The role of genetics in susceptibility to environmentally-induced myopia

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Summary

Myopia is a common ocular disorder with complex and yet unidentified causes. Studies in animal models of myopia have revealed substantial variation in the degree of myopia induced by a uniform regimen of visual manipulation. This study investigated the role of genetics on susceptibility to environmentally-induced myopia by means of a selective breeding experiment.

Chicks with high or low susceptibility to monocular form deprivation (FD) were selected from an outbred population that showed considerable variation in the response to FD. After two rounds of selection, the High and Low susceptibility selected lines exhibited an evident divergence in their ocular responses to FD. Chicks from the High line developed twice the degree of myopia compared to those from the Low line. This difference was not due to visual disability or immaturity of the visual system in Low line chicks. Thus, susceptibility to form deprivation in chicks has a strong genetic component.

In estimating the heritability, approximately 50% of the variation in the chicks' susceptibility to FD-induced myopia was attributable to additive genetic effects. However, the genetic variants that control the normal variation in eye size appear to be distinct from the variants that determine susceptibility to FD due to no evidence of pleiotropic genetic effects between these traits.

When chicks from the High and Low lines were tested for their responses to lens-induced visual defocus, a significant difference between the two selected lines was observed for minus lens wear, but not for plus lens wear. Thus, there must be some shared mechanism(s) in the ocular responses to FD and minus lens wear, but different mechanisms in the responses to plus lens wear and FD.

Compared to the chicken, the mouse model of myopia has the advantages of a closer evolutionary relationship to humans and features of primate myopia. Using a novel swept-source OCT system, it was found that OCT showed good repeatability and accuracy in measuring axial ocular components in mouse. In addition, axial ocular components in mice were found to be mainly controlled by additive genetic effects.

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Chapter 1

Introduction

1.1. Myopia

Myopia is a common ocular condition which arises from a mismatch between the refractive power and axial length of the eye. In emmetropic eyes with accommodation relaxed, parallel light rays from an object at optical infinity pass through the ocular media (cornea, aqueous, lens, vitreous) to focus on the retina with the generation of a clear image. However, in myopic eyes, the corneal or lens curvature is too strong, or the length of the eye is too long, so that parallel light rays focus in front of the retina rather than on the retina, which results in a blurred image (Miller et al., 2005) (Figure 1.1).

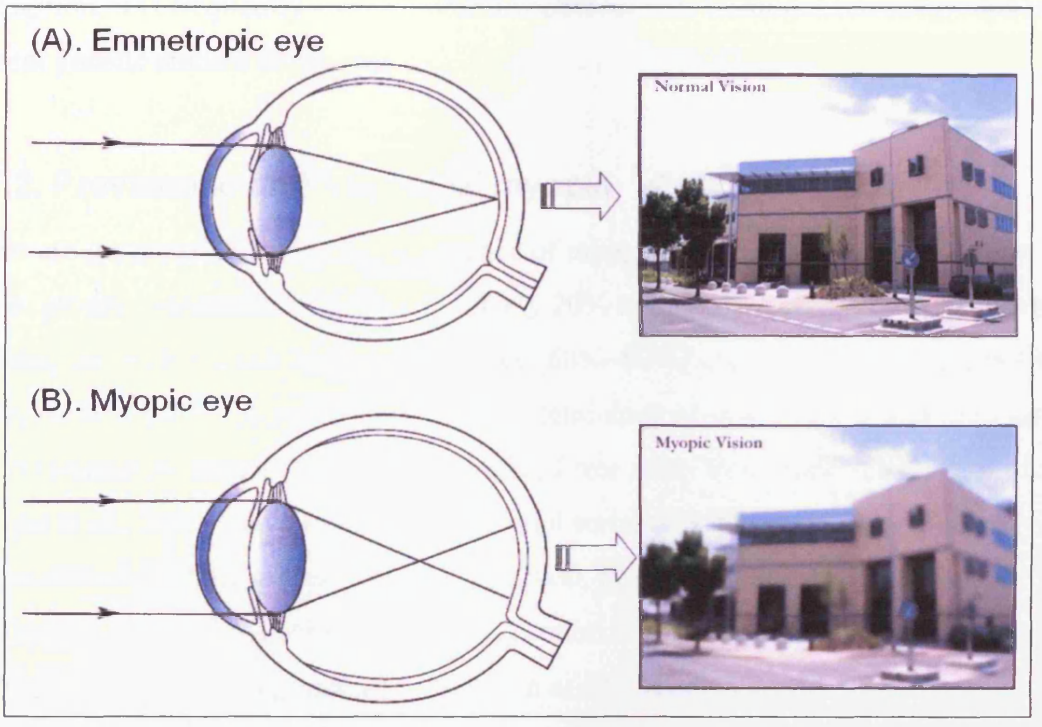


Figure 1.1 Images generated from an emmetropic eye and a myopic eye.
(A). In an emmetropic eye, parallel light rays from infinity focus on the retina with the generation of a clear image. (B). In a myopic eye, parallel light rays from infinity focus in front of the retina with the generation of a blurred image. (Illustration modified from Miller, Albert et al. (2005))

1.1.1. Classification of myopia

Many classification systems have been used to divide myopia into different categories based on clinical characteristics, in order to have a better understanding of the underlying

aetiologies. Amongst these classification systems, Curtin (1985) classified myopia into physiologic (simple), intermediate and pathologic myopia on the basis of aetiology, degree of myopia and age of onset. Another classification of myopia based on prevalence and age of onset was introduced by Grovernor (1987). Using easily-verifiable information without assumptions about the aetiology, it includes four categories: congenital, youth-onset, early adult-onset and late adult-onset. Apart from these two major classification systems, three categories defined by the degree of myopia, i.e. low myopia (0 D to -1.50 D), moderate myopia (-1.50 D to -6.00D) and high myopia (-6.00D or more) (Fredrick, 2002b), are commonly adopted to facilitate comparisons amongst epidemiological studies of myopia. In addition, it is frequently used to indicate aetiologically homogenous subgroups in current genetic studies of myopia.

1.1.2. Prevalence and impact of myopia

There are great variations in the prevalence of myopia across different populations and ethnic groups. Myopia affects approximately 20% to 25% of individuals in Western populations, with a much higher prevalence (60%~80%) in certain Asian regions (Saw, 2003; Hyman, 2007). A series of careful epidemiological studies using a unified sampling and measurement strategy, i.e. the Refractive Error Study in Children (RESC) protocol (Negrel et al., 2000), have revealed substantial variations in the prevalence of myopia across different ethnic groups (Zhao et al., 2000, Pokharel et al., 2000, Maul et al., 2000, Dandona et al., 2002, Murthy et al., 2002, Naidoo et al., 2003). Similar results were found in the Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error (CLEERE) Study (Kleinstein et al., 2003) with the highest prevalence rate in Asians. Furthermore, these significantly different rates of myopia amongst ethnic groups still existed even after controlling for age and sex.

Although discrepancies in myopia prevalence rates have been shown across populations and ethnic groups, there has been an observable, worldwide trend of increasing prevalence of myopia over the past few decades (Framingham Offspring Eye Study Group, 1996; Rose et al., 2001; Bar Dayan et al., 2005), particularly in East Asia (Lin et al., 1999; Saw et al., 2001; Lin et al., 2004). For example, in young males conscripted into the military in Singapore, the myopia rate has progressively increased from 26% in the 1970s to 79% in

the 1990s (Saw et al., 2001). In a recent study comparing the prevalence of myopia in the United States between 1971-1972 and 1999-2004, a substantially higher prevalence of myopia was revealed in the more recent as compared to the earlier period of time (41.6% vs 25.0%) using similar survey methods (Vitale, Sperduto and Ferris, 2009).

This gradually increasing trend in the prevalence of myopia is particularly noteworthy due to an aggravating global burden on not only the cost of myopic correction, but also the care of myopia-related complications. Since a myopic eye with its characteristic axial elongation is a vulnerable eye, especially at levels beyond -6 D, it is susceptible to a range of ocular pathologies, including glaucoma (Mitchell et al., 1999), cataract (Younan et al., 2002) and retinal detachment (Ogawa and Tanaka, 1988; Wang et al., 2005), as well as myopic macular degeneration (Tano, 2002; Vongphanit, Mitchell and Wang, 2002), which is a leading cause of monocular blindness in East Asian countries (Hsu et al., 2004; Iwase et al., 2006). Recently, myopic macular degeneration was also indicated as the 4th leading cause of blindness in a U.K. study (Bamashmus, Matlhaga and Dutton, 2004). Generally, the higher the degree of myopia, the greater is the risk of pathological complications. In East Asian countries such as Singapore and Taiwan, where the increase in the prevalence of myopia is most pronounced, there has been a concomitant increasing shift towards higher degrees of myopia (Lin et al., 2004), meaning that myopia is becoming an increasingly frequent cause of irreversible vision loss.

1.1.3. Therapeutic interventions for myopia

The main treatment options for myopia include single vision spectacle lenses, contact lenses and refractive surgery (Gwiazda, 2009). Nevertheless, these treatments can only correct myopic refractive errors, but not restore the ocular axial elongation and its associated pathological changes. A number of "myopia control" treatment strategies have been tested, with the aim of curtailing further myopia progression (Saw et al., 2002b, Saw et al., 2002c, Gwiazda, 2009). Whilst several of these treatments have shown a statistically significant effect in slowing myopia progression, none has been shown to provide an effective, long-term solution.

In terms of pharmacological treatment, atropine eye drops have been widely investigated and shown to be effective in slowing myopia progression and ocular axial elongation in low and moderate myopia (Chua et al., 2006). However, a recent follow-up study revealed higher rates of myopia progression after cessation of atropine drops (a rebound effect) compared to the control group (Tong et al., 2009). In addition, the side effects associated with atropine, such as blurred near vision, photophobia, and potential risks of macular degeneration and cataract due to ultraviolet light exposure through a dilated pupil, make its future clinical use debatable (Fredrick, 2002a). A similarly effective result has been shown with another muscarinic receptor antagonist, Pirenzepine (Siatkowski et al., 2004; Tan et al., 2005; Siatkowski et al., 2008). Nevertheless, the short follow-up time, unknown rebound effect, as well as systemic and ocular side effects still make its clinical applications debatable. Therefore, a clearer understanding of the aetiology and underlying mechanism(s) of myopic development is essential to find effective approaches to arrest the progression of myopia and excessive ocular axial elongation.

1.2 Aetiology of myopia

There has been a long-standing debate on the relative importance of nature versus nurture in the development of myopia (Wallman, 1994; Mutti, Zadnik and Adams, 1996; Mutti, 2010). Although the precise mechanisms of myopia development are still unclear, it is widely accepted that a complex interplay between genetic factors and environmental factors influences refractive development (Rose et al., 2002).

1.2.1 Genetic factors

There is abundant evidence of the contribution of genetic factors to the development of myopia. From family correlation studies in myopia, it has been found that the risk of myopia for a child is increased if the parents or other siblings are myopic (Zadnik et al., 1994, Wu and Edwards, 1999, Pacella et al., 1999, Mutti et al., 2002, Farbrother et al., 2004, Jones et al., 2007). For example, Pacella et al. (1999) found that children with two myopic parents were 6.42 times as likely to become myopic as children with one or no myopic parents. Twin studies also provide evidence of a genetic component to myopia, revealing higher within-pair correlations for myopia in monozygotic twin pairs compared

to dizygotic twin pairs (Teikari et al., 1991; Hammond et al., 2001; Lyhne et al., 2001; Dirani et al., 2006). In addition, high heritability estimates of myopia derived from family studies indicate an important role for genetic inheritance in the development of myopia (Rose et al., 2002). Furthermore, many myopia genetic loci have been identified recently by linkage and association studies, due to technological advances in disease gene mapping (Hornbeak and Young, 2009). Additional evidence supporting genetic factors in the development of myopia comes from a variety of hereditary systemic and ocular disorders, such as Stickler (Snead and Yates, 1999), Marfan (Dietz et al., 1991, Robinson and Godfrey, 2000), Ehler-Danlos (Callewaert et al., 2008), Down (da Cunha and Moreira, 1996; Patterson, 2009) and Weill-Marchesani syndromes (Faivre et al., 2003), as well as congenital stationary night blindness (Pusch et al., 2000).

In searching for myopia susceptibility genes, many loci linked to myopia have been identified to date from family-based linkage studies, as shown in Table 1.1. Some of these loci have been successfully replicated in independent linkage studies, including MYP1, MYP2, MYP3, MYP6, MYP8, MYP10, MYP11, MYP12, MYP13, MYP14 and MYP17. In addition, axial length is generally considered to be the main determinant of refractive error and an endophenotype of myopia (Young, Metlapally and Shay, 2007; Hornbeak and Young, 2009; Meng et al., 2009). Two studies have also revealed evidence of linkage for axial length to 2p24 (Biino et al., 2005) and 5q (Zhu et al., 2008), suggesting that these loci may play a role in the development of refractive error. Hyperopia has been less studied in the literature due to its lower prevalence and ocular morbidity. Although two loci, 11p (Othman et al., 1998) and 11q23 (Sundin et al., 2005), have been found to be associated with nanophthalmos and high hyperopia, no loci have been identified with linkage to nonsyndromic hyperopia to date.

In candidate gene studies, numerous genes have been examined and have revealed positive associations with myopia and/or hyperopia due to their possible biological functions related to the development of refractive error and/or their position within or close to MYP loci (Table 1.2). Although there are usually multiple biological functions and, in addition, gene-gene interaction may exist between these candidate genes, they can roughly be categorised into three main groups according to the plausible biological mechanisms

associated with the development of refractive error. Firstly, PAX6 and SOX2 are considered master regulatory genes for eye development. Mutations in PAX6 or SOX2 can lead to nanophthalmos or anophthalmos (Fantes et al., 2003; Tsonis and Fuentes, 2006). Furthermore, an interaction between PAX6 and SOX2 genes has been revealed in lens development (Kondoh, Uchikawa and Kamachi, 2004), suggesting a potential role in the development of refractive error. Han et al. (2009) and Ng et al. (2009) have reported positive associations between PAX6 and high myopia, but in contrast, a large British cohort study did not reveal such an association (Simpson et al., 2007). In addition, the SOX2OT gene, which may play a regulatory role in SOX2 expression, has been revealed to be associated with refractive error (Andrew et al., 2008), although no association of refractive error with SOX2 has been found (Simpson et al., 2007). The second group comprises genes associated with scleral remodelling. In this process, changes in the constituents of the extracellular matrix lead to the alteration of scleral biomechanic properties, such as scleral elasticity and creep rate, which can result in different rates of ocular growth and hence a difference in susceptibility to the development of refractive error (McBrien, Jobling and Gentle, 2009). The genes involved in extracellular matrix remodelling that have been shown to be linked to refractive error development include COL1A1, COL2A1, TGFB1, TGIF, LUM, MYOC, MMP, HGF, and c-MET (Table 1.2). Moreover, alteration in retinal signalling after visual manipulation is believed to modify sclera remodelling and lead to changes in eye growth and refractive status (section 1.3.2). Thus, genes associated with the retinal signalling cascade constitute another category of myopia candidate genes. For instance, an M1 selective antagonist, Pirenzepine, has been shown to prevent myopia progression in animal models of myopia (section 1.3.2.1). Polymorphisms within the cholinergic muscarinic receptor-1 (CHRM1) gene were found to be associated with the development of high myopia (Lin et al., 2009). Additionally, RASGRF1 and GJD2 have recently been suggested as candidate genes associated with refractive error in genome-wide association studies (Hysi et al., 2010; Solouki et al., 2010) because both of them are involved in visual signal transmission and processing in the retina (Deans et al., 2002; Fernandez-Medarde et al., 2009). Furthermore, RASGRF1 expression is regulated by muscarinic receptors (Mattingly and Macara, 1996) and retinoic acid (Tonini et al., 1999), both of which have been implicated in the control of eye growth and the development of refractive errors in animal studies (section 1.3.2.1).

In addition to these three main categories of myopia candidate genes, a genome-wide association study for high myopia in a Japanese population has recently identified a susceptibility region at 11q24.1 and suggested BLID as a potential myopia susceptibility gene (Nakanishi et al., 2009). Moreover, in a study of replication and fine mapping of the MYP 8 region, Andrew et al. (2008) discovered the association of MFN1 and PARL genes with the extreme phenotypes in the distribution of refractive error. Although these three genes are involved in the mitochondrial regulatory processes in the retina, their relationship to the mechanisms of refractive error development is still unclear. Thus, these results provide novel insight into the molecular mechanisms of the development of myopia.

Table 1.1 Summary of the identified myopia loci.

Locus	OMIM	Location	Ethnicity	Myopia severity	Inheritance	Reference	Replication
MYP 1	310460	Xq28	Caucasian	-	XR	Schwartz et al. (1990)	Li et al. (2009), Guo *et al.* (2010)
MYP 2	160700	18p11.31	Caucasian, Asian	≤ -6.00D	AD	Young et al. (1998b)	Heath et al. (2001), Lam et al. (2003b)
MYP 3	603221	12q21-q23	Caucasian	≤ -6.00D	AD	Young et al. (1998a)	Farbrother et al. (2004), Nurnberg et al. (2008), Li et al. (2009), Wojciechowski et al. (2009b)
MYP 4	608367	7q36	Caucasian, African	≤ -6.00D	AD	Naiglin et al. (2002)	-
MYP 5	608474	17q21-q22	Caucasian	≤ -5.00D	AD	Paluru et al. (2003)	-
MYP 6	608908	22q12	Caucasian	≤ -1.00D	QTL	Stambolian et al. (2004)	Stambolian et al. (2006), Klein et al. (2007), Li et al. (2009)
MYP 7	609256	11p13	Caucasian	< 0 D	QTL	Hammond et al. (2004)	-
MYP 8	609257	3q26	Caucasian	< 0 D	QTL	Hammond et al. (2004)	Andrew et al. (2008)
MYP 9	609258	4q12	Caucasian	< 0 D	QTL	Hammond et al. (2004)	-
MYP 10	609259	8p23	Caucasian	< 0 D	QTL	Hammond et al. (2004)	Stambolian et al. (2005)
MYP 11	609994	4q22-q27	Asian	-5.00D to -20.00D	AD	Zhang et al. (2005)	Li et al. (2009), Wojciechowski et al. (2009b)
MYP 12	609995	2q37.1	Caucasian	≤ -6.00D	AD	Paluru et al. (2005)	Chen et al. (2007), Li et al. (2009)
MYP 13	300613	Xq23-q25	Asian	≤ -6.00D	XR	Zhang et al. (2006)	Zhang et al. (2007)
MYP 14	610320	1p36	Caucasian	≤ -1.00D	QTL	Wojciechowski et al. (2006)	Li et al. (2009), Wojciechowski et al. (2009a), Wojciechowski et al. (2009b)
MYP 15	612717	10q21.1	Caucasian	≤ -5.00D	AD	Nallasamy et al. (2007)	-
MYP 16	612554	5p15.33-p15.2	Asian	≤ -6.00D	AD	Lam et al. (2008a)	-
MYP 17	608367	7p15	Caucasian, African	≤ -5.00D	QTL	Paget et al. (2008a)	Wojciechowski et al. (2009b)
MYP 18	613626	14q22.1-q24.2	Asian	≤ -6.00D	AR	Yang et al. (2009)	-

OMIM: Online Mendelian inheritance in man, AD: Autosomal dominant, AR: Autosomal recessive, XR: X-linked recessive, QTL: Quantitative trait locus.

Table 1.2 Summary of the candidate genes showing a positive association with refractive error.

Gene Symbol	Gene Name	Plausible associated function	Location	Linkage Locus	Phenotype	Reference
PAX6	Paired Box 6	Eye development and growth	11p13	MYP 7	High Myopia	Han et al. (2009), Ng et al. (2009)
SOX2OT	SOX2 Overlapping Transcript	Eye development and growth	3q26.3-q27	-	Myopia, Hyperopia	Andrew et al. (2008)
COL1A1	Collagen Type1 α-1	Scleral remodelling	17q21.31-q22	MYP 5	High Myopia	Inamori et al. (2007)
COL2A1	Collagen Type2 α-1	Scleral remodelling	12q13.11-q13.2	-	Myopia	Mutti et al. (2007a), Metlapally et al. (2009)
TGFB1	Transforming Growth Factor β-1	Scleral remodelling	19q13.1	-	Myopia	Lin et al. (2006), Zha et al. (2009), Khor et al. (2010)
TGIF	Transforming Growth Factor β-induced Factor	Scleral remodelling	18p11.3	MYP 2	High Myopia	Lam et al. (2003a)
LUM	Lumican	Scleral remodelling	12q21.3-q22	MYP 3	High Myopia	Wang et al. (2006), Chen et al. (2009), Lin et al. (2010)
MYOC	Myocilin	Scleral remodelling	1q24.3-q25.2	-	High Myopia	Tang et al. (2007)
MMP 1	Matrix Metalloproteinase 1	Scleral remodelling	11q22-q23	-	Myopia, Hyperopia	Wojciechowski et al. (2010)
MMP 2	Matrix Metalloproteinase 2		16q13		Myopia, Hyperopia	Wojciechowski et al. (2010)
MMP 3	Matrix Metalloproteinase 3		11q23		Myopia	Hall et al. (2009)
MMP 9	Matrix Metalloproteinase 9		20q11.2-q13.1		Myopia	Hall et al. (2009)
HGF	Hepatocyte Growth Factor	Scleral remodelling	7q21.1	-	Myopia, Hyperopia	Han et al. (2006), Yanovitch et al. (2009), Veerappan et al. (2010)
c-MET	HGF Receptor	Scleral remodelling	7q31	-	Myopia	Khor et al. (2009)
CHRM1	Cholinergic Receptor, Muscarinic 1	Retinal signalling cascade and/or scleral remodelling	11q13	-	High Myopia	Lin et al. (2009)
IGF1	Insulin-like Growth Factor 1	Retinal signalling cascade	12q22-q24.1	MYP 3	Myopia	Metlapally et al. (2010)
MFN1	Mitofusin-1	Mitochondrial regulatory processes in the retina	3q26	MYP 8	Myopia, Hyperopia	Andrew et al. (2008)
PARL	Presenilin-associated Rhomboid-like protein	Mitochondrial regulatory processes in the retina	3q26	MYP 8	Myopia, Hyperopia	Andrew et al. (2008)
Candidate genes suggested by results from genome-wide association studies						
BLID	BH3-like Motif-containing Cell Death Inducer	Mitochondrial regulatory processes in the retina	11q24.1	-	High Myopia	Nakanishi et al. (2009)
RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1	Retinal signalling cascade	15q25	-	Myopia, Hyperopia	Hysi et al. (2010)
GJD2	Gap Junction Protein δ-2	Retinal signalling cascade	15q14	-	Myopia, Hyperopia	Solouki et al. (2010)
ACTC1	Actin, α Cardiac Muscle 1	Unkown	15q14	-	Myopia, Hyperopia	Solouki et al. (2010)

1.2.2 Environmental factors

Numerous epidemiologic studies provide evidence in support of an environmental contribution to myopia. As mentioned earlier (section 1.1.2.), the rather rapid increase in the prevalence of myopia recently, particularly in East Asia, indicates a significant role of environmental factors in the development of myopia, because the genetic background has not changed dramatically over this short period (Seet et al., 2001, Rose et al., 2002, Morgan and Rose, 2005). For instance, a higher myopia prevalence was found in younger compared with older cohorts, as was a weaker association with myopia between siblings with increasing sibling age difference (Framingham Offspring Eye Study Group 1996).

Several environmental risk factors, such as extensive near work, educational attainment, intelligence, higher socioeconomic status and urbanization, have been associated with the development of myopia in epidemiologic studies (Saw et al., 1996, Saw, 2003, Saw et al., 2001). Since there are general associations amongst education level, socioeconomic status, intelligence, and near work activity, education level could be considered a surrogate factor associated with myopia (Saw et al., 2001). Recently, an intriguing and reproducible finding regarding environmental factors and myopia was an association with outdoor activity (Jones et al., 2007, Rose et al., 2008, Dirani et al., 2009). Light intensity, other than physical activity per se, was postulated to explain this protective effect (Rose et al., 2008). This hypothesis was tested in the chicken model of myopia, and it was found that high illuminance retarded the development of myopia induced by form deprivation and hyperopic defocus (Ashby, Ohlendorf and Schaeffel, 2009; Ashby and Schaeffel, 2010).

1.2.3 Interaction between genes and the environment

Both genetic and environmental factors are deemed to play important roles in the development of myopia. However, only limited available data support this conjecture. In a twin study, Chen et al. (1985) found significant association between concordance for myopia and the interaction between zygosity and reading habits in Chinese twin pairs. Lyhne et al. (2001) investigated refractive error in 114 Caucasian twin pairs and revealed a statistically significant correlation between the intrapairwise sum of ocular refraction and the absolute value of the intrapairwise difference amongst 53 monozygotic twin pairs,

using the Jinks and Fulker Test (Jinks and Fulker, 1970). In addition, Saw et al. (2001) found that near work (measured by the number of books read per week) interacted with parental myopia to influence the risks of moderate to high myopia (SE $\leq$ -3.00 D) in Singaporean children. Nonetheless, caution is needed in interpreting evidence of gene-environment interaction, due to the difficulty of classifying each factor as a pure genetic factor or a pure environmental factor. For instance, parental myopia history could denote a genetic factor, a common lifestyle, or both. Recently, the GEM twin study (Dirani, Shekar and Baird, 2008) revealed that genes (additive genetic effects) explained 69% of the variance in educational level, which was significantly associated with myopic refraction, and suggested that educational level should not be considered as a purely environmental risk factor.

The high prevalence rates of myopia observed in Chinese and Japanese populations might suggest the influence of genes on the development of myopia (Saw, 2003). Notably, differences in the prevalence of myopia between populations with similar genetic backgrounds living in urban and rural environments also suggested a role of environment factors (Zhang et al., 2000; Xu et al., 2005). Nevertheless, in the studies of myopia prevalence in subjects originating from different ethnic groups, but growing up in the same country, Asians, particular those of Chinese origin, exhibited a higher prevalence of myopia than other ethnic groups after adjusting for confounding factors, such as education, age and sex (Au Eong, Tay and Lim, 1993; Kleinstein et al., 2003; Saw et al., 2006). Genetic differences between ethic groups, cultural differences, or both could be responsible for this difference in prevalence rates between the ethnic groups. In addition, the involvement of gene-environment interaction in the development of myopia may need to be considered.

1.3 Animal models of myopia

The eyes of neonates in human and newborn animals are usually ametropic, but show a shift towards emmetropia during maturation (Figure 1.2). This developmental process of actively matching the eye length to the optical power is commonly known as emmetropisation (Grosvenor and Flom, 1991; Wildsoet, 1997; Wallman and Winawer, 2004), and myopia might represent a failure of this normal process. In order to know how myopia develops, it would be valuable to understand the mechanisms behind the process of emmetropisation and explore the possible pathways that shift emmetropia to myopia. Animal models make this possible by testing the hypothesised mechanisms related to emmetropisation under defined conditions.

Since Wiesel and Raviola (1977) discovered myopia development after neonatal lid fusion in monkeys, there is now widespread use of this technique of manipulation of early visual experience to disrupt emmetropisation and produce refractive errors in animals. Myopia has been successfully induced in a variety of animals, such as chick (Wallman, Turkel and Trachtman, 1978; Wallman and Adams, 1987), tree shrew (Sherman, Norton and Casagrande, 1977), monkey (Wiesel and Raviola, 1977), marmoset (Troilo and Judge, 1993), mouse (Tejedor and de la Villa, 2003), guinea pig (McFadden, Howlett and Mertz, 2004) and fish (Shen, Vijayan and Sivak, 2005).

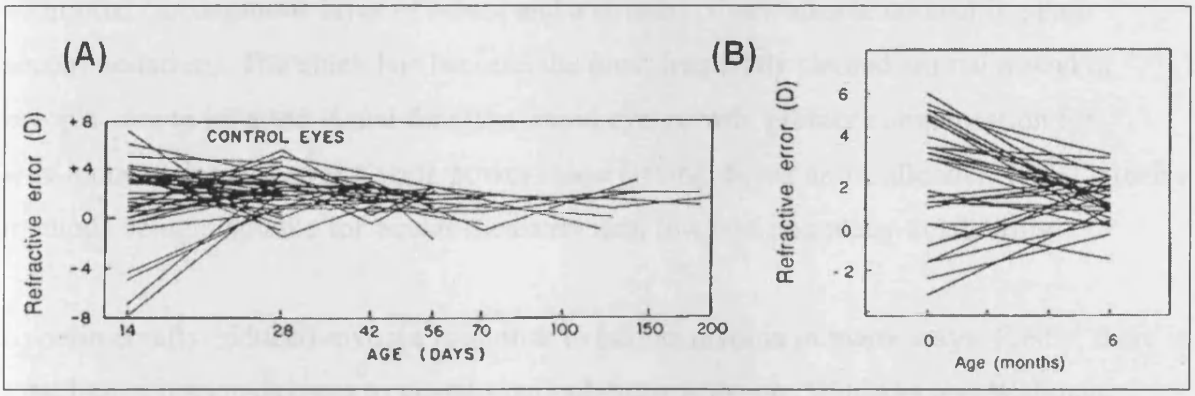


Figure 1.2 Emmetropisation in chicks and human neonates.

(A). Changes in distribution of refractive error of chicks at different ages. (B). Changes in distribution of cycloplegic refractive error in infants measured at birth and 6 months old. (from Grosvenor and Flom (1991))

It would be ideal to study an animal with spontaneous myopia because such a model is closer to early and late-onset human myopia. However, spontaneous myopia in animals is rare. Naturally occurring myopia due to an elongated vitreous chamber has been found in the Labrador retriever (Mutti, Zadnik and Murphy, 1999), but there are some limitations, such as the high cost and the difficulty of obtaining and housing an adequate number of animals for good experimental designs. In addition, Jiang et al. (2009) found an approximately 13% prevalence of spontaneous axial myopia from screening refractive error in a wild-type guinea pig strain. Nonetheless, these guinea pigs with spontaneous myopia also showed some deficits in visual functions, such as a lack of light-induced pupil responses and no signs of accommodation, although a test of visual acuity using an optomotor drum did not reveal any significant differences compared to previous studies in other wild-type guinea pigs.

Amongst the animal models of myopia, primates are ideal due to their evolutionary similarity to humans, and their parallel ocular anatomy, such as retinal vascular structure and fovea. However, this model suffers from limited availability, a longer treatment period to induce myopia and the high expense for large scale studies. The mouse model is also limited due to its nocturnal nature, poor visual function and the lack of reliable methods for the ocular measurement of small eyes (Schmucker and Schaeffel, 2004b) despite its obvious advantages for genomic studies. Despite certain differences in ocular structures in chicks compared to human eyes (for instance, chicks have an avascular retina, an additional cartilaginous layer of sclera and a striated ciliary muscle controlling their accommodation). The chick has become the most frequently studied animal model of myopia, due to its good visual function, rapid eye growth, prompt compensation for lens-induced defocus over a wide power range (Irving, Sivak and Callender, 1992), precise methods being available for ocular measurement, low cost and ready availability.

Experimentally-induced myopia is similar to human myopia in many ways. Firstly, there is a decline in responsiveness to visual manipulations with age (Wildsoet and Wallman, 1995), i.e. it has been found that the younger the animal, the higher the degree of induced refractive error (Siegwart and Norton, 1998, Wallman and Adams, 1987). Secondly, both experimental myopia and human myopia have a characteristic feature of an increase in the

axial length of the eye, particularly in the vitreous chamber depth (Wallman and Adams, 1987; McBrien and Adams, 1997). Furthermore, the changes in retina and sclera due to the development of myopia observed in human (Curtin, 1988) have also been found in animal models, such as retinal and scleral thinning and retinal lesions, observable as lacquer cracks (McBrien and Gentle, 2003, Montiani-Ferreira et al., 2004, Hirata and Negi, 1998). Moreover, form-deprivation myopia was found to have an equivalent in human infants with deprivation of vision through congenital cataract or disorders of eyelids (von Noorden and Lewis, 1987). Although studies in animals have the advantage of enabling manipulations of experimental conditions, the results need to be examined carefully before they can be extended to humans.

1.3.1 Experimental visual manipulations

There are two well-established methods to manipulate early visual experience and induce myopia in animals, namely, "form-deprivation myopia" and "lens-induced myopia".

1.3.1.1. Form-deprivation myopia

"Form-deprivation myopia" can be induced in a variety of animal species by suturing the eyelids or placing a translucent diffuser in front of the eye (Wallman, 1990). It produces a reduction in both the contrast and sharpness of the retinal image and results in eye elongation. The form-deprived eye becomes progressively more and more myopic with time, as a result of a continual inability to obtain sharp images on the retina. This is known as the "open-loop" characteristic of form-deprivation myopia. In addition, removal of the diffusers from animals with form-deprivation myopia has been found to suppress the eye elongation, and recovery from form-deprivation myopia occurs in chickens (Wallman and Adams, 1987) and tree shrews (Siegwart and Norton, 2001) as long as the animal is still young enough to emmetropise.

1.3.1.2. Lens-induced myopia

Schaeffel et al. (1988) first fitted chicks with lenses over their eyes and found myopia and hyperopia were induced by minus and plus lenses, respectively. Later, Irving et al. (1992) observed that the eyes of young chicks were able to compensate for lens powers from

+15D to -10D completely (incomplete compensation was observed up to +30D and -20D). "Lens-induced myopia" was also demonstrated in other animals, for example, tree shrews (Shaikh, Siegwart and Norton, 1999) and young monkeys (Hung, Crawford and Smith, 1995). By fitting a minus lens in front of the eye, the eye elongation occurs as a result of the emmetropisation system's attempt to match the length of the eye to the changed optical power. Such an eye will attain "functional emmetropia" with the spectacle lens in place, but will be myopic once the lens is removed. This model has "closed loop" characteristics because the eye only continues to maintain its altered rate of growth until it has compensated for the refractive power of the spectacle lens. Conversely, fitting a plus lens in front of the eye decreases the rate of eye growth and results in hyperopia (Hung et al., 1995; Irving, Callender and Sivak, 1995). These changes are also presumed to involve the emmetropisation process, in that they lead to the movement of the retina towards the altered plane of focus.

1.3.2. Mechanisms associated with the control of eye growth

From studies of animal models of myopia, the control of eye growth has been found to be mediated principally by a local ocular mechanism. Evidence comes from experimental studies which used optic nerve section to isolate the retina from the brain, or tetrodotoxin to block neural signalling between the retina and brain. Neither treatment prevented form-deprivation myopia (Troilo, Gottlieb and Wallman, 1987; Norton, Essinger and McBrien, 1994; McBrien et al., 1995). Wallman et al. (1987) discovered that when "regional" form deprivation was imposed, eye growth and myopic development were limited to the deprived regions. Similarly, Diether and Schaeffel (1997) fitted hemi-field lenses to produce visual defocus across either the nasal or the temporal visual field of the eyes in chicks, and found defocus imposed on local retinal areas produced local changes in eye growth. These findings thus implicate a local ocular growth control signalling pathway. Furthermore, Ehrlich et al. (1990) investigated the relationship between the extent of retinal cell damage and eye growth after treatment with different selective neurotoxins. They concluded that the photoreceptors play an important part in FD-induced growth of the eye due to the varied form-deprivation responses observed after differing extents of retinal cell damage caused by kainic acid, quisqualic acid or tunicamycin.

Similarly, after inducing blindness in chicks through damaging the retinal pigment epithelium and photoreceptor layers by formoguanamin, form-deprivation myopia could no longer be induced (Oishi and Lauber, 1988).

The different responses of the choroid and sclera in response to visual manipulation with minus and plus lenses are shown in Figure 1.3 (Wallman et al., 1995; Wildsoet and Wallman, 1995). Fitting minus lenses results in increased ocular elongation and thinning of the choroid. In contrast, fitting plus lenses decreases ocular elongation and thickens the choroid. These results suggest that, in some as yet unknown way, the visual system has the ability to distinguish between defocus caused by minus and plus lenses, and to adjust the rate of eye growth to compensate for that defocus. Because the changes in ocular growth and refraction are locally regulated by visual processing, the retinal circuitry is deemed to play a major role in the emmetropisation process (Wallman, 1993; Crewther, 2000). Therefore, many molecular signals from the retina have been investigated and revealed to be involved in the control of eye growth.

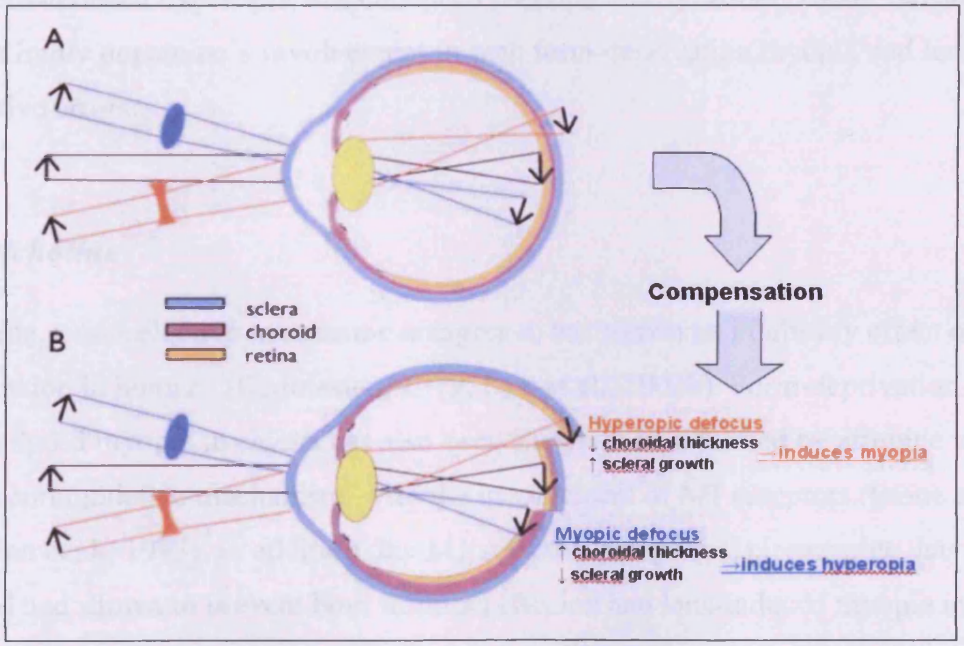


Figure 1.3 Ocular compensation for lens-induced defocus in chicks.
(A). The relative position of images relative to the retina, produced by plus and minus lenses. (B) Compensatory choroidal and scleral responses to defocus induced by plus and minus lenses, and the consequent refractive error after removal of lenses (reproduced from Wallman and Winawer (2004))

1.3.2.1. Retinal molecular signalling related to eye growth regulation

Several neurotransmitters or modulators found in the retina have been linked to the control of eye growth:

Dopamine

Dopamine, released by amacrine cells, was revealed to inhibit form-deprivation myopia through D2 receptors in chicks and primates (Stone et al., 1989; Iuvone et al., 1991; Rohrer, Spira and Stell, 1993; Schaeffel et al., 1995; Schmid and Wildsoet, 2004). However, there was inconsistency in dopamine levels after lens-induced refractive errors were produced in chickens. Bartman et al. (1994) found no significant change in retinal dopamine level after lens treatment, whereas Guo et al (1995) found the level of retinal dopamine was reduced by hyperopic defocus and increased by myopic defocus. Different lens powers and treatment periods were argued to produce this inconsistency (Guo et al., 1995). Furthermore, intravitreal injection of apomorphine, a nonselective dopamine receptor agonist, inhibited axial eye growth and myopia induced by minus lenses, while plus lens-induced hyperopia was enhanced (Schmid and Wildsoet, 2004). Notably, these results imply dopamine's involvement in both form-deprivation myopia and lens-induced refractive errors.

Acetylcholine

Atropine, a nonselective muscarinic antagonist, has shown an inhibitory effect on myopia progression in humans (Bedrossian, 1979; Saw et al., 2002b). Form-deprivation and lens-induced myopia in chicks has also been shown to be inhibited by atropine via a non-accommodative mechanism, with the involvement of M1 receptors (Stone et al., 1991, McBrien et al., 1993). In addition, the M1 selective antagonist, pirenzepine, has been studied and shown to prevent both form-deprivation and lens-induced myopia in chicks (Stone, Lin and Laties, 1991; Leech, Cottriall and McBrien, 1995; Cottriall et al., 1999). Pirenzepine also has been used in human clinical trials and shown to be effective in slowing the progression of myopia (Siatkowski et al., 2004; Tan et al., 2005; Siatkowski et al., 2008), at least in the short term. However, evidence from recent studies favour an inhibitory effect via M4 muscarinic receptor blockade due to the likely absence of M1

receptors in the eyes of chick (Yin, Gentle and McBrien, 2004) and an inhibitory effect on form-deprivation myopia by an M4 selective antagonist (Cottriall, Truong and McBrien, 2001). Furthermore, the actual site of action (Fischer et al., 1998) (e.g. retina versus sclera) of anti-muscarinics in inhibiting myopic eye growth needs to be confirmed by further study.

ZENK-Glucagon

It was found that glucagon-containing amacrine cells respond differentially to the sign of defocus and may thus mediate changes in ocular growth and refraction (Fischer et al., 1999). The expression of the immediate-early gene product ZENK increased in this cell population in eyes fitted with plus lenses and decreased in chicks wearing minus lens. Moreover, a bidirectional regulation of glucagon mRNA levels has been observed. Glucagon gene expression correlated with the sign of the imposed defocus, i.e. downregulation with minus lenses, and upregulation with plus lenses, suggesting that glucagon may act as a growth inhibiting signal (Buck et al., 2004).

Retinoic Acid

Retinoic acid, the active metabolite of vitamin A, is a potent regulator of cellular differentiation and growth. It plays an important role in retinal development at early embryonic stages and has been found to be a candidate signalling molecule in the visual control of eye growth (Seko et al., 1996, Bitzer et al., 2000, Mertz and Wallman, 2000, McFadden et al., 2004). Retinoic acid levels increase in the retina after form-deprivation and minus lens-induced myopia in both chicks and guinea pig. However, retinoic acid levels in the choroid and sclera declined during myopia development in chicks, but increased in guinea pig (McFadden et al., 2004). Retinoic acid has been shown to suppress the rate of scleral glycosaminoglycan synthesis, providing a clear causal link between changes in the choroid and changes in the sclera of chicks (Mertz and Wallman, 2000) and primates (Troilo et al., 2006). Hence, the different direction of changes in retinoic acid levels in choroid and sclera between chicks and guinea pig is hypothesized to be the result

of species differences in scleral structures and its response associated with ocular growth (Rada, Shelton and Norton, 2006).

1.3.2.2. Remodeling of the sclera during induced eye growth

The structure of mammalian sclera is different from the sclera of chicks. The fibrous mammalian sclera thins during the development of myopia, which is associated with a reduction in proteoglycan content, the rate of incorporation of precursors into glycosaminoglycans, and collagen synthesis (Norton and Rada, 1995; Gentle et al., 2003). Furthermore, scleral matrix metalloproteinase-2 (MMP-2) activity increases (Guggenheim and McBrien, 1996) and tissue inhibitor of metalloproteinases (TIMP-1) mRNA is decreased (Siegwart and Norton, 2002). Form deprivation has been shown to slow or reverse the normal process of extracellular matrix accumulation in mammalian sclera, which may allow the sclera to become more distensible, permitting vitreous chamber elongation, with resultant myopia (Norton and Rada, 1995). However, the sclera of chicks is composed of two layers, an outer fibrous layer and an inner cartilaginous layer. The outer fibrous layer becomes thinner in the posterior pole of myopic chicks while the inner cartilaginous thickens when myopia is induced (Gottlieb, Joshi and Nickla, 1990; Marzani and Wallman, 1997). Increased levels of gelatinase A (MMP-2) mRNA and decreased tissue inhibitor of metalloproteinases (TIMP-2) in the sclera have been discovered in form-deprived chick eyes (Rada et al., 1999). In addition, decreased basic fibroblast growth factor (bFGF) and increased transforming growth factor beta 2 (TGF-β2) were revealed in the posterior sclera of eyes with form deprivation (Seko, Shimokawa and Tokoro, 1995). In vitro, bFGF may act as a potent growth stimulator and TGF-β as a growth regulator of scleral chondrocytes and scleral fibroblasts (Seko, Tanaka and Tokoro, 1995). Typically, the changes in the chicken cartilaginous sclera occur in the opposite direction to those seen in the mammalian sclera and the chick fibrous sclera (McBrien and Gentle, 2003).

In summary, myopia is often considered as a failure in the process of emmetropisation. Emmetropisation involves defocus detection at the retina, diffusion of signals across the retina and choroid, and alteration of the sclera. Better understanding of these molecular

signalling pathways from retina to choroid and sclera may lead to the discovery of potential therapeutic targets to prevent the development of myopia.

1.3.3. From animal models of myopia to human myopia

There are clear parallels between human myopia and animal models of myopia. Firstly, the process of emmetropisation has been observed in both human and animal models (Grosvenor and Flom, 1991). Secondly, form-deprivation myopia has also been observed in children with congenital ptosis (O'Leary and Millodot, 1979; Hoyt et al., 1981), cataract (Rasooly and BenEzra, 1988) and phlyctenular keratitis (Meyer et al., 1999). Furthermore, both ocular axial elongation in animal models of myopia and progression of myopia in human are mainly the result of an increase of vitreous chamber depth (Wiesel and Raviola, 1977; Wallman et al., 1987; Saw et al., 2005). Nevertheless, some arguments have been raised about differences between human myopia and induced myopia in animals. For instance, imposed visual defocus is compensated by dramatic modulation of choroidal thickness in chicks, whereas mammals show a greatly reduced choroidal response (Zadnik and Mutti, 1995, Nickla and Wallman, 2009). In addition, the bi-directional ocular growth for compensation to visual defocus produced by moderate powered minus and plus lenses has been observed in monkeys (Hung et al., 1995, Smith and Hung, 1999). However, undercorrection of myopia (i.e. myopic defocus) for more than one year did not prevent the progression of myopia in children (Chung et al., 2002, Adler and Millodot, 2006), although a small but significant increase or decrease in axial length induced by short-term hyperopic and myopic defocus, respectively, was observed in the eyes of young adult human subjects (Read, Collins and Sander, 2010). Moreover, the sensitive period for myopia varies between species (Zadnik and Mutti, 1995). In humans, myopia usually develops during school age. However, the sensitive periods for induced myopia are at hatching in chicks (Wallman and Adams, 1987) and at 15 days after eye opening in tree shrew (McBrien and Norton, 1992) with a gradual decrease in sensitivity thereafter. Therefore, interpretation of the results from animal studies must be applied to human myopia with caution.

1.3.4. Evidence of a genetic role in animal models of myopia

Since myopia in animal models is induced through manipulation of early visual experience, most studies stress the importance of environmental influences on the development of myopia. Nevertheless, there is considerable inter-animal variation in the degree of induced myopia after a uniform regimen of form deprivation (Wallman and Adams, 1987, Troilo et al., 1995, Schmid and Wildsoet, 1996, Stone et al., 1995, Smith and Hung, 2000, Guggenheim et al., 2002). Measurement errors and environment factors seem unlikely to fully explain this variation. Troilo et al. (1995) found significant differences in ocular response to visual deprivation between two strains of White Leghorn chicks, the Cornell-K and Washington H&N, and indicated the potential role of genetic differences. Guggenheim et al. (2002) compared susceptibility to form-deprivation myopia in three strains of chicks, White Leghorn, Brown Leghorn and Broiler. They suggested the potential for mapping quantitative trait loci (QTL) underlying the difference between strains in the response to form deprivation, but that this would be difficult due to the considerable within-strain variation. Perhaps most compelling, Saltarelli et al. (2004) discovered a significant correlation in the magnitude of axial elongation in individual chicks when they were exposed to two sequential periods of visual deprivation, with a recovery interval in-between (Figure 1.4). This finding suggests that the differential susceptibility to experimentally induced myopia is likely to be genetic in origin. So far, however, there have been no studies to delineate the variation in the susceptibility of myopia induced in animal models.

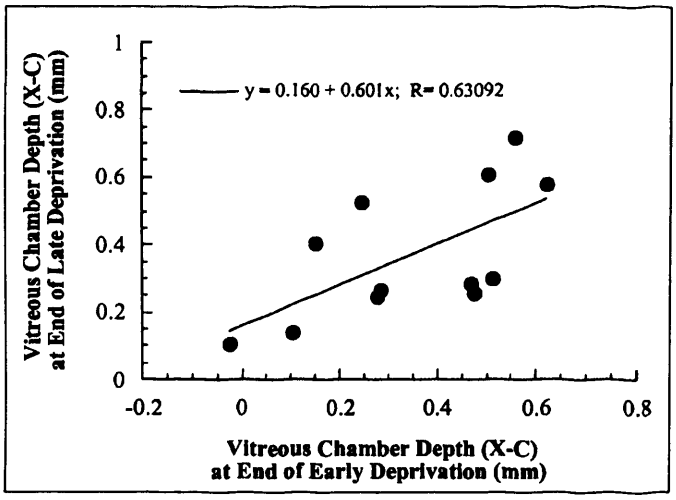


Figure 1.4 Scatter plot of the changes in vitreous chamber depth induced by monocular visual deprivation in two sequential episodes of visual deprivation. There is a significant correlation between the changes in the two separate periods. (from Saltarelli et al. (2004))

Studies investigating the normal variation in eye size in mice indicated a genetic component in the control of eye growth. Zhou and Williams (1999b) measured eye weight, lens weight and retinal area in 507 mice from 50 different strains. After accounting for sex, age and body size, heritability estimates of eye weight, lens weight and retinal area were 0.31, 0.25 and 0.41, respectively. Subsequently, they mapped two quantitative trait loci, namely Eye1 and Eye2, which influenced the normal variation in eye weight of 26 BXD recombinant inbred mouse lines (Zhou and Williams, 1999a). Although quantitative trait loci involved in the control of eye size might be expected to influence susceptibility to induced myopia, there could be distinct loci that influence this susceptibility as well. Therefore, further investigation of the variation in the ocular response to induced myopia is essential to clarify the mechanisms regulating the emmetropisation process. This issue is the main focus of my thesis.

1.4 Quantitative Genetics

1.4.1 Quantitative traits

A trait denotes a phenotype that differs between individuals in a species and shows some stability across time and situations (Plomin, Haworth and Davis, 2009). Quantitative traits are those that show a continuously distributed variation (usually a normal distribution) which results from the combined effects of multiple genes, environmental factors and gene-environment interactions (Hirschhorn and Daly, 2005). Examples are height, weight and blood pressure. Myopia is a common, complex disease, and abundant evidence of polygenic influences on its development comes from linkage and association studies (Hornbeak and Young, 2009). Therefore, investigation of myopia as a quantitative trait makes it possible to elucidate the genetic and environmental contributions to the variation of myopia in the population. Furthermore, it may lead to an understanding of the genetic architecture for the variation in the degree of myopia and clarify the underlying mechanisms in its development.

1.4.2 Heritability

The concept of heritability summarizes how heritable a particular phenotype is, primarily with reference to the resemblance of offspring and parents (Visscher, Hill and Wray, 2008). Since the resemblance between relatives derives from shared genes and/or environments, heritability aims to delineate to what extent the variation in a trait of interest arises from genetic and/or environmental factors. In other words, heritability is an analysis of variation to explain the degree to which a trait varies in a population due to genetic factors. Hence, estimating heritability allows an assessment of the strength of genetic influence on a particular trait, which determines the potential efficiency of gene-mapping studies. In addition, it provides important information to predict the response to selection in animals and plants.

1.4.2.1 Components of variance

Variance is a measure of the amount of variation. It is the mean of the square values of the individual differences from the population mean (Falconer and Mackay, 1996). Thus the phenotypic variance (V_P), i.e. the total variance, denotes the variation of a trait, which is the sum of the genetic variance (V_G) and environmental variance (V_E) under the assumption of no correlation or interaction between genotypes and environments. The genetic variance includes additive genetic variance (V_A) and non-additive genetic variance which can be further broken down into variance due to dominance effects (V_D) and gene-gene interaction (V_I), so that:

$$V_P = V_G + V_E$$
$$= V_A + V_D + V_I + V_E$$

1.4.2.2 Definition of heritability

Heritability is a ratio to estimate the degree of genetic determination of a particular trait in a population, measured at a particular time or age. The "broad sense" heritability (H^2) is defined as the proportion of the phenotypic variance (V_P) that is attributable to the total genetic variance (V_G), whereas heritability in the "narrow sense" (h^2) is the ratio of additive genetic variance (V_A) to the phenotypic variance (V_P) (Falconer and Mackay, 1996). Thus,

$$H^2 = V_G / V_P$$
$$h^2 = V_A / V_P$$

Broad sense heritability thus describes the total genetic contribution to the trait of interest. However, narrow sense heritability is frequently of more interest in practice, because additive genetic effects are passed on reliably from parents to offspring, allowing the response to selection to be predicted.

1.4.2.3. Estimation of heritability

Heritability can be estimated from the degree of resemblance between relatives, for example, parent-offspring regression or correlation between siblings. For the parent-offspring relationship, heritability is (a) twice the regression coefficient of offspring

and one parent, or (b) the regression coefficient of offspring and the mean of the two parents. For siblings, heritability is (a) twice the correlation coefficient of full sibs, or (b) four times the correlation coefficient of half sibs. In twin pairs, a significantly higher correlation of the trait in monozygotic twins compared to that in dizygotic twins indicates a genetic influence on the trait. The crude heritability can be obtained from twice the difference between the intrapair correlations of the monozygotic and dizygotic twins. However, this estimate is easily biased due to the assumption of the same environmental components of variance in the two types of twins (Falconer and Mackay, 1996).

Amongst these relationships, half-sib correlation and regression of offspring on father are more reliable due to less bias by maternal effects or common environment. Nevertheless, these estimates are unbiased only under the assumption of a balanced study design. When the study is unbalanced, or complex pedigrees are examined, variance components and heritability can be estimated more accurately by maximum likelihood statistical procedures which can accommodate any structure of genetic relationship, and also selection studies (Falconer and Mackay, 1996). In addition, the sampling error, which is a function of the sample size and pedigree structure, also influences the accuracy of heritability estimates (Visscher et al., 2008). Recruiting more families, more individuals with diverse relationships in a family, and a larger sample size, will reduce the sampling variance. Thus, the standard error (SE) of a heritability estimate will be decreased and the accuracy of the heritability estimate will also be improved.

1.4.2.4. Heritability estimates of ocular traits

A number of studies have explored the heritability of ocular component dimensions and refractive error in humans (Table 1.3) as a first step towards mapping quantitative trait loci (QTL). Although most studies reveal relatively high heritability estimates for these ocular traits, and achieve a consensus of genetic influences in these traits, there is still a wide range of heritability estimates, for instance, ranging from 0.20 to 0.94 for axial length. In addition to different populations in different environments, the variation in heritability estimates may result from sampling error, differences in the distribution of age and gender between populations and different measurement methods.

These potential confounding factors for ocular traits can be minimised in laboratory animals, and thus more precise estimates of variance components can be obtained. This provides a powerful setting for detecting the genetic variants controlling ocular traits. However, the heritability of ocular traits has rarely been studied in either wild or laboratory animal populations. As mentioned above, Zhou and Williams (1999a) measured eye weights in approximately 700 mice from 26 BXD recombinant inbred mouse lines, and estimated the heritability to be 0.48 for eye weight. Subsequently they mapped two quantitative trait loci (QTL), Eye1 and Eye 2, for eye weight. Other researchers have taken these laboratory-based findings forward, resulting in hepatocyte growth factor (HGF), a candidate gene at the Eye1 locus, being identified as harbouring common genetic variants associated with susceptibility to human high myopia and refractive error (Han et al., 2006; Yanovitch et al., 2009; Veerappan et al., 2010).

For dissecting the genetics of complex traits or disorders, the most straightforward method would be mapping the QTLs directly in human. Nonetheless, this strategy may be undermined by the complications of varied environmental exposures and population substructure in human subjects, and consequent losses in the power to detect QTLs. Using animal models to map the QTLs allows for the control of not only a uniform environment, but also the genetic variation within the animal population, such as advanced intercross lines and selected lines (Hunter and Crawford, 2008). Although application of results from QTL mapping in animal models to human complex disorders is not always straightforward, it does provide powerful and complementary information for identifying candidate genes for complex traits in humans. Several disease-susceptibility genes in man have been identified using the mouse-to-human strategy, such as TNFSF4 influencing atherosclerosis susceptibility (Wang et al., 2005b), CTLA4 contributing to autoimmune disorders (Ueda et al., 2003) and HGF for susceptibility to high myopia as describe above. Given this evidence, QTL mapping in animals may be a powerful approach to supplement the search for myopia susceptibility genes in humans.

Table 1.3 Heritability of ocular traits in human subjects.

Study design	Ethnicity	Sample size	Reference	Corneal curvature	Corneal thickness	Anterior Chamber depth	Lens thickness	Axial length	Refractive error
Parent-offspring	UK population	28 families	(Sorsby, Leary and Fraser, 1966)	0.76	-	0.53	0.31	0.63	0.45
Parent-offspring	Not stated	300 families	(Mash, Hegmann and Spivey, 1975)	0.89	-	-	-	-	
Parent-offspring	Eskimos	931 subjects	(Alsbirk, 1979)	0.64	-	0.56	-	0.76	0.14
Twin study	Caucasian	109 pairs	(Teikari et al., 1991)	-	-	-	-	-	0.58 (myopia*)
Twin study	Caucasian	114 pairs	(Lyhne et al., 2001)	0.90	-	0.94	0.93	0.94	0.89 - 0.94
Twin study	UK population	506 pairs	(Hammond et al., 2001)	-	-	-	-	-	0.84 - 0.86
Parent-offspring	Caucasian	201 families	(Biino et al., 2005)	0.57	-	0.44 (M) 0.47 (F)	-	0.60 (M) 0.31 (F)	0.18 -0.27
Twin study	Caucasian	256 pairs	(Toh et al., 2005)	-	0.95	-	-	-	-
Sib-sib correlation	Mixed	241 families	(Wojciechowski et al., 2005)	-	-	-	-	-	0.61
Twin study	Caucasian	612 pairs	(Dirani et al., 2006)	0.50 (M) 0.60 (F)	-	0.51 (M) 0.78 (F)	-	0.94 (M) 0.92 (F)	0.88 (M) 0.75 (F)

*Myopia was treated as a binary trait.

Table 1.3 Heritability of ocular traits in human subjects. (continuation)

Study design	Ethnicity	Sample size	Reference	Corneal curvature	Corneal thickness	Anterior Chamber depth	Lens thickness	Axial length	Refractive error
Full pedigrees	Not stated	132 families	(Chen et al., 2007)	0.16	-	0.78	-	0.73	0.50
Sib-sib correlation	Amish	269 families	(Peet et al., 2007)	-	-	-	-	-	0.70
Twin study	Asian	449 pairs	(Zheng et al., 2008)	-	0.88 (M) 0.91 (F)	-	-	-	-
Twin study	Caucasian	433 pairs	(Zhu et al., 2008)	-	-	-	-	0.81	-
Full pedigrees	Mixed	55 families	(Paget et al., 2008b)	-	-	-	-	0.20	0.20
Full pedigrees	Caucasian	189 families	(Klein et al., 2009)	0.95	-	0.78	-	0.67	0.58
Parent-offspring	Caucasian	33 families	(Landers et al., 2009)	-	0.68	-	-	-	-
Twin study	UK population	2301 pairs	(Lopes et al., 2009)	-	-	-	-	-	0.77
Full pedigrees	Caucasian	125 families	(Vitart et al., 2010)	0.84	0.75	0.59	0.37	0.37	0.01
Full pedigrees	Caucasian	>136 families	(Vitart et al., 2010)	0.52	0.71	0.45	0.32	0.74	0.17

1.4.2.5. Correlations between traits

When there is a correlation between two quantitative traits, i.e. a phenotypic correlation (ρ_P), it can arise from genetic correlation (ρ_G) and/or environmental correlation (ρ_E). Formally, these relationships can be quantified as:

$$\rho_P = \rho_G (h_1^2 \times h_2^2)^{1/2} + \rho_E ((1 - h_1^2) \times (1 - h_2^2))^{1/2}$$

where h_1^2 and h_2^2 denote the heritabilities of trait 1 and trait 2, respectively (Lynch and Walsh, 1998). If both traits have high heritabilities, then the genetic correlation is more important in determining the phenotypic correlation and vice versa (Falconer and Mackay, 1996). These correlations may be positive or negative. For example, Klein et al (2009) revealed a positive genetic correlation of 0.4 between corneal curvature and axial length using data from the Beaver Dam Eye Study, which means genes causing an increase in corneal curvature also produced a simultaneous increase in axial length. Nonetheless, the genetic correlation between spherical equivalent and axial length was -0.30, which denoted that genes causing an increase in axial length tend to produce a corresponding decrease in spherical equivalent. The main cause of genetic correlations is pleiotropy, which means a gene has multiple phenotypic effects. Another cause of genetic correlation is genetic linkage, which occurs when the genes controlling two different traits tend to be transmitted together. The important difference between these two causes is the persistent genetic correlation in pleiotropy over evolutionary time because functional constraints of an individual protein might not be able to be dissociated. In contrast, genetic linkage can be broken and produce new associations between alleles (Russell, 2006). Thus, genetic correlation indicates the extent to which two quantitative traits are influenced by common genes. In regard to environmental correlation, it comprises the correlations of not only environmental deviations but also non-additive genetic deviations. Consequently a genetic correlation provides more explicit and valuable information than does an environmental correlation.

1.4.3. Artificial selection

Selection is a process which alters the genetic structure of a population via either a natural process or artificial intervention. It depends on the presence of genetic variation. In addition, the amount and type of genetic variation are important in determining the rate of

change in genetic structure (Russell, 2006). Moreover, differences in fertility among the selected individuals and differences in the viability of their offspring also have a crucial influence on the change of genetic structure in the population. Usually, the details of genetic loci controlling a quantitative trait are unknown. Thus, the selection of subjects and observation of selection effects for the trait are restricted mainly to the phenotypic value and to changes in the trait's population mean (Falconer and Mackay, 1996).

1.4.3.1. Types of selection

There are three main types of selection, namely, directional, stabilizing and disruptive selection (Figure 1.5) (Thoday, 1972). Directional selection is selection for a phenotypic value away from the population mean. It is important in the improvement of domesticated animals and plant breeds, for example, chickens with higher egg production rate. In stabilizing selection, selection favours some intermediate phenotypic value against deviation to the extreme values. This type of selection is the common mechanism of action for natural selection. Disruptive selection is selection for the extreme phenotypic values, in which the variance of the trait increases and the population will gradually split into two distinct groups. Disruptive selection is frequently adopted in the design of selection experiments to investigate the genetic components of particular traits. Selection experiments lasting less than five generations enable researchers to obtain information regarding the genetic variance and heritability of a trait in the population (Hill and Caballero, 1992).

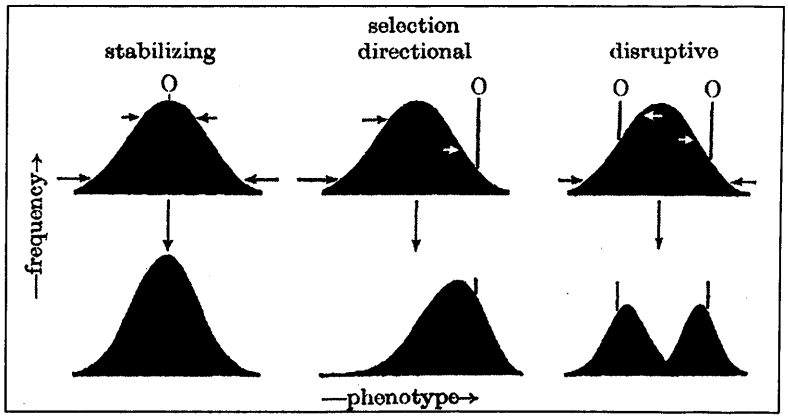


Figure 1.5 The main types of selection and corresponding effects. Horizontal arrows denote the directions and relative magnitudes of the selection forces. "O" indicates optimum mean value of phenotype after selection (from Thoday (1972)).

1.4.3.2. The response to selection and realized heritability

The effects of selection are usually observed from the changes of population mean. Hence, the selection response (R) is defined as the difference in the mean phenotypic value between the offspring of selected parents and the mean of the parental generation. The selection differential (S) denotes the difference in the mean phenotypic value between the selected parents and the overall mean in their generation. Since the response to selection depends on genetic components of the selected trait, which encompasses the number, effect and frequencies of the genes influencing the trait, the response to selection can be used to estimate the heritability. Therefore, the ratio of selection response to selection differential is defined as the realized heritability (h2), thus:

$$\mathbf{h^2 = R / S}$$

Nevertheless, realized heritability needs to be interpreted cautiously because of the possible influence of maternal effects on the response to selection and variation of the generation-to-generation mean phenotypic value by random genetic drift, sampling errors and environmental perturbations (Falconer and Mackay, 1996).

1.4.3.3. Applications

Artificial selection in animals provides information about phenotypic changes over the course of time and the dynamics of these changes reflect the underlying genetic architecture. Apart from the improvement in breeds of domesticated animals and plants, experimental selection provides a useful tool to investigate the genetic architecture of quantitative traits of interest based on the way a population responds to selection over one or several generations (Hill and Caballero, 1992). For example, disruptive selection can be used to investigate whether genetic components have an influence on a quantitative trait, as shown in Figure 1.6. If the phenotypic variance is completely due to environmental factors, the phenotypic distributions of the two groups of offspring will resemble the distribution of the parental generation (blue curves). However, the two groups of offspring will significantly differ from each other in their phenotypic distributions (red curves) when genetic variance contributes to the phenotypic variance (Griffiths et al., 2007). Furthermore, highly divergent lines produced by disruptive selection and their crosses are a valuable resource for study of the biochemical and physiological basis of the traits.

Moreover, these selected lines are valuable for identifying and mapping the genes affecting the trait.

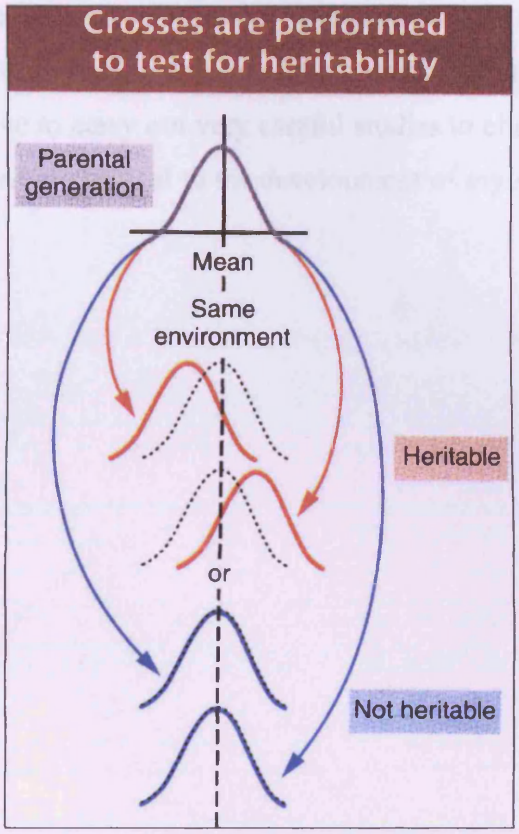


Figure 1.6 Standard method for testing for heritability in experimental selections. (From Griffiths et al. (2007))

1.5. Outline of the study

Because substantial inter-animal variation in response to an identical regimen of form deprivation has been observed in several studies (see section 1.3.4), genetic differences in the visual control of eye growth may exist. The purpose of our study was to test the hypothesis that the inter-animal variability in susceptibility to myopia is genetically determined. To test the hypothesis, we carried out a selective breeding experiment in chickens which were either (a) highly susceptible to, or (b) poorly susceptible to, environmentally-induced myopia. If the inter-animal variability in susceptibility is genetic in origin, then the selected lines will gradually diverge in their susceptibility. Then the extent to which the variation in susceptibility is due to genetics can be obtained from

estimating its heritability. Through selective breeding techniques, the genes involved in the control of ocular development in chicks can be isolated, and their relationship to the visual control of eye growth can be fully explored. If the variability is completely due to environmental variability, the selected lines will not diverge in their susceptibility and it will provide the incentive to carry out very careful studies to elucidate precisely which environmental conditions are critical to the development of myopia.

Chapter 2

Materials and Methods

2.1 Materials

Experimental myopia was induced in Lohmann strain White Leghorn chicks (Gallus gallus domesticus) obtained from Lohmann Tierzucht GMBH, Germany, as fertilized eggs. This Lohmann strain has been maintained by random mating of a very large population group undergoing selection for production traits by the breeding company and was expected to exhibit a high level of genetic diversity for eye traits.

Chicks were chosen for this experiment for several reasons. Firstly, chicks are highly visual animals with a cone-dominated retina that provides good visual acuity (Meyer and May, 1973; Schmid and Wildsoet, 1998; Dawkins and Woodington, 2000). Secondly, chicks are the most well established animal model of myopia, and highly precise ocular measurement techniques have been developed for them. The visually-guided eye growth of the chick has also been shown to use molecular mechanisms similar to that of primates (Wallman and Winawer, 2004). In addition, the considerable variability in the response to form-deprivation myopia in outbred strains (Wallman and Adams, 1987; Troilo et al., 1995; Schmid and Wildsoet, 1996; Guggenheim et al., 2002) implies that there is sufficient natural variation to offer the potential for significant genetic selection. Finally, chickens have been used successfully in several other selective breeding experiments (Kean et al., 1994; Lakshmanan, Gavora and Lamont, 1997; Shlosberg et al., 1998; Kjaer, Sørensen and Su, 2001).

For the first 4 days post-hatch, the chicks were raised in temperature-controlled (25–27°C) brooders with transparent Plexiglas sides and lid. After visual restriction was induced, chicks were transferred to a wire-mesh/transparent Plexiglas floor pen with a suspended infra-red heat lamp. Illumination in the brooder and floor pen was 250–300 lx with a 12:12-hour light/dark cycle. As the selected chicks grew, they were paired and transferred to individual, large floor pens maintained at ambient room temperature. Food and water were provided ad libitum. All experimental procedures involving animals complied with the U.K. legislation (Animals Act 1986), the European Communities Council Directive 86/609/EEC (1986) and were carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

2.2 Methods

2.2.1 Method to induce myopia: Form deprivation (FD)

To meet the requirements of the wide range of potential response for selective breeding, form deprivation was used as the method to induce experimental myopia in chicks due to the open-loop characteristics of FD-induced eye growth (section 1.3.1.1). Under general anaesthesia by an intramuscular injection of ketamine 50 mg/kg and xylazine 3.5 mg/kg, monocular deprivation of sharp vision was implemented by suturing a diffuser to the skin around the orbit of the treated eye with monofilament nonabsorbable suture material (Ethilone 4-0; Ethicon, Johnson & Johnson Intl., Norderstedt, Germany). After recovery from anaesthesia, the treated eye was observed to be able to open freely. The translucent diffusers were made from a sheet of 0.8-mm-thick polypropylene with an absorbance of 0.07 log units. The polypropylene sheet was heated and compression moulded into appropriately sized hemispheres. All diffusers were checked by eye for flaws in an attempt to ensure uniformity of shape and translucency. A diffuser was fitted to the treated eye by using three Ethilone sutures in the superior, inferior and lateral position of periorbital skin for the following reasons (Figure 2.1). Firstly, sutures provided better fixation of the diffusers compared to Velcro, which allowed the diffusers to be removed or decentred by chicks' claws. Three sutures provided enough strength to accurately fix the diffuser in place and yet allowed some space between the diffuser and the underlying feathers for evaporation of moisture, preventing it from "misting up" the diffuser. Secondly, Ethilone is a non-absorbable nylon suture material that causes minimal tissue reaction and provides enduring suture knots. On the other hand, using glue to attach the Velcro can result in tissue inflammation around the eye which might disturb the ocular response to form deprivation. Moreover, tissue damage from removal of Velcro was a common complication after treatment. In contrast, the sutures could be easily removed by cutting the suture knots, without detectable tissue damage. This was important for avoiding pecking each other for chicks kept for breeding.

In chicks, the ocular changes induced by visual deprivation are rapid early in life and evident after only 3 days of treatment (Wallman and Adams, 1987). Therefore, form

deprivation was induced in 4 days old chicks and maintained for 4 days. Under this regimen, the inter-animal variability in response to form deprivation would be evident enough to be discriminated after the 4-day period of form-deprivation treatment, while at the same time ensuring that the form-deprivation response had not yet reached its plateau.

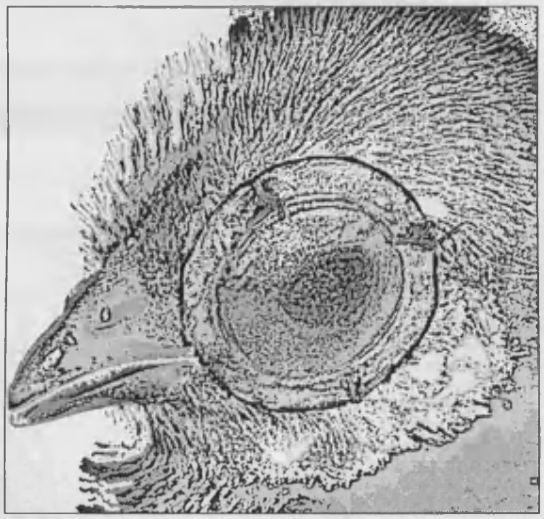


Figure 2.1 A diffuser fitted over the left eye of a chick.

2.2.2 Measurements and quantification of myopia susceptibility

2.2.2.1 High-frequency A-scan ultrasonography

Ocular component dimensions in chicks were measured using high-frequency A-scan ultrasonography. In this technique, a pulse of electricity is sent to the probe tip where a crystal element vibrates and emits a sound wave at a specific frequency. As the sound beams passes through the eye, it undergoes partial reflection at each interface of different acoustic impedance (i.e. at each ocular surface) as a series of echoes. Then, the echoes are received by the probe tip and converted back into electrical impulses, and displayed as a series of echo spikes. Therefore, each spike represents an interface between two media of different densities, which, in the eye, are comprised of the anterior corneal surface, the posterior corneal surface/aqueous interface, the aqueous/anterior lens surface, the posterior lens capsule/anterior vitreous, the posterior vitreous/retinal surface, the retina/choroid interface and the choroid/anterior scleral surface (Figure 2.2 A). Because the velocity of sound is determined completely by the density of the medium through which it passes, the distance between the spikes can be measured based on how long it takes the sound to

travel at a given velocity. By calculating the distance from different spikes, each ocular component dimension can be measured (Atta, 1996).

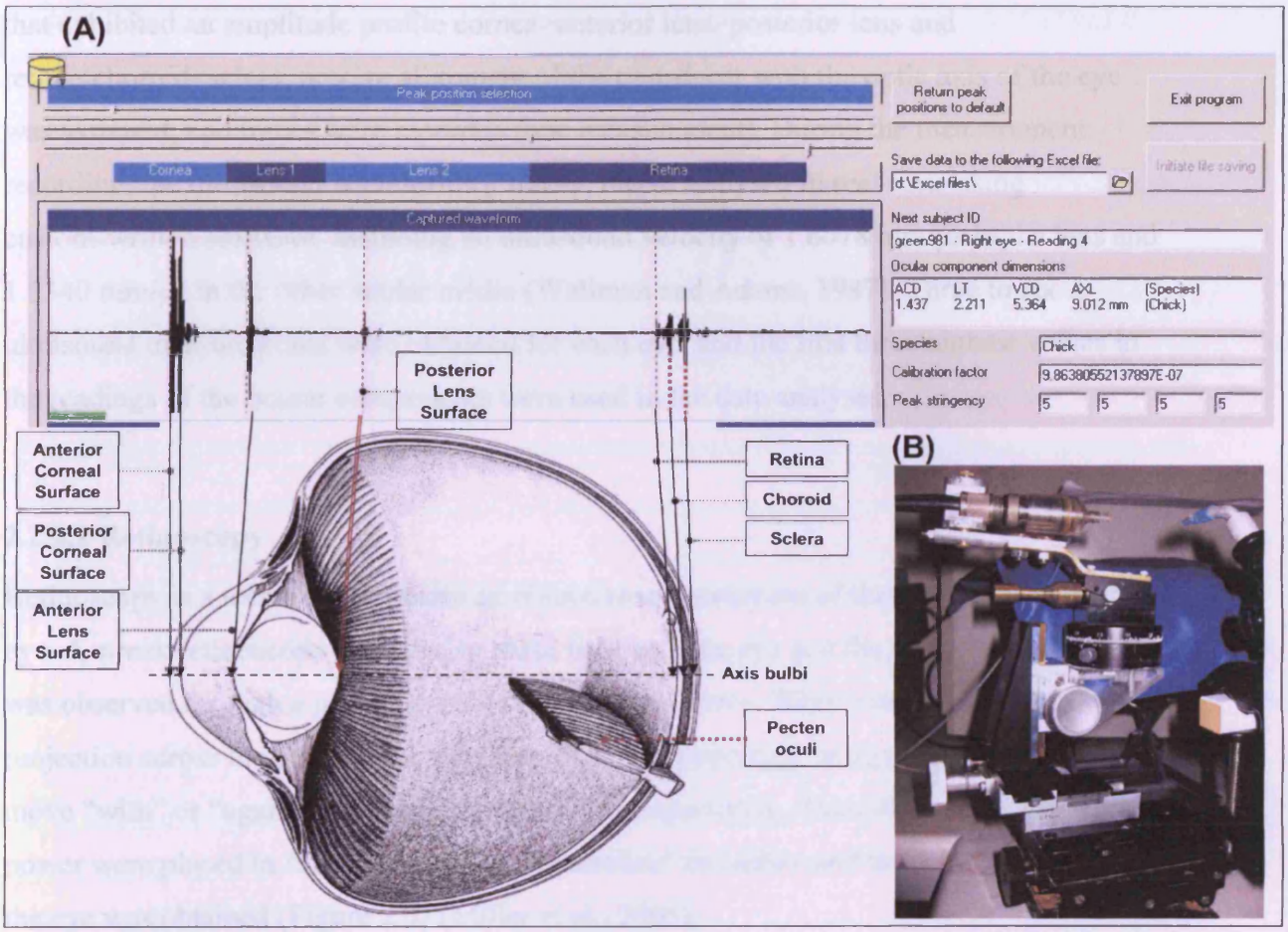


Figure 2.2 High-frequency A-scan ultrasonography.

(A) Ocular component dimension of chick's eye with corresponding echo spikes in A-scan ultrasonography. (B) Custom-made holding device for the transducer to facilitate the alignment of transducer and the optic axis. (The picture of a horizontal section of the chick eye was modified from Evans (1996))

The A-scan ultrasound system which was used in this study consisted of a 20 MHz transducer of focal length 25 mm fitted with a saline stand-off of 15 mm perfused at a rate of 0.15 l/min, a Panametrics model 5073PR pulser-receiver and a personal computer fitted with an Acqiris DP-110 data acquisition card. Traces were sampled at 100 MHz and files saved after averaging 50 traces. During the measurement process, the transducer was aligned with the optic axis of the eye with the assistance of a custom-made holding device,

which allowed translational movements of the transducer along the X, Y and Z axes of the eye position, plus rotational movement in the vertical (pitch) and horizontal (yaw) axes (Figure 2.2 B). When clear echo spikes of the ocular component interfaces were displayed that exhibited an amplitude profile cornea>anterior lens>posterior lens and retina<choroid<sclera, precise alignment of the transducer with the optic axis of the eye was assumed, and traces were recorded (one measurement). During the measurement recording, the ultrasound waveforms ("traces") were analysed in real-time using custom-written software, assuming an ultrasound velocity of 1.6078 mm/μs in the lens and 1.5340 mm/μs in the other ocular media (Wallman and Adams, 1987). Three to six ultrasound measurements were obtained for each eye, and the first three highest values in the readings of the ocular components were used in the data analyses.

2.2.2.2 Retinoscopy

Retinoscopy is a technique to obtain an objective measurement of the refractive state of the eye. A streak retinoscope was used to shine light into the eye and the reflex from the retina was observed through a peephole in the retinoscope mirror. When moving the streak projection across the pupil of the eye, the reflex of a hyperopic or myopic eye appears to move "with" or "against" the projection motion, respectively. Then, lenses of sufficient power were placed in front of the eye to "neutralize" the reflex and the refractive state of the eye was obtained (Figure 2.3) (Miller et al., 2005).

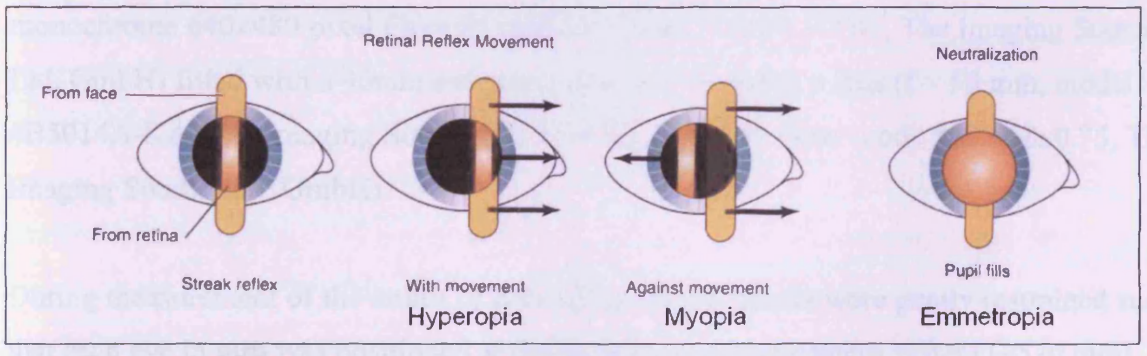


Figure 2.3 Retinal reflex movement in eyes with different refractive status. (Miller et al., 2005)

During measurement of refractive error, awake chicks were gently restrained such that each eye in turn was positioned approximately perpendicular to the light beam of the retinoscope at a distance of 50 cm. In the last generation only, post-treatment refractive error was measured in chicks under anaesthesia after eyelid removal. The final refractive error was obtained from the gross retinoscopy value minus the power of the corresponding working distance (50 cm → 2 dioptres). A hypermetropic artefact of retinoscopy has been disclosed in measuring animals with small eyes, possibly due to an optical reflection from the retina/vitreous interface (Glickstein and Millodot, 1970; Hughes, 1979). However, Mutti et al. (1997) found no significant difference in the refractive errors measured by retinoscopy and visual evoked potentials, suggesting that the source of the optical reflection in retinoscopy was near to the photoreceptors and thus, that the artefact from measuring small eyes by retinoscopy was smaller than previously assumed. In addition, the relative change in refraction between the treated and control eyes was the primary concern of this study. Thus, no correction for the small eye artefact was made for the measurements of refractive error.

2.2.2.3 Videokeratometry

The radius of corneal curvature (RCC) was measured using a modified videokeratometer in awake birds. This videokeratometer, which was based on the design of Schaeffel and Howland (Schaeffel and Howland, 1987), consisted of a 520 mm ring of 6 infrared LEDs (5 mm diameter, 875 nm; code #497-0486, RS Components Ltd, Corby, UK) attached to a monochrome 640x480 pixel Firewire camera (model #DMK 21F04, The Imaging Source Ltd, GmbH) fitted with a 40mm extension tube, 2X extender, a lens (f = 50 mm, model #B5014A-KA, The Imaging Source Ltd, GmbH) and an IR filter (code #092-58x0.75, The Imaging Source Ltd, GmbH).

During measurement of the radius of corneal curvature, chicks were gently restrained such that each eye in turn was positioned at the focal plane of the camera system (~520 mm) (Figure 2.4 A). The ring of reflected LED point sources was centred with respect to the centre of the cornea, whose position was gauged from the IR reflectance of the feathers surrounding the chick's eye. Images of the LED point sources reflected by the cornea were sampled in real time and used to calculate corneal curvature in the two principle meridians

using custom-written software (Figure 2.4 B). Data were automatically saved to an Excel spreadsheet. Six measurements were obtained for each eye, and mean keratometry readings were used in the data analyses. The videokeratometer was calibrated daily using a series of precision steel balls of known diameter. This modified instrument was only built in the final year of the project, and thus RCC measurements were only carried out in the selectively bred chicks of the third generation.

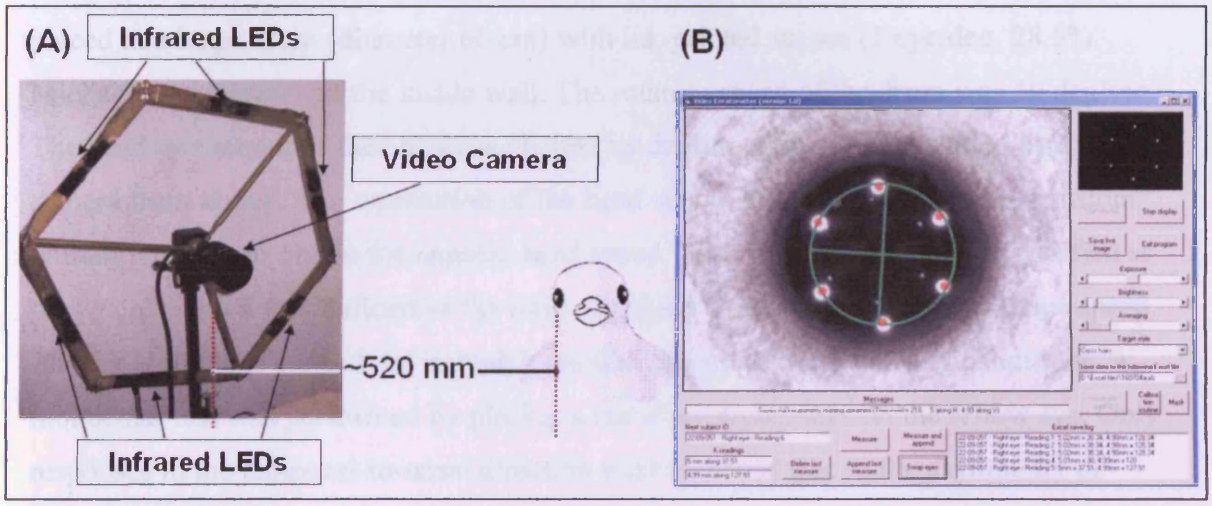


Figure 2.4 Videokeratometer for measurement of the radius of corneal curvature. (A) The modified videokeratometer consisted of a ring of 6 infrared LEDs and an infrared-sensitive video camera. (B) Corneal curvature was obtained by analysing the reflected LED points on the cornea using custom-written software.

2.2.2.4. Optokinetic nystagmus responses

Optokinetic nystagmus (OKN) is a visuomotor reflex stimulated by moving objects in a constant direction of the visual field. This eye movement consists of a slow phase in the tracking direction and a fast phase in the opposite direction for eye resetting. It has been observed in fish (Easter, 1972), birds (Gioanni et al., 1981, Wallman and Velez, 1985) and primates (Koerner and Schiller, 1972) with variations in the pattern of response between species. For example, head tracking movements have been detected in chicks due to their limited range of eye movement. In addition, OKN has been used as a method to measure visual function in chick (Schmid and Wildsoet, 1998) and human (Lewkonia, 1969).

During the process of emmetropisation, regulation in the growth of the ocular component dimensions is dependent on visual input. A difference in the rate of eye growth may occur as a result of disparity in visual function. Therefore, a modified optokinetic nystagmus paradigm (Diether and Schaeffel, 1999; Feldkaemper et al., 1999) was used to test if there was any deficit in visual function in chicks from the two selected lines (Figure 2.5). A black leather pad (approximately 15x 5mm) with two white spots was attached on top of the head in chicks to represent the orientation of the head. Then, chicks were individually placed in a large drum (diameter 66 cm) with ink-printed stripes (1 cyc/deg, 28.5% Michaelson contrast) on the inside wall. The rotating speed of the drum was 50 deg/sec. The head movement of the chickens elicited by drifting stripes was recorded by a video camera from above. The orientation of the head was tracked and analysed by a custom-written program to obtain the angular head speed. The visual function was quantified as the "gain", which was defined as the ratio of angular head speed to angular stripe speed. The OKN test was carried out in both binocular and monocular viewing conditions. A monocular test was performed by placing a translucent occluder on the fellow eye. Only responses in the temporal-to-nasal direction were recorded due to the asymmetry of monocularly elicited OKN in chick (Wallman and Velez, 1985). (This work was carried out at the Institute for Ophthalmic Research, Department of Pathophysiology of Vision and Neuro-ophthalmology, Tübingen, Germany during a lab-exchange.)

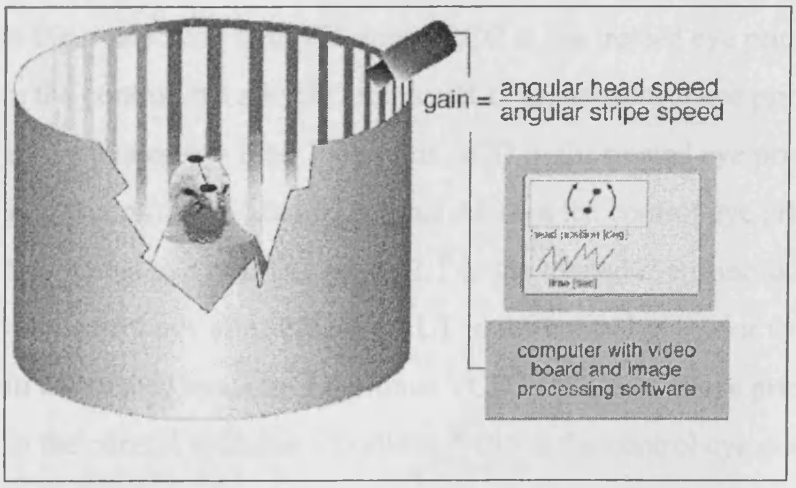


Figure 2.5 The optokinetic nystagmus testing paradigm. Optokinetic nystagmus responses in chicks were determined by recording the smooth head pursuit that was elicited by drifting stripe patterns inside the drum (reproduced from Feldkaemper et al. (1999)).

2.2.2.5. Quantification of myopia susceptibility

Ocular measurements were carried out before and after the period of form deprivation, with the experimenter masked regarding the line (High or Low) from which the chicks were derived. High-frequency A-scan ultrasonography was used to measure ocular component dimensions, including anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL), in chickens under anesthesia. The refractive state of each eye was measured using non-cycloplegic streak retinoscopy, immediately after removal of the occluder. The radius of corneal curvature (RCC) was measured using a modified videokeratometer. Changes in ocular component dimensions after form deprivation were compared between the treated and control eyes of each chick as follows:

$$\Delta RCC = \Delta RCC_T - \Delta RCC_C$$

$$\Delta ACD = \Delta ACD_T - \Delta ACD_C$$

$$\Delta LT = \Delta LT_T - \Delta LT_C$$

$$\Delta VCD = \Delta VCD_T - \Delta VCD_C$$

$$\Delta AL = \Delta AL_T - \Delta AL_C$$

$$\Delta RX = RX_T - RX_C$$

Where,

ΔRCC_T= RCC in the treated eye after FD minus RCC in the treated eye prior to FD

ΔRCC_C= RCC in the control eye after FD minus RCC in the control eye prior to FD

ΔACD_T= ACD in the treated eye after FD minus ACD in the treated eye prior to FD

ΔACD_C= ACD in the control eye after FD minus ACD in the control eye prior to FD

ΔLT_T = LT in the treated eye after FD minus LT in the treated eye prior to FD

ΔLT_C = LT in the control eye after FD minus LT in the control eye prior to FD

ΔVCD_T= VCD in the treated eye after FD minus VCD in the treated eye prior to FD

ΔVCD_C= VCD in the control eye after FD minus VCD in the control eye prior to FD

ΔAL_T = AL in the treated eye after FD minus AL in the treated eye prior to FD

ΔAL_C = AL in the control eye after FD minus AL in the control eye prior to FD

RX_T = RX in the treated eye after FD

RX_C = RX in the control eye after FD

According to previous studies of experimentally-induced myopia in chickens, the experimentally-induced myopia is mainly the result of an increase in the vitreous chamber depth of the eye (Hayes et al., 1986; Wallman and Adams, 1987; Wallman et al., 1987). In addition, axial length reflects the summation of the changes in all ocular components during the emmetropisation process. Therefore, the relative changes in AL, VCD and RX (i.e. ΔAL, ΔVCD, ΔRX) were chosen to quantify myopia susceptibility. Since ΔAL, ΔVCD and ΔRX were highly correlated, ΔAL was used as the primary indicator of myopia susceptibility when selecting chicks. However, for chicks in whom ΔAL values were similar, those with the "better" ΔVCD and ΔRX values were chosen.

2.2.3 Sex identification in chicks

A PCR-restriction enzyme digest assay using DNA extracted from a blood sample was used to identify the sex of young chicks (Guggenheim et al., 2002) due to the difficulty of sexing them by their appearance. The PCR method is a test based on two conserved Chromo-Helicase-DNA-binding (CHD) genes (Griffiths, Daan and Dijkstra, 1996; Griffiths et al., 1998; Nota and Takenaka, 1999). CHD genes are located on the avian sex chromosomes of all birds. In chicks, the sex chromosomes are Z and W (instead of X and Y in mammals). Furthermore, males are homozygous ZZ, and females are heterozygous ZW. The CHD-W gene is located on the W chromosome and is unique to females, whereas the other gene, CHD-Z, is found on the Z chromosome and hence occurs in both sexes.

2.2.3.1 DNA extraction

Blood samples were taken from chicks either during form-deprivation surgery (from a wing vein) or after selection (by cardiac puncture after injection of an overdose of Euthatal). A 100μl aliquot of 200mM ethylene diamine tetraacetic acid (EDTA) was drawn into a syringe to mix with the collected blood sample of approximately 2ml. The role of EDTA was to prevent clotting (0.1ml 200mM EDTA to 2ml blood). DNA was extracted from blood with the following protocol:

A. A 15µl aliquot of EDTA-mixed blood was put in a 1.5ml microfuge tube with 800µl TES solution (250mM Tris, 25mM EDTA and 2% Sodium dodecyl sulfate, pH=8.0) to lyse cells. This mixture was pipetted gently up and down until the solution was homogeneous.
B. RNA was removed by adding 1.5µl RNAse solution (100mg/ml stock, RNase A, QIAGEN) at 37°C incubation for 30 minutes.
C. After cooling the solution to room temperature, proteins were precipitated by adding 200µl cold ammonium acetate (7.5M, 4°C) and 100µl chloroform, followed by vortex mixing for 20 seconds and centrifuging at maximum speed for 3 minutes (14,000g in a microcentrifuge).
D. The upper phase of the liquid was harvested into a fresh 1.5ml tube. DNA was precipitated after adding 700µl isopropanol to the harvest with gentle mixing and centrifuging at 14,000g for 2 minutes.
E. After pouring off the supernatant, the DNA pellet was washed with 200µl 70% ethanol and centrifuged at 14,000g for 2 minutes. After pouring off the supernatant, the DNA pellet was allowed to air dry for at least 15 minutes.
F. The DNA pellet was re-suspended in 100µl TE solution (10mM Tirs, 1mM EDTA) by incubation overnight at 37°C.

2.2.3.2 Polymerase chain reaction and restriction fragment length polymorphism (RFLP)

The Polymerase Chain Reaction (PCR) technique was developed to provide highly efficient amplification of DNA sequences of interest. PCR entails the use of a pair of primers which are complementary to a defined sequence on each of the two strands of the DNA. These primers are extended by a DNA polymerase so that a copy is made of the designated sequence. After making this copy, the newly synthesized DNA sequences can also be used as templates and the same primers can be used again for the subsequent cycles. This leads to an exponential amplification of the DNA sequences of interest.

After extracting DNA from chicks' blood, PCR was implemented to amplify parts of the CHD-W and CHD-Z sequences. Each PCR reaction was performed using 10µl of approximately 10µg/ml DNA solution (i.e. 100ng DNA) plus 20µl of a master mix

solution which contained 4.0μl 10X PCR Buffer (QIAGEN), 4.0μl 2mM dNTP mix, 3.2μl 25mM MgCl2, 2.0μl 20μM CHD-UP1 and CHD-DN1 primers, 13.8μl PCR water and 1.0μl 1000U/ml Taq DNA polymerase (New England BioLabs). PCR programs comprised a step at 95°C for 5 min followed by 35 cycles of 94°C for 1min, 52°C for 1min and 72°C for 1min. A final extension step of 72°C for 5 min was carried out for all reactions. PCR was performed in a PROGENE machine (Techne-Cambridge).

The PCR primer sequences were:

CHD-UP1 5' CTC CCG AGG ATG AGA AAC TG,

CHD-DN1 5' TCT GCA TCA CTA AAT CCT TT

Unfortunately, the size of PCR product of the CHD-Z sequence (345 base pairs) is similar to that of the PCR product of the CHD-W sequence (362 base pairs). Thus, these two PCR products cannot easily be differentiated by gel electrophoresis. Therefore, an RFLP method with HaeIII was carried out to differentiate these PCR products. HaeIII is a restriction enzyme whose recognition sequence is GGCC (Figure 2.6 A). There is a restriction site for HaeIII in the CHD-Z sequence, but not in CHD-W. So this restriction enzyme, HaeIII, can selectively cut a fragment from the CHD-Z products before gel electrophoresis. In the process of enzyme digestion, PCR reactant (30μl) was mixed with 3.55μl restriction enzyme solution (0.35μl HaeIII buffer (New England BioLabs), 0.2μl 10,000U/ml Hae III (New England BioLabs) and 3.0μl distilled water) and incubated at 37°C for at least 3 hours. After digestion of the PCR product by restriction enzyme, the sizes of the major products from the CHD-Z amplicons are 280 and 65 base pairs, which can therefore easily be distinguished from the 362 base pair CHD-W product by gel electrophoresis. After adding 10μl of loading buffer (2.4μl of 15% Ficoll 400, 0.5% xylene cyanol FF, 10mM EDTA, 1:50 dilution of SYBR Green I stock solution (Molecular Probes Ltd, Paisley, UK) and 8.6μl distilled water) to the digested PCR products, they were separated on 2% agarose gels and visualised under UV light. Finally, the sex of each chick could be identified as two visible DNA bands in female samples, but only a single band in male samples (Figure 2.6 B). Note that the 65bp fragment was barely visible on the gel.

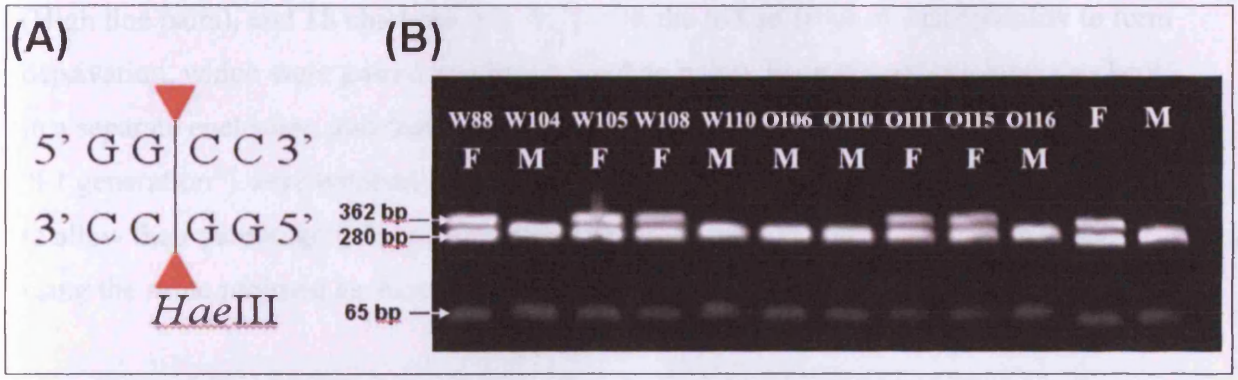


Figure 2.6 PCR-RFLP for sex identifying in chicks.

(A) The recognition sequence of restriction enzyme HaeIII. (B) There were two visible DNA bands in female and only one in male on the gel electrophoresis after digesting PCR products by HaeIII. Internal standards (labelled "F" and "M") were included to ensure the RFLP protocol had occurred successfully.

2.3. Selective breeding procedures

The outbred, Lohmann strain White Leghorn chickens used in the initial round (R1) of selection were obtained from Lohmann Tierzucht Ltd (Cuxhaven, Germany) as fertilized eggs, as described above. Eggs were hatched in batches of approximately 20-30 chicks. The outbred chickens were assumed to be unrelated to one another since they were sourced from an extremely large WL breeding population. As prescribed previously, form deprivation was induced in chicks beginning at 4 days post-hatch and maintained for 4 days. Subsequently, the susceptibility to form deprivation of each chick was quantified and compared amongst chickens in the same batch and the sample of chickens that had already been selected from previous batches. Chickens with highest and lowest degree of susceptibility to form deprivation were retained for breeding. The sex of each chick was determined by the PCR-RFLP method, as described above (an exception was that a small number of chickens were kept until adulthood, in whom sex was apparent from secondary sexual characteristics). The selective breeding process is summarized in Figure 2.7.

From 232 outbred chickens treated in the first round of selection (R1), 36 chickens (18♂, 18♀) were retained for breeding. These were comprised of 18 chickens (9♂, 9♀) with the highest level of susceptibility to form deprivation, which were paired together

(High line pairs), and 18 chickens (9♂, 9♀) with the lowest level of susceptibility to form deprivation, which were paired together (Low line pairs). Each pair of chickens was kept in a separate enclosure, and their eggs were labelled when collected (daily). Chicks (the "F1 generation") were hatched individually in hatching boxes and tagged with a wing band to allow their parentage to be ascertained. The F1 generation chicks were form deprived using the same regimen as above (selection round R2).

A total of 267 F1 chicks were assessed in R2 (144 chicks from the High line and 123 from the Low line). Since susceptibility to form-deprivation myopia is partially dependent on sex (Zhu et al., 1995; Schmid and Wildsoet, 1996), we aimed to screen at least 5 male and 5 female offspring from each set of parents. Furthermore, to maximise genetic diversity and reduce inbreeding depression, we aimed to select one male and one female from each set of parents, thus ensuring each set contributed equally to the next generation. Within these caveats, the 18 F1 chickens (9♂, 9♀) with the highest degree of induced myopia from the High line parents were selected for breeding, as were the 16 F1 chickens (8♂, 8♀) with the lowest degree of form-deprivation myopia from the Low line parents. The chickens within each susceptibility group were paired, making sure that the male and female of each pair were unrelated to one another. Offspring (the F2 generation) from the F1 parents were hatched as described above. In R3, a total of 392 F2 chicks were form deprived and their susceptibility to form deprivation was assessed (200 and 192 chickens from the High and Low lines, respectively).

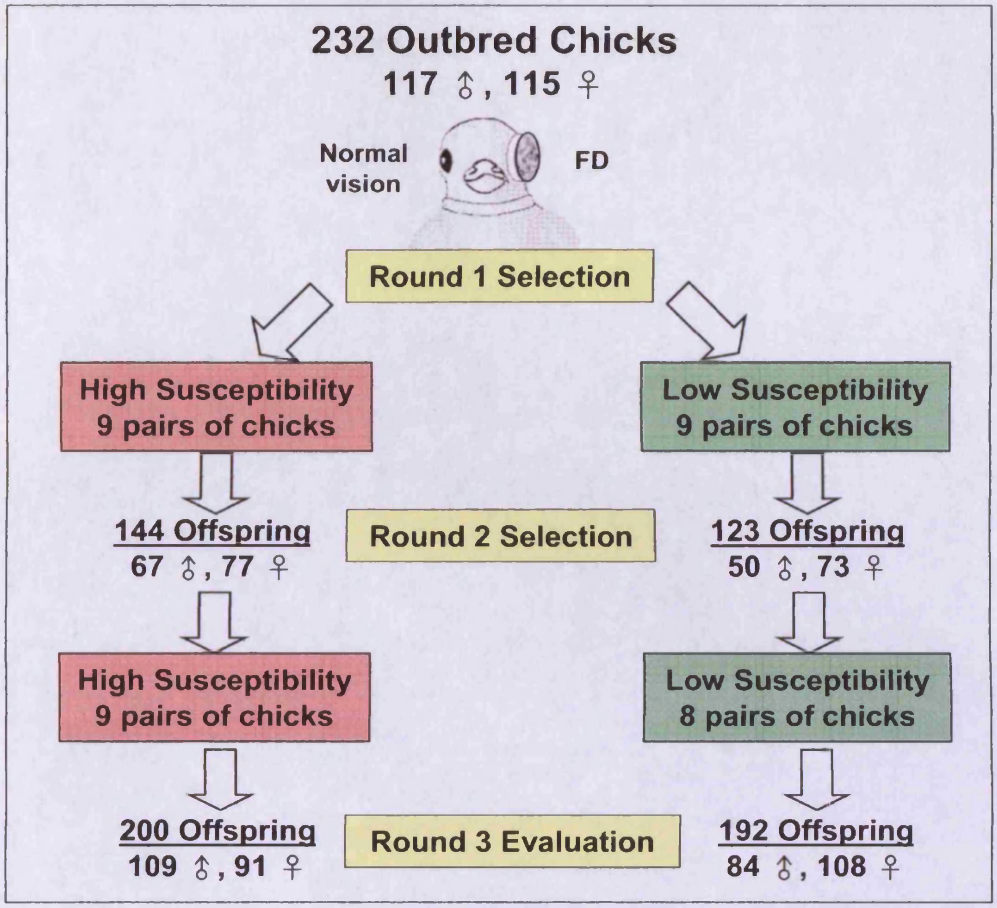


Figure 2.7 The selective breeding process for chicks with high or low susceptibility to form-deprivation myopia.

Potential limitations of this selective breeding strategy are lack of a control strain and replication. A control strain derived by randomly selecting animals for breeding according to the same breeding system would be a useful control for the effects of genetic drift and environmental fluctuations from the assessment of selection response. Running the replication concurrently with the main breeding experiment would provide information regarding the impact of genetic drift on this breeding population (Falconer and Mackay, 1996). Although limited capacity in our animal facility prevented us from setting up a control strain or undertaking replication, an increase in the numbers of chickens measured (> 200 chickens per generation) and selected (36 and 34 chickens in first and second generations) is likely to have reduced the variation due to genetic drift. In addition, the environmental fluctuations (batch-to-batch variability) were also taken into account in the data analysis using general linear model (section 3.2.2) and a household effect in the SOLAR program (section 2.4.2.2).

2.4. Statistical analysis

2.4.1. Analysis of the changes in ocular component dimensions and refractive error

All data for the pre-treatment ocular component dimensions and the changes after form deprivation, such as ΔACD, ΔLT, ΔVCD, ΔAL and ΔRX were tested for normality using the Kolmogorov-Smirnov test. Due to a non-normal distribution of ΔRX, the differences in ΔRX between the High and Low lines were compared using the Mann–Whitney U test. Comparisons of the ocular component dimensions and their FD-induced changes between the two selected lines were made using independent samples *t*-test. Pearson's (parametric) correlation coefficient was used throughout, except for tests involving the change in refractive error, in which case Spearman's (non-parametric) correlation coefficient was used. All analyses were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL).

2.4.2. Quantitative genetic analysis of heritability and genetic correlations

2.4.2.1. Variance components analysis

For polygenic quantitative traits, the total phenotypic variance (V_P) is composed of additive genetic variance (V_A), dominance genetic variance variance (V_D), gene-interaction variance (V_I) and environmental variance (V_E) (Falconer and Mackay, 1996):

$$V_P = V_A + V_D + V_I + V_E$$

Narrow sense heritability is defined as the proportion of phenotypic variance that is attributed to additive genetic variance (Falconer and Mackay, 1996):

$$h^2 = V_A / V_P$$

Before obtaining heritability estimates, it is essential to partition the total phenotypic variance into these components. Thus, variance components analysis was carried out on account of the random-effect character of these components. This analysis is an extension of the analysis of variance by using a random effects model to investigate the amount of

variance of a dependent variable that can be explained by one or more random-effects variables. It decomposes the phenotypic variance mainly into genetic variance and environmental variance which includes measurement errors and residual variance. The genetic variance can be further broken down into additive genetic variance and dominant genetic variance. Therefore, the heritability can be obtained from the ratio of additive genetic variance to total phenotypic variance. To estimate these variance components, maximum likelihood (ML) statistical procedures were used in this study for two reasons. Firstly, ML-based variance components analysis can take all the genetic relationships of the data into account. In addition, balanced designs are not required in this procedure. Hence, it can be applied to selection data, and will utilise all of the available genetic information to improve the accuracy of heritability estimation. Briefly, ML procedures calculate the probability that observed data can be obtained, given specific numerical starting values for the parameters and iteratively optimising the parameter values to identify those which give the maximum probability (Lange, Westlake and Spence, 1976; Elston and Rao, 1978; Falconer and Mackay, 1996).

2.4.2.2. Univariate and bivariate genetic analysis

Both univariate and bivariate genetic analyses were carried out using variance components analysis with the software package Sequential Oligogenic Linkage Analysis Routines (SOLAR, version 4.2.7) (Almasy and Blangero, 1998). Univariate analysis was used to calculate heritability estimates for ocular traits and susceptibility to form deprivation. The total phenotypic variance of the trait is partitioned into an additive genetic component and an environmental component that includes the non-additive genetic component, environmental factors and measurement errors. The narrow sense heritability was estimated as the proportion of total phenotypic variance of the trait due to additive genetic effects. Optimizations of parameter estimation were carried out using the maximum likelihood method implemented in SOLAR, with sex included as a covariate as well as estimation of household effects to take batch-to-batch variability into account..

In bivariate genetic analysis, the total phenotypic correlation between two traits (ρ_P) is partitioned into a genetic correlation (ρ_G) and an environmental correlation (ρ_E) (Lynch and Walsh, 1998):

$$\rho_P = \rho_G (h_1^2 \times h_2^2)^{1/2} + \rho_E ((1 - h_1^2) \times (1 - h_2^2))^{1/2}$$

where h_1^2 and h_2^2 denote the heritabilities of trait 1 and trait 2, respectively. The pairwise genetic correlation between two traits indicates the degree of shared genetic determination. Again, sex and household effects were taken into consideration in the analyses.

Chapter 3

The effects of sex, body weight and eye size on susceptibility to form-deprivation myopia

3.1 Introduction

3.1.1 The relationships amongst sex, body stature and myopia in humans

Association amongst sex, body stature and myopia has been observed in human epidemiological studies, although some results are conflicting. Differences in myopia prevalence between the sexes are frequently found (Table 3.1). For instance, Matsumura and Hirai (1999) reported a significant gender difference in the change of refractive error during a 6-year follow-up in a mass ophthalmic survey of Japanese students, and Saw et al. (2008) recently revealed that being of female sex was significantly associated with myopia (OR=1.87) in the Singapore Malay population. In addition, sex was found to be a significant covariate in estimating heritabilities for refractive error and ocular component dimensions in the Genes in Myopia family study (Chen et al., 2007). However, some studies did not find a gender difference in the prevalence of myopia (Multi-Ethnic Pediatric Eye Disease Study Group, 2010, Anton et al., 2009, Junghans and Crewther, 2003).

In terms of body stature, height and body weight have been revealed to relate to the ocular component dimensions, and to refractive error, after adjustment for confounding factors. However, the results have been inconsistent across studies (Table 3.2). Wong et al. (2001a) found height was positively correlated to ocular component dimensions, but did not appear to influence refraction in adults. Saw et al. (2002a) observed that taller children tended towards myopia with longer axial length after controlling for age, sex, parental myopia, reading, school, and body weight. These studies suggest that emmetropisation mechanisms may relate to body growth. In addition, both studies revealed that subjects with heavier body weight tended to be more hyperopic. Krause et al. (1982) disclosed a similar trend that myopic children were taller, but they were also heavier than the others. Teikari (1987) revealed that myopic subjects were taller than the non-myopic ones in only males, and that no association between myopia and body weight was observed in a Finnish population. Nonetheless, myopic males were found to be shorter in height and lighter in weight in Israeli military recruits (Rosner, Laor and Belkin, 1995). Moreover, neither height nor body weight was correlated significantly with myopia in a population of Australian

children, although taller children did have longer axial lengths and flatter corneas (Ojaimi et al., 2005).

Generally speaking, axial length appears to be an important determinant of refraction (Saw et al., 1996; Ip et al., 2007; Olsen et al., 2007). Children with myopic parents have longer premyopic eyes and an increased risk of myopia (Zadnik et al., 1994; Lam et al., 2008b). In addition, axial length has been revealed to correlate with body stature significantly (Wong et al., 2001a; Saw et al., 2002a; Eysteinsson et al., 2005; Ojaimi et al., 2005; Wu et al., 2007; Lee et al., 2009). Furthermore, ocular component dimensions have also been found to differ by sex (Wong et al., 2001b; Shufelt et al., 2005). Males are also generally taller and heavier than females. Nonetheless, as noted above, a higher prevalence of myopia in females than in males has been reported in some, but not all, studies (Table 3.1). Hence the interrelationships amongst sex, body stature and ocular dimension on refractive error appear to be complex and intertwined. The true relationships amongst them are still veiled- probably due to inconsistent results from epidemiological studies, which could have arisen from sampling variations (sample size, age difference), differences in genetic background across populations, methodology (data collection), confounding variables and environments. Despite the fact that animal models of myopia may be not totally equivalent to human myopia, the relationships between sex, body size, eye size and refractive error can be studied and clarified under controlled experimental conditions in animal models.

Table 3.1 Prior studies have disclosed a sex difference in the prevalence of myopia in human subjects.

Study	Ethnicity	Definition of myopia	Sample size (M/F)	age	Myopia prevalence (M/F)	Study type
Goldschmidt (1968)	Caucasian	<-1.50D	9,243 (4,605 / 4,638)	13~14	Total: 5.1% / 6.7% Academic: 7.3% / 9.4%	Cohort
Krause et al. (1982)	Caucasian	SE <-0.25D, Cycloplegia: (+): 84.6%; (-): 15.4%	1939 (717 / 1,222)	~15	11.88% / 24.14%	Cohort
Matsumura and Hirai (1999)	Asian	Mean spherical power <-0.50D	346	3~17	Boys: 40.4% →66.0% Girls: 46.3% →66.7% Higher progression rate in boys (P<0.0001)	Cohort
Lin et al. (1999)	Asian	SE <-0.25D, Cycloplegia (+)	11,178 (5,676 / 5,502)	7~18	50%/58%	Cross-sectional
Wong et al. (2000)	Asian	SE <-0.50D, Cycloplegia (-) High Myopia: SE<-5.00D)	1,113 (500 / 613)	40~79	Myopia: 33.0% / 36.5% High Myopia: 5.2% / 8.3%	Cross-sectional
Midelfart et al. (2002)	Caucasian	SE <-0.50D, Cycloplegia (-)	3137 (1418 / 1719)	20~25 & 40~45	20~25 y/o: 33.2% / 36.4%; 40~45 y/o: 28.1% / 32.3%	Cross-sectional
Zhao et al. (2002)	Asian	SE <-0.50D, Cycloplegia (+)	4621 (2384 / 2237)	5~13	Myopic shift > 0.50D associated with female sex (OR= 1.89)	Cohort
Bourne et al. (2004)	Asian	SE <-0.50D, Cycloplegia (-)	11189 (5489 / 5700)	>30	Male: 26.3% Female: 21.0%	Cross-sectional
He et al. (2007)	Asian	SE <-0.50D, Cycloplegia (+)	2400 (1222 / 1178)	13~17	Higher prevalence in females (OR= 2.15)	Cross-sectional
Xu et al. (2005)	Asian	SE <-0.50D, Cycloplegia (-)	4319 (2424 / 1895)	40~90	Higher prevalence in females (P<0.001)	Cohort
Saw et al. (2008)	Asian	SE <-0.50D, Cycloplegia(-)	2974 (1427 / 1547)	40~79	22.2% / 26.8% (OR=1.87, P=0.015)	Cross-sectional
Vitale et al. (2008)	Mixed	SE <-1.00D, Cycloplegia (-)	12010 (5790 / 6220)	>20	32.6% / 39.9% (P<0.001)	Cross-sectional

D: Dioptre, OR: odds ratio, SE: Spherical Equivalents.

Table 3.2 Prior studies investigated on the association between body stature and ocular biometry/myopia in human subjects.

Study	Ethnicity	Sample size	Age	Myopia	Height		Body weight		BMI & Myopia	Data collection	Confounders control
					Myopia	Ocular Biometry	Myopia	Ocular Biometry			
Goldschimidt (1966)	Caucasians (military recruits)	3511 (male only)	18~20 mainly	≤-0.50D	Non-significant	-	-	-	-	Measurements	social class (occupation)
Krause et al. (1982)	Caucasians	1939	~15	≤-0.25D	**taller**	-	**heavier**	-	-	Questionnaires, Medical records	Sex, social class
Teikari (1987)	Caucasians (twins)	790	30~31	≤-0.25D	**taller** (only in male)	-	Non-significant	-	**smaller** (only in male)	Questionnaires, prescription	Sex
Rosner et al. (1995)	Israelis (military recruits)	106926 (male only)	17~19	≤-0.25D	**shorter**	-	**lighter**	-	**smaller**	Measurements	Sex, education, intelligence
Wong et al. (2001a)	Asians	951	40~81	Continuous variable*	Non-significant	Taller: deeper ACD, longer VCD & AL, thinner Lens, flatter Cornea	Non-significant (Heavier persons tended to be more hyperopic)	Non-significant	Non-significant (Persons with higher BMI tended to be more hyperopic)	Measurements	Age, sex, education, occupation, housing type, income
Saw et al. (2002a)	Asians	1449	7~9	Continuous variable*	**taller**	Taller: deeper ACD, longer VCD & AL, thinner Lens, flatter Cornea	Non-significant (Heavier children tended to be more hyperopic)	Heavier boys: shorter VCD & AL Heavier girl: longer VCD & AL, thinner Lens, flatter Cornea	Non-significant (Children with higher BMI tended to be more hyperopic)	Measurements	Age, sex, parental myopia, reading, school
Ojaimi et al. (2005)	Mixed	1740	6~9	Continuous variable*	Non-significant	Taller: longer AL, flatter Cornea	Non-significant	Non-significant	Non-significant	Measurements	Age, sex, parental myopia
Wu et al. (2007)	Asians	2418	≥40	Continuous variable*	Non-significant	Taller: deeper ACD, longer VCD & AL, flatter Cornea	**Lighter**	Heavier: deeper ACD, longer VCD & AL, flatter Cornea	**smaller**	Measurements	Age, sex
Jacobsen et al. (2007)	Mixed (military recruits)	4681 (male only)	18	≤-0.50D	Non-significant	-	Non-significant	-	Non-significant	Medical records	Sex
Dirani et al. (2008)	Caucasians (Twins)	2448	18~86	≤-0.50D	Non-significant	-	**Heavier** (only in female)	-	Non-significant	Measurements	Age, sex, education

Significant associations between body stature and myopia are marked in bold. "*": Refraction treated as a continuous variable.

3.1.2. The effect of sex, eye size and body size on animal models of myopia

Animal studies reveal consistently positive correlations between eye size and body size in fish (Shen and Sivak, 2007), mouse (Zhou and Williams, 1999b) and chicken (Prashar et al., 2009). Howland et al. (2004) analysed data from the literature and also disclosed that axial length of vertebrate eyes followed a logarithmic relationship with body weight. Additionally, differences in body size between males and females can be found in nearly all animals (Fairbairn, 1997). Zhou et al. (1999a) reported that approximately 57% of the variance in eye weight can be explained by age, sex, body weight and brain weight concurrently in the mice of 26 BXD strains. Similarly, Prashar et al. (2009) found that body size and sex together predicted 51~56% of the variation in ocular biometry, except lens thickness, in an advanced intercross line of Broiler and White Leghorn chickens.

When myopia is induced by a uniform regimen of form deprivation, considerable inter-animal variability in the degree of developed myopia is often observed in animal models (Troilo et al., 1995; Schmid and Wildsoet, 1996; Guggenheim et al., 2002; Shen and Sivak, 2007). In chickens, this differential susceptibility to myopia occurs not only between strains (Table 3.3), but also within strains (Troilo et al., 1995, Schmid and Wildsoet, 1996, Guggenheim et al., 2002). Little is known about what causes this variability. Zhu et al. (1995) found that male chickens normally developed eyes with slightly longer anterior and vitreous chambers and were more susceptible to form-deprivation myopia than females. Guggenheim et al. (2002) also discovered minor sex differences in the response to visual deprivation in three chicken strains. Males developed approximately 0.2mm longer vitreous chamber depths and axial lengths than females, but no significant difference in the degree of induced myopia was found. However, Schmid and Wildsoet (1996) did not observe a sex difference in susceptibility to visual deprivation after lid suture for two weeks in White Leghorn chickens. Furthermore, studies in fish suggested that body weight influenced susceptibility to form-deprivation myopia (Shen and Sivak, 2007).Yet, it should be noted that this result could be subject to confounding, since weight and age are correlated, and susceptibility to form-deprivation myopia was deemed to decrease with age. The inconsistency in these results of susceptibility to

environmentally-induced myopia could be due to sampling variations (small sample size), different genetic backgrounds and different visual deprivation methods (occluder or lid suture).

The first round selection from an outbred population of White Leghorn chickens under a uniform regimen of form deprivation provided a good sample size to examine the effects of sex, body weight and eye size on susceptibility to form-deprivation myopia. Moreover, the relationships between these parameters were also explored using potential causal models.

Table 3.3 Prior studies examining susceptibility to form-deprivation myopia in different chicken strains.

Chicken strains in the study		No.	Visual deprivation	Body weight	Ocular Biometry		Induced Myopia
					Control eyes	Treated eyes	
Troilo et al. (1995)							
White Leghorns	Cornell-K	31	Occluder (full field, monocular)	No significant difference	Flatter cornea, thicker lenses, longer VCD & AL,	2 weeks: flattening of cornea 4 weeks: Less VCD elongation	2weeks: Less myopic 4 weeks: No significant difference
	Washington H & N	14			Steeper cornea, thinner lenses, shorter VCD & AL	2 weeks: Steepening cornea 4 weeks: More VCD elongation	2weeks: More myopic 4 weeks: No significant difference
Stone et al. (1995)							
White Leghorns (only the group under 12h light)	Cornell-K	13	Lid-suture (monocular) for 2 weeks	-	Thicker lenses , longer VCD & AL	Less ACD, VCD & AL elongation, thicker lenses	Less myopic
	Truslow	11			Thinner lenses , shorter VCD & AL	More ACD, VCD & AL elongation, thinner lenses	More myopic
Schmid and Wildsoet (1996)							
White Leghorns		8/ 8	Lid-suture / occluder (monocular) for 2 weeks	Lighter	Steeper cornea, shallower ACD, thicker lenses, shorter VCD & AL, more hyperopic	Steepening cornea, More ACD, VCD & AL elongation	More myopic
Broiler cross		6/ 6		Heavier	Flatter cornea, deeper ACD, thinner lenses, Longer VCD & AL, Less hyperopic	Flattening cornea, Less ACD, VCD & AL elongation	Less Myopic
Guggenheim et al. (2002)							
White Leghorn		10	Occluder (monocular) for 2 weeks	Lighter	Steeper cornea, shorter ACD, VCD & AL, more hyperopic	Thicker lenses	No significant difference
Brown leghorn		9		Midway	Thicker lenses	Thinner lenses, Less VCD elongation	
Broiler		11		Heavier	Flatter cornea, longer ACD , VCD & AL, thinner lenses, less hyperopic	More VCD elongation	

3.2 Materials and Methods

3.2.1 Subjects and inducing myopia

Monocular deprivation of sharp vision was carried out in an outbred population of White Leghorn chickens for 4 days, beginning when the chicks were 4 days old (section 2.2.1). Ocular component dimensions and body weight were measured before and after form deprivation (section 2.2.2.1). Ocular refraction was measured only after the treatment period (section 2.2.2.2). Myopia susceptibility was quantified using the relative changes in vitreous chamber depth (ΔVCD), axial length (ΔAL) and refraction (ΔRX) between the treated and control eyes (section 2.2.2.5). Because of the limited capacity in our animal facility, the experiment was performed in 15 separate hatchings (batches) of chicks. The number of chicks in each batch varied from 10 to 25 (mean=15). In total, 232 chicks were studied, comprising 117 males and 115 females. The sex of the chickens was determined by a PCR-restriction fragment length polymorphism method in 175 chicks (section 2.2.3), by observation of secondary sexual characteristics, such as larger comb and stature in the males and egg-laying in the females, in 32 chicks that were allowed to mature; and by both methods in 25 chicks. For the latter group of 25 chicks, the results of sex identification were fully concordant.

3.2.2 Statistical Analysis

The Kolmogorov-Smirnov test was used to test for the normality of the frequency distributions of measurements. The data for ΔRX and body weight were found to be non-normally distributed. Thus, the Spearman correlation coefficient was used to test the relationships involved ΔRX and body weight. Comparisons between the ocular component dimensions before and after form deprivation were made using paired *t*-test, and the Pearson's correlation coefficient was used to investigate the relationship between susceptibility to form deprivation and sex or initial ocular component dimensions (ocular component dimensions before form deprivation). Differences in ocular component dimensions between males and females were compared using independent *t*-test. The Mann-Whitney U test was used to test the difference in ΔRX and body weight between the sexes. A general linear model (GLM) was used to adjust measures of susceptibility to form deprivation for the effect of hatch-to-hatch variability (a "batch effect"). Multiple linear

regression analysis was performed to investigate the association between sex, body weight, eye size and myopia susceptibility.

3.2.3 Fitting of the potential causal models

Three potential causal models explaining the relationship between sex, initial eye size, and myopia susceptibility were proposed and examined by the partial correlation coefficients from the regression analysis (details described in section 3.3.4.1).

Structural Equation Modelling (SEM)

Structural equation modelling (SEM) is a statistical methodology that takes a confirmatory, i.e. hypothesis-testing, approach to the analysis of a structural theory bearing on some phenomenon. SEM integrates principles of factor analysis, path analysis and estimation techniques for model fitting. Thus, it is used for the analysis of multivariate data to measure underlying hypothetical constructs and their interrelationships (Violato and Hecker, 2007; Byrne, 2009).

In SEM, latent variables are the theoretical constructs which cannot be observed or measured directly. Nonetheless, some observable or measurable variables, which are linked and presumed to represent latent variables, can serve as their indicators. For example, the susceptibility to form-deprivation myopia is a latent variable because it is not directly measurable. Hence, we use the relative changes in VCD, AL and refraction after form deprivation to represent this susceptibility. These latent and observed variables constitute ***the measurement model*** which describes how the latent variables are measured by those observed variables. Another model in SEM is ***the structural model***, which delineates the relationships between latent variables and other observed variables that are not indicators of latent variables (Violato and Hecker, 2007; Byrne, 2009). For instance, the structural model consists of interrelationships amongst sex, eye size and susceptibility to form-deprivation myopia in our SEM, as shown in Figure 3.1.

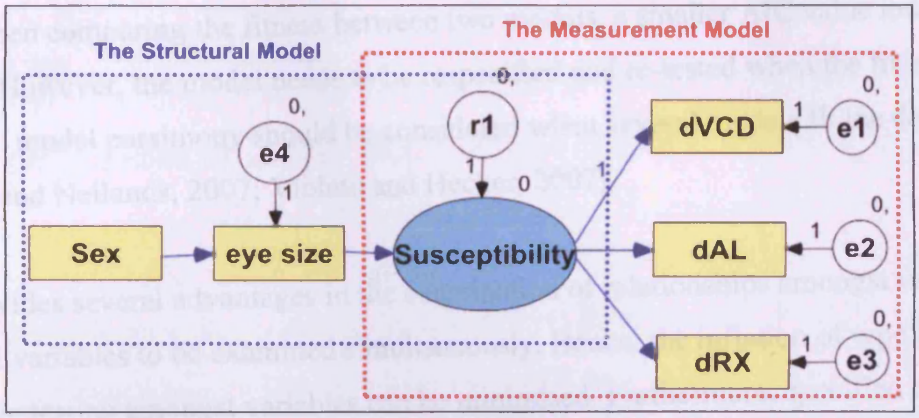


Figure 3.1 An example of a structural equation model depicting the relationship between the measurement model and the structural model.

The first step in SEM is model specification and identification. The theoretical models should be built on the basis of the research question, theory, and/or previous research findings. In addition, the available information in the data needs to be equal to or exceed the information being estimated in order to assess the models properly. After that, the model is estimated and an assessment is made regarding how it fits the observed data based on the extent to which the model-implied covariance matrix is equivalent to the data-derived covariance matrix. Maximum likelihood is most frequently used to estimate parameters that maximize the probability that the predicted model fits the observed model.

Several indicators are usually used to assess how well the collected data fit the hypothesized model, such as the chi-square value (χ^2), the comparative fit index (CFI), and the root mean square error of approximation (RMSEA). The chi-square value indicates the discrepancy between the the data-derived covariance matrix and the model-implied covariance matrix. Although some of the fit indices are based on the chi-square value, it is not a good fit index in practice due to substantial influence by sample size. The CFI is based on a ratio of the chi-square of the tested model and the null or independence model, indicating the extent to which the tested model is better than the null model. The RMSEA takes into account the error of approximation in the population and assesses the misfit per degree of freedom. Generally a model with CFIs ≥ 0.90, and RMSEA <0.10 is considered as a good fit (Violato and Hecker, 2007). Additionally, comparison between different models can be examined with the Akaike information criterion (AIC). The AIC addresses the issue of parsimony in the assessment of model fit, and thus both statistical goodness-of-fit and the number of estimated parameters are taken into account (Byrne,

2009). When comparing the fitness between two models, a smaller AIC value indicates a better fit. However, the model needs to be respecified and re-tested when the fit is poor. Moreover, model parsimony should be considered when several models fit the data (Buhi, Goodson and Neilands, 2007; Violato and Hecker, 2007).

SEM provides several advantages in the examination of relationships amongst variables. It allows all variables to be examined simultaneously. Hence, the inflation of type I error due to multiple testing amongst variables can be minimised. Furthermore, specified causal relationships, including indirect, direct and total effects across variables can be efficiently tested and estimated in complex models. Moreover, the relationships amongst latent variables with multiple observed variables can be examined while measurement error in each observed variable and residual error of latent variables are taken into account in the modelling process. Therefore, more accurate and stronger relationships between the variables can be obtained from SEM than from multivariate methods, such as multiple regressions. However, there is a need for caution in using SEM because of its limitations. The models should build on a well-developed theory and empirical evidence. In addition, good reliability and validity of the measurements and adequate sample size (e.g. at least 200 cases) are required to obtain reliable results (Buhi et al., 2007; Violato and Hecker, 2007; Byrne, 2009).

The models delineating interrelationships amongst sex, initial eye size, and myopia susceptibility were also examined using structural equation modelling. In this chapter, statistical analyses and structural equation modelling were carried out with SPSS 14.0 and AMOS 5.0 (SPSS Inc., Chicago, IL, USA).

3.3 Results

3.3.1 Relationships amongst sex, body weight and eye size

Before form-deprivation treatment, all ocular component dimensions were significantly larger in the male chicks than in the females (all $P < 0.001$; Table 3.4). Body weight between sexes did not differ significantly before form deprivation ($P = 0.139$; Table 3.4). However, significant differences between male and female in body weight and its change after form deprivation were observed ($P = 0.005$ and 0.002, respectively; Table 3.4). In addition, there was a significant correlation between initial eye size (average value of axial length of right and left eyes) and body weight before treatment (Spearman's rho = 0.43, $P<0.001$). Chickens with heavier body weight had larger eye size before form deprivation.

Table 3.4 Ocular component dimensions and body weight before and after form deprivation (FD), stratified by sex. (Values show mean ± standard deviation).

Sex (Number of subjects)	Male (N = 117)	Female (N = 115)	P-value
Ocular component dimension (mm)			
Both eyes prior to FD			
Anterior chamber depth	1.27 ± 0.04	1.25 ± 0.04	<0.001[a]
Lens thickness	1.83 ± 0.04	1.81 ± 0.03	<0.001[a]
Vitreous chamber depth	5.09 ± 0.12	4.99 ± 0.13	<0.001[a]
Axial length	8.19 ± 0.14	8.05 ± 0.15	<0.001[a]
Treated eye after FD			
Anterior chamber depth	1.38 ± 0.10	1.33 ± 0.08	<0.001[a]
Lens thickness	1.97 ± 0.04	1.96 ± 0.06	0.07[a]
Vitreous chamber depth	5.62 ± 0.17	5.43 ± 0.19	<0.001[a]
Axial length	8.96 ± 0.25	8.71 ± 0.23	<0.001[a]
Control eye after FD			
Anterior chamber depth	1.40 ± 0.04	1.36 ± 0.04	<0.001[a]
Lens thickness	1.97 ± 0.04	1.96 ± 0.06	0.11[a]
Vitreous chamber depth	5.15 ± 0.13	5.03 ± 0.13	<0.001[a]
Axial length	8.52 ± 0.15	8.33 ± 0.16	<0.001[a]
Body weight (BW) (gm)			
BW prior to FD	50.30 ± 4.09	49.41 ± 4.55	0.139[b]
BW after FD	72.66 ± 7.25	69.91 ± 7.73	0.005[b]
The change in BW during FD	22.36 ± 4.76	20.50 ± 5.09	0.002[b]

[a] *t*-test for a difference between sexes.
[b] Mann-Whitney U-test for a difference between sexes.

3.3.2 Variation in susceptibility to form deprivation

The changes in ocular component dimensions induced by form deprivation are summarized in Table 3.5. After form deprivation for 4 days, there were significant differences in the changes of anterior chamber depth, vitreous chamber depth, and axial length between the treated and control eyes (paired *t*-test, all $P < 0.001$), but this was not the case with lens thickness ($P = 0.39$). Relative to control eyes, treated eyes showed large increases in vitreous chamber depth and axial length and a small decrease in anterior chamber depth. Thus, the axial elongation induced by form deprivation was mainly the result of an increase in vitreous chamber depth.

Table 3.5 Comparison of the changes in ocular component dimensions between the treated eye and control eye after form deprivation for 4 days in all subjects. (Values show mean ± standard deviation).

Ocular component	Treated Eye (N=232)	Control Eye (N=232)	P-value
Anterior chamber depth (mm)	0.09 ± 0.08	0.11 ± 0.04	<0.001
Lens thickness (mm)	0.14 ± 0.04	0.14 ± 0.05	0.39
Vitreous chamber depth (mm)	0.50 ± 0.15	0.03 ± 0.06	<0.001
Axial length (mm)	0.73 ± 0.20	0.28 ± 0.07	<0.001

P-values relate to a test for a difference in ocular component dimensions between the treated and control eyes (paired *t*-test).

Myopia susceptibility was quantified as the difference in eye growth between the treated and control eyes during form deprivation (parameters ΔVCD and ΔAL) and as the difference in refraction between the treated and control eyes after form deprivation (parameter ΔRX). There was substantial variability in myopia susceptibility in this outbred Lohmann strain of chicks (Table 3.6). The coefficients of variation were: ΔVCD, 30%; ΔAL, 42%; ΔRX, 23%. Both ΔVCD and ΔAL were highly correlated with ΔRX ($r = 0.55$ and $r = 0.68$, respectively; both $P < 0.001$). After form deprivation, the male chicks still had significantly longer eyes than did the female chicks. Specifically, both the treated and control eyes of the male chicks had deeper anterior and vitreous chambers, and longer overall axial length than did the corresponding eyes of the females (all $P < 0.001$;

Table 3.4). By contrast, lens thickness was no longer significantly greater in the males than in the females after form deprivation in either the treated or control eye (Table 3.4). In terms of susceptibility to from-deprivation myopia, only the relative change in vitreous chamber depth and axial length (i.e., ΔVCD and ΔAL) were significantly different between the sexes, with males again showing a greater relative increase in ocular component dimensions than females (Table 3.6). Although males developed slightly more myopia after form deprivation (approximately -0.27D on average) than did females, this difference did not reach statistical significance (P = 0.573).

In summary, male chicks had larger eyes before form deprivation, larger eyes after form deprivation, and developed a greater degree of FD-induced myopic eye growth than the females. An important finding, however, was that the males did not develop more form-deprivation myopia per se than did the females (Table 3.6).

Table 3.6 Myopia susceptibility (differences between the treated and control eyes due to 4 days of form deprivation) stratified by sex. (Values show mean ± standard deviation).

Sex (Number of subjects)	All (N = 232)	Male (N = 117)	Female (N = 115)	P-value
Parameter				
Δ*VCD* (mm)	0.47 ± 0.14	0.50 ± 0.13	0.44 ± 0.14	<0.001[a]
Δ*AL* (mm)	0.45 ± 0.19	0.49 ± 0.19	0.41 ± 0.17	0.002[a]
Δ*RX* (D)	-13.47 ± 3.12	-13.60 ± 3.30	-13.33 ± 2.93	0.573[b]

[a] *t*-test for a difference between sexes.
[b] Mann-Whitney U Test

3.3.3 Eye size, sex, body weight, and susceptibility to form deprivation

Eye size before form deprivation was found to be a predictor of the effects on ocular growth response of form deprivation. Specifically, there were significant correlations between initial anterior chamber depth and ΔVCD, initial vitreous chamber depth and ΔVCD, and initial axial length and ΔVCD (Table 3.7). Nevertheless, initial lens thickness did not correlate significantly with ΔVCD. Similar to these associations with ΔVCD, there were significant correlations between initial anterior chamber depth and ΔAL, as well as

initial axial length and ΔAL (Table 3.7). However, initial ocular component dimensions did not correlate significantly with ΔRX.

In addition, the chick's sex also predicted its susceptibility to experimentally-induced eye growth. The male chickens had greater ΔVCD and ΔAL after form deprivation than did the females (Table 3.6). The correlation between sex and ΔVCD was $r = 0.26$, $P < 0.001$. Thus, in univariate analyses, both sex and the initial eye size were significant predictors of susceptibility to ΔVCD (accounting for 6.4% and 3.8% of the variance in response, respectively). Similarly sex and the initial eye size accounted for 4.7% and 1.7% of the variance in ΔAL. However, body weight did not have any significant correlation with any parameters representing susceptibility to visual deprivation.

In summary, both sex and eye size had significant correlations with relative ocular growth in vitreous chamber depth and axial length after visual deprivation. Eye size also differed significantly between males and females. However, there was no significant correlation between body weight and the three parameters denoting susceptibility to form-deprivation myopia. Furthermore, the changes in refraction did not correlate significantly with sex, eye size or body weight. Therefore, further analysis was carried out to test whether sex exerts its effects on susceptibility to myopic eye growth by virtue of the eye size differences between the sexes, and/or by an independent effect.

Table 3.7 Correlations amongst sex, eye size, body weight and susceptibility to FDM

Correlation coefficients	Susceptibility to FDM		
	ΔVCD (mm)	ΔAL (mm)	ΔRX (Dioptres)
Sex	$r_p = 0.26$, $P < 0.001$*	$r_p = 0.23$, $P = 0.001$*	$r_s = -0.05$, $P = 0.466$
Initial ACD (mm)	$r_p = 0.22$, $P = 0.001$*	$r_p = 0.25$, $P < 0.001$*	$r_s = -0.10$, $P = 0.151$
Initial LT (mm)	$r_p = -0.03$, $P = 0.645$	$r_p = -0.04$, $P = 0.584$	$r_s = 0.09$, $P = 0.175$
Initial VCD (mm)	$r_p = 0.19$, $P = 0.004$*	$r_p = 0.10$, $P = 0.116$	$r_s = -0.10$, $P = 0.148$
Initial AL (eye size, mm)	$r_p = 0.21$, $P = 0.002$*	$r_p = 0.15$, $P = 0.027$*	$r_s = -0.07$, $P = 0.315$
Body Weight (gm)	$r_s = 0.04$, $P = 0.531$	$r_s = 0.03$, $P = 0.614$	$r_s = -0.01$, $P = 0.897$

"r_p": Pearson correlation coefficient, "r_s": Spearman's rank correlation coefficient,
"*": significant correlation with $P < 0.05$

3.3.4 Hypothesized causal models testing

3.3.4.1 Multiple regression models

When sex, initial eye size and body weight were included in a multiple regression model, sex was the only significant predictor of the rate of eye enlargement (ΔVCD and ΔAL) (Table 3.8). Body weight was the least plausible predictor of the rate of myopic eye growth in this regression model. Similar results were found when two alternative methods of defining the degree of myopic eye growth were used in the same regression model (Table 3.9). Hence, the findings were not dependent on any one set of definitions, which provided greater confidence to remove body weight in the potential causal models.

Table 3.8 Multiple regression analysis to identify predictive variables associated with the rate of myopic eye growth. The relative changes in vitreous chamber depth (ΔVCD) and axial length (ΔAL) resulting from 4 days of form deprivation were examined as a function of sex, initial eye size (i.e. axial length prior to treatment) and initial body weight. All variables were included in the starting model (A), followed by backward removal of variables to identify more parsimonious models (B, C).

Dependent variable	Model	Adjusted R^2	Predictors	B [a]	P-value	Correlations	
						Zero-order	Partial
ΔVCD	A	0.068	Sex	0.204	0.004	0.260	0.187
			Eye size	0.141	0.078	0.206	0.116
			Body weight	-0.050	0.492	0.036	-0.045
	B	0.070	Sex	0.210	0.003	0.260	0.193
			Eye size	0.115	0.102	0.206	0.108
	C	0.064	Sex	0.260	<0.001	0.260	-
ΔAL	A	0.041	Sex	0.201	0.006	0.226	0.182
			Eye size	0.057	0.480	0.145	0.047
			Body weight	0.001	0.986	0.048	0.001
	B	0.045	Sex	0.201	0.005	0.226	0.183
			Eye size	0.058	0.419	0.145	0.053
	C	0.047	Sex	0.226	0.001	0.226	-

[a] Standardised regression coefficient.

Table 3.9 Two alternative methods (A and B) of defining myopic eye growth were explored to evaluate whether the choice of definition altered the results of multiple regression analyses examining the relative importance of the predictor variables sex, initial eye size and initial body weight. The methods of defining the rate of myopic eye growth for A and B are shown below.

Model	Dependent variable	Predictor variable	Standardised regression coefficient	P-value
		Sex	0.202	0.006
A	**VCD_1**	Eye size	0.053	0.514
		Body weight	-0.043	0.555
		Sex	0.213	0.003
B	**VCD_2**	Eye size	0.053	0.509
		Body weight	-0.047	0.522
		Sex	0.189	0.010
A	**AL_1**	Eye size	0.020	0.811
		Body weight	-0.028	0.978
		Sex	0.196	0.007
B	**AL_2**	Eye size	0.019	0.818
		Body weight	-0.006	0.931

Method A: Relative ratio change

$$VCD_1 = \left(\frac{postVCD_T}{postVCD_C}\right) - \left(\frac{preVCD_T}{preVCD_C}\right)$$

$$AL_1 = \left(\frac{postAL_T}{postAL_C}\right) - \left(\frac{preAL_T}{preAL_C}\right)$$

Method B: Growth ratio change

$$VCD_2 = \left(\frac{postVCD_T - preVCD_T}{preVCD_T}\right) - \left(\frac{postVCD_C - preVCD_C}{preVCD_C}\right)$$

$$AL_2 = \left(\frac{postAL_T - preAL_T}{preAL_T}\right) - \left(\frac{postAL_C - preAL_C}{preAL_C}\right)$$

where,

$preVCD_C$ = VCD in the control eye prior to FD,

$postVCD_C$ = VCD in the control eye after FD,

$preVCD_T$ = VCD in the treated eye prior to FD,

$postVCD_T$ = VCD in the treated eye after FD,

$preAL_C$ = AL in the control eye prior to FD,

$preAL_T$ = AL in the treated eye prior to FD,

$postAL_C$ = AL in the control eye after FD,

$postAL_T$ = AL in the treated eye after FD.

Because the directions of the potential causal relationships between the chick's sex, initial eye size, and susceptibility to myopia were unambiguous (e.g., it would not be logical for either myopia susceptibility or eye size to determine sex), three potential causal effect models were proposed to explain the interrelationships among sex, eye size, and the susceptibility to myopic eye enlargement (Figure 3.2).

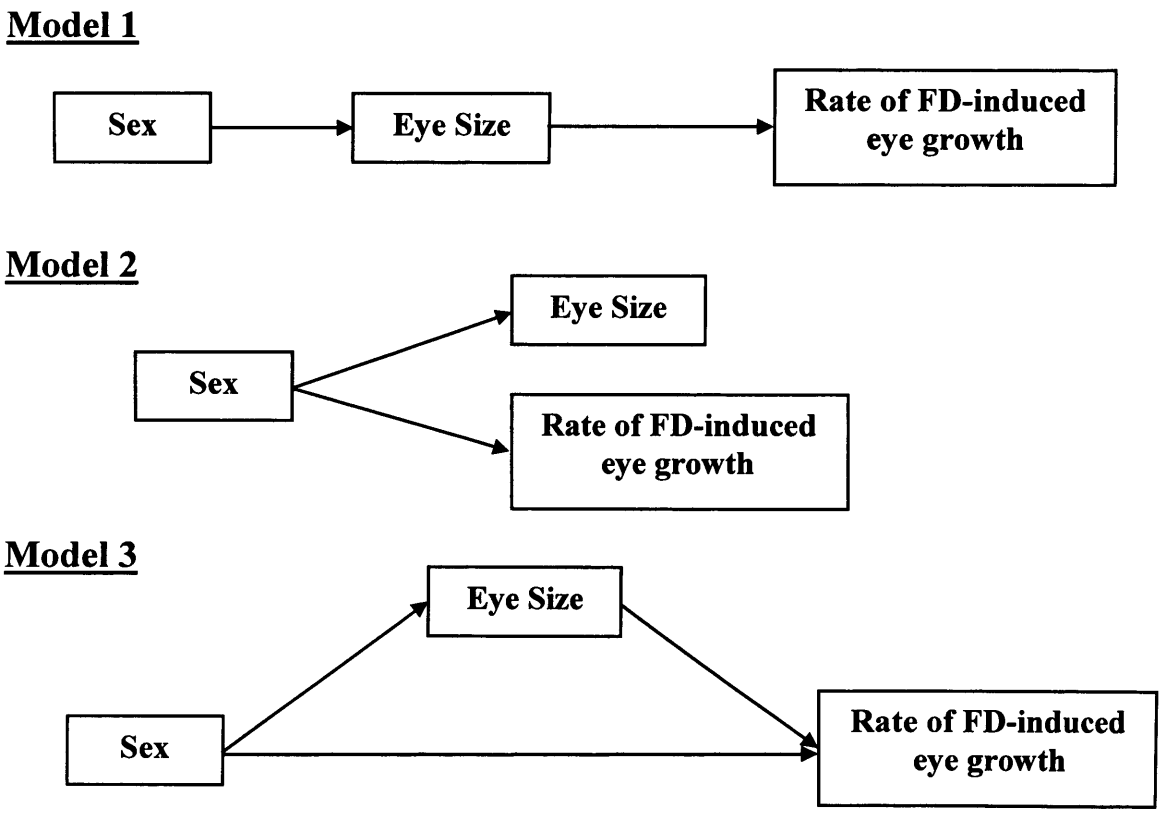


Figure 3.2 Potential models describing the relationships amongst sex, eye size prior to visual deprivation, and the rate of FD-induced eye growth. Arrows show the direction of causal effects.

According to model 1, the chick's sex exerted its effects on the rate of eye enlargement solely by virtue of its having produced a difference in initial eye size between the sexes. In model 2, sex influenced susceptibility independent of its effects on initial eye size, and in model 3, sex influenced susceptibility both directly (independent of eye size) and indirectly, as a result of producing differences in initial eye size.

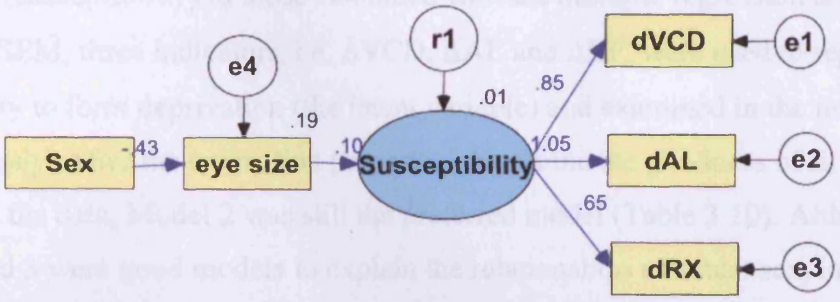
If model 1 were correct, then controlling for eye size should remove the correlation between the chick's sex and the rate of myopic eye growth. However, this was not the case. The partial correlation between sex and ΔVCD with initial axial length held constant and was still significant ($r = 0.193$, $P = 0.003$), demonstrating that model 1 was incorrect. If model 3 were correct, then controlling for sex should not remove the correlation between initial eye size and the rate of myopic eye growth, because an independent causal link between these latter two variables should remain. However, there was no longer a significant partial correlation between initial axial length and ΔVCD, with sex held constant ($r = 0.11$, $P = 0.10$), suggesting that model 2 might be most consistent with these observations. Nonetheless, the result obtained when controlling for sex could have been strongly influenced by a lack of power due to the modest sample size. Hence, model 3 could not be ruled out completely.

Thus, in the analysis of multiple regression models, sex appeared to exert its influence on eye size and the rate of myopic eye growth independently. However, there might be a possibility of an indirect sex effect on the rate of myopic eye growth through eye size.

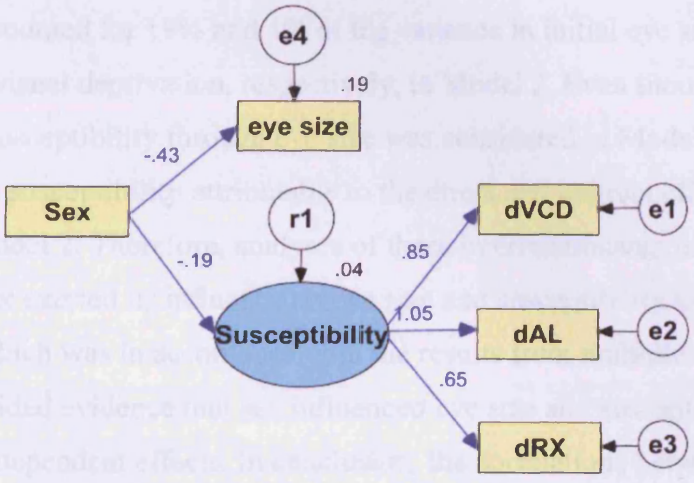
3.3.4.2 Structural equation modelling (SEM)

These three potential causal models were also examined using SEM, because of the advantages of SEM (section 3.2.3). The main difference between SEM and multiple regression analysis was the measurement model used in SEM, which allowed all the measurements representing susceptibility to form deprivation (the latent variable) to be examined simultaneously. Thus, type I error due to multiple testing was minimised in SEM. In addition, comparisons amongst the models could be carried out easily in SEM by comparing the model fit indices between models, such as the Akaike information criterion (AIC). Therefore, the three causal models shown in Figure 3.3 were examined by SEM.

Model 1



Model 2



Model 3

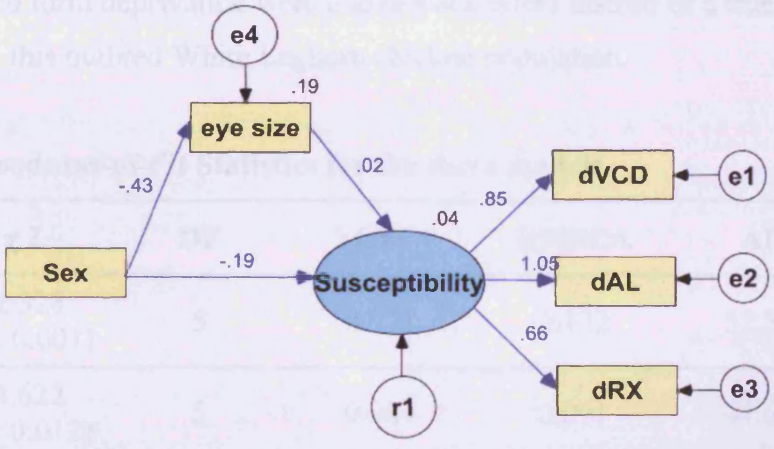


Figure 3.3 Three structural equation models describing the relationships amongst sex, eye size prior to visual deprivation, and susceptibility to form-deprivation myopia. (dVCD= ΔVCD, dAL= ΔAL, dRX= ΔRX; e1, e2, e3 and e4 denotes measurement error in ΔVCD, ΔAL, ΔRX and eye size, respectively; r1 (residual) represents error in the prediction of myopia susceptibility from three parameters, i.e. ΔVCD, ΔAL, and ΔRX; numbers alongside the blue single-headed arrows represent standardised regression coeffecients; numbers alongside the boxes denote squared multiple correlations.)

These three models expressed the similar relationships of sex, eye size and FD-induced eye growth (susceptibility) to those examined with the multiple regression analysis models. In SEM, three indicators, i.e. ΔVCD, ΔAL and ΔRF, were used to represent susceptibility to form deprivation (the latent variable) and examined in the model simultaneously. After the estimation process to determine the goodness of fit between the models and the data, Model 2 was still the preferred model (Table 3.10). Although both Model 2 and 3 were good models to explain the relationships amongst sex, initial eye size and susceptibility to form deprivation, Model 2 had a better fit to the data than did Model 3. Sex accounted for 19% and 4% of the variance in initial eye size and susceptibility to visual deprivation, respectively, in Model 2. Even though the indirect effect of sex on susceptibility through eye size was considered in Model 3, the proportion of the variance in susceptibility attributable to the direct and indirect effect of sex was still the same as in Model 2. Therefore, analyses of these interrelationship models by SEM confirmed that sex exerted its influence on eye size and susceptibility to form deprivation independently, which was in accordance with the results from multiple regression analysis. Both results provided evidence that sex influenced eye size and susceptibility to form deprivation by independent effects. In conclusion, the correlations between eye size and susceptibility to form deprivation were due to a sex effect instead of a true causal relationship in this outbred White Leghorn chicken population.

Table 3.10 Goodness-of-Fit Statistics for the three models.

	$\chi 2$	DF	CFI	RMSEA	AIC	Preferable Model
Model 1	22.328 ($P < 0.001$)	5	0.971	0.122	52.328	
Model 2	14.622 ($P = 0.012$)	5	0.984	0.091	44.622	√
Model 3	14.534 ($P = 0.006$)	4	0.982	0.107	46.534	

χ^2: chi-square value; DF: degrees of freedom; CFI: comparative fit index;
RMSEA: root mean square error of approximation; AIC: Akaike information criterion.

3.4 Discussion

The study in outbred chicks showed a varied response to form deprivation, as has been noted many times in other White Leghorn and non-White Leghorn lines (Section 3.1.2 and Table 3.3). One important result was that the level of induced myopia was not significantly different between the male and female chickens. Rather, the eyes of the male chicks elongated to a greater degree in response to form deprivation than did the eyes of the female chicks (which implies that the anterior segments of the sexes must also have been different). The finding that approximately 4~6% of the intersubject variability in FD-induced eye growth could be predicted by knowing the sex of the animal is consistent with the hypothesis that the differential susceptibility to form deprivation is partially genetic in origin (Troilo et al., 1995, Schaeffel and Howland, 1991, Saltarelli et al., 2004). Initial eye size (i.e., axial length before form deprivation) was also a statistically significant predictor of the rate of myopic eye growth. However, multiple regression analysis and SEM suggested that this relationship was likely due to one of "guilt by association"—that is, it represented a noncausal relationship produced by virtue of the tendency for the male chicks to have larger eyes than the female chicks prior to form deprivation.

Inconsistent results in studies comparing the ocular responses to visual deprivation between male and female chicks have been observed (Table 3.11). Schmid and Wildsoet (1996) found similar responses to form deprivation in male and female White Leghorn chickens. Guggenheim et al. (2002) revealed only minor sex differences in ocular response to visual deprivation in three different strains chickens. However, Zhu et al. (1995) identified a higher susceptibility to form-deprivation myopia in male White Leghorn chicks. These conflicting results may be due to the different genetic backgrounds of the chicken lines concerned, or chance effects due to sampling variation (in view of the limited extent of sex's influence on myopia susceptibility). The results regarding the independent effects of sex on eye size and FD-induced eye growth suggest that the genes responsible for the increased eye size in males are not necessarily the same genes that give rise to the enhanced rate of myopic eye growth in males. In contrast to the findings of Shen et al. (2005) that susceptibility to form-deprivation myopia in fish was strongly related to initial body weight, there was no such relationship in our chickens (Table 3.8 and Table 3.9).

Table 3.11 Studies examining differences in ocular biometry and susceptibility to form-deprivation myopia between male and female chickens.

	Sample size	Visual deprivation	Ocular Biometry		Induced myopia
			Control eyes	Treated eyes	
Zhu et al. (1995)					
White Leghorns (Truslow)	23 males 24 females	Lid-suture (monocular) for 2 weeks	Males: larger eyes (LT, VCD and AL) No significant difference in ACD	Males: more increase in ACD and AL No difference in LT, VCD	No significant difference
	26 males 30 females	Occluder (monocular) for 2 weeks		Males: more increase in ACD, VCD and AL No difference in LT	Males: more myopic
Schimid and Wildsoet (1996)					
White Leghorns	18 males 17 females	Lid-suture (monocular) for 2 weeks	No difference before 7 weeks of age Male: larger eyes (ACD, VCD, AL) after 7 weeks of age	No significant difference	No significant difference
Guggenheim et al. (2002)					
White Leghorns, Brown Leghorns and Broilers	30	Occluder (monocular) for 2 weeks	Males: more hyperopic, thicker LT	Males: slightly more increase in VCD and AL, No significant differences in corneal curvature, ACD and LT	No significant difference

There is an extensive body of statistical literature on the difficulty of attributing specific anatomic differences in scale to the sex of the subject (Bishop and Wahlsten, 1999). Usually, the problem is one of deciding whether a given morphologic region of interest (ROI) is larger in males simply by virtue of males being larger than females in general, or because of a specific enlargement of the ROI in males over and above the general size difference between the sexes. Thus, the question is one of relative scaling (for example, the size of the ROI relative to overall body size). The question addressed in this study was that the greater myopic eye growth of male chicks was due to their larger initial eye size, or due to an effect of sex over and above this. The way in which the relative size of an ROI is defined can profoundly influence the results obtained when testing for an effect of sex. Because of this, two alternative methods of defining the degree of myopic eye growth were tested (Table 3.9), and the results were in line with those using my original definitions for ΔVCD and ΔAL (Table 3.8). Hence, these findings provided a measure of confidence that the conclusion was not dependent on any one set of definitions.

In humans, differences in myopia prevalence between the sexes are frequently, although not always, found (Table 3.1). In addition, two genetic loci for high myopia have been mapped to the X chromosome to date: MYP1 at Xq28 (Schwartz et al., 1990) and MYP13 at Xq23–25 (Zhang et al., 2006, Zhang et al., 2007), as has a genetic locus for "low" (common) myopia (Stambolian et al., 2005). However, the genetics of sex determination in mammals and birds are very different. Whereas it is males who are the heterogametic sex in mammals (males carry X and Y chromosomes, females two X chromosomes), in birds it is females who are heterogametic (males ZZ, females ZW). Thus, while gene dosage appears to be important in sex determination in both mammals and birds (Zhao et al., 2010), there is little or no synteny between the human X chromosome and the chicken Z chromosome (Figure 3.4; specifically, the chicken Z chromosome is syntenic with regions of human chromosomes 5, 8, 9, and 18) (Nanda et al., 2002; International Chicken Genome Sequencing Consortium, 2004). Since the degree of induced myopia in the chickens was not significantly different between the sexes, my results are not consistent with prior findings of sex-related differences in the prevalence of human myopia. Furthermore, since the chicken Z and human X chromosomes are not syntenic, findings in this study do not implicate genes on the X chromosome as being especially likely to

modulate axial eye growth differently in males and females. (Instead, human chromosomes 5, 8, 9, and 18 may be interesting to study in regard to possible sex differences in humans.)

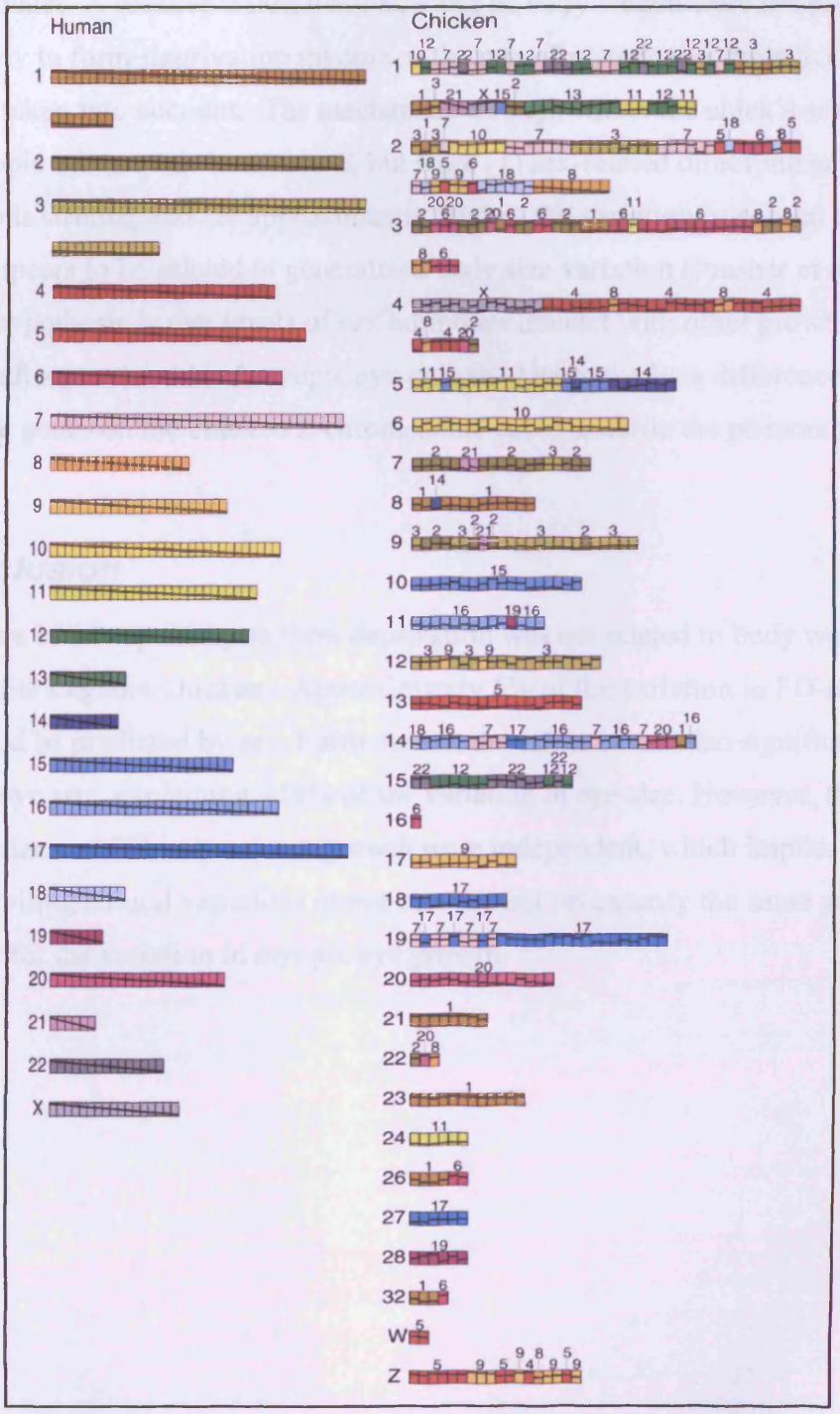


Figure 3.4 The arrangement of 586 synteny blocks in human and chicken genomes. The chicken W and Z chromosome showed conserved synteny mainly with human chromosomes 5, 8, and 9. (From International Chicken Genome Sequencing Consortium (2004))

In this study, the sex of White Leghorn chicks was found to influence the rate of FD-induced myopic eye growth, with the males having a greater degree of axial elongation than the females. A chicken's initial eye size and its body weight were not predictive of susceptibility to form-deprivation myopia or the rate of myopic eye growth, once the effect of sex was taken into account. The mechanism through which the chick's sex affects the rate of myopic eye growth is unknown, but since (1) sex-related dimorphism of body size in chickens is striking and (2) approximately 50% of the variation in normal eye size in chickens appears to be related to generalized body size variation (Prashar et al., 2009), one appealing hypothesis is that levels of sex hormones interact with other growth-promoting stimuli to influence the rate of myopic eye growth. Alternatively, a difference in dosage for one or more genes on the chicken Z chromosome could underlie the phenomenon.

3.5 Conclusion

The variation in susceptibility to form deprivation was not related to body weight in these outbred White Leghorn chickens. Approximately 6% of the variation in FD-induced eye growth could be predicted by sex. Furthermore, the effect of sex also significantly influenced eye size, explaining ~19% of the variation in eye size. However, the effects of sex on eye size and FD-induced eye growth were independent, which implies that the genes controlling natural variations in eye size are not necessarily the same genes as those responsible for the variation in myopic eye growth.

Chapter 4

A selective breeding experiment for susceptibility to form-deprivation myopia

4.1 Introduction

A considerable variation in the degree of myopia induced by a uniform regimen of visual deprivation has been found not only between chicken strains but also within each strain (section 3.1.2 and Table 3.3). However, there is very little understanding about the causes of this variability in the literature. Troilo et al.(1995) found significant differences in both normal ocular development and the ocular response to visual deprivation between two strains of White Leghorn chickens and suggested there may be a role of genetics involved in the visual control of eye growth. Saltarelli et al. (2004) also indicated the possible effect of genetics in the susceptibility to form-deprivation myopia due to the significant correlation between vitreous chamber elongation induced over two successive periods of form-deprivation treatment (section 1.3.4). Because phenotypic changes through artificial selection can reflect the underlying genetic architecture (section 1.4.3.3), a selective breeding experiment was carried out to test the hypothesis that the inter-animal variability in susceptibility to myopia was genetically determined. If the variation in susceptibility between chickens was completely due to environmental factors, the High and Low susceptibility selected lines would show similar susceptibility to form deprivation. However, the two selected lines would gradually diverge in their susceptibility if the inter-animal variability in susceptibility was genetic in origin.

The previous chapter described the selection of chickens with high or low susceptibility to form deprivation, starting from an outbred White Leghorn chicken stock. There was substantial variability in both the degree of myopia and the extent of eye growth induced by form deprivation (section 3.3.2). In this chapter, the role of genetics in susceptibility to form-deprivation myopia in White Leghorn chickens was examined directly by the selective breeding for chickens with High and Low susceptibility to form-deprivation myopia.

4.2 Materials and Methods

4.2.1 Subjects and identification of susceptibility to form-deprivation myopia

Monocular form deprivation with diffusers for 4 days was carried out in 4-day old chickens (section 2.2.1). Ocular component dimensions were measured before and after form deprivation using high-frequency A-scan ultrasonography (section 2.2.2.1). Retinoscopy was used to measure the refraction status in both the treated and the control eye after the treatment period (section 2.2.2.2). Sex identification of the chickens was determined by the PCR-RFLP method mainly (section 2.2.3) and by observation of secondary sexual characteristics in 32 chickens (section 3.2.1). Susceptibility to form-deprivation myopia was quantified by the relative change in vitreous chamber depth (ΔVCD), axial length (ΔAL) and refractive error (ΔRX) between the treated and control eyes after form deprivation for 4 days (section 2.2.2.5). Corneal curvature was measured before and after form deprivation using a videokeratometer, in the third generation, to investigate the relationship between corneal curvature and susceptibility to form deprivation (section 2.2.2.3).

4.2.2 Selection process

After quantification of the susceptibility to form-deprivation myopia of each chicken, those with the highest and lowest susceptibility were kept separately for breeding. Selective breeding was carried out for three generations as described in section 2.3.

4.2.3 Visual function testing in selectively bred chickens

An optokinetic response was tested on 7 day old chicks in the third generation, to examine if there was any difference in visual function between the two selected lines. Seven chickens (four and three from the High and Low susceptibility selected lines, respectively) were examined for their optokinetic nystagmus response (section 2.2.2.4).

4.2.4 A longer period of form deprivation in selectively bred chickens

To test if there was any difference between the two selected lines in their ocular response to form deprivation over a longer period of time, sixty-four chickens from the third generation (33 and 31 from the High and Low lines, respectively) underwent monocular form deprivation for a longer period. Refractive error, corneal curvature and ocular component dimensions were measured prior to treatment when the chicks were 4 days old and then again after 4 and 10 days of form deprivation. In addition, forty-four chickens from the third generation (22 and 22 from the High and Low lines, respectively) without any treatment were raised under the same environment (including ocular measurements) to investigate normal ocular growth and refractive status in the two selected lines.

4.2.5 Statistical analysis

All data for ocular component dimensions before and after form deprivation and the relative changes (ΔACD, ΔLT, ΔVCD, ΔAL and ΔRX) were tested for normality using the Kolmogorov-Smirnov test. Due to a non-normal distribution of ΔACD, ΔLT, and ΔRX, differences between the High and Low lines were compared using the Mann–Whitney U test. Other comparisons of the ocular component dimensions and their FD-induced changes between the two selected lines were made using independent samples *t*-test. The optokinetic nystagmus responses, quantified as the "gain" in chickens from high and low susceptibility selected lines, were compared using the Mann-Whitney U test. All analyses were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL).

4.3 Results

4.3.1. Ocular components dimensions before form deprivation

The numbers of chicks used at each stage of the experiment is shown in Figure 2.7. In the three generations of chicks studied during the selective breeding experiment, there were significant differences in ocular component dimensions (except lens thickness) before form deprivation between the High and Low susceptibility selected lines (Table 4.1 and Figure 4.1). In the second generation, chickens from the High line had a deeper vitreous chamber and a longer axial length than did those from the Low line. These differences became more evident (and the anterior chamber depth before form deprivation was also deeper) in chickens from the High line than from the Low line in the third generation. Furthermore, corneal curvature was steeper and refraction was more hyperopic in Low line chickens compared to High line ones in the third generation.

Table 4.1 Ocular component dimensions and refractive status before form deprivation in the three generations of chickens from the High and Low selected lines. (Values show mean ± standard deviation.) Chicks were aged 4 days old.

	1st Generation (N=232)	2nd Generation (N=267: L 123, H 144)			3rd Generation (N=392: L 192, H 200)		
RCC (mm)‡	-	-			L	2.77±0.05	P=0.016*
					H	2.79±0.04	
ACD (mm)	1.26±0.04	L	1.25±0.04	P=0.427	L	1.25±0.03	P<0.001*
		H	1.26±0.04		H	1.27±0.03	
LT (mm)	1.82±0.04	L	1.81±0.03	P=0.155	L	1.83±0.03	P=0.092
		H	1.80±0.03		H	1.83±0.03	
VCD (mm)	5.04±0.13	L	4.98±0.10	P=0.002*	L	4.97±0.12	P<0.001*
		H	5.02±0.12		H	5.03±0.12	
AL (mm)	8.12±0.16	L	8.04±0.13	P=0.034*	L	8.05±0.15	P<0.001*
		H	8.08±0.15		H	8.12±0.15	
RX (Dioptres)§	-	-			L	4.43±0.75	P<0.001*
					H	4.22±0.66	

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth, "*": significant difference (P<0.05). ‡RCC was measured only in Generation 3 (N=226: L=104, H=122), § RX before treatment was measured only in Generation 3 (N=216: L=105, H=111).

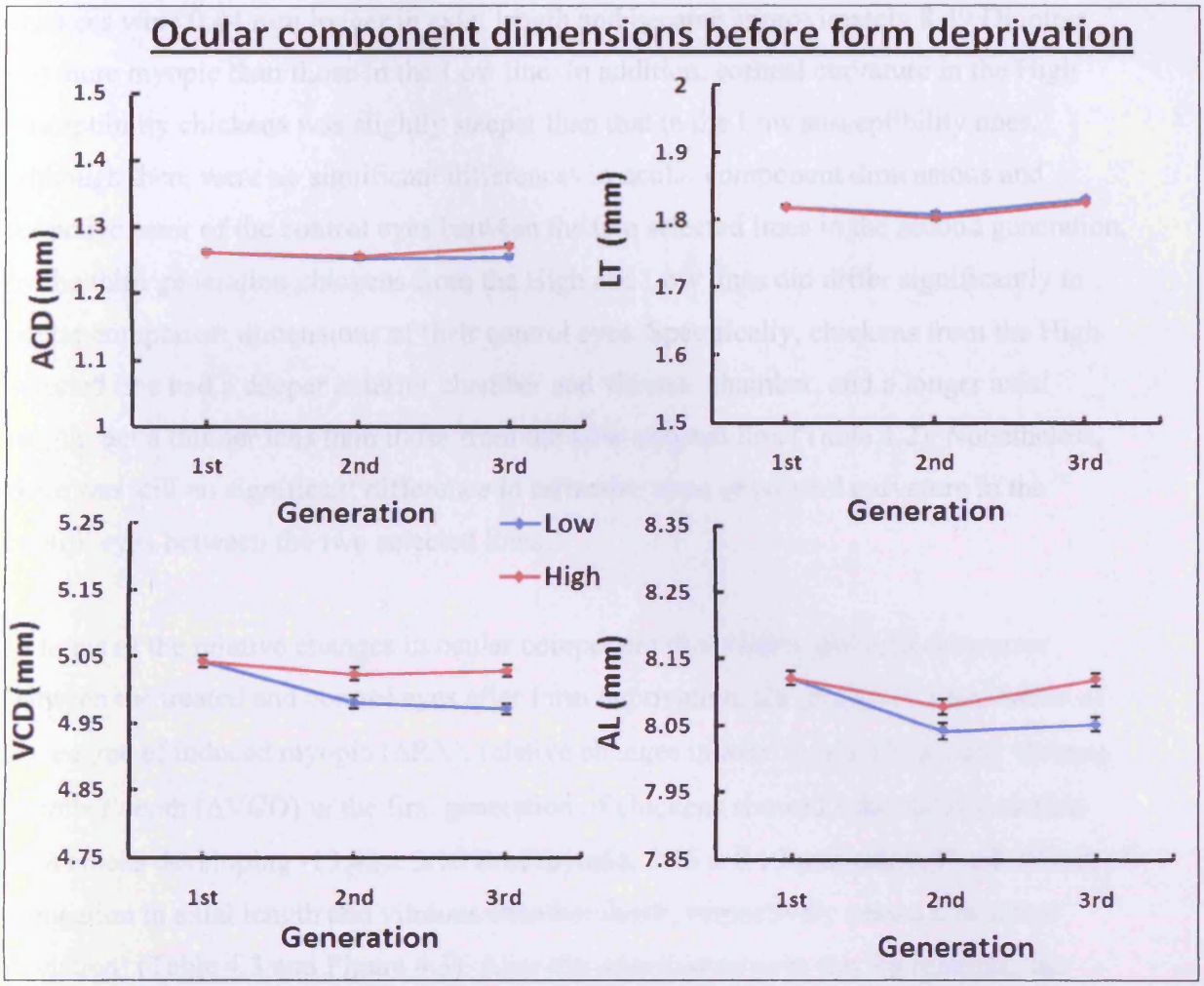


Figure 4.1 Ocular component dimensions before form deprivation in the three generations of the selectively bred chickens. There were significant differences, and a slight divergence in anterior chamber depth (ACD), vitreous chamber depth (VCD) and axial length (AL), but not lens thickness (LT) between the High and Low lines. Error bars show standard errors. Blue and red lines denote Low and High line chickens, respectively.

4.3.2. Ocular components dimensions and refractive error induced after form deprivation

Chickens from the High and Low lines showed significantly different and obviously divergent ocular responses to form deprivation in the second and third generations (Table 4.2 and Figure 4.2). Chickens selected for high susceptibility to form-deprivation myopia developed greater dimensions in all ocular components and became more myopic than did those selected for low susceptibility. In the third generation, the treated eyes in High line

chickens were 0.44 mm longer in axial length and became approximately 8.49 Dioptres (D) more myopic than those in the Low line. In addition, corneal curvature in the High susceptibility chickens was slightly steeper than that in the Low susceptibility ones. Although there were no significant differences in ocular component dimensions and refractive error of the control eyes between the two selected lines in the second generation, by the third generation chickens from the High and Low lines did differ significantly in ocular component dimensions of their control eyes. Specifically, chickens from the High selected line had a deeper anterior chamber and vitreous chamber, and a longer axial length, but a thinner lens than those from the Low selected line (Table 4.2). Nonetheless, there was still no significant difference in refractive error or corneal curvature in the control eyes between the two selected lines.

In terms of the relative changes in ocular component dimensions and refractive error between the treated and control eyes after form deprivation, the frequency distribution of the degree of induced myopia (ΔRX), relative changes in axial length (ΔAL) and vitreous chamber depth (ΔVCD) in the first generation of chickens showed substantial variation, with chicks developing -13.42 ± 3.16 D of myopia, 0.45 ± 0.19 mm and 0.47 ± 0.14 mm of elongation in axial length and vitreous chamber depth, respectively (mean ± standard deviation) (Table 4.3 and Figure 4.3). After the selection process (i.e. by retaining the animals in the tails of the distribution for breeding for two rounds), there was an obvious divergence in the distribution of these ocular changes between the High and Low lines, especially in ΔVCD, ΔAL, and ΔRX (Table 4.3, Figure 4.3 and Figure 4.4). After form deprivation, chickens from the High line developed approximately twice the degree of myopia and three times the axial length growth than those from the Low line in the third generation. The High susceptibility chickens had an average relative axial length growth (ΔAL) of 0.54 ± 0.16 mm and a level of induced myopia of -15.27 ± 3.47 D, whilst Low susceptibility birds had an average axial length growth of only 0.16 ± 0.16 mm and a level of induced myopia of -6.88 ± 3.35 D.

Table 4.2 Refraction and ocular component dimensions (values show mean ± standard deviation) in chicks monocularly form deprived for 4 days. For ocular component dimensions, P-values relate to a test for a difference in trait means between High line versus Low line chickens (*t*-test). For refraction, P-values relate to a Mann-Whitney U test between the High versus the Low line.

	Generation 1 (N=232)	**Generation 2 (N=267: L=123, H=144)**			**Generation 3 (N=392: L=192, H=200)**		
	Treated eyes	Treated eyes			Treated eyes		
	Outbred	High	Low	P-value	High	Low	P-value
RX (D)	-9.26±3.10	-8.97±3.62	-4.42±3.36	<0.001	-10.92±3.83	-2.43±3.53	<0.001
RCC (mm)‡	-	-	-		2.92±0.05	2.93±0.06	0.045
ACD (mm)	1.35±0.09	1.35±0.09	1.29±0.07	<0.001	1.37±0.09	1.23±0.07	<0.001
LT (mm)	1.96±0.05	1.95±0.04	1.94±0.04	0.035	1.98±0.04	1.97±0.04	<0.001
VCD (mm)	5.53±0.20	5.60±0.19	5.45±0.19	<0.001	5.67±0.20	5.39±0.19	<0.001
AL (mm)	8.84±0.27	8.88±0.26	8.66±0.23	<0.001	9.01±0.25	8.57±0.23	<0.001
	Control eyes	Control eyes			Control eyes		
	Outbred	High	Low	P-value	High	Low	P-value
RX (D)	4.17±1.26	4.98±0.55	5.05±0.40	0.276	4.34±1.29	4.45±0.90	0.886
RCC (mm)‡	-	-	-		2.92±0.05	2.92±0.06	0.777
ACD (mm)	1.38±0.04	1.39±0.05	1.38±0.05	0.492	1.38±0.05	1.36±0.05	<0.001
LT (mm)	1.96±0.05	1.95±0.05	1.95±0.04	0.853	1.97±0.03	1.99±0.04	<0.001
VCD (mm)	5.09±0.14	5.08±0.15	5.07±0.12	0.489	5.13±0.15	5.08±0.13	0.001
AL (mm)	8.42±0.18	8.41±0.18	8.40±0.16	0.617	8.47±0.19	8.41±0.16	0.001

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth. ‡RCC was measured only in the 3rd Generation (N=178: L=84, H=94).

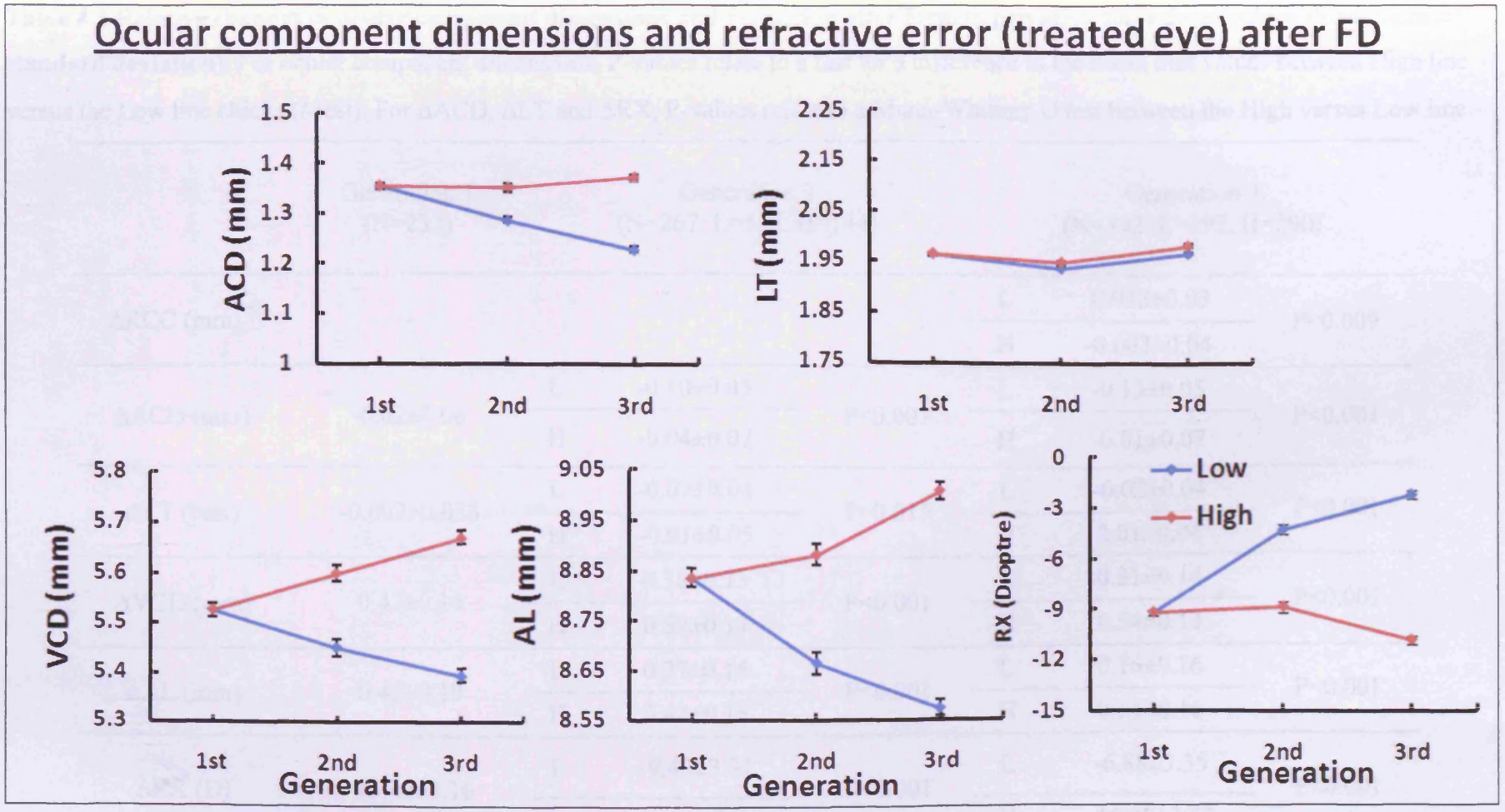


Figure 4.2 Significant difference and divergence in ocular component dimensions and refraction in response to form deprivation for 4 days after two rounds of selective breeding. Error bars show standard error. Blue and red lines denote Low and High line chickens, respectively.

Table 4.3 Relative changes in ocular component dimensions and refraction after form deprivation for 4 days (values show mean ± standard deviation). For ocular component dimensions, P-values relate to a test for a difference in the mean trait values between High line versus the Low line chicks (*t*-test). For ΔACD, ΔLT and ΔRX, P-values relate to a Mann-Whitney U test between the High versus Low line.

	Generation 1 (N=232)		Generation 2 (N=267: L=123, H=144)		Generation 3 (N=392: L=192, H=200)		
ΔRCC (mm) ‡	-		-		L	0.012±0.03	P=0.009
					H	-0.003±0.04	
ΔACD (mm)	-0.02±0.08	L	-0.10±0.05	P<0.001	L	-0.13±0.05	P<0.001
		H	-0.04±0.07		H	-0.01±0.07	
ΔLT (mm)	-0.002±0.038	L	-0.02±0.04	P=0.015	L	-0.02±0.04	P<0.001
		H	-0.01±0.05		H	0.01±0.04	
ΔVCD (mm)	0.47±0.14	L	0.38±0.13	P<0.001	L	0.31±0.14	P<0.001
		H	0.52±0.13		H	0.54±0.13	
ΔAL (mm)	0.45±0.19	L	0.27±0.15	P<0.001	L	0.16±0.16	P<0.001
		H	0.47±0.16		H	0.54±0.16	
ΔRX (D)	-13.42±3.16	L	-9.47±3.31	P<0.001	L	-6.88±3.35	P<0.001
		H	-13.95±3.59		H	-15.27±3.47	

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth. ‡RCC was measured only in the 3rd Generation (N=178: L=84, H=94).

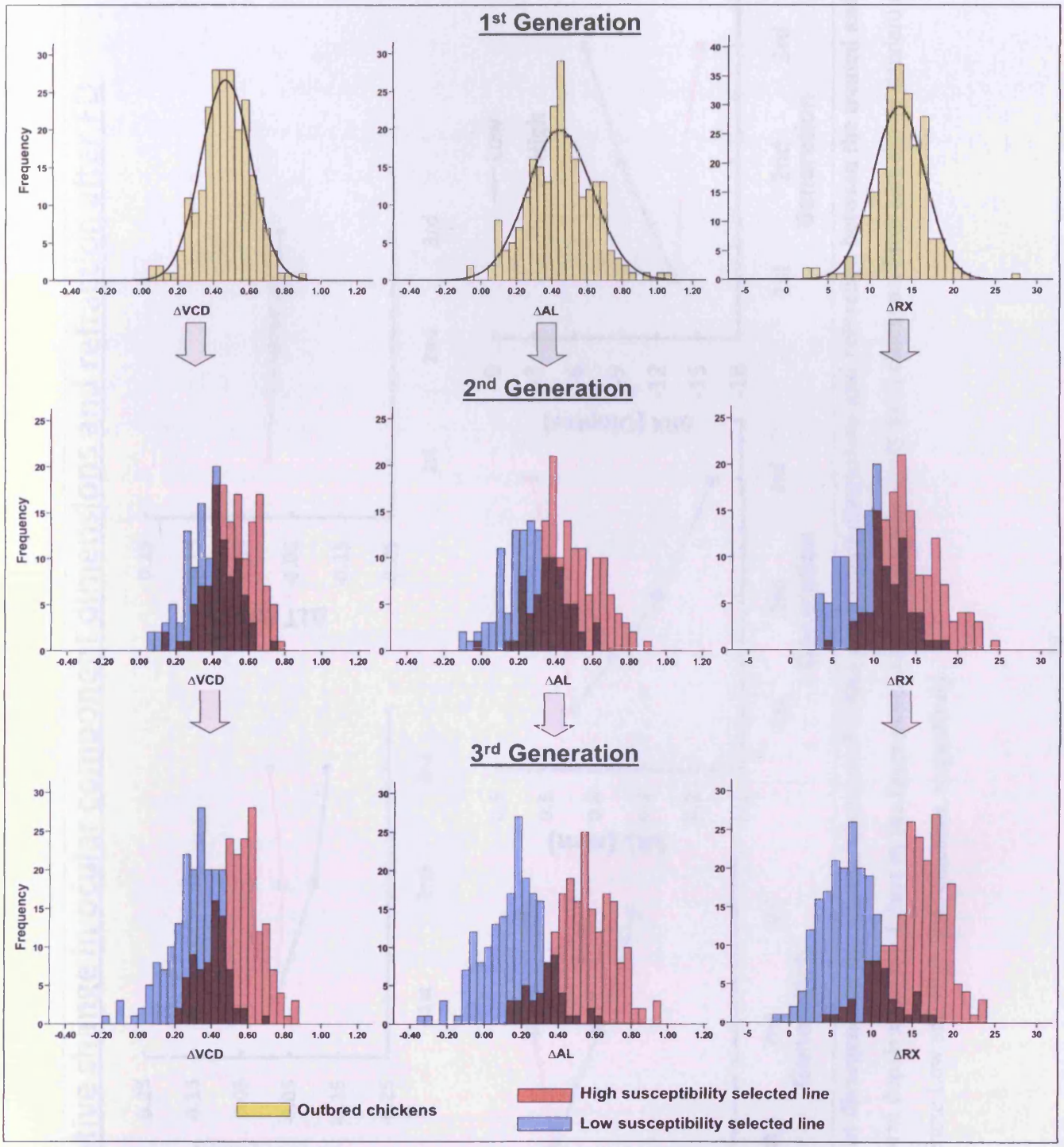


Figure 4.3 Frequency distribution of three parameters (ΔVCD, ΔAL, and ΔRX) used to quantify susceptibility to form-deprivation myopia in the three generations. An obvious divergence in the distributions of ΔVCD, ΔAL, and ΔRX between the High and Low line was observed.

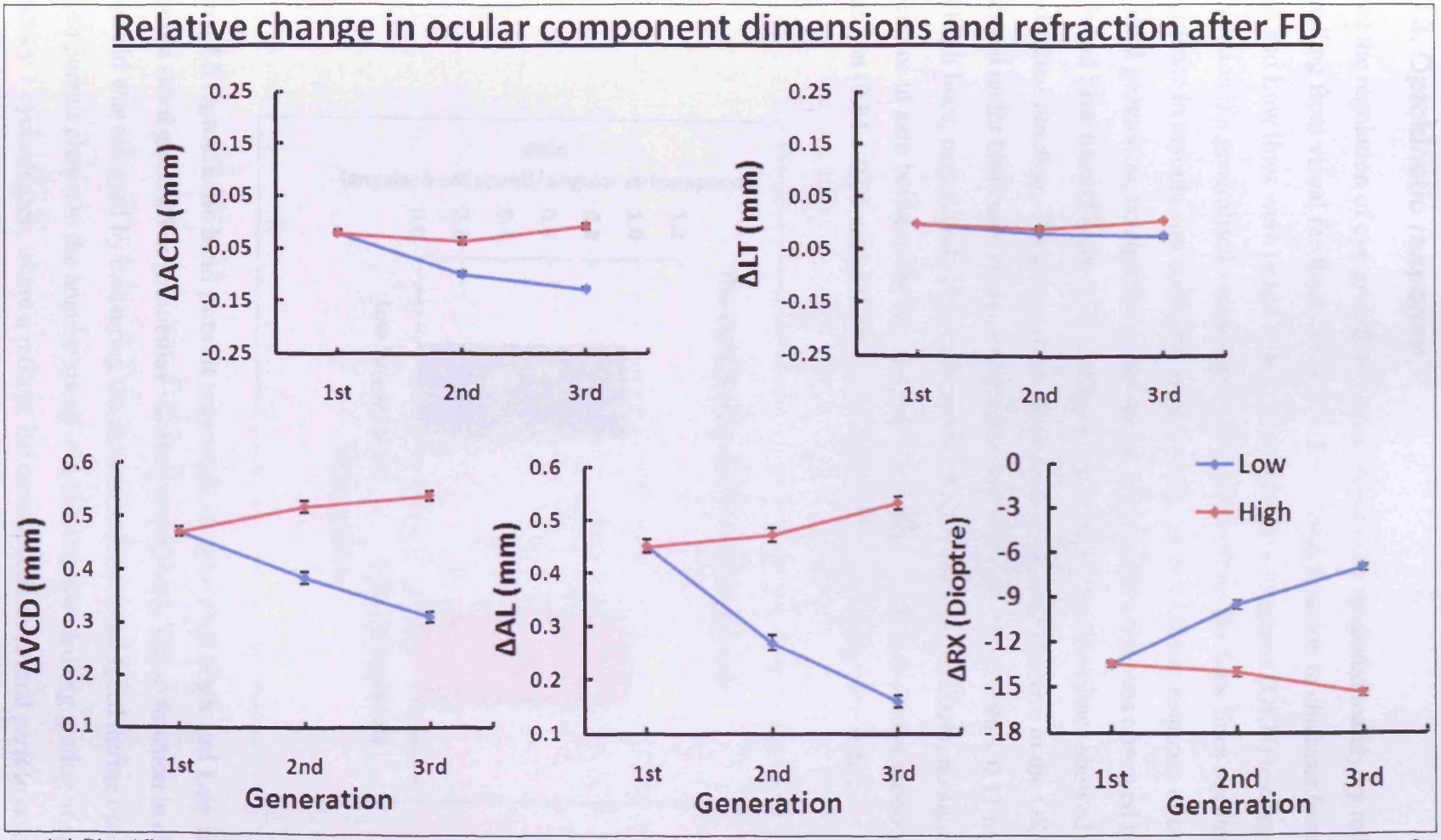


Figure 4.4 Significant divergence in the relative changes of ocular component dimensions and refraction between the treated and control eyes after form deprivation for 4 days in the three generations of the selectively bred chickens. Error bars showe standard error. Blue and red lines denote Low and High lines chickens, respectively.

4.3.3. Optokinetic responses

Since the regulation of eye growth has been found to be mediated mainly by retinal signalling from visual feedback (section 1.3.2), visual function in chickens from both the High and Low lines were tested using an optokinetic nystagmus (OKN) testing paradigm to examine if a generalised visual deficit in chickens from the Low lines resulted in the divergence in myopia susceptibility. After testing the optokinetic response of chickens in the third generation, no significant difference in visual function was observed between the High and Low lines (Figure 4.5). Chickens from the two selected lines showed similarly good visual function. The gain (which quantified visual performance in the OKN test) observed under binocular viewing condition was 0.95 ± 0.11 and 0.96 ± 0.11 in the Low and High lines, respectively (Mann-Whitney U test; P=0.72). Similarly, no significant difference in gain between the two selected lines was detected under the monocular testing condition (0.84 ± 0.04 versus 0.88 ± 0.11, Mann-Whitney U test; P = 0.86).

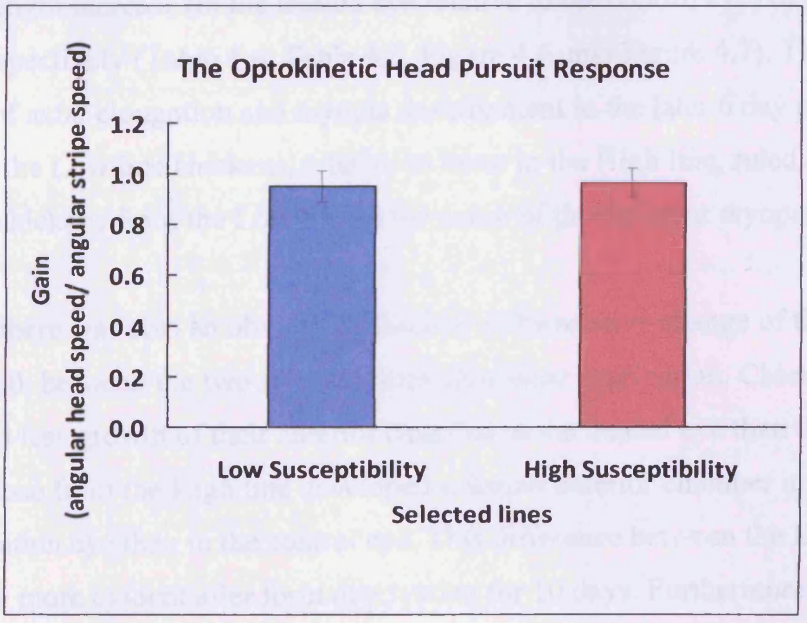
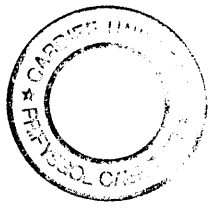


Figure 4.5 Optokinetic head pursuit responses in untreated High and Low line chicks from the third generation (binocular viewing condition). Visual function in chicks aged 7 days old was assessed by measuring the ratio of angular head speed during optokinetic smooth pursuit phases to the angular speed of a low-contrast drifting grating of spatial frequency 1 cycle/degree, where a ratio of 1.0 corresponds to normal performance and a ratio of zero corresponds to no visual function. Error bars show standard error. Blue and red bars denote Low and High lines chickens, respectively.

4.3.4. Susceptibility to form deprivation for 10 days in selectively bred chicks

The quantification of myopia susceptibility in the selection process was based on the ocular response to form deprivation for only 4 days. Thus, there could have been a delayed response in chickens from the Low line, which would have been the cause of the significantly different susceptibility observed between the two selected lines. Hence, form deprivation for 10 days was implemented in chicks from the High and Low lines to test whether the different ocular responses between them was still evident after form deprivation for a longer period of time. Chickens from the High line developed an average of -12.88 D of myopia after form deprivation for the first 4 days and -20.53 D after 10 days, and exhibited an average daily increase in axial length (in the treated eye relative to the control eye) of 0.12 mm and 0.11 mm over the first 4 and the subsequent 6 days. Nonetheless, chickens from the Low line developed only an average of -5.21 D and -7.61 D of myopia after form deprivation for 4 and 10 days, and showed an average daily axial length increase (in the treated eye relative to the control eye) of 0.02 and 0.05 mm, respectively (Table 4.4, Table 4.5, Figure 4.6, and Figure 4.7). The continued slower rate of axial elongation and myopia development in the later 6 day period of treatment in the Low line chickens, relative to those in the High line, ruled out a delayed response in chickens from the Low line as the cause of the different myopia susceptibility.

In addition, there was also an obvious difference in the relative change of the anterior chamber depth between the two selected lines after form deprivation. Chickens from the Low line had less growth of their anterior chamber in the treated eye than the control eye. However, those from the High line developed a deeper anterior chamber in the form-deprivation eye than in the control eye. This difference between the High and Low lines became more evident after form deprivation for 10 days. Furthermore, the degree of myopia induced in the later 6 days was less than that in the first 4 days in both the Low and High lines, despite the relative changes in axial length (ΔAL) being greater in the later 6 days than in the first 4 days. This may be due to the relationship between the relative scaling of eye size and refractive error. To develop the same degree of myopia, a greater increase in axial length is required in a larger eye than in a smaller one. However, the crucial finding was that chickens from the Low line did not catch up with those from the High line in their degree of induced myopia, even after a longer period of form deprivation.



When chickens in the third generation were left untreated, there were only small differences in lens thickness and vitreous chamber depth between those from the High and Low lines at an age of 8 days (the age corresponding to the treated group after form deprivation for 4 days). Furthermore, no significant difference in any ocular component dimension between the lines was found at 14 days of age (the age corresponding to the treated group after form deprivation for 10 days). Refractive status did not differ significantly between the two selected lines either at 8 days old or at 14 days old (Table 4.4).

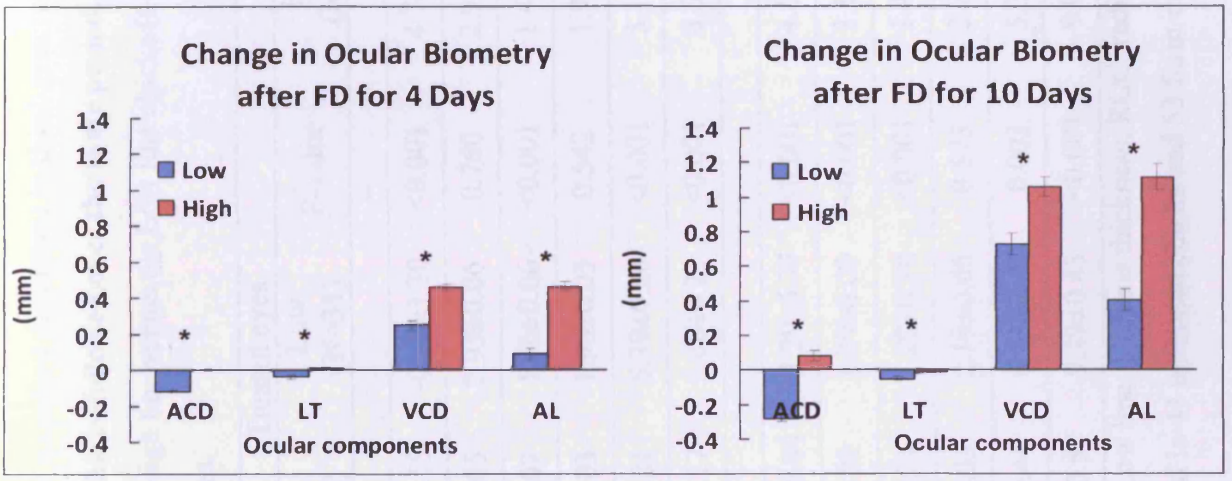


Figure 4.6 The relative changes in ocular components in High and Low line chickens in the third generation after form deprivation for 4 and 10 days. Bars show change in treated eye minus change in control eye over the treatment period, error bars represent standard error (" * ": significant difference, P<0.05).

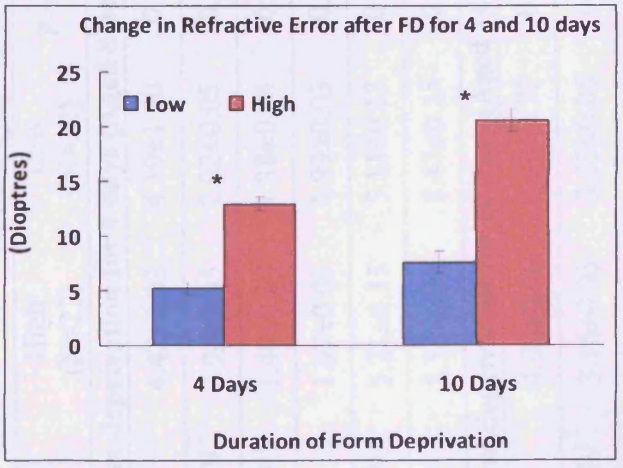


Figure 4.7 The relative changes in refraction in High and Low line chickens in the third generation after form deprivation for 4 and 10 days. Bars show change in control eye minus change in treated eye over the treatment period, error bars represent standard error (" * ": significant difference, P<0.05).

Table 4.4 Refraction and ocular component dimensions (mean ± standard deviation) in third-generation chicks monocularly deprived of sharp vision for 10 days and in (the right eyes of) untreated chicks followed over the same period. For ocular component dimensions, P-values relate to a test for a difference in trait means between the High line versus the Low line chicks (*t*-test). For refraction, P-values relates to a Mann-Whitney U test between the High versus Low lines.

	Normal eyes			Treated eyes			Control eyes		
	High (N=22)	Low (N=22)	P-value	High (N=33)	Low (N=31)	P-value	High (N=33)	Low (N=31)	P-value
After form deprivation for 4 days (Aged 8 days old)									
RX (D)	4.42±1.03	4.19±1.50	0.720	-8.12±3.59	-0.74±3.30	<0.001	4.76±0.73	4.47±1.25	0.595
RCC (mm)‡	2.94±0.05	2.92±0.05	0.209	2.93±0.05	2.93±0.06	0.760	2.92±0.05	2.92±0.06	0.928
ACD (mm)	1.40±0.04	1.38±0.04	0.077	1.41±0.07	1.26±0.06	<0.001	1.41±0.04	1.39±0.03	0.129
LT (mm)	1.97±0.03	1.99±0.03	0.016	1.97±0.03	1.96±0.03	0.542	1.96±0.03	2.00±0.04	<0.001
VCD (mm)	5.21±0.15	5.11±0.13	0.032	5.60±0.21	5.39±0.20	<0.001	5.15±0.16	5.13±0.13	0.693
AL (mm)	8.56±0.18	8.47±0.15	0.072	8.97±0.27	8.60±0.23	<0.001	8.50±0.19	8.51±0.16	0.939
After form deprivation for 10 days (Aged 14 days old)									
RX (D)	4.02±0.66	4.23±0.72	0.114	-15.91±6.09	-3.29±5.75	<0.001	4.62±0.76	4.32±0.74	0.220
RCC (mm)‡	3.16±0.05	3.13±0.05	0.197	3.14±0.09	3.29±0.09	<0.001	3.14±0.06	3.15±0.07	0.496
ACD (mm)	1.48±0.05	1.46±0.04	0.214	1.55±0.21	1.20±0.10	<0.001	1.48±0.06	1.48±0.05	0.899
LT (mm)	2.21±0.05	2.22±0.05	0.580	2.19±0.05	2.19±0.05	0.573	2.20±0.04	2.24±0.06	0.009
VCD (mm)	5.46±0.16	5.38±0.12	0.063	6.45±0.44	6.13±0.40	0.003	5.39±0.19	5.39±0.15	0.959
AL (mm)	9.14±0.19	9.05±0.15	0.100	10.17±0.58	9.49±0.43	<0.001	9.06±0.24	9.08±0.18	0.628

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth. ‡RCC was measured in 44 untreated chicks and 53 form-deprived chicks (L=24, H=29).

Table 4.5 Relative changes in ocular component dimensions and refraction after form deprivation for 10 days (values show mean ± standard deviation change in treated eye minus control eye). For ocular component dimensions, P-values relate to a test for a difference in trait means between the High line versus the Low line chicks (*t*-test). For ΔACD, ΔLT and ΔRX, P-values relate to a Mann-Whitney U test between the High versus Low line.

		Changes in the first 4 days	P value	Changes in the latter 6 days	P value	Changes in 10 days	P value
ΔRCC (mm)	L	0.01 ± 0.03	0.732	0.12 ± 0.06	<0.001	0.14 ± 0.06	<0.001
	H	0.01 ± 0.05		-0.01 ± 0.06		0.003 ± 0.08	
ΔACD (mm)	L	-0.12 ± 0.04	<0.001	-0.15 ± 0.07	<0.001	-0.28 ± 0.09	<0.001
	H	0.002 ± 0.05		-0.07 ± 0.15		0.08 ± 0.19	
ΔLT (mm)	L	-0.04 ± 0.05	<0.001	-0.01 ± 0.07	0.328	-0.05 ± 0.07	0.029
	H	0.01 ± 0.03		-0.02 ± 0.06		-0.01 ± 0.05	
ΔVCD (mm)	L	0.25 ± 0.15	<0.001	0.48 ± 0.26	0.077	0.73 ± 0.35	<0.001
	H	0.46 ± 0.11		0.60 ± 0.27		1.06 ± 0.31	
ΔAL (mm)	L	0.09 ± 0.16	<0.001	0.31 ± 0.25	<0.001	0.40 ± 0.36	<0.001
	H	0.47 ± 0.14		0.65 ± 0.35		1.12 ± 0.44	
ΔRX (D)	L	-5.21 ± 2.71	<0.001	-2.40 ± 3.58	<0.001	-7.61 ± 5.59	<0.001
	H	-12.88 ± 3.54		-7.65 ± 3.76		-20.53 ± 5.89	

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth.

4.3.5. Relationship between corneal curvature and susceptibility to form deprivation in selectively bred chicks

In the third generation of selectively bred chickens, the radius of corneal curvature before and after treatment was measured in 226 and 178 chickens, respectively. There were small but significant differences in corneal curvature before and after treatment between the High and Low lines (Table 4.1, Table 4.2 and Table 4.3). The corneal curvature in chickens from the High line was flatter before form deprivation and became steeper after treatment compared to those from the Low line. This difference in corneal curvature between the two selected lines became more evident after form deprivation for 10 days (Table 4.4 and Table 4.5). In addition, sex, eye size before treatment and the relative change in axial length after treatment were all found to correlate with pre-treatment corneal curvature (Table 4.6). Nonetheless, significant correlations could only be found between sex and pre-treatment corneal curvature, and between pre-treatment eye size and corneal curvature, when the selected lines (High versus Low line) were controlled for. Thus, multiple regression analysis was carried out to test whether corneal curvature before form deprivation was a significant predictive variable for susceptibility to form-deprivation myopia after taking other potential predictors into consideration.

In the multiple regression analysis, sex, selected line, eye size and corneal curvature before form deprivation were examined for their effects on susceptibility to form-deprivation myopia. Selected line had the most significant influence on susceptibility to form-deprivation myopia (standardised regression coefficient ranged from 0.626 to 0.810) in these models. Sex was also a significant predictor of susceptibility to form-deprivation myopia. However, only a borderline effect of eye size before treatment on susceptibility to form-deprivation myopia was observed. No significant effect on susceptibility to form-deprivation myopia from corneal curvature before treatment was found in any model (Table 4.7). Thus, corneal curvature is not a predictor of susceptibility to form-deprivation myopia in this White Leghorn chicken population. This robust result from the multiple regression analysis suggested that further analysis using structural equation modelling was not warranted.

Table 4.6 Correlations amongst sex, eye size, susceptibility to form-deprivation myopia and radius of corneal curvature.

Correlation coefficients	Sex	Initial AL (eye size, mm)	Susceptibility to FDM		
			ΔVCD (mm)	ΔAL (mm)	ΔRx (Dioptres)
RCC_T before FD (mm) (N=226) **(Bivariate correlation)**	$r_p = 0.39$, $P < 0.001$*	$r_p = 0.76$, $P < 0.001$*	$r_p = 0.11$, $P = 0.107$	$r_p = 0.14$, $P = 0.034$*	$r_s = 0.09$, $P = 0.166$
RCC_T before FD (mm) (partial correlation)	$r = 0.38$, $P < 0.001$*	$r = 0.76$, $P < 0.001$*	$r = 0.03$, $P = 0.613$	$r = 0.07$, $P = 0.329$	$r = -0.04$, $P = 0.519$

FDM: form-deprivation myopia, RCC_T: Radius of Corneal curvature in the treated eye, r_p: Pearson correlation coefficient, r_s: Spearman's rank correlation coefficient, r: partial correlation coefficient "after controlling for selected line (High versus Low)", "*": significant correlation with $P < 0.05$.

Table 4.7 Multiple regression analysis to test whether corneal curvature (RCC) before form deprivation was a significant predictive variable for susceptibility to form-deprivation myopia. The parameters to denote susceptibility to form-deprivation myopia, i.e.ΔVCD, ΔAL and ΔRx, were examined as a function of sex, selected line, eye size and corneal curvature before form deprivation. All variables were included in the starting model (A), followed by backward removal of variables to identify more parsimonious models (B or C).

Dependent variable	Model	Adjusted R2	Predictors	B[a]	P-value
ΔVCD	A	0.455	Sex	0.290	<0.001
			Selected line	0.630	<0.001
			Eye Size	-0.183	0.026
			RCC	0.053	0.495
	B	0.456	Sex	0.293	<0.001
			Selected line	0.626	<0.001
			Eye Size	-0.143	0.012
ΔAL	A	0.642	Sex	0.238	<0.001
			Selected line	0.767	<0.001
			Eye Size	-0.108	0.104
			RCC	0.031	0.614
	B	0.643	Sex	0.240	<0.001
			Selected line	0.765	<0.001
			Eye Size	-0.084	0.069
	C	0.639	Sex	0.203	<0.001
			Selected line	0.748	<0.001
ΔRx	A	0.675	Sex	0.183	<0.001
			Selected line	0.806	<0.001
			Eye Size	-0.065	0.303
			RCC	-0.047	0.433
	B	0.675	Sex	0.180	<0.001
			Selected line	0.810	<0.001
			Eye Size	-0.101	0.023

[a] Standardised regression coefficient.

4.4 Discussion

After three rounds of selective breeding for high and low susceptibility to form deprivation, not only the degree of myopia induced after form deprivation but also the relative changes in ocular components showed significant differences and an evident divergence between the High and Low lines. Since the changes in ocular components induced by form deprivation are vision-dependent, one potential explanation for the reduced susceptibility of chicks from the Low line would be the inheritance of an allele or alleles causing generalised visual disability. However, as assessed using an optokinetic nystagmus testing paradigm, visual function was similarly good in both the High and Low line animals, ruling out a generalised visual deficit as the cause of the divergence in susceptibility to form-deprivation myopia.

Another plausible cause of this divergence was that chicks from the Low line exhibited their reduced levels of induced myopia because of a relative immaturity either of the retinal circuitry necessary to detect and respond to image blur or some other aspect of their eyes' vision-dependent regulatory growth pathway. Nevertheless, there were significant differences in the relative changes of ocular component dimensions and induced myopia between chickens from the High and Low lines treated with monocular form deprivation for a longer period of 10 days. The slower rate of axial elongation and myopia development in both the first 4 days and later 6 days of treatment in the Low line chicks, relative to those in the High line, ruled out this experiment having selected chicks on the basis of the maturity of their visually-guided growth regulation system. Instead, the results are consistent with the idea that chicks were selected dependent on the "gain" (Tepelus and Schaeffel, 2010) of their eye growth regulatory system. Therefore, the evident divergence in the susceptibility to form-deprivation myopia in the three generations of selective breeding provided evidence that there is a strong genetic component in the development of environmentally-induced myopia.

The myopia induced by form deprivation is primarily brought about by an increase in vitreous chamber depth (Hayes et al., 1986; Wallman and Adams, 1987). It is also interesting to note that chicks from the Low line showed a significant decrease in the rate

of anterior chamber deepening over the treatment period, whilst they developed less vitreous chamber elongation than chicks from the High line. This effect was particularly pronounced after form deprivation for 10 days. As a result of this relatively slow rate of anterior chamber deepening, the combined refractive power of the cornea and crystalline lens would be increased, thus tending to make the Low line chickens more myopic than would otherwise be the case and representing a counter-intuitive result. In addition, there was also a significant difference in corneal curvature between the High and Low lines. Although the High line chickens had flatter corneas than those from the Low line before treatment, corneal curvature in the High line chickens became significantly steeper than that in Low line ones, especially after 10 days of treatment. The coupling of influences on the rate of growth of both the anterior and posterior segments of the eye in both lines of chickens during form deprivation suggested alleles with pleiotropic effects on the growth of both anterior and posterior segments of the eye may have been subject to selection.

Furthermore, refractive error and ocular component dimensions differed significantly between the High and Low line chickens even before treatment was initiated. At 4 days of age, chickens from the Low line were an average of +0.21 D more hyperopic than their High line counterparts (+4.43 versus +4.22 D, respectively, $P<0.001$) and had shorter eyes (8.05 versus 8.12 mm, respectively, $P<0.001$). Similar phenomena have been observed in some studies of myopia development in humans. Zadnik et al. (1994) carried out an initial cross-sectional study of 716 schoolchildren and found that non-myopic children with non-myopic parents have shorter eyes and a more hyperopic refractive error than do non-myopic children with myopic parents, after controlling for grade in school and near work. In a longitudinal follow-up study, they found cycloplegic refractive error in the third grade could predict the onset of juvenile myopia with a sensitivity of 86.7% and a specificity of 73.3%, which could be slightly improved upon by adding information on corneal power, lens power and axial length (Zadnik et al., 1999). In addition, children destined to become myopic have been shown to have a lower level of hyperopia in infancy than do those destined to remain emmetropic (Gwiazda et al., 1993). Mutti et al. (2007b) also revealed that longer eyes and more negative refractive errors were evident 2 to 4 years before the onset of myopia in a longitudinal study of refractive error in schoolchildren. However, a cross-sectional survey in Chinese children suggested that the growth rate of

the eye, instead of its size before the onset of myopia, was influenced by parental history of myopia (Lam et al., 2008b). As well as these pre-treatment differences in eye size and refraction between the High and Low lines, the fellow control eyes of chicks undergoing form deprivation also demonstrated subtly different "yoked" (Zhu and Wallman, 2009) responses in anterior chamber depth, vitreous chamber depth and axial length between the two lines (Table 4.2). Moreover, corneal curvature was measured in the third generation and significant differences between the High and Low lines were revealed. However, pre-treatment corneal curvature was not a significant predictor of susceptibility to form-deprivation myopia. Instead, in multiple regression analyses of selectively bred chickens, sex was found to be a significant variable capable of predicting susceptibility, which was in accordance with the results from outbred chickens (Chapter 3). Selected line (High versus Low) showed a substantial effect on the susceptibility to form-deprivation myopia in the multiple regression analyses, which confirmed again the effect of this selection strategy on susceptibility to environmentally-induced myopia.

In spite of the longer axial length and less hyperopia in the pre-treatment eyes of chickens from the High line compared to those from the Low line, these significant differences between the two lines did not persist when chickens were left untreated until 14 days of age. It is intriguing to note that selection only showed its effects in untreated chicks at age 4 days, maybe suggesting that specific alleles that had been selected had an effect at this age more than at other ages. Another possible explanation could be that the effect of emmetropisation minimized this difference during the process in natural growth of the eye. However, the process of emmetropisation was disrupted when form deprivation was introduced with the result of different responses in ocular growth and induced refractive error between the two selected lines, which demonstrated that susceptibility to environmentally-induced myopia in White Leghorn chickens was strongly genetic in origin.

4.5 Conclusion

This selective breeding experiment showed an obvious divergence in the response of both ocular components and the level of myopia induced by form deprivation. This significant divergence was neither due to a generalised visual deficit nor a delayed response to form deprivation in chickens from the Low line. Therefore, the results of the selective breeding experiment convincingly demonstrated that susceptibility to environmentally-induced myopia in chickens is substantially genetically determined.

Chapter 5

Susceptibility to lens-induced visual defocus in chicks selectively bred for high and low susceptibility to form-deprivation myopia

5.1 Introduction

Schaeffel et al. (1988) first found that chicks fitted with a spectacle lens in front of one eye were able to modify the growth of the eye and its refractive development according to the sign and power of the defocusing lens. Thus, minus powered lenses that shifted the focal plane of the eye behind the retina caused the eye to elongate at a faster rate than usual, whilst plus lenses that shifted the focal plane in front of the retina caused the eye to slow its normal rate of growth. Since the original study of Schaeffel et al. (1988), this treatment paradigm has been widely used to study the underlying mechanisms in the development of refractive error. Irving et al. (1992) revealed that hatchling chicks could compensate for lens powers ranging from -10 to +15 Dioptres in one week. In addition, they found an asymmetry of response to the sign of defocus, namely, a faster response to plus lens than to minus lens treatment, suggesting there might be different underlying mechanisms controlling the responses to plus and minus lenses. Furthermore, glucagon-containing amacrine cells in the retina have been found to respond differentially to the sign of defocus (section 1.3.2.1). Moreover, different responses to minus and plus lenses in choroid and sclera have been observed (Wildsoet and Wallman, 1995). Hyperopic defocus produced by minus lens wear has been found to stimulate thinning of the choroid, growth of the sclera and elongation of axial length, with resultant myopia. In contrast, myopic defocus generated by plus lens wear has been found to cause thickening of the choroid and deceleration of the growth in sclera and axial length, with induced hyperopia. Zhu et al. (2009) further used the rate of the responses in choroidal thickness and ocular length to plus and minus lens wear in chickens to infer the temporal properties of the underlying growth signals induced by defocus. Similar "rising rates" were observed after plus and minus lens treatments, yet slower "declining rates" were found in plus lens wear compared to minus lens wear. These results suggested that the underlying mechanisms for the ocular responses to minus and plus lenses might be different.

Furthermore, although both form deprivation and hyperopic defocus (by minus lens wear) produce elongation of axial length and induce myopia, there has been accumulating evidence that different underlying mechanisms exist. For instance, 6-hydroxydopamine, which is a neurotoxin impairing the function of dopaminergic neurons in the retina,

inhibits axial myopia induced by form deprivation, but not by minus lens wear (Schaeffel et al., 1994). In addition, form deprivation resulted in a decline in retinal dopamine and its metabolite (3, 4-dihydroxyphenylacetic acid, DOPAC) (Stone et al., 1989, Bartmann et al., 1994). However, this was not the case when myopia was induced by minus lenses. Retinal dopamine and DOPAC levels remained unchanged even after full compensation for the lenses had been achieved (Bartmann et al., 1994). Bitzer et al. (2000) found that intravitreal injection of disulfiram, an inhibitor of retinoic acid synthesis, inhibited myopia induced by form deprivation, but not by minus lenses. Moreover, constant light exposure suppressed the development of form-deprivation myopia, but did not influence the refractive error induced by lenses (Bartmann et al., 1994). On the other hand, diurnal growth rhythms of the eyes in chickens were both changed during form deprivation and lens treatment. Additionally, intravitreal injection of reserpine, which depletes dopamine stores, suppressed both the elongation of axial length induced by form deprivation and minus lenses. Therefore, Schaeffel et al. (1995) suggested there might be some common characteristics between the responses to form deprivation and lenses treatment due to the above observations. However, it remains unclear whether there are shared mechanisms or pathways in the ocular responses to form deprivation and hyperopic defocus.

A significant divergence in susceptibility to form-deprivation myopia between the High and Low lines was observed in the third generation of chickens selectively bred for this response (Chapter 4). In this chapter, susceptibility to visual defocus induced by lens wear was examined in the two selected lines, in order to test whether they differed in their susceptibility to these visual cues. If a significant difference in the response to lens-induced defocus between the two selected lines was observed, there must be some shared mechanism(s) or pathway(s) between form deprivation and lens treatment controlled by one or more gene variants which had been selected for during the process of selective breeding. If the High and Low lines did not differ in their response to lenses, different underlying mechanisms or pathways must exist, at least in part.

5.2 Materials and Methods

5.2.1 Subjects and visual defocus imparted by lenses

Fifty-six and 52 third-generation chickens from the High and Low lines (section 4.2), respectively, were randomly assigned to monocular treatment with a plano, +10 D or -15 D lens at age 4 days old. Plano, +10 D plano-convex and -15 D plano-concave glass lenses (diameter 12mm) were fitted inside short (8mm) lengths of soft, translucent silicone tubing (i.d. 12mm), which were in turn each attached to a Velcro ring. A mating ring of Velcro was glued to the feathers around the eye to attach the lens (Figure 5.1). Lenses were cleaned twice daily to avoid occurrence of visual deprivation effect due to unclean lenses during continuous treatment for 4 days.

Since full compensation for a wide range of lens powers has been observed in one week in hatchling chicks (section 5.1), lenses with a power of either +10 D or -15 D were used in treating the chickens, to avoid full compensation (a situation which would make it difficult to differentiate the inter-animal variation in susceptibility to lens treatment).

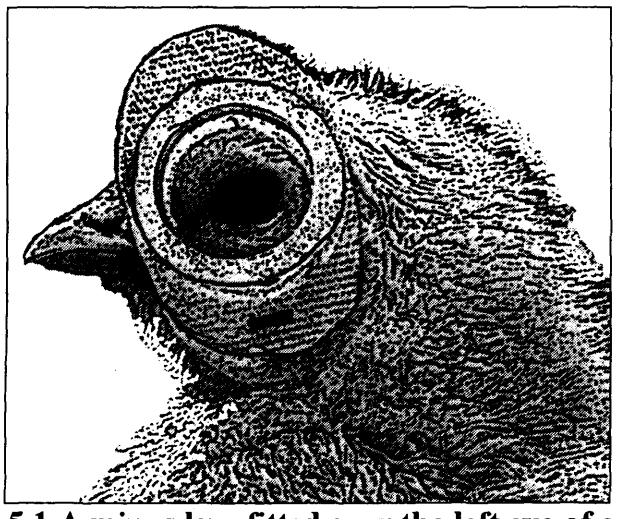


Figure 5.1 A minus lens fitted over the left eye of a chick.

5.2.2 Ocular measurements and quantification of susceptibility to lens-indcued defocus

Ocular component dimensions, corneal curvature and refractive error were measured by A-scan ultrasonography, videokeratometry and retinoscopy, respectively, before and after 4 days continuous lens wear (section 2.2.2). Susceptibility to lens-induced defocus was quantified in each chicken by the relative changes in vitreous chamber depth, axial length and refractive error between the treated and control eyes (ΔVCD, ΔAL and ΔRX) as the parameters to quantify myopia susceptibility, as described in section 2.2.2.5. After treatment, susceptibility to lens-induced defocus was compared between the High and Low lines to find if there were any differences between them.

5.2.3 Statistical analysis

All data for ocular component dimensions before and after lens treatment, and the relative changes (ΔRCC, ΔACD, ΔLT, ΔVCD, ΔAL and ΔRX), were tested for normality using the Kolmogorov-Smirnov test. Due to a non-normal distribution of ΔRX, differences between the High and Low lines were compared using the Mann–Whitney U test. Other comparisons of the ocular component dimensions and relative changes after treatment between the two selected lines were made using independent samples *t*-test. All analyses were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL).

5.3 Results

5.3.1 Ocular components dimensions before lens treatment

Before treatment, there were significant differences in ocular component dimensions between the High and Low lines, including corneal curvature, anterior chamber depth, vitreous chamber depth and axial length. Chickens from the High line had a flatter cornea, a deeper anterior chamber, vitreous chamber and a longer axial length than did the Low lines birds (Table 5.1). They also differed significantly in refractive status: specifically, a less hyperopic refraction was observed in chickens from the High line than the Low line. These results were in accordance with those from chickens tested for susceptibility to form

deprivation in the third generation of the selective breeding experiment (Table 4.1), suggesting that both populations from the third generation had the same ocular characteristics.

Table 5.1 Ocular component dimensions and refractive status before lens treatment in chickens from the third generation of selective breeding (mean ± standard deviation, independent samples *t*-test, "*": significant difference (P<0.05), § Mann-Whitney U test).

Selected lines	Subject number	RCC (mm)	ACD (mm)	LT (mm)	VCD (mm)	AL (mm)	RX (Dioptres) §
Low	52	2.77±0.05	1.26±0.04	1.84±0.03	4.96±0.13	8.05±0.15	4.50±0.50
High	56	2.81±0.06	1.28±0.03	1.83±0.03	5.09±0.13	8.19±0.16	4.17±0.73
P value		0.002*	0.001*	0.609	<0.001*	<0.001*	0.006*

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line,
LT: lens thickness, RCC: radius of corneal curvature, RX: refraction,
VCD: vitreous chamber depth.

5.3.2. The relative changes in ocular component dimensions and refractive error produced by wearing lenses

There were 29, 39 and 40 chickens treated by plano lenses, +10 D lenses and -15 D lenses, respectively. The changes in ocular component dimensions and refraction (compared to the fellow control eye) after lens treatment for 4 days are shown in Table 5.2, Figure 5.2 and Figure 5.3. In the plano-lens treated group, there were small differences in the relative changes in anterior chamber depth (ΔACD) and axial length (ΔAL) between the High and Low selected lines. In chickens treated with +10 D lenses for 4 days, only a significant difference in ΔACD was observed between the two lines. As in the plano-treated birds, the Low line chickens had a shorter anterior chamber than the High line birds. No significant difference in refractive error induced by +10 D lenses wear between the High and Low lines was observed (+6.90 D vs +8.13 D of induced hyperopia, P= 0.093). However, in the group treated with –15 D lenses, the relative changes in all ocular components except lens thickness (ΔLT) differed significantly between the two selected lines. Compared to chickens from the Low line, chickens from the High line had a steeper cornea, a deeper

anterior chamber, deeper vitreous chamber, longer axial length and became more myopic after treatment. Specifically, after wearing -15 D lenses for 4 days, chickens from the High line developed more than twice the degree of myopia and exhibited three times the axial length growth than did those from the Low line. The High susceptibility chickens had an average relative axial length growth (ΔAL) of 0.45 ± 0.14 mm and a level of induced myopia of -11.14 ± 2.87 D, whilst the Low susceptibility ones had an average axial length growth of only 0.15 ± 0.13 mm and a level of induced myopia of -4.80 ± 3.20 D (both P<0.001). The differences between the High and Low lines in the ocular response to visual defocus by minus lenses were similar to the responses to form deprivation.

Table 5.2 Relative changes in ocular component dimensions and refractive error after plano, +10D and -15D lens treatment in chickens (mean ± standard deviation, independent samples *t*-test, "*": significant difference (P<0.05), § Mann-Whitney U test).

	ΔRCC (mm)	ΔACD (mm)	ΔLT (mm)	ΔVCD (mm)	ΔAL (mm)	ΔRx (Dioptre) §
Plano Lens treatment						
Low (N=14)	0.020±0.04	-0.08±0.06	-0.03±0.07	0.01±0.08	-0.09±0.07	-0.43±1.14
High (N=15)	0.004±0.04	-0.01±0.07	-0.01±0.05	0.004±0.07	-0.02±0.09	-0.17±0.72
P value	0.308	0.014*	0.257	0.729	0.029*	0.683
+10 D Lens treatment						
Low (N=19)	-0.01±0.04	-0.11±0.04	-0.05±0.04	-0.28±0.12	-0.44±0.11	8.13±2.16
High (N=20)	-0.01±0.03	-0.04±0.05	-0.03±0.03	-0.31±0.11	-0.38±0.12	6.90±2.43
P value	0.799	<0.001*	0.108	0.454	0.114	0.093
-15 D Lens treatment						
Low (N=19)	0.03±0.04	-0.08±0.05	-0.05±0.05	0.28±0.12	0.15±0.13	-4.80±3.20
High (N=21)	-0.01±0.06	0.02±0.06	-0.03±0.09	0.44±0.11	0.45±0.14	-11.14±2.87
P value	0.029*	<0.001*	0.440	<0.001*	<0.001*	<0.001*

ACD: anterior chamber depth, AL: axial length, H: High line, L: Low line,
LT: lens thickness, RCC: radius of corneal curvature, RX: refraction,
VCD: vitreous chamber depth.

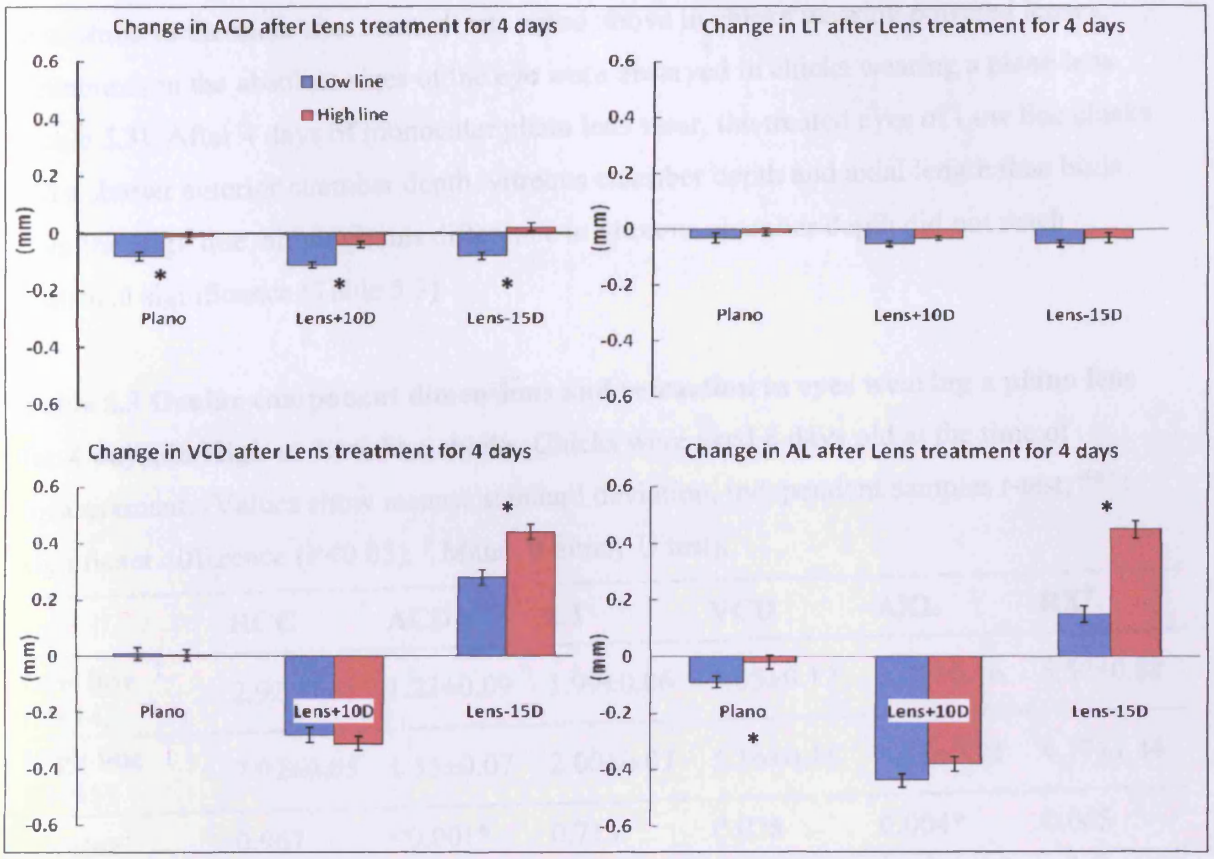


Figure 5.2 The relative changes in ACD, LT, VCD and AXL between the treated and control eyes after lens treatment for 4 days in the High and Low line chicks.

(" * ": significant difference ($p<0.05$); error bars represent standard error.)

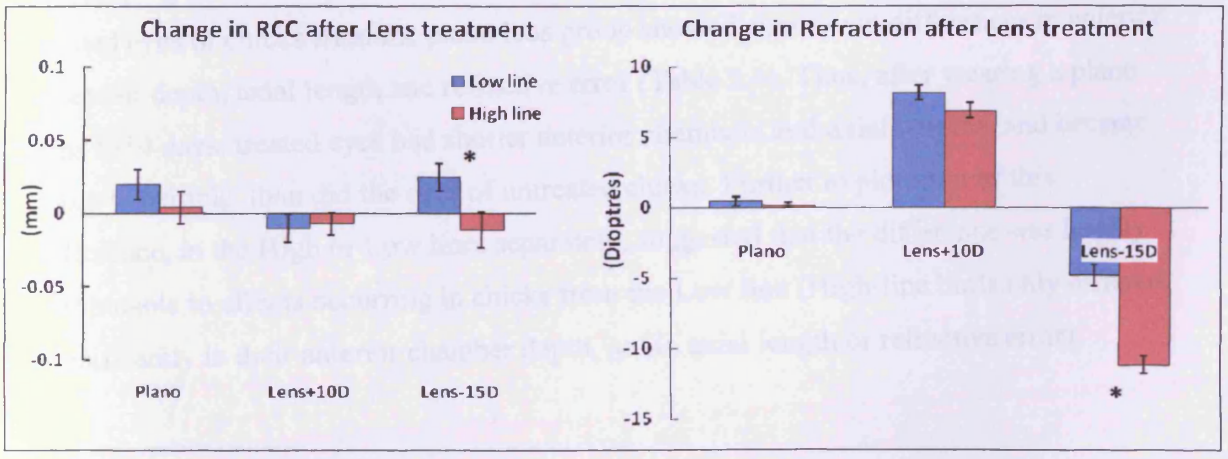


Figure 5.3 The relative changes in corneal curvature (ΔRCC) and refractive error (ΔRX) between the treated and control eyes induced by 4 days of monocular lens wear in the High and Low line chicks. (" * ": significant difference ($p<0.05$), error bars represent standard error.)

In addition to the main treatment effects noted above in chicks wearing powered lenses, differences in the absolute sizes of the eye were observed in chicks wearing a plano lens (Table 5.3). After 4 days of monocular plano lens wear, the treated eyes of Low line chicks had a shorter anterior chamber depth, vitreous chamber depth and axial length than birds from the High line, although this difference in vitreous chamber depth did not reach statistical significance (Table 5.3).

Table 5.3 Ocular component dimensions and refraction in eyes wearing a plano lens for 4 days, in High or Low line chicks. Chicks were aged 8 days old at the time of measurement. (Values show mean ± standard deviation, independent samples *t*-test, "*": significant difference (P<0.05), § Mann-Whitney U test).

	RCC	ACD	LT	VCD	AXL	RX§
Low line (N=14)	2.92±0.08	1.23±0.09	1.99±0.06	5.05±0.12	8.25±0.16	5.50±0.88
High line (N=15)	2.92±0.05	1.35±0.07	2.00±0.07	5.16±0.18	8.48±0.21	4.77±1.44
P value	0.967	<0.001*	0.713	0.078	0.004*	0.065

ACD: anterior chamber depth, AL: axial length, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth.

In comparison to the ocular component dimensions of age-matched untreated chicks, the treated eyes of chicks from the plano lens group showed significant differences in anterior chamber depth, axial length and refractive error (Table 5.4). Thus, after wearing a plano lens for 4 days, treated eyes had shorter anterior chambers and axial lengths, and became more hyperopic, than did the eyes of untreated chicks. Further exploration of this difference, in the High or Low lines separately, suggested that the difference was largely attributable to effects occurring in chicks from the Low line (High-line birds only differed significantly in their anterior chamber depth, not in axial length or refractive error).

Table 5.4 Ocular component dimensions and refraction in the treated eyes of chicks from the plano lens group and in the right eyes of untreated chicks. Chicks were aged 8 days old at the time of measurement. (Values show mean ± standard deviation, independent samples *t*-test, "*": significant difference (P<0.05), §Mann-Whitney U test).

	RCC	ACD	LT	VCD	AL	RX§
High and Low lines together						
Plano lens wearing eyes (N=29)	2.92±0.06	1.29±0.10	1.99±0.06	5.11±0.16	8.37±0.22	5.12±1.24
Untreated right eyes (N=44)	2.93±0.05	1.39±0.04	1.98±0.03	5.16±0.15	8.52±0.17	4.31±1.28
P value	0.677	<0.001*	0.477	0.163	0.002*	0.001 *
Low line only						
Plano lens wearing eyes (N=14)	2.92±0.08	1.23±0.09	1.99±0.06	5.05±0.12	8.25±0.16	5.50±0.88
Untreated right eyes (N=22)	2.92±0.05	1.38±0.04	1.99±0.03	5.11±0.13	8.47±0.15	4.19±1.50
P value	0.818	<0.001*	0.679	0.191	<0.001*	0.001 *
High line only						
Plano lens wearing eyes (N=15)	2.92±0.05	1.35±0.07	2.00±0.07	5.16±0.18	8.48±0.21	4.77±1.44
Untreated right eyes (N=22)	2.94±0.05	1.40±0.04	1.97±0.03	5.21±0.15	8.56±0.18	4.42±1.03
P value	0.344	0.003*	0.210	0.377	0.199	0.164

ACD: anterior chamber depth, AL: axial length, LT: lens thickness, RCC: radius of corneal curvature, RX: refraction, VCD: vitreous chamber depth.

5.3.3. Susceptibility to lens-induced defocus in selectively bred chickens

In terms of susceptibility to lens-induced defocus, the frequency distributions of the three parameters used to quantify ocular growth responses are shown in Figure 5.4. The susceptibility to plus lens wear did not differ significantly between the High and Low lines. The frequency distributions of ΔVCD, ΔAL, and ΔRX were largely overlapping between the two selected lines, and no significant difference was found ($p=0.454$, 0.114, 0.093, respectively). However, there was an obvious divergence in the distributions of ΔVCD, ΔAL, and ΔRX after minus lens treatment for 4 days between the two selected lines. Chickens from the High line had more growth in vitreous chamber depth, axial length and developed more myopia after wearing minus lenses compared to those from the Low line (all $p<0.001$). The High and Low lines differed significantly in their susceptibility to hyperopic defocus by minus lenses, which was similar to the response to form deprivation.

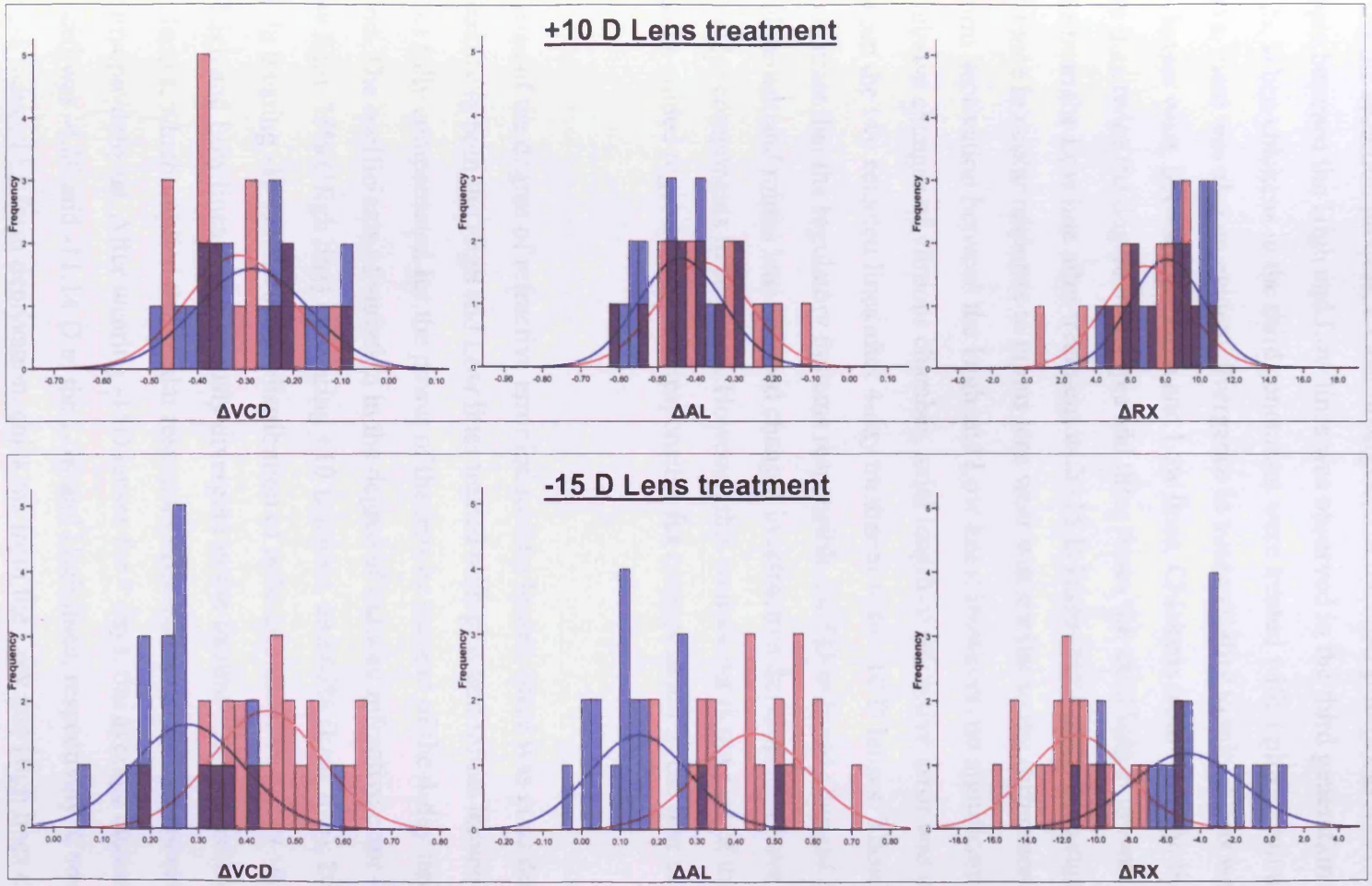


Figure 5.4 Frequency distribution of the three parameters (ΔVCD, ΔAL, and ΔRX) used to quantify susceptibility to lens-induced refractive development. The upper and lower graphs show the frequency distributions of susceptibility to +10 D and -15 D lens-induced defocus in chickens from the High (red bars) and Low (blue bars) lines, respectively. The overlapping regions are shown as purple bars.

5.4 Discussion

During the selection process, a clear divergence in the susceptibility to form-deprivation myopia between the High and Low lines was observed in the third generation (section 4.3.2). When chickens in the third generation were treated with a plus or minus lens for 4 days, there was also an evident divergence in susceptibility to minus lens wear, but not plus lenses wear, between the High and Low lines. Chickens from the High line developed more than twice the degree of myopia and three times the axial length growth compared to those from the Low line after treatment with -15 D lenses for 4 days. This significant difference in ocular response to minus lens wear was similar to the difference in response to form deprivation between the High and Low lines. However, no significant difference in the relative changes of vitreous chamber, axial length or refractive error was observed between the two selected lines after 4-day treatment with +10 D lenses. These findings demonstrate that the regulatory systems responsible for FD-induced changes in refractive development and minus lens-induced changes in refractive development have one or more molecular components in common. However, this component is not part of the visually-guided regulatory system responsible for compensation to the blur caused by plus lenses.

In terms of the degree of refractive error induced by lenses, there was considerable variability in both the High and Low lines treated with plus and minus lenses, and few chicks fully compensated for the power of the lens by the end of the 4-day treatment period. The coefficients of variation in the degree of induced refractive error were 27% (Low line), 35% (High line) in wearing +10 D lenses, and 67% (Low line), 26% (High line) in wearing -15 D lenses. The distribution of induced refractive error (ΔRX) between the High and Low lines was obviously divergent in the treatment of minus lens, but not plus lenses, which implied that ocular responses differed significantly between hyperopic and myopic defocus. After wearing -15D lenses for 4 days, the average degree of myopia induced was -4.80 and -11.14 D in the Low and High lines, respectively. Compared to myopia induced by form deprivation, chickens from the Low and High lines developed -6.88 and -15.27 D after a 4-day treatment (section 4.3.2). Thus, form deprivation induced more myopia than did wearing -15D lenses in these selectively bred chickens during

4 days. However, Wildsoet and Wallman (1995) found wearing -15D lenses produced more myopia than did form deprivation in White Leghorn chickens. This discrepancy could be explained by the different genetic background of the two chicken populations and that the chickens involved in this study had been selected for susceptibility to form deprivation. Furthermore, the effective power of -15D lenses might be different in the two studies, because lenses were fitted in a tube with a length of 8 mm in this study. Therefore, the effective power of -15 D lenses was only -13.39 D when vertex distance was taken into account by the equation: $Pe=P/(1 + d \times P)$ (Keirl and Christie, 2007), where Pe: effective power, P: lens power, d: vertex distance. Moreover, different study designs could also lead to inconsistent results. For example, the duration of treatment with diffusers or lenses was 4 days in this study, but 5 days in Wildsoet's experiment. The diffusers used in both studies might also differ in the degree of image degradation and transparency.

Surprisingly, eyes wearing a plano lens developed a shallower anterior chamber during the treatment period than did the eyes of untreated, normally developing birds (Table 5.4). This effect occurred in chicks from both the High and Low lines. Furthermore, for the Low line only, plano lens-wearing eyes became shorter overall than the eyes of untreated chicks, resulting in a less hyperopic refractive error (Table 5.4). A potential explanation for this result is that form deprivation might have arisen in the peripheral retina due to the restriction of the visual field caused by the short silicon tube sleeve that held the (plano) lens. (Such an effect would also occur in eyes wearing plus or minus lenses, as well). An effect restricted to the peripheral visual field in this manner would be possible, since local eye growth is known to be controlled by local retinal image clarity (Wallman et al., 1987; Diether and Schaeffel, 1997). In previous studies, Wallman and Turkel (1978) investigated the influence of restriction of peripheral vision on the development of refractive error in chicks, using a similar system to the lens holder used here (a 7 mm opaque vinyl tube sleeve) yet found no significant difference in anterior chamber depth, axial length or refractive error between treated and untreated, normal birds. Analogously, Stone et al. (2006) used a series of diffusers with central apertures of 5, 8 or 10 mm to evaluate the effect of different local patterns of form deprivation on changes in eye shape and refractive error in chicks. Eyes treated for 2 weeks with any of the three diffuser designs showed a similar magnitude of *equatorial* eye expansion, which differed significantly from that in

fellow control eyes. Moreover, as the size of central aperture increased, the degree of axial eye growth decreased, and the refractive error became less myopic. Indeed, no significant difference in *axial* eye growth or refractive error between treated and control eyes was found in chicks wearing diffusers with a central aperture of 10 mm. Schippert and Schaeffel (2006) investigated the influence of peripheral defocus on the development of refractive error in chicks using lenses with a central aperture. They found that on-axis refraction remained unaffected, although refractive error at an angle of 45 degree to the visual axis showed partial compensation for the lens power, with a negative correlation between the magnitude of induced refractive error and the size of the aperture. In contrast to the above findings in chicks, peripheral form deprivation was reported to affect eye growth and refractive error along the visual axis in infant monkeys (Smith et al., 2005). These conflicting results may be due to the species differences and/or dissimilarities in the study design.

In the present study, the presence of the lens holder around the orbit primarily affected the development of the anterior chamber, and was most evident in chicks from the Low line. Plausibly, as with the studies cited above, this may have been due to form deprivation in the peripheral retina, i.e. the anterior part of the eye. Consistent with this explanation, a reduction in the rate of anterior chamber deepening similar to that noted above was observed in form-deprivation chicks from the Low line (Table 4.4). Specifically, after 4 days of form deprivation, treated eyes in chicks from the Low line had an anterior chamber depth of 1.26 ± 0.06 mm compared to 1.38 ± 0.04 mm in the eyes of untreated, age-matched, normal Low line chicks ($P < 0.001$). No such effect was observed in High line chicks (1.41 ± 0.07 versus 1.40 ± 0.04 mm in form-deprived and untreated eyes, respectively). In addition, normal untreated chicks from the High line had a longer vitreous chamber than those from the Low line, at 8 days of age (Table 4.4). Thus, peripheral form deprivation due to the lens holder may have resulted in a change in eye shape towards a more oblate shape (i.e. relative elongation equatorially) in Low line chicks, compared with more uniform eye expansion in the High line birds. Without commensurate axial growth, this change in eye shape in chicks from the Low line may have led to a decrease in anterior chamber depth, thereby resulting in a reduction in axial elongation and producing a more hyperopic refraction than in normal chicks. Nevertheless, in chicks wearing a powered lens

in front of their treated eye, the emmetropisation process would still function, resulting in compensation for the imposed defocus along the visual axis.

Both form deprivation by diffusers and hyperopic defocus produced by minus lens wear drive the eye to grow longer and develop myopia. Other similarities in these responses have been observed in previous studies, such as choroidal thinning (Wallman et al., 1995), decreased ZENK synthesis (Fischer et al., 1999), inhibitory effects of atropine and apomorphine (Schmid and Wildsoet, 2004) and suppressed axial elongation by reserpine (Schaeffel et al., 1995). Most recently, Ashby et al. (2010) found similar time-courses and strengths of response in the retinal expression of ZENK and pre-proglucagon RNA transcript levels in chickens after form deprivation and minus lenses wear, suggesting that similar molecular pathways were involved in the ocular response to form deprivation and minus lenses. These similarities could be attributed to some shared causal mechanisms. However, there have also been some dissimilarities noted in the ocular responses to diffusers and minus lenses in previous reports. As described in the introduction (section 5.1), differences in dopamine metabolism (Schaeffel et al., 1995) and the effect of constant light (Bartmann et al., 1994) suggest that there could also be some different mechanisms or pathways underlying the responses to diffusers and minus lenses. With regard to the ocular responses to myopic defocus produced by plus lens wear and hyperopic defocus produced by minus lens wear, not only the different changes to the choroid and sclera, but also altered ZENK expression have been observed after exposure to the two types of lenses, which implies distinct underlying mechanisms (Wildsoet and Wallman, 1995; Beresford et al., 2001; Bitzer and Schaeffel, 2002). The results found in the present study provide powerful evidence that there must be shared mechanisms or pathways in the ocular responses to form deprivation and minus lens wear. Nonetheless, the mechanisms or biochemical pathways underlying the ocular responses to form deprivation and plus lens wear must exhibit at least one difference.

5.5 Conclusion

By selecting for susceptibility to form deprivation, susceptibility to minus lens wear, but not plus lens wear, was also altered. Therefore, shared underlying mechanisms/ biochemical pathways for the ocular responses to form deprivation and minus lens wear must exist. Moreover, there must be at least one distinct mechanism or one unique component of a biochemical signalling pathway underlying the ocular responses to form deprivation and plus lens wear.

Chapter 6

Quantitative genetic influence on ocular traits and susceptibility to form-deprivation myopia

6.1 Introduction

Myopia is a complex disease with important contributions from both genetic variants and the environment (section 1.2). Refractive errors can potentially result from mismatches between the relative dimensions or refractive indices of any the eye's component parts, but most often an excessive axial length of the eye is the cause of myopia (Sorsby, Leary and Richards, 1962; Wildsoet, 1998). Thus, myopic eyes tend to have a longer axial length compared with emmetropic eyes, with most of the increase in the vitreous chamber (Gwiazda et al., 2002). Consequently, researchers interested in the genetics of refractive error have suggested that polymorphisms affecting the size and shape of the ocular components – particularly axial length – may play a role in the inheritance of refraction (Sorsby, Benjamin and Bennett, 1981; Biino et al., 2005; Meng et al., 2009; Prashar et al., 2009; Meng et al., 2010; Vitart et al., 2010).

Heritability is an analysis of variation designed to explain the strength of genetic influence on a particular trait, which determines the potential efficiency of gene-mapping studies (section 1.4.2). A number of researchers have explored the heritability of ocular component dimensions and refractive error in humans (Table 1.3) as a first step towards mapping quantitative trait loci (QTL). By contrast, the heritability of ocular component dimensions has rarely been studied in either wild or laboratory animal populations. In the latter group – laboratory animals – environmental influences on ocular morphology can be minimised. This provides a powerful setting for detecting the genetic variants controlling natural variations in eye size and shape (Zhou and Williams, 1999b, Prashar et al., 2009). Zhou and Williams exploited this line of reasoning by estimating the heritability of eye weight and crystalline lens weight in mice (Zhou and Williams, 1999b) and subsequently mapped 2 QTL for eye weight (Zhou and Williams, 1999a) (section 1.4.2.4).

As described in Chapter 4, a significant divergence in susceptibility to form-deprivation myopia was observed between two lines of chicks selectively bred for a difference in myopia susceptibility, thus suggesting a strong genetic component in determining this susceptibility. In this chapter, heritabilities were estimated to determine the extent to which genetic factors contributed to (a) the development of normal ocular component dimensions

and (b) susceptibility to form deprivation. Furthermore, genetic correlations between the ocular components and susceptibility to form deprivation were examined via bivariate genetic analysis, to find whether there was any pleiotropic effect between ocular component dimensions and susceptibility to form deprivation.

6.2 Materials and methods

6.2.1 Subjects and ocular measurements

From the experiment of selective breeding for susceptibility to form deprivation, all three generations of chickens treated with monocular diffuser wear for 4 days were included (section 2.3). The data were comprised of ocular component dimensions, corneal curvature and refraction measured using A-scan ultrasonography (section 2.2.2.1), videokeratometry (section 2.2.2.3) and retinoscopy (section 2.2.2.2), respectively. Data for the ocular component dimensions before form deprivation, and the relative changes in axial length (ΔAL), vitreous chamber depth (ΔVCD) and refractive error (ΔRX) after form deprivation [i.e. variables quantifying susceptibility to form-deprivation myopia (section 2.2.2.5)], were collected and used for univariate and bivariate genetic analysis to estimate the genetic contribution to these ocular traits. Information regarding the degree of relatedness between individuals in the study population is a prerequisite for estimating heritability and genetic correlations. For our chickens, the pedigree information of each individual chicken was known from records kept during the breeding process (section 2.3), apart from those chickens in the first, outbred generation, who were assumed to be unrelated to one another (since the outbred chickens were sourced from an extremely large White Leghorn breeding population). The known pedigree structure was imported into the genetic analysis software as a pedigree file.

6.2.2 Statistical analysis

Statistical analyses of the ocular traits were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). For pre-treatment trait measurements, outlier detection and removal proceeded as follows. Firstly, utilizing the finding that the bilateral ocular traits were highly correlated (range of Pearson correlation coefficients 0.82 to 0.94, all $P<0.001$),

data points that fell outside the 99.9% confidence intervals of a fitted regression line in a scatter plot of trait values in right versus left eyes were set as missing values. Secondly, after taking the average trait value of the bilateral traits, trait values three standard deviations beyond the mean, were also set as missing values. All ocular traits were deemed to be normally distributed by the Kolmogorov-Smirnov test, except relative change in refractive error (ΔRX). Hence, transformation of ΔRX was carried out using the *rn*-transform function in the GenABEL software package for R (Aulchenko et al., 2007) prior to heritability analysis.

6.2.3 Heritability estimation and genetic correlation

Heritability estimates were obtained using variance components analysis implemented in the SOLAR program (version 4.2.7) (section 2.4.2). Briefly, the total phenotypic variance of the ocular traits was partitioned into an additive genetic component and an environmental component that included non-additive genetic effects, environmental factors and measurement errors. Parameters estimation was performed by a maximum likelihood method. Then, a likelihood ratio test was performed to test whether the estimated additive genetic variance was significantly different from zero, i.e. the null hypothesis. Thus, minus two times the difference in the log likelihood between these two models provided a test statistic which was distributed as a chi-squared statistic with one degree of freedom. The portion of the total phenotypic variance accounted for by the additive genetic variance is the "narrow sense" heritability (h^2). The heritabilities of all ocular traits were estimated using a polygenic model with sex as a covariant and estimation of household effects to take batch-to-batch variability into account.

Bivariate genetic analysis was also carried out using SOLAR. The total phenotypic correlation (ρ_P) between two traits was partitioned into a genetic correlation (ρ_G) and an environmental correlation (ρ_E) (section 2.4.2). Similar to univariate genetic analysis, the significance of the genetic and environmental correlations between two traits was also tested using a likelihood ratio test after parameter estimation using maximum likelihood. A significant non-zero genetic correlation implies that the extent of trait covariation is due to shared genes (i.e. pleiotropy).

6.3 Results

6.3.1 Descriptive statistics and familial relatedness

Descriptive statistics for the ocular traits are shown in Table 6.1. All of the ocular component dimensions were found to be significantly larger in males than in females ($P<0.001$) for the 4-day old chicks (Table 6.2). Males also had flatter corneas and higher susceptibility to form-deprivation myopia (i.e. greater changes in ΔVCD, ΔAL and ΔRX) than did females ($P=0.001$, Mann-Whitney U test for ΔRX; $P<0.001$, independent *t*-test for all other ocular traits). Of the 891 chickens in the selective breeding experiment, there were 695 related individuals. The other 196 individuals were outbred birds in the first generation that were not selected for breeding. The 695 related chickens could be assigned to either a High susceptibility or Low susceptibility 3-generation pedigree. In total, there were 36 founders, 6349 sibling pairs, 948 half-sib pairs, 8530 cousins pairs, 1318 parent-offspring pairs, 1568 grandparent-grandchild pairs, and 10778 avuncular pairs. The variance components analysis took all of these relationships into account in estimating heritability.

Table 6.1 Descriptive statistics of ocular traits in the chickens.

Ocular Trait		N	Mean	SD
Before form deprivation				
Corneal Curvature (mm)		374	2.79	0.05
Anterior Chamber Depth (mm)		887	1.26	0.04
Lens Thickness (mm)		886	1.82	0.03
Vitreous Chamber Depth (mm)		883	5.01	0.12
Axial Length (mm)		886	8.09	0.15
After form deprivation				
Susceptibility to form deprivation	ΔVCD (mm)	887	0.45	0.16
	ΔAL (mm)	888	0.39	0.22
	ΔRX (Dioptres)	891	-11.98	4.62

Table 6.2 Comparison of ocular traits between male and female chickens (all $p<0.001$ in the independent *t*-test for all other ocular traits, and $P = 0.001$ in Mann-Whitney U test for ΔRX).

Ocular trait		Sex	N	Mean	SD
Before form deprivation					
Corneal Curvature (mm)		M	184	2.80	0.05
		F	190	2.77	0.05
Anterior Chamber Depth (mm)		M	425	1.27	0.04
		F	462	1.25	0.03
Lens Thickness (mm)		M	424	1.83	0.03
		F	462	1.81	0.03
Vitreous Chamber Depth (mm)		M	423	5.07	0.11
		F	460	4.96	0.11
Axial Length (mm)		M	423	8.16	0.13
		F	463	8.02	0.13
After form deprivation					
Susceptibility to form deprivation	ΔVCD (mm)	M	427	0.48	0.15
		F	460	0.42	0.16
	ΔAL (mm)	M	426	0.43	0.22
		F	462	0.35	0.21
	ΔRX (Dioptres)	M	427	-12.58	4.48
		F	464	-11.42	4.68

6.3.2 Heritability estimates

Heritability estimates for ocular components before form deprivation and susceptibility to form-deprivation myopia are shown in Table 6.3. Ocular components, including corneal curvature, anterior chamber depth, lens thickness, vitreous chamber depth and axial length exhibited significant heritabilities, ranging from 0.36 to 0.57 (all $P<0.001$). In terms of susceptibility to form deprivation, heritability estimates for all three parameters (ΔVCD, ΔAL and ΔRX) were 0.38, 0.46, and 0.51, respectively (all $P<0.001$). The household effect (batch) accounted for 9~18% of the variation in ocular components before form deprivation, but only 2~3% of the variation in FD-induced eye growth (ΔVCD and ΔAL). Approximately 6% of the variation in form-deprivation myopia arose from a household (batch) effect. In addition, the covariate sex, explained 6~23% and 4% of the variation in ocular components before and after form deprivation, respectively. However, only 1% of the variation of in myopia susceptibility could be explained by sex.

Table 6.3 Heritabilities of ocular traits in chickens. Standard errors are shown in brackets. All heritability estimates were significantly greater than zero ($P<0.001$). A statistically significant household effect was observed for all ocular traits before form deprivation and refractive change (ΔRX) (all $P<0.001$, except $^{+}$ $P= 0.01$ for ΔVCD and ‡ $P=0.07$ (not significant) for ΔAL in estimating household effect).

Ocular trait		N	Heritability estimate	Household effect	Sex effect
Before form deprivation					
Corneal Curvature (mm)		374	0.48 (0.15)	0.14 (0.05)	0.08
Anterior Chamber Depth (mm)		887	0.41 (0.07)	0.17 (0.04)	0.08
Lens Thickness (mm)		886	0.36 (0.08)	0.18 (0.04)	0.06
Vitreous Chamber Depth (mm)		883	0.57 (0.07)	0.09 (0.03)	0.20
Axial Length (mm)		886	0.52 (0.07)	0.15 (0.04)	0.23
After form deprivation					
Susceptibility to form deprivation	ΔVCD (mm)	887	0.38 (0.04)	0.03 (0.02) $^{+}$	0.04
	ΔAL (mm)	888	0.46 (0.04)	0.02 (0.01) ‡	0.04
	ΔRX (gm)	891	0.51 (0.05)	0.06 (0.02)	0.01

6.3.3 Ocular trait correlations

The phenotypic correlations between ocular traits in all chickens are shown in Table 6.4. There were significant correlations between the ocular components before form deprivation, except the correlation between lens thickness and vitreous chamber depth. High correlations were also observed between the three parameters (ΔVCD, ΔAL and ΔRX) used to quantify susceptibility to form deprivation (absolute value, all ≥ 0.75). The correlations between individual ocular components before form deprivation and these three susceptibility parameters, were all statistically significant, but low in magnitude, ranging from -0.16 to 0.23.

After decomposing phenotypic correlations into genetic and environmental correlations (Table 6.5), high pairwise genetic correlations were observed between corneal curvature, anterior chamber depth, vitreous chamber depth, and axial length. The genetic correlations ranged between 0.43 for anterior chamber depth and vitreous chamber depth, to 0.98 for vitreous chamber depth and axial length (all $P<0.05$). By contrast, non-significant correlations were found when lens thickness was compared to any of the other traits, except anterior chamber depth (Table 6.5). In the case of environmental correlations, there were also significant pairwise correlations between the ocular components before form deprivation, including anterior chamber depth, vitreous chamber depth and axial length. Nonetheless, corneal curvature correlated only with axial length. Lens thickness also exhibited a significant environmental correlation with anterior chamber depth only. In addition, both genetic and environmental correlations between ΔVCD, ΔAL and ΔRX were all highly significant. However, there was no significant genetic correlation between the three myopia susceptibility parameters and the ocular components before form deprivation.

Table 6.4 Pairwise phenotypic correlations between ocular traits. (Pearson's correlation coefficient, except Spearman correlation coefficient for relationships between ΔRX and other ocular traits; The significant levels of the correlations are written in bold and indicated as "*", "**", "***", corresponding to P<0.05, P<0.01, and P<0.001, respectively). "Note that anterior chamber depth, lens thickness, vitreous chamber depth and axial length were measured prior to form deprivation."

	Anterior Chamber Depth	Lens Thickness	Vitreous Chamber Depth	Axial Length	ΔVCD	ΔAL	ΔRX
Corneal Curvature	**0.42*** **	**0.17** **	**0.64*** **	**0.68*** **	**0.18*** **	**0.21*** **	**-0.16** **
Anterior Chamber Depth		**0.07***	**0.48*** **	**0.62*** **	**0.12*** **	**0.20*** **	**-0.13*** **
Lens Thickness			0.06	**0.27*** **	**-0.11** **	**-0.10** **	**0.07***
Vitreous Chamber Depth				**0.95*** **	**0.21*** **	**0.23*** **	**-0.15*** **
Axial Length					**0.17*** **	**0.21*** **	**-0.13*** **
ΔVCD						**0.92*** **	**-0.75*** **
ΔAL							**-0.86*** **

Table 6.5 Genetic correlations (above the diagonal) and environmental correlations (below the diagonal) between pairs of ocular traits in chickens. (Standard errors of correlations are shown in brackets and significant parameters are written in bold. The significances of the correlations different from zero are indicated as " * ", "**", "***", corresponding to P<0.05, P<0.01,and P<0.001, respectively).

Ocular trait	Corneal Curvature	Anterior Chamber Depth	Lens Thickness	Vitreous Chamber Depth	Axial Length	ΔVCD	ΔAL	ΔRX
Corneal Curvature	-	**0.68**** (0.17)	-0.11 (0.37)	**0.89***** (0.07)	**0.96***** (0.04)	0.26 (0.16)	0.26 (0.15)	-0.26 (0.15)
Anterior Chamber Depth	0.11 (0.16)	-	**-0.57**** (0.16)	**0.43**** (0.12)	**0.44***** (0.11)	0.08 (0.13)	0.15 (0.11)	-0.10 (0.12)
Lens Thickness	0.23 (0.19)	**0.22*** (0.10)	-	0.07 (0.16)	0.10 (0.17)	-0.13 (0.14)	-0.18 (0.12)	0.14 (0.13)
Vitreous Chamber Depth	0.34 (0.13)	**0.40**** (0.10)	-0.19 (0.12)	-	**0.98***** (0.01)	0.20 (0.12)	0.18 (0.11)	-0.14 (0.11)
Axial Length	**0.40*** (0.13)	**0.73***** (0.08)	0.21 (0.11)	**0.88***** (0.02)	-	0.17 (0.12)	0.15 (0.11)	-0.12 (0.11)
ΔVCD	-0.15 (0.11)	-0.04 (0.08)	-0.10 (0.08)	-0.06 (0.09)	-0.09 (0.09)	-	**0.88***** (0.03)	**-0.86***** (0.05)
ΔAL	-0.13 (0.11)	0.04 (0.08)	-0.05 (0.08)	-0.10 (0.09)	-0.10 (0.10)	**0.89***** (0.02)	-	**-0.97***** (0.02)
ΔRX	**0.24*** (0.12)	0.06 (0.09)	-0.04 (0.08)	0.08 (0.09)	0.09 (0.10)	**-0.51***** (0.05)	**-0.66***** (0.04)	-

6.4 Discussion

In this selective breeding experiment, moderate-to-high heritabilities were observed for the ocular components before form deprivation and for the myopia-related changes in vitreous chamber, axial length and refractive error (ΔVCD, ΔAL and ΔRX). These findings represent evidence for a major genetic contribution to the control of natural variation in ocular component dimensions and in the susceptibility to form deprivation in chickens. Additive genetic effects explained 36~57% of the variation in the ocular components and 38~51% of the variation in susceptibility to form deprivation in this population of White Leghorn chickens. In addition, sex explained 23% and 4% of the variation in eye size before form deprivation and form-deprivation induced eye growth, respectively, which was in accordance with the results from outbred chickens (~20% and ~5%) in Chapter 3 (section 3.3.4).

The heritability estimates for ocular component dimensions in White Leghorn chickens at the age of 4 days in this study are consistent with ocular component heritability estimates from parent-offspring and full-pedigree studies in human subjects (Table 1.3). However, numerous environmental factors are known to be associated with – and possibly influence – ocular biometric traits as children growup (e.g., socio-economic status, level of education and level of outdoor activity) (Wong et al., 2002; Rose et al., 2008), whereas in laboratory animal studies, environmental factors can be controlled and such variations minimised. In this respect, my heritability estimates may represent the upper limits likely to be obtained in natural populations. Zhou et al. (1999ab) investigated the heritability of eye weight in mice and subsequently showed the utility of this laboratory-based approach by successfully mapping two quantitative trait loci (QTL) regulating this trait, which they termed Eye1 and Eye2. Other researchers have taken these laboratory-based findings forward, resulting in hepatocyte growth factor, a candidate gene at the Eye1 locus, being identified as harbouring common genetic variants associated with susceptibility to human high myopia and refractive error (Han et al., 2006, Yanovitch et al., 2009, Veerappan et al., 2010). Hence, the moderate-to-high heritability estimates in ocular components and susceptibility to form deprivation provide a promising basis for mapping the responsible genes.

Bivariate genetic analyses in this study disclosed high genetic correlations across corneal curvature, vitreous chamber depth, and axial length (ρ_G range 0.89 to 0.98). This is

indicative of a common source of genetic influence ("pleiotropy"). Thus, an eye with a flatter cornea generally has a deeper vitreous chamber and longer axial length. A similar finding has been made previously in a population-based study in humans. In the Beaver Dam Eye Study, Klein et al. (2009) found a significant genetic correlation between axial length and corneal curvature (ρ_G=0.40, P<0.001) in a sample of 715 subjects from 189 pedigrees. The reason for the much lower genetic correlation in the Beaver Dam Eye Study compared to that found here (ρ_G=0.40 vs. 0.96) could reflect a species difference, but it is also likely to be influenced by the differential exposure to environmental sources of variation in refractive development mentioned above. In complete contrast to the pleiotropic genetic variants that were found to control overall eye size in these White Leghorn chickens, non-significant genetic correlations were found when lens thickness was compared to all other ocular traits. Thus, even though lens thickness is controlled in part by genetic variation (heritability= 0.36), the polymorphisms concerned may be distinct, in that they do not influence the dimensions of most of the other ocular components (except anterior chamber depth). This result is in accordance with previous reports. For example, Sivak et al. (1989) found a constancy of the optical properties of the developing chick lens from 14-day embryo to post-hatching 15 days and suggested that lens development was independent of globe development. Furthermore, no significant differences in lens properties, including dimensions, weight, morphology and focal length, were found in the eyes with induced refractive errors by manipulations of visual experience by form deprivation, hyperopic or myopic defocus in chickens (Priolo et al., 2000).

In terms of susceptibility to form deprivation, significant heritability estimates and extremely high genetic correlations (absolute value) were observed across the three parameters, ΔVCD, ΔAL and ΔRX, which implied these parameters can be taken as the appropriate endophenotypes for susceptibility to form deprivation to map the responsible genes. The "positive" genetic correlation between ΔVCD and ΔAL suggested pleiotropic genetic variants influenced both of them in the same direction. When vitreous chamber grows deeper, axial length also becomes longer. Nonetheless, the "negative" genetic correlations between ΔVCD and ΔRX, and between ΔAL and ΔRX, suggested the action of pleiotropic genetic variants in different directions on the two traits concerned. Thus, as vitreous chamber grows deeper or axial length grows longer, the refractive error becomes more myopic.

There are two lines of evidence suggesting that some of the genetic variants controlling the natural variation in eye size also influence susceptibility to myopia. For instance, variants in the HGF gene, which was originally implicated through its discovery as a candidate regulator of eye size in mice (Zhou and Williams, 1999a), have been found to influence susceptibility to human high myopia (Han et al., 2006, Yanovitch et al., 2009, Veerappan et al., 2010). Furthermore, eye size in non-myopic children has been observed in some studies to be a predictor of future myopia development (Zadnik et al., 1994, Mutti et al., 2007). However, there were no significant genetic correlations between ocular component dimensions before form deprivation and susceptibility to form deprivation in this study. This suggests that some genetic variants that determine susceptibility to form deprivation must be distinct from those that normally control the natural variation in ocular components. Nevertheless, this does not rule out the possibility of an undetectable genetic correlation due to the different direction in action of the genetic variants in a common set of genes that regulate eye size and myopia susceptibility. Regarding the environmental correlation, this reflects common sources of variation affecting pairs of traits other than that due to additive genetic effects. In spite of significant environmental correlations across ocular components, and for the three parameters representing susceptibility to form deprivation, our study was unable to identify whether this correlation arose from dominant genetic effects, emmetropisation-related visual cues, or some other source.

In summary, significant genetic contributions were found not only in the ocular components but also in the ocular growth and refractive error induced by form deprivation. Approximately 51% of the variation in the degree of form-deprivation myopia could be explained by an additive genetic effect, which implied that susceptibility to environmentally-induced myopia is considerably determined by genetics. In addition, insignificant genetic correlations between ocular components before form deprivation and susceptibility to form deprivation suggested there must be some distinct genetic variants in controlling ocular size and myopia susceptibility. These results provide promise for further genetic investigation such as QTL mapping to identify genes responsible for susceptibility to environmentally-induced myopia in White Leghorn chickens.

6.5 Conclusions

Moderate-to-high heritability estimates for ocular components and susceptibility to form deprivation suggested a significant genetic contribution in controlling the variation in these traits. In White Leghorn chickens, additive genetic effects explained 36~57% and 38~51% of the variation in (a) ocular component dimensions and (b) susceptibility to form deprivation, respectively. Furthermore, high genetic correlations were observed between (a) corneal curvature, vitreous chamber depth, and axial length, and, between (b) the three parameters representing susceptibility to form deprivation, i.e. ΔVCD, ΔAL and ΔRX, which implied the action of pleiotropic genetic effects on these traits. However, the genetic correlations between ocular components before form deprivation and susceptibility to form deprivation were not significant. Thus, the genetic variants that control the normal variation in eye size in White Leghorn chickens appear to be distinct from the variants that determine susceptibility to myopia.

Chapter 7

Heritability of ocular biometric traits in mice

7.1 Introduction

The mouse model is a powerful tool in genetic studies of many human diseases not only due to its close similarity to human, in physiology and genome, but also the ready availability of techniques to manipulate and analyse the mouse genome, such as transgenic and gene knockout mice (Kim, Shin and Seong, 2010). In addition, the mouse genome has been fully sequenced (Waterston et al., 2002). Together this makes functional genomic studies in the mouse feasible, which provides an important platform to reveal the genetic basis of human traits and diseases. The mouse has been used as a model for investigating genetic influences on several ocular traits and diseases. For instance, the quantitative trait loci (QTL), Eye1 and Eye2, were first discovered by mapping eye weight in mice (Zhou and Williams, 1999a). Later, the hepatocyte growth factor (HGF) gene, a strong candidate for Eye1, was found to be associated with susceptibility to human high myopia and refractive error (Han et al., 2006; Yanovitch et al., 2009; Veerappan et al., 2010). Furthermore, several transgenic mouse models with the feature of abnormal retinal lipofuscin accumulation have been used for studying Age-Related Macular Degeneration (AMD) (Rakoczy et al., 2006). Moreover, early growth response protein-1 (Egr-1) has been implicated in the visual feedback mechanisms of ocular growth and myopia development. Egr-1 gene knockout mice were found to have longer eyes and relative myopia, compared to wild-type mice, which strengthened the evidence for the involvement of Egr-1 in the control of eye growth (Schippert et al., 2007).

The development of refractive error is associated with a failure in the coordination of the growth in ocular components to achieve emmetropia, i.e. emmetropisation (section 1.3). Thus, understanding the genetic control of the growth in ocular components may unveil the underlying mechanisms in myopic development. Mouse QTL mapping has proven a promising method to dissect the genetics of biometric traits (Flint et al., 2005; Hunter and Crawford, 2008). In addition, to achieve high-resolution mapping, a proven strategy is to study relatively outbred mouse populations and to obtain accurate and precise phenotypic measurements on hundreds or even thousands of mice (Mott et al., 2000; Yalcin et al., 2004; Mott and Flint, 2008). Therefore, in the process of dissecting the genetics of the growth control in ocular components in mice, it is essential to obtain accurate and precise measurements of ocular component dimensions, in order to improve mapping resolution. Nonetheless, one of the disadvantages in studying ocular traits in mice is the difficulty of measuring each ocular component and its changes during myopia development with high accuracy and precision, due to the small eye size of mice.

Several methods have been used to measure the ocular dimensions in mice. Callipers (Shupe et al., 2006; Barathi et al., 2008) and video imaging morphometry of histological sections (Tejedor and de la Villa, 2003; Schmucker and Schaeffel, 2004b; Barathi et al., 2008) can only measure the enucleated eye and have limited resolution. Furthermore, ocular component dimensions might potentially change soon after death, and this situation could become even worse in the histological processing of the enucleated eyes (Tattersall et al., 2010). For instance, histological processing can result in a loss of the original mass of the eye in mice, ranging from 6% to 25% (Zhou and Williams, 1999b; Shupe et al., 2006). This could further hamper the accuracy and precision of the measurement results. Although laser micrometry (Wisard et al., 2010) provides high levels of resolution (less than 0.77μm) on the measurement of ocular dimensions, it still suffers from the same limitations of ex vivo measurement. Ultrasound biomicroscopy (Brown et al., 2005) and magnetic resonance imaging (Tkatchenko, Shen and Tkatchenko, 2009) can achieve in vivo measurements of ocular component dimensions in mice, yet there are some limitations of both methods, such as being time consuming and providing poor resolution. Methods based on interferometric techniques, including optical low-coherence interferometry (OLCI) (Schmucker and Schaeffel, 2004a; Puk et al., 2006; Schippert et al., 2007; Barathi et al., 2008) and optical coherence tomography (OCT) (Zhou et al., 2008; Wang et al., 2010), provide rapid measurements of ocular dimensions in vivo with good resolution. While the ocular components of mice have been measured successfully using OLCI, the dimensions of lens thickness and vitreous chamber depth were not always obtained because the reflection from the posterior lens surface was infrequently detected. Furthermore, OCT can generate two- or even three-dimensional images, which provide additional information in measuring ocular traits. Therefore, OCT appears to be an optimal method for ocular phenotyping in genetic studies of mice because of its potential for high-throughput measurements with high levels of accuracy and precision.

In this chapter, an OCT device expressly designed to provide accurate, high-throughput measurements of mouse ocular component dimensions (Wang et al., 2010) was used to study the genetic influence on ocular traits in mice.

7.2 Material and methods

7.2.1 Subject

MF-1 outbred (albino) mice were obtained from Harlan Ltd (Oxon, UK) at age 7 weeks old. Males and females were housed separately in groups of 4-5. At age 8 weeks, ocular component dimensions were measured using OCT (section 7.2.2.2) in 22 mice (11 males, 11 females) followed by the implantation of an RFID chip for the purpose of identification. One week later, male and female mice were paired (using a random number generator to assign the pairings), and each pair was housed separately. When the offspring from the pairings were 3 weeks old, 4 female offspring from each litter were selected at random to be kept for phenotyping (their littermates were removed and not studied further). Ocular component dimensions for the 4 females from each litter were assessed at age 8 weeks old. There was 1 litter that contained only 3 female offspring. Hence, the total number of offspring phenotyped was 43. When phenotyping the offspring mice, the experimenters were masked as regards the ocular component dimensions of the parental mice. All experimental procedures involving animals complied with the U.K. legislation (Animals Act 1986), the European Communities Council Directive 86/609/EEC (1986) and were carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

7.2.2 Measurements of ocular traits

7.2.2.1 Optical coherence tomography

Optical coherence tomography (OCT) is a non-invasive technique to produce high-resolution, cross-sectional imagines for morphological investigation of the tissue in biological systems (Huang et al., 1991; Fujimoto et al., 1995). This technique is analogous to ultrasound B scans, except light waves are used instead of sound waves. It is based on the concept of low-coherence interferometry for measuring the properties of a new wave pattern produced by the superposition of light backscattered from tissue, and the fact that light travels a known distance in respect of the time delay through a reference path (Drexler and Fujimoto, 2008). Combining low-coherence interferometry with lateral point beam scanning, OCT produces two-dimensional images with a depth-profile of the sample structure (Huang et al., 1991). In conventional (Time-Domain) OCT, the depth information of the sample is obtained by scanning the reference arm in the axial direction. For more rapid image acquisition and improvement of image resolution, Spectral-Domain OCT (one

type of Fourier-Domain OCT) analyzes the interference signals between light backscattered from the sample and light travelling along a fixed reference arm, using a spectrometer (Figure 7.1) (Coscas, 2009).

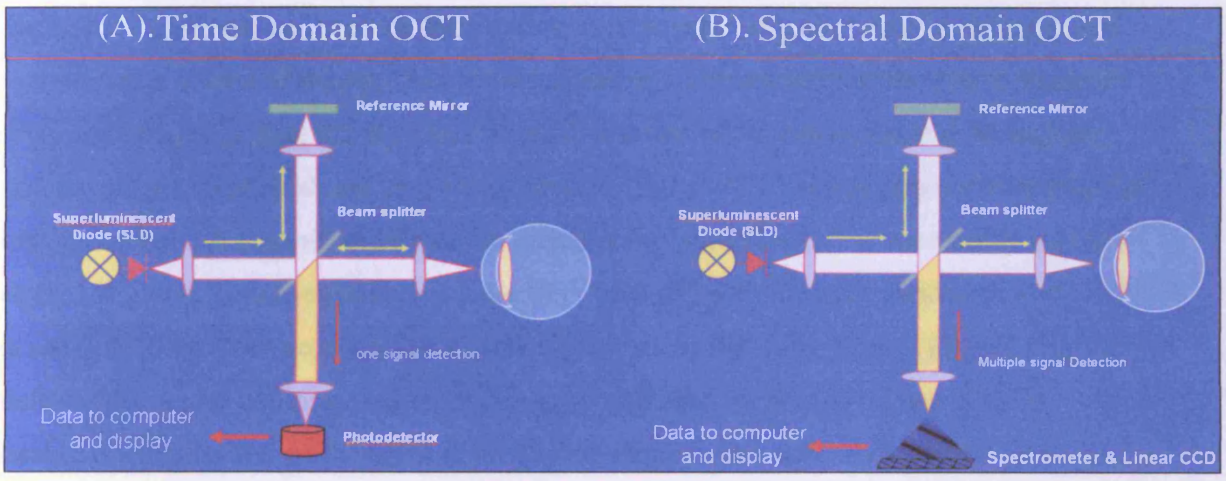


Figure 7.1 Two main types of OCT system.

(A). Time-Domain OCT: the depth information of the ocular components is obtained from analysing the interference signals between light backscattered from the eye and light travelling along a reference arm, which changes by moving the reference mirror in the axial direction (scanning). (B) Spectral-Domain OCT: the reference arm is fixed and the depth information is obtained from analysing the interference signals by a spectrometer, instead of moving the reference mirror. (Coscas, 2009)

In the present study, another type of Fourier-Domain OCT, a swept-source OCT system designed by Dr. Ling Wang (Wang et al., 2010), was used to measure ocular biometry in mice due to its advantages of better sensitivity with imaging depth and longer imaging range, compared with Spectral-Domain OCT (Potsaid et al., 2010). This system was comprised of a wavelength tunable laser (HSL-1000, Santec, Japan), a free space Michelson interferometer and a fibre-optic Mach-Zehnder interferometer. The laser had a centre wavelength of 1056 nm and a monodirectional scanning range of ~70 nm (flat-top shaped), operating at a speed of 28 kHz with 8.2 mW output power. The axial resolution of the OCT system was ~17.6μm in air within ± 4.0 mm depth range. I was not involved in the design or construction of the OCT system.

7.2.2.2 Measurements of ocular component dimensions by OCT

After measuring their body weight, mice were anaesthetised by an intraperitoneal injection of ketamine (125 mg/kg) and xylazine (8 mg/kg) and carefully placed inside a 50ml

centrifuge tube (w x h; 28 x 115mm; model FB55959, Fisher Scientific, Loughborough, UK) whose conical end was recessed to allow free access to both eyes. The tube was mounted on a custom-designed stage that allowed one eye to be positioned at the centre of rotation of a 5-axis positioning system (Figure 7.2 A). Real-time OCT B-scans through the eye in the transverse and sagittal planes were used to precisely align the OCT instrument with the optical axis of the eye, this being judged by the symmetry of the eye in the cross-sectional images (Figure 7.2 B). Once aligned, a series of 50 transverse and 50 sagittal scans were acquired, with the zero delay position of the OCT system positioned within the crystalline lens. A second set of measurements was obtained with the zero-delay position altered slightly. Both eyes of each mouse were imaged, with the eye scanned first chosen at random. After post-processing and image-analysis by Dr. Ling Wang (Figure 7.2 C), data for the ocular component dimensions were obtained for statistical and genetic analysis. Irregularities of the corneal surface developed after anaesthesia in two eyes of two mice, which influenced the quality of the scanned images and precision of the measurements. Thus, data of the eyes with irregularities of the corneal surface were excluded. In addition, axial ocular component dimensions were measured in 12 mice on two consecutive days to investigate the day-to-day reproducibility of measurements with the swept-source OCT device.

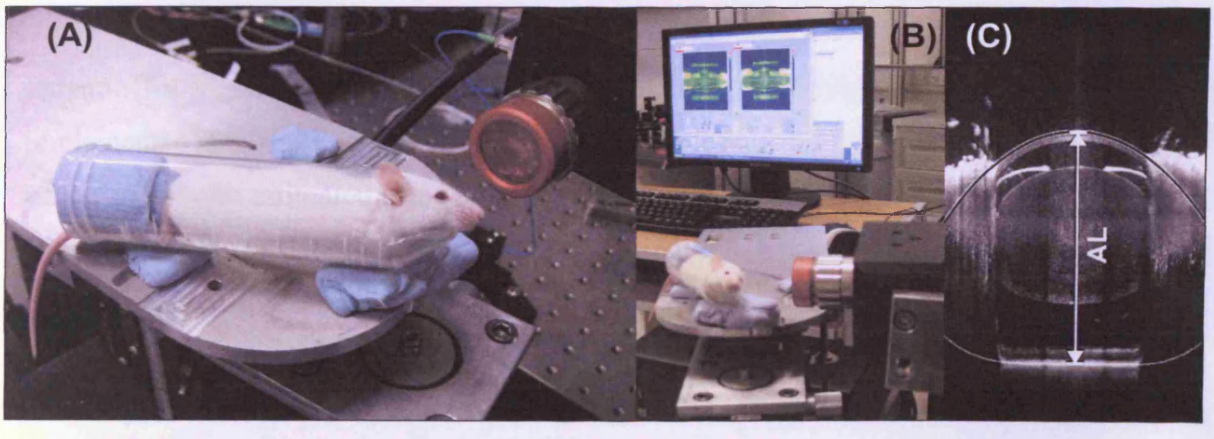


Figure 7.2 Positioning the mouse during OCT scan and measurement of ocular component dimensions by analysing the scanned images.

(A). The mouse was placed into a centrifuge tube (recessed conical end) mounted on a custom-designed stage. (B). Real-time OCT B-scans through the eye in the transverse and sagittal planes were displayed on the computer monitor to align the OCT instrument precisely with the optical axis of the eye. (C) Ocular image after post-processing was used to calculate the ocular component dimensions.

7.2.3 Statistical analysis and heritability estimation

Statistical analyses of the ocular traits were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Day-to-day reproducibly was assessed using the 'limits of agreement' method of Bland and Altman (Bland and Altman, 1986). Outliers detection and removal proceeded as follows. Firstly, utilizing the finding that the bilateral ocular traits were highly correlated (range of Pearson correlation coefficients 0.72 to 0.98, all $P<0.001$), data points that fell outside the 99.9% confidence intervals of a fitted regression line in a scatter plot of trait values in right versus left eyes were set as missing values. For the 2 occasions that only one eye of a mouse provided images suitable for extraction of biometric data, the trait values obtained from this single eye were also excluded from further analysis. This resulted in the removal of data for 3, 2, 2, 3, 2, and 3 individuals for the traits corneal thickness, anterior chamber depth, lens thickness, vitreous chamber depth, axial length, and corneal radius of curvature, respectively. Secondly, after taking the average trait value of the bilateral traits, trait values beyond three standard deviations from the mean, were also set as missing values. This led to the removal of a further 1 mouse from the lens thickness analysis. All ocular traits were deemed to be normally distributed by the Kolmogorov-Smirnov test. Hence, no transformation was made for univariate and bivariate genetic analysis. For mice that were phenotyped on two occasions, the readings from the first measurement day were used in the univariate and bivariate genetic analyses.

Parent-offspring heritability estimates were calculated using variance components analysis implemented in SOLAR software (version 4.2.7) (section 2.4.2). The heritabilities of all ocular traits were estimated using a polygenic model with sex included as a covariate. Bivariate genetic analysis was also carried out using SOLAR to estimate pairwise genetic correlations, which describe the extent to which two traits co-vary because of shared genetic effects (i.e. pairs of traits that are significantly genetically correlated to one another are likely to be controlled by a common set of genetic variants) (section 2.4.2).

7.3 Results

7.3.1 Descriptive statistics

Ocular component dimensions were measured in 65 mice, including 11 pairs of males and females in the parental generation and 43 females in the offspring generation. There were high, statistically significant correlations between ocular traits' values in right and left eyes, ranging from 0.72 to 0.98 (all $p<0.001$) (Table 7.1). After removal of the outliers (section 7.2.3), descriptive statistics for the ocular traits are shown in Table 7.2.

Table 7.1 Pearson correlation between ocular traits' values between right and left eyes and average standard deviations of ocular traits measurements.

Trait	Pearson correlation between fellow eyes‡		Average SD (μm)§
	Correlation	P value	
Corneal thickness	0.91	<0.001	2.6 ± 1.5
Anterior chamber depth	0.89	<0.001	1.5 ± 2.1
Lens thickness	0.98	<0.001	3.4 ± 1.5
Vitreous chamber depth	0.88	<0.001	5.2 ± 4.3
Axial length	0.94	<0.001	3.6 ± 2.4
Radius of corneal curvature	0.72	<0.001	32.3 ± 27.0

‡ All subjects (N=65) were included in the correlation coefficient test.

§ Average of the standard deviations of measurements from the readings of 4 scanning images (2 transverse and 2 sagittal images) per eye.

Table 7.2 Descriptive statistics for the ocular traits after removal of outliers.

Trait	Sample size	Mean (μm)	SD (μm)	Range (μm)
Corneal thickness	62	124. 2	9.1	108.6 ~ 146.0
Anterior chamber depth	63	449.5	25.3	395.80 ~ 498.95
Lens thickness	62	1805.8	28.0	1740.56 ~ 1871.13
Vitreous chamber depth	62	904.0	26.2	853.14 ~ 977.36
Axial length	63	3279.4	53.8	3167.52 ~ 3369.22
Radius of corneal curvature	62	3009.5	80.4	2788.63 ~ 3231.25

7.3.2 Measurement repeatability

To evaluate the repeatability of OCT measurement, measurements of ocular component dimensions were taken on two consecutive days in 12 mice. Because of high correlations between ocular traits' values in right and left eyes (Table 7.1), data from the measurements of the right eye on two consecutive days were used for this analysis. The repeatability of the measurements was evaluated by the 95% limits of agreement method of Bland and Altman plot (Bland and Altman, 1986). The results of the day-to-day repeatability analysis in 12 mice are summarised in Table 7.3 and Figure 7.3. There were significant correlations between measurements on Day 1 and Day 2 in all ocular traits with Pearson correlation coefficients ranging from 0.62 to 0.99. The highest correlation was for lens thickness ($r = 0.99$, $p<0.001$) whilst measurements of corneal curvature had lower correlations between 2 consecutive days ($r = 0.62$, $p= 0.032$). Furthermore, the best repeatability was observed in the measurements of lens thickness and corneal thickness, in which the 95% limits of agreement were within the range of 10.5μm and 18.1μm, respectively. The measurements of corneal curvature had the worst repeatability, with a range of 95% limits of agreement beyond 250μm. These findings suggested that this OCT system was able to reliably assess axial ocular components, including corneal thickness, anterior chamber depth, lens thickness, vitreous chamber depth and axial length, but not corneal curvature.

Table 7.3 Measurement repeatability for mice measured on consecutive days.

Trait	N (eyes)	Pearson correlation		Mean difference [a] (μm)	95% Limits of Agreement [a] (μm)
Corneal thickness	12	0.72	P=0.008	-1.5	-10.5 to 7.6
Anterior chamber depth	12	0.74	P=0.007	2.5	-19.0 to 24.1
Lens thickness	12	0.99	P<0.001	3.6	-1.6 to 8.9
Vitreous chamber depth	12	0.91	P<0.001	1.9	-10.0 to 13.8
Axial length	12	0.94	P<0.001	6.6	-13.2 to 26.4
Radius of corneal curvature	12	0.62	P=0.032	32.2	-128.6 to 193.0

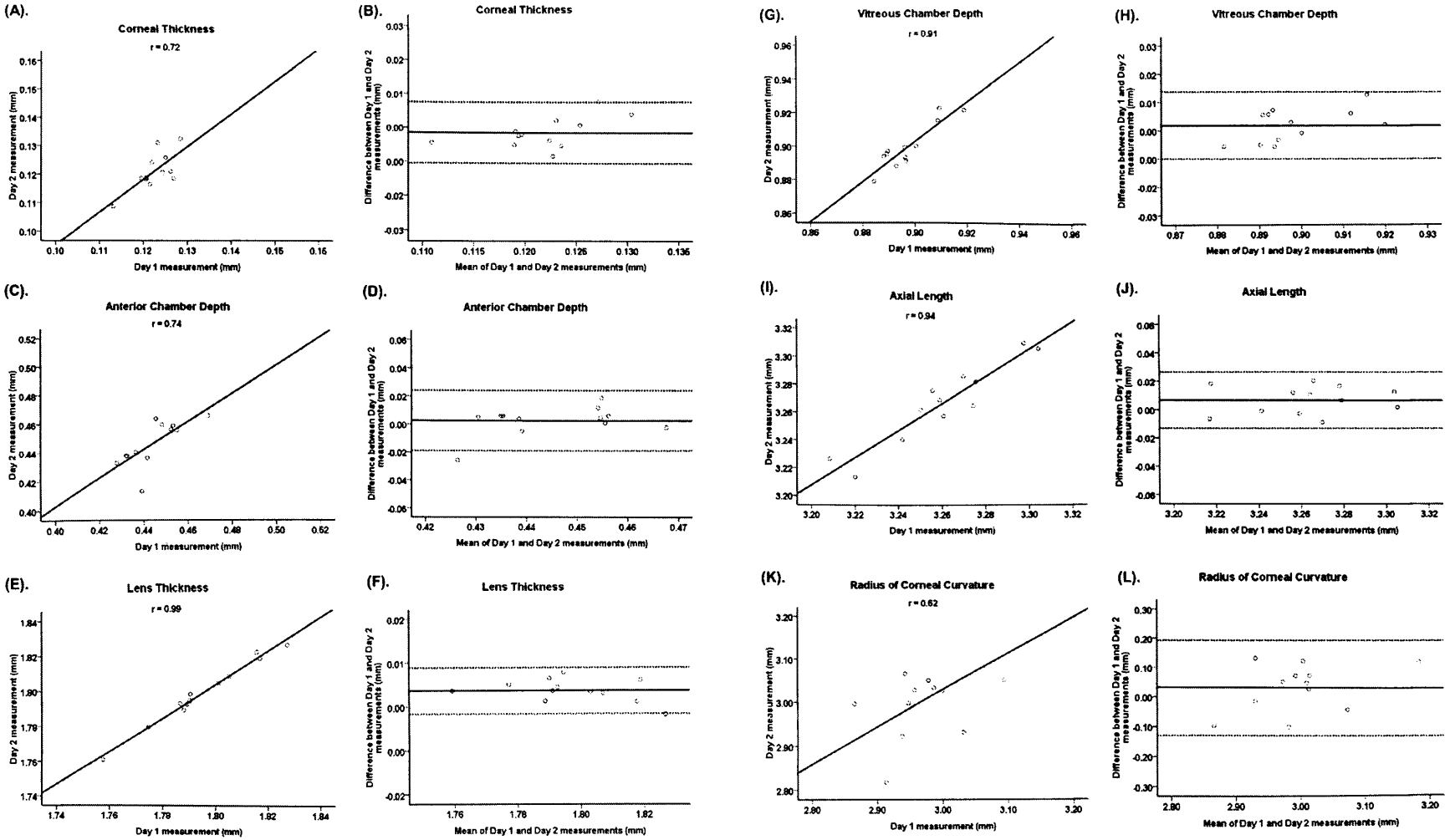


Figure 7.3 The reproducibility of OCT measurements. Evaluation of the reproducibility in the OCT measurements of ocular traits, using scatter plots of readings obtained on day 1 against those on day 2 with a fitted regression line (Panels A, C, E, G, I, and K), and Bland and Altman plots (Panels B, D, F, H, J and L; the solid horizontal line shows the mean difference, whilst the two dashed lines show the 95% limits of agreement).

7.3.3. Heritability estimates and correlations of mouse ocular traits

Heritability estimates for mouse ocular component dimensions from parent-offspring analyses are summarised in Table 7.4. The ocular traits, including anterior chamber depth, lens thickness, vitreous chamber depth and axial length, exhibited high heritability (h^2 = 0.61~0.80), which implies that, under laboratory conditions, eye size in outbred albino mice is largely determined by additive genetic effects. However, the ocular traits related to cornea, such as corneal thickness and corneal curvature, showed low (insignificant) heritability estimates of 0.14 and 0.24, respectively, suggesting these low values might reflect a low influence of additive genetic effects on corneal traits.

Table 7.4 Heritability estimates for ocular traits in outbred MF1 mice.

Trait	Midparent-offspring heritability				
	N	Heritability	SE	P-value	Sex effect
Corneal thickness	62	0.14	0.23	0.24	0.049
Anterior chamber depth	63	0.67	0.16	<0.001	0.001
Lens thickness	62	0.72	0.16	<0.001	<0.001
Vitreous chamber depth	62	0.61	0.19	<0.001	0.004
Axial length	63	0.80	0.13	<0.001	<0.001
Radius of corneal curvature	62	0.24	0.17	0.06	0.079

SE: Standard errors.

The phenotypic correlations between the mouse ocular traits are shown in Table 7.5. There were significant correlations between axial length and all other traits. Apart from that, corneal curvature correlated significantly with anterior chamber depth and lens thickness. Furthermore, corneal thickness exhibited significant correlation with lens thickness. After decomposing phenotypic correlations into genetic and environmental correlations (Table 7.6), axial length had significant genetic correlations with other traits (ρ_G = 0.63~0.98), except corneal thickness. In addition, there was a significant genetic correlation between lens thickness and corneal curvature (ρ_G = 0.88). However, there was only one statistically significant environmental correlation, that between axial length and corneal curvature (ρ_E = 0.80).

Table 7.5 Pairwise phenotypic correlations between ocular traits in outbred MF1 mice. (Pearson's correlation coefficient; the significances of the correlations are written in bold and indicated as "*", "**", "***", corresponding to P<0.05, P<0.01, and P<0.001, respectively.)

Traits	Anterior chamber depth	Lens thickness	Vitreous chamber depth	Axial length	Radius of corneal curvature
Corneal thickness	-0.17	**0.35****	0.08	**0.32***	-0.01
Anterior chamber depth		0.17	0.17	**0.65*****	**0.43****
Lens thickness			0.03	**0.68*****	**0.52*****
Vitreous chamber depth				**0.55*****	0.19
Axial length					**0.57*****

Table 7.6 Pairwise genetic correlations (above the diagonal) and environmental correlations (below the diagonal) between ocular traits in outbred MF1 mice. (Standard errors of correlations are shown in brackets and significant parameters are written in bold. The significances of the correlations different from zero are indicated as " * ", "**", "***", corresponding to P<0.05, P<0.01,and P<0.001, respectively.)

Traits	Corneal thickness	Anterior chamber depth	Lens thickness	Vitreous chamber depth	Axial length	Radius of corneal curvature
Corneal thickness	-	-0.28 (0.68)	0.56 (0.32)	-0.75 (1.58)	0.31 (0.43)	-0.45 (0.82)
Anterior chamber depth	-0.19 (0.28)	-	0.13 (0.30)	0.41 (0.29)	**0.69**** (0.19)	0.69 (0.27)
Lens thickness	0.34 (0.28)	0.03 (0.39)	-	0.07 (0.27)	**0.71***** (0.12)	**0.88*** (0.30)
Vitreous chamber depth	0.32 (0.22)	-0.36 (0.26)	-0.02 (0.32)	-	**0.63**** (0.16)	0.46 (0.41)
Axial length	0.50 (0.23)	0.41 (0.33)	0.66 (0.23)	0.42 (0.26)	-	**0.98**** (0.28)
Radius of corneal curvature	0.21 (0.21)	0.38 (0.21)	0.39 (0.23)	0.10 (0.22)	**0.80**** (0.28)	-

7.4 Discussion

Axial ocular component dimensions in MF-1 can be measured reliably using swept-source OCT with good day-to-day repeatability. This system can assess not only the mouse eye in vivo but also obtain all ocular component dimensions in detail, which overcomes the problems of changes in ocular component dimensions post-mortem or after histological processing and the incapability of previous OLCI systems of detecting the reflection from the posterior lens surface consistently. From the analysis of the ocular component dimensions in mice obtained from this OCT system, highly significant heritability estimates were observed for anterior chamber depth, lens thickness, vitreous chamber depth and axial length, ranging from 0.61 to 0.80. In addition, pleiotropic effects on ocular components were also revealed between axial length and other axial ocular components, as well as lens thickness and corneal curvature. Nonetheless, heritability estimates for cornea-associated traits, such as corneal curvature and corneal thickness, did not show significant results.

The mouse has been recognised as a promising model to study genetic influences in eye growth and development of refractive error (Schaeffel et al., 2003; Schaeffel et al., 2004) since the discovery of the quantitative loci influencing eye weight in mice (Zhou and Williams, 1999ba). In spite of the obstacles due to relative poor optical quality and the small size of the mouse eye, as well as poor visual acuity (about 0.5 cycles per degree in C57BL/6J mice (Prusky, West and Douglas, 2000; Abdeljalil et al., 2005)), several studies successfully induced form-deprivation myopia in mice (Tejedor and de la Villa, 2003; Schaeffel et al., 2004; Barathi et al., 2008; Tkatchenko, Shen and Tkatchenko, 2010). However, some studies suffered from the problem of reliability in measuring the small changes in ocular component dimensions by histological sectioning after myopia development. With advances in the phenotypic assessment of mouse eyes, such as the ACMaster and high resolution MRI, the small changes in ocular component dimensions during the development of refractive error can be detected reliably (Barathi et al., 2008; Tkatchenko et al., 2010). Nonetheless, there are some limitations to both methods, such as being time consuming and the poor image resolution for MRI and incapability of the ACMaster to determine the dimensions of lens thickness and vitreous chamber depth consistently. The swept-source OCT used in this study produced reproducible and precise results without the above limitations. Hence, it is an ideal method to study eye growth during the development of refractive error in mice.

For the purpose of high-throughput phenotyping of mouse ocular biometry for genetic studies, this swept-source OCT system showed the potential to provide high accuracy and repeatability in the measurement of axial ocular components with rapid scanning speed. The high repeatability was revealed by high correlations (0.72~0.99) and the narrow ranges of the 95% limits of agreement (±10~20μm) for the day-to-day measurements of axial ocular components. Although the day-to-day repeatability analysis would underestimate measurement precision if eye size or shape truly varies from one day to the next, due to normal growth or diurnal effects, it has the advantage of incorporating all sources of measurement variation, including potential misalignment of the eye with the axis of the OCT system, and scan-to-scan and intra-scan variability, e.g. due to movement of the animal from breathing or pulse rhythms. In addition, this OCT system was able to distinguish the relative axial ocular component dimensions of individual mice from the same outbred strain, as judged by a level of day-to-day repeatability that was smaller than the natural range of the trait values (e.g. axial length ranged from 3167.52μm to 3369.22μm, i.e. a range of 200μm, while the 95% limits of repeatability for this trait were approximately ± 20μm). This inference was confirmed by the detection of highly significant heritability estimates for the axial traits. In comparison with previous studies, it provided results which were at least as accurate and precise as the OLCI system (ACMaster) (Schmucker and Schaeffel, 2004a) and the time-domain OCT system of Zhou et al. (2008). For axial length, the correlation between the measurements taken on two consecutive days was 0.94 with swept-source OCT, which was comparable to 0.87 obtained using the time-domain OCT system (Zhou et al., 2008). When two axial length measurements were taken consecutively during phenotyping, this swept-source OCT system exhibited a standard deviation of 3.6 ± 2.4μm, which was also comparable to 8.0 ± 2.9μm obtained using the ACMaster (Schmucker and Schaeffel, 2004a). In terms of the speed to acquire a single measurement, it took approximately 2 seconds for 50 transverse and 50 sagittal scans, which was similar to the amount of time required for the ACMaster (approximately 1 second), but much faster than that needed for the time-domain OCT system of Zhou et al. (2008) (approximately 1 minute).

There were limitations in this swept-source OCT system to measure the ocular traits in mice. Although this system was able to assess the curvature of the cornea during the measurement without needing a separate, specialised instrument, the accuracy and repeatability of corneal curvature was poor in comparison to those of the axial ocular components. This could result from magnifying systematic errors (e.g. the residual

distortion in the scan) during the process of edge detection for the corneal surface when fitting short circular arcs to calculate the radius of corneal curvature, because the image contrast was higher at the corneal apex than in the periphery. Apart from that, the time-consuming task for manual intervention during the post-acquisition image processing procedure is a limit to high throughput.

In terms of additive genetic influences on mouse ocular component dimensions, high heritability estimates (h^2 = 0.61~0.80) were observed for all of the ocular traits, except corneal thickness and corneal curvature. Previous studies of mouse eye weight produced estimates of heritability of 0.31~0.48, which led to the mapping of QTLs modulating eye size (Zhou and Williams, 1999ba). Nonetheless, mouse eye weight was measured from perfusion-fixed specimens in these two studies, which may increase variation in the phenotype measurements. The mouse ocular traits in this study were measured in vivo. Hence, the phenotype data should more closely reflect the physiological condition, resulting in higher heritability than previous studies. These results suggest that eye size in mice is mainly determined by additive polygenic effects, which should make these traits amenable to QTL mapping. In addition, there were high pairwise genetic correlations between the traits, lens thickness and corneal curvature, and between axial length and other ocular traits, except corneal thickness. This provides evidence of pleiotropic effects between these pairs of traits, i.e. a single group of genetic variants influencing multiple traits. A conflict result between borderline significant heritability estimate of corneal curvature (h^2 = 0.24, P = 0.06) and significantly high genetic correlation between axial length and corneal curvature (ρ_G = 0.98, P<0.001) could be explained by measurement error in corneal curvature due to the wide range of 95% limits of agreement for day-to-day measurements (~ ± 140μm), which could cause the heritability of corneal curvature to be underestimated. Similar shared genetic regulation of corneal curvature and axial length has been observed in chickens (section 6.3.3) and humans (Klein et al., 2009). In comparison with genetic correlations between ocular traits in chickens (section 6.3.3), there were significant genetic correlations between axial length and other ocular traits (except lens thickness) in both chickens and mice. However, the high genetic correlations between axial length and lens thickness, as well as corneal curvature and lens thickness, were only found in mice. Furthermore, significant genetic correlations amongst corneal curvature, anterior chamber depth and vitreous chamber depth in chickens were not discovered in mice, suggesting that the genetic regulation of lens thickness in mice differs from that in chickens. These results might reflect these species differences. For instance, the lens

occupies most of the eye volume in mice (Schmucker and Schaeffel, 2004b), but the vitreous chamber does so in chickens (Avila and McFadden, 2010).

In summary, mouse axial ocular component dimensions can be rapidly measured using this new OCT system, with a narrow range of 95% limits of agreement for day-to-day measurements. The OCT device provides reliable phenotype data, yielding high heritability estimates and genetic correlations between pairs of ocular component dimensions. Overall, these results imply that the development of ocular components in mice is influenced substantially by additive genetic effects.

7.5 Conclusion

High heritability estimates for axial ocular components suggest that eye size in MF 1 mice is mainly determined by additive genetic effects. Pleiotropic genetic effects were also observed between axial ocular components. These results provide a strong basis for further mapping QTL controlling the development of ocular component dimensions in mice. In addition, the swept-source OCT system can be used as a reliable and fast method to measure the axial ocular component dimensions in mice.

Chapter 8

General discussion and future work

8.1 General discussion

Studies in animal models demonstrate conclusively that perturbations in early visual experience can disrupt emmetropisation to produce refractive errors in a wide range of species (section 1.3). The myopia induced in animal models shares some characteristic features with the naturally-occurring myopia of humans. For instance, an increase in the axial length of the eye with induced myopia is due principally to elongation of the vitreous chamber. Nonetheless, substantial variations have been noted in the degree of myopia induced by a uniform regimen of visual manipulation in animal models (section 3.1.2). In the chicken myopia model, such variations have been observed not only between strains but also within strains (section 3.1.2 and Table 3.3). Although Saltarelli et al. (2004) suggested a possible role of genetics in determing susceptibility to form-deprivation myopia in chicks, due to significant correlations between the changes in vitreous chamber depth in individual birds over two sequential periods of form deprivation, the extent of a genetic influence has never been investigated. Selective breeding has been used as a standard method to test for heritability of traits of interest, i.e. the role of genetics in controlling specific traits. Thus, a selective breeding experiment for high and low susceptibility to form deprivation was carried out in this study, to test whether this variability was genetically determined in chickens.

During the initial selection process in outbred White leghorn chickens, considerable variability in the susceptibility to form deprivation was observed (coefficients of variation 23% to 42%), which allowed the investigation of several potentially important variables (sex, eye size and body weight) in predicting susceptibility to form deprivation. Previous studies have shown conflicting results regarding the differences in susceptibility to form deprivation between male and female chickens (Table 3.11). In this study, sex was found to play a role in the susceptibility to form-deprivation induced eye growth in the analyses of multiple regression models and structural equation modelling. Approximately 6% of the intersubject variability could be explained by sex. Although the mechanism underlying the sex effect on the rate of myopic eye growth is unknown, potential hypotheses are the influence of sex hormones and/or the dosage effect for one or more genes on the chicken Z chromosome. However, the degree of myopia induced by form deprivation did not differ significantly between males and females. In addition, there was no statistically significant correlation between body weight and susceptibility to form deprivation. Furthermore, the significant correlation between eye size and the susceptibility to form-deprivation induced

eye growth arose from the association between sex and eye size, instead of a true causal relationship.

After two rounds of selection, the third generation of selectively bred High and Low lines exhibited a highly significant divergence in susceptibility to form deprivation. Chicks from the High line developed approximately twice the degree of myopia (-15.27 D versus -6.88 D) and the three times axial length growth (0.54 mm versus 0.16 mm) compared to those from the Low line in the third generation. Furthermore, statistically significant differences between the High and Low lines were also revealed in the changes of other ocular components after 4-day form deprivation, including anterior chamber depth, lens thickness and vitreous chamber depth, as well as the ocular component dimensions before form deprivation (except lens thickness). Since form-deprivation induced eye growth is vision dependent, two hypotheses regarding the reduced susceptibility of chickens from the Low line were (a) inheritance of an allele or alleles causing generalised visual disability, or (b) a relative immaturity either of the retinal circuitry necessary to detect and respond to image blur or some other aspect of their eyes' vision dependent regulatory growth pathway. However, chickens from both the High and Low lines showed similarly good responses in the evaluation of visual function using an optokinetic nystagmus testing paradigm. In addition, the difference in susceptibility to form deprivation between High and Low lines persisted and became even greater after a longer period of form deprivation. Hence, the above two hypotheses were rejected. Instead it was apparent that it was the "gain" of the chicks' eye growth regulatory system which had been selected for. Accordingly, the marked divergence in susceptibility to form deprivation between High and Low lines observed in this selective breeding suggests that susceptibility to form deprivation in White Leghorn chickens has a strong genetic component.

The extent to which susceptibility to form deprivation in White Leghorn chickens was determined by genetics was quantified by calculating its heritability. Data from chickens in all three generations were included in this calculation, and also that for the heritability of ocular component dimensions. Estimates of heritability of ocular component dimensions before form deprivation ranged from 36 to 57%, and sex (as a covariant in the analysis) explained 8~23% of the variation of these traits. The three parameters used to quantify susceptibility to form deprivation, ΔVCD, ΔAL and ΔRX, exhibited heritabilities of 38, 46 and 51%, respectively. Thus, approximately 50% of the variation in form-deprivation

myopia was attributable to additive genetic variance. In addition, sex explained 4% of the variation in form-deprivation induced eye growth and only 1% of variation in the degree of myopia induced, which was in line with the results observed in outbred chickens. Although batch-to-batch variability was taken into account in estimating heritability, this may have reduced differences between High and Low line chicks due to uneven numbers of chickens from each selected line in each batch. Thus, the heritability estimates above might be underestimated. The overall results of (a) divergence in susceptibility to form deprivation between the High and Low lines, and (b) highly significant heritability estimates of the ocular traits representing myopia susceptibility in this selective breeding experiment demonstrate conclusively that susceptibility to environmentally-induced myopia in White Leghorn chickens is predominantly genetically determined. This finding is entirely novel.

Shared genetic effects (pleiotropy) on eye size and susceptibility to myopia have been suggested in previous studies (section 6.4). To search for evidence of pleiotropic genetic effects on eye size and myopia susceptibility, bivariate genetic analysis was carried out to calculate pairwise genetic correlations between these traits. Although the pre-treatment eye size traits, corneal curvature, anterior chamber depth and vitreous chamber depth, exhibited high pairwise genetic correlations amongst one another, no significant genetic correlation was revealed between pre-treatment eye size traits and the traits representing susceptibility to form deprivation. Thus, the genetic variants that control the normal variation in eye size appear to be distinct from the variants that determine susceptibility to myopia in this chicken population. Nevertheless, the possibility that different genetic variants in a common set of genes regulate eye size and myopia susceptibility can not be totally ruled out. In addition, the lack of a significant genetic correlation between eye size and susceptibility to form deprivation was consistent with the result that eye size was not a significant predictor for the eye growth induced by form deprivation as determined from multiple regression analysis in outbred chickens.

Lens defocus has been used in animal models as an alternative to form deprivation for studying the underlying mechanisms in the regulation of eye growth and the development of refractive error. Distinct ocular responses to plus and minus lens wear have been observed, which suggests that there might be different underlying mechanisms between them (Irving et al., 1992; Wildsoet and Wallman, 1995; Fischer et al., 1999). However, controversial results regarding the underlying mechanisms of ocular responses to form deprivation and hyperopic defocus by minus lens wear have been observed, despite the

fact that myopia is induced by both visual perturbations (Bartmann et al., 1994; Schaeffel et al., 1994; Bitzer et al., 2000). When susceptibility to lens-induced visual defocus was tested in chickens from the High and Low lines, significantly different ocular responses were observed between the two selected lines after minus lens wear, but not plus lens wear. Chickens from the High line developed more than twice the degree of myopia compared to those from the Low line after -15 D lens wear for 4 days. Thus, by selecting for susceptibility to form deprivation, susceptibility to hyperopic defocus by minus lens was also altered in this chicken population. Nonetheless, this was not the case for susceptibility to myopic defocus induced by plus lens wear. The results suggest that there must be some shared mechanisms or biochemical pathways underlying the ocular responses to form deprivation and minus lens wear. Furthermore, it also confirms that ocular responses to plus lens wear possess different mechanisms or biochemical pathways compared to the response to minus lens wear or diffusers.

Compared to the chicken model of myopia, the mouse model has the advantages of a closer evolutionary relationship to humans and the ready availability of techniques to manipulate and analyse its genome. Previous studies revealed that a candidate gene regulating eye weight in mice, the HGF gene (Zhou and Williams, 1999a), was associated with susceptibility to human high myopia (Han et al., 2006; Yanovitch et al., 2009; Veerappan et al., 2010). Furthermore, myopia induced in mice shared some features of primate myopia (Tkatchenko et al., 2010). For instance, myopia is associated with elongation of the vitreous chamber of the eye in both mice and primates. Hence, the mouse model is also a potential model to unveil the genetic control of myopia development. However, the small eye size in mice creates difficulties regarding the accuracy of measurements of ocular component dimensions. From measuring ocular component dimensions in two generations of MF-1 outbed mice using a noval swept-source OCT system in this study, good repeatability in the measurements of axial ocular components was observed, with high correlations and a narrow range for the 95% limits of agreement. Additionally, apart from corneal thickness, these axial ocular components exhibited statistically significant, high heritabilities ($h^2 = 0.61\sim0.80$), as well as genetic correlations between axial length and other ocular components ($\rho_G = 0.63\sim0.71$). These findings were similar to the results of additive genetic effects on ocular traits from selectively bred chickens, suggesting ocular component dimensions were mainly genetically determined and with shared (pleotropic) additive genetic effects amongst them in both species. Notably, a very high genetic correlation between corneal curvature and axial length was

revealed both in chicken ($\rho_G = 0.96$) and mouse ($\rho_G = 0.98$). Moreover, a significant correlation between corneal curvature and axial length has been previously observed in humans ($\rho_G = 0.40$) (Klein et al., 2009). The lower level of this genetic correlation in humans compared to animal models may be due to species differences or the exposure to a more complex environment for human subjects. Nonetheless, this common pattern in the genetic control of ocular components could be useful in the search for underlying mechanisms or pathways controlling ocular growth.

8.2 Future work

Myopia is a complex eye disorder that results from the interplay of genetic and environmental factors. Although the application of results from animal models to human myopia is not always straightforward, they do provide useful information to complement observations in human populations. In addition, the effects of environmental factors can be limited in carefully designed animal studies. Hence, it is often easier to isolate and identify the genetic variants controlling traits such as myopia susceptibility in animals than in humans. From the selective breeding experiment, the hypothesis that susceptibility to environmentally-induced myopia is genetically determined in chickens has been supported conclusively. Approximately 50% of the variation in susceptibility to form-deprivation myopia was found to be determined by additive genetic effects, which provides a promising basis for the search for the genes responsible. Therefore, further work will be identifying specific genetic variants that are associated with myopia susceptibility using quantitative trait locus (QTL) mapping. After genotyping the DNA samples from the selectively bred chicken pedigrees, an analysis could be carried out from the combined information of the genotype data and phenotype data. If there is a difference in the mean value of susceptibility to myopia (such as ΔAL or ΔRX) amongst marker genotype classes, then the presence of a QTL linked to the marker can be inferred. Nonetheless, mapping a QTL only crudely localizes the chromosomal location (to within approximately 20cM regions) of a genetic variant that influences a trait (Falconer and Mackay, 1996). The responsible gene or genes for myopia susceptibility may be identified within such QTL regions using a candidate gene fine mapping approach. By elucidating the function of genes identified in this manner, a better understanding of the underlying molecular mechanisms of myopia development can be achieved and probably lead to the discovery of potential therapeutic targets to slow down or stop the progression of myopia (Figure 8.1).

In dissecting the genetics of myopia via genetic association studies, factors relating to environmental exposures, such as reading and outdoor activity, are mostly ignored. For complex diseases, this could hamper the statistical power to identify candidate genes if the environmental factors were not taken into consideration in the association studies (Khoury and Wacholder, 2009). Williamson et al. (2010) showed that a joint test for a gene-disease association can have greater statistical power by allowing for a gene-environment interaction than a study that does not consider interactions. Given the evidence from this selective breeding experiment that susceptibility to environmentally-induced myopia was substantially determined by additive genetic effects, it suggested the existence of the influence of an interaction between genetic effects and environmental factors on the development of myopia. Hence, the statistical power of a genetic association study in the search for myopia candidate genes may increase when gene-environmental interaction is considered. For instance, individuals with the same SNP can be further categorised into subgroups according to levels of environmental exposure, such as the time spent doing near work. Then the association between myopia and the genetic effect of this SNP modified by the environmental factor can be tested. Hence, incorporation of environmental exposure information into genetic association studies could provide greater statistical power to detect the genetic variants influencing the development of myopia compared to studies ignoring environmental factors.

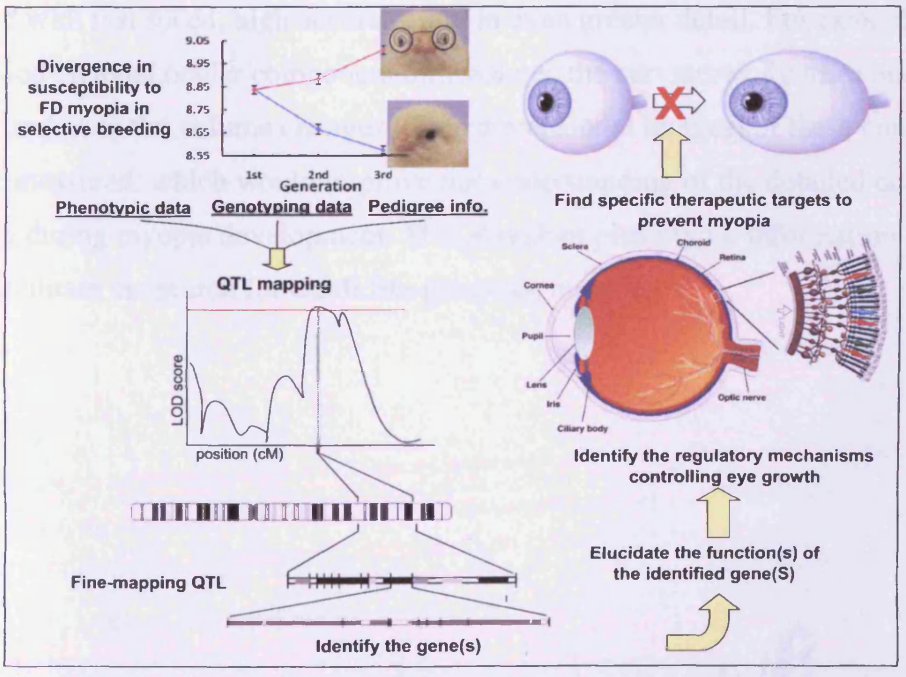


Figure 8.1 Prospective path from the selective breeding towards identification of the responsible genes and the discovery of therapeutic targets to prevent myopia. (The picture of a schematic section of the eye is from Kolb (2007).)

For measuring axial ocular components in mice, the noval swept-source OCT tested here showed reliable results with fast speed and high accuracy. In addition, axial ocular components in mice were revealed to exhibit high heritabilities (the first time this has been achieved). Myopia can be successfully induced in mice (Tkatchenko et al., 2010). With information from the completely sequenced genome of the mouse (Waterston et al., 2002), the mouse model of myopia has great potential in the search for myopia susceptibility genes. However, some potential limits are insufficient genetic diversity in inbred mice strains and poor visual function in mice (especially albino strains) which may result in poor responses to visual manipulations. One possibility for QTL mapping of myopia susceptibility in mice would be a cross between two inbred strains, such as the BXD recombinant inbred mouse strains which have been used successfully to identify QTLs influencing eye weight (Zhou and Williams, 1999a). If variability in susceptibility to myopia induced by a uniform treatment regimen can be found in the mice from a cross between two inbred strains, then QTL mapping for myopia susceptibility loci is feasible. After finding candidate gene/genes, the mechanisms or pathways that regulate myopia development can be further studied through the manipulation of the mouse genome, for example, via gene knockouts. This may also provide valuable information about potential strategies to prevent the development or progression of myopia. With further advances in OCT technology, the ocular components in mouse or even in chicken may be able to be measured with fast speed, high accuracy and in even greater detail. For example, information on axial ocular component dimensions, the curvature of cornea and lens surfaces and even the volume changes (three dimensional images) of these components could be measured, which would improve our understanding of the detailed ocular changes occurring during myopia development. This abundant phenotypic information would further facilitate the search for candidate genes for myopia.

References

Abdeljalil J, Hamid M, Abdel-mouttalib O, Stephane R, Raymond R, Johan A, Jose S, et al. (2005) The optomotor response: A robust first-line visual screening method for mice. *Vision Research* 45: 1439-1446.

Almasy L, and Blangero J (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. *American Journal of Human Genetics* 62: 1198-1211.

Alsbirk P H (1979) Refraction in adult West Greenland Eskimos. A population study of spherical refractive errors, including oculometric and familial correlations. *Acta Ophthalmologica (Copenhagen)* 57: 84-95.

Andrew T, Maniatis N, Carbonaro F, Liew S H, Lau W, Spector T D, and Hammond C J (2008) Identification and replication of three novel myopia common susceptibility gene loci on chromosome 3q26 using linkage and linkage disequilibrium mapping. *PLoS Genetics* 4: e1000220.

Ashby R, Kozulin P, Megaw P L, and Morgan I G (2010) Alterations in ZENK and glucagon RNA transcript expression during increased ocular growth in chickens. *Molecular Vision* 16: 639-649.

Ashby R, Ohlendorf A, and Schaeffel F (2009) The effect of ambient illuminance on the development of deprivation myopia in chicks. *Investigative Ophthalmology & Visual Science* 50: 5348-5354.

Ashby R S, and Schaeffel F (2010) The Effect of Bright Light on Lens-Compensation in Chicks. *Investigative Ophthalmology & Visual Science* 50: 5247-5253.

Atta H R (1996) *Ophthalmic ultrasound : a practical guide*. New York: Churchill Livingstone.

Au Eong K G, Tay T H, and Lim M K (1993) Race, culture and Myopia in 110,236 young Singaporean males. *Singapore Medical Journal* 34: 29-32.

Aulchenko Y S, Ripke S, Isaacs A, and van Duijn C M (2007) GenABEL: an R library for genome-wide association analysis. *Bioinformatics* 23: 1294-1296.

Avila N V, and McFadden S A (2010) A detailed paraxial schematic eye for the White Leghorn chick. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* 196: 825-840.

Bamashmus M A, Matlhaga B, and Dutton G N (2004) Causes of blindness and visual impairment in the West of Scotland. *Eye* 18: 257-261.

Bar Dayan Y, Levin A, Morad Y, Grotto I, Ben-David R, Goldberg A, Onn E, et al. (2005) The changing prevalence of myopia in young adults: a 13-year series of population-based prevalence surveys. *Investigative Ophthalmology & Visual Science* 46: 2760-2765.

Barathi V A, Boopathi V G, Yap E P, and Beuerman R W (2008) Two models of experimental myopia in the mouse. *Vision Research* 48: 904-916.

Bartmann M, Schaeffel F, Hagel G, and Zrenner E (1994) Constant light affects retinal dopamine levels and blocks deprivation myopia but not lens-induced refractive errors in chickens. *Visual Neuroscience* 11: 199-208.

Bedrossian R H (1979) The effect of atropine on myopia. *Ophthalmology* 86: 713-719.

Beresford J A, Crewther S G, Kiely P M, and Crewther D P (2001) Comparison of refractive state and circumferential morphology of retina, choroid, and sclera in chick models of experimentally induced ametropia. *Optometry & Vision Science* 78: 40-49.

Biino G, Palmas M A, Corona C, Prodi D, Fanciulli M, Sulis R, Serra A, et al. (2005) Ocular refraction: heritability and genome-wide search for eye morphometry traits in an isolated Sardinian population. *Human Genetics* 116: 152-159.

Bishop K M, and Wahlsten D (1999) Sex and species differences in mouse and rat forebrain commissures depend on the method of adjusting for brain size. *Brain Research* 815: 358-366.

Bitzer M, Feldkaemper M, and Schaeffel F (2000) Visually induced changes in components of the retinoic acid system in fundal layers of the chick. *Experimental Eye Research* 70: 97-106.

Bitzer M, and Schaeffel F (2002) Defocus-induced changes in ZENK expression in the chicken retina. *Investigative Ophthalmology & Visual Science* 43: 246-252.

Bland J M, and Altman D G (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307-310.

Bourne R R, Dineen B P, Ali S M, Noorul Huq D M, and Johnson G J (2004) Prevalence of refractive error in Bangladeshi adults: results of the National Blindness and Low Vision Survey of Bangladesh. *Ophthalmology* 111: 1150-1160.

Brown A S, Zhang M, Cucevic V, Pavlin C J, and Foster F S (2005) In vivo assessment of postnatal murine ocular development by ultrasound biomicroscopy. *Current Eye Research* 30: 45-51.

Buck C, Schaeffel F, Simon P, and Feldkaemper M (2004) Effects of positive and negative lens treatment on retinal and choroidal glucagon and glucagon receptor mRNA levels in the chicken. *Investigative Ophthalmology & Visual Science* 45: 402-409.

Buhi E R, Goodson P, and Neilands T B (2007) Structural equation modeling: a primer for health behavior researchers. *American Journal Health Behavior* 31: 74-85.

Byrne B M (2009) *Structural Equation Modeling with AMOS*. 2nd ed. New York: Routledge Academic.

Callewaert B, Malfait F, Loeys B, and De Paepe A (2008) Ehlers-Danlos syndromes and Marfan syndrome. *Best Practice & Research. Clinical Rheumatology* 22: 165-189.

Chen C J, Cohen B H, and Diamond E L (1985) Genetic and environmental effects on the development of myopia in Chinese twin children. *Ophthalmic Paediatrics and Genetics* 6: 353-359.

Chen C Y, Stankovich J, Scurrah K J, Garoufalis P, Dirani M, Pertile K K, Richardson A J, et al. (2007) Linkage replication of the MYP12 locus in common myopia. *Investigative Ophthalmology & Visual Science* 48: 4433-4439.

Chen Z T, Wang I J, Shih Y F, and Lin L L (2009) The association of haplotype at the lumican gene with high myopia susceptibility in Taiwanese patients. *Ophthalmology* 116: 1920-1927.

Chua W H, Balakrishnan V, Chan Y H, Tong L, Ling Y, Quah B L, and Tan D (2006) Atropine for the treatment of childhood myopia. *Ophthalmology* 113: 2285-2291.

Coscas G (2009) *Optical coherence tomography in age-related macular degeneration: OCT in AMD*. 2nd ed. Berlin: Springer Verlag.

Cottriall C L, McBrien N A, Annies R, and Leech E M (1999) Prevention of form-deprivation myopia with pirenzepine: a study of drug delivery and distribution. *Ophthalmic and Physiological Optics* 19: 327-335.

Cottriall C L, Truong H T, and McBrien N A (2001) Inhibition of myopia development in chicks using himbacine: a role for M(4) receptors? *Neuroreport* 12: 2453-2456.

Crewther D P (2000) The role of photoreceptors in the control of refractive state. *Progress in Retinal and Eye Research* 19: 421-457.

Curtin B J (1985) *The Myopias, Basic Science and Clinical Management.* Philadephia: Harper and Row.

Curtin B J (1988) Pathologic myopia. *Acta Ophthalmologica Supplement* 185: 105-106.

da Cunha R P, and Moreira J B (1996) Ocular findings in Down's syndrome. *American Journal of Ophthalmology* 122: 236-244.

Dawkins M S, and Woodington A (2000) Pattern recognition and active vision in chickens. *Nature* 403: 652-655.

Deans M R, Volgyi B, Goodenough D A, Bloomfield S A, and Paul D L (2002) Connexin36 is essential for transmission of rod-mediated visual signals in the mammalian retina. *Neuron* 36: 703-712.

Diether S, and Schaeffel F (1997) Local changes in eye growth induced by imposed local refractive error despite active accommodation. *Vision Research* 37: 659-668.

Diether S, and Schaeffel F (1999) Long-term changes in retinal contrast sensitivity in chicks from frosted occluders and drugs: relations to myopia? *Vision Research* 39: 2499-2510.

Dirani M, Chamberlain M, Shekar S N, Islam A F, Garoufalis P, Chen C Y, Guymer R H, et al. (2006) Heritability of refractive error and ocular biometrics: the Genes in Myopia (GEM) twin study. *Investigative Ophthalmology & Visual Science* 47: 4756-4761.

Dirani M, Islam A, and Baird P N (2008) Body stature and myopia-The Genes in Myopia (GEM) twin study. *Ophthalmic Epidemiology* 15: 135-139.

Dirani M, Shekar S N, and Baird P N (2008) The role of educational attainment in refraction: the Genes in Myopia (GEM) twin study. *Investigative Ophthalmology & Visual Science* 49: 534-538.

Drexler W, and Fujimoto J G (2008) *Optical coherence tomography: technology and applications.* Berlin: Springer.

Easter S S, Jr. (1972) Pursuit eye movements in goldfish (Carassius auratus). *Vision Research* 12: 673-688.

Ehrlich D, Sattayasai J, Zappia J, and Barrington M (1990) Effects of selective neurotoxins on eye growth in the young chick. *Ciba Foundation Symposium* 155: 63-84; discussion 84-68.

Elston R C, and Rao D C (1978) Statistical modeling and analysis in human genetics. *Annual Review of Biophysics and Bioengineering* 7: 253-286.

Evans H E (1996) Anatomy of the budgerigar and other birds.*Diseases of Cage and Aviary Birds*. Baltimore: Williams & Wilkins.

Eysteinsson T, Jonasson F, Arnarsson A, Sasaki H, and Sasaki K (2005) Relationships between ocular dimensions and adult stature among participants in the Reykjavik Eye Study. *Acta Ophthalmologica Scandinavica* 83: 734-738.

Fairbairn D J (1997) ALLOMETRY FOR SEXUAL SIZE DIMORPHISM:Pattern and Process in the Coevolution of Body Size in Males and Females. *Annual Review of Ecology and Systematics* 28: 659-687.

Faivre L, Dollfus H, Lyonnet S, Alembik Y, Megarbane A, Samples J, Gorlin R J, et al. (2003) Clinical homogeneity and genetic heterogeneity in Weill-Marchesani syndrome. *American Journal of Medical Genetics Part A* 123A: 204-207.

Falconer D S, and Mackay T F C (1996) *Introduction to quantitative genetics*. Fourth ed. London: Longman.

Fantes J, Ragge N K, Lynch S A, McGill N I, Collin J R, Howard-Peebles P N, Hayward C, et al. (2003) Mutations in SOX2 cause anophthalmia. *Nature Genetics* 33: 461-463.

Farbrother J E, Kirov G, Owen M J, Pong-Wong R, Haley C S, and Guggenheim J A (2004) Linkage analysis of the genetic loci for high myopia on 18p, 12q, and 17q in 51 U.K. families. *Investigative Ophthalmology & Visual Science* 45: 2879-2885.

Feldkaemper M, Diether S, Kleine G, and Schaeffel F (1999) Interactions of spatial and luminance information in the retina of chickens during myopia development. *Experimental Eye Research* 68: 105-115.

Fernandez-Medarde A, Barhoum R, Riquelme R, Porteros A, Nunez A, de Luis A, de Las Rivas J, et al. (2009) RasGRF1 disruption causes retinal photoreception defects and associated transcriptomic alterations. *Journal of Neurochemistry* 110: 641-652.

Fischer A J, McGuire J J, Schaeffel F, and Stell W K (1999) Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. *Nature Neuroscience* 2: 706-712.

Fischer A J, Miethke P, Morgan I G, and Stell W K (1998) Cholinergic amacrine cells are not required for the progression and atropine-mediated suppression of form-deprivation myopia. *Brain Research* 794: 48-60.

Flint J, Valdar W, Shifman S, and Mott R (2005) Strategies for mapping and cloning quantitative trait genes in rodents. *Nature Reviews. Genetics* 6: 271-286.

Framingham Offspring Eye Study Group (1996) Familial aggregation and prevalence of myopia in the Framingham Offspring Eye Study. The Framingham Offspring Eye Study Group. *Archives of Ophthalmology* 114: 326-332.

Fredrick D (2002a) Discussion on Review of Myopia Interventions. *Ophthalmology* 109: 422-423.

Fredrick D R (2002b) Myopia. *British Medical Journal (Clinical Research ed.)* 324: 1195-1199.

Fujimoto J G, Brezinski M E, Tearney G J, Boppart S A, Bouma B, Hee M R, Southern J F, et al. (1995) Optical biopsy and imaging using optical coherence tomography. *Nature Medicine* 1: 970-972.

Gentle A, Liu Y, Martin J E, Conti G L, and McBrien N A (2003) Collagen gene expression and the altered accumulation of scleral collagen during the development of high myopia. *The Journal of Biological Chemistry* 278: 16587-16594.

Glickstein M, and Millodot M (1970) Retinoscopy and eye size. *Science* 168: 605-606.

Goldschmidt E (1966) MYOPIA AND HEIGHT. *Acta Ophthalmologica* 44: 751-761.

Goldschmidt E (1968) On the etiology of myopia. An epidemiological study. *Acta Ophthalmologica (Copenhagen)* 98(Suppl): 1-172.

Gottlieb M D, Joshi H B, and Nickla D L (1990) Scleral changes in chicks with form-deprivation myopia. *Current Eye Research* 9: 1157-1165.

Griffiths A J F, Wessler S R, Lewontin R C, and Carroll S B (2007) *Introduction to Genetic Analysis*. 9th Revised ed. New York: W.H.Freeman & Co Ltd.

Griffiths R, Daan S, and Dijkstra C (1996) Sex identification in birds using two CHD genes. *Proceedings. Biological sciences* 263: 1251-1256.

Griffiths R, Double M C, Orr K, and Dawson R J (1998) A DNA test to sex most birds. *Molecular Ecology* 7: 1071-1075.

Grosvenor T (1987) A review and a suggested classification system for myopia on the basis of age-related prevalence and age of onset. *American Journal of Optometry and Physiological Optics* 64: 545-554.

Grosvenor T, and Flom M C (1991) *Refracitve anomalies: research and clinical applications*. Stoneham: Butterworth-Heinemann.

Guggenheim J A, Erichsen J T, Hocking P M, Wright N F, and Black R (2002) Similar genetic susceptibility to form-deprivation myopia in three strains of chicken. *Vision Research* 42: 2747-2756.

Guggenheim J A, and McBrien N A (1996) Form-deprivation myopia induces activation of scleral matrix metalloproteinase-2 in tree shrew. *Investigative Ophthalmology & Visual Science* 37: 1380-1395.

Guo S S, Sivak J G, Callender M G, and Diehl-Jones B (1995) Retinal dopamine and lens-induced refractive errors in chicks. *Current Eye Research* 14: 385-389.

Guo X, Xiao X, Li S, Wang P, Jia X, and Zhang Q (2010) Nonsyndromic high myopia in a Chinese family mapped to MYP1: linkage confirmation and phenotypic characterization. *Archives of Ophthalmology* 128: 1473-1479.

Gwiazda J (2009) Treatment options for myopia. *Optometry & Vision Science* 86: 624-628.

Gwiazda J, Marsh-Tootle W L, Hyman L, Hussein M, and Norton T T (2002) Baseline refractive and ocular component measures of children enrolled in the correction of myopia evaluation trial (COMET). *Investigative Ophthalmology & Visual Science* 43: 314-321.

Gwiazda J, Thorn F, Bauer J, and Held R (1993) Emmetropization and the progression of manifest refraction in children followed from infancy to puberty. *Clinical Vision Sciences* 8: 337-344.

Hall N F, Gale C R, Ye S, and Martyn C N (2009) Myopia and polymorphisms in genes for matrix metalloproteinases. *Investigative Ophthalmology & Visual Science* 50: 2632-2636.

Hammond C J, Andrew T, Mak Y T, and Spector T D (2004) A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of dizygotic twins. *American Journal of Human Genetics* 75: 294-304.

Hammond C J, Snieder H, Gilbert C E, and Spector T D (2001) Genes and environment in refractive error: the twin eye study. *Investigative Ophthalmology & Visual Science* 42: 1232-1236.

Han W, Leung K H, Fung W Y, Mak J Y, Li Y M, Yap M K, and Yip S P (2009) Association of PAX6 polymorphisms with high myopia in Han Chinese nuclear families. *Investigative Ophthalmology & Visual Science* 50: 47-56.

Han W, Yap M K, Wang J, and Yip S P (2006) Family-based association analysis of hepatocyte growth factor (HGF) gene polymorphisms in high myopia. *Investigative Ophthalmology & Visual Science* 47: 2291-2299.

Hayes B P, Fitzke F W, Hodos W, and Holden A L (1986) A morphological analysis of experimental myopia in young chickens. *Investigative Ophthalmology & Visual Science* 27: 981-991.

He M, Huang W, Zheng Y, Huang L, and Ellwein L B (2007) Refractive error and visual impairment in school children in rural southern China. *Ophthalmology* 114: 374-382.

Heath S, Robledo R, Beggs W, Feola G, Parodo C, Rinaldi A, Contu L, et al. (2001) A novel approach to search for identity by descent in small samples of patients and controls from the same mendelian breeding unit: a pilot study on myopia. *Human Heredity* 52: 183-190.

Hill W G, and Caballero A (1992) Artificial Selection Experiments. *Annual Review of Ecology and Systematics* 23: 287-310.

Hirschhorn J N, and Daly M J (2005) Genome-wide association studies for common diseases and complex traits. *Nature Reviews. Genetics* 6: 95-108.

Hornbeak D M, and Young T L (2009) Myopia genetics: a review of current research and emerging trends. *Current Opinion in Ophthalmology* 20: 356-362.

Howland H C, Merola S, and Basarab J R (2004) The allometry and scaling of the size of vertebrate eyes. *Vision Research* 44: 2043-2065.

Hoyt C S, Stone R D, Fromer C, and Billson F A (1981) Monocular axial myopia associated with neonatal eyelid closure in human infants. *American Journal of Ophthalmology* 91: 197-200.

Hsu W M, Cheng C Y, Liu J H, Tsai S Y, and Chou P (2004) Prevalence and causes of visual impairment in an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology* 111: 62-69.

Huang D, Swanson E A, Lin C P, Schuman J S, Stinson W G, Chang W, Hee M R, et al. (1991) Optical coherence tomography. *Science* 254: 1178-1181.

Hughes A (1979) The artefact of retinoscopy in the rat and rabbit eye has its origin at the retina/vitreous interface rather than in longitudinal chromatic aberration. *Vision Research* 19: 1293-1294.

Hung L F, Crawford M L, and Smith E L (1995) Spectacle lenses alter eye growth and the refractive status of young monkeys. *Nature Medicine* 1: 761-765.

Hunter K W, and Crawford N P (2008) The future of mouse QTL mapping to diagnose disease in mice in the age of whole-genome association studies. *Annual Review of Genetics* 42: 131-141.

Hyman L (2007) Myopic and hyperopic refractive error in adults: an overview. *Ophthalmic Epidemiology* 14: 192-197.

Hysi P G, Young T L, Mackey D A, Andrew T, Fernandez-Medarde A, Solouki A M, Hewitt A W, et al. (2010) A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. *Nature Genetics*.

Inamori Y, Ota M, Inoko H, Okada E, Nishizaki R, Shiota T, Mok J, et al. (2007) The COL1A1 gene and high myopia susceptibility in Japanese. *Human Genetics* 122: 151-157.

International Chicken Genome Sequencing Consortium (2004) Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432: 695-716.

Ip J M, Huynh S C, Kifley A, Rose K A, Morgan I G, Varma R, and Mitchell P (2007) Variation of the contribution from axial length and other oculometric parameters to refraction by age and ethnicity. *Investigative Ophthalmology & Visual Science* 48: 4846-4853.

Irving E L, Callender M G, and Sivak J G (1995) Inducing ametropias in hatchling chicks by defocus--aperture effects and cylindrical lenses. *Vision Research* 35: 1165-1174.

Irving E L, Sivak J G, and Callender M G (1992) Refractive plasticity of the developing chick eye. *Ophthalmic and Physiological Optics* 12: 448-456.

Iuvone P M, Tigges M, Stone R A, Lambert S, and Laties A M (1991) Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. *Investigative Ophthalmology & Visual Science* 32: 1674-1677.

Iwase A, Araie M, Tomidokoro A, Yamamoto T, Shimizu H, and Kitazawa Y (2006) Prevalence and causes of low vision and blindness in a Japanese adult population: the Tajimi Study. *Ophthalmology* 113: 1354-1362.

Jacobsen N, Jensen H, and Goldschmidt E (2007) Prevalence of myopia in Danish conscripts. *Acta Ophthalmologica Scandinavica* 85: 165-170.

Jiang L, Schaeffel F, Zhou X, Zhang S, Jin X, Pan M, Ye L, et al. (2009) Spontaneous axial myopia and emmetropization in a strain of wild-type guinea pig (Cavia porcellus). *Investigative Ophthalmology & Visual Science* 50: 1013-1019.

Jinks J L, and Fulker D W (1970) Comparison of the biometrical genetical, MAVA, and classical approaches to the analysis of human behavior. *Psychological Bulletin* 73: 311-349.

Kean R P, Cahaner A, Freeman A E, and Lamont S J (1994) Direct and correlated responses to multitrait, divergent selection for immunocompetence. *Poultry Science* 73: 18-32.

Keirl A, and Christie C (2007) *Clinical optics and refraction : a guide for optometrists, contact lens opticians and dispensing opticians*. Oxford: Elsevier Butterworth-Heinemann.

Khor C C, Fan Q, Goh L, Tan D, Young T L, Li Y J, Seielstad M, et al. (2010) Support for TGFB1 as a susceptibility gene for high myopia in individuals of Chinese descent. *Archives of Ophthalmology* 128: 1081-1084.

Khor C C, Grignani R, Ng D P, Toh K Y, Chia K S, Tan D, Goh D L, et al. (2009) cMET and refractive error progression in children. *Ophthalmology* 116: 1469-1474, 1474 e1461.

Khoury M J, and Wacholder S (2009) Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. *American Journal of Epidemiology* 169: 227-230; discussion 234-225.

Kim I Y, Shin J H, and Seong J K (2010) Mouse phenogenomics, toolbox for functional annotation of human genome. *BMB Reports* 43: 79-90.

Kjaer J B, Sørensen P, and Su G (2001) Divergent selection on feather pecking behaviour in laying hens (Gallus gallus domesticus). *Applied Animal Behaviour Science* 71: 229-239.

Klein A P, Duggal P, Lee K E, Klein R, Bailey-Wilson J E, and Klein B E (2007) Confirmation of linkage to ocular refraction on chromosome 22q and identification of a novel linkage region on 1q. *Archives of Ophthalmology* 125: 80-85.

Klein A P, Suktitipat B, Duggal P, Lee K E, Klein R, Bailey-Wilson J E, and Klein B E (2009) Heritability analysis of spherical equivalent, axial length, corneal curvature, and anterior chamber depth in the Beaver Dam Eye Study. *Archives of Ophthalmology* 127: 649-655.

Kleinstein R N, Jones L A, Hullett S, Kwon S, Lee R J, Friedman N E, Manny R E, et al. (2003) Refractive error and ethnicity in children. *Archives of Ophthalmology* 121: 1141-1147.

Koerner F, and Schiller P H (1972) The optokinetic response under open and closed loop conditions in the monkey. *Experimental Brain Research* 14: 318-330.

Kolb H (2007) Simple Anatomy of the Retina. *accessed 13 October 2010,* <*http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=webvision&part=A34#A35*>.

Kondoh H, Uchikawa M, and Kamachi Y (2004) Interplay of Pax6 and SOX2 in lens development as a paradigm of genetic switch mechanisms for cell differentiation. *The International Journal of Developmental Biology* 48: 819-827.

Krause U, Krause K, and Rantakallio P (1982) Sex differences in refraction errors up to the age of 15. *Acta Ophthalmologica (Copenhagen)* 60: 917-926.

Lakshmanan N, Gavora J S, and Lamont S J (1997) Major histocompatibility complex class II DNA polymorphisms in chicken strains selected for Marek's disease resistance and egg production or for egg production alone. *Poultry Science* 76: 1517-1523.

Lam C Y, Tam P O, Fan D S, Fan B J, Wang D Y, Lee C W, Pang C P, et al. (2008a) A genome-wide scan maps a novel high myopia locus to 5p15. *Investigative Ophthalmology & Visual Science* 49: 3768-3778.

Lam D S, Fan D S, Lam R F, Rao S K, Chong K S, Lau J T, Lai R Y, et al. (2008b) The effect of parental history of myopia on children's eye size and growth: results of a longitudinal study. *Investigative Ophthalmology & Visual Science* 49: 873-876.

Lam D S, Lee W S, Leung Y F, Tam P O, Fan D S, Fan B J, and Pang C P (2003a) TGFbeta-induced factor: a candidate gene for high myopia. *Investigative Ophthalmology & Visual Science* 44: 1012-1015.

Lam D S, Tam P O, Fan D S, Baum L, Leung Y F, and Pang C P (2003b) Familial high myopia linkage to chromosome 18p. *Ophthalmologica* 217: 115-118.

Landers J A, Hewitt A W, Dimasi D P, Charlesworth J C, Straga T, Mills R A, Savarirayan R, et al. (2009) Heritability of central corneal thickness in nuclear families. *Investigative Ophthalmology & Visual Science* 50: 4087-4090.

Lange K, Westlake J, and Spence M A (1976) Extensions to pedigree analysis. III. Variance components by the scoring method. *Annals of Human Genetics* 39: 485-491.

Lee K E, Klein B E, Klein R, Quandt Z, and Wong T Y (2009) Association of age, stature, and education with ocular dimensions in an older white population. *Archives of Ophthalmology* 127: 88-93.

Leech E M, Cottriall C L, and McBrien N A (1995) Pirenzepine prevents form deprivation myopia in a dose dependent manner. *Ophthalmic and Physiological Optics* 15: 351-356.

Lewkonia I (1969) Objective assessment of visual acuity by induction of optokinetic nystagmus. *The British Journal of Ophthalmology* 53: 641-644.

Li Y-J, Guggenheim J A, Bulusu A, Metlapally R, Abbott D, Malecaze F, Calvas P, et al. (2009) An international collaborative family-based whole-genome linkage acan for high-grade myopia. *Investigative Ophthalmology Visual Science* 50: 3116-3127.

Lin H J, Wan L, Tsai Y, Chen W C, Tsai S W, and Tsai F J (2009) Muscarinic acetylcholine receptor 1 gene polymorphisms associated with high myopia. *Molecular Vision* 15: 1774-1780.

Lin H J, Wan L, Tsai Y, Chen W C, Tsai S W, and Tsai F J (2010) The association between lumican gene polymorphisms and high myopia. *Eye* 24: 1093-1101.

Lin H J, Wan L, Tsai Y, Tsai Y Y, Fan S S, Tsai C H, and Tsai F J (2006) The TGFbeta1 gene codon 10 polymorphism contributes to the genetic predisposition to high myopia. *Molecular Vision* 12: 698-703.

Lin L L, Shih Y F, Hsiao C K, and Chen C J (2004) Prevalence of myopia in Taiwanese schoolchildren: 1983 to 2000. *Annals Academy of Medicine Singapore* 33: 27-33.

Lin L L, Shih Y F, Tsai C B, Chen C J, Lee L A, Hung P T, and Hou P K (1999) Epidemiologic study of ocular refraction among schoolchildren in Taiwan in 1995. *Optometry & Vision Science* 76: 275-281.

Lopes M C, Andrew T, Carbonaro F, Spector T D, and Hammond C J (2009) Estimating heritability and shared environmental effects for refractive error in twin and family studies. *Investigative Ophthalmology & Visual Science* 50: 126-131.

Lyhne N, Sjolie A K, Kyvik K O, and Green A (2001) The importance of genes and environment for ocular refraction and its determiners: a population based study among 20-45 year old twins. *The British Journal of Ophthalmology* 85: 1470-1476.

Lynch M, and Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sunderland: Sinauer Associates Inc.

Marzani D, and Wallman J (1997) Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. *Investigative Ophthalmology & Visual Science* 38: 1726-1739.

Mash A J, Hegmann J P, and Spivey B E (1975) Genetic analysis of indices of corneal power and corneal astigmatism in human populations with varying incidences of strabismus. *Investigative Ophthalmology & Visual Science* 14: 826-832.

Matsumura H, and Hirai H (1999) Prevalence of myopia and refractive changes in students from 3 to 17 years of age. *Survey of Ophthalmology* 44 Suppl 1: S109-115.

Mattingly R R, and Macara I G (1996) Phosphorylation-dependent activation of the Ras-GRF/CDC25Mm exchange factor by muscarinic receptors and G-protein beta gamma subunits. *Nature* 382: 268-272.

McBrien N A, and Adams D W (1997) A longitudinal investigation of adult-onset and adult-progression of myopia in an occupational group. Refractive and biometric findings. *Investigative Ophthalmology & Visual Science* 38: 321-333.

McBrien N A, and Gentle A (2003) Role of the sclera in the development and pathological complications of myopia. *Progress in Retinal and Eye Research* 22: 307-338.

McBrien N A, Jobling A I, and Gentle A (2009) Biomechanics of the sclera in myopia: extracellular and cellular factors. *Optometry & Vision Science* 86: E23-30.

McBrien N A, Moghaddam H O, Cottriall C L, Leech E M, and Cornell L M (1995) The effects of blockade of retinal cell action potentials on ocular growth, emmetropization and form deprivation myopia in young chicks. *Vision Research* 35: 1141-1152.

McBrien N A, and Norton T T (1992) The development of experimental myopia and ocular component dimensions in monocularly lid-sutured tree shrews (Tupaia belangeri). *Vision Research* 32: 843-852.

McFadden S A, Howlett M H C, and Mertz J R (2004) Retinoic acid signals the direction of ocular elongation in the guinea pig eye. *Vision Research* 44: 643-653.

Meng W, Butterworth J, Malecaze F, and Calvas P (2009) Axial length: an underestimated endophenotype of myopia. *Medical Hypotheses* 74: 252-253.

Meng W, Butterworth J, Malecaze F, and Calvas P (2010) Axial Length of Myopia: A Review of Current Research. *Ophthalmologica* 225: 127-134.

Mertz J R, and Wallman J (2000) Choroidal retinoic acid synthesis: a possible mediator between refractive error and compensatory eye growth. *Experimental Eye Research* 70: 519-527.

Metlapally R, Ki C S, Li Y J, Tran-Viet K N, Abbott D, Malecaze F, Calvas P, et al. (2010) Genetic association of insulin-like growth factor-1 polymorphisms with high-grade myopia in an international family cohort. *Investigative Ophthalmology & Visual Science* 2010: 30.

Metlapally R, Li Y J, Tran-Viet K N, Abbott D, Czaja G R, Malecaze F, Calvas P, et al. (2009) COL1A1 and COL2A1 genes and myopia susceptibility: evidence of association and suggestive linkage to the COL2A1 locus. *Investigative Ophthalmology & Visual Science* 50: 4080-4086.

Meyer C, Mueller M F, Duncker G I, and Meyer H J (1999) Experimental animal myopia models are applicable to human juvenile-onset myopia. *Survey of Ophthalmology* 44 Suppl 1: S93-102.

Meyer D B, and May H C, Jr. (1973) The topographical distribution of rods and cones in the adult chicken retina. *Experimental Eye Research* 17: 347-355.

Midelfart A, Kinge B, Midelfart S, and Lydersen S (2002) Prevalence of refractive errors in young and middle-aged adults in Norway. *Acta Ophthalmologica Scandinavica* 80: 501-505.

Miller K M, Albert D L, Asbell P A, Atebara N H, Schechter R J, Wang M X, and Morse C (2005) *Clinical optics*. 2005-2006 ed. San Francisco: American Academy of Ophthalmology.

Mitchell P, Hourihan F, Sandbach J, and Wang J J (1999) The relationship between glaucoma and myopia: the Blue Mountains Eye Study. *Ophthalmology* 106: 2010-2015.

Mott R, and Flint J (2008) Prospects for complex trait analysis in the mouse. *Mammalian Genome* 19: 306-308.

Mott R, Talbot C J, Turri M G, Collins A C, and Flint J (2000) A method for fine mapping quantitative trait loci in outbred animal stocks. *Proceedings of the National Academy of Sciences of the United States of America* 97: 12649-12654.

Mutti D O (2010) Hereditary and environmental contributions to emmetropization and myopia. *Optometry & Vision Science* 87: 255-259.

Mutti D O, Cooper M E, O'Brien S, Jones L A, Marazita M L, Murray J C, and Zadnik K (2007a) Candidate gene and locus analysis of myopia. *Molecular Vision* 13: 1012-1019.

Mutti D O, Hayes J R, Mitchell G L, Jones L A, Moeschberger M L, Cotter S A, Kleinstein R N, et al. (2007b) Refractive error, axial length, and relative peripheral refractive error before and after the onset of myopia. *Investigative Ophthalmology & Visual Science* 48: 2510-2519.

Mutti D O, Ver Hoeve J N, Zadnik K, and Murphy C J (1997) The artifact of retinoscopy revisited: comparison of refractive error measured by retinoscopy and visual evoked potential in the rat. *Optometry & Vision Science* 74: 483-488.

Mutti D O, Zadnik K, and Adams A J (1996) Myopia. The nature versus nurture debate goes on. *Investigative Ophthalmology & Visual Science* 37: 952-957.

Mutti D O, Zadnik K, and Murphy C J (1999) Naturally occurring vitreous chamber-based myopia in the Labrador retriever. *Investigative Ophthalmology & Visual Science* 40: 1577-1584.

Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idder A, Rascol O, Malecaze F, et al. (2002) A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *Journal of Medical Genetics* 39: 118-124.

Nakanishi H, Yamada R, Gotoh N, Hayashi H, Yamashiro K, Shimada N, Ohno-Matsui K, et al. (2009) A genome-wide association analysis identified a novel susceptible locus for pathological myopia at 11q24.1. *PLoS Genetics* 5: e1000660.

Nallasamy S, Paluru P C, Devoto M, Wasserman N F, Zhou J, and Young T L (2007) Genetic linkage study of high-grade myopia in a Hutterite population from South Dakota. *Molecular Vision* 13: 229-236.

Nanda I, Haaf T, Schartl M, Schmid M, and Burt D W (2002) Comparative mapping of Z-orthologous genes in vertebrates: implications for the evolution of avian sex chromosomes. *Cytogenetic and Genome Research* 99: 178-184.

Ng T K, Lam C Y, Lam D S, Chiang S W, Tam P O, Wang D Y, Fan B J, et al. (2009) AC and AG dinucleotide repeats in the PAX6 P1 promoter are associated with high myopia Association of PAX6 polymorphisms with high myopia in Han Chinese nuclear families. *Molecular Vision* 15: 2239-2248.

Norton T T, Essinger J A, and McBrien N A (1994) Lid-suture myopia in tree shrews with retinal ganglion cell blockade. *Visual Neuroscience* 11: 143-153.

Norton T T, and Rada J A (1995) Reduced extracellular matrix in mammalian sclera with induced myopia. *Vision Research* 35: 1271-1281.

Nota Y, and Takenaka O (1999) DNA extraction from urine and sex identification of birds. *Molecular Ecology* 8: 1237-1238.

Nurnberg G, Jacobi F K, Broghammer M, Becker C, Blin N, Nurnberg P, Stephani U, et al. (2008) Refinement of the MYP3 locus on human chromosome 12 in a German family with Mendelian autosomal dominant high-grade myopia by SNP array mapping. *International Journal of Molecular Medicine* 21: 429-438.

O'Leary D J, and Millodot M (1979) Eyelid closure causes myopia in humans. *Experientia* 35: 1478-1479.

Ogawa A, and Tanaka M (1988) The relationship between refractive errors and retinal detachment--analysis of 1,166 retinal detachment cases. *Japanese Journal of Ophthalmology* 32: 310-315.

Oishi T, and Lauber J K (1988) Chicks blinded with formoguanamine do not develop lid suture myopia. *Current Eye Research* 7: 69-73.

Ojaimi E, Morgan I G, Robaei D, Rose K A, Smith W, Rochtchina E, and Mitchell P (2005) Effect of stature and other anthropometric parameters on eye size and refraction in a population-based study of Australian children. *Investigative Ophthalmology & Visual Science* 46: 4424-4429.

Olsen T, Arnarsson A, Sasaki H, Sasaki K, and Jonasson F (2007) On the ocular refractive components: the Reykjavik Eye Study. *Acta Ophthalmologica Scandinavica* 85: 361-366.

Othman M I, Sullivan S A, Skuta G L, Cockrell D A, Stringham H M, Downs C A, Fornes A, et al. (1998) Autosomal dominant nanophthalmos (NNO1) with high hyperopia and

angle-closure glaucoma maps to chromosome 11. *American Journal of Human Genetics* 63: 1411-1418.

Paget S, Julia S, Vitezica Z G, Soler V, Malecaze F, and Calvas P (2008a) Linkage analysis of high myopia susceptibility locus in 26 families. *Molecular Vision* 14: 2566-2574.

Paget S, Vitezica Z G, Malecaze F, and Calvas P (2008b) Heritability of refractive value and ocular biometrics. *Experimental Eye Research* 86: 290-295.

Paluru P, Ronan S M, Heon E, Devoto M, Wildenberg S C, Scavello G, Holleschau A, et al. (2003) New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. *Investigative Ophthalmology & Visual Science* 44: 1830-1836.

Paluru P C, Nallasamy S, Devoto M, Rappaport E F, and Young T L (2005) Identification of a novel locus on 2q for autosomal dominant high-grade myopia. *Investigative Ophthalmology & Visual Science* 46: 2300-2307.

Patterson D (2009) Molecular genetic analysis of Down syndrome. *Human Genetics* 126: 195-214.

Peet J A, Cotch M F, Wojciechowski R, Bailey-Wilson J E, and Stambolian D (2007) Heritability and familial aggregation of refractive error in the Old Order Amish. *Investigative Ophthalmology & Visual Science* 48: 4002-4006.

Plomin R, Haworth C M, and Davis O S (2009) Common disorders are quantitative traits. *Nature Reviews. Genetics* 10: 872-878.

Potsaid B, Baumann B, Huang D, Barry S, Cable A E, Schuman J S, Duker J S, et al. (2010) Ultrahigh speed 1050nm swept source / Fourier domain OCT retinal and anterior segment imaging at 100,000 to 400,000 axial scans per second. *Optics Express* 18: 20029-20048.

Prashar A, Hocking P M, Erichsen J T, Fan Q, Saw S M, and Guggenheim J A (2009) Common determinants of body size and eye size in chickens from an advanced intercross line. *Experimental Eye Research* 89: 42-48.

Priolo S, Sivak J G, Kuszak J R, and Irving E L (2000) Effects of Experimentally Induced Ametropia on the Morphology and Optical Quality of the Avian Crystalline Lens. *Investigative Ophthalmology & Visual Science* 41: 3516-3522.

Prusky G T, West P W, and Douglas R M (2000) Behavioral assessment of visual acuity in mice and rats. *Vision Research* 40: 2201-2209.

Puk O, Dalke C, Favor J, de Angelis M H, and Graw J (2006) Variations of eye size parameters among different strains of mice. *Mammalian Genome* 17: 851-857.

Pusch C M, Zeitz C, Brandau O, Pesch K, Achatz H, Feil S, Scharfe C, et al. (2000) The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein. *Nature Genetics* 26: 324-327.

Rada J A, Perry C A, Slover M L, and Achen V R (1999) Gelatinase A and TIMP-2 expression in the fibrous sclera of myopic and recovering chick eyes. *Investigative Ophthalmology & Visual Science* 40: 3091-3099.

Rada J A, Shelton S, and Norton T T (2006) The sclera and myopia. *Experimental Eye Research* 82: 185-200.

Rakoczy P E, Yu M J, Nusinowitz S, Chang B, and Heckenlively J R (2006) Mouse models of age-related macular degeneration. *Experimental Eye Research* 82: 741-752.

Rasooly R, and BenEzra D (1988) Congenital and traumatic cataract. The effect on ocular axial length. *Archives of Ophthalmology* 106: 1066-1068.

Read S A, Collins M, and Sander B (2010) Human optical axial length changes in response to defocus. *Investigative Ophthalmology & Visual Science* 51: 6262-6269.

Rohrer B, Spira A W, and Stell W K (1993) Apomorphine blocks form-deprivation myopia in chickens by a dopamine D2-receptor mechanism acting in retina or pigmented epithelium. *Visual Neuroscience* 10: 447-453.

Rose K, Smith W, Morgan I, and Mitchell P (2001) The increasing prevalence of myopia: implications for Australia. *Clinical & Experimental Ophthalmology* 29: 116-120.

Rose K A, Morgan I G, Ip J, Kifley A, Huynh S, Smith W, and Mitchell P (2008) Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology* 115: 1279-1285.

Rose K A, Morgan I G, Smith W, and Mitchell P (2002) High heritability of myopia does not preclude rapid changes in prevalence. *Clinical & Experimental Ophthalmology* 30: 168-172.

Rosner M, Laor A, and Belkin M (1995) Myopia and stature: findings in a population of 106,926 males. *European Journal of Ophthalmology* 5: 1-6.

Russell P J (2006) *iGenetics- A Molecular Approach.* Second ed. San Francisco: Pearson Education Inc.

Saltarelli D, Wildsoet C, Nickla D, and Troilo D (2004) Susceptibility to form-deprivation myopia in chicks is not altered by an early experience of axial myopia. *Optometry & Vision Science* 81: 119-126.

Saw S M (2003) A synopsis of the prevalence rates and environmental risk factors for myopia. *Clinical & Experimental Optometry* 86: 289-294.

Saw S M, Chan Y H, Wong W L, Shankar A, Sandar M, Aung T, Tan D T, et al. (2008) Prevalence and risk factors for refractive errors in the Singapore Malay Eye Survey. *Ophthalmology* 115: 1713-1719.

Saw S M, Chua W H, Gazzard G, Koh D, Tan D T, and Stone R A (2005) Eye growth changes in myopic children in Singapore. *The British Journal of Ophthalmology* 89: 1489-1494.

Saw S M, Chua W H, Hong C Y, Wu H M, Chia K S, Stone R A, and Tan D (2002a) Height and its relationship to refraction and biometry parameters in Singapore Chinese children. *Investigative Ophthalmology & Visual Science* 43: 1408-1413.

Saw S M, Gazzard G, Au Eong K G, and Tan D T (2002b) Myopia: attempts to arrest progression. *The British Journal of Ophthalmology* 86: 1306-1311.

Saw S M, Goh P P, Cheng A, Shankar A, Tan D T, and Ellwein L B (2006) Ethnicity-specific prevalences of refractive errors vary in Asian children in neighbouring Malaysia and Singapore. *The British Journal of Ophthalmology* 90: 1230-1235.

Saw S M, Katz J, Schein O D, Chew S J, and Chan T K (1996) Epidemiology of myopia. *Epidemiologic Reviews* 18: 175-187.

Saw S M, Wu H M, Seet B, Wong T Y, Yap E, Chia K S, Stone R A, et al. (2001) Academic achievement, close up work parameters, and myopia in Singapore military conscripts. *The British Journal of Ophthalmology* 85: 855-860.

Schaeffel F, Bartmann M, Hagel G, and Zrenner E (1995) Studies on the role of the retinal dopamine/melatonin system in experimental refractive errors in chickens. *Vision Research* 35: 1247-1264.

Schaeffel F, Burkhardt E, Howland H C, and Williams R W (2004) Measurement of refractive state and deprivation myopia in two strains of mice. *Optometry & Vision Science* 81: 99-110.

Schaeffel F, Glasser A, and Howland H C (1988) Accommodation, refractive error and eye growth in chickens. *Vision Research* 28: 639-657.

Schaeffel F, Hagel G, Bartmann M, Kohler K, and Zrenner E (1994) 6-Hydroxy dopamine does not affect lens-induced refractive errors but suppresses deprivation myopia. *Vision Research* 34: 143-149.

Schaeffel F, and Howland H C (1987) Corneal accommodation in chick and pigeon. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* 160: 375-384.

Schaeffel F, Simon P, Feldkaemper M, Ohngemach S, and Williams R W (2003) Molecular biology of myopia. *Clinical & Experimental Optometry* 86: 295-307.

Schippert R, Burkhardt E, Feldkaemper M, and Schaeffel F (2007) Relative axial myopia in Egr-1 (ZENK) knockout mice. *Investigative Ophthalmology & Visual Science* 48: 11-17.

Schippert R, and Schaeffel F (2006) Peripheral defocus does not necessarily affect central refractive development. *Vision Research* 46: 3935-3940.

Schmid K, and Wildsoet C (1996) Breed- and gender-dependent differences in eye growth and form deprivation responses in chick. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* 178: 551-561.

Schmid K L, and Wildsoet C F (1998) Assessment of visual acuity and contrast sensitivity in the chick using an optokinetic nystagmus paradigm. *Vision Research* 38: 2629-2634.

Schmid K L, and Wildsoet C F (2004) Inhibitory effects of apomorphine and atropine and their combination on myopia in chicks. *Optometry & Vision Science* 81: 137-147.

Schmucker C, and Schaeffel F (2004a) In vivo biometry in the mouse eye with low coherence interferometry. *Vision Research* 44: 2445-2456.

Schmucker C, and Schaeffel F (2004b) A paraxial schematic eye model for the growing C57BL/6 mouse. *Vision Research* 44: 1857-1867.

Schwartz M, Haim M, and Skarsholm D (1990) X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clinical Genetics* 38: 281-286.

Seko Y, Shimokawa H, and Tokoro T (1995) Expression of bFGF and TGF-beta 2 in experimental myopia in chicks. *Investigative Ophthalmology & Visual Science* 36: 1183-1187.

Seko Y, Tanaka Y, and Tokoro T (1995) Influence of bFGF as a potent growth stimulator and TGF-beta as a growth regulator on scleral chondrocytes and scleral fibroblasts in vitro. *Ophthalmic Research* 27: 144-152.

Shaikh A W, Siegwart J T, Jr., and Norton T T (1999) Effect of interrupted lens wear on compensation for a minus lens in tree shrews. *Optometry & Vision Science* 76: 308-315.

Shen W, and Sivak J G (2007) Eyes of a lower vertebrate are susceptible to the visual environment. *Investigative Ophthalmology & Visual Science* 48: 4829-4837.

Shen W, Vijayan M, and Sivak J G (2005) Inducing form-deprivation myopia in fish. *Investigative Ophthalmology & Visual Science* 46: 1797-1803.

Sherman S M, Norton T T, and Casagrande V A (1977) Myopia in the lid-sutured tree shrew (Tupaia glis). *Brain Research* 124: 154-157.

Shlosberg A, Bellaiche M, Berman E, Perk S, Deeb N, Neumark E, and Cahaner A (1998) Relationship between broiler chicken haematocrit-selected parents and their progeny, with regard to haematocrit, mortality from ascites and bodyweight. *Research in Veterinary Science* 64: 105-109.

Shufelt C, Fraser-Bell S, Ying-Lai M, Torres M, and Varma R (2005) Refractive error, ocular biometry, and lens opalescence in an adult population: the Los Angeles Latino Eye Study. *Investigative Ophthalmology & Visual Science* 46: 4450-4460.

Shupe J M, Kristan D M, Austad S N, and Stenkamp D L (2006) The eye of the laboratory mouse remains anatomically adapted for natural conditions. *Brain, Behavior and Evolution* 67: 39-52.

Siatkowski R M, Cotter S, Miller J M, Scher C A, Crockett R S, and Novack G D (2004) Safety and efficacy of 2% pirenzepine ophthalmic gel in children with myopia: a 1-year, multicenter, double-masked, placebo-controlled parallel study. *Archives of Ophthalmology* 122: 1667-1674.

Siatkowski R M, Cotter S A, Crockett R S, Miller J M, Novack G D, and Zadnik K (2008) Two-year multicenter, randomized, double-masked, placebo-controlled, parallel safety and efficacy study of 2% pirenzepine ophthalmic gel in children with myopia. *Journal of the American Association for Pediatric Ophthalmology and Strabismus* 12: 332-339.

Siegwart J T, Jr., and Norton T T (2001) Steady state mRNA levels in tree shrew sclera with form-deprivation myopia and during recovery. *Investigative Ophthalmology & Visual Science* 42: 1153-1159.

Siegwart J T, Jr., and Norton T T (2002) The time course of changes in mRNA levels in tree shrew sclera during induced myopia and recovery. *Investigative Ophthalmology & Visual Science* 43: 2067-2075.

Simpson C L, Hysi P, Bhattacharya S S, Hammond C J, Webster A, Peckham C S, Sham P C, et al. (2007) The Roles of PAX6 and SOX2 in Myopia: lessons from the 1958 British Birth Cohort. *Investigative Ophthalmology & Visual Science* 48: 4421-4425.

Sivak J G, Ryall L A, Weerheim J, and Campbell M C (1989) Optical constancy of the chick lens during pre- and post-hatching ocular development. *Investigative Ophthalmology Visual Science* 30: 967-974.

Smith E L, 3rd, Kee C S, Ramamirtham R, Qiao-Grider Y, and Hung L F (2005) Peripheral vision can influence eye growth and refractive development in infant monkeys. *Investigative Ophthalmology & Visual Science* 46: 3965-3972.

Snead M P, and Yates J R (1999) Clinical and Molecular genetics of Stickler syndrome. *Journal of Medical Genetics* 36: 353-359.

Solouki A M, Verhoeven V J, van Duijn C M, Verkerk A J, Ikram M K, Hysi P G, Despriet D D, et al. (2010) A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nature Genetics*.

Sorsby A, Benjamin B, and Bennett A G (1981) Steiger on refraction: a reappraisal. *The British Journal of Ophthalmology* 65: 805-811.

Sorsby A, Leary G A, and Fraser G R (1966) Family studies on ocular refraction and its components. *Journal of Medical Genetics* 3: 269-273.

Sorsby A, Leary G A, and Richards M J (1962) Correlation ametropia and component ametropia. *Vision Research* 2: 309-313.

Stambolian D, Ciner E B, Reider L C, Moy C, Dana D, Owens R, Schlifka M, et al. (2005) Genome-wide scan for myopia in the Old Order Amish. *American Journal of Ophthalmology* 140: 469-476.

Stambolian D, Ibay G, Reider L, Dana D, Moy C, Schlifka M, Holmes T, et al. (2004) Genomewide linkage scan for myopia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 22q12. *American Journal of Human Genetics* 75: 448-459.

Stambolian D, Ibay G, Reider L, Dana D, Moy C, Schlifka M, Holmes T N, et al. (2006) Genome-wide scan of additional Jewish families confirms linkage of a myopia susceptibility locus to chromosome 22q12. *Molecular Vision* 12: 1499-1505.

Stone R A, Lin T, Desai D, and Capehart C (1995) Photoperiod, early post-natal eye growth, and visual deprivation. *Vision Research* 35: 1195-1202.

Stone R A, Lin T, and Laties A M (1991) Muscarinic antagonist effects on experimental chick myopia. *Experimental Eye Research* 52: 755-758.

Stone R A, Lin T, Laties A M, and Iuvone P M (1989) Retinal dopamine and form-deprivation myopia. *Proceedings of the National Academy of Sciences of the United States of America* 86: 704-706.

Stone R A, Pendrak K, Sugimoto R, Lin T, Gill A S, Capehart C, and Liu J (2006) Local patterns of image degradation differentially affect refraction and eye shape in chick. *Current Eye Research* 31: 91-105.

Sundin O H, Leppert G S, Silva E D, Yang J M, Dharmaraj S, Maumenee I H, Santos L C, et al. (2005) Extreme hyperopia is the result of null mutations in MFRP, which encodes a Frizzled-related protein. *Proceedings of the National Academy of Sciences of the United States of America* 102: 9553-9558.

Tan D T, Lam D S, Chua W H, Shu-Ping D F, and Crockett R S (2005) One-year multicenter, double-masked, placebo-controlled, parallel safety and efficacy study of 2% pirenzepine ophthalmic gel in children with myopia. *Ophthalmology* 112: 84-91.

Tang W C, Yip S P, Lo K K, Ng P W, Choi P S, Lee S Y, and Yap M K (2007) Linkage and association of myocilin (MYOC) polymorphisms with high myopia in a Chinese population. *Molecular Vision* 13: 534-544.

Tano Y (2002) Pathologic myopia: where are we now? *American Journal of Ophthalmology* 134: 645-660.

Tattersall R J, Prashar A, Singh K D, Tokarczuk P F, Erichsen J T, Hocking P M, and Guggenheim J A (2010) Ex vivo magnetic resonance imaging of crystalline lens dimensions in chicken. *Molecular Vision* 16: 144-153.

Teikari J M (1987) Myopia and stature. *Acta Ophthalmologica (Copenhagen)* 65: 673-676.

Teikari J M, O'Donnell J, Kaprio J, and Koskenvuo M (1991) Impact of heredity in myopia. *Human Heredity* 41: 151-156.

Tejedor J, and de la Villa P (2003) Refractive changes induced by form deprivation in the mouse eye. *Investigative Ophthalmology & Visual Science* 44: 32-36.

Tepelus T C, and Schaeffel F (2010) Individual set-point and gain of emmetropization in chickens. *Vision Research* 50: 57-64.

Thoday J M (1972) Disruptive selection. *Proceedings. Biological sciences* 182: 109-143.

Tkatchenko T V, Shen Y, and Tkatchenko A V (2009) Analysis of postnatal eye development in the mouse with high-resolution small animal magnetic resonance imaging. *Investigative Ophthalmology & Visual Science* 51: 21-27.

Tkatchenko T V, Shen Y, and Tkatchenko A V (2010) Mouse experimental myopia has features of primate myopia. *Investigative Ophthalmology & Visual Science* 51: 1297-1303.

Toh T, Liew S H, MacKinnon J R, Hewitt A W, Poulsen J L, Spector T D, Gilbert C E, et al. (2005) Central corneal thickness is highly heritable: the twin eye studies. *Investigative Ophthalmology & Visual Science* 46: 3718-3722.

Tong L, Huang X L, Koh A L, Zhang X, Tan D T, and Chua W H (2009) Atropine for the treatment of childhood myopia: effect on myopia progression after cessation of atropine. *Ophthalmology* 116: 572-579.

Tonini R, Mancinelli E, Balestrini M, Mazzanti M, Martegani E, Ferroni A, Sturani E, et al. (1999) Expression of Ras-GRF in the SK-N-BE neuroblastoma accelerates retinoic-acid-induced neuronal differentiation and increases the functional expression of the IRK1 potassium channel. *The European journal of neuroscience* 11: 959-966.

Troilo D, Gottlieb M D, and Wallman J (1987) Visual deprivation causes myopia in chicks with optic nerve section. *Current Eye Research* 6: 993-999.

Troilo D, and Judge S J (1993) Ocular development and visual deprivation myopia in the common marmoset (Callithrix jacchus). *Vision Research* 33: 1311-1324.

Troilo D, Li T, Glasser A, and Howland H C (1995) Differences in eye growth and the response to visual deprivation in different strains of chicken. *Vision Research* 35: 1211-1216.

Troilo D, Nickla D L, Mertz J R, and Summers Rada J A (2006) Change in the synthesis rates of ocular retinoic acid and scleral glycosaminoglycan during experimentally altered eye growth in marmosets. *Investigative Ophthalmology & Visual Science* 47: 1768-1777.

Tsonis P A, and Fuentes E J (2006) Focus on molecules: Pax-6, the eye master. *Experimental Eye Research* 83: 233-234.

Veerappan S, Pertile K K, Islam A F, Schache M, Chen C Y, Mitchell P, Dirani M, et al. (2010) Role of the hepatocyte growth factor gene in refractive error. *Ophthalmology* 117: 239-245 e231-232.

Violato C, and Hecker K G (2007) How to use structural equation modeling in medical education research: a brief guide. *Teaching and Learning in Medicine* 19: 362-371.

Visscher P M, Hill W G, and Wray N R (2008) Heritability in the genomics era--concepts and misconceptions. *Nature Reviews. Genetics* 9: 255-266.

Vitale S, Ellwein L, Cotch M F, Ferris F L, 3rd, and Sperduto R (2008) Prevalence of refractive error in the United States, 1999-2004. *Archives of Ophthalmology* 126: 1111-1119.

Vitale S, Sperduto R D, and Ferris F L, 3rd (2009) Increased prevalence of myopia in the United States between 1971-1972 and 1999-2004. *Archives of Ophthalmology* 127: 1632-1639.

Vitart V, Bencic G, Hayward C, Herman J S, Huffman J, Campbell S, Bucan K, et al. (2010) Heritabilities of ocular biometrical traits in two croatian isolates with extended pedigrees. *Investigative Ophthalmology & Visual Science* 51: 737-743.

von Noorden G K, and Lewis R A (1987) Ocular axial length in unilateral congenital cataracts and blepharoptosis. *Investigative Ophthalmology & Visual Science* 28: 750-752.

Vongphanit J, Mitchell P, and Wang J J (2002) Prevalence and progression of myopic retinopathy in an older population. *Ophthalmology* 109: 704-711.

Wallman J (1990) Retinal influences on sclera underlie visual deprivation myopia. *Ciba Foundation Symposium* 155: 126-134; discussion 135-141.

Wallman J (1993) Chapter 6 Retinal control of eye growth and refraction. *Progress in Retinal Research* 12: 133-153.

Wallman J (1994) Nature and nurture of myopia. *Nature* 371: 201-202.

Wallman J, and Adams J I (1987) Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Research* 27: 1139-1163.

Wallman J, Gottlieb M D, Rajaram V, and Fugate-Wentzek L A (1987) Local retinal regions control local eye growth and myopia. *Science* 237: 73-77.

Wallman J, Turkel J, and Trachtman J (1978) Extreme myopia produced by modest change in early visual experience. *Science* 201: 1249-1251.

Wallman J, and Velez J (1985) Directional asymmetries of optokinetic nystagmus: developmental changes and relation to the accessory optic system and to the vestibular system. *The Journal of Neuroscience* 5: 317-329.

Wallman J, Wildsoet C, Xu A, Gottlieb M D, Nickla D L, Marran L, Krebs W, et al. (1995) Moving the retina: choroidal modulation of refractive state. *Vision Research* 35: 37-50.

Wallman J, and Winawer J (2004) Homeostasis of eye growth and the question of myopia. *Neuron* 43: 447-468.

Wang I J, Chiang T H, Shih Y F, Hsiao C K, Lu S C, Hou Y C, and Lin L L (2006) The association of single nucleotide polymorphisms in the 5'-regulatory region of the lumican gene with susceptibility to high myopia in Taiwan. *Molecular Vision* 12: 852-857.

Wang L, Hofer B, Chen Y P, Guggenheim J A, Drexler W, and Povazay B (2010) Highly reproducible swept-source, dispersion-encoded full-range biometry and imaging of the mouse eye. *Journal of Biomedical Optics* 15: 0460041-0460046.

Wang N K, Tsai C H, Chen Y P, Yeung L, Wu W C, Chen T L, Lin K K, et al. (2005) Pediatric rhegmatogenous retinal detachment in East Asians. *Ophthalmology* 112: 1890-1895.

Waterston R H, Lindblad-Toh K, Birney E, Rogers J, Abril J F, Agarwal P, Agarwala R, et al. (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* 420: 520-562.

Wiesel T N, and Raviola E (1977) Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature* 266: 66-68.

Wildsoet C (1998) *Structural correlates of myopia. In: Rosenfield, M. Gilmartin, B. eds. Myopia and nearwork.* Oxford: Butterworth-Heinemann.

Wildsoet C, and Wallman J (1995) Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Research* 35: 1175-1194.

Wildsoet C F (1997) Active emmetropization--evidence for its existence and ramifications for clinical practice. *Ophthalmic and Physiological Optics* 17: 279-290.

Williamson E, Ponsonby A L, Carlin J, and Dwyer T (2010) Effect of including environmental data in investigations of gene-disease associations in the presence of qualitative interactions. *Genetic Epidemiology* 34: 552-560.

Wisard J, Chrenek M A, Wright C, Dalal N, Pardue M T, Boatright J H, and Nickerson J M (2010) Non-contact measurement of linear external dimensions of the mouse eye. *Journal of Neuroscience Methods* 187: 156-166.

Wojciechowski R, Bailey-Wilson J E, and Stambolian D (2009a) Fine-mapping of candidate region in Amish and Ashkenazi families confirms linkage of refractive error to a QTL on 1p34-p36. *Molecular Vision* 15: 1398-1406.

Wojciechowski R, Bailey-Wilson J E, and Stambolian D (2010) Association of matrix metalloproteinase gene polymorphisms with refractive error in Amish and Ashkenazi families. *Investigative Ophthalmology & Visual Science* 51: 4989-4995.

Wojciechowski R, Congdon N, Bowie H, Munoz B, Gilbert D, and West S K (2005) Heritability of refractive error and familial aggregation of myopia in an elderly American population. *Investigative Ophthalmology & Visual Science* 46: 1588-1592.

Wojciechowski R, Moy C, Ciner E, Ibay G, Reider L, Bailey-Wilson J E, and Stambolian D (2006) Genomewide scan in Ashkenazi Jewish families demonstrates evidence of linkage of ocular refraction to a QTL on chromosome 1p36. *Human Genetics* 119: 389-399.

Wojciechowski R, Stambolian D, Ciner E, Ibay G, Holmes T N, and Bailey-Wilson J E (2009b) Genomewide linkage scans for ocular refraction and meta-analysis of four populations in the Myopia Family Study. *Investigative Ophthalmology & Visual Science* 50: 2024-2032.

Wong T Y, Foster P J, Hee J, Ng T P, Tielsch J M, Chew S J, Johnson G J, et al. (2000) Prevalence and risk factors for refractive errors in adult Chinese in Singapore. *Investigative Ophthalmology & Visual Science* 41: 2486-2494.

Wong T Y, Foster P J, Johnson G J, Klein B E, and Seah S K (2001a) The relationship between ocular dimensions and refraction with adult stature: the Tanjong Pagar Survey. *Investigative Ophthalmology & Visual Science* 42: 1237-1242.

Wong T Y, Foster P J, Johnson G J, and Seah S K (2002) Education, socioeconomic status, and ocular dimensions in Chinese adults: the Tanjong Pagar Survey. *The British Journal of Ophthalmology* 86: 963-968.

Wong T Y, Foster P J, Ng T P, Tielsch J M, Johnson G J, and Seah S K (2001b) Variations in ocular biometry in an adult Chinese population in Singapore: the Tanjong Pagar Survey. *Investigative Ophthalmology & Visual Science* 42: 73-80.

Wu H M, Gupta A, Newland H S, Selva D, Aung T, and Casson R J (2007) Association between stature, ocular biometry and refraction in an adult population in rural Myanmar: the Meiktila eye study. *Clinical & Experimental Ophthalmology* 35: 834-839.

Xu L, Li J, Cui T, Hu A, Fan G, Zhang R, Yang H, et al. (2005) Refractive error in urban and rural adult Chinese in Beijing. *Ophthalmology* 112: 1676-1683.

Yalcin B, Willis-Owen S A, Fullerton J, Meesaq A, Deacon R M, Rawlins J N, Copley R R, et al. (2004) Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. *Nature Genetics* 36: 1197-1202.

Yang Z, Xiao X, Li S, and Zhang Q (2009) Clinical and linkage study on a consanguineous Chinese family with autosomal recessive high myopia. *Molecular Vision* 15: 312-318.

Yanovitch T, Li Y J, Metlapally R, Abbott D, Viet K N, and Young T L (2009) Hepatocyte growth factor and myopia: genetic association analyses in a Caucasian population. *Molecular Vision* 15: 1028-1035.

Yin G C, Gentle A, and McBrien N A (2004) Muscarinic antagonist control of myopia: a molecular search for the M1 receptor in chick. *Molecular Vision* 10: 787-793.

Younan C, Mitchell P, Cumming R G, Rochtchina E, and Wang J J (2002) Myopia and incident cataract and cataract surgery: the blue mountains eye study. *Investigative Ophthalmology & Visual Science* 43: 3625-3632.

Young T L, Metlapally R, and Shay A E (2007) Complex trait genetics of refractive error. *Archives of Ophthalmology* 125: 38-48.

Young T L, Ronan S M, Alvear A B, Wildenberg S C, Oetting W S, Atwood L D, Wilkin D J, et al. (1998a) A second locus for familial high myopia maps to chromosome 12q. *American Journal of Human Genetics* 63: 1419-1424.

Young T L, Ronan S M, Drahozal L A, Wildenberg S C, Alvear A B, Oetting W S, Atwood L D, et al. (1998b) Evidence that a locus for familial high myopia maps to chromosome 18p. *American Journal of Human Genetics* 63: 109-119.

Zadnik K, and Mutti D O (1995) How applicable are animal myopia models to human juvenile onset myopia? *Vision Research* 35: 1283-1288.

Zadnik K, Mutti D O, Friedman N E, Qualley P A, Jones L A, Qui P, Kim H S, et al. (1999) Ocular predictors of the onset of juvenile myopia. *Investigative Ophthalmology & Visual Science* 40: 1936-1943.

Zadnik K, Satariano W A, Mutti D O, Sholtz R I, and Adams A J (1994) The effect of parental history of myopia on children's eye size. *The Journal of the American Medical Association* 271: 1323-1327.

Zha Y, Leung K H, Lo K K, Fung W Y, Ng P W, Shi M G, Yap M K, et al. (2009) TGFB1 as a susceptibility gene for high myopia: a replication study with new findings. *Archives of Ophthalmology* 127: 541-548.

Zhang M Z, Saw S M, Hong R Z, Fu Z F, Yang H, Shui Y B, Yap M K, et al. (2000) Refractive errors in Singapore and Xiamen, China--a comparative study in school children aged 6 to 7 years. *Optometry & Vision Science* 77: 302-308.

Zhang Q, Guo X, Xiao X, Jia X, Li S, and Hejtmancik J F (2005) A new locus for autosomal dominant high myopia maps to 4q22-q27 between D4S1578 and D4S1612. *Molecular Vision* 11: 554-560.

Zhang Q, Guo X, Xiao X, Jia X, Li S, and Hejtmancik J F (2006) Novel locus for X linked recessive high myopia maps to Xq23-q25 but outside MYP1. *Journal of Medical Genetics* 43: e20.

Zhang Q, Li S, Xiao X, Jia X, and Guo X (2007) Confirmation of a genetic locus for X-linked recessive high myopia outside MYP1. *Journal of Human Genetics* 52: 469-472.

Zhao D, McBride D, Nandi S, McQueen H A, McGrew M J, Hocking P M, Lewis P D, et al. (2010) Somatic sex identity is cell autonomous in the chicken. *Nature* 464: 237-242.

Zhao J, Mao J, Luo R, Li F, Munoz S R, and Ellwein L B (2002) The progression of refractive error in school-age children: Shunyi district, China. *American Journal of Ophthalmology* 134: 735-743.

Zheng Y, Ge J, Huang G, Zhang J, Liu B, Hur Y M, and He M (2008) Heritability of central corneal thickness in Chinese: the Guangzhou Twin Eye Study. *Investigative Ophthalmology & Visual Science* 49: 4303-4307.

Zhou G, and Williams R W (1999a) Eye1 and Eye2: gene loci that modulate eye size, lens weight, and retinal area in the mouse. *Investigative Ophthalmology & Visual Science* 40: 817-825.

Zhou G, and Williams R W (1999b) Mouse models for the analysis of myopia: an analysis of variation in eye size of adult mice. *Optometry & Vision Science* 76: 408-418.

Zhou X, Xie J, Shen M, Wang J, Jiang L, Qu J, and Lu F (2008) Biometric measurement of the mouse eye using optical coherence tomography with focal plane advancement. *Vision Research* 48: 1137-1143.

Zhu G, Hewitt A W, Ruddle J B, Kearns L S, Brown S A, Mackinnon J R, Chen C Y, et al. (2008) Genetic dissection of myopia: evidence for linkage of ocular axial length to chromosome 5q. *Ophthalmology* 115: 1053-1057 e1052.

Zhu X, Lin T, Stone R A, and Laties A M (1995) Sex differences in chick eye growth and experimental myopia. *Experimental Eye Research* 61: 173-179.

Zhu X, and Wallman J (2009) Temporal properties of compensation for positive and negative spectacle lenses in chicks. *Investigative Ophthalmology & Visual Science* 50: 37-46.

List of Publications

Publication arising from this work

CHEN, Y. P., PRASHAR, A., HOCKING, P. M., ERICHSEN, J. T., TO, C. H., SCHAEFFEL, F. & GUGGENHEIM, J. A. (2010) Sex, eye size, and the rate of myopic eye growth due to form deprivation in outbred white leghorn chickens. *Investigative Ophthalmology & Visual Science* 51, 651-7.

Other publications produced during the PhD period

MCMAHON, G., ZAYATS, T., **CHEN, Y. P.,** PRASHAR, A., WILLIAMS, C. & GUGGENHEIM, J. A. (2009) Season of birth, daylight hours at birth, and high myopia. *Ophthalmology* 116, 468-73.

WANG, L., HOFER, B., **CHEN, Y. P.,** GUGGENHEIM, J. A., DREXLER, W. & POVAZAY, B. (2010) Highly reproducible swept-source, dispersion-encoded full-range biometry and imaging of the mouse eye. *Journal of Biomedical Optics* 15, 0460041-0460046.