Selective Breeding for Susceptibility to Myopia Reveals a Gene–Environment Interaction

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PURPOSE. To test whether the interanimal variability in susceptibility to visually induced myopia is genetically determined.

METHODS. Monocular deprivation of sharp vision (DSV) was induced in outbred White Leghorn chicks aged 4 days. After 4 days' DSV, myopia susceptibility was quantified by the relative changes in axial length and refraction. Chicks in the extreme tails of the distribution of susceptibility to DSV were kept and paired for breeding (high- and low-susceptibility lines). A second round of selection was then performed. The third generation of chicks, derived from the selected parents, was assessed after either monocular DSV (4 or 10 days) or lens wear.

RESULTS. After two rounds of selective breeding, the chicks from the high-susceptibility line developed approximately twice as much myopia in response to 4 days' DSV as did those from the low-susceptibility line ($P < 0.001$). All ocular component dimensions differed significantly ($P < 0.001$) between the two selected lines, both before treatment and in the responses of the treated eye. When DSV was conducted for 10 days, the relative changes in axial length and refractive error were still significantly different between the high and low lines ($P < 0.001$). The chicks bred for high or low susceptibility to DSV also showed significantly different responses to minus lens wear, but not to plus lens wear. Additive genetic effects explained ~50% of the interanimal variability in response to DSV.

CONCLUSIONS. Genes and environment interact to shape refractive development in chicks. (*Invest Ophthalmol Vis Sci.* 2011; 52:4003–4011) DOI:10.1167/iovs.10-7044

Myopia results from a mismatch between the optical power of the eye and its length, causing light to be focused in front of the retina and leading to blurred vision. It is one of the most prevalent eye disorders worldwide,[1,2] and high-degree myopia is a leading cause of untreatable loss of sight resulting from retinal and choroidal degeneration.[3–5] Studies in both humans and animals have demonstrated that the rate of postnatal eye growth is regulated by visual experience, whereas twin and family studies have shown that refractive errors are highly heritable.[6,7] Thus, myopia is largely considered a multifactorial, complex disorder.[8,9] Although recent genome-wide association studies for refractive error have successfully tagged several causal genetic variants,[10,11] the list of identified genetic and environmental risk factors is able to explain only a small proportion of the variance in refractive error in the populations studied.[7,12–14] Gene–environment interactions represent an attractive explanation for the missing heritability[15] and the seemingly paradoxical evidence supporting both strongly genetic and strongly environmental determination of refractive error. However, to date, the influence of gene–environment interactions in complex disorders such as myopia is almost entirely unknown.[16]

Myopia that occurs due to the deprivation of sharp vision (DSV; also known as form deprivation)[17] during early life has been found to occur in birds, fish, and mammals, including man.[18] DSV in the chicken has become a well-established animal model of myopia.[19,20] Interestingly, 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,[21–24] yet little is understood about the causes of this variability. Troilo et al.[21] found significant differences in both normal ocular development and the ocular response to visual deprivation between two strains of White Leghorn chickens and suggested that genetics may play a role in the visual control of eye growth. Saltarelli et al.[25] also indicated a possible effect of genetics in the susceptibility to DSV after finding a significant correlation between vitreous chamber elongation induced over two successive periods of treatment. Stimulated by these investigations using the chicken myopia model, we adopted a novel, selective breeding strategy to test directly the importance of gene–environment interactions in refractive development.

MATERIALS AND METHODS

All experimental procedures involving animals complied with the U.K. Home Office legislation and the European Communities Council Directive 86/609/EEC (1986) and were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

Deprivation of Sharp Vision: Treatment and Selective Breeding

The outbred White Leghorn chickens used in the initial round of selection (generation 1) were obtained from Lohmann Tierzucht GmBH (Cuxhaven, Germany) as fertilized eggs. The Lohmann Company has maintained this strain by random mating of a large breeding population undergoing selection for production traits and thus the population was expected to exhibit a high level of genetic diversity for eye traits. The eggs were hatched in batches of 20 to 30 chicks. For the first 4 days after hatching, the chicks were housed in a clear-sided, thermoregulated (25–27°C) Perspex brooder. After ocular measurements when the chicks were 4 days old, they were transferred to a wire-mesh/Perspex-sided floor pen with a suspended infrared heat lamp. Illumination in the brooder and floor pen was 250 to 300 lux with a 12:12-hour light/dark cycle. The chicks were fed commercial chick starter and given access to water ad libitum.

Myopia was induced in one eye of 4-day-old chicks by monocular DSV. Translucent diffusers were sutured to the skin around the orbit of one eye with monofilament nonabsorbable suture material (Ethilone 4-0; Ethicon, Johnson and Johnson Intl., Norderstedt, Germany) while the animal was under general anesthesia (produced by an intramuscular injection of ketamine 50 mg/kg and xylazine 3.5 mg/kg). Diffusers were removed after 4 days' DSV by removal of the sutures. Ocular measurements were performed before and after DSV to quantify the myopia susceptibility of each chicken (details of the ocular measurement procedures and myopia quantification calculations are given below). Please refer to Chen et al.[26] for a more detailed description of the procedures used. The sex of each chicken was determined via a PCR-restriction enzyme digest assay, using DNA extracted from a blood sample, as described previously[24] (an exception was for a small number of chickens from generation 1, which were kept until adulthood, in whom sex was apparent from secondary sexual characteristics).

From 232 outbred chickens treated in generation 1, 36 chickens were selected and retained for breeding. These comprised the nine male and nine female chicks with the highest level of susceptibility to DSV, which were paired together (high-line pairs), and the nine male and nine female chicks with the lowest level of susceptibility to DSV, which were paired together (low-line pairs). Because the experiment was performed using batches of chicks, as each new batch was assessed, any male chick in the pool of selected chicks with a less extreme phenotype than that of a newly phenotyped male chick was replaced by the chick with the more extreme phenotype. Similarly, any female chick in the selected pool was replaced if a female chick in a new batch was identified as having a more extreme phenotype, until all the batches had been examined. Because pedigree information was not available for the outbred chicks treated in generation 1, we assumed that these chicks were unrelated to one another.

Each pair of selected chickens was kept in a separate enclosure, and their eggs were labeled when collected (daily). Chicks (the F_1 generation, or generation 2) were hatched individually in hatching boxes and tagged with a wing band to allow their parentage to be ascertained. Chicks from the second generation were deprived of sharp vision using the same regimen as above.

A total of 267 F_1 chicks were assessed in generation 2 (144 chicks from the high line and 123 from the low line). Chicks from the high-

and low-susceptibility lines were hatched and raised together to ensure the uniformity of their environment. Since susceptibility to DSV is partially dependent on sex,[23,27] we sought to screen at least five male and five female offspring from each set of parents. Furthermore, to maximize genetic diversity and reduce inbreeding depression, we selected one male and one female from each set of parents. Within these caveats, the 18 F_1 chickens (nine male, nine female) with the highest degree of induced myopia from the high-susceptibility line parents were selected for breeding, as were the 16 F_1 chickens (eight male, eight female) with the lowest degree of induced myopia from the low-susceptibility 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 from the F_1 parents (the F_2 generation, or generation 3) were hatched as described above. A total of 392 F_2 chicks were assessed after 4 days' DSV in generation 3 (200 and 192 chicks from the high- and low-susceptibility lines, respectively). Table 1 lists the number of chicks used for this and the remaining experiments.

Ocular Measurements and Quantification of Myopia Susceptibility

Measurements were performed by examiners masked to the identity (i.e., high- or low-line status) of the animals. 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 before and after the period of DSV.[26,28] The refractive state (RX) of each eye was measured by using streak retinoscopy immediately after removal of the diffuser. In the third generation, the radius of corneal curvature (RCC) was measured with a custom-built infrared videokeratometer based on the design of Schaeffel and Howland.[29] Changes in ocular component dimensions resulting from DSV were compared between the treated and control eye of each chick. The relative changes in AL, VCD, and RX were chosen to quantify myopia susceptibility[26]:

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

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

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

$$\Delta RX = RX_T - RX_C$$

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

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

where,

ΔAL_T = AL in the treated eye after DSV − AL in the treated eye before DSV;

ΔAL_C = AL in the control eye after DSV − AL in the control eye before DSV;

TABLE 1. Number of Animals Used in Each Experimental Group

Generation	Line	DSV (4 Days Only/4 and 10 Days)	Lens Wear (Plano/Plus/Minus)	Untreated	AS-OCT (10 Days DSV)	OKN (Untreated)	Total
1	Outbred	232 (232/0)	0	0	0	0	232
2	High	144 (144/0)	0	0	0	0	144
2	Low	123 (123/0)	0	0	0	0	123
3	High	200 (167/33)	56 (15/20/21)	22	6	4	288
3	Low	192 (161/31)	52 (14/19/19)	22	6	3	275

DSV, deprivation of sharp vision; AS-OCT, anterior segment optical coherence tomography; OKN, optokinetic nystagmus testing.

and similarly for ΔVCD_T, ΔVCD_C, ΔLT_T, ΔLT_C, ΔACD_T, ΔACD_C, ΔRCC_T, and ΔRCC_C and where,

RX_T = RX in the treated eye after DSV;
RX_C = RX in the control eye after DSV.

Since ΔAL, ΔVCD, and ΔRX correlated highly, ΔAL was used as the primary indicator of myopia susceptibility when selecting chicks. However, for chicks in which ΔAL values were similar, those with the more extreme ΔVCD and ΔRX values were chosen (i.e., for high-susceptibility line chicks, those with higher ΔVCD and ΔRX values, and for low-susceptibility line chicks, those with lower ΔVCD and ΔRX values).

Deprivation of Sharp Vision for 10 Days

Sixty-four chicks from the third generation (33 and 31 from the high- and low-susceptibility lines, respectively) underwent monocular DSV for 10 days. Refractive error, corneal curvature and ocular component dimensions were measured before treatment when the chicks were 4 days old and then again after 4 and 10 days of DSV. An additional 44 chicks (22 from the high-susceptibility line and 22 from the low-susceptibility line) were randomly chosen to serve as untreated controls and measured alongside those undergoing the 10-day DSV treatment.

Visual Defocus by Lenses

Plano, +10-D plano-convex and −15-D plano-concave glass lenses (diameter, 12 mm) were fitted inside short (8 mm) lengths of soft, translucent silicone tubing (inner diameter, 12 mm), which were in turn each attached to a Velcro ring. A mated ring of Velcro was glued to the feathers around the eye to attach the lens. Fifty-six third-generation chicks from the high-susceptibility line and 52 third-generation chicks from the low-susceptibility line were randomly assigned to wear a plano, +10-D or −15-D lens. Refractive error, corneal curvature and ocular component dimensions were measured before and after 4 days of continuous lens wear, with treatment starting at 4 days of age, as for DSV. The lenses were cleaned twice daily.

Anterior Segment Imaging

Anterior segment imaging was performed on both eyes of a sample of third-generation chicks (six from the high-susceptibility line and six from the low-susceptibility line) after DSV treatment for 10 days, before ultrasound biometry. Images were obtained with a custom-built swept source optical coherence tomography (SS-OCT) system that used the fast dispersion encoded full-range (DEFR) algorithm,[30] to double the usable depth range. Details of the biometry system have been presented elsewhere.[31]

Assessment of Visual Function Using Optokinetic Responses

Optokinetic responses were tested on 7-day-old untreated chickens from the third generation. Details of the optokinetic nystagmus testing paradigm have been reported previously.[32,33] Briefly, seven randomly selected chickens (4 from the high-susceptibility line and three from the low-susceptibility line) were individually placed inside a large drum with printed stripes (1 cyc/deg, 28.5% contrast) on the inner wall. The rotating speed of the drum was 50 deg/s. The head movement of the chickens, elicited by the drifting grating, was recorded from above by a video camera and analyzed with custom-written software after digitization of the video frames. Visual function was quantified as the ratio of angular head speed to angular stripe speed. The OKN test was performed under both binocular and monocular viewing conditions. When monocular testing was performed (achieved by placing an opaque occluder over one eye) only responses in the temporal-to-nasal direction were recorded, because of the asymmetry of monocularly elicited OKN in chicks.[34] The relative performance (ratio of angular head speed to angular stripe speed) of chicks from the high- and low-susceptibility lines was tested with the Mann-Whitney U test.

Statistical Analysis

All data for the pretreatment ocular component dimensions and the posttreatment traits ΔVCD, ΔAL, and ΔRX were tested for normality with the Kolmogorov-Smirnov test. Due to a non-normal distribution of ΔRX, differences between the high- and low-susceptibility lines were compared by using the Mann-Whitney U test. Comparisons of the ocular component dimensions and their DSV-induced changes between the two selected lines were made using an independent-samples *t*-test (SPSS ver. 14.0; SPSS Inc., Chicago, IL).

Heritability Estimates for Susceptibility to Deprivation of Sharp Vision

For each of the traits of interest (ΔRX, ΔAL, and ΔVCD), outliers, defined as the trait values beyond three standard deviations from the mean,[35] were identified and removed. Outlier removal is common practice in quantitative genetics, since extreme values are potentially informative, but have a high risk of being the result of a measurement error.[36] The removal of falsely identified outliers (extreme trait values that are not due to measurement errors) causes a small, downward bias in heritability estimation, such that the result becomes conservative. However, failure to remove true outliers (extreme values resulting from measurement errors) can dramatically bias heritability estimates, giving very misleading results.[36,37] The number of outliers removed was 2, 3, and 4 for the traits ΔRX, ΔAL, and ΔVCD, respectively (i.e., <0.5% of the total sample for all three traits). Next, the frequency distributions of all ocular traits were tested for normality by the Kolmogorov-Smirnov test. Only the trait ΔRX was non-normally distributed. Therefore, a normal quantile transformation of ΔRX was made, as described previously.[38,39] (This method assigns a normal deviate value to each subject according to its phenotypic rank.) Heritability estimates for susceptibility to DSV were calculated by using variance components analysis with the software package Sequential Oligogenic Linkage Analysis Routines (SOLAR, version 4.2.7).[40] Here, the total phenotypic variance of the trait is partitioned into an additive genetic component and an environmental component that includes the nonadditive genetic component, environmental factors and measurement errors. The narrow sense heritability (h^2) was estimated as the proportion of total phenotypic variance of the trait due to additive genetic effects. SOLAR uses maximum likelihood to optimize the model parameters. Batch-to-batch variability was taken into account using the house (household effects) command, and sex was included as a covariate.

RESULTS

Myopia Susceptibility in Selectively Bred Chicks

The frequency distribution of the degree of induced myopia (ΔRX) in the first generation of chickens was approximately Gaussian, with the chicks ($n = 232$) developing -13.42 ± 3.16 D of myopia (mean $\pm$ SD). The animals in the tails of the distribution were retained for breeding, and the selection process was repeated (Fig. 1A; Tables 2, 3). After two rounds of selection, the animals in the third generation showed a clear divergence in their susceptibility to DSV-induced myopia (Figs. 1B–G). The chickens in this generation with parents that had been selected for high susceptibility to myopia developed an average of -15.27 ± 3.47 D, whereas those with parents selected for low susceptibility developed only -6.88 ± 3.35 D ($n = 392$; $P < 0.001$; Fig. 2A). There were similarly significant differences between the two lines in the extent to which each of the treated eye's ocular components changed relative to the untreated fellow (control) eye in response to DSV (Figs. 2C–F; Tables 2, 3).

Remarkably, refractive error and ocular component dimensions were also found to differ significantly between the high- and low-susceptibility line chicks in the third generation, even before treatment was initiated (Table 4). At 4 days of age, the
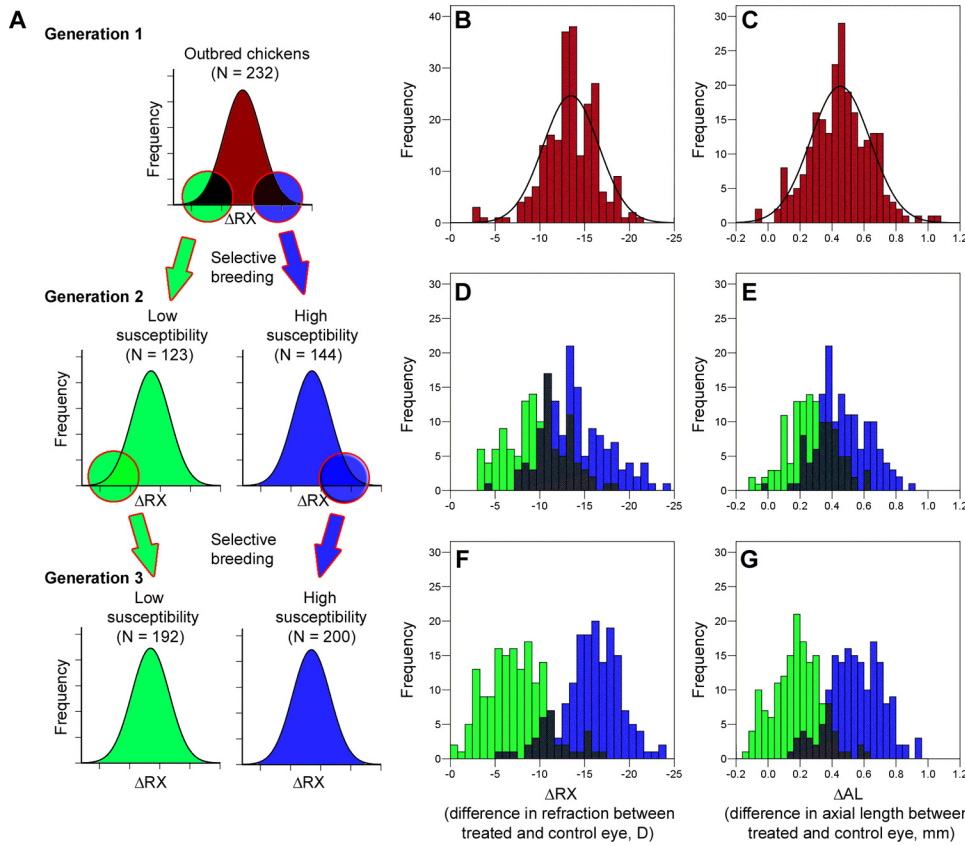
FIGURE 1. Selective breeding for myopia susceptibility. Myopia was induced in one eye of outbred chickens (generation 1) by monocular deprivation of sharp vision, and individuals from the tails of the induced-myopia frequency distribution were retained for breeding. Offspring (generation 2) from the parents with the highest myopia susceptibility (high line) and the lowest myopia susceptibility (low line) were hatched and raised together, and subjected to monocular DSV treatment. The degree of induced myopia was assessed in the generation 2 chicks, and again, the most and least susceptible chicks were retained for breeding a third generation. The degree of induced myopia was assessed in the generation 3 chicks, with observers again being masked to the high- or low-line status of the animals. (**A**) The breeding scheme. *n*, number of animals. (**B**, **D**, **F**) Distribution of degree of induced myopia (ΔRX) in generations 1, 2, and 3, respectively. (**C**, **E**, **G**) Distribution of degree of induced axial eye growth (ΔAL) in generations 1, 2, and 3, respectively. (**B–G**) *Red*, outbred; *green*, low line; *blue*, high line; *black*, high/low overlap.

chicks from the low-susceptibility line were an average of +0.21 D more hyperopic than their high-susceptibility line counterparts (+4.43 vs. +4.22 D, respectively, *P* < 0.001) and had shorter eyes (8.05 vs. 8.14 mm, respectively, *P* < 0.001).

Assessment of Visual Function Using Optokinetic Responses

Since DSV-induced changes in eye growth are vision dependent, visual function in chickens from the high- and low-susceptibility lines were tested using an optokinetic nystagmus

(OKN) testing paradigm to examine whether a generalized visual deficit in the chickens from the low lines could explain the divergence in myopia susceptibility. After the optokinetic response of the chickens in the third generation was tested, no significant difference in visual function was observed between the high and low lines (Fig. 2F). The 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-susceptibility lines, respec-

TABLE 2. Refraction and Ocular Component Dimensions in Chicks after Monocular DSV for 4 Days

	Round R1 (*n* = 232)	Round R2 (*n* = 267: L = 123, H = 144)			Round R3 (*n* = 392: L = 192, H = 200)		
		High	**Low**	**P**	**High**	**Low**	**P**
Treated eyes							
RX	−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*	—	—	—		2.92 ± 0.05	2.93 ± 0.06	0.045
ACD	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	1.96 ± 0.05	1.94 ± 0.04	1.95 ± 0.04	0.035	1.98 ± 0.04	1.97 ± 0.04	< 0.001
VCD	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	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							
RX	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*	—	—	—		2.92 ± 0.05	2.92 ± 0.06	0.777
ACD	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	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	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	8.42 ± 0.18	8.41 ± 0.18	8.40 ± 0.16	0.617	8.47 ± 0.19	8.41 ± 0.16	0.001

Data are expressed as the mean ± SD. For ocular component dimensions, *P* relates to the difference in trait means between high- and low-line chicks, by *t*-test. For refraction, *P* relates to the difference between the high- and low-line chicks, by Mann-Whitney U test. RX, refraction; RCC, radius of corneal curvature; ACD, anterior chamber depth; LT, length thickness; VCD, vitreous chamber depth; AL, axial length.

* Measured in generation 3 only (*n* = 178: L = 84, H = 94).

TABLE 3. Changes in Refraction and Ocular Component Dimensions after 4 Days of DSV

	Generation 1 (*n* = 232)		Generation 2 (*n* = 267: L = 123, H = 144)			Generation 3 (*n* = 392: L = 192, H = 200)	
ΔRCC*	—		—		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* = 0.101	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	

Data are the mean ± SD change in treated eye minus control eye. H, high line; L, low line; other abbreviations and probabilities are as described in Table 2.

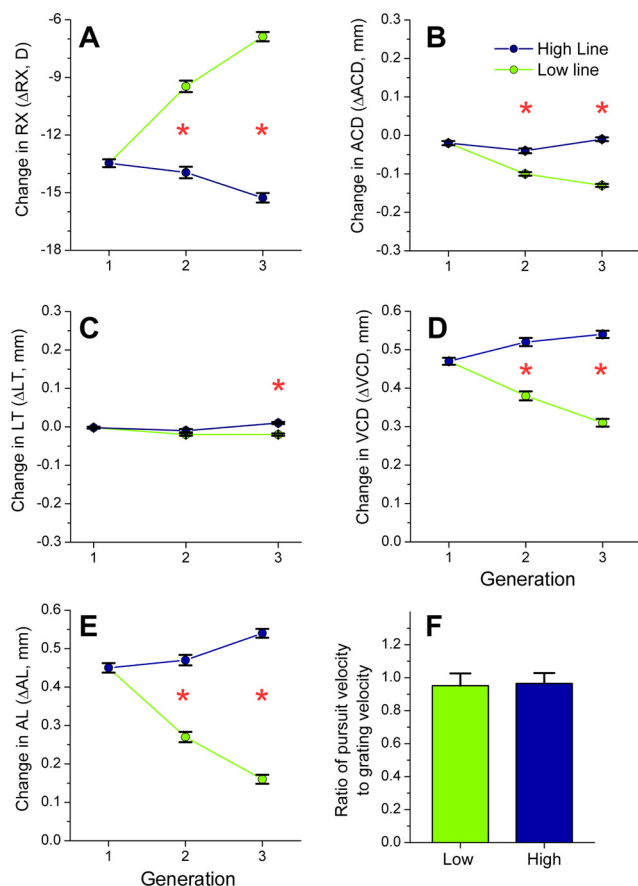* Measured in generation 3 only (both pre- and posttreatment data available for *n* = 178: L = 84, H = 94).

FIGURE 2. (**A–E**) Changes in refraction and ocular component dimensions induced by 4 days' DSV (treated eye minus control eye) in high- and low-line chicks. Error bars, SE. *Significant difference between the high and low lines (*P* < 0.001). (**F**) Optokinetic head pursuit responses in untreated high- and low-line chicks in the third generation. Visual function in chicks aged 7 days 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 cyc/deg, where a ratio of 1.0 corresponds to normal performance and a ratio of 0 corresponds to no visual function. Error bars show SE.

tively (mean ± SE; 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 vs. 0.88 ± 0.11, Mann-Whitney U test; *P* = 0.86).

Deprivation of Sharp Vision for 10 Days

In investigating whether the divergence in myopia susceptibility was due to a delayed response to DSV in the chicks from the low-susceptibility line, we deprived chicks from the high- and low-susceptibility lines of sharp vision monocularly, starting at 4 days of age, as previously, but for a period of 10 days. Refractive error, corneal curvature, and ocular component dimensions were measured before treatment and after 4 (Table 4) and 10 (Tables 5, 6) days of DSV. The chicks from the high-susceptibility line developed an average of −12.88 D of myopia after the first 4 days' DSV 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 and 0.11 mm over the first 4 and the subsequent 6 days, respectively. The animals from the low-susceptibility line developed an average of −5.21 and −7.61 D of myopia after 4 and 10 days of DSV, and showed an average daily axial length increase (treated eye relative to control eye) of 0.02 and 0.05 mm, respectively (Figs. 3A, 3C). The continued slower rate of axial elongation and myopia development in the later 6-day period of treatment in the low-line chicks, relative to those in the high line, ruled out our having selected chicks on the basis of the maturity of their visually guided growth regulation system.

Interestingly, while developing less vitreous chamber elongation than chicks from the high-susceptibility line, the chicks from the low-susceptibility line showed a significant DSV-induced decrease in their rate of anterior chamber deepening over the treatment period, combined with an increased rate of corneal flattening. This effect was particularly pronounced after 10 days of DSV (Figs. 3B, 3D; Tables 5, 6).

Visual Defocus by Lenses

Chicks from the high- and low-susceptibility lines were treated with a plano, +10-D, or −15-D lens over one eye for 4 days. The degree of refractive compensation for minus lenses differed significantly between the high and low lines. However, this was not the case with plus or plano lens wear (Fig. 4A). Specifically, minus lens wear induced −11.14 and −4.80 D of myopia in the high and low lines, respectively (*P* < 0.001). Plus lens wear induced +6.90 and +8.13 D of hyperopia in the high and low lines, respectively (*P* = 0.093). Whereas the

TABLE 4. Refraction and Ocular Component Dimensions in 4-Day-Old Chicks before DSV Treatment

	Generation 1 (n = 232)		Generation 2 (n = 267: L = 123, H = 144)			Generation 3 (n = 544: L = 266, H = 278)	
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 ± 0.04	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	1.834 ± 0.032	P = 0.010
		H	1.80 ± 0.03		H	1.828 ± 0.030	
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	5.04 ± 0.13	
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	8.14 ± 0.15	
RX, D†	—		—		L	4.43 ± 0.75	P < 0.001
					H	4.22 ± 0.66	

Data are expressed as the mean ± SD. Measurements in the right and left eyes of each chick were averaged. Probabilities and abbreviations are as described in Table 2.

* Measured in generation 3 only (pretreatment data available for n =226: L = 104, H = 122).

† Measured only in generation 3, before treatment (n = 216: L = 105, H = 111).

frequency distributions of ΔRX in the two selected lines were largely overlapping after plus lens wear, there was an obvious divergence in the distributions after minus lens wear (Fig. 4B).

Magnitude of the Genetic Influence toward Myopia Susceptibility

A variance components analysis of all the chickens used in the selective breeding experiment yielded heritability estimates for susceptibility to DSV of 0.51 ± 0.05, 0.46 ± 0.04, and 0.38 ± 0.04 (mean ± SE) when quantified using the traits ΔRX, ΔAL, and ΔVCD, respectively (all $P < 0.001$). These results demonstrate that susceptibility to a completely environmental cause of myopia was strongly dependent on genetic background in these chickens.

DISCUSSION

Substantial variations have been observed in the degree of myopia induced by a uniform regimen of DSV in outbred White Leghorn chickens.[21-24] After selective breeding for high and low susceptibility to DSV, the degree of myopia induced by

DSV showed a highly significant difference between the high- and low-susceptibility lines, as did the DSV-induced changes in ocular component dimensions.

Plausible explanations for this divergence in susceptibility to DSV between the selected lines would be (1) a genetic effect, (2) the inheritance of an allele or alleles causing generalized visual disability in the low-line chicks because of the vision dependency of DSV-induced myopia, and (3) a relative immaturity of the pathway of vision-dependent regulatory eye growth in the low-susceptibility line. 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 generalized visual deficit as the cause of the divergence in susceptibility to DSV. Furthermore, there were significant differences in the relative changes in ocular component dimensions and induced myopia between the chickens from the high- and low-lines treated with monocular DSV over a longer deprivation 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-susceptibility line chicks, relative to those in the high-susceptibility line, ruled

TABLE 5. Refraction and Ocular Component Dimensions in Third-Generation Chicks Monocularly Deprived of Sharp Vision for 10 Days and in the Right Eyes of Untreated Chicks Observed during the Same Period

	Untreated Right Eyes			Treated Eyes			Control Eyes		
	High (n = 22)	Low (n = 22)	P	High (n = 33)	Low (n = 31)	P	High (n = 33)	Low (n = 31)	P
After 4 Days' DSV									
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	2.94 ± 0.07	0.337	2.92 ± 0.05	2.93 ± 0.06	0.582
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 10 Days' DSV									
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	0.411
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

Data are expressed as the mean ± SD. Probabilities and abbreviations are as described in Table 2.

TABLE 6. Relative Changes in Ocular Component Dimensions and Refraction in Third-Generation Chicks during Monocular DSV for 10 Days

		Changes in the First 4 Days	P	Changes in the Latter 6 Days	P	Changes in 10 Days	P
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	
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	

Data are expressed as the mean ± SD change in treated eye minus control eye. For high line, $n = 33$; for low line $n = 31$. For ΔRCC, ΔVCD, and ΔAL, P relates to the difference in trait means between high line and low line chicks, by t-test. For ΔACD, ΔLT, and ΔRX, P relates to the difference between the high- and low-line chicks, by Mann-Whitney U test. L, low-susceptibility line; H, high-susceptibility line; other abbreviations are as described in Table 2.

out our having selected the chicks on the basis of the maturity of their visually guided growth regulation system. Instead, the results are consistent with the idea that the chicks were selected dependent on the gain[41] of their eye growth regulatory system. Therefore, the evident divergence in the susceptibility to DSV in the three generations of selective breeding provided evidence of a strong genetic component in the development of environmentally induced myopia. Indeed, genetic effects explained ∼50% of the variation in susceptibility to DSV in this population of White Leghorn chickens. The most likely molecular determinants underlying this genetic effect are multiple DNA polymorphisms, each having a small individual effect[42] (although, conceivably, epigenetic changes could also contribute to this heritable variation in response to DSV[43]).

The myopia susceptibility (ΔRX) of both lines of the selected chicks diverged away from that of the outbred starting population (both directions, $P < 0.001$). However, as seen strikingly in Figure 2, the low-susceptibility line responded to a much greater extent than the high-susceptibility line. Thus, comparing the chicks in generation 3 against those in the starting population (generation 1), birds in the low line developed, on average, 49% less induced myopia, whereas the chicks from the high line developed just 14% more. Because the stringency of selection was the same for the two lines, and the frequency distribution of ΔRX in generation 1 was not markedly skewed, this asymmetric divergence is likely to represent a biologically driven phenomenon rather than a statistically driven one. According to quantitative genetics theory, several interrelated factors can create an asymmetry in the response to selection, including directional dominance (for instance, a tendency for the majority of alleles that confer low myopia susceptibility to be recessive) and directional gene frequencies (for instance, a tendency for the majority of the alleles conferring low myopia susceptibility to be at a relatively low frequency in the population).[44] However, these causes are rarely evident after just two generations of selective breeding. Hence, we speculate that the principal reason for the reduced divergence of the high-susceptibility line is that the rate of myopic eye growth is limited to a finite, maximum level.

The myopia induced by DSV is primarily brought about by an increase in vitreous chamber depth.[45,46] Interestingly, however, the chicks from the low-susceptibility line showed significant anterior and posterior segment changes over the treatment period. Thus, at the same time that they were developing less vitreous chamber elongation than chicks from the high line, treated eyes in chicks from the low line developed unusually flat corneas and unusually shallow anterior chambers. These effects were particularly pronounced after DSV for 10 days (Fig. 3D). As a result of this relatively slow rate of anterior chamber deepening and faster rate of corneal flattening, we calculated[47,48] the equivalent refractive power of the combined cornea and crystalline lens to be approximately +153 D in the treated eyes of 14-day-old low-line chicks, compared with +156 D in the untreated, normal eyes of chicks from this line at this age (assuming a total lens power of +61.83 D and a first principal point distance for the lens of 0.93 mm, for 15 day old chicks[48]). Thus, the anterior segment changes in the treated eyes of low-line chicks also served to make them less myopic, augmenting the effect of slow vitreous chamber elongation. The most straightforward explanation for this apparent
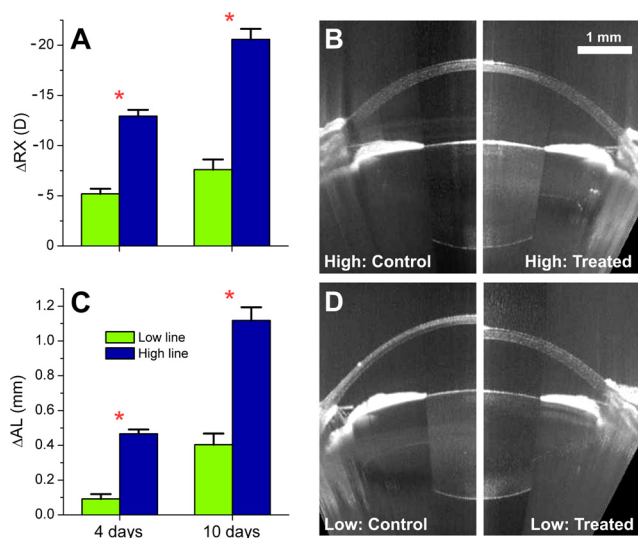

FIGURE 3. (**A**, **C**) Changes in refraction (ΔRX) and axial length (ΔAL) in high- and low-line chicks in the third generation after 4 and 10 days of DSV. Bars show change in the treated eye minus the change in the control eye over the treatment period, error bars, SE. *Significant difference between the high and low lines ($P < 0.001$). (**B**, **D**) Representative in vivo anterior segment images of the treated and control eyes of chicks from the (**B**) high and (**D**) low lines after 10 days of DSV.
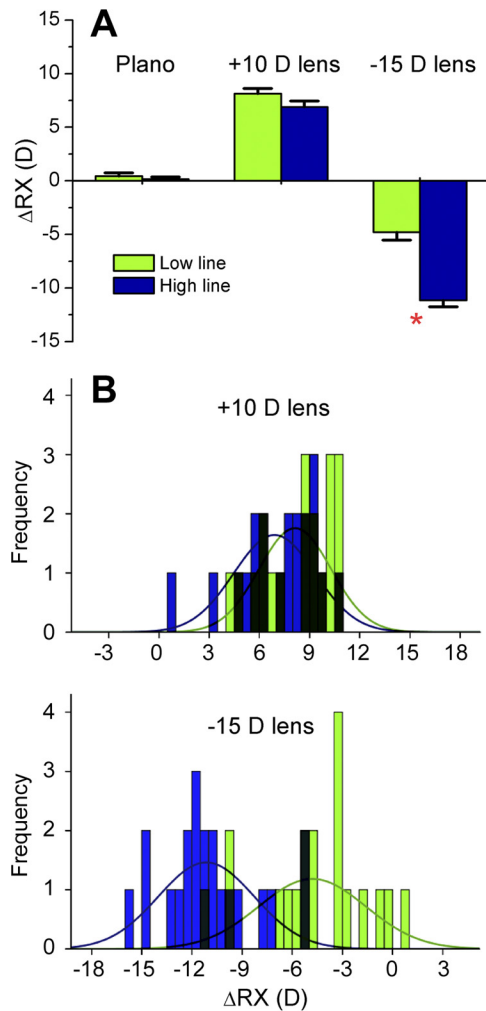
FIGURE 4. Change in refraction (ΔRX) induced by 4 days of monocular lens wear in high- and low-line chicks in the third generation. (**A**) Change in refraction after plano, +10-D, and −15-D lens wear in the high and low lines. Error bars, SE. *Significant difference between the high and low lines (*P* < 0.001). (**B**) Frequency distribution of the change in refraction after +10-D or −15D lens wear in the high and low lines. *Black*: high/low overlap.

coupling of influences on the rate of growth of both the anterior and posterior segments of the eye is that separate groups of alleles, some regulating the response of the anterior segment to DSV and other regulating the response of the posterior segment, were subject to selection. However, an alternative, albeit speculative, explanation is that alleles with pleiotropic effects on the growth of both the anterior and posterior segments were subject to selection (in support of the latter idea, we recently observed pleiotropic genetic effects in the control of axial length and corneal curvature in normally developing chicks[49]).

Chicks raised wearing a spectacle lens in front of one eye are able to modify their refractive development in a sign of defocus-dependent manner.[50] When the chicks from the high and low lines were fitted with a plano, +10-D, or −15-D lens over one eye, the degree of refractive compensation for minus lenses differed significantly between the two selected lines, as was the case for DSV (Fig. 4). However, this was not the case with plus (or plano) lens wear. These findings suggest that the regulatory systems responsible for DSV-induced changes in refractive development and minus lens-induced changes in refractive development have one or more molecular compo-

nents in common. However, this component is not part of the visually guided regulatory system responsible for compensation for the blur caused by plus lenses. Although previous studies[51-54] have reported similarities in the responses to minus lenses and diffusers, an important aspect of the similarity found here is that it can be more directly attributed to a shared *causal* mechanism (but note that we cannot argue this point with certainty, since it is possible that the −15-D lenses we used were beyond the range for which chicks of this age could emmetropize, and thus the minus lenses may have produced form deprivation rather than simply hyperopic defocus). Our results support previous work in demonstrating that the molecular pathways underlying emmetropization in response to plus lens wear and DSV must be distinct to at least some extent. More important, however, the difference in susceptibility to myopia induced by minus lens wear in the selected chicken lines suggests that a single genetic gain control system may determine a chick's susceptibility to all types of visually induced myopic eye growth, and thus have crucial relevance to human myopia.

In conclusion, a selective breeding experiment demonstrated that susceptibility to environmentally induced myopia in chickens is strongly genetic in origin. If gene–environment interactions of this kind are also important in human myopia, then projects seeking to identify myopia-predisposing gene variants will benefit by considering genetic and lifestyle risk factors jointly.

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