. Participants were asked to manipulate the colour of the skin portions of colour calibrated facial photographs along oxygenated and deoxygenated blood colour axes to optimise healthy appearance. Single axis transforms allowed the participants to manipulate the images along oxygenated and deoxygenated blood colour axes independently, while two dimensional trials allowed the manipulation of both axes simultaneously. In the single axis transforms, participants increased blood colour, especially oxygenated, to optimise healthy appearance. In the two-dimensional trials, participants removed deoxygenated blood colour and added oxygenated blood colour. Thus, they increased the blood colouration of faces, but altered the ratio of oxygenated to deoxygenated blood colour in favour of increased oxygenation.

These results fit the predictions that I made from a body of literature covering several aspects of blood perfusion. Skin blood perfusion is determined by vascularisation and vasodilation (Zonios *et al.*, 2001). Increased vascularisation and vasodilation are associated with increased levels of sex hormones (Charkoudian *et al.*, 1999; Thornton, 2002), physical fitness (Johnson, 1998) and absence of certain illnesses (Charkoudian, 2003).

Skin blood colour acts as a socio-sexual signal in non-human primates, with various species using blood-based skin reddening to signal dominance (Setchell & Dixson,

2001; Setchell & Wickings, 2005), testosterone levels (Rhodes *et al.*, 1997) and reproductive status (Setchell *et al.*, 2006), and being preferred by the opposite sex (Setchell, 2005; Waitt *et al.*, 2003, 2006).

Increased blood oxygenation is associated with increased aerobic fitness (Armstrong & Welsman, 2001), while blood deoxygenation is associated with a range of mainly cardiac and respiratory illnesses (Ponsonby *et al.*, 1997).

I have shown, therefore, that humans use the skin blood colouration of human faces as a perceptible cue to health. This cue is likely to reflect aspects of hormonal and reproductive status, aerobic fitness and absence of certain illnesses. Since apparent health is an important aspect of attractiveness (Jones *et al.*, 2004; Jones *et al.*, 2005), it is likely that these cues are used in mate selection, and therefore may be sexually selected and impact on evolution.

In Section 5, I investigated the effects of melanin and carotenoid colour on the apparent health of human faces. Participants were asked to manipulate the melanin and carotenoid colour of the skin portions of colour calibrated facial photographs to optimise healthy appearance. Single axis transforms allowed participants to manipulate the faces along the carotenoid and melanin colour axes independently, while two-dimensional trials allowed participants to manipulate the faces along both melanin and carotenoid colour axes simultaneously. In the single axis transforms, participants increased both the carotenoid and melanin colour of the faces to optimise healthy appearance. However, participants chose to increase the carotenoid colour more than the melanin colour. In the two-dimensional transforms, participants

increased the carotenoid colour by a large amount and increased the melanin colour by a much smaller amount. This suggests that melanin may appear healthy only because of its similarity in appearance with carotenoid colour (they are both yellow pigments).

The healthy appearance of carotenoid colour was predicted from a body of literature. Colourful, condition dependent, carotenoid based ornaments are exhibited by many species of birds (Saks *et al.*, 2003) and fish (Pike *et al.*, 2007a). Larger and brighter ornaments are associated with increased health (Blount *et al.*, 2003), and are preferred by the opposite sex (Amundsen & Forsgren, 2001; Hill, 1990; Massaro *et al.*, 2003). Carotenoids are also associated with immune and reproductive health in humans and other animals (Coffey & Britt, 1993; Hughes, 1999, 2001; Schweigert *et al.*, 2003). Melanin, on the other hand, has a more equivocal relationship with health. Melanin provides protection from UV radiation, reducing the risk of sunburn and skin cancer (Daniels *et al.*, 1973; Robins, 1991), and preventing the photolysis of folate (thereby reducing the risk of neural tube defects in offspring; Branda & Eaton, 1978). However, melanin also prevents the photosynthesis of vitamin D in the skin, increasing the risk of rickets (Murray, 1934).

I have shown that carotenoid colour provides a perceptible cue to health in human faces. This colouration provides information about the diet (fruit and vegetable and carotenoid intake) of the individual, and may also provide information about the immune and reproductive status of the individual. Melanin also increases the apparent health of human faces, though this may be primarily due to its similarity in colour to carotenoids. Since apparent health is closely related to attractiveness (Jones *et al.*,

2004; Jones *et al.*, 2005), carotenoid (and maybe also melanin) colour may be sexually selected and affect the evolution of human skin colour.

In Section 6, I examined the effect of ethnicity on the preference for skin colour in human faces. There was no effect of face ethnicity on the amount of yellowness and lightness change that was applied to the faces to optimise healthy appearance. More redness was added to East Asian faces to optimise healthy appearance, though this could be attributed to the less red initial colour of the East Asian faces. Faces of all ethnicities lay along the same regression line relating initial facial redness to amount of red change applied to optimise healthy appearance of the faces. The ethnicity of the participants did not affect the amount of yellowness or redness applied to faces to optimise healthy appearance. African participants lightened faces more than did UK participants. I attributed this to the greater racial stratification of South African society than UK society (Treiman *et al.*, 1996; Yach & Harrison, 1995). This hypothesis suggests that, since wealth and health care provision is disproportionately held by the white population, people in this society may associate lightness of skin with health (Jones, 2000). However, this hypothesis warrants further study.

7.1 Effects on Evolution

Since the apparent health of human faces is very closely associated with attractiveness (Jones *et al.*, 2004, 2005), the preferences discussed in this thesis may have affected, and indeed may still be affecting the sexual selection of human skin colour, driving selection for yellower and redder skin, containing more carotenoid and more oxygenated blood. There is a tendency to associate sexually selected traits solely with greatly exaggerated, sexually dimorphic traits that are the result of runaway (Fisherian)

sexual selection. Such an association may lead to the conclusion that the preferences described in this thesis are unlikely to have been subject to sexual selection, as human skin is not bright red or bright yellow. This conclusion would be unwarranted for a number of reasons.

Firstly, in order for an exaggerated trait to evolve, sexual selection must initially act on a much less exaggerated form of the trait. Fisher (1958) noted that a trait such as slightly longer tail feathers in a bird species may confer on a male a survival and foraging advantage via improved agility. Females who mate with males with the longer tail feathers would therefore obtain the direct benefits of improved male provisioning, as well as the indirect benefits of obtaining alleles for slightly longer tail feathers for her offspring. In this way, selection for female choice for an advantageous trait begins the process of the coevolution of female preference and male trait in a classic Fisherian process (Andersson, 1994; Fisher, 1958). The preference for carotenoid colour in human faces may be analogous to the early stages of this process. Individuals who consume more carotenoids and more fruit and vegetables in the normal diet display more carotenoid in their skin and therefore have yellower skin (Section 2). Serum carotenoid levels, which correlate with skin carotenoid levels (Stahl *et al.*, 1998), are affected by the efficiency of uptake of carotenoids from the gut after consumption of fruit and vegetables (Stahl & Sies, 1992), which can be disrupted by gut parasites (Horak *et al.*, 2004). Increased carotenoid levels are thought to have a number of health benefits, including improved reproductive (Brief & Chew, 1985; Schweigert *et al.*, 2003) and immune function (Hughes, 1999, 2001) and photoprotection (Matthews-Roth *et al.*, 1974), and individuals with increased carotenoid levels may historically have been better foragers and be less affected by

gut parasites (Horak *et al.*, 2004). Individuals who prefer to mate with individuals who have this health benefit would therefore obtain the direct benefits of improved provisioning and reduced exposure to parasites. They may also obtain indirect benefits of genes for parasite resistance and improved carotenoid uptake for their offspring. This would provide a selection pressure for sexual choice for increased carotenoid colour. Support is provided for this hypothesis by Section 5, which shows that people perceive increased carotenoid colour as healthy. Since apparent health and attractiveness are very closely related (Jones *et al.*, 2004, 2005), this may indicate mate choice for individuals displaying more carotenoid colour.

It may be asked at this stage why human skin is not therefore bright yellow, as are bird carotenoid ornaments. As mentioned above, we may be at an early stage in the evolution of bright carotenoid ornaments. Carotenoids are deposited in the highest concentrations in the top layer of skin where they are most visible (Edwards & Duntley, 1939; La Placa *et al.*, 2000), but we are not yet bright yellow. Additionally, sexually selected traits are limited in their size and extravagance by natural selection, by competing forms of sexual selection, and by limiting factors in the environment. In guppies, males with larger carotenoid-based orange spots are preferred by females. Why then have the spots not expanded to cover the entire organism? Beyond a certain size, the attractiveness of the orange spots levels off (Andersson, 1994; Houde, 1987). Similarly, in humans, it may be that beyond a certain level of yellowness, additional carotenoid colour is no longer preferred. This is supported by Section 5, which shows that participants do not select the highest possible amount of carotenoid colour, but rather choose a less exaggerated amount of colouration. This may be related to the preference for averageness (Langlois & Roggman, 1990) and for a slight exaggeration

of attractive traits away from average, but not for greatly exaggerated traits very far from the population mean (Perrett *et al.*, 1994).

A balance of selection pressures has been suggested to explain the gradient of skin lightness from very dark near the equator to very light far from the equator (Jablonski & Chaplin, 2000). Under this hypothesis, dark skin is selected for its UV protective properties near the equator, whilst light skin is selected for its increased ability to synthesise vitamin D in the absence of large amounts of UV light at high latitudes. The carotenoid colour of the skin could be sexually selected by a preference for carotenoid coloured faces, while at the same time other selection pressures act to constrain the degree of carotenoid colouration in the skin. The amount of carotenoid colour in the skin is limited by the amount of carotenoids that can be obtained from the diet (Stahl *et al.*, 1998), which is in turn constrained by the amount of carotenoid-rich foods available and the other dietary needs of the body. A diet composed exclusively of carotenoid-rich fruits and vegetables (which would exclude meat, fish, dairy products and potatoes/grains) could lead to deficiencies of various nutrients or caloric deficiency. Indeed, vegetarians are at increased risk of a number of nutritional deficiencies (Lowik *et al.*, 1990). Further, displaying the carotenoids in the skin means that they are unavailable for use in immune defence and the other processes of the body (Saks *et al.*, 2003). It may be expected that selection pressures exist for an increased preference for the consumption of carotenoid-rich foods, and improved uptake of carotenoids in the gut.

Similarly, skin blood perfusion and oxygenation are known to be positively associated with aspects of health, such as physical fitness (Armstrong & Welsman, 2001;

Johnson, 1998) and an absence of certain illnesses (Charkoudian, 2003; Panza *et al.*, 1990). Yet here too it could be asked why human faces are not extreme in redness like the red patches of mandrills' faces. It could be noted that the lips of human faces are extreme in redness in light skinned groups (and many shades of lipstick are even more extreme in redness; Low, 1979). As well as the limits on skin redness caused by the preference for averageness, as discussed above, the amount of oxygenated blood colour in the skin could be limited by natural selection. Vasodilation, increasing skin blood perfusion, is a mechanism for rapid heat loss during exercise or other periods of heat stress (Johnson, 1998). Having large amounts of blood in the skin could cause excessive heat loss, which would be disadvantageous in temperate environments and also at night. Temporary flushing of the skin in a greatly exaggerated manner causes a rapid drop in blood pressure and could lead to fainting. This could potentially explain why flushing associated with social embarrassment, sexual arousal or anger takes place primarily in the face (and upper body to a lesser extent), where it will have the maximal impact as a social signal, whilst not reducing blood pressure to a dangerous extent. It is possible that evolution could select for mechanisms that would allow high levels of skin blood perfusion without excessive heat loss, or a significant drop in blood pressure.

7.2 Conclusions

This thesis has investigated the effects of skin colour on the apparent health of human faces, as well as the pigment basis of these effects. I have established that lightness, redness and yellowness appear healthy in faces, and that the skin levels of blood, especially oxygenated blood, and carotenoids are the basis of these effects. I have also linked carotenoid colour in the skin to natural dietary intake of carotenoids and fruit

and vegetables. The colour of carotenoids in the skin therefore has associations with the real health of the individual, as well as increasing the apparent health of the individual. Carotenoid colour provides a perceptible cue to human health. Blood colour, especially oxygenated blood colour also has associations with the actual health of the individual, as well as the apparent health of the individual, providing a perceptible cue to human health. These pigments, affecting healthy appearance (and therefore attractiveness; Jones *et al.*, 2004, 2005) may affect mate selection, and therefore may be subject to sexual selection, affecting the evolution of human skin colour.

References

Alaluf, S., Atkins, D., Barrett, K., Blount, M., Carter, N., & Heath, A. (2002a). Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin. *Pigment Cell Research, 15*, 112-118.

Alaluf, S., Heinrich, U., Stahl, W., Tronnier, H., & Wiseman, S. (2002b). Dietary Carotenoids Contribute to Normal Human Skin Color and UV Photosensitivity. *J. Nutr., 132*(3), 399-403.

Alaluf, S., Atkins, D., Barrett, K., Blount, M., Carter, N., & Heath, A. (2002c). The impact of epidermal melanin on objective measurements of human skin colour. *Pigment Cell Research, 15*, 119-126.

Alaluf, S., Heath, A., Carter, N., Atkins, D., Mahalingham, H., Barrett, K., et al. (2001). Variation in melanin content and composition in type V and VI photoexposed and photoprotected human skin: the dominant role of DHI. *Pigment Cell Research, 14*, 337-347.

Alexander, M., Newmark, H., & Miller, R. G. (1985). Oral β-carotene can increase the number of OKT4+ cells in human blood. *Immunology Letters, 9*, 221-224.

Allen, G. (1879). *The Colour-sense: Its Origin and Development*. Massachussetts: Houghton, Osgood and Company.

Amundsen, T., & Forsgren, E. (2001). Male mate choice selects for female colouration in a fish. *Proceedings of the National Academy of Science, 98*(23), 13155-13160.

Andersson, M. (1994). *Sexual Selection*. Princeton: Princeton University Press.

Aoki, K. (2002). Sexual selection as a cause of human skin colour variation: Darwin's hypothesis revisited. *Annals of Human Biology, 29*, 589-608.

Armstrong, N., & Welsman, J. R. (2001). Peak oxygen uptake in relation to growth and maturation in 11-17-year-old humans. *European Journal of Applied Physiology, 85*, 546-551.

Baird, D. D., Wilcox, A. J., Weinberg, C. R., Kamel, F., McConnaughey, D. R., Musey, P. I., et al. (1997). Preimplantation hormonal differences between the conception and non-conception menstrual cycles of 32 normal women. *Human Reproduction Update, 12*(12), 2607-2613.

Barber, N. (1995). The Evolutionary Psychology of Physical Attractiveness: Sexual Selection and Human Morphology. *Ethology and Sociobiology, 16*, 395-424.

Bateman, A. J. (1948). Intra-sexual selection in Drosophila. *Heredity, 2*, 349-368.

Beadle, P. C. (1977). The epidermal biosynthesis of cholecalciferol (vitamin D3). *Photochemistry and Photobiology, 25*, 519-527.

Bendich, A., & Olson, J. A. (1989). Biological actions of carotenoids. *FASEB Journal, 3*, 1927-1932.

Biard, C., Surai, P. F., & Moller, A. P. (2005). Effects of carotenoid availability during laying on reproduction in the blue tit. *Oecologia, 144*, 32-44.

Billingham, R. E. (1948). Dendritic cells. *Journal of Anatomy, 82*, 93-109.

Blair, J. R., Schatzki, R., & Orr, K. D. (1957). Sequellae to cold injury in one hundred patients. *Journal of the American Medical Association, 163*, 1203-1208.

Blount, J. D., Metcalfe, N. B., Birkhead, T. R., & Surai, P. F. (2003). Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science, 300*, 125-127.

Bogels, S. M., Alberts, M., & de Jong, P. J. (1996). Self-consciousness, self-focussed attention, blushing propensity and fear of blushing. *Personality and Individual Differences, 21*(4), 573-581.

Boman, H. G., & Hultmark, D. (1987). Cell-Free Immunity in Insects *Annual Review of Microbiology, 41*(1), 103-126. doi:10.1146/annurev.mi.41.100187.000535.
Booth, A., & Dabbs, J. (1993). Testosterone and men's marriages. *Social Forces, 72*, 463-477.
Borgerhoff Mulder, M. (1988). Kipsigis bridewealth payments. In Betzig, L.L., Borgerhoff Mulder, M., Turke, P.W. (Eds) *Human Reproductive Behaviour* (pp 65-82). Cambridge: Cambridge University Press.
Bortolotti, G. R., Negro, J. J., Surai, P. F., & Pricto, P. (2003). Carotenoids in eggs and plasma of red-legged partridges: effects of diet and reproductive output. *Physiological and Biochemical Zoology, 76*(3), 367-374.
Bourne, G. R., Breden, F., & Allen, T. C. (2003). Females prefer carotenoid coloured males as mates in the pentamorphic livebearing fish, *Poecilia parae*. *Naturwissenschaften, 90*, 402-405.
Branda, R., & Eaton, J. (1978). Skin color and nutrient photolysis: an evolutionary hypothesis. *Science, 201*(4356), 625-626. 10.1126/science.675247.
Brief, S., & Chew, B. P. (1985). Effects of vitamin A and β-carotene on reproductive performance in gilts. *Journal of Animal Science, 60*(4), 998-1004.
Brown, J. L. (1997). A theory of mate choice based on heterozygosity. *Behavioral Ecology, 8*, 60-65.
Brown, J. L. (1999). The new heterozygosity theory of mate choice and the MHC. *Genetica, 104*, 215-221.
Brown, W. M., Price, M. E., Kang, J., Pound, N., Zhao, Y., & Yu, H. (2008). Fluctuating asymmetry and preferences for sex-typical bodily characteristics. *PNAS, 105*, 12938-12943.
Burkhart, C. G., & Burkhart, C. N. (2005). The mole theory: primary function of melanocytes and melanin may be antimicrobial defense and immunomodulation (not solar protection). *International Journal of Dermatology, 44*(4), 340-342. doi:10.1111/j.1365-4632.2004.02556.x.
Burt, D. M., & Perrett, D. I. (1995). Perception of Age in Adult Caucasian Male Faces: Computer Graphic Manipulation of Shape and Colour Information. *Proceedings: Biological Sciences, 259*(1355), 137-143.
Buss, D. M. (1989). Sex differences in human mate preferences: evolutionary hypotheses tested in 37 cultures. *Behavioral and Brain Sciences, 12*, 1-49.
Buss, D.M. & Dedden, L.A. (1990) Derogation of competitors. *Journal of Social and Personal Relationships, 7*, 395-422.
Byard, P. J. (1981). Quantitative genetics of human skin colour. *Yearbook of Physical Anthropology, 24*, 123-137.
Carroll, J., Murphy, C. J., Neitz, M., ver Hoeve, J. N., & Neitz, J. (2001). Photopigment basis for dichromatic colour vision in the horse. *Journal of Vision, 1*, 80-87.
Chagnon, N. (1968). Yanomamo social organisation and welfare. In M. Fried, M. Harris & R. Murphy (Eds.), *War: The Anthropology of Armed Conflict and Aggression*. Garden City: The Natural History Press.
Changizi, M. A., Zhang, Q., & Shimojo, S. (2006). Bare skin, blood and the evolution of primate colour vision. *Biology Letters, 2*(2), 217-221.
Charkoudian, N. (2003). Skin Blood Flow in Adult Human Thermoregulation: How It Works, When It Does Not, and Why. *Mayo Clin Proc, 78*(5), 603-612 603.

Charkoudian, N., Stephens, D. P., Pirkle, K. C., Kosiba, W. A., & Johnson, J. M. (1999). Influence of female reproductive hormones on local thermal control of skin blood flow. *J Appl Physiol, 87*(5), 1719-1723.

Chew, B. P. (1993). Effects of supplemental beta-carotene and vitamin A on reproduction in swine. *J. Anim Sci., 71*(1), 247-252.

Chung, S.-J., Hoerr, S., Levine, R., Coleman, G. (2006). Processes underlying young women's decisions to eat fruits and vegetables. *Journal of Human Nutrition and Dietetics, 19,* 287-298.

Coffey, M. T., & Britt, J. H. (1993). Enhancement of sow reproductive performance by beta-carotene or vitamin A. *J. Anim Sci., 71*(5), 1198-1202.

Cronk, L., Dunham, B. (2007). Amounts spent on engagement rings reflect aspects of male and female mate quality. *Human Nature, 18,* 329-333

Crook, J. H. (1972). Sexual selection, dimorphism and social organisation in the primates. In B. Campbell (Ed.), *Sexual Selection and the Descent of Man* (pp. 231-281). London: Heinemann.

Cunningham, M. R., Roberts, A. R., Barbee, A. P., Druen, P. B., & Wu, C.-H. (1995). "Their ideas of beauty are, on the whole, the same as ours": Consistency and variability in the cross-cultural perception of female physical attractiveness. *Journal of Personality and Social Psychology, 68*(2), 261-279.

Daniels, F. J. W., van der Leun, K. C., & Johnson, B. E. (1973). Sunburn. In C. L. Brace & J. Metress (Eds.), *Man in Evolutionary Perspective*. Toledo.

Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*.

DeBruine, L. M., Jones, B. C., Unger, L., Little, A. C., & Feinberg, D. R. (2007). Dissociating averageness and attractiveness: Attractive faces are not always average. *Journal of Experimental Psychology: Human Perception and Performance, 33*(6), 1420-1430.

Dietary Guideline Advisory Committee (2005) Nutrition and Your Health: Dietary Guidelines for Americans. www.health.gov/dietaryguidelines

Dixson, A.F. (1983) Observations on the evolution and behavioural significance of "sexual skin" in female primates. *Advances in the Study of Behaviour, 13,* 63-106.

Dobzhansky, T. (1970). *Genetics of the Evolutionary Process*. New York: Columbia University Press.

Dominy, N. J., Garber, P. A., Bicca-Marques, J. C., & Azevedo-Lopes, M. A. d. O. (2003). Do female tamarins use visual cues to detect fruit rewards more successfully than do males? *Animal Behaviour, 2003,* 829-837.

Drummond, P. D., & Quah, S. H. (2001). The effect of expressing anger on cardiovascular reactivity and facial blood flow in Chinese and Caucasians. *Psychophysiology, 38,* 190-196.

Dulai, K. S., von Dornum, M., Mollon, J. D., & Hunt, D. M. (1999). The evolution of trichromatic colour vision by opsin gene duplication in New World and Old World primates. *Genome Research, 9,* 629-638.

Edwards, E. A., & Duntley, S. Q. (1939). The pigments and color of living human skin. *American Journal of Anatomy, 65*(1), 1-33.

Elias, P. M., & Feingold, K. R. (2003). Skin as an organ of protection. In I. M. Freedberg, A. Z. Eisen, K. Wolff, K. F. Austen, L. A. Goldsmith & S. Katz (Eds.), *Fitzpatrick's Dermatology in General Medicine* (pp. 164-174). Philadelphia: McGraw-Hill.

Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution, 34,* 76-91.

Feather, J. W., Ellis, D. J., & Leslie, G. (1988). A portable reflectometer for the rapid quantification of cutaneous haemoglobin and melanin. *Physics in Medicine and Biology, 33*, 711-722.
Feinmann, S., & Gill, G. W. (1978). Sex differences in physical attractiveness preferences. *Journal of Social Psychology, 105*, 43-52.
Fernandez, A. A., & Morris, M. R. (2007). Sexual selection and trichromatic colour vision in primates: statistical support for the preexisting-bias hypothesis. *American Naturalist, 170*, 10-20.
Fink, B., Grammer, K., & Matts, P. J. (2006). Visible skin color distribution plays a role in the perception of age, attractiveness and health in female faces. *Evolution and Human Behavior, 27*(6), 433-442.
Fink, B., Grammer, K., & Thornhill, R. (2001). Human (*Homo sapiens*) facial attractiveness in relation to skin texture and colour. *Journal of Comparative Psychology, 115*(1), 92-99.
Fisher, R. A. (1958). *The Genetical Theory of Natural Selection*. New York: Dover Publications.
Fisher, M.,L. (2004) Female intrasexual competition decreases female facial attractiveness. *Proceedings of the Royal Society of London B (Supplement), 271*, S283-S285
Folstad, I., & Karter, A. J. (1992). Parasites, bright males and the immunocompetence hypothesis. *American Naturalist, 139*, 603-622.
Forman, M., Beecher, G., Muesing, R., Lanza, E., Olson, B., Campbell, W., et al. (1996). The fluctuation of plasma carotenoid concentrations by phase of the menstrual cycle: a controlled diet study. *Am J Clin Nutr, 64*(4), 559-565.
Fox, C. A., Wolf, H. S., & Baker, J. A. (1970). Measurement of intra-vaginal and intra-uterine pressures during human coitus by radio-telemetry. *Journal of Reproduction and Fertility, 22*, 243-251.
Friis, H., Gomo, E., Kastel, P., Ndhlovu, P., Nyazema, N., Krarup, H., et al. (2001). HIV and other predictors of serum {beta}-carotene and retinol in pregnancy: a cross-sectional study in Zimbabwe. *Am J Clin Nutr, 73*(6), 1058-1065.
Furnham, A., Moutafi, J., & Baguma, P. (2002). A cross-cultural study on the role of weight and waist-to-hip ratio on female attractiveness. *Personality and Individual Differences, 32*, 729-745.
Furnham, A., & Nording, R. (1998). Cross-cultural differences in preferences for specific male and female body shapes. *Personality and Individual Differences, 25*, 635-648.
Gangestad, S. W., Thornhill, R., & Garver-Apgar, C. E. (2005). Women's sexual interests across the ovulatory cycle depend on primary partner developmental instability. *Proceedings of the Royal Society B: Biological Sciences, 272*(1576), 2023-2027.
Gangestad, S. W., Thornhill, R., & Yeo, R. A. (1994). Facial attractiveness, developmental stability, and fluctuating asymmetry. *Ethology and Sociobiology, 15*, 73-85.
Getty, T. (2002). Signalling health versus parasites. *American Naturalist, 159*, 363-371.
Gibson, R. M. (1996). Female choice in sage grouse: The roles of attraction and active comparison. *Behavioral Ecology and Sociobiology, 39*, 55-59.
Godin, G., & Shephard, R. J. (1997). Godin Leisure Time Exercise Questionnaire. *Medicine and Science in Sports and Exercise, 29 June Supplement*, S36-S38.

Gonzalez, G., Sorci, G., & de Lope, F. (1999). Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology, 46*, 117-122.
Goodwin, T. W. (1984). *The Biochemistry of Carotenoids*. London: Chapman and Hall.
Goswami, R., Gupta, N., Goswami, D., Marwaha, R. K., Tandon, N., & Kochupillai, N. (2000). Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *Am J Clin Nutr, 72*, 472-475.
Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology, 144*, 517-546.
Grammer, K., Fink, B., Moller, A. P., & Thornhill, R. (2003). Darwinian aesthetics: sexual selection and the biology of beauty. *Biology Review, 78*, 385-407.
Grammer, K., & Thornhill, R. (1994). Human (Homo sapiens) facial attractiveness and sexual selection: The role of symmetry and averageness. *Journal of Comparative Psychology, 108*(3), 233-242.
Gurven, M. (2004). To give and to give not: the behavioural ecology of human food transfers. *Behavioural and Brain Sciences, 27*, 543-583.
Halberstadt, J. & Rhodes, G. (2000) The attractiveness of nonface averages: Implications for an evolutionary explanation of the attractiveness of average faces. *Psychological Science, 11*, 285-289.
Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science, 218*, 384-387.
Hamilton, W. J. (1973). *Life's Colour Code*: McGraw-Hill.
Hasselquist, D., Marsh, J. A., Sherman, P. W., & Wingfield, J. C. (1999). Is avian humoral immunocompetence suppressed by testosterone? *Behavioral Ecology and Sociobiology, 45*, 167-175.
Hedrick, P. W., & Black, F. L. (1997). HLA and mate selection: no evidence in south Amerindians. *American Journal of Human Genetics, 61*, 505-511.
Hennessy, A., Oh, C., Diffey, B., Wakamatsu, K., Ito, S., & Rees, J. (2005). Eumelanin and pheomelanin concentrations in human epidermis before and after UVB irradiation. *Pigment Cell Research, 18*, 220-223.
Hill, G. E. (1990). Female house finches prefer colourful males: Sexual selection for a condition-dependent trait. *Animal Behaviour, 40*(3), 563-572.
Hill, G. E. (1991). Plumage coloration in a sexually selected indicator of male quality. *Nature, 350*, 337-339.
Hill, H. Z., & Hill, G. J. (2000). UVA, pheomelanin and the carcinogenesis of melanoma. *Pigment Cell Research, 13(Supp. 8)*, 140-144.
Hill, R. A., & Barton, R. A. (2005a). Psychology Red enhances human performance in contests. *435*(7040), 293.
Hill, R. A., & Barton, R. A. (2005b). Reply: Seeing red? Putting sportswear in context. *Nature, 437*, E10-E11.
Hiramatsu, C., Melin, A. D., Aureli, F., Schaffner, C. M., Vorobyev, M., Matsumoto, Y., et al. (2008). Importance of Achromatic Contrast in Short-Range Fruit Foraging of Primates. *PLoS ONE, 3*(10), e3356.
Hong, G., Luo, M. R., & Rhodes, P. A. (2001). A study of digital camera colorimetric characterization based on polynomial modeling. *Color Research & Application, 26*(1), 76-84.
Horak, P., Saks, L., Karu, U., Ots, I., Surai, P. F., & McGraw, K. J. (2004). How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology, 73*, 935-947.

Hoskinson, C. D., Chew, B. P., & Wong, T. S. (1989). Effects of β-carotene (BC) and vitamin A (VA) on mitogen-induced lymphocyte proliferation in the pig *in vivo*. *Federation of American Societies for Experimental Biology Journal, 3*, 663.

Hoskinson, C. D., Chew, B. P., & Wong, T. S. (1992). Effects of injectable β-carotene and vitamin A on lymphocyte proliferation and polymorphonuclear neutrophil function in piglets. *Biology of the Neonate, 62*, 325.

Houde, A. E. (1987). Mate choice based on naturally occurring color patern variation in a guppy population. *Evolution, 41*, 1-10.

Hughes, D. A. (1999). Effects of carotenoids on human immune function. *Proceedings of the Nutrition Society, 58*, 713-718.

Hughes, D. A. (2001). Dietary carotenoids and human immune function. *Nutrition, 17*(10), 823-827.

Hulse, F. S. (1967). Selection for skin colour among the Japanese. *American Journal of Physical Anthropology, 27*, 143-156.

Hussain, Z., Roberts, N., Whitehouse, G., Garcia-Finana, M., & Percy, D. (1999). Estimation of breast volume and its variation during the menstrual cycle using MRI and stereology. *Br J Radiol, 72*(855), 236-245.

Ihara, Y., Aoki, K., Tokumaga, K., Takahashi, K., & Juji, T. (2000). HLA and human mate choice: tests on Japanese couples. *Anthropological Science, 108*, 199-214.

Institute of Medicine (2000) Food and Nutrition Board: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington DC: National Academy Press.

Iozumi K, Hoganson GE, Pennella R, Everett MA & Fuller BB. (1993) Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. *Journal of Investigative Dermatology, 100*, 806–811.

Jablonski, N. G. (2006). *Skin: A Natural History*. California: University of California Press.

Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin colour. *Journal of Human Evolution, 39*, 57-106.

Jacobs, G. H. (1993). The distribution and nature of colour vision among the mammals. *Biological Reviews of the Cambridge Philosophical Society, 68*, 413-471.

Jimbow, K., Pathak, M. A., Szabo, G., & Fitzpatrick, T. B. (1974). Ultrastructural changes in human melanocytes after ultraviolet radiation. In T. B. Fitzpatrick (Ed.), *Sunlight and Man* (pp. 195-215). Tokyo: University of Tokyo Press.

Johnson, J. M. (1998). Physical training and the control of skin blood flow. *Medicine and Science in Sports and Exercise, 30*, 382-386.

Johnston, V. S., & Franklin, M. (1993). Is beauty in the eye of the beholder? *Ethology and Sociobiology, 14*, 183-199.

Jones, B. C., Little, A. C., Penton-Voak, I. S., Tiddeman, B.P., Burt, D.M., Perrett, D.I. (2001) Facial symmetry and judgements of apparent health: Support for a "good genes" explanation of the attractiveness-symmetry relationship. *Evolution and Human Behavior, 22*, 417-429.

Jones, B. C., Little, A. C., Burt, D. M., & Perrett, D. I. (2004). When facial attractiveness is only skin deep. *Perception, 33*, 569-576.

Jones, B. C., Perrett, D. I., Little, A. C., Boothroyd, L., Cornwell, R. E., Feinberg, D. R., et al. (2005). Menstrual cycle, pregnancy and oral contraceptive use alter

attraction to apparent health in faces. *Proceedings of the Royal Society B: Biological Sciences, 272*(1561), 347-354.
Jones, D. (1995). Sexual selection, physical attractiveness and facial neoteny: cross-cultural evidence and implications. *Current Anthropology, 36*(5), 723-748.
Jones, D. (2000). Physical attractiveness, race and somatic prejudice in Bahia, Brazil. In L. Cronk, N. Chagnon & W. Irons (Eds.), *Adaptation and Human Behaviour: An Anthropological Perspective* (pp. 133-152).
Karino, K., & Haijima, Y. (2004). Algal-diet enhances sexual ornament, growth and reproduction in the guppy. *Behaviour, 141*, 585-601.
Kirkpatrick, M., & Ryan, M. J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature, 350*, 33-38.
Knight, C.D. (1991). *Blood Relations. Menstruation and the Origins of Culture.* New Haven and London: Yale University Press.
Kollias, N. (1995). The physical basis of skin color and its evaluation. *Clinics in Dermatology, 13*, 361-367.
Kollias, N., Sayer, R. M., Zeise, L., & Chedekel, M. R. (1991). Photoprotection by melanin. *Journal of Photochemistry and Photobiology, 9*, 135-160.
Krinsky, N. I., Cornwell, D. G., & Oncley, J. L. (1958). The transport of vitamin A and carotenoids in human plasma. *Archives of Biochemistry and Biophysics, 73*, 233-246.
La Placa, M., Pazzaglia, M., & Tosti, A. (2000). Lycopenaemia. *Journal of the European Academy of Dermatology & Venereology, 14*(4), 311-312.
Laland, K. N. (1994). Sexual selection with a culturally transmitted mating preference. *Theoretical Population Biology, 45*, 1-15.
Langlois, J. H., Kalakanis, L., Rubenstein, A. J., Larson, A., Hallam, M., & Smoot, M. (2000). Maxims or myths of beauty? A meta-analytic and theoretical review. *Psychological Bulletin, 126*(3), 390-423.
Langlois, J. H., Ritter, J. M., Roggman, L. A., & Vaughn, L. S. (1991). Facial diversity and infant preferences for attractive faces. *Developmental Psychology, 27*(1), 79-84.
Langlois, J. H., & Roggman, L. A. (1990). Attractive faces are only average. *Psychological Science, 1*(2), 115-121.
Langlois, J. H., Roggman, L. A., & Musselman, L. (1994). What is average and what is not average about attractive faces? *Psychological Science, 5*(4), 214-220.
Law Smith, M. J., Perrett, D. I., Jones, B. C., Cornwell, R. E., Moore, F. R., Feinberg, D. R., et al. (2006). Facial appearance is a cue to oestrogen levels in women. *Proceedings of the Royal Society B: Biological Sciences, 273*(1583), 135-140.
Le Boeuf, B. J. (1974). Male-male competition and reproductive success in elephant seals. *American Zoologist, 14*, 163-176.
Leary, M. R., Britt, T. W., Cutlip, W. D., & Templeton, J. L. (1992). Social blushing. *Psychological Bulletin, 112*(3), 446-460.
Leary, R. F., Allendorf, F. W., & Knudsen, K. L. (1985). Inheritance of meristic variation and the evolution of developmental stability in rainbow trout. *Evolution, 39*, 308-314.
Lindstrom, K. M., Krakower, D., Lundstrom, J. O., & Silverin, B. (2001). The effects of testosterone on a viral infection in greenfinches (*Carduelis chloris*): an experimental test of the immunocompetence handicap hypothesis. *Proceedings of the Royal Society B: Biological Sciences, 268*, 207-211.

Lipson, S. F., & Ellison, P. T. (1996). Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Human Reproduction, 11*(10), 2090-2096.

Little, A. C., Burt, D. M., Penton-Voak, I., & Perrett, D. I. (2001). Self-perceived attractiveness influences human female preferences for sexual dimorphism and symmetry in male faces. *Proceedings of the Royal Society B: Biological Sciences, 268*, 39-44.

Little, A. C., Jones, B. C., Burt, D. M., & Perrett, D. I. (2007). Preferences for symmetry in faces change across the menstrual cycle. *Biological Psychology, 76*, 209-216.

Little, A. C., Jones, B. C., Penton-Voak, I. S., Burt, D. M., & Perrett, D. I. (2002). Partnership status and the temporal context of relationships influence human female preferences for sexual dimorphism in male face shape. *Proceedings of the Royal Society B: Biological Sciences, 269*, 1095-1100.

Liu, D., Moberg, E., Kollind, M., Lins, P.-E., Adamson, U., & Macdonald, I. A. (1992). Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. *Diabetologia, 35*(3), 287-290.

Loomis, W. F. (1967). Skin-pigment regulation of vitamin-D biosynthesis in man. *Science, 157*(788), 501-506.

Low, B. S. (1979). Sexual selection and human ornamentation. In N. Chagnon & W. Irons (Eds.), *Evolutionary Biology and Human Social Behaviour*. North Slituate, MA: Duxbury Press.

Lowik, M. R., Schrijver, J., Odink, J., van den Berg, H., & Wedel, M. (1990). Long-term effects of a vegetarian diet on the nutritional status of elderly people (Dutch Nutrition Surveillance System). *Journal of the American College of Nutrition, 9*(6), 600-609.

Lozano, G. A. (1994). Carotenoids, Parasites, and Sexual Selection. *Oikos, 70*(2), 309-311.

Lucas, P. W., Darvell, B. W., Lee, P. K. D., Yuen, T. D. B., & Choong, M. F. (1998). Colour cues for leaf food selection by long-tailed macaques (*Macaca fascicularis*) with a new suggestion for the evolution of trichromatic colour vision. *Folia Primatologica, 69*, 139-152.

Lucas, P. W., Dominy, N. J., Riba-Hernandez, P., Stoner, K. E., Yamashita, N., Loria-Calderon, E., et al. (2003). Evolution and function of routine trichromatic vision in primates. *Evolution, 57*(11), 2636-2643.

MacDougall, A. K., & Montgomerie, R. (2003). Assortative mating by carotenoid-based plumage colour: a quality indicator in American goldfinches, *Carduelis tristis*. *Naturwissenschaften, 90*, 464-467.

Maddox, K. B., & Gray, S. A. (2002). Cognitive representations of black Americans: reexploring the role of skin tone. *Personality and Social Psychology Bulletin, 28*, 250-259.

Manning, J. T., Scutt, D., & Lewis-Jones, D. I. (1998). Developmental stability, ejaculate size, and sperm quality in men. *Evolution and Human Behaviour, 19*, 273-282.

Manning, J. T., Scutt, D., Whitehouse, G. H., Leinster, S. J., & Walton, J. M. (1996). Asymmetry and the menstrual cycle in women. *Ethology and Sociobiology, 17*(2), 129-143.

Margetts, B. M., Cade, J. E., & Osmond, C. (1989). Comparison of a Food Frequency Questionnaire with a Diet Record. *Int. J. Epidemiol., 18*(4), 868-873. 10.1093/ije/18.4.868.

Martinez-Padilla, J., Mougeot, F., Perez-Rodrigues, L., & Bortolotti, G. R. (2007). Nematode parasites reduce carotenoid-based signalling in male red grouse. *Biology Letters, 3*(2), 161-164.

Martinkauppi, B. Face Colour Under Varying Illumination – Analysis and Applications. Oulo Yliopisto, Oulu.

Massaro, M., Davis, L., & Darby, J. (2003). Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (Megadyptes antipodes). *Behavioral Ecology and Sociobiology, 55*(2), 169-175.

Matthews-Roth, M. M., Pathak, U. A., Fitzpatrick, T. B., Harber, L. C., & Kass, E. H. (1974). Beta-carotene as an oral photoprotective agent in erythropoietic protoporphyria. *Journal of the American Medican Association, 228*(8), 1004-1008.

Matts, P. J., Fink, B., Grammer, K., & Burquest, M. (2007). Colour homogeneity and visual perception of age, health and attractiveness of female facial skin. *J Am Acad Dermatol, 57*(6), 977-984.

Mazur, A., & Booth, A. (1998). Testosterone and dominance in men. *Behavioural and Brain Sciences, 21*, 353-397.

McClarty, J. W., Holiday, D. B., Girard, W. M., Yanagihara, R. H., Kummet, T. D., & Greenberg, S. D. (1995). β-carotene, vitamin A, and lung cancer chemoprevention: results of an intermediate endpoint study. *American Journal of Clinical Nutrition, 62*, 1S-8S.

McGraw, K. J., & Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: An experimental test. *The American Naturalist, 162*(6), 704-712.

McGraw, K. J., & Hill, G. E. (2000). Differential effects of endoparasitism on the expression of carotenoid and melanin-based ornamentation colouration. *Proceedings of the Royal Society B: Biological Sciences, 267*, 1525-1531.

McGraw, K. J., Stoehr, A. M., Nolan, P. M., & Hill, G. E. (2001). Plumage redness predicts breeding onset and reproductive success in the house finch: a validation of Darwin's theory. *Journal of Avian Biology, 32*, 90-94.

Mealey, L., Bridgstock, R., & Townsend, G. C. (1999). Symmetry and perceived facial attractiveness: A monozygotic co-twin comparison. *Journal of Personality and Social Psychology, 76*(1), 151-158.

Meggitt. (1977). *Blood is their Argument: Warfare among the Mae Enga Tribesmen of the New Guinea Highlands*. Palo Alto: Mayfield.

Melia, J., & Bulman, A. (1995). Sunburn and tanning in a British population. *Journal of Public Health Medicine, 17*, 223-229.

Mikhail, G. R. (1963). Hair neogenesis in rat skin. *Archives of Dermatology, 88*, 713-728.

Miller, E. S. (1937). A precise method, with detailed calibration for the determination of absorption coefficients; the quantitative measurement of the visible and ultraviolet absorption spectra of alpha carotene, beta carotene, and lycopene. *Plant Physiology, 12*, 667-684.

Moller, A. P., Christe, P., & Lux, E. (1999). Parasitism, host immune function, and sexual selection. *Quarterly Review of Biology, 74*, 3-20.

Møller, A. P., Soler, M., & Thornhill, R. (1995). Breast asymmetry, sexual selection, and human reproductive success. *Ethology and Sociobiology, 16*(3), 207-219.

Moller, A. P., & Thornhill, R. (1998). Bilateral Symmetry and Sexual Selection: A Meta-Analysis. *The American Naturalist, 151*(2), 174-192. doi:10.1086/286110.
Monk, B. (1983). Carotenemia. *International Journal of Dermatology, 22*(6), 376-377.
Mougeot, F., Irvine, J. R., Seivwright, L., Redpath, S. M., & Piertney, S. (2004). Testosterone, immunocompetence, and honest sexual signalling in male red grouse. *Behavioural Ecology, 15*, 930-937.
Mueller, U., & Mazur, A. (1998). Facial dominance in *Homo sapiens* as honest signalling of male quality. *Behavioral Ecology, 8*, 569-579.
Muhe, L., Oljira, B., Degefu, H., Jaffar, S., & Weber, M. W. (2000). Evaluation of clinical pallor in the identification and treatment of children with moderate and severe anaemia. *Tropical Medicine & International Health, 5*(11), 805-810. doi:10.1046/j.1365-3156.2000.00637.x.
Mulvey, M., Keller, G. P., & Meffe, G. K. (1994). Single and multiple-locus genotypes and life-history responses of *Gambusia holbrooki* reared at two temperatures. *Evolution, 48*, 1810-1819.
Murray, F. G. (1934). Pigmentation, sunlight and nutritional disease. *American Anthropologist, 36*(3), 438-448.
Navarra, K. J., & Hill, G. E. (2003). Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration. *Behavioural Ecology, 14*(6), 909-916.
Neer, R. M. (1975). The evolutionary significance of vitamin D, skin pigment, and ultraviolet light. *American Journal of Physical Anthropology, 43*, 409-416.
Nolan, P. M., Hill, G. E., & Stoehr, A. M. (1998). Sex, size, and plumage redness predict house finch survival in an epidemic. *Proceedings: Biological Sciences, 265*, 961-965.
Ober, C. (1999). Studies of HLA, fertility and mate choice in a human isolate. *Human Reproduction Update, 5*, 103-107.
Oinonen, K. A., & Mazmanian, D. (2007). Facial symmetry detection ability changes across the menstrual cycle. *Biological Psychology, 75*(2), 136-145.
Olson, V. A., & Owens, I. P. F. (1998). Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution, 13*(12), 510-514.
Olsson, M., Wapstra, E., Madsen, T., & Silverin, B. (2000). Testosterone, ticks and travels: a test of the immunocompetence handicap hypothesis in a free-ranging male sand lizards. *Proceedings of the Royal Society B: Biological Sciences, 267*, 2339-2343.
Omaye, S. T. (1993). Nutrient deficiencies and pregnancy outcome. In R. P. Sharma (Ed.), *Dietary Factors and Birth Defects*. San Francisco.
Panza, J., Quyyumi, A., Brush, J., & Epstein, S. (1990). Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med, 323*(1), 22-27.
Park, E. A. (1932). Some aspects of rickets. *Canadian Medical Association Journal, 26*, 3-5.
Parker, G. A. (1983). Mate quality and mating decisions. In P. Bateson (Ed.), *Mate Choice* (pp. 141-166). Cambridge: Cambridge University Press.
Parker, G. A., & Simmons, L. W. (1996). Parental investment and the control of sexual selection: predicting the direction of sexual competition. *Proceedings of the Royal Society B: Biological Sciences, 263*, 315-321.
Pawson, I. G., & Petrakis, N. L. (1975). Comparisons of breast pigmentation among women of different racial groups. *Human Biology, 47*, 441-450.

Penn, D. J. (2002). The scent of genetic compatibility: sexual selection and the MHC. *Ethology, 801*, 1-21.
Penn, D. J., & Potts, W. K. (1999). The evolution of mating preferences and major histocompatibility genes. *American Naturalist, 153*, 145-164.
Penton-Voak, I. S., Jones, B. C., Little, A. C., Baker, S., Tiddeman, B., Burt, D. M., et al. (2001). Symmetry, sexual dimorphism in facial proportions and male facial attractiveness. *Proceedings of the Royal Society B: Biological Sciences, 268*(1476), 1617-1623.
Penton-Voak, I. S., Little, A. C., Jones, B. C., Burt, D. M., Tiddeman, B. P., & Perrett, D. I. (2003). Female condition influences preferences for sexual dimorphism in faces of male humans (Homo sapiens). *Journal of Comparative Psychology, 117*(3), 264-271.
Penton-Voak, I. S., & Perrett, D. I. (2000). Female preference for male faces changes cyclically: Further evidence. *21*(1), 39-48.
Penton-Voak, I. S., Perrett, D. I., Castles, D. L., Kobayashi, T., Burt, D. M., Murray, L. K., et al. (1999). Menstrual cycle alters face preference. *399*(6738), 741-742.
Perez-Rodrigues, L., & Vinuela, J. (2008). Carotenoid-based bill and eye ring colouration as honest signals of condition: an experimental test in the red-legged partridge (*Alectoris rufa*). *Naturwissenschaften, 95*(9), 821-830.
Perrett, D. I., Burt, D. M., Penton-Voak, I. S., Lee, K. J., Rowland, D. A., & Edwards, R. (1999). Symmetry and Human Facial Attractiveness. *20*(5), 295-307.
Perrett, D. I., Lee, K. J., Penton-Voak, I., Rowland, D., Yoshikawa, S., Burt, D. M., et al. (1998). Effects of sexual dimorphism on facial attractiveness. *394*(6696), 884-887.
Perrett, D. I., May, K. A., & Yoshikawa, S. (1994). Facial shape and judgements of female attractiveness. *368*(6468), 239-242.
Peskin, M., & Newell, F. N. (2004). Familiarity breeds attraction: Effects of exposure on the attractiveness of typical and distinctive faces. *Perception, 33*, 147-157.
Peters, A., Denk, A. G., Delhey, K., & Kempenaers, B. (2004). Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *Journal of Evolutionary Biology, 17*, 1111-1120.
Pierard, G. E. (1998). Continuing medical education. *Journal of the European Academy of Dermatology and Venereology, 10*(1), 1-11. doi:10.1111/j.1468-3083.1998.tb00921.x.
Pike, T. W., Blount, J. D., Bjerkeng, B., Lindstrom, J., & Metcalfe, N. B. (2007a). Carotenoids, oxidative stress and female mating preference for longer lived males. *Proceedings of the Royal Society B: Biological Sciences, 274*, 1591-1596.
Pike, T. W., Blount, J. D., Lindstrom, J., & Metcalfe, N. B. (2007b). Availability of non-carotenoid antioxidants affects the expression of a carotenoid-based sexual ornament. *Biology Letters, 3*, 353-356.
Polsinelli, M. L., Rock, C. L., Henderson, S. A., & Drewnowski, A. (1998). Plasma carotenoids as biomarkers of fruit and vegetable servings in women. *Journal of the American Dietetic Association, 98*(2), 194-196.
Ponsonby, A.-L., Dwyer, T., & Couper, D. (1997). Sleeping position, infant apnea, and cyanosis: a population-based study. *Pediatrics, 99*, e3,1.
Post, P. W., Szabo, G., & Keeling, M. E. (1975). A quantitative and morphological study of the pigmentation system of the chimpanzee with light and electron microscope. *American Journal of Physical Anthropology, 43*, 435-444.

Prince, M. R., & Frisoli, J. K. (1993). Beta-carotene accumulation in serum and skin. *American Journal of Clinical Nutrition, 57*, 175-181.
Rasmussen, K. (1931). *The Netsilik Eskimos: Social Life and Spiritual Culture*. Copenhagen.
Regan, B. C., Julliot, C., Simmen, B., Vienot, F., Charles-Dominique, P., & Mollon, J. D. (2001). Fruits, foliage and the evolution of primate colour vision. *Philosophical Transactions of the Royal Society of London B, 356*, 229-283.
Rhodes, G., Chan, J., Zebrowitz, L. A., & Simmons, L. W. (2003). Does sexual dimorphism in human faces signal health? *Proceedings of the Royal Society B: Biological Sciences, 270*(0), S93-S95.
Rhodes, G., Sumich, A., & Byatt, G. (1999). Are Average Facial Configurations Attractive Only Because of Their Symmetry? *Psychological Science, 10*(1), 52-58.
Rhodes, G., Yoshikawa, S., Clark, A., Lee, K., McKay, R., & Akamatsu, S. (2001a). Attractiveness of facial averageness and symmetry in non-Western cultures: In search of biologically based standards of beauty. *Perception, 30*, 611-625.
Rhodes, G., Yoshikawa, S., Palermo, R., Simmons, L. W., Peters, M., Lee, K., et al. (2007). Perceived health contributes to the attractiveness of facial symmetry, averageness, and sexual dimorphism. *Perception, 36*, 1244-1252.
Rhodes, G., Zebrowitz, L. A., Clark, A., Kalick, S. M., Hightower, A., & McKay, R. (2001b). Do facial averageness and symmetry signal health? *Evolution and Human Behaviour, 22*(1), 31-46.
Rhodes, L., Argersinger, M., Gantert, L., Friscino, B., Hom, G., Pikounis, B., et al. (1997). Effects of administration of testosterone, dihydrotestosterone, oestrogen and fadrozole, an aromatase inhibitor, on sex skin colour in intact male rhesus macaques. *J Reprod Fertil, 111*(1), 51-57. 10.1530/jrf.0.1110051.
Riba-Hernandez, P., Stoner, K. E., & Osorio, D. (2004). Effect of polymorphic colour vision for fruit detection in the spider monkey *Ateles geoffroyi*, and its implications for the maintenance of polymorphic colour vision in platyrrhine monkeys. *Journal of Experimental Biology, 207*, 2465-2470.
Richardson, D. (1987). Effects of tobacco smoke inhalation on capillary blood flow in human skin. *Archives of Environmental Health, 42*, 19-25.
Rikowski, A., & Grammer, K. (1999). Human body odour, symmetry and attractiveness. *Proceedings of the Royal Society B: Biological Sciences, 266*(1422), 869-874.
Roberts, S. C., Havlicek, J., Flegr, J., Hruskova, M., Little, A. C., Jones, B. C., et al. (2004). Female facial attractiveness increases during the fertile phase of the menstrual cycle. *Biology Letters, 271*, S270-S272.
Roberts, S. C., Little, A. C., Gosling, L. M., Perrett, D. I., Carter, V., Jones, B. C., et al. (2005). MHC-heterozygosity and human facial attractiveness. *Evolution and Human Behaviour, 26*, 213-226.
Robins, A. H. (1991). *Biological Perspectives on Human Pigmentation*. Cambridge: Cambridge University Press.
Rosentreich, S. J., Rich, C., & Volwiler, W. (1971). Depostion in and release of vitamin D3 from body fat: evidence for a storage site in the rat. *Journal of Clinical Investigation, 50*, 679-687.
Rowe, C., Harris, J. M., & Roberts, S. C. (2005). Seeing red? Putting sportswear in context. *Nature, 437*, E10.
Rubenstein, A. J., Langlois, J. H., & Roggman, L. A. (2001). What makes a face attractive and why: the role of averageness in defining facial beauty. In G.

Rhodes & L. A. Zebrowitz (Eds.), *Facial Attractiveness: Evolutionary, Cognitive and Social Perspectives*. Westport, CT: Ablex Publishing.
Russell, R. (2003). Sex, beauty and the relative luminance of facial features. *Perception, 32*, 1093-1107.
Russell, R. (2008). Cosmetics exaggerate a sex difference in facial contrast. *Personal Communication.*
Saino, N., Moller, A. P., & Bolzern, A. M. (1995). Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behavioral Ecology, 6*, 397-404.
Saks, L., Ots, I., & HÃµrak, P. (2003). Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia, 134*(3), 301-307.
Sarna, T., & Swartz, H. M. (1988). The physical properties of melanin. In J. J. Nordlund (Ed.), *The Pigmentary System*. Oxford: Oxford University Press.
Scheib, J. E., Gangestad, S. W., & Thornhill, R. (1999). Facial attractiveness, symmetry and cues of good genes. *Proceedings of the Royal Society B: Biological Sciences, 266*(1431), 1913-1917.
Schultz, F. W. (1932). The clinical significance of vitamin D in infancy and childhood. *Journal of the American Medical Association, 99*, 384-388.
Schweigert, F. J., Steinhagen, B., Raila, J., Siemann, A., Peet, D., & Buscher, U. (2003). Concentrations of carotenoids, retinol and {alpha}-tocopherol in plasma and follicular fluid of women undergoing IVF. *Hum. Reprod., 18*(6), 1259-1264. 10.1093/humrep/deg249.
Seifter, E., Rettura, G., & Levenson, S. M. (1981). Carotenoids and cell mediated immune responses. In G. Charamblois & G. Inglett (Eds.), *The Quality of Foods and Beverages: Chemistry and Technology*. New York: Academic Press.
Serne, E. H., Gans, R. O., ter Maaten, J. C., Tangelder, G. J., Donker, A. J., & Stehouwer, C. D. (2001). Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. *Hypertension, 38*, 238-242.
Service, R. F. (1998). New role for oestrogen in cancer? *Science, 279*, 1631-1633.
Setchell, J. M. (2005). Do female mandrills prefer brightly coloured males? *International Journal of Primatology, 26*(4), 715-735.
Setchell, J. M., & Dixson, A. F. (2001). Changes in the Secondary Sexual Adornments of Male Mandrills (Mandrillus sphinx) Are Associated with Gain and Loss of Alpha Status. *Hormones and Behavior, 39*(3), 177-184.
Setchell, J. M., Jean Wickings, E., & Knapp, L. A. (2006). Signal content of red facial coloration in female mandrills (Mandrillus sphinx). *Proceedings of the Royal Society B: Biological Sciences, 273*(1599), 2395-2400.
Setchell, J. M., & Wickings, J. (2005). Dominance, status signals and coloration in male mandrills (*Mandrillus sphinx*). *Ethology, 111*, 25-50.
Shaw, N. J. (2003). Vitamin D Deficiency Rickets. In Z. Hochberg (Ed.), *Vitamin D and Rickets*: Karger Publishers.
Shriver, M. D., & Parra, E. J. (2000). Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. *American Journal of Physical Anthropology, 112*, 17-27.

Singh, D. (1993). Adaptive significance of female physical attractiveness: role of waist-to-hip ratio. *Journal of Personality and Social Psychology, 65*(2), 293-307.
Singh, D. (1995). Female health, attractiveness, and desirability for relationships: role of breast asymmetry and waist-to-hip ratio. *Ethology and Sociobiology, 16*, 465-481.
Siskind, J. (1973). *To Hunt in the Morning*. New York: Oxford University Press.
Skinner, A. M. J., & Watt, P. J. (2006). Phenotypic correlates of spermatozoon quality in the guppy. *Behavioural Ecology, 18*, 47-52.
Slate, J., & Pemberton, J. M. (2002). Comparing molecular measures for detecting inbreeding depression. *Journal of Evolutionary Biology, 15*, 20-31.
Smith, G. D. (2000). Learning to live with complexity: ethnicity, socioeconomic position, and health in Britain and the United States. *American Journal of Public Health, 90*(11), 1694-1698.
Smith, R. L. (1984). Human sperm competition. In R. L. Smith (Ed.), *Sperm Competition and the Evolution of Animal Mating Systems*.
Stahl, W., Heinrich, U., Jungmann, H., Sies, H., & Tronnier, H. (2000). Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induces erythema in humans. *American Journal of Clinical Nutrition, 71*, 795-798.
Stahl, W., Heinrich, U., Jungmann, H., von Laar, J., Schietzel, M., Sies, H., et al. (1998). Increased Dermal Carotenoid Levels Assessed by Noninvasive Reflection Spectrophotometry Correlate with Serum Levels in Women Ingesting Betatene. *J. Nutr., 128*(5), 903-907.
Stahl, W., & Sies, H. (1992). Uptake of lycopene and its geometrical isomers is greater from heat processed than from unprocessed tomato juice in humans. *Journal of Nutrition, 122*, 2161-2166.
Stamatas, G. N., Zmudzka, B. Z., Kollias, N., & Beer, J. Z. (2004). Non-Invasive Measurements of Skin Pigmentation In Situ. *Pigment Cell Research, 17*(6), 618-626. doi:10.1111/j.1600-0749.2004.00204.x.
Stewart, D., Overstreet, J., Nakajima, S., & Lasley, B. (1993). Enhanced ovarian steroid secretion before implantation in early human pregnancy. *J Clin Endocrinol Metab, 76*(6), 1470-1476. 10.1210/jc.76.6.1470.
Stokes, M., Fairchild, M. D., & Berns, R. S. (1992). Precision requirements for digital color reproduction. *ACM Trans. Graph. , 11* (4), 406-422 http://doi.acm.org/10.1145/146443.146482
Strauss, M. S. (1979). Abstraction of prototypical information by adults and 10-month-old infants. *Journal of Experimental Psychology: Human Learning and Memory, 5*, 618-632.
Surridge, A. K., Osorio, D., & Mundy, N. I. (2003). Evolution and selection of trichromatic vision in primates. *Trends in Ecology & Evolution, 18*(4), 198-205.
Swaddle, J. P., & Cuthill, I. C. (1994). Preference for symmetric males by female zebra finches. *367*(6459), 165-166.
Swaddle, J. P., & Cuthill, I. C. (1995). Asymmetry and Human Facial Attractiveness: Symmetry May not Always be Beautiful. *Proceedings of the Royal Society B: Biological Sciences, 261*(1360), 111-116.
Swaddle, J. P., & Reierson, G. W. (2002). Testosterone increases perceived dominance but not attractiveness in human males. *Proceedings of the Royal Society B: Biological Sciences, 269*, 2285-2289.
Symons, D. (1979). *The Evolution of Human Sexuality*: Oxford University Press.

Tadokoro, T., Kobayashi, N., Zmudzka, B. Z., Ito, S., Wakamatsu, K., Yamaguchi, Y., et al. (2003). UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin. *FASEB Journal, 17*, 1177-1179.

Tankersley, C. G., Smolander, J., Kenney, W. L., & Fortney, S. M. (1991). Sweating and skin blood flow during exercise: effects of age and maximal oxygen uptake. *J Appl Physiol, 71*(1), 236-242.

Tarr, M. J., Kersten, D., Cheng, Y., & Rossion, B. (2001). It's Pat! Sexing faces using only red and green. *Journal of Vision, 1*, 337.

Taylor, C. R., Stern, R. S., Leyden, J. J., & Gilchrest, B. A. (1990). Photoaging, photodamage and photoprotection. *Journal of the American Academy of Dermatology, 22*, 1-15.

Thornhill, R. (1992). Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (Panorpa japonica: Mecoptera). *Behav. Ecol., 3*(3), 277-283. 10.1093/beheco/3.3.277.

Thornhill, R., & Gangestad, S. W. (1993). Human facial beauty: Averageness, symmetry, and parasite resistance. *Human Nature, 4*, 237-269.

Thornhill, R., & Gangestad, S. W. (2006a). Facial sexual dimorphism, developmental stability, and susceptibility to disease in men and women. *27*(2), 131-144.

Thornhill, R., & Gangestad, S. W. (2006b). Facial sexual dimorphism, developmental stability, and susceptibility to disease in men and women. *Evolution and Human Behavior, 27*(2), 131-144.

Thornhill, R., Gangestad, S. W., & Comer, R. (1995). Human female orgasm and mate fluctuating asymmetry. *Animal Behaviour, 50*(6), 1601-1615.

Thornhill, R., Gangestad, S. W., Miller, R., Scheyd, G., McCollough, J. K., & Franklin, M. (2003). Major histocompatibility complex genes, symmetry, and body scent attractiveness in men and women. *Behav. Ecol., 14*(5), 668-678. 10.1093/beheco/arg043.

Thornhill, R., & Grammer, K. (1999). The body and face of a woman: one ornament that signals quality? *Evolution and Human Behaviour, 20*, 105-120.

Thornton, M. J. (2002). The biological actions of estrogens on skin. *Experimental Dermatology, 11*(6), 487-502. doi:10.1034/j.1600-0625.2002.110601.x.

Tjoelker, L. W., Chew, B. P., Tanaka, T. S., & Daniel, L. R. (1990). Effect of dietary vitamin A and β-carotene on polymorphonuclear leukocyte and lymphocyte function in dairy cows during the early dry period. *Journal of Dairy Science, 73*, 1017-1022.

Toda, K., Pathak, M. A., Parrish, J. A., & Fitzpatrick, T. B. (1972). Alterations of racial differences in melanosome distribution in human epidermis after exposure to ultraviolet light. *Nature, 236*, 143-145.

Treiman, D. J., McKeever, M., & Fodor, E. (1996). Racial differences in occupational status and income in South Africa, 1980 and 1991. *Demography, 33*, 111-132.

Trivers, R. L. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual Selection and the Descent of Man, 1871-1971* (pp. 136-179). Chicago, IL: Aldine.

Utami, S., Goossens, B., Bruford, M. W., de Ruiter, J. R., & van Hoof, J. A. R. A. M. (2002). Male bimaturism and reproductive success in Sumatran orang-utans. *Behavioural Ecology, 15*, 643-652.

van den Berghe, P. L., & Frost, P. (1986). Skin colour preference, sexual dimorphism and sexual selection: a case of gene-culture coevolution? *Ethnic and Racial Studies, 9*, 87-119.

Van Valen, L. (1962). A study of fluctuating asymmetry. *Evolution, 16*, 125-142.

Veblen, T. (1899). *The Theory of the Leisure Class: an Economic Study of Institutions*. New York: Penguin.
Waitt, C., Gerald, M. S., Little, A. C., & Kraiselburd, E. (2006). Selective attention toward female secondary sexual color in male rhesus macaques. *American Journal of Primatology, 68*, 738-744.
Waitt, C., Little, A. C., , Wolfensohn, S., , Honess, P., et al. (2003). Evidence from rhesus macaques suggests that male coloration plays a role in female primate mate choice. *Proceedings of the Royal Society B: Biological Sciences, 270*(0), S144-S146.
Waynforth, D. (1998). Fluctuating asymmetry and human male life-history traits in rural Belize. *Proceedings of the Royal Society B: Biological Sciences, 265*(1405), 1497-1501.
Wedekind, C., Seeback, T., Bettens, F., & Paepke, A. J. (1995). MHC-dependent mate preferences in humans. *Proceedings of the Royal Society B: Biological Sciences, 260*, 245-249.
Wolf, N. (1991). *The Beauty Myth*: Anchor.
Wolfenbarger, L. L. (1999). Red coloration of male northern cardinals correlates with mate quality and territory quality. *Behav. Ecol., 10*(1), 80-90. 10.1093/beheco/10.1.80.
Woolard, I. (2002). *An overview of poverty and inequality in South Africa*.
Yach, D., & Harrison, D. (1995). Inequalities in health: determinants and status in South Africa. In K. van der Velden (Ed.), *Health Matters: Public Health in North-South Perspective*. Amsterdam: Houten-Diegem.
Zahavi, A. (1975). Mate selection - a selection for a handicap. *Journal of Theoretical Biology, 53*, 205-214.
Zahavi, A., & Zahavi, A. (1997). *The Handicap Principle*. Oxford: Oxford University Press.
Zonios, G., Bykowski, J., & Kollias, N. (2001). Skin Melanin, Hemoglobin, and Light Scattering Properties can be Quantitatively Assessed In Vivo Using Diffuse Reflectance Spectroscopy. *117*(6), 1452-1457.

Appendix A: Graphs showing relationship between correlation coefficients and carotenoid absorption spectra

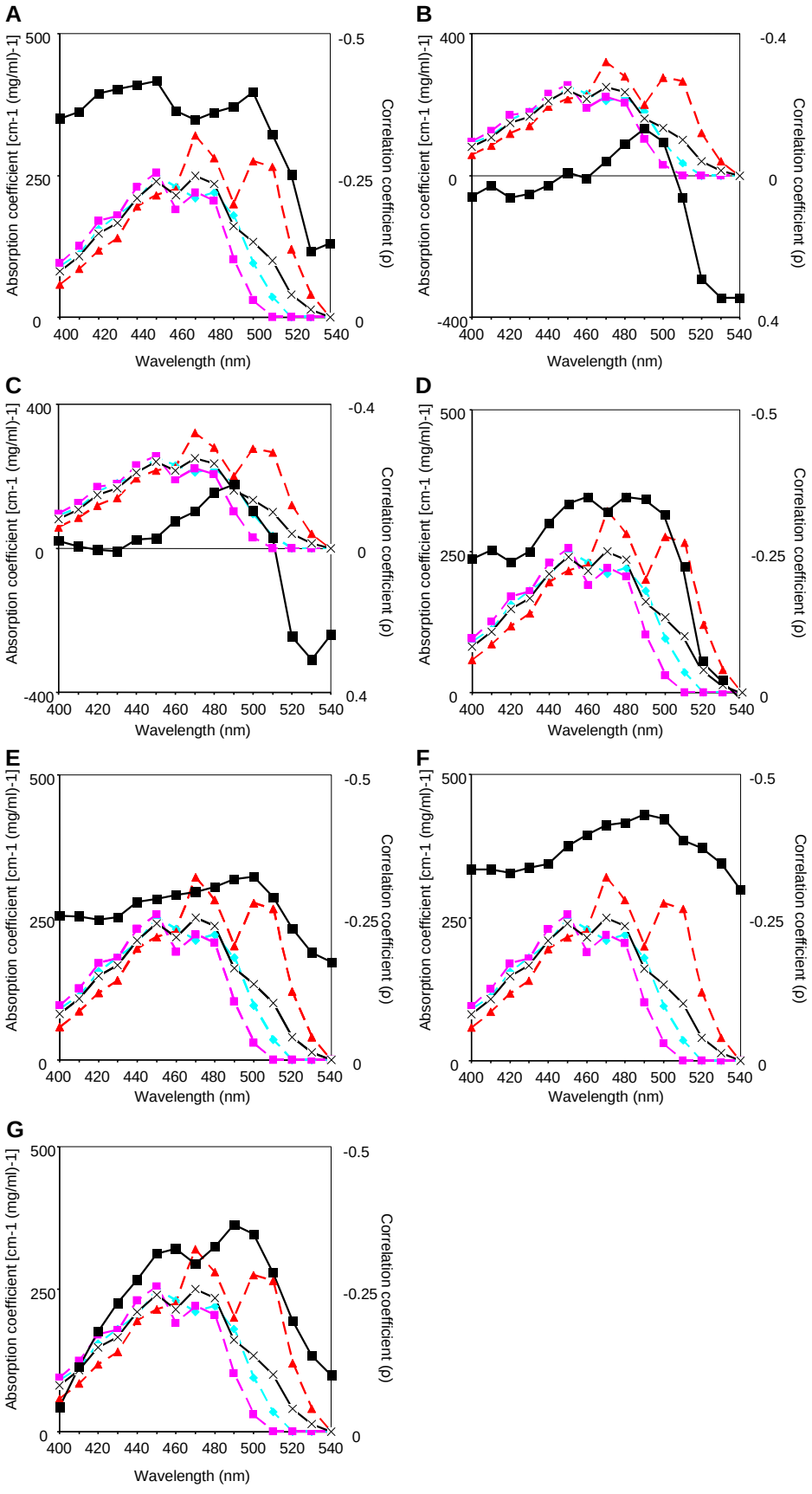


Fig. A.1: Correlation coefficients (solid black line) of the relationship between dietary β-carotene intake and skin reflectance values at 10nm intervals. Dashed lines show absorption spectra for common carotenoids. Red=lycopene, blue=β-carotene, purple=α-carotene. Black dashed line shows mean absorption spectrum for the three carotenoids. **(A)** Inner Arm, **(B)** Right Cheek, **(C)** Left Cheek, **(D)** Forehead, **(E)** Upper Arm, **(F)** Shoulder, **(G)** Palm.

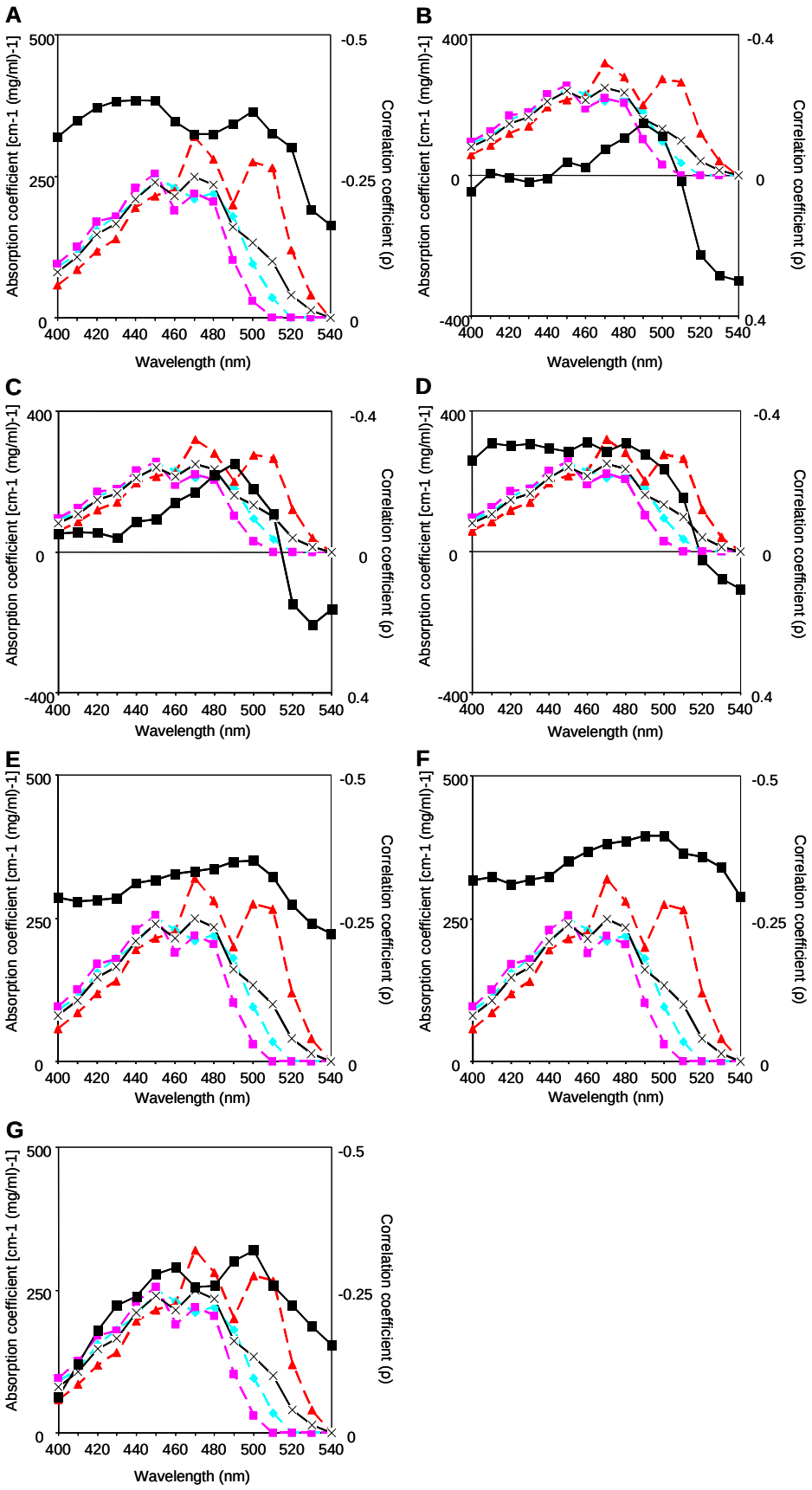


Fig. A.2: Correlation coefficients (solid black line) of the relationship between dietary fruit and vegetable intake and skin reflectance values at 10nm intervals. Dashed lines show absorption spectra

for common carotenoids. Red=lycopene, blue=β-carotene, purple=α-carotene. Black dashed line shows mean absorption spectrum for the three carotenoids. **(A)** Inner Arm, **(B)** Right Cheek, **(C)** Left Cheek, **(D)** Forehead, **(E)** Upper Arm, **(F)** Shoulder, **(G)** Palm.

Appendix B: Display Accuracy

Mean CIELab colour values for the skin portion of each calibrated, unmanipulated facial photograph were derived (as described in Section 3). Even-coloured patches were displayed on the monitor. The Spyder2Pro's colorimeter function was used to measure the colour displayed on the screen.

Spectrophotometer measurements were made of both cheeks of 53 participants (31 female, 22 male; aged 18-26; also drawn from the University of St Andrews) who were not underweight or obese (18<BMI<30). Mean CIELab values for the cheeks of each individual were calculated.

No difference was found between the spectrophotometer measurements and displayed lightness or redness for female (L* t_{41}=0.417; p=0.820; a* t_{41}=0.858; p=0.180) or male (L* t_{41}=0.658, p=0.446; a* t_{41}=0.578, p=0.560) faces. Male faces showed no difference in yellowness (t_{41}=0.959; p=0.052; Δb*=1.147), whereas displayed female faces were less yellow than spectrophotometer measurements (t_{41}=0.980; p=0.026). However, this difference was small (Δb*=0.864). While this may cause a slight overestimation of yellowness preferences, this is considerably smaller than the amount of yellow added to optimise healthy appearance in Section 3 (Δb*=5.25 to optimise healthy appearance) and well within the acceptable colour error range (Stokes *et al.*, 1992). Images lay within the colour range of the population (Fig B.1). The images displayed on the monitor were thus consistent with the real-world colour

of faces drawn from the same population, and participants were presented with realistic images to manipulate.

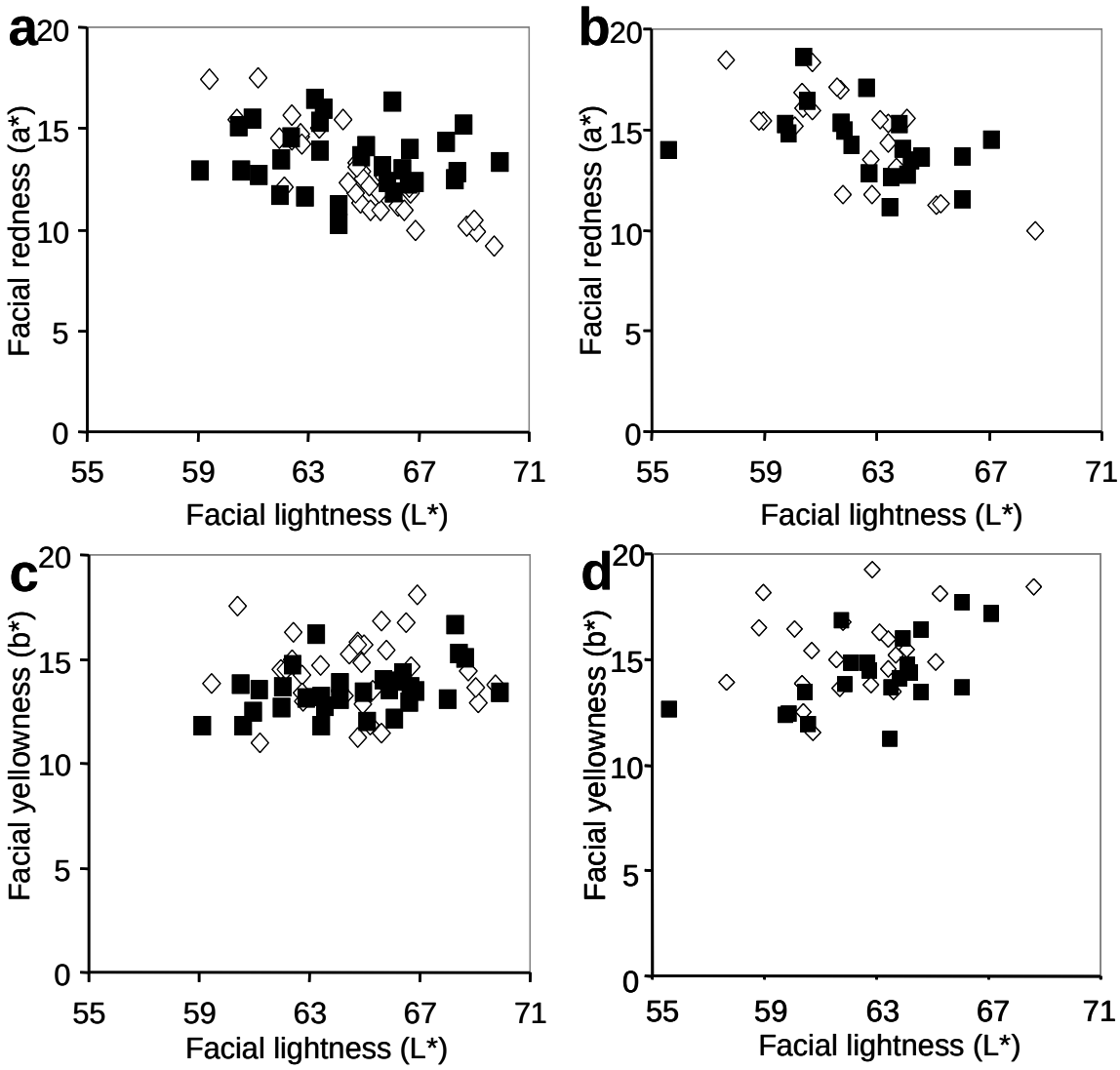


Figure B.1: The relationship between skin colour measured from the monitor screen (solid symbols) and the spectrophotometer measurements of cheek skin (hollow symbols) in another sample from the same population. a) Facial lightness (L*) versus facial redness (a*) for female faces. b) Facial lightness (L*) versus facial redness (a*) for male faces. c) Facial lightness (L*) versus facial yellowness (a*) for female faces. d) Facial lightness (L*) versus facial yellowness (b*) for male faces.