Effect of Long-Term Selection for Non-Destructive Deformation on Egg Shape in White Leghorns

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Several conventional traits, including eggshell thickness, are commonly being improved genetically as a means to increase eggshell strength. At the same time, researchers have come to recognize that factors related to egg geometry, such as egg shape, are important determinants of the variability remaining in eggshell strength, after conventional traits have been considered. Therefore, given that the value of the egg shape index –the egg's width to length ratio– depends highly on the hen strain, it is necessary to examine the relationship between eggshell strength and shape index more closely in a variety of breeds. From this perspective, by using REML methodology under a five-trait animal model, we analyzed a two-way selection experiment for non-destructive eggshell deformation in 31 generations of White Leghorns, to evaluate the effect of selection for eggshell strength on egg shape. In the strong line, which refers to the line that was selected for decreased non-destructive deformation value, the genetic correlation between eggshell breaking strength and shape index was 0.285 ± 0.055, whereas that between non-destructive deformation and shape index was -0.021 ± 0.063. In the weak line, these values were 0.244 ± 0.055 and -0.093 ± 0.060, respectively. The heritability estimates were 0.381 ± 0.033 for non-destructive deformation, 0.349 ± 0.029 for eggshell breaking strength, and 0.544 ± 0.027 for shape index in the strong line, and 0.408 ± 0.031, 0.468 ± 0.032, and 0.484 ± 0.028, respectively, in the weak line. The genetic correlation between eggshell breaking strength and shape index suggests that rounder eggs are somewhat more resistant to breakage than more elongated eggs. The moderately high heritability estimates for shape index indicate the potential to improve egg shape through genetic gain.

Key words: egg shape, long-term selection, non-destructive deformation, shape index, White Leghorn

Introduction

Poor eggshell quality is a recurring problem in the poultry industry. It results in substantial economic losses (Nys *et al*., 2011) and poses a potential threat to food safety (Bain *et al*., 2006). For this reason, decades of research have been devoted to reducing shell breakage; this has been achieved in large part by improving eggshell thickness and eggshell breaking strength, two traits generally believed to be good indicators of eggshell strength (Tyler and Geake, 1961; Mertens *et al*., 2006).

However, it is often pointed out that other structural properties, which are also essential indicators of eggshell quality, must be taken into account (De Ketelaere *et al*., 2003; Bain, 2005; Yan *et al*., 2014). One example is egg

shape, a trait that is typically measured by using the egg shape index, as defined by the egg width to length ratio (Nedomová *et al*., 2009). One of the reasons why the shape index is used is that factors such as eggshell thickness, specific gravity, shell percentage, and egg weight only partly explain eggshell strength (Frank *et al*., 1964); factors related to egg geometry, including the shape index, help account for a substantial part of the remaining variability (Richards and Swanson, 1965). Several recent studies have also confirmed that eggshell strength is highly dependent on shape index (Anderson *et al*., 2004; Altuntaş and Şekeroğlu, 2008).

In parallel with this, the literature also indicates that egg shape is dependent on strain (Monira *et al*., 2003), implying that more research needs to be conducted in a variety of breeds to better evaluate the relationship between eggshell strength and shape index. Here, we therefore examined how long-term selection for a single eggshell trait –non-destructive deformation– affected shape index in a population of White Leghorns.

Materials and Methods

Experiment

The experiment analyzed in this study was conducted at the Institute of Livestock and Grassland Science (Tsukuba, Japan) in accordance with Japan's Act on Welfare and Management of Animals. We applied a divergent selection procedure based on eggshell strength to 31 generations of White Leghorns. To properly assess the effect of selection for eggshell strength on egg shape, a single trait, non-destructive deformation, was used for selection instead of introducing a selection index. The two lines created through the selection process are therefore based on low and high non-destructive deformation values; in this paper, they are hereafter referred to as the strong line and the weak line, respectively. For technical reasons, however, eggshell breaking strength was used as a selection criterion instead of non-destructive deformation for generation 2. No substantial impact is however expected from this inconsistency, given the number of generations and the high correlation between non-destructive deformation and eggshell breaking strength.

Selection Methods

From generation 1 to generation 13, selection was based on individual performance; 80 females and 10 males were selected per generation. Although female data only was used for the model, the full-sib mean was used for male selection given that they did not have their own records. From generation 14 to generation 17, to prevent the coefficient of inbreeding from increasing, a within-family selection procedure was used, and from generation 18 onward, individuals were randomly chosen and mated within their lines, which were both maintained until generation 31.

In total, the strong line consisted of 6519 records (210 records per generation on average), whereas the weak line consisted of 5903 (190 records per generation on average). Each record was calculated by taking the mean of three measurements, performed on three different eggs within 24 h after laying, for each female between 36 and 38 weeks of age.

Model Parameters

Apart from the error term, five random effects were included in the model: non-destructive deformation (in μ m/kg), eggshell breaking strength (in kg), egg width (in cm), egg length (in cm), and shape index (as a percentage). Non-destructive deformation was measured for a standard load of 1kg, applied on the minor axis. The same axis was also used to measure the shell's breaking strength. In addition to these five (random) animal effects, the model also included one fixed effect, the generation effect.

Statistical Analysis

The breeding values were calculated by using best linear unbiased prediction (BLUP) methodology with a multivariate animal model (Henderson, 1975; Henderson and Quaas, 1976). The restricted maximum likelihood (REML) approach (Patterson and Thompson, 1971) was used to estimate genetic parameters and variance components. The breeding values and genetic parameters were estimated under the R environment (R Core Team, 2014), by using ASReml-R (version 3.0) (Butler et al., 2009).

Results and Discussion

Phenotypic Value Analysis

To better understand how selection for eggshell strength affected the shape index, as a first step we examined the effect of selection for non-destructive deformation on the length of each egg axis. We plotted the evolution of the generation mean of egg width and egg length in the weak and strong lines over the course of the experiment (Fig. 1). The size of each axis decreased visibly in both lines over the generations ($P<0.01$), indicating a reduction in egg size in both lines.

The most likely explanation for this decrease in egg size in both lines lies in the fact that the base population used in this study stemmed from a strain of White Leghorns that was originally bred for commercial purposes, and was thus selected for a variety of traits, including large egg size. Given that in this research selection was based on eggshell strength only rather than on a selection index, which would have included other traits such as egg size or egg weight, it is therefore probable that, through natural selection, this experiment allowed the generation mean to gradually move
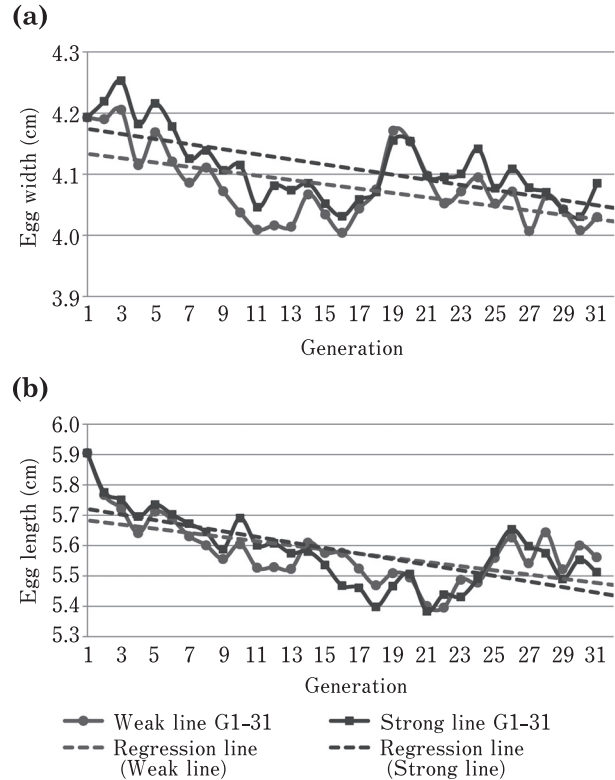
(a)

(b)

Fig. 1. **(a) Change over time of the generation mean of egg width in the weak and strong lines. (b) Change over time of the generation mean of egg length in the weak and strong lines.**

Table 1. **Phenotypic correlation between shape index and traits related to eggshell strength**

Gen	Strong line		Weak line	
	NDD	BS	NDD	BS
1	N/A	0.153	N/A	0.153
2	−0.036	0.237	−0.005	0.141
3	−0.105	0.304	−0.083	0.214
4	−0.110	0.331	0.083	0.221
5	−0.094	0.270	−0.039	0.307
6	−0.097	0.248	−0.122	0.304
7	0.076	0.256	−0.126	0.326
8	0.073	0.269	−0.075	0.272
9	−0.042	0.372	−0.208	0.343
10	−0.024	0.266	0.045	0.212
11	−0.107	0.346	−0.049	0.305
12	−0.073	0.363	−0.049	0.266
13	−0.253	0.429	0.061	0.140
14	0.053	0.283	−0.182	0.330
15	−0.131	0.386	−0.035	0.230
16	−0.098	0.273	−0.170	0.168
17	−0.040	0.166	−0.128	0.238
18	0.032	0.136	−0.081	0.297
19	−0.082	0.228	−0.166	0.226
20	−0.131	0.239	−0.167	0.153
21	−0.190	0.379	−0.159	0.305
22	−0.232	0.289	−0.122	0.083
23	−0.027	0.261	−0.218	0.201
24	−0.098	0.299	−0.200	0.207
25	−0.300	0.472	−0.178	0.166
26	−0.294	0.475	0.006	0.099
27	−0.270	0.466	−0.204	0.242
28	−0.104	0.228	−0.052	0.191
29	−0.040	0.144	−0.018	0.115
30	−0.235	0.459	−0.221	0.244
31	−0.292	0.466	−0.209	0.251
Mean	−0.109	0.306	−0.102	0.224
All[1]	−0.227	0.357	−0.067	0.086

BS, Eggshell breaking strength; Gen, Generation; NDD, Non-destructive deformation; N/A, Not available.
[1] The values in this row represent the correlation coefficient calculated using all of the records (the entire data set for each line).

eggshell strength increased. From an initial value of 71.06% for generation 1, the shape index had increased to 74.15% by generation 31. Use of the entire data set, encompassing all generations, gave a correlation coefficient between non-destructive deformation and shape index of −0.227. This trend was illustrated even more clearly by the phenotypic correlation between eggshell breaking strength and shape index: all of the generations independently displayed a moderately strong positive correlation (with a mean of 0.306), indicating that somewhat round eggs were stronger than eggs with a more elongated end; for the entire data set, the correlation coefficient between the two traits was 0.357. Selection for strong eggshells through non-destructive deformation therefore made the eggs more round. This result is also illustrated by the relationship between eggshell breaking strength and shape index: the regression coefficient calculated for the strong line showed that for a 1-kg increase in eggshell breaking strength, the shape index could be expected to increase by 1.2 ($P < 0.0001$), predicting eggs with a shape index of 76% at a breaking strength of 5.97 kg. It is not possible from these results to make conclusive comments about the exact relationship between egg shape and breaking strength for eggs whose shape index is above 76%, given that the data from this experiment does not include that range of values. Nevertheless, these findings are in line with the results of Altuntaş and Şekeroğlu (2008), who found that greater force is required to break eggs with high shape index values.

The results observed in the weak line, for which the selection process decreased egg shape index –i.e. made the eggs more ovoid– are consistent with those in the strong line. The generation mean of the phenotypic correlation between non-destructive deformation and shape index was −0.102, whereas between eggshell breaking strength and shape index it was 0.224.

There were also interesting phenotypic correlations between the length of each axis and the traits related to eggshell strength (non-destructive deformation and eggshell breaking strength). As indicated in Table 2, in the strong line, within each generation, the correlation coefficient between eggshell breaking strength and egg width was positive, whereas it was negative between eggshell breaking strength and egg length. This confirms the results described above in which, within each generation, rounder eggs were stronger. Nevertheless, for the entire data set, encompassing all of the generations, the correlation coefficient between eggshell breaking strength and egg width was negative, indicating that over the course of the experiment, egg width decreased as breaking strength increased. Although this result may sound paradoxical, it indicates that, despite the positive correlation observed in each generation, the natural pressure to reduce egg size (and thereby egg width) described above was not counterbalanced by selection for stronger eggshell only.

The same trend was found in the weak line. At the generational level, weaker eggshell was correlated with a greater egg length; indeed, the correlation coefficient

back towards lower values for egg size. It is indeed unlikely that the selection process would influence both lines so distinctly given the lack of evidence of a correlation between egg size –expressed as egg weight– and eggshell breaking strength (De Ketelaere *et al.*, 2002).

From this perspective, we next analyzed the effect of the selection process on egg shape index. Table 1 shows the phenotypic correlation between non-destructive deformation and shape index, as well as between eggshell breaking strength and shape index, in both lines.

In the strong line, 26 of 30 generations displayed a negative relationship between non-destructive deformation and shape index. This implies that shape index increased as the value observed for non-destructive deformation decreased, meaning that the eggs became more round as

Table 2.   **Phenotypic correlation between axis length and traits related to eggshell strength**

	Strong line				Weak line			
	NDD		BS		NDD		BS	
Gen	EW	EL	EW	EL	EW	EL	EW	EL
1	N/A	N/A	0.178	−0.015	N/A	N/A	0.178	−0.015
2	0.041	0.067	0.186	−0.093	−0.090	−0.063	0.204	0.007
3	−0.002	0.107	0.253	−0.135	0.024	0.112	0.095	−0.163
4	0.077	0.177	0.129	−0.231	−0.014	−0.099	0.316	0.042
5	0.017	0.113	0.161	−0.165	−0.058	−0.010	0.296	−0.085
6	0.074	0.161	0.115	−0.133	−0.070	0.087	0.266	−0.137
7	0.146	0.052	0.090	−0.188	−0.054	0.101	0.186	−0.215
8	0.187	0.085	0.046	−0.198	−0.125	−0.047	0.325	0.028
9	0.080	0.106	0.170	−0.241	−0.122	0.144	0.250	−0.180
10	−0.011	0.016	0.218	−0.084	0.031	−0.024	0.196	−0.072
11	0.122	0.202	0.089	−0.280	0.009	0.061	0.270	−0.093
12	0.161	0.204	0.158	−0.256	−0.067	0.001	0.279	−0.050
13	−0.092	0.217	0.262	−0.266	0.180	0.100	0.013	−0.148
14	0.112	0.035	0.088	−0.197	−0.200	0.021	0.366	−0.040
15	−0.065	0.119	0.342	−0.218	0.029	0.063	0.153	−0.120
16	0.079	0.171	0.263	−0.050	−0.004	0.200	0.094	−0.125
17	0.005	0.052	0.133	−0.079	0.026	0.149	0.091	−0.178
18	0.189	0.122	0.085	−0.078	−0.092	0.031	0.267	−0.153
19	0.053	0.140	0.178	−0.075	−0.044	0.142	0.063	−0.195
20	0.141	0.234	0.075	−0.178	0.134	0.294	−0.159	−0.296
21	0.009	0.229	0.187	−0.249	0.227	0.329	0.021	−0.302
22	0.015	0.261	0.205	−0.151	0.142	0.354	−0.111	−0.258
23	0.110	0.119	0.144	−0.118	−0.100	0.159	0.245	−0.023
24	0.047	0.147	0.064	−0.245	−0.078	0.166	0.177	−0.107
25	−0.049	0.319	0.178	−0.404	0.095	0.257	0.006	−0.168
26	−0.075	0.255	0.145	−0.398	0.134	0.115	0.066	−0.065
27	−0.065	0.259	0.294	−0.278	0.106	0.330	0.055	−0.236
28	−0.014	0.097	0.118	−0.146	0.103	0.141	−0.014	−0.237
29	−0.103	−0.037	0.175	−0.007	0.213	0.217	−0.058	−0.173
30	−0.127	0.165	0.362	−0.218	−0.103	0.169	0.108	−0.190
31	−0.095	0.264	0.180	−0.385	−0.122	0.180	0.228	−0.169
Mean	0.032	0.149	0.170	−0.186	0.004	0.123	0.144	−0.133
All[1]	0.202	0.376	−0.205	−0.488	−0.309	−0.196	0.377	0.225

BS, Eggshell breaking strength; EL, Egg length; EW, Egg width; Gen, Generation; NDD, Non-destructive deformation; N/A, Not available.
[1] The values in this row represent the correlation coefficient calculated using all of the records (the entire data set for each line).

between eggshell breaking strength and egg length was negative (−0.133 on average), suggesting that eggs became more elongated as eggshell strength decreased. Nevertheless, for the whole data set, the correlation coefficient was positive (0.225), indicating on the contrary that as breaking strength decreased, so did egg length, putting pressure on the shape index to increase. In other words, even though over the entire experiment selection for weak eggshell was expected to make egg length increase (and the shape index decrease) to remain consistent with the observations made at a generational level, the effect of natural selection to reduce egg size was stronger, and thus reduced egg length.

Breeding Values and Genetic Parameters

Breeding values were estimated with BLUP methodology. In both lines, the evolution of generation means over the course of the experiment is presented for the following traits: non-destructive deformation, egg width, egg length, and shape index (Fig. 2).

The selection process for non-destructive deformation successfully formed two lines, with an apparent asymmetry in the selection response. This asymmetry was also visible in the changes in the breeding values of the shape index over time. In the strong line, the generation mean for shape index increased gradually, with small ups and downs, to produce at generation 31 individuals whose offspring were expected to lay eggs with a shape index of 71.91%. In the weak line, the generation mean sharply decreased in the first ten generations before increasing again and stabilizing at 69.78%. The value of 71.91% estimated in the strong line is consistent with previous indications that the ideal value of shape index likely lies in the 70% to 80% range (Havlíček et al., 2008).

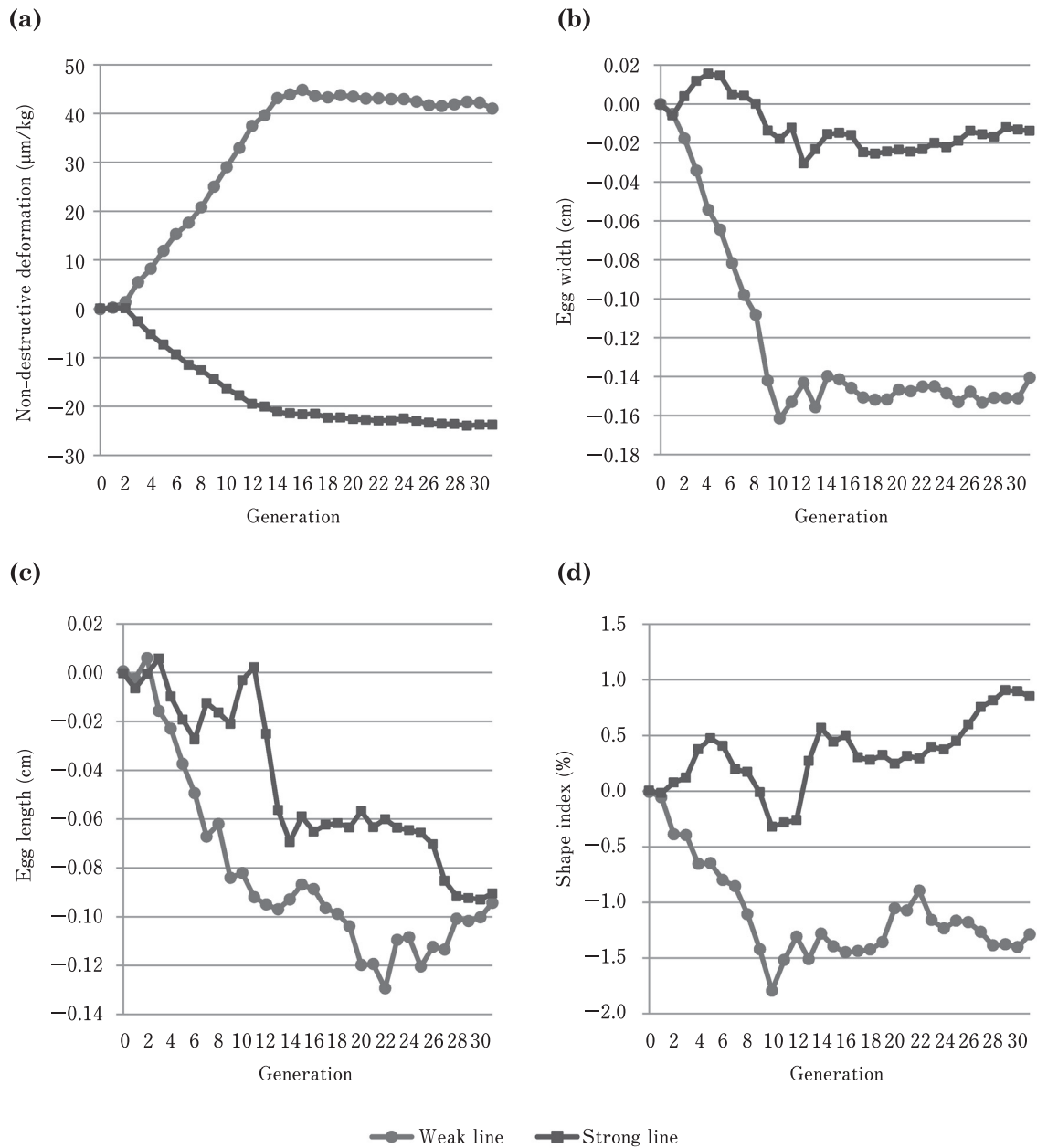In the strong line, it can be inferred from Figure 2 that the

(a)

(b)

(c)

(d)

Weak line   Strong line

Fig. 2.   **(a) Generation means of the estimated breeding values for non-destructive deformation for the weak and strong lines. (b) Generation means of the estimated breeding values for egg width for the weak and strong lines. (c) Generation means of the estimated breeding values for egg length for the weak and strong lines. (d) Generation means of the estimated breeding values for shape index for the weak and strong lines.**

Table 3.   Genetic correlation between eggshell breaking strength and traits related to egg shape, calculated as the Pearson product-moment correlations between the breeding values

	Strong line			Weak line		
Gen	EW	EL	SI	EW	EL	SI
0	0.369	0.011	0.318	0.489	0.268	0.252
1	0.292	0.036	0.236	0.392	0.240	0.188
2	0.149	−0.093	0.218	0.376	0.274	0.055
3	0.292	−0.031	0.237	0.308	0.090	0.209
4	0.243	−0.134	0.366	0.544	0.210	0.270
5	0.151	−0.117	0.226	0.548	0.135	0.370
6	0.235	0.194	0.026	0.567	0.212	0.271
7	0.106	−0.072	0.179	0.482	−0.024	0.423
8	−0.012	−0.163	0.224	0.640	0.203	0.546
9	0.028	−0.282	0.359	0.554	0.077	0.544
10	0.268	−0.186	0.447	0.471	0.308	0.231
11	0.010	−0.320	0.383	0.538	0.176	0.411
12	0.298	−0.239	0.456	0.412	0.160	0.321
13	0.307	−0.121	0.356	0.214	0.074	0.154
14	0.224	0.032	0.144	0.492	0.189	0.361
15	0.457	0.061	0.275	0.271	0.056	0.254
16	0.245	0.052	0.149	0.188	0.029	0.183
17	0.103	0.074	−0.008	0.323	0.174	0.150
18	0.057	0.082	−0.041	0.493	0.090	0.354
19	0.095	0.145	−0.084	0.396	0.014	0.293
20	0.230	0.054	0.154	0.135	−0.234	0.306
21	0.337	−0.017	0.347	0.020	−0.171	0.167
22	0.348	0.026	0.292	0.102	−0.127	0.190
23	0.170	0.085	0.102	0.406	0.185	0.160
24	0.070	−0.022	0.115	0.454	0.134	0.179
25	0.053	−0.368	0.425	0.297	0.168	0.103
26	0.303	−0.147	0.410	0.329	0.289	0.006
27	0.391	−0.001	0.366	0.335	0.266	0.037
28	0.426	0.094	0.293	0.276	0.161	0.099
29	0.440	0.236	0.127	0.286	0.078	0.199
30	0.544	0.156	0.345	0.382	0.114	0.216
31	0.356	−0.199	0.487	0.478	0.151	0.235
Mean	0.237	−0.037	0.248	0.381	0.124	0.242
All[1]	−0.082	−0.282	0.218	0.678	0.426	0.363

EL, Egg length; EW, Egg width; Gen, Generation; SI, Shape index.
[1] The values in this row represent the correlation coefficient calculated using all of the records (the entire data set for each line).

increase in shape index was due mostly to a decrease in egg length, whereas egg width remained relatively stable. In this line, the overall genetic correlations, calculated as the Pearson product-moment correlations between the breeding values, were −0.082 between eggshell breaking strength and egg width, and −0.282 between eggshell breaking strength and egg length (Table 3). Nevertheless, as with the phenotypic correlations, the genetic correlation within each generation between eggshell breaking strength and egg width was on average positive (0.237), confirming the hypothesis that stronger eggs tended to be more round. In addition, the genetic correlation of shape index with non-destructive deformation was −0.113, whereas with eggshell breaking strength it was 0.218.

In the weak line, the observed decrease in shape index appeared mostly due to the substantial drop in egg width (Fig. 2). The genetic correlations were 0.678 between eggshell breaking strength and egg width, and 0.426 between eggshell breaking strength and egg length. For shape index, the genetic correlation was −0.296 with non-destructive deformation and 0.363 with eggshell breaking strength.

Genetic correlations were also calculated together with heritabilities by REML using variance/covariance component correlations (Table 4). Between eggshell breaking strength and shape index, the genetic correlation coefficients were 0.285 (±0.055) for the strong line and 0.244 (±0.055) for the weak line, whereas the genetic correlations between non-destructive deformation and shape index were −0.021

Table 4. **Heritability (±standard error), genetic correlation (±standard error), and phenotypic correlation (±standard error) estimates calculated using REML, for the five traits studied (Generation 1 to 31). For each line, the heritability estimates are indicated by the diagonals elements, the genetic correlations by the entries above the diagonal, and the phenotypic correlations by the entries below the diagonal.**

Strong line					
Trait	NDD	BS	EW	EL	SI
NDD	0.381±0.033	−0.771±0.032	0.039±0.059	0.059±0.060	−0.021±0.063
BS	−0.676±0.009	0.349±0.029	0.224±0.055	−0.097±0.057	0.285±0.055
EW	0.031±0.019	0.180±0.017	0.628±0.026	0.436±0.039	0.416±0.039
EL	0.121±0.019	−0.168±0.017	0.364±0.016	0.605±0.026	−0.636±0.028
SI	−0.089±0.019	0.303±0.016	0.450±0.015	−0.666±0.010	0.544±0.027

Weak line					
Trait	NDD	BS	EW	EL	SI
NDD	0.408±0.031	−0.851±0.021	−0.137±0.055	−0.039±0.060	−0.093±0.060
BS	−0.763±0.008	0.468±0.032	0.332±0.050	0.057±0.058	0.244±0.055
EW	−0.033±0.019	0.198±0.018	0.628±0.025	0.404±0.042	0.520±0.037
EL	0.077±0.019	−0.083±0.019	0.300±0.017	0.519±0.028	−0.571±0.034
SI	−0.096±0.018	0.229±0.018	0.513±0.014	−0.663±0.011	0.484±0.028

BS, Eggshell breaking strength; EL, Egg length; EW, Egg width; NDD, Non-destructive deformation; SI, Shape index.

(±0.063) and −0.093 (±0.060), respectively. Regardless of the method used, the genetic correlation of shape index with non-deformation appeared smaller than that with eggshell breaking strength.

In the strong line, the heritability estimates were 0.381 (±0.033) for non-destructive deformation, 0.349 (±0.029) for eggshell breaking strength, 0.628 (±0.026) for egg width, 0.605 (±0.026) for egg length, and 0.544 (±0.027) for shape index. In the weak line, the respective heritability estimates were 0.408 (±0.031), 0.468 (±0.032), 0.628 (±0.025), 0.519 (±0.028), and 0.484 (±0.028). The moderately high values observed for shape index, which were slightly higher than those found by Singh *et al*. (2000) in a commercial strain of White Leghorns (0.459±0.104), indicate the potential for improving egg shape through genetic gain.

Conclusion

Although we know that egg shape is a determinant of eggshell strength, much is still unknown about the exact relationship between these two traits, especially given that differences in shape exist among breeds.

The results of this two-way selection experiment for non-destructive deformation conducted in White Leghorns over 31 generations showed that selection for eggshell strength effectively influenced the shape index. The average shape index in generation 1 was 71.06%; the analysis of the breeding values revealed that, in the strong line, the individuals of generation 31 were expected to produce offspring with significantly rounder eggs, with an average shape index of 71.91%. Furthermore, the genetic correlation coefficient between eggshell breaking strength and shape index, as estimated using REML methodology, was 0.285±0.055 for

the strong line and 0.244±0.055 for the weak line. These coefficients indicate that rounder eggs tended to be more resistant than more elongated eggs to breakage for the range of values studied. The moderately high heritability estimates for shape index found in this experiment (0.544±0.027 in the strong line and 0.484±0.028 in the weak line) indicate that it is possible to enhance shape index through genetic improvement. Additional studies on other breeds are however needed to better understand the relationship between eggshell strength and egg shape.

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