Heritability of Ocular Component Dimensions in Chickens: Genetic Variants Controlling Susceptibility to Experimentally Induced Myopia and Pretreatment Eye Size Are Distinct

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Purpose. To investigate the extent to which shared genetic variants control (1) multiple ocular component dimensions and (2) both normal eye length and susceptibility to visually induced myopic eye growth.

Methods. Two laboratory-reared populations of chicks were examined. The first was a three-generation pedigree of White Leghorn (WL) birds used in a selective breeding experiment testing susceptibility to monocular deprivation of sharp vision (DSV). The chicks were assessed before (age, 4 days) and after 4 days of treatment with diffusers. The second was the 10th generation of an advanced intercross line (AIL) derived from a broiler-layer cross (age, 3 weeks). Variance components analysis was used to estimate heritability and to assess the evidence for shared genetic determination.

Results. All measured ocular components were moderately or highly heritable (range, 0.36–0.61; all $P < 0.001$) in both chick populations, and there were strong genetic correlations across the traits, corneal curvature, vitreous chamber depth, and axial length. The genetic correlations between eye size and myopia susceptibility traits were not significantly different from 0.

Conclusions. The genetic variants controlling ocular component dimensions in chicks are shared across some ocular traits (corneal curvature, vitreous chamber depth, and axial length) but distinct for others (lens thickness and corneal thickness). The genetic variants controlling susceptibility to visually induced myopia in chicks are different from those controlling normal eye size. (*Invest Ophthalmol Vis Sci.* 2011;52:4012–4020) DOI:10.1167/iovs.10-7045

Twin and family studies suggest that ocular refraction is a multifactorial trait with important contributions from both genetic variants and the environment.[1–7] Refractive errors can result from mismatches between the relative dimensions or refractive indices of any the eye's component parts, but most often, it is an axial length imbalance that is the major structural cause of myopia and hyperopia.[8,9] Researchers interested in the genetics of refractive error have therefore suggested that polymorphisms affecting the size of the ocular components—particularly axial length—may play a role in the inheritance of refraction.[10–13] Several studies have explored the heritability of ocular component dimensions in humans,[12–19] as a first step toward mapping quantitative trait loci (QTL).

In contrast to the extensive literature for human subjects, 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 minimized, which provides a powerful setting for detecting the individual genetic variants responsible for natural variations in eye size.[11,20] Zhou and Williams[21] explored this line of reasoning by estimating the heritability of eye weight and crystalline lens weight in mice and subsequently mapped 2 QTL for eye weight, termed *Eye1* and *Eye2*. In these experiments, eye weight was measured in approximately 500 mice from 46 different subspecies, strains, and substrains. After accounting for sex, age, and body size, the authors estimated the heritability to be 0.31 for eye weight and 0.25 for lens weight.[20] In a less diverse selection of 26 BXD recombinant inbred mouse lines,[21] they reported the heritability of eye weight to be 0.48. Zhou and Williams[21] highlighted the hepatocyte growth factor (*Hgf*) gene on mouse chromosome 5 as a promising candidate gene at the *Eye1* locus. Subsequently, common polymorphisms in the human homologue of this gene, *HGF*, were found to influence the risk of high myopia in humans[22–24] (albeit, not in all replication studies[25]).

As reviewed by Wildsoet,[9] many ocular traits correlate with one another, suggesting either shared genetic determination or coordinated growth regulated by environmental stimuli (e.g., as would occur during active, visually guided emmetropization). Several research groups have sought to quantify these relative sources of influence. Using data from the Beaver Dam Eye Study, Klein et al.[18] reported a significant genetic correlation between axial length and corneal curvature ($\rho_G = 0.40$, $P < 0.001$) in a sample of 715 subjects from 189 pedigrees. A significant genetic correlation such as this implies that a shared

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set of genetic variants contribute to the natural variation in both corneal curvature and axial length (one way of conceptualizing genetic correlations is shown in Fig. 1). Likewise, in a large twin study, He et al.[26] found that ~22% (89% of 25%) of the natural variation in anterior chamber depth was determined by genetic variants that also controlled axial length. In a second twin study, Dirani et al.[27] found that ~50% of the natural variation in refractive error and axial length is jointly determined by a common set of genetic variants.

Together, the above studies suggest that: (1) the dimensions of many ocular components share a common source of genetic regulation (i.e., they are determined by a common set of genetic variants), and (2) some of these genetic variants also influence the risk of developing refractive errors such as myopia.

The chicken is a frequently studied animal model of myopia.[7] We recently performed two experiments in laboratory-reared populations of chickens,[11,28] each of which provided the opportunity to estimate the heritability of ocular component dimensions and the extent to which pairs of ocular traits share a common source of genetic determination. In one of these populations, we also collected data on susceptibility to myopia induced by alterations to the visual environment, and thus we could evaluate the shared genetic determination of ocular component dimensions and myopia susceptibility.

Methods

Animals and Ocular Measurements

All experimental procedures involving animals complied with U.K. Home Office regulations and were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. For both sets of experiments, eggs were hatched in batches of approximately 20 to 30 chicks. Initially, hatchlings were housed in a clear-sided Perspex brooder at 25°C to 27°C before being transferred to a wire-mesh and Perspex-sided floor pen with a suspended infrared heat lamp. Illumination in the brooder and floor pen was 250 to 300 lux. Chicks were given access to water and fed commercial chick starter ad libitum. The sex of each chicken was determined with a PCR-restriction enzyme digest assay, using DNA extracted from a blood sample, as described previously[29] (except for a small number of chickens that were kept until adulthood, in whom sex was apparent from secondary sexual characteristics). A brief description of the groups of chickens examined is given in Table 1.

White Leghorn (WL) Population. These chickens comprised three generations of birds used in a selective breeding experiment designed to test whether susceptibility to myopia induced by the monocular deprivation of sharp vision (DSV; also known as form deprivation) is genetically determined. Methodological details of the selective breeding experiment are reported elsewhere.[30] Briefly, A-scan ultrasonography was performed on a sample of 232 outbred WL birds, before treatment (when the birds were 4 days old). The measurements were obtained while the chicks were anesthetized, and their lids were kept open with a speculum. The ultrasonography procedure provided measures of anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL). After 4 days of monocular DSV treatment, the chicks were refracted using retinoscopy, and the A-scan ultrasonography measurements were repeated. Pairs of chicks with the highest degree of induced myopia (n = 9 pairs; high line) and the lowest degree of induced myopia (n = 9 pairs; low line) were raised to sexual maturity. Offspring from the two lines (generation 2; high line, n = 144; low line, n = 123) were phenotyped before and after DSV treatment, as above. Chicks from the second generation that developed either a high or low level of induced myopia (high line, n = 9 pairs; low line, n = 8 pairs) were retained for breeding a third generation.[30] At 4 days of age and after 4 days of monocular DSV, chicks from the third generation were assessed with retinoscopy, infrared video-keratometry, and A-scan ultrasonography (generation 3; high line, n = 200; low line, n = 192). For measurements that were performed on more than one generation of birds, the same instrumentation and procedures were used to obtain the measurements.

Once hatched, batches of WL chicks were raised together, and the experimenters were masked to each bird's high- or low-line status during treatment and phenotypic assessment.

AIL Population. The derivation of the AIL population and details of the majority of the assessment methods used to phenotype these birds are described in previous publications.[11,31] Briefly, a cross be-

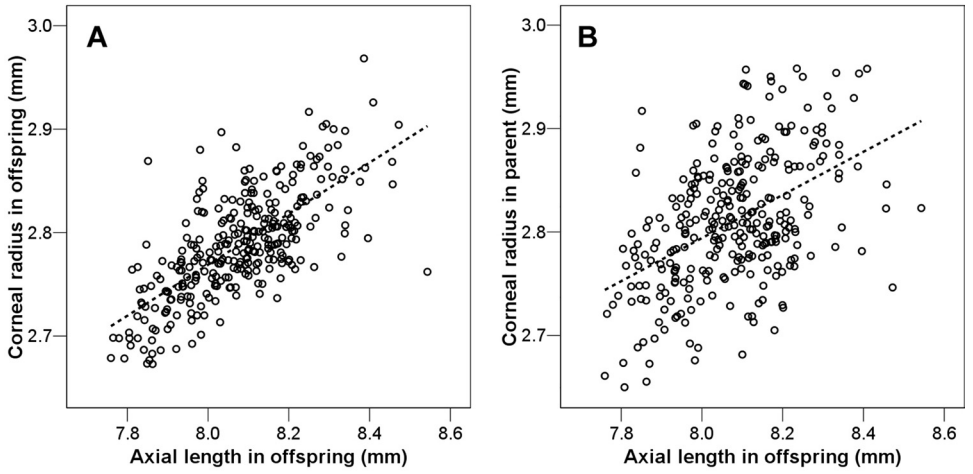

Figure 1. Visualizing the meaning of genetic correlation. (**A**) The relationship between two traits, corneal curvature and axial length, measured in the same set of individuals. Such a relationship could be quantified using a conventional correlation coefficient. In genetics, it is frequently of interest to plot graphs showing the relationship between *a single trait* measured in a set of parents and in their offspring, to visualize the heritability of the trait. By analogy with this approach, in (**B**) the corneal curvature in a set of parents is plotted against the axial length of their offspring. Here, the relationship between *the two trait*s can be envisioned as a genetic correlation. (Note that (1) in practice, genetic correlations are not calculated in this manner, and (2) the data plotted here were generated for illustrative purposes only and thus do not represent true relationships).

TABLE 1. Details of the Animal Groups

Group	Genetic Composition	Treatment	Pretreatment Measurements	Posttreatment Measurements
WL Selective Breeding Population				
Age			4 days	8 days
Generation 1	Outbred (n = 232)	DSV	U	U, R
Generation 2	High line (n = 144) Low line (n = 123)	DSV	U	U, R
Generation 3	High line (n = 200) Low line (n = 192)	DSV	U, R, K	U, R, K
Advanced Intercross Line				
Age			19–21 days	
Generation 10	Intercross (n = 510)	None	U, U2, K, E, W	N/A

U, A-scan ultrasonography of major components (ACD, LT, VCD and AL); U2, A-scan ultrasonography of central corneal thickness (CCT); R, retinoscopy; K, keratometry; E, equatorial eye diameter measurement; W, eye weight measurement.

tween broiler and layer chickens was established, and their offspring were intercrossed for 10 generations. At 3 weeks of age, chicks from the 10th intercross generation were weighed, anesthetized, and examined by video keratometry and high-resolution A-scan ultrasonography. After the chicks were killed with a lethal overdose of pentobarbital sodium, the eyes were removed and cleaned of extraneous muscle and conjunctival and fatty tissue under a dissecting microscope. Equatorial diameter was measured with a custom-designed video camera system, and eye weight was measured with a digital balance.

In addition to the ultrasound measurements for the major ocular component dimensions, a further set of readings were taken on the AIL birds to measure central corneal thickness (CCT). These latter ultrasound waveforms were acquired within a 50% shorter time interval than for the whole-eye readings (10,000 samples acquired during a 10-μs window, with 50 waveforms averaged per acquisition), to provide higher resolution. Peaks corresponding to the front and back surfaces of the cornea were detected in real time using custom software, and CCT was computed assuming an ultrasound velocity[32] of 1534 m/s.

Calculation of the Degree of Relatedness

Heritability calculations require knowledge of the degree of relatedness between individuals in a study population. For the WL population, pedigree information was available, apart from those chickens in the first, outbred generation, who were assumed to be unrelated to one another (this assumption was deemed tenable, since the outbred chickens were sourced from a large WL breeding population). To enable the WL pedigree information to be collected during the selective breeding experiment, stable pairs of chickens were housed separately from other pairs, eggs were labeled when they were laid, and chicks were hatched singly in hatching boxes and tagged with numbered wing bands. The known pedigree structure was imported into the genetic analysis software as a pedigree file.

The degree of relatedness of AIL chicks was determined using a molecular genetics approach in preference to a pedigree-based approach, because of the complexity of the AIL pedigree. Specifically, we used genome-wide single-nucleotide polymorphism (SNP) genotyping to calculate kinship coefficients and identify familial relationships—as is commonly done in studies of animal populations in the wild and in human genome-wide association studies (to identify cryptic relatedness). DNA samples from the AIL were genotyped for a panel of 3061 SNPs distributed across the chicken genome with a custom assay (details available on request; GoldenGate; Illumina, San Diego, CA). Genotype data cleaning was performed with the GenABEL software package[33] for R. Of the total sample of 510 AIL chicks that were phenotyped and genotyped, one batch of 23 was excluded because of poor genotyping quality. Specifically, the average number of SNP genotypes called as "missing" was significantly higher for this batch than for the other batches (missing calls in batch 14 = 214.2; 95% CI, 139.9–288.5 versus missing calls in all other batches = 35.3; 95% CI, 28.5–42.1; $P < 0.001$). This was probably due to poor-quality DNA, since the DNA samples for each batch were extracted together. One additional chick from another batch was also excluded due to poor-quality genotyping, as were two additional chicks detected as having an unusually high level of heterozygosity. Two further chicks identified as duplicates (presumably due to a sample mix-up) were also removed, leaving a total of 482 AIL chicks available for analysis. Pairwise kinship coefficients were calculated using the ibs function of GenABEL. This approach utilizes *observed* levels of genetic sharing, and thus for extremely complex pedigrees such as the 10-generation AIL population, it is superior to methods based on the known pedigree, which rely on *expected* levels of allele sharing.[13,34] A jack-knife resampling assessment (not shown) indicated that the number of markers was sufficient for accurate kinship estimation. The AIL kinship matrix was modified in R so that it conformed to the format used by SOLAR (sequential oligogenic linkage analysis routines),[35] by converting kinship coefficients to phi2 coefficients (phi2 coefficient = 2 × kinship coefficient), setting the diagonals of the matrix to 1, and setting negative kinship values to 0 (as this indicated pairs of individuals that were less closely related than randomly selected individuals in the population[36]). The modified "phi2" file was imported into SOLAR using the "matcrc" and "loadkin" commands.

Statistical Analysis

Statistical analyses of the ocular traits were performed with commercial software (SPSS ver. 14.0; SPSS Inc., Chicago, IL). Outlier detection and removal proceeded as follows. First, using the finding that the bilateral ocular traits correlated highly in fellow eyes (range of Pearson correlation coefficients, 0.82–0.97; all $P < 0.001$), data points that fell outside the 99% CI of a fitted regression line in a scatter plot of trait values in right versus left eyes were set as missing values. Second, 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. For the WL population, this resulted in the removal of data for 1, 4, 5, 8, and 5 individuals for the traits, radius of corneal curvature, anterior chamber depth, lens thickness, vitreous chamber depth, and axial length, respectively. Similarly, in the AIL population, 7, 5, 6, 11, 8, 6, 7, and 3 individuals were removed for the traits, radius of corneal curvature, anterior chamber depth, lens thickness, vitreous chamber depth, axial length, corneal thickness, eye diameter, and eye weight, respectively. As well as these trait values that were deliberately excluded, a small number of chickens were missing phenotype information for certain traits (e.g., due to equipment failure). The final number of WL and AIL chicks used in the analysis of each trait is shown in Table 2, broken down by sex. All ocular traits were deemed to be normally

TABLE 2. Comparison of Ocular Traits between Male and Female Chickens

Ocular Trait	Sex	WL Population (4 Days Old)			AIL Population (3 Weeks Old)		
		n	Mean	SD	*n*	Mean	SD
Corneal curvature, mm	M	184	2.80	0.05	244	3.42	0.08
	F	190	2.77	0.05	231	3.36	0.08
Corneal thickness, mm	M	—	—	—	237	0.26	0.01
	F	—	—	—	231	0.24	0.01
Anterior chamber depth, mm	M	425	1.27	0.04	244	1.60	0.05
	F	462	1.25	0.03	227	1.58	0.04
Lens thickness, mm	M	424	1.83	0.03	244	2.38	0.05
	F	462	1.81	0.03	228	2.34	0.05
Vitreous chamber depth, mm	M	423	5.07	0.11	241	5.92	0.19
	F	460	4.96	0.11	228	5.82	0.19
Axial length, mm	M	423	8.16	0.13	243	9.91	0.21
	F	463	8.02	0.13	231	9.74	0.22
Eye diameter, mm	M	—	—	—	243	13.27	0.29
	F	—	—	—	231	13.02	0.29
Eye weight, g	M	—	—	—	246	0.86	0.05
	F	—	—	—	233	0.81	0.05

In each population, males and females had significantly different dimensions for all ocular traits ($P < 0.001$; *t*-test). Not all traits were measured in the WL chickens.

distributed (by the Kolmogorov-Smirnov test), and hence no transformations of the traits were made before heritability analysis.

Heritability Estimation

Univariate heritability estimates were obtained using variance components analysis (VCA) with SOLAR, version 4.2.7.[35] VCA uses the principle that the total phenotypic variance of a trait can be partitioned into an additive genetic component and an environmental component that includes nonadditive genetic effects, environmental factors and measurement errors. The (narrow sense) heritability (h^2) is estimated as the proportion of the total phenotypic variance of the trait due to the additive genetic component. SOLAR uses a maximum-likelihood method to estimate variance component parameters. Batch-to-batch variability (household effects) was taken into account using the "house" command, and sex was included as a covariate. *z*-Tests were used to assess whether there were significant differences in the heritability estimates of the same trait between different populations (AIL versus WL). The *z*-scores were calculated from the difference between the two heritability estimates divided by the square root of the sum of the squares of their standard errors.

Bivariate genetic analysis[37] was also performed with SOLAR. This partitions the total phenotypic correlation between two traits (ρ_P) into a genetic correlation (ρ_G) and an environmental correlation (ρ_E), as described by Lynch and Walsh[38]:

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

where h_1^2 and h_2^2 denote the heritability of traits 1 and 2, respectively.

Because the WL population was undergoing selection for a trait related to eye size and because untreated, 4-day-old, selectively bred chicks from the low-myopia-susceptibility line had slightly shorter axial lengths than their high-susceptibility-line counterparts,[30] we tested whether heritability estimates and genetic correlations differed between the high and low lines. However, after the two lines were analyzed separately, no between-line differences were detected. Hence, only the results for analyses of the whole WL population are reported below.

RESULTS

Descriptive Statistics and Familial Relatedness

Descriptive statistics for the ocular traits in the WL and AIL populations are presented in Table 2. All the ocular trait dimensions were found to be larger in males than in females ($P < 0.001$) for both the 4-day old WL chicks and the 3-week-old AIL chicks. Of the 891 WL chickens with phenotypic data, there were 695 related individuals (the other 196 were outbred birds phenotyped in generation 1 that were not used for breeding). The 695 related WL chickens could be assigned to one of two three-generation pedigrees, comprising the lines with high and low susceptibility to myopia, respectively. In total, there were 36 founders, 6,349 sibling pairs, 948 half-sib pairs, 8,530 cousin pairs, 1,318 parent-offspring pairs, 1,568 grandparent–individual pairs, and 10,778 avuncular pairs. Variance components analysis takes all these relationships into account in calculating heritability. All 482 chickens in the single generation of the AIL population included in the heritability calculations were potentially related to one another, because they were partially inbred. Genotype-inferred kinship coefficients suggested that 466 of the AIL chickens were full siblings, with >35 sibships in total.

Heritability Estimates

The ocular trait heritability estimates in the 4-day-old WL population were moderate to high for all the ocular traits (Table 3; range, 0.36–0.57; all significantly greater than zero; $P < 0.001$) and exceeded the heritability for body weight at this age (h^2 =

TABLE 3. Heritabilities of Ocular Traits and Body Weight in the AIL and WL Populations

Trait	WL Population (4 Days Old)	AIL Population (3 Weeks Old)
Corneal curvature, mm	0.48 (0.15)	0.50 (0.06)
Corneal thickness, mm	—	0.48 (0.07)
Anterior chamber depth, mm	0.41 (0.07)	0.36 (0.07)
Lens thickness, mm	0.36 (0.09)	0.57 (0.06)
Vitreous chamber depth, mm	0.57 (0.07)	0.61 (0.06)
Axial length, mm	0.52 (0.07)	0.52 (0.06)
Eye diameter, mm	—	0.41 (0.06)
Eye weight, mg	—	0.59 (0.06)
Body weight, g	0.33 (0.05)	0.29 (0.07)

Standard errors are shown in parentheses. All heritabilities were significantly greater than zero ($P < 0.001$). Not all traits were measured in the WL chickens.

0.33; $P < 0.001$). In the 3-week old AIL population, the heritability of ocular component dimensions were of a similar magnitude (Table 3) except for lens thickness, which had a heritability of 0.57 in the AIL (compared with only 0.36 in the WL chicks). However, the difference in heritability estimates of lens thickness between the two populations was not statistically significant ($P > 0.05$). The heritability of body weight was comparable to that reported previously,[39,40] providing further confidence in the ocular trait heritability estimates.

Pairwise Correlations between Ocular Traits

Pairwise correlations between the various ocular traits were similar in magnitude in the WL population (Table 4) and the AIL population.[31] In addition, all these pairwise correlations were statistically significant, except for the correlation between lens thickness and vitreous chamber depth (WL: $r = 0.06$, $P = 0.10$; AIL: $r < 0.01$, $P = 0.93$) and between lens thickness and anterior chamber depth in the AIL birds ($r = 0.07$, $P = 0.14$). The general trend was that ocular components were highly and positively correlated with one another, except that, as observed previously,[11,31] lens thickness was poorly correlated with the other ocular traits. Of interest, the correlation between corneal curvature and axial length was significantly higher in the AIL than in the WL chickens (AIL: $r = 0.91$, WL: $r = 0.68$; Fisher's z-score $= 10.01$, $P < 0.001$), suggestive of a possible change with age.

Genetic Correlations between Morphologic Traits

Significant pairwise genetic correlations were observed between corneal curvature, anterior chamber depth, vitreous chamber depth, and axial length in the WL chickens (Table 5A; range, 0.43–0.98; all $P < 0.01$). In the AIL birds, significant genetic correlations were seen for the traits corneal curvature, anterior chamber depth, vitreous chamber depth, axial length, eye diameter, and eye weight (Table 5B; range 0.54–0.99; all $P < 0.001$). In particular, the genetic correlations between corneal curvature, vitreous chamber depth, and axial length were strikingly high in both populations (range, 0.89–0.98; all $P < 0.001$). There was a statistically significant negative genetic correlation between anterior chamber depth and lens thickness in the WL chicks ($\rho_G = -0.57$, $P < 0.01$), but a nonsignificant correlation between these two traits in the AIL birds ($\rho_G = -0.15$, $P = 0.24$). In contrast, a statistically significant negative genetic correlation was observed between lens thickness and vitreous chamber depth in the AIL population ($\rho_G = -0.34$, $P < 0.01$), but not in the WL group ($\rho_G = 0.07$, $P = 0.65$). Apart from these results, nonsignificant genetic correlations were found when either lens thickness or corneal thickness was compared to any of the other traits (Table 5). In addition, eye diameter and eye weight (traits that were only measured in AIL chickens) were observed to have high genetic correlations with the other eye-size–related ocular traits (range, 0.71–0.99; all $P < 0.001$) but to correlate weakly with lens thickness and corneal thickness.

There were significant pairwise genetic correlations between body weight and the ocular traits corneal curvature, anterior chamber depth, vitreous chamber depth, and axial length in both the WL and AIL chickens (Table 5; range, 0.37–0.89; all $P < 0.01$). However, nonsignificant or very weak correlations were found between body weight and lens thickness or corneal thickness.

Thus, in summary, the natural variations in the traits that represent or govern eye size (i.e., corneal curvature, vitreous chamber depth, and axial length) are determined by a common set of genetic variants. A proportion (30%–60%) of this same group of genetic variants also determines the natural variation in body size.

Genetic Correlations between Eye Size and Myopia Susceptibility

Myopia susceptibility was assessed only in the WL population. As reported separately,[30] genetic factors were the major determinant of susceptibility to DSV. Approximately 50% of the variation in susceptibility to DSV was due to additive polygenic effects. In bivariate genetic analysis, the two related myopia susceptibility traits ΔRx (refractive error in the DSV-treated eye, relative to that in the control eye) and ΔAL (relative change in axial length between the DSV-treated eye and the control eye), as defined in Chen et al.,[30] had a high genetic correlation with each other ($\rho_G = -0.97$; $P < 0.001$). To test whether the same set of genetic variants control the natural variation in eye size and in susceptibility to DSV-induced myopia, genetic correlations were calculated for such pairs of traits (Table 6). However, none of the eye size traits measured before DSV treatment was genetically correlated with either ΔRx or ΔAL (all $\rho_G < 0.3$ [absolute value]; all $P > 0.05$). Moreover, there were no significant genetic correlations between body weight and myopia susceptibility (ΔRx and ΔAL; $\rho_G < 0.2$ [absolute value]; both $P > 0.05$).

Discussion

Heritability of Ocular Traits in Chickens

The moderate-to-high heritabilities observed in this study, especially those for corneal curvature, vitreous chamber depth, axial length, equatorial eye diameter, and eye weight, represent evidence of a major genetic contribution to the control of natural variation in chicken ocular component dimensions. Despite their different genetic backgrounds and the different

Table 4. Phenotypic Pairwise Pearson Correlations between Ocular Traits in the WL Population

	Anterior Chamber Depth	Lens Thickness	Vitreous Chamber Depth	Axial Length	Body Weight
Corneal curvature	0.42 $P < 0.001$	0.17 $P = 0.001$	0.64 $P < 0.001$	0.68 $P < 0.001$	0.45 $P < 0.001$
Anterior chamber depth		0.07 $P = 0.03$	0.48 $P < 0.001$	0.62 $P < 0.001$	0.46 $P < 0.001$
Lens thickness			0.06 $P = 0.10$	0.27 $P < 0.001$	0.21 $P < 0.001$
Vitreous chamber depth				0.95 $P < 0.001$	0.45 $P < 0.001$
Axial length					0.53 $P < 0.001$

Pairwise correlations between ocular traits in the AIL population can be found in a previous article.[31]

TABLE 5. Genetic Correlations between Pairs of Traits in the WL and AIL Populations

A. WL Population

Trait	Anterior Chamber Depth	Lens Thickness	Vitreous Chamber Depth	Axial Length	Body Weight
Corneal curvature	0.68 (0.17) $P < 0.01$	−0.11 (0.37) NSD	0.89 (0.07) $P < 0.001$	0.96 (0.04) $P < 0.001$	0.89 (0.10) $P < 0.001$
Anterior chamber depth	—	−0.57 (0.16) $P < 0.01$	0.43 (0.12) $P < 0.01$	0.44 (0.11) $P < 0.01$	0.37 (0.12) $P < 0.01$
Lens thickness	—	—	0.07 (0.16) NSD	0.10 (0.17) NSD	−0.10 (0.18) NSD
Vitreous chamber depth	—	—	—	0.98 (0.01) $P < 0.001$	0.43 (0.11) $P < 0.001$
Axial length	—	—	—	—	0.41 (0.10) $P < 0.001$

B. AIL Population

Trait	Anterior Chamber Depth	Lens Thickness	Vitreous Chamber Depth	Axial Length	Corneal Thickness	Eye Diameter	Eye Weight	Body Weight
Corneal curvature	0.54 (0.11) $P < 0.001$	−0.01 (0.12) NSD	0.92 (0.02) $P < 0.001$	0.95 (0.01) $P < 0.001$	0.91 (0.12) NSD	0.90 (0.03) $P < 0.001$	0.94 (0.02) $P < 0.001$	0.64 (0.10) $P < 0.001$
Anterior chamber depth	—	−0.15 (0.13) NSD	0.62 (0.10) $P < 0.001$	0.70 (0.08) $P < 0.001$	0.10 (0.14) NSD	0.78 (0.08) $P < 0.001$	0.71 (0.08) $P < 0.001$	0.49 (0.15) $P < 0.01$
Lens thickness	—	—	−0.34 (0.10) $P < 0.01$	−0.18 (0.11) NSD	−0.003 (0.12) NSD	−0.06 (0.12) NSD	−0.16 (0.11) NSD	0.01 (0.15) NSD
Vitreous chamber depth	—	—	—	0.96 (0.01) $P < 0.001$	0.13 (0.12) NSD	0.89 (0.03) $P < 0.001$	0.93 (0.02) $P < 0.001$	0.56 (0.11) $P < 0.001$
Axial length	—	—	—	—	0.21 (0.12) NSD	0.96 (0.02) $P < 0.001$	0.99 (0.01) $P < 0.001$	0.62 (0.10) $P < 0.001$
Corneal thickness	—	—	—	—	—	0.18 (0.12) NSD	0.19 (0.11) NSD	0.15 (0.15) NSD
Eye diameter	—	—	—	—	—	—	0.99 (0.01) $P < 0.001$	0.62 (0.10) $P < 0.001$
Eye weight	—	—	—	—	—	—	—	0.60 (0.09) $P < 0.001$

Standard errors are shown in parentheses. NSD indicates the correlation is not significantly different from zero.

ages at which they were phenotyped, heritability estimates were similar in the two chicken populations. Our findings are consistent with pedigree-based heritability estimates for ocular components in human subjects.[12–18] However, numerous environmental factors are known to be associated with—and potentially influence— ocular biometric traits as children grow up (e.g., socioeconomic status, level of education and level of outdoor activity),[41,42] whereas in laboratory animal studies, environmental factors can be controlled and such variations minimized. In this respect, studies in chickens offer an interesting alternative to those in mammals, because groups of unrelated chicks can be incubated and hatched together under standardized conditions and then reared in the absence of their own, or foster, parents. This limits the influence of intrauterine and maternal effects in chick studies, which otherwise serve as additional sources of familial resemblance.[43,44] An interesting exception to the general rule for ocular components to show similar heritabilities in chicks and in pedigree-based human studies, was central corneal thickness. In chicks, the heritability of CCT was ~0.48, whereas in human subjects, higher estimates of 0.71, 0.75, 0.68, and 0.6 to 0.7 have been obtained.[13,45,46] A potential explanation for this discrepancy is the relatively low-resolution method we used to measure corneal thickness (ultrasonography with a 20-MHz probe) compared to the methods used in the human studies. Thus, imprecise measurements may have resulted in an artificially low heritability of CCT in our chick sample, by virtue of measurement error being partitioned as a source of nongenetic variation (i.e., an environmental effect during the heritability analysis).

TABLE 6. Genetic Correlations between Eye Size Traits, Body Weight, and Myopia Susceptibility Traits

Trait	ΔAL	ΔRx
Corneal curvature	0.26 (0.15) NSD	−0.26 (0.15) NSD
Anterior chamber depth	0.15 (0.11) NSD	−0.10 (0.12) NSD
Lens thickness	−0.18 (0.12) NSD	0.14 (0.13) NSD
Vitreous chamber depth	0.18 (0.11) NSD	−0.14 (0.11) NSD
Axial length	0.15 (0.11) NSD	−0.11 (0.11) NSD
Body weight	0.16 (0.11) NSD	−0.13 (0.12) NSD
ΔAL	—	−0.97 (0.02) $P < 0.001$

Standard errors are shown in parentheses. NSD, correlation is not significantly different from zero.

Shared Genetic Determination of Ocular Traits

Our bivariate genetic analyses disclosed extremely high genetic correlations across the five traits corneal curvature, vitreous chamber depth, axial length, eye diameter, and eye weight (ρ_G range, 0.89–0.99). This result is indicative of a common source of genetic influence (pleiotropy). The reason for the much lower genetic correlation between corneal curvature and axial length in human subjects participating in the

Beaver Dam Eye Study[18] compared with those found here (ρ_G = 0.40 vs. 0.95–0.96) could reflect a species difference, but it is also likely to be influenced by the variable exposure to environmental sources of variation in refractive development mentioned above.[47] In complete contrast to the pleiotropic genetic variants that were found to control overall eye size in our chicken populations, small or nonsignificant genetic correlations were found when either corneal thickness or lens thickness were compared to all other ocular traits. Thus, even though these two traits are controlled in part by genetic variation (heritability, 0.36–0.57), the polymorphisms concerned appear to be distinct, in that they have only a minimal influence on the dimensions of the other ocular traits we measured.

For the ocular components that were assessed in both the AIL and WL populations, genetic correlations were comparable (the exceptions, as discussed below, being the lens thickness versus anterior chamber depth and lens thickness versus vitreous chamber depth relationships). These results suggest that the genetic co-regulation of the two traits with the strongest influence on refractive error, corneal curvature and axial length, is present both at an early stage (day 4) and a later stage (3 weeks) of chicken ocular development and thus that this shared genetic regulation of corneal and axial eye growth may be a consistent feature of eye maturation in the chicken. There was a significant negative genetic correlation between lens thickness and anterior chamber depth ($\rho_G = -0.57$; $P < 0.01$), but a nonsignificant genetic correlation between lens thickness and vitreous chamber depth ($\rho_G = 0.07$; $P = 0.65$) in the WL population. However, the significance of these two pairwise genetic correlations was reversed in the AIL population, with a significant genetic correlation between lens thickness and vitreous chamber depth ($\rho_G = -0.34$; $P < 0.01$), but a nonsignificant genetic correlation between lens thickness and anterior chamber depth ($\rho_G = -0.15$; $P = 0.24$). This could be related to the different strains used[47] (e.g., an influence of broiler genetic variants, which were present in the AIL but not the WL chickens) or the different ages studied (since separate groups of genetic variants could be operating at the earlier and later time points). Usually, negative genetic correlations are of special evolutionary interest, in that they suggest the action of selective pressure in different directions on the two traits concerned (i.e., if the magnitude of the first trait is increased, there is a selective advantage in the magnitude of the second trait being diminished). Here, however, a more mundane explanation is likely: In an eye with a relatively thins lens, either the depth of the anterior chamber (e.g., in the WL population) or vitreous chamber (e.g., in the AIL population) naturally tends to be deeper.

Our previous analysis of the AIL chickens showed that body size (specifically, head width, body length or body weight) predicted 45% to 49% of the variation in eye size,[11] in keeping with the significant correlations between body stature and ocular traits, such as vitreous chamber depth, axial length, and corneal curvature reported in several population-based studies in humans.[48–50] Here, the strong genetic correlations between body weight and eye size traits in both the AIL and WL populations extend these earlier observations by providing further evidence that this body size versus eye size association is driven by pleiotropy.

Thus, our findings suggest that eye size in chickens is governed by (1) a set of genetic variants that scale the majority of ocular component dimensions with body size, (2) a separate set of genetic variants that scale these same ocular component dimensions, independent of body size, but that still maintain coordinated scaling among the components themselves, (3) a third set of genetic variants that selectively scales the size of the lens (i.e., with little coordination between lens size and overall eye or body size), and (4) environmental influences (that from previous studies[7] are known to include a system of visual feedback that fine tunes and coordinates growth of the ocular components).

Lack of Shared Genetic Determination of Eye Size and Myopia Susceptibility

We found no evidence to support the theory that the genetic variants regulating normal eye size also determine susceptibility to environmentally induced myopia. This result was surprising, given some of the prior findings—namely, that in (1) some[51–53] but not all[54] longitudinal studies in humans, investigators have observed that axial length in nonmyopic children is a predictor of myopia development in later life; (2) the *HGF* gene, which was chosen for study based on its hypothesized role in regulating normal eye size,[21] has been reported to confer susceptibility to high myopia in humans[22–24]; and (3) evidence has been reported in a human twin study[27] that axial length and refractive error are determined in part by a shared set of genetic variants.

One potential explanation for points (1) and (2) is that *different genetic variants in the same genes* may regulate eye size and myopia susceptibility. For instance in the case of *HGF*, certain *HGF* polymorphisms may increase eye size in such a way that axial length and corneal curvature remain well balanced, to give rise to large, but nonmyopic eyes. Meanwhile, a separate set of *HGF* polymorphisms may influence susceptibility to high myopia—for instance, by producing, in response to visual or other cues, axial elongation that is not offset by balancing changes to the curvature of the cornea. If this type of situation were widespread, then eye size and myopia susceptibility would not show a significant genetic correlation. Point (3) is most likely related to the different experimental designs of our study and the twin study by Dirani et al.[27] Thus, whereas Dirani et al. found evidence for shared genetic determination of the "final" axial lengths of eyes and their "final" refractive error, we were interested in the relationship between *pretreatment* eye size and *susceptibility* to a change in refractive error. In this sense, the respective studies were investigating very different phenomena.

Several previous studies in animal models have explored research questions related to those that we investigated. First, in tree shrews, Siegwart and Norton[55] reported evidence that the eye has an intrinsically defined "preference" to attain and maintain a particular absolute size. The genetically orchestrated growth of the major ocular components that we observed suggests that genetic "hard-wiring" may facilitate this attainment of an appropriately proportioned globe. Second, Tepelus and Schaeffel[56] tested whether the precise set point of the emmetropization system in chicks, which varies subtly from bird to bird, is actively attained and maintained at its individual-specific level. Finding that, after a period of experimentally induced ametropia, chicks recovered to a level of refraction similar to their baseline level, the authors concluded that the refractive set point was indeed endogenously defined. As with the results of Siegwart and Norton,[55] this is indicative of a coordinated endpoint that the eye is striving to reach, and because individual chicks emmetropize to different set points, despite experience of the same visual environment, genetic involvement is an attractive explanation for the individual-specific effects.[56] Third, Saltarelli et al.[57] discovered that chicks subjected to two periods of form deprivation, with an intervening recovery period, developed similar degrees of myopia in each deprivation period. They concluded that chicks have an individual specific level of susceptibility to induced myopia, which is likely to be genetically determined. In similar experiments using sequential periods of lens wear in chicks, Tepelus and Schaeffel[56] found only borderline evidence for

such an effect. However, our own experiments with form deprivation[30] again highlighted genetics as the major determinant of susceptibility to myopia in chicks.

In conclusion, we found moderate to high heritability estimates for all ocular component dimensions in two independent populations of chickens. Furthermore, there was evidence of extremely tight genetic co-regulation of the five traits corneal curvature, vitreous chamber depth, axial length, eye diameter, and eye weight, which implies the involvement of a particular set of genetic variants in controlling overall eye size. In keeping with our prior findings, distinct sets of genetic variants appeared to control the natural variation in lens thickness (and similarly, corneal thickness). We found no evidence of shared genetic determination of ocular component dimensions and susceptibility to experimentally induced myopia.

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