UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

GENETIC AND PHENOTYPIC CUES ASSOCIATED WITH FACIAL ATTRACTIVENESS AND HEALTH

by

Vinet Coetzee

Submitted in partial fulfillment of the requirements for the degree

Magister Scientiae

In the Faculty of Natural of Agricultural Sciences

Department of Genetics

University of Pretoria

Pretoria

June 2006

Under the supervision of Prof. J. M. Greeff, Dr. L. Barrett and Prof. S. P. Henzi.

DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree M.Sc. to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.

Vinet Coetzee

June 2006

Preface

This dissertation is mostly about mate choice, more specifically human mate choice and the cues associated with facial attractiveness. It all started with the observation that woman find heterozygous men at the human leococyte complex (HLA), more attractive than more HLA homozygous men (Roberts *et al*. 2005). This was a fascinating find in the study of human mate choice: direct evidence that genes associated with the immune response, influence human mate choice. But is this true of all populations? Roberts *et al*. (2005) used a British population, who compared to other populations worldwide, have relatively few pathogens that routinely challenge their immune response. Wouldn't this effect be stronger in a population with a higher pathogen load? Also, what other factors play a role in mate choice based on facial features and will men show the same preferences for female faces? To answer these and other questions we set out to do this study in an African population, a population frequently challenged by a multitude of infectious diseases.

Chapter 1 of this dissertation comprises a brief overview of the literature relevant to our current knowledge of mate choice, the HLA, selective forces driving the HLA and facial attractiveness. We highlight the role of the various HLA based mating preferences and propose a unified view incorporating the different mating preferences. Special attention is also paid to the role of male mate choice in humans, a topic previously largely disregarded.

Chapter 2 contains a detailed study of the role of heterozygote advantage and frequency dependent selection on various health measures. This chapter also

looks at the effect of heterozygote advantage, frequency dependent selection and a third variable, inbreeding avoidance, in predicting facial attractiveness.

Chapter 3 examines the role of three of the major facial attractiveness cues (symmetry, sexual dimorphism and neoteny) in predicting facial attractiveness and the correlation amongst these cues in both sexes.

Chapter 4 considers the role of ethnic recognition in our study population. The South African population is a heterogenous mix of nine different African ethnic groups. If recognition exists between these different groups, it could serve as a basis for ethnic preference, a possible confounding factor in attractiveness studies.

Chapter 5 examines the role of condition dependent factors on choosiness in both sexes. The study specifically tests the difference between male and female choice and the function of self esteem, self perceived attractiveness and relationship status on mate choice.

Chapter 6 focuses specifically on the role of self-esteem in sexually risky behavior. Because HIV is extremely prevalent in the South African population, we tested the role of self esteem in predicting behaviour, thereby higligting some new directions for HIV prevention campains.

Acknowledgements

I would like to thank the following people and organizations for their assistance with this project:

- My supervisors, Prof. Jaco M. Greeff, Dr. Louise Barrett and Prof. Peter S. Henzi for their invaluable guidance and insights.
- Prof. Dave I. Perrett for his financial assistance and advice.
- My fellow students, Nicole Creux, Minique de Castro, Therese de Castro, Christoff Erasmus, Tracey-Leigh Hatherall, Aret Meyer, Ronnie Nelson, Duncan Newman, Jason Pienaar and Isa-Rita Russo for their valuable help with data collection.
- The National Research Foundation of South Africa (NRF), for their financial assistance through grants held by JMG and PSH.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

Introduction

1.1. Sexual selection and mate choice

"This depends, not on a struggle for existence, but on a

struggle between the males for possession of the females:

the result is not death to the unsuccessful competitor, but few or no offspring"

Charles Darwin, 1859

In his classic 1859 book, *On the Origin of Species by Means of Natural Selection*, and in more detail in *The Descent of Man and Selection in relation to Sex* (1871) Darwin proposed sexual selection in addition to natural selection as a driving force behind evolution. Sexual selection was proposed to explain secondary sexual characters that seem to decrease the individual's fitness if only survival is taken into account. For example, the male guppies enlarged tail with colourful markings that renders them more conspicuous to predators (Gould and Gould, 1989). According to Darwin, sexual selection not only favours traits that enhance combat ability between same sex individuals but also traits that enhance an individual's attractiveness to the opposite sex. In the guppies, females prefer and selectively mate with males that have more elaborate tails (Houde, 1987).

The strength of sexual selection depends on the ratio of sexually receptive individuals between the two sexes (Emlen and Oring, 1977) and on the reproductive investment of each sex (Borgia, 1979). Imagine a population with only a few females and a massive excess of males. One would expect that there would be severe, even deadly, competition between the males to secure a female. According to Brown (1975) almost all males in a monogamous system find females to mate with if the sex ratio is even. But as in other sexually reproducing species, females invest more in their offspring than males, since eggs are more costly to produce than sperm. This

discrepancy is exacerbated by gestation in humans, as the females incur further costs by carrying the baby to birth (Barrett, Dunbar and Lycett, 2002).

1.1.1. Selection within and between the sexes

Since in humans, females invest more than males reproductively, females are expected to be choosy about their prospective partner (female choice). This choosiness fuels competition between males to win the favour of the female (male-male competition). Although most discussions on sexual selection do not refer to male choice and female competition, particular care is taken not to exclude them either. However, in monogamous species with biparental care, paternal investment does not end after conception. According to Trivers study on birds (1972) if the male plans to raise her young “he should be about as discriminating as she is towards him”.

Paternal care involves supplying the offspring with resources. In humans, this amounts to an enormous financial cost since the offspring only become independent later in life. Women are expected to compete for men with a high socio-economic status, which can burden the cost of paternal care with more ease. In turn, these men are expected to be more discriminating in the females they choose (Barrett, Dunbar and Lycett, 2002). Male choice has already been shown in various species including mice (Yamazaki *et al.* 1976) and monogamous birds (Griggio *et al.* 2005). Since choice is no longer only restricted to the females (female choice) and competition to males (male-male competition), they can be more accurately described as intersexual and intrasexual selection.

Intrasexual selection occurs when members of one sex compete to gain access to members of another sex (Barrett, Dunbar and Lycett, 2002). This is most often observed in male-male competition, where males traditionally compete for access to a female or group of females (Brown, 1975). In order to compete, males

develop costly weapons such as antlers, horns and large canines but are also judged by other males by their large body size or stature. In some species, competition does not end after copulation as sperm competition between the sperm of two or more males also play a role (Trivers, 1972). Even after birth, infants fathered by another male are sometimes killed by the new resident male as seen in the langur (*Presbytis entellus*) (Borries *et al.* 1999).

Intersexual selection, or mate choice, occurs when members of one sex choose members of another sex, based on certain characteristics they possess, which will increase the number and quality of offspring. As discussed earlier, choosiness is mostly observed in females, where she chooses males based on the direct or indirect benefits they can provide her with. Female choice does not necessarily end after copulation; in some species females can still use cryptic selection within the female reproductive tract (Rülicke *et al.* 1998) and after birth (see Sheldon, 2005 for review).

1.1.2. Direct and Indirect Benefits

Direct benefits include the ability of the male to provide the female with resources, such as food, shelter, paternal care and protection from parasite due to his own reduced parasite load (Ryan, 1997). These benefits have an immediate effect on the females' reproductive success (Ryan, 1997).

The female also indirectly benefits from choosing males with traits that indicate their "high quality", which are passed on to the offspring, especially the sons (Fedorka and Mousseau, 2004), enabling them to be of "high quality" themselves. These "high quality" traits need not necessarily correlate with survival ability. As long as females show a preference for it, it will increase the males mating success (Brown 1975; Houde 1987). Fisher (1958) initially dealt with the issue of female preference evolution without direct selection. He showed that a "runaway process" could occur if

a female preference was linked to a male trait. Initially this trait has to have some advantage not due to female preference, however small in magnitude. An additional female preference for this trait is also necessary for the “runaway process” to occur. If the trait is heritable, the offspring will possess the trait or preference depending on the sex of the offspring. Daughters will have the preference and an increased mating success because of their preference, while the sons will be more attractive because they possess the trait. Females will choose males with ever more exaggerated traits and the speed of “exaggeration” will increase exponentially with time. This process will only be stopped when the survival ability of the male is reduced sufficiently by the exaggerated trait. Natural selection therefore counters sexual selection eventually. Another theory that links up closely with Fishers “runaway selection” is the “sexy son” hypothesis proposed by Weatherhead and Robertson (1979). They based their theory on the observation that even though females compete for the most attractive men, those that win and mate with them reared fewer offspring, because these males provided little or no paternal care. They proposed that the fitness loss due to fewer offspring is countered by the fitness gain due to the fact that the “sexy sons” produced from this union have a higher fitness than their competitors. These females ultimately have a higher fitness than the females that mate with less attractive males (but see Cameron, Day and Rowe, 2003).

According to the “good genes” hypothesis, the other major point of view, females choose certain male traits because these traits indicate superior genetic quality concerning the males’ ability to survive. These traits serve as an honest signal of the males’ genetic quality. Zahavi’s (1975) handicap principle proposed that the female assess the male genetic quality by the size of the handicap they can survive with. Many sexually dimorphic traits increase male mortality (Ryan, 1997) and if the males can survive with these traits it indicates a superior genetic quality. Low quality males simply cannot afford the handicap. An alternative to the Zahavi (1975)

Handicap principle is the Condition-dependent handicap principle (Ryan, 1997) whereby a male varies his investment in his handicap based on his condition. He optimizes the trade off between sexual selection and natural selection in order to maximise his fitness. Hamilton and Zuk (1982) proposed that these “good genes” could relate to disease resistance in the male. Disease resistance can be judged by a wide array of traits including bright plumage, symmetry or even a vigorous courtship displays. If the male could not cope with the continuous onslaught of pathogens his plumage would dull and his display would be somewhat less thrilling. These genes will be passed on to the offspring reducing their inherent resistance to disease. In the constant arms race between host and pathogen, the environment is ever changing. Pathogens evolve at an alarming rate and the host needs to keep up in order to remain healthy (Jeffery and Bangham, 2000). One of the most important and best studied weapons in the vertebrate host’s arsenal is the highly polymorphic Major Histocompatibilty complex (MHC). It is an extremely polymorphic set of genes (Hedrick, 2004) and is constantly changing in order to keep up with pathogens. We will deal with the MHC in more detail later in this Chapter.

1.1.3. Balancing inbreeding and outbreeding avoidance

The grossest blunder in sexual preference, which we can conceive of an animal making, would be to mate with a species different from its own and with which the hybrids are either infertile or, through the mixture of instincts and other attributes appropriate to different courses of life, at so serious a disadvantage as to leave no descendants.

Ronald A. Fisher, 1958

Inbreeding and outbreeding avoidance are often overlooked in discussions of mate choice. When choosing a mate, it is necessary for any organism to balance how closely the prospective partner is related to it, coined optimal outbreeding by Bateson (1983). At the one severe end of the continuum, the partner can be from another species, and a mating can result in infertile offspring if any. Less severe outbreeding results in outbreeding depression, defined as the fitness decline of progeny resulting from outbreeding. This reduction in fitness is mainly due to a loss of local adaptation both behaviourally and genetically (Pusey and Wolf, 1996). However contentious the subject, there is some empirical evidence that certain species choose mates of intermediate relatedness thereby avoiding the cost of outbreeding (see Pusey and Wolf, 1996 for a review). For instance, Waser and Price (1994) showed in Mountain Delphinium that significantly more seedlings survived from crosses between plants that were located 10m apart, as opposed to plants that were selfed or located 1000m apart. Outbreeding also involves dispersal, which exposes the individual to foreign parasites and the cost involved with travelling (Bateson, 1983).

At the other end of the spectrum we find inbreeding. Inbreeding depression, or the reduction of fitness associated with inbreeding, has been extensively studied in captive populations, but also in some natural populations (Pusey and Wolf 1996; Arkush *et al.* 2002). The level of inbreeding depression varies between and within species (Keller and Waller, 2002) but is essentially caused by unmasking of recessive deleterious alleles, due to increased homozygosity and an associated decrease in heterozygosity. This brings about the loss of heterozygote advantage in the population (Charlesworth and Charlesworth, 1987). Inbreeding depression is more severe in natural populations due to environmental stress and competitive environments (Miller, 1994). In species where inbreeding depression is severe, inbreeding avoidance mechanisms have evolved. These include dispersal from natal site, extra-pair copulations, kin recognition and delayed maturation (Pusey and Wolf, 1996). In a study by Meagher, Penn and Potts (2000) inbred and outbred house mice

were allowed to compete in seminatural enclosures. They found that inbred males had fewer offspring with lower survivorship than outbred males. Inbred females also produced significantly fewer offspring.

The impact of inbreeding and outbreeding avoidance on mate choice will undoubtedly differ between species and even populations within a species. Bateson (1983) showed that Japanese Quail have a very small optimum range of relatedness, while other species might have broader ranges. Inbreeding and outbreeding avoidance limits the playing field within which individuals can seek a partner.

1.2. The Major Histocompatibility complex

The Major Histocompatibility Complex (MHC) was first discovered for its role in transplant rejection (Parham and Ohta 1996; Hauptmann and Bahram 2004). Since then it has been identified in birds, reptiles, amphibians, bony and cartilaginous fishes and every mammalian species studied so far (Kasahara *et al.* 1995). The only vertebrate exception is the jawless fish (hagfish and lamprey) in which no MHC genes have been found despite extensive scrutiny (Flajnik and Kasahara, 2001). In humans this complex is referred to as the Human Leucocyte Antigen (HLA) and the H-2 complex in mice. The MHC plays an essential role in both humoral and cell mediated immune response where they serve as antigen presenting molecules (Marieb 1995; Penn and Potts 1999)

1.2.1. Genetic map of MHC

In humans, the MHC is located on chromosome 6 and spans over 3.6 megabases (Mb) (The MHC sequencing consortium, 1999). It consists of three classes (Flajnik and Kasahara, 2001) of which class I and II are structurally and functionally related (Bjorkman *et al.* 1987a)(Figure 1).

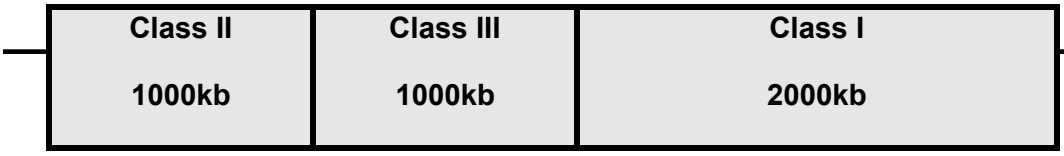


***Figure 1**: Diagrammatic representation of the Human Leucocyte antigen complex (HLA) classes located on chromosome 6.*

1.2.1.1. Class I genes

Class I HLA genes code for glycoproteins that are located on most nucleated cells although the level of expression varies between different cell types. They are highly prevalent on lymphocytes and myeloid cells, common on liver lung and kidney cells and also occur in low quantities on skeletal muscle and neural cells (Levine *et al.* 1991; Roitt and Delves 2001). These glycoproteins play a crucial role in the immune response where they present self and non-self peptides to CD8+ (predominantly cytotoxic) T cells (Gao *et al.* 1997). These T-cells are then activated to produce clones that destroy similarly infected cells (Penn and Potts, 1999). Class I genes are divided into classical (HLA-A, B and C) and non-classical genes (HLA-E, F, G, H, J, X, MICA-MICE) (Figure 2). The function of the non-classical genes is still largely unknown, although some of them are pseudogenes and therefore do not code for a protein product. Other non-classical genes such as HLA-G and H are limited to certain cell types and have highly specialised functions (Roitt and Delves, 2001). The recently discovered MIC gene complex, that forms part of the non-classical group, is expressed in low levels on epithelial cells and is induced by heat and other stimuli. Their function is however still unknown (Goldsby, Kindt and Osborne, 2000).

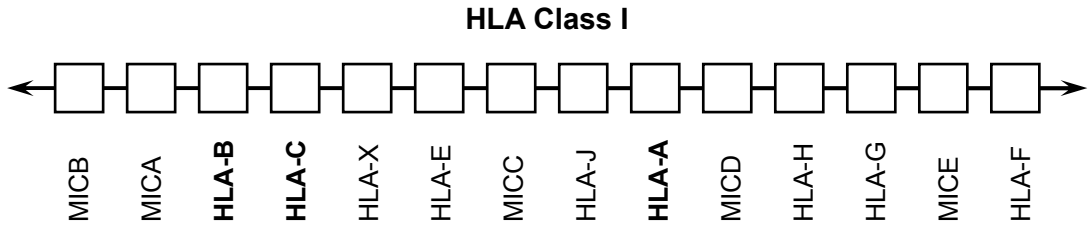


***Figure 2**: Comprehensive map of the HLA class I region. This region consists of 14 known genes, each coding for the extra cellular α regions of the HLA class I molecule. Classical genes are indicated in bold.*

We will focus mainly on the classical genes. Each of the classical genes, for example HLA-A, consists of a leader exon at the 5' end, followed by a short signal peptide and 5 to 6 exons at the 3' end. Of these last exons, the first three, exons 1, 2 and 3 encode the extra cellular α1, α2 and α3 domains of the HLA molecule. Exon 4 codes for the transmembrane region of the molecule, while exon 5 and 6 encode the cytoplasmic domain (Malissen, Malissen and Jordan, 1982) (Figure 4).

1.2.1.2. Class II genes

Class II HLA genes encode glycoproteins that are predominantly located on antigen presenting cells, such as macrophages, dendritic cells and B cells (Brown *et al.* 1993). Here they present processed peptides to CD4+ (predominantly helper) T cells. Once the CD4+ T-cell binds to the MHC complex, the T-cells proliferate activating the macrophages and triggering antibody secretion by B-cells (Penn and Potts, 1999). As with class I genes, class II genes are divided into classical (HLA-DR, DP and DQ) and non-classical (HLA-DM, DN and DO) regions (Figure 3). The non-classical HLA-DM molecules facilitate the loading of peptides into class II molecules, while HLA-DO molecules have a regulatory function. The function of HLA-DN is still uncertain. All three classical regions (HLA-DR, DP and DQ) consist of 4-5 genes each that encode either α or β chains (Goldsby, Kindt and Osborne, 2000)(Figure 3).

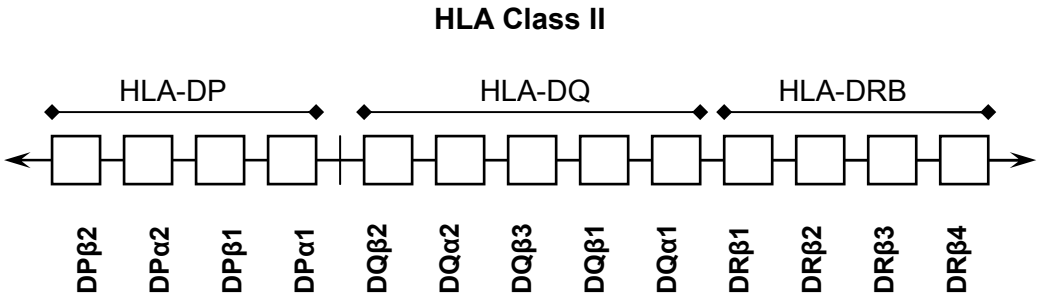


***Figure 3**: Comprehensive genomic map of HLA class II region. Non-classical genes are not shown but reside between the HLA-DP and DQ regions. The classical genes shown encode either an α or β region of the class II molecule.*

Each class II gene, for example DRβ1, consists of a leader exon on the 5' side followed by exon 1 and 2, and finally the transmembrane exon and one or more cytoplasmic exons on the 3' side. Exon 1 and 2 encode the extra cellular glycoprotein α or β chains depending on the gene. In the case of DRβ1, both exon 1 and 2 encode a single β chain, which then combines with the DRα chain to form a class II HLA glycoprotein (Janeway and Travers, 1994) (Figure 5).

1.2.1.3. Class III genes

Class III HLA is a heterogeneous collection of genes that are functionally and structurally unrelated to class I and II (Hauptmann and Bahran, 2004). They do however play a critical role in the immune system where they code for several genes involved in the activation cascades of the complement system (C2, factor B, C4), hormonal synthesis (steroid 21-hydroxylases, CYP21), heat shock proteins (HSPA1A, HSPA1B, HSPA1L), inflammation and cell stress (Tumour necrosis Factor (TNF) α and β) and extra cellular matrix organisation (Tenascin and immunoglobulin superfamily members 1C7, G6f and G6b) (Flajnik and Kasahara, 2001). The remainder of the genes in class III have no apparent function in the immune system (Hauptmann and Bahram, 2004).

1.2.1.4. Linkage Disequilibrium

Two curious attributes of the HLA complex is the enormous amount of polymorphisms (Hedrick, 2004) noted to date and the high degree of Linkage disequilibrium (LD) within this complex (Cullen *et al.* 1997; Sanchez-Mazas *et al.* 2000; Slatkin 2000; Cao *et al.* 2004). We will discuss the polymorphic and polygenic nature of the complex in detail later in this chapter. Linkage disequilibrium is defined as the non-random association of alleles at different loci (Gibson and Muse 2001; Nordborg and Tavaré 2002). In practice this means that different loci tend to segregate together as opposed to randomly. Due to the high LD and codominant expression of HLA, offspring mostly express the maternal and paternal allele combinations without any recombination.

1.2.2. Structure of HLA molecules

1.2.2.1. Class I molecules

Class I molecules are composed of a large α chain and a small β_2-microglobulin chain (Bjorkman *et al.* 1987a; Gao *et al.* 1997). The α chain is encoded by exons 1-3 of the classic class I genes, HLA-A, B and C, while β_2-microglobulin is the product of a gene outside the MHC complex. The α chain consists of three external domains, α1, α2 and α3 (relating to exons 1,2 and 3), a hydrophobic transmembrane domain, followed by a cytoplasmic anchor (Malissen, Malissen and Jordan, 1982)(Figure 4).

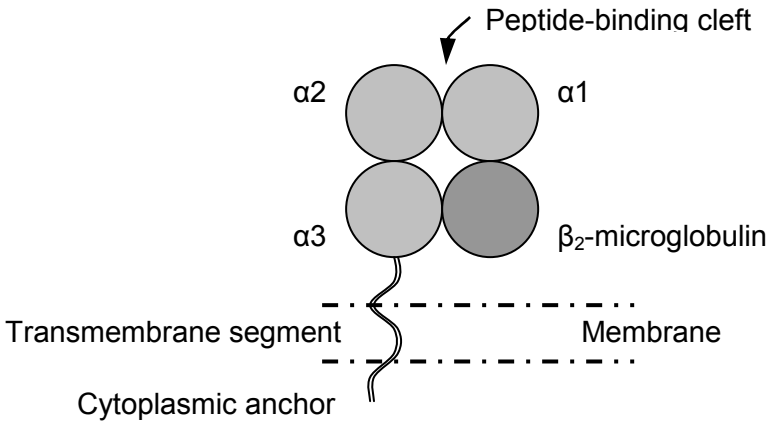


***Figure 4**: Diagrammatic representation of a class I HLA molecule. β_2-microglobulin is covalently bound to the three extra cellular α chain domains, α1, α2 and α3. The peptide binding cleft, which binds both self and non-self peptides, is formed between α1 and α2. Class I HLA molecules are connected to the cells by one hydrophobic transmembrane segment and a cytoplasmic anchor.*

Two of the α domains, α1 and α2 interact to form the peptide-binding cleft, while α3 interacts with the CD8+ T cell (Gao *et al.* 1997). β_2-microglobulin connects with all the α domains and is essential for protein folding and the presentation of the class I molecule on the cell membrane (Goldsby, Kindt and Osborne, 2000).

As noted earlier, the main function of the class I molecule is to present self and non-self peptides to the CD8+ T cells (Gao *et al.* 1997). This is accomplished by binding a peptide in the peptide binding cleft. Class I molecules usually accommodate peptides that are only 8 to 10 amino acids (aa) in length, though some molecules can accommodate up to 13 aa (Rammensee, 1995). This size restriction is primarily due to a blockage of the peptide binding cleft at both ends. Peptides are derived from endogenous intracellular proteins, digested within the cytosol (Kasahara *et al.* 1995; Zhang, Anderson and DeLisi 1998). As a rule class I molecules require "anchor residues" near both ends of the peptide for binding (Falk *et al.* 1991) but see (Zhang, Anderson and DeLisi, 1998). These "anchor residues" insert into pockets in

the peptide binding cleft at both ends and bend away from the molecule in the middle (Guo *et al.* 1992). This presumably facilitates better peptide recognition by T cells.

1.2.2.2. Class II molecules

A class II molecule is composed of a non-covalently bound α and β chain. Both chains consist of two extra cellular domains (α1, α2 for the α chain and β1, β2 for the β chain), a transmembrane segment and a cytoplasmic anchor (Brown *et al.* 1993).

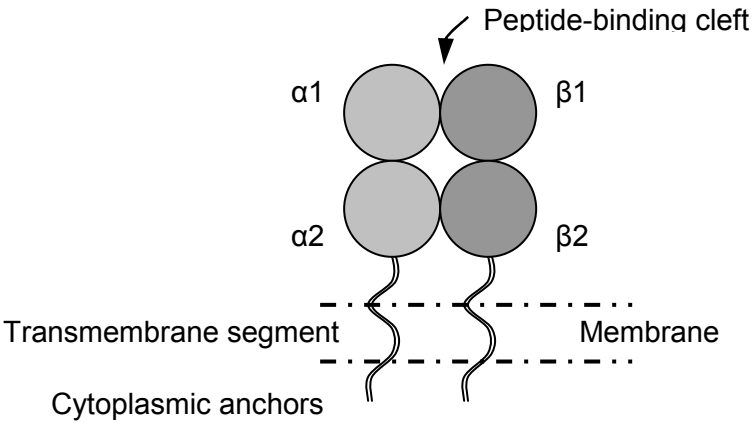


***Figure 5**: Diagrammatic representation of a class II molecule. The molecule consists of a non-covalently bound α and β chain. Two of the extra cellular domains, α1 and β1, interact to form the peptide-binding cleft, while two transmembrane segments and two cytoplasmic anchors connect the molecule to the cell.*

The α1 and β1 domains interact to form the peptide binding cleft (Figure 5). Class II molecules can bind longer peptides (12-25 amino acids) than class I molecules as the class II peptide-binding cleft is open at both ends (Brown *et al.* 1993). Since class II molecules are present on antigen presenting cells such as macrophages, peptides are obtained from self and non-self exogenous proteins, as opposed to the endogenous proteins of class I (Kasahara *et al.* 1995). These exogenous proteins are internalised by the antigen presenting cells, either through

phagocytosis or receptor mediated endocytosis, digested and displayed on the cell surface. Some of these exogenous peptides include peptides once bound to class I molecules. Peptides need not have “anchor residues” to facilitate binding in the peptide binding cleft. Peptide binding is instead facilitated by the central 9 amino acids of the peptide and as a consequence the peptide maintains a constant elevation from the peptide-binding cleft floor (Brown *et al.* 1993; Rammensee 1995). Both class I (Bjorkman *et al.* 1987b; Guo *et al.* 1992) and class II molecules (Brown *et al.* 1993) bind peptides tenaciously.

1.2.3. HLA Diversity

In the battle to ward of disease, the HLA complex must keep up with the rapidly evolving array of pathogens out there. There are several ways in which this is accomplished and we will discuss them in turn.

1. **HLA is polygenic**. There are several different class I (HLA-A, B and C) and class II (HLA-DR, DP and DQ) genes performing the same or overlapping functions (Janeway and Travers, 1994).
2. **HLA is extremely polymorphic**. At the time of writing, 396 HLA-A and 699 HLA-B alleles have been identified. Class II β chains are also highly polymorphic (695 alleles identified so far), while class II α chains (70 alleles identified) and β_2-microglobulin are less so (IMGT/HLA sequence database, 2005).
3. **Class II rearrangements**. In class II molecules diversity is greatly increased by the association of multiple β with α chains (Janeway and Travers, 1994). For instance in the HLA-DR complex four different β chains can associate with the single α chain.

4. **Co-dominant expression**. In a heterozygous individual both parental alleles are expressed on all the cells (Janeway and Travers 1994; Roitt and Delves 2001).
5. **Multiple Peptide binding**. Each HLA molecule can bind a set of different peptides and the same peptide can often be bound by more than one HLA molecule (Falk *et al.* 1991; Zhang, Anderson and DeLisi 1998).
6. **Cellular occurrence**. HLA molecules are well represented on many different cells. In one study it was estimated that there are approximately 10^5 HLA molecules per allele on each cell. In a heterozygous individual they found more than 2000 distinct peptides bound with a frequency of 100 to 4000 copies per cell, while only 100 copies is sufficient to illicit an immune response (Goldsby, Kindt and Osborne, 2000).

1.3. Selective forces driving the MHC

The maintenance of the extreme polymorphism in the MHC has been intensely debated for decades (Takahata and Nei 1990; Wills 1991; Hughes 2000). According to Takahata and Nei (1990) there are four main characteristics of MHC polymorphisms that should be adequately explained in order to clarify the maintenance of this extreme polymorphism. Firstly as previously mentioned, the level of polymorphism is exceptionally high. Secondly, there is a large amount of nucleotide differences between alleles. Some alleles differ by as much as 100 nucleotides (Li, 1997). This indicates that these alleles diverged from each other long ago and has been maintained across generations. Thirdly, the rate of nonsynonymous mutations is substantially higher than the rate of synonymous mutations in the peptide binding domain (Hughes and Nei 1988; Hughes and Nei, 1989) a clear indication of positive selection (Hughes, 2000). Lastly, some

polymorphisms are shared among species (transspecies polymorphism) (Kasahara *et al.* 1995; Danchin *et al.* 2003). These polymorphisms most likely occurred before the speciation event and were maintained for a long period (Li, 1997).

1.3.1. Neutral theory

It was initially thought that the high degree of polymorphism is maintained in a balance between a high mutation rate and random genetic drift (neutral theory of molecular evolution) (Kimura, 1968). Under the neutral theory, the rate of synonymous mutations is predicted to be higher than the rate of nonsynonymous mutations (Edwards and Hedrick, 1998). However, in the MHC the opposite is true, nonsynonymous mutations outnumber synonymous mutations in the peptide binding domain (Hedrick 1999; Bernatchez and Landry 2003). Furthermore, according to a model by Takahata and Nei (1990), neutral polymorphisms fail to explain the antiquity of MHC polymorphisms as they are not expected to be maintained for such long periods across speciation events. A high mutation rate alone would thus not be able to explain the transpecies polymorphisms observed, nor the amount of nucleotide differences between alleles (Takahata and Nei, 1990).

1.3.2. Pathogen-driven selection

The positive selection on the peptide binding domain (Hughes, 2000) and the crucial function this domain plays in the immune function has led to the widely held view that the unprecedented polymorphism is maintained, at least in part, by pathogen-driven selection (see reviews by Potts and Wakeland 1993; Zavazava and Eggert 1997; Hedrick 1999; Jeffery and Bangham 2000; Bernatchez and Landry 2003; Trowsdale 2005). The major histocompatibility complex has been associated with Marek's disease in chickens (Briles, Stone and Cole, 1977), lymphocytic choriomeningitis virus (LCMV) in mice (Zinkernagel *et al.* 1985), bacterial infection in Atlantic salmon

(Langefors *et al.* 2001) and various infective agents in Chinook salmon (Arkush *et al.* 2002). In humans, the HLA complex has been associated with more than a 100 diseases (Shiina, Inoko and Kulski, 2004). These include resistance to hepatitis (Thursz *et al.* 1997), malaria (Hill *et al.* 1991; Gilbert *et al.* 1998) and the onset of AIDS (Carrington *et al.* 1999). Studies have also shown a positive correlation between the HLA complex and susceptibility to autoimmune diseases (Salvetti *et al.* 2000), hepatitis B (Almarri and Batchelor, 1994), and herpes (Lekstrom-Himes *et al.* 1999). The two main hypothesis of how pathogens might maintain MHC polymorphisms is heterozygote advantage and frequency dependent selection (see reviews by Potts and Wakeland 1993; Zavazava and Eggert 1997; Hedrick 1999; Jeffery and Bangham 2000; Bernatchez and Landry 2003). Other discussions on the topic have included fluctuating selection, originally proposed by Hedrick, Thomson and Klitz (1987), as a possible third driving force (Hedrick 1999, Thursz *et al.* 1997).

1.3.2.1. Heterozygote advantage

According to the heterozygote advantage, or overdominant hypothesis, heterozygotes have a higher fitness than homozygotes because they can present a broader array of antigen peptides to the immune system. This enables the heterozygous individual to resist a broader array of pathogens (Doherty and Zinkernagel, 1975). Naturally, the extent of the benefit depends on the amount of overlap between presented antigen peptides (Jeffery and Bangham, 2000). Consequently not all heterozygotes are equally resistant to disease, but as a rule heterozygotes are more resistant than homozygotes. Indirect evidence in support of this hypothesis comes from studies showing a statistical significant deficiency of homozygotes (excess of heterozygotes) in several populations (Hedrick, Whittam and Parham 1991; Markow *et al.* 1993). A significant excess of MHC heterozygotes were also detected in natural populations of pheasants (Von Schantz *et al.* 1996) and

mice (Ritte *et al.*1991). Conversely, a recent study on desert bighorn sheep found no MHC homozygote deficiency (Boyce *et al.* 1997; Hedrick and Parker 1998). The fact that only some populations seem to have an excess of heterozygotes might depend on the pathogen load the population is exposed to. Hings and Billingham (1981) found a significant excess of heterozygous male offspring, but this excess disappeared when they treated the mice with antibiotics. In mice, the number of heterozygote offspring also increases when the population is exposed to mouse hepatitis virus (Rülicke *et al.* 1998).

More direct evidence for heterozygote advantage comes from studies correlating MHC heterozygosity and specific diseases. Chinook salmon, heterozygous for class II MHC loci, had a higher survival rate than homozygotes when exposed to infectious haematopoietic necrosis virus (Arkush *et al.* 2002). Carrington *et al.* (1999) found that maximally heterozygous HIV positive individuals had a delayed onset of AIDS symptoms compared to more homozygous HIV positive individuals. An increase in class II heterozygosity was also correlated with less persistent infections of hepatitis B (Thursz *et al.* 1997). When it comes to offspring viability, heterozygote leghorn hens produce more viable offspring than their homozygous inbred parents (Sato *et al.* 1992). According to Takahata and Nei (1990), heterozygote advantage is the most likely candidate to explain the maintenance of MHC polymorphism. Their models show that heterozygote advantage can account for all four features associated with MHC polymorphism.

1.3.2.2. Frequency dependent selection

The most popular model of frequency dependent selection, or rare allele advantage, states that new mutant alleles will have a fitness advantage because the pathogens haven't had time to adapt to them (Bodmer 1972; Parham and Ohta 1996). Once these alleles become more common the pathogens will adapt to them and their

frequency will decrease (Gilbert *et al.* 1998). It is exactly for this reason that Takahata and Nei (1990) favoured heterozygote advantage above frequency dependent selection because according to them these alleles would eventually be lost due to genetic drift. Frequency dependent selection could explain a high degree of polymorphism but not the longevity of MHC alleles (Takahata and Nei, 1990). A more recent form of frequency dependent selection is the minority advantage (Wills, 1991). This hypothesis assumes that once the old allele becomes rare enough, the pathogens adapted to it will become very scarce. These alleles then once again have the selective advantage and increase in frequency. In short this hypothesis predicts a more dynamic polymorphism (Slade and McCallum, 1992), although Hughes and Nei (1992) argue that there is no reason to believe that these frequency changes are cyclic in the MHC.

Several studies suggested evidence for frequency dependent selection. In a study on Atlantic salmon Langefors *et al.* (2001) showed that one class II MHC allele was significantly linked to higher resistance against the bacterium *Aeromonas salmonicida.* They did not find any correlation between increased heterozygosity and increased resistance. Specific alleles were also linked to resistance for *Plasmodium falciparum* (Gilbert *et al.* 1998) and intestinal nematodes in Soay sheep (Paterson, Wilson and Pemberton, 1998). Paterson, Wilson and Pemberton (1998) found that the rarer alleles were associated with increased resistance and the more common alleles with decreased resistance. The correlation between specific alleles and resistance was not consistent between lambs and yearlings. They concluded that different alleles were important at different life stages and that heterozygotes showed the highest overall fitness.

Although the debate on the relative importance of heterozygote advantage vs. frequency dependent selection still rages on, it is becoming obvious that both hypotheses play a role in the maintenance of MHC polymorphism (Slade and McCallum, 1992).

1.3.2.3. Fluctuating selection

Fluctuating selection, originally proposed by Hedrick, Thomson and Klitz (1987), has not received much attention as a possible explanation for the maintenance of MHC polymorphism. This is surprising, since in its essence it proposes what a lot of authors are saying at the moment, that both frequency dependent selection and heterozygote advantage are important selective forces for the MHC (Slade and McCallum, 1992). Fluctuating selection proposes that a specific allele (or a few specific alleles) confer resistance to a specific parasite. This specific parasite confers a variable pressure on the host population over time, in other words its frequency fluctuates. When two such parasites are present in the host population at the same time, there is a heterozygote advantage (Hedrick, Thomson and Klitz, 1987). This hypothesis takes into account that most major human diseases of the past were epidemic (Hedrick, 1999), and thus the pathogen pressure fluctuated over time.

1.3.3. Sexual selection

Two additional major forces shaping MHC polymorphism is MHC based mating preferences and selective block of pregnancy (Clarke and Kirby 1966; Zavazava and Eggert 1997; Eggert, Müller-Ruchholtz and Ferstl 1999)(Figure 6). These two mechanisms were once thought too far-fetched as possible explanations for the maintenance of MHC polymorphism. However, the extreme polymorphism observed in the MHC complex, although unique among vertebrate loci, is rivalled by loci that control the mating patterns in plants (self-incompatibility loci)(Haring *et al.* 1990). MHC based mating preferences and selective block of pregnancy will be discussed in turn.

1.3.3.1. MHC based mating preferences

The second major driving force behind MHC polymorphism is MHC based mating preferences (for review see Potts and Wakeland 1993; Zavazava and Eggert 1997; Hedrick 1999; Penn and Potts 1999; Bernatchez and Landry 2003). Yamazaki *et al.* (1976) first discovered MHC based dissasortative mating preferences (a preference for dissimilar MHC genotype/alleles) in inbred mice. Since then several studies on mice have shown MHC based dissasortative mating preferences in the laboratory (Yamazaki *et al.* 1978; Egid and Brown 1989) and in a semi natural population (Potts, Manning and Wakeland, 1991). MHC based dissasortative mating preferences have also been shown in savannah sparrows (Freeman-Gallant *et al.* 2003), Atlantic salmon (Landry *et al.* 2001) and humans (Ober *et al.* 1997). Although similar studies did not find MHC based dissasortative mating preferences in Soay sheep (Paterson and Pemberton, 1997) and three other human populations (Rosenberg, Cooperman and Payne 1983; Jin, Speed and Thomson 1995; Hedrick and Black 1997). It is evident that the topic of MHC based mating preferences is far from resolved.

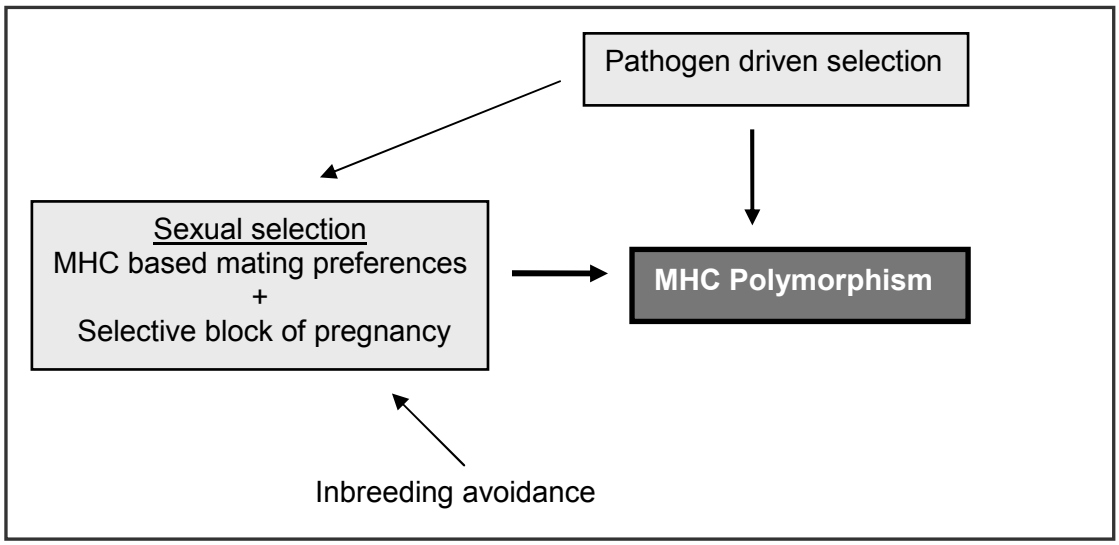


***Figure 6**. Primary driving forces of MHC polymorphism. The three driving forces behind MHC polymorphism is pathogen driven selection, MHC based mating preferences and selective fertilization/abortion. Inbreeding avoidance and pathogen*

driven selection both favour MHC based mating preferences. Figure adapted from Potts and Wakeland (1993)

MHC based mating preferences are driven by pathogen driven selection, consisting of heterozygote advantage and frequency dependent selection, and inbreeding avoidance. (for a review see Potts and Wakeland 1993) (Figure 6, 7). In pathogen driven selection, heterozygote advantage shapes mating preferences in two ways: Firstly, heterozygote advantage favours dissasortative mating preferences, since a dissimilar mate will produce more heterozygous offspring (Figure 7). According to Brown (1996) an individual should choose a mate that complements his/her genes in such a way as to increase the heterozygosity of their offspring (Heterozygous offspring hypothesis). Secondly, individuals are also expected to prefer a heterozygous mate, since such an individual will confer direct benefits due to their increased health and resistance to infectious diseases (Figure 7). What is more, mating with a heterozygous mate will produce more heterozygous offspring at most allele frequencies (Mitton *et al.* 1993)(Heterozygous mate hypothesis). The latter hypothesis does not necessarily facilitate dissasortative mating, as individuals will prefer a heterozygote mate whether or not they share alleles with them (Figure 7). According to Nowak, Tarczy-Hornoch and Austyn (1992) maximum MHC heterozygosity might not be the optimal strategy. During thymic selection of MHC molecules, molecules that present self peptides are eliminated to avoid an autoimmune response. If an individual is maximally heterozygous, more MHC molecules are eliminated, thereby reducing the T cell repertoire. It follows that an intermediately heterozygous individual will strike a better equilibrium between antigen presentation and T cell repertoire.

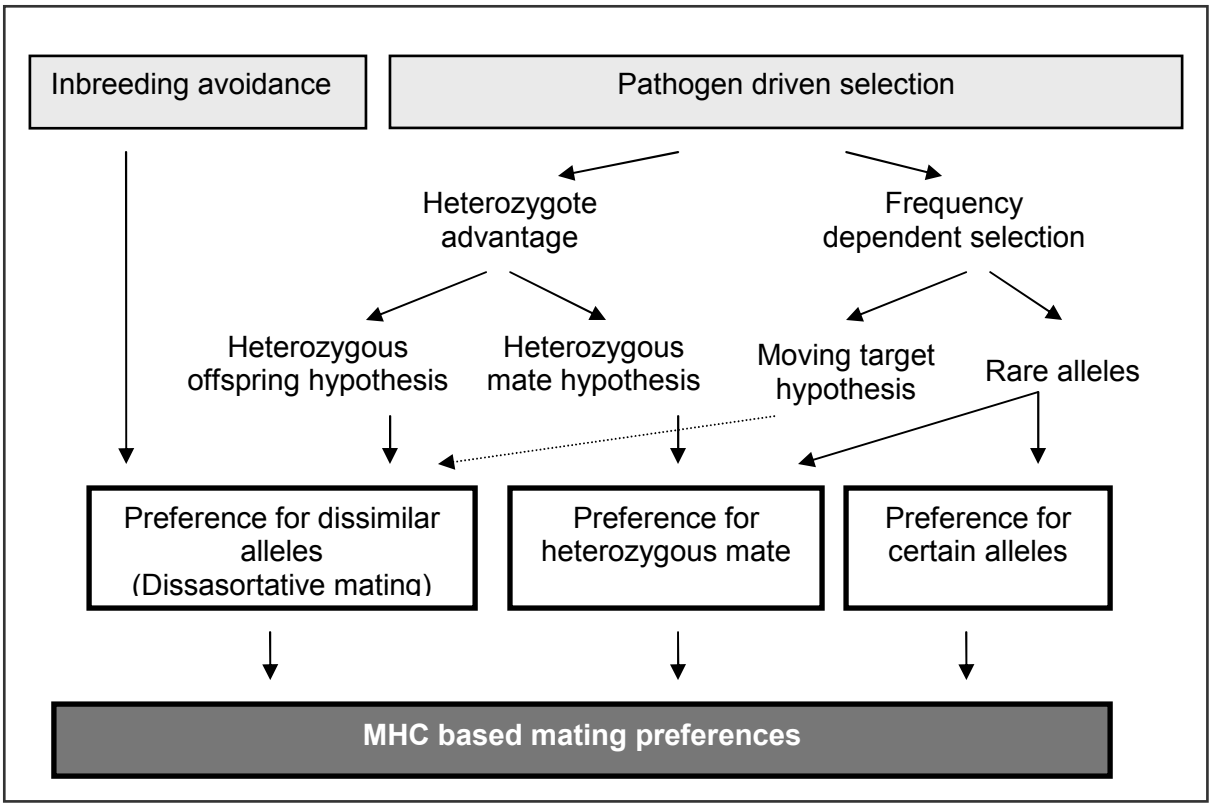


Figure 7. *Distinct MHC based mating preferences. MHC based mating preferences consists of (i) a preference for dissimilar alleles (Dissasortative mating preference) to avoid inbreeding, produce heterozygous offspring and provide a moving target against pathogens (ii) a preference for a heterozygous mate, to increase the direct benefits, increase the number of heterozygous offspring and to benefit from rare alleles (iii) a preference for certain beneficial alleles.*

Frequency dependent selection also favours dissasortative mating preferences. According to the moving target hypothesis, proposed by Penn and Potts (1999) MHC dissasortative mating preferences provide a moving target to pathogens that successfully evade immune recognition. As the pathogen adapts to the parental MHC type, dissasortative mating produces offspring dissimilar to the parental type, thereby increasing offspring survival (Figure 7). This is especially important in the light of the fact that most important pathogens driving MHC diversity are suspected to be transmitted vertically (Klein and O'Huigin, 1994). But this is not the only way in

which frequency dependent selection shapes MHC based mating preferences. In the moving target hypothesis the offspring benefits due to a “rare genotype” compared to the parental genotypes. Our second form of frequency dependent selection focuses on the benefit associated with rare alleles. As mentioned earlier, rare alleles, especially new ones, confer a selective advantage because very few, if any, pathogens are adapted to them. Due to their inherent low frequency, these alleles will most probably be present in a heterozygote (Thornhill *et al.* 2003), thereby favouring a preference for a heterozygous mate (Figure 7). Furthermore, Hill *et al.* (1991) and Paterson *et al.* (1998) showed that specific alleles increase resistance to certain diseases (Figure 7). If MHC recognition is allele-specific, a preference for these specific alleles might develop. However to my knowledge, no previous studies have shown a preference for specific alleles.

Finally, the extreme polymorphism of MHC makes it ideally suited as a marker for inbreeding avoidance. If individuals share MHC alleles, they are most likely related (Penn and Potts, 1999). Thereby, by using MHC as markers to mate disassortatively, individuals generally avoid mating with close kin (Partridge 1988; Hedrick 1992) (Figure 7).

According to Howard (1991) MHC dissasortative mating preferences are primarily due to pathogen driven selection, since these mating preferences are potentially perfect at preventing homozygosity (heterozygote advantage) but imperfect at preventing inbreeding. Even though this is true, the situation is slightly more complex. The relative importance of these factors in the mating preference is context dependent. In a population with substantial inbreeding load and/or a high likelihood of kin meeting as potential mates, inbreeding avoidance will play an important role. Similarly, if the pathogen load is substantial, pathogen driven selection will be a more essential factor in mate preference (Potts and Wakeland, 1993). Even after taking context into account, most studies only test for MHC based

dissasortative mating. It should be apparent by now that MHC based mating preferences are more than just MHC based dissasortative mating. In order to understand the discrepancies between these studies, we need to examine them in more detail.

A brief review of MHC based mating preferences

In this section we will restrict our discussion to studies measuring MHC based preferences directly, through actual matings and/or pairbonding. Yamazaki *et al.* (1976, 1978) found that males preferentially mate with MHC dissimilar females. Egid and Brown (1989) found a similar preference in female mice, as did Potts, Manning and Wakeland (1991) in a semi natural population. According to Potts, Manning and Wakeland (1991) and Hedrick (1992) these MHC based dissasortative mating preferences are strong enough to account for the diversity observed in mice populations. Penn and Potts (1999) cautioned that these studies should not be extrapolated to wild populations, since some assays that determine mating preference indirectly, are done in highly inbred populations and control dominance in male mice. Despite these cautionary notes, few authors dispute the role of MHC based dissasortative mating preferences in mice.

Studies in other species have been less conclusive. In savannah sparrows, female yearlings avoid pairing with MHC similar mates. Females are also more prone to cheat if her social male is MHC similar (Freeman-Gallant *et al.* 2003 but see Kleven and Lifjeld, 2005). However, once they grow older they start pairing at random (Freeman-Gallant *et al.* 2003). This discrepancy is probably due to a decrease in choosiness with increased age (Penn and Potts 1998a; Lynch *et al.* 2005). Landry *et al.* (2001) tested for heterozygote advantage and overall inbreeding avoidance in Atlantic salmon. They found that Atlantic salmon preferentially choose mates in order to increase their offspring's heterozygosity and not to avoid inbreeding. Paterson and Pemberton (1997) found no MHC based mating preference

in Soay sheep, but noted that there seems to be very little female choice. Mating was mostly as a result of a dominance hierarchy established by male-male interaction. In a subsequent study they did however find certain MHC alleles to be important in survival and resistance to intestinal nematodes (Paterson, Wilson and Pemberton, 1998). This relates to frequency dependent selection, and since they only tested for the number of alleles shared in their first study they would not have picked up a preference for certain alleles. In humans, detecting dissasortative mating preferences is even more challenging. Studies on human mating preferences are often contradictory. Ober *et al.* (1997) found a dissasortative mating preference in the Hutterites, a highly inbred, religiously isolated society of European descent. Hedrick and Black (1997), on the other hand, found no dissasortative mating preference in South Amerindians, nor a heterozygote excess at the MHC. Earlier studies also found no dissasortative mating preference (Rosenberg, Cooperman and Payne, 1983). It is not immediately obvious why these studies should produce contradictory results, but Beauchamp and Yamazaki (1997) warns that dissasortative mating may be very weak and difficult to detect in humans. We have several cultural and biological factors that conceal mating preferences and even among inbred mice not all strains show dissasortative mating preferences. The high degree of inbreeding in the Hutterite population probably contributed greatly to the mating preferences observed. Inbreeding avoidance is bound to be important in humans because of the high cost of inbreeding depression in our species (May, 1979) and the real chance of encountering close kin as a mating partner (Ober *et al.* 1997). It seems that most of the disparity can be explained by a lack of actual mate choice. Factors such as dominance, lack of choosiness, biological and cultural factors and a limited choice of partners must be taken into account. It is evident that the topic of MHC based mating preferences still requires extensive future work. In these studies, particular care must also be taken to differentiate between the different MHC based preferences.

1.3.3.2. Selective block of pregnancy

The third and last major driving force behind MHC polymorphism is a form of postcopulatory mate choice, selective block of pregnancy (Clarke and Kirby 1966; Zavazava and Eggert 1997; Eggert, Müller-Ruchholtz and Ferstl 1999). According to this hypothesis, females selectively reject the sperm, or abort the offspring, of specific males (cryptic female choice)(Eberhard and Cordero, 1995). Studies indicate that HLA sharing between couples tend to result in more spontaneous abortions (Thomas *et al.* 1985; Ober 1995; Black and Hedrick 1997). Homozygous offspring are apparently selectively aborted, to increase offspring heterozygosity and to reduce inbreeding (Potts, Manning and Wakeland, 1991). Abortional selection is generally assumed to be due to interactions between the mother and foetus, or sperm (Penn and Potts, 1999), through two proposed mechanisms (Thomas *et al.* 1985).

(i) Immune hypothesis: An adequate maternal immune response against the paternal antigens in the foetus seems to be essential for proper implantation and survival of the embryo.

(ii) Genetic hypothesis: Foetuses with similar HLA alleles, which are presumably linked to recessive lethal alleles, are more frequently aborted.

Despite various studies on the topic, the association between HLA similarity and spontaneous abortions is not completely resolved. Not all studies find that HLA similarities cause spontaneous abortions (for review see Beydoun and Saftlas, 2005). Abortional selection might depend on the female infection status, as virally infected female mice produce more MHC heterozygous offspring (Rülicke *et al.* 1998). Spontaneous abortions might also be due to non HLA loci, linked to the HLA. (Thomas *et al.* 1985; Ober 1995).

1.4. Recognising the MHC genotype

MHC based mating preferences was initially discredited, due to the lack of correlation between the known role of the MHC in antigen presentation and the mating preferences observed (Potts and Wakeland 1993; Hughes and Hughes 1995). Since the mid-1970's a steady stream of evidence revealed a correlation between odour and MHC genotype (for review see Penn and Potts, 1998b). Mice and rats can differentiate between the odours of congenic individuals differing only at their MHC loci (Yamazaki *et al.* 1983; Singh, Brown and Roser 1987). Female mice prefer the odour of MHC similar females as communal nesting partners (Manning, Wakeland and Potts, 1992), while preferring the odour of MHC dissimilar males as mates (Egid and Brown 1989; Roberts and Gosling 2003). On the contrary, a follow up study by Eklund, Egid and Brown (1991) found no MHC based dissasortative preference in males. A more recent study by Beauchamp, Curran and Yamazaki, (2000) showed that male mice can distinguish between pregnant females carrying foetuses with differing MHC type, and preferentially associate with females carrying more dissimilar foetuses. In fish, juvenile arctic char prefer water scented by MHC identical siblings (Olsén *et al.* 1998), while gravid female three-spined sticklebacks prefer water scented by very MHC heterozygous males (Reusch *et al.* 2001). Odours might also play a role in spontaneous abortions, since women find it more difficult to maintain pregnancies when exposed to certain odours (Penn and Potts, 1999)

Due to our supposed feeble sense of smell, odour based mating preferences were initially somewhat disregarded in humans (Potts and Wakeland, 1993). Yet, humans are extremely sensitive to some odours and there are strong links between smell, physiology and emotions (Cain 1977; Stoddart 1980; Stoddart 1990). Not only can we smell comparatively well, we are also the most richly endowed with scent producing glands among the primates (Stoddart, 1990). Several studies have shown

a link between odour and MHC type in humans. Humans can differentiate between two congenic mouse strains, differing only in their MHC type (Gilbert *et al.* 1998). Women prefer the odours of more MHC dissimilar men (Wedekind *et al.* 1995; Wedekind and Furi 1997 but see Hedrick and Loeschcke 1996). A follow up study by Thornhill *et al.* (2003) did not find a preference for MHC dissimilarity in women but they did in men. Instead they found a preference for MHC heterozygosity in women. Individuals even prefer the smell of perfumes with a similar odour as their own HLA type, presumably to amplify their own body odour (Milinski and Wedekind, 2001). All these studies indicate that we do use odour in mating preferences and kin recognition, but we are not necessarily aware of the fact (Jacob *et al.* 2002).

In order to identify MHC dissimilar individuals, there needs to be some reference to own MHC type. Self-recognition can be accomplished by using close kin as referents (familial imprinting) or by self-inspection. Several cross fostering studies have shown familial imprinting (Beauchamp *et al.* 1988; Yamazaki *et al.* 1988; Eklund 1997; Penn and Potts 1998c; Yamazaki *et al.* 2000; Olsén *et al.* 2002). But, self-imprinting should not be entirely disregarded (Yamazaki *et al.* 2000; Jacob *et al.* 2002). It is still unsure if MHC based odour perception is allele-specific or genotype-specific (Potts and Wakeland, 1993), but there is at least some evidence that individuals can smell specific alleles (Jacob *et al.* 2002).

MHC class I and II molecules are present in blood serum of healthy individuals (Zavazava and Eggert, 1997) and is excreted in urine, faeces and sweat (Singh, Brown and Roser 1987; Brown and Eklund 1994; Zavazava, Westphal and Müller-Ruchholtz 1990; Pearse-Pratt *et al.* 1999; Yamazaki, Singer and Beauchamp 1999; Toivanen, Vaahtovuo and Eerola 2001). There are several hypotheses explaining how MHC molecules influence odour (for review see Brown and Eklund 1994; Penn and Potts 1998c). We will discuss them only briefly:

1. The MHC molecule hypothesis: MHC molecules or the fragments thereof are the odorants (Singh, Brown and Roser. 1987).

2. The peptide hypothesis: Peptides once bound by MHC molecules, dislodge from the MHC molecules and become odorant precursors (Singer, Beauchamp and Yamazaki, 1997).
3. Microflora hypothesis: The MHC shapes the commensal microflora population, which in turn produces odorants (Toivanen, Vaahtovuo and Eerola 2001).
4. Carrier hypothesis: MHC molecules bind a unique cocktail of volatiles produced by the commensal microflora (Pearse-Pratt *et al.* 1999; Singh 1999; Yamazaki, Singer and Beauchamp 1999).
5. Peptide microflora hypothesis: MHC molecules alter the pool of available peptides, and their degraded products are made volatile by commensal microflora (Penn and Potts, 1998c)

Olfactory cues are not the only cues that could play a role in MHC-based preferences. Visual and acoustic cues have been somewhat overlooked when it comes to MHC-based preferences, although they play a prominent role in mate choice. This is perhaps not surprising, as the mechanism linking these senses to the known immunological function of MHC is less than obvious (Roberts *et al.* 2005a).

Nevertheless, several studies have shown MHC based preferences based on visual cues alone. In pheasants, spur length is linked to the MHC, and females show a preference for males with longer spurs (Von Schantz *et al.* 1997). Similarly in white-tailed deer, antler size is significantly correlated with the MHC genotype and parasite load (Ditchkoff *et al.* 2001). In humans, facial attractiveness and an attractive scent is positively correlated in women (Rikowski and Grammer 1999; Thornhill and Gangestad, 1999; Thornhill *et al.* 2003) and in men if the female rater is in her fertile phase (Thornhill and Gangestad 1999; Rikowski and Grammer, 1999). Two recent studies also showed MHC based preferences for facial features in humans (Roberts

et al. 2005a; Roberts *et al.* 2005b). To my knowledge, no such studies have yet been done for acoustic cues.

1.5. Facial attractiveness

Humans utilize both physical and psychological cues when selecting a prospective partner (Miller and Todd, 1998). Unfortunately for some, psychological cues take longer to access (Miller and Todd, 1998). First impressions therefore are mainly based on physical attractiveness (Walster *et al.* 1966; Kurzban and Weeden 2005), and men are no longer the only gender believed to be taking notice. Although physical attractiveness in the opposite sex is undoubtedly exceptionally important for the male gender (Feingold 1990; Buss and Schmitt 1993; Furnham, Mistry and McClelland 2004), both sexes use physical attractiveness to some extend when selecting a partner (Buss 1994; Kurzban and Weeden 2005).

The human face portrays a considerable number of visual cues, some of which signal attractiveness. Facial attractiveness is currently a vibrant field of research, combining biological, psychological, cognitive and even computational image processing viewpoints. We will focus mainly on the biological and psychological perspectives.

Facial attractiveness preferences are shared across different cultures (Cunningham *et al.* 1995; Langlois *et al.* 2000), ages (Zebrowitz, Olson and Hoffman 1993; Kissler and Bäuml 2000; Ramsey *et al.* 2004) and sexes (Fink and Penton-Voak 2002). Both sexes prefer to associate with and date facially attractive individuals (Walster *et al.* 1966; Dion, Berscheid and Walster 1972). This preference is warranted, at least in part, by the increased fecundity of attractive individuals (Furnham, Mistry and McClelland, 2004). Facially attractive men have significantly higher semen quality (Soler *et al.* 2003) and female physical attractiveness is seen as the principal indicator of fecundity (Symons, 1979). Not only do they have a higher

fecundity but attractive individuals are considered more successful, happier and a better marital partners than more unattractive individuals. Interestingly, individuals with average attractiveness are rated as the best parents (Dion, Berscheid and Walster, 1972). Facial attractiveness is also positively correlated with perceived health in both sexes (Kalick *et al.* 1998) and self reported health in females but not males (Hume and Montgomerie, 2001). Studies seeking a correlation between facial attractiveness and actual health have had mixed results. Facial attractiveness is positively correlated with longevity (Henderson and Anglin, 2003) and fewer physical symptoms (Shackelford and Larsen, 1999) but not with adolescent and future health (Kalick *et al.* 1998).

There are four main features that contribute to facial attractiveness: symmetry, averageness, hormone markers and age. These will be discussed in turn.

1.5.1. Symmetry

Symmetry is a reliable indicator of the ability to resist certain perturbations during development. According to the evolutionary advantage viewpoint, symmetry advertises mate quality and is therefore important for mate choice, whilst it also plays a role in other social interactions such as reciprocal altruism (Polak and Trivers, 1994). Another view, the perceptual bias view, states that there is a preference for perceptual stimuli in general, since symmetrical stimuli are more easily processed. However, Little and Jones (2003) provided evidence against a simple perceptual bias view. Symmetry, or rather the lack thereof, is commonly measured by fluctuating asymmetry (FA), a specific form of asymmetry. Fluctuating asymmetry is defined as random deviations from perfect symmetry in bilaterally paired traits (Van Valen 1962; Thornhill and Gangestad 1996). According to Polak and Trivers (1994) FA might be the best measure of phenotypic quality we have. Fluctuating asymmetry serves as a reliable indicator of developmental instability (Palmer and Strobeck, 1986) and is

increased by genetic (mutation, homozygosity etc) and environmental stresses (habitat destruction, infection by parasites, maternal disease etc) during development (Møller 1992; Parsons 1992; Markow and Martin 1993; Manning and Chamberlain 1994; Møller 1997).

Fluctuating asymmetry is negatively correlated with facial and bodily attractiveness in both sexes (Grammer and Thornhill 1994; Mealey, Bridgestock and Townsend 1999; Rikowski and Grammer 1999; Hume and Montgomerie 2001; Penton-Voak *et al.* 2001), but is more prominent in sexually dimorphic traits, especially male sexually dimorphic traits (Manning and Chamberlain 1994; Møller, Soler and Thornhill 1995; Manning, Koukourakis and Brodie 1997; Møller and Thornhill 1998; Simmons *et al.* 2004). This is especially true in monogamous species, in which males contribute significantly to offspring (Ryan, 1997). Males with decreased FA have higher body mass (Manning, 1995), mating success, fecundity, (Watson and Thornhill, 1994; Møller 1997; Møller and Thornhill 1998; Waynforth 1998) and their partners' even orgasm more often than less symmetrical males (Thornhill, Gangestad and Comer, 1995). Fluctuating asymmetry is also negatively correlated with mating success and fertility in females (Møller, Soler and Thornhill 1995; for review see Watson and Thornhill 1994; Thornhill and Møller, 1997).

Manning, Koukourakis and Brodie (1997) found a positive correlation between FA and resting metabolic rate in males but not in females. The resting metabolic rate is defined as the energy expenditure when at rest. This implies that individuals with a high FA spend more of their energy just to "idle", where more symmetrical individuals can invest this extra energy for other things such as sexual secondary characteristics and combating disease. As with facial attractiveness, fluctuating asymmetry is negatively linked to perceived health (Schakelford and Larsen 1997; Rhodes *et al.* 2001). Fluctuating asymmetry has also been negatively linked to genetic, psychological, emotional, physiological health (Shackelford and Larsen 1997; Milne *et al.* 2003) and positively linked with morbidity (Livshits and Kobvlianski 1991:

Watson and Thornhill 1994; Thornhill and Møller 1997; Waynforth 1998). On the contrary Rhodes *et al.* (2001) found no correlation between facial FA and health measures.

Symmetry is not the only factor predicting facial attractiveness. Scheib, Gangestad and Thornhill (1999) tested the effect of symmetry, by presenting only one side of the face. They found that more symmetrical faces are still rated as more attractive. This they took as evidence that a correlate of symmetry, and not symmetry *per se*, drives facial attractiveness. It is true that male and female sexually dimorphic traits (Scheib, Gangestad and Thornhill 1999 but see Penton-Voak *et al.* 2001), averageness (Baudouin and Tiberghein, 2004), apparent healthiness of facial skin in males (Jones *et al.* 2004) and even the body mass index (BMI) in females (Hume and Montgomerie, 2001) all correlate with facial symmetry and all predict facial attractiveness independent of symmetry. But, this should not necessarily exclude symmetry as a predictor of facial attractiveness. Even when only the left side of the face is presented, there are still indicators of symmetry around the original midline (D. I. Perret, personal communication).

1.5.2. Averageness

Langlois and Roggman (1990, quoted in Grammer and Thornhill 1994) showed that computer composite averaged faces are more attractive than most of the individual faces it is composed from. But as Alley and Cunningham (1991, quoted in Grammer and Thornhill 1994) pointed out, these composites are also more symmetrical and have a more homogenous skin tone. According to O'Toole *et al.* (1999) shape normalisation attribute more to facial attractiveness than texture normalisation. Another study by Grammer and Thornhill (1994) found that only female composites appear significantly more attractive, while male averaged faces appear less attractive. They attribute this discrepancy to the fact that certain secondary sexual

characteristics are preferred when large, such as the masculine jaw. Average faces also appear younger (O'Toole *et al.* 1999), an attribute preferred by the male gender. However, Perrett, May and Yoshikawa (1994) and Perrett *et al.* (1998) did not find a correlation between facial attractiveness and facial averageness in females. It is evident that facial averageness as predictor of facial attractiveness is still a highly contentious subject.

Halberstadt and Rhodes (2000) identified three main reasons why average faces would appear more attractive:

1. Average faces are more familiar and familiarity is perceived as more attractive (Langlois *et al.* 1994 in Halberstadt and Rhodes 2000)
2. Average faces indicate increased health due to the presumed relationship between averageness and heterozygosity (Fink and Penton-Voak 2002)
3. Averageness is attractive because it is easier to process.

Halberstadt and Rhodes (2000; 2003) found that average animal objects are not usually perceived as more familiar, though wristwatches and automobiles are. On the other hand, facial averageness is associated with increased perceived health and signals true health in females (Grammer and Thornhill 1994; Rhodes *et al.* 2001) and males (Rhodes *et al.* 2001). Furthermore, deviations of facial averageness is characteristic of certain chromosomal disorders (for review see Thornhill and Møller, 1997). The hypothesis that averageness is generally more attractive, not only relating to identifying high quality partners cannot be excluded either. Dogs, birds, watches, and fish are all rated more attractive when average (Halberstadt and Rhodes 2000; Halberstadt and Rhodes 2003). Based on these studies, it is evident that none of the abovementioned hypothesis can be excluded safely.

1.5.3. Hormone markers

1.5.3.1. Testosterone

Testosterone is the principal hormone responsible for male secondary sexual characteristics and reproductive function. The level of testosterone-to-oestrogen determines the lateral growth of the cheekbones, jaws and chin, the forward growth of the bones of the eyebrow ridges and the lengthening of the lower face (Miller and Todd 1998; Fink and Penton-Voak 2002). In essence, it is responsible for the masculinisation of the male face.

Facial masculinity is positively correlated with perceived dominance (Perrett *et al.* 1998), a sexually selected trait (Kenrick, 1989), but not reliably with facial attractiveness (Keating 1985; Perrett *et al.* 1998). Some studies have shown a preference for masculine faces (Cunningham, Barbee and Pike 1990; Grammer and Thornhill 1994; Johnston *et al.* 2001) while others have not (Perrett *et al.* 1998). According to recent studies the preference for masculinity is condition dependent. Women in the fertile phase of their menstrual cycle prefer more masculine men for short-term relationships than women in the less fertile phase (Penton-Voak and Perrett 2000; Johnston *et al.* 2001).

Whether or not women find them more attractive, facially masculine men are perceived healthier. Nevertheless, there is only a modest correlation between facial masculinity and actual health (Rhodes *et al.* 2003). It is evident that testosterone's role in the immune system is an intricate one (for a review see Ahmed and Talal, 1990). There are four hypotheses regarding the action of testosterone in the immune system. According to the immunocompetence handicap hypothesis first proposed by Folstad and Karter (1992), only high quality males with a good immune response can overcome the immunosuppressive effect of testosterone and display audaciously. This hypothesis was long held as the central dogma of testosterone's role in the immune system. In 1994, Wedekind and Folstad proposed the resource allocation

hypothesis suggesting that testosterone suppresses the immune system in favour of the production of secondary sexual characteristics. However, according to Hillgarth and Wingfield (1997) the risk associated with immune suppression would be immense compared to the metabolic resources saved by immunosupression. Instead, Hillgarth, Ramenofsky and Wingfield (1997) proposed the sperm protection hypothesis, which states that testosterone suppresses the immune response in order to protect antigenic spermatozoa. All three these hypothesis inherently assume that testosterone is wholly immunosuppressive. Indeed, it has long been known that men are more susceptible to a variety of infections that women (Alexander and Stimson, 1988). According to convention, testosterone suppresses both the humoral and cell mediated immune response (Alexander and Stimson, 1988). But several recent studies have brought the supposed immunosuppressive effect of testosterone into question (for review see Braude, Tang-Martinez and Taylor, 1999). It is into this milieu that the immuneredistribution hypothesis was born. According to this hypothesis, immune cells are shunted to different areas of the body, depending on the need. Testosterone presumably shunts the immune cells away from the intestinal tract to areas of potential injury such as the skin (Braude, Tang-Martinez and Taylor, 1999)

1.5.3.2. Oestrogen

Both oestrogen and progesterone play an important role in female reproductive biology. Yet, oestrogen is alone responsible for the production of female secondary sexual characteristics and the maturation of the reproductive organs at puberty (Marieb, 1995). The oestrogen-to-testosterone level signals female fertility and determines the relative hairlessness and smooth skin, smaller and lower face (Miller and Todd 1998; Fink, Grammer and Thornhill 2001; Fink and Penton-Voak 2002) and lighter skin colour in females (Manning, Bundred and Mather 2004 but see Fink,

Grammer and Thornhill 2001). Facial femininity in females is correlated with attractiveness and perceived health but not actual health (Rhodes *et al.* 2003). Oestrogen seemingly inhibits the cell mediated immune response, while stimulating the humoral immunity (Alexander and Stimson, 1988). Despite the generally favourable effect of oestrogen on the immune response, oestrogen might be immunosuppressive in high quantities (Grossman, 1984).

1.5.4. Age

Perceived youthfulness plays an important role in female attractiveness. Neonate features such as large eyes, a small nose, small chin, prominent cheekbones and narrow cheeks are considered very attractive in women (Cunningham, 1986). These features presumably indicate that the woman is in her fertile years (Buunk *et al.* 2001). According to Menken, Trussell and Larsen (1986) men place more importance on perceived youthfulness in their female partners because women have a limited reproductive window. Men on the other hand are fertile through most of their adult lives, and one might expect that perceived youthfulness might not be as important for their attractiveness (Symons, 1979). Yet, Korthase and Trenholme (1982) found that perceived youthfulness is important for both sexes, albeit more important for female attractiveness.

1.5.5. Evaluating multiple features

Thornhill and Møller (1997) cautions that physical attractiveness features should not be studied in isolation. Observers use more than one feature to assess a potential partner (Kurzban and Weeden, 2005). One notable example is male sexual ornamentation. More often than not males have multiple sexual ornaments. How females use all these ornaments to assess the male's genetic quality is still a contentious subject. The multiple message hypothesis states that each ornament

signals a different aspect of the individual's condition (e.g. food intake, immune system etc). Another hypothesis, the redundant signal hypothesis suggests that each ornament provides a partial account of overall condition. By evaluating more than one ornament against each other, the error associated with each ornament can be minimised. The unreliable signal hypothesis differs from the previous two hypotheses in the fact that this hypothesis assumes that many ornaments no longer reliably signal mate quality. These ornaments are only maintained because they are cheap and there is still a weak female choice for them (for review see Møller and Pomiankowski, 1993).

References

Ahmed, S. A. and Talal, N. (1990) Sex hormones and the immune system-part 2. Animal data. *Baillières Clinical Rheumatology*, 4: 13-31.

Alexander, J. and Stimson, W. H. (1988) Sex hormones and the course of parasitic infection. *Parasitology Today*, 4: 189-193.

Almarri, A. and Batchelor, J. R. (1994) HLA and hepatitis B infection. *The Lancet*, 344: 8931.

Arkush, K. D., Giese, A. R., Mendonca, H. L., McBride, A. M., Marty, G. D. and Hedrick, P. W. (2002) Resistance to three pathogens in the endangered winter-run chinook salmon (*Oncorhynchus tshawytscha*): effects of inbreeding and major histocompatibility complex genotypes. *Canadian journal of fisheries and aquatic sciences*, 59: 966-975.

Barrett, L., Dunbar, R. and Lycett, J. (2002) *Human Evolutionary Psychology*. New York, Palgrave

Bateson, P. (1983) *Mate Choice*. Cambridge, Cambridge University Press.

Baudouin, J.-Y. and Tiberghein, G. (2004) Symmetry, averageness, and feature size in the facial attractiveness of women. *Acta Psychologica*, 117: 313-332.

Beauchamp, G. K., Curran, M. and Yamazaki, K. (2000) MHC-mediated fetal odourtypes expressed by pregnant females influence male associative behaviour. *Animal Behaviour*, 60: 289-295.

Beauchamp, G. K. and Yamazaki, K. (1997) HLA and mate selection in humans: commentary. *American Journal of Human Genetics*, 61: 494-496.

Beauchamp, G. K., Yamazaki, K., Bard, J. and Boyse, E. A. (1988) Preweaning experience in the control of mating preferences by genes of the major histocompatibility complex of the mouse. *Behavior Genetics*, 18: 537-547.

Bernatchez, L. and Landry, C. (2003) MHC studies in nonmodel vertebrates : what have we learned about natural selection in 15 years? *Journal of Evolutionary Biology*, 16: 363-377.

Beydoun, H. and Saftlas, A. F. (2005) Association of human antigen sharing with recurrent spontaneous abortions. *Tissue Antigens*, 65: 123-135.

Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. and Wiley, D. C. (1987a) Structure of the human class I histocompatibility antigen, HLA-A2. *Nature*, 329: 506-512.

Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. and Wiley, D. C. (1987b) The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature*, 329: 512-518.

Black, F. L. and Hedrick, P. W. (1997) Strong balancing selection at HLA loci: evidence from segregation in South Amerindian families. *Proceedings of the National Academy of Science, USA,* 94: 12452-12456.

Bodmer, W. F. (1972) Evolutionary significance of the HL-A system. *Nature*, 237: 139-145.

Borries, C., Launhardt, K., Epplen, J. T. and Winkler, P. (1999) DNA analysis support the hypothesis than infanticide is adaptive in langur monkeys. *Proceedings of the Royal Society of London*, B 266: 901-904.

Borgia, G. (1979) Sexual selection and the evolution of mating systems. In: Blum, M. S. and Blum, N. A. eds. *Sexual Selection and Reproductive Competition in Insects*. New York, Academic Press, pp.19-80.

Boyce, W. M., Hedrick, P. W., Muggli-Cockett, N. E., Kalinowski, S., Penedo, M. C. T. and Ramey II, R. R. (1997) Genetic variation of major histocompatibility complex and microsatelite loci: a comparison in bighorn sheep. *Genetics*, 145: 421-433.

Braude, S., Tang-Martinez, Z. and Taylor, G. T. (1999) Stress, testosterone, and the immunoredistribution hypothesis. *Behavioral Ecology*, 10: 345-350.

Briles, W. E., Stone, H. A. and Cole, R. K. (1977) Marek's disease: effects of B histocompatibility alloalleles in resistant and susceptible chicken lines. *Science*, 195: 193-195.

Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L. and Wiley, D. C. (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*, 364: 33-39.

Brown, J. L. (1975) *The Evolution of Behavior*. New York, W. W. Norton & Company.

Brown, J. L. (1996) A theory of mate choice based on heterozygosity. *Behavioral Ecology*, 8: 60-65.

Brown, J. L. and Eklund, A. (1994) Kin recognition and the major histocompatibility complex: an integrative review. *The American Naturalist*, 143: 435-461.

Buss, D. M. (1994). *The Evolution of Desire: Strategies of Human Mating*. New York: Basic Books.

Buss, D. M. and Schmitt, D. P. (1993) Sexual strategies theory: an evolutionary perspective on human mating. *Psychological Review*, 100: 204-232.

Buunk, B. P., Dijkstra, P., Kenrick, D. T. and Warntjes, A. (2001) Age preferences for mates as related to gender, own age, and involvement level. *Evolution and Human Behavior*, 22: 241-250.

Cain, W. S. (1977) Physical and cognitive limitations on olfactory processing in human beings. In: Müller-Schwarze, D. and Mozell, M. M. eds. *Chemical Signals in Vertebrates*. New York, Plenum Press, pp. 287-303.

Cameron, E., Day, T. and Rowe, L. (2003) Sexual conflict and indirect benefits. *Journal of Evolutionary Biology,* 16:1055-1060.

Cao, K., Moormann, A. M., Lyke, K. E., Masaberg, C., Sumba, O. P., Doumbo, O. K., Koech, D., Lancaster, A., Nelson, M., Meyer, D., Single, R., Hartzman, R. J., Plowe, C. V., Kazura, J., Mann, D. L., Sztein, M. B., Thomson, G. and Fernández-Viña, M. A. (2004) Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. *Tissue Antigens*, 63: 293-325.

Carrington, M., Nelson, G. W., Martin, M. P., Kissner, T., Vlahov, D., Goedert, J. J., Kaslow, R., Buchbinder, S., Hoots, K. and O'Brien, S. J. (1999) HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science*, 283: 1748-1752.

Charlesworth, D. and Charlesworth, B. (1987) Inbreeding depression and its evolutionary consequences. *Annual Review in Ecology and Systematics,* 18: 237-268.

Clarke, B. and Kirby, D. R. S. (1966) Maintenance of histocompatibility polymorphism. *Nature*, 211: 999-1000.

Cullen, M., Noble, J., Erlich, H., Thorpe, K., Beck, S., Klitz, W., Trowsdale, J. and Carrington, M. (1997) Characterization of recombination in the HLA class II region. *American Journal of Human Genetics*, 60: 397-407.

Cunningham, M. R. (1986) Measuring the physical in physical attractiveness: quasi-experiments on the socio-biology of female facial beauty. *Journal of Personality and Social Psychology*, 50: 925-935.

Cunningham, M. R., Barbee, A. P. and Pike, C. L. (1990) What do women want? Facialmetric assessment of multiple motives in the perception of male facial

physical attractiveness. *Journal of Personality and Social Psychology*, 59: 61-72.

Cunningham, M. R., Roberts, A. R., Barbee, A. P., Druen, P. B. and 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: 261-279.

Danchin, E. G. J., Abi-Rached, L., Gilles, A. and Pontarotti, P. (2003) Conservation of the MHC-like region throughout evolution. *Immunogenetics*, 55: 141-148.

Darwin, C. (1859) *The Origin of Species*. London, Murray.

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

Dion, K., Berscheid, E. and Walster, E. (1972) What is beautiful is good. *Journal of Personality and Social Psychology*, 24: 285-290.

Ditchkoff, S. S., Lochmiller, R. L., Masters, R. E., Hoofer, S. R. and Van Den Bussche, R. A. (2001) Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer *(Odocoileus virginianus)*: evidence for good-genes advertisement. *Evolution*, 55: 616-625.

Doherty, P. C. and Zinkernagel, R. M. (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*, 256: 50-52.

Ebenhard, W. G. and Cordero, C. (1995) Sexual selection by cryptic female choice on male seminal products – a new bridge between sexual selection and reproductive physiology. *Trends in Ecology and Evolution*, 10: 493-496.

Edwards, S. V. and Hedrick, P. W. (1998) Evolution and ecology of MHC molecules: from genomics to sexual selection. *Trends in Ecology and Evolution*, 13: 305-311.

Eggert, F., Müller-Ruchholtz, W. and Ferstl, R. (1999) Olfactory cues associated with the major histocompatibility complex. *Genetica*, 104: 191-197.

Egid, K. and Brown, J. L. (1989) The major histocompatibility complex and female mating preference in mice. *Animal Behaviour*, 38: 548-549.

Eklund, A. (1997) The effects of early experience on MHC-based mate preferences in two B10.W strains of mice (*Mus domesticus*). *Behavior Genetics*, 27: 223-229.

Eklund, A., Egid, K. and Brown, J. L. (1991) The major histocompatibility complex and mating preferences of male mice. *Animal Behaviour*, 42: 693-694.

Emlen, S. T. and Oring, L. W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science,* 197: 215-223.

Falk, K., Rötzschke, O., Stevanovic, S., Jung, G. and Rammensee, H.-G. (1991) Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature,* 351:290-296.

Fedorka, K. M. and Mousseau, T. A. (2004) Female mating bias results in conflicting sex-specific offspring fitness. *Nature*, 429: 65-67.

Feingold, A. (1990) Gender differences in effects of physical attractiveness on romantic attraction: a comparison across five research paradigms. *Journal of Personality and Social Psychology*, 59: 981-993.

Fink, B., Grammer, K. and Thornhill, R. (2001) Human (*Homo sapiens*) facial attractiveness in relation to skin texture and color. *Journal of Comparative Psychology*, 115: 92-99.

Fink, B. and Penton-Voak, I. (2002) Evolutionary psychology of facial attractiveness. *Current Directions in Psychological Science*, 11: 154-158.

Fisher, R. A. (1958) *The Genetical Theory of Natural Selection*. New York, Dover.

Flajnik, M. F. and Kasahara, M. (2001) Comparative genomics of the MHC: glimpses into the evolution of the adaptive immune system. *Immunity,* 15: 351-362.

Folstad, I. and Karter, A. J. (1992) Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, 139: 603-622.

Freeman-Gallant, C. R., Mecuerdichian, M., Wheelwright, N. T. and Sollecito, S. V. (2003) Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, 12: 3077-3083.

Furnham, A., Mistry, D. and McClelland, A. (2004) The influence of age of the face and the waist to hip ratio on judgements of female attractiveness and traits. *Personality and Individual Differences,* 36: 1171-1185.

Gao, G. F., Tormo, J., Gerth, U. C., Wyer, J. R., McMichael, A. J., Stuart, D. I., Bell, J. I., Jones, Y., and Jakobsen, B. K. (1997) Crystal structure of the complex between human CD8αα and HLA-A2. *Nature,* 387: 630-634.

Gibson, G. and Muse, S. V. (2001) *A Primer of Genome Science*. North Carolina, Sinauer Associates.

Gilbert, S. C., Plebanski, M., Gupta, S., Morris, J., Cox, M., Aidoo, M., Kwiatkowski, D., Greenwood, B. M., Whittle, H. C. and Hill, A. V. S. (1998) Association of malaria parasite population structure, HLA, and immunological antagonism. *Science*, 279: 1173-1177.

Goldsby, R. A., Kindt, T. J. and Osborne, B. A. (2000) *Kuby Immunology*. 4th edition. New York, W. H. Freeman and Company.

Gould, J. L. and Gould, C. G. (1989) *Sexual Selection*. New York, Scientific American Library.

Grammer, K. and Thornhill, R. (1994) Human (Homo sapiens) facial attractiveness and sexual selection: the role of symmetry and averageness. *Journal of Comparative Psychology*, 108: 233-242.

Greeff, J. M. and Bennett, N. C. (2000) Causes and consequences of incest avoidance in the cooperatively breeding mole-rat, *Cryptomys darlingi* (Bathyergidae). *Ecology Letters*, 3: 318-328.

Griggio, M., Valera, F., Casas, A. and Pilastro, A. (2005) Males prefer ornamented females: a field experiment of male choice in the rock sparrow. *Animal Behaviour,* 69: 1243-1250.

Grossman, C. J. (1984) Regulation of the immune system by sex steroids. *Endocrine Reviews*, 5: 435-455.

Guo, H.-C., Jardetzky, T. S., Garrett, T. P., Lane, W. S., Strominger, J. L. and Wiley, D. C. (1992) Different length peptides bind to HLA-Aw68 similarly at their ends but bulge out in the middle. *Nature*, 360: 364-366.

Halberstadt, J. and Rhodes, G. (2000) The attractiveness of nonface averages: implications for an evolutionary explanation of the attractiveness of average faces. *Psychological Science*, 11: 285-289.

Halberstadt, J. and Rhodes, G. (2003) It's not just average faces that are attractive: computer-manipulated averageness makes birds, fish and automobiles attractive. Psychonomic Bulletin and Review, 10: 149-156.

Hamilton, W. D. and Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science,* 218: 384-387.

Haring, V., Gray, J. E., McCure, B. A., Anderson, M. A. and Clarke, A. E. (1990) Self-incompatibility: a self-recognition system in plants. *Science*, 250: 937-941.

Hauptmann, G. and Bahram, S. (2004) Genetics of the central MHC. *Current Opinion in Immunology,* 16: 668-672.

Hedrick, P. W. (1992) Female choice and variation in the major histocompatibility complex. *Genetics*, 132: 575-581.

Hedrick, P. W. (1999) Balancing selection and MHC. *Genetica*, 104: 207-214.

Hedrick, P. (2004) Foxy MHC selection story. *Heredity*, 93: 237-238.

Hedrick, P. W. and Black, F. L. (1997) HLA and mate selection: no evidence in south Amerindians. *American Journal of Human Genetics*, 61: 505-511.

Hedrick, P. and Loescke, V. (1996) MHC and mate selection in humans. *Trends in Ecology and Evolution*, 11: 24-25.

Hedrick, P. W. and Parker, K. M. (1998) MHC variation in the endangered gila topminnow. *Evolution*, 52: 194-199.

Hedrick, P. W., Thomson, G. and Klitz, W. (1987) Evolutionary genetics and HLA: another classic example. *Biological Journal of the Linnean Society*, 31: 311-331.

Hedrick, P. W., Whittam, T. S. and Parham, P. (1991) Heterozygosity at individual amino sites: extremely high levels for HLA-A and –B genes. *Proceedings of the National Academy of Sciences, USA*, 88: 5897-5901.

Henderson, J. J. A. and Anglin, J. M. (2003) Facial attractiveness predicts longevity. *Evolution and Human Behavior*, 24: 351-356.

Hill, A. V. S., Allsopp, C. E. M., Kwaitkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. and Greenwood, B. M. (1991) Common West African HLA antigens are associated with protection from severe malaria. *Nature*, 352: 595-600.

Hillgarth, N., Ramenofsky, M. and Wingfield, J. (1997) Testosterone and sexual selection. *Behavioral Ecology*, 8: 108-109.

Hillgarth, N. and Wingfield, J. C. (1997) Parasite-mediated sexual selection: endocrine aspects. In: Clayton, D. H. and Moore, J. eds. *Host-parasite evolution: general principles and avian models*. Oxford, Oxford University Press, pp. 78-104.

Hings, I. M. and Billingham, R. E. (1981) Splenectomy and sensitization of Fischer female rats favors histoincompatibility of R_2 back-cross progeny. *Transplantation Proceedings*, 13: 1253-1255.

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

Howard, J. C. (1991) Disease and evolution. *Nature*, 352: 565-567.

Hughes, A. L. (2000) Evolution of introns and exons of class II major histocompatibility complex genes of vertebrates. *Immunogenetics*, 51: 473-486.

Hughes, A. L. and Hughes, M. K. (1995) Natural selection on the peptide-binding regions of major histocompatibility complex molecules. *Immunogenetics*, 42: 233-243.

Hughes, A. L. and Nei, M. (1988) Patterns of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature*, 335: 167-170.

Hughes, A. L. and Nei, M. (1989) Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. *Proceedings of the National Academy of Science*, 86: 958-962.

Hughes, A. L. and Nei, M. (1992) Models of host-parasite interaction and MHC polymorphism. *Genetics*, 132: 863-864.

Hume, D. K. and Montgomerie, R. (2001) Facial attractiveness signals different aspects of “quality” in women and men. *Evolution and Human Behavior*, 22: 93-112.

IMGT/HLA Sequence Database. [Internet], EMBL-EBI European Bionformatics Institute. Available from: <http://www.ebi.ac.uk/imgt/hla/stats.html> [Accessed: 29 August 2005].

Jacob, S., McClintock, M. K., Zelano, B. and Ober, C. (2002) Paternally inherited HLA alleles are associated with women’s choice of male odor. *Nature Genetics*, 30: 175-179.

Janeway, C. A. and Travers, P. (1994) *Immuno Biology: The Immune System in Health and Disease.* Oxford, Blackwell.

Jeffery, K. J. M. and Bangham, C. R. M. (2000) Do infectious diseases drive MHC diversity? *Microbes and Infection*, 2: 1335-1341.

Jin, K., Speed, T. P. and Thomson, G. (1995) Tests of random mating for a highly polymorphic locus: application to HLA data. *Biometrics*, 51: 1064-1076.

Johnston, V. S., Hagel, R., Franklin, M., Fink, B. and Grammer, K. (2001) Male facial attractiveness: evidence for hormone-mediated adaptive design. *Evolution and Human Behavior*, 22: 251-267.

Jones, B. C., Little, A. C., Feinberg, D. R., Penton-Voak, I. S., Tiddeman, B. P. and Perrett, D. I. (2004) The relationship between shape symmetry and perceived skin condition in male facial attractiveness. *Evolution and Human Behavior*, 25: 24-30.

Kalick, S. M., Zebrowitz, L. A., Langlois, J. H. and Johnston, R. M. (1998) Does human facial attractiveness honestly advertise health? *Psychological Science*, 9: 8-13.

Kasahara, M., Flajnik, M. F., Ishibashi, T. and Natori, T. (1995) Evolution of the major histocompatibility complex: a current overview. *Transplant Immunology,* 3: 1-20.

Keating, C. F. (1985) Gender and the physiognomy of dominance and attractiveness. *Social Psychology Quarterly*, 48: 61-70.

Keller, L F. and Waller, D. M. (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution,* 17: 230-241.

Kenrick, D. T. (1989) Bridging social psychology and socio-biology: the case of sexual selection. In: Bell, R. W. and Bell, N. J. eds. *Sociobiology and the Social Sciences.* Texas, Texas Tech University Press, pp 5-23.

Kissler, J and Bäuml, K.-H. (2000) Effects of the beholder's age on the perception of facial attractiveness. *Acta Psychologica*, 104: 145-166.

Klein, J. and O'Huigin, C. (1994) MHC polymorphism and parasites. *Philosophical Transactions of the Royal Society of London, B*, 346: 351-358.

Kleven, O. and Lifjeld, J. T. (2005) No evidence for increased offspring heterozygosity from extrapair mating in the reed bunting (*Emberiza schoeniclus*). *Behavioral Ecology*, 16: 561-565.

Kurzban, R. and Weeden, J. (2005) Hurrydate: mate preferences in action. *Evolution and Human Behavior*, 26: 227-244.

Kimura, M. (1968) Evolutionary rate at the molecular level. *Nature*, 217: 624-626.

Korthase, K. M. and Trenholme, I. (1982) Percieved age and perceived physical attractiveness. *Perceptual and Motor Skills*, 54: 1251-1258.

Landry, C., Garant, D., Duchesne, P. and Bernatchez, L. (2001) 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London, B,* 268: 1279-1285.

Langefors, A., Lohm, J., Grahn, M., Anderson, Ø. von Schantz, T. (2001) Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. *Proceedings of the Royal Society of London, B*, 268: 479-485.

Langlois, J. H., Kalakanis, L., Rubenstein, A. J., Larson, A., Hallam, M. and Smoot, M. (2000) Maxims or myths of beauty? A meta-analytic and theoretical review. *Psychological Bulletin*, 126: 390-423.

Lekstrom-Himes, J. A., Hohman, P., Warren, T., Wald, A., Nam, J., Simonis, T., Corey, L. and Straus, S. E. (1999) Association of major histocompatibility complex determinants with the development of symptomatic and asymptomatic genital herpes simplex virus type 2 infections. *The Journal of Infectious Diseases,* 179: 1077-1085.

Levine, B., Hardwick, J. M., Trapp, B. D., Crawford, T. O., Bollinger, R. C. and Griffin, D. E. (1991) Antibody-mediated clearance of alphavirus infection from neurons. *Science*, 254: 856-860.

Li, W.-H. (1997) *Molecular Evolution*. Sunderland, Sinauer Associates. Inc.

Little, A. C. and Jones, B. C. (2003) Evidence against perceptual bias views for symmetry preferences in human faces. *Proceedings of the Royal Society of London, B,* 270: 1759-1763.

Livshits, G. and Kobyliansky, E. (1991) Fluctuating asymmetry as a possible measure of developmental homeostasis in humans: a review. *Human Biology*, 63: 441-466.

Lynch, K. S., Rand, S. R., Ryans, M. J., Wilczynski, W. (2005) Plasticity in female mate choice associated with changing reproductive states. *Animal Behaviour*, 69: 689-699.

Malissen, M., Malissen, B. and Jordan, B. R. (1982) Exon/intron organization and complete nucleotide sequence of an HLA gene. *Proceedings of the National Academy of Sciences, USA,* 79: 893-897.

Manning, J. T. (1995) Fluctuating asymmetry and body weight in men and women: implications for sexual selection. *Ethology and Sociobiology*, 16: 145-153.

Manning, J. T., Bundred, P. E. and Mather, F. M. (2004) Second and fourth digit ratio, sexual selection, and skin colour. *Evolution and Human Behavior*, 25: 38-50.

Manning, J. T. and Chamberlain, A. T. (1994) Fluctuating asymmetry in gorilla canines: a sensitive indicator of environmental stress. *Proceedings of the Royal Society of London, B*, 255: 189-193.

Manning, J. T., Koukourakis, K. and Brodie, D. A. (1997) Fluctuating asymmetry, metabolic rate and sexual selection in human males. *Evolution and Human Behavior*, 18: 15-21.

Manning, C. J., Wakeland, E. K. and Potts, W. K. (1992) Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature*, 360: 581-583.

Marieb, E. N. (1995) *Human Anatomy and Physiology*. 3rd edition. California, Benjamin/Cummings Publishing Company.

Markow, T., Hedrick, P. W., Zuerlein, K., Danilovs, J., Martin, J., Vyvial, T. and Armstrong, C. (1993) HLA polymorphism in the Havasupai: evidence for balancing selection. *American Journal of Human Genetics*, 53: 943-952.

Markow, T. A. and Martin, J. F. (1993) Inbreeding and developmental stability in a small human population. *Annals of Human Biology*, 20: 389-394.

May, R. M. (1979) When to be incestuous. *Nature*, 279: 192-194.

Meagher, S., Penn, D. J. and Potts, W. K. (2000) Male-male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Science, USA,* 97: 3324-3329.

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

Menken, J., Trussell, J. and Larsen, U. (1986) Age and infertility. *Science*, 233: 1389-1394.

Milinski, M. and Wedekind, C. (2001) Evidence for MHC-correlated perfume preferences in humans. *Behavioral Ecology*, 12: 140-149.

Miller, P.S. (1994) Is inbreeding depression more severe in a stressful environment? *Zoo Biology,* 13: 195-208.

Miller, G. F. and Todd, P. M. (1998) Mate choice turns cognitive. *Trends in Cognitive Science*, 2: 190-198.

Milne, B. J., Belsky, J., Poulton, R., Thomson, W. M., Caspi, A. and Kieser, J. (2003) Fluctuating asymmetry and physical health among young adults. *Evolution and Human Behavior*, 24: 53-63.

Mitton, J. B., Schuster, W. S. F., Cothran, E. G. and De Fries, J. C. (1993) Correlation between the individual heterozygosity of parents and their offspring. *Heredity*, 71: 59-63.

Møller, A. P. (1992) Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters. *Journal of Evolutionary Biology*, 5: 691-699.

Møller, A. P. (1997) Developmental stability and fitness: a review. *The American Naturalist*, 149: 917-932.

Møller, A. P. and Pomiankowski, A. (1993) Why have birds got multiple ornaments? *Behavioral Ecology and Sociobiology*, 32: 167-176.

Møller, A. P., Soler, M. and Thornhill, R. (1995) Breast Asymmetry, Sexual Selection, and Human Reproductive Success. *Ethology and Sociobiology*, 16: 207-219.

Møller, A. P. and Thornhill, R. (1998) Bilateral symmetry and sexual selection: a meta-analysis. *The American Naturalist*, 151: 174-192.

Nordborg, M. and Tavaré, S. (2002) Linkage disequilibrium: what history has to tell us. *Trends in Genetics,* 18: 83-90.

Nowak, M. A., Tarczy-Hornoch, K. and Austyn, J. M. (1992) The optimal number of major histocompatibility complex molecules in an individual. *Proceedings of the National Academy of Sciences, USA*, 89: 10896-10899.

Ober, C. (1995) Current topic: HLA and reproduction: lessons from studies in the Hutterites. *Placenta*, 16: 569-577.

Ober, C., Weitkamp, L. R., Cox, N., Dytch, H., Kostyu, D. and Elias, S. (1997) HLA and mate choice in humans. *American Journal of Human Genetics*, 61: 497-504.

Olsén, K. H., Grahn, M., Lohm, J. and Langefors, A. (1998) MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behaviour*, 56: 319-327.

Olsén, K. H., Grahn, M. and Lohm, J. (2002) Influence of mhc on sibling discrimination in arctic char, *Salvelinus alpinus* (L.). *Journal of Chemical Ecology*, 28: 783-795.

O'Toole, A. J., Price, T., Vetter, T., Bartlett, J. C. and Blanz, V. (1999) 3D shape and 2D surface textures of human faces: the role of "averages" in attractiveness and age. *Image and Vision Computing*, 18: 9-19.

Palmer, A. R. and Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review in Ecology and Systematics*, 17: 391-421.

Parham, P. and Ohta, T. (1996) Population biology of antigens presentation by MHC class I molecules. *Science*, 272: 67-74.

Parsons, P.A. (1992) Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity*, 68: 361-364.

Partridge, L. (1988) The rare-male effect: what is its evolutionary significance? *Philosophical Transactions of the Royal Society of London, B*, 319(1196): 525-539.

Paterson, S. and Pemberton, J. M. (1997) No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proceedings of the Royal Society of London, B*, 264: 1813-1819.

Paterson, S., Wilson, K. and Pemberton, J. M. (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries L.*). *Proceedings of the National Academy of Sciences, USA*, 95: 3714-3719.

Pearse-Pratt, R., Schellinck, H., Brown, R., Singh, P. B. and Roser, B. (1999) Soluble MHC antigens and olfactory recognition of genetic individuality: the mechanism. *Genetica*, 104: 223-230.

Penn, D. and Potts, W. (1998a) MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society of London, B,* 265: 1299-1306.

Penn, D. and Potts, W. K. (1998b) Chemical signals and parasite-mediated sexual selection. *Trends in Ecology and Evolution*, 13(10): 391-396.

Penn, D. and Potts, W. (1998c) How do major histocompatibility complex genes influence odor and mating preference? *Advances in Immunology*, 69: 411-436.

Penn, D. J. and Potts, W. K. (1999) The evolution of mating preferences and major histocompatibility complex genes. *The American Naturalist,* 153: 145-164.

Penton-Voak, I. S., Jones, B. C., Little, A. C., Baker, S., Tiddeman, B., Burt, D. M. and Perrett, D. I. (2001) Symmetry, sexual dimorphism in facial proportions and male facial attractiveness. *Proceedings of the Royal Society of London, B*, 268: 1617-1623.

Penton-Voak, I. S. and Perrett, D. I. (2000) Female preference for male faces changes cyclically: further evidence. *Evolution and Human Behavior*, 21: 39-48.

Perrett, D. I., Lee, K. J., Penton-Voak, I., Rowland, D., Yoshikawa, S., Burt, D. M., Henzi, S. P., Castles, D. L. and Akamatsu, S. (1998) Effects of sexual dimorphism on facial attractiveness. *Nature*, 394: 884-887.

Perrett, D. I., May, K. A. and Yoshikawa, S. (1994) Facial shape and judgements of female attractiveness. *Nature*, 368: 239-242.

Polak, M. and Trivers, R. (1994) The science of symmetry in biology, *Trends in Ecology and Evolution*, 9: 122-124.

Potts, W. K., Manning, C. J. and Wakeland, E. K. (1991) Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature*, 352: 619-621.

Potts, W. K. and Wakeland, E. K. (1993) Evolution of MHC genetic diversity: a tale of incest, pestilence and sexual preference. *Trends in Genetics*, 9(12): 408-412.

Pusey, A. and Wolf, M. (1996) Inbreeding avoidance in animals. *Trends in Ecology and Evolution,* 11: 201-206.

Rammensee, H.-G. (1995) Chemistry of peptides associated with MHC class I and class II molecules. *Current Opinion in Immunology*. 7: 85-96.

Ramsey, J. L., Langlois, J. H., Hoss, R. A., Rubenstein, A. J. and Griffin, A. M. (2004) Origins of a stereotype: categorization of facial attractiveness by 6-month-old infants. *Developmental Science*, 7: 201-211.

Reusch, T. B. H., Häberli, M. A., Aeschlimann, P. B. and Milinski, M. (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, 414: 300-302.

Rhodes, G., Chan, J., Zebrowitz, L. A. and Simmons, L. W. (2003) Does sexual dimorphism in human faces signal health? *Proceedings of the Royal Society of London, B (Supplement)*, 270: S93-S95.

Rhodes, G., Zebrowitz, L. A., Clark, A., Kalick, S. M., Hightower, A. and McKay, R. (2001) Do facial averageness and symmetry signal health? *Evolution and Human Behavior*, 22: 31-46.

Rikowski, A. and Grammer, K. (1999) Human body odour, symmetry and attractiveness. *Proceedings of the Royal Society of London, B*, 266: 869-874.

Ritte, U., Neufeld, E., O'hUigin, C., Figueroa, F. and Klein, J. (1991) Origins of *H-2* polymorphism in the house mouse. II. Characterization of a model population and evidence for heterozygous advantage. *Immunogenetics*, 34: 164-173.

Roberts, S. C. and Gosling, L. M. (2003) Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics*, 35: 103-106.

Roberts, S. C., Little, A. C., Gosling, L. M., Jones, B. C., Perrett, D. I., Carter, V. and Petrie, M. (2005a) MHC-assortative facial preferences in humans. *Biology Letters*, 1: 400-403.

Roberts, S. C., Little, A. C., Gosling, L. M., Perrett, D. I., Carter, V., Jones, B. C., Penton-Voak, I. and Petrie, M. (2005b) MHC-heterozygosity and human facial attractiveness. *Evolution and Human Behavior*, 26: 213-226.

Roitt, I. M. and Delves, P. J. (2001) *Roitt's Essential Immunology*. 10th edition. Oxford, Blackwell.

Rosenberg, L. T., Cooperman, D. and Payne, R. (1983) HLA and mate selection. Immunogenetics, 17: 89-93.

Rülicke, T., Chapuisat, M., Homberger, F. R., Macas, E. and Wedekind, C. (1998) MHC-genotype of progeny influenced by parental infection. *Proceedings of the Royal Society of London, B,* 265: 711-716.

Ryan, M. J. (1997) Sexual selection and mate choice. In: Krebs, J. R. and Davies, N. B. eds. *Behavioural Ecology: An Evolutionary Approach*. 4th edition. Oxford, Blackwell, pp. 179-202.

Sato, K., Abplanalp, H., Napolitano, D. and Reid, J. (1992) Effects of heterozygosity of major histocompatibility complex haplotypes on performance of leghorn hens sharing a common inbred background. *Poultry Science*, 71: 18-26.

Salvetti, M., Ristori, G., Bomprezzi, R., Pozzilli, P. and Leslie, R. D. G. (2000) Twins: mirrors of the immune system. *Review Immunology Today*, 21: 342-347.

Sanchez-Mazas, A., Djoulah, S., Busson, M., Le Monnier de Gouville, I., Poirier, J.-C., Dehay, C., Charron, D., Excoffier, L., Schneider, S., Langaney, A., Dausset, J. and Hors, J. (2000) A linkage disequilibrium map of the MHC region based on the analysis of 14 loci haplotypes in 50 French families. *European Journal of Human Genetics,* 8: 33-41.

Scheib, J. E., Gangestad, S. W. and Thornhill, R. (1999) Facial attractiveness, symmetry and cues of good genes. *Proceedings of the Royal Society of London, B,* 266: 1913-1917.

Shackelford, T. K. and Larsen, R. J. (1997) Facial asymmetry as an indicator of psychological, emotional and physiological distress. *Journal of Personality and Social Psychology*, 72: 456-466.

Sheldon, B. C. (2005) Differential allocation: tests, mechanisms and implications. *Trends in Ecology and Evolution*, 15(10): 397-402.

Shiina, T., Inoko, H. and Kulski, J. K. (2004) An update of the HLA genomic region, locus information and disease association: 2004. *Tissue Antigens*, 64: 631-649.

Simmons, L. W., Rhodes, G., Peters, M. and Koehler, N. (2004) Are human preferences for facial symmetry focused on signals of developmental instability? *Behavioral Ecology*, 15: 864-871.

Singer, A. G., Beauchamp, G. K. and Yamazaki, K. (1997) Volatile signals of the major histocompatibility complex in male mouse urine. *Proceedings of the National Academy of Science, USA*, 94: 2210-2214.

Singh, P. B. (1999) The present status of the 'carier hypothesis' for chemosensory recognition of genetic individuality. *Genetica*, 104: 231-233.

Singh, P. B., Brown, R. E. and Roser, B. (1987) MHC antigens in urine as olfactory recognition cues. *Nature*, 327: 161-164.

Slade, R. W. and McCallum, H. I. (1992) Overdominant vs. frequency-dependent selection at MHC loci. *Genetics*, 132: 861-862.

Slatkin, M. (2000) Balancing selection at closely linked, overdominant loci in a finite population. *Genetics*, 154: 1367-1378.

Soler, C., Núñez, M., Gutiérrez, R., Núñez, J., Medina, P., Sancho, M., Álvarez, J. and Núñez, A. (2003) Facial attractiveness in men provides clues to semen quality. *Evolution and Human Behavior*, 24: 199-207.

Stoddart, D. M. (1980) *The Ecology of Vertebrate Olfaction*. New York, Chapman and Hall.

Stoddart, D. M. (1990) *The Scented Ape*. Cambridge, Cambridge University Press.

Symons, D. (1979) *The Evolution of Human Sexuality*. Oxford, Oxford University Press.

Takahata, N. and Nei, M. (1990) Allelic geneology under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. *Genetics*, 124: 967-978.

The MHC sequencing consortium. (1999) Complete sequence and gene map of the major histocompatibility complex. *Nature,* 401: 921-923.

Thomas, M. L., Harger, J. H., Wagener, D. K., Rabin, B. S. and Gill, T. J. (1985) HLA sharing and spontaneous abortion in humans. *American Journal of Obstetrics and Gynacology*, 151: 1053-1058.

Thornhill, R. and Gangestad, S. W. (1996) The evolution of human sexuality. *Trends in Ecology and Evolution*, 11: 98-102.

Thornhill, R. and Gangestad, S. W. (1999) The scent of symmetry: a human sex pheromone that signals fitness? *Evolution and Human Behavior*, 20: 175-201.

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

Thornhill, R., Gangestad, S. W., Miller, R., Scheyd, G., McCollough, J. K. and Franklin, M. (2003) Major histocompatibility complex genes, symmetry, and body scent attractiveness in men and women. *Behavioral Ecology*, 14: 668-678.

Thornhill, R. and Møller, A. P. (1997) Developmental stability, disease and medicine. *Biological Reviews*, 72: 497-548.

Thursz, M. R., Thomas, H. C., Greenwood, B. M. and Hill, A. V. S. (1997) Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nature Genetics*, 17: 11-12.

Toivanen, P., Vaahtovuo, J. and Eerola, E. (2001) Influence of major histocompatibility complex on bacterial composition of fecal flora. *Infection and Immunity*, 69: 2372-2377.

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

Trowsdale, J. (2005) HLA genomics in the third millennium. *Current Opinion in Immunology*, 17: 498-504.

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

Von Schantz, T., Wittzell, H., Göransson, G. and Grahn, M. (1997) Mate choice, male condition-dependent ornamentation and MHC in the pheasant. *Hereditas*, 127: 133-140.

Von Schantz, T., Wittzell, H., Göransson, G., Grahn, M. and Persson, K. (1996) MHC genotype and male ornamentation: genetic evidence for the Hamilton-Zuk model. *Proceedings of the Royal Society of London, B,* 263: 265-271.

Walster, E., Aronson, V., Abrahams, D. and Rottman, L. (1966) Importance of physical attractiveness in dating behavior. *Journal of Personality and Social Psychology*, 4: 508-516.

Waser, N. M. and Price, M. V. (1994) Crossing-distance effects in *Delphinium nelsonii*: outbreeding and inbreeding depression in progeny fitness. *Evolution,* 48: 842-852.

Watson, P. J. and Thornhill, R. (1994) Fluctuating asymmetry and sexual selection. *Trends in Ecology and Evolution*, 9: 21-25.

Waynforth, D. (1998) Fluctuating asymmetry and human male life-history traits in rural Belize. *Proceedings of the Royal Society of London, B*, 265: 1497-1501.

Weatherhead, P.J. and Robertson, R. J. (1979) Offspring quality and the polygyny threshold: the sexy son hypothesis. *The American Naturalist*, 113: 201-208.

Wedekind, C. and Folstad, I. (1994) Adaptive and non-adaptive immunosuppression by sex hormones. *The American Naturalist,* 143: 936-938.

Wedekind, C. and Füri, S. (1997) Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society of London, B,* 264: 1471-1479.

Wedekind, C., Seebeck, T., Bettens, F. and Paepke, A. J. (1995) MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London, B*, 260: 245-249.

Wills, C. (1991) Maintenance of multiallelic polymorphism at the MHC region. *Immunological Reviews*, 124: 165-220.

Yamazaki, K., Beauchamp, G. K., Curran, M., Bard, J. and Boyse, E. A. (2000) Parent-progeny recognition as a function of MHC odortype identity. *Proceedings of the National Academy of Sciences, USA*, 97(19): 10500-10502.

Yamazaki, K., Beauchamp, G. K., Kupniewski, D., Bard, J., Thomas, L. and Boyse, E. A. (1988) Familial imprinting determines H-2 selective mating preferences. *Science*, 240: 1331-1332.

Yamazaki, K., Beauchamp, G. K., Wysocki, C. J., Bard, J., Thomas, L. and Boyse, E. A. (1983) Recognition of H-2 types in relation to the blocking of pregnancy in mice. *Science*, 221: 186-188.

Yamazaki, K., Boyse, E. A., Miké, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Boyse, J., Zayaz, Z. A. and Thomas, L. (1976) Control of mating preferences in mice by genes in the major histocompatibility complex. *The Journal of Experimental Medicine*, 144: 1324-1335.

Yamazaki, K., Singer, A. and Beauchamp, G. K. (1999) Origin, function and chemistry of H-2 regulated odorants. *Genetica*, 104: 235-240.

Yamazaki, K., Yamaguchi, M., Andrews, P. W., Peake, B. and Boyse, E. A. (1978) Mating preferences of F_2 segregrants of crosses between MHC-congenic mouse strains. *Immunogenetics*, 6: 253-259.

Zahavi, A. (1975) Mate selection: a selection for the handicap. *Journal of Theoretical Biology,* 67: 603-605.

Zavazava, N. and Eggert, F. (1997) MHC and behaviour. *Trends Immunology Today*, 18: 8-10.

Zavazava, N., Westphal, E. and Müller-Ruchholtz, W. (1990) Characterization of soluble HLA molecules in sweat and quantitative HLA differences in serum of healthy individuals. *Journal of Immunogenetics*, 17; 387-394.

Zebrowitz, L. A., Olson, K. and Hoffman, K. (1993) Stability of babyfaceness and attractiveness across the life span. *Journal of Personality and Social Psychology*, 64: 453-466.

Zhang, C., Anderson, A. and DeLisi, C. (1998) Structural principles that govern the peptide-binding motifs of class I MHC molecules. *Journal of Molecular Biology,* 271: 929-947.

Zinkernagel, R. M., Leist, T., Hengartner, H. and Althage, A. (1985) Susceptibility to lymphocytic choriomeningitis virus isolates correlates directly with early and high cytotoxic T cell activity, as well as with footpad swelling reaction, and all three are regulated by H-2D. *The Journal of Experimental Medicine*, 162: 2125-2141.

CHAPTER 2

COMMON HLA ALLELES ASSOCIATED WITH HEALTH, BUT NOT WITH FACIAL ATTRACTIVENESS.

Vinet Coetzee[1*], Louise Barrett[2], Jaco M. Greeff[1], Peter S. Henzi[2], Dave I. Perrett[3] and Ahmed A. Wadee[5]

[1]Department of Genetics, University of Pretoria, Pretoria, South Africa; [2]Department of Psychology, University of Central Lancashire, Preston, UK; [3]Department of Psychology, University of St. Andrews, St. Andrews, Scotland; [4]Department of Immunology, University of the Witwatersrand, Johannesburg, South Africa.
**Corresponding author vcoetzee@tuks.co.za.*

Contributors

This chapter consists of a manuscript that has been prepared for submission to a peer-reviewed journal (*Proceedings of the Royal Society of London*). The study was designed by myself, Prof. Jaco M Greeff and Dr. Louise Barrett. I conducted all data collection, except for the assistance noted in the acknowledgements. I also extracted DNA and prepared samples for HLA typing. Prof. Ahmed A Wadee and staff typed most of the HLA samples, except for a few samples sent to the University of Cape Town. I conducted all data analyses and drafted the first manuscript. Prof. Dave I Perrett provided financial assistance.

Abstract

Three adaptive hypotheses have been proposed to explain the link between HLA, health measures and facial attractiveness: inbreeding avoidance, heterozygote advantage and frequency-dependent selection. This paper reports the role of these adaptive hypotheses in health and facial attractiveness. HLA heterozygosity did not significantly predict health measures in women, but allele frequency did. Women with more common HLA alleles reported better health. To our knowledge, this is the first study to show a positive correlation between HLA allele frequency and general health measures. We propose that certain common HLA alleles confer resistance to prevalent pathogens. Nevertheless, neither HLA heterozygosity nor allele frequency significantly predicted rated health or attractiveness. Three non-mutually exclusive explanations are put forward to explain this finding.

Introduction

Mate choice has long fascinated, and baffled, evolutionary and behavioural ecologists. Essentially, mate choice predicts that individuals should choose partners that will increase the number and quality of offspring produced, and that such choices will be based on phenotypic characteristics associated with the ability to supply offspring with resources (direct benefits) or which indicate a superior genotype (indirect benefits). Conventionally females are considered the choosier sex, because of their excessive reproductive and gestational investment in offspring (Trivers, 1972). However, humans have a resource-based mating system and men invest substantially in offspring, especially since offspring only become independent relatively late in life. Women, therefore, prefer men that can shoulder such a large resource-based burden, and these men in turn are choosier about the women they select as partners (Trivers 1972; Kenrick 1989, Johnstone, Reynolds and Deutsch 1996). Thus, in humans at least, both sexes prefer partners that can provide them with direct and indirect benefits. Though males traditionally provide most of the direct benefits in the form of food, shelter and protection against parasites (Ryan, 1997), both sexes benefit indirectly from choosing “high genetic quality” partners, as these “good genes” are inherited by offspring, and confer survival and/or reproductive advantages.

But what exactly is “genetic quality”? According to Neff and Pitcher (2005) “genetic quality” can be defined as the sum of two components: additive genetic effects or “good genes” and non-additive genetic effects or “compatible genes”. Specific alleles that increase fitness independently of the rest of the genome contribute to additive genetic benefits, while heterozygote advantage, inbreeding avoidance and epitasis contribute to non-additive genetic benefits. Both additive and non-additive genetic effects are considered important for “genetic quality” and subsequently mate choice (for a review, see Neff and Pitcher, 2005).

One of the best-studied genetic based mating systems in vertebrates is the major histocompatibility complex (MHC). The MHC plays an essential role in immune response, where it serves as antigen presenting cells (Marieb 1995; Penn and Potts, 1999). Several previous studies have linked the MHC to mating preferences in mice (Yamazaki *et al.* 1976; Yamazaki *et al.* 1978; Egid and Brown 1989), savannah sparrows (Freeman-Gallant *et al.* 2003), Atlantic salmon (Landry *et al.* 2001) and humans (Ober *et al.* 1997), although there are also studies that show no such effects (Soay sheep: Paterson and Pemberton, 1997; humans: Rosenberg, Cooperman and Payne 1983; Jin, Speed and Thomson 1995; Hedrick and Black 1997).

According to past research MHC based mating preferences are primarily driven by inbreeding avoidance, heterozygote advantage and frequency dependent selection (for a review see Potts and Wakeland, 1993). Since specific rare alleles confer fitness benefits under frequency-dependent selection, and such alleles would still confer these benefits without the contribution of any other alleles, we postulate that frequency-dependent selection is as a component of additive genetic effects.

In a recent study, Roberts *et al.* (2005) linked a form of “genetic quality”, HLA heterozygosity, to a sexually selected trait, facial attractiveness. They also linked HLA heterozygosity to perceived male health in the same study. A previous study by Thornhill *et al.* (2003) did not find a link between HLA heterozygosity and facial attractiveness among males. The preference for HLA heterozygosity observed by Roberts *et al.* (2005) could be due to three, non-mutually exclusive, HLA based mating preferences. First, individuals might prefer heterozygous mates because such individuals will confer direct benefits in the form of increased health and therefore better resource-holding potential, or indirect benefits in the form of more heterozygous offspring (Mitton *et al.* 1993). Secondly, the preference for heterozygosity could be a by-product of a preference for “rare alleles”, because heterozygous individuals are more likely to possess such rare alleles than homozygous individuals (Thornhill *et al.* 2003). Lastly, the preference for a

heterozygous partner potentially could be due to inbreeding avoidance, especially if the population is inbred. The more inbred the population, the more individuals are expected to be homozygous. Heterozygosity, therefore, should serve as an indicator that the individual is more outbred relative to the rest of the population.

Our objectives were to (a) investigate the health benefits associated with the two pathogen-driven selection pressures: frequency dependent selection and heterozygote advantage and (b) the role of inbreeding avoidance, in conjunction with heterozygote advantage and frequency dependent selection, in predicting HLA based facial preferences. Our study differs from previous studies in three important respects, first we tested male choice of female partners, rather than female preferences for males. Second, our sample population group is largely outbred and has a much higher pathogen load that previously studied populations. Due to the high pathogen load one might expect strong selection for the pathogen-driven selective force that provides the most protection against pathogens. Third, we also looked at genetic distance rather than just heterozygosity *per se*. Since the amount of overlap between HLA molecules (and therefore the genetic distance between these molecules) dictate the extend of the benefit associated with heterozygosity (Jeffery and Bangham, 2000), genetic distance will serve as a more sensitive measure of heterozygosity.

Materials and Methods

Sample group

Our sample group consisted of 59 female volunteers (age, Mean = 19.8, S.D. = 1.56, Range = 18-26). All participants identified themselves as Tswana, the most abundant ethnic group at the University of Pretoria (V. Coetzee, unpublished data). Each volunteer signed a subject information and consent form before taking part in the study.

Data collection

Participants completed a questionnaire, providing information on their sex, age, hormonal contraceptive use (Q1, Appendix), sexual preference (Q2, Appendix), self-rated health (Q3, Appendix), number of illnesses in the previous year (Q4, Appendix) and the number of colds and flu bouts per year (Q5-6, Appendix). All but two of the participants did not report hormonal contraceptive use in the previous year. Three participants reported being homosexual, but participants generally struggled comprehending the concept of homosexuality, which leads to uncertainty about the validity of their answers.

Buccal cells were collected by scraping the inside of the mouth for a minimum of ten strokes with a sterile nylon bristle cytology brush. Brush tips were then stored in 300μl cell lysis solution and kept at room temperature for a maximum of 36 hours, well within the recommended storage period.

All participants were photographed in full colour with a Sony Cybershot DSC P72 (default settings with 3.1 Mega pixels fine, Soft light flash) under standard lighting conditions. Participants were asked to maintain a neutral expression and were seated at a fixed distance from the camera. Two photographs were taken of each participant and the best one used for analyses. Slight lateral tilting of individual faces was corrected by rotation around the facial midline using vertical guidelines and cropped 5cm from each side to standardize size in Coral Photo-Paint v. 10 (Coral Corporation, Ontario, Canada). Next, faces were masked to eliminate confounding factors (e.g., hairstyles) in Coral Knockout v. 1.5 (Coral Corporation, Ontario, Canada) and Coral Photo-Paint v. 10. All images were saved in 24-bit RGB colour at 72 dpi.

Masked photographs were used to compile 24 full colour presentations consisting of 5 female photographs each. Presentations were rated by 59 Tswana

male (age, Mean = 21.1, S.D. = 2.11, Range = 18-26) participants from the University of the Witwatersrand, to minimise the probability of familiarity. Raters assessed opposite sex individuals for attractiveness (Q7, Appendix), perceived health (Q8, Appendix) and whether or not they knew the rated individual (Q9, Appendix). Raters indicated rated attractiveness and perceived health on a continuous line (142 cm), with markings to indicate the ends, centre and quarter points. They were instructed to place their mark anywhere along the line. Individuals that recognised any of the rated individuals were given another presentation set to rate. Rated attractiveness and health ratings were averaged to yield an index of facial attractiveness and health respectively. All volunteers were provided with lunch in return for participation.

HLA typing

Genomic DNA was extracted with the Puregene® DNA Purification kit (Gentra Systems, Inc., Minneapolis, USA), according to the manufacturers recommended protocol. After extraction, DNA was resuspended in 20µl low TE (10mM Tris, 0.1mM EDTA). Twenty eight samples with a total DNA content > 480ng were typed for HLA-A and HLA-B using the Dynal RELI™ SSO typing kit (Invitrogen Corporation, California, USA) according to manufacturers instructions. An additional 13 samples (total DNA content > 200ng) were typed with the INNO-LiPA HLA-A/B kits (Innogenetics group, Gent, Belgium) as this method requires less total DNA. Collectively, these methods resolved allele types on the two-digit allele group level for 41 participants. Three individuals showed ambiguous allele classifications for HLA-B, such that one of the HLA-B alleles could belong to one of two allele groups. Contrary to previous studies we focused specifically on only two HLA loci, HLA-A and HLA-B because these loci are important in all nucleated cells, not just certain cells of the immune system.

To test the first hypothesis that HLA heterozygosity positively influences health and facial attractiveness, we calculated two measures of heterozygosity. The first measure, as previously defined by Roberts *et al.* (2005), was the number of alleles shared at both loci. Individuals that were heterozygous at both loci were grouped as heterozygous, while individuals that were homozygous at one or both loci were grouped as homozygous. The three ambiguous allele classifications were all heterozygous for HLA-B and were grouped accordingly.

For our second measure of heterozygosity, we calculated the genetic distance between the two alleles of each locus and averaged the value over both loci. Genetic distance should serve as a more sensitive measure of heterozygosity than heterozygosity/ homozygosity *per se*. To calculate genetic distance, we downloaded sequence data for all alleles (4-digit) within a specific allele group (2-digit) from the IMGT/HLA sequence database (IMGT/HLA sequence database, 2005). We excluded null alleles, unconfirmed alleles and alleles that were shorter than the bulk of the sequence data (HLA-A < 1098bp, HLA-B < 1089bp). Homozygous individuals were assumed to have 4-digit alleles identical by descent. Alleles were aligned with Clustal W (Thompson, Higgins and Gibson, 1994) available in BioEdit Sequence Alignment editor v.7.0.5.2 (Hall, 1999) and the genetic distance calculated using DNADist v.3.5c also available in BioEdit Sequence Alignment Editor v.7.0.5.2. Separate general linear models (GLM) were performed for each dependent variable and both measures of heterozygosity.

To test the second hypothesis that rare alleles are positively correlated with health measures, we calculated the allele frequency of HLA-A and HLA-B alleles in our sample group. Allele frequencies were averaged across both loci to produce a combined allele frequency. Separate GLM analyses were performed for each measure of health and facial attractiveness independently.

All analyses were performed in SPSS version 13.0 (Chicago, USA). In each analysis, we calculated Cook's values to identify influential outliers (Cook's values >

0.11). Once identified, influential outliers were removed from analyses. We report only results obtained after the removal of influential outliers, unless there was a difference in statistical significance, in which case we report both results.

Results

HLA heterozygosity, health and facial attractiveness

We found no significant difference in any of the health or attractiveness measures between the heterozygous and homozygous groups. Heterozygous females did not rate themselves as significantly healthier than homozygotes (t_{39} = -0.323, p = 0.748), nor did they report fewer illnesses in the previous year (t_{38} = 0.028, p = 0.977) or colds and flu bouts per year (t_{37} = 0.087, p = 0.931; Discrepancies in degrees of freedom are due to omitted answers on illnesses in the previous year [1 participant] and number of colds and flu's per year [2 participants]). Male raters also did not rate heterozygous females as being significantly healthier (t_{39} = -0.130, p = 0.897) or more attractive (t_{39} = 1.186, p = 0.243).

As a second measure of heterozygosity we tested associations between HLA genetic distance (as a continuous measure of HLA heterozygosity) and health and attractiveness measures. Genetic distance did not significantly predict self-rated health ($F_{1,37}$ = 1.229, R^2 = 0.032, p = 0.275), number of illnesses in the previous year ($F_{1,35}$ = 0.356, R^2 = 0.010; p = 0.555) or number of cold and flu bouts per year ($F_{1,34}$ = 0.242, R^2 = 0.007; p = 0.626). In addition, larger genetic distances did not predict how healthy ($F_{1,38}$ = 0.532, R^2 = 0.014, p = 0.470) or attractive ($F_{1,37}$ = 0.017, R^2 < 0.001; p = 0.897) females were rated by the opposite sex.

HLA allele frequency, health and facial attractiveness

Combined allele frequency (averaged for HLA-A and HLA-B) significantly predicted the number of cold and flu bouts per year (equation: Cold and flu bouts = 5.536 –

18.743 × allele frequency, $F_{1,34}$ = 5.618, R^2 = 0.142, p = 0.024)(Figure 1), but only predicted self-rated health and number of illnesses in the previous year after the removal of influential outliers (Self-rated health: equation: Self-rated health = 74.715 + 288.08 × allele frequency, $F_{1,37}$ = 6.587, R^2 = 0.151, p = 0.014; Figure 2)(Number of illnesses: equation: Ill per year = 4.089 – 16.245 × allele frequency, $F_{1,35}$ = 5.014, R^2 = 0.125, p = 0.032; Figure 3)(Table 1), and not before (Self-rated health: $F_{1,39}$ = 2.221, R^2 = 0.054, p = 0.144)(Number of illnesses: $F_{1,38}$ = 2.357, R^2 = 0.058, p = 0.133). Interestingly, these results show that females with high combined allele frequencies (i.e., those with more common alleles) considered themselves healthier and reported fewer illnesses.

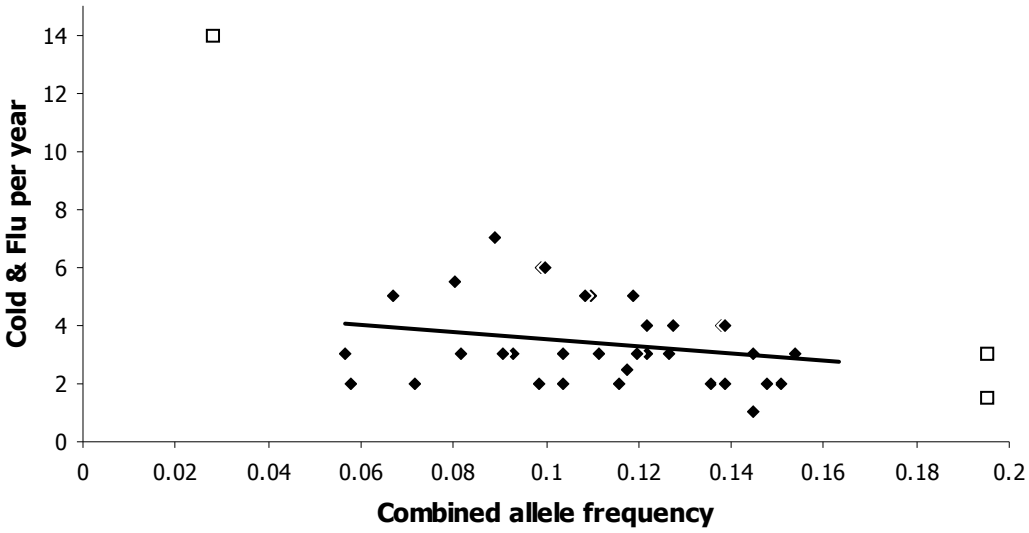


Figure 1. *The relationship between combined allele frequency and the number of self reported colds and flu's per year. Outliers are indicated as squares*

Conversely, combined allele frequencies did not significantly predict how healthy or attractive females were rated (Health: $F_{1,36}$ = 0.175, R^2 = 0.005, p = 0.678; attractiveness: $F_{1,34}$ = 2.421, R^2 = 0.066, p = 0. 129)(Table 1).

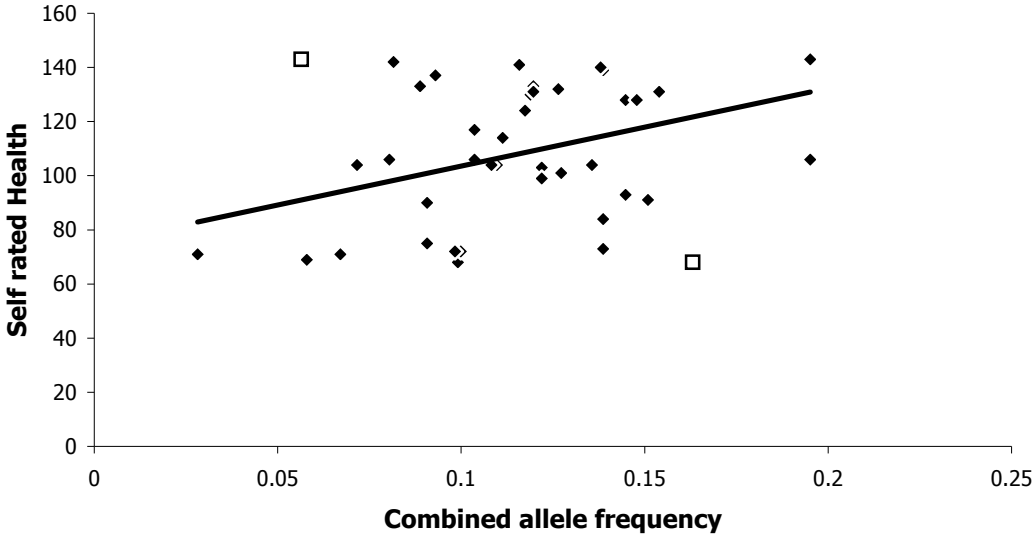


Figure 2. *The relationship between combined allele frequency and self-rated health. Outliers are indicated as squares.*

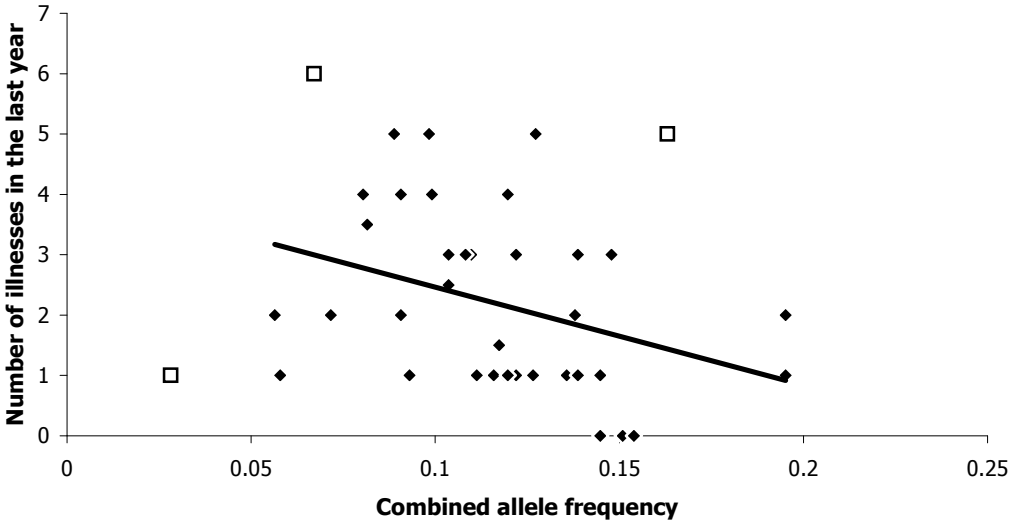


Figure 3. *The relationship between combined allele frequency and the number of self reported illnesses in the previous year. Outliers are indicated as squares.*

Next, we tested the influence of the allele frequency of the separate loci (HLA-A and HLA-B) on the predictive power of the combined allele frequency. HLA-B did not significantly predict self-rated health ($F_{1,35}$ = 1.185, R^2 = 0.033, p = 0.284),

while HLA-A significantly predicted self-rated health after the removal of outliers (equation: Self-rated health = 71.347 + 329.534 × allele frequency, $F_{1,37} = 8.946$, $R^2 = 0.195$, $p = 0.005$)(Figure 4) but not before ($F_{1,39} = 3.390$, $R^2 = 0.080$, $p = 0.073$). On the other hand, HLA-B significantly predicted the number of cold and flu bouts per year (equation: Colds & Flu's = 5.079 – 13.686 × allele frequency, $F_{1,34} = 5.829$, $R^2 = 0.146$, $p = 0.021$)(Figure 5), while HLA-A did not ($F_{1,36} = 0.038$, $R^2 = 0.001$, $p = 0.847$). Neither HLA-A nor HLA-B significantly predicted the number of illnesses in the previous year (HLA-A, $F_{1,35} = 3.182$, $R^2 = 0.083$, $p = 0.083$)(HLA-B, $F_{1,35} = 2.937$, $R^2 = 0.077$, $p = 0.095$).

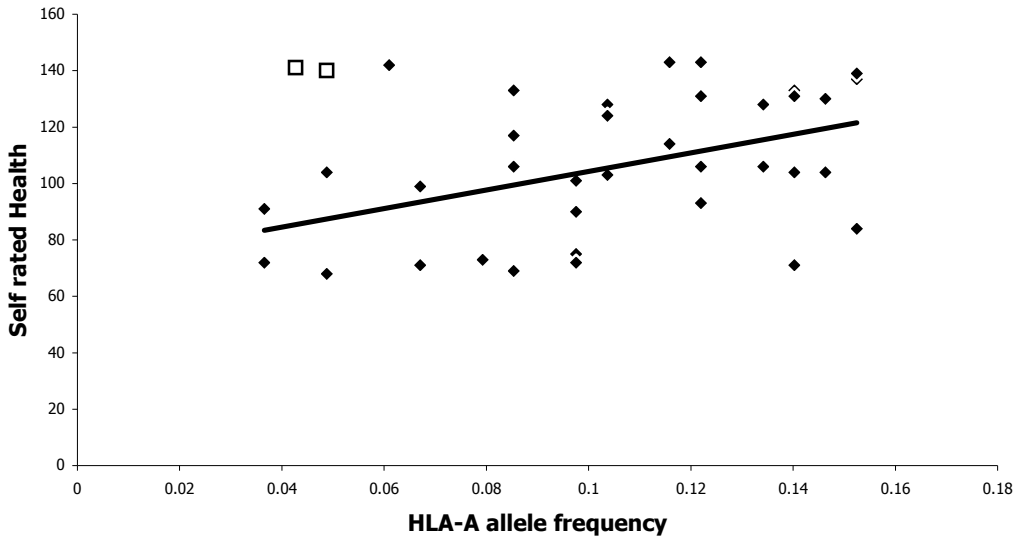


Figure 4*. The relationship between HLA-A allele frequency and self reported health. Outliers are indicated as squares.*

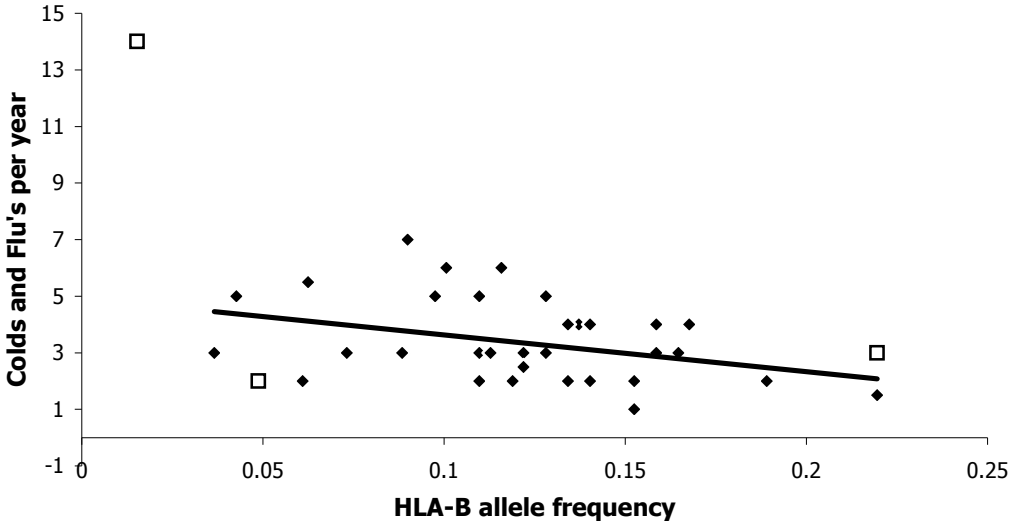


***Figure 5**. The relationship between HLA-B allele frequency and the number of self reported colds and flu's per year. Outliers are indicated as squares.*

Neither HLA-A allele frequency nor HLA-B allele frequency significantly predicted health or attractiveness ratings by males (Health: HLA-A: $F_{1,35} = 1.412$, $R^2 = 0.039$, $p = 0.243$; HLA-B: $F_{1,36} = 0.458$, $R^2 = 0.013$, $p = 0.503$; attractiveness: HLA-A: $F_{1,37} = 0.751$, $R^2 = 0.020$, $p = 0.392$; HLA-B: $F_{1,36} = 0.114$, $R^2 = 0.003$, $p = 0.738$).

***Table 1**. HLA allele frequency as a predictor of health and attractiveness measures. Significant predictors are indicated in bold.*

Source	df	F	Sign	R^2
Self-rated health				
Both loci	**1, 37**	**6.587**	**0.014**	**0.151**
HLA-A	**1, 37**	**8.946**	**0.005**	**0.195**
HLA-B	1, 35	1.185	0.284	0.033
Ill last year				
Both loci	**1, 35**	**5.014**	**0.032**	**0.125**
HLA-A	1, 35	3.182	0.083	0.083
HLA-B	1, 35	2.937	0.095	0.077
Colds & Flu's per year				
Both loci	**1, 34**	**5.618**	**0.024**	**0.142**
HLA-A	1, 36	0.038	0.847	0.001
HLA-B	**1, 34**	**5.829**	**0.021**	**0.146**
Rated health				
Both loci	1, 36	0.175	0.678	0.005
HLA-A	1, 35	1.412	0.243	0.039
HLA-B	1, 36	0.458	0.503	0.013
Rated attractiveness				
Both loci	1, 34	2.421	0.129	0.066
HLA-A	1, 37	0.751	0.392	0.020
HLA-B	1, 36	0.114	0.738	0.003

Discussion

Our results show that HLA heterozygous women do not consider themselves healthier than more homozygous women, nor do they report fewer illnesses. We did find that women with more common HLA class I alleles rated themselves healthier and reported fewer illnesses, although the class I loci responsible for the beneficial effects differed between different health measures. To our knowledge, this is the first study to directly report a positive correlation between HLA allele frequency and general health measures.

One plausible explanation for this novel finding is that, contrary to what might normally be expected, certain common alleles might increase an individual's ability to resist specific pathogens. Several previous studies have shown a correlation between specific HLA class I alleles and resistance to chronic Hepatitis B (Almarri and Batchelor, 1994), malaria (Hill *et al.* 1991) and delayed progression to AIDS (Lopez-Vazquez *et al.* 2005). In all three studies, resistance was conferred by alleles that were common in the respective study populations. The precise mechanism explaining the conference of resistance by common alleles remains unclear. Nevertheless, it is possible that common alleles that confer resistance are the result of the host out-competing pathogens in the host-parasite coevolutionary arms race. Any allele that confers resistance to a high-frequency pathogen, especially in a population with a high pathogen load, will increase in frequency rapidly.

Although we found no evidence for heterozygote advantage, we do not wish to discredit heterozygote advantage as a form of pathogen-driven selection. Our results show only that the benefit associated with the different adaptive hypotheses is likely to be context-specific. HLA heterozygote advantage is expected to be less beneficial in a situation where the pathogen load is high, and most ailments are caused by a few specific pathogens. In African populations, most of the infectious diseases are caused by a few common pathogens (Statistics South Africa, 2006). In such a scenario, one might expect an exceptional health benefit from alleles that confer resistance to these major pathogens. HLA heterozygosity undoubtedly contributes to general resistance to pathogens, but the specific alleles that confer specific resistance provide more protection under these conditions.

Though women with more common alleles reported better health, men did not rate them as being significantly healthier or more attractive. There are three, non-mutually exclusive, explanations for these findings. First, it is unlikely that there are allele-specific facial cues to resistance and attractiveness, and even more unlikely that such cues can be reliably recognised. Second, the HLA-based mating facial

preference observed in Roberts *et al.* (2005) could also be partly due to inbreeding avoidance. In African populations, selection for inbreeding avoidance is bound to be reduced because of high genetic diversity. In fact, there is more diversity within the African population than between Africans and Eurasians (Yu *et al.* 2002). Third, contrary to previous studies, this study focused specifically on rating female subjects as opposed to males. Despite male resource based contributions to offspring, selection on males may not have been strong enough for them to detect more subtle individual variations in attractiveness, as opposed to more obvious features such as facial neoteny, averageness and symmetry. However men place exceptional importance on female physical attractiveness and men in our study population have been shown to be as choosy as women (Chapter 5).

In conclusion, we have found evidence for context specific relationships between HLA, health and facial attractiveness in a South African population. In this population allele frequency positively predicts health, presumably through specific common alleles that confer resistance to parasites. However, cues to health cannot readily be recognised in facial features, and healthier individuals are therefore not rated to be either healthier or more attractive.

Acknowledgements

We would like to thank Nicole Creux, Minique de Castro, Therese de Castro, Christoff Erasmus, Tracey-Leigh Hatherall, Aret Meyer, Ronnie Nelson, Duncan Newman, Jason Pienaar and Isa-Rita Russo for their valuable help with data collection. This material is based, in part, upon work supported by the National Research Foundation under Grant number 2053809 to JMG. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research

Foundation. This project was cleared by the ethics committees of the University of Pretoria and the University of the Witwatersrand respectively.

References

Almarri, A. and Batchelor, J. R. (1994) HLA and hepatitis B infection. *The Lancet*, 344: 8931.

Egid, K. and Brown, J. L. (1989) The major histocompatibility complex and female mating preference in mice. *Animal Behaviour*, 38: 548-549.

Freeman-Gallant, C. R., Mecuerdichian, M., Wheelwright, N. T. and Sollecito, S. V. (2003) Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, 12: 3077-3083.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95-98.

Hedrick, P. W. and Black, F. L. (1997) HLA and mate selection: no evidence in south Amerindians. *American Journal of Human Genetics*, 61: 505-511.

Hill, A. V. S., Allsopp, C. E. M., Kwaitkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. and Greenwood, B. M. (1991) Common West African HLA antigens are associated with protection from severe malaria. *Nature*, 352: 595-600.

IMGT/HLA Sequence Database. [Internet], EMBL-EBI European Bionformatics Institute. Available from: <http://www.ebi.ac.uk/imgt/hla/allele.html> [Accessed: 22 February 2006].

Jeffery, K. J. M. and Bangham, C. R. M. (2000) Do infectious diseases drive MHC diversity? *Microbes and Infection*, 2: 1335-1341.

Jin, K., Speed, T. P. and Thomson, G. (1995) Tests of random mating for a highly polymorphic locus: application to HLA data. *Biometrics*, 51: 1064-1076.

Johnstone, R. A., Reynolds, J. D. and Deutsch, J. C. (1996) Mutual mate choice and sex differences in choosiness. *Evolution*, 50: 1382-1392.

Kenrick, D. T. (1989) Bridging social psychology and socio-biology: the case of sexual selection. In: Bell, R. W. and Bell, N. J. eds. *Sociobiology and the Social Sciences.* Texas, Texas Tech University Press, pp 5-23.

Landry, C., Garant, D., Duchesne, P. and Bernatchez, L. (2001) 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London, B,* 268: 1279-1285.

Lopez-Vazquez, A., Mina-Blanco, A., Martinez-Borra, J., Njobvu, P. D., Suarez-Alvarez, B., Blanco-Gelaz, M. A., Gonzalez, S., Rodrigo, L., Lopez-Larrea, C. (2005) Interaction between KIR3DL1 and HLA-B*57 supertype alleles influences the progression of HIV-1 infection in a Zambian population. *Human Immunology,* 66: 285-289.

Marieb, E. N. (1995) *Human Anatomy and Physiology*. 3rd edition. California, Benjamin/Cummings Publishing Company.

Mitton, J. B., Schuster, W. S. F., Cothran, E. G. and De Fries, J. C. (1993) Correlation between the individual heterozygosity of parents and their offspring. *Heredity*, 71: 59-63.

Neff, B. D. and Pitcher, T. E. (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, 14: 19-38.

Ober, C., Weitkamp, L. R., Cox, N., Dytch, H., Kostyu, D. and Elias, S. (1997) HLA and mate choice in humans. *American Journal of Human Genetics*, 61: 497-504.

Paterson, S. and Pemberton, J. M. (1997) No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proceedings of the Royal Society of London, B*, 264: 1813-1819

Penn, D. J. and Potts, W. K. (1999) The evolution of mating preferences and major histocompatibility complex genes. *The American Naturalist,* 153: 145-164.

Potts, W. K. and Wakeland, E. K. (1993) Evolution of MHC genetic diversity: a tale of incest, pestilence and sexual preference. *Trends in Genetics*, 9(12): 408-412.

Roberts, S. C., Little, A. C., Gosling, L. M., Perrett, D. I., Carter, V., Jones, B. C., Penton-Voak, I. and Petrie, M. (2005) MHC-heterozygosity and human facial attractiveness. *Evolution and Human Behavior*, 26: 213-226.

Rosenberg, L. T., Cooperman, D. and Payne, R. (1983) HLA and mate selection. *Immunogenetics,* 17: 89-93.

Ryan, M. J. (1997) Sexual selection and mate choice. In: Krebs, J. R. and Davies, N. B. eds. *Behavioural Ecology: An Evolutionary Approach*. 4th edition. Oxford, Blackwell, pp. 179-202.

Statistics South Africa, Mortality and causes of death in South Africa, 1997-2003. [Internet]. Available from: <http://www.statssa.gov.za > [Accessed: 12 May 2006].

Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22: 4673-4680.

Thornhill, R., Gangestad, S. W., Miller, R., Scheyd, G., McCollough, J. K. and Franklin, M. (2003) Major histocompatibility complex genes, symmetry, and body scent attractiveness in men and women. *Behavioral Ecology*, 14: 668-678.

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

Yamazaki, K., Boyse, E. A., Miké, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Boyse, J., Zayaz, Z. A. and Thomas, L. (1976) Control of mating preferences in mice by genes in the major histocompatibility complex. *The Journal of Experimental Medicine*, 144: 1324-1335.

Yamazaki, K., Yamaguchi, M., Andrews, P. W., Peake, B. and Boyse, E. A. (1978) Mating preferences of F_2 segregrants of crosses between MHC-congenic mouse strains. *Immunogenetics*, 6: 253-259.

Yu, N., Chen, F.-C., Ota, S., Jorde, L. B., Pamilo, P., Patthy, L., Ramsay, M., Jenkins, T., Shyue, S.-K. and Li, W.-H. (2002) Larger genetic differences within Africans than between Africans and Eurasians. *Genetics,* 161*:* 269-274.

Appendix

Initial male/ female questionnaire:

1. Are you currently using the pill or injection as contraception?
2. Female questionnaire: Are you heterosexual? (Do you prefer men as your sexual partners)
3. How healthy are you in general?
4. How many times were you ill in the last year?
5. How many times per year do you get the flu?
6. How many times per year do you get a cold?

Image scoring questionnaire:

7. Please indicate how attractive you think this woman is.
8. Please indicate how healthy you think this woman is.
9. Do you know any of these women?

CHAPTER 3

FACIAL ATTRACTIVENESS: THE ROLE OF SYMMETRY, SEXUAL DIMORPHISM AND PERCEIVED AGE

Vinet Coetzee[1*], Louise Barrett[2], Jaco M. Greeff[1] and Peter S. Henzi[2] .

*[1]Department of Genetics, University of Pretoria, Pretoria, South Africa; [2]Department of Psychology, University of Central Lancashire, Preston, UK. *Corresponding author vcoetzee@tuks.co.za.*

Contributors

This chapter consists of a manuscript that has been prepared for submission to a peer-reviewed journal (*Evolution and Human Behavior*). The study was designed by myself, Prof. Jaco M Greeff and Dr. Louise Barrett. I conducted all data collection, except for the assistance noted in the acknowledgements. I also conducted all data analyses and drafted the first version of the manuscript.

Abstract

Facial attractiveness plays a crucial role in human mate choice. Facial symmetry, sexual dimorphic traits and neoteny all play a role in predicting facial attractiveness, but tend to be studied independently of each other. Here, we have combined all three facial features in one study in order to evaluate their relative roles in predicting facial attractiveness and the relationship between them. Our results show that feminine facial traits in females and perceived age in both sexes correlated significantly with facial attractiveness, but not with facial symmetry. Overall, perceived age or “neoteny” was the best predictor of facial attractiveness. Younger looking individuals of both sexes were rated more attractive. Our findings support the hypothesis that preference for femininity is a by-product of preference for neotenous cues.

Introduction

Facial attractiveness plays a crucial role in human mate choice, since both sexes prefer to date and associate with facially attractive individuals (Walster *et al.* 1966; Dion, Berscheid and Walster 1972). By itself, facial attractiveness serves as an important indicator of genetic quality, because it indicates both components of fitness: survivorship (Henderson and Anglin, 2003) and fecundity (Symons 1979; Soler *et al.* 2003). Previous studies have identified four facial features that contribute to facial attractiveness: averageness, symmetry, hormone markers and age or "neoteny". In this study, we focus only on the last three. First, symmetry, or rather the lack thereof, fluctuating asymmetry (FA), is a reliable indicator of developmental stability (Palmer and Strobeck, 1986). Previous studies have shown a negative correlation between FA and facial attractiveness in both sexes (Grammer and Thornhill 1994; Mealey, Bridgestock and Townsend 1999; Rikowski and Grammer 1999; Hume and Montgomerie 2001; Penton-Voak *et al.* 2001), but this correlation is especially prominent in sexually dimorphic male traits (Manning and Chamberlain 1994; Møller, Soler and Thornhill 1995; Manning, Koukourakis and Brodie 1997; Møller and Thornhill 1998; Simmons *et al.* 2004).

Second, the level of sexual dimorphism is determined by the ratio of testosterone-to-oestrogen. Higher levels of testosterone-to-oestrogen leads to facial masculinisation with lateral growth of the cheekbones, lateral growth of the jaws and chin, forward growth of the bones of the eyebrow ridges and the lengthening of the lower face (Miller and Todd 1998; Fink and Penton-Voak 2002). Masculine faces are longer, with wider jaws and smaller eyes (Penton-Voak *et al*. 2001; Gangestad and Thornhill 2003). Structural facial femininity, on the other hand, stems from a lack of masculine growth, which leaves faces shorter, with narrower jaws and larger eyes compared to the face size (Penton-Voak *et al*. 2001; Gangestad and Thornhill 2003). Gangestad and Thornhill (2003) also identified horizontal lip width as a facial sexually

dimorphic trait, with wider lips associated with males. In addition to structural features, feminine faces are characterized by relatively hairless, smooth skin (Fink, Grammer and Thornhill, 2001) and lighter skin colour in females (Manning, Bundred and Mather 2004 but see Fink, Grammer and Thornhill 2001). In males, facial masculinity correlates positively with perceived dominance (Perrett *et al.* 1998), but not reliably with facial attractiveness. Some studies have found a preference for masculine male faces (Cunningham, Barbee and Pike 1990; Grammer and Thornhill 1994; Johnston *et al.* 2001) while others have shown a preference for feminised male faces (Perrett *et al.* 1998). The situation is much simpler in females, where feminine faces are consistently rated as more attractive (Perrett, May and Yoshikawa, 1994; Perrett *et al*. 1998; Rhodes *et al.* 2003). The influence of prenatal testosterone on facial features can also be assessed indirectly via measures of the 2nd digit to 4th digit ratio (2D: 4D ratio)(Garn *et al.* 1975). Even though the 2D: 4D ratio shows an irregular correlation with circulating testosterone levels (Manning *et al.* 1998), prenatal testosterone presumably serves to "organise" male facial features and therefore serves as a predictor of facial masculinity (Neave *et al.* 2003).

Body mass index (BMI) serves as an important indicator of female attractiveness (Kurzban and Weeden, 2005). Women with low BMI are generally rated more attractive, even facially (Hume and Montgomerie, 2001). In males, a higher BMI is preferred, in part because this implies increased muscle mass, a signal of masculinity (Halbertadt and Rhodes, 2000).

Lastly, age, or rather the perception of age, plays an important role in female attractiveness. Men prefer neotenous facial features in women (Cunningham, 1986), presumably because it indicates that the women is young and therefore fertile (Buunk *et al.* 2001). According to Menken, Trussell and Larsen (1986) men place more importance on perceived youthfulness in their female partners because women have a limited reproductive window. Men, on the other hand, are fertile throughout most of their adult lives, and one might expect that perceived youthfulness might not be as

important for their attractiveness (Symons, 1979). However, Korthase and Trenholme (1982) found that perceived youthfulness is important for both sexes, albeit more important for female attractiveness. As neotenous features such as large eyes, small nose and small chin are also more feminine, there is also a positive correlation between perceived youthfulness and femininity in both sexes (Berry and McArthur 1985; Perrett *et al.* 1998).

Although all these facial features predict facial attractiveness independently, previous studies have studied them in isolation. The aim of this study is to determine the role of these three facial features, symmetry, sexual dimorphism and perceived age, in predicting facial attractiveness in both males and females.

Materials and Methods

Sample group

Our sample group consisted of 54 male (age, Mean = 21.1, S.D. = 2.17, Range = 18-26) and 59 female volunteers (age, Mean = 19.8, S.D. = 1.56, Range = 18-26). All participants identified themselves as Tswana, the most abundant ethnic group at the University of Pretoria (V. Coetzee, unpublished data). To minimise the possibility of familiarity between rated and rater individuals we recruited participants from two different Universities. Females were recruited from the University of Pretoria and males from the University of the Witwatersrand. Each volunteer signed a subject information and consent form before taking part in the study.

Data collection

Participants completed a questionnaire, providing information on their sex, age, hormonal contraceptive use (females only)(Q1, Appendix) and sexual preference (Q2-3, Appendix). All but two of the females did not report hormonal contraceptive use in the last year. Three females and one male reported being homosexual, but

many participants had difficulty comprehending the concept of homosexuality, which leads to some uncertainty about the validity of their answers. Next, we measured each participant's weight and height directly to calculate their body mass index (BMI = body mass/height^2). For male participants, the 2D: 4D ratio was measured with steel Vernier callipers to the nearest millimetre. Measurements were taken on the ventral side of the finger, from the centre of the finger crease proximal to the palm to the tip of the finger. Both hands were measured and the ratio averaged. According to Manning (2002) such soft tissue measurements correlate well with measurements taken from the bones of the finger as observed on X-rays. None of the male participants reported any injuries to their 2nd or 4th digits.

Participants from both sexes were photographed and their images masked according to the protocol described in chapter 2. Masked photographs were used to compile 12 full colour presentations consisting of 10 male photographs each, and 24 full colour presentations consisting of 5 female photographs each, to account for the difference in the number of photographs and raters. Presentations were rated by 53 females (age, Mean = 20.1, S.D = 1.74, Range = 18-26) and 59 males (age, Mean = 21.1, S.D. = 2.11, Range = 18-26). Raters assessed opposite sex individuals for attractiveness (Q4, Appendix), perceived age (Q5, Appendix) and whether or not they knew the rated individual (Q6, Appendix). Raters indicated rated attractiveness on a continuous line (142 cm), with markings to indicate the ends, centre and quarter points. They were instructed to place their mark anywhere along the line. Individuals that recognised any of the rated individuals were given another presentation set to rate. Rated attractiveness was averaged to yield an index of facial attractiveness. All volunteers were provided with lunch in return for participation.

Fluctuating asymmetry measurements

To calculate fluctuating asymmetry (FA), we first positioned eight points by eye on standard facial features (Farkas 1994; Fink, Grammer and Thornhill 2001)(Figure 1). Four linear measurements were taken: biocular width (ex-ex), intercanthal width (en-en), nose width (al-al) and mouth width (ch-ch). All measurements were taken using ImageJ version 1.36b available from the American National Institute of Health (Image J, Image Processing and Analysis in Java, 2006). We computed the plane of symmetry (PS) by averaging the midpoints of all linear measurements. The asymmetries between the left and right side of the face were calculated by measuring the distance between PS and the four traits on each side of the face. We specifically focused on traits in the centre of the face, since facial masking inherently compromises points on the facial periphery. Similar left and right-sided traits were not measured sequentially thus the two sides were measured "blind" relative to each other.

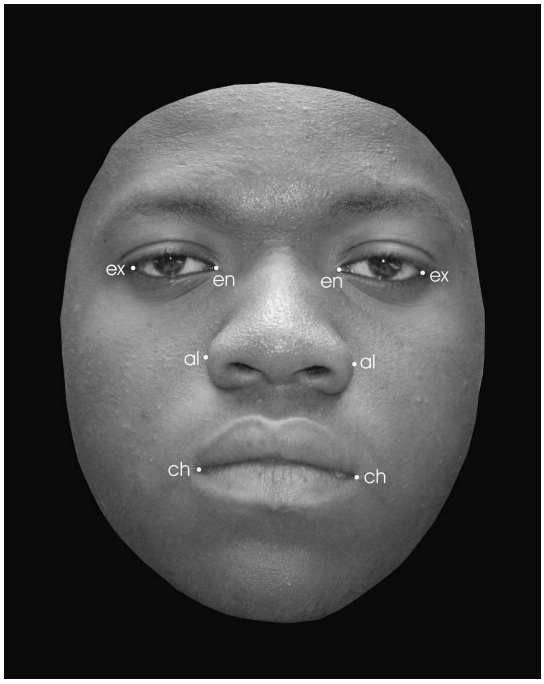


Figure 1. *Craniofacial points used to calculate fluctuating asymmetry: exocanthion (ex) the most lateral corner of the orbital fissure where the eyelids meet;*

endocanthion (en) the most medial corner of the orbital fissure where the eyelids meet; alare (al) the most lateral point of the nasion; cheilion (ch) the most lateral corner of the oral fissure where the lips meet.

Separate measurements were taken of 30 participants to access the repeatability (intraclass correlation coefficient) of FA measurements. None of the four unsigned traits showed significant directional asymmetry (DA)(t_{70} < 0.949, p > 0.346 in all cases) and therefore qualified as true FA traits. Traits also showed little kurtosis. We observed no significant difference in FA measures between the sexes (t < 0.355, p > 0.724 in all cases) therefore data were combined for both sexes for further analyses.

Signed asymmetries were calculated as the difference between the right (R) and left (L) traits. All signed traits had high (mean = 0.886, range = 0.743-0.975, N = 4) and statistically significant repeatability's ($F_{1,28}$ > 6.791, p < 0.005 in each case).To correct for discrepancies in face size, we calculated the percentage difference between the right and left traits, by dividing the unsigned asymmetries with the maximum width between the two points (|R-L|/|R+L|)*100. These percentages were divided by the average trait size for each trait to produce a composite trait measure that would contribute equally to a composite index ((|R-L|/|R+L|)*100)/ average|R-L|). Separate composite trait measures were summed to produce the composite index (CFA 2 in Leung, Forbes and Houle, 2000) on the assumption that no traits are better indicators of developmental stability than others. Eleven females and 10 males were excluded from the FA analyses because of slight facial tilting that influenced FA measures.

Sexual dimorphism

To calculate the level of sexual dimorphism we positioned 22 points by eye on standard facial features (Figure 2). These points have previously been shown to be

reliably identifiable facial features (Farkas 1994; Fink, Grammer and Thornhill 2001). From these we measured bilateral eye height (e1-e2, averaged for both eyes), bilateral eye width (en-ex, averaged for both eyes), face height (tr-gn), face width (eu-eu), lip width (ch-ch), jaw width (j1-j1) and lower face length (e1-gn, averaged for both sides). Measures were specifically chosen from previous research to represent major contributors to sexual dimorphism (Penton-Voak *et al.* 2001; Gangestad and Thornhill 2003). We corrected traits for slight facial size differences by dividing them with the appropriate facial measure. The four facial traits were: (1) lip width/ face width, (2) jaw width/ face width, (3) lower face height/ face height and (4) eye size/ face size. Eye size was calculated as 2(|e1-e2|/2)(|en-ex|/2) and face size as 2(|tr-gn|/2)(|eu-eu|/2). Although there is a discrepancy between true eye and face size and our estimations of these two parameters, the difference is expected to be constant across images. Spearman's correlation analyses were used to correlate facial attractiveness and sexually dimorphic traits, as transformations of BMI did not sufficiently alter skew. Separate measurements were taken of 30 participants to access the repeatability (intraclass correlation coefficient) of sexually dimorphic measurements. Repeatability's were high (mean = 0.983, range = 0.936-1.000, N = 7) and statistically significant ($F_{1,28} > 30.263$, $p < 0.005$ in each case).

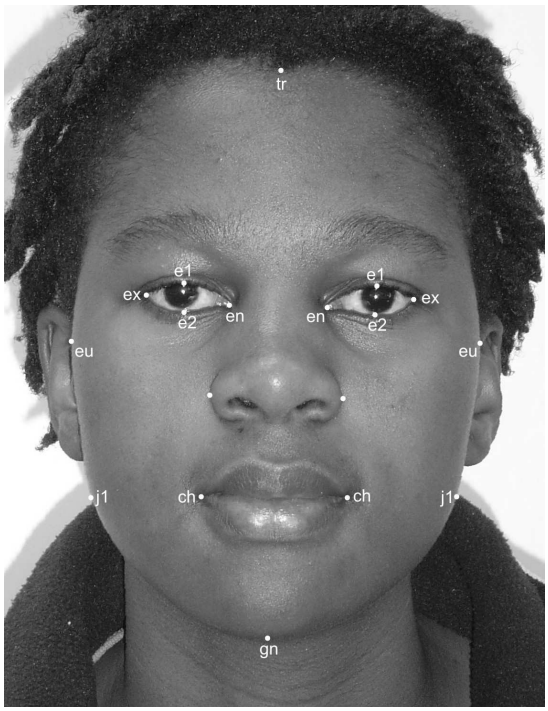


***Figure 2**. Additional craniofacial points used to calculate sexually dimorphic traits: tr (trichion) the midpoint of the hairline; eu (eurion) the most lateral point on the head; (j1) the most lateral point on the horizontal cheilion plane; gn (gnathion) in the midline, the lowest point on the lower border of the chin; (e1) the most superior point of the orbital white; (e2) the most inferior point of the orbital white.*

Age and perceived age

Perceived age was averaged for each individual. In order to test whether true or perceived age were better predictors of facial attractiveness we used GLM models (Facial attractiveness = true age)(Facial attractiveness = perceived age) separately for each sex. We also tested if individuals rated opposite sex individuals they perceived to be older than themselves to be more attractive, by calculating the perceived age difference for female (male perceived age – female true age) and male raters (female perceived age – male true age).

Predictors of facial attractiveness

The correlations between FA, sexual dimorphic measures, age and perceived age were tested with a Pearson correlation analysis. To test the role of symmetry, sexual dimorphism, true and perceived age as predictors of facial attractiveness we performed separate GLM analyses for each sex (Facial attractiveness = CFA2 + eye size/ face size + lip width/ face width + lower face height/ face height + true age+ perceived age). We included one measure of symmetry, the CFA2, because it accurately represented the fluctuating asymmetry of all traits. Sexually dimorphic traits were included separately (eyes/ face, lips width/ face, lower face/ face and jaw/ face) because of the low correlation between traits. We analysed the relationship using a GLM in a backwards and forwards stepwise manner, both methods yielded identical results. All analyses were performed in SPSS version 13.0 (Chicago, USA), alpha was set at 0.05 and all reported tests are two tailed. In each test, we identified influential outliers as data points with a Cook's value of 0.11 or higher. Once identified, influential outliers were removed, except in FA analyses where outliers are considered important. We report only results obtained after the removal of influential outliers, unless there was a difference in statistical significance, in which case we report both results.

Results

Fluctuating asymmetry

To test if unsigned asymmetries were shared between traits, we examined correlations among unsigned asymmetries of all traits for each subject. Four of the six (67%) between-trait correlations were significantly correlated after Bonferroni correction ($r > 0.384$, $p < 0.005$ in all cases). All correlations were in the positive direction. Because of the high positive between-trait correlations, we could safely

combine traits to form a composite index (CFA2), which according to Leung, Forbes and Houle (2000) is a better indicator of FA than separate traits.

This composite index (CFA2) was not significantly correlated with facial attractiveness ($r = 0.103$, $N = 90$, $p = 0.333$).

Sexual dimorphic traits

Three of the five traits (eye size/ face size, jaw width/face width and lower face height/face height) differed significantly between the sexes, even after sequential Bonferroni correction ($t_{109} > 3.001$, $p < 0.003$). The other two traits, lip width/ face width ($t_{109} = 2.062$, $p = 0.042$) and BMI logged ($t_{109} = -2.248$, $p = 0.027$) were not significant at the Bonferroni significance level of $\propto = 0.01$. As expected, males had smaller eyes, longer lower faces and larger jaws compared to women. However, these traits showed very little between-trait correlations within sexes. In males, only one of the 15 traits (13%) were significantly correlated at the Bonferroni significance level of $\propto = 0.003$. In females, none of the 10 traits were significantly correlated at the Bonferoni significance level of $\propto = 0.005$. Discrepancies in trait numbers between the sexes are due to an extra trait in males (2D: 4D). Because of the low between-trait correlations and the difference in direction, sexual dimorphic measures were used separately and not combined to form a composite index.

In males, none of the sexually dimorphic measures correlated significantly with facial attractiveness (eye size/ face size, $r = 0.051$, $N = 53$, $p = 0.717$; lips width/ face width, $r = -0.013$, $N = 52$, $p = 0.927$; jaw width/ face width, $r = 0.024$, $N = 50$, $p = 0.871$; lower face height/ face height, $r = -0.202$, $N = 52$, $p = 0.075$; BMI, $r = -0.148$, $N = 53$, $p = 0.291$; 2D: 4D, $r = 0.156$, $N = 52$, $p = 0.270$). In females, lip width/ face width correlated significantly with facial attractiveness after the removal of influential outliers ($r = -0.327$, $N = 56$, $p = 0.014$)(Figure 3) but not before ($r = -0.216$, $N = 58$, $p = 0.104$). Females with narrower lips were considered more attractive. None of the

other sexual dimorphic traits significantly correlated with female facial attractiveness (eyes/face, r = 0.007, N = 56, p = 0.959; jaw/face, r = -0.236, N = 57, p = 0.077; lower face/face, r = 0.156, N = 56, p = 0.251; BMI, r = -0.164, N = 56, p = 0.228).

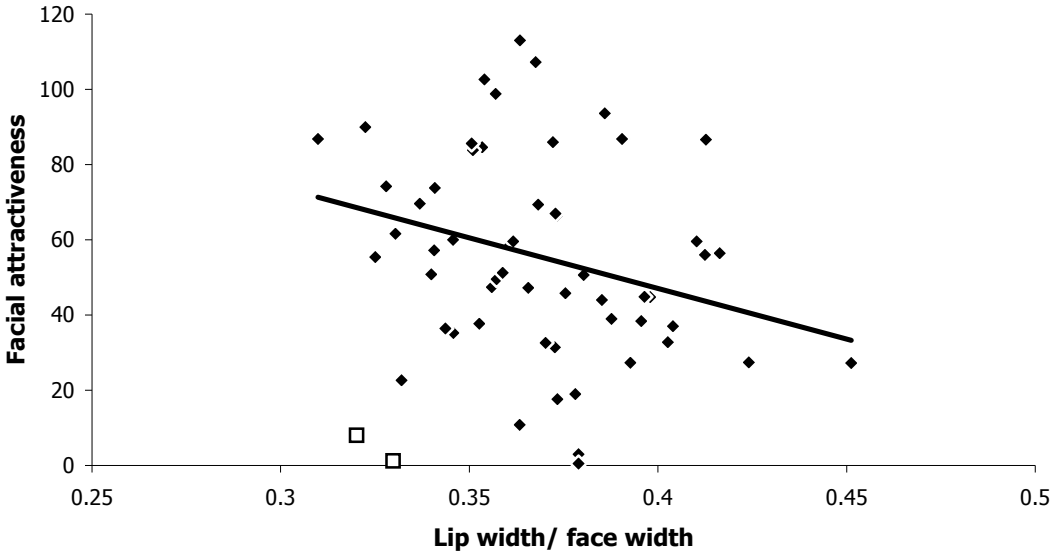


***Figure 3**. Spearman's correlation between rated facial attractiveness and lip width in relation to face width. Outliers are indicated as squares.*

Age and perceived age

Separate GLM analyses were done for each sex. In females, true age did not significantly predict rated attractiveness ($F_{1,56}$ = 2.238, R^2 = 0.038, p = 0.140), but perceived age did (equation: Rated attractiveness = 248.631 – 8.929 x perceived age; $F_{1,55}$ = 14.438, R^2 = 0.208, p < 0.0005)(Figure 4). Males therefore rated females to be more attractive if they also perceived them to be younger.

True age marginally predicted rated attractiveness in males (equation: Rated attractiveness = 126.488 – 3.470 x true age, $F_{1,50}$ = 4.117, R^2 = 0.076, p = 0.048). Perceived age, however, explained more variance and predicted rated attractiveness better (equation: Rated attractiveness = 277.376 – 9.837 x perceived age, $F_{1,36}$ = 11.083, R^2 = 0.235, p = 0.002)(Figure 5).

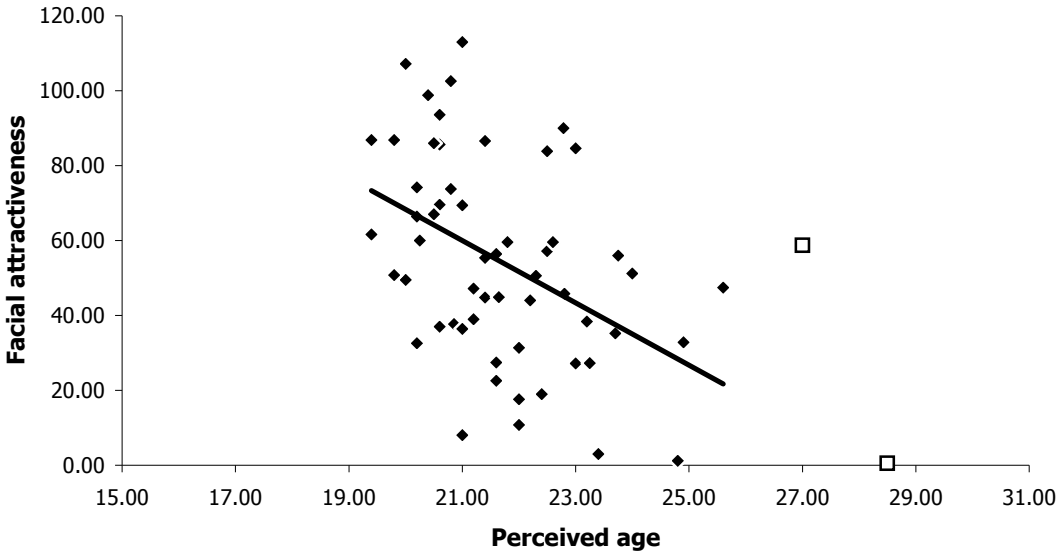


***Figure 4**. Pearson's correlation between rated facial attractiveness and perceived age in females. Outliers are indicated as squares.*

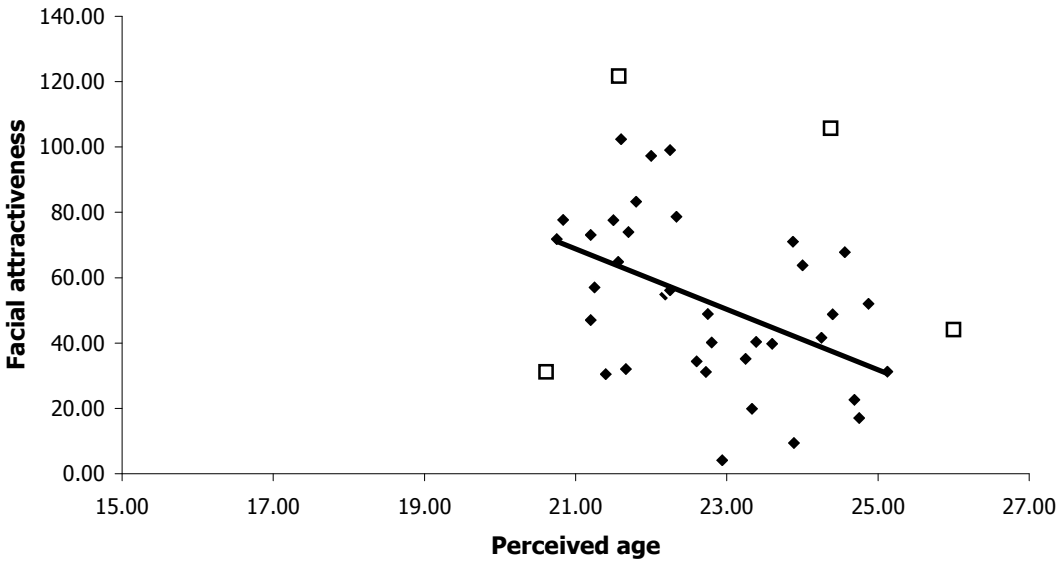


***Figure 5**. Pearson's correlation between rated facial attractiveness and perceived age in males. Outliers are indicated as squares.*

Further evidence for the importance of perceived age over true age, came from a full GLM model containing both true and perceived male age as explanatory

variables (Facial attractiveness = true age + perceived age). In this model only perceived age significantly predicted facial attractiveness. Therefore, if perceived age is taken into account, true age is unimportant. Interestingly, the association between male perceived age and facial attractiveness is negative. The negative association implies that females prefer males they perceive to be younger. However, in our sample size males were significantly older than females (t_{109} = 3.665, p < 0.0005). To check if the preference for younger looking men was not actually a preference for similar aged men, we calculated the difference between female true age and male perceived age directly for each separate rating. Females rated men they perceived to be older than themselves to be less attractive (equation: Rated attractiveness = 60.630 – 2.103 x perceived age difference, $F_{1,241}$ = 5.093, R^2 = 0.021, p = 0.025) (Figure 6).

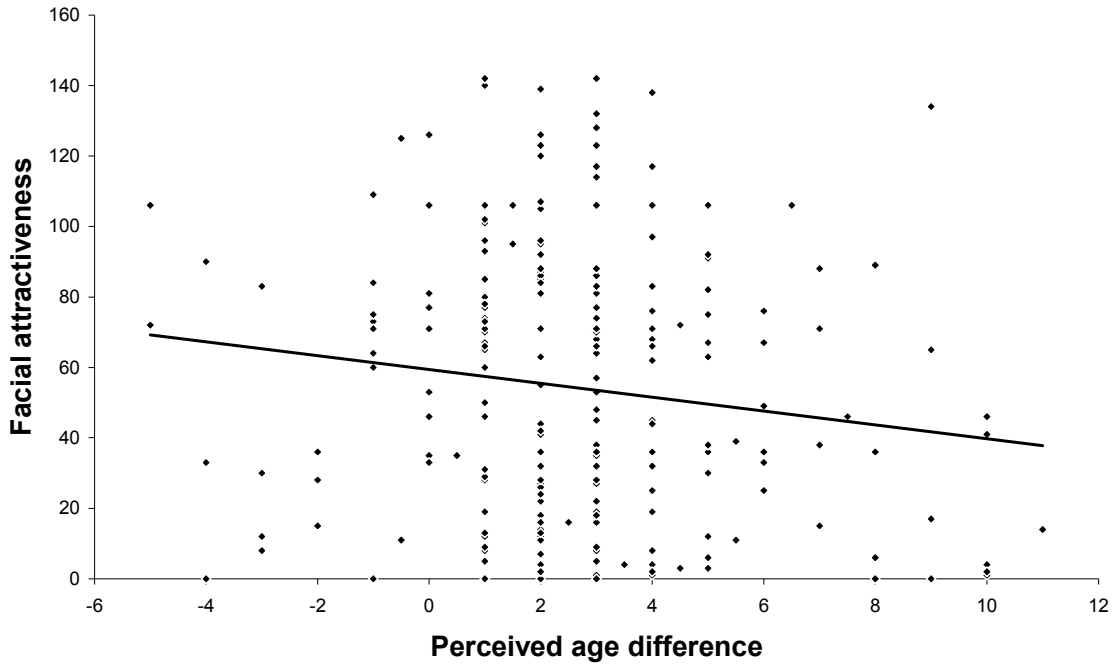


***Figure 6**. Perceived male age as a predictor of male facial attractiveness. Women rate men more attractive if they perceive the men to be younger than themselves (p = 0.021).*

Predictors of facial attractiveness

Both age and perceived age correlated significantly with three of the four sexual dimorphic traits, though the correlation was stronger for perceived age (*Age*: lip width/ face, r = 0.207, N = 90, p = 0.050; jaw/ face, r = 0.215, N = 90, p = 0.042; lower face/ face, r = 0.220, N = 90, p = 0.037; eyes/ face, r = 0.003, N= 90, p = 0.979; *perceived age*: lip width/ face, r = 0.329, N = 78, p = 0.003; jaw/ face, r = 0.438, N = 78, p < 0.0005; lower face/ face, r = 0.204, N = 78, p = 0.073; eyes/ face, r = -0.231, N = 78, p = 0.041). In all cases, younger individuals or individuals that were perceived to be younger were also perceived as more feminine. There was also a strong correlation between age and perceived age, such that younger individuals were also perceived as younger looking (r = 0.436, N = 78, p < 0.0005). We observed no significant correlation between FA and any of the sexual dimorphic features, age or perceived age (*Sexual dimorphic*: eyes/ face, r = -0.149, N = 90, p = 0.162; lip width/ face, r = 0.023, N = 90, p = 0.833; jaw/ face, r = 0.037, N = 90, p = 0.727; lower face/ face, r = 0.060, N = 90, p = 0.574; *age*: r = -0.086, N = 90, p = 0.419; *perceived age*: r = 0.032, N = 78, p = 0.778)(Table 1)

In the male full GLM model (Facial attractiveness = CFA2 + eye size/ face size + lip width/ face width + lower face height/ face height + true age+ perceived age), perceived age was the only significant predictor of facial attractiveness (equation: Rated attractiveness = 179.874 – 5.696 x perceived age, $F_{1,76}$ = 9.865, R^2 = 0.115, p = 0.002).

Table 1. *Significance of correlations between FA, sexual dimorphic traits, age and perceived age. Significant correlations are indicated in bold.*

	CFA2	Eyes/ face	Lip width/ face	Jaw/ face	Lower face/ face	Age	Perceived age
CFA2	-	0.162	0.833	0.727	0.574	0.419	0.778
Eyes/ face		-	-	-	-	0.979	**0.041**
Lip width/ face			-	-	-	**0.050**	**0.003**
Jaw/ face				-	-	**0.042**	**< 0.0005**
Lower face/ face					-	**0.037**	0.073
Age						-	**< 0.0005**
Perceived Age							-

Discussion

Our results show that symmetry by itself did not correlate significantly with facial attractiveness. One of the measures of sexual dimorphism, lip width, did significantly correlate with facial attractiveness in females. Women with more feminine narrow lips were rated as more attractive. Furthermore, trends in the data indicated a preference for femininity in both sexes. A previous study by Perrett *et al.* (1998) also showed a preference for femininity in male faces.

True age did not significantly correlate with facial attractiveness in women, but it did marginally correlate with male facial attractiveness, with younger men rated as more attractive. However, the best measure of facial attractiveness was not

femininity or true age, but perceived age. Perceived age in both sexes showed a highly significant correlation with facial attractiveness. Both sexes preferred "neotenous" features, or "baby-facedness" in the opposite sex. It is easy to comprehend why men might prefer younger looking women. From an evolutionary standpoint, perceived age and true age are highly correlated, and younger women are more likely to be fertile. What is more difficult to comprehend is why women find younger men more attractive, especially because men are fertile through most of their adult lives. One plausible explanation is Cunningham, Druen and Barbee's (1997) "Multiple fitness model". According to this model women prefer men with "neotenous" features because they evoke feelings of nurturance, and because they are perceived to have more vigour required to raise children. Another plausible explanation is one originally proposed by Perrett *et al*. (1998). They found that masculine faces are associated with negative personality attributes, such as lower perceived warmth, emotionality, honesty, cooperativeness and quality as a parent. More feminine faces might therefore be rated more attractive because they are associated with more positive personal attributes.

Boothroyd *et al.* (2005) proposed that the variation in preferences for sexual dimorphism could just be a by-product of variation in preferences for "neotenous" cues. Our results support this hypothesis for the following reasons. First, of all the features, perceived age correlated the strongest with facial attractiveness. Second, trends in the data suggest that both sexes rate feminine members of the opposite sex more attractive. As "neotenous" features are inherently more feminine, this weaker preference for femininity could easily be due to a correlation between perceived age and femininity. In fact, we did find a strong significant correlation between perceived age and three of the four measures of femininity. Moreover, the major alternative to the "neoteny" hypothesis, the immunocompetence handicap hypothesis (Folstad and Karter, 1992) predicts a positive correlation between masculinity and facial attractiveness, something we did not find. Finally, FA, the only facial feature that did

not correlate significantly with perceived age, was also the only facial feature that did not significantly predict facial attractiveness.

In conclusion, we showed that cues to age, or rather perceived age, are recognised significantly better than cues to symmetry or sexual dimorphism and serve as the basis for preferences in facial attractiveness in both sexes.

Acknowledgements

We would like to thank Nicole Creux, Minique de Castro, Therese de Castro, Christoff Erasmus, Tracey-Leigh Hatherall, Aret Meyer, Ronnie Nelson, Duncan Newman, Jason Pienaar and Isa-Rita Russo for their valuable help with data collection. This material is based, in part, upon work supported by the National Research Foundation under Grant number 2053809 to JMG. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation. This project was cleared by the ethics committees of the University of Pretoria and the University of the Witwatersrand respectively.

References

Berry, D. and McArthur, L. (1985) Some components and consequences of a babyface. *Journal of Personality and Social Psychology*, 48: 312-323.

Boothroyd, L. G., Jones, B. C., Burt, D. M., Cornwell, R. E., Little, A. C., Tiddeman, B. P. and Perrett, D. I. (2005) Facial masculinity is related to perceived age but not perceived health. *Evolution and Human Behavior*, 26: 417-431.

Buunk, B. P., Dijkstra, P., Kenrick, D. T. and Warntjes, A. (2001) Age preferences for mates as related to gender, own age, and involvement level. *Evolution and Human Behavior*, 22: 241-250.

Cunningham, M. R. (1986) Measuring the physical in physical attractiveness: quasi-experiments on the socio-biology of female facial beauty. *Journal of Personality and Social Psychology*, 50: 925-935.

Cunningham, M. R., Barbee, A. P. and Pike, C. L. (1990) What do women want? Facialmetric assessment of multiple motives in the perception of male facial physical attractiveness. *Journal of Personality and Social Psychology*, 59: 61-72.

Cunningham, M., Druen, P. and Barbee, A. (1997) Angels, mentors, and friends: trade-offs among evolutionary, social and individual variables in physical appearance. In: J. A. Simpson and D. T. Kenrick eds. *Evolutionary Social Psychology*. New Jersey, Lawrence Erlbaum Associates, pp. 109-140.

Dion, K., Berscheid, E. and Walster, E. (1972) What is beautiful is good. *Journal of Personality and Social Psychology*, 24: 285-290.

Farkas, L. G. (1994) *Anthropometry of the head and face*. 2nd edition. Raven Press, New York.

Fink, B., Grammer, K. and Thornhill, R. (2001) Human (*Homo sapiens*) facial attractiveness in relation to skin texture and color. *Journal of Comparative Psychology*, 115: 92-99.

Fink, B. and Penton-Voak, I. (2002) Evolutionary psychology of facial attractiveness. *Current Directions in Psychological Science*, 11: 154-158.

Fink, B., Grammer, K. and Thornhill, R. (2001) Human (*Homo sapiens*) facial attractiveness in relation to skin texture and color. *Journal of Comparative Psychology*, 115: 92-99.

Folstad, I. and Karter, A. J. (1992) Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, 139: 603-622.

Gangestad, S. W. and Thornhill, R. (2003) Facial masculinity and fluctuating asymmetry. *Evolution and Human Behavior*, 24: 231-241.

Garn, S. M., Burdi, A. R., Babler, W. J. and Stinson, S. (1975) Early prenatal attainment of adult metacarpalphalangeal rankings and proportions. *American Journal of Physical Anthropology*, 43: 327-332.

Grammer, K. and Thornhill, R. (1994) Human (Homo sapiens) facial attractiveness and sexual selection: the role of symmetry and averageness. *Journal of Comparative Psychology*, 108: 233-242.

Halberstadt, J. and Rhodes, G. (2000) The attractiveness of nonface averages: implications for an evolutionary explanation of the attractiveness of average faces. *Psychological Science*, 11: 285-289.

Henderson, J. J. A. and Anglin, J. M. (2003) Facial attractiveness predicts longevity. *Evolution and Human Behavior*, 24: 351-356.

Hume, D. K. and Montgomerie, R. (2001) Facial attractiveness signals different aspects of “quality” in women and men. *Evolution and Human Behavior*, 22: 93-112.

Image J, Image Processing and Analysis in Java. [Internet], National Institute of Health. Available from: <http://rsb.info.nih.gov/ij/ > [Accessed: 1 May 2006].

Johnston, V. S., Hagel, R., Franklin, M., Fink, B. and Grammer, K. (2001) Male facial attractiveness: evidence for hormone-mediated adaptive design. *Evolution and Human Behavior*, 22: 251-267.

Korthase, K. M. and Trenholme, I. (1982) Percieved age and perceived physical attractiveness. *Perceptual and Motor Skills*, 54: 1251-1258.

Kurzban, R. and Weeden, J. (2005) Hurrydate: mate preferences in action. *Evolution and Human Behavior*, 26: 227-244.

Leung, B., Forbes, M. R. and Houle, D. (2000) Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *The American Naturalist,* 155: 101-115.

Manning, J. T. (2002) Digit ratio: a pointer to fertility, behavior and health. New Jersey: Rutgers University Press. In Manning. J. T.. Bundred. P. E. and

Mather, F. M. (2004) Second to fourth digit ratio, sexual selection, and skin colour. *Evolution and Human Behavior,* 25: 38-50.

Manning, J. T., Bundred, P. E. and Mather, F. M. (2004) Second and fourth digit ratio, sexual selection, and skin colour. *Evolution and Human Behavior*, 25: 38-50.

Manning, J. T. and Chamberlain, A. T. (1994) Fluctuating asymmetry in gorilla canines: a sensitive indicator of environmental stress. *Proceedings of the Royal Society of London, B*, 255: 189-193.

Manning, J. T., Koukourakis, K. and Brodie, D. A. (1997) Fluctuating asymmetry, metabolic rate and sexual selection in human males. *Evolution and Human Behavior*, 18: 15-21.

Manning, J. T., Scutt, D., Wilson, J. and Lewis-Jones, D. I. (1998) The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human Reproduction*, 13(11): 3000-3004.

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

Miller, G. F. and Todd, P. M. (1998) Mate choice turns cognitive. *Trends in Cognitive Science*, 2: 190-198.

Møller, A. P., Soler, M. and Thornhill, R. (1995) Breast Asymmetry, Sexual Selection, and Human Reproductive Success. *Ethology and Sociobiology*, 16: 207-219.

Møller, A. P. and Thornhill, R. (1998) Bilateral symmetry and sexual selection: a meta-analysis. *The American Naturalist*, 151: 174-192.

Neave, N., Laing, S., Fink, B. and Manning, J. T. (2003) Second to fourth digit ratio, testosterone and perceived male dominance. *Proceedings of the Royal Society of London, B*, 270: 2167-2172.

Palmer, A. R. and Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review in Ecology and Systematics*, 17: 391-421.

Penton-Voak, I. S., Jones, B. C., Little, A. C., Baker, S., Tiddeman, B., Burt, D. M. and Perrett, D. I. (2001) Symmetry, sexual dimorphism in facial proportions and male facial attractiveness. *Proceedings of the Royal Society of London, B*, 268: 1617-1623.

Perrett, D. I., Lee, K. J., Penton-Voak, I., Rowland, D., Yoshikawa, S., Burt, D. M., Henzi, S. P., Castles, D. L. and Akamatsu, S. (1998) Effects of sexual dimorphism on facial attractiveness. *Nature*, 394: 884-887.

Perrett, D. I., May, K. A. and Yoshikawa, S. (1994) Facial shape and judgements of female attractiveness. *Nature*, 368, 239-242.

Rhodes, G., Chan, J., Zebrowitz, L. A. and Simmons, L. W. (2003) Does sexual dimorphism in human faces signal health? *Proceedings of the Royal Society of London, B (Supplement)*, 270: S93-S95.

Rikowski, A. and Grammer, K. (1999) Human body odour, symmetry and attractiveness. *Proceedings of the Royal Society of London, B*, 266: 869-874.

Simmons, L. W., Rhodes, G., Peters, M. and Koehler, N. (2004) Are human preferences for facial symmetry focused on signals of developmental instability? *Behavioral Ecology*, 15: 864-871.

Soler, C., Núñez, M., Gutiérrez, R., Núñez, J., Medina, P., Sancho, M., Álvarez, J. and Núñez, A. (2003) Facial attractiveness in men provides clues to semen quality. *Evolution and Human Behavior*, 24: 199-207.

Symons, D. (1979) *The Evolution of Human Sexuality*. Oxford, Oxford University Press.

Walster, E., Aronson, V., Abrahams, D. and Rottman, L. (1966) Importance of physical attractiveness in dating behavior. *Journal of Personality and Social Psychology*, 4: 508-516.

Appendix

Initial male/ female questionnaire:

1. Are you currently using the pill or injection as contraception?
2. Female questionnaire: Are you heterosexual? (Do you prefer men as your sexual partners)
3. Male questionnaire: Are you heterosexual? (Do you prefer women as your sexual partners)

Image scoring questionnaire:

4. Please indicate how attractive you think this man/ woman is.
5. How old do you think he/she is?
6. Do you know any of these men?

CHAPTER 4

FACIALLY BASED ETHNIC RECOGNITION IN SOUTH AFRICA

Vinet Coetzee[1*], Louise Barrett[2], Jaco M. Greeff[1] and Peter S. Henzi[2].

*[1]Department of Genetics, University of Pretoria, Pretoria, South Africa; [2]Department of Psychology, University of Central Lancashire, Preston, UK [3]. *Corresponding author vcoetzee@tuks.co.za.*

Contributors

This chapter consists of a manuscript that has been prepared for submission to a peer-reviewed journal (*Evoution and Human Behavior*). The study was designed by myself and Prof. Jaco M Greeff. I conducted all data collection, except for the assistance noted in the acknowledgements. Data analyses were done by me and Prof. Jaco M Greeff and I drafted the first version of the manuscript.

Abstract

Mate choice preferences based on aspects of facial attractiveness are well-studied phenomena, and there is high cross-cultural agreement on attractiveness. However, there is also evidence to suggest that individuals are adept at identifying ethnic and racial groups from facial data, which could influence actual mate choice patterns via preference for the facial features of one's own ethnic group. To date, most data on this issue have considered widely separated populations, both geographically and culturally, making it hard to disentangle effects of familiarity with an ability to identify ethnic groups *per se*. We used data from a highly intermixed population of Bantu-speaking peoples from South Africa to test whether individuals could correctly differentiate between facial images of two ethnic groups, the Tswana and Pedi. Individuals could not assign ethnicity better than expected by chance, and there was no significant difference between the sexes in accuracy of assignment. Interestingly, we also observed a trend that individuals of mixed ethnic origin were better at assigning ethnicity to Pedi and Tswana's, than individuals more closely related to the two groups. This result supports the hypothesis that ethnic recognition is based on the visual expertise gained with exposure to different ethnic groups.

Introduction

The human face reveals an enormous wealth of information, most importantly on identity, age, gender and ethnicity (Farkas, 1994), and plays an important role in mate preferences (see Fink and Penton-Voak, 2002 for a review). Cross-cultural studies, for example, have shown that people generally agree on attractiveness ratings across different ethnic groups (e.g., Perrett, May and Yoshikawa 1994; Jones 1995). However, it is also the case that individuals can recognise individuals belonging to different races and ethnic groups (Hajniš *et al*. 1994) (where ethnic group refers to distinct populations within a particular racial grouping, e.g., comparing Germans to Americans within the Caucasian grouping), that faces from the same race as the observer illicit more brain activity in regions linked to face recognition (Golby *et al*. 2001) and that recognition of one's own ethnic group is better than that for other ethnic groups (Malpass & Kravitz 1969; O'Toole *et al*. 1994). One plausible explanation for superior recognition of same race and same ethnic group faces is exposure. Most people, especially young people, have more exposure to their own ethnic group (Chance, Turner and Goldstein, 1982). This variation in exposure can contribute to the development of visual expertise for same group faces (Golby *et al*. 2001). If individuals are exposed more frequently to different ethnic groups, one might expect their visual expertise to include other ethnic groups as well. In fact, two recent studies showed that individuals from minority ethnic groups are better at recognising other ethnic groups in their area than individuals from majority ethnic groups (Golby *et al*. 2001; Tanaka, Kiefer and Bukach 2004). Thus, despite agreement on attractiveness across races, there may remain a significant element of ethnic recognition, and potential preference, within particular racial categories that potentially may influence mate preferences and subsequent mate choice.

To date, however, studies comparing differences within ethnic groups have shown a significant separation of culture and geography (North America, Germany

and the Czech Republic: Hajniš *et al*. 1994). This means one cannot discount an influence of environmental and/or sociocultural factors on facial morphology and/or greater familiarity with faces of one's own ethnicity compared to other groups. In order to resolve these issues, we tested whether recognition is also possible in a population where there is a large overlap of both culture and geography between the different ethnic groups. Specifically, we tested for ethnic recognition within the South African Bantu-speaking population.

According to ethnological, linguistic and genetic data (Levitas 1983; Schapera 1962; Lane *et al*. 2002), the South African Bantu-speaking people can be divided into two major groups: the Nguni and the Sotho group. Autosomal and Y-chromosome data group the Xhosa, Zulu, Swazi and Ndebele into the Nguni group, while the Tswana, Pedi and Sotho form part of the Sotho group. Venda and Tsonga group separately (Lane *et al*. 2002). These groupings are also observed in the linguistic data, except for the Tsonga, which group with the Nguni. Despite this clear separation of ethnic groups, there is, however, a high degree of intermixing both between and within major groups, especially in urban areas (V. Coetzee, unpublished data).

In this study we focused specifically on the Pedi and Tswana to determine whether individuals from the same and other groups could assign ethnicity based on facial features alone. These groups have a similar cultural background as members of the Sotho major group and they also show a very minor geographic separation: the hub of the Tswana and Pedi populations are located in the neighbouring North-West and Northern Province of South Africa respectively (Schapera 1962; Lane *et al*. 2002). We also assessed levels of ethnic intermixing in our own sample to confirm that we were drawing subjects from a population in which individuals of different ethnicities had significant experience of each other.

Materials and Methods

Full colour photographs of 39 individuals (14 male and 25 female) belonging to the Pedi or Tswana groups were taken with a Sony Cybershot DSC P72 (default settings with 3.1 Mega pixels fine, Soft light flash and -1.0EV) under standard lighting conditions. Participants were asked to maintain a neutral expression. All volunteers were students at the University of Pretoria and signed a subject information and consent form briefly explaining the study. Their ethnicity and that of both parents were self-reported. Slight lateral tilting of individual faces were corrected by rotation around the facial midline using vertical guidelines and cropped 5cm from each side to standardize size in Coral Photopaint v.10. Next, faces were masked to eliminate confounding factors in Coral Knockout v. 1.5 (Figure 1).

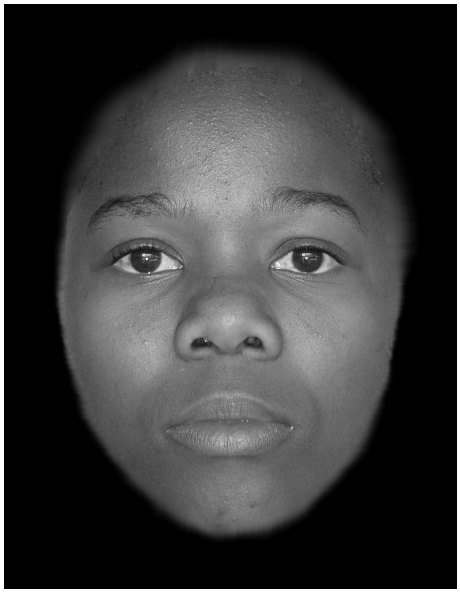


Figure 1*. Individual photos were rotated around the facial midline, cropped and masked to eliminate confounding factors.*

Twenty nine individuals (16 Pedi and 13 Tswana) of whom both parents belonged to the same ethnic group were used to compile 40 full colour presentations; each presentation contained 4 photographs (1 Pedi male, 1 Pedi female, 1 Tswana male and 1 Tswana female) in random order for each sex. These presentations were

then displayed to 100 individuals (50 male and 50 female) of known ethnic origin. Each subject was asked to assign ethnicity to each of the images in the presentations as a forced choice between Tswana and Pedi. Thirteen participants (10 male and 3 female) were excluded from the study since both their parents were not originally from South Africa and one female participant was excluded for falling outside the age range of 18-26. Data were analyzed using a binomial test in SPSS version 13.0 (Chicago, IL). To test whether Tswana's and Pedi's can be correctly identified we compared the frequencies of correctly rated images to the frequencies expected under the binomial distribution, with a probability parameter of 0.5. Each sex was tested separately. Secondly, we compared the frequencies of correct raters per sex to the frequencies expected under the binomial distribution, to determine if one sex was better at recognising ethnicity. Lastly, we tested whether rater ethnicity had a significant effect on the accuracy of their ethnicity recognition. Raters were divided into four main groups: (a) both parents belonging to the Sotho major group (Sotho major), (b) both parents belonging to the Nguni major group (Nguni major), (c) one parent belonging to the Sotho major and one parent belonging to the Nguni major group (Mixed major) and (d) one or both parents belonging to the Venda or Tsonga ethnic groups (Venda/ Tsonga group). Each of these groups observed frequencies were compared with the expected frequencies of 0.5.

Results

Our rater population was ethnically mixed, with 43% of the 86 raters used in the study being of mixed ethnic origin (i.e., had parents belonging to two different ethnic groups). Within the mixed origin group, 19% had one parent belonging to the Nguni and one parent belonging to the Sotho major groups, while 71% had both parents belonging to the same major group but different ethnic groups. The remaining 10% had one parent that belonged to either the Venda or Tsonga group. Overall, then,

ethnic groupings were fluid and many individuals were exposed to different ethnic groups within as well as between families.

Our results revealed no significant deviation from the expected 50% for the correct assignment of Tswana's and Pedi's. Both male (Observed frequency = 0.49, p = 1.000, N = 87) and female images (Observed frequency = 0.59, p = 0.133, N = 87) could not be recognised better than expected by chance alone. We also observed no significant deviation from the expected 50% for male and female raters. Neither of the rater sexes (male: observed frequency = 0.54, p = 0.576, N = 80; Female: observed frequency = 0.54, P= 0.470, N = 94) recognised Tswana/ Pedi images better than expected at random. Interestingly, mixed major and Venda/Tsonga raters correctly identified Tswana and Pedi images 62.5% of the time, more frequently than Sotho or Nguni major groups who only correctly assigned ethnicity in 51% and 50% of the time respectively. However, this result did not significantly differ from the random expectation of 50%.

Discussion

A high degree of intermixing between the different ethnic groups was observed (43%) in our sample, and is undoubtedly due to drawing subjects from an urban area. Our study population therefore have high level exposure to individuals of different ethnicity, especially those individuals whose parents belong to different major ethnic groups.

Our results show that individuals from our study population cannot significantly differentiate between facial features of Tswana and Pedi individuals. To our knowledge, this is the first study to test ethnic recognition in two such closely related ethnic groups, with similar environmental and socio cultural histories. We also show that neither males nor females can differentiate between Tswana and Pedi images significantly better than random. This suggests that perceptual or

morphological differences are negligible between the sexes and it is therefore unlikely that there has been selection for one sex to be better at recognising or displaying ethnicity than the other.

Previous studies showed that individuals are better at recognising their own ethnic group (Malpass and Kravitz, 1969). However, we found that Sotho major group individuals were comparatively poor at recognising own group faces. Instead, individuals whose parents belonged to different major ethnic groups were better at recognising Tswana/ Pedi images. This discrepancy can most probably be attributed to the variation in exposure. Ethnically mixed individuals are likely to have been exposed to a greater variety of ethnic groups within their family environment. However, despite the observed tendency, mixed major ethnic group individuals did not assign ethnicity correctly more than expected at random. Consequently, our results need further investigation and such a study should increase the proportion of mixed major individuals.

In conclusion, our study showed that Bantu speaking South Africans couldn't reliably differentiate between Tswana and Pedi individuals, based on facial features alone. We cannot exclude the possibility that ethnic recognition is possible based on whole body features, but our results suggest that the Tswana and Pedi can be used simultaneously in facial preference studies. We also showed a tendency for more ethnically mixed individuals to be better at recognising Tswana and Pedi faces, presumably because of their heightened exposure to a variety of different ethnic groups. However, more research is needed to unravel the correlation between exposure and ethnic intermixing.

Acknowledgements

We thank Ronnie Nelson and Christoff Erasmus for their invaluable assistance with data collection. VC was supported by an NRF studentship under Grant number

2053809 to JMG and LB was supported by a Leverhulme Trust Research Fellowship during the writing of this paper. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation.

References

Chance, J. E., Turner, A. L. and Goldstein, A. G. (1982) Development of differential recognition for own-and other-race faces. *Journal of Psychology*, 112: 29-37.

Farkas, L. G. (1994) *Anthropometry of the Head and Face*. 2nd edition. New York, Raven Press.

Fink, B. and Penton-Voak, I. (2002) Evolutionary psychology of facial attractiveness. *Current Directions in Psychological Science*, 11: 154-158.

Golby, A., Gabrieli, J., Chiao, J. and Eberhardt, J. (2001) Differential responses in the fusiform region to same-race and other-race faces. *Nature Neuroscience*, 4: 845-850.

Hajniš, K., Farkas, L. G., Ngim, R. C. K., Lee, S. T. and Venkatadri, G. (1994) Racial and ethnic morphometric differences in the craniofacial complex. In: Farkas, L. G. ed. *Anthropometry of the Head and Face*. 2nd edition. New York, Raven Press.

Jones, D. (1995) Sexual selection, physical attractiveness, and facial neoteny. *Current Anthropology*, 35: 723-748.

Lane, A.B., Soodyall, H., Arndt, S., Ratshikhopha, M.E., Jonker, E., Freeman, C., Young, L., Morar, B. and Toffie, L. (2002) Genetic substructure in South African bantu-speakers: evidence from autosomal DNA and Y-chromosome studies. *American Journal of Physical Anthropology*, 119: 175-185.

Levitas, B. (1983). *Ethnology: An Introduction to the Peoples and Cultures of Southern Africa*. Cape Town, Oxford University Press.

Malpass, R. and Kravitz, J. (1969) Recognition for faces of own and other race. *Journal of Personality and Social Psychology*, 13: 330-334.

O'Toole, K., Deffenbacher, D., Valentin, D. and Abdi, H. (1994) Structural aspects of face recognition and the other race effect. *Memory and Cognition*, 22: 208-224.

Perrett, D. I., May, K. A. and Yoshikawa, S. (1994) Facial shape and judgements of female attractiveness. *Nature*, 368: 239-242.

Schapera, I. (1962) *Bantu-speaking tribes of South Africa: An Ethnographical survey*. London, Routledge & Kegan Paul Ltd.

Tanaka, J. W., Kiefer, M. and Bukach, C. M. (2004) A holistic account of the own-race effect in face recognition: evidence from a cross-cultural study. *Cognition*, 93: B1-B9.

CHAPTER 5

THE ROLE OF AGE, SELF-ESTEEM, SELF-PERCEIVED ATTRACTIVENESS AND RELATIONSHIP STATUS ON MATE CHOICE

Vinet Coetzee[1*], Louise Barrett[2], Jaco M. Greeff[1] and Peter S. Henzi[2] .

*[1]Department of Genetics, University of Pretoria, Pretoria, South Africa; [2]Department of Psychology, University of Central Lancashire, Preston, UK [3]. *Corresponding author vcoetzee@tuks.co.za.*

Contributors

This chapter consists of a manuscript that has been prepared for submission to a peer-reviewed journal (*Evolution and Human Behavior*). The study design and data collection were done by me, except for the assistance noted in the acknowledgements. I also did the data analyses and drafted the first version of the manuscript.

Abstract

Mate choice strategies are condition dependant with low quality individuals lowering their demands on prospective partners. We studied the effects of three condition dependent factors, age, self-perceived attractiveness and relationship status on male and female choosiness in a traditionally polygamous society, the Tswana. Our study shows that condition dependent factors affect choosiness in a context specific manner. Women who perceive themselves as more attractive rate members of the opposite sex less attractive, an indication of their increased choosiness; while both self-perceived attractiveness and to some extend age positively predict how choosy they are of potential short term partners. Choosiness in regard to potential long-term partners is determined only by their current relationship status. None of the condition dependant factors significantly affected male choosiness and self-esteem had no significant effect on choosiness in either sex. Contrary to popular belief we also found no significant difference between male and female choosiness, an indication that male choice is crucial in human populations. Both sexes were choosier of long than short-term partners, presumably because they have more to loose by choosing an unsuitable long-term partner.

Introduction

Selection favours individuals that maximise their fitness. One obvious way of increasing one's fitness is by choosing a high quality member of the opposite sex as a mate (Symons, 1979). By choosing a high quality mate, individuals gain direct and indirect benefits, increasing their survival and reproductive potential (Ryan, 1997). Unfortunately, not everyone can acquire the fittest mate. Mate choice is a frequency dependent market where high fitness individuals are in demand and can choose among potential mates, while low fitness individuals struggle to obtain a mate (Pawlowski and Dunbar 1999; Buston and Emlen 2003). However, opposite sex and same sex individuals are not the only individuals judging a person's fitness. Individuals assess their own fitness based on their perceived condition, as defined by their particular circumstances and their perceived market value (Barrett, Dunbar and Lycett, 2002). Both these factors fluctuate on a temporal scale. For instance, a woman who was in high demand in her twenties may not be in such high demand in her forties. If an individual perceives his/her condition to be less than optimal, one solution is to be less choosy (Pawlowski and Dunbar 1999; Barrett, Dunbar and Lycett, 2002). By lowering their standards, such individuals potentially increase their fitness by obtaining more lower quality mates.

Lower fitness individuals are also less likely to successfully employ their "preferred mating strategy". According to the sexual strategies theory originally proposed by Buss and Schmitt (1993) optimal mating strategies differ between men and women, but both sexes benefit from short and long term strategies. These strategies are not consciously planned or articulated, but stem from desires, carefully honed over millions of years of evolution (Miller and Fishkin, 1997). Buss and Schmitt (1993) argue that men benefit especially from a short term mating strategy, where their reproductive success is constrained only by the number of fertile women they can identify and inseminate (but see Miller, Putcha-Bhagavatula and Pedersen,

2002). This is not to say that men cannot benefit from a long term mating strategy (Buss and Schmitt, 1993). Disregarding forced sex, high quality women can usually only be acquired if the man proves that he is willing to commit his resources to her exclusively. By investing in a long-term partner, men can monopolize their partner's lifetime reproductive success, benefit from prolonged economic cooperation with the women and form alliances with her kin (Buss and Schmitt 1993; Buss 1994).

In contrast, women require a long-term mate in order to secure paternal investment for her offspring. She might also benefit from short-term relationships with high quality men, as long as it does not impinge on her long-term strategy. Women are generally regarded as the choosier sex, due to the high cost of oocyte production, gestation, lactation and other care. However, in societies with biparental care, paternal investment does not end after conception. Human babies only become independent fairly late in life and need a huge amount of resources in order to survive. Traditionally, men provide most of the resources, and several studies have shown that paternal investment correlates positively with offspring survival (Pennington and Harpending 1988; Marlowe 2000). Thus, in environments where offspring need considerable resources to survive, men can increase their own fitness by increasing paternal care. But, because paternal care comes at a price, males that plan to invest in offspring will be choosier about prospective long-term partners (Trivers 1972; Kenrick 1989; Johnstone, Reynolds and Deutsch 1996). We predict that men are choosier that previously expected, especially for long-term partners.

There are several factors influencing conditional mate choice strategies. We will focus on three, namely, age, self-perceived attractiveness and relationship status. Women only have a limited "fertile window" (Menken, Trussell and Larsen 1986; Symons 1979), therefore men prefer younger women still in their fertile years (Cunningham 1986; Buunk 2001; De Sousa Campos, Otta and de Oliveira Siqueira 2002). This preference is especially pronounced in more traditional societies, where according to Pawlowski and Dunbar (1999) female market value peaks earlier. Fertile

women can afford to be choosier of their prospective partners, as they have more potential candidates to choose from. This is true for both long and short-term relationships. Men, on the other hand, are fertile through most of their adult lives. Therefore, age should not play a role in a man's value as a mate (Symons, 1979). De Sousa Campos, Otta and de Oliveira Siqueira (2002) did find an age-related preference for men. However, the preference is for slightly older men, and this preference is due to the positive association between age and status and/or resources (Pawlowski and Dunbar, 1999).

Individuals in an existing dating relationship, rate members of the opposite sex less attractive (Simpson, Gangestad and Lerma, 1990). They are therefore expected to be choosier when they are in an existing relationship. Not only does their relationship status signal their mate value, but they also have more to lose, whether they choose to have an affair or move on to a new partner. For women the cost is especially severe, as their long-term mate value will be severely reduced if they are labelled promiscuous, particularly in a traditionally polygamous society, such as the Tswana. According to traditional Tswana law, women are punished for infidelity, while infidelity in men is tolerated, as long as they do not neglect their duties towards their wives (Shapera, 1970). It follows that individuals in a relationship are expected to be choosier of a prospective partner, and the effect is likely to be more pronounced in women.

Individuals that perceive themselves to be of better mate value are expected to be choosier (Pawlowski and Dunbar 1999; Buston and Emlen 2003). However, what is considered valuable in a mate differs between the sexes. Women are mostly valued for their physical attractiveness, as their attractiveness signals their health and fertility (Symons 1979; Furnham, Mistry and McClelland 2004). This is true for both long and short-term relationships, although men are expected to be less discriminating when choosing a short-term partner (Buss and Schmitt 1993; Buss 1994). According to Pawlowski and Dunbar (1999), western women reach their peak

market value in their late 20s, while women from more traditional societies should tend to peak earlier. Men, on the other hand, are valued mostly for their status, resources and probability of future pair bond termination, since all serve as signals of paternal investment (Buss and Schmitt 1993; Buss 1994; Pawlowski and Dunbar 1999). Women are more demanding of paternal investment for long-term relationships. Since men first have to accrue resources, western men only reach their peak market value in their late 30s. According to Pawlowski and Dunbar (1999) men's market value in a traditional society should peak later because of the high mortality in younger cohorts.

Lastly, self-esteem is believed to serve as an index of self-perceived mate value (Shackelford, 2001). Individuals with high self esteem presumably have a higher self-perceived mate value and might therefore be choosier.

In this study we will test the association between four condition dependent factors, age, self-esteem, self-perceived attractiveness and relationship status and three measures of choosiness. The first measure of choosiness is attractiveness ratings of members of the opposite sex. According to a study by Pennebaker (1979) both sexes rate members of the opposite sex as more attractive if their own chances of acquiring a mate decrease. Pennebaker (1979) asked sober individuals in a singles bar to rate same and opposite sex individuals for attractiveness, at three different time periods during the night. They found that same sex individuals were rated consistently, while opposite sex individuals were rated more attractive as the night progressed. They concluded that individuals rated members of the opposite sex more attractive as their chances of going home alone increased. Our other two measures are choosiness with respect to potential partners for short-term and long-term relationships.

Since most studies on mating behaviour focus on predominately monogamous western populations, we conducted our study on a traditionally polygamous society, the Tswana.

Materials and Methods

Sample group

Our sample group consisted of 58 male volunteers, with a mean age of 21 (range 18-26) and 42 female Tswana volunteers, with a mean age of 20 (range 18–26). The Tswana is one of the nine official African ethnic groups in South Africa, and the most abundant African ethnic group at the University of Pretoria (V. Coetzee, unpublished data). Males were selected from the University of the Witwatersrand and females from the University of Pretoria. Each volunteer signed a subject information and consent form before completing the questionnaire.

Data collection

The questionnaire included questions on: self-perceived attractiveness (Q2-3, Appendix), self-esteem (Q4-6, Appendix), relationship status (Q7, Appendix) and age (Q1, Appendix). Participants indicated self-perceived attractiveness and self-esteem on a continuous line (142 cm), with markings to indicate the ends, centre and quarter points. They were instructed to place their mark anywhere along the line. Participants from both sexes were photographed and their images masked according to the protocol described in chapter 2. These masked photographs were then used to compile 12 full colour presentations (10 male photographs each) and 24 full colour presentations (5 female photographs each) to account for the difference in the number of photographs and raters. Presentations were rated by opposite sex individuals for attractiveness (Q8, Appendix), and whether or not they would consider the individual for a short term or a long-term mate (Q9-10, Appendix). All volunteers were provided with a lunch in return for their participation.

Data Analysis

We tested the difference in choosiness between the two sexes by comparing the means of three different measurements of choosiness using a t-test. The first measure of choosiness was calculated as the group average for each sex, in how attractive they rated members of the opposite sex (ATTRACT). Secondly, we measured the average number of times a gender reported they would not consider a member of the opposite sex as a short-term partner (SHORT). The same was done for potential long-term partners as the third variable of choosiness (LONG).

To test the relationship between choosiness and the four independent variables, age, self-perceived attractiveness (SP attractiveness), self-esteem and relationship status, we defined the measures of choosiness slightly differently than before. All three measures of choosiness were defined as the difference between the average ratings for each rater and the average ratings for the particular images they were exposed to. More specifically, for a given rater we took his/her mean rating and subtracted the mean rating across all raters for the images that specific rater was exposed to. Higher values therefore indicated a decrease in choosiness. We analysed the relationship between choosiness and all three independent variables (age, relationship status and self-image) in a hierarchical fashion, using a general linear model for all three measures of choosiness individually. Only statistically significant variables ($p \leq 0.05$) were included in the final model. Outliers were considered influential if they had high Cook's values (Cook's value > 0.11) or were located on the extreme edge of the plot (Figures 2a-2d). Once identified, influential outliers were removed from analyses. We report results obtiained before and after the removal of influential outliers, only if the was a difference in statistical significance between the two. All analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL).

Results

Male and female choosiness

There was no significant difference between the choosiness of male and female volunteers, although women had a slightly higher mean for all three measurements of choosiness (Table 1). As expected however, both males (t_{114}=-4.103, $p < 0.0005$) and females (t_{90}=-4.103, $p < 0.0005$) are choosier about long term than short-term partners (Table 1, Figures 1.1. 1.2).

***Table 1**. Male and female choosiness. There is no significant difference between the sexes in (a) how attractive they rate members of the opposite sex (ATTRACT) and their choosiness for (b) short (SHORT) and (c) long term partners (LONG). Both sexes are choosier with regards to long term (LONG) than short-term (SHORT) partners.*

Measure	Sex	Mean	Std. Dev
ATTRACT	MALE	53.74	22.88
	FEMALE	54.83	22.02
SHORT	MALE	3.59	1.23
	FEMALE	3.65	1.16
LONG	MALE	4.35	0.95
	FEMALE	4.48	0.72

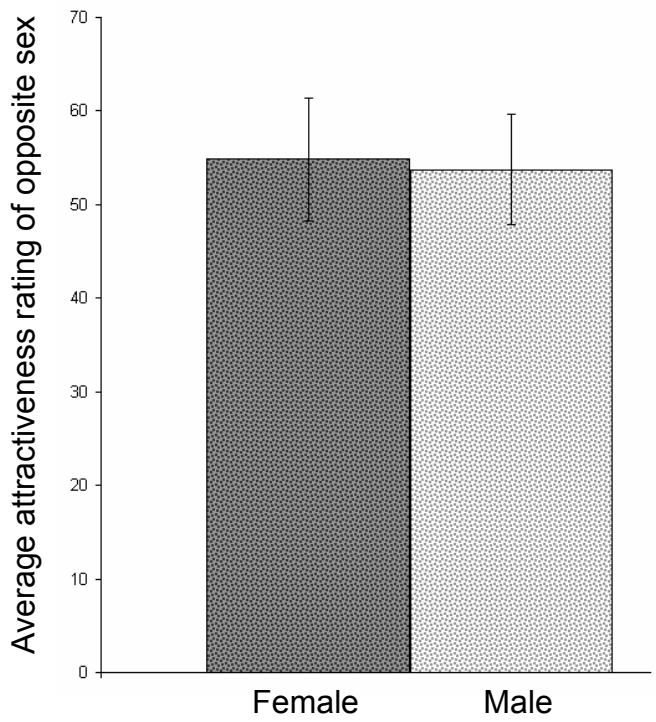


Figure 1.1. *Difference in how attractive they rate the opposite sex. There is no significant difference in how attractive the sexes rate members of the opposite sex. Bars indicate 95% confidence intervals.*

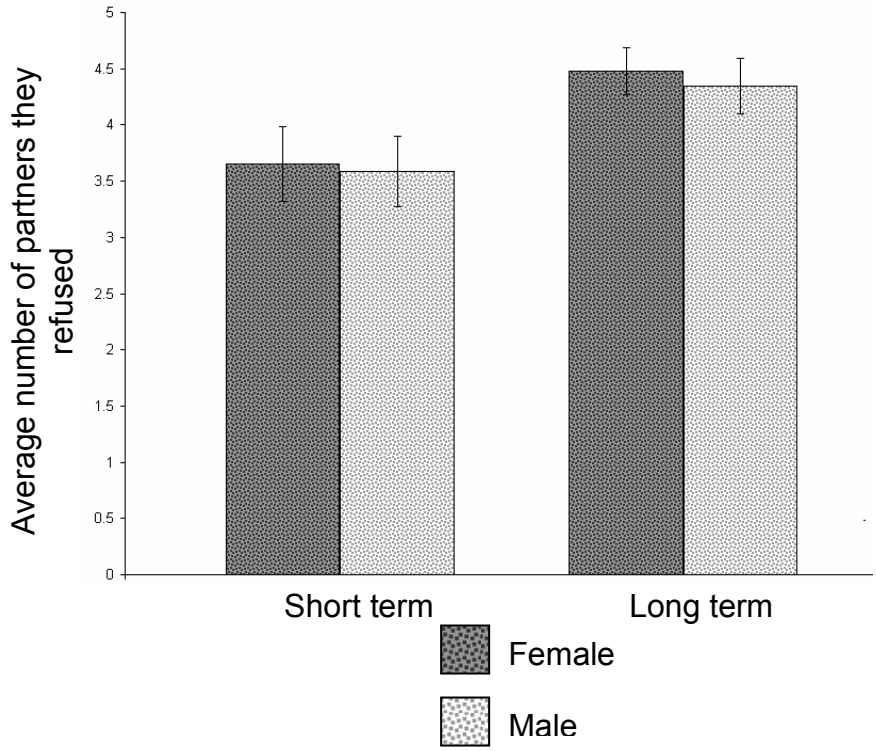


Figure 1.2. *Choosiness for short and long term partners. Both sexes are significantly choosier of long term than short-term partners ($p < 0.0005$), but there is no significant difference in choosiness between the sexes. Bars indicate 95% confidence intervals*

Self image, age and relationship status as predictors of choosiness

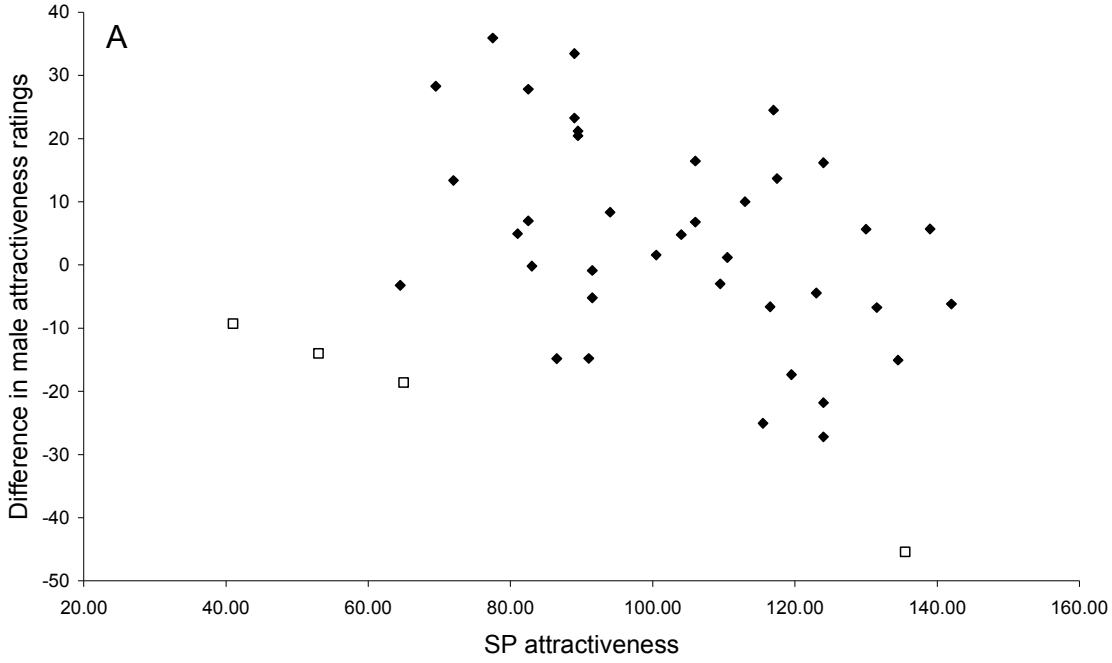
We ran separate GLM analysis for men and women. For women, age ($F_{1,39}$ = 6.391, R^2 = 0.198, p = 0.016) and SP attractiveness ($F_{1,39}$ = 4.431, R^2 = 0.198, p = 0.042) were initially significant predictors of how attractive they rated the opposite sex (ATTRACTDIFF)(equation: ATTRACTDIFF = - 55.385 - 0.221 × SP attractiveness + 3.970 × Age). Age lost significance after the exclusion of influential outliers, but SP attractiveness remained a highly significant predictor (equation: ATTRACTDIFF = 38.937 - 0.336 × SP attractiveness, $F_{1,36}$ = 8.188, R^2 = 0.185, p = 0.007) (Table 2, Figure 2a). Relationship status and self-esteem did not significantly affect ATTRACTDIFF.

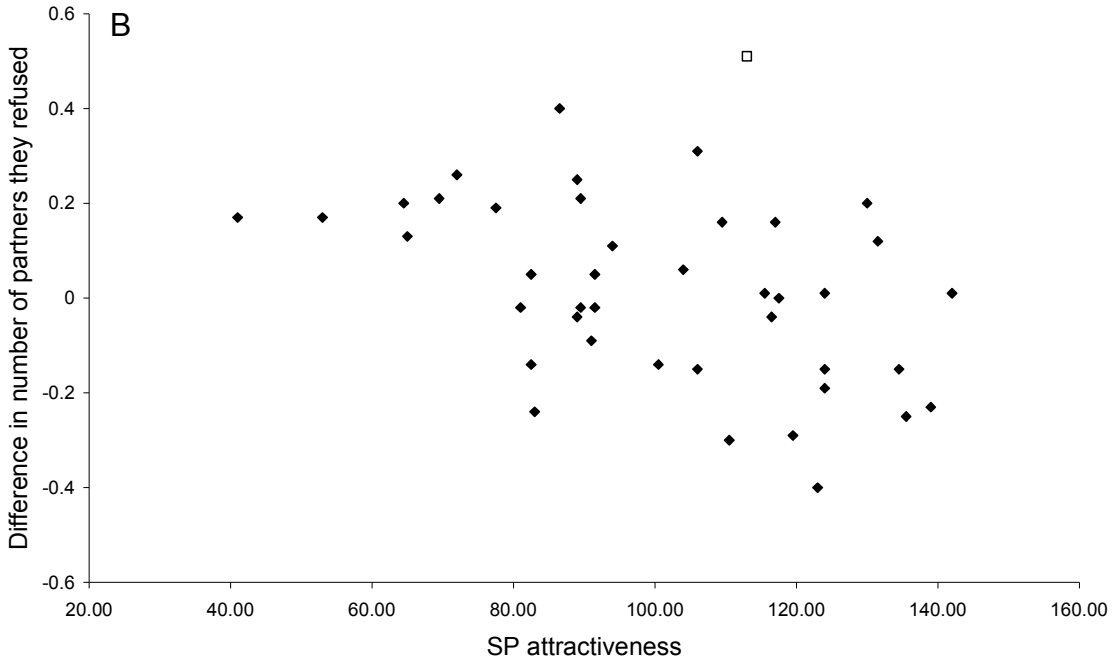
Before removing influential outliers only SP attractiveness (equation: SHORTDIFF = 0.353 - 0.003 × SP attractiveness, $F_{1,40}$ = 7.440, R^2 = 0.157, p = 0.009) significantly predicted choosiness for a short-term mate (SHORTDIFF). After removing outliers, age gained some significance and both SP attractiveness ($F_{1,37}$ = 12.581, R^2 = 0.298, p = 0.001)(Figure 2b) and to some extent age ($F_{1,37}$ = 4.307, R^2 = 0.298, p = 0.045)(Figure 2c) significantly predicted SHORTDIFF (equation: SHORTDIFF = - 0.411 – 0.004 × SP attractiveness + 0.040 × Age)(Table 2). We removed age to test the effect of age on the full model. Self-perceived attractiveness lost some significance and the amount of variance explained by the model dropped substantially (equation: SHORTDIFF = 0.370 – 0.004 × SP attractiveness, $F_{1,38}$ = 10.468, R^2 = 0.216, p = 0.003). This indicates that although age has a very small effect size of its own, it increases the explanatory power of SP attractiveness. Relationship status and self-esteem did not significantly affect SHORTDIFF.

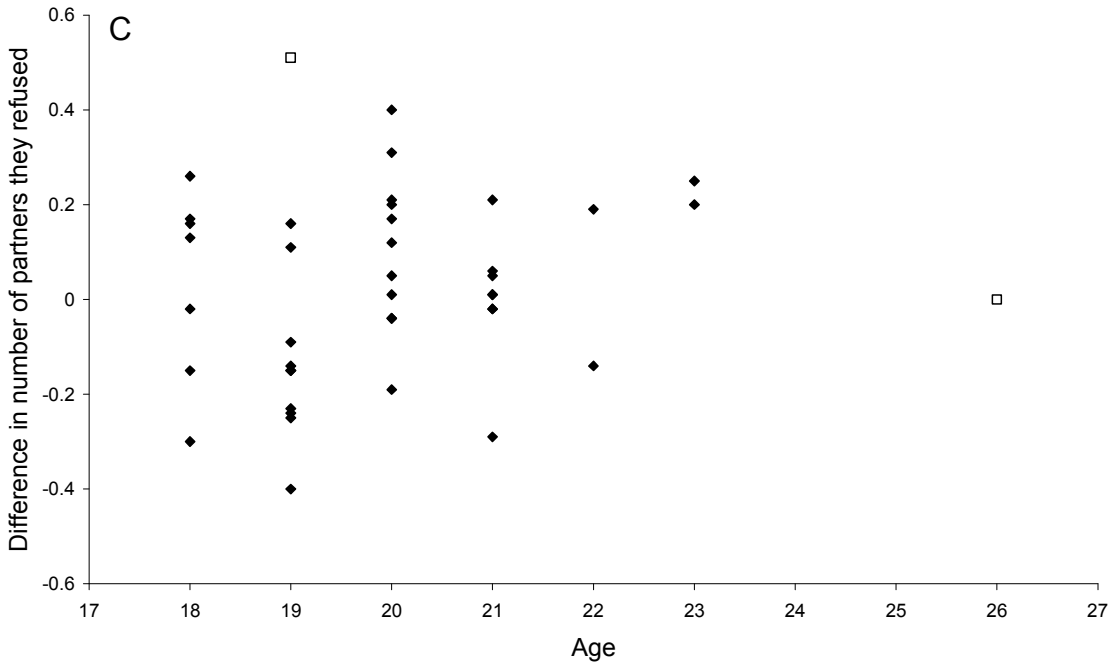
Before removing outliers, both SP attractiveness ($F_{1,39}$ = 4.182, R^2 = 0.219, p = 0.048) and relationship status ($F_{1,39}$ = 4.817, R^2 = 0.219, p = 0.034) significantly predicted how choosy individuals were of long-term partners (LONGDIFF)(equation: LONGDIFF = 0.281 - 0.001 × SP attractiveness – 0.078 × Relationship status).

However, after outlier exclusion only relationship status remained significant (equation: LONGDIFF = 0.094 – 0.061 × relationship status, $F_{1,36} = 4.429$, $R^2 = 0.110$, $p = 0.042$)(Table 2, Figure 2d). These tests all have comparatively low R^2 values.

In men, age significantly predicted LONGDIFF before removal of outliers (equation: LONGDIFF = - 0.416 + 0.019 × age, $F_{1,55} = 5.068$, $R^2 = 0.084$, $p = 0.028$), but lost significance after the removal of one influential outlier. Furthermore, none of the other measures of male choosiness were significantly correlated with age, SP attractiveness or relationship status (Table 2).







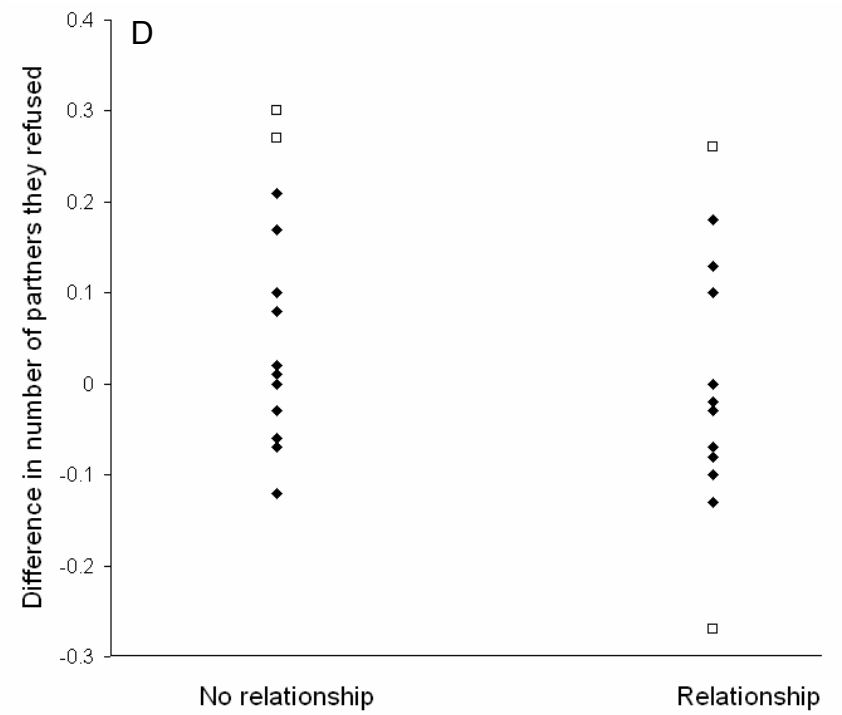


Figure 2. *Conditional factors significantly influencing choosiness. (a) The role of female self-perceived attractiveness in attractiveness ratings of the opposite sex. Women who perceive themselves to be less attractive rate men significantly (p = 0.007) more attractive than their peers. (b) The role of female self-perceived attractiveness in choosiness of short-term partners. Self-perceived attractiveness significantly (p = 0.001) predicts how choosy women are of short term partners. (c) The role of female age in choosiness of short-term partners. Age is a moderately significant (p = 0.045) predictor of how choosy women are of short-term partners. (d) The role of relationship status in choosiness of long-term partners. In women, relationship status significantly predicts (p = 0.042) how choosy they are of long-term partners. High values on the y axis indicate a decrease in choosiness. Influential outliers are indicated as squares.*

Table 2. *The role of self-esteem, SP attractiveness, age and relationship status on female choosiness. In women, SP attractiveness significantly predicts how attractive they find members of the opposite sex. Both SP attractiveness and to some extend age predicts how choosy they are of short-term partners, while only relationship status significantly predicts how choosy they are of long term partners. Variables were calculated after removal of influential outliers.*

Source	df	F	Sign	R^2
Female ATTRACTDIFF				
SP attractiveness	1, 36	8.188	0.007	0.185
Age			NS	
Relationship Status			NS	
Female SHORTDIFF				
SP attractiveness	1, 37	12.581	0.001	0.298
Age	1,37	4.307	0.045	0.298
Relationship Status			NS	
Female LONGDIFF				
SP attractiveness			NS	
Age			NS	
Relationship Status	1,36	4.429	0.042	0.110

Discussion

Our results show that there is no significant difference in choosiness between the two sexes. This confirms our prediction that men are choosier than previously expected; in fact, almost exactly as choosy as women. One might argue that we measured self-reported preferences and not actual mating behaviour, but preferences should be a better indication of evolutionary based desires. In reality, when choosing a mate there are several confounding factors that mask one's desires such as the preferences of family and friends, religion, politics and the media. Self reported preferences are less affected by these confounding factors and thus serves as a

better indication of true desires. One might argue that self-esteem or SP attractiveness also serves as confounding factors in this regard. However, information on SP attractiveness and self-esteem are most likely stored in the subconscious, while opinions of family, friends and the media are stored in a preconscious or conscious level. For instance, when deciding if you want to date a particular man, one might easily recall what your best friend said about him, or the fact that he doesn't exactly share the same religious beliefs as you. These factors are all factored into a sort of mental calculation whether you should date him or not. On the contrary, very few people, if any, would think: "I'm not very attractive and have low self-esteem; therefore he is the best partner I can get under the present circumstances". Instead most of us simply think: "I'm attracted to him". Mental calculations about your present condition and how this affects your market value are most probably accomplished subconsciously, leaving us with the desire, but without insight into the reasoning behind it.

Another factor that might influence our results is the fact that our male student population consisted mainly of men with above average earning potential. Although these men have not yet accrued their resources, they can easily do so in the near future. They are therefore expected to be choosier than the general population since they will be able to provide sufficient paternal investment in the near future. Our female student population also has above average earning potential, but earning potential and resources are not the main predictors of female market value. Instead, their market value is influenced more by their physical attractiveness. It follows that male choosiness might be artificially elevated in our population, but the study still shows that both sexes are choosy. This is a crucial point, as male choice is often disregarded in mate choice studies. We also found that both sexes are choosier of long term than of short-term partners. This is not unexpected as both sexes lose more by investing in an unsuitable long-term partner.

In the second part of our study, we tested the relationship between choosiness and four condition dependent factors, age, SP attractiveness, self-esteem and relationship status. Our results show that female SP attractiveness is the only explanatory variable that significantly predicts women's attractiveness ratings of the opposite sex. Women, who perceived themselves as more attractive, rated men less attractive than those women who perceived themselves as unattractive. Pennebaker (1979) showed that people rate members of the opposite sex more attractive as their chances of acquiring a mate decreased. Therefore, it is reasonable to assume, that women who see themselves as less attractive rate men more attractive due to a decrease in choosiness. We propose that what people view as attractive is merely the unacknowledged result of a subconscious calculation. Who they find attractive depends on their perceptions about themselves and the subconsciously calculated "obtainable ideal mate". Evolutionary speaking, individuals who pursued the highest quality obtainable mate, had the largest fitness benefits. Individuals that chased after unobtainable higher quality mates or settled for lower quality mates lost out reproductively. Therefore it pays to find the highest quality obtainable mate most attractive. Individuals that find the "obtainable ideal mate" most attractive, are more likely to choose them as partners, and as a consequence, have a higher fitness. None of the other variables significantly affected how attractive either sex rated members of the opposite sex.

In women, SP attractiveness also significantly predicted choosiness for a short-term partner in women. It is clear that SP attractiveness is a very important predictor of female choosiness. This makes intuitive sense, as female attractiveness is the main predictor of female market value. Female age also plays a small role in choosiness of a short-term partner, but this is probably mainly because age and SP attractiveness share information. This does not exclude age as a significant predictor for short and long-term partners in the general population. Our sample population has a limited age span (18-26), which are well within the "fertile window" for women.

In order to test the effect of age on short and long-term choosiness, it would be better to test women nearing the end of their reproductive life.

Relationship status significantly affected choosiness for a long term but not a short-term partner in women. Female volunteers that were in a current relationship were choosier of long-term partners but not of short-term partners. This makes intuitive sense as women who are currently in a relationship need to terminate the current relationship in order to move on to a new long-term relationship. This entails a high cost on their part if the new relationship does not pan out. This is not necessarily true of a short-term relationship, as they can potentially hide the short-term relationship from their current partner. We did not find a significant effect of male relationship status on short or long-term choosiness. This difference between the sexes can be explained by the difference in cost of promiscuity. Women suffer a severe cost to their long-term mate value if they are labelled promiscuous. Since men can never be entirely sure of their paternity, promiscuous women are shunned as mates. Women are therefore expected to be very careful of new relationships, especially if they stand a chance of being labelled promiscuous. Promiscuity in men is generally less frowned upon, especially in polygamous societies such as the Tswana. Except for the historically polygamous nature of Tswana marriages, men were also allowed concubines under traditional law (Schapera, 1970). Despite the recent conversion to monogamy, African women still show a relaxed response to male infidelity (Meyer-Weitz *et al.* 1998). It follows that men need not be very choosy of potential new mates as the cost is minimal on their current relationship.

Interestingly, we found no significant effect of self-esteem on choosiness. One possible explanation for this surprising finding is that all our subjects had relatively high self-esteem. Future studies might benefit from including subjects with a more diverse background and socio-economic status, to increase variability in self-esteem.

This study served as a preliminary investigation into the role of age, SP attractiveness, self-esteem and relationship status and on male and female choosiness. Our study illustrates their value in predicting choosiness, but since they only explain a modest amount of variation other potential factors should also be pursued. Future studies should also benefit from increasing the age span of subjects to include volunteers outside the "fertile window". This is especially true for female subjects that have a smaller "fertile window'. Self-esteem might also have a more potent influence on choosiness in older subjects. Furthermore, current relationships should be judged as long or short-term relationships. We predict that both men and women should be more wary of leaving a long-term partner than a short-term partner. Another interesting question would be the difference in choosiness of men in polygamous and monogamous historical backgrounds.

Acknowledgements

We would like to thank Nicole Creux, Minique de Castro, Therese de Castro, Christoff Erasmus, Tracey-Leigh Hatherall, Aret Meyer, Ronnie Nelson, Duncan Newman, Jason Pienaar and Isa-Rita Russo for their valuable help with data collection. This material is based, in part, upon work supported by the National Research Foundation under Grant number 2053809 to JMG. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation. This project was cleared by the ethics committees of the University of Pretoria and the University of the Witwatersrand respectively.

References

Barrett, L., Dunbar, R. and Lycett, J. (2002). *Human Evolutionary Psychology*. New York, Palgrave.

Buss, D. M. (1994) *The Evolution of Desire: Strategies of Human Mating*. New York, Basic Books.

Buss, D. M. and Schmitt, D. P. (1993) Sexual strategies theory: an evolutionary perspective on human mating. *Psychological Review,* 100: 204-232.

Buston, P. M. and Emlen, S. T. (2003) Cognitive processes underlying human mate choice: the relationship between self-perception and mate preference in Western society. *Proceedings of the National Academy of Sciences, USA,* 100: 8805-8810.

Buunk, B. P., Dijkstra, P., Kenrick, D. T. and Warntjes, A. (2001) Age preferences for mates as related to gender, own age, and involvement level. *Evolution and Human Behavior,* 22: 241-250.

Cunningham, M. R. (1986) Measuring the physical in physical attractiveness: quasi-experiments on the socio-biology of female facial beauty. *Journal of Personality and Social Psychology*, 50: 925-935.

De Sousa Campos, L., Otta, E. and de Oliveira Siqueira, J. (2002) Sex differences in mate selection strategies: content analyses and responses to personal advertisements in Brazil. *Evolution and Human Behavior,* 23: 395-406.

Furnham, A., Mistry, D. and McClelland, A. (2004) The influence of age of the face and the waist to hip ratio on judgements of female attractiveness and traits. *Personality and Individual Differences,* 36: 1171-1185.

Johnstone, R. A., Reynolds, J. D. and Deutsch, J. C. (1996) Mutual mate choice and sex differences in choosiness. *Evolution*, 50: 1382-1392.

Kenrick, D. T. (1989) Bridging social psychology and socio-biology: the case of sexual selection. In: R. W. Bell and N. J. Bell eds. *Sociobiology and the Social Sciences.* Texas, Texas Tech University Press, pp. 5-23.

Marlowe, F. (2000) Paternal investment and the human mating system. *Behavioural Processes,* 51: 45-61.

Meyer-Weitz, A., Reddy, P., Weijts, W., Van der Borne, B. and Kok, G. (1998) The socio-cultural contexts of sexually transmitted diseases in South Africa: implications for health education programmes. *AIDS Care*, S1: 39-56.

Menken, J., Trussell, J. and Larsen, U. (1986) Age and infertility. *Science*, 233: 1389-1394.

Miller, L. C. and Fishkin, S. A. (1997) On the dynamics of human bonding and reproductive success: seeking windows on the adapted-for human-environmental interface. In: J. A. Simpson and D. T. Kenrick eds. *Evolutionary Social Psychology*. New York, Lawrence Erlbaum Associates, pp. 197-236.

Pawlowski, B. and Dunbar, R. I. M. (1999) Impact of market value on human mate choice decisions. *Proceedings of the Royal Society of London, B,* 266: 281-285.

Pennebaker, J. W. (1979) Truckin with country-western psychology. *Psychology Today*, November, pp. 18-19.

Pennington, R. and Harpending, H. (1988) Fitness and fertility among Kalahari !Kung. *American Journal of Physical Antropology,* 77: 303-319.

Ryan, M. J. (1997) Sexual selection and mate choice. In: J. R. Krebs and N. B. Davies eds. *Behavioural Ecology: An Evolutionary Approach*. 4th edition. Oxford, Blackwell, pp. 179-202.

Schapera, I. (1970) *A Handbook of Tswana Law and Custom*. London, Frank Cass and Company Limited.

Shackelford, T. K. (2001) Self-esteem and marriage. *Personality and Individual Differences,* 30: 371-390.

Simpson, J. A., Gangestad, S. W. and Lerma, M. (1990) Perception of physical attractiveness: mechanisms involved in the maintenance of romantic relationships. *Journal of Personality and Social Psychology*, 59: 1192-1201.

Symons, D. (1979) *The Evolution of Human Sexuality*. Oxford: Oxford University Press.

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

Appendix

Initial male/female questionnaire:

1. How old are you?
2. Do you think you have an attractive face?
3. Do you think you have an attractive body?
4. How important do you think you are?
5. How much do you like yourself?
6. Do you think you possess the necessary skills for life?
7. Do you have a girlfriend/wife at the moment? (Male questionnaire)

   Do you have a boyfriend/husband at the moment? (Female questionnaire)

Image scoring questionnaire

8. Please indicate how attractive you think this man/woman is?
9. Would you consider having a short term relationship with this man/women?
10. Would you consider having a long term relationship with this man/woman?

CHAPTER 6

THE ROLE OF SELF-ESTEEM IN SEXUALLY RISKY BEHAVIOR

Vinet Coetzee[1*], Louise Barrett[2], Jaco M. Greeff[1] and Peter S. Henzi[2].

*[1]Department of Genetics, University of Pretoria, Pretoria, South Africa; [2]Department of Psychology, University of Central Lancashire, Preston, UK [3]. *Corresponding author vcoetzee@tuks.co.za.*

Contributors

This chapter consists of a manuscript that has been prepared for submission to a peer-reviewed journal (*Social Science and Medicine*). The study was designed and data collected by myself, except for the assistance noted in the acknowledgements. I also did the data analyses and drafted the first version of the manuscript.

Abstract

Self-esteem influences sexual behaviour and, consequently, HIV infection risk. We studied the correlation between self-esteem and sexually risky behaviour with specific reference to (a) age of sexual onset, (b) number of sexual partners per active year and (c) the propensity to engage in extra pair copulations among South African youths. Our results show that men with high self-esteem are more likely to be sexually active after the age of 18 compared to men with low self-esteem. However, men with low self-esteem are more likely to start sexual activity prematurely. No significant correlation was observed between self-esteem and sexual behaviour in women. Our second objective was to test the correlation between self-esteem and self-perceptions, specifically perceived physical attractiveness and socio-economic status. We showed that female self-esteem is significantly correlated with perceived attractiveness, while male self-esteem is significantly correlated with perceived socio-economic status and not perceived attractiveness. The factors influencing self-esteem, therefore, are gender specific and correlate well with the self-perceived market value of the individual.

Introduction

Human immunodeficiency virus (HIV) infection is extremely prevalent in sub Saharan Africa (Mynhardt 2002; Eaton, Flisher and Aarø 2003). This region is home to 70% of all HIV positive individuals, despite the fact that only around 10% of the world's population live in sub Saharan Africa (UNAIDS 2004 Global report, 2004). South Africa alone houses 20% of Sub-Saharan Africa's HIV positive population (Dorrington *et al*. 2004). Despite various educational and HIV prevention campaigns, HIV risky behaviour remains prevalent, especially amongst youths (Eaton, Flisher and Aarø, 2003). The high incidence is mainly attributed to sexually risky behaviors, such as infrequent condom use and the pervasive practice of copulation with multiple partners (Eaton, Flisher and Aarø, 2003; Blum 2004). According to a recent report, up to 90% of the rural population fail to use condoms (Mynhardt, 2002). Naturally, a history of sexual activity also serves as a HIV risk factor (Taffa, Sundby and Bjune, 2003) with higher incidence of HIV infection amongst sexually experienced individuals (Blum, 2004). Youths that start sexual activity earlier therefore face increased risk of HIV infection, a risk that is exacerbated by the sexual violence often accompanying early sexual debuts (Finkelhor and Dziuba-Leatherman 1994; Wu, Berenson and Wiemann 2003). In South Africa, youths tend to become sexually active at a very early age, with an estimated 50% of South African youths sexually active by the age of 16 (Eaton, Flisher and Aarø, 2003). Boys start sexual activity earlier than girls and amongst both sexes African youths start sexual activity earlier than other ethnic groups (Weber *et al*. 1989; Benson and Torpy 1995; Eaton, Flisher and Aarø 2003; Ruangkanchanasetr *et al*. 2005).

Knowledge of HIV infection and risk factors can decrease the incidence of infection, but knowledge alone fails to bring about the appropriate behavioural change (Archer 1989; Perkel 1991). Individuals persist with risky behaviour due to underestimation of personal risk and personal factors such as low self-esteem (Baron

and Byrne 2004; Ethier *et al*. 2006). Self-esteem is based largely on specific experiences and the opinion of others (Baron and Byrne, 2004). Factors such as socio-economic status, religion, family structure, sibling position, parental interest (Rosenberg, 1965) and physical attractiveness (Perkel, 1991) all influence self-esteem. Low self-esteem affects the need for conformation and external affirmation (Perkel, 1991): a need that can be realized by pleasing partners and peers. Several studies showed that youths, especially girls, with low self-esteem are prone to early onset of sexual activity (Orr *et al*. 1989) presumably to prove their fertility and avoid displeasing their partners (Perkel, 1991). However, a study by Benson and Torpy (1995) found no significant effect of self-esteem with respect to the early onset of sexual activity. Two studies have even found a correlation between high self-esteem and early sexual activity in New Zealand girls (Paul *et al*. 2000) and American boys (Jessor and Jessor, 1975). Paul *et al*. (2000) attributed their finding to the fact that early sexual onset could be seen as the norm across all socio-economic groups in the New Zealand population.

The objectives of this study were firstly to test the correlation between self-esteem and sexually risky behaviour with specific reference to age of sexual onset, the number of sexual partners per active year and the propensity to engage in extra-pair copulations in African youths of South Africa. Individuals with low self-esteem are predicted to engage in more sexually risky behavior (e.g. earlier sexual onset, multiple partners and more extra-pair copulations) than individuals with high self-esteem. However, if one or more of the sexually risky behaviors is considered the cultural norm, individuals with high self-esteem are also expected to partake in these risky behaviors.

Our second objective was to test the correlation between self-esteem and self-perceptions; specifically, perceived physical attractiveness and socio-economic status. We propose that female self-esteem in terms of mate value should be correlated with their perceived physical attractiveness, since this is the main criterion

for female market value (Feingold 1990; Buss and Schmitt 1993; Furnham, Mistry and McClelland 2004). More attractive females might therefore be expected to engage in less risky sexual behavior, since they are less likely to need external affermation from their sexual partners. Male market value, on the other hand, is determined mainly by socio-economic status (Buss and Schmitt 1993; Thornhill and Gangestad 1996), thus their self-esteem should be correlated with perceived wealth and status and not physical attractiveness. Men with more resources and a higher social standing might therefore be less likely to engage in sexually risky behaviour, unless the sexual behavior is considered the cultural norm.

Materials and Methods

Our initial sample group consisted of 60 male and 69 female volunteers. All volunteers belonged to the Tswana ethnic group, the most abundant African ethnic group at the University of Pretoria. (V. Coetzee, unpublished data). Males were selected from the University of the Witwatersrand and females from the University of Pretoria. Each volunteer signed a subject information and consent form before completing the questionnaire. The questionnaire included questions on: self-esteem (Q 1-3, Appendix) perceived facial and bodily attractiveness (Q 4, 5, Appendix) and sexual history (Q 6-9, Appendix) and was rated on a continuous scale from 0-142. Subjects were allowed to omit questions if they felt uncomfortable. To increase the sample size we recruited fifty additional female African volunteers from the University of Pretoria to complete a questionnaire with identical questions on self-esteem, perceived facial and bodily attractiveness and sexual history. Furthermore, we asked 50 additional male students from the University of Pretoria to complete a similar questionnaire with questions on socio-economic status (Q 10, 11, Appendix) instead of perceived facial and bodily attractiveness.

Data Analyses

Analyses were performed with SPSS version 13.0. Separate analyses were performed for each sex. We tested the correlation between self-esteem and three variables of sexual history, with age partialed out in all correlations. The sexual history variables included: (a) their age of sexual onset, (b) the total number of times they had engaged in extra pair copulations with individuals other than their partners (e.g. reported cheating on their partners) and (c) the number of sexual partners per sexually active year. The number of sexual partners per active year was log transformed in both sexes to correct for a right hand skew. Because of the binary nature of the fourth variable of sexual history (d) whether or not they are sexually active, we tested the association using a GLM model, with age as a covariate.

Next, we tested the correlation between perceived attractiveness and self-esteem in both sexes. For the men, we added an additional correlation between self-esteem and socio-economic status. Self-esteem was squared in both sexes to correct for a left hand skew. For all analyses, we calculated Cook's values to identify influential outliers (Cook's values > 0.11). Once identified, influential outliers were removed from analyses. We report only results obtained after the removal of influential outliers, unless there was a difference in statistical significance, in which case we report both results.

Results

A total of 89 completed questionnaires were collected for males (age, Mean = 21.63, S.D. = 2.71, Range = 18-34) and 103 for females (age, Mean = 20.65, S.D. = 2.40, Range = 18-31). At the time of sampling, 82% of males reported being sexually active, while only 59% of females reported sexual activity. On average, male students reported starting sexual intercourse at a significantly younger age than female students ($t = -5.750$, $N = 132$, $p < 0.0005$) and engaging in extra pair

copulations significantly more ($t = 3.335$, $N = 122$, $p < 0.001$). On the other hand, both sexes reported a fairly similar number of sexual partners per active year ($t = 0.537$, $N = 132$, $p = 0.592$)(Table 1).

***Table 1**. Descriptive statistics of sexual history.*

Variable	Mean	SD	N	Range
Females				
Age of sexual onset	18.082	1.646	61	14- 21
Sexual partners/ active year	1.246	0.813	61	0.22- 4
Extra-pair copulation	0.7414	1.25	58	0- 5
Males				
Age of sexual onset	16.014	2.372	73	7-20
Sexual partners/ active year	1.330	0.969	73	0.14-5
Extra-pair copulation	1.803	2.121	66	0-10

We tested the association between each variable of sexual history and self-esteem separately. Because the probability of being sexually active, age of sexual onset, number of extra pair copulations and sexual partners per active year were all expected to correlate with age, we partialed age out of all analyses. With the effect of age statistically controlled, sexual activity in men was significantly associated with self-esteem ($F_{1,34} = 9.784$, $R^2 = 0.119$, $p = 0.002$) (Figure 1). Age of sexual onset in men was also marginally significantly correlated with self-esteem ($r = 0.242$, $N = 67$, $p = 0.045$)(Figure 2). None of the other sexual history variables were significantly correlated with self-esteem at the two-tailed level, in either sex.

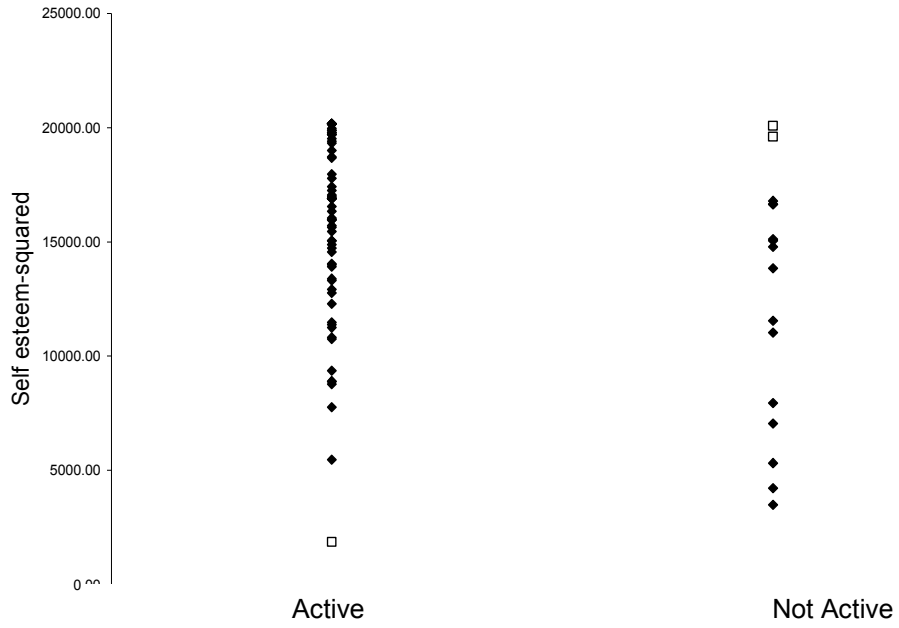


Figure 1. *The association between self-esteem and sexual activity. Men with high self-esteem are significantly (p = 0.002) more likely to be sexually active than those with a low self-esteem. Influential outliers are indicated as squares.*

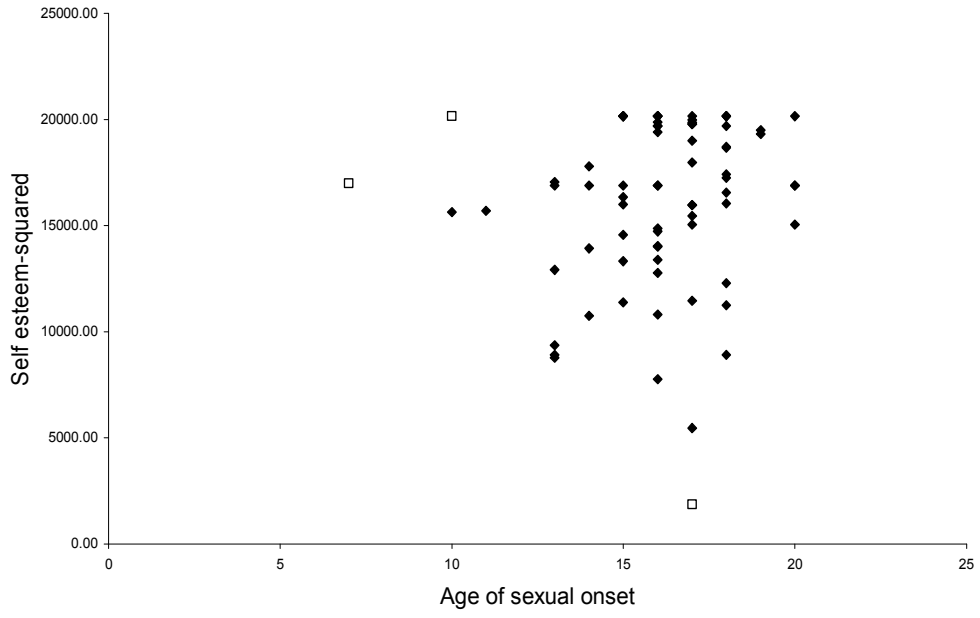


Figure 2. *The partialed correlation between self-esteem and age of sexual onset in men. Men with high self-esteem tend to delay the initiation of sexual activity (p = 0.045).*

As expected, perceived attractiveness and self-esteem was significantly correlated in females (r = 0.303, N = 102, p = 0.002)(Figure 3). In males, we initially observed a marginally significant correlation between self-esteem and perceived attractiveness (r = 0.291, p = 0.040, N = 50), but this correlation disappeared after the removal of one influential outlier (r = 0.255, p = 0.077, N = 49). Perceived socio-economic status were however significantly correlated with self-esteem (r = 0.444, p = 0.006, N = 37)(Figure 4).

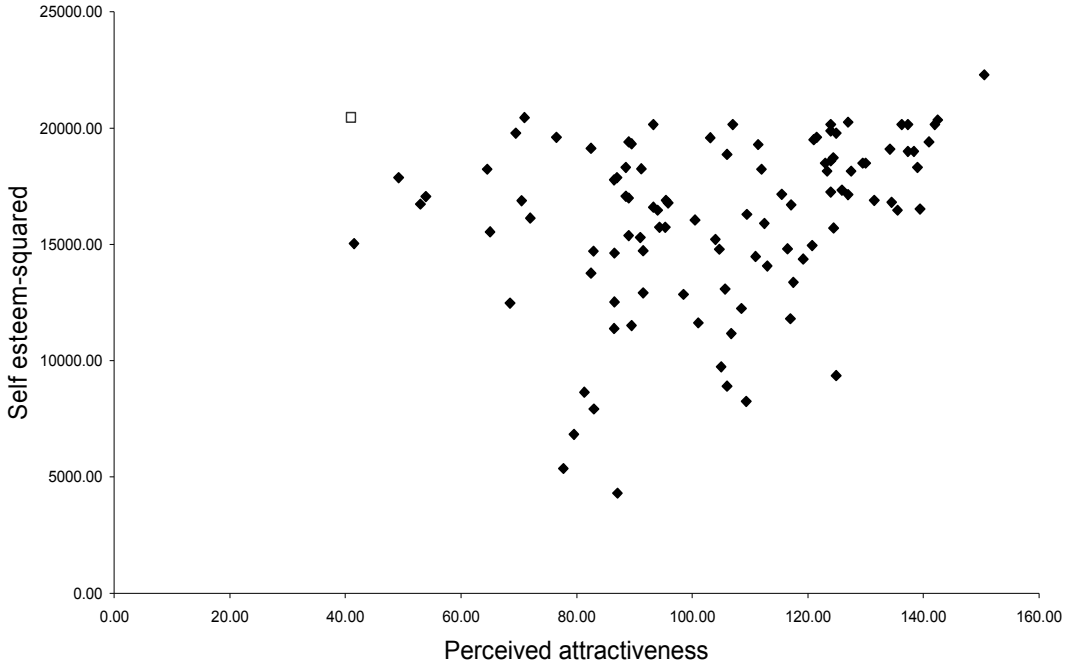


***Figure 3**. Correlation between self-esteem and perceived attractiveness in women. Women with high self-esteem tend to view themselves as significantly more attractive than women with low self-esteem (p = 0.002).*

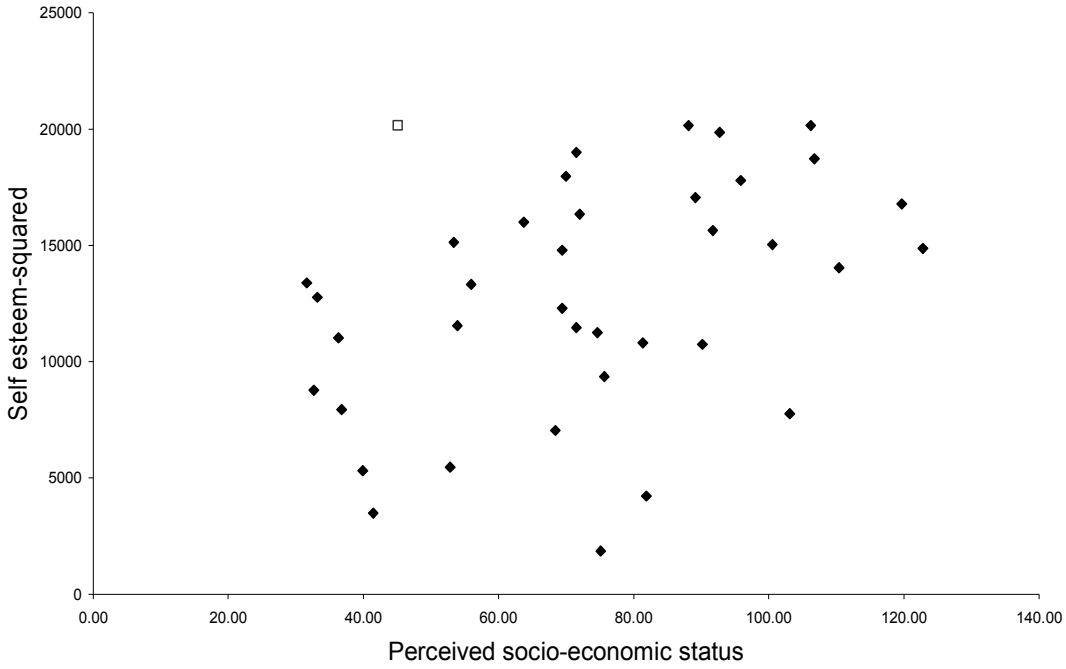


***Figure 4**. Correlation between self-esteem and socio-economic status. Men with a high self-esteem also perceive their socio-economic status as significantly higher (p = 0.006).*

Discussion

Our study showed a marked difference in sexual behaviour between the genders. Men were significantly more likely to be sexually active and lost their virginity at a significantly earlier age than women. Males also reported engaging in significantly more extra-pair copulations than females and had more partners per sexually active year, although not significantly so. On the whole, men in our study cohort reported more sexual risky behaviour than the women, since they started sexual activity younger and had more sexual intercourse with individuals other than their partners. Coupled with the low condom usage reported by Eaton, Flisher and Aarø (2003) their behaviour presents a high risk for the transmission of sexually transmitted diseases, such as HIV. The discrepancy between the sexes might be somewhat inflated by the fact that the sexual history information was self-reported. Even though all information

was kept extremely confidential, female students might have underreported their sexual encounters for fear of being labelled promiscuous. Men on the other hand, might have exaggerated their sexual exploits. Nevertheless, youths have been shown to respond honestly to sensitive questions, such as alcohol addiction (Campanelli, Dielman and Shope, 1987). Despite possible under and over reporting, we feel that the difference between the sexes is sufficiently large to present a robust difference.

Although abstinence remains the ideological ideal of many HIV prevention campains, our study together with previous studies (Eaton, Flisher and Aarø, 2003) show that sexual activity is the cultural norm amongst African youths, especially male African youths. We found a significant association between male sexual activity and self-esteem, a finding that correlates well with a study by Jessor and Jessor (1975). Men with high self-esteem were significantly more likely to be sexually active than men with low self-esteem. Men with low self-esteem presumably had trouble acquiring a sexual partner, since the cultural norm for South African youths is to be sexually active by the age of 18 (Eaton, Flisher and Aarø, 2003), and our study group were all 18 or older. Because sexual activity after the age of 18 is considered a cultural norm in our study population, and individuals with high self-esteem act according to the cultural norms (Paul *et al.* 2000), HIV prevention programs would benefit more by focusing on behavioral interventions other than abstinence. Condom usage is the most common alternative behavioural intervention and has proven beneficial in the past (Jemmott and Jemmott, 2000). By focusing on condom usage as the main behavioural intervention, HIV risk will be reduced without facing the complex task of challenging cultural norms.

Although sexual activity after the age of 18 can be considered a cultural norm in the South African population, early sexual onset cannot. Amongst sexually active men, those with low self-esteem started sexual activity significantly earlier than those with high self-esteem. It is unclear whether self-esteem is antecedent to or a

consequence of early sexual experiences. Either way, the correlation between low self-esteem and early sexual onset was previously only considered important in girls. Our study suggests that attention should also be paid to boys with low self-esteem.

It is clear from the results that only some of the sexual risky behaviors are linked to self-esteem in males. Self-esteem does not affect the number of sexual partners nor the propensity to engage in extra-pair copulations in males. In addition, none of the sexual risky behaviours correlate with female self-esteem. There are several plausible explanations for this finding, but the most plausible is probably the fact that other personal and environmental factors also play a role in predicting sexual risky behaviors. Self-esteem, therefore, plays a limited but crucial role in predicting sexual risky behaviors.

We also showed that self-perceived correlates of self-esteem are gender specific. Women show a significant correlation between self-esteem and physical attractiveness. High self-esteem women therefore tend to have a higher estimation of their own attractiveness. Conversely, men's self-esteem correlates significantly with perceived socio-economic status but not with perceived physical attractiveness.

In summary, then, our findings focus attention on the importance of self-esteem in male sexual behaviour and HIV prevention programs. In addition, the correlation between female self-esteem and sexual onset proves to be a complicated one, with both negative (Paul *et al*. 2000) and positive (Benson and Torpy, 1995) correlations reported previously. These conflicting results most likely reflect cultural differences in cultural norms. Moreover, we showed direct, gender specific, correlations between predictors of market value and self-esteem. This study uncovered some of the intricate cultural and gender specific correlates of self-esteem and their role in sexual behaviour, a field of study that will undoubtedly yield valuable results for HIV prevention.

Acknowledgements

We would like to thank Nicole Creux, Minique de Castro, Therese de Castro, Christoff Erasmus, Tracey-Leigh Hatherall, Aret Meyer, Ronnie Nelson, Duncan Newman, Jason Pienaar and Isa-Rita Russo for their valuable help with data collection. This material is based upon work supported by the National Research Foundation under Grant number 2053809 to JMG. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation. This project was cleared by the ethics committees of the University of Pretoria and the University of the Witwatersrand respectively.

References

Archer, V. E. (1989) Psychological defences and control of AIDS. *American Journal of Public Health*, 79: 876-878.

Baron, R. A. and Byrne, D. (2004) *Social Psychology*. Tenth edition. USA, Pearson Education.

Benson, M. D. and Torpy, E. J. (1995) Sexual behavior in junior high school students. *Sexual Behavior in Students*, 85: 279-284.

Blum, R. (2004) *Risk and protective factors affecting adolescent reproductive health in developing countries* [Internet]. World Health Organization, Geneva. Available from: <http://www.who.int/child-adolescent-health/publications> [Accessed 7 June 2006].

Buss, D. M. and Schmitt, D. P. (1993) Sexual strategies theory: an evolutionary perspective on human mating. *Psychological Review*, 100: 204-232.

Campanelli, P. C., Dielman, T. E. and Shope, J. T. (1987) Validity of adolescents' self-reports of alcohol use and misuse using a bogus pipeline procedure. *Adolescence*, 22: 7-22.

Dorrington, R. E., Bradshaw, D., Johnson, L., and Budlender, D. (2004) *The Demographic Impact of HIV/AIDS in South Africa: National indicators for 2004*. Cape Town, Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa.

Eaton, L., Flisher, A. J. and Aarø, L. E. (2003) Unsafe sexual behaviour in South African youth. *Social Science and Medicine*, 56: 149-165.

Ethier, K. A., Kershaw, T. S., Lewis, J. B., Milan, S., Niccolai, L. M. and Ickovics, J. R. (2006) Self-esteem, emotional distress and sexual behavior among adolescent females: Inter-relationship and temporal effects. *Journal of Adolescent Health*, 38:268-274.

Feingold, A. (1990) Gender differences in effects of physical attractiveness on romantic attraction: a comparison across five research paradigms. *Journal of Personality and Social Psychology*, 59: 981-993.

Finkelhor, D. and Dziuba-Leatherman, J. (1994) Children as victims of violence: a national study. *Pediatrics*, 94: 413-420.

Furnham, A., Mistry, D. and McClelland, A. (2004) The influence of age of the face and the waist to hip ratio on judgements of female attractiveness and traits. *Personality and Individual Differences,* 36: 1171-1185.

Jemmott, J. B. and Jemmott, L. S. (2000) HIV risk reduction behavioural interventions with heterosexual adolescents. *AIDS*, 14: S40-S52.

Jessor, S. L. and Jessor, R. (1975) Transition from virginity to non-virginity among youth: A social-psychological study over time. Developmental Psychology, 11: 473-484.

Mynhardt, J. C. (2002) *South African Supplement to Social Psychology*. Cape Town, Pearson Education.

Orr, D. P., Wilbrandt, M. L., Brack, C. J., Rauch, S. P. and Ingersoll, G. M. (1989) Reported sexual behaviors and self-esteem among young adolescents. *American Journal of Disease in Children*, 143: 86-90.

Paul, C., Fitzjohn, J., Herbison, P. and Dickson, N. (2000) The determinants of sexual intercourse before age 16. *Journal of Adolescent Health*, 27: 136-147.

Perkel, A. K. (1991) Psychosocial variables in the transmission of AIDS. *Ph.D. thesis, University of the Western Cape.*

Rosenberg, M. (1965) *Society and the Adolescent Self-Image*. Princeton, Princeton University Press.

Ruangkanchanasetr, S., Plitponkarnpim, A., Hetrakul, P. and Kongsakon, R. (2005) Youth risk behavior survey: Bangkok, Thailand. *Journal of Adolescent Health*, 36: 227-235.

Taffa, N., Sundby, J. and Bjune, G. (2003) Reproductive health perceptions, beliefs and sexual risk-taking among youth in Addis Ababa, Ethiopia. *Patient Education and Counselling*, 49: 165-169.

Thornhill, R. and Gangestad, S. W. (1996) The evolution of human sexuality. *Trends in Ecology and Evolution*, 11: 98-102.

UNAIDS 2004 Global Report (2004) *Red Ribbon* [Internet]. South Africa. Available from: <http://0-www.redribbon.co.za> [Accessed 27 January 2006].

Weber, F. T., Elfenbein, D. S., Richards, N. L., Davis, A. B. and Thomas, J. (1989) Early sexual activity of delinquent adolescents. *Journal of Adolescent Health Care*, 10: 398-403.

Wu, Z. H., Berenson, A. B. and Wiemann, C. M. (2003) A profile of adolescent females with a history of sexual assault in Texas: familial invironment, risk behaviors, and health status. *Journal of Pediatric and Adolescent Gynecology*, 16: 207-216.

Appendix

Initial male/female questionnaire:

1. How important do you think you are?

2. How much do you like yourself?
3. Do you think you possess the necessary skills for life?
4. Do you think you have an attractive face?
5. Do you think you have an attractive body?
6. Are you sexually active?
7. If yes, at what age did you have sex for the first time?
8. How many sexual partners have you had in your lifetime?
9. Have you ever cheated on a boyfriend/husband? (female questionnaire)

   Have you ever cheated on a girlfriend/wife? (Male questionnaire)
10. How wealthy do you think you are?
11. How high do you judge your status in the community?

SUMMARY

Genetic and Phenotypic cues associated with Facial attractiveness and Health

Vinet Coetzee

Supervised by ***Prof. Jaco M. Greeff, Dr. Louise Barrett and Prof. Peter S. Henzi***

Submitted in partial fulfillment of the requirements for the degree ***Magister Scientiae***

Department of Genetics

University of Pretoria

Summary

Facial attractiveness plays a crucial role in human mate choice, with individuals from both sexes using facial attractiveness cues to some degree when choosing a partner. Although some of the general facial attractiveness preferences have been studied in cross-cultural populations, most of the research focused specifically on Western populations. Most previous studies also approached facial attractiveness solely from a psychological point of view. One notable exception was a recent study by Roberts *et al.* (2005) in which the authors linked the Human Leococyte Antigen (HLA) system to cues for health and facial attractiveness in males. This study provides fascinating evidence that genes involved in the immune response also signal attractiveness and health. But is this true cross-culturally and across genders? Roberts *et al*. (2005) used a British population, who compared to other populations worldwide, have relatively few pathogens that routinely challenge their immune response.

The first objective of our study was to test the role of the HLA system in an African female population with a high pathogen load. We found that common HLA alleles, that seemingly provide resistance against common pathogens, play a more important role in health measures than heterozygosity *per se*. However, our results showed these individuals were not necessarily rated more attractive. So which facial cues do individuals from our study population find attractive in the opposite sex? According to this study individuals from both sexes prefer neotenous features in the opposite sex. Interestingly, we found no preference for facial symmetry and only a slight preference for femininity in females. Our findings support the hypothesis by Boothroyd *et al.* (2005) that preference for femininity is a by-product of preference for neotenous cues. To test if ethnic preference could not play a confounding role in facial attractiveness ratings of the ethnically mixed South African population, we tested ethnic recognition in two abundant South African ethnic groups. Our results showed that individuals from both sexes could not reliably assign ethnicity to facial images of the two groups. Ethnic preference could therefore not play a role in our study.

But mate choice does not only depend on cues displayed by the person being observed. Conditional dependent factors, inherent to the observer, influence how choosy they are of potential partners and therefore how attractive they rate members of the opposite sex. We tested the role of three condition dependent factors, age self-perceived attractiveness and relationship status in both sexes. We observed no significant difference in choosiness between males and females. Male choice therefore plays a more important role in human mate choice than previously expected. Furthermore, our study showed that condition dependent factors affect choosiness differently in males and females. Females are generally more sensitive to condition dependent factors, especially self-perceived attractiveness, while males showed no correlation between any of the condition-dependent factors and choosiness.

Since HIV is so prevalent in the South African population, we also tested the role of self esteem in predicting sexual risky behaviour. Our results showed that high self-esteem males were more likely to be sexually active after the age of 18, but that males with low self-esteem were more likely to start sexual activity prematurely. We observed no significant correlation for females. These results indicate that HIV prevention campaigns should focus more on behavioural outcomes other than abstinence, instead of challenging the cultural norms, as indicated by the behavior of high self-esteem individuals.

In conclusion, this dissertation is based on the first comprehensive study of genetic and conditional cues associated with facial attractiveness and health in an African population. This African population, with its high pathogen load, high diversity and novel cultural background provided many novel findings, which would hopefully contribute to a more universal view of human mate choice.